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# *Nannochloris eucaryotum* growth: Kinetic analysis and use of 100% $CO_2$

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**Abstract.** Microalgae are receiving an increasing attention because of their potential use as $CO_2$ capture method and/or as feedstock for biofuels production. On the other hand the current microalgae-based technology is still not widespread since it is characterized by technical and economic constraints that hinder its full scale-up. In such contest the growth kinetics of *Nannochloris eucaryotum* (a relatively unknown marine strain) in batch and semi-batch photobioreactors is quantitatively investigated with the aim of obtaining the corresponding kinetic parameters suitable for process engineering and its optimization. In particular the maximum growth rate was evaluated to be 1.99 $10^{-3}$ $h^{-1}$. Half saturation concentrations for nitrates ($K_N$) and phosphates uptake ($K_P$) were evaluated as 5.4 $10^{-4}$ $g_N$ $L^{-1}$ and 2.5 $10^{-5}$ $g_P$ $L^{-1}$, respectively. Yield factors for nitrogen ($Y_N$) and phosphorus ($Y_P$) resulted to be 5.9 $10^{-2}$ $g_N$ $g^{-1}_{biomass}$ and 6.0 $10^{-3}$ $g_P$ $g^{-1}_{biomass}$, respectively. The possibility of using 100% (v/v) $CO_2$ gas as carbon source is also evaluated for the first time in the literature as far as *N. eucaryotum* is concerned. The strain showed a good adaptability to high concentrations of dissolved $CO_2$ as well as to low pH. The lipid content under 100% $CO_2$ is about 16.16 %wt $wt^{-1}$ and the fatty acid methyl esters composition of the extracted oil is in compliance with the European regulation for quality biodiesel.

**Keywords:** microalgae; kinetics; Nannochloris eucaryotum; lipid content; biofuels; $CO_2$ capture

## 1. Introduction

The production of biofuels from renewable resources is well known to be highly critical to guarantee a sustainable economy and face global climate changes (Cheng *et al*. 2011). In recent years, microalgae have been recognized to be a promising alternative source for biofuel-convertible lipids (Halim *et al*. 2011). In fact, when compared to first generation biofuels, microalgae are characterized by higher growth rates and larger bio-oil productivities. In addition,

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cultivation of microalgae does not compromise arable lands (Chisti 2007) thus avoiding “food for fuel” concerns. Moreover, biological fixation of $CO_2$ can be carried out much more effectively by using autotrophic microalgae rather than terrestrial crops (Chisti 2007, Usui and Ikenouci 1997, Borowitza 1999). For these reasons, the potential use of microalgae as renewable feedstock for the massive production of liquid biofuels is receiving a rising interest mostly driven by the global concerns related to the depletion of fossil fuels supplies and the increase of $CO_2$ levels in the atmosphere (Olgun 2003, Mulbry *et al*. 2008). The high potential of algae based biofuels is confirmed by the number of recent papers available in the literature (Francisco *et al*. 2010, Huang *et al*. 2010, Li *et al*. 2011, Mallik *et al*. 2011, Phukan *et al*. 2011, Sasi *et al*. 2011, Singh *et al*. 2011) on the subject, the growing investments of private companies and governments (Chen *et al*. 2011, Mata *et al*. 2010, Sheehan *et al*. 1998, Torrey 2008) as well as the increasing number of filed patents (Cao and Concas 2008, 2010).

Despite such interest, the current microalgae-based technology is still not widespread since it is characterized by technical and economic constraints that might hinder its full scale-up. In particular, the main barriers are related to the extensive land's areas needs as well as the estimated high costs of the operating phases of microalgae cultivation, harvesting and lipid extraction (Chen *et al*. 2011). Thus, one of the main targets of the scientific community is to identify, and/or create, new microalgae strains which are intrinsically characterized by high biomass productivity and lipid content as well as the capability of capturing $CO_2$ from flue gases (Cao and Concas 2008, 2010, Mazzuca 2010).

In such contest the most attractive scientific challenge is the genetic manipulation of existing strains with the aim of increasing their photosynthetic efficiency (Tetali *et al*. 2007, Mitra and Melis 2008, Melis 2009) and/or regulate their metabolism (Dorval *et al*. 2009, Radakovits *et al*. 2010) in order to achieve an abundant production of lipids coupled with high biomass accumulation. A further goal is to suitably exploit mathematical models and process engineering techniques to identify the operating conditions of photobioreactors (i.e., light supply, mass transfer, culture media, etc.) that maximize lipid productivity and $CO_2$ fixation as well as the economic viability of the technique (Concas *et al*. 2010, 2012).

In particular, one of the most impacting cost item is related to the need of a continuous replenishment of macronutrients (mainly $CO_2$, nitrogen and phosphorus) during algal cultivation (Jiang *et al*. 2011). In fact, as rule of thumb, about 1.8 kg of $CO_2$, 0.33 kg of nitrogen and 0.71 kg of phosphate are consumed to produce 1 kg of microalgal biomass (Amaro *et al*. 2011, Yang *et al*. 2011). Since large scale cultivation of microalgae implies the consumption of huge amounts of such macronutrients, the economic feasibility of the entire process could be seriously affected by the erroneous evaluation of their depletion kinetics. Therefore, in view of industrial scaling-up, the effect of nutrients concentration in the medium on biomass composition and productivity should be quantiatively evaluated. In addition, changes in nutrients concentration can result in conflicting effects on the process economics. For instance, a decrease of nitrogen concentration in the cultivation broth typically results in higher lipid contents counteracted by lower growth rates. This inverse relationship between biomass productivity and lipid content makes the process optimization in terms of lipid productivity not straightforward.

