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# Light and growth medium effect on *Chlorella vulgaris* biomass production

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ABSTRACT

Algae can serve as feedstock for many high value bioproducts and biofuels production. The key to economic algal biomass production is to optimize the growth conditions. This study presents the effect of light wavelengths and growth medium composition on the growth of *Chlorella vulgaris*. Different light wavelengths [blue, clear (white), green, and red] were used to test their effect on algal growth. Growth media formulations were varied to optimize the growth media composition for maximized algal biomass production. Experimental study was conducted in three phases to evaluate: (1) the effect of different light wavelengths; (2) the effect of the recommended growth medium at 25%, 50%, and 100% of suggested composition; and (3) the effect of nutrient concentrations (nitrogen and phosphorous). The effect of these factors was evaluated through specific algal growth rates and volumetric biomass productivities during the entire growth period. In this study, blue light performed better (higher growth rate and biomass productivity) at longer growth periods (10–14 days) compared to clear, red and green light wavelengths. The growth media and nutrient effect results indicate that the growth of *C. vulgaris* is higher at 50% suggested growth media composition compared to 100% growth media composition which results in approximately 50% potential reduction in chemical costs for large scale algal production.



## Introduction

Algae represent a renewable and environmental-friendly feedstock for production of a variety of high value bio-products and biofuels [1]. Specific advantage with microalgae is that they contribute to carbon sequestration during their production. The production of algae is carbon-neutral process since for every pound of algal biomass produced; about 1.8 pounds of $CO_2$ are sequestered from the atmosphere [2]. A variety of biofuels can be derived from different algal species which depends on their cell composition. Algae have been investigated for the production of different biofuels including biodiesel, bio-oil, bio-syngas, bio-electricity and bio-hydrogen [1–7].

The algal cultivation and harvesting/processing systems for high value bio-products seem to be profitable for producers due to high cost of the final product, but the same is not true when biofuel production (biogas, bioethanol and biodiesel) is considered [3,8]. Algal cultivation and harvesting/processing are both energy- and cost-intensive [9,10], especially for algal biofuel production, existing cultivation and processing methods are not economical or sustainable [4,5]. For algal biomass production, suitable growth medium with nutrients is essential [9,10]. Current methods utilize large quantities of nutrients which results in overall imbalance of energy and environmental benefits. Considering tight profit margin of the algae feedstock for biofuel production, algal growth optimization with minimum nutrients is warranted.

Algae essentially require light (energy), carbon source ($CO_2$ for autotrophic metabolism), growth medium (water) and nutrients (nitrogen and phosphorous) for reproduction. Some algae species can utilize waste organic sources such as municipal and industrial wastewaters as carbon source through heterotrophic metabolism. Among the requirements for algal growth, nutrients are the most cost-involving as their production involves natural resource and energy utilization. Optimizing the nutrients required for algal growth can mitigate their production costs and significantly improve the downstream process economics.

While carbon dioxide is available abundantly in the atmosphere as well as from anthropogenic sources, the availability of light is very important for the algae growth. Since, photoautotrophic microalgae depend on light source to obtain energy and convert it into chemical energy such as adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADP), it is worthwhile to study the effect of different light spectrum on the algal growth [11]. Generally, microalgae use light of wavelengths from 400 to 700 nm for photosynthesis. The wavelengths absorbed by microalgae differ depending on the species. For instance, green microalgae absorb light energy for photosynthesis through chlorophylls as a major pigment absorbing light energy in the range of 450–475 nm and 630–675 nm and carotenoids as an accessory pigment absorbing light energy of 400–550 nm. Several

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studies reported the growth of microalgae in different light wavelengths. Red (600–700 nm) and blue lights (400–500 nm) stimulate the growth of microalgae, and the growth rates and lipid content of the microalgae differ with light intensity [12–15]. Microalgal cell growth rates and lipid content are affected by environmental parameters such as temperature, light intensity and frequency, gas composition, and nutrient level in the culture system [16–18]. Some studies focused on the temperature and nitrogen concentration effect on the growth of fresh algae and *Nannochloropsis oculata* [19–21].

This research is aimed to study the effect of light (different wavelengths) and different nutrient concentrations on the growth of microalgae *Chlorella vulgaris.* Three major experimental tasks were undertaken to study the effect of: (1) different light spectrum, i.e., blue, clear (white), green and red light under the suggested growth medium, (2) overall growth medium composition (i.e., culture conditions); and (3) specific effect of nitrogen and phosphorous concentrations on the growth of microalgae. The nutrient effect study was conducted to verify the suitability of the suggested growth media by the supplier to grow microalgae *C. vulgaris.* As this growth media was a generalized nutrient formula, specific nutrient composition was obtained by evaluating the influence of the overall media concentration and as well as concentrations of nitrogen and phosphorus individually. Algal cell growth was evaluated through cell density measurements, daily volumetric biomass productivity and specific growth rates. The effect of nitrogen and phosphorous concentrations individually on the growth of *C. vulgaris* was also discussed.

## Materials and methods

### *Algal growth medium*

The microalgae *C. vulgaris* was purchased from Connecticut Valley Biological Supply Co. Inc., Southampton, MA. The medium used in the cultivation of the microalgae was Bold's basal medium (BBM). This medium stimulates the growth of *Chlorella sp.* preventing the growth of others so the population of *C. vulgaris* is increased by cultivations. The algae growth media composition is as follows: $CaCl_2$ (25 mg/L), NaCl (25 mg/L), $NaNO_3$ (250 mg/L), $MgSO_4$ (75 mg/L), $KH_2PO_4$ (105 mg/L), $K_2HPO_4$ (75 mg/L), and 3 mL of trace metal solution with the following concentration was added to the 1000 mL of the above solution: $FeCl_3$ (0.194 g/L), $MnCl_2$ (0.082 g/L), $CoCl_2$ (0.16 g/L), $Na_2MoO_4{\cdot}2H_2O$ (0.008 g/L), and $ZnCl_2$ (0.005 g/L). These values are shown in Tables 1a (nutrients) and 1b (trace mineral composition). This growth medium is similar to the standard microalgae media 3N-Basal Bold medium (3N-BB) except that 3N-BB has higher nitrate concentrations. This nutrient formula was optimized in general to be suitable for most green, yellow-green, golden-brown, and red algae and dinoflagellates. However, it was unclear if this growth medium was optimized to grow *C. vulgaris*, thus lacking optimized growth media for maximized cell growth. Algae growth was monitored by measuring the optical density of the algal medium with Spectronic®20 Genesys spectrophotometer at a wavelength of 650 nm. Measurements were taken daily and three replicates were measured per each reactor.

