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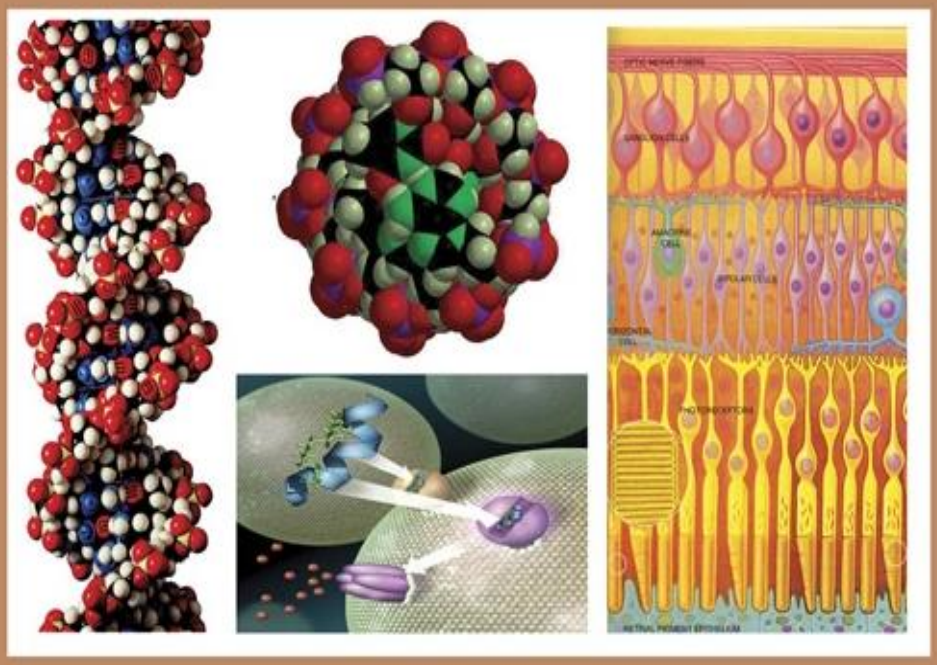

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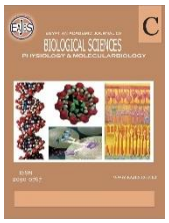

# Prediction of Microalgae Growth Kinetics In Semi-Continuous Culture From Batch Culture Experiments

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**ABSTRACT**

Microalgae have recently attracted considerable interest worldwide, due to their extensive application potential in the renewable energy, biopharmaceutical, and nutraceutical industries. For microalgal production, the choice of the photobioreactor, the method of cultivation used, and the harvesting regime adopted (batch, semi-continuous or continuous cultures) are very important. In this work, we examined the growth rate and productivity of a small volume experimental culture in batch and semi-continuous mode. Several microalgae species have been investigated for their potential as value-added products, thus we have chosen two species: green microalga (*Nannochloropsis gaditana*) and a cyanobacterium (*Arthrospira platensis*). This study develops a simple model by which biomass values in semi-continuous operation can be predicted from kinetic growth parameters obtained from a shorter batch experiment. Based on results, biomass concentrations and volumetric productivity in semi-continuous operation were successfully predicted.

## INTRODUCTION

The microalgae play an important role in the worldwide biofuel demand, together with the production of high-value-added products used in the pharmaceutical, nutraceutical and cosmetic industries (Obando, 2015; Khan *et al.*, 2018).

The chemical composition of microalgae can vary considerably depending on the measurement methods used (Barbarino and Lourenço, 2005), their physiological state (Grobbelaar, 2014), the age of the culture (Paes *et al.*, 2016), and the experimental conditions applied, such as temperature (Durmaz *et al.*, 2009), light intensity (Lourenço *et al.*, 2008), culture medium (Huerlimann *et al.*, 2010) and culture method (Benvenuti *et al.*, 2016). Indeed, when producing the metabolites necessary for the manufacture of biofuel, an important aspect must be evaluated, namely; the harvesting regime adopted (batch, semi-continuous and continuous cultures) (Benvenuti *et al.*, 2016).

