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Biomass production and biochemical variability of marine microalgae *Isochrysis galbana* in relation to light intensity

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Abstract
Isochrysis galbana is a marine microalga that has a great potential as a source of animal feed, human nutrition and biofuel. Light intensity is one of the key limiting factors for the growth of dense culture of photosynthetic microalgae both in indoor and outdoor culture. In this study, we investigated the effect of different light intensity (35, 125 and 275 µmol photons m^{-2} s^{-1}) on the biomass production and its biochemical composition (protein carbohydrate, lipid and pigments). Growth is calculated daily by measuring optical density at 710nm and dry weight of filtered cells was incinerated at 550ºC in a muffle oven. The biochemical components protein, lipid, carbohydrate and pigment were measured by Lowry, Bligh and Dryer, Dubois and Strickland and Parsons Method respectively. In this study, reported culture (LL) grown under low light have maximum amount of protein content (44.73+0.50%DCW) and chlorophyll a (4.67+0.28mg/L) whereas culture (HL) grown under high light intensity were highest in the lipids contents (30.66 ± 0.30 % of DCW) and carbohydrates (16.06 ± 0.70% of DCW). Maximum accumulation of total carotenoids (4.89 ± 0.25 mg/L) reported in culture (HL) grown under high light and with decreased light carotenoids decreases. In conclusion result reported that varied light intensity have a significant impact on growth, dry weight and biochemical composition of *Isochrysis galbana* which have wide application in aquaculture, nutraceutical, pharmaceutical and biofuel industry.

Keywords: *Isochrysis galbana*, light intensity, biochemical composition, pigments

1. Introduction
Isochrysis galbana is one of the common marine algae have been used in mariculture to feeds for bivalves & larva of fish, crustaceans, mollusks in aquaculture [1]. Its high nutritional value, small size, higher growth rate and simpler growth requirement, attracts the nutritionist and biotechnologist. They have the ability to grow in extreme environmental condition and in order to adopt these conditions they accumulates varieties of bioactive compounds that can be used in bioremediation [2], biofuel [3], biofertilizer [4], human food [5], animal feed [6], and pharmaceutical industry [7]. In order to have microalgae with high nutritional value various researches have being conducted around world. The nutritional value of microalgae relates to its biochemical composition which varies with growth phase and culture conditions like light, temperature, nitrogen concentration or salinity [8]. Among various environmental factors, light is one of the key factors that control the various physiological processes and thereby their growth and biomass production. Quantity and quality of light determines the amount of energy available to photosynthetic organisms to conduct their metabolic activities [9]. Therefore, it is the most important factor affecting the photosynthesis kinetics productivity of microalgae [10]. In large scale cultivation, dense culture of microalgae limits the penetration of light which affects the photosynthesis and growth of microalgae. Previous studies have shown that under stress they alter their metabolic pathway from growth promoting to energy saving pathway [10]. Thereby it changes their biomass yield, growth rates and biochemical composition which have diverse application in the aquaculture, pharmaceutical, nutraceutical, cosmetic, and biofuel industry. Variation of irradiance and depletion of nutrients is the critical event occurs in both indoor and outdoor cultivation which affects biomass production and its composition. The objective of this study is to point out how change in light intensity in batch culture affects the growth and biochemical composition of microalgae. As microalgae is valuable bio-resources for animal feed, human nutrition, nutraceutical and biofuel industry on the world market, it raises the need of improved knowledge of its composition, growth and chemical variability for higher production of particular valuable metabolites with high mass productivity. Therefore it is important to investigate the effect of light limitation on growth and biochemical composition of microalgae.

2. Materials and Methods
2.1 Algal culture
The starter culture of marine microalgae *Isochrysis galbana* was obtained from Center of Marine and Fishery Research Institute (CMFRI) kochi, India.

