**Original Research Article**

DOI: 10.26479/2018.0406.29

EFFECT OF TEMPERATURE AND pH VARIATION ON BIOMASS AND LIPID PRODUCTION OF *AUXENOCHLORELLA PYRENOIDOSA***B R D'Mello^{1,2*}, M S Chemburkar¹**

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ABSTRACT: Algae are efficient producers of natural oils, sequester carbon dioxide thereby reducing greenhouse gases, and do not compromise a food stock or deplete soil nutrients. When put into nutrient starvation conditions, algae may switch carbon allocation from reproduction to oil production. Finding high lipid producing strains and selecting the appropriate culturing and processing conditions are critical to realize the potential and largescale adoption of advanced algal biofuels. The present study was aimed at studying the effect of temperature and pH variation on biomass and lipid production by a newly isolated microalga *Auxenochlorella pyrenoidosa* under autotrophic conditions. It was found that the optimum temperature for biomass and lipid production was 30°C at which the microalga produced 1.196 g/L of biomass and 4.6% lipid per gram of dry biomass in presence of nitrogen source and produced 0.691g/L of biomass and 9.6% lipid per gram of dry biomass in absence of nitrogen. The optimum pH for biomass production and lipid production was found to be 7. This thermotolerant strain can be further optimised for large scale lipid production.

KEYWORDS: *Auxenochlorella pyrenoidosa*, nitrogen starvation, lipid, biodiesel.

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1. INTRODUCTION

Algae are efficient producers of natural oils, sequester carbon dioxide thereby reducing greenhouse gases, and do not compromise a food stock or deplete soil nutrients. When put into stressful environments (e.g. nutrient starvation), algae may switch carbon allocation from reproduction to oil production [1-3]. This oil from algae can be extracted and turned into biodiesel through a chemical

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Peer review under responsibility of Life Science Informatics Publications

2018 Nov – Dec RJLBPCS 4(6) Page No.378

process called transesterification [4]. Finding high lipid producing strains and selecting the appropriate culturing and processing conditions are critical to realize the potential and largescale adoption of advanced algal biofuels [5-7]. An increase in microalgal lipid content can also be induced as response to unexpected change of growth conditions. For example, during nitrogen deprivation and constant light exposure of microalgal cultures, cell division ceases and cells begin to accumulate lipids, leading to a 2–3-fold increase in lipid content [8]. Temperature is a major factor for growth in algae cultures [9]. In addition, temperature stability should be maintained at $\pm 2^{\circ}\text{C}$, particularly for marine strains which are less tolerant to temperature variations [10]. Due to the increasing demand for microalgal biomass, a process-oriented strain selection is essential in order to make large scale production economical. Process-oriented strain selection supports the use of either indigenous strains from the respective production site, or strains adapted to stress, which due to fast growth rates are able to outcompete predators or weed algae. Besides light exposure and nutrient availability, the temperature influences growth efficiency significantly. Due to high and favourable illumination the summer months usually lead to high biomass productivities [11-15]. The optimal growth temperature for common laboratory strains of microalgae varies among different species, but is usually stated to be between 20 and 30 $^{\circ}\text{C}$. Higher temperature conditions in greenhouses or outdoor cultivation settings during summer months may negatively affect growth of many microalgal species [16]. In greenhouses temperatures can reach up to 55 $^{\circ}\text{C}$, resulting in maximum culture temperatures exceeding 35 $^{\circ}\text{C}$ [17]. For outdoor cultivation similar temperatures surpassing 35 $^{\circ}\text{C}$ and even 40 $^{\circ}\text{C}$ were reported [18-20]. Whereas temperatures below the optimum lead to a retained biomass production, temperatures above the optimum results in a steep decrease in productivity and possibly the total loss of the culture. The degradation and inactivation of enzymes involved in the photosynthetic process caused by heat stress results in the inhibition of growth or even programmed cell death [21]. Construction of temperature-controlled environments for cultivation of microalgae would be ideal, but has been proven to not be sustainable due to high initial investment and operation costs. Therefore, acquiring microalgae strains with the ability to grow and propagate in these severe heat conditions, especially during summer temperatures, is of utmost importance [22]. Utilizing thermophilic organisms would minimize the amount of energy expended on cooling, thereby contributing to the overall efficiency of the system. The higher optimal growth temperature lowers the likelihood of competing species and may enable them to grow in the high-temperature waste water emitted from industrial plants [23]. Therefore, the present study was aimed at finding out the effect of temperature and pH for biomass and lipid production for a newly isolated thermotolerant strain of *Auxenochlorella pyrenoidosa* in presence and absence of a nitrogen source in the medium.

