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Brazilian Journal
of Chemical
Engineering

ISSN 0104-6632
Printed in Brazil
www.abeq.org.br/bjche

Vol. 33, No. 04, pp. 773 - 781, October - December, 2016
dx.doi.org/10.1590/0104-6632.20160334s20150016

SIMULATION OF MICROALGAL GROWTH IN A CONTINUOUS PHOTOBIOREACTOR WITH SEDIMENTATION AND PARTIAL BIOMASS RECYCLING

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(Submitted: January 12, 2015 ; Revised: June 7, 2015 ; Accepted: June 24, 2015)

Abstract - Microalgae are considered as promising feedstocks for the third generation of biofuels. They are autotrophic organisms with high growth rate and can stock an enormous quantity of lipids (about 20 – 40% of their dried cellular weight). This work was aimed at studying the cultivation of *Scenedesmus obliquus* in a two-stage system composed of a photobioreactor and a settler to concentrate and partially recycle the biomass as a way to enhance the microalgae cellular productivity. It was attempted to specify by simulation and experimental data a relationship between the recycling rate, kinetic parameters of microalgal growth and photobioreactor operating conditions. *Scenedesmus obliquus* cells were cultivated in a lab-scale flat-plate reactor, homogenized by aeration, and running in continuous flow with a residence time of 1.66 day. Experimental data for the microalgal growth were used in a semi-empirical simulation model. The best results were obtained for $F_w = 0.2F_I$, when R = 1 and k_d = 0 and 0.05 day^{-1}, with the biomass production in the reactor varying between 8 g L^{-1} and 14 g L^{-1}, respectively. The mathematical model fitted to the microalgal growth experimental data was appropriate for predicting the efficiency of the reactor in producing *Scenedesmus obliquus* cells, establishing a relation between cellular productivity and the minimum recycling rate that must be used in the system.
Keywords: Continuous photobioreactor; *Scenedesmus obliquus*; Recovering; Sedimentation; Recycling rate; Downstream.

INTRODUCTION

Microalgae have gained much attention for biofuel production due to their high capability of storing value-added energy compounds. The chemical composition of such compounds encompasses starches and highly saturated fatty acids convertible to neutral lipids, which play an important role in production of bioethanol and biodiesel. This feature, along with high growth rates and ease of cultivation, make microalgae very promising when compared to higher plants (Rawat *et al.*, 2013; Khan; Bahadar, 2013; Silva and Bertucco, 2016).

Scenedesmus obliquus is a microalga that has been widely studied because of its high cellular productivity and accumulation of value-added energy compounds. Several works relying on its cultivation have investigated a variety of aspects, including types of culture medium and substrate, which have been tested in bench or continuous systems, also employing different intensities of light. However, studies on the efficiency of biomass recycling coupled to the photobioreactor either through simulation or experimental data are still required (Vigeolas *et al.*, 2012; Yin-Hu *et al.*, 2012; Baky *et al.*, 2013; Wang *et al.*, 2013; Wu *et al.*, 2013; Lee *et al.*, 2013;

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Kim *et al*., 2014). In order to study the sustainability of the cellular production process, a full analysis of the steps subsequent to the reactor is necessary, i.e., from the upstream to downstream sections, in such a way that the whole process can be optimized and consolidated.

Sedimentation, centrifugation, conventional- and ultra-filtration, floculation and flotation are the most used unit operations for cellular biomass recovery (Mata *et al*., 2010). Gravitational sedimentation particularly has many advantages in comparison to other unit operations, for instance, low cost for achieving a controlled process, margin for scaling-up, and ease of separating supernatant with minimum operating cost, mainly when pumping is involved. Conversely, it is a time-consuming operation that gives rise to a probability of biomass deterioration occurring during the process (Rawat *et al*., 2013).

Partial mass recycling could be used to reduce costs associated with the inoculum preparation and shorten the production time, as well as obtain high cellular concentration in the reactor. Nevertheless, the recycling rate must be carefully taken into account by considering operating kinetic parameters of the microalgal culture, integrating both separation and production systems.

In this work, *Scenedesmus obliquus* was used as a model microorganism for investigating microalgal growth in a two-stage photobioreactor-settler system with partial biomass recycling. The main objective was to develop a mathematical model capable of predicting the recycling rate as a function of kinetic parameters of the microalgal growth and operating conditions of the bioreactor.

