Research Article

Optimization of Culture Conditions for Growth of Marine Phytoplankton

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ABSTRACT

The present study investigated the optimum levels of ambient temperature, salinity and light intensity for the growth of marine diatoms and microalgae. The growth of marine diatoms *Thalassiosira subtilis*, *Entomoneis paludosa* and microalgae *Isochrysis galbana* were optimized. Phytoplankton subcultures were prepared in filtered natural seawater enriched with f/2 media for diatoms and Conway media for *I. galbana*. Cultures were grown under three different levels of parameters such as temperature, salinity and light intensity consisting of 27 combinations in 96-well plates. Ten replicates of cultures were maintained for each combination of ambient levels. The algal density was determined by spectrometric absorbance of culture at 680 nm. Likewise, the growth was estimated from the rate of increase in the absorbance values over a period of time. The duration of growth differed between the species. The *I. galbana* was grown for prolonged culture duration of 15 days followed by *E. paludosa* with 12 days and *T. subtilis* with 8 days. Ambient temperature and light intensity are the driving parameters for optimum growth of the species studied while the optimum salinity of 30 psu was observed for all species. Ambient levels of 28°C, 30 psu and 2500 lux were found optimum for the growth of *T. subtilis* and *E. paludosa* attained its optimum growth at 24°C, 30 psu and 2500 lux. Higher light intensity (4500 lux) at 24°C and 30 psu has enhanced the growth of *I. galbana*.

Keywords: Diatom, Growth Optimum levels, Temperature, Salinity, Light

Introduction

Phytoplankton is being explored for their potential in many fields concerned with ecological and industrial aspects. Therefore, constant efforts are being made since last century towards the micro algal culturing techniques [1]. Microalgae were cultured for aquaculture during beginning of the last century [2]. Many recent studies have reported the advantages of plankton culture under controlled conditions for attaining greater quality and quantity of biomass at lesser duration. Phytoplankton are potentially important for environmental impact assessment studies, synthesis of bioactive compounds, pigments, biofuels and applications in phytoremediation of contaminated wa-

ter [3–8]. Most of the phytoplankton are sensitive to the ambient environmental conditions hence their community structure and biodiversity depends on ambient levels of nutrients, temperature, salinity, pH and light [9]. There are more than 5000 marine phytoplankton species identified of which less than 10% of the species can be cultured under laboratory conditions and only a few of them on a mass scale [10]. The cultivation of plankton depends on the identification of optimum conditions for specific groups/species. The levels of nutrients and minerals were reported for most of the marine microalgae and diatoms as culture media recipes [11, 12]. These media recipes are described with different level of macronutrients

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(C, N, P, S, K, Na, Fe, Mg, and Ca) and micronutrients (B, Cu, Mn, Zn, Mo, Co, V, and Se). Most of these media are suitable for culturing a group of plankton like f/2 media for diatoms, Conway and B11 media for green microalgae [13]. Unlike media composition, the optimum ambient environmental parameters such as temperature, salinity, light intensity and photoperiod differs among the species [14]. Therefore, optimizing environmental factors for linear growth rate is essential for the conduct of bioassays to derive toxicity values towards environmental impact assessments. Temperature, salinity and light intensities control the rates of all chemical reactions related to algal growth, metabolism and biochemical profile of microalgae [15, 16, 17].

Few microalgal species have been extensively studied for attaining better quantity and quality in biomass and biochemical composition by altering of culture conditions [18, 19]. However, reports on optimum conditions for the crucial parameters like growth, biomass and biochemical profile are scarce for most of common diatoms and occasional phytoplankton. Diatoms *Thalassiosira subtilis* [20] and haptophyte microalga *Isochrysis galbana* [21] have wide distribution and are extensively studied for their various applications such as nutrition, biofuels and environmental impact assessment. The benthic diatom *Entomoneis paludosa* [22] is a rare cultured species and is largely unexplored for growth characteristics and biochemical profiles. Hence the present study was conducted for the identification of optimum culture conditions of temperature, salinity and light intensity for the growth of diatoms and microalgae.

