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

C. E. de Farias Silva*, B. Gris and A. Bertucco

Department of Industrial Engineering, University of Padova, via Marzolo 9, 35131, Padova, Italy.
E-mail: carloseduardo.defariassilva@studenti.unipd.it

(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 $F_r = 0.2F_f$, 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;
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, flocculation 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 unlimited 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 CO₂ 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).](image1.png)

![Figure 2: Schematic of the flat-plate photobioreactor.](image2.png)

**Table 1: Variables of maintenance of the photobioreactor.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>θ (day) - Residence Time Reactor</td>
<td>1.66</td>
</tr>
<tr>
<td>I (µE m⁻² s⁻¹) - Lighting</td>
<td>300</td>
</tr>
<tr>
<td>F_l (m³ day⁻¹) - Volumetric flow rate of Culture Medium</td>
<td>1.5x10⁻⁴</td>
</tr>
<tr>
<td>F_g (m³ day⁻¹) - Gas volume flow</td>
<td>1.2x10⁻³</td>
</tr>
<tr>
<td>C^c_O₂ (%) – Concentration of CO₂ (Mixed-CO₂-air)</td>
<td>5</td>
</tr>
<tr>
<td>T - Reactor Temperature (°C)</td>
<td>22-24</td>
</tr>
</tbody>
</table>
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 $\lambda = 445$ 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.

![Flat-Plate Reactor](image)

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

It was assumed that $C_s^I = C_p^I = C_x^S = 0$, 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:

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

where $\Delta C$ is the difference in concentration ($C$) between the entrance ($C^i$) and exit ($C^o$) of the reactor tank ($\Delta C = C^o - C^i$), $\theta$ 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 ($r_c$), given by the Monod’s equation, minus the cellular death rate ($r_{cd}$), 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):

$$r_{x,d} = r_c - r_{x,cd}$$  \hspace{1cm} (2)

$$r_s = \frac{k \cdot C_s \cdot C}{K_M + C_s}$$  \hspace{1cm} (3)

$$r_{x,cd} = kd \cdot C_s$$  \hspace{1cm} (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 $C_c$ 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):

$$Y_{x/s} = \frac{\Delta C_s}{-\Delta C_s} = \frac{C_{x,\text{out}} - C_{x,\text{in}}}{(C_{s,\text{in}} - C_{s,\text{out}})}$$  \hspace{1cm} (5)

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

$$r_s = \frac{1}{Y_{x/s}} \left( \frac{k \cdot C_s \cdot C}{K_M + C_s} \right)$$  \hspace{1cm} (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):

$$R = \frac{F_R}{F_i}$$  \hspace{1cm} (7)

The solid retention time (SRT) or biomass age ($\theta_c$) 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.

$$\theta_c = \frac{V_c \cdot C_s^I}{F_w \cdot C_s^E}$$  \hspace{1cm} (8)
where \( V_r \) is the effective volume of the reactor.

The concept of wash-out time, \( \theta_c^{\text{wo}} \), is very important in the analysis of continuous bioprocesses. \( \theta_c^{\text{wo}} \) is defined as the minimum residence time that allows biomass maintenance in the system. This means that \( \theta_c^{\text{wo}} \) 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 \( \frac{1}{\theta_c} = r_x - k_d \) (biomass balance over the system), the wash-out time \( \theta_c^{\text{wo}} \) can be determined when \( c_x^{\text{dc}} \) is minimum and \( \mu = \frac{r_x}{C_x} \) is maximum for \( C_x = C_x^{l} \). Thus:

\[
\theta_c^{\text{wo}} = \frac{(K_M + C_x^{l})}{((k - k_d) \cdot C_x^{l} - K_M k_d)} \quad (9)
\]

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

\[
R_{\text{min}} = \frac{F_w \cdot (\theta - \theta_c^{\text{wo}})}{\theta_c^{\text{wo}} \cdot F_w - \theta \cdot F_{\text{ij}}} \quad (10)
\]

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

\[
\theta = \frac{V_r}{F_{\text{ij}}} \quad (11)
\]

A relationship between \( \theta \) and \( \theta_c \) can be found and written as Equation (12):

\[
\theta_c = \frac{\theta}{1 + R \left( \frac{1 + R \cdot F_{\text{ij}}}{F_w} \right)} \quad (12)
\]

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):

\[
C_x^{U} \left( \frac{g}{L} \right) = \frac{1 + R}{1 + R \cdot \frac{\theta_c}{\theta}} C_x^{L} \quad (15)
\]

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

### RESULTS AND DISCUSSION

The \( S. obliquus \) cultivation experiments were carried out using \( \text{CO}_2 \) and nitrate \( (\text{NO}_3^-) \) solutions as carbon and nitrogen sources, respectively, for the microalgae growth. \( \text{CO}_2 \) was pumped in excess into the system while \( \text{NO}_3^- \) solution was chosen as the limiting substrate. Table 3 displays the values of \( \text{NO}_3^- \) concentration at the entrance and exit of the reactor tank \( (C_x^{I} \text{ and } C_x^{U} \text{ respectively}) \), biomass concentration (dried weight) at the reactor exit \( c_x^{\text{dc}} \) and maximum specific growth rate \( (r_