



# Optimization of physical parameters for the growth and lipid production in *Nannochloropsis gaditana* (Lubian, 1982)

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## ABSTRACT

Microalgae produce a wide range of compounds including pigments, protein, starch, and lipids, which have been extensively used for various applications. In the current scenario, microalgal lipids are considered as a promising source for the production of next-generation bioenergy, and a huge productivity is needed to meet the demand. Thus, to increase the production of biomass and lipid content, physical conditions play an important role and necessary to be optimized. The present study made such an attempt to optimize the physical factors for the growth and production of lipid from *Nannochloropsis gaditana*. The study aimed to determine the effect of physical parameters such as pH, temperature, light intensity, and salinity. The results showed that the maximum growth rate of the *N. gaditana* was noticed in the salinity of 25 ppt, pH of 8, temperature of 25°C, and 2,000 lux of light intensity. The highest lipid content of the *N. gaditana* was noticed in the salinity of 30 ppt, pH of 8, and temperature of 30°C with 2,000 lux light intensity. After optimization, above 40% of lipid yield was obtained, and it can be effectively utilized in bioenergy production.

## 1. INTRODUCTION

Bioenergy is an alternative and eco-friendly energy with the highest potential to fulfill global energy demand [1]. Major sources of bioenergy are agricultural residues, which include straw, wood waste, sugarcane, manure, and many agro-wastes [2]. One major drawback of these sources is that the cultivation and farmland exploitation will affect food security and prices [3]. As increased demand for bioenergy, microalgae have been identified as an excellent source of bioenergy, which has been widely utilized for various applications. Compared to agro-wastes, the advantage of using microalgae are its high photosynthesis efficiency, fast growth, high lipid content, no competition with food crop for farmland, and least water demand [4,5]. Among primary metabolites produced from marine microalgae, the lipid has been extensively used as a major source for the production

of bioenergy [6,7]. Mass scale microalgal cultivation with improved lipid content is required for the generation of a large amount of biomass for bioenergy production [8]. In general, the environmental and culturing condition differences can alter the growth and lipid production as well as chemical compositions of microalgae. These conditions can be optimized by regulating environmental parameters, such as salinity [9,10], temperature [11,12], and pH [11,13].

There are many species of microalgae that have been reported with high lipid content which includes *Botryococcus braunii*, *Chlorella vulgaris*, and *Scenedesmus obliquus* [7]. Since many microalgal species are needed to be identified and explored for lipid production. *Nannochloropsis* species are also called picoplanktonic algae, belonging to the class *Eustigmatophyceae* reported to produce lipid [14] and shown to be suitable for bioenergy, especially biofuel production due to its ease of growth with high oil content. The productivity of these algae mainly depends on nutrient content, pH, salinity, temperature, and light. Some researchers documented that the environmental factors influence the biochemical profile of *Nannochloropsis* sp. [15,16].

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The present study aimed to enhance the growth and lipid content of *Nannochloropsis gaditana* through the optimization of physical parameters.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Marine Water Sample

The isolation of microalgae was carried out from the marine water sample. The sample was collected from the seashore water from different coastal areas of Rajakkamangalam in Kanyakumari district. The water samples were taken from both the surface and middle of the water column. The collected samples were packed in transparent plastic bottles and properly labeled and transported to the laboratory for further analysis.

### 2.2. Isolation and Identification of Marine Microalgae

Microalgae were isolated by serial dilution technique which was previously described by Perumal *et al.* [17]. The culture was grown in a conical flask (500 ml) containing 250 ml of sterile Conway media, and the flasks were maintained at a temperature of 25°C ± 1°C with a continuous illumination of 1,000 lux with 16:8 light and dark cycle.

The morphological characterization of microalgae was identified based on the technique which was previously described [18–20].

### 2.3. Optimization of Physical Parameters for the Growth and Lipid Production

The influences of various physical parameters were optimized. The microalga was cultivated in Conway medium with the influence of different values of salinity (20, 25, 30, 35, and 40 ppt), pH (4, 5, 6, 7, 8, 9, and 10), temperature (20°C, 25°C, 30°C, 35°C, and 40°C), and light (1,000, 2,000, 3,000, 4,000, and 5,000 lux). They were individually optimized for growth and lipid production.

The individually optimized parameters were combined and subjected to another experiment to enhance the growth and lipid production of *N. gaditana*.

