# STATISTICAL MODELS PROPOSED FOR ALGAL GROWTH IN OPEN SYSTEM TO OPTIMIZE THE CULTIVATION TECHNOLOGY
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# *MODELE STATISTICE PROPUSE PENTRU OPTIMIZAREA TEHNOLOGIEI DE CULTIVAREA ALGELOR ÎN SYSTEM DESCHIS*

**Nedelcu A., Cârdei P., Ciupercă R., Ştefan V., Zaica A.** [1]
[1)] National Institute of Research - Development for Machines and Installations Designed to Agriculture and Food Industry
INMA Bucharest / Romania
*Tel: 0740883936; E-mail: nedelcu.ancuta@yahoo.com, E-mail: petru_cardei@yahoo.com*
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## ABSTRACT

*In this article, the authors present the results of research on Chlorella Vulgaris algae growing technology in open system with cascade type pilot installation, Bold Basal Medium (BBM), culture agitation by recirculation and ambient temperature (with minimal warming). In the experiments were identified monitoring elements necessary to provide information that can lead to mathematical models of algal growth which can demonstrate the viability of the cultivation system and which can be used to optimize algae culture growing technology. In this article, an elementary statistical model for prediction, by linear regression, of algal biomass concentration is also presented. Prediction can be the basis for creating a more complex, largely deterministic model. The article presents results that will support mathematical models that can optimize the cultivation systems of these algae. Statistical models (linear or nonlinear regressions) can be useful and precise in automated production systems only when used in working intervals corresponding to the experimental ones.*

## REZUMAT

*În acest articol, autorii prezintă unele rezultatele ale cercetărilor privind o tehnologie de cultivare a algelor Chlorella Vulgaris, cu o instalație pilot tip cascadă în sistem deschis, într-un mediu nutritiv Bold Basal Medium, cu agitarea culturii prin recirculare și la temperatura ambiantă (cu control minim asupra încălzirii). În cadrul experiențelor au fost identificate elementele de monitorizare necesare pentru a furniza informațiile necesare pentru crearea unor modele matematice de creștere a algelor care să demonstreze viabilitatea sistemului de cultivare și care ar putea fi utilizate pentru optimizarea tehnologiei de cultivare a algelor. Acest articol prezintă, de asemenea, un model statistic elementar pentru predicția, prin intermediul regresiei liniare, a concentrației de biomasă a algelor. Predicţia poate constitui baza pentru crearea unui model mai complex, în mare măsură determinist. Articolul prezintă rezultate care vor susține modele matematice care pot optimiza sistemele de cultivare a acestor alge. Modelele statistice (regresiile liniare sau neliniare) pot fi utile și precise în sistemele automatizate de producție, numai atunci când sunt utilizate în intervale de lucru corespunzătoare celor experimentale.*

## INTRODUCTION

Climate change is a serious threat to our planet and one of the causes is the increase of carbon dioxide emissions, due to the use of fossil fuels (*Eggleston et al., 2006*).

According to some authors (*Grevé et al, 2011*) about 89% of the commercially produced energy comes from fossil fuels such as oil, coal or natural gas. The decrease of natural reserves, as well as the fact that the largest reserves of fossil fuels are in sensitive geopolitical areas, were also reasons that contributed to the orientation towards biofuels. The use of biofuels is currently expanding across the globe, as it offers more advantages than fossil fuels (they are nontoxic, biodegradable and renewable) (*Sână et al, 2011*). Also, according to European legislation and the literature, in the future limiting the amount of fuel from crops is intended and focusing on biofuels from non-food sources such as waste and algae.

In this context, biofuels produced from waste and residues have a lower impact on the environment, have no effect on food prices and do not replace other crops or forests.

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[1] *Nedelcu A., Ph.D. Eng.; Cârdei P., Mat., Ciupercă R., Ph.D. Eng.; Ştefan V., Ph.D. Stud. Eng.; Zaica A., Ph.D. Stud. Eng.*

Also, one of the variants tested in the last years for the production of biofuels are the algae.

