Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 7 (19): 2848-2855, 2007
ISSN 1812-5654

An Algorithm for Modeling and Simulation of Microalgea Production

[1]O. Akpolat and [2]S. Eriştürk
[1]Department of Bioengneering, Faculty of Engineering, Ege University, 35100, Bornova, Izmir, Turkey
[2]Graduate School of Natural and Applied Sciences, Bioengineering Branch,
Ege University, 35100, Bornova, Izmir, Turkey

Abstract: *Heamatococcus pluvialis* is a green microalgea to have a great interest for production of natural astaxhantin and it can be cultivated in a closed photobiorector system under controlled conditions. Biomass composition, growth rate and high value product spectra like polyunsaturated fatty acids, pigments, poly saccariydes or vitamins depend on strongly cultivation parameters. These are composition of cultivation medium, mixing model and aeration rate, hydrodynamic stress of medium which can be changed by adding some chemicals, cultivation temperature, pH, carbon dioxide and oxygen supply and most important of all: illumination. Cultivation kinetics, cultivation process and scale-up of the process are able to be modeled as a function of biomass concentration and average irradiance in the reactor for the simulation of all the process. In this study based on the simulation of the *Heamatococcus pluvialis* production, biomass measurements of the cultivation were carried out experimentally to determine kinetic parameters for modeling of the process. All study consists of three parts; these are a basic for mathematical equations stated cultivation kinetics, cultivation process and scale-up of the process, an algorithm for the solution of the model equations and illustration of the simulation results.

Key words: Photobiorector, microalgea, simulation

INTRODUCTION

Microalgea has a great importance by producing of high value chemicals and their proven antiviral activities and by ecologically role in climate modeling as the biggest primary producers of oxygen worldwide (Csögör *et al*., 1999). Microalgea can synthesis themselves organic materials to need for cell activities by photosynthesis. Intermediate metabolites by photosynthesis are basically proteins, fatty acids, keratenoides, vitamins, antibiotics and lots of high value chemicals. *Heamatococcus pluvialis* is also a green microalgea grown authotrofically and it has a four cell type following each other along its life cycle.

These are phase of microzoides, phase of macrozoides with big flagelies, phase of immobile palmella form and phase of hematokists having bigger red cells with thick cell membrane. As the culture medium having lower level concentration of nutrients during the cultivation, the number of cells in palmella phase change their structure and they go into hematokist phase and then astaxhantin accumulation in the cells follows this phase. Astaxhantin is a ketakeratoneid with red color and high value pigments to give pink color to bodies of lots of sea animals like salmon, shrimp or crab (Eriştürk and Akpolat, 2005).

During the cultivation of algae like *Heamatococcus pluvialis* growth of culture is determined by quantitative and qualitative methods. Qualitative methods are basically cell numbering, optical density, dry end wet weight, amount of chlorophyll and directly measuring of carbon context (Eriştürk and Akpolat, 2005; Boussiba, 2000). Biomass composition, that means growth rate and product spectra depend strongly on the cultivation conditions. These are composition of medium, temperature, pH, carbon dioxide and oxygen supply and most important of all: illumination. Microalgeas as photosynthetic organisms need carbon dioxide and light within the Photosynthetically Active Radiation (PAR) to obtain energy. The wave length range of the PAR is between 400 to 700 nm, which is equal to visible light. Additionally it could be remembered that the use of excessive light and oxygen in cultivation has harm full effects. The radiation can be quantified adequately by the photon flux density (quantum of photons per area and time) (Csögör *et al*., 1999; Molina *et al*., 2001).

As to modeling of the cultivation kinetics, the cultivation process and the scale up of the process, because of the complex interactions between the production parameters of microalgea there is a big demand to develop a model which predicts the behavior of the whole system and a software algorithm by using

Corresponding Author: O. Akpolat, Department of Bioengneering, Faculty of Engineering, Ege University, 35100, Bornova, Izmir, Turkey

numerical and optimization techniques for solving mathematical equations in the model (Csögör *et al*., 1999; Lübbert *et al*., 2000, 2001; Bailey and Ollis, 1986; Smith, 1970; Türker, 2005).