### 2.4. Harvesting of Microalgae

Algal biomass was collected by centrifuging the algal cultures at 8,000 rpm for 10 minutes on the log and stationary phase of growth. To obtain the dried algal biomass, the supernatant was discarded, and the pellets were oven-dried at 50°C for at least 12 hours. The total biomass was then determined gravimetrically and expressed in g l<sup>-1</sup> [21].

### 2.5. Estimation of Lipid

The lipid content was estimated using Bligh and Dyer [22] method. A mixture of 2 ml of methanol and 1 ml of chloroform was prepared and added to 1 g of oven-dried algal biomass. It was kept for 24 hours, at room temperature to dissolve the lipids properly. The mixture was centrifuged at 3,000 rpm for 10 minutes. The supernatant was separated, and 2 ml of chloroform was again added to the pellets and shaken properly. It was again centrifuged at 3,000 rpm for 5 minutes, and the supernatant was separated. After adding

2 ml of 1% KCL to the supernatant, two layers were formed. The lower layer was pipetted out and weighed [23].

Lipid Content (%) = weight of lipid (g) × 100/weight of culture (g)

## 3. RESULTS AND DISCUSSION

### 3.1. Identification of Isolated Microalgae

The cultivation of lipid-rich microalgae has received much interest among researchers. Lipid plays an important role in the metabolism of microalgae and also supplies energy for many biological activities such as DNA replication, division of nuclear, and development of daughter cells [7]. In this study, marine water samples were collected from Rajakkamangalam Sea, near Kanyakumari district, Tamil Nadu, India. During the isolation process, eight microalgal cultures were isolated from the marine water sample, and they were identified as follows: *Nannochloropsis* sp., *Nitzschia* sp., *Coscinodiscus* sp., *Navicula* sp., *Chlorella* sp., *Tetraselmis* sp., *Amphora* sp., and *Chaetoceros* sp. The maximum yield of lipid was observed in *Nannochloropsis* sp. with 29.03% of lipid content. Nearly 24.89% of lipid yield was observed in *Chaetoceros* sp., and this was also observed in *Nitzschia* sp. with 22.30%, both *Coscinodiscus* sp. and *Navicula* sp. with 21.00%, and *Chlorella* sp. with 19.09% of lipid content. Other microalgae species such as *Tetraselmis* sp. and *Amphora* sp. showed the least lipid yield of 17%. Among the eight isolates, based on the maximum lipid content, *N. gaditana* was selected for further studies. The morphology of *N. gaditana* was characterized under the light microscope during the exponential growth phase. The cells showed simple morphology with cylindrical-to-oval shapes. The cells also exhibited refractile granules and granular inclusions.

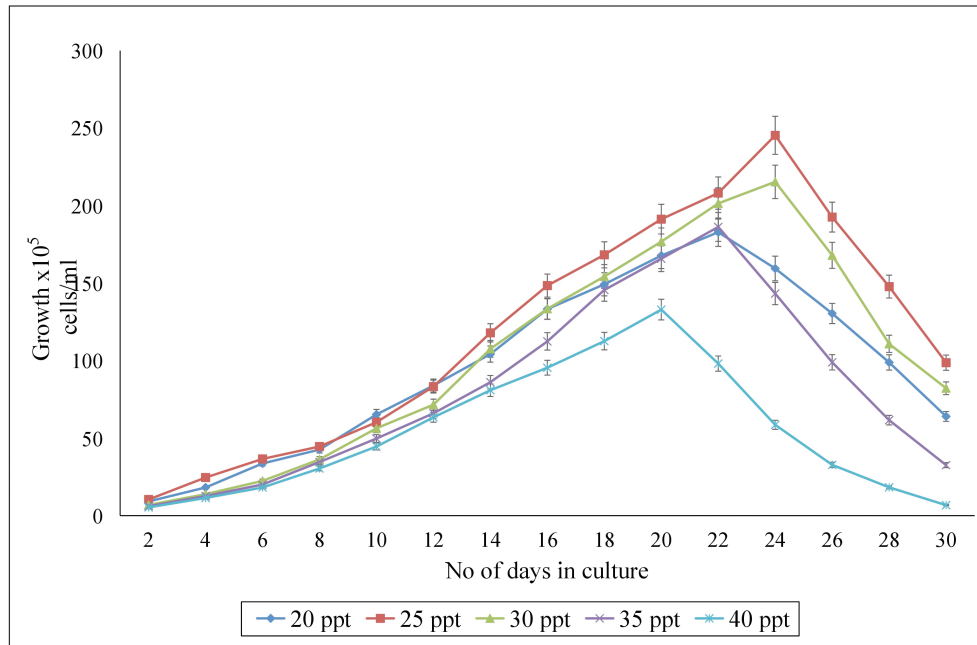
### 3.2. Effect of a Physical Factor on Growth and Lipid Production

