# Kinetic parameters of *Scenedesmus obliquus* (Chlorophyceae) growth and yield under different cultivation conditions *in vitro*

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## ABSTRACT

The aim of the present study is to assess the kinetic growth and yield of microalgae belonging to the species *Scenedesmus obliquus* (Kützing, 1833) grown under different macronutrient concentrations (N:P:K), pH and temperature. A three-level three-variable Box-Behnken factorial design was developed to test the influence of environmental factors on the growth parameters applied to a microalgal crop, namely: maximum cell density ($N_{max}$), specific growth rate ($\mu$, $d^{-1}$), doubling time ($T_2$) and maximum yield ($P_{max}$). A classical logistic growth model was applied to estimate the kinetic behavior of a *S. obliquus* culture in comparison to other studies in the literature. Maximum cell density ($N_{max}$) significantly changed between $32.3 \pm 0.84$ and $101.5 \pm 12.5 \times 10^5$ cells $mL^{-1}$ ($p = 0.004$). Specific growth rates ($\mu$) and $P_{max}$ showed significant mean variations between $0.28 \pm 0.10$ day$^{-1}$ and $6.21 \pm 4.57$ cells $mL^{-1}$ $d^{-1}$, respectively. All growth curves were adjusted to the proposed logistic model and range variation was ($0.65 \leq r^2 \leq 0.99$, $p = 0.01$). Maximum microalgae cell density per crop was found at temperature of 24 °C ($N_{max} = 49.0 \times 10^5$ cells $mL^{-1}$) ($p = 0.01$), in 20 mL Nitrogen, Phosphorus, and Potassium (NPK) dilution ($N_{max} = 46.5 \times 10^5$ cells $mL^{-1}$) ($p = 0.02$). Despite the significant variation in pH values ($5.0 \leq pH \leq 9.0$, $p = 0.05$), it did not have strong influence on plant growth responses. In conclusion, the kinetic study applied to *S. obliquus* growth and yield parameters emerged as reference for this species' kinetic behavior and bioprospecting under controlled and experimentally standardized conditions, and as support for pilot projects on bioreactors.

**Subjects** Agricultural Science, Biotechnology, Microbiology, Plant Science, Freshwater Biology
**Keywords** Microalgae, NPK, Growth kinetics

## INTRODUCTION

Microalgae are a source of organic compounds that present high potential for bioprospecting and biotechnological applications, mainly for producing both biodiesel

and dietary supplements used in human and animal nutrition (*Breuer et al., 2012*; *Moreno-Garcia et al., 2017*). *Silveira Júnior, Faustino & Cunha (2019)* conducted a literature review to analyze the role played by microalgae in bioprospecting and their application as dietary supplement and immunostimulant at regional and global level, with emphasis on aquaculture in the Brazilian Amazon. They assessed the primary advantages of applying microalgae bioactive compounds and found gaps in the knowledge about this topic. These gaps assumingly hinder the economic and biotechnological exploitation of these microalgae.

The increased production of microalgal biomass grown in cultivation ponds (open systems) and in photobioreactors (closed systems) has been a trend for some decades (*Posten, 2009*; *Silveira Júnior, Faustino & Cunha, 2019*; *Wen et al., 2016*). These crops have enhanced the use of these microalgae in the food, aquaculture, and bioenergy industries (*Adams et al., 2013*; *Hempel, Petrick & Behrendt, 2012*; *Mallick et al., 2016*; *Wen et al., 2016*). However, despite such a production trend, knowledge on its high operational costs and on these organisms' industrial use, mainly on their yield and effective biomass production, remains scarce (*Tan & Lee, 2016*; *Wang, Sheng & Yang, 2017*). Conditioning factors associated with these processes need to be better understood to allow foreseeing the specific parameters to make its sustainable production feasible, mainly when it comes to its multiple biotechnological applications (*Barbera et al., 2016*).

Microalgal production stages must be optimized in order to achieve appropriate harvesting, drying and biomass extraction conditions (*Valverde et al., 2016*). However, yield optimization and associated cost reductions remain a challenge due to difficulties in controlling basic conditions and microalgal growth specifications (*Lee, Jalalizadeh & Zhang, 2015*; *Silveira Júnior, Faustino & Cunha, 2019*) that, in their turn, limit and hinder the development of new research. Cultivation method optimization has evolved over the last few years (*Barbera et al., 2016*; *Bohnenberger & Crosseti, 2014*; *Hodaifa et al., 2010*; *Sipaúba-Tavares et al., 2017*; *Xu et al., 2012*), but kinetic studies carried out with microalgae must be encouraged (*Silveira Júnior, Faustino & Cunha, 2019*). These authors acknowledge the urgency in building a biotechnological basis, such as microalgal growth kinetic analysis, to support the development of photobioreactor projects. In addition, kinetic parameters are the starting point for process design and control, since they allow better operational cultivation performance and yield (*Lee, Jalalizadeh & Zhang, 2015*; *Surendhiran et al., 2015*).

Some classical studies on microalgae kinetic parameters are associated with both single and multiple factors that, in their turn, influence microalgal growth. Light intensity, pH, nutrient availability, dissolved $CO_2$ and $O_2$ concentration, and temperature are essential parameters for microalgae crops (*Hodaifa et al., 2010*; *Krzemińska et al., 2014*; *Lee, Jalalizadeh & Zhang, 2015*; *Li, Gao & Lin, 2015*). Assessing these parameters' influence on microalgae growth kinetics is a basic condition to reach a successful cultivation, since they define the optimal or most appropriate conditions for different cultivation alternatives. Understanding constraints imposed by these key parameters is the way to accomplish better biomass growth and yield rates (*Arumugam et al., 2013*; *Baumgartner et al., 2013*; *Tan & Lee, 2016*).

*S. obliquus* has been extensively investigated in bioprospective studies. *Zaharieva et al. (2022)* assessed the effect of luminosity on specific growth rates and biocompound production in *S. obliquus* biomass. They observed higher content of polyphenols under red light, whereas the green light favored flavonoids accumulation in the biomass. These compounds enhanced antimicrobial effects against foodborne pathogens when they were associated with penicillin, fluoroquinolones or with oregano essential oil, at concentrations ranging from 0.01 to 0.05 mg/mL. *Kaewkannetra, Enmak & Chiu (2012)* assessed salinity effect on *S. obliquus* cultures in the presence of NaCl, at concentrations of 0.05, 0.2, and 0.3 M. The highest lipid accumulation was found in cells grown under saline stress in comparison to that recorded in non-saline medium, and it led to 36% lipid content. According to *Gorrini et al. (2020)*, *S. obliquus* biomass production can be significantly enhanced by properly manipulating control variables, such as incident light, biomass and substrate concentrations. These parameters work as natural and effective control strategy for optimization issues linked to this species' culture.

The main hypothesis tested in the present study lied on finding the basic limits for parameters applied to control the kinetic growth behavior of *S. obliquus* grown in photobioreactors, at bench scale. The tested kinetic model had to keep first-order reaction features and they can be defined through a classic logistic model, at relatively high reliability ($r^2 > 75\%$). Experimental adjustments to data of the proposed model pointed out that the kinetic growth behavior of the assessed microalgal species can be compared to behaviors observed in similar studies available in the literature (*Arumugam et al., 2013*; *Barbera et al., 2016*; *Goswami & Kalita, 2011*; *Nayak, Thirunavoukkarasu & Mohanty, 2016*; *Sipaúba-Tavares et al., 2017*; *Xin et al., 2010*). Therefore, it was safe and replicable at pilot scale level, if one takes into consideration similar and controlled environments.

The aim of the current investigation was to test a classical logistic growth model to represent *S. obliquus* growth kinetics at adequate significance level. This microalgae species is naturally distributed in Amazonian ecosystems. The main aims of the present study were to test growth and yield rate variations, and to assess different cultivation conditions (Nitrogen, Phosphorus, and Potassium (NPK) concentration and dilution, pH and temperature) as limiting or potentiating factors influencing kinetic microalgal growth in crop systems, *in vitro*, to assess its sustainable production at industrial scale.

## MATERIALS & METHODS

### *Scenedesmus obliquus* isolation and culture activation

*Scenedesmus obliquus* isolation was performed based on both capillarity and successive dilution techniques (*Lourenço, 2006*). The experiment was carried out at the Algae Cultivation and Bioprospecting Laboratory (LACAL) of Federal University of Amapá (UNIFAP), after the species was subjected to taxonomic identification through optical microscopy. Phytoplankton samples collected in a natural aquatic ecosystem (Lagoa dos Índios, Amapá, Brazil, 0°01′54.260″N 51°06′09.665″W) were used in the study. Isolated cells were kept in test tubes filled with liquid synthetic culture medium (NPK 15:05:05) (Table 1). Cells were incubated at 23 °C, under continuous photoperiod.