Compared to other alternative sources of energy, algae have a number of advantages: they grow fast, they do not compete with food sources for agricultural land, they do not harm the environment if they are stored randomly, they do not affect the fresh water reserves, they are biodegradable and do not need fresh water to grow.

Algae fuel is part of the new-generation biofuel class. Algae are high-yield raw materials for biofuel production. They produce 30 times more energy per $m^2$ than crops on land (*Sână et al, 2011*). Taking into account the increasing prices of fossil fuels (oil), there is a growing interest in the algae culture for biofuel production.

For large-scale utilization and exploitation of algae, the major growth obtained by cultivating them is necessary. Algae cultivation allows producing algal biomass that can be used in practice.

Algae cultivation can be done under open conditions - in the field and closed - in the laboratory, in freshwater or saline waters that cannot be used for agriculture. The following culture systems are used to produce microalgal biomass: open, closed and hybrid (*Nedelcu et al, 2017*).

In the research of these cultivation systems in the laboratory, for the production of algal biomass required in advanced biofuel technology, it has been essential to achieve experimental conditions close to natural conditions and to find cost-effective solutions for increasing cell density.

In recent years, algae have begun to be studied and cultivated because of their high protein intake (*Guccione et al, 2014*) applications in medicine and biofuel production, (*Blinová et al, 2015; Lammers et al, 2017*). Algae are renewable and virtually inexhaustible sources. With the discovery of the medical potential of these algae, interest in this topic has grown enormously, there being thousands of articles and millions of searches on Internet networks (*Fu et al, 2016*). In fact, there are journals devoted to algae growth and exploitation, at the best publishing houses, (*Huesemann et al, 2016; 't Lamet al, 2017*). The prediction of algae cultures development is a topic frequently addressed in the literature on algae culture, (*Blanken et al, 2016; Huesemann et al, 2016*) and, also the extraction of useful substances from them, (*Jamsa et al, 2017; Posada et al, 2016*). Algal growth processes are also an interesting subject for the mathematical modelling, (*Malek et al, 2016; Surendhiran et al, 2015; Yang et al, 2017*).

## MATERIAL AND METHOD

For the laboratory study of the algae culture development under different growing conditions, but as close as possible to the natural ones (nutritive medium, lighting regime, agitation mode, ambient temperature), was created a functional plant model for algae cultivation in open system, presented in Figure 1, (*Nedelcu et al, 2018*). The aim of the research was to identify new hypothesis, to find simple, economical technical solutions applicable to industrial plants for obtaining algal biomass as a source of non-food raw material for the production of alternative biofuels. These activities will be completed by theoretical and experimental studies, such as (*Graham T., 2013* and *Posada et al, 2016*).

With this installation, is studied in the laboratory, the microalgae cultivation technology in open system, cascade type, in which culture agitation is carried out continuously by recirculating and sliding it in a thin layer on a transparent barrier plane so that all cells receive the amount of light needed for photosynthesis.

The algae culture, agitated by recirculation and sliding on flat surfaces and over barriers, forming small cascades, is exposed to light and heat in a uniform manner avoiding the phenomenon of self-shading. Monitoring and adjusting the pH of the culture in the plant to normal values is achieved with a pH controller, digital pH meter and $CO_2$ feed system.

The research was carried out in a technology for the cultivation of *Chlorella Vulgaris* algae, AICB 555 strain in a *Bold Basal Medium (BBM)* and under various cultivation conditions.

During the experiments the following activities were carried out:

- measurement of environmental parameters in which the experiments are carried out: air temperature and humidity, degree of illumination;

- measurement of nutritive medium parameters: pH, temperature, turbidity, conductivity, salinity;

In collaboration with the Faculty of Biotechnical Systems Engineering (ISB Bucharest), the following determinations were made for algae culture: at certain time intervals were harvested samples of algae culture grown in open system, cascade type plant, and were determined the density [g/cm$^3$], the dynamic viscosity [mPa s], biomass [g/100ml], absorbance, algal density [number of cells/ml].

