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Effect of Light Intensity and pH on Cell Density Assessed by Spectrophotometry for the Unicellular Algae *Chlorella vulgaris*

Nishat Tasnim[1], Debabrata Karmakar[1], Rakibul Hasan[1], Rashadul Islam[1], Saddam Hossain[1], Aftab Ali Shaikh[1,2], Rezaul Karim[1*]

[1]Bangladesh Council of Scientific and Industrial Research, Dhanmondi (BCSIR), Dhaka, Bangladesh
[2]Department of Chemistry, University of Dhaka, Dhaka, Bangladesh
Email: *ittibcsir2020@gmail.com

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Abstract

In this study, an effective environment for *Chlorella vulgaris* growth is sought after. As a substitute source of food and feed, increasing the cell density of *Chlorella* culture is one of the keys to ensuring sustainability. It can be showed from different studies that optimum light intensity and pH could increase cell density. In this study, the effects of light and pH on the growth rate of *C. vulgaris* were observed in photobioreactor. A specific wavelength (682 nm) was determined by UV-Vis Spectrophotometry to carry out the further analysis. The light intensities were set at 7409, 9261 and 11,113 lux; pH values were set at 7, 8 and 9 respectively. The experimental results depicted the light intensity of 9261 lux as the best due to the higher number of cells (48.56×10^6 cells/mL) obtained using this intensity. In terms of pH, without pH control, cell numbers were found to be highest under the light intensity of 9261 lux. When pH was controlled, it was found that under the optimum light intensity, pH control between 7.0 and 7.5 was the optimum range for the growth of *C. vulgaris*. Moreover, this method of study may possibly be a promising source of low cost culture for *Chlorella vulgaris*.

Keywords

Chlorella vulgaris, Light Intensity, pH, Cell Density

1. Introduction

By 2050, Bangladesh will have 200 million people, an increase in population of around 1.08%, which will provide problems for the country's sustainability overall as well as the provision of food and energy. Bangladesh is experiencing "se-

rious localized food insecurity" as a result of various issues, such as financial difficulties, flooding, exorbitant costs for key foods, etc. For uplifting the population and economy, food and energy need to be not only sustained but also greatly enhanced. In many nations, academics and policymakers are searching for fossil fuel substitutes; biofuel is one of the more promising possibilities. Microalgae have been touted as a promising source for the environmentally friendly production of feed, fuels, and chemicals for a number of years [1].

Bangladesh has diverse biodiversity of natural resources [2]. Microalgae are a component of the abundant diversity in addition to other plants and animals. The small microorganisms known as microalgae can flourish in fresh, salt, marine, and waste water. Microalgae that are grown photo autotrophically outdoors go through regular day-night cycles of sunlight and CO_2 absorption. As a result, they might be referred to as "microplants", and the products they produce can simply be called vegetables [3].

Chlorella vulgaris is one of the microalgae that can withstand all environmental changes. It is a spherical, single-celled green alga that is 3 to 8 microns in size [4]. Being a cosmopolitan microalga, *C. vulgaris* can thrive in humid environments and be lowered on the ground [5]. Additionally, *Chlorella* has the capacity to inhibit the growth of pathogenic microorganisms [6]. It is imperative to establish the best growing methods for chlorella to get biomass in the shortest period of time.

The biomass of *C. vulgaris* comprises significant chemical components like total lipid content, proteins, chlorophyll, and carbohydrates. The economic value of *C. vulgaris* is considerable, and it contains nutrients such as fat, which has a concentration of 14% - 22%, as well as protein, carbs, etc. [7] [8]. The production of maximum algal biomass using the photobioreactors by continuous culture has been used worldwide [9]. It is standard practice to continuously evaluate the density of the grown algae in terms of cells per milliliter or dry weight (g/L). Both are time-consuming. Simply recording the optical density (absorbance) of culture samples and determining the algal density using a reference regression line of optical density (OD) to cell density by employing spectrophotometry absorbance values can substantially simplify the monitoring of culture density [10].

So, improving the cell density of microalgal culture has great significance for industrialization which can be potentially used as both feeds and nutrient-dense meals (supplements) (feed for larvae, fish and shrimp). Numerous studies have demonstrated that environmental variables like intensity of light and pH have an impact on the growth of microalgae. In addition to controlling biological processes, light intensity also affects biomass composition and output [11]. The photosynthesis of microalgae is influenced by light intensity, which in turn impacts their growth rate. Up to a certain point, depending on the particular microalgae species, increased light intensity often accelerates microalgal growth. However, photoinhibition may result from excessive light intensity up to the point of saturation [12]. When light levels exceed physiological saturation, photoinhibition

occurs when there is a surplus of photons that cannot be disposed of by photosynthesis, carbon fixation or fluorescence. On the other hand, microalgae growth will be constrained if light intensity is below the saturation point [13]. According to numerous research, chlorella may thrive in a wide pH range, with alkaline circumstances producing the highest biomass yield [14].

