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Research Article

Evaluation of Media and Nitrogen:Phosphorous Ratios for Optimal Growth of Biotechnologically Important Unicellular Microalgae

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Abstract

This study was conducted to assess the impact of varying nitrogen:phosphorous ratios of liquid culture media on biomass productivity in microalgal strains of biotechnological importance. Four strains of microalgae were grown in standing cultures at 28°C for up to 14 days with a light:dark photoperiod of 15 h:9 h. A commercially available freshwater algal growth medium (AG), modified blue-green 11 (mBG11), modified Bold (mBold), and modified Chu#13 (mChu) were used as the liquid media. Algal species *Botryococcus braunii* (UTEX 572) cultured in AG, *Neochloris oleoabundans* (UTEX 1185) cultured in mChu, and *Scenedesmus obliquus* (UTEX 1450) cultured in mChu attained the shortest doubling times of 5.2, 3.3, and 3.6 days, respectively for each media/algae combination. Doubling times for *Scenedesmus obliquus* (UTEX 393) in AG and mChu media were similar, 3.5 +/- 0.1 and 3.6 +/- 0.1 days, respectively. An N:P mass ratio of 5.5:1 (mChu) and 30.5:1 (AG) promoted the most rapid biomass production, while the two media with ratios of 2.5:1 (mBold) and 47.6:1 (mBG11) had lower biomass productivities. Macronutrients were above minimum concentrations required for growth in each media; therefore, N:P ratio is an important consideration, and media with N:P ratios within the range of 5.5:1 to 30.5:1 are the most desirable for green algae biomass production.

Keywords: Biofuels; Microalgae; Renewable Fuels; Bioenergy

1. Introduction

Unicellular microalgae are rapidly growing photoautotrophic microorganisms that have many commercial applications in biotechnology and human and animal nutrition (Spolaore et al., 2006). For example, they have been investigated for their potential as lipid-producers and liquid biofuel feedstocks (Chisti, 2007); as biomass feedstock for co-firing in power plants (Kadam, 2002); as

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catalysts for the removal of nutrients from wastewater (Martínez et al., 2000; An et al., 2003); and as organisms that reutilize and sequester carbon (Doucha et al., 2005). The greatest benefits from microalgae may be realized through the implementation of a comprehensive system for carbon reutilization, renewable energy production, and wastewater treatment. For both energy- and environmentally-related applications, a high biomass production rate is desirable.

Like other biological processes, optimal biomass production by microalgae requires the proper combination of input macro and micronutrients, as well as suitable physical conditions, such as temperature and illumination. Several species of microalgae, including *Botryococcus spp.* (Chu, 1942; Weetall, 1985; Yamaguchi et al., 1987; An et al., 2003; Dayananda et al., 2007; Shen et al., 2008;), *Neochloris oleoabundans* (Tornabene et al., 1983; Gouveia and Oliveira, 2008; Li et al., 2008; Gouveia et al., 2009; Pruvost et al., 2009; Murray et al., 2011), and *Scenedesmus spp.* (Goldman and Graham, 1981; Kim et al., 2007; Gouveia and Oliveira, 2008;) have been investigated because of their potential in energy and environment related applications. The effects of nutrient composition on the growth of *Botryococcus* sp. were investigated by Chu (Chu, 1942), who reported that nitrate (NO_3^-) was a better source of nitrogen (N) than ammonium (NH_4^+) nitrogen. Additionally, it was reported that higher NO_3-N concentrations did not necessarily correlate with higher growth rates; and N and phosphorus (P) deficiencies limited *Botryococcus* growth. In another study (An et al., 2003), biomass yields of *B. braunii* (UTEX 572) were examined while varying the N concentration from 10 $mg \cdot L^{-1}$ to 1,020 $mg \cdot L^{-1}$ and P concentrations from 2.2 $mg \cdot L^{-1}$ to 211.8 $mg \cdot L^{-1}$ in a modified Chu13 media. The specific growth rate was highest (0.034 h^{-1}) when the initial N was 204 $mg \cdot L^{-1}$ or 510 $mg \cdot L^{-1}$ and initial P was 10.4 $mg \cdot L^{-1}$ (An et al., 2003). Biomass yields of *B. braunii* (UTEX 572) were later examined (Dayananda et al., 2007) using five variations of the Chu13, BG11, and two variations of the Bold Basal Medium (BBM). Different strengths of the Chu13 media were examined by adjusting the component concentrations by factors of 0.25, 0.5, 0.75, 1.00, and 2.00. It was found that biomass yields were not considerably different; thus, growth yield for the organism in Chu-type media was not limited by nutrients such as N or P (Dayananda et al., 2007) but may have been limited by other conditions (e.g., pH or carbon source). In another study using three strains of *Scenedesmus* spp. (Kim et al., 2007), biomass yields were examined in two variations of modified BBM at 24°C, a light:dark photoperiod of 14 h:10 h, light intensity of 115 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with continuous aeration. It was determined that growth rates were 3-fold higher in the modified BBM containing 3% fermented swine wastewater (Kim et al., 2007).