The results of these experiments were presented in the Experimental Report and synthetically in the web page of the project *PN 16 24 04 04/2016* (*INMA, 2016*) and in the paper (*Nedelcu et al, 2018*). These culture parameters, which in model (1) - (2) do not appear, will be included in an extended model in a future study, after further research.

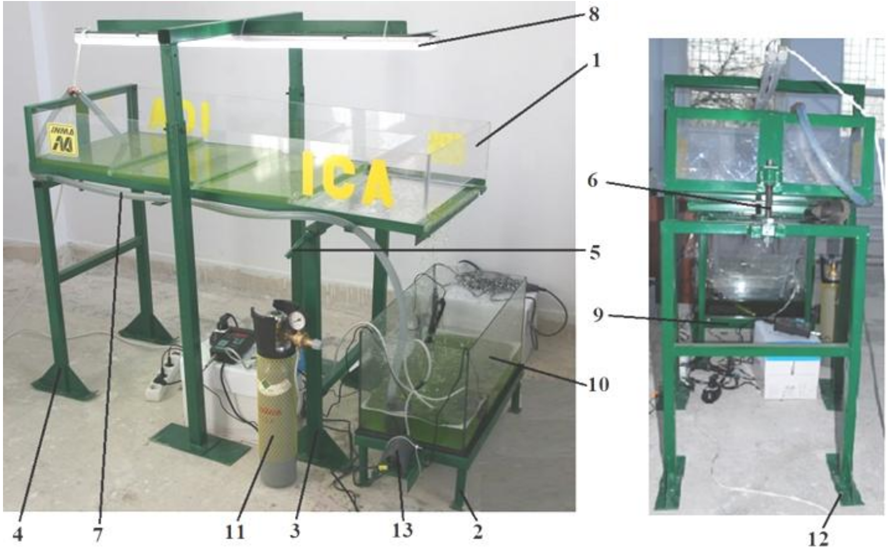


**Fig.1 - Plant for algae in open system, cascade type** (*Nedelcu et al, 2018*)

*1 - Cascade compartment; 2 - Collector compartment holder; 3 - Frame foot; 4 - Compartment support; 5 - Bolt 16; 6 - Adjustment screw; 7 - Recirculation system; 8 - Lighting system; 9 - Mounting bar; 10 - Collection compartment; 11 - $CO_2$ AAA Feed system + pH controller; 12 - Anchor bolt; 13 – Agitator*

## RESULTS

In this chapter are presented the main statistical characteristics of the experimental data, namely those that research the possible links between the variables. Experimental results are presented only graphically. Finally, a statistical model for prediction by linear regression of the algal biomass concentration is presented. The usefulness of data for constructing a deterministic mathematical model of the process, as well as the necessity of refining the experiments, is emphasized.

*1. Analysing the correlations between the amount of biomass and the process parameters*

The variation of the main process parameters during the experiments is shown graphically in Figure 2. To represent the experimental data as economical and unitary as possible, they were scaled to the maximum value, each.