MATERIALS AND METHODS

Here it is discussed the biomass production of algae experimentally and its mathematical modeling. Improved algorithm to solve the model equations has some main and sub algorithms to be explained details later on.

Experimental: In this study *Heamatococcus pluvialis* was cultivated for modeling purposes in a panel glass photobioreactor of 1.5 L volume shown in the experimental set-up on Fig. 1 under 75 μE m^{-2} s^{-1} light flux at the vessel surface at 25°C room temperature. The width of the reactor is 5 cm and the air sending to the reactor consists of CO_2 of 1.5% V. The aeration rate was measured as 1.5 L min^{-1} by flow meter and *Heamatococcus pluvialis* strain U was used for inoculation in BG11 medium given in Table 1 (Sukatar, 2002).

Strains stored in agar at carrying glasses was firstly inoculated on solid agar with BG11 medium and incubated along 15 days. Later stock culture was prepared by transferring of inoculated strain into tube of 70 mL, flask of 250 mL and pyrex glasses of 1 and 5 L, respectively.

The sample of the volume to need for inoculation of the reactor medium was taken into sterilized flask firstly. After removing supernatant phase by precipitation of cells, fresh medium was added on the cells. This inoculated culture was grown along 4 days under 75 μE m^{-2} s^{-1} light flux and 1 L min^{-1} aeration rate conditions. Starting culture is 4×10^5 cell numbers L^{-1} or 0.48 g L^{-1} for all experiments to do as three parallels. Reactors were sterilized by sodium hypo chloride solution of 1% w/v and cell accounting, biomass, chlorophyll-a, pH and viscosity measurements were repeated at the same time interval for all cultivation. Biomass measurements for the reaction conditions were given in Table 2 (Eriştürk and Akpolat, 2005).

Biomass amounts measured experimentally were evaluated statistically using main programs coded 01 and related sub programs explained in modeling algorithm in part 2.4 and growth curve of the cultivation was lined. Statistically evaluation of the experimental results was also carried out by student t-test in a probability level of 95%. This test for a sample group with N elements consists of basically following steps (Ikiz *et al*., 1996).

- Zero hypothesis (H_0): It is test hypothesis, here, x_m, x_0, d_f, P_b, σ, t-Calc and t-test show mean value, median value, degrees of freedom, probability level, standard deviation, calculated t value (or distribution function) and theoretical test t value from t-Table, respectively.

Table 1: Composition of the culture medium

Chemical	Concentration (mg L^{-1})
$NaNO_3$	1500.00
Na_2CO_3	20.00
K_2HPO_4	40.00
H_3BO_3	2.86
$MgSO_4.7H_2O$	75.00
$MnCl_2.4H_2O$	1.81
$CaCl_2.2H_2O$	36.00
$ZnSO_4.7H_2O$	0.22
Sitrik Asit	6.00
$Na_2MoO_4.2H_2O$	0.39
Amonium Ferric Sitrate	6.00
$CuSO_4.5H_2O$	0.08
EDTA-Na_2	1.00
$Co(NO_3)_2.6H_2O$	0.05

$$x_m = \frac{\Sigma x_i}{N} \quad (1)$$

$$d_f = N\text{-}1 \quad (2)$$

For N<30

$$\sigma = \sqrt{(x_i - x_m)/N - 1} \quad (3)$$

$$t - calc = \frac{x_m - x_0}{\frac{\sigma}{\sqrt{N}}} \quad (4)$$

- Test statistics: t-statistics value (t-test) is determined from t-test table related to degrees of freedom and probability level.
- If t-Calc<t-test, (H_0), is on an acceptable area and the sample group has a meaningful statistically.
- Regression analysis is done for this sample group at last.