The effects of varying N:P ratios on biomass yields of *Neochloris oleoabundans* (UTEX 1185) were examined in work reported by Li et al. (2008). The authors reported that varying the N concentration from 42 to 280 $mg \cdot L^{-1}$ and maintaining a constant P concentration of 100 $mg \cdot L^{-1}$ in a modified soil extract media resulted in the greatest biomass productivity (0.63 g dry cell weight$\cdot L^{-1} \cdot day^{-1}$) using initial N concentrations of 140 $mg \cdot L^{-1}$ (Li et al., 2008).

The aforementioned work has provided insights into chemical and physical parameters that affect the growth of single algal strains of biotechnological interest. However, few side-by-side comparative studies exist that examine the effects of these factors (e.g., medium composition, N:P ratios, effects of wastewater amendments) on these algal strains. It is known that N and P must be properly balanced to promote plant growth, and that the Redfield ratio (7.2:1) serves as a benchmark for the N:P mass ratio (Hall et al., 2005). Nutritional requirements for macronutrients for microalgae could be discerned from a C-normalized general molecular formula for microalgal

biomass reported as $CH_{1.83}O_{0.48}N_{0.11}P_{0.01}$ (Chisti, 2007), with an N:P mass ratio of 5.0:1. A C-normalized molecular formula for *Neochloris oleoabundans,* $CH_{1.715}O_{0.427}N_{0.148}S_{0.014}P_{0.012}$, with a N:P mass ratio of 5.6:1 was reported by others (Tornabene et al., 1983; Pruvost et al. 2009).

In this study, we investigate the growth of biotechnologically important algae on a variety of liquid media. Our objective was to compare the biomass productivity of microalgae for various media and assess the N:P ratios most suitable for promoting microalgal growth. Specifically, this study describes a side-by-side comparative study of the growth of four oleaginous strains of algae (*B. braunii* UTEX 572, *N. oleoabundans* UTEX 1185 and *S. obliquus* strains UTEX 393 and UTEX 1450) on several liquid media in order to identify the growth medium that supports the highest biomass yields. The effects of different N:P ratios in media on growth is also investigated.

2. Methods

2.1 Algal strains and growth medium

Four microalgal species – *Botryococcus braunii* (UTEX 572), *Neochloris oleoabundans* (UTEX 1185), *Scenedesmus obliquus* (UTEX 393), and *Scenedesmus obliquus* (UTEX 1450) were obtained from the University of Texas Culture Collection (UTEX). Loops of solid media containing cells were taken from 40 mL slants, each of which was aseptically transferred into 5 mL of deionized (DI) water; 0.1 mL of cell suspension in water was used as inoculum for growth studies in flasks.