The growth of microalgae is highly dependent on the environmental conditions, and variables in the culture condition are different from one species to another. However, the most studied variables are salinity, pH, temperature, and light.

#### 3.2.1. Salinity

Salinity is one of the main factors for the process of plants' life cycle and can cause a delay in central metabolic activities including photosynthesis [24]. Microalgae differ in their adaptability toward salinity, and based on their tolerance, they are grouped into halophilic and halotolerant [25]. In this study, the influence of salinity on the growth and lipid production of microalgae was studied in different salinities such as 20, 25, 30, 35, and 40 ppt at a temperature of 25°C ± 1°C with the continuous illumination of 1,000 lux with 16:8 light and dark cycle. *N. gaditana* reached the highest growth rate of 245 × 10<sup>5</sup> cells/ml on the 24th day at 25 ppt salinity, and the maximum oil yield of 35.8% was recorded in 30 ppt (log phase) (Fig. 1 and Table 1).

In this study, the algae showed a maximum growth in 25 ppt, and the growth was lowered when the salinity increases. This may be due to the algal cells that can tolerate a lower NaCl concentration, and thus, it grows in the normal way but with an increase in NaCl



**Figure 1:** Influence of salinity on the growth of *N. gaditana*. Values are represented as mean  $\pm$  standard deviation ( $n = 3$ ).

