EFFECT OF PHYSICAL AND NUTRITIONAL STRESSES ON GROWTH BIOPIGMENT AND LIPID CONTENT OF *DUNALIELLA SALINA* TEOD

[1]Priyanka Sharma*, [2]Dr. Komal Lata Nagpal

[1]Research Scholar, [2]Supervisor

[1-2]Department of Botany, SunRise University, Alwar, Rajasthan, India

Email ID: priyankakataria.pk.pk@gmail.com

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Abstract

The present study deals with the green algae Dunaliella salina isolated from Sambhar salt lake, Rajasthan. This study is being carried out with an object to optimization of inorganic media, physical stresses like salinity, pH, temperature and light intensities along with nutritional stresses like nitrogen (nitrate), phosphorus (phosphate), sulphur (sulphate) on growth, biopigments and lipid accumulation in D. salina.

Paper Identification

Introduction

Microalgae are considered as a solution to many of the environmental problems we face now a days. Microalgae represent a unique experimental system to study stress responses of photosynthetic organisms. The study of stress physiology and adaptation of microalgae also has an important application in further development of the biotechnology for mass culturing of microalgae. Microalgal growth is highly dependent on the environmental conditions where the variables that affect the growth rates are different from one species to another. However, the most studied variables are salinity, pH, temperature, light as well as the nutrition. Combination of several environmental stresses in one culture medium could become a novel strategy for enhancing the metabolites production during single growth cycle.

The unicellular green alga (Chlorophyceae) Dunaliella salina is among the most widely spread eukaryotic organism in hypersaline environments. It's physiological responses to different environmental conditions have attracted a great deal of scientific interest. The halophytic properties of Dunaliella confer to this alga an important advantage for outdoor cultivation. Dunaliella salina is considered as one of the best commercial source of natural carotenoids in the world. Among eukaryotic microalgae, the relatively unique ability to accumulate carotenoids in response to stress has made this alga the star of the algal biotechnology.

Moreover, the unique feature of Dunaliella cells is that it lacks rigid cell wall, a feature distinguishing them from other unicellular green algae. Presence of tough cell wall increases the costing of extraction and purification of pigments. On the other hand absence of cell wall in Dunaliella makes disruption of cells much easier than other algae. These traits make Dunaliella salina an attractive "cell factory" for the commercial production of carotenoids. So above described beneficial properties of Dunaliella salina clearly indicate that the alga possess inherent abilities. The ecological adaptation and flexibility make Dunaliella salina as a promising candidate for commercial exploitation.

The algal cultures of Dunaliella were purified and maintained as unialgal cultures in Department of Botany, University of Rajasthan, Jaipur. The cultures were grown in suitable culture medium. Various experiments were also conducted to optimize nutrient medium, various stresses like salinity, pH, temperature, light intensities, nitrogen (nitrate), phosphorus (phosphate) and sulphur (sulphate) to obtain high biomass and efforts were also made to enhance the biopigments and total lipid content. The results obtained from this study have been summarized as follows.

1. Evaluation of inorganic requirement for optimum growth of D. salina

The establishment of suitable nutrient medium is prime requirement for raising the cultures of the algae. It is very interesting that growth of algae is effected by chemical composition of various inorganic media used in the culture. Nutrient requirements of Dunaliella salina has been worked out employing four inorganic media varying in their chemical composition and pH. Among various inorganic media tested for D.salina, the best growth was found in Artificial Sea Water medium followed by De Walne's, Modified Johnson's respectively and least growth was observed in Bold's Basal medium. Algal growth in above mentioned media was assessed by optical density, dry weight and growth rate of the cultures and these parameters also supported these findings.

At the end of Vth week of experiment the accumulated biopigments (chlorophyll and carotenoids) and total lipid content evaluated. Maximum accumulated biopigment, total chlorophyll (1.625%), carotenoids content (1.443%) and total lipid content (15.5%dcw) were observed in Artificial Sea Water Medium followed by De Walne's and Modified Johnson's media respectively. While least biopigments accumulation, total chlorophyll content (1.405%), carotenoids content (0.524%) and lipid content (9.06%dcw) were recorded in Bold's Basal medium.

Hence, Artificial Sea Water medium for D.salina not only rendered optimum growth but also improved the pigments and lipid content of the cultures.