MATERIALS AND METHODS

Experimental Part

S. obliquus cultivation was performed using BG-11 medium (Rippka *et al*., 1979) with doubled concentration and unlimitated nutrients (Figure 1). Microalgae cultures were sustained in solid medium by adding agar (10 g L^{-1}) to the BG-11 medium. Pre-inocula of *S. obliquus* were cultivated in flasks at approximately 100 μE m^{-2} s^{-1} and held in the exponential phase. The simplified plant was fed with CO_2 in excess conditions (5% in air), while maintaining pH at 8 with 10 mM HEPES buffer in order to prevent the culture medium from acidifying. *S. obliquus* growth was monitored each 24 h by means of optical density measurements at λ = 750 nm (UV-visible Spectro, Spectronic Unicam), cell counting in a Burker counting chamber (HGB®, Germany), and dried weight determinations (Sforza *et al*., 2012).

The inoculum cellular concentration within the reactor tank had an optical density of 0.5 at λ = 750 nm. The reactor was illuminated with a LED lamp (Light Source SL 3500, Photon System Instruments) whose effective light intensity was measured for both continuous and bench operations with a DeltaOhm HD2102.1 radiometer positioned at same distance as between the reactor and lamp. The photobioreactor used in the experiments had a flat-plate layout which is depicted in Figure 2. The experimental parameters are summarized in Table 1.

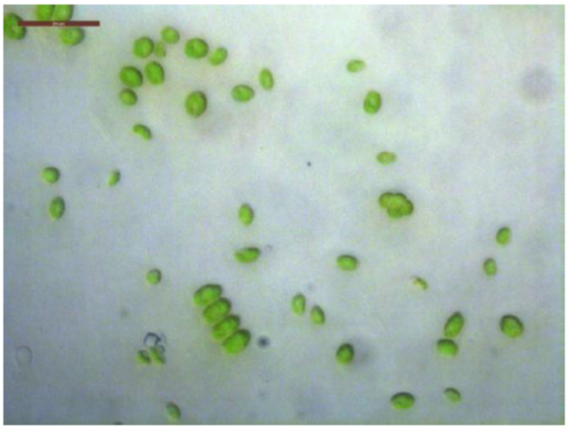


Figure 1: Optical microscopic image of *Scenedesmus obliquus* (magnification of 100x).

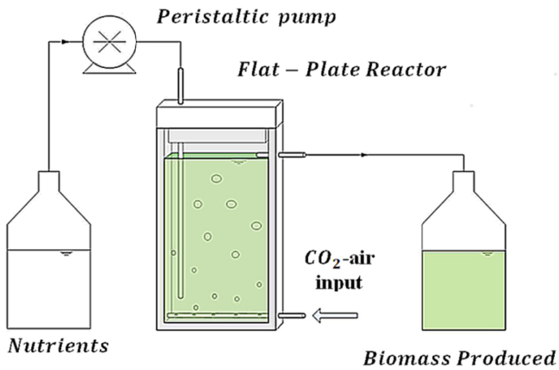


Figure 2: Schematic of the flat-plate photobioreactor.

Table 1: Variables of maintenance of the photobioreactor.

Variables	Values
θ (day) - Residence Time Reactor	1.66
I (μE m^{-2} s^{-1}) - Lighting	300
F_l (m^3 day^{-1}) – Volumetric flow rate of Culture Medium	1.5x10^{-4}
Fg (m^3 day^{-1}) – Gas volume flow	1.2x10^{-3}
$C^g_{CO_2}$ (%) – Concentration of CO_2 (Mixed-CO_2-air)	5
T - Reactor Temperature (°C)	22-24

The nitrate concentration (used as reference substrate) was determined using a Kit Idrimetre St. Carlo Erba Reagenti. The colorimetric reaction consists of an initial reduction of nitrate to nitrite, which forms a diazo after reacting with sulfuric acid. The subsequent reaction between the diazonium salt and gentisic acid (2,5-dihydroxybenzoic acid) forms a diazo dye. The absorbance of the samples was spectrophotometrically measured at the selected wavelength of λ = 445 nm. The analytical curve was made using different $NaNO_3$ solutions.