2. MATERIALS AND METHODS

2.1 Inoculum

Auxenochlorella pyrenoidosa, isolated from Sativali hot springs of Palghar district in Maharashtra was used for this study. The microalgal strain was inoculated in 100ml of BG 11 Broth and cultured for 14 days at 25°C with 16:8 h period of light: dark where artificial illumination of 8000 lux was provided using fluorescent lights. The microalgal culture was then homogenized and used as inoculum for further studies.

2.2 Effect of temperature for algal biomass and lipid production

To determine the optimum temperature required for algal biomass production the isolated microalgae was grown in 250ml flasks containing 100 ml of BG 11 broth which were incubated at different temperature. Two set of BG 11 media were used one containing the nitrogen source and the other devoid of the nitrogen source (NaNO₃). The temperatures selected for the study were 25°C, 30°C, 40°C, 50°C, 55°C and 60°C. The flasks were provided with 16:8 h period of light: dark where artificial illumination of 8000 lux was provided using the fluorescent lights and incubated for 7 days. After the incubation period, the algal biomass produced was harvested by centrifuging at 5000 rpm for 15 minutes. The harvested biomass was transferred to a pre-weighed evaporating dish and dried at 70°C in an oven till constant Dry cell weight (DCW) was obtained. All experiments were run in triplicates.

2.2 Effect of pH for algal biomass and lipid production

To determine the optimum pH required for algal biomass production the isolated microalgae was grown in 250ml flasks containing 100 ml of BG 11 broth which were incubated at different temperature. Two set of BG 11 media were used one containing the nitrogen source and the other devoid of the nitrogen source (NaNO₃). The pH selected for the study were 5,6,7,8 & 9.

The pH of the media was adjusted using 0.1N HCl/0.1N NaOH. The flasks were provided with 16:8 h period of light: dark where artificial illumination of 8000 lux was provided using the fluorescent lights and incubated for 7 days. After the incubation period, the algal biomass produced was harvested by centrifuging at 5000 rpm for 15 minutes. The harvested biomass was transferred to a pre-weighed evaporating dish and dried at 70°C in an oven till constant Dry cell weight (DCW) was obtained. All experiments were run in triplicates.

2.3 Extraction of lipids from microalgal biomass

The dry biomass was extracted with chloroform/methanol/water system (1:1:0.9) as described by Isik et al. (1999) [24]. The mass of the lipids extracted was measured gravimetrically by evaporating the solvent.

3. RESULTS AND DISCUSSION

3.1 Effect of temperature on biomass and lipid production by *Auxenochlorella pyrenoidosa*.

The process parameter of temperature was assessed for biomass production and lipid production for the newly isolated strain of *Auxenochlorella pyrenoidosa*. The microalga was cultured autotrophically at different temperatures. The biomass and lipid produced at the end of the incubation was dried and estimated gravimetrically. It was found that the optimum temperature for biomass and lipid production was in between 30°C (Fig 1). The microalgae showed considerable growth and lipid production at 40°C also. Although the growth decreased with the increase in temperature, the microalgal isolate was able to tolerate a temperature of 60°C with a low biomass productivity (Fig 1). Lipid content was higher in the microalgae cultured in media devoid of nitrogen source.