A commercially available freshwater algal growth medium (Alga-Grow, AG) was used in this study. Triplicate samples of AG were analyzed by a commercial laboratory (Pollution Control Services, Universal City, TX) to allow for calculation of total nitrogen (TN) and total phosphorus (TP), and the corresponding N:P mass ratio. NO_3-N, Ammonia-N (ISE), total Kjeldahl-N, and total Phosphorus concentrations were determined using EPA 352.1, SM 4500-NH_3 D, SM 4500-N B/E, SM 4500-P/B/E methods, respectively. Resulting TN was 23.1 ± 1.95 mg L^{-1} and TP was 0.757 ± 0.010 mg L^{-1}, which corresponds to a N:P mass ratio of 30.5:1.

BG11 (Stanier et al., 1971) has a N:P mass ratio of 47.6:1, and was slightly modified (mBG11, Table 1) by substituting EDTA disodium salt for EDTA disodium magnesium salt, and substituting citric acid monohydrate for citric acid.

Bold's medium was originally formulated for the growth of soil algae (Bold, 1970) and has more recently been used as BBM (Tornabene et al., 1983; Pruvost et al., 2009) for planktonic algae, which has a N:P mass ratio of 0.9:1. BBM was slightly modified to mBold (Table 1) by increasing N and P concentrations approximately 3-fold and substituting trace-metal mix A5 (Stanier et al., 1971) for the BBM trace nutrients, which resulted in a N:P mass ratio of 2.5:1.

Chu #13 media (Chu, 1942) was formulated for planktonic algae and has a N:P mass ratio of 5.3. Chu #13 media was slightly modified to mChu (shown in Table 1) for this study by increasing N and P components approximately 4-fold, substituting citric acid monohydrate and ferric ammonium citrate for ferric chloride, and substituting trace-metal mix A5 (Stanier et al., 1971) for the micrometabolic elements solution (Chu, 1942), which resulted in a N:P mass ratio of 5.5:1.

The four growth media were prepared in distilled and deionized water using reagent grade chemicals according to Table 1. The liquid media were sterilized by autoclaving for 30 minutes prior to inoculation. To test growth in different N:P ratios, mChu medium was made up with N:P

Table 1 Growth media formulations.

Component, mg L^{-1}	*AG	mBG11	mBold	mChu
$NaNO_3$	NA	1,500	750	--
KNO_3	NA	--	--	200
KH_2PO_4	NA	--	175	--
K_2HPO_4	NA	40	75	40
$MgSO_4 \cdot 7H_2O$	NA	75	75	100
$CaCl_2 \cdot 2H_2O$	NA	36	25	80
NaCl	NA	--	25	--
citric acid monohydrate	NA	6	--	100
ferric ammonium citrate	NA	6	--	10
Na_2CO_3	NA	20	--	--
EDTA (disodium salt)	NA	1	--	--
trace components	NA	**	**	**
N:P mass ratio	30.5:1	47.6:1	2.5:1	5.5:1

*AG: commercial freshwater algal growth medium (Alga-Grow), NA indicates that concentrations are not available.

**trace components include (mg L^{-1}): H_3BO_3, 2.86; $MnCl_2 \cdot 4H_2O$, 1.81; $ZnSO_4 \cdot 7H_2O$, 0.222; $Na_2MoO_4 \cdot 2H_2O$, 0.390; $CuSO_4 \cdot 5H_2O$, 0.079; $Co(NO_3)_2 \cdot 6H_2O$, 0.049.

ratios of 2.5:1, 10:1 and 20:1 to assess the effect of varied N:P ratios on algal growth (the N:P ratio of mChu medium is 5.3:1). In all cases, the phosphate concentration was the same as mChu medium and nitrate levels were changed to achieve the desired N:P ratio. In order to evaluate the effects of altered N:P ratios on growth, phosphate concentrations were kept constant and only KNO_3 concentrations were altered in the growth media. This approach was taken in order to avoid growth rate effects resulting from changes in media pH: since the pH of mChu medium is governed by the concentration of potassium phosphate (as dibasic potassium phosphate K_2HPO_4), changing potassium phosphate concentrations would, in addition to altering phosphate in N:P ratio analyses, also result in differences in media pH. And changes in media pH have been shown to alter growth and other properties of algae (Santos et al., 2014).