**Table 1  Culture medium used for *Scenedesmus obliquus* microalgae culture.**

| Medium | Reagent | NPK dilution (mg/L) | | |
|---|---|---|---|---|
| | | **12** | **16** | **20** |
| NPK | Nitrogen (water-soluble N) | 0.64 g/L | 0.86 g/L | 1.12 g/L |
| | Phosphorus (water-soluble $P_2O_5$) | 0.19 g/L | 0.25 g/L | 0.32 g/L |
| | Potassium (water-soluble $K_2O$) | 0.26 g/L | 0.35 g/L | 0.44 g/L |

**Table 2  Variables used in Box-Behnken factorial experimental planning.**

| Factor | Variable | Level | | |
|---|---|---|---|---|
| | | **−1** | **0** | **+1** |
| $X1$ | pH | 5 | 7 | 9 |
| $X2$ | Temperature (°C) | 21 | 24 | 28 |
| $X3$ | NPK dilution (mg/L) | 12 | 16 | 20 |

Isolates ($11.7 \pm 0.3 \times 10^4$ cells mL$^{-1}$) were transferred to activation crops in Erlenmeyer (200 mL) flasks until the tests were run.

## Experimental design and *S. obliquus* cultivation in photobioreactor

A three-level three-variable Box-Behnken factorial design was developed to determine the temperature (21 °C, 24 °C and 28 °C), pH (5.0, 7.0 and 9.0) and NPK dilution (12 mg/L, 16 mg/L and 20 mg/L) influence on *S. obliquus* cultivation (Table 2). The pH range was defined according to conditions naturally observed in Amazonian ecosystems (*Barros et al., 2021*). STATISTICA® software (version 10, Statesoft–Inc., Tulsa, USA, trial version, 2011) was used in the experimental design for matrix elaboration. Independent variables were divided into X1, X2 and X3.

Inoculates (200 mL) were transferred to bioreactors—2L Erlenmeyer vials (*in vitro*) filled with NPK culture medium at different dilutions (12, 16 and 20 mg/L distilled water)—at the beginning of the experiments (Table 2). Initial axenic inoculum consisted of $11.7 \pm 0.3 \times 10^4$ cells mL$^{-1}$ in all assays. All experiments were simultaneously conducted in triplicate.

Inoculates were grown and kept under continuous photoperiod (28.5 $\mu$mol m$^{-2}$s$^{-1}$), as already reported for *S. obliquus* (*Breuer et al., 2013*; *Girard et al., 2014*). Constant aeration was carried out by $CO_2$ (14 L min$^{-1}$) air diffusion through pumping (1/2 HP). Temperature in the growing room was controlled and monitored with the aid of digital thermometers ($21 \pm 0.4$ °C, $24 \pm 0.6$ °C, $28 \pm °0.9$ °C). Different pH values were determined by adding HCl 1N and NaOH 1N ($5 \pm 0.7$, $7 \pm 0.5$ and $9 \pm 0.3$) solutions. The pH in the culture medium was measured every 48 h with the aid of a pH-meter.

## Cell density and yield

Cell density in the culture (average recorded for the three replicates) was measured on a daily basis in Neubauer hemocytometer by counting microalgal cells in optical microscope (*Lourenço, 2006*). Density values were used to calculate specific growth rate ($\mu$), maximum cell concentration (N$_{max}$) and maximum yield (P$_{max}$).

Specific growth rate ($\mu$, $d^{-1}$) was calculated based on the logarithmic phase regression of the cell growth curve. Maximum cell concentration ($N_{max}$, cells $mL^{-1}$) was represented by the maximum concentration value recorded in the logarithmic phase. Maximum yield ($P_{max}$, cel $mL^{-1}$ $d^{-1}$) was calculated through Eq. (1):

$$p_{max} = \frac{(X_1 - X_0)}{(t_1 - t_0)} \qquad (1)$$

wherein, $X_1$ is cellular concentration (cells $mL^{-1}$) at time $t_1$ (d) and $X_0$ (cells $mL^{-1}$) is cellular concentration at time $t_0$ (d) (*Schmidell et al., 2001*). Doubling time per day ($T_2$) was calculated through Eq. (2):

$$T_2 = \frac{ln2}{\mu}. \qquad (2)$$

## Cellular density logistic model adjustment

Microalgae growth kinetics can often be modeled through the Verhulst logistic equation (*Mazumdar et al., 2018*; *Yang et al., 2011*), which is a model based on three important parameters: initial cell density at $T_0$ (cells $mL^{-1}$), theoretically reached maximum cell density (cells $mL^{-1}$) and specific growth rate ($\mu$, $day^{-1}$).

Cell density ($N_t$), at any time (t), based on $N_o$ as the initial cell density is given by Eq. (3) in this model:

$$N_t = \frac{N_0 . N_{Max}}{N_0 + (N_{Max} - N_0)e^{-\mu t}}. \qquad (3)$$

A transformed version of this Eq. (3) was used to estimate the growth parameters. It was based on adjusting the experimental raw data in the model of a nonlinear function (logistics) through the least square's method (*Mazumdar et al., 2018*; *Weisberg, 2005*), which is expressed in Eq. (4):

$$N_t = \frac{N_{Max}}{1 + e^{(\theta + \mu t)}} \qquad (4)$$

wherein, $N_t$ is cell count at time t, t is time after experiment has started, $N_{Max}$ isthe maximum estimated cell count, $N_0$ is the initial cell count, $\mu$ is maximum specific growth rate - $\theta$ was defined through Eq. (5):

$$\theta = \ln(\frac{N_0}{N_{Max} - N_0}). \qquad (5)$$

## Statistical treatment

Model significance was tested in the R Statistic (version *R Core Team, 2016*) software. Logistic regression analysis was applied to assess the explainability and significance of models adjusted based on Eqs. (2) and (3).

Shapiro–Wilk normality test, followed by Levene/Bartlett tests, was carried out to assess experimental data homogeneity and homoscedasticity and to model the assessed kinetic parameters. Analysis of variance applied to repeated measurements was performed through

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**Table 3 Maximum cell density ($N_{max}$), daily growth rate ($\mu$), doubling time ($T_2$) and maximum yield ($P_{max}$) recorded for *Scenedesmus obliquus* under different growing conditions.** Different letters in the same column indicate significant difference at $p < 0.05$ (Dunn's Test).

| Order | Experiment | $N_0$ (cel mL$^{-1}$ × 10$^4$) | $N_{max}$ (cel mL$^1$ × 10$^5$) | $\mu$ (day$^{-1}$) | $T_2$ (day) | $P_{max}$ (cel mL$^{-1}$ × 10$^5$ d$^{-1}$) |
|---|---|---|---|---|---|---|
| 1 | E1pH$_7$T$_{21}$C$_{12}$ | 11.7 | 48.5$^{abc}$ | 0.30$^a$ | 2.28$^{ac}$ | 5.46$^{ac}$ |
| 2 | E2pH$_7$T$_{24}$C$_{16}$ | 11.7 | 44.0$^{ab}$ | 0.29$^a$ | 2.36$^a$ | 6.64$^{ac}$ |
| 3 | E3pH$_5$T$_{24}$C$_{12}$ | 11.7 | 39.7$^{ab}$ | 0.22$^a$ | 3.07$^{ab}$ | 3.85$^{ab}$ |
| 4 | E4pH$_7$T$_{28}$C$_{12}$ | 11.7 | 42.1$^{ab}$ | 0.24$^a$ | 2.81$^a$ | 4.37$^{ab}$ |
| 5 | E5pH$_9$T$_{21}$C$_{16}$ | 11.7 | 38.8$^{ab}$ | 0.13$^b$ | 5.27$^b$ | 5.75$^a$ |
| 6 | E6pH$_5$T$_{21}$C$_{20}$ | 11.7 | 56.2$^{cd}$ | 0.36$^{ac}$ | 1.92$^{ac}$ | 10.7$^{cd}$ |
| 7 | E7pH$_9$T$_{28}$C$_{16}$ | 11.7 | 34.1$^b$ | 0.21$^a$ | 3.27$^a$ | 2.64$^b$ |
| 8 | E8pH$_5$T$_{28}$C$_{20}$ | 11.7 | 32.6$^a$ | 0.18$^a$ | 3.84$^b$ | 2.83$^b$ |
| 9 | E9pH$_9$T$_{24}$C$_{20}$ | 11.7 | 101.5$^d$ | 0.48$^c$ | 1.44$^c$ | 17.9$^d$ |
| | Median | 11.7 | 42.1 | 0.24 | 2.81 | 5.46 |
| | *p*-value | – | $p < 0.05$ | $p < 0.05$ | $p < 0.05$ | $p < 0.05$ |

Kruskal–Wallis test, followed by *post-hoc* dunn test, to find significant differences in growth parameters, in different experiments, since data distribution rejected normality. All tests were considered significant at $\alpha < 0.05$.