2. Impact of different physical stresses on growth, biopigments and lipid content in D.salina

a. Effect of salinity stress

Salinity has diverse effects on species and varies greatly across habitats. NaCl is an inexpensive and readily available source of nutrients, adjusting the concentration of NaCl. Microalgal species can tolerate salinity stress up to an extent. D.salina shows a remarkable degree of adaptation to a wide variety of salt concentrations, which makes this alga the most halotolerant eukaryote known. Its extreme salinity tolerance simplifies the maintenance of unialgal cultures, free of competitors, pathogens and predators.

In our present investigation impact of NaCl concentration on the growth, biopigments and lipid content of an algal species Dunaliella salina has been

evaluated. Dunaliella salina was exposed to different concentrations of NaCl ranging from 1.0 M to 5.0 M along with control over a period of 30 days. Salt concentration clearly affected growth pattern in Dunaliella salina. It was found that the algal biomass yield was highest at 2 M NaCl concentration as compared to control and then it subsequently decreases with increase in NaCl concentration. Highest growth in terms of optical density dry weight and growth rate at 2 M concentration of NaCl, followed by at 1M, 3M, 4M respectively and the least growth was observed at 5M concentration of NaCl.

Further biopigments (chlorophyll) accumulation in Dunaliella salina also correlates with the growth of the cultures. Total chlorophyll content of the algal species decreases as the salt concentration was increased when compared with control. Chlorophyll accumulation observations were also in favour of growth record. Maximum total chlorophyll content was recorded by cultures grown at 2M salinity (i.e. 1.692 %), followed by at 1 M, 3M, 4M respectively and the least (i.e. 0.890%) observed at 5M salinity. Salinity stress can further stimulate the production of total carotenoids. So it is interesting to note that carotenoids content increased with increase in concentrations of NaCl as compared to control for the culture studied. Maximum carotenoids content were found in cultures grown at 4M salinity (3.561%), followed by at 3M, 2M, 1M and minimum was observed at 5M salinity (0.501%). Moreover total lipid content at different salt concentration correlates with the carotenoids accumulation. Maximum lipid contents were found in cultures grown at 4 M salinity (35.4% dcw), followed by at 3M, 2M, 1M respectively and minimum was observed at 5M salinity (5.10%dcw).

In conclusion the effect of various concentrations of NaCl on the microalga Dunaliella salina showed, increased biomass yield at 2M NaCl concentration as compared to control and then it subsequently decreases with increase in NaCl concentration. Further Total chlorophyll content decreased with higher concentrations of NaCl as compared to control for the culture studied. While carotenoids along with lipid content increases with increase in NaCl concentration. Initial increase of NaCl concentration from 1M to 4 M increased the lipid accumulation from 5.81 to 9.13% dcw. These beneficial properties indicated that, adaptation of the alga to salinity was characterized by the accumulation of carotenoids and lipids.

b. Effect of pH stress

The hydrogen ion concentration (pH) of the culture medium is one of the most important factors that seriously affect the optimal growth of algal cultures. pH is very important for the character of metabolism of microorganisms and hence for the biosynthesis of the bioactive products as secondary metabolites. Dunaliella species have a wide range of pH tolerance from 0 to 11 but the optimum pH for D. salina is between 8 and 11. In our present investigation to observe the effect of different pH regimes on the growth, biopigment composition and lipid content of D. salina. In this study cells of D.salina were transferred from pH 6 to pH 10 along with the control in the ASWM (ARTIFICIAL SEA WATER MEDIUM).

Wide range of pH tested, the highest growth was obtained at pH 8 as compared to control. At extremes of pH, i.e., pH 6 and 10, a sharp decline in growth of algal cell was observed. Highest growth rate was recorded at pH 8 followed by pH 7, pH 6, pH 9 respectively and in comparison to all the pH regimes tested, pH 10 was found least effective in promoting the growth of D. salina. The least growth rate of alga was recorded at this pH value.

With regard to the pigment accumulation in D.salina subjected to a pH range, pH 8 exhibited the highest accumulation values of total chlorophyll content. The chlorophyll content significantly decreased as the pH shifted towards the acidic or alkaline sides. The lowest values of the total chlorophyll pigment were attained at pH 10. The maximum biopigment (total chlorophyll) content were found at 8 pH value as compared to control. Total chlorophyll content was recorded in culture grown at 8 pH (1.58%) followed by pH 7, pH 6, pH 9 and least chlorophyll content was recorded at pH 10 (0.63%). On the contrary, the accumulation of total Carotenoids content was observed highest value at 9 pH (2.648%) as compared to control, followed by pH 8, 6 pH, 7 pH and least carotenoids was found at pH 10 regime (0.252%).

