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Batch Growth Kinetics of *Nannochloris Eucaryotum* and its Cultivation in Semi-Batch Photobioreactors under 100 %v/v CO_2: Experimental and Modeling Analysis

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The growth kinetics of Nannochloris eucaryotum in batch reactors is quantitatively investigated in this work with the purpose to obtain the main kinetic parameters needed to design photobioreactors for its cultivation at the industrial scale. Specifically, maximum growth rate, half saturation constants and yields coefficients for nitrates and phosphates, respectively, are determined by fitting the experimental data. The reliability of the obtained parameter values is then successfully tested by predicting further experimental data through the model. Finally, the effects resulting from the use of 100 % (v/v) CO_2 gas as carbon source on the growth and lipid production are investigated.

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 (Scarsella et al., 2010). In recent years, microalgae have been recognized to be a promising alternative source for biofuel-convertible lipids (Concas et al., 2010). The high potential of algae based biofuels is confirmed by the number of recent papers available in the literature on the subject, the growing investments of private companies and governments as well as the increasing number of filed patents. 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 (Concas et al., 2012). 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 (Cicci and Bravi, 2014). Specifically, one of the most impacting cost items is related to the need of a continuous replenishment of macronutrients (mainly CO_2, nitrogen and phosphorus) during algal cultivation. 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. 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 productivity should be quantitative evaluated. Moreover, 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 evaluated for the microalgae strains candidate to industrial exploitation. This way, macronutrients concentrations might be precisely controlled during cultivation. Furthermore, 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

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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*, also known as *Pseudochloris Whilemi* (Somogy et al., 2013) shows high adaptability to extreme environmental conditions such as high salinity, low irradiance and elevated CO_2 levels. While these aspects make this 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. For these reasons, 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. 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* to capture CO_2 from sources characterized by high concentration values of this gas.

2. Materials and Methods

2.1 Batch experiments

The marine algal strain *Nannochloris eucaryotum* (SAG 55.87) was investigated in this work. Growth experiments were performed in 250-mL Pyrex bottles in contact with atmospheric air. Culture volumes of 200 mL were continuously agitated by means of a magnetic bar and maintained at room temperature under a photon flux density of 84 $\mu E\ m^{-2}\ s^{-1}$ provided by suitable lamps for a light/dark photoperiod of 12 h. In the base case experiment the growth medium was characterized by the concentrations of nutrients shown in Table 1.

Table 1 Composition of the culture medium used in the base case experiments (Lutzu et al., 2012)

Component	Concentration (gL^{-1})	Component	Concentration (gL^{-1})
KNO_3	$2.0\cdot10^{-1}$	$CoCl_2\cdot6H_2O$	$3.5\cdot10^{-5}$
K_2HPO_4	$2.0\cdot10^{-2}$	$CuSO_4\cdot5H_2O$	$8.0\cdot10^{-5}$
$MgSO_4\cdot7H_2O$	$2.0\cdot10^{-2}$	$Na_2MoO_4\cdot2H_2O$	$2.3\cdot10^{-4}$
H_3BO_3	$2.86\cdot10^{-3}$	EDTA-Na_2	$2.98\cdot10^{-2}$
$MnCl_2\cdot4H_2O$	$1.81\cdot10^{-3}$	$FeSO_4\cdot7H_2O$	$2.49\cdot10^{-2}$
$ZnSO_4\cdot7H_2O$	$2.22\cdot10^{-4}$		

The initial concentrations of nitrates and phosphates adopted in the base case experiment (Table 1), will be hereafter indicated by the symbols N_0 and P_0 respectively. Further experiments were then performed using different initial concentrations of nitrates and phosphates to investigate their effect on the kinetic behaviour of the cultures.