2.2 Growth condition
The experiment was carried out in 1litre conical flask containing 200-300ml of growth media. The growth media contained autoclaved filtered natural seawater, supplemented with f/2 culture medium [11]. The f/2 culture medium was composed of 75 mg L^{-1} $NaNO_3$, 5 mg L^{-1} NaH_2PO_4H2O, 1ml L^{-1} trace metal solution (3.15 mg L^{-1} $FeCl_36H2O$, 4.36 mgL^{-1} Na_2EDTA, 0.0098 mgL^{-1} $CuSO_45H_2O$, 0.0063 mgL^{-1} Na_2MoO_42H2O, 0.022 mgL^{-1} $ZnSO_47H2O$, 0.01 mg L^{-1}

$CoCl_2$6H2O, 0.18 mgL^{-1} $MnCl_2$4H2O) and 0.5 ml/L of vitamin solution (0.001 mg L^{-1} vitamin B12, 0.2 mgL^{-1} vitamin B_1, 0.001 mg $biotin^{-1}$) were added. In the laboratory, *Isochrysis galbana* grown in a batch culture at temperature of 22-25 ^{0}C, pH 8-8.2 and under different light intensity: 35 µmol photon m^{-2} s^{-1} (low), 125 µmol photon m^{-2} s^{-1} (medium), and 275 µmol photon m^{-2} s^{-1} (high), provided by white fluorescent tube for 24:0 h light–dark period. All the glassware and media were always sterilized prior to inoculation. All the experiments were carried out after 5th of cultivation and in triplicates. For this biomass was harvested after 5 days by centrifugation at 10,000 rpm for 10 min, washed twice with distilled water.

2.3 Measurement of Algal growth

The growth of *Isochrysis galbana* is calculated by measuring optical density (O.D.) and dry weight. Optical density was measured daily at 710nm by UV/visible spectrophotometer. For dry weight measurements, 50ml of culture samples were centrifuged at 5000 rpm for 5 min. After rinsing twice with distilled water, the pellets were dried 6hr in an oven at 110^{0}C and then cooled down in desiccators before weighing. The difference between the initial and final weight were taken as the dry weight of algal biomass (mg/l).

2.4 Biochemical Analysis

The crude protein was determined by modified Lowery method [12]. The absorbance of the sample was taken at 650nm and the concentration was determined using standard curve: Total Protein Content = wt. of protein (from curve) X 100/ dry cell mass (mg).The content of carbohydrate is estimated by the modified by the phenol-sulfuric acid method of Dubois [13]. The optical density of the sample was determined against the blank at 490 nm in a UV-visible spectrophotometer. Carbohydrate Content (%) = wt. of carbohydrate (from Glucose standard curve) X 100/ dry cell mass (g) and total lipid contents were analyzed gravimetrically after extraction with chloroform–methanol (2:1) modified by Bligh and Dyer [14]. Pigments were extracted in acetone (90 %) at 4 °C overnight and measured by spectrophotometric methods [15].

2.5 Data Analysis/Statistical Analysis

All experiments were done with three replicates and data represent the means ± SD. They were analyzed by one- way ANOVA and significant differences between treatments were tested using Duncan's multiple range test (DMRT). *P*-values <0.05 were considered significant. Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) 10.0.

3. Results

3.1 Growth pattern

Light depletion is one of the key limiting factor affecting the growth and biomass production. To evaluate the effect of light intensity on biomass productivity absorbance at710nm was measured. Figure 1 illustrated the growth curve of *Isochrysis galbana* under different intensities of light. It elicited that growth was significantly (>0.05) higher in culture under high light intensity. Growth measured in term of dry weight was shown in figure 2. There was a significant difference (>0.05) between all the cultures under different light intensity. The culture (ML) grown under medium light attained a highest dry weight (0.97±0.028g/L) followed by culture HL (0.83±0.042g/L). At end of cultivation (5day), the dry weight increased from 0.6 mg/L to 0.97 mg/L with increased light intensity from 35 µmol photons m^{-2} s^{-1} to 125 µmol photons m^{-2} s^{-1} but further increase of light intensity from 125 to 275 µmol photons m^{-2} s^{-1} decreased dry weight from 0.97mg/L to 0.83mg/L had observed.