Furthermore, the data obtained concerning the effect of pH on lipid contents of D. salina revealed that pH 9 seems to be the most suitable pH for the accumulation of lipids in Dunaliella cells. At acidic or alkaline pH values, the contents of total lipids significantly increased. In the range of pH studied, pH 10 recorded the lowest values of lipid content in the cells of this micro alga. The maximum total lipid content was calculated at 9 pH (28.9%dcw) as compared to control, followed by 8 pH, 6 pH, 7 pH and least lipid content was found at pH 10 regime (4.33%dcw).

c. Effect of temperature stress

The effect of temperature on biochemical reactions makes it one of the most important environmental factors influencing the biochemical composition of algae. It was also found that decreases in growth when temperature below the optimal range. In addition, low temperatures induce cellular accumulation of polyols and amino acids or amino acid derivatives as compatible solutes, which might contribute to the sensitivity or tolerance of microalgae to chilling. Temperature plays an important role in the accumulation of carotenoids within microalgal cells. Higher temperatures result in increased accumulation of carotenoids in microalgal species due to increased photo-oxidative stress. Both low and high temperatures are preferred for attaining higher lipid profiles, depending on the species. A decrease in growth temperature below an optimal level generally increases the degree of unsaturation of lipids in membrane systems.

In our present investigation to evaluate the influence of different temperatures on the growth, biopigment accumulation and lipid content of Dunaliella salina. To observe the influence of temperature on growth biopigments and lipid accumulation, the cultures were subjected to different temperatures i.e. from 20oC to 40oC (at an interval of 4o C) at 2500 lux light intensity.

The optimum temperature for growth of D. salina was around 28oC as compared to control. There was a significant decrease in growth with increasing in temperature. It attained maximum growth at 28oC, followed by at 32oC, 24oC, 20oC, 36oC respectively and least growth was observed at 40oC. Dunaliella salina showed a wide range of temperature from 20oC to 40oC and at 40oC growth was dropped down.

In biopigment accumulation total recorded chlorophyll content were also in favor of growth record. Maximum chlorophyll contents were observed by cultures grown at 28oC as compared to control. Higher temperature had opposite effects on total chlorophyll accumulation so it is found increasing temperature, caused chlorophyll content to decrease. Total chlorophyll content was recorded in culture grown at 28oC (1.62%) followed by 32oC, 24oC, 20oC, 36oC and least

chlorophyll content was recorded at 40oC (0.82%). On the contrary, the maximum accumulation of carotenoids content was found in cultures grown at 36oC as compared to control. Carotenoids content showed an increasing pattern with increase in temperature. Highest carotenoids was observed at 36oC (2.035%), followed by 32oC, 20oC, 24oC, 28oC and least carotenoids was found at 40oC (0.202%). Total lipid content at different temperature, correlates with the carotenoids accumulation. Maximum Lipid content was found cultures grown at 36oC (27.5%dcw) followed by 32oC, 20oC, 24oC, 28oC and least lipid content was observed in cultures grown at 40oC (4.11%dcw).

Further, it seems that in many microalgal species temperature changes from low to high increases the lipids productivity. High temperature also favors biofuel properties. Therefore, temperature is a crucial stress factor to be taken in to account for optimizing lipids and biomass productivity.

d. Effect of light intensities

Light is not only the primary source for photosynthesis energy conversion but also an essential regulatory factor for microbial growth. Effects of light on biochemical composition of photosynthetic algae are largely controlled by the process called photoacclimation or photoadaptation. Light intensity as well as photoperiod affect growth, biomass, carotenoids, lipids and other metabolites of interest in diverse microalgae species.

In our present investigation Dunaliella salina was undertaken to evaluate the different light intensity conditions for growth, biopigment accumulation and lipid content. To observe the influence of light intensity on growth pigment and lipid accumulation, the cultures were subjected to different light intensities i.e. from 800 lux to 4800 lux (at interval of 800 lux) at a temperature of 25±2oC. The growth and growth rate of D.salina cultures steadily depends on light irradiances. At low light intensities, the growth of cultures increases but further at higher irradiances, the growth was very slow and gradual decline in growth rate was recorded. Highest growth was shown at 2400 lux as compared to control followed by cultures grown at 1600 lux, 800 lux, 3200 lux, 4000 lux and least growth rate was found in cultures at grown at 4800. After 4000 lux or further increase in light intensities, sharp reduction in growth was calculated.