2.2 Semi-batch experiments under continuous flux of 100% CO_2

The possibility of exploiting 100 % (v/v) CO_2 gas as carbon source for the growth of *N. eucaryotum* was also investigated. To this aim the photobioreactor, whose schematic representation is reported elsewhere (Concas et al., 2012), was employed. It consisted 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 filled with 1 L of growth medium and then mechanically stirred at 400 rpm through a rotating blade powered by an electrical engine. Cultures were maintained at 25 °C by a thermostatic bath (GD120 series) and illuminated by a photon flux density of 100 $\mu E\ m^{-2}\ s^{-1}$ provided by suitable lamps 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 Biomass concentration and pH measurements

The growth of microalgae was monitored through spectrophotometric measurements of the culture media optical density (OD) at 560 nm wavelength (D560) with 1 cm light path. Biomass concentration X ($g\ L^{-1}$) was calculated from OD measurements using a suitable X vs. OD calibration curve. The pH was daily measured by pHmeter (KNICK 913).

2.4 Analysis of fatty acids methyl esters of extracted lipids

Lipid extraction was performed according to the procedure reported by Concas et al. (2014). The fatty acids methyl esters composition of extracted lipid was determined after transesterification with methanol-acetyl chloride according to the procedure reported by Steriti et al. (2014).

3. Mathematical model

The material balance for the microalgal biomass, used to quantitatively evaluate the kinetic parameters related to *N. eucaryotum* in the batch photobioreactor, is reported as follows (Concas et al., 2013):

$$\frac{dX}{dt} = \mu X = \mu_0(CO_2, T, I, pH) \prod_{i=N,P} \frac{C_i}{K_i + C_i} X \quad i = N, P \tag{1}$$

where X represents the cell mass (g L^{-1}), t is the time (h), μ (h^{-1}) is the growth rate while μ_0 (h^{-1}) is the maximum growth rate under the temperature level T, the light intensity I, the dissolved CO_2 concentration and the pH conditions of the adopted experimental set-up. The symbol K_i (g L^{-1}) represents the half saturation constant for nitrogen and phosphorus, respectively. 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 cultivation time, to the biomass concentration X through the following relationship:

$$C_i = C_{0,i} - Y_i(X - X_0) \quad i = N, P \tag{2}$$

where $C_{0,i}$ (g L^{-1}) is the initial concentration of nitrogen and phosphorus while Y_i (/) represent the yield coefficient for the same nutrients. In order to solve Eqs. (1) and (2) and thus to interpret the experimental results, the values of five parameters (μ_0, K_N, K_P, Y_N, Y_P) are needed. The strategy adopted to fit the above mentioned kinetic parameters is illustrated in what follows. By assuming that μ remains constant during exponential growth phase, Eq. (1) can be integrated along with the initial condition $X = X_0$ at $t = 0$ to give the following relationship between the microalgae mass concentration and time:

$$\ln\left(\frac{X}{X_0}\right) = \mu t \tag{3}$$

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. (3) in order to obtain the value of μ_0. While maintaining fixed the above reported value of μ_0, the kinetic parameter K_N and Y_N were evaluated by coupling the numerical integration of Eqs. (1) and (2) 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 μ_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 the case where phosphorus limitation phenomena took place. The reliability of the fitted parameters were then evaluated by successfully predicting suitable 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.

4. Results and discussion

4.1 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. It can be observed from Fig. 1a that, for the case where $N_{init} = N_0$ and $P_{init} = P_0$ (i.e. for the base case experiment) *N. eucaryotum* grows exponentially with time up to the end of the cultivation period. Thus, 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 caused by microalgal uptake. The experimental data obtained using $N_{init} = N_0$ and $P_{init} = P_0$ can be fitted through Eq. (3) by means of a constant growth rate (i.e. $\mu = \mu_0$) equal to 1.99×10^{-3} (h^{-1}), under the selected experimental conditions. The comparison between model results and experimental data shown in Fig. 1a 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 available in the medium due to microalgae absorbance. The fitted value of μ can be then regarded as the