**Table 1:** Influence of physical parameters on lipid production from *N. gaditana*.

Factors	Log phase			Stationary phase		
	Dry wt. g/l	Lipid g/l	Lipid %	Dry wt. g/l	Lipid g/l	Lipid %
Salinity (ppt)						
20	0.94 $\pm$ 0.05	0.25 $\pm$ 0.53	27.0 $\pm$ 1.87	1.12 $\pm$ 0.08	0.20 $\pm$ 0.08	18.4 $\pm$ 2.40
25	1.06 $\pm$ 0.13	0.30 $\pm$ 0.58	28.7 $\pm$ 0.98	1.25 $\pm$ 0.78	0.17 $\pm$ 0.08	13.5 $\pm$ 2.89
30	1.15 $\pm$ 0.33	0.41 $\pm$ 0.28	35.8 $\pm$ 2.35	1.38 $\pm$ 0.16	0.32 $\pm$ 0.02	23.6 $\pm$ 1.62
35	0.75 $\pm$ 0.06	0.14 $\pm$ 0.37	19.1 $\pm$ 2.45	1.02 $\pm$ 0.25	0.07 $\pm$ 0.03	7.6 $\pm$ 0.90
40	0.37 $\pm$ 0.72	0.04 $\pm$ 0.38	11.6 $\pm$ 2.65	0.49 $\pm$ 0.12	0.03 $\pm$ 0.03	6.8 $\pm$ 1.06
pH						
4	0.59 $\pm$ 0.06	0.08 $\pm$ 0.01	14.0 $\pm$ 1.00	0.79 $\pm$ 0.08	0.07 $\pm$ 0.01	9.2 $\pm$ 0.45
5	0.84 $\pm$ 0.36	0.16 $\pm$ 0.04	19.4 $\pm$ 2.20	1.15 $\pm$ 0.03	0.17 $\pm$ 0.09	15.5 $\pm$ 1.67
6	1.05 $\pm$ 0.08	0.25 $\pm$ 0.07	24.0 $\pm$ 2.62	1.22 $\pm$ 0.25	0.26 $\pm$ 0.07	21.6 $\pm$ 2.09
7	1.10 $\pm$ 0.05	0.32 $\pm$ 0.09	29.4 $\pm$ 1.87	1.11 $\pm$ 0.75	0.29 $\pm$ 0.09	26.4 $\pm$ 1.04
8	1.02 $\pm$ 0.35	0.35 $\pm$ 0.13	34.6 $\pm$ 1.39	1.10 $\pm$ 0.04	0.32 $\pm$ 0.00	29.7 $\pm$ 2.23
9	0.34 $\pm$ 0.06	0.05 $\pm$ 0.01	15.7 $\pm$ 1.76	0.89 $\pm$ 0.43	0.06 $\pm$ 0.00	7.0 $\pm$ 0.47
10	0.47 $\pm$ 0.00	0.03 $\pm$ 0.04	11.1 $\pm$ 0.87	0.73 $\pm$ 0.03	0.04 $\pm$ 0.00	5.7 $\pm$ 0.60
Light (lux)						
1,000	0.43 $\pm$ 0.38	0.04 $\pm$ 0.01	9.9 $\pm$ 0.18	0.69 $\pm$ 0.18	0.03 $\pm$ 0.00	5.1 $\pm$ 0.25
2,000	1.13 $\pm$ 0.08	0.38 $\pm$ 0.05	33.8 $\pm$ 1.34	1.28 $\pm$ 0.21	0.23 $\pm$ 0.09	18.0 $\pm$ 1.04
3,000	1.09 $\pm$ 0.05	0.30 $\pm$ 0.09	27.8 $\pm$ 2.56	1.25 $\pm$ 0.08	0.17 $\pm$ 0.83	14.1 $\pm$ 1.67
4,000	0.65 $\pm$ 0.12	0.07 $\pm$ 0.03	10.8 $\pm$ 1.98	0.83 $\pm$ 0.02	0.05 $\pm$ 0.00	7.1 $\pm$ 1.94
5,000	0.76 $\pm$ 0.38	0.06 $\pm$ 0.00	8.8 $\pm$ 0.23	0.97 $\pm$ 0.28	0.04 $\pm$ 0.01	5.0 $\pm$ 1.96
Temperature ( $^{\circ}$ C)						
20	0.78 $\pm$ 0.08	0.07 $\pm$ 0.05	12.4 $\pm$ 1.06	1.10 $\pm$ 0.33	0.18 $\pm$ 0.05	16.4 $\pm$ 1.50
25	0.95 $\pm$ 0.03	0.28 $\pm$ 0.09	29.4 $\pm$ 1.97	1.30 $\pm$ 0.21	0.31 $\pm$ 0.06	23.5 $\pm$ 2.03
30	1.28 $\pm$ 0.25	0.42 $\pm$ 0.30	32.9 $\pm$ 2.65	1.30 $\pm$ 0.80	0.39 $\pm$ 0.03	28.6 $\pm$ 1.26
35	1.10 $\pm$ 0.48	0.30 $\pm$ 0.08	27.1 $\pm$ 1.24	0.98 $\pm$ 0.28	0.07 $\pm$ 0.00	7.6 $\pm$ 1.51
40	0.68 $\pm$ 0.06	0.06 $\pm$ 0.00	9.6 $\pm$ 2.60	0.69 $\pm$ 0.03	0.04 $\pm$ 0.09	6.3 $\pm$ 1.03

Values are represented as mean  $\pm$  standard deviation ( $n = 3$ ).

concentration. Chlorophyll degradation takes place, which leads to lower cell growth or cell death. An earlier report of Monika et al. [23] stated that the positive trend in the growth rate of the microalga was observed when the NaCl concentration was in 0.2 M, but a negative trend in the growth rate was observed when the NaCl concentration was in 0.5 M. An earlier study also confirms that the *Chlorella* sp. reached the highest growth rate in 25 ppt [26].

In this study, the maximum oil yield was recorded in 30 ppt, and this may be due to the stress developed during the salinity increase. Thus, the stress significantly induces lipid accumulation in the microalgae. An earlier report of Bartley et al. [27] stated that the salinity increase from 22 to 34 PSU can cause a significant increase in lipid content of 35% by weight, and a recent work of the previous researcher from our laboratory reported that the *Skeletonema* sp. obtained the maximum oil yield of 35.69 ml/100 g in 35 ppt [28].

3.2.2. pH

The pH is a very important factor in algal culture as it determines the solubility and availability of CO<sub>2</sub> and other nutrients. They also significantly impact on algal metabolism [29,30]. Thus, the influence of pH for growth and lipid production was studied using pH ranging from 4 to 10 at a temperature of 25°C ± 1°C with the continuous illumination of 1,000 lux with 16:8 light and dark cycle (Fig. 2 and Table 1). *Nannochloropsis gaditana* reached the highest growth rate of 218 × 10<sup>5</sup> cells/ml on the 20th day at pH 8, and the maximum oil yield of 34.6% was recorded in the same pH (log phase).