*Nannochloropsis gaditana* is a photosynthetic unicellular microalgae considered one of the most interesting marine algae to produce biofuels and food additives due to its rapid growth rate and high lipid accumulation (Cecchin *et al.,* 2020). The cyanobacterium *Arthrospira platensis* is attracting more attention in basic biotechnology research because of its economic, ecological and nutritional importance (Eriksen, 2008). For these reasons, these two strains were selected; they were grown in two successive steps, first in batch and then in semi-continuous reactor.

In the present study, a simple mathematical model has been developed whereby a scenario of semi-continuous growth of the selected microalgae can be predicted from shorter batch experiments.

## MATERIALS AND METHODS

### Nomenclatures

P volumetric productivity ($M.L^{-3}.T^{-1}$)
Q flow rate ($L^3T^{-1}$)
t time (T)
T time (T)
$T_d$ doubling time (T)
$V_e$ volume taken from reactor / volume of medium added in reactor ($L^{-3}$)
$V_R$ reactor volume ($L^{-3}$)
X momentary concentration of microorganisms ($M.L^{-3}$)
$X_0$ initial cell concentration ($M.L^{-3}$)
$X_f$ final biomass concentration achieve in semi-continuous reactor ($M.L^{-3}$)
$X_i$ initial biomass concentration in semi-continuous reactor ($M.L^{-3}$)
$X_m$ maximum concentration that the system can achieve in batch ($M.L^{-3}$)
$\mu$ maximum specific growth rate ($T^{-1}$)
$\theta$ hydraulic retention time in the reactor (T)

### Microalgae and Culture Medium:

The *A. platensis* strain used comes from the crystalline massif of Hoggar (Tamanrasset, South Algeria). It has been cultivated in the spirulina medium (Robert, 2005). The *N. gaditana* strain was supplied to us by the Laboratory of Wastewater Photobioremediation (University of Cadiz, Spain). It was cultivated in the f/2 Guillard medium (Robert, 2005). The experiments were carried out in triplicate. The photobioreactor (PBR) used are column type with a useful volume of 2 liters. The cultures were carried out in a culture chamber at a controlled temperature of 20 ± 1°C.

### Reactors set-up and Experiment:

The culture was mixed and aerated using an air pump. The air was injected from the bottom of the reactor through a sterile filter (cellulose ester: 0.45 µm). The air exits from the top of the reactor through a sterile filter (cellulose ester: 0.45 µm). Illumination was provided by eight fluorescent lamps (3 Sylvania Luxline Plus F58W lamps and 2 Philips TL-D 58W lamps) placed on one side of the PBR. The incident light intensity was of 190 ± 10 $\mu mol^{-2}s^{-1}$ (measured by the Hansatech QRT1 Quantitherm photometer) with a photoperiod of 14:10 h light/dark cycle. The initial absorbance (measured with the spectrophotometer at 680 nm) of the PBR culture was 0.15.

During batch culture, the culture medium was introduced at the beginning of the experiment. At the end of the batch culture mode, the PBRs operated in semi-continuous mode. Part of the culture was harvested (17-18% of the culture volume) and immediately reconstituted with a sterile culture medium. pH and temperature in the PBRs were controlled daily using a Multi-Parameter Hach Lange. At the end of the experiments, the biomass was harvested and the resulting paste was frozen in freeze-drying flasks for less than 24 h and then subjected directly to freeze-drying (Telstar-LyoAlfa 15 Selecta®) for 72 h (0.183 mBar, -76°C). The dry biomass obtained was crushed using a mortar to homogenize it. The samples were then labeled and stored in a dry place until analysis.

### Parameters of Growth Kinetics:

### Batch phase:

Verhulst's Kinetic Logistic Model (Verhulst, 1838) was used to model the evolution of the experimental biomass concentration in reactors. This model is a substrate-independent equation and can

accurately describe biomass growth under the different culture conditions that occur in many batch bioreactors (Gong and Lun, 1996) (Fig. 1).