**Table 1a**
Algal growth medium composition (major nutrients).

| Compound | Conc. (mg/L) | Compound | Conc. (mg/L) |
|---|---|---|---|
| **Algal growth medium composition (recommended by the supplier)** | | | |
| $CaCl_2$ | 25 | $MgSO_4$ | 75 |
| NaCl | 25 | $KH_2PO_4$ | 105 |
| $NaNO_2$ | 250 | $K_2HPO_4$ | 75 |

**Table 1b**
Algal growth medium composition (trace mineral composition).

| Compound | Conc. (mg/L) | Compound | Conc. (mg/L) |
|---|---|---|---|
| **Trace mineral solution** | | | |
| $FeCl_3$ | 194 | $CoCl_2$ | 160 |
| $MnCl_2$ | 82 | $Na_2MoO4$ | 8 |
| $ZnCl_2$ | 5 | | |

### *Light and overall growth medium effect tests*

Four identical photo-bioreactors were constructed to test the light and overall growth medium effects on the algal growth. Photo-bioreactors setup includes 3.8 gallon clear tanks filled to a depth of 6 cm (six liters) with algal growth medium and constant agitation provided by two Thermolyne Cimarec magnetic stir plates. Each photobioreactor was enclosed in a custom-made cardboard container to isolate each other from external light as well as peer-light interference. 60 W (276 μmol/$m^2$ s) CFL bulbs with the following wavelengths were used for the four photo-bioreactors: red (650 nm), blue (475 nm), green (510 nm) and clear light. They were placed at 20 cm distance from the photo-bioreactors in order to eliminate the temperature effect that may be caused by the illumination. Approximately 0.07 g/L of algal cell density were introduced initially in the algae growth reactors. The aforementioned nutrient growth medium was used in these tests. To evaluate the effect of overall nutrient medium, we have tested 25%, 50% and 100% of the original suggested medium concentrations for algal growth. The experimental setup was similar to that of the light effect test. Growth medium concentrations are shown in Table 1. The pH of the samples was adjusted to 7 during these tests.

### *Nitrogen and phosphorous concentration effect test*

For these tests, sixteen 250 mL Erlenmeyer Flasks, each filled with 200 mL of different growth medium concentrations were used as algae growth reactors. An air pump supplied 1 vvm (air volume/medium/minute) into sixteen separate air flow tubes into the vessels via a custom-made splitter [9]. Agitation (mixing) was supplied by a New Brunswick Classic C-Line incubator shaker set at 100 rpm and a temperature of 25 °C. Clear light was supplied at a rate of 60 W (lamp suspended 20 cm above the system) while the shaker was isolated from external light by cardboard enclosures. The experimental set up and nutrient scheme are shown in Fig. 1 and Table 2, respectively.

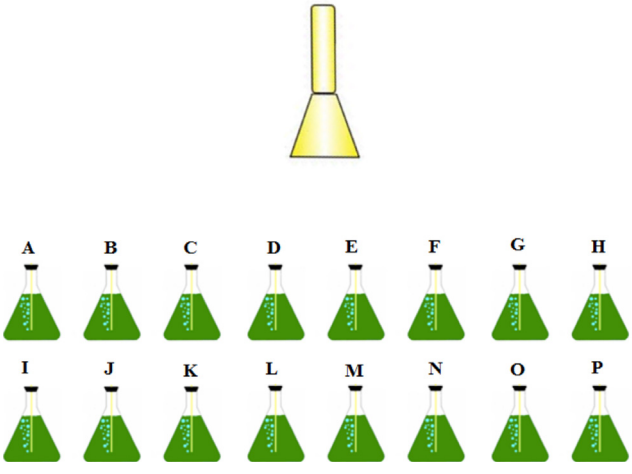


**Fig. 1.** Experimental set-up for the nutrient effect experiments.

*Growth evaluation*

Algal growth was evaluated daily by optical density measurements at 650 nm in three replicates, which was converted into dry cell weight per liter of culture by a regression equation derived previously. Specific growth rate (GR) and volumetric biomass productivity (VBP) were calculated using the cell density (g/L).

Specific growth rate μ ($d^{-1}$) was calculated as follows [15]:

$$\mu = \frac{\ln[X_2/X_1]}{t_2 - t_1} \quad (1)$$

Volumetric biomass productivity, $r_X$ ($g\,l^{-1}\,d^{-1}$) was calculated as follows:

$$r_X = \frac{X_2 - X_1}{t_2 - t_1} \quad (2)$$

where $X_1$ and $X_2$ were the biomass concentration ($g\,l^{-1}$) on days $t_1$ and $t_2$, respectively.

## Results and discussion

*Effect of light wavelength*

Microalgae photosynthesize, i.e., they assimilate inorganic carbon for conversion into organic matter. Light is the source of energy which drives this reaction and in this regard intensity and spectral quality need to be considered. Considering light as the most important energy source for the photoautotrophic algae, many studies have focused on the effect of light intensity. However, much of the experimental work conducted on the effect of monochromatic light exposure on microalgae photosynthesis has been conducted over a very short photoperiod of between 30 s and 1 day [15]. We have conducted this test for seven days with the suggested growth medium. The goal of this test was to determine the most suitable wavelength for photosynthetic rate in *C. vulgaris*. A higher light absorption rate would allow for the chloroplast to manufacture increased amounts of usable chemical energy which would result in an increase in algal growth (biomass). The cell density measurements for algal medium under blue, clear, green and red light exposures are shown in Fig. 2A. It can be noted that the clear light resulted in the highest absorption percentage over the test duration indicating the largest amount of algal culture along with the quickest growth rate. On the other hand, the green and red wavelengths of light were least absorbed by the *C. vulgaris* chloroplast, resulting in almost identically lower absorption percentages, algal cell density, and growth rates. From Fig. 2A, it is also worth noting that the growth rates for all wavelengths of light follow closely to that of a linear relationship (high $R^2$ values for linear growth trend). High $R^2$ (determination coefficient) values mean that the growth rate strongly adhered to the linear function indicating the growth trend to be linear in the test period for the given experimental conditions. The algae growths in clear light and blue light wavelengths have closely fit the linear function compared to the green and red wavelengths. Over the seven day test these growth rates, with respect to light, vary only by magnitude and not exponential degree. Blue light is seen to be a significant improvement from the Red and Green light absorption percentages, but fails to approach or exceed clear light. In previous studies, the red and blue lights were reported to have the capability to induce the photosynthesis even at low levels of exposure. In some other studies, the combination of different wavelengths has reportedly increased the photosynthesis rates.