2.2 Algal strain cultivation and analysis of growth

Growth studies were designed to test experimental objectives in small volume flasks in the laboratory. These conditions were similar to those of previous studies to allow for comparison of biomass production under various N:P mass ratios. In order to evaluate the different growth media with each algal strain, a metal utility rack was used to support six 1.22 m (48-inch) long 34-W, T12 fluorescent light bulbs positioned horizontally approximately 32 cm above the growth media surface. The lighting system was controlled by a timer set to cycle on and off each day to create a

light:dark photoperiod of 15 h:9 h. This arrangement created low-light conditions (~ 100 □mol·m^{-2}·s^{-1} PAR), within the range of photosaturation observed in studies of light intensity (Wahal and Viamajala, 2010). Temperature of the room housing the indoor growth system was maintained at 28°C.

Triplicate cultures were prepared for 16 media/algae combinations, each in a 250 mL sidearm flask, for a total of 48 flasks. Each flask contained 30 mL of the designated medium and was inoculated using 0.1 mL of inoculum prepared from the slants, as previously described. Standing cultures of algae were grown at 28°C with a light:dark photoperiod of 15 h:9 h. The cultures were not supplemented with CO_2; thus, atmospheric CO_2 (0.0385%) was the primary source of carbon introduced only by atmospheric exchange. Cultures for N:P ratio studies were prepared similarly.

Optical densities of the cultures were measured as absorbance at 600 nm during the test period at approximately 24 hour intervals, as in other growth and biomass productivity studies (Koch, 1970; Goldman and Graham, 1981; Li et al., 2008). Prior to obtaining measurements, culture flasks were swirled to mix cells to homogeneity and absorbances were determined using a Spectronic 20 spectrophotometer. Changes in biomass densities (expressed as A_{600}) were used to model exponential growth for the calculation of growth rates and doubling times for each algae/media combination. Specific growth rates (μ) and doubling times (t_d) were calculated using Equations (1) and (2), respectively:

Eq. 1, for calculation of specific growth rate:

$$\mu = \left(\frac{1}{t_2 - t_1}\right) \ln\left(\frac{N_2}{N_1}\right) \quad (1)$$

Eq. 2, for calculation of doubling time:

$$t_d = \frac{\ln 2}{\mu} \quad (2)$$

where μ is specific growth rate (day^{-1}), t_2 is end time (day), t_1 is start time (day), N_2 is optical density or biomass concentration at t_2, N_1 is optical density or biomass concentration at t_1, and t_d is doubling time in days.

3. Results and Discussion

The absorbance of *B. braunii* cultures steadily increased throughout the cultivation period (Fig. 1). Absorbance was highest on the last day of cultivation (day 8) in mBold (0.090), followed by AG (0.058), mChu (0.054), and mBG11 (0.050). Specific growth rate and t_d calculations show that *B. braunii* had the shortest mean t_d (5.2 ± 2.5 days) in AG media; however, due to the high variability of the A_{600} triplicate measurements (shown as standard error bars in Fig. 1), the t_d values for *B. braunii* did not differ substantially (Table 2).

Optical density of *N. oleoabundans* in mBold and mChu steadily increased throughout the cultivation period (Fig. 2); however, *N. oleoabundans* in AG and mBG11 reached stationary phase after five days of cultivation. Absorbance was highest on the last day of cultivation (day 14) in mChu (1.281), followed by mBold (1.223), mBG11 (0.737), and AG (0.467). Specific growth rate and t_d calculations show that *N. oleoabundans* had the shortest mean t_d (3.3 ± 0.1 days) in mChu media; however, the variability of A_{600} triplicate measurements (standard error bars in Fig. 2) were high enough to result in equivalent t_d values of *N. oleoabundans* in mChu and mBold (Table 2).