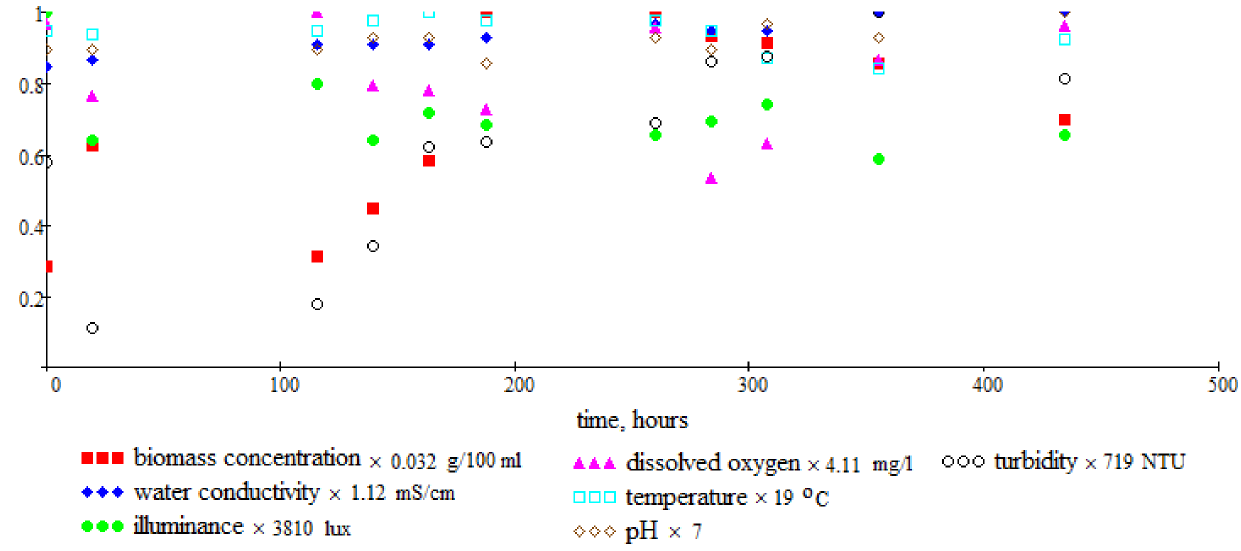


**Fig. 2 - Variation of process parameters over the observation time interval**

The variation of *Chlorella* algae biomass in relation to the main process parameters considered can be characterized in a first approximation using the main statistical characteristics.

**Table 1**

**Estimators of the relationship between biomass and process parameters**

| | Correlation | Covariance | Pearson correlation | Square of Pearson correlation | Slope | Intercept |
|---|---|---|---|---|---|---|
| Time | 0.631 | 0.671 | 0.631 | 0.398 | 0.000 | 0.014 |
| pH | 0.081 | 0.000 | 0.081 | 0.007 | 0.003 | 0.006 |
| Conductivity | 0.630 | 0.000 | 0.630 | 0.396 | 0.100 | -0.082 |
| Turbidity | 0.634 | 1.036 | 0.634 | 0.403 | 0.000 | 0.011 |
| Dissolved oxygen | -0.511 | -0.002 | -0.511 | 0.262 | -0.007 | 0.046 |
| Temperature | -0.237 | -0.002 | -0.237 | 0.056 | -0.002 | 0.063 |
| Salinity | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.022 |
| Illumination degree | -0.580 | -1.928 | -0.580 | 0.337 | 0.000 | 0.054 |

*1.1 Correlation (Pearson correlation)*

Correlation coefficient (Pearson) is a measure of the linear association of two variables, in other words, the extent to which the bivariate representation in the form of a dispersion diagram approaches a straight line. As it can be seen from Table 1, the correlation coefficient between biomass and time is positive. This shows that algae biomass concentration increases over time. Its value is 0.631, which, according to (Colton, 1974), includes the relation between the concentration of *Chlorella* algae biomass and time in the category of moderate relations. The form of dependence is, very likely, quite far from a linear one.

*1.2 Covariance*

The covariance shows that between biomass concentration and time, as well as turbidity, there is a direct connection (they increase and decrease simultaneously). The biomass concentration, according to the covariance values, is also connected by an inverse relationship to the soluble oxygen concentration, temperature and degree of illumination.

*1.3 Square of Pearson correlation*

This estimator shows to what extent the independent variable concerned explains the behaviour of the dependent variable. For example, time dependence explains in proportion of 39.8% the variation of the biomass concentration of *Chlorella* algae, in the conditions of the experiments performed. In a similar proportion (39.6%), the explanation of the evolution process of *Chlorella* algae biomass concentration is given by the behaviour of the solution conductivity. The same behaviour of the biomass concentration is explained more than 40% by the turbidity variation. As it can be seen in Table 1, significant explanations on the dependent variable behaviour (the concentration of *Chlorella* algae biomass) are also given by the degree of illumination (over 30%) and the dissolved oxygen concentration.