Regarding growth rates, clear, blue and red lights have shown the highest growth rates on day 3 and they are as follows: 0.369 $d^{-1}$, 0.235 $d^{-1}$, and 0.140 $d^{-1}$, respectively (Fig. 2B). The green light had the highest growth rate on day 2 which is 0.137 $d^{-1}$. The green light was reported to be most effective in production of cellular carbohydrate (i.e., $CH_2O$) derived from $CO_2$. Also, green photon has 20% more energy than one red photon (i.e., 680 nm) and 15.5% less energy than a blue photon (i.e., 470 nm). Therefore, higher green light exposure may have some inhibitory effect due to higher energy supplied. While it can be seen that the blue light has more energy than green photon, blue photon was reported to support the algae growth. The volumetric biomass productivity is shown in Fig. 2C. The highest volumetric biomass productivity was 0.038 g/L-d, 0.0199 g/L-d, and 0.0096 g/L-d for clear, blue and red lights, respectively, on day 3, but for green light, it was 0.0088 g/L-d on day 2.

It appeared that the seven day results did not represent the complete growth cycle of the algae except for red and green light which have consistently shown lower growth rates prolonging the growth periods which is not ideal for practical applications. Another set of experiments was conducted using blue and clear lights for 12 days. The results are shown in Fig. 2D. The growth curves were somewhat similar (linear growth) to the trend observed in seven days growth profiles (Fig. 2A). It can be seen that the algae growth rate was comparable between blue and clear light wavelengths within the growth period 0–10 days. But it can be noticed that the algae growth rate for blue light has overtaken the algae growth rate for clear light (after 10 days). This shows that algae growth (*C. vulgaris*) rate can be enhanced under blue light after a period of acclimatization. This result is in agreement with Das et al. who have shown that the blue light was optimal for growth of *Nannochloropsis* species [15]. It should be noted that Matthijs et al. [22] reported a similar result but for the red light wavelength for *Chlorella pyrenoidosa* reproduction. They explained that microalgae are good at absorbing red light through their green pigment, chlorophyll [22]. In our study, the red light did not show higher growth rates. This can be explained by the observations made by previous researchers who reported that pure red light could actually cause cell damage which can be recovered by slow and low exposure to blue light [23]. The same applied to blue light at higher intensities as observed by Kebede and Ahlgren [24]. Also, it should be noted that the specific light wavelength absorbance capacity depends on the type of alga meaning its cellular composition, growth medium and physiological conditions as explained earlier. Yan et al. reported that the clear (white) and red light have shown higher growth rates of *C. vulgaris*, however, their study was conducted between 6 and 10 days and their growth medium was synthetic high-strength wastewater [25]. Also, the growth characteristics depend on the light intensities [26]. In another study by Barghbani et al., [27] higher biomass production

**Table 2**
Different compositions of the algal growth medium.

| Compound | Conc. (mg/L) | | | Compound | Conc. (mg/L) | | |
|---|---|---|---|---|---|---|---|
| | 100% | 50% | 25% | | 100% | 50% | 25% |
| **Algal growth medium composition (100% = recommended by the supplier)** | | | | | | | |
| $CaCl_2$ | 25 | 12.5 | 6.25 | $MgSO_4$ | 75 | 37.5 | 18.75 |
| NaCl | 25 | 12.5 | 6.25 | $KH_2PO_4$ | 105 | 52.5 | 26.25 |
| $NaNO_2$ | 250 | 125 | 62.25 | $K_2HPO_4$ | 75 | 37.5 | 18.75 |

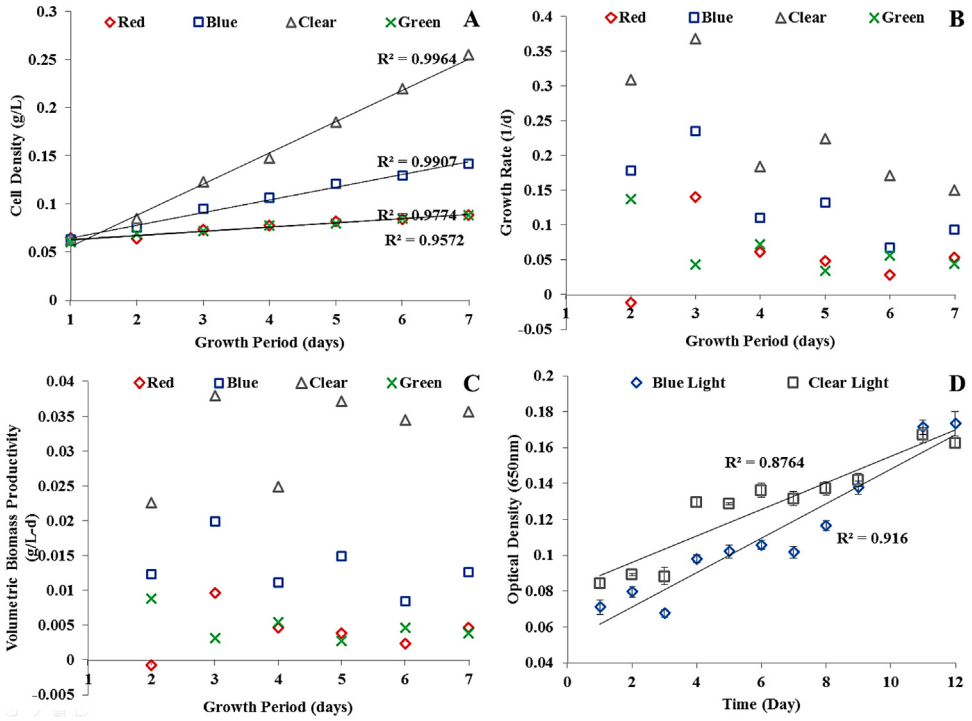


**Fig. 2.** Effect of light wavelength on: (A) cell density; (B) growth rate; (C) volumetric biomass productivity; and (D) optical density for 12 day period.

was obtained when blue lights were used. This observation from our study is in agreement with their study.