Therefore, since nutrients concentration and supplies are among the most controllable factors in microalgae cultivation, at least the main macronutrients (i.e., nitrogen and phosphorus) uptake rates need to be quantitatively evaluated for the microalgae strains candidate to industrial exploitation. This way, macronutrients concentrations might be precisely controlled during cultivation. Hence, biomass production can be optimized with respect to the required process

end-products by means of suitable growth kinetics and broth composition. Moreover, the exploitation of costless feedstocks such as seawater and flue gas as sources of micronutrients and $CO_2$, might greatly improve the economic feasibility of the microalgae-based technology while simultaneously producing a positive impact on important environmental concerns such as water and air pollution. In addition, marine strains capable to survive under elevated $CO_2$ concentration might represent suitable candidate for the industrial cultivation of microalgae for biofuels production and $CO_2$ capture. Among such strains the unicellular marine eukaryotic green alga *Nannochloris eucaryotum* (Menzel and Wild 1989), also known as *Nanochlorum eucaryotum* (Wilhelm and Wild 1982) or *Picochlorum eucaryotum* (Henley *et al*. 2004), shows high adaptability to extreme environmental conditions such as high salinity, low irradiance and elevated $CO_2$ levels (Geisert *et al*. 1987). It has been also found that the lipid content of strains belonging the same genus (i.e., Nannochloris) can be close to about 50% (Negoro *et al*. 1991). While these aspects make this microalga strain a suitable candidate for large-scale biofuel production and $CO_2$ capture, it is important to note the lack of information available in the literature about its growth kinetics and lipid content. Thus, such strain seems to be worthy of further and deeper investigations.

Along the lines of our recent work (Lutzu *et al*. 2012) on this subject, the growth kinetics of *N. eucaryotum* in batch photobioreactors is quantitatively investigated in this paper with the aim of determining useful kinetic parameters which might be used for process engineering and its optimization. In particular, the Monod's model for multiple nutrients limitation is adopted to quantitatively describe the growth of this microalga as a function of nitrogen and phosphorus concentrations. The maximum growth rate, the half saturation constants and yields coefficients for nitrate and phosphate uptake are also determined by suitably fitting the experimental data by Lutzu *et al*. (2012). The reliability of the obtained parameter values is than tested by suitably predicting new experimental data.

Finally, the possibility of using 100% (v/v) $CO_2$ gas as carbon source in a semi-batch photobioreactor is also investigated in this work with the aim of verifying the capability of *N. eucarytoum* of capturing $CO_2$ from sources characterized by high concentration values of this gas. Lipid content and fatty acid composition is also evaluated in order to assess the potential exploitability of *N. eucaryotum* as feedstock for biofuel production. It should be noted that, to the best of our knowledge, all these aspects have not been addressed in the literature as far as this microalga strain is concerned.

## 2. Materials and methods

### *2.1 Microorganism*

The marine algal strain Nannochloris eucaryotum (strain N° 55.87) obtained from the Sammlung von Algenkulturen at the University of Göttingen (SAG), Germany, was investigated in this work. Stock cultures were propagated and maintained in Erlenmeyer flasks with a Brackish Water Medium (BWM) under incubation conditions of 25°C (SAG 2008), a photon flux density of 98 $\mu$mol $m^{-2}$ $s^{-1}$ provided by four 15 W white fluorescent tubes and a light/dark photoperiod of 12 h was assured. Flasks were continuously shaken at 100 rpm (Universal Table Shaker 709).