Simulation Model

A photobioreactor coupled to a settler with partial mass recycling was considered for the simulation of *S. obliquus* cultivation in a continuous system, as depicted in Figure 3. Operating conditions at steady-state were simulated according to the literature (Sundstrom and Klei, 1979) with some modifications.

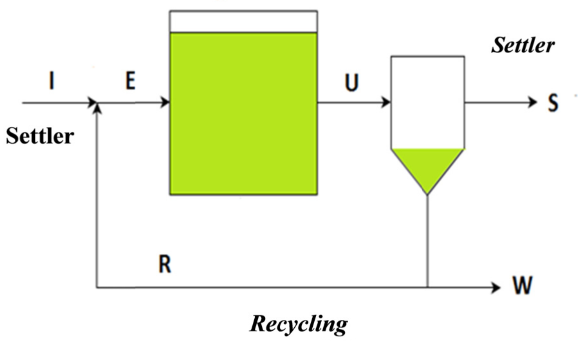


Figure 3: Schematic of continuous *S. obliquus* cultivation process.

It was assumed that $Cx^I = Cp^I = Cx^S = 0$, i.e., there were no product and biomass as process inputs, and that biomass exiting from the sedimentator's top was approximately zero. The flat-plate photobioreactor was modeled as a continuous stirred tank reactor (CSTR) by Sforza *et al.* (2013). For a CSTR the mass balance takes the general form:

$$\frac{\Delta C}{\theta} = r \quad (1)$$

where ΔC is the difference in concentration (C) between the entrance (C^{in}) and exit (C^{out}) of the reactor tank ($\Delta C = C^{out} - C^{in}$), θ is the residence time, and r is the rate of production or consumption of component i.

The net biomass production rate is assumed to be equal to the growth rate (r_x), given by the Monod's equation, minus the cellular death rate ($r_{x,d}$), which is linearly proportional to the cellular concentration (Borzani, 2001). Hence, it is possible to establish relationships between the variables and obtain Equations (2), (3) and (4):

$$r_{x,t} = r_x - r_{x,d} \quad (2)$$

$$r_x = \frac{k \cdot C_x \cdot C_s}{K_M + C_s} \quad (3)$$

$$r_{x,d} = kd \cdot C_x \quad (4)$$

where K_M is the Monod saturation constant for substrate S (g L^{-1}), k is the maximum specific growth rate (day^{-1}), k_d is the specific rate of cell death (day^{-1}), whereas C_s and C_x represent the concentrations of substrate and biomass, respectively. The apparent yield coefficient for substrate-to-biomass conversion $(Y_{x/s})$ is defined by Equation (5):

$$Y_{x/s} = \frac{\Delta C_x}{-\Delta C_s} = \frac{C_{x,out}}{(C_{s,in} - C_{s,out})} \quad (5)$$

A relationship can be found between the biomass growth rate and the substrate consumption rate, as given by Equation (6):

$$r_s = -\frac{1}{Y_{x/s}} \cdot \left(\frac{k.C_x.C_s}{K_M + C_s} \right) \quad (6)$$

The recycling rate (R) is defined as a relation between the recycling flow rate (F_R) and the inlet flow rate (F_I), which is given by Equation (7):

$$R = \frac{F_R}{F_I} \quad (7)$$

The solid retention time (SRT) or biomass age (θ_c) is a relation between the biomass quantity in the reactor tank and the biomass quantity that is removed from the system (Equation (8)) (Von Sperling, 2001). The SRT is considered to be adequate when it warrants high process efficiency, i.e., there is sufficient time for the process so that microorganisms can metabolize the most part of the raw-material existing in the reactor.

$$\theta_c = \frac{V_r \cdot C_x^U}{F_w \cdot C_x^R} \quad (8)$$

where V_r is the effective volume of the reactor.