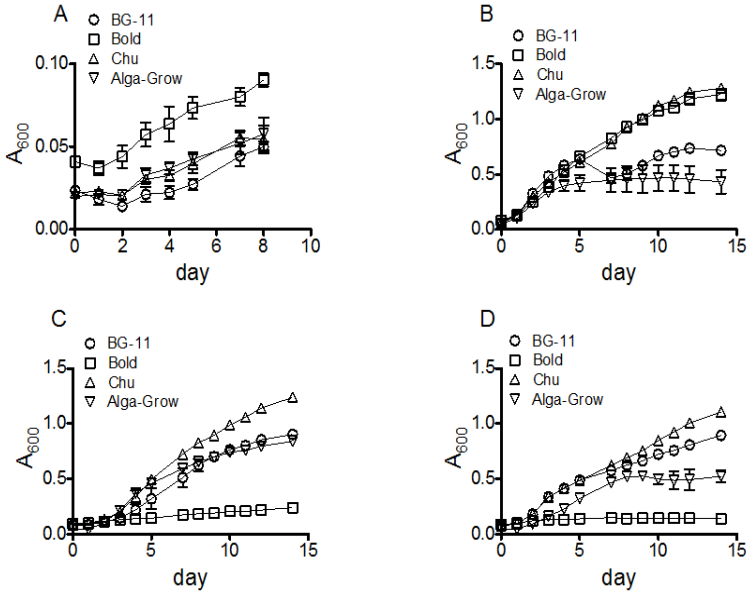

Fig. 1. Growth kinetics of four strains of microalgae on four different liquid growth media. Growth was measured spectrophotometrically at an absorbance of 600 nm. Data are presented as the average of three independent measurements, vertical bars represent standard error of the mean. A, *Botryococcus braunii* UTEX 572; B, *Neochloris oleoabundans* UTEX 1185; C, *Scenedesmus obliquus* UTEX 393; D, *Scenedesmus obliquus* UTEX 1450.

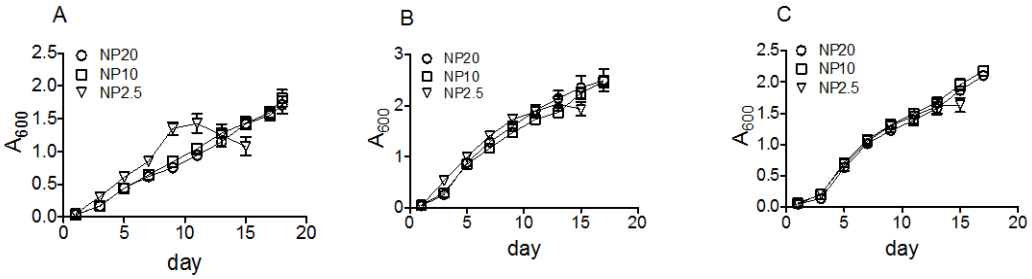

Fig. 2. Growth kinetics of three strains of microalgae on Chu4X medium with varying N:P ratios. NP2.5, N:P of 2.5:1; NP10, N:P of 10:1; NP20, N:P of 20:1. A, *Neochloris oleoabundans* UTEX 1185; B, *Scenedesmus obliquus* UTEX 393; C, *Scenedesmus obliquus* UTEX 1450.

Optical density of *S. obliquus* (UTEX 393) cultures steadily increased throughout the cultivation period (Fig. 3), with the exception of *S. obliquus* (UTEX 393) in mBold that exhibited only a slight increase in optical density over 14 days. Absorbance was highest on the last day of cultivation (day 14) in mChu (1.238), followed by mBG11 (0.903), AG (0.840), and mBold (0.145). Specific growth rate and t_d calculations show that *S. obliquus* (UTEX 393) had the shortest mean t_d (3.5 ± 0.0 days) in AG media despite the substantially higher optical density for *S. obliquus* in mChu (Fig. 3). Considering the variability of the A_{600} triplicate measurements, the t_d of *S. obliquus* (UTEX 393) in mChu and AG were equivalent (Table 2). Optical density of *S. obliquus* (UTEX 1450) in mBG11 and mChu steadily increased throughout the cultivation period (Fig. 4). Optical density of *S. obliquus* (UTEX 1450) in mBold was nearly stationary during the entire cultivation period and stationary in AG after day 8. Absorbance was

