CHEMICAL ENGINEERING TRANSACTIONS
VOL. 57, 2017
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Sauro Pierucci, Jiří Jaromír Klemeš, Laura Piazza, Serafim Bakalis

ISBN 978-88-95608- 48-8; **ISSN** 2283-9216

Experiments and Modeling of the Growth of C. sorokiniana in Lab Batch and BIOCOIL Photobioreactors for Lipid production

Alessandro Concas*[a], Veronica Malavasi[b], Massimo Pisu[a], Santina Soru[b], Giacomo Cao[b,c].

[a]Center for Advanced Studies, Research and Development in Sardinia (CRS4), Loc. Piscina Manna, Building 1, 09010 Pula (CA), Italy
[b] Interdepartmental Center of Environmental Science and Engineering (CINSA), University of Cagliari, Via San Giorgio 12, 09124 Cagliari, Italy
[c] Department of Mechanical, Chemical and Materials Engineering, University of Cagliari, Piazza d'Armi, 09123 Cagliari, Italy
aconcas@crs4.it

A novel mathematical model for the quantitative assessment of the effect of dissolved nitrogen on the autotrophic batch-growth and lipid accumulation of *C. sorokiniana*, is proposed in this work. Model results have been validated through comparison with suitable experimental data performed in lab photobioreactors. Further experiments have been then performed using a BIOCOIL operated in fed-batch mode. The experimental results have been successfully predicted through the proposed model. Therefore, the model might represent a first step toward the development of a tool for the scale-up and optimization of the operating conditions of BIOCOIL photobioreactors. Furthermore, the fatty acid methyl esters obtained by trans-esterification of lipids extracted from *C. sorokiniana*, have been analysed in view of the assessment of their usability for producing biofuels. Subsequently, on the basis of the fatty acids profile, a wide range of biodiesel fuel properties have been predicted through suitable software.

1. Introduction

Microalgae represent one of the most promising renewable feedstocks for the production of a wide range of consumer goods among which biofuels, nutraceuticals, pharmaceuticals, bioplastics and functional food. Nevertheless, the current lipid productivity of microalgae should be increased in order to make the technology economically viable.
Several methods are currently being investigated for boosting lipid biosynthesis in microalgae. Today nitrogen starvation in the culture seems to be the only feasible strategy for boosting lipid productivity on the large scale in an economically sustainable fashion (Concas et al., 2016a). However, likewise what happen to all the techniques for lipid accumulation based on the induction of stress conditions, while from one side the lipid content of microalgae is increased, on the other one, the growth rate is reduced and thus the resulting lipid productivity is similar to the one which might be obtained by cultivating algae under normal conditions.
Since the increase of lipid productivity is the target of the nitrogen starvation strategy, it is apparent that the identification of the trade-off value of nitrogen concentration is a key issue in view of the implementation of this technique at the industrial scale. For these reasons, further and deeper investigations about the effect of nitrogen depletion on lipid productivity are required especially for strategic microalgal strains such as *C. sorokiniana*. Despite the fact that the latter one is a very promising strain in view of the production of biofuels through microalgae only few papers have been so far devoted to the systematic investigation of the effect of nitrogen starvation on the lipid productivity and lipid quality of *C. sorokiniana*, especially when grown under photoautotrophic conditions (Orsini et al., 2016). Such a lack represents one among the different causes which have limited the exploitation of such cultivation strategy to improve bio-oil yields at the large scale.
Accordingly, the aim of the present work is to propose a mathematical model to quantitatively describe the growth of *C. sorokiniana* and its capability to accumulate lipid in response to nitrogen starvation operating conditions. Specific experiments in batch photobioreactors have been then performed in order to validate

model results. In these experiments, particular attention has been devoted to the assessment of the effect of nitrogen concentrations on the final quality of FAMEs (Fatty Acid Methyl Esters) obtained by transesterification of lipids. Subsequently, on the basis FAMEs profile, a wide range of biodiesel fuel properties have been predicted through suitable software. Finally, once the optimal nitrogen concentration identified, the possibility to scale-up the investigated process in a BIOCOIL photobioreactor has been evaluated by cultivating *C. sorokiniana* for the first time in such reactor. The obtained experimental results have been suitably predicted through the proposed model.