The concept of wash-out time, θ_c^{wo}, is very important in the analysis of continuous bioprocesses. θ_c^{wo} is defined as the minimum residence time that allows biomass maintenance in the system. This means that θ_c^{wo} is an operating limit, below which the biomass cannot be maintained in the system because the wash-out rate is higher than the growth rate. From the fact that $\frac{1}{\theta_c} = \frac{r_x}{C_x} - k_d$ (biomass balance over the system), the wash-out time θ_c^{wo} can be determined when θ_c is minimun and $\mu = \frac{r_x}{C_x}$ is maximum for $C_s = C_s^I$. Thus:

$$\theta_c^{wo} = \frac{\left(K_M + C_s^I\right)}{\left(\left(k - k_d\right) \cdot C_S^I - K_M k_d\right)} \tag{9}$$

The minimum recycling rate (R_{min}) can be determined by combining Equations (7), (8) and (9):

$$R_{min} = \frac{F_w \cdot \left(\theta - \theta_c^{wo}\right)}{\left(\theta_c^{wo} \cdot F_w - \theta \cdot F_I\right)} \tag{10}$$

Considering that the residence time in the reactor tank (θ), or hydraulic retention time (HRT) is given by Equation (11):

$$\theta = \frac{V_r}{F_I} \tag{11}$$

A relationship between θ and θ_c can be found and written as Equation (12):

$$\theta_c = \frac{\theta}{1+R} \cdot \left(1 + \frac{R.F_I}{F_w}\right) \tag{12}$$

From an analysis of mass balance over the system and over the settler, the substrate concentration at the exit of the reactor and the biomass concentration at the exit and recycling line of the reactor are calculated by Equations (13), (14) and (15):

$$C_s^U\left(\frac{g}{L}\right) = \frac{K_M \cdot (1 + k_d \cdot \theta_c)}{\left(\left(k - k_d\right) \cdot \theta_c - 1\right)} \tag{13}$$

$$C_x^U\left(\frac{g}{L}\right) = \frac{Y_{x/s} \cdot (C_s^I - C_s^U)}{(1 + k_d \cdot \theta_c)} \cdot \frac{\theta_c}{\theta} \tag{14}$$

$$C_x^R\left(\frac{g}{L}\right) = \frac{\left(1 + R - \frac{\theta}{\theta_c}\right) \cdot C_x^U}{R} \tag{15}$$

The simulation was performed using the proposed mathematical model, introducing experimental data of specific growth for *S. obliquus*. The cultivation experiments of this microalga in the flat-plate reactor allowed one to calculate the C_s^I, C_s^U, C_x^U and k coefficients. The value of 1.66 day for θ was used in the steady-state. The other parameters used in the simulation model are listed in Table 2. The variables $C_s^U, C_x^U \, \text{e} \, C_x^U$ were predicted by varying the recycling rate (R) between $R = R_{min}$ and $R = 1$ using the MATLAB R2011 software.

Table 2: Selected parameters for *S. obliquus* growth simulation.

Variable/Parameter	Value
F_I (m^3 day^{-1}) – Inlet volumetric flow rate	1.0
F_w (m^3 day^{-1}) – Volumetric flow rate of cell purge	0.1, 0.2, 0.3
K_M (g L^{-1}) – Monod saturation constant for substrate	0.8
k_d (day^{-1}) – specific death rate	0 and 0.05
V_r (m^3) – Reactor volume	1.66
R – Recycling rate	R_{min} to 1

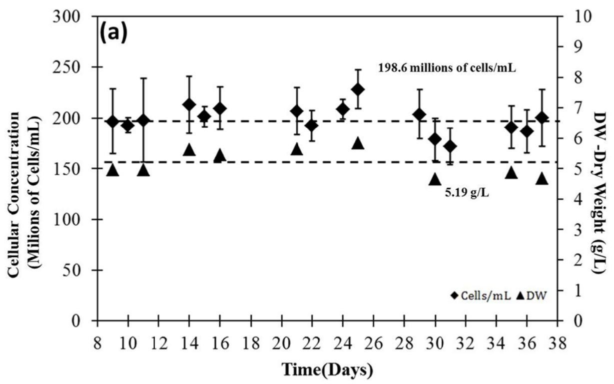
RESULTS AND DISCUSSION

The *S. obliquus* cultivation experiments were carried out using CO_2 and nitrate (NO_3^-) solutions as carbon and nitrogen sources, respectively, for the microalgae growth. CO_2 was pumped in excess into the system while NO_3^- solution was chosen as the limiting substrate. Table 3 displays the values of NO_3^- concentration at the entrance and exit of the reactor tank (C_s^I and C_s^U respectively), biomass concentration (dried weight) at the reactor exit (C_x^U), maximum specific growth rate (k) and yield ($Y_{x/s}$). It is noted that the NO_3^- comsumption was approximately 79%.