From Fig. 2D, it can be observed that the growth rate under blue light ($R^2$ = 0.916) seems to follow linear trend more closely than the clear light ($R^2$ = 0.8764) or even indicating close exponential growth phase trend. Although, the exponential growth phase is desirable for practical reasons, blue light required significant time lag to reach exponential growth. Thus, the slow growth rate under blue light is not ideal for practical applications because the faster the exponential phase is reached, the higher will be the biomass production, the shorter will be the pond or photobioreactor volumes for algae production and the shorter will be the harvesting time. Nevertheless, since clear (white) light has resulted in highest algal growth, we have continued the growth medium and nutrient concentration experiments with clear light.

*Effect of the algal growth medium*

Media composition has significant effect on both the growth rate and the final concentration of microalgae. Microalgae are known to grow more abundantly in nutrient rich (eutrophic) waters leading frequently to algal blooms [28]. However, for large scale production, it poses an economical challenge to provide excess nutrients. The effect of algal growth medium composition on algae growth was tested experimentally by considering three different compositions, i.e., the strength of the original suggested medium was reduced to 25% and 50% which was compared to 100% of the suggested medium. Table 2 shows the actual compositions used in these experiments. This is implemented to determine the suitable concentration that will enhance the growth of the algae. It is intuitive that the higher the nutrient availability, the higher will be the growth of microalgae but the results from this study suggest a different pattern.

Fig. 3A shows the optical density measurements of the algae medium for three different growth medium concentrations. The tests were conducted on a 12 day growth period. From Fig. 3B, it is very interesting to note that the cell density also follow an exponential growth phases while the growth trend under the light effect were mostly linear indicating the effect of different wavelengths. The $R^2$ values are shown in Fig. 3A. While 50% and 25% growth media composition have clearly established the exponential growth phases (determination coefficient, $R^2$ values for 50% and 25% nutrients are 0.9864 and 0.9388, respectively, for the experimental data), the 100% composition did not quite establish such a pattern ($R^2$ = 0.8359). This suggests that the suggested media is over formulated for the growth of *C. vulgaris*. Fig. 3A also shows that 50% formula was able to keep the exponential growth phase even on day 12, while 25% formula has just entered the stationary phase on day 11. This test suggests a 50% savings in the growth media composition for the growth of *C. vulgaris*.

Algae growth was described to follow five different phases [29]. They are: (1) lag or acclimatization phase; (2) log growth phase; (3) declining growth phase; (4) stationary phase; and (5) death (lysis) phase. At the 25% medium composition, the growth curve closely resembles this normal curve of development. From Fig. 3B, the lag phase occurs between 0 and 4 days, exponential from 4 to 8 days, stationary from 8 to 10 days, and lysis from 10 to 12 days. At 25% the recommended media composition, this nutrient composition takes 12 days to completely cycle through the expected growth phases. When applying the typical algal growth curve to the 50% growth media composition, it can be seen that at the 12 day mark the respective algal culture has only completed the lag phase (within 0–3 days), and continues to stay in the log growth phase and has yet to reach the stationary and lysis phases.

Fig. 3C and D show the volumetric biomass productivity and growth rates of *C. vulgaris* in three different media compositions. The maximum volumetric biomass productivities of 0.0475 g/L-d (day 4), 0.0525 g/L-d (day 8), and 0.0893 g/L-d (day 7) were recorded for 100%, 50% and 25% growth media compositions, respectively. Highest growth rates of 0.384 $d^{-1}$ (day 4), 0.382 $d^{-1}$ (day 4), and 0.374 $d^{-1}$ (day 7) were recorded for 100%, 50% and 25% growth media compositions, respectively. 50% composition has shown consistent growth rate throughout the experimental

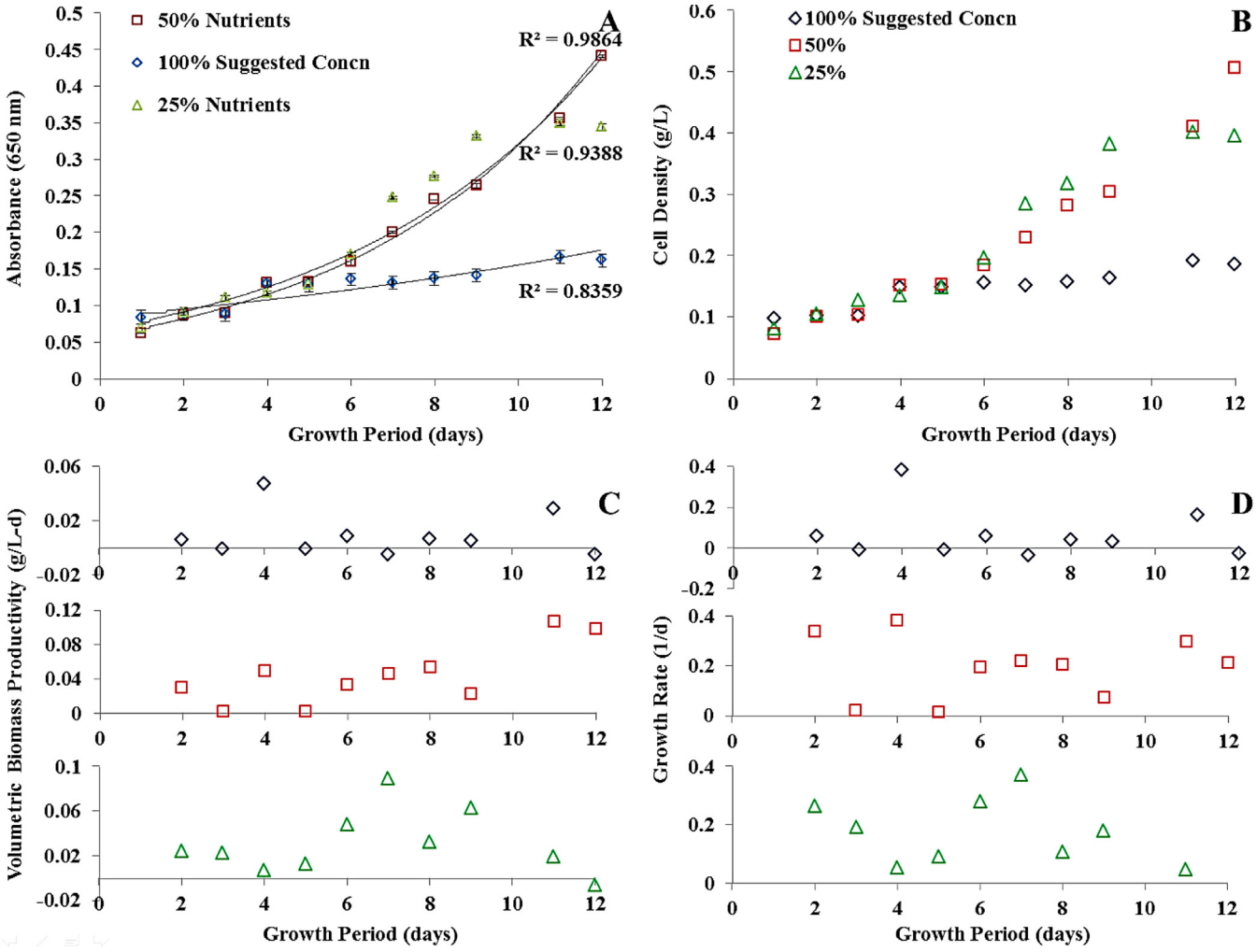


**Fig. 3.** Effect of growth medium on: (A) optical density; (B) cell density; (C) volumetric biomass productivity; and (D) growth rate.