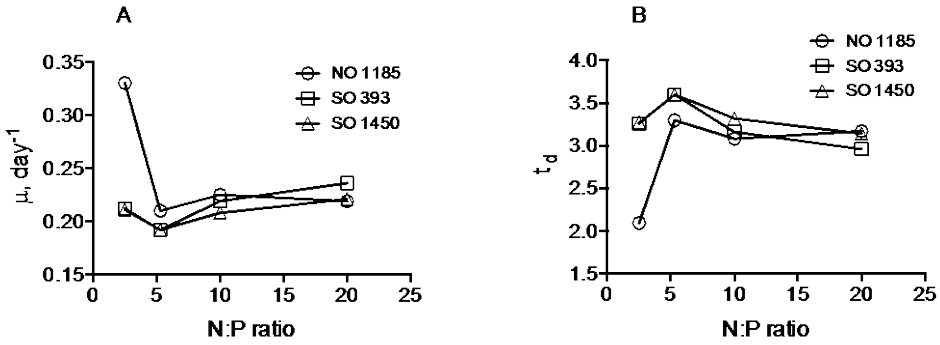

Fig. 3. Specific growth rates (A) and doubling times (B) of algal isolates over varying N:P ratios in mChu medium. NO 1185, *N. oleoabundans* UTEX 1185; SO 393, *S. obliquus* UTEX 393; SO 1450, *S. obliquus* UTEX 1450. Specific growth rates and doubling times were calculated using Eqs. (1) and (2), respectively.

highest in mChu (1.105) on day 14, followed by mBG11 (0.894) on day 14, AG (0.524) on day 9, and mBold (0.149) on day 7. Specific growth rate and t_d calculations show that *S. obliquus* (UTEX 1450) clearly had the shortest mean t_d (3.6 ± 0.1 days) in mChu media (Table 2).

Table 2 Cultivation period doubling times (days) for media/algae combinations.

	AG	mBG11	mBold	mChu
B. braunii UTEX572	5.2 ± 2.5	7.4 ± 1.7	7.0 ± 0.1	6.2 ± 1.8
N. oleoabundans UTEX1185	4.6 ± 0.4	3.9 ± 0.1	3.6 ± 0.2	3.3 ± 0.1
S. obliquus UTEX393	3.5 ± 0.0	4.0 ± 0.0	10.2 ± 0.2	3.6 ± 0.1
S. obliquus UTEX1450	4.0 ± 0.5	4.0 ± 0.0	20.2 ± 0.8	3.6 ± 0.1

Since the highest growth rates for all strains were observed when the organisms were cultured in mChu medium, this medium was selected to investigate effects of varying N:P ratios on growth properties. Growth of *N. oleoabundans* UTEX 1185, *S. obliquus* UTEX 393 and *S. obliquus* UTEX 1450 in mChu medium with four different N:P ratios is shown in Fig. 2. Further studies using *B. braunii* (UTEX 572) were discontinued as very low growth rates were observed with all media tested (see

below). Growth kinetics were similar for both strains of *S. scenedesmus* for each N:P ratio tested. Growth rates were lower for *N. oleoabundans* UTEX 1185 compared with the growth of the *S. obliquus* strains. Notably, growth of *N. oleoabundans* UTEX 1185 in mChu medium with N:P ratio of 2.5:1 reached a plateau at an A_{600} value of about 1.5, suggesting that the organism may have become limited for nitrogen after 10 days. Doubling times for *S. obliquus* UTEX 393 in media with N:P ratios of 2.5:1, 10:1 and 20:1 were 3.26, 3.16, and 2.96 days, respectively; doubling times for *S. obliquus* UTEX 1450 in media with N:P ratios of 2.5:1, 10:1 and 20:1 were 3.27, 3.60 and 3.14 days, respectively. Doubling times for *N. oleoabundans* UTEX 1185 in the same media were lower for N:P ratios of 2.5:1 and 10:1, with observed values of 2.09, 3.08, respectively. In mChu medium with N:P ratio of 20:1, the doubling time was larger than that of the *S. obliquus* strains at the same ratio, with an observed value of 3.17 days. These data are shown in Fig. 3A.