Modeling of cultivation kinetics and cultivation process: A photosynthetic reaction in a microalgea cell is basically as follows:

$$6\,CO_2 + 6\,H_2O + light \rightarrow C_6H_{12}O_6 + 6\,O_2$$

Glucose produced by this reaction is used for energy to need later either in anabolic or katabolic reactions or other cell activities by breaking down into CO_2 and H_2O, or directly starting materials for smaller molecules like alcohols and acids produced enzymatically in catabolic path ways or for larger molecules like amino acids and pigments produced enzymatically in anabolic path ways (Bailey and Ollis, 1986; Türker, 2005).

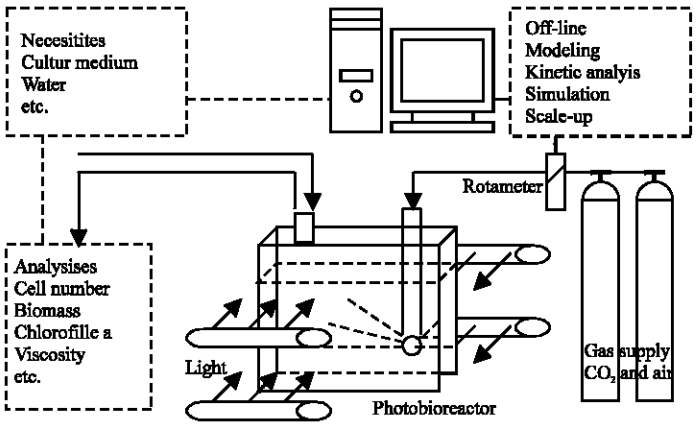

Fig. 1: Schematic view of the experimental set-up

Table 2: Biomass measurements for the cultivation of *Heamatococcus pluvialis*

Time (day)	0	1	2	3	4	5	6	7	8	9	10
Biomass (g L^{-1})	0.48	0.50	0.84	1.32	1.80	2.52	2.76	3.72	4.08	4.20	3.96

Biomass productivity or specific growth rate (μ) (h^{-1}) for microalgea was expressed as follow kinetic model basically depending on average irradiance ($\mu E\ m^{-2}\ s^{-1}$) (Molina, 2001)

$$\mu = \mu_{max} \frac{I_{AV}^{\ n}}{I_{AV}^{\ n} + I_{AK}^{\ n}} \quad (5)$$

where, μ_{max}: maximum specific growth rate (h^{-1}), I_{AV}: an average irradiance constant ($\mu E\ m^{-2}\ s^{-1}$), I_{AK}: a constant dependent on algal species and cultivation conditions ($\mu E\ m^{-2}\ s^{-1}$) and n is an empirically established exponent.

Modeling of cultivation bases on batch process according to cell and cultivation rate in this model is shown by an ordinary differential equation as an initial value problem.

$$\frac{dX}{dt} = \mu X \quad (6)$$

where, μ: specific growth rate (h^{-1}), X: measured biomass concentration (g L^{-1}) and dX/dt: cultivation rate (g $L^{-1}\ h^{-1}$) (Erişturk and Akpolat, 2005).

Euler method for numerical solution of these types of equations is the simplest integration technique, which goes on following steps.

$$t_{i+1} = t_i + \Delta t \quad (7)$$

$$X_{i+1} = X_i + (\mu X_i)\Delta t \quad (8)$$

where, $t_i = t_0$ and $X_i = X_0$ are initial values of the problem and Δt is the time interval chosen for the numerical solution (Lübert, 2000). Calculated values of the variables by solving this differential equation depending on the initial values are used to plot simulation curves of the process.