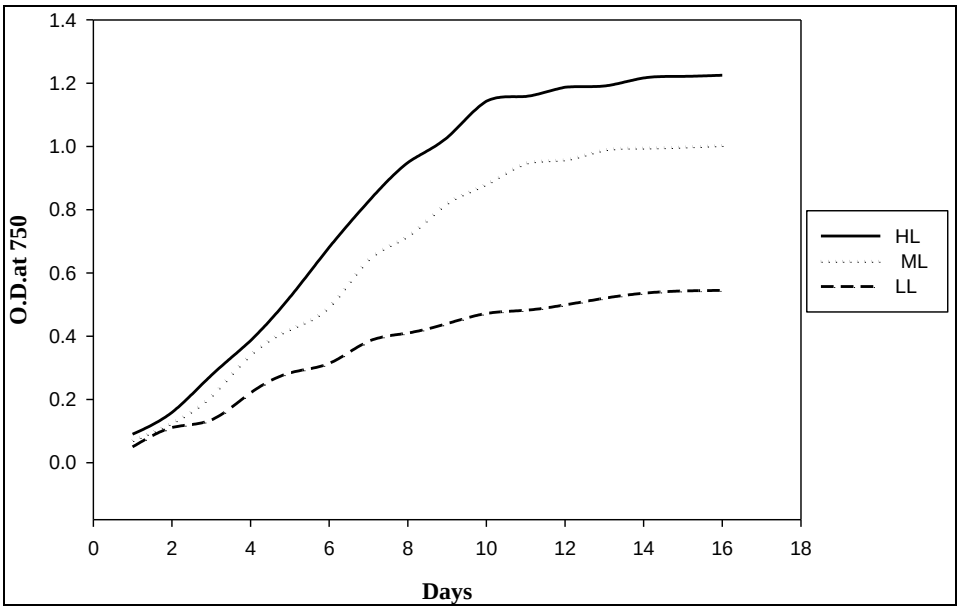

Fig 1: Growth pattern of *Isochrysis galbana* under different light condition

a) high light intensity of 35 µmol photon m^{-2} s^{-1}, b) medium light intensity of 125 µmol photon m^{-2} s^{-1}, c) low light intensity of 275 µmol photon m^{-2} s^{-1}.

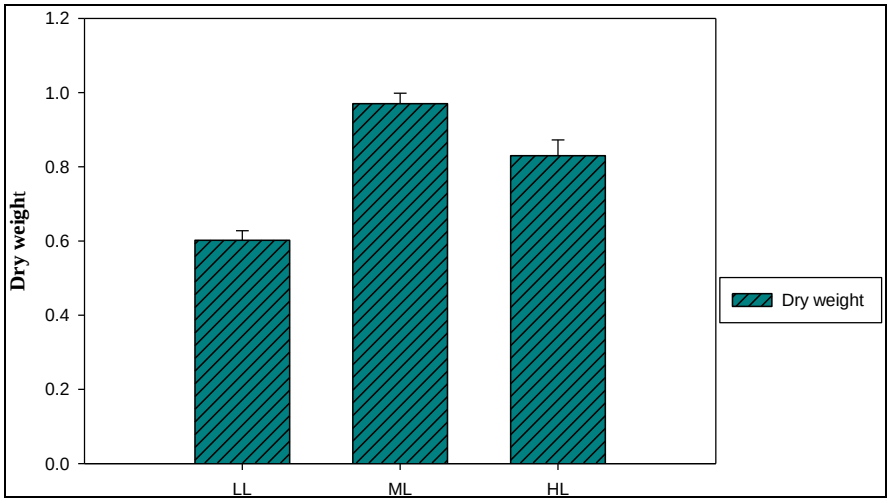

Fig 2: Effect of light on biomass yield of *I galbana.*

3.2 Biochemical composition & Pigment

In this study, culture were grown in three light regime (LL,ML,HL) and its effect on biochemical composition and pigment content were shown in table1. It had reported that in each light regime, all biochemical parameters were significant different ($p<0.05$). The variations of the determined biochemical compositions during the cultivation of *I. galbana* under different light intensity are illustrated in figure 3. With increased light intensity, carbohydrate and lipid accumulates from 13.2% to 18.8% and 16.06 % to 30.66% respectively. Under low light intensity, culture (LL) attained highest protein content 44.73% and lowest carbohydrate and lipid content13.2% and 16.06% respectively. The pigments content *of I galbana* significantly (>0.05%) affected by light intensity in all cultures. With increased light intensities photosynthetic pigment chl a & chl c decreased from 4.67±0.28 to 2.99 ±0.032 mg/L and 3.82±0.35 to 2.74±0.24 mg/L respectively. Whereas carotenoid content increases from 3.24 ± 0.63 to 4.89+0.25 mg/L. Similar trend is found in other studies *Dunaliella salina* [16],*Chlorella zofingiensis* [17], *Isochrysis galbana* [18].