A benchmark study of the influence of growth media on planktonic algae (Chu, 1942) was re-examined to determine that the N:P mass ratios ranged from 0.1:1 to 532.6:1 for *Botryococcus* growth media. Biomass production by *Botryococcus* was greatest when the N:P mass ratio was 2.7:1 using a modified Chu #2 formulation with a KNO_3 concentration of 25 mg L^{-1} and K_2HPO_4 concentration of10 mg L^{-1} (Chu, 1942). However, the low biomass production by *Botryococcus* when the N:P mass ratio was 5.3:1, as reported by Chu (1942), was likely due to a P deficiency. In another study, biomass yields of *B. braunii* (UTEX 572) were examined while varying the N concentration from 10 mg L^{-1} to 1020 mg L^{-1} and P concentrations from 2.2 mg L^{-1} to 211.8 mg L^{-1} in a modified Chu13 media (An et al., 2003). Upon re-examination of the An et al. (2003) study, it was determined that the N:P mass ratio ranged from 0.2:1 to 98.0:1 and average specific growth rate was highest when the N:P mass ratio was 19.6:1 or 49.0:1(An et al., 2003). Biomass yields of *B. braunii* (UTEX 572) were also evaluated by Dayananda et al. (2007) using five variations of the Chu13, BG11, and two variations of the BBM media. Our re-examination of the Dayananda et al. (2007) study shows that the N:P mass ratio ranged from 3.3:1 to 47.6:1, and biomass yield was highest for a modified BG11 media with a N:P mass ratio of 47.6:1.

The variability of optical density measurements for *B. braunii*, in our study, does not allow us to report a substantially higher growth rate in any of the media; growth rates are mathematically equivalent for *B. braunii* in each media tested. However, the shortest mean t_d (5.2 ± 2.5 days) for *B. braunii* was observed in AG media, which had a N:P mass ratio of 30.5:1. This N:P mass ratio is similar in magnitude to the N:P mass ratios that promoted the best relative growth rates in the previous studies (Chu, 1942; An et al., 2003; Dayananda et al., 2007) described above. The lower growth rates obtained for *B. braunii* were likely due to culturing the organism at 28°C, in comparison with other studies which were done at ≤ 25°C (see for example, Chu, 1942; Dayananda et al., 2007).

A re-examination of the Li et al. (2008) study allowed for comparison of *N. oleoabundans* biomass production versus N:P mass ratios in the range of 0.4:1 to 2.8:1. Biomass productivity was highest when the initial N was 140 mg L^{-1} and initial P was 100 mg L^{-1}, which equated to an N:P mass ratio of 1.4:1(Li et al., 2008). Due to the variability of optical density measurements for *N. oleoabundans*, in our study, the doubling time was mathematically equivalent in mChu (t_d = 3.3 ± 0.1 days) and mBold (t_d = 3.6 ± 0.2 days) media, which had N:P mass ratios of 5.5:1 and 2.5:1, respectively. These N:P mass ratios are most similar in magnitude to the N:P mass ratio of 1.4:1 that promoted the best relative growth rate in the Li et al. (2008) study.