2. Materials and methods

2.1 Microorganism and culture medium

An authentic fresh water strain *Chlorella sorokiniana* was obtained from the University of Göttingen (SAG 211-8k) and then investigated in this work. The BBM growth medium, whose composition is reported elsewhere (Concas et al 2016a), was used for the base case batch experiment. The initial concentration of nitrogen in the BBM medium, hereafter indicated by the symbol C_N^0, was equal to 250 $g_{NaNO_3} m^{-3}$. However, it was varied in the framework of suitable experiments performed with the aim of assessing the effect of nitrogen starvation on lipid productivity of *C. sorokiniana*. Specifically, the effects arising from the reduction by one-fifth (1/5N-BBM) and the increase by five times (5N-BBM), respectively the initial nitrogen concentration, were investigated. The choice of the levels of nitrogen concentration subjected to investigation were made on the basis of the results of preliminary experiments of short duration which highlighted how the levels 1/N and 5N would have permitted to well assess the effect of nitrogen on biomass and lipid productivity even in long duration experiments. The growth medium used during the experiments in the BIOCOIL was the standard BBM specified above.

2.2 Culture conditions

Batch growth experiments were performed in 1L Pyrex bottles under axenic conditions. During cultivation, the culture was agitated at 300 rpm. A photon flux density of 100 µE m^{-2} s^{-1} provided by six 11 W white fluorescent tubes and a light/dark photoperiod of 12 h. Further experiments were then carried out through the BIOCOIL photobioreactor, which consisted of a 6 L helical tubular photobioreactor coupled with a degasser system, as described in the literature (Steriti et al., 2014). It was internally illuminated by three 60W white fluorescent lamps providing a light intensity of 100 µE m^{-2} s^{-1} for a light-dark photoperiod of 12 h. Liquid circulation in the light collector was assured by a peristaltic pump. Once the culture approached the stationary growth phase, the photobioreactor was operated in fed-batch mode. Specifically, after the 14th cultivation day, 800 mL of culture were daily withdrawn and then replaced by an equal volume of fresh BBM medium. The withdrawals made during the operation in fed-batch mode were then used for lipid extraction. The growth was monitored through spectrophotometric measurements of the culture media optical density (OD) at 663 nm with 1 cm light path. The biomass concentration Cb (gdw L-1) was calculated from OD measurements using a suitable Cb vs OD calibration curve. The pH was measured daily by pH-meter.

2.3 Lipid colorimetric quantification, cell disruption, lipid extraction and FAMEs analysis

The colorimetric method recently proposed in the literature (Mishra et al., 2014) and based on the use of Sulpho-Phospho-Vanillin was adopted to quantify the lipid content of microalgae during cultivation. Subsequently, once the culture in the photobioreactor reached the stationary growth phase, microalgae were first harvested and then centrifuged to obtain a concentrated pellet of wet biomass. Next, wet pellets containing known amounts of dry biomass were subjected to the cell disruption procedure proposed by Concas et al., (2015) which consisted of contacting them with selected volumes of an aqueous solution of H_2O_2 (0.5 M) within a falcon flask that was kept sealed and continuously shaken at 300 rpm at room temperature for a suitably prolonged time. Neutral lipid extraction was then performed directly on the wet disrupted biomass by taking advantage of ethanol and hexane according to a method consisting of a slight modification of a technique proposed in the literature (Fajardo et al., 2007). Finally, the composition of FAMEs, obtained by transesterification of the extracted lipids with methanol-acetyl-chloride, was determined by chromatographic means according to the European regulation EEC n° 2568 (1991).

3. Mathematical model

According to the present model, the cell is considered to consist of three distinct compartments, i.e. the free fatty acid fraction (f), the lipid fraction (ℓ) and finally the functional fraction (x) which represents the bio-synthetic apparatus and is constituted by all the molecules having functions different from the energy storage one. On the contrary, the fractions (f) and (ℓ) account for all the intracellular compounds whose role is to store the carbon (and thus energy) excess deriving from photosynthesis under nitrogen starvation. The sum of the