The effect of light intensities on pigment accumulation of D.salina was illustrated. There was found an inverse relationship between chlorophyll content and light intensities. Total chlorophyll content was also favors of growth records. Maximum chlorophyll contents were observed by cultures grown at 2400 lux light intensities (i.e. 1.60%) followed by cultures grown at 1600 lux, 800 lux, 3200 lux, 4000 lux and minimum chlorophyll content was found in cultures at grown at 4800 (0.80%). On the other hand, carotenoids contents showed a different trend, a stimulating impact of light intensities on carotenoids accumulation was clearly observed. Carotenoids increased with increasing light intensities, being maximum at 4000 lux (2.205%) followed by 3200 lux, 2400 lux, 1600 lux, 800 lux and least carotenoids content was observed in cultures grown at 4800 lux (0.20%). Further total lipid content at different light intensities, correlates with the carotenoids accumulation. Lipid accumulation increases at increased light irradiance. Maximum Lipid content was found cultures grown at 4000 lux (25.8% dcw) followed by 3200 lux, 2400 lux, 1600 lux, 800 lux and least lipid content was observed in cultures grown at 4800 lux (4.02 % dcw).

However in response to high light intensity chlorophyll and other pigments directly involved in photosynthesis decrease, while the secondary carotenoids which serve

as photoprotective agents, increase. Further strong light intensity was observed to increase the total lipid accumulation in D.salina. It may be feasible to use high light intensities for enhanced production of lipids, biomass as well as suitable fatty acid profile for improving biofuel potential. Lipids productivity is therefore found to be influenced under high light stress. A serious concern is that the cells experience photoinhibition at high light. This strategy may improve the overall biomass and lipid productivity and addresses appropriately the concern of photoinhibition.

3. Impact of different nutritional stresses on growth, biopigments and lipid content of D.salina

Nutritional stress

Nutrient availability has a significant impact on growth and propagation of microalgae and broad effects on their biochemical composition. When the nutritional requirements of mass cultured algae are satisfied and the environmental conditions are not growth-limiting, mixing designed to create a turbulent flow constitutes the most important requisite for consistently obtaining high yields of algal mass.

a) Effect of nitrogen stress (as in nitrate form)

Of all the macronutrients in the culture medium, nitrogen, which accounts for 1–10% of the total dry matter in microalgae is quantitatively the most important nutrient affecting growth and lipid accumulation in various algae. A variety of organic N compounds are utilized by algae, several of which can serve as the only source of N. Ammonia nitrogen is often the preferred N-source for microorganisms and the assimilation of either NO3+ or NH + is related to the pH of the growth media. When ammonia is used as the sole source of N, the pH could drop significantly during active growth, due to the release of H+ ions.

Absence or low levels of nutrients including nitrogen stimulate rapid physiological responses, which further trigger the secondary biosynthetic pathways. Until cell nitrogen falls below a threshold value, photosynthesis still continues, though at a reduced rate. The flow of carbon, fixed in photosynthesis, under these circumstances is diverted from the path of protein synthesis to that leading to either lipid-or carbohydrate synthesis. Thus, nitrate levels must be controlled throughout the growth in culture to maintain the nutrient contents of a medium stable and thereby prevent damage to the cell membrane, loss of biomass, and other changes in cell composition.

In our present investigation different concentration of Nitrogen (as nitrate), viz. Low, moderate and high along the control was experimented upon. To evaluate nutritional stresses (nitrate) for the growth, biopigments and lipid content of D.salina, the microalga was grown in normal media(ASWM) further treated with excess concentration of nitrate (8 mM of KNO3), moderate concentration of nitrate (5mM of KNO3) and low concentration of nitrate /nitrogen starvation stress (2mM of KNO3).

The growth pattern increased with increasing Nitrogenous nutrient in media, which demonstrate that this alga preferred elevated concentration of Nitrate nutrients rather than low concentration. Highest growth rate (i.e. 0.320 divisions/day) was recorded in the fifth week of the experimental period, at excess concentration (8 mM) of Nitrate, followed by Moderate concentration(5 mM) of nitrates (0.252 divisions/day) and least growth rate was recorded at Low concentration of nitrate (2 mM) and it was 0.012 divisions/day.