growth period of 14 days. It suggested that if the maximum growth rate and volumetric biomass production are delayed, then the cultivation pond size would be increased.

*Individual effect of nitrogen and phosphorous*

The results from the overall growth media tests were intriguing and further experiments were conducted to study the actual effect of the individual nutrient concentrations. The trace metal concentrations were not tested since they are applied in very small concentrations. The experimental scheme is shown in Table 3. Nitrogen and phosphorus composition was varied in these experiments. Since 50% growth medium resulted in higher growth rates, we tested the effect of nitrogen and phosphorous individually on 50% as well as 100% compositions. Sixteen experimental vessels were used for these tests as shown in

**Table 3**
Experimental scheme to study the effect of nutrients.

| Compound | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| **50% base (mg/L)** | | | | | | | | |
| $CaCl_2$ | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 |
| NaCl | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 |
| $NaNO_2$ | 62.5 (25%) | 125 (50%) | 250 (100%) | 500 (200%) | 125 | 125 | 125 | 125 |
| $MgSO_4$ | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 |
| $KH_2PO_4$ | 52.5 | 52.5 | 52.5 | 52.5 | 26.25 (25%) | 52.5 (50%) | 105 (100%) | 210 (200%) |
| $K_2HPO_4$ | 37.5 | 37.5 | 375 | 37.5 | 18.75 (25%) | 37.5 (50%) | 75 (100%) | 150 (200%) |
| Compound | I | J | K | L | M | N | O | P |
| **100% base (mg/L)** | | | | | | | | |
| $CaCl_2$ | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| NaCl | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| $NaNO_2$ | 62.5 (25%) | 125 (50%) | 250 (100%) | 500 (200%) | 250 | 250 | 250 | 250 |
| $MgSO_4$ | 75 | 75 | 75 | 75 | 75 | 75 | 75 | 75 |
| $KH_2PO_4$ | 105 | 105 | 105 | 105 | 52.5 (25%) | 105 (50%) | 210 (100%) | 420 (200%) |
| $K_2HPO_4$ | 75 | 75 | 75 | 75 | 37.5 (25%) | 75 (50%) | 150 (100%) | 300 (200%) |

Fig. 1. And the details are discussed further in this section. First eight vessels were tested with 50% growth media (A–H) and the remaining eight vessels were filled with 100% growth media (I–P). As shown in Table 3, the vessels A, B, C, and D are filled with 50% growth media composition except for nitrate concentration which was varied between 25% and 200%. These experiments were conducted in 18 day growth periods. The cell density variations over 18 day growth period is shown in Fig. 4A for A (N = 25%), B (N = 50%), C (N = 100%), and D (N = 200%). It can be seen that C and D have shown higher dry biomass concentrations (g/L) and A developed the least cell density over the 17 day test. This indicates that the algal growth was directly proportional to the amount of nitrogen present in the growth media. Additionally, at a 50% media, the algae growth followed the described algal growth pattern as discussed earlier. For this data the lag phase is relatively short (0–3 days), exponential relatively long (3–11 days), followed by a long stationary phase (11–14/16 days depending on sample) before moving into the lysis phase. Between C (N = 100%) and D (N = 200%); C appears to have performed better than D, meaning that there is an optimum N concentrations for maximum algal growth.

At the 50% growth media composition, the phosphorous effect test presented in Fig. 4B indicates that the cell density or algal growth was independent of phosphate amount until a certain threshold is met. Samples E (P = 25%), F (P = 50%), and G (P = 100%) range from a 25% to 100% P concentration levels and yielded the same amount of algal biomass which progresses quickly through the described algae growth curve. This results in a short lag phase, a condensed exponential growth phase, maximizing algal growth around the 10 day period, and a long lysis phase for samples E–G. Further, at the 200% P concentration, a threshold is met where the amount of phosphate severely lags growth. This can be seen by reviewing sample H's (P = 200%) growth curve. In general, H's cell density is significantly lower during the beginning and middle portions of the test indicating an extremely long lag phase (0–8 days). As the test continues, H picks up exponential growth phase ranging from day 8 to the end of the test and possibly beyond, indicating that the growth curve common with algae is significantly delayed at high phosphate levels and independent at lower levels.

I–L nitrogen concentration tests indicated similar results as the A–D test. From Fig. 4C, it can be seen that samples K and L yielded the greatest amount of cell densities while I yielded the least. This leads to the same conclusion that nitrogen concentration increases the cell growth to a maximum. At the 100% saturation level the algal growth curve followed much the same pattern as Fig. 4A but with a shorter exponential growth (0–9 days) and an elongated stationary phase (10–17 days). A comparison between the A, B, C and I, J, K shows that with 100% growth media, the exponential growth phase is attained more quickly than 50% growth media. However, the difference is minimal and cell densities were almost similar. This suggests that 50% growth media with 100% nitrogen concentration is the best combination. Fig. 4D shows the relationship between cell densities and growth period for different phosphate amounts in a 100% suggested growth medium. Similar to Fig. 4B phosphate amounts did not show a positive effect on the growth rate. The cell densities were lower than A–D tests and the M–P growth pattern is very different in nature from all others.