masses of the above fractions returns the total microalgal biomass (b). Moreover, the growth of microalgae is conceptually divided into three phases wherein different phenomena rule the synthesis of functional biomass or lipids depending on the light intensity and nitrogen availability in solution. In quantitative terms, the net carbon fixation rate $\mathrm{P}\ (\mathrm{g_C\ g_{dw}^{-1}\ min^{-1}})$ can be evaluated as a Poisson function of average light intensity $\mathrm{I_{av}}\ (\mu\mathrm{E\ m^{-2}\ min^{-1}})$ as follows:

$$P = P_C^{\max} \cdot \left(1 - \frac{Q_N^{\min}}{q_N}\right) \cdot \left[1 - \exp\left(-\frac{\alpha \cdot \Phi \cdot q_{Chla} \cdot I_{av}}{P_C^{sat}}\right)\right] \tag{1}$$

Where $\mathrm{P_C^{max}}\ (\mathrm{g_C\ g_{dw}^{-1}\ min^{-1}})$ is the carbon-specific maximum photosynthetic rate, $\mathrm{q_{Chla}}\ (\mathrm{g_{Chl}\ g_{dw}^{-1}})$ and $\alpha\ (\mathrm{m^2\ g_{Chl}^{-1}})$ represent, respectively, the intracellular content of chlorophyll-a and its optical cross section, while $\Phi\ (\mathrm{g_C\ \mu E^{-1}})$ is the quantum efficiency of photosynthesis. The symbol $\mathrm{q_N}(\mathrm{g_N\ g_{dw}^{-1}})$ represents the intracellular quota of nitrogen while $\mathrm{Q_N^{min}}\ (\mathrm{g_N\ g_{dw}^{-1}})$ is the minimum content of nitrogen that allows the cell to survive. Depending upon the availability in solution, as well as from the intracellular content, nitrogen is continuously absorbed by the cell and used, along with the intracellular carbon, to build up functional biomass (x). Since the nitrogen uptake by algae leads to its consumption in the growth medium, the following mass balance can be written to describe the time evolution of its concentration in the bulk liquid phase of the batch system under investigation:

$$\frac{dC_N}{dt} = -\left[\nu_N^{\max}\left(\frac{Q_N^{\max} - q_N}{Q_N^{\max} - Q_N^{\min}}\right) \cdot \frac{C_N}{K_N + C_N}\right] \cdot C_x + D \cdot \left(C_N^0 - C_N\right) \tag{2}$$

along with the corresponding initial condition: $C_N = C_N^0 = MW_N[NO_3^-]^0$ at $t = 0$. The symbol $\mathrm{C_N}$ represents the total mass concentration of nitrogen in the bulk liquid, C_x is the concentration of the functional fraction of algal biomass and $[NO_3^-]^0$ is the initial molar concentration of nitrates in the growth medium. The symbol $D\ (min^{-1})$ represents the dilution ratio adopted during the fed batch cultivation of the BIOCOIL while it is set equal to zero when simulating the experiments performed in batch reactors. The symbol v_N^{max} ($\mathrm{g_N\ g_{dw}^{-1} min^{-1}}$) represents the maximum uptake rate of nitrogen by microalgae while the term within square brackets in Eq. 2 represents the corresponding actual uptake rate and is related to the nitrogen concentration in solution while it is down-regulated by a linear satiation function of the cell quota $\mathrm{q_N}$ as cells approach their maximum allowed content of nitrogen. On the other hand, q_N varies according to the ordinary differential equation:

$$\frac{dq_N}{dt} = \nu_N - \mu \cdot q_N \tag{3}$$

along with the initial condition $q_N = q_N^0$ at $t = 0$. In Eq. 3 the product $\mu \cdot q_N\ (g_N\ g_{dw}^{-1}\ min^{-1})$ represents the rate at which nitrogen is consumed within the cell to produce functional proteins. The material balance for the functional microalgal biomass in batch reactors can be then written as follows:

$$\frac{dC_x}{dt} = \left(\mu - k_d - D\right) \cdot C_x \tag{4}$$

along with the corresponding initial condition $C_x = C_x^0$ at $t = 0$. The specific rate $\mu\ (min^{-1})$ at which the functional biomass grows, can be limited either by nitrogen or by the intracellular carbon that, in the last resort, depends from light intensity. Therefore, according to the Liebig's law it can be evaluated as follows:

$$\mu = \min\left\{\mu_{\max}\left(1 - \frac{Q_N^{\min}}{q_N}\right);\ \frac{P}{\gamma_{c/x}}\right\} \tag{5}$$