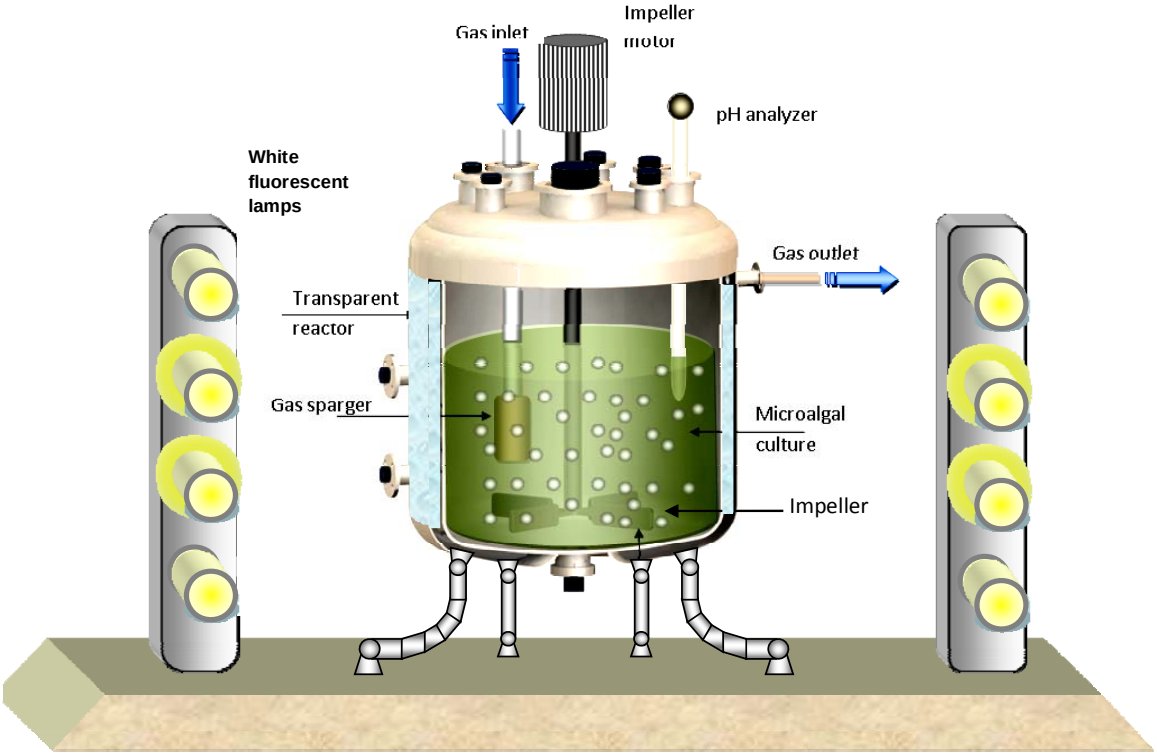


Fig. 1 Schematic representation of the semi-continuous photobioreactor used for cultivating *N. eucaryotum* under high $CO_2$ concentration levels

### *2.2 Culture conditions*

The experimental data used to fit the kinetic parameters of the Monod's equation shown in the next section were obtained in a recent work (Lutzu *et al*. 2012) from which the description of the experimental set up and procedure, also used to obtain novel data in this work, might be seen.

In addition, the possibility of exploiting 100% (v/v) $CO_2$ gas as carbon source was evaluated using the photobioreactor whose schematic representation is reported in Fig. 1. It consists of a cylindrical glass photobioreactor (9.5 cm diameter and 21 cm height) with a volumetric capacity of 1.5 L and operated in semi batch mode (i.e. batch mode for the liquid phase and continuous mode for the gas one).

The reactor was then filled with 1 L of growth medium and then mechanically stirred at 400 rpm through a rotating blade powered by an electrical engine (GZ high power overhead stirrer). Cultures were maintained at 25°C by a thermostatic bath (GD120 series) and illuminated by a photon flux density of 100 $\mu$mol $m^{-2}$ $s^{-1}$ provided by eight 11 W white fluorescent bulbes with a light/dark photoperiod of 12 h. A gas consisting of pure $CO_2$ (100% v/v) from a cylinder was continuously supplied through suitable spargers at a flow rate of 40 ml $min^{-1}$. The inlet pressure of $CO_2$ was equal to 1.6 bar.

### *2.3 Culture medium*

The culture medium to perform the experiments with pure $CO_2$ was prepared by considering the following components and proportions: natural sea water (Mediterranean sea, lat. 39° 11" N - long. 09° 10' E) centrifuged at 4000 rpm for 15 min (Thermo Fisher Scientific Inc. Waltham) and then filtered by means of 0.45 $\mu$m filter, 455 ml $L^{-1}$; de-ionized water, 450 ml $L^{-1}$; soil extract (SAG 2008) 30 ml $L^{-1}$; macronutrients aqueous solution ($KNO_3$ 10 g $L^{-1}$; $K_2HPO_4$ 1 g $L^{-1}$; $MgSO_4 \cdot 7H_2O$ 1 g $L^{-1}$), 20 ml $L^{-1}$; micronutrients aqueous solution (SAG 2008) 5 ml $L^{-1}$. The volumetric and chemical composition of the growth medium is reported in Table 1. Cultivations were then performed with different initial concentrations of nitrates ($N_0$) and phosphates ($P_0$).

### *2.4 Biomass concentration and pH measurements*

The growth of microalgae was monitored through daily measurements of the culture media optical density (OD) (Genesys 20 spectrophotometer, Thermo Fisher Scientific Inc. Waltham) at 560 nm wavelenght ($D_{560}$) with 1 cm light path. The biomass concentration ($X$) was calculated from OD measurements using an $X$ vs. OD calibration curve (Lutzu *et al*. 2012). The latter was obtained by gravimetrically evaluating the biomass concentration of known volumes of culture medium which were previously centrifuged at 4000 rpm for 15 min and dried at 105°C for 24 h. pH was daily measured by pHmeter (KNICK 913). For the sake of reproducibility, each experimental condition was investigated at least in duplicate.

### *2.5 Extraction of lipids and their content evaluation*

In order to evaluate the lipid content of *N. eucaryotum*, the microalgae were first harvested and then centrifuged to obtain a wet biomass pellet characterized by a water concentration of about 90 %wt/wt. Subsequently, the pellet was contacted for a suitable amonut of time with a solution containing Fenton's reactant in order to break the algae cell wall according to a novel procedure recently investigated which will be the subject of future works. Such pre-treatment step was aimed to increase the lipid extraction efficiency. Finally, lipids were extracted to evaluate their content by following a procedure based on that one proposed by Fajardo *et al*. (2007).