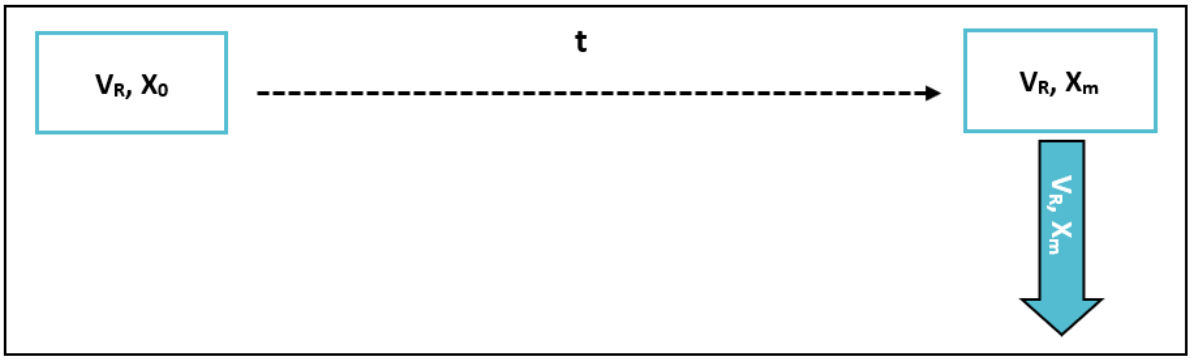


**Fig 1.** Operation of the reactor in batch mode

According to Ruiz *et al.* (2012), the following equation will allow us to predict the growth kinetics from the experimental results:

$$X = \frac{X_0 X_m e^{\mu t}}{X_m - X_0 + X_0 e^{\mu t}} \quad \text{(Eq.1)}$$

The strain doubling time was calculated by the equation proposed in the work of Madkour *et al.* (2012) :

$$T_d = \frac{Ln\ 2}{\mu} \quad \text{(Eq. 2)}$$

Volumetric productivity is an important parameter to consider in microalgae culture technology, as it shows the capacity of a reactor to produce biomass under specific operating conditions and is defined as the biomass produced per reactor volume and per unit of time. Reactor volumetric productivity was calculated as follows (Ruiz *et al.*, 2012):

$$P = \frac{\mu\,(0,9X_m - 1,1X_0)}{Ln(\frac{9(X_m - 1,1X_0}{1,1X_0})} \quad \text{(Eq. 3)}$$

**Semi-Continuous Phase:**

The kinetic growth parameters obtained in the batch phase are not only useful for comparison between different experimental conditions and between different species in batch mode but can also be used to predict growth in semi-continuous operation. Figure (2) shows the semi-continuous mode :

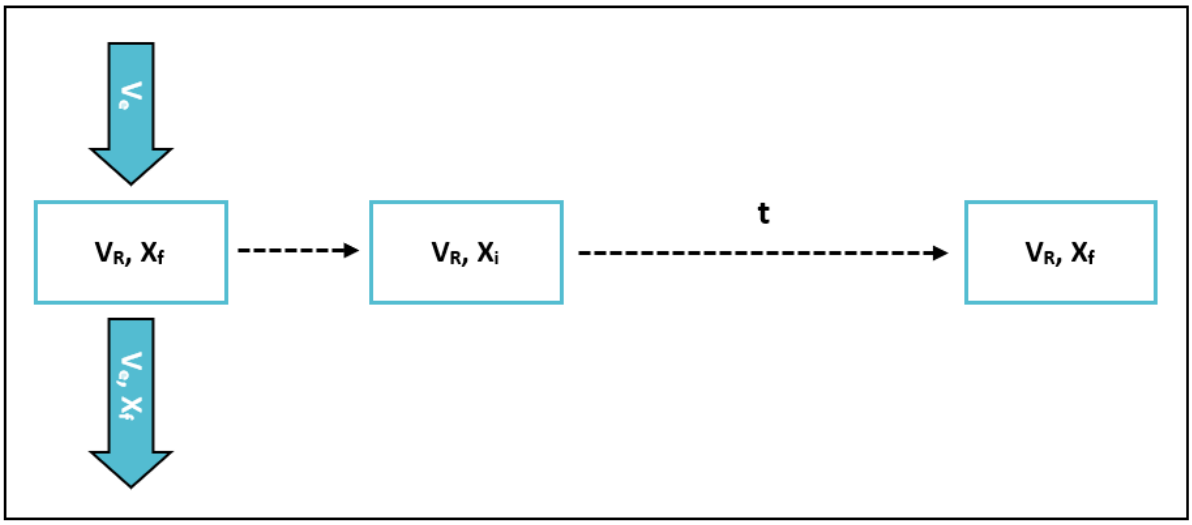


**Fig 2.** Operation of the reactor in semi-continus mode.

According to Ruiz *et al.* (2013), the determination of the sample volume (Ve) allows maximum productivity in the PBR. To calculate Ve, we must first calculate the hydraulic retention time, which is the hydraulic residence time of the culture in the photobioreactor. It was calculated as follows (Ruiz *et al.*, 2012) :