where $\gamma_{c/x}$ ($\mathrm{g_C\ g_{dw}^{-1}}$) is the carbon content of the functional fraction of microalgae. Under nitrogen starvation and, if light intensity is sufficient, internal carbon is used to produce functional biomass at a rate ($\mu \cdot \gamma_{c/x}$) which is lower than that one (P) at which carbon is fixed by photosynthesis. As a result, an excess of carbon is internalized with respect to the minimum amount needed to synthesize functional biomass. The carbon excess is then stored by the cells in the form of lipids through an intermediate step consisting of the synthesis of fatty acids. According to to Concas et al. (2016a), the rate at which the excess carbon is converted into fatty acids is thus equal to $\left(P - \gamma_{c/x}\,\mu\right) \cdot C_x / \gamma_{c/f}$ and, accordingly, the mass balance for the fatty acids is given by:

$$\frac{dC_f}{dt} = \frac{\left(P - \gamma_{c/x}\mu\right)C_x}{\gamma_{c/f}} - \Gamma_{f \to \ell} - D \cdot C_f \tag{6}$$

along with the initial condition $C_f = C_f^0$ at $t = 0$. The term $\gamma_{c/f}$ $(g_C \cdot g_{dw}^{-1})$ is the average carbon content of microalgal fatty acids while $\Gamma_{f\to\ell}$ $(g_{dw} \cdot m^{-3} \cdot min^{-1})$ is the rate at which they are converted into lipids (tryacylglicerols). The latter one is assumed to depend in a linear fashion from the concentration of free fatty acids, as well as from an additional saturation term that takes into account the decrease in algal oil production as cells become saturated with oil, i.e $\Gamma_{f\to\ell} = \Gamma_{f\to\ell} C_f (1 - q_\ell / q_\ell^{max})$. Therefore, according to Concas et al., (2016a) the mass balance for the lipidic fraction of biomass C_ℓ can be written as follows:

$$\frac{dC_\ell}{dt} = k_{f\to\ell} \cdot C_f \cdot \left(1 - \frac{q_\ell}{q_\ell^{\max}}\right) - D \cdot C_\ell \qquad (7)$$

along with the initial condition $C_\ell = q_\ell^0 C_b^0$ at $t = 0$. where q_ℓ is the lipid cell quota which can be evaluated at each cultivation time as $q_\ell = C_\ell / C_b$, being C_b the total biomass concentration, i.e. the sum of the three compartment concentrations $C_b = C_\ell + C_f + C_x$. The symbols with the suffix "0" refers to the corresponding initial values. Finally, in order to complete the set of equations of the model it should be noted that the term q_{Chla} in Eq. 1 is the amount of chlorophyll (Chla) per unit of microalgal biomass, namely the chlorophyll cell quota $(g_{Chla} \cdot g_{dw}^{-1})$, which varies with time according to the following equation (Concas et al., 2016a):

$$\frac{dq_{Chla}}{dt} = \theta_{Chla}^{N,\max} \cdot \left(\frac{P}{\alpha \cdot \Phi \cdot q_{Chla} \cdot I_{av}}\right) \cdot v_N - \mu \cdot q_{Chla} \qquad (8)$$

along with the initial condition $q_{Chla} = q_{Chla}^0$ at t = 0. The symbol $\theta_{Chla}^{N,max}(g_{Chla} \cdot g_{dw}^{-1})$ represents the maximum ratio at which nitrogen can be used for chlorophyll synthesis. The average light intensity (I_{av}) within the culture has been evaluated according to the procedure described elsewhere (Concas et al., 2013; 2016b).

4. Results and discussion

Specific experiments were carried out in batch stirred bottles while changing the initial concentration of dissolved nitrogen. In particular, the growth and lipid accumulation kinetics of *C. sorokiniana*, occurring when using nitrogen concentration equal to the typical one of BBM, i.e. 41.6 $g_N m^{-3}$, was first investigated as a base case. The obtained results are shown in Figure 1a in terms of algal biomass concentration evolution and in Figure 1b in terms of lipid content evolution with the tag BBM.