Table 1: Effect of Light Intensity on Biochemical composition & Pigments

Biochemical Composition	Low light	Medium light	High light
Dry Weight	0.602±0.025	0.97±0.028	0.83±0.042
Protein	44.73+.50	35.26 ±3.15	27.06± 0.35
Carbohydrate	13.2 ±.36	15.8 +0.62	18.8± 0.20
Lipid	16.06 ±0.70	23.33±0.611	30.66 ± 0.30
Chl a	4.67+0.28	3.555+0.35	2.99+0.03
Chl c	3.82+0.354	2.23+0.24	2.74+0.24
Total Carotenoid	3.24+0.636	3.35+0.12	4.89+0.256
Car/T Chl	0.38+0.03	0.578+0.56	0.853+0.17

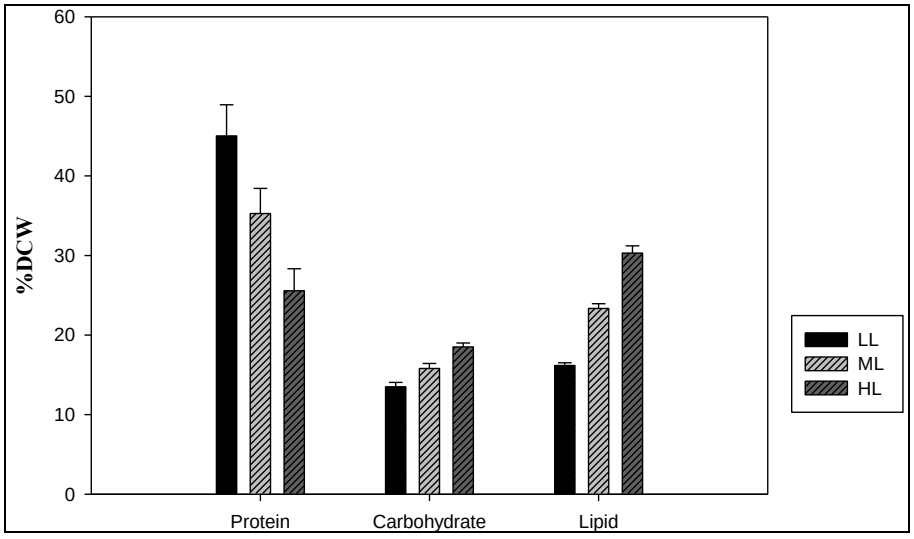

Fig 3: Biochemical variation under different light intensity

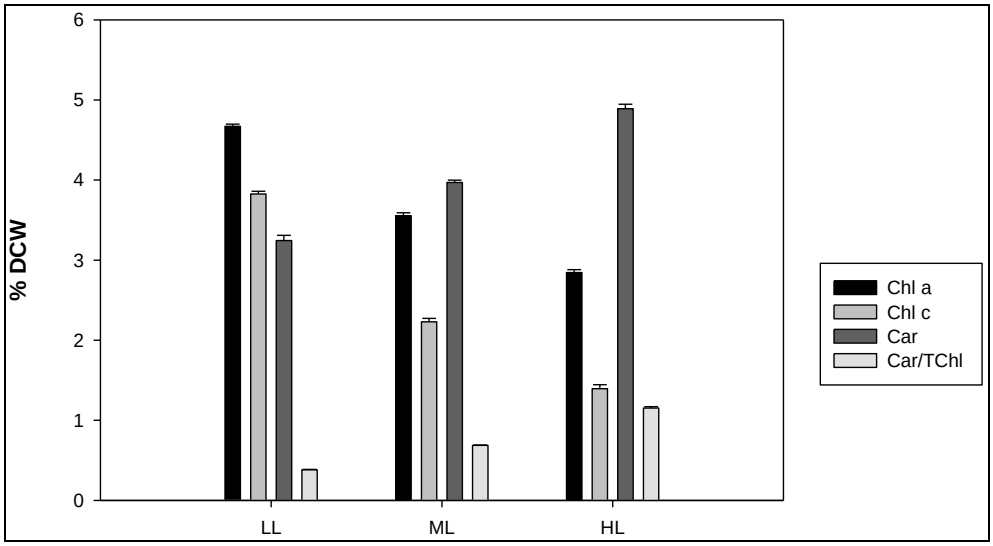

Fig 4: Variation of pigments under different variables

4. Discussion

4.1 Growth

Since *I galbana* is a photosynthetic microalgae, light is determinant factor for their growth and photosynthetic activity [19, 20]. The growth curve of culture under low light intensities has shorter lag phase and longer stationary phase then other treatments. Therefore, the stationary phase may easily to achieve within its cultures. Growth pattern was investigated from optical density reveals that high light intensity stimulates the growth the *Isochrysis galbana* more efficiently than low light intensity. This could be because light is the energy source for photoautotroph's. They utilize light energy to fix carbon dioxide (Co_2) to organic compounds. It also have been seen under stress such as high light intensities microalgae tend to increase in size in order to survive in stressful environments [21]. When the culture becomes very dense the growth of phytoplankton is usually slowed by light limitation [22]. Possibly excess of cells produces self shading in culture [23]. In this study *I. galbana* achieved highest growth rate under medium light intensity while further increase in light intensity lowers specific growth rate. It is in consistent with other finding in which *I galbana* culture achieved a high growth rate at light intensities of 25–100 μmolm^{-2} S^{-1} and light inhibitory effect occurred at 200 μmol photonm−2 s−1[24]. In our study, between three light intensities culture under medium light intensity of 125 µmol photons m^{-2} s $^{-1}$had the highest biomass density suggesting that the optimum light regime for *Isochrysis galbana* is 125 µmol photons m^{-2} s $^{-1}$. This implies that high light intensity may result in photoinhibition of *I. galbana*, This result were consistent with previouswork found irradiance level 175 µmol photons m^{-2} s^{-1} saturates photosynthesis and growth of all *Isochrysis galbana* cultures [25]. Maximum level of *Nannochloropsis sp*. growth was obtained by increasing light intensity up to 10 000 Lux (135 µmol photons.m^{-2}s^{-1}) which was in harmony with findings of this study (125 µmol photons.m^{-2}s^{-1} [26]. Similar result are comparable with the finding of other groups also reported light intensities in the range of 45-120 µmol photons m^{-2}s^{-1} saturating the growth of most of microalgae species [27]. The biomass content significantly decreased with depletion of light from 0.97g/L to 0.602g/L. This may be due to decrease in photosynthesis, cell size and growth rates [28, 29]. The lowest dry weight have been found in cell grown in low light intensity (LL) resulted. 602 ± 0.062 g/L DW.