Calculating the data of simulation needs to determine kinetic parameters in the kinetic model proposed for the experimental results in this work, which are μ_{max}, I_{AV}, I_{AK} and n, but I_{AV} was only measured directly here. For that reason, an optimization function based on biomass concentration was written depending on μ_{max}, I_{AK} and n values and minimized this function to give the sum of differences between the calculated (or simulated) by the solution of the differential equation and the experimental results.

$$P = \sqrt{\sum_{i=1}^{m} (X_{Calculated} - X_{Experimental})^2} \quad \rightarrow \quad Min \quad (9)$$

During the optimization of the calculated values for P function related with the case depending on chosen μ_{max}, I_{AK} and n values for minimization, minimal P to be found is optimal case and the chosen values for it are the kinetic parameters in the kinetic model proposed for the experimental results. Additionally, the optimization function results for all cases were evaluated statistically by student t-test as described earlier.

Finally, biomass-time simulation curves for different initial biomass concentration were plotted by using the

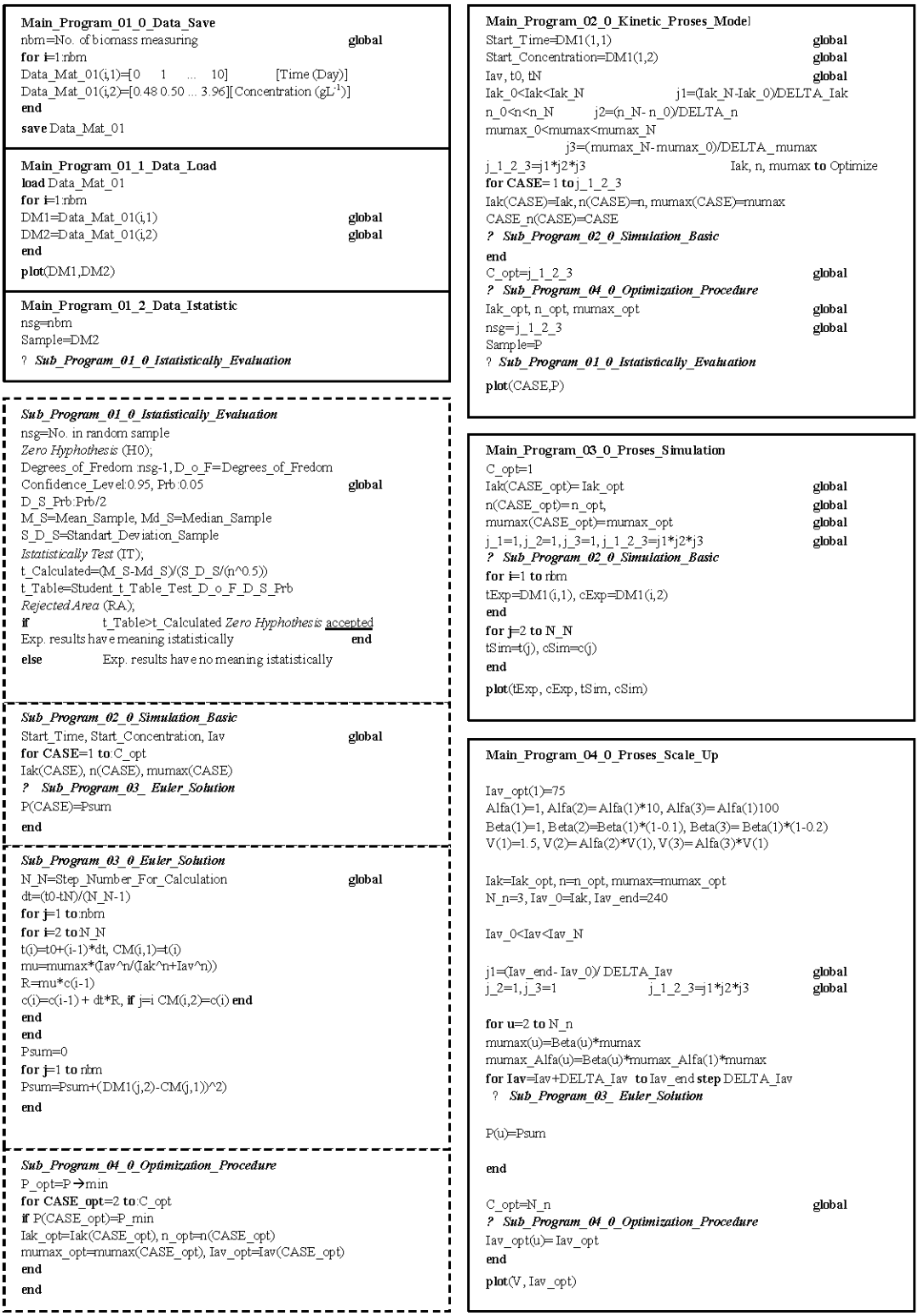

Fig. 2: Algorithm for modeling

kinetic parameters optimized. Here it is accepted that maximal productivities all simulations are the same as that of the experimental but reached in the shorter time than those of the experimental linearly. Calculation of the kinetic parameters, statistically evaluation of the optimization results and simulation of the cultivation process were carried out by using main programs coded 02 and 03 and related sub programs in modeling algorithm.

Modeling of scale-up: Because the objectives are different, operating conditions and/or reactor types are usually different for laboratory and large scale units and the global rate from the laboratory reactor can not be used directly for large scale apparatus. If all resistances to all transfers, internal or external, like heat and mass are significant, there will be a different global rate in the commercial-scale reactor for the conversion to be same as that of laboratory reactor (Smith, 1970).