The high value of biomass concentration seen in Table 3 is typical of *S. obliquus*, which is referred as one of the most promising microalga for biofuel production. This result is in agreement with values previously reported in the literature. For instance, Baky *et al.* (2012) found a dried weight of 1.651 g L^{-1} for *S. obliquus* cultivated at 25 °C in N-9 culture medium at 200 μE m^{-2} s^{-1} and 9% CO_2 in the gas line. Wang *et al.* (2013) obtained 4 - 5 g L^{-1} of biomass after 6 days of *Scenedesmus dimorphus* cultivation using BG-11 culture medium at 510 μE m^{-2} s^{-1}. Breuer *et al*. (2013) obtained a biomass content of 6 - 7 g L^{-1} by cultiving *S. obliquus* in the range 300 - 500 μE m^{-2} s^{-1}. All these results show that the *Scenedesmus* genus, *S. obliquus* in particular, is resistant against increases of light intensity. These data also show that both relative growth and photosynthetic efficiency decrease for concentrations of approximately 800 – 1000 μE m^{-2} s^{-1}, which have been examined in details by Sforza *et al*. (2014). Figure 4 displays values of cellular concentration at the steady-state. It can be verified that the steady-state conditions were successfully maintained for 1 month, enabling reproducibility of the system with high cellular concentration.

Table 3: Experimental data for the bench *S. obliquus* cultivation process.

Parameter	Value
C_s^I (g L^{-1})	1.78 ± 0.18
C_s^U (g L^{-1})	0.38 ± 0.07
C_x^U (g L^{-1})	5.19 ± 0.47
k (day^{-1})	0.49
$Y_{x/s} = \dfrac{C_x^U}{(C_s^I - C_s^U)}$	3.86



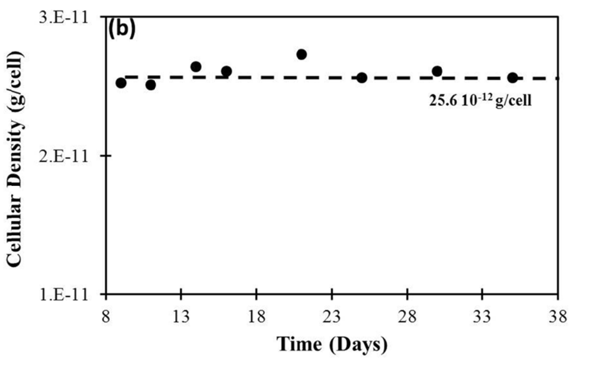


Figure 4: Steady-state data for the photobioreactor operation. (a) Cellular concentration. (b) Cellular density.

Simulations were run with the variables/parameters listed in Table 2 and 3, and the resulting parameters are summarized in Tables 4 and 5. Data were plotted and exhibited in Figures 5, 6 and 7.

Table 4: Simulation results for $k_d = 0$.

Parameter	Value		
F_w (m^3 day^{-1})	**0.1**	**0.2**	**0.3**
R_{min}	0.10	0.25	0.50
θ_c (day)*	9.0	5.0	3.6
C_x^U (g/L)*	32.0	14	6.0
C_x^R (g/L)*	58.0	22.5	9.0
θ_c^{wo} (day)	2.97		

*Maximum values.

Table 5: Simulation results for k_d = 0.05 day^{-1}.

Parameter	Value		
F_w (m^3 day^{-1})	**0.1**	**0.2**	**0.3**
R_{min}	0.14	0.38	0.90
θ_c (day)*	9	5	3.6
C_x^U (g/L)*	19	8	0.9
C_x^R (g/L)*	35	14	1.5
θ_c^{wo} (day)	3.49		

*Maximum values.

The values of R_{min} are satisfactory for $k_d = 0$ (absence of cell death) when cell purge flow rates (F_w) of 0.1 and 0.2 m^3 day^{-1} were used. The values of C_x^U and C_x^R also suggest good operational conditions. However, when k_d is 0.05 day^{-1} (see Table 5), it is observed that F_w strongly influences the cellular concentration at the steady state. R_{min} increases significantly, making the conditions for F_w = 0.3 inapplicable for the process.