STATISTICA software® (*version 10, Statesoft - Inc., Tulsa, USA, test version, 2011*) was used for data analysis. Analysis of variance (ANOVA) was applied to transformed data to assess influence significance and interactions between independent variables, namely: temperature, pH and NPK dilution. Pareto graphs were plotted to find the significance of tested variables and to accomplish modeling through response surfaces.

# RESULTS

## Cell density and microalgal yield

The effect of different culture medium dilution, temperature and pH levels on *S. obliquus* cultivation was tested based on logistic models defined through Eqs. (2) and (3). Significant differences were observed in all assessed microalgal growth measurements between experiments. Maximum cell density ($N_{max}$) ranged from 32.6 to 101.5 × 10$^5$ cells mL$^{-1}$; median was 42.1 × 10$^5$ cells mL$^{-1}$. This variation led to significantly higher $N_{max}$ in experiments 9 and 6, which were respectively defined as E9pH$_9$T$_{24}$C$_{20}$ (101.5 × 10$^5$ cells mL$^{-1}$) and E6pH$_5$T$_{21}$C$_{20}$ (56.2 × 10$^5$ cells mL$^{-1}$) ($p = 0.04$ and $p = 0.004$) (Table 3). Similarly, specific growth rates ($\mu$) and maximum yield (Pmax) recorded medians of 0.24 d$^{-1}$ and 5.46 cells mL$^{-1}$ d$^{-1}$, respectively. Significant and higher values were also observed in experiments 9 and 6, respectively: E9pH$_9$T$_{24}$C$_{20}$ and E6pH$_5$T$_{21}$C$_{20}$ ($p = 0.002$ and $p = 0.005$).

Different experiments presented median cell-doubling time of 2.81 days. The longest doubling time ($T_2$) was recorded for experiment 5, *i.e.,* E5pH$_9$T$_{21}$C$_{16}$ (5.2 days) ($p = 0.005$) - values were similar to those observed for assays 3 and 8, respectively: E3pH$_5$T$_{24}$C$_{12}$ (3.07 days) and E8pH$_5$T$_{28}$C$_{20}$ (3.8 days) (Table 3). The shortest cell doubling time was observed for experiment 9, *i.e.,* E9pH$_9$T$_{24}$C$_{20}$ (1.4 ± 0.17 days) ($p = 0.002$) - values were similar to
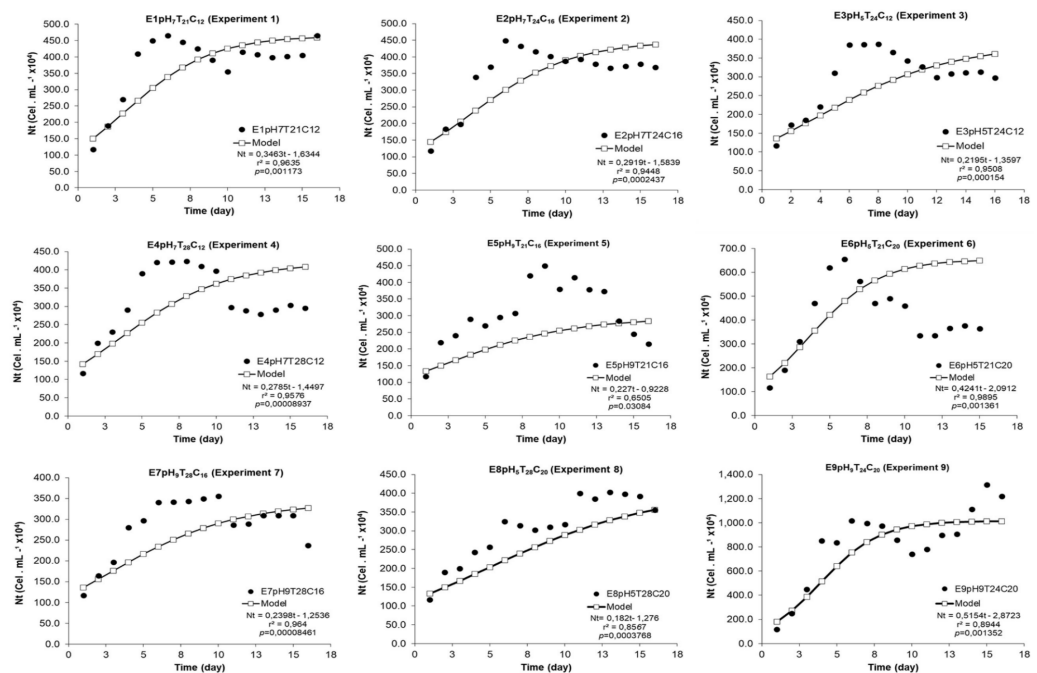
**Figure 1** Experimental and adjusted growth curve of *Scenedesmus obliquus* grown under different cultivation conditions.

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those recorded for experiments 1 and 6, respectively: E1pH$_7$T$_{21}$C$_{12}$ (2.0 ± 0.14 days) and E6pH$_5$T$_{21}$C$_{20}$ (1.6 ± 0.19 days).

## Adjustment to the logistic model applied to microalgae cell density

The estimated model significantly adjusted itself to the experimental data, since r$^2$ ranged from 0.65 (experiment 5) to 0.99 (experiment 6) (Fig. 1). All modeled growth curves presented noticeably short or, practically, no *lag* phase (growth induction or latency). All experiments presented exponential growth phase between the 2nd and 7th experiment days (Figs. 1 and 2). The end of the stationary phase and the start of the decline phase (cell death) in the crop were observed between the 10th and 16th day of microalgal culture. The maximum concentration phase was detected when N$_{Max}$ was also observed.

The logistic curve adjustment showed significant differences between Nt responses recorded for the nine models tested under different pH, temperature and N:P:K concentration conditions. Experiments E9pH$_9$T$_{24}$C$_{20}$ (N$_{max}$ = 101.5 × 10$^5$ cel mL$^{-1}$) and E6pH$_5$T$_{21}$C$_{20}$ (N$_{max}$ = 56.2 × 10$^5$ cel.mL$^{-1}$) ($p$ = 0.002 and $p$ = 0.007) recorded the highest cell density growth expression. (Fig. 2A).

The adjusted curves showed significant differences when the different factors (temperature × pH × medium dilution) (Figs. 2B, 2C and 2D) used for factorial planning worked in the same microalgal crop growth parameters. Higher microalgaceate cell density was observed in microalgal growth at 24 °C (N$_{max}$ = 49.0 × 10$^5$ cells mL$^{-1}$) ($p$ = 0.01), in 20 mg/L culture-medium dilution (N$_{max}$ = 46.5 × 10$^5$ cells mL$^{-1}$) ($p$ = 0.02). Crops stored at pH 9 tended to present higher cell density ($p$ = 0.05).
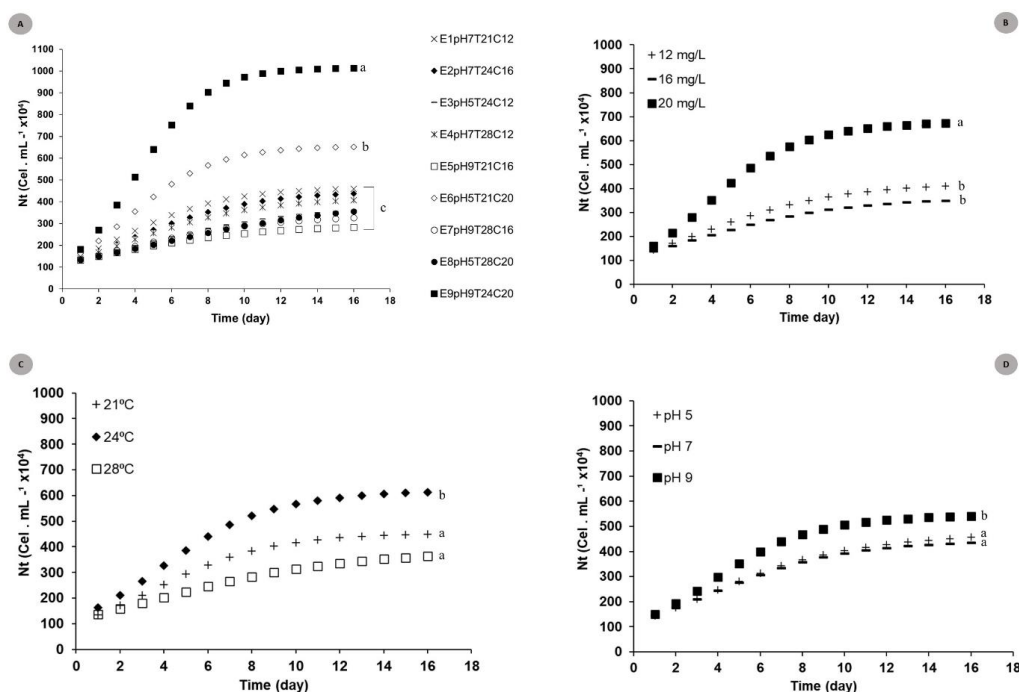
**Figure 2  Theoretical logistic growth curves estimated for *S. obliquus* cultivated under different conditions.** (A) General: curves adjusted to all experiments together; (B) Exclusive concentration influence: curves adjusted under the influence of different medium dilutions; (C) Exclusive temperature influence: curves adjusted based on the influence of different temperatures; (C) Exclusive pH influence: curves adjusted based on the influence of different pH values. Different lower-case letters indicate significant differences at $p < 0.05$.