$$\boldsymbol{\theta = \frac{2}{\mu}} \qquad \text{(Eq. 4)}$$

According to Fogler (2005), the hydraulic retention time can also be calculated according to the following equation:

$$\theta = \frac{V_R}{Q} \qquad \text{(Eq. 5)}$$

The volume flow is expressed in volume per unit of time,

$$Q = \frac{V_e}{t} \qquad \text{(Eq. 6)}$$

Combining Eq. 5 and Eq. 6 we obtain the following equation which allows us to calculate the daily withdrawal volume (Ve):

$$\boldsymbol{V_e = \frac{V_R \times t}{\theta}} \qquad \text{(Eq. 7)}$$

During a semi-continuous culture, we obtain a kinetic represented by a constant initial and final daily biomass. From the parameters of the growth kinetics obtained during the batch culture, we calculated the predicted initial biomass ($X_i$) as follows:

From a mass balance in the reactor (**Fig. 2**), we have:

Initial biomass content = final biomass content + biomass removed from the reactor

Therefore:

$$V_R \cdot X_f = V_R \cdot X_i + V_e \cdot X_f \qquad \text{(Eq. 8)}$$

So:

$$X_i = X_f \cdot \left(1 - \frac{V_e}{V_R}\right) \qquad \text{(Eq. 9)}$$

As shown in Eq. 5 and Eq. 6, the hydraulic retention time can be calculated as follows (Fogler, 2005):

$$\theta = \frac{V_R}{Q} = \frac{V_R}{\left(\frac{V_e}{t}\right)} \qquad \text{(Eq. 10)}$$

So :

$$\frac{V_e}{V_R} = \frac{t}{\theta} \qquad \text{(Eq. 11)}$$

Combining Eq. 9 and Eq. 11 we obtain :

$$\boldsymbol{X_i = X_f \cdot \left(1 - \frac{t}{\theta}\right)} \qquad \text{(Eq. 12)}$$

The predicted final biomass ($X_f$) was calculated as follows:

According to Ruiz *et al.* (2013) we have:

$$X_{medium} = X_m \cdot \left(1 - \frac{1}{\theta \cdot \mu}\right) \qquad \text{(Eq. 13)}$$

And that:

$$X_{medium} = \frac{X_f + X_i}{2} \qquad \text{(Eq. 14)}$$

So :

$$\frac{X_f + X_i}{2} = X_m \cdot \left(1 - \frac{1}{\theta \cdot \mu}\right) \qquad \text{(Eq. 15)}$$

If we combine Eq. 12 and Eq. 15 we obtain the following equation:

$$\mathbf{X_f = X_m \cdot \frac{2 \cdot (\mu \cdot \theta - 1)}{\mu \cdot (2 \cdot \theta - t)}} \qquad \text{(Eq. 16)}$$

For the optimal volumetric productivity in the semi-continuous culture of the reactor, we used the equation of Ruiz *et al.* (2012):

$$P = \frac{X_f}{\theta} \qquad \text{(Eq. 17)}$$

Substituting Eq. 16 in Eq. 17:

$$\boldsymbol{P = \left(\frac{X_m}{\theta}\right) \cdot \frac{2 \cdot (\mu \cdot \theta - 1)}{\mu \cdot (2 \cdot \theta - t)}} \qquad \text{(Eq. 18)}$$

**Analysis of Biomass:**

Daily biomass concentration was measured gravimetrically as dry weight according to the standardized 2540-D method (APHA, AWWA, WEF, 1992).

**Data Analysis:**

Descriptive statistics (mean ± standard deviations) were used to describe the overall results. The statistical processing was carried out using the STATISTICA 6 software. To verify all descriptive observations, a factorial analysis of variance ANOVA was applied to the experimental data (Tukey's test; $P < 0.05$).