Therefore, the primary goal of the study is to calibrate a regression model to estimate algal density as well as the impact of light intensity and pH on *Chlorella vulgaris* in order to get high cell density.

2. Materials and Methods

The microalga was collected from The University of Texas at Austin's Culture Collection of Algae in the United States. In this experiment *Chlorella vulgaris* 2714 was tested. The alga was grown axenically using Bold's Basal Medium [15] and continuous illumination.

C. vulgaris was grown using a certain method on a closed laboratory bioreactor of 6 L capacity (Nano, Synoxis algae, France) which provides the creation of a vertical spiral movement generated by an Air lift (by air injection) in a tubular system. The spiral movement promotes light exposure and gas or culture medium exchange. It thus brought an appropriate environment for the development of microalgae and the culture was homogenous. This technology is combined with a control system to automate the cultures.

C. vulgaris were cultured 10 - 12 days, with bold basal medium in the photobioreactor with three replicates under different light intensities. Prior to cultivation, the culture media and all containers were sterilized in an autoclave for 15 minutes at 121˚C to reduce the risk of contamination. The inoculum algae density was ~ 0.5 - 1.0 × 10^6 cells/mL.

The following equation was used to calculate the microalgae's growth rate:

$$\text{Cell Density} = m * \text{optical density} + C,$$

where, m denotes the slope of curve and C denotes the intercept of Y axis.

The light intensities were set at 100 μE /m^2/s (7409 lux), 125 μE /m^2/s (9261 lux) and 150 μE /m^2/s (11,113 lux). The light/dark period was 12 h/12h. The intent of this variation in light intensity was to examine how light intensity affected the *C. vulgaris* cell density. During its 12-day growth phase, this microalga was cultured at a temperature of 25˚C ± 1˚C. Air pipes that were fed with continuously filtered air by a compressor with an average flow rate of 10 lpm at 1.2 bar pressure were used to aerate the vessels. The pH was set at 7, 8 and 9 respectively and pH adjustment method included with and without pH control. The method of pH control involves daily adjustment of pH using CO_2 gas and the method of without pH control refers to only setting up the value of pH at the beginning of cultivation.

To determine the influence of these conditions, the concentration of cells (observation of algae growth) was observed by an Inverted Biological Microscope Raxvision Y100 (**Figure 1**) and UV-vis Spectrophotometry (Hach, DR-6000) was

used to examine the optical density of the suspension.

3. Results and Discussion

3.1. Relation between Absorbance and Cell Density of *Chlorella vulgaris*

To estimate algal concentration, the method of direct cell count is followed by obtaining the mean number of cells. An equal mixture of cell suspension and trypan blue solution is used to determine the cell number using a hemocytometer.

By using a UV-Vis Spectrophotometer to scan a culture sample between 600 and 800 nm, the maximum absorbance was determined. Measurements of the spectra at various growth stages revealed a nearly comparable pattern. In this study, the algal species exhibited prominent peak at 682 nm (Figure 2).

Direct cell counts, measurements of the chlorophyll content, and absorbance

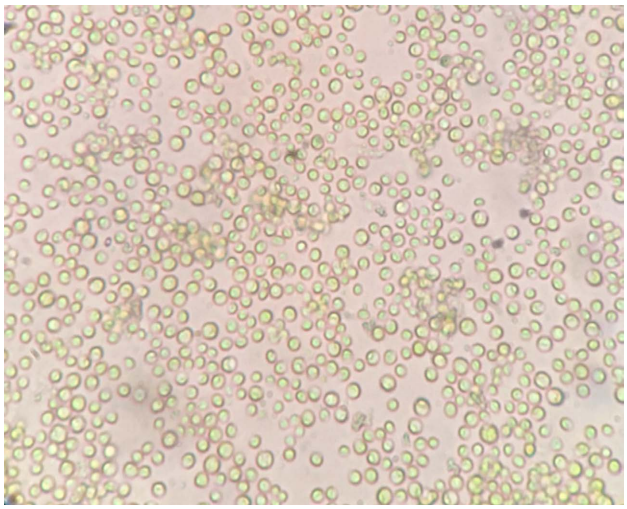

Figure 1. Cells of *Chlorella vulgaris* (Microscopic image 40× magnification).