Basically the conversions of chemical or biochemical reaction in the experimental conditions can be done also for larger volume reactors by scale up. In this part modeled of the scale up for microalgea cultivation because of the changes in reaction rates along with scale-up, as being the volumes larger by α_i times according to that of the experimentally the specific growth rates were accepted to be diminished by γ_i% according to that of laboratory conditions for the same conversions or the same growth rates of the microalgea cultivation. Model equations for this case are as follows, (i = 1 to m and m = 3);

$$\alpha_{i+1} = \alpha_i * 10 \quad (10)$$

$$V_{i+1} = \alpha_i V_i \quad (11)$$

$$(X)_{\alpha i+1} = (X)_{\alpha i} \quad (12)$$

$$(\mu)_{\alpha i+1} = (\mu)_{\alpha i} \quad (13)$$

$$\left(\frac{dX}{dt}\right)_{\alpha i+1} = \left(\frac{dX}{dt}\right)_{\alpha i} \quad (14)$$

$$(\mu)_{\alpha i} = (\mu_{max})_{\alpha i} * \frac{\left[(I_{AV})_{\alpha i}\right]^n}{I_{AK}{}^n + \left[(I_{AV})_{\alpha i}\right]^n} \quad (15)$$

$\gamma_1 = 0$ and $\beta_1 = 1$ $$\beta_{i+1} = \beta_i\left[1 - (\gamma_i + 0.1)\right] \quad (16)$$

$$(\mu_{max})_{\alpha i+1} = \beta_i (\mu_{max})_{\alpha i} \quad (17)$$

Calculating of I_{AV} depending on changes of volume and of maximum specific growth rate during scale-up can be carried out by optimizing of I_{AV} similar to those of I_{AK} and n calculating. The optimization procedure was given earlier in details.

The numerical solution of the mathematical model equations explained for scale-up were conducted by using main programs coded 03 and related sub programs in modeling algorithm.

Algorithm for modeling: An algorithm for modeling which of steps given in earlier for algea cultivation kinetics, cultivation process and scale-up of the process was shown in Fig. 2.