In this study, the algae showed the maximum growth, and oil yield was recorded in alkaline pH. The reason may be that the alkaline

pH reduces the cell release, thereby inducing lipid accumulation. An earlier report of Guckert and Cooksey [31] on chlorella CHLOR-1 stated that the alkaline pH forms autospore, and thus, it reduces the cell release, thereby inducing lipid accumulation. The earlier reports of Rodolfi et al. [32] found that the *N. gaditana* showed a maximum lipid content ranged from 24.4% to 35.7% at pH 8. The study of Monika et al. [23] also stated the same result.

3.2.3. Light intensity

In this study, the different light intensities for the growth and lipid production of microalgae were tested. The cultures were maintained at different light intensities such as 1,000, 2,000, 3,000, 4,000, and 5,000 lux at a temperature of 25°C ± 1°C with 16:8 light and dark cycle with medium pH 7. Among these, the highest growth (228 × 10<sup>5</sup> cells/ml) was noticed in 2,000 lux light intensities, and the maximum lipid yield of 33.8% was recorded in the same (Fig. 3 and Table 1).

In this study, the microalgae recorded a maximum growth and the lipid yield was observed in 2,000 lux, and the growth and lipid content was lowered when the light intensity was more than 2,000 lux. The reason for this may be due to the damages that occurred in photosynthetic antenna system and PSII reaction center on exposure to excess light, accompanied by the photobleaching of pigments. Bhandar and Sharma [33] also stated that the chlorophyll and carotenoids are necessary for light-harvesting and defending oxidative stress, respectively. Li et al. [34] study confirmed that both cellular chlorophyll and carotenoid content decreased as the irradiance level increased. Sukenik et al. [35] and Monika et al. [23] stated that the *Chlorella* sp. showed the maximum growth rate only up to a certain limit of light intensity, i.e., 2,700 lux, and the maximum biomass 1.050 g l<sup>-1</sup> was obtained at 2,700 lux light intensity, and moreover, when the culture was grown under 3,300