maximum growth rate μ_0 (cf. Eq. (1)) under the temperature, light intensity, CO_2 concentration and pH conditions available in the case where $N_{init} = N_0$ and $P_{init} = P_0$. The effect of initial nitrogen concentration was investigated (Concas et al., 2013) by reducing it to one half and one fourth of N_0, (i.e. N_{init} = ½ N_0 and N_{init} = ¼ N_0), while maintaining constant the initial phosphate concentration (i.e. $P_{init} = P_0$). From the experimental data reported in Figure 1a, it clearly appeared that for N_{init} = ½ N_0 the growth curve approached a stationary phase after about 720 h, thus indicating the occurrence of nitrogen starvation phenomena. Consequently, by maintaining fixed the above reported value of μ_0, the kinetic parameter 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 verified in the base case experiment. Model results are compared with experimental data in Fig.1a. The best fitting value for the half saturation constant K_N was equal to 5.2 x 10^{-4} (g_N L^{-1}) while the corresponding one of the nitrogen yield Y_N was 5.9 x 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 Figure 1a clearly show that when the initial content of P was reduced to ¼ P_0, the cells mass concentration increased during the first 400-500 h of cultivation. Then a stationary phase was reached and maintained up to 700 h of cultivation. This fact indicates that for P_{init} = ¼ P_0 phosphorus becomes a limiting nutrient after a specific culture time. Hence, by considering that C_N is much greater than K_N under these experimental conditions and maintaining fixed the values of μ_0 already obtained, the kinetic parameters K_P and Y_P were obtained by non linearly fitting the experimental data obtained when $N_{init} = N_0$ and P_{init} = ¼ P_0 in the time interval 0-700 (h). Model results are compared with experimental data in Fig. 1a. In particular, the best fitting value of 2.5 x 10^{-5} (g L^{-1}) is obtained for the half saturation constant K_P while the corresponding value of 6.0 x 10^{-3} ($g_P/g_{biomass}$) is obtained for the phosphorus yield Y_P.

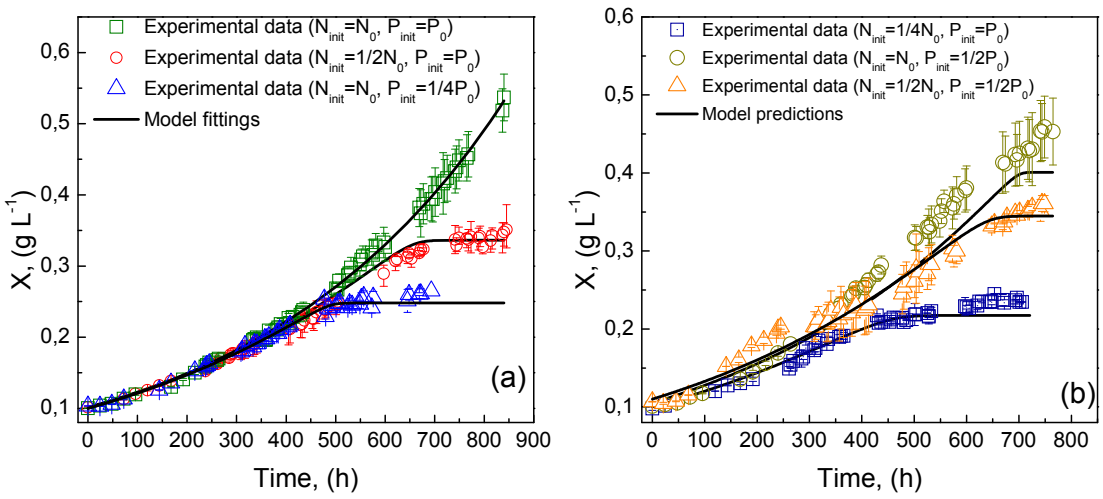

Figure 1. Comparison between experimental data in terms of cells concentration as a function of time and (a) model fittings results and (b) model predictions results.

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} = ¼ N_0 and $P_{init} = P_0$) and only the initial phosphorus concentration was halved (i.e. $N_{init} = N_0$ and P_{init} = ½ P_0), were performed. Figure 1b illustrates the comparison between experimental data and model results which were obtained by maintaining fixed the kinetic 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 were simultaneously reduced, experimental data related to the case where N_{init} = ½ N_0 and P_{init} = ½ P_0 were also predicted. As it can be observed from Figure 1b, also in this case a quite good matching between model predictions and experimental data was achieved thus confirming the reliability of the obtained kinetic parameters.