*Growth rates and volumetric biomass productivities*

Fig. 5 shows the specific growth rates for the sixteen experiments with standard error bars. The highest specific growth rates of 0.885 $d^{-1}$ (day 7); 0.864 $d^{-1}$ (day 4); 0.658 $d^{-1}$ (day 2); 0.557 $d^{-1}$ (day 5) were calculated for A, B, C, D vessels, respectively. Similarly, 1.074 (4) $d^{-1}$; 0.811 (2) $d^{-1}$; 0.922 (3) $d^{-1}$; 0.31 (3) $d^{-1}$, respectively, were calculated for E, F, G, H vessels for 50% growth media composition. From Fig. 4A, it appears that C and D produced higher cell densities, this is mainly due to consistent growth that was achieved by the growth media composition. It can be concluded from Fig. 4A that 50% growth media with 100% nitrogen concentration is ideal for algal growth. Fig. 5C shows that higher growth rates are obtained with 100% nitrogen concentration within 0–8 days consistently which is a desired trend for successful algal harvesting. Similarly from Fig. 5G, it can be noted that the phosphorous concentration at 100% promotes higher growth rates.

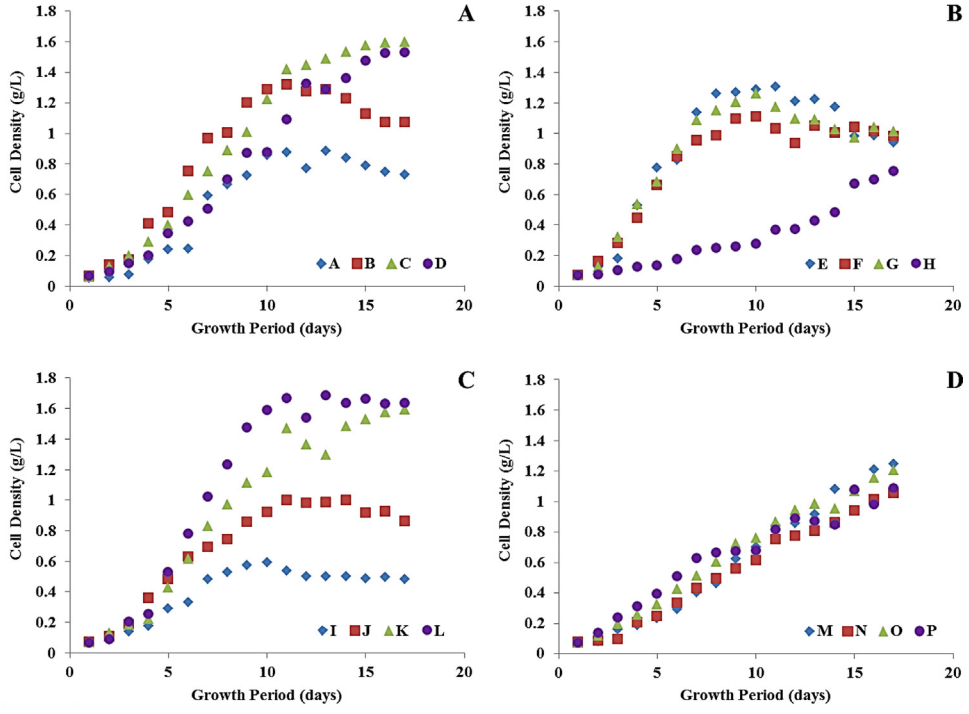


**Fig. 4.** The nutrient concentration effect on cell density.

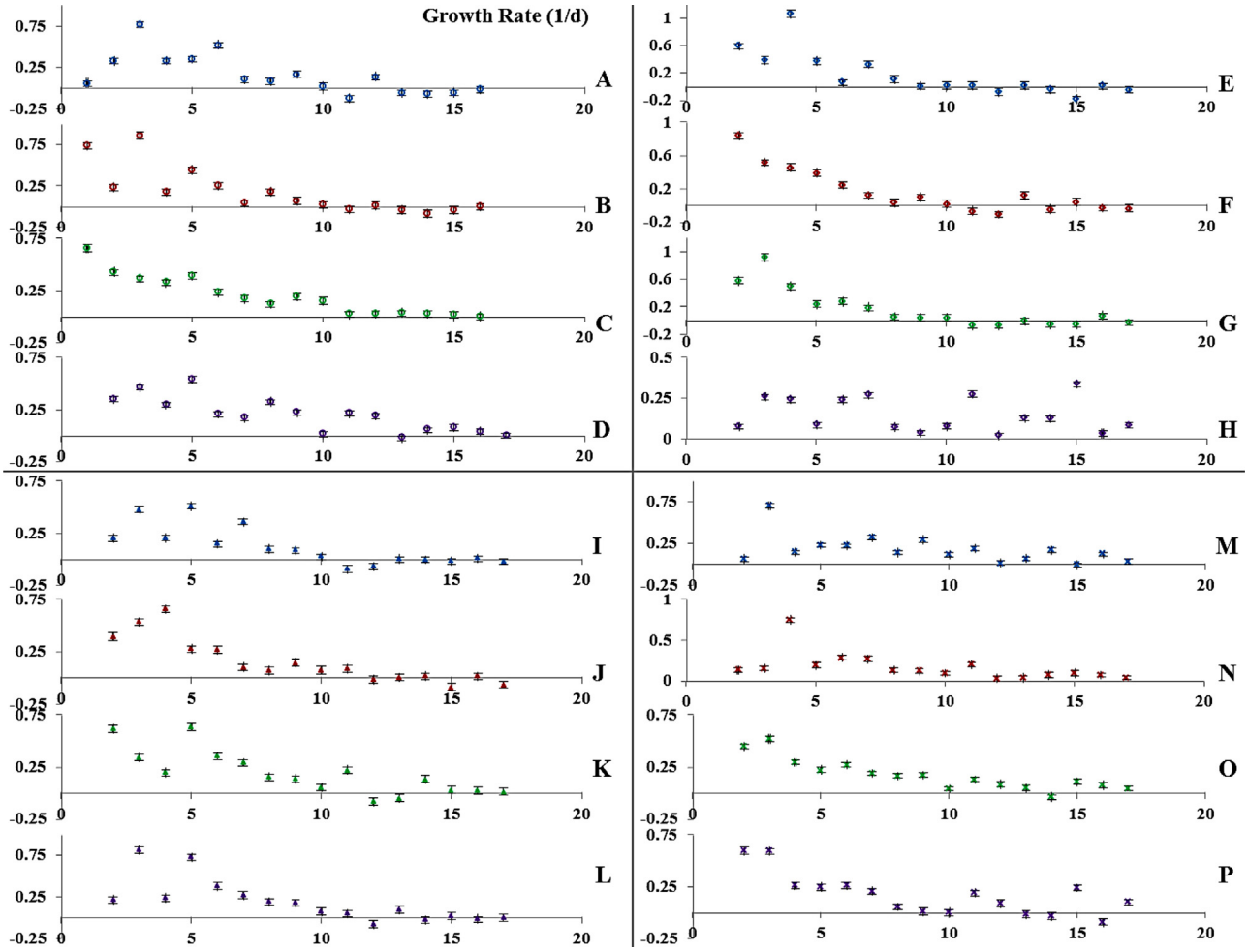


**Fig. 5.** The nutrient concentration effect on growth rate (growth period (days) vs. growth rate (L/d)).

From Fig. 5I–L and M–P, it is clear that growth rates were lower than those for Fig. 5A–D and E–H. This reveals that specific growth rate can be maximized by optimizing the growth medium concentrations.