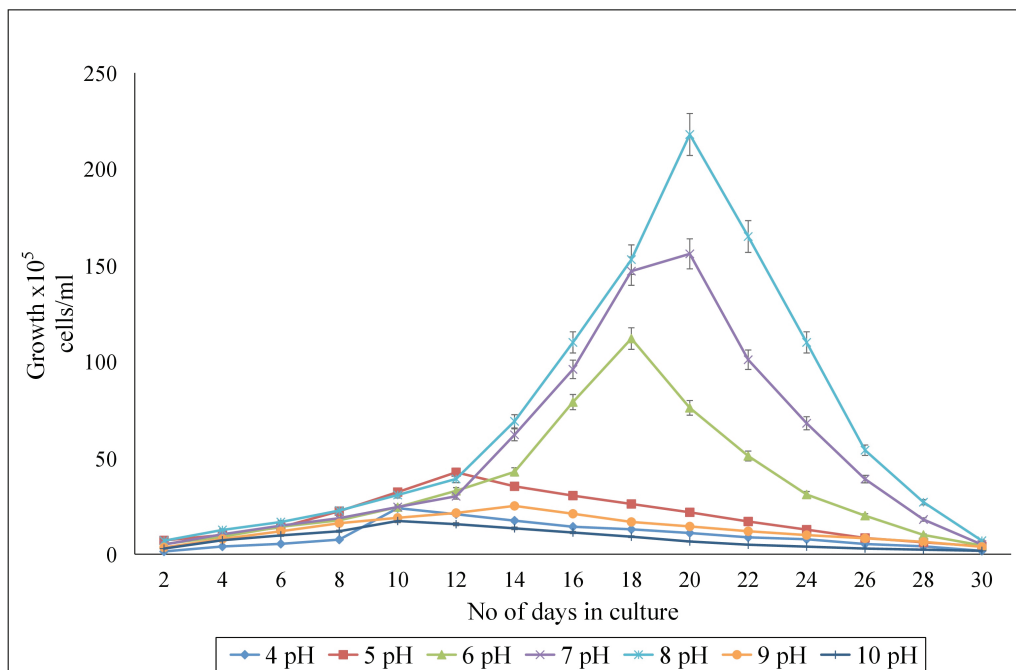
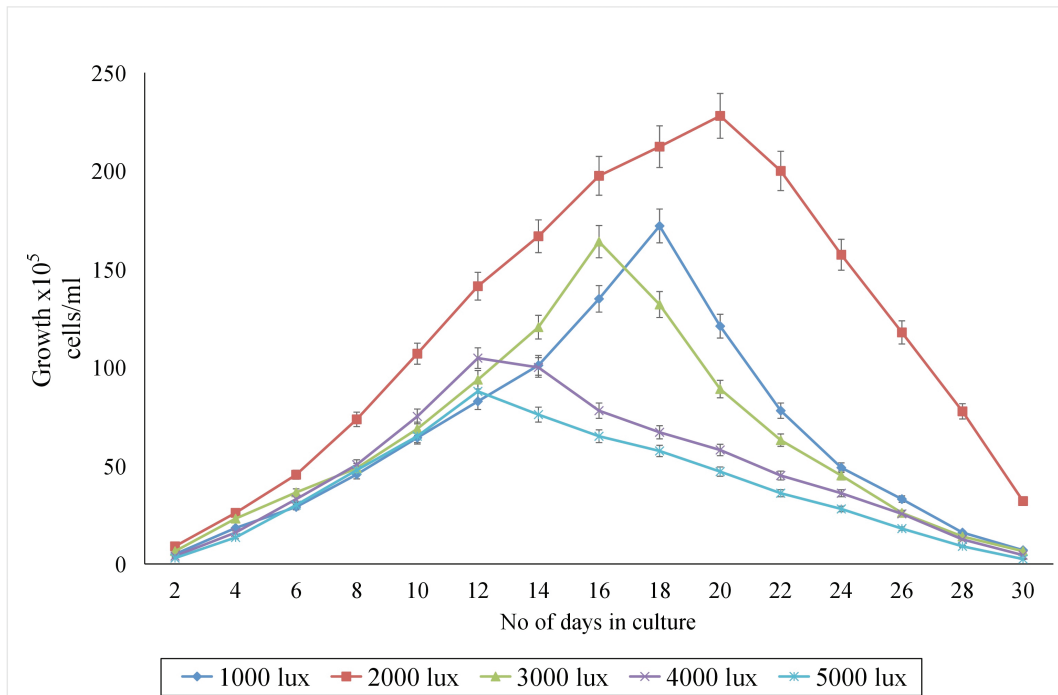
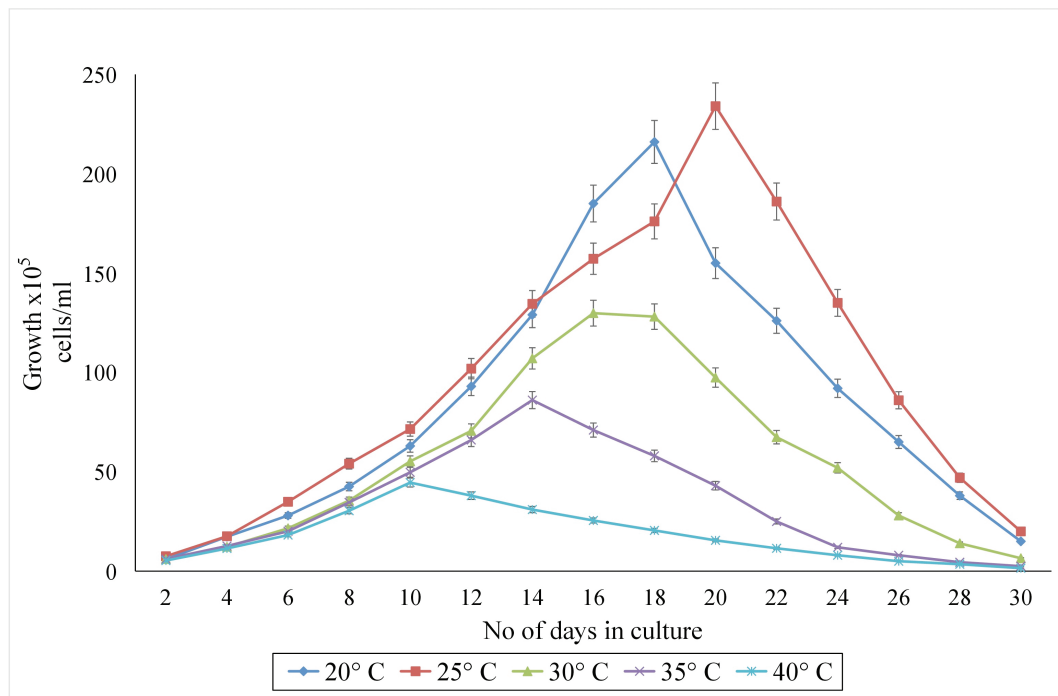