Table 1: Effect of temperature on Biomass, Biomass Productivity and lipid production by *Auxenochlorella pyrenoidosa* in BG 11 medium with/without Nitrogen source

Sr. no.	Temperature	BG11 with Nitrogen source			BG11 without Nitrogen source		
		Biomass g/L	Biomass productivity g/L/day	Lipid produced (%)	Biomass g/L	Biomass productivity g/L/day	Lipid produced (%)
1	25°C	0.885	0.126	4.4	0.574	0.082	9.3
2	30°C	1.196	0.171	4.6	0.691	0.098	9.6
3	40°C	0.944	0.134	3.9	0.644	0.092	9.1
4	50°C	0.796	0.114	3.6	0.471	0.067	8.15
5	55°C	0.605	0.086	3.1	0.356	0.050	6.84
6	60°C	0.436	0.062	1.8	0.235	0.033	4.2

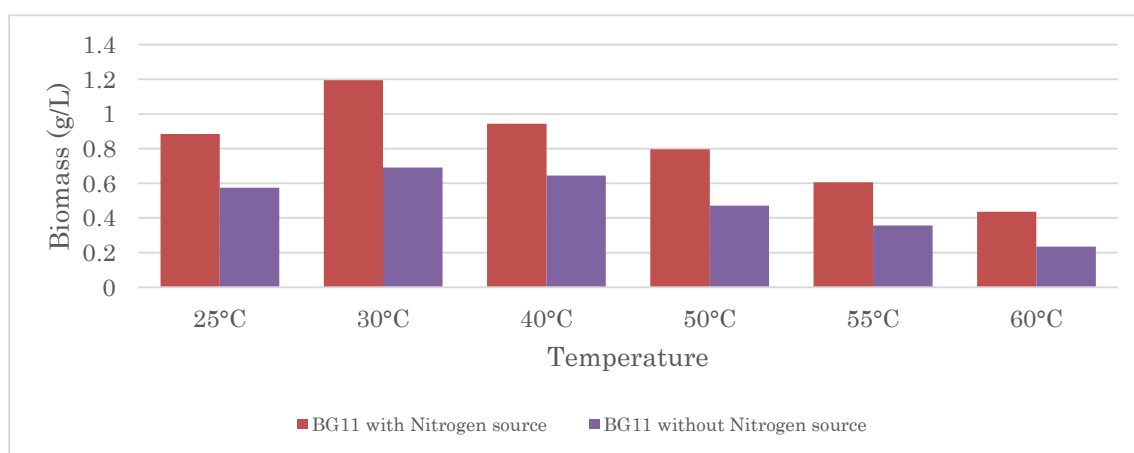


Figure 1: Effect of temperature on biomass production by *Auxenochlorella pyrenoidosa* in BG 11 medium with/without Nitrogen source

Ma X et al (2014) reported that optimal temperature for cell growth and lipid accumulation for *C. vulgaris* was between 25 and 30 °C [25]. In 2009, Converti et al stated that the lipid content of microalgae was strongly influenced by the variation of tested parameters; indeed, an increase from 25 to 30 °C brought about a decrease of the lipid content of *C. vulgaris* from 14.71 to 5.90%.

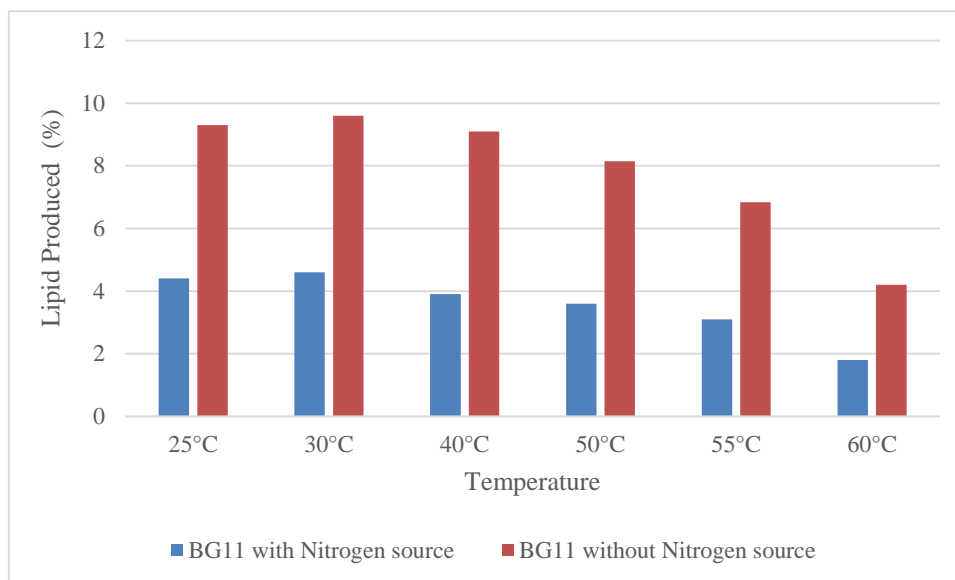


Figure 2: Effect of temperature on lipid production in *Auxenochlorella pyrenoidosa*