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## Assessing *S. obliquus* cultivation based on response surface modeling

Factorial planning allowed assessing the influence of different factors on response variables: doubling time ($T_2$), maximum cell density ($N_{max}$), maximum yield ($P_{max}$) and specific growth rate ($\mu$, day$^{-1}$). Pareto graphs pointed towards the strong effect of NPK medium dilution on variables $T_2$ (Fig. 3A) ($p = 0.02$), $P_{max}$ (Fig. 3B) ($p = 0.03$) and $\mu$(day$^{-1}$) (Fig. 3C) ($p = 0.01$). Analysis of variance (ANOVA) applied to these variables' model showed coefficients of determination ($r^2$) equal to 0.83, 0.79 and 0.87, respectively. Similarly, there was strong crop pH ($p = 0.03$) effect on $N_{max}$ response in the *S. obliquus* culture (Fig. 3D). ANOVA applied to the model of variable $N_{max}$ presented coefficient of determination ($r^2$) equal to 0.87.

The response surface analysis (Figs. 4 and 5) showed that pH ranging from 8.5 to 9.0 had strong influence on biomass yield based on variable $P_{max}$. Wide temperature range (21 to 28 °C) affected *S. obliquus* yield (Fig. 4A). The higher the NPK concentration in the crop, the higher the biomass yield in comparison to the effect of different pH values (Figs. 4B and 4C).

According to the response surfaces, the highest NPK concentrations had more influence on cell doubling time $T_2$ than crop pH (Fig. 4D). Temperature ranging from 24 °C to 29 °C
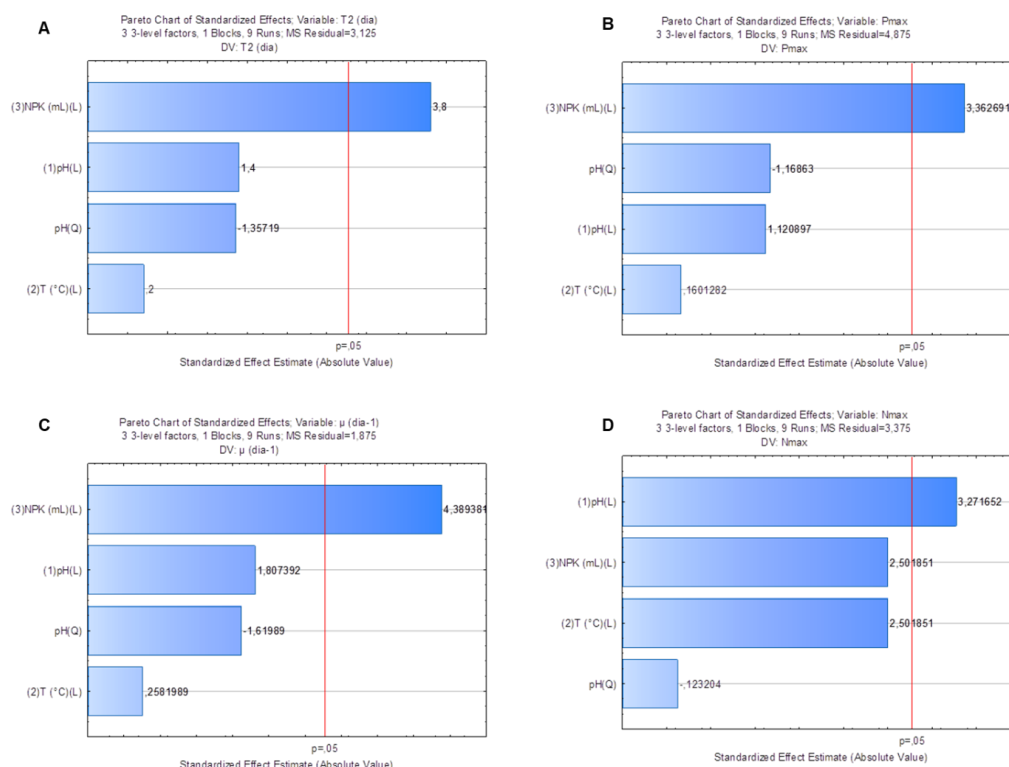
**Figure 3** Pareto graph to find the significance ($p < 0.05$) of $T_2$ (3A), $N_{max}$ (3B), Pmax (3C) and $\mu$ (day$^{-1}$) (3D) as variable response to *Scenedesmus obliquus* cultivation under different pH, medium dilution and temperature conditions.

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had strong effect on *S. obliquus* cell doubling when it was conditioned in 24 mL/L NPK (Fig. 4E). Temperature highly interacted with $T_2$ when temperature ranged from 24 °C to 28 °C. The pH value had influence on $T_2$ when it was higher than 8.5 (Fig. 4F).

The pH > 9.0 in association with temperatures higher than 24 °C had strong influence on *S. obliquus* specific growth rate ($\mu$, day$^{-1}$) (Fig. 5A). Growth rate was assessed based on NPK dilution and temperature (Fig. 5B), as well as on NPK and pH dilution (Fig. 5C). Higher NPK concentrations had strong influence on the specific growth rate in both response surfaces. Temperatures between 24 °C and 29 °C, in association with pH higher than 9, affected *S. obliquus* growth rate (Figs. 5B and 5C).

Response surface applied to maximum cell density ($N_{max}$) based on crop temperature and pH showed interaction between temperatures > 27 °C and pH > 9.0 (Fig. 5D). Higher $N_{max}$ was observed when temperature ranging from 27 °C to 29 °C was associated with higher NPK concentrations in *S. obliquus* culture (Fig. 5E). Crops conditioned at pH ranging from 7.5 to 9.5 presented high cell density when they were conditioned under the highest NPK concentration (Fig. 5F).
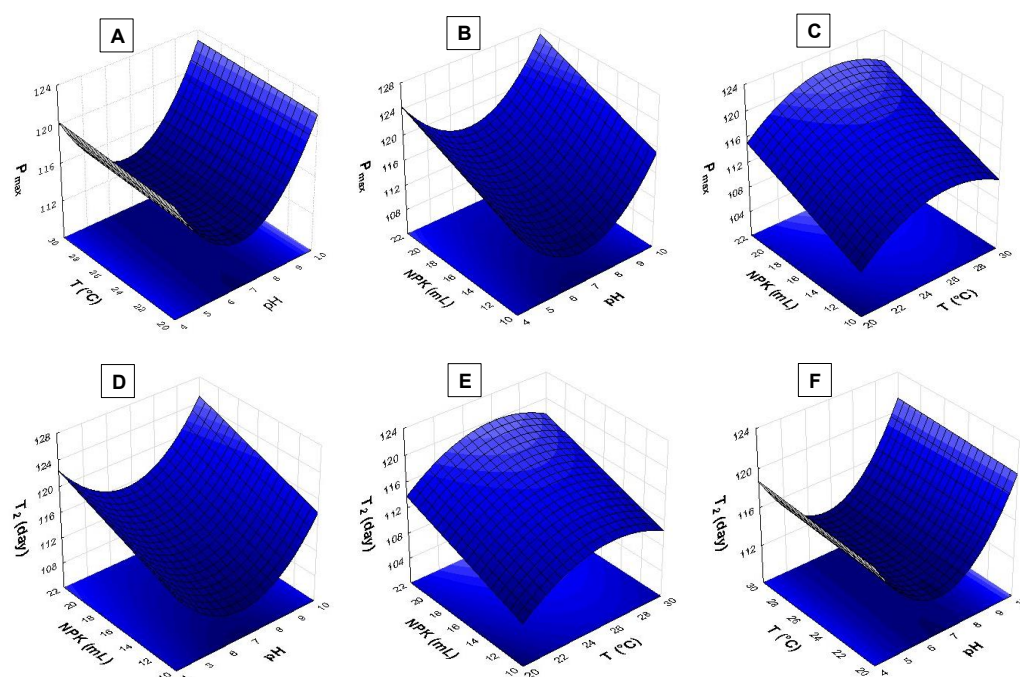
**Figure 4** Response surface recorded for *S. obliquus* culture (A) $P_{max}$ based on temperature and pH; (B) $P_{max}$ based on pH value and NPK dilution; (C) $P_{max}$ based on temperature and NPK dilution; (D) $T_2$ based on pH value and NPK dilution; (E) $T_2$ based on temperature and NPK dilution; (F) $T_2$ based on temperature and pH.