*1.4 Linear regression slope*

The linear regression slope of algae biomass relative to each of the process parameters considered has low values, indicating that the algal biomass concentration variation is slow relative to each of these parameters. Positive slope values indicate an increasing global variation over the parametric range considered, while the negative values indicate inverse variations in algal biomass concentration relative to the respective process parameter. The only positive value that draws attention in the table is the positive slope of the biomass concentration relative to the conductivity of the solution. However, the way the water conductivity varies shows that it is not controlled, but it takes values caused by effective control parameters and biomass concentration. The inverse variation of algal biomass concentration with the dissolved oxygen concentration is thus confirmed.

*1.5 Linear regression intercept*

The intercept corresponds to what in mathematics is called y-coordinate at the origin and means the value of the biomass concentration at the minimum level of the parametric interval considered, referring to each parameter in part. In our case, the meaning doesn't have an important physical sense; sometimes it does not even have a physical sense (the minimum value of intercepting the algal biomass concentration in relation to the conductivity of the solution).

*2. Variation over time of algae biomass concentration*

The variation over time of algae biomass concentration in the plant varied over 435 hours according to the curve in Figure 3 (also in Figure 2). Algal biomass growth curves, with this form, also appear in (*Apel et al, 2017; Fu et al, 2016; Jamsa et al, 2017; Lammers et al, 2017; Malek et al, 2016*). The polynomial approximations of the biomass concentration variation confirm the fact that the curve in Figure 1 belongs to the family of curves that characterizes the development of algal culture over time, as defined by the literature.

The calculation of biomass concentration variation over time is important for predicting the production process, for calculating productivity, generally for economic calculation. In fact, the main factors influencing the growth of algae culture (as well as of the living matter in general) are not represented by the time but by the real physical factors: temperature, conductivity, oxygen concentration, pH, degree of illumination, etc. Polynomial regressions of the 1$^{st}$, 2$^{nd}$, 3$^{rd}$ and 4$^{th}$ degree, of algal biomass concentration dependence on time, are given in Figure 4.

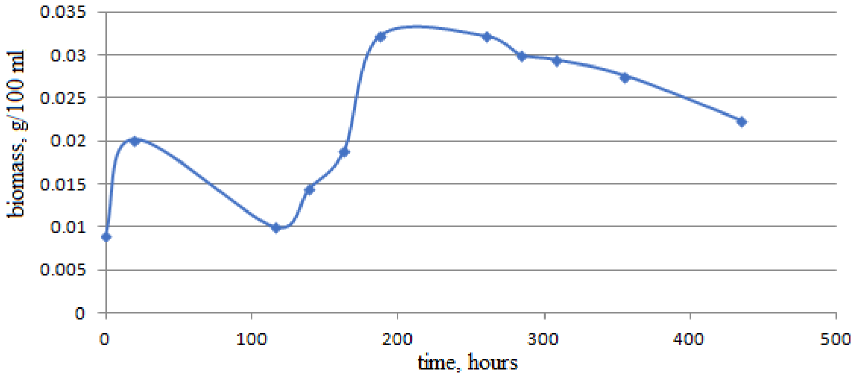


**Fig. 3 - *Chlorella* algae biomass variation over time**

Starting from the primary statistical conclusions given in Table 1, a relation of the following form is assumed for the biomass concentration function (Eq.1):

$$c = c(K, L, O, \theta, pH) \tag{1}$$

where *c* is the concentration of algal biomass, *K* is the conductivity of the solution, *L* is the degree of illumination, *θ* is the temperature, and *pH* is the pH of the solution. We chose the parameters on which the algal biomass concentration depends, relative to the correlation and the square of the Pearson correlation, in descending order. We eliminated turbidity, because we considered that, at least in the experiments performed, turbidity does not influence the process, but algae development influences the solution turbidity value, as well as salinity, which was constant during the process. The linear regression calculated starting from the experimental data gives the following linear approximation of biomass concentration, depending on the control parameters mentioned above:

$$c = -0.034 + 0.123K - 1.169 \cdot 10^{-6} L - 6.112 \cdot 10^{-3} O + 5.107 \cdot 10^{-4} \theta - 8.961 \cdot 10^{-3} pH \tag{2}$$

The distribution of Chlorella vulgaris algal biomass concentration values provided by the linear regression (1) in relation to the distribution of the experimental values for the corresponding data sets of the process control parameters (arguments of the function (Eq.2)) are given numerically and graphically in Figure 5.