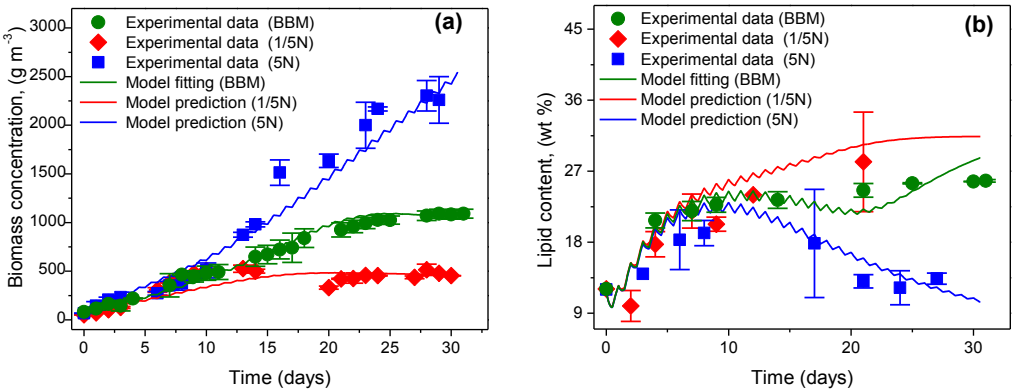

Figure 1. Comparison between model results and experimental data in terms of biomass concentration (a) and lipid content (b).

In the same Figures, even the model simulation results, obtained when nitrogen concentration was the one typical of BBM, are shown. It should be noted that, in this case (cf. tag BBM) all model parameters are taken from the literature (cf. Concas et al., 2016a) except for the optical cross section of chlorophyll-a (α) and the kinetic rate constant $(k_{f\to\ell})$ of conversion of fatty acids into lipids, which have been suitably tuned through a nonlinear fitting procedure in order to quantitatively interpret the experimental data. Their corresponding values result to be 7.5·101 $m^2 g_{Chla}^{-1}$, and 3.0·10^{-4} min^{-1}, respectively. From the Figures, it can be observed that, in general, the proposed model quantitatively captures both the growth trend and the lipid accumulation dynamics from day to day. In order to confirm the model capabilities, further experiments were performed and simulated. In particular, further experiments were performed by setting the initial concentration of dissolved nitrogen equal to 5 times and 1/5 times the corresponding value in the BBM medium, respectively (cf tags 5N and 1/5N the Figures). It could be observed that, when nitrogen concentration is augmented (cf. 5N) biomass concentrations grows continuously while lipid content of microalgae decreases after the 10th cultivation day

while under nitrogen starvation (1/5 N), the biomass concentration reaches a plateau at the 15th cultivation and the corresponding lipid content increases up to about 30 %wt/wt. As it can be observed form Figure 1a and 1b, the proposed model is capable to reproduce these phenomena and in particular to predict the experimental data quite well without tuning any model parameter. Lipid productivity under batch operation can be evaluated as $\pi_\ell = q_\ell^f C_b^f / t^f$ where the suffix "f" refers to the final value at the time of batch discharge t^f. Since the lipid productivity obtained using the BBM medium is quite higher than the one obtained when using 5N or 1/5N medium, the former growth medium (i.e. the BBM) was used to perform the experiments in the BIOCOIL reactor. In this regard, the evolution of biomass concentration during cultivation of *C. sorokiniana* in the BIOCOIL photobioreactor is shown in Figure 2a. It can be observed that, such strain is capable to grow effectively in this device showing a growth dynamics quite similar to the one already observed in the 1L batch photobioreactors, except for the lower value of biomass concentration achieved at the steady state, i.e. 900 Vs 1000 g m^{-3} respectively. However, this lower biomass concentration achieved at the apparent steady state is the result of the fed batch operation of the reactor starting from the 14th cultivation day, i.e before the actual steady state could be reached.