On the other hand, nitrogen limitation in the medium increased the lipid production of *C. vulgaris* from 5.90 to 16.41% [13]. Bamba *et al.* (2015) reported that *C. vulgaris* growth was similar at 25 and 30 °C, while at temperatures above 30 °C, growth was notably slower [27]. Similar results were obtained by El-Sheekh et al (2017) for *Scenedesmus acutus*, where the microalgae showed maximum growth at 30°C and the growth reduction was less pronounced at 40°C [28]. Also, the differences in biomass productivity did not vary significantly between incubation temperatures of 25°C and 30°C. Fogg and Thake (1987) stated that low growth rate of microalgae could be a result of the increase in respiration due to rise in temperature above the species optimum level. [29]

3.2 Effect of pH on biomass and lipid production of *Auxenochlorella pyrenoidosa*

The optimum pH for the biomass and lipid production for the microalgae was found to be 7 (Fig 1&2). The microalgal culture showed reduced production of biomass and lipid at acidic pH when compared to that of alkaline pH. Sakarika M *et al* (2016) reported similar results for *Chlorella vulgaris* where optimal pH for biomass growth and lipid accumulation was found to be 7.5 [30]. Feifei H et al (2013) reported that the best growth performance was achieved by controlling pH at 7; lipid contents of the *Chlorella pyrenoidosa* cells can be increased under the nitrogen-limitation conditions [31]. Zhang et al. (2014) reported that optimal pH value for lipid accumulation of *Chlorella sp.* HQ should be between 7.0 and 9.0 [32]. Rodolfi et al. (2009) observed better growth of *C.vulgaris* at pH 6.5 and 7.0, and accumulated lipid at pH 7 and 8.5, so optimal for growth and lipid accumulation of *C.vulgaris* was at pH7.0 [6]. Alkaline pH increases the flexibility of the cell

wall of mother cells, which prevents its rupture and inhibits autospore release, thus increasing the time for cell cycle completion which leads to lipid accumulation [33]. Karatay et al. (2011) reported maximum lipid production for three microalgae in BG-11 medium was within the pH range of 7-9 [34].

Table 2: Effect of pH on Biomass, Biomass Productivity and lipid production by *Auxenochlorella pyrenoidosa* in BG 11 medium with/without Nitrogen source

Sr. no.	pH	BG11 with Nitrogen source			BG11 without Nitrogen source		
		Biomass g/L	Biomass productivity g/L/day	Lipid produced (%)	Biomass g/L	Biomass productivity g/L/day	Lipid produced (%)
1	5	0.566	0.081	3.41	0.375	0.054	4.81
2	6	0.754	0.108	3.85	0.503	0.072	7.1
3	7	1.184	0.169	4.62	0.785	0.112	9.6
4	8	1.021	0.146	4.3	0.702	0.100	9.44
5	9	0.711	0.102	3.64	0.511	0.073	6.87