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## DISCUSSION

Microalgae biomass production, mainly for bioprospecting and for identifying compounds with potential to be used for several purposes, has become an attractive factor in recent decades. The growth of these microalgae is associated with their likely large-scale cultivation, based on their biomass extraction and on their ability to accumulate and store secondary metabolites that, in their turn, are important for their strategic applications, such as in aquaculture, food and pharmaceutical industries, and for power generation (*Krzemińska et al., 2014*; *Ota et al., 2015*; *Santos et al., 2013*).

Microalgae cultivation, mainly at commercial scale, is not yet economically viable or sustainable (*Álvarez-Díaz et al., 2015*; *Silveira Júnior, Faustino & Cunha, 2019*). Its large-scale cultivation, even in pilot projects, presents severe limitations when it comes to controlling specific environmental crop conditions, such as temperature, light, carbon dioxide concentration, pH and nutrient availability - mainly nitrogen (*Bohnenberger & Crosseti, 2014*). The selection of an adequate microalgae species and its optimization depends on growing–environment conditions, such as using kinetic parameters and assessing microalgal biomass at semi-industrial or industrial scale, since they can trigger a series of interdependent processes (*Breuer et al., 2013*).

*S. obliquus* was assessed due to its potential use for different purposes, and it explains the scientific and prospective interest in it. This interest justifies its technological assessment
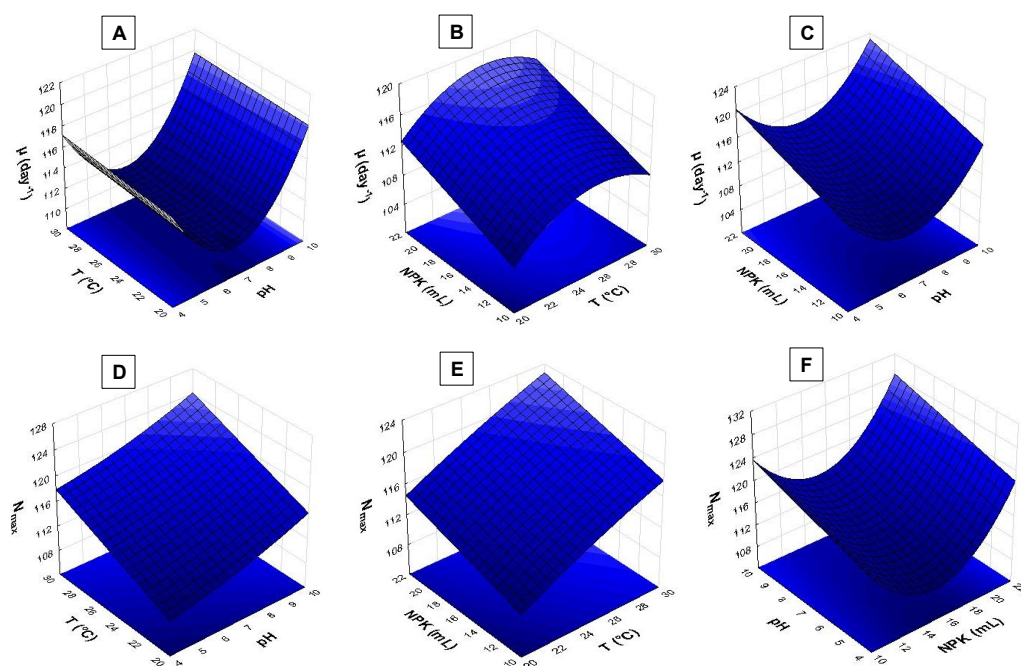
**Figure 5 Response surface recorded for *S. obliquus* culture.** (A) $\mu(day^{-1})$ based on temperature and pH; (B) $\mu(day^{-1})$ based on temperature and NPK dilution; (C) $\mu(day^{-1})$ based on pH and NPK dilution; (D) $N_{max}$ based on temperature and pH; (E) $N_{max}$ based on temperature and NPK dilution; (F) $N_{max}$ based on pH and NPK dilution.

and the kinetic analysis applied to its growth and yield rates under different temperature, pH and NPK concentration conditions. Specific growth rate in the current analysis ranged from $0.13 \pm 0.003$ to $0.52 \pm 0.5$ day$^{-1}$, maximum cell density was close to $101.5 \pm 12.5 \times 10^5$ cells mL$^{-1}$ and mean doubling time was approximately $2.78 \pm 1.07$ days. These parameters were useful to define and analyze microorganisms' biomass-production stages and processes. In addition, these parameters can be applied to any reactor or production system type. Yet, their usefulness is essential for the development of industrial–scale projects (*Lee, Jalalizadeh & Zhang, 2015*; *Novoveská et al., 2016*).

*Li, Gao & Lin (2015)* cultivated *S. obliquus* in Blue-Green medium 11 (BG11 medium) at initial inoculum of $5 \times 10^4$ cell mL$^{-1}$, and recorded specific growth rate ranging from 0.65 to 0.14 day$^{-1}$, under light intensity of 60 and 10 µmol of photons m$^{-2}$ s$^{-1}$, respectively. In the present study, *S. obliquus* inoculants were grown under 28.5 µmol of photons m$^{-2}$ s$^{-1}$ in all experiments.

*Scenedesmus obliquus* cultivated in culture medium diluted in wastewater from olive oil production showed mean specific growth rate of 0.57 d$^{-1}$ (*Hodaifa et al., 2010*). This rate is similar to the maximum rate (0.52 d$^{-1}$) found for the inorganic medium herein cultivated at 24 °C, in alkaline pH. Similar mean rates were observed for other microalgae species, namely: *chlorella sp.* (0.57 day$^{-1}$), *Coelastrum microporum* (0.29 day$^{-1}$), *Rhodomonas* sp. (0.26 day$^{-1}$), *S. pectinatus* (0.23 day$^{-1}$) and *S. dimorphus* (0.14 day$^{-1}$) (*Bruno, Udhaya*

*& Sandhya, 2013*; *Gour et al., 2016*; *Huerlimann, de Nys & Heimann, 2010*; *Mohammadi, Arabian & Khalilzadeh, 2016*).

Mean maximum *S. obliquus* yield ($6.21 \pm 4.5 \times 10^5$ cells mL$^{-1}$ d$^{-1}$) was similar to that observed for *S. acuminatus* crops grown in different media, including NPK (*Baumgartner et al., 2013*). On the other hand, the maximum yield rate of $0.99 \times 10^4$ cells mL$^{-1}$ d$^{-1}$ was observed for *S. dimorphus* grown in BG11 urea-enriched medium (*Goswami & Kalita, 2011*). These different outcomes can be associated with the mean cell doubling time observed in the present study ($2.78 \pm 1.07$ days), *i.e.*, it was shorter than that observed for other species belonging to genus *Scenedesmus*, such as *S. dimorphus* (4.91 days) *and S. quadricauda* (5.73 days)—both cultivated in BG11 medium (*Gour et al., 2016*). Doubling time was shorter than that observed for *Ankistrodesmus gracilis* (4.4 days) cultivated in NPK medium and in NPK+macrophytes (*Sipaúba-Tavares et al., 2017*).

The NPK medium confirmed the shorter doubling time, which resulted in higher growth rate and microalgae yield under the specific temperature and pH conditions used in the present study. This finding can be related to the phosphorus-nitrogen-potassium combination, which always produces higher microalgae density (*Sipaúba-Tavares et al., 2017*). Similar result was observed by Ammar (*Ammar, 2016*) for cultivar *Chlorella vulgaris* grown in NPK medium, whose doubling time was of approximately 0.52 days. Microalgae yield can increase when nitrogen and phosphorus are efficiently used for microalgal growth (*Hamouda & Abou-El-Souod, 2018*). Previous studies have shown that the use of NPK as single source of nutrients (nitrogen, phosphorus, and potassium) can be beneficial in comparison to the growth and biomass yield of microalgae belonging to genus *Scenedesmus* (*Xin et al., 2010*). The application of fertilizers, such as NPK, is a cost-effective and attractive replacement adopted to meet microalgae nutritional requirements in different cultivation processes (*Nayak, Thirunavoukkarasu & Mohanty, 2016*).