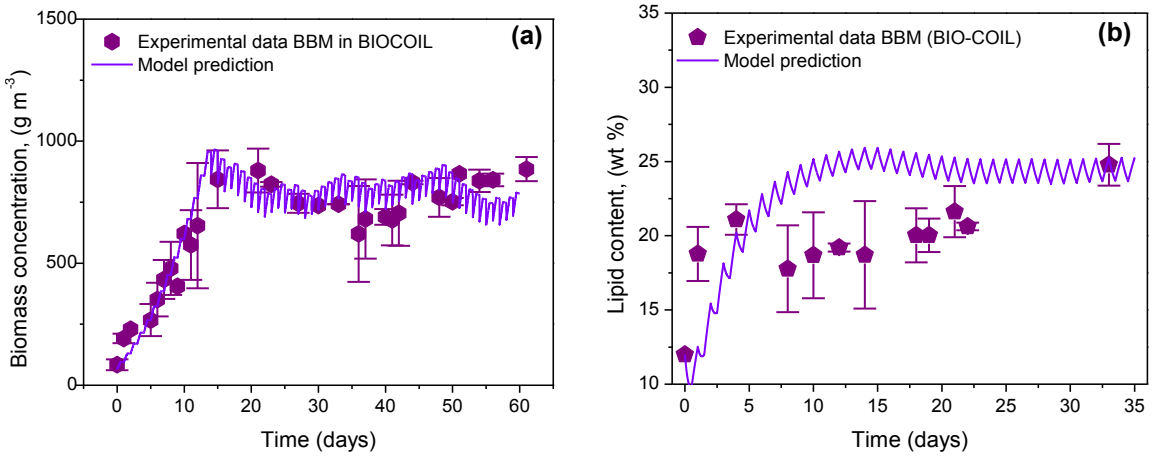

Figure 2. Comparison between model predictions and experimental data in terms of biomass (a) and lipid (b) evolution within the BIOCOIL photobioreactor using the BBM growth medium.

Apart this aspect, the observed growth in the BIOCOIL demonstrates that the photobioreactor can be suitably operated in fed-batch mode using a dilution ratio of about 0.133 day^{-1} while ensuring the culture stability for very prolonged periods of time thus confirming the potential of this device in view of the production of microalgae at the large scale. As it can be observed from Figure 2a, the corresponding experimental results are well predicted by the proposed model even when considering the fed-batch operation mode of the reactor. The saw-tooth trend of model results after the 14th days are due to the simulation of the operation mode. The dynamic evolution of lipid content during growth within the BIOCOIL is shown in Figure 2b. In this case, lipid accumulation is a bit lower than the one already observed in the small-scale reactors. Such slight difference might be due to the accumulation of oxygen in the tubes of the BIOCOIL which might damage the photosystem II of microalgae and trigger lipid peroxidation phenomena (Concas et al., 2015). In Figure 2b, the comparison between experimental data and model predictions, is also shown. As it can be observed, while the general trend of lipid content evolution is enough well captured by the model, the experimental data regarding the period of time ranging from 5 to 20 days are over estimated by the proposed model, probably due to the fact that the latter one neglects the potential lipid oxidation phenomena discussed above. However, in general terms, it can be stated that even with reference to the lipid accumulation dynamics, the results obtained in the laboratory photobioreactors are well reproduced in the BIOCOIL, thus confirming the scalability of the obtained results also in terms of lipid productivity.

In order to verify whether the different cultivation conditions had affected the quality of microalgal lipids, the content of FAMEs obtained after lipid extraction and transesterification was analyzed (Concas et al., 2016a). Different biodiesel characteristics had been then evaluated on the base of the FAMEs profile by taking advantage of specific mathematical relationships implemented in the software Biodiesel Analyzer© Ver. 2.2 (Talebi et al., 2013; Ramírez-Verduzco et al., 2012). The estimated values of the main characteristics affecting the quality of biodiesel obtained by transesterification of oils obtained from the different experiments are shown in Table 1. It should be noted that, some critical parameters, such as SFA, IV, CN, OS, ν and ρ, of the

biodiesel obtained from oil produced in the BIOCOIL, comply with the range of values prescribed by the ASTM D6751-12 standard for quality biodiesel.