## RESULTS AND DISCUSSION

### Batch Phase:

We compared the growth of two species *N. gaditana* and *A. platensis* cultivated according to two modes of culture, it means batch and semi-continuous culture. A typical four-phase evolution of a batch culture was observed (Clément-Larosière, 2012; Ruiz *et al.*, 2012; Mennaa *et al.*, 2015). Growth in *N. gaditana* and *A. platensis* was characterized by a latency phase between 1 and 3 days. The stationary phase was reached after 16 days in *N. gaditana* and 18 days in *A. platensis* (Fig. 3). This phase depends on the species. It was achieved due to the absence of fundamental growth elements (nitrogen, phosphorus, light, etc.) (Clément-Larosière, 2012).

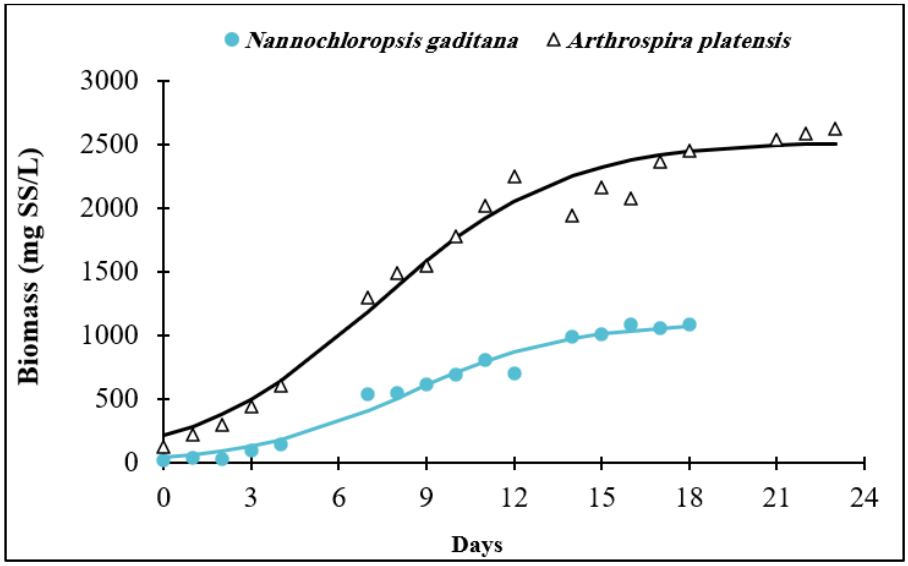


**Fig 3.** Biomass evolution of *N. gaditana* and *A. platensis* during batch operation (The continuous lines represent the values predicted by the Verhulst logistic model).

The kinetic parameters obtained from the model are listed in table 1. from our results, we can see that the maximum biomass concentration was significantly different ($p < 0.05$) between the two strains studied. Indeed, *A. platensis* reached the highest $x_m$ at the end of the batch culture 2463.46 ± 59.02 Mg SS/L, whereas in the case of *N. gaditana*, the $X_m$ obtained was 1107 ± 26.58 Mg SS/L. Statistical analysis indicates no significant difference ($P < 0.05$) between the specific growth rates obtained. These results corroborate those reported by huerlimann *et al*. (2010), Madkour *et al*. (2012), Xu and Boeing (2014) and Da Silva *et al.* (2016). However, higher specific growth rates were reported by mennaa *et al.* (2015) and praharyawan *et al.* (2016). This difference is due to the ability of the strain to acclimatize to the culture medium and/or culture conditions (Mennaa *et al.*, 2015; Devasya, 2017).

**Table 1.** Parameters of the Verhulst growth kinetics in batch reactor**.**

| Growth parameters | *N. gaditana* | *A. platensis* |
|---|---|---|
| $X_m$ (mg SS/L) | 1107 ± 26,58[a] | 2463,46 ± 59,02[b] |
| $\mu$ ($d^{-1}$) | 0,36 ± 0,03[a] | 0,34 ± 0,01[a] |
| Strain duplication time (d) | 1,92 ± 0,16[a] | 2,03 ± 0,07[a] |
| $R^2$ | 0,97 ± 0,01 | 0,97 ± 0,01 |

Values marked by different indices indicate a significant difference ($P < 0.05$) according to Tukey's test.