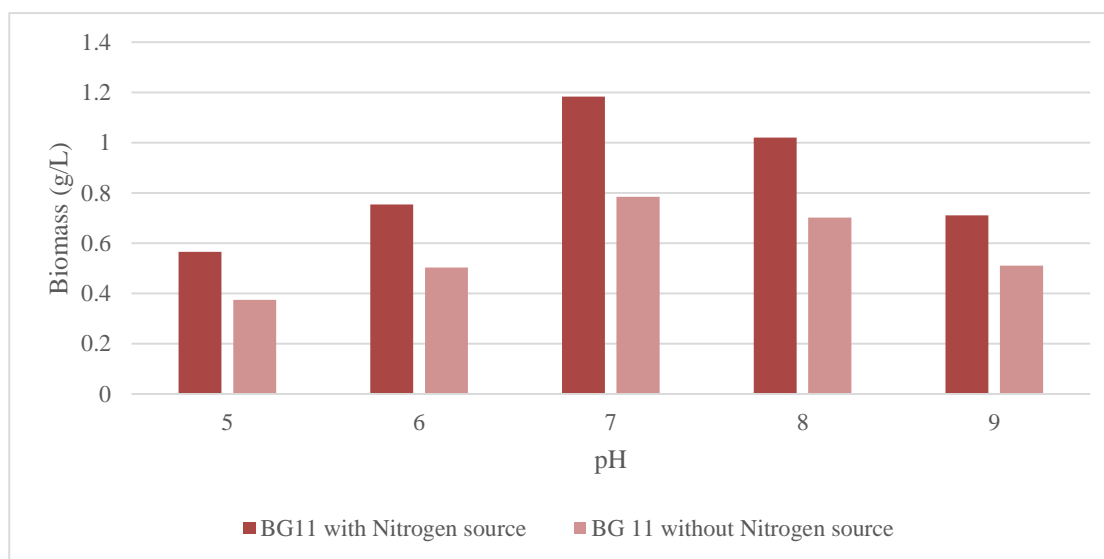


Figure 3: Effect of pH on biomass production by *Auxenochlorella pyrenoidosa* in BG 11 medium with/without Nitrogen source

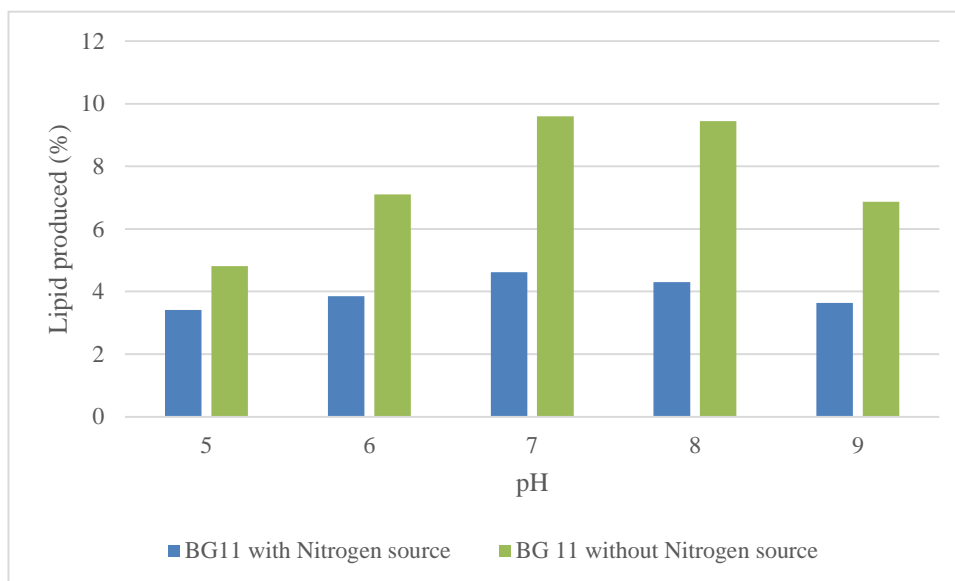


Figure 4: Effect of pH on lipid production in presence/absence of nitrogen source for *Auxenochlorella pyrenoidosa*

4. CONCLUSION

The variation of parameters tested (temperature and pH) strongly influenced the lipid content of microalgae. Both the stress conditions investigated led not only to the accumulation of lipids, but also to a reduction in microalgae growth, thereby affecting the lipids productivity. In particular, the growth of *Auxenochlorella pyrenoidosa* was not significantly influenced by temperature. This may be because the microalgal culture was isolated from hot springs which have a temperature of around 55-56°C and so it is well acclimatized to higher temperatures. The microalgal culture showed maximum biomass and lipid production at a pH of 7 but it was not significantly affected by increase in the pH of the medium. For the use of microalgae in the production of biodiesel, large scale biomass production needs to be carried out where temperature and pH are the limiting factors. To make the process more economical, thermotolerant microalgae can be used which would circumvent the need to maintain lower temperatures. Thus, the newly isolate strain of *Auxenochlorella pyrenoidosa* can be used in future for large scale biofuel production.

ACKNOWLEDGEMENT

The authors would like to thank the help and support of Department of Botany, Bhavans College, Mumbai and Department of Biotechnology, VIVA College, Maharashtra.

CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

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