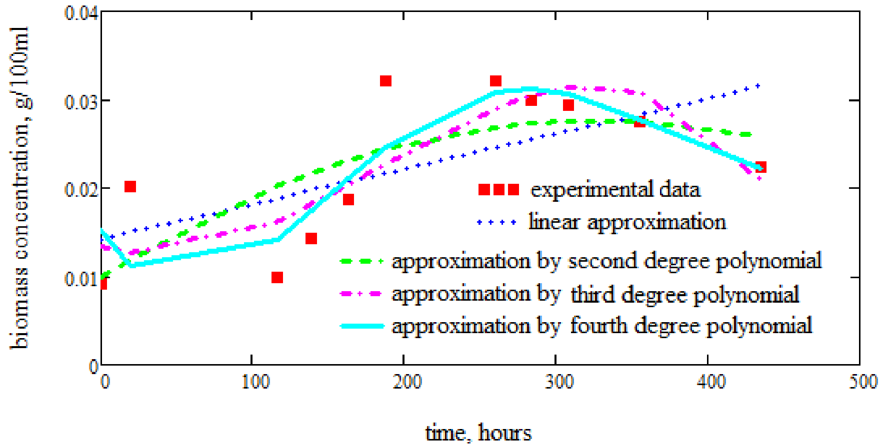


**Fig. 4 - Polynomial approximations of the variation over time of biomass concentration in the solution**

The values of algal biomass concentration, obtained in the experiments carried out in this project, fall within the limits given in the literature, (*Gokul A., 2013*).

experimental data

| | |
|---|---|
| 0 | $9.1 \cdot 10^{-3}$ |
| 1 | 0.02 |
| 2 | 0.01 |
| 3 | 0.014 |
| 4 | 0.019 |
| 5 | 0.032 |
| 6 | 0.032 |
| 7 | 0.03 |
| 8 | 0.029 |
| 9 | 0.028 |
| 10 | 0.022 |

linear approximation

| | |
|---|---|
| 0 | $7.646 \cdot 10^{-3}$ |
| 1 | 0.017 |
| 2 | 0.016 |
| 3 | 0.02 |
| 4 | 0.021 |
| 5 | 0.029 |
| 6 | 0.024 |
| 7 | 0.034 |
| 8 | 0.026 |
| 9 | 0.03 |
| 10 | 0.023 |

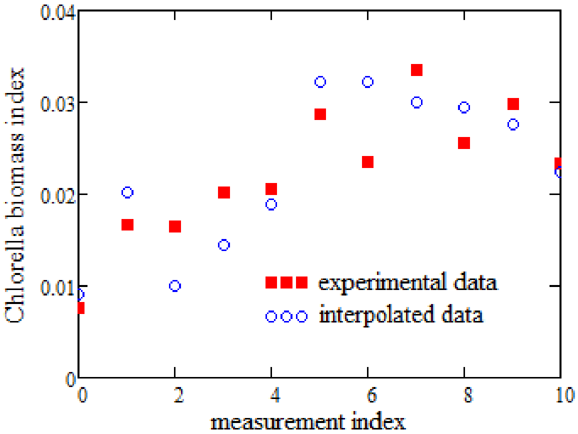


**Fig. 5 - Distribution of experimental data compared to that of multiple linear interpolation data**

The form of regression, which gives the biomass concentration dependence on to the control parameters, shows only that the increase of temperature and conductivity is favourable to the growth of algal biomass, while the increase of other parameters does not favour the algae culture development. A more detailed study can be made on culture growth rate. Throughout the experiments referred to in this article, the temperature was below 19°C. This value is, according to (*Lammers et al, 2017)*, appreciably below the optimal growing values of *Chlorella* algae culture (around 26°C – 34°C). Therefore, it is normal for temperature increase to favour the development of algal biomass in our experiments. If the experiments had been carried out in an atmosphere with the temperature values over 35°C, then probably the temperature coefficient would have been negative in (2). Decreasing the degree of illumination over almost the entire observation interval is also involved in decreasing the biomass concentration in the solution. The influence of light is confirmed by (*Lammers et al, 2017)*, although in other terms. The correlation coefficient between the algal biomass concentration and the degree of illumination (illumination) is significant and negative, so it confirms the emitted hypothesis, that of inverse variation of the algal biomass concentration with the illumination, at least on the observation interval, and we refer to the multidimensional range which also includes the variation intervals of the other process parameters.