4.2 Effects of using 100% (v/v) CO_2 on cell growth, pH evolution and lipid content

The effect of high CO_2 concentration on the growth of *N. eucaryotum* in semi-batch photobioreactors was also investigated. To this aim, 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 whose chemical composition is reported in Table 1. The semi-batch photobioreactor described by Concas et al., (2012) was used. From

Figure 2a it can be observed that, under these 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 Figure 2a with the corresponding ones (i.e. $N_{init} = N_0$ and $P_{init} = P_0$) obtained as shown above, it can be observed that when pure CO_2 is used as carbon source, an higher initial growth rate can be observed. Such behaviour is probably due to the better availability of dissolved CO_2 which results in the increase of the specific growth rate μ_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, when using CO_2 from the atmosphere microalgae keep growing almost exponentially up to 840 (h) of cultivation. Once the steady state was attained, the possibility to operate the photobioreactor in fed-batch mode (Coelho et al., 2014) was evaluated. In fact starting from the 16th 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 of about 1.5 x 10^{-3} (h^{-1}). As shown in Figure 2a, 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 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. Therefore, the photobioreactor can be suitably operated in fed-batch mode while assuring the culture stability with a dilution ratio (D) of 1.5 x 10^{-3} (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) was evaluated, through the equation $P_b = DX_s$, 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 assuring reactor stability. This might allow one to obtain higher biomass productivities.

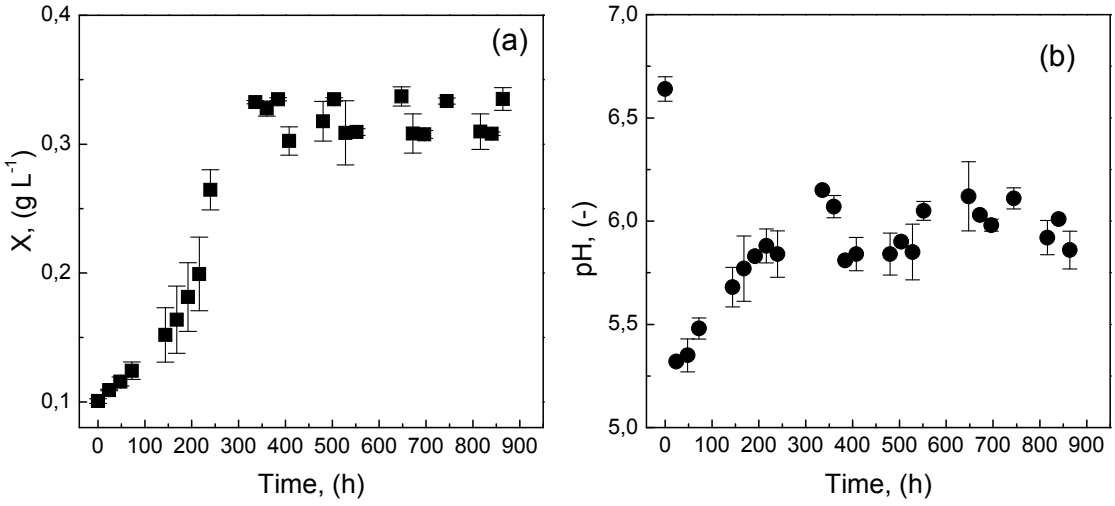

Figure 2 Growth of N. eucaryotum in the semi-batch photobioreactor in terms of (a) microalgae concentration and (b) pH as a function of time. Culture conditions: 100 % (v/v) CO_2, aeration rate = 40 mL min^{-1}, agitation speed = 400 rpm and 25 °C.

Finally, it is worth noting that this result is obtained under extreme operating conditions such as elevated CO_2 levels and low pH (cf. Figure 2b) at which most of the algal strains investigated so far in the literature have been shown to grow with strongly reduced rate or not to grow at all (Papazi et al., 2008). Figure 2b shows the pH evolution during the experiment. It can be observed that when the culture is started, pH suddenly drops to the value of 5.32, as a result of the CO_2 inlet. Despite 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 behaviour 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. 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.