It is worth mentioning that, in some cases the biomass concentration at the exit of the reactor is very high, which is not encountered in real conditions. The cellular growth depends on the light intensity; nevertheless high concentrations (usually greater than 8 g L^{-1}) are not true conditions because there is obstruction of the light throughout the reactor when the concentration reaches high levels. This is due to the shading caused by cells of the light source (so-called self-shading effect). The absorption of light by cells located farther from the light source is then reduced, thereby decreasing the productivity of the reactor. In this work, transmission and absorption of light were not taken in account since a better energy efficiency of *S. obliquus* cultivation in a similar flat-plate reactor at 300 μE m^{-2} s^{-1} had been previously demonstrated (Sforza *et al*., 2014). A limiting concentration of 8 g L^{-1} is often reported in studies where cellular concentrations expressed in dried weight do not exceed this value for applied light intensities between 300 – 1500 µE m^{-2} s^{-1} (Breuer *et al*., 2013; Wang *et al*., 2013). Furthermore, the conditions for self-shading also depend on reactor geometry.

It has been reported that cellular concentrations of 1.33 g L^{-1} and 9.66 g L^{-1} are found for light intensities fixed at 150 µE m^{-2} s^{-1} and 1000 µE m^{-2} s^{-1}, respectively, for cultivation of *Scenedesmus obliquus* in a photobioreactor with similar geometry and dimensions. The energy conversion efficiency decreased from 24% to 13%, which was probably due to self-shading (Beraldi, 2013).

From Figure 7 and Tables 4 and 5, it can be noted that R_{min} increases and C_x^U decreases as the biomass removal is increased in the system. This is likely because the amount of cell that remains in the system does not provide sufficient active biomass for an effective microalgae growth. It shows that for the operating conditions in F_w = 0.3 for values of k_d and F_w = 0.1 or for k_d = 0 are unreal in terms of operation, due to either low cellular growth in the former condition, or physical limitation of the reactor for microalgae growth in the latter condition as it provides a huge cellular concentration value of 32 g L^{-1}.

Ho *et al*. (2013) reported a hydraulic retention time (HRT) of 150 hours (6.25 days) for the cultivation of *Scenedesmus obliquus* in a continuous system, which exceeds the HRT used in this work. This can be explained by the lower CO_2 concentration (1.5 vvm) and light intensity (240 µE m^{-2} s^{-1}) used by the authors, which justifies the longer retention time used to achieve better growth parameters. In this work, a CO_2 concentration of 2% was used and a light intensity of 300 µE m^{-2} s^{-1}, reaching consequently a HRT of 1.66 day (approximately 40 h).

It can be observed from Figures 5, 6, 7 and 8 that the operating conditions diverge significantly when varying the biomass removal rate and cellular residence time. This denotes the importance of simulating the process before laboratory tests, by obeying the microalgae growth kinetics as well as the cellular maintenance and the biomass removal.

The use of recycling caused an increment in the cellular concentration because the experimental conditions without recycling led to a cellular concentration of 5.19 g L^{-1}, whereas values between 6 and 14 g L^{-1} were obtained for the simulated data. The simulated values seem to be applicable for validating the model.