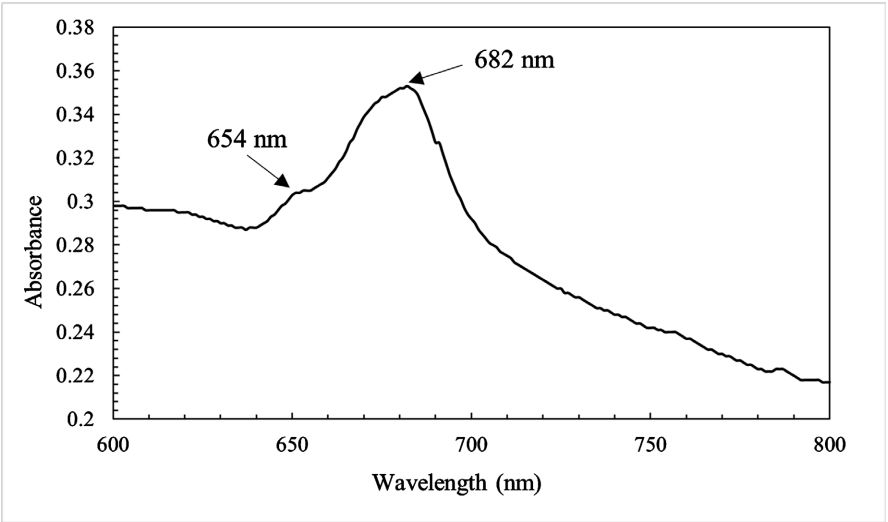

Figure 2. Light absorbance pattern for a *C. vulgaris* solution screened between 600 and 800 nm.

can all be used to calculate the concentration of algae [16]. If cell concentration is to be obtained through spectrophotometry, then a wavelength of 750 nm is more preferable [17], although values of 665 nm, 680 nm are also used [18]. Typically, 680 nm is associated with chlorophyll absorption and has the possibility of interfering with results whereas 750 nm is above chlorophyll absorption [19].

The pattern of light absorption for a solution of *Chlorella vulgaris* screened between 600 and 800 nm is shown in **Figure 2**. Although two peaks (654 and 682) could be seen, the wavelength with the highest sensitivity to quantify *C. vulgaris* was obtained at 682 nm, where the highest absorbance was also obtained. Consequently, similar wavelength was used to read all subsequent analyses of samples.

Figure 3 depicts the association between *C. vulgaris* absorbance and cell density.

The line represents the absorbance equation:

$$y = 15.485\ x + 0.0168\ (R^2 = 0.9992)$$

Here, y = Cell density (Millions)

x =Absorbance (682 nm)

3.2. Optimization of Light Intensity on Cell Density of *Chlorella vulgaris*

According to Dean [20], a variety of parameters, particularly light intensity, influence the growth of microalgae. For microalgal autotrophic growth and photosynthetic activity, light is an essential source [21]. It stimulates cell division, respiration, and photosynthesis [22]. To produce ATP and NADPH as well as for the synthesis of essential molecules needed for growth, microalgae require light [23]. Varied microalgae species have different optimal light levels for growth and biomass production, which also depend on other parameters including temperature, pH, and the nutrients availability in the culture medium

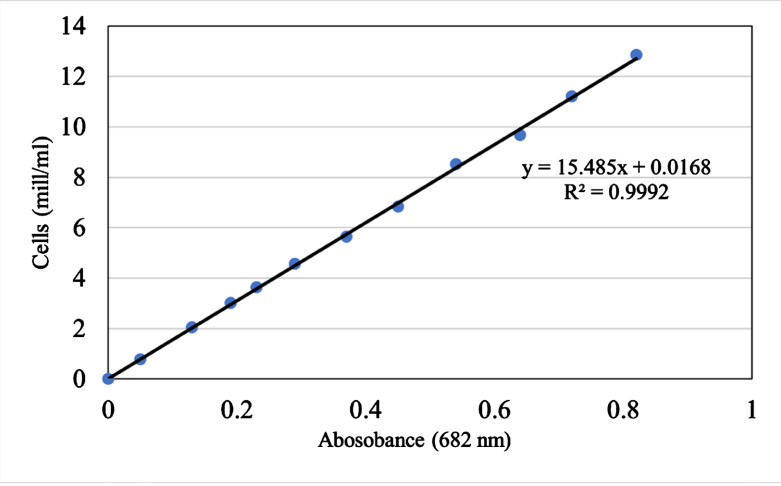

Figure 3. Relationship between *C. vulgaris* cell density and absorbance (682 nm).

[24]. As light intensity increases as a result of photosynthetic activity, microalgal growth also increases. On the other hand, photo-inhibition occurs at high light intensities, exceeding the saturation point [25].