The variation of the solution conductivity and pH is influenced by the culture medium according to *(Lizzul et al, 2014),* and are seen in this article as effects of algal growth and not, first of all, as factors of influence for the development of algal culture. We can observe situations in which the conductivity and pH of the solution, the algae develops in, grow or decrease and they can also have approximately constant limits. On the other hand, many of the articles study the influence of process parameters on the content, in certain substances, of algae obtained, according to the purpose they are produced for. Some conditions and technologies favour the content of substances favourable to transforming algae into biofuels, others into food or medicine. In (*Qiu et al, 2017)*, for example, an optimal value of 6.0 is found for pH.

Obviously, regressions with higher-grade polynomials were more efficient, but for these we do not have enough data, more experimental records being necessary.

The fact that the variation of the curve that gives biomass concentration dependence over time (Figures 3 and 4) decreases towards the end of the observation interval, may also be due to the slight increase in the solution pH from 6.25, at the beginning of the process, to 6.5 and even 7 at its end. This hypothesis is suggested by the results presented in (*Ma et al, 2016,* Figure 3, p. 439).

A full discussion about the influences of all the important parameters on algae growing processes depends on the technology adopted. In this respect, important systematizations have already been made. Algae and water are the raw material, carbon dioxide and nutrients are the feed parameters, sunlight or other light, but also temperature, pH, conductivity, and possibly water salinity, are parameters for controlling and adjusting the process. Systematization also includes bioremediation processes related to the production of biofuel from algae. Finally, obviously, any discussion will lead to the estimation of the used technology efficiency. Comparative terms are found in an extremely rich literature.

## CONCLUSIONS

1. A first conclusion is that the technology is functional, the final product is made and, depending on the process control parameters, the algae production can be increased.
2. The main parameters influencing the growth of algae culture are the feed parameters (the amount of BBM introduced and the amount of carbon dioxide introduced in the solution) and the environmental parameters: temperature, pH, culture illumination. The control parameters can be conductivity and turbidity of the solution. Water salinity can influence the development of the culture, but since it has been kept constant throughout the process, no conclusions can be drawn about its role.
3. Thermal control has been reduced as much as possible in a period with ambient temperatures of 6-18°C. As shown in the literature, an advantage of the algae culture growth is to maintain an optimal temperature (26-34°C). For the next level of experimentation, it will be proposed to scan the optimal temperature range and the adjacent intervals. We will compare quantitatively and qualitatively the productions obtained under these conditions. Then, by making the report between the process cost and the quantitative and qualitative results, it is possible to choose an improved cultivation technology.
4. The comparisons made between these experiments and the ones described in the literature, as well as in the literature on algal growth mathematical modelling, show that monitoring the nutrient and carbon dioxide supply in the algae environment is also necessary. These requirements are very important to obtain the information needed to build mathematical models that can be used to optimize algal culture technologies. Algal culture growth monitoring must be done at a resolution that records nutrient and carbon dioxide supply events, as well as the significant variations in field parameters involved in the process.
5. The formulation of a primary mathematical model, but comprehensive enough, following these experiments, will facilitate a sufficiently complete planning of the following experiments that must be performed in order to obtain an optimal and efficient algae culture technology. Statistical models such as linear or nonlinear regressions can be very useful and precise, so they can be used in automated production systems only when used in small enough dimension working intervals. These models can be elaborated after the complex mathematical modelling which takes into account the influences of all the parameters of the algal growth process and precisely determines those intervals.

## ACKNOWLEDGEMENT

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