This algorithm consists of six main programs and four sub programs. The programs in the algorithm are as follows;

Main_Program_01_0_Data_Save
Main_Program_01_1_Data_Load
Main_Program_01_2_Data_Statistics
Main_Program_02_0_Kinetic_Process_Model
Main_Program_03_0_Process_Simulation
Main_Program_04_0_Process_Scale_Up
Sub_Program_01_0_Statistics_Evaluation
Sub_Program_02_0_Simulation_Basic
Sub_Program_03_0_Euler_Solution
Sub_Program_04_0_Optimization_Procedure

RESULTS

A computation program based on the modeling algorithm given earlier can be written by any software like Visual Basic versions, MatLab versions, Matematica versions or etc. Calculated results by the algorithm were explained as follows.

Statistically evaluation of microalgea growth: The growth rate of *Heamatococcus pluvialis* plotted by using the data experimentally was given in Fig. 3. It is understood that biomass amounts in the 9th day reaches to a maximal of 4.2 g L^{-1} by an initial cell concentration of 0.48 g L^{-1} accepting of an average irradiance inside the reactor of 75 μE m^{-2} s^{-1} to be the same as that of on the surface of the reactor and it tends toward being lower following the 9th day. Measured all biomass values experimentally was evaluated by student-t-test for 0.95 probability and it was found them meaningful statistically.

Simulation of cultivation kinetics and cultivation process: As explained earlier details, the values of maximum specific growth rate (μ_{max}; h^{-1}), irradiance

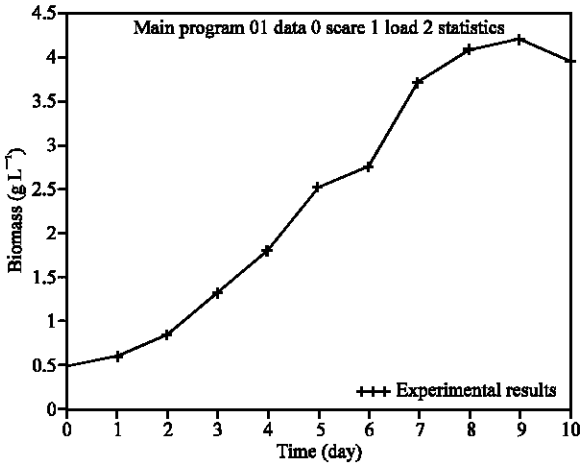

Fig. 3: The growth rate of microalgea for ten days

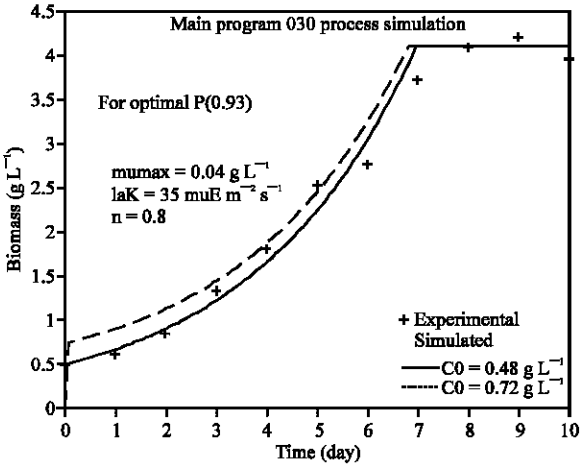

Fig. 5: Simulation curves of the microalgea productivity for different initial biomass concentrations