All growth curves in the present case fit the estimated logistic model ($0.65 \le r^2 \le 0.99$, $p < 0.05$). Curves presented very short, or no lag phase, which is marked by the adaptation of cells in a pre-existing culture (N:P:K inoculum) to new environmental conditions—biomass is almost imperceptible and it can take hours or days to happen (*Baumgartner et al., 2013*; *Lourenço, 2006*). In addition, the lag phase at this growth stage, can be delayed due to the presence of non-viable cells or to physiological adjustments to the new environmental conditions (*Lee, Jalalizadeh & Zhang, 2015*). This finding highlight that the *S. obliquus* inoculate used in the current research presented excellent adaptation to the NPK medium and was well conditioned to the assessed environmental control variables. This very same effect was observed for *S. obliquus* cultivated in wastewater medium, which showed good adaptability to the different tested and developed cultivation conditions (*Hodaifa et al., 2010*).

Overall, microalgal growth presented *lag* phase (exponential) between days 2 and 7. Day 7 showed the maximum cell density recorded in the exponential growth phase. Inoculate ($2.1 \times 10^5$ cells mL$^{-1}$) of *S. obliquus* cultivated in Bold's Basal medium (BBM) medium got richer due to elements characteristic of Zn, Mn, Co and Mo. Inoculate cultivated in artificial medium (Provasolli, Schlosser and Zarrouk) showed the highest maximum growth at 10th and 15th cultivation

days, respectively (*Rinanti et al., 2013*; *Toyub et al., 2008*). The inoculum of *A. gracilis* ($2 \times 10^5$ cel m$^{-1}$) cultivated in NPK medium reached the maximum exponential density at the 11th cultivation day (*Sipaúba-Tavares et al., 2017*). *Scenedesmus acuminatus* cultivated in medium enriched with microelements presented exponential growth phase between the 3rd and 14thcultivation day (*Baumgartner et al., 2013*). *Scendesmus dimorphus* and *S. quadricauda* cultures took 16 days to reach the stationary phase. In addition to factors such as pH and crop temperature, the exponential phase duration mainly depends on the availability of essential nutrients (macroelements) and light (*Lourenço, 2006*).

*S. obliquus* growth and yield kinetics seemed to be strongly affected by both temperature and the medium dilution applied to the culture (N:P:K). Higher cell density was observed at the end of the exponential phase and at beginning of the stationary phase, within a short period-of-time (6 and 7 days) during experiments conditioned in 20 mL/L NPK, added with 1.12 g/L N, 0.32 g/L $P_2O_5$ and 0.44 g/L $K_2O$. Lower NPK concentrations (20:20:20) enriched with micronutrient and vitamin B triggered the stationary phase at the 5th day of the *C. vulgaris* culture, whereas crops subjected to 0.06 and 0.08 g/L NPK started their stationary phase at the 6th and 7th cultivation days (*Ammar, 2016*). *Scenedesmus* sp. recorded satisfactory growth at NPK concentration of 10:26:26 added with 0.17 g/L N, 0.32 g/L P and 0.32 g/L K. This process accelerated the specific growth rate; however, it only reached the stationary phase at the 18th cultivation day (*Nayak, Thirunavoukkarasu & Mohanty, 2016*), *i.e.*, the exponential phase was late in comparison to that observed in the present study.

Temperature exerted strong influence on the *S. obliquus* culture grown in NPK medium. Experiments cultivated at < 24 °C showed higher mean growth (0.35 day$^{-1}$) and yield rates (9.98 cells mL$^{-1}$ $\times$ 10$^5$ d$^{-1}$). *Hodaifa et al. (2010)* found that *S. obliquus* grown in mineral medium added with 0.14 g/L N and 0.16 g/L P presented better growth rate at 29 °C, *i.e.*, 4 degrees above the maximum temperature observed for *S. obliquus* in the present study. Higher specific growth rate (1.12 day$^{-1}$) was observed for *S. obliquus* cultivated at 30 °C in phosphorus-enriched medium; however, the highest biomass yield was recorded at 20 °C (*Martínez, Jiménez & El Yousfi, 1999*). *Breuer et al. (2013)* observed higher initial yield and final biomass concentration at 27 °C in *S. obliquus* culture. However, this same microalgae species grown in modified BG11 medium added with 0.15 gL$^{-1}$ NaNO$_3$, 0.10 gL$^{-1}$ K$_2$HPO$_4$·3H$_2$O and 0.05 gL$^{-1}$ Na$_2$SiO$_3$·9H$_2$O showed no significant differences in growth parameters at temperature ranging from 14 °C to 20 °C and 30 °C (*Xu et al., 2012*). The optimum temperature-range for *S. obliquus* growth is relatively wide, because its growth rates tend to do not significantly change between thermal amplitudes ranging from 10 °C to 30 °C (*Mata et al., 2013*; *Xin, Hong-ying & Yu-ping, 2011*). A significant fraction of microalgae, including *S. obliquus*, are mesophilic organisms and they can develop at temperatures ranging from 15 °C to 40 °C (*Martínez, Jiménez & El Yousfi, 1999*).

A synthesis of *S. obliquus* kinetic growth and yield is described in Table 4. The aim is to better compare the kinetic parameters recorded in the present research to those available in the literature. Microalgae cultivation in NPK medium at temperature <24 °C allowed plotting a growth curve presenting higher and/or similar maximum cell density, specific growth rate and maximum yield in comparison to data in the literature. Such data are also

**Table 4 Comparative synthesis between the growth parameters of *S. obliquus* assessed in the present study and results in studies available in the literature.**

| Species | Cultivation conditions | | | Growth parameters | | | | | Present research/ References |
|---|---|---|---|---|---|---|---|---|---|
| | Medium | T (°C) | pH | $N_0$ ($\times 10^4$ cel mL$^{-1}$) | $N_{max}$ ($\times 10^5$ cel mL$^{-1}$) | $\mu$ (day$^{-1}$) | $T_2$ (dia) | $P_{max}$ ($\times 10^5$ cel mL$^{-1}$ d$^{-1}$) | |
| *Scenedesmus obliquus* | NPK medium | 24.0 | 9.0 | 11.7 | 101.5 | 0.52 | 1.40 | 17.9 | ←**Present research** |
| *Scenedesmus obliquus* | NPK medium | 24.0 | 5.0 | 11.7 | 65.5 | 0.42 | 1.60 | 10.7 | |
| *Ankistrodesmus gracilis* | NPK medium | 22.0 | | 44.0 | 16.2 | 0.22 | 4.47 | | *Sipaúba-Tavares et al. (2017)* |
| *Haematococcus pluvialis* | NPK medium | 23 | | 2.6 | 1.4 | 0.17 | | | *Sipaúba-Tavares et al. (2017)* |
| *Scenedesmus bijugatus* | Soil extract | | | | | | | | *Arumugam et al. (2013)* |
| *Scenedesmus dimorphus* | BG11 medium | 25 | 7.5 | 2,250 | | 0.14 | 4.91 | 0.099 | *Goswami & Kalita (2011)* |
| *Scenedesmus dimorphus* | BG11 medium | 24 | | 10–12 | | 0.14 | 4.68 | | *Gour et al. (2016)* |
| *Scenedesmus obliquus* | BG11 medium | 23 | | 500 | | 0.56 | | | *Barbera et al. (2016)* |
| *Scenedesmus obliquus* | Vitamin B enriched medium | 27.5 | 7.0 | | | | | | *Breuer et al. (2012)* |
| *Scenedesmus obliquus* | Bold's basal | 25 | 6.8 | | | 0.48 | | | *Chalifour & Juneau (2011)* |
| *Scenedesmus obliquus* | Bold's basal | 22.5 | | 100 | 250.0 | 0.26 | | | *Girard et al. (2014)* |
| *Scenedesmus obliquus* | Zarrouk medium | 25 | | | 3.1 | | | | *Rinanti et al. (2013)* |
| *Scenedesmus quadricauda* | BG11 medium | 25 | 7.5 | 3,500 | | 0.35 | 1.93 | 0.077 | *Goswami & Kalita (2011)* |
| *Scenedesmus quadricauda* | BG11 medium | 24 | | 10–12 | | 0.12 | 5.41 | | *Gour et al. (2016)* |
| *Scenedesmus sp.* | BG11 medium | 25 | 8.0 | | | 0.36 | | | *Bakuei et al. (2015)* |
| *Scenedesmus sp.* | BG11 medium | 25 | 9.0 | 50–200 | 130.0 | 0.20 | 3.00 | | *Difusa et al. (2015)* |
| *Scenedesmus sp.* | NPK medium | 25 | 7.0–7.5 | | | 0.24 | | | *Nayak, Thirunavoukkarasu & Mohanty (2016)* |
| *Scenedesmus sp.* | BG11 medium | 25 | | 65 | | 0.38 | | | *Xin et al. (2010)* |

**Notes.**

$N_0$, Initial cell density; $N_{max}$, Maximum cell density; $\mu$, daily growth rate; $T_2$, doubling time; $P_{max}$, maximum yield.

compared to those in studies that used initial inoculums higher than that in the present study (*Difusa et al., 2015*; *Girard et al., 2014*; *Goswami & Kalita, 2011*; *Sipaúba-Tavares et al., 2017*; *Xin, Hong-ying & Yu-ping, 2011*). Medium pH did not have strong influence on the kinetic growth and yield of *S. obliquus* grown in inorganic fertilizer (NPK). This finding followed the wide range of pH values used in crops of different microalgae species, mainly in those of species belonging to genus *Scenedesmus*, as reported in the literature.