**Semi-Continuous Phase:**

At the end of the batch mode culture, the PBR operated in semi-continuous mode. To plot the growth kinetics, we used the following biomass concentration calibration :

Biomass of *N. gaditana* (mg SS/L) = (373.68 Abs 680 nm) + 11.996; ($R^2$ = 0.96044)

Biomass of *A. platensis* (mg SS/L) = (669.05 Abs 680 nm) + 195.9; ($R^2$ = 0.96896)

Figure 4 shows the growth kinetics of semi-continuous culture. The results of the predicted $X_i$ and $X_f$ in *N. gaditana* and *A. platensis* are also shown. We can first note that the adaptation time to the semi-continuous culture system was practically long in both microalgae. If we compare the results obtained with the estimated theoretical values, we can clearly see that the $X_i$ and $X_f$ concentrations of the last 4 cycles fall within the theoretical ranges for each species.

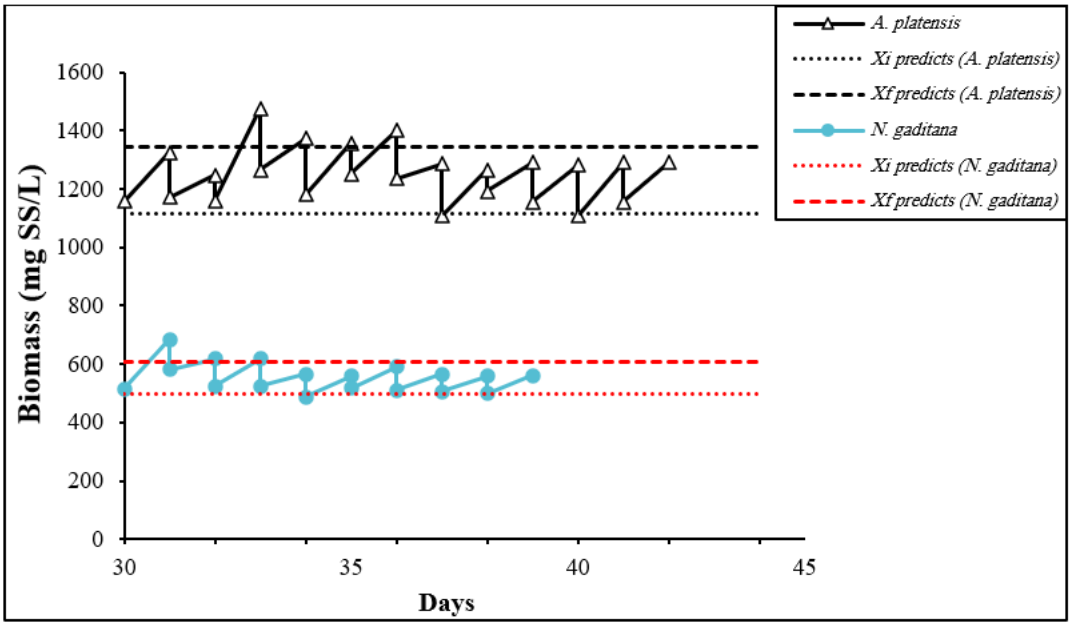


**Fig 4.** Biomass evolution of *N. gaditana* and *A. platensis* during semi-continuous operation. The point lines represent the predicted initial and final biomass concentrations.

An overview of all parameters of steady-state kinetics is shown in Table 2. We can see that the hydraulic retention time varies between 5.53 and 5.87 $d^{-1}$ in the two species studied. The results also show that the final biomass concentration ($X_f$) obtained in *A. platensis* was 1346.45 ± 30.51 mg SS/L and in *N. gaditana* this concentration was 609.09 ± 12.42 mg SS/L..