Material and Methods

Diatom and microalgae culture

Phytoplankton samples were collected from the coastal waters of Ennore (Lat. 13.232190; Long. 80.330068) by horizontal towing of plankton net (20µ mesh size). The samples were transferred into polypropylene bottles and transported to laboratory. The diatoms *T. subtilis* and *E. paludosa* were identified using the morphological identification characteristics [23]. The diatoms were isolated by filtration and serial dilution methods. The haptophyte microalgae *I. galbana* was obtained from micro algae culture laboratory, NIOT, Chennai, Tamil Nadu. The mono-species

cultures were maintained under controlled laboratory conditions in filtered sterilized seawater enriched with f/2 media [24] and Conway media [25] for diatoms and microalgae respectively.

Experimental design

Growth of two species of marine diatoms and *I. galbana* were examined with 27 combinations of temperature, salinity and light intensity experiments. The 27 probable combinations were made from three different levels of temperature (24, 28 and 30°C), salinity (28, 30, and 33 psu) and light intensity (1500, 2500, and 4500 lux which are equivalent to 20, 35 and 60 µmol photon m⁻² s⁻¹ respectively). Separate subcultures for each species were made in 100 ml of media with three different salinities. Culture media in different salinities without plankton cells were used as blank. Totally nine 96 well plates (Nunc, Denmark) with cultures and blanks were used for the experiment. Every plate was loaded with 250µl of culture of each species in different salinities and blanks. 90 wells were loaded in each plate consisting of ten replicated cultures (30 wells/species × 3 salinity = 90 wells) and remaining 6 wells for media blanks in duplicate. The plates were separated into 3 sets and each set of plates were placed at different light intensities in three different temperature-controlled rooms. The different light intensities were obtained by placing the plates at different distances from the light source of white fluorescent lamps. Photoperiod was maintained at 12 h light and 12 h dark cycle. The plates were measured for the growth of alga/diatoms at 24 h interval.

Measurement of physico-chemical parameters

Environmental parameters such as temperature, salinity and light intensity were maintained during the growth experiment. The temperature in the experimental chamber was measured twice a day using a glass mercury thermometer with 0.1°C resolution. Salinity was measured during the preparation of the test media using refractometer (Make: ATAGO, Serial No. 0144294). The light intensity was measured by placing photoreceptor cell of a digital lux meter (Make: Lutron, Model: LX-101) on the culture plates.

Growth estimation

The absorbance of culture at 24 h interval was used for the estimation of growth [26]. The blanks

and cultures in the wells were mixed thoroughly using micropipette for homogenization. Absorbance was measured at 680 nm using multi-mode microplate reader (BioTek Instruments, Synergy H1). The absorbance of blank was subtracted and absorbance data of cultures was acquired from Gen5™ software.

Statistical analysis

Analysis of variance (ANOVA) with multi-variance analysis was performed for each species to differentiate the cultures on the basis of growth patterns between the various combinations of environmental parameters employed.

Results and Discussion

Growth characteristics of *T. subtilis*, *E. paludosa*, and *I. galbana* under different levels of temperature, salinity and light intensity were studied for their entire culture period from lag phase to decline phase. The absorbance of culture was measured at 24 h intervals at 680 nm in multi-mode microplate reader. The initial absorbance of cultures was recorded in the range of 0.077-0.080 for *T. subtilis*, 0.066-0.069 for *E. paludosa* and 0.090-0.100 for *I. galbana*. Subsequently, the cultures reached their maximum absorbance values at different durations among the combinations and the species. The growth curves are plotted using the absorbance values of culture over a period of time for each species and combinations (Figure 1 and 2). Then the growth curves were statistically compared by using ANOVA with multi-variance analysis (Figure 3). Significant changes in the growth curves were observed under different levels in ambient parameters. Few combinations yielded higher growth with maximum absorbance value during the experiment (0.13, 0.21 and 0.35 in cultures of *T. subtilis*, *E. paludosa*, and *I. galbana* respectively). All the three parameters influenced differently on the growth of diatoms and microalgae. Five most optimum level combinations of parameters were picked out based on the statistical analysis of the growth under all the combinations examined (Figure 4). The optimum levels were in the order of MTMSHL, MTHSHL, HTHSHL, LTMSHL and HTMSHL for the growth of *T. subtilis*; LTMSML, HTLSHL, HTMSHL, HTHSHL and HTLSML for the growth of *E. paludosa*; LTMSHL, MTLSHL, MTHSHL, MTHSML and MTMSHL for the growth of *I. galbana* (Figure 4)