A re-examination of the Kim et al. (2007) study allowed for comparison of *S. obliquus* biomass production versus N:P mass ratios in the range of 0.8:1 to 1.2:1. A modified BBM supplemented with treated swine wastewater, resulting in a N concentration of 6.8 mg L^{-1} and P concentration of 5.6 mg L^{-1} (N:P mass ratio of 1.2:1), had a higher biomass growth rate (Kim et al., 2007). Due to the variability of optical density measurements for *S. obliquus* (UTEX 393), in our study, the t_d was mathematically equivalent in AG (t_d = 3.5 ± 0.0 days) and mBold (td = 3.6 ± 0.1 days) media, which had N:P mass ratios of 30.5:1 and 5.5:1, respectively. Specific growth rate and t_d calculations show that *S. obliquus* (UTEX 1450) clearly had the shortest mean t_d (3.6 ± 0.1 days) in mChu media, which had a N:P mass ratio of 5.5:1. The N:P mass ratios promoting the best growth, in our study, were higher in magnitude than the N:P mass ratio of 1.2:1 that promoted the best relative growth rate in the Kim et al. (2007) study. This may be due to strain differences, *S. obliquus* in our study versus the *Scenedesmus spp.* isolate used by Kim et al. (2007); or may reflect the narrow N:P range and potentially low (i.e., deficient) N and P concentrations used in the Kim et al. (2007) study.

It is also of interest to note that, taken together, the differences in the optimal N:P ratios we observed for *N. oleoabundans* UTEX 1185 and *S. scenedesmus* strains UTEX 393 and UTEX 1450 can be viewed from the standpoint of differences in their respective habitats and the nutrient and resource levels available to the strains in those habitats. Consequently one might predict that the physiological ecology of the organisms should reflect the properties of their respective habitats. For example, *N. oleoabundans* is a terrestrial algal species originally isolated from sand samples in the Saudi Arabian desert (Chantanachat & Bold, 1962); and the relatively lower N:P optima observed for *N. oleoabundans* from among the N:P ratios examined is consistent with the nutrient-poor properties of these types of ecosystems. Moreover, in a study conducted by Baldisserotto et al. (2012), while it was shown that growth of *N. oleoabundans* was improved in media with higher salinity, growth rates and cell yields in medium containing 2 mM nitrate were indistinguishable from media containing 5 mM nitrate or even 10 mM nitrate. Similarly, for *Scenedesmus obliquus* isolates, as the *S. obliquus* group is a rather diverse one, it would be predicted that a wide range of N:P optima might be observed for a diverse species group such as *S. obliquus*. In this case, diversification of the *S. obliquus* group has resulted in the emergence of related but distinct strains adapted to a variety of niches with different resource and nutrient levels. This would explain how similar species exhibit different N:P optima for growth. Taxonomic and phylogenetic studies of the *Scenedesmus* group have confirmed the diverse nature of the *Scenedesmus* clade in general and the *S. obliquus* group in particular. For example, differences were found between the nucleotide sequences of the ITS-2 internal transcribed spacer regions for *S. scenedesmus* strains used in this present study UTEX 393 and UTEX 1450 (van Hannen et al., 2000).

4. Conclusion

The results of our study indicate that under fixed photoperiod light:dark regimes of 15 h light:9 h dark, the ratios of the macronutrients phosphorous and nitrogen have a significant influence on the biomass productivity values of the species of microalgae tested. And significantly, the N:P ratios that support the highest biomass productivity rates differ among different microalgal species. This observation may reflect fundamental differences in the physiological ecology of the organisms and metabolic adaptations to their natural environments. The strains used in this study were *Botryococcus braunii* (UTEX 572), *Neochloris oleoabundans* (UTEX 1185), *Scenedesmus obliquus* (UTEX 393) and *Scenedesmus obliquus* (UTEX 1450). From among the various media and N:P ratios

examined for each strain, the shortest doubling times observed were 5.2 days (*B. braunii* AG medium, N:P ratio 30.5:1), 3.3 days (*N. oleoabundans* mChu medium, N:P ratio 5.5:1), 3.5 days (*S. obliquus* UTEX 393 AG medium, N:P ratio 30.5:1), and 3.6 days (*S. obliquus* UTEX 1450 mChu medium N:P ratio 5.5:1). These results therefore provide media formulations and N:P ratios that support growth and high biomass productivity that are important for applications such as the production of algal biomass for use as, e.g., feedstocks, and for the creation of biocatalysts for the production of fuels and other chemicals.

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