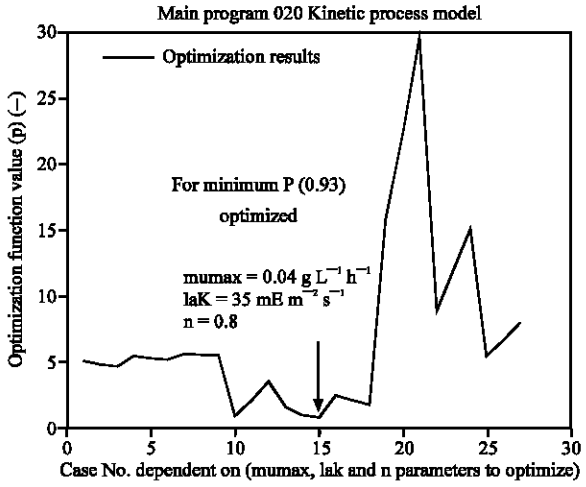

Fig. 4: Optimization function results

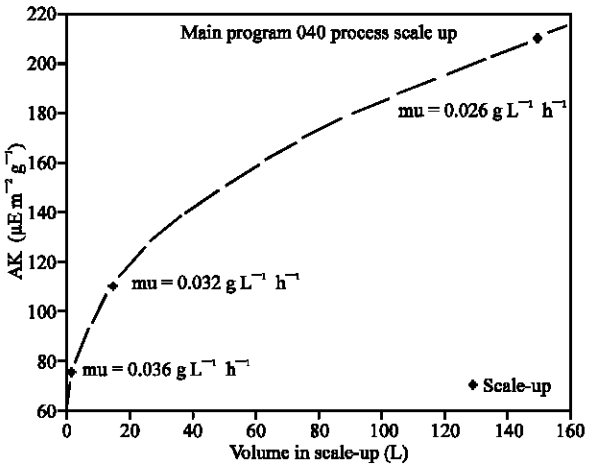

Fig. 6: Variation of average irradiation amounts depends on volumes changed in scale-up

constant dependent on algal species and cultivation conditions (I_{AK}: µE m^{-2} s^{-1}) and empirically established exponent (n) for the kinetic model were chosen for the parameter groups in optimization procedure and each group were numbered optimization case. Biomass amounts were simulated and optimization function values for all cases were calculated using by simulation results. The parameters in the case with minimal p-value are optimal for kinetic model proposed for the experimental results. The graphic of P function values dependent on kinetic parameters was plotted on Fig. 4.

Chosen parameters for optimization were in the following intervals.

$$0.02 \le \mu_{max} \le 0.06 \quad (18)$$

$$20 \le I_{AK} \le 50 \quad (19)$$

$$0.6 \le n \le 1 \quad (20)$$

As understood from the Fig. 4 determined kinetic parameters for optimal P (minimal P) value are μ_{max} = 0.04 g L^{-1}, I_{AK} = 35 and n = 0.8. Additionally the group of the calculated optimization function values was evaluated by student-t-test for 0.95 probability level and it was found that it has meaningful statistically.

Simulation curves of the microalgea productivity for different initial biomass concentrations based on determined kinetic parameters earlier were lined on Fig. 5. As shown in Fig. 5 initial biomass concentration was increased by 50% from 0.48 to 0.72 g L^{-1} depending on that of experimental.

Scale-up: Chosen reactor volumes for scale up were 1.5, 15 and 150 L increasing by 10 times and it was accepted

that the specific growth rates depending on the changed reactor volumes decrease by 10% for each step of scale up. Calculated average irradiation amounts for the same conversions or the same growth rates for three different volumes of the reactors by scale up were given in Fig. 6 and these were 75, 110 and 210 μE m^{-2} s^{-1}, respectively, that means they increases of 30 and 50% approximately in average irradiations.

CONCLUSION

In this study biomass amounts were measured for the production *Heamatococcus pluvialis*, a green microalgea, in an experimental reactor of 0.5 L volume with an initial cell concentration 0.48 g L^{-1} and under an average irradiance of 75 μE m^{-2} s^{-1}. First a kinetic model as follows was proposed as given in literature

$$\mu = \mu_{max} \frac{I_{AV}^{\ n}}{I_{AV}^{\ n} + I_{AK}^{\ n}}$$

then the cultivation process was mathematically modeled as a function of growth rate and biomass production expressed by an ordinary differential equation depending on an initial microalgea concentration as follows

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

Simulation curves of biomass production were lined solving the kinetic and the process model equations numerically to optimize μ or μ_{max}, I_{AK} and n by minimization of P function consisting of their different values in a chosen interval. The optimization function was stated as the sum of differences between calculated and experimental biomass values as follows:

$$P = \sqrt{\sum_{i=1}^{m} (X_{Calculated} - X_{Experimental})^2} \quad \rightarrow \quad Min$$

Additionally a scale-up modeling based on experimentally results was improved and finally, an algorithm was written for all numerical solution and statistically evaluation.