Data in previous studies (Table 4) corroborated the similarity between *S. obliquus* kinetic and yield parameters in the present study. However, *S. obliquus* specific growth and yield rates recorded in the present study were higher than the averages reported in previous studies: $0.28 \pm 0.14$ d$^{-1}$ and $0.09 \pm 0.01 \times 10^5$ cells mL$^{-1}$. d$^{-1}$, respectively. *S. obliquus* cell doubling time ($T_2$) was shorter and more effective, since it recorded mean $T_2$ of 2.7 days, which was 30% shorter than the mean doubling time observed in studies available in the literature ($4.07 \pm 1.31$ days).

## CONCLUSIONS

The kinetic study applied to microalgae belonging to species *S. obliquus* has revealed predictable and estimable growth behavior based on simple logistic models. This finding

made the development of industrial-scale cultivation easier, as well as the use of these microalgae in bioprospective products. The pH ranges from 8.5 to 9.0 had stronger influence on biomass yield (Pmax). Yet, wide temperature ranges from 21 to 28 °C affected biomass yield, and lower temperatures (<24 °C) often resulted in higher mean growth and yield rates. In addition, higher NPK concentrations often led to higher biomass yield, specific growth rate and maximum cell density. This evidence becomes even more compelling if microalgal biomass is used as raw material for different biotechnological applications, mainly in aquaculture, renewable biomass energy, wastewater treatment, and in the pharmaceutical and cosmetic industries. Finally, pilot biotechnological studies conducted with *S. obliquus* deserve closer attention, since they shine light over this species' relevant potential for valuing and bioprospecting other Amazonian species.

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The authors declare there are no competing interests.

### Author Contributions

- Arialdo M. Silveira Júnior conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Silvia Maria Mathes Faustino conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

- Fabrício Holanda e Holanda analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Irlon Maciel Ferreira conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Alan Cavalcanti da Cunha conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

## Data Availability

The following information was supplied regarding data availability:

Raw data are available as a Supplementary Files.

## Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.17879#supplemental-information.

## REFERENCES

**Adams C, Godfrey V, Wahlen B, Seefeldt L, Bugbee B. 2013.** Understanding precision nitrogen stress to optimize the growth and lipid content trade-off in oleaginous green microalgae. *Bioresource Technology* **131**:188–194 DOI 10.1016/j.biortech.2012.12.143.

**Álvarez-Díaz PD, Ruiz J, Arbib Z, Barragán J, Garrido-Pérez MC, Perales JA. 2015.** Wastewater treatment and biodiesel production by Scenedesmus obliquus in a two-stage cultivation process. *Bioresource Technology* **181**:90–96 DOI 10.1016/j.biortech.2015.01.018.

**Ammar SH. 2016.** Cultivation of microalgae Chlorella vulgaris in Airlift photobioreactor for biomass production using commercial NPK nutrients. *Al-Khwarizmi Engineering Journal* **12**:90–99.

**Arumugam M, Agarwal A, Arya MC, Ahmed Z. 2013.** Influence of nitrogen sources on biomass productivity of microalgae Scenedesmus bijugatus. *Bioresource Technology* **131**:246–249 DOI 10.1016/j.biortech.2012.12.159.

**Bakuei N, Amini G, Najafpour GD, Jahanshahi M, Mohammadi M. 2015.** Optimal cultivation of Scenedesmus sp. microalgae in a bubble column photobioreactor. *Indian Journal of Chemical Technology* **22**:20–25.

**Barbera E, Sforza E, Kumar S, Morosinotto T, Bertucco A. 2016.** Cultivation of Scenedesmus obliquus in liquid hydrolysate from flash hydrolysis for nutrient recycling. *Bioresource Technology* **207**:59–66 DOI 10.1016/j.biortech.2016.01.103.

**Barros ADS, Ferreira ACS, Bastos GLL, Faustino SMM, Cunha AC, Silveira Júnior AM. 2021.** Cyanobacteria in the aquatic ecosystem of the environmental protection area (EPA) of Curiaú River, Amapá State, Amazon Region, Brazil. *Nature and Conservation* **14(2)**:12–21.

**Baumgartner TRS, Burak JAM, Kogikoski ME, Sebastien NY, Arroyo PA. 2013.** Avaliação da produtividade da microalga Scenedesmus acuminatus (Lagerheim) Chodat em diferentes meios de cultivo. *Brazilian Journal of Biological Sciences* **11**:250–255.

**Bohnenberger JE, Crosseti LO. 2014.** Influence of temperature and nutrient content on lipid production in freshwater microalgae cultures. *Anais Da Academia Brasileira de Ciencias* **86**:1239–1248 DOI 10.1590/0001-3765201420130136.

**Breuer G, Lamers PP, Martens DE, Draaisma RB, Wijffels RH. 2012.** The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains. *Bioresource Technology* **124**:217–226 DOI 10.1016/j.biortech.2012.08.003.

**Breuer G, Lamers PP, Martens DE, Draaisma RB, Wijffels RH. 2013.** Effect of light intensity, pH, and temperature on triacylglycerol (TAG) accumulation induced by nitrogen starvation in Scenedesmus obliquus. *Bioresource Technology* **143**:1–9 DOI 10.1016/j.biortech.2013.05.105.

**Bruno LB, Udhaya R, Sandhya S. 2013.** Biomass and lipid productivity by four fresh water microalgae in photoreactor. *Journal of Modern Biotechnology* **2**:82–88.

**Chalifour A, Juneau P. 2011.** Temperature-dependent sensitivity of growth and photosynthesis of Scenedesmus obliquus, Navicula pelliculosa and two strains of Microcystis aeruginosa to the herbicide atrazine. *Aquatic Toxicology* **103**:9–17 DOI 10.1016/j.aquatox.2011.01.016.

**Difusa A, Talukdar J, Kalita MC, Mohanty K, Goud VV. 2015.** Effect of light intensity and pH condition on the growth, biomass and lipid content of microalgae Scenedesmus species. *Biofuels* **6**:37–44 DOI 10.1080/17597269.2015.1045274.

**Girard J-M, Roy M-L, Ben HM, Gagnon J, Faucheux N, Heitz M, Tremblay R, Deschênes J-S. 2014.** Mixotrophic cultivation of green microalgae Scenedesmus obliquus on cheese whey permeate for biodiesel production. *Algal Research* **5**:241–248 DOI 10.1016/j.algal.2014.03.002.

**Gorrini FA, Zamudio Lara JM, Biagiola SI, Figueroa JL, Hernández Escoto H, Hantson A-L, Wouwer AV. 2020.** Experimental study of substrate limitation and light acclimation in cultures of the microalgae Scenedesmus obliquus—parameter identification and model predictive control. *Processes* **8**:2–23 DOI 10.3390/pr8121551.

**Goswami RCD, Kalita MC. 2011.** Scenedesmus dimorphus and Scenedesmus quadricauda: two potent indigenous microalgae strains for biomass production and CO2 mitigation—a study on their growth behavior and lipid productivity under different concentration of urea as nitrogen source. *Journal of Algal Biomass Utilization* **2**:42–49.

**Gour RS, Chawla A, Singh H, Chauhan RS, Kant A. 2016.** Characterization and screening of native Scenedesmus sp. isolates suitable for biofuel feedstock. *PLOS ONE* **11**:1–16 DOI 10.1371/journal.pone.015532.

**Hamouda RAE, Abou-El-Souod GW. 2018.** Influence of various concentrations of phosphorus on the antibacterial, antioxidant and bioactive components of green microalgae scenedesmus obliquus. *International Journal of Pharmacology* **14**:99–107 DOI 10.3923/ijp.2018.99.107.

**Hempel N, Petrick I, Behrendt F. 2012.** Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production. *Journal of Applied Phycology* **24**:1407–1418 DOI 10.1007/s10811-012-9795-3.

**Hodaifa G, Martínez ME, Órpez R, Sánchez S. 2010.** Influence of hydrodynamic stress in the growth of Scenedesmus obliquus using a culture medium based on olive-mill wastewater. *Chemical Engineering and Processing: Process Intensification* **49**:1161–1168 DOI 10.1016/j.cep.2010.08.017.

**Huerlimann R, De Nys R, Heimann K. 2010.** Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. *Biotechnology and Bioengineering* **107**:245–257 DOI 10.1002/bit.22809.