### *2.6 Fatty acids methyl esters analysis*

After lipid extraction the total amount of saponifiable lipids and fatty acid composition of extracted lipid was determined after transesterification with methanol-acetyl chloride (Lepage and Roy 1986). Gas chromatographic analysis was carried out according to EEC N° 2568/91 (EEC 1991) using a flame ionization detector (FID) (Thermo Trace Ultra, GC-14B) and a RTX-WAX column $T$ (fused silca, 0.25 mm × 60 m × 0.25 $\mu$m) maintained at 180°C. Helium was used as carrier gas at a flow rate of 1 ml $min^{-1}$.

### *2.7 Model equations and parameters evaluations*

The relevant material balance used to quantitatively evaluate the kinetic parameters related to *N. eucaryotum* in the batch photobioreactor is reported as follows

$$\frac{dX}{dt} = \mu X \tag{1}$$

where $X$ represents the cell mass [g $L^{-1}$], $t$ is the time [h], and $\mu$ the growth rate [$h^{-1}$], which is typically a function of nutrients concentration, pH, temperature, light and other culture conditions. Moreover the Monod's model for multiple nutrients limitation was adopted to simultaneously take into account the effect of nitrogen and phosphorus concentration on the growth rate of *N. eucaryotum* (Bailey and Ollis 1986)

$$\mu = \mu_0(CO_2, T, I, pH) \prod_{i=N,P} \frac{C_i}{K_i + C_i} \quad i = N, P \tag{2}$$

where $\mu_0$ can be regarded as the maximum growth rate under the temperature level $T$, the light intensity $I$, the $CO_2$ mass transport and the pH conditions of the adopted experimental set-up while $K_i$ represents the half saturation constant.

Since the photobioreactor was operated in batch mode, the mass concentration $C_i$ (g $L^{-1}$) of nitrogen and phosphorus in the medium may be related, at any cultivating time, to the biomass concentration $X$ through the following relationship

$$C_i = C_{0,i} - Y_i(X - X_0) \quad i = N, P \tag{3}$$

where $C_{0,i}$ is the initial concentration of the limiting nutrients, and $Y_i$ the yield coefficient of nitrogen and phosphorus. On the basis of Eqs. (1), (2), and (3), the values of five parameters ($\mu_0$, $K_N$, $K_P$, $Y_N$, $Y_P$) are needed to quantitatively interpret the experimental results. The strategy adopted to fit the above mentioned kinetic parameters is illustrated in what follows. By assuming $\mu$ as a constant during the phase of exponential growth, Eq. (1) can be integrated with the initial condition $X = X_0$ at $t = 0$ to give the following relationship between the microalgae mass concentration and time

$$\ln\left(\frac{X}{X_0}\right) = \mu t \tag{4}$$

Experimental data obtained in the case where the exponential growth took place without being affected by nutrient or light limitation phenomena (i.e., $\mu = \mu_0$), are then linearly fitted through Eq. (4) in order to obtain the value of $\mu_0$. While maintaining fixed the above reported value of $\mu_0$, the kinetic parameter $K_N$ and $Y_N$ were evaluated by coupling the numerical integration of Eqs. (1), (2), and (3) with a non-linear fitting of the experimental data related to the case where nitrogen limitation phenomena took place. Numerical integration was performed using standard IMSL (International Mathematics and Statistics Library) routines. Finally, by maintaining fixed the fitted values of $\mu_0$, $K_N$ and $Y_N$, the kinetic parameters $K_P$ and $Y_P$ were obtained by non-linearly fitting the experimental data obtained in case where phosphorus limitation phenomena took place. The reliability of the fitted parameters were then evaluated by successfully predicting novel experimental results obtained in this work when nitrogen and phosphorus starvation phenomena occurred both simultaneously or separately, albeit at different concentration levels with respect to the experimental data used during the fitting procedure.

## 3. Results and discussion

### *3.1 Evaluation of kinetic parameters related to the growth of N. eucaryotum*

A series of batch experiments were carried out recently (Lutzu *et al*. 2012) to evaluate the effect of the initial concentration of nitrogen ($N_{init}$) and phosphorus ($P_{init}$) on the growth of *N. eucaryotum* by varying the initial content of potassium nitrate and potassium biphosphate in the culture medium. The initial concentrations of nitrogen and phosphorus of the "base case" experiment will be hereafter indicated as $N_0$ and $P_0$, respectively (cf. Table 1).

It can be observed from Fig. 2 that, for the case where $N_{init} = N_0$ and $P_{init} = P_0$, *N. eucaryotum* grows exponentially with time up to the end of the cultivation period. Since microalgae growth causes nutrients depletion, it can be stated that in this case the growth rate does not seem to be significantly affected by the diminishing nitrates and phosphates concentrations. Thus, the experimental data obtained for the case $N_{init} = N_0$ and $P_{init} = P_0$ can be linearly fitted through Eq. (4) as it may be seen from Fig. 2, by means of a constant growth rate (i.e., $\mu = \mu_0$) equal to 0.00199 $h^{-1}$ under the selected experimental conditions.