Fig. 6 shows the volumetric biomass productivities for the sixteen experiments with standard error bars. A comparison of the volumetric biomass productivities (g/L-d) for different algal species from other studies is summarized in Table 4 [43–46]. Algae growth rate in this study is comparable to other studies involving different algal species (*Haematococcus pluvialis*, *Nannochloropsis* sp., *Pleurochrysis carterae*, and *Tetraselmis secuia*). It should be noted that the algae growth in this study is optimized at 50% growth medium composition. The chlorella species growth study by Barghbani et al. [27] is optimized by Taguchi method and they have reported a range of biomass productivities (0.11–0.51) and only one experiment showing 0.51 g/L-d of biomass productivity while the rest of the conditions showing significantly lower productivities. Additionally the air flow rate in that study is higher compared to this study. It should also be noted that the growth rates depend on the type of algal species as well. Volumetric biomass productivity of 50% media Fig. 6C (N = 100%) is more consistent within 0–10 days compared to all others (A–D) and the similar observation can be made for Fig. 6G. At 100% growth media composition, the biomass productivities are slightly higher (Fig. 6K and L) but are not consistent or significant. However, from Fig. 6(M–P), phosphorous concentrations did not seem to affect the growth of the microalgae. From these observations, it can be concluded that suggested growth medium was over formulated for the growth of microalgae *C. vulgaris*, and the optimum growth media would be 50% of the suggested media with 100% nitrogen concentration and 50–100% phosphorous concentration.

Recently, many studies focused on the growth of Chlorella species; these studies have investigated the effect of $NaHCO_3$, $CO_2$ bubbling and fixation, NaCl, and inorganic carbon, compounds and nutrient uptake [27,30–34]. A change of the concentration of nitrates (as $KNO_3$) in the culture medium, when growing different strains of *Chlorella*, had little effect on the biomass concentration and lipid production possibly due to the fact that the lowest concentration tested (1.24 g/L) was already high enough to promote growth [30]. Addition of $NaHCO_3$ to Basal media, showed an increase of biomass production by *C. vulgaris* in a concentration range of 0.1–1.6 g/L, as the concentration of $NaHCO_3$ increased so did the biomass production, reaching its maximum (0.6 g/L of biomass) at a concentration of 1.2 g/L of $NaHCO_3$ [32]. In this study, we found that (From Figs. 5 and 6) lower nutrient concentrations (i.e., N and P) in the range 50–100% are sufficient for maximized algal growth. Also lower nutrient concentrations have shown to increase the biomass as well as lipid productivities in *C. vulgaris* [35].

### *Significance of the present study*

Optimizing the growth media is a critical step to develop an economical route for sustainable algal biomass production. This

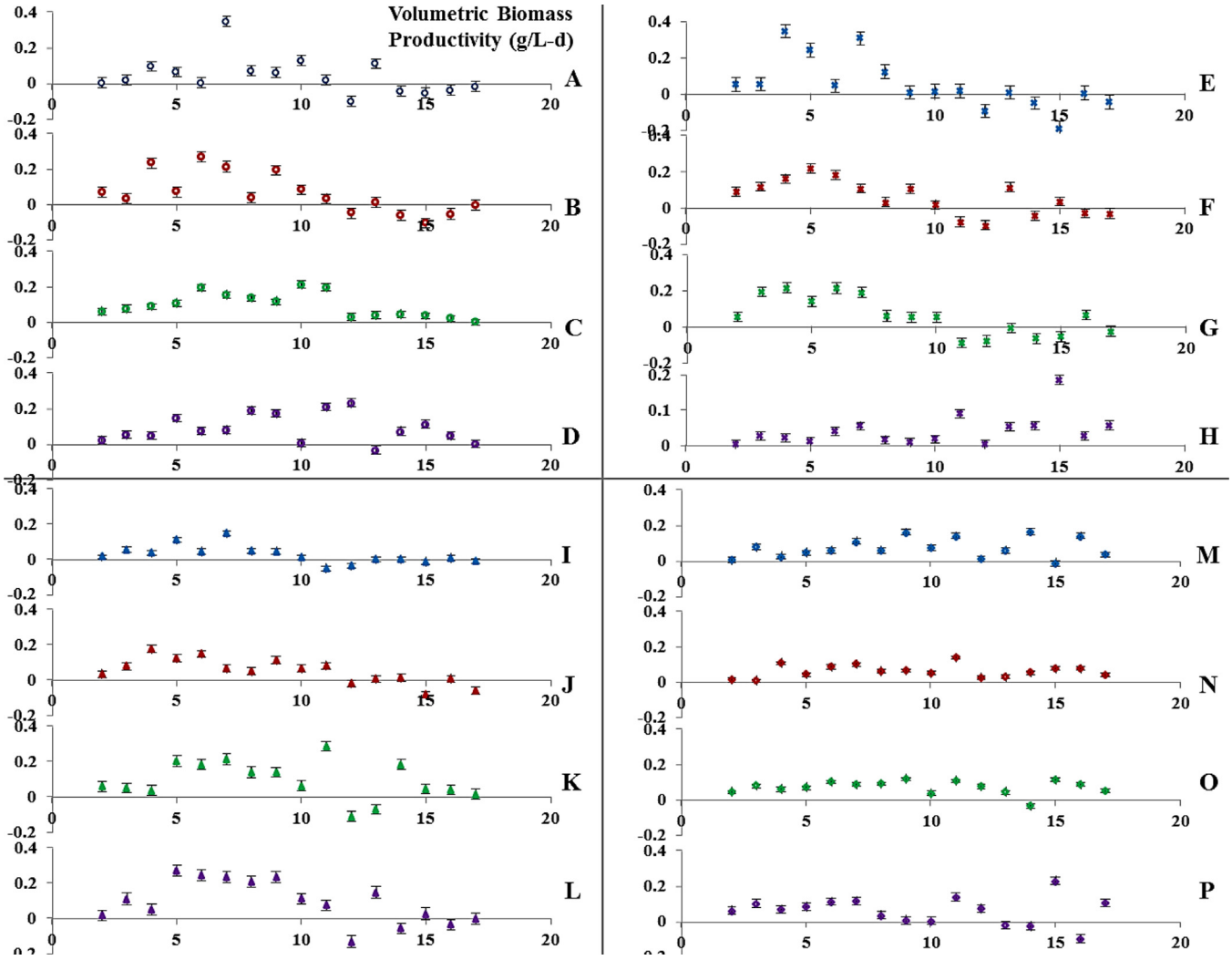


**Fig. 6.** The nutrient concentration effect on volumetric biomass productivity (growth period (days) vs. volumetric biomass productivity (g/L-d)).