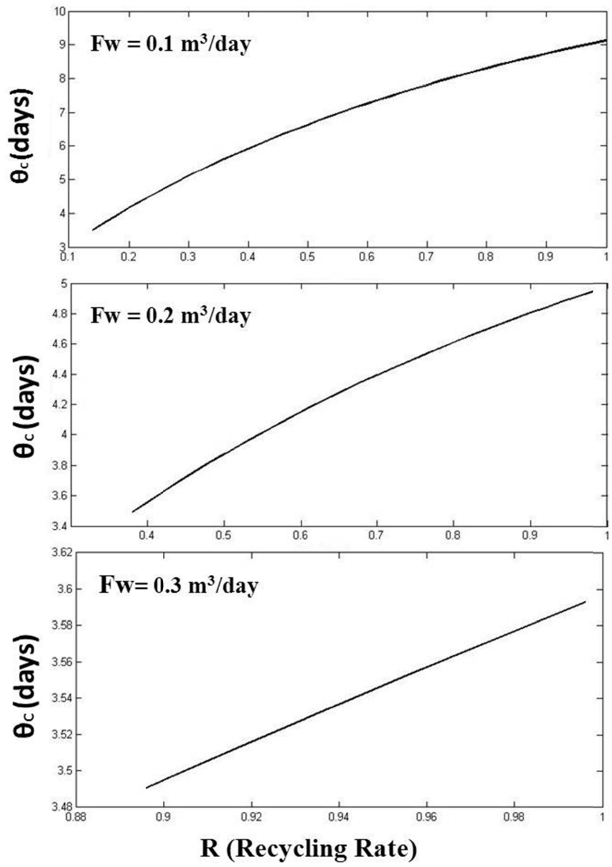


Figure 5: Plot of θ_c as a function of recycling rate (R).

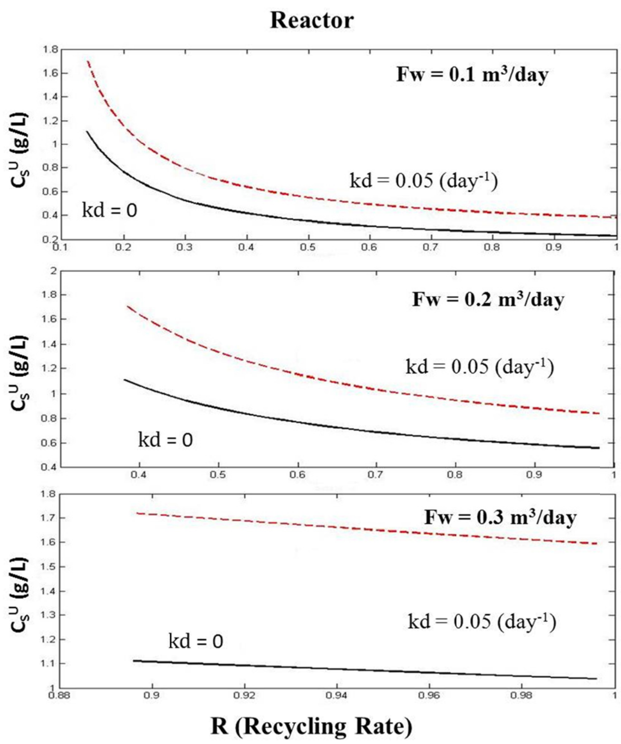


Figure 6: Plot of C_s^U as a function of recycling rate (R).

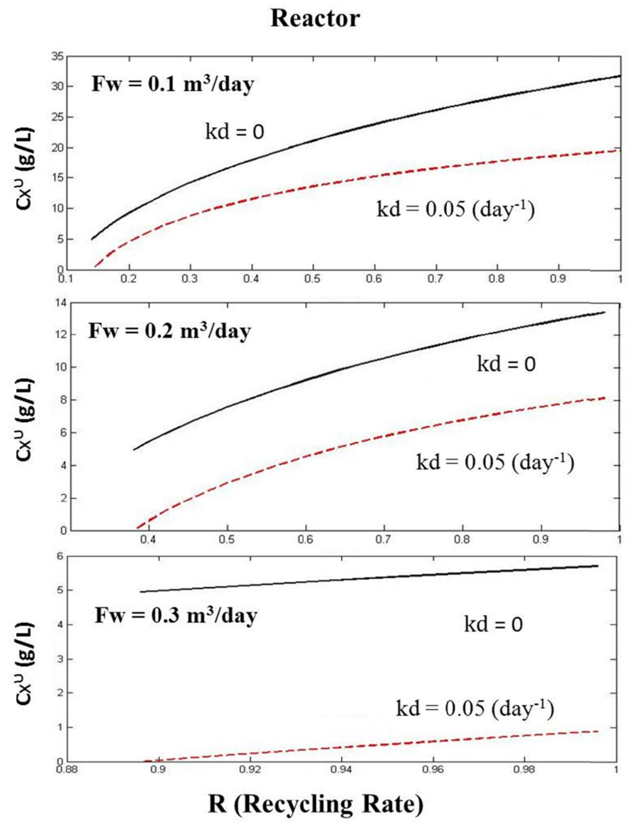


Figure 7: Plot of C_x^U as a function of recycling rate (R).