In the current study, it was observed that *C. vulgaris* growth was significantly impacted by light intensity. Other studies, like [25] and [26], have also shown faster growth rates for *C. vulgaris* under higher light intensities. All light intensity batches reached their peak growth on the 12th day, according to the findings of this study. Once the microalga's growth had reached its peak and had transitioned into its stationary phase, it was harvested.

According to Muchammad [27], *C. vulgaris* can grow when given adequate light intensity, which was in the range of 500 - 5000 lux. Febrieni [28] reported that 10,000 lux showed a different response where the growth remained high [28]. However, this study was different at initial light intensity. In this study, we measured the algal growth at three different light intensities: 7409 lux, 9261 lux and 11,113 lux. It was observed that these different light intensities didn't give the maximum growth, where they are set as the initial light intensity. The microalgae cells failed to survive with these high light intensities at the initial period. Moreover, in this study, we maintained a definite light intensity (7409 lux) upto a specific cell density (~2 - 3 × 10^6 cells/mL obtained within Day 2) and then from that certain point of growth, by counting the number of cells, we were able to determine how those light intensities affected the rate of algal growth.

With a temperature of 25˚C, aeration, and a day/night cycle of 12:12h, an initial light intensity of 7409 lux was set for each batch to observe the impact on the growth rate of *C. vulgaris*. During the experiment, when we set the initial light intensity at 9261 lux, its growth relatively decreased so as for 11,113 lux. That might have happened because the light that obtained was not the best for absorbing photon energy, which may reduce photosynthesis. Wu H [29] reported that very high light can reduce the rate of photosynthesis and also the growth [29]. So, we maintained the initial light intensity of 7409 lux for 2 days and reached the certain growth. After that we set the light intensity at 9261 lux and 11,113 lux sequentially to observe the effect. The cell numbers were 40.21, 48.56 and 43.37 × 10^6 cells/mL under the light intensity of 7409 lux, 9261 lux and 11,113 lux respectively. Although, while observing the algal growth started from an initial light intensity, *C. vulgaris* showed higher response to light intensity of 9261 lux. The rate of growth persisted up to 48.56 × 10^6 cells/mL which was ~1.2 times greater than the rest of the two (**Figure 4** & **Figure 5**).

From **Figure 5**, the differences in cell density could be seen under the three light intensities by the bar diagrams.

3.3. Optimization of pH on Cell Density of *Chlorella vulgaris*

Qitao G [30] reported that, the cell densities had very small differences in various initial pH levels under the same light intensity [30]. At three different light intensities, without pH control, the cell density under the light intensity of 9261

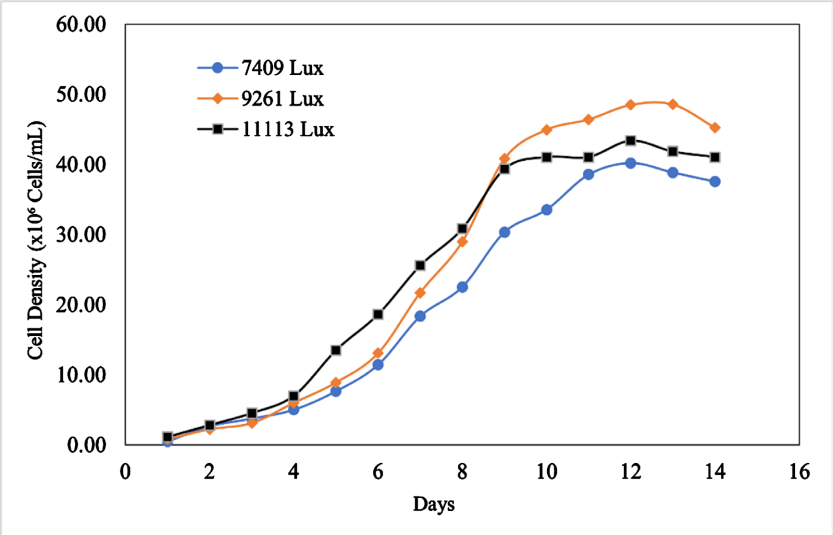

Figure 4. Growth curve of *C. vulgaris* under various light intensities.