**Kaewkannetra P, Enmak P, Chiu T. 2012.** The effect of CO2 and salinity on the cultivation of Scenedesmus obliquus for biodiesel production. *Biotechnology and Bioprocess Engineering* **17**:591–597 DOI 10.1007/s12257-011-0533-5.

**Krzemińska I, Pawlik-Skowrońska B, Trzcińska M, Tys J. 2014.** Influence of photoperiods on the growth rate and biomass productivity of green microalgae. *Bioprocess and Biosystems Engineering* **37**:735–741 DOI 10.1007/s00449-013-1044-x.

**Lee E, Jalalizadeh M, Zhang Q. 2015.** Growth kinetic models for microalgae cultivation: a review. *Algal Research* **12**:497–512 DOI 10.1016/j.algal.2015.10.004.

**Li M, Gao L, Lin L. 2015.** Specific growth rate, colonial morphology and extracellular polysaccharides (EPS) content of Scenedesmus obliquus grown under different levels of light limitation. *International Journal of Limnology* **51**:329–334 DOI 10.1051/limn/2015033.

**Lourenço SO. 2006.** Cultivo de microalgas marinhas: princípios e aplicações. Rima Editora, São Carlos.

**Mallick N, Bagchi SK, Koley S, Singh AK. 2016.** Progress and challenges in microalgal biodiesel production. *Frontiers in Microbiology* **7**:1–11 DOI 10.3389/fmicb.2016.01019.

**Martínez ME, Jiménez JM, El Yousfi F. 1999.** Influence of phosphorus concentration and temperature on growth and phosphorus uptake by the microalga Scenedesmus obliquus. *Bioresource Technology* **67**:233–240 DOI 10.1016/S0960-8524(98)00120-5.

**Mata TM, Melo AC, Meireles S, Mendes AM, Martins AA, Caetano NS. 2013.** Potential of microalgae Scenedesmus obliquus grown in brewery wastewater for biodiesel production. *Chemical Engineering Transactions* **32**:901–906 DOI 10.3303/CET1332151.

**Mazumdar N, Novis PM, Visnovsky G, Gostomski PA. 2018.** Effect of nutrients on the growth of a new alpine strain of Haematococcus (Chlorophyceae) from New Zealand. *Phycological Research* DOI 10.1111/pre.12344.

**Mohammadi FS, Arabian D, Khalilzadeh R. 2016.** Investigation of effective parameters on biomass and lipid productivity of Chlorella vulgaris. *Period. Biol* **118**:123–129 DOI 10.18054/pb.2016.118.2.3197.

**Moreno-Garcia L, Adjallé K, Barnabé S, Raghavan GSV. 2017.** Microalgae biomass production for a biorefinery system: recent advances and the way towards sustainability. *Renewable and Sustainable Energy Reviews* **76**:493–506 DOI 10.1016/j.rser.2017.03.024.

**Nayak M, Thirunavoukkarasu M, Mohanty RC. 2016.** Cultivation of freshwater microalga Scenedesmus sp. using a low-cost inorganic fertilizer for enhanced biomass and lipid yield. *Journal of General and Applied Microbiology* **62**:7–13 DOI 10.2323/jgam.62.7.

**Novoveská L, Zapata AKM, Zabolotney JB, Atwood MC, Sundstrom ER. 2016.** Optimizing microalgae cultivation and wastewater treatment in large-scale offshore photobioreactors. *Algal Research* **18**:86–94 DOI 10.1016/j.algal.2016.05.033.

**Ota M, Takenaka M, Sato Y, Smith Jr RL, Inomata H. 2015.** Effects of light intensity and temperature on photoautotrophic growth of a green microalga, Chlorococcum Littorale. *Biotechnology Reports* **7**:24–29 DOI 10.1016/j.btre.2015.05.001.

**Posten C. 2009.** Design principles of photo-bioreactors for cultivation of microalgae. *Engineering in Life Sciences* **9**:165–177 DOI 10.1002/elsc.200900003.

**R Core Team.** R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. *Available at* https://www.R-project.org/.

**Rinanti A, Kardena E, Astuti DI, Dewi K. 2013.** Growth response and chlorophyll content of Scenedesmus obliquus cultivated in different artificial media. *Asian Journal of Environment & Ecology* **1**:1–9 DOI 10.13140/RG.2.1.3370.7926.

**Santos MO, Martins MA, Coimbra JSR, Gates RS, Corrêdo LP. 2013.** Rheological behavior of Chlorella sp. e Scenedesmus sp. cultures in different biomass concentrations. *Engenharia Agrícola* **33**:1063–1071 DOI 10.1590/S0100-69162013000500017.

**Schmidell W, Lima AU, Aquarone E, Borzani W. 2001.** *Biotecnologia Industrial*. 2nd edition. Edgard Blücher LTDA, São Paulo.

**Silveira Júnior AM, Faustino SMM, Cunha AC. 2019.** Bioprospection of biocompounds and dietary supplements of microalgae with immunostimulating activity: a comprehensive review. *PeerJ* **7**:e7685 DOI 10.7717/peerj.7685.

**Sipaúba-Tavares LH, Segali AMDL, Berchielli-Morais FA, Scardoeli-Truzzi B. 2017.** Desenvolvimento de meios de cultura de baixo custo para Ankistrodesmus gracilis à base de fertilizante inorgânico e macrófita. *Acta Limnologica Brasiliensia* **29**:1–9 DOI 10.1590/S2179-975X3916.

**Surendhiran D, Vijay M, Sivaprakash B, Sirajunnisa A. 2015.** Kinetic modeling of microalgal growth and lipid synthesis for biodiesel production. *3 Biotech* **5**:663–669 DOI 10.1007/s13205-014-0264-3.

**Tan KWM, Lee YK. 2016.** The dilemma for lipid productivity in green microalgae: importance of substrate provision in improving oil yield without sacrificing growth. *Biotechnology for Biofuels* **255**:3–14 DOI 10.1186/s13068-016-0671-2.

**Toyub M, Miah M, Habib M, Rahman M. 2008.** Growth performance and nutritional value of Scenedesmus obliquus cultured in different concentrations of sweetmeat factory waste media. *Bangladesh Journal of Animal Science* **37**:86–93 DOI 10.3329/bjas.v37i1.9874.

**Valverde F, Romero-Campero FJ, León R, Guerrero MG, Serrano A. 2016.** New challenges in microalgae biotechnology. *European Journal of Protistology* **55**:95–101 DOI 10.1016/j.ejop.2016.03.002.

**Wang X, Sheng L, Yang X. 2017.** Pyrolysis characteristics and pathways of protein, lipid and carbohydrate isolated from microalgae Nannochloropsis sp. *Bioresource Technology* **229**:119–125 DOI 10.1016/j.biortech.2017.01.018.

**Weisberg S. 2005.** *Applied linear regression.* John Wiley & Sons, Vervuert Verlagsge- sellschaft DOI 10.31819/9783865278043-002.

**Wen X, Du K, Wang Z, Peng X, Luo L, Tao H, Xu Y, Zhang D, Geng Y, Li Y. 2016.** Effective cultivation of microalgae for biofuel production: a pilot-scale evaluation of a novel oleaginous microalga Graesiella sp. WBG-1. *Biotechnology for Biofuels* **9**:123 DOI 10.1186/s13068-016-0541-y.

**Xin L, Hong-ying H, Ke G, Ying-xue S. 2010.** Effects of different nitrogen and phos- phorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga Scenedesmus sp. *Bioresource Technology* **101**:5494–5500 DOI 10.1016/j.biortech.2010.02.016.

**Xin L, Hong-ying H, Yu-ping Z. 2011.** Growth and lipid accumulation properties of a freshwater microalga Scenedesmus sp. under different cultivation temperature. *Bioresource Technology* **102**:3098–3102 DOI 10.1016/j.biortech.2010.10.055.

**Xu K, Jiang H, Juneau P, Qiu B. 2012.** Comparative studies on the photosynthetic re- sponses of three freshwater phytoplankton species to temperature and light regimes. *Journal of Applied Phycology* **24**:1113–1122 DOI 10.1007/s10811-011-9741-9.

**Yang J, Rasa E, Tantayotai P, Scow KM, Yuan H, Hristova KR. 2011.** Mathematical model of Chlorella minutissima UTEX2341 growth and lipid production under photoheterotrophic fermentation conditions. *Bioresource Technology* **102**:3077–3082 DOI 10.1016/j.biortech.2010.10.049.

**Zaharieva MM, Zheleva-Dimitrova D, Rusinova-Videva S, Ilieva Y, Brachkova A, Balabanova V, Gevrenova R, Kim TC, Kaleva M, Georgieva A, Mileva M, Yoncheva K, Benbassat N, Najdenski H, Kroumov AD. 2022.** Antimicrobial and antioxidant potential of Scenedesmus obliquus microalgae in the context of integral biorefinery concept. *Molecules* **27**:2–25 DOI 10.3390/molecules27020519.