Calculated kinetic parameters by the algorithm were found as μ_{max} = 0.026 h^{-1}, I_{AK} = 35 μE m^{-2} s^{-1} and n = 0.8.

ACKNOWLEDGMENT

This study as a part of master thesis was financially supported by Ege University Research Fund, project number 2003 Muh 040. We also thankfully acknowledge for the financial support to Ege University.

NOTATION

d_f	:	Degrees of freedom (-)
H_0	:	Zero hyphothesis (-)
I_{AK}	:	Irradience constant (μE m^{-2} s^{-1})
I_{AV}	:	Average irradience constant (μE m^{-2} s^{-1})
N	:	No. of element in sample group (-)
n	:	Emprically estabilished exponent (-)
P_b	:	Probability (%)
P	:	Optimization function (-)
t-Calc	:	Calculated t-value (-)
t-test	:	Test t-value (-)
t	:	Time (Day)
V	:	Volume (L)
x	:	Conversion (%)
X	:	Biomass concentration (g L^{-1})
x_m	:	Mean value (-)
x_0	:	Median value
α	:	Proportion invariable (-)
β	:	Proportion invariable (-)
Δ	:	Delta symbol (-)
σ	:	Standard deviation (-)
Σ	:	Sum operator
μ	:	Specific growth rate (h^{-1})
μ_{max}	:	Maximum specific growth rate (h^{-1})

REFERENCES

Bailey, E.B. and D. Ollis, 1986. Biochemical Engineering Fundamentals. 2nd Edn., McGraw Hill Book Company.

Boussiba, S., 2000. Carotenogesis in the green algea *Heamatococcus Pluvialis*: Cellular physiology and stressres*ponse*. Physiologyia Pllantorum, 108: 111-117.

Csögör, Z., M. Herrenbauer, I. Perner, K. Schmidt and C. Posten, 1999. Design of a photo-biorector for modeling purposes. Chem. Eng. Proc., 38: 517-523.

Eriştürk, S. and O. Akpolat, 2005. Optimization And Simulation Of Physical Parameters Affecting Production Of *Haematococcus pluvialis*. In: The Photobiorectors. MS Thesis, Institute of National and Applied Science, Branch of Bioengineering Ege University, Izmir.

Ikiz, F., H. Püskülcü and Ş. Eren, 1996. Introduction to Statistics (Istatistiğe GiriŞ), 4th Edn., Barış Yayın. Fakülteler Kitabevi, Bornova.

Lübbert, A., R. Simutis, N. Volk and V. Galvanauskas, 2000. Hands on Course on Biochemical Process Optimization and Control, Martin Luther Universität Halle Wittenberg.

Lübbert, A., P. Kieran and M. Beroviè, 2001. Optimization of Bioprocesses, Bioprocesses Engineering Course. The Europian Federation of Biotechnology, Faculty of Chemistry Technology, pp: 286-302.

Molina, E., F.G. Acièn Fernandez and Y. Chisti, 2001. Tubular photobioreactor design for algal cultures. J. Biotechnol., 92: 113-131.

Smith, J.M., 1970. Chemical Engineering Kinetics, 2nd Edn., McGraw Hill Book Company.

Sukatar, A., 2002. Methods of Algea Culturing (Alg Kültür Yöntemleri), Ege Üniversitesi Yayınları, pp: 184-168.

Türker, M., 2005. Bioreaction Engineering (Biyoreaksiyon Mühendisliği), Su Vakfı Yayınları, Istanbul.