This finding confirms that in this case the growth rate is not significantly affected by the diminishing nitrogen and phosphorus concentrations as well as by the decreasing light intensity inside the medium due to microalgae absorbance. In addition, during this experiment (Lutzu *et al*. 2012), the medium pH varied from the initial value of 7.0 to 8.8. Thus, the growth rate does not appear to depend on pH in the above mentioned range. The fitted value of $\mu$ can be then regarded as the maximum growth rate $\mu_0$ (cf. Eq. (6)) under the temperature, light intensity, $CO_2$ transfer

Table 1 Volumetric and chemical composition of the culture medium used in this work

| Component | units | ($N_0$, $P_0$) |
|---|---|---|
| Volumetric composition | | |
| De-ionized water | ml $L^{-1}$ | 450 |
| Seawater | ml $L^{-1}$ | 455 |
| Soil extract | ml $L^{-1}$ | 30 |
| Macronutrients aqueous solution | ml $L^{-1}$ | 20 |
| Micronutrients aqueous solution | ml $L^{-1}$ | 5 |
| Resulting chemical composition | | |
| $KNO_3$ | g $L^{-1}$ | $2.0 \cdot 10^{-1}$ |
| $K_2HPO_4$ | g $L^{-1}$ | $2.0 \cdot 10^{-2}$ |
| $MgSO_4 \cdot 7H_2O$ | g $L^{-1}$ | $2.0 \cdot 10^{-2}$ |
| $H_3BO_3$ | g $L^{-1}$ | $2.86 \cdot 10^{-3}$ |
| $MnCl_2 \cdot 4H_2O$ | g $L^{-1}$ | $1.81 \cdot 10^{-3}$ |
| $ZnSO_4 \cdot 7H_2O$ | g $L^{-1}$ | $2.22 \cdot 10^{-4}$ |
| $CoCl_2 \cdot 6H_2O$ | g $L^{-1}$ | $3.5 \cdot 10^{-5}$ |
| $CuSO_4 \cdot 5H_2O$ | g $L^{-1}$ | $8.0 \cdot 10^{-5}$ |
| $Na_2MoO_4 \cdot 2H_2O$ | g $L^{-1}$ | $2.3 \cdot 10^{-4}$ |
| EDTA-$Na_2$ | g $L^{-1}$ | $2.98 \cdot 10^{-2}$ |
| $FeSO_4 \cdot 7H_2O$ | g $L^{-1}$ | $2.49 \cdot 10^{-2}$ |

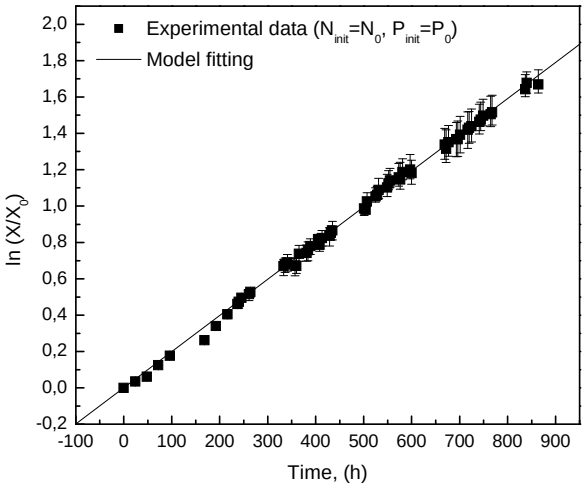


Fig. 2 Comparison between model results and experimental data (Lutzu *et al*. 2012) in terms of cells mass as a function of time to obtain the *N. eucaryotum* maximum growth rate ($\mu_0$)

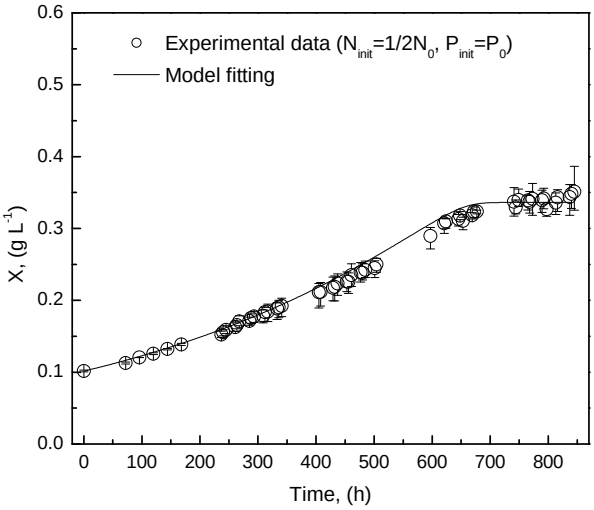


Fig. 3 Comparison between model results and experimental data (Lutzu *et al*. 2012) in terms of cells mass as a function of time to obtain the half-saturation constant ($K_N$) and yield coefficient ($Y_N$) for nitrates uptake by *N. eucaryotum*

and pH conditions available in the case where $N_{init} = N_0$ and $P_{init} = P_0$.

The effect of initial nitrogen concentration was also investigated (Lutzu *et al*. 2012) by reducing it to one half and one fourth of $N_0$, while maintaining constant the initial phosphate concentration (i.e., $P_{init} = P_0$). From the experimental data reported in Fig. 3, it clearly appeared that for $N_{init} = 1/2\ N_0$ growth curve approached a stationary phase after about 720 h thus indicating the occurrence of nitrogen starvation phenomena. Thus by maintaining fixed the above reported value of $\mu_0$, the kinetic parameters $K_N$ and $Y_N$ were evaluated with the proposed model by fitting the experimental

data. It is worth noting that, in this case $C_P$ is assumed to be much greater than $K_P$, since for $P_{init} = P_0$, phosphorus does not limit the algae growth as it may be seen from Fig. 2. Model results are compared with experimental data in Fig. 3.