Table 1. Ranges of temperature, salinity and light intensity and details of acronyms

	Low	Medium	High
Temperature (°C)	24 (LT)	28 (MT)	30 (HT)
Salinity (psu)	28 (LS)	30 (MS)	33 (HS)
Light Intensity (lux)	1500 (LL)	2500 (ML)	4500 (HL)

and (Table 1).

Salinity and light intensity were the major influencing factors for the growth of *T. subtilis*. The reduction in the growth of *T. subtilis* was observed at 24°C to 28°C combined with lower salinity of 28 psu. Growth of *E. paludosa* depends on temperature and light intensities at higher levels. The temperature of 30°C, light intensities of 35 and 60 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ along with the salinity ranges of 28, 30 and 33 psu were found to be the optimum levels for the growth of *E. paludosa*. All the combinations with higher temperature inhibited the growth of *I. galbana*. Maximum growth inhibition of diatom *T. subtilis* was observed at lesser temperature and higher salinities. Contrastingly, maximum growth inhibition in *E. paludosa* was found at higher temperature level and lower light intensity. Lower temperature and salinity were responsible for the growth reduction in *I. galbana* cultures.

Culture optimization of phytoplankton is important to explore their potential applications. The ambient levels of temperature, salinity and light intensity were studied for their impact on the growth of *T. subtilis*, *E. paludosa*, and *I. galbana*. Apparent optimum levels of these parameters were revealed by growth characteristics under different combinations (Figure 1 and 2). The significant differences in growth pattern of each species indicated the combined effect of temperature, salinity and light intensity at different levels (Figure 3). Among the three selected parameters, temperature and light intensity were found to be more significant parameters than salinity. Growth of diatoms and *I. galbana* demonstrated that light and temperature are influencing the culture in combination. Higher light intensities combined with lesser salinities promoted the growth. Diatoms exhibited their tolerance to higher levels of temperature, salinity and light intensity. The haptophyte microalga, *I. galbana* is sensitive to higher tempe-

Figure 1. Growth curves of diatoms under different levels of temperature, salinity and light intensity in different combinations. Growth of *T. subtilis* at varying salinity and light intensities with 24°C, 28°C and 30°C of temperatures respectively (a, b, and c); Growth of *E. paludosa* at varying salinity and light intensities with 24°C, 28°C and 30°C of temperatures respectively (d, e, and f)

Figure 2. Growth of *I. galbana* at 24°C (a), 28°C (b), and 30°C (c) of temperatures respectively with varying salinity and light intensities.

Figure 3. Significant difference under different levels of environmental parameters among the combinations

perature (30°C) and requires higher light intensity ($60 \mu\text{mol photon m}^{-2}\cdot\text{s}^{-1}$) for optimum growth. The reduced exponential phases in diatom cultures were observed for the combination of temperature, salinity and light intensity at higher levels. The diatoms *T. subtilis* and *E. paludosa* attained their maximum growth on fourth and sixth day and thereafter it declined at higher levels of temperature, salinity, and light.