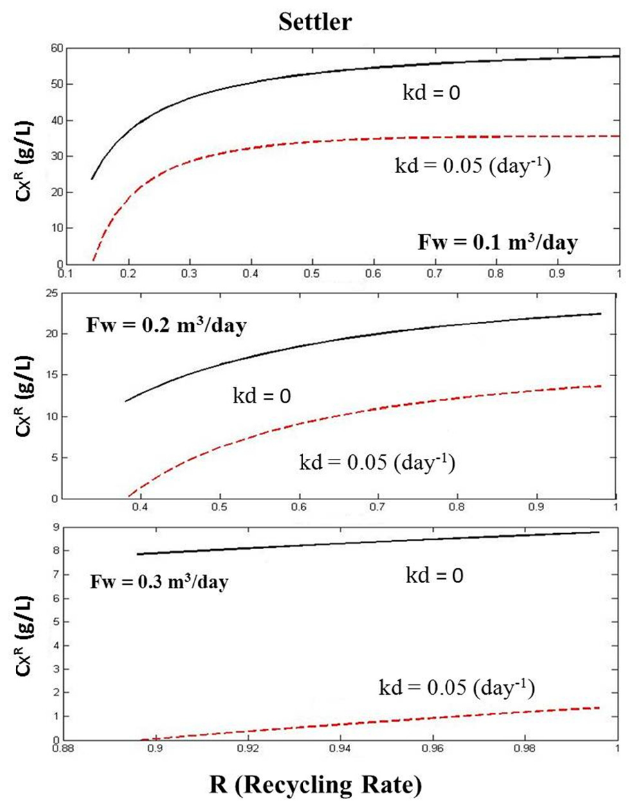


Figure 8: Plot of C_x^R as a function of recycling rate (R).

The model developed in this work did not take into account the sedimentation rates; however, they can be further controlled by centrifugation because sedimentation conditions depend on the microalgae species. However, the proposed model was useful for simulating the two stage photobioreactor-settler system with partial biomass recycling. The model also provided insights into the process behavior as θ_c^{wo} and R_{min} were changed, not only in terms of kinetic data, for instance the cellular residence time, but also in terms of operating conditions such as the biomass purge flow rate (F_w). Finally, the simulation results indicated 0.1 and 0.2 m^3 day^{-1} as the more appropriate values of F_w for the continuous cultivation of *Scenedesmus obliquus*.

CONCLUSIONS

A mathematical model to simulate the efficiency of *Scenedesmus obliquus* cultivation in a photobioreactor with partial biomass recycling was developed in this work. It was verified that the values of R_{min} become applicable when cell death is not considered ($k_d = 0$) and when the cell purge flow rate (F_w) lies in the range of 0.1 and 0.2 m^3 day^{-1}. However, the recycled biomass concentration decreases to insufficient levels for an adequate microalgae growth as F_w increases. Finally the microalgae sedimentation behavior was not taken into account in the proposed mathematical model. It is noteworthy that the sedimentation conditions are dependent on the microalgae species, in such a way that the sedimentation rate should be measured to examine whether sedimentation is able to affect the entering biomass rate.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge CAPES for all support provided to this work. The opportunity and welcome from the Università degli Studi di Padova is also acknowledged. This research was partially presented in oral form at the XXXVI ENEMP-Maceiò-Alagoas-Brazil.

LIST OF SYMBOLS

C	Concentration of component i (g L^{-1})
θ	Residence time or hydraulic retention time (HRT) (day)

r	Rate of production or consumption of component i (g L^{-1} day^{-1})
K_M	Monod saturation constant for substrate (g L^{-1})
k	Maximun specific growth rate (day^{-1})
k_d	Specific rate of cell death (day^{-1})
F_w	Cell purge flow rate (m^3 day^{-1})
F_R	Recycling flow rate (m^3 day^{-1})
F_1	Inlet flow rate (m^3 day^{-1})
θ_c	Solid retention time (SRT) (day)
θ_c^{wo}	Wash-out time (day)
$Y_{x/s}$	Apparent yield coefficient for substrate-to-biomass conversion (g g^{-1})
V_r	Effective volume of the reactor (m^3)
R_{min}	Minimum recycling rate (-)

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