The best fitting value for the half saturation constant $K_N$ was equal to 5.2 $10^{-4}$ $g_N$ $L^{-1}$ while the corresponding value of the nitrogen yield $Y_N$ was 5.9 $10^{-2}$ $g_N/g_{biomass}$.

As far as the effect of phosphorus depletion on the growth kinetic of *N. eucaryotum* is concerned, the experimental data reported in Fig. 4 clearly show that when the initial content of *P* was reduced to $1/4\ P_0$, the cells mass concentration increased only during the first 400 h of cultivation. Then a stationary phase was reached up to 700 h of cultivation. This fact indicates that for $P_{init} = 1/4\ P_0$ phosphorus becomes a limiting nutrients after a specific culture time. Hence, by assuming that $C_N$ is much greater than $K_N$ under these experimental conditions and maintaining fixed the values of $\mu_0$ already obtained, the kinetic parameters $K_P$ and $Y_P$ were obtained by non-linearly fitting the experimental data for the case when $N_{init} = N_0$ and $P_{init} = 1/4\ P_0$ in the time interval 0-700 h. Model results are compared with experimental data in Fig. 4.

In particular, the best fitting value of 2.5 $10^{-5}$ g $L^{-1}$ is obtained for the half saturation constant $K_P$ while the corresponding value of 6.0 $10^{-3}$ $g_P/g_{biomass}$ is obtained for the phosphorus yield $Y_P$.

With the aim of testing the predictive capability of the adopted growth model as well as the reliability of the fitted parameters, numerical simulation of new experimental runs where only the initial nitrogen was further reduced (i.e., $N_{init} = 1/4\ N_0$ and $P_{init} = P_0$) and only the initial phosphorus concentration was halved (i.e., $N_{init} = N_0$ and $P_{init} = 1/2\ P_0$) were performed. Fig. 5 illustrates the comparison between experimental data and model results which were obtained by maintaining fixed the corresponding parameters obtained through the fitting procedure described above.

To further test the predictive capability of the model when both the initial nitrogen and phosphorus concentrations are simultaneously reduced, new experimental data have been obtained

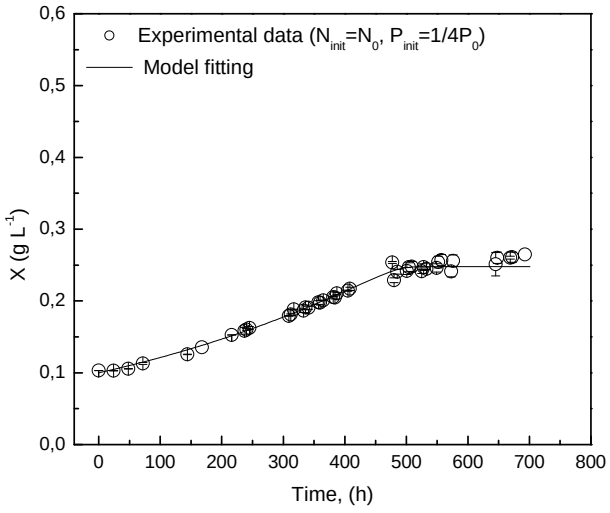


Fig. 4 Comparison between model results and experimental data (Lutzu *et al*. 2012) in terms of cells mass as a function of time to obtain the half-saturation constant ($K_P$) and yield coefficient ($Y_P$) for biphosphates uptake by *N. eucaryotum*

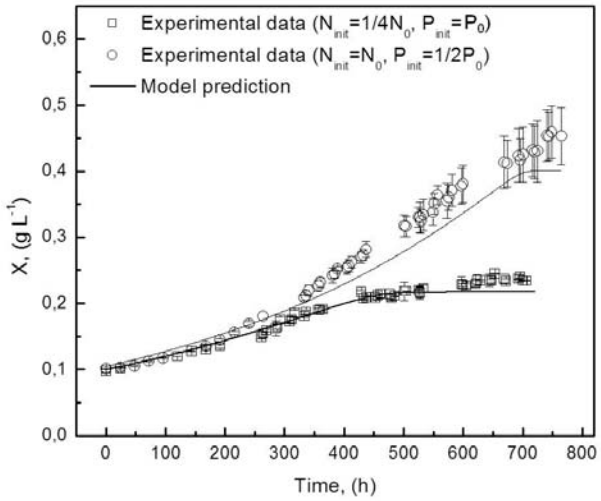


Fig. 5 Comparison between model predictions and experimental data (Lutzu *et al*. 2012) in terms of cells mass as a function of time

in this work for the case where $N_{init}$ = *1/2* $N_0$ and $P_{init}$ = *1/2* $P_0$, following the procedure described in the literature (Lutzu *et al*. 2012). Experimental results are compared with model predictions in Fig. 6, from which it can be seen that also in this case the model permits to predict the culture behavior at varying initial nitrate and phosphate concentrations in the medium with a reasonable accuracy.

It should be mentioned however, that the so called phenomenon of "diaouxic growth" that occurred after prolonged culture times (Lutzu *et al*. 2012) was not simulated in this work.