4.3 Lipid content and FAME profile

The microalgae collected after each withdrawal during the fed-batch operation of the photobioreactor, were subjected to the lipid extraction procedure reported in Concas et al. (2014). The obtained average value of lipids extracted from *N. eucaryotum* cultivated under the above operating conditions was about 16.2 % ($wt/wt_{biomass}$). Moreover, the fatty acid methyl esters composition of the biodiesel obtained by transesterfication of the extracted lipids were characterized by a cumulative amount of FAMEs having carbon numbers from C16 to C18 of about 71.2 % wt/wt as well as a very low content of linolenic acid, i.e. 0.18% 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.

5. Concluding remarks

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. The maximum growth rate, half saturation concentrations for nitrate (K_N) and phosphate uptake (K_P) were evaluated as well as the corresponding yields, namely Y_N and Y_P. The predictive capability of the adopted growth model along with the fitted kinetic parameters was also tested with good results. 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. The strain showed a good adaptability to high concentrations of dissolved CO_2 as well as to low pH, thus being potentially useful for the CO_2 capture from flue gases. Finally, the fatty acids methyl esters (FAME) composition of the oil extracted from the microalga cultivated under 100 % CO_2 is in compliance with the European regulation for quality biodiesel.

References

Cicci A. and Bravi M., 2014. Production of the Freshwater Microalgae Scenedesmus Dimorphus and Arthrospira Platensis by Using Cattle Digestate. Chem. Eng. Trans. 38, 85-90

Coelho R. S., Vidotti A. D., Reis É. M., Franco T. T. 2014. High Cell Density Cultures of Microalgae under Fed-batch and Continuous Growth. Chem. Eng. Trans., 38, 313-318.

Concas A., Pisu M., Cao G., 2010, Novel simulation model of the solar collector of BIOCOIL photobioreactors for CO_2 sequestration with microalgae, Chem. Eng. J., 157, 297-303.

Concas A., Lutzu G. A, Pisu M., and Cao G., 2012, Experimental analysis and novel modeling of semibatch photobioreactors operated with Chlorella vulgaris and fed with 100 %(v/v) CO_2, Chem. Eng. J., 213, 203–213.

Concas A., Lutzu G. A, Locci A. M., and Cao G., 2013. Nannochloris eucaryotum growth in batch photobioreactors: kinetic analysis and use of 100%(v/v) CO_2. Adv. Env. Res., an International Journal, 2, 19-33

Concas A., Steriti A., Pisu M., and Cao G., 2014, Comprehensive modeling and investigation of the effect of iron on the growth rate and lipid accumulation of Chlorella vulgaris cultured in batch photobioreactors, Bioresource Technol., 153, 340-350.

Damiani M.C., Popovich C.A., Constenla D., Leonardi P.I. 2010, Lipid analysis in Haematococcus pluvialis to assess its potential use as a biodiesel feedstock. Bioresour Technol, 101, 3801–3807.

Geisert M., Rose T., Bauer W. and Zahn R.H., 1987. Occurence of carotenoids and sporopollenin in Nanochlorum eucaryotum, a novel marine alga with unusual characteristics. Biosystems, 20, 133-142.

Lutzu, G. A., Locci, A. M., Cao, G. 2012. Effect of Medium Composition on the Growth of Nannochloris eucaryotum in Batch Photobioreactors. Journal of Biobased Materials and Bioenergy, 6(1), 94-100.

Papazi A., Makridis P., Divanach P., Kotzabasis K., 2008. Bioenergetic changes in the microalgal photosynthetic apparatus by extremely high CO_2 concentrations induce an intense biomass production. Physiol. Plantarum, 132, 338–349.

Scarsella, M., Belotti, G., De Filippis, P., and Bravi, M., 2010. Study on the optimal growing conditions of Chlorella vulgaris in bubble column photobioreactors. Chem. Eng. Trans., 20, 85-90.

Somogyi, B., Felföldi, T., Solymosi, K., Flieger, K., Márialigeti, K., Böddi, B., Vörös,, L. 2013. One step closer to eliminating the nomenclatural problems of minute coccoid green algae: Pseudochloris wilhelmii, gen. et sp. nov.(Trebouxiophyceae, Chlorophyta). Eur. J. Phycol., 48, 427-436.

Steriti A., Rossi R., Concas A. and Cao, G., 2014. A novel cell disruption technique to enhance lipid extraction from microalgae. Bioresource Technol., 164, 70-77.