The Biopigment composition of algae correlates with the growth of D.salina. Maximum biopigment chlorophyll content was found at excess concentration

(8mM) of nitrate. Total chlorophyll content was recorded in culture grown in excess concentrations of nitrate (1.68%) followed by moderate concentration (5mM) of nitrate (1.63%) and least chlorophyll content was recorded at the deprived condition (2mM) of nitrate (i.e. 0.12%).While Total carotenoids content was observed highest value in deprived condition (3.506%), followed by excess concentration of nitrate nutrients (2.812%) and least carotenoids was found at a moderate concentration of nitrate (1.855%). Further total lipid content of algae was calculated at different nitrogenous component concentrations (excess nitrate, moderate nitrate, and deprived condition of nitrate). Highest lipid content was observed in deprived conditions of nitrate and it was (31.5%dcw), followed by excess concentration of nitrate (25.4%dcw) and least lipid content was found at a moderate concentration of nitrate (20.3%dcw).

Thus slightly high concentrations of nitrates supported the biomass, growth and chlorophyll content of the alga while reduced the carotenoids and lipid accumulation. And under nutrients deprivation negative effects exhibit on growth and total chlorophyll content while positive effects recorded on carotenoids accumulation and lipid contents. To reduce the negative influence of nitrate starvation on resulting growth, intermediate nitrate concentrations can be applied.

b) Effect of phosphate stress (as in phosphate form)

Phosphorus has a significant impact on the growth and metabolism of microalgae. Phosphorous makes up considerably less than 1% of total algal biomass (~0.03–0.06%) and yet is an essential component in the medium for sustainable growth and development of microalgae. Absence of phosphorus in a medium results in repression of photosynthesis and affects the growth of microalgae. However, phosphorus has a significant impact on the growth and metabolism of microalgae.

In our present investigation to evaluate nutritional stresses (phosphate) for the growth, biopigments and lipid content of D.salina, the microalga was grown in normal media(ASWM) further treated with excess concentration of phosphate (0.5 mM of KH2PO4), moderate concentration of phosphate (0.2 mM of KH2PO4) and low concentration of phoshate/phosphorus starvation stress (0.05 mM of KH2PO4).

The growth pattern increased with increasing phosphorus nutrient in media, which demonstrate that this alga preferred elevated concentration of phosphate nutrients rather than low concentration. Highest growth rate (i.e. 0.202 divisions/day) was recorded in the fifth week of the experimental period, at excess concentration (0.5mM) of phosphate followed by moderate concentration (0.2 mM) of phosphate (0.175 divisions/day). While least growth rate was recorded at low concentration of phosphate (0.05 mM) and it was 0.020 divisions/day.

The Biopigment composition of algae correlates with the growth of D.salina. Maximum biopigment chlorophyll content was found at excess concentration (0.5mM) of phosphate. Total chlorophyll content was recorded in culture grown in excess concentrations of phosphate (1.62%) followed by moderate concentration (0.2 mM) of phosphate (1.45%) and least chlorophyll content was recorded at the deprived condition (0.05 mM) of phosphate (i.e. 0.15%). While Total carotenoids content was observed highest value in deprived condition (2.125%), followed by excess concentration of phosphate nutrients (1.216%) and least carotenoids was found at a moderate concentration of phosphate (0.251%). Further Total lipid content of algae was calculated at different

phosphorus component concentrations (excess, moderate, and deprived condition of phosphate). Highest lipid content was observed in deprived conditions of phosphate and it was (25.1%dcw), followed by excess concentration (18.4%dcw) and least lipid content was found at a moderate concentration of phosphate (15.5%dcw).

Thus slightly high concentrations of phosphate promote the biomass, growth and chlorophyll content of the alga while reduced the carotenoids and lipid accumulation. And under low concentration of phosphate or phosphate starvation shows adverse effects on growth and total chlorophyll content while positive effects recorded on carotenoids accumulation and lipid contents. To reduce the negative influence of phosphate starvation on resulting growth, intermediate phosphate concentrations can be applied.

c) Effect of sulphur stress (as in sulphate form)

The oceans represent huge reservoirs of sulphur as dissolved sulphate, with typical concentrations around 29 mM. Therefore, in spite of the high and invariant sulphur availability, marine algae must be ready to modulate their sulphur acquisition and metabolism to respond to disturbances in the interactions between sulphur-related pathways and the rest of the cell activities. Aquatic environments are very different from one another with respect to sulphur content: while in the oceans sulphate concentration is constantly high, freshwaters are characterized by daily and seasonal variations and by a wide range of sulphur concentration.