### *3.2 Effects of using of 100% (v/v) $CO_2$ on cell growth and pH evolution*

Subsequently, the effect of high $CO_2$ concentration on the growth of *N. eucaryotum* in batch photobioreactors was also investigated in this work. Specifically, specific experiments were carried out where $CO_2$ (100% v/v) was continuously bubbled at a flow rate of 40 ml $min^{-1}$ into the growth medium when $N_{init}$ = $N_0$ and $P_{init}$ = $P_0$. To this aim the semi-batch photobioreactor shown schematically in Fig. 1 was used. From Fig. 7 it can be observed that, under the above mentioned conditions, microalgae start growing with a modest lag phase, which probably indicates the intrinsic affinity of *N. eucaryotum* for high dissolved $CO_2$ concentration in the growth medium. Moreover, when comparing the experimental results of Fig. 7 with the corresponding ones (i.e., $N_{init}$ = $N_0$ and $P_{init}$ = $P_0$) obtained in our previous work (Lutzu *et al*. 2012) using $CO_2$ available in the atmosphere, an higher initial growth rate can be observed. Such behavior is due to the higher availability of dissolved $CO_2$ which results in the increase of the specific growth rate $\mu_0$ *($CO_2$, pH, I)*, thus suggesting that its dependence upon dissolved $CO_2$ concentration should be also taken into account through Monod's type kinetics. In fact $CO_2$ is the main macronutrient for triggering photosynthesis in microalgae. On the contrary, a stationary phase is attained after about 350 h of cultivation when the biomass concentration was about 0.35 g $L^{-1}$, while using $CO_2$ from the atmosphere microalgae keep growing almost exponentially up to 840 h of cultivation (Lutzu *et al*.

2012). Once the steady state was attained, the possibility to operate the photobioreactor in fed-batch mode was evaluated. In fact starting from the 16$^{th}$ day of culture, 150 mL of culture were withdrawn every 5 days and then replaced by an equal volume of fresh medium, thus imposing a dilution rate ($D$) (Novik and Szilard 1950, Fogle 2006) of about 0.0015 $h^{-1}$. As shown in Fig. 7, after each withdrawal, the biomass concentration decreases and then starts increasing as a result of nutrient availability and the diminished concentration of toxic catabolites. In particular, 4 cycles of

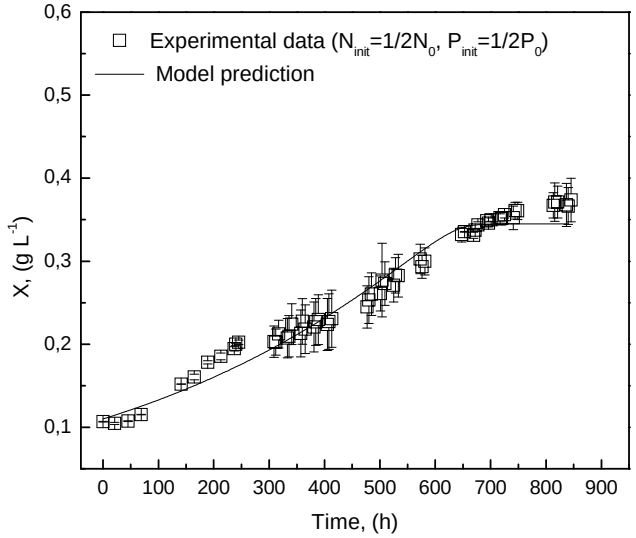


Fig. 6 Comparison between model predictions and experimental data (this work) in terms of cells mass as a function of time

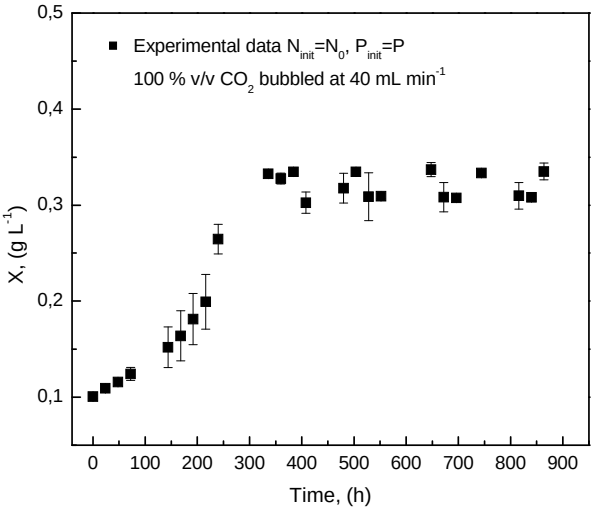


Fig. 7 Growth of *N. eucaryotum* in the batch photobioreactor depicted in Fig. 1 in terms of cells mass as a function of time. Culture conditions: 100% (v/v) $CO_2$, aeration rate = 40 ml min$^{-1}$, agitation speed = 400 rpm and 25°C

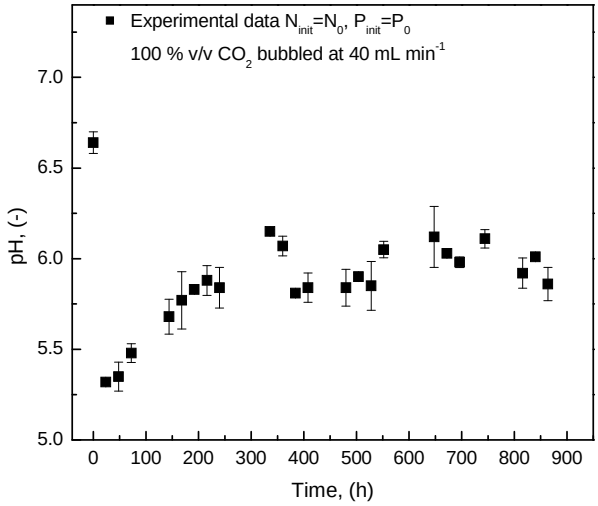


Fig. 8 pH evolution as a function of time during the growth of *N. eucaryotum* in the batch photobioreactor depicted in Fig. 1. Culture conditions: 100% (v/v) $CO_2$, aeration rate = 40 ml $min^{-1}$, agitation speed = 400 rpm and 25°C