Table 1. Estimated values of the main parameters of the biodiesels obtained in the experiments

Symbol	Biodiesel Parameter	Units	BBM (COIL)	BBM (Batch)	5N (Batch)	1/5N (Batch)
SFA	Saturated Fatty Acid	%	36,857	33,04	13,067	26,963
MUFA	Mono Unsaturated Fatty Acid	%	15,124	14,832	9,286	22,975
PUFA>1double	Poly Unsaturated Fatty Acid	%	47,967	52,011	77,558	50,062
DU	Degree of Unsaturation		111,058	118,854	164,403	123,098
SV	Saponification Value	mg/g	204,928	205,642	202,772	204,693
IV	Iodine Value	-	113,805	126,275	193,153	131,697
CN	Cetane number	-	47,328	44,429	29,758	43,333
LCSF	Long Chain Saturated Factor		8,742	6,018	2,229	6,16
CFPP	Cold Filter Plugging Point	°C	10,988	2,43	-9,475	2,875
CP	Cloud Point	°C	11,088	9,473	0,822	5,947
PP	Pour Point	°C	5,216	3,463	-5,928	-0,365
APE	Allylic Position Equivalent		107,916	113,553	161,235	115,839
BAPE	Bis-Allylic Position Equivalent		63,064	72,476	125,006	74,127
OS	Oxidation Stability	hours	5,056	4,863	4,114	4,946
HHV	Higher Heating Value		39,338	39,257	39,208	39,329
υ	Kinematic Viscosity	mm^2/s	3,596	3,469	3,151	3,488
ρ	Density	g/cm^3	0,879	0,88	0,888	0,881

Overall, the oil produced in the BIOCOIL shows the best quality in view of its utilization for producing blends with fossil diesel to be used in the automotive sector. On the other hand, some other parameters values, in particular CP, CFPP etc., do not comply with the corresponding ones foreseen by the ASTM biodiesels standard. Therefore, hydrogenation treatment or blending with specific additives should be performed before using the obtained biodiesel in the transportation sector. Research activity is on the way along these lines.

5. Conclusions

Growth and lipid accumulation of *C. sorokiniana* in batch and BIOCOIL photobioreactors have been experimentally investigated and simulated through a suitable mathematical model. By comparing model results with experimental data a good matching is obtained. The model thus permits to develop suitable optimization strategies for the scale-up of the photobioreactors. The FAMEs composition of lipids extracted from *C. sorokiniana* would permit the production of a biodiesel, which, once treated or blended with specific additives, might be used in the automotive sectors.

References

Concas A., Malavasi V., Costelli C., Fadda P., Pisu M., Cao G., 2016a, Autotrophic growth and lipid production of Chlorella sorokiniana in lab batch and BIOCOIL photobioreactors: Experiments and modelling, Bioresour. Technol. 211, 327-338.

Concas A., Pisu M., Cao G., 2016b, A novel mathematical model to simulate the size-structured growth of microalgae strains dividing by multiple fission. Chem. Eng. J. 287, 252–268.

Concas A., Pisu M., Cao G., 2015. Disruption of microalgal cells for lipid extraction through Fenton reaction: Modeling of experiments and remarks on its effect on lipids composition, Chem. Eng. J. 263, 392–401.

Concas A., Pisu M., Cao, G., 2013, Mathematical modelling of Chlorella vulgaris growth in semi-batch photobioreactors fed with pure CO2, Chemical Engineering Transactions, 32, 1021-1026.

Fajardo A.R., Cerdan L.E., Medina A.R., Fernández F.G.A., Moreno P.A.G., Grima E.M., 2007, Lipid extraction from the microalga Phaeodactylum tricornutum, Eur. J. Lipid Sci. Technol. 109, 120–126.

Mishra S.K., Suh W.I., Farooq W., Moon M., Shrivastav A., Park M.S., Yang J.-W., 2014, Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method, Bioresour. Technol 155, 330–333.

Orsini M., Cusano R., Costelli C., Malavasi V., Concas A., Angius A., Cao G., 2016, Complete genome sequence of chloroplast DNA (cpDNA) of Chlorella sorokiniana, Mitochondrial DNA Part A 27(2), 838-839.

Ramírez-Verduzco L. F., Rodríguez-Rodríguez J. E., del Rayo Jaramillo-Jacob A., 2012, Predicting cetane number, kinematic viscosity, density and higher heating value of biodiesel from its fatty acid methyl ester composition, Fuel 91, 102-111.

Talebi A. F., Mohtashami S. K., Tabatabaei M., Tohidfar M., Bagheri A., Zeinalabedini M., Bakhtiari S., 2013, Fatty acids profiling: a selective criterion for screening microalgae strains for biodiesel production, Algal Research, 2, 258-267.