study has shown that a 50% of the suggested media was adequate to grow microalgae *C. vulgaris* without compromising the biomass productivities. For large scale applications, costs associated with growth media and nutrients can be very significant in the overall algal bioenergy or bio-product processes. Commercial algal farms require sources of water, nutrients and carbon dioxide which contribute to 10–30% of total production costs [36]. Growth medium recycling which is commonly practiced in commercial farms may reduce algal productivity due to increased contamination by algal pathogens and/or accumulation of inhibitory secondary metabolites [37–39]. Since nutrients are required in large quantities, utilization of cheap nutrient sources such as municipal wastewater and other industrial co-products is ideal for algal growth economics with some pretreatment. Utilizing wastewater resources to replace growth media may alleviate the cultivation costs to some extent [40]. Algae are able to remove the nutrients from wastewater successfully and the lipid production is found to be similar to pure culture conditions [41,42]. Algae growth can be integrated with wastewater treatment and nutrient removal processes. Traditional wastewater treatment facilities release large amount of $CO_2$ to the atmosphere during degradation of organic pollutants. Large quantities of $CO_2$ are released per every ton of wastewater treated [47]. The $CO_2$ emissions can be captured (or from other industrial sources) and supplied to the algal ponds as substrate for the algal cell growth. Algae have a stoichiometric composition of C:N:P ratios of 50:8:1 while domestic wastewater has a composition of (C:N:P) 20:8:1 [48]. With addition of carbon source ($CO_2$), wastewater can serve as excellent medium for algal growth. Since the wastewater has adequate nutrient composition, algae growth using wastewater may completely eliminate the

**Table 4**
Volumetric biomass productivity comparison with other studies.

| Strain of microalgae | Volumetric productivity (g/L-d) | Aeration rate (v/v/m) | Volume ($m^3$) | Reference |
|---|---|---|---|---|
| *H. pluvialis* | 0.68 | – | 0.22 | [39] |
| Nannochloropsis sp. | 0.35 | 0.1 | 0.14 | [40] |
| *P. carterae* | 0.39 | 1.17 | 0.003 | [41] |
| *T. secuia* | 0.42 | 0.21 | 0.12 | [42] |
| *C. vulgaris* | 0.11–0.51 | 1 (1 L/min) | – | [25] |
| *C. vulgaris* | 0.35 | 1 | 0.006 | This study |

**Table 5**
Comparison of operating costs for open pond algae cultivation system.

| Input | Cost (per kg) | Amount required per hectare (kg) | Cost per hectare ($)[a] | Wastewater as source | This study |
|---|---|---|---|---|---|
| **Cost per hectare per year ($)**[a] | | | | | |
| $CO_2$ | 0.035 | 246 400 | 8624 | 5174.4 | 8624 |
| N as $NH_3$ | 0.25 | 5936 | 1484 | 0 | 742 |
| P, as superphosphate | 0.9 | 560 | 504 | 0 | 252 |
| Fe, as $FeSO_4$ | 0.5 | 560 | 280 | 280 | 280 |
| **Electricity costs (at $ 0.05883/kWh)** | | | | | |
| Mixing (kWh) | | 10 729 | 631 | 631 | 631 |
| Nutrient supply (kWh) | | 521 | 31 | 0 | 15 |
| Total operating costs per year ($/yr) | | | 11 554 | 6086 | 10 545 |
| Biomass cost ($/ton) | | | 103 | 54 | 94 |

[a] 112 tons of biomass produced per hectare per year.

nutrient costs. In the present study, we have identified a potential for reducing the nutrient requirements by optimizing the growth medium as well as the concentrations of nitrogen and phosphorous. A comparison on process operating costs for different scenarios (conventional algal cultivation system, wastewater as growth medium and this study-50% nutrient cost reduction) for nutrient supplies is shown in Table 5 [49,50]. It shows that wastewater has the potential to completely eliminate the nutrient related costs and $CO_2$ costs significantly while this study can reduce the nutrient costs by 50%. The nutrient costs for conventional, wastewater and this medium were $31, $0, $15, respectively, while the total operating costs per ton of biomass were $103, $54 and $94, respectively, for the three growth medium sources. When the costs for $CO_2$ supplies are excluded in all cases, the nutrient cost reduction can become significant. While this study did not focus on the lipid composition of the algal cells, the primary objective was to study the general growth of algae with minimized nutrient requirements. Besides biodiesel production, algal biomass can be used to produce biogas, bioethanol, bioelectricity [3,6,7] or can be used as organic source in many environmental remediation and other applications [7]. This study reveals that identifying suitable growth media can mitigate unnecessary costs in large scale algal biomass production.

## Conclusions

This study has shown that light wavelength has a noticeable effect on the algal growth rates. Red and green light did not show a positive trend in the growth rates compared to clear and blue light wavelengths. Nutrient concentrations can promote exponential growth when formulated correctly. At the recommended (100%) growth media composition, the algal growth was observed to be linear, however at 50% composition; the growth followed an exponential trend. Further tests on nutrient concentration effect (nitrogen and phosphorous) have shown that low nitrogen concentrations can also stimulate algal growth. At 50% the recommended growth media, algal growth is independent of phosphate concentration from 25% to 100% levels, (i.e., low or no effect) and severely delayed at 200% Phosphorous concentration. It is concluded from this study that by optimizing the growth media composition, algal growth can be maximized at lower chemical costs by approximately 50% in large scale applications. However, in large scale algal growth, some other critical operational parameters may affect the growth kinetics/dynamics and biomass productivities which need to be addressed case by case.

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