withdrawal and replacement with fresh medium were performed and, after 5 days from each withdrawal, the biomass always reached the concentration corresponding to the steady state. Such behavior demonstrates that the photobioreactor can be suitably operated in fed-batch mode while assuring the culture stability with a dilution ratio ($D$) of 0.0015 $h^{-1}$. By indicating with $X_s$ the microalgae concentration at the steady state, i.e., 0.35 g $L^{-1}$, the potential biomass productivity ($P_b$) (Mazzuca and Chisti 2010) was evaluated, through the equation $P_b = D \cdot X_s$, to be about 12.6 mg $L^{-1}$ $day^{-1}$. It should be noted that, given the high growth rate observed during the initial phase, higher dilution rates could be probably used while guaranteeing reactor stability. This could allow us to obtain higher biomass productivities.

Finally, it is worth noting that such result is obtained under extreme operating conditions such as elevated $CO_2$ levels and low pH (cf. Fig. 8) at which very many of the algal strains investigated so far in the literature have been shown to grow with strongly reduced capability (Papazi *et al*. 2008) or not to grow at all (Watanabe *et al*. 1992). Fig. 8 shows the pH evolution during the experiment. It can be observed that when the culture is started, pH drops to the value of 5.32, as a result of the $CO_2$ inlet. Although such low value of initial pH, microalgae start growing exponentially while pH increases as a result of the photosynthetic activity. According to Geisert *et al*. (1987) this behavior confirms that *N. eucaryotum* could survive under very low pH values. In fact, even though the optimal pH for *N. eucaryotum* is in the range between 5 and 7, cell growth can take place at pH equal to 4 and 9, respectively (Geisert *et al*. 1987). Such result is very important in view of the utilization of such strain to capture $CO_2$ from sources where its concentration is quite high. In fact, such microalga grows not only at low pH but also at a higher rate during the initial growth phase with respect to the corresponding one observed when lower $CO_2$ levels are used.

### *3.3 Lipid content and FAME profile*

It is worth noting in passing that under the above mentioned experimental conditions the lipid content of *N. eucaryotum* is evaluated to be 16.2% wt/$wt_{biomass}$. While details of the extraction procedure will be reported in a subsequent publication, it should be noted that the cumulative amount of fatty acid methyl esters (FAME) having carbon numbers from C16 to C18 is about 71.2% wt/wt. Thus it can be stated that, at least from a qualitative point of view (Damiani *et al*. 2010) lipids extracted from *N. eucaryotum* could be suitably exploited for the production of biodiesel.

## 4. Conclusions

In this work the Monod's growth model for multiple nutrients limitation was adopted in order to evaluate the kinetic parameters related to the growth of *N. eucaryotum* under the experimental conditions of our recent work (Lutzu *et al*. 2012). The maximum growth rate was evaluated to be 1.99 $10^{-3}$ $h^{-1}$. Half saturation concentrations for nitrate ($K_N$) and phosphate uptake ($K_P$) were evaluated as 5.4 $10^{-4}$ $g_N L^{-1}$ and 2.5 $10^{-5}$ $g_P L^{-1}$, respectively. Yield factors for nitrogen ($Y_N$) and phosphorus ($Y_P$) resulted to be 5.9 $10^{-2}$ $g_N/g_{biomass}$ and 6.0 $10^{-3}$ $g_P/g_{biomass}$, respectively. Predictive capability of the adopted growth model along with the fitted kinetic parameters was also tested with good results. It is worth noting that these results represent a first step for developing useful mathematical models to simulate and optimize the growth of *N. eucaryotum* in large-scale photobioreactors.

Subsequently, the possibility to grow *N. eucaryotum* in a semi batch photobioreactor fed with a gaseous stream of pure (100% v/v) $CO_2$ was experimentally demonstrated for the first time in this work. The strain showed a good adaptability to high concentrations of dissolved $CO_2$ as well as to low pHs thus being potentially useful for the $CO_2$ capture from flue gases. Finally, although the potential biomass productivity is not high, the lipid content of *N. eucaryotum*, grown under elevated $CO_2$ levels, is relatively good (i.e., 16.16% wt/wt) and the fatty acids methyl esters (FAME) composition of the extracted oil is in compliance with the European regulation for quality biodiesel. This aspect represents an interesting result since the oil extracted from the majority of microalgal strains is characterized by FAMEs composition that is not suitable for the production of biodiesel through simple transesterification processes.

Although further analyses should be performed to evaluate the potential exploitability of *N. eucaryotum* as feedstock for biofuels production, the obtained results allow to state that, at least from a qualitative point of view, the oil extracted from this strain seems to be suitable for the production of biodiesel. On the other hand the low biomass productivity might severely affect its exploitability at the industrial level. For these reasons the optimization of operating conditions should be performed by means of suitable mathematical models where the kinetic parameters obtained in this work are needed in order to achieve this target.

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