**Table 2.** Parameters of growth kinetics in semi-continuous reactor.

| Growth parameters | *N. gaditana* | *A. platensis* |
|---|---|---|
| Reactor volume ($V_R$) (ml) | 1600 | 1600 |
| Hydraulic retention time (θ) ($d^{-1}$) | 5,53 ± 0,47 | 5,87 ± 0,19 |
| Volume taken from the culm (Ve) (ml) | 290 | 270 |
| $X_i$ (mg SS/L) | 498,48 ± 15,50 $^a$ | 1117,01 ± 28,92 $^b$ |
| $X_f$ (mg SS/L) | 609,09 ± 12,42 $^a$ | 1346,45 ± 30,51 $^b$ |

Values marked by different indices indicate a significant difference ($P < 0.05$) according to Tukey's test.

**Volumetric Productivity:**

Even though the specific growth rate can vary considerably, it does not allow us to properly compare between species. For this, it is important to use another useful parameter that helps to compare microalgae species under a single value that incorporates growth kinetic parameters : volumetric productivity in the PBR (Mennaa *et al.*, 2015).

If we compare the results of volumetric productivity in *A. platensis* and *N. gaditana*, we can observe a very significant difference ($P < 0.0002$) and this was valid in both batch and semi-continuous experiments (Fig. 5). In the PBRs operating in semi-continuous mode, the biomass was constantly in an exponential growth phase, because nutrients were supplied on a daily basis, whereas in batch culture mode the microalgae depleted their nutrients after a few days (Ruiz *et al.*, 2013). The results are consistent with those obtained by McGinn *et al.* (2012) at *Scenedesmus sp.* where batch volumetric productivity was almost half of that obtained in semi-continuous culture. According to several researchers, this difference in volumetric productivity was due to the accumulation of toxic metabolite and/or depletion of certain limiting nutrients during batch growth (Yang *et al.*, 2011; Ahii Chia *et al.*, 2013). Moreover, in semi-continuous cultures, a part of the cells was eliminated daily. This decrease in cell density allows an increase in the availability of light to the cell and thus an increase in volumetric productivity (Clément-Larosière, 2012).

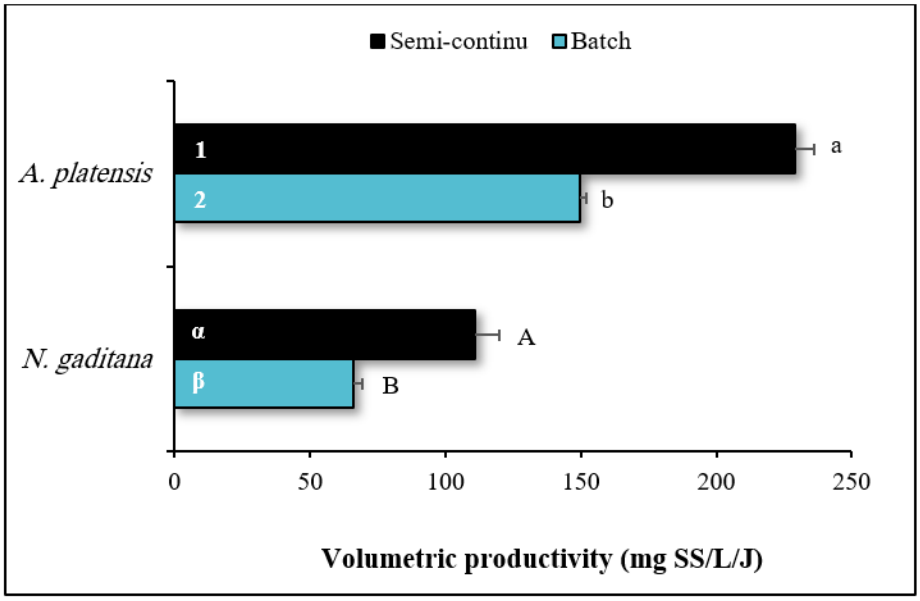


**Fig 5.** Volumetric productivity of *N. gaditana* and *A. platensis* in batch and semi-continuous reactors. Histograms marked with different indices indicate a significant difference ($P < 0.05$) according to Tukey's test.

**Conclusion**

Based on batch kinetic growth parameters, the biomass concentrations and the productivities under semi-continuous conditions can be satisfactorily predicted. Our results also allowed us to conclude that the biomass concentration and volumetric productivity in the reactor is higher in *A. platensis* than in *N. gaditana* in both batch and semi-continuous culture modes.

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