The temperature dependence of the growth rate can differ considerably among species [27]. The optimum temperature range was found to be 24 to 30°C for the growth of diatoms *T. subtilis* and *E. paludosa*. Similarly, an optimum temperature of 24 to 28°C was identified for *I. galbana*. It is important to discuss the temperature dependency that has been reported previously in comparison to the present results. An optimum temperature of 25-30°C has been reported as an ideal range for the growth of diatom *Chaetoceros* sp. [28] and 20-25°C was suitable for the growth of *C. pyrenoidosa* [29]. Equally, 30°C was found as optimum for higher growth of *Chlorella minutissima* [30] and for maximum biomass and lipid production by *Chlorella pyrenoidosa* [31]. Diatoms

Figure 4. Optimum levels of environmental parameters for the growth of diatoms and *I. galbana* (Each data points with different alphabets are significantly different ($p \leq 0.05$) among the optimum levels and combinations).

Chaetoceros calcitrans and *Skeletonema costatum* also exhibited high growth at 30°C, above which the algal culture crash was observed [32]. Likewise, growth inhibition and depletion of biomass production in *Chlorella sorokiniana* was recorded at elevated dissolved oxygen and temperature [33]. The optimum level of temperature is related to their habitat ranges. The rise in temperature above the species optimum level could result in low growth rate of phytoplankton [34]. Creswell [35] stated that the tropical and sub-tropical phytoplankton species grow best at temperatures ranging from 16 to 27°C. Therefore, it is pertinent to note that, the diatoms are sustained in a wide range of temperature and microalgae prefer to grow at slightly lower temperature than the diatoms. However, the influence of physiological and water quality parameters cannot be overlooked.

In the present study, salinity of 28-30 psu was found to be optimum for the growth of *T. subtilis*, *E. paludosa*, and *I. galbana*. The higher salinity of 33 psu affected the growth of diatoms and microalgae. Lower salinity led to maximum growth, cell density, biomass and chlorophyll content of *Chaetoceros calcitrans* [18, 36]. Increasing salinity from 40 to 60 psu decreases the growth of marine *Chlorella saccharophila* [37]. Similarly, salinity

of 15 to 25 psu is reported to be optimum for the maximum growth of diatom *Skeletonema costatum* and *Chaetoceros* sp. [38]. In contrast, the diatom species such as *Thalassionema eccentrica* and *Pseudo-nitzschia seriata* were able to survive at hypersaline conditions up to 150 psu [39]. Therefore, respiration, photosynthesis and growth are affected at hypersaline conditions above the optimum levels due to the overloading of the osmoregulatory mechanism [40, 41]. Similarly, salinity at higher levels demonstrated the reduced chlorophyll resulting to limitation in assimilation rate [42].

Light is required for photochemical production of Adenosine triphosphate (ATP) and Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH). Equally, the light and dark photoperiod is essential for the synthesis of biochemical molecules [43]. Hence, the intensity of light and its duration is the major influencing factor for the growth of phytoplankton. The results exhibited that the growth of diatoms and *I. galbana* is optimum in the light intensity of 35 to 60 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$. Light intensity of 60 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ is optimum for the growth of diatom *T. subtilis* and microalga *I. galbana*. Lesser light intensity may provide insufficient photon energy for the photo-

synthesis; hence some phytoplankton demonstrated their adaptation by increasing the size of photo-harvesting apparatus chloroplast [44, 45]. On the other hand, higher light intensities can harm the algae by damaging the photosynthetic pigments and photo-oxidation of biomolecules [46, 47]. Thus, the amount of light and duration of photoperiod is unequal among the phytoplankton species [48].

Conclusion

The study of growth characteristics of microalgae and diatoms are essential for various applications. Prolonged exponential growth phase with linear growth rate is necessary for the utilization of particular species for bioassay experiments. Perhaps, fastest growth is desirable for employing the microalgae for remediation studies. Similarly, attaining the highest quality of biochemical profile in algal biomass is the objective for utilization of algae to nutrition and bio-fuel production. In this study, five optimum conditions were identified for linear growth rate, and highest biomass production in lesser durations for diatoms and haptophyte microalgae *I. galbana*. Further studies on biochemical profiles and mass scale production are suggested for better utilization of these species to a maximum extent.

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