4.2 Biochemical Composition &Pigments

Light availability is one of the chief limiting factors for growth, biomass and production of various metabolites [30]. Light quality and quantity determinates the amount of light energy available to photosynthetic organisms to conduct their metabolic activities Under high light intensity, carbohydrate and lipid in *Isochrysis galbana* accumulates significantly in order to avoid photo-oxidative damage excess light is converted to chemical energy [31, 32, 33]. The accumulation of energy-rich compounds, such as protein, lipids and carbohydrates could occur in many microalgae species under stress conditions such as light, nutrient limitation [34, 35, 36]. Stress such as high light and nitrate depletion affects the metabolic activity of cell and divert the cell from protein synthesis to carbohydrate and lipid accumulation. Lipids and carbohydrates are the preferred storage products in various stress conditions because they are hydrophobic in nature and have highly reduced states [37]. In addition they efficiently packed in small compartment of cells and can also be used during adverse conditions for cell survival and proliferation. The result of this study is in consistent with other studies reported high light intensity before photoinhibition (400nm) leads to accumulation of lipid and carbohydrates [38, 39]. Although, some studies found no significant effects of irradiances on lipid and carbohydrate accumulation [40] It have been seen under high light intensities carotenoids accumulates to prevent the absorption of surplus light energy by the photosynthetic machinery and thereby protect the photosynthetic apparatus from oxidative stress. In our study, at high light intensity, *I galbana* synthesis less photosynthetic unit (chlorophyll a or accessory pigments) and accumulate carotenoids attributed to prevent photo damage and to boost photoprotective action**.**

5. Conclusion

The result of our study indicated that the light intensity is capable to manipulate the biochemical composition of *I. galbana*, producing either valuable proteins under low light intensity or producing carbohydrates and lipids under higher light intensities. Therefore, optimization of environmental condition enhanced the accumulation of particular metabolites which could be used for aquaculture, human nutrition, biofuel and other industrial application. On the basis of our findings we concluded that the optimum light intensity for *I galbana* is 125 µmol photon m^{-2} s^{-1} shows maximum biomass dry weight. High light intensity promotes the accumulaton of carotenoids, lipid and carbohydrates to protect the cell from damaging effect of high light and shifts metabolic physiology from protein synthesis to energy reserve.

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