In our present investigations to evaluate nutritional stresses (sulphate) for the growth, biopigments and lipid content of D.salina, the microalga was grown in normal media (ASWM) further treated with excess concentration of sulphate (8 mM of MgSO4), moderate concentration of sulphate (5mM of MgSO4) and low concentration of sulphate/sulphur starvation stress (2mM of MgSO4).

The results obtained from this experiments regarding the growth pattern of

D. salina cultured at different concentrations of sulphate nutrient tested (i.e., Excess, moderate & low). The growth pattern increased with increasing sulphur containing nutrient in media, which demonstrate that this alga preferred elevated concentration of sulphur nutrients rather than low concentration. Highest growth rate (0.280 divisions/day) was recorded in the fifth week of the experimental period, at excess concentration (8 mM) of Sulphate followed by moderate concentration (5 mM) of sulphate (0.214 divisions/day). While least growth rate of the alga was recorded at Low concentration of sulphate (2 mM) and it was 0.012 divisions/day.

The Biopigment composition of algae correlates with the growth of D.salina. Maximum biopigment chlorophyll content was found at excess concentration (8mM) of sulphate. Total chlorophyll content was recorded in culture grown in excess concentrations of sulphate (1.52%) followed by moderate concentration (5mM) of sulphate (1.46%) and least chlorophyll content was recorded at the deprived condition (2mM) of sulphate (i.e. 0.12%).While Total carotenoids content was observed highest value in deprived condition (3.104%), followed by excess concentration of sulphate nutrients (2.520%) and least carotenoids was found at a moderate concentration of sulphate (1.605%). Further Total lipid content of algae was calculated at different concentrations of sulphur containing component MgSO4 (excess, moderate and deprived condition of sulphate). Highest lipid content was observed in deprived conditions of sulphate and it was (22.8%dcw), followed by excess concentration of

sulphate (20.0%dcw) and least lipid content was found at a moderate concentration of sulphate (17.4%dcw).

Thus slightly high concentrations of sulphate promote the biomass, growth and chlorophyll content of the alga while reduced the carotenoids and lipid accumulation. And under low concentration of sulphate or sulphate starvation shows adverse effects on growth and total chlorophyll content while positive effects recorded on carotenoids accumulation and lipid contents. To reduce the negative influence of sulphate starvation on resulting growth, intermediate sulphate concentrations can be applied.

Furthermore, compared with all three nutrient components, on D.salina cultures, the concentrations of nitrogen (nitrate) had a maximum impact on algal growth, biopigments and lipid accumulation. In addition, the relative importance and abundance of Nitrate compared with Phosphate and Sulphate could explains that D. salina growth is more effective under (+N or –N), followed by phosphate concentration (+P or –P) and next one is sulphate concentration (+S or –S). However, Environmental stresses are a type of metabolic imbalance that requires biochemical and metabolic adjustments before a new state of growth can be established. Stressful conditions can stimulate the accumulation of carotenoids and lipid content in some microalgae. Dunaliella can adapt to environmental stresses by alter the various physiological mechanism.

In conclusion, Our results indicates that the moderate parameters of physical stresses like salt, pH, temperature and light intensities utilized in the experiments were directly related to support algal growth, biomass, photosynthesis mechanism and chlorophyll accumulation but at low and high dose of these stresses, inhibited growth rates and increased enzymatic defense and directly related to enhances the carotenoids and lipid accumulation in treated algal cells. On the other hand, when we treated algal cells with nutritional stress like nitrate, phosphate and sulphate we found that both excess and moderate concentrations of these nutrients promote better growth, biomass and chlorophyll content, while low concentration of any nutrient (nutrient starvation or deprivation) enhance the carotenoids accumulation and total lipid content in the algal cells.

As far as we know, the observations presented here provided a baseline as an aspect of relationship between accumulated carotenoids as well as lipid content and the physical likewise nutritional stresses. The enhancement achieved in biomass production, chlorophyll, carotenoids and lipid accumulation of algae by this study would be economically viable since the objective of algal biotechnology is to produce specific products which have more substantial commercial value and importance.

This research depicts some general trends of the cellular responses of microalgae, in terms of cell composition, to the major environmental factors, and then cause how manipulation of algal cultures with various environmental factors could achieve specific biotechnological goals.

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