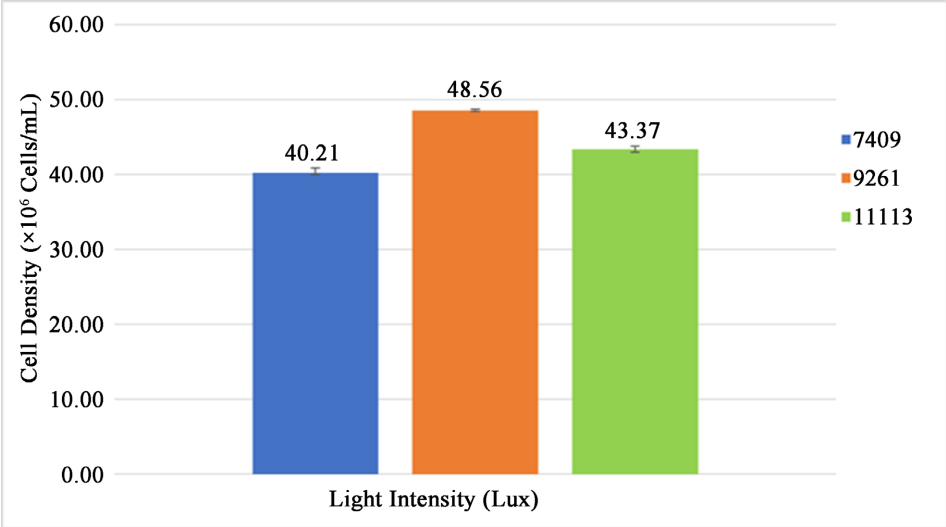

Figure 5. The effect of different light intensity on cell density of *C. vulgaris.*

lux was the highest 48.56 × 10^6 cells/mL (**Figure 5**). In this study, cells under 9261 lux grew rapidly with initial pH at 7.0 (**Figure 6(a)**).

When experimented with different pH values such as, 7.0, 8.0 and 9.0, it was seen that, pH control between 7.0 and 7.5 was the optimal range for the growth of *Chlorella vulgaris* under the light intensity of 9261 lux. When the pH of the culture suspension was set at 8 and 9 respectively, they showed a gradual decrease in cell numbers with the days passed by. The cells had a significant decrease since the 7^{th} to 8^{th} day (**Figure 6(a)**). Consequently, the mentioned day had to be chosen as the stationary phase. The microalga suffers death during the stationary phase was reported by Widyaningrum [31]. **Figure 6(b)** showed the differences of cell density with different pH under the same light intensity. Moreover, with the initial pH at 7.0, showed good results for microalgae cell proliferation (49.72 × 10^6 cells/mL) under the light intensity of 9261 lux.

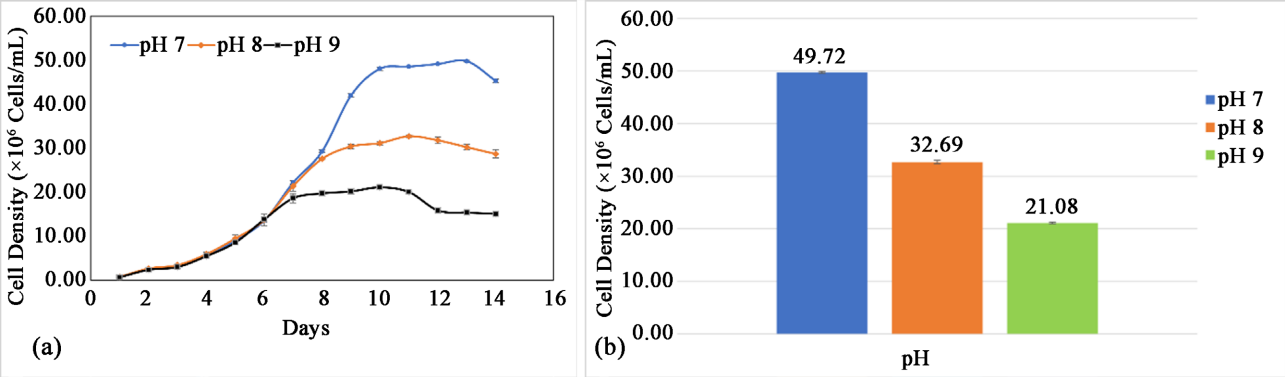

Figure 6. Effect of pH on *C. vulgaris* cell density. (a) Comparison of different pH values with days and (b) Bar diagram showing the changes in cell numbers with different pH values.

4. Conclusion

The growth of *Chlorella vulgaris* was significantly influenced by the light's wavelength and intensity. The results indicated that this study offers the advantages of having a higher cell density with totally controlled light and temperature in a small spaced photobioreactor. The best productivity was obtained with a pH between 7.0 and 7.5 under the light intensity of 9261 lux using the photobioreactor system. *Chlorella vulgaris* is environmentally friendly and cultivating this alga in a commercial scale will lead us to the sustainable food or feed supplements for our upcoming future. We have studied this system to be able to grow algae throughout the year especially in fish hatchery production seasons.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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