Figure 2: Influence of pH on the growth of *N. gaditana*. Values are represented as mean ± standard deviation (n = 3).



**Figure 3:** Influence of light intensity on the growth of *N. gaditana*. Values are represented as mean  $\pm$  standard deviation ( $n = 3$ ).



**Figure 4:** Influence of temperature on the growth of *N. gaditana*. Values are represented as mean  $\pm$  standard deviation ( $n = 3$ ).

lux, the biomass decreased to  $0.6743 \text{ g l}^{-1}$ , but the light intensity variation may be due to species specificity. Thus, the study of Monika research team supports these present findings.

#### 3.2.4. Temperature

The impact of temperature changes the rate of chemical reaction and the stability of cellular components [36]. Brock and Brock

[37] stated that most of the microbes respond to increases in temperature with increased exponential growth rates until reaching their optimum. In the present study, the role of temperature for the growth and lipid production was tested at different temperatures ranging from  $20^\circ\text{C}$ ,  $25^\circ\text{C}$ ,  $30^\circ\text{C}$ ,  $35^\circ\text{C}$ , and  $40^\circ\text{C}$  with medium pH 7 and light illumination of 1,000 lux with 16:8 light and dark cycle. *N. gaditana* showed a maximum growth of  $227 \times 10^5 \text{ cells/}$

ml at 25°C and the maximum lipid yield of 32.9% at 30°C (log phase) (Fig. 4 and Table 1).

In this study, the microalgae recorded the maximum growth at 25°C, and the maximum lipid yield of 32.9% was observed at 30°C. This may be due to temperature changes which induced the rate of chemical reaction and the stability of cellular components, thus inducing cell proliferation and changes in cell metabolic activities in response to environmental stress [36,38]. An earlier worker also reported that the *Nannochloropsis salina* culture reached their maximum specific growth rate at 26°C, whereas no growth was seen above 35°C [39]. It was also recorded that the *Chlorella capsulata* showed the best growth at 25°C [26].

The range of all influential parameters on maximum lipid yield was studied separately based on the optimization result. The factors such as 30 ppt of salinity, pH 8, temperature of 30°C, and 2,000 lux light intensity with 16:8 light and dark cycle were selected for large-scale cultivation under 5-LI plastic container. *N. gaditana* showed a maximum growth of  $293 \times 10^5$  cells/ml and an oil yield of 40%. Thus, the result showed that the optimized condition enhanced the lipid content up to 40%.

#### 4. CONCLUSION

From the observation, it is concluded that the microalgae prefer different physical parameters to attain their maximum growth and lipid yield. The lipid yield obtained from *N. gaditana* was 40% with the optimized condition of 30 ppt salinity, pH 8, temperature 30°C with 2,000 lux light intensity and it can be a promising candidate for bioenergy production. To maximize the lipid yield, the physical parameters optimized in this study can be applied. Further research will be carried out for purification and characterization of lipid from *N. gaditana* and incorporating them in various applications.

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#### CONFLICT OF INTEREST

Markose Shyni, Chellappan Ajan, Thangamani Praba, Geroge Subilal, Thangaswamy Selvaraj, Thavasimuthu Citarasu, and Mariavincent Michaelbabu – The authors have no conflict of interests.

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#### AUTHORS CONTRIBUTIONS

Markose Shyni – conceptualized the idea, designed the experiments, drafted the manuscript, and conducted a part of the experiment; Chellappan Ajan, Thangamani Praba, and Geroge Subilal – conducted a part of the experiment; Thangaswamy Selvaraj and Thavasimuthu Citarasu - assisted in Statistics, Mariavincent

Michaelbabu – supervised the work and assisted in drafting of the manuscript. All authors approved the manuscript and agreed to the authorship and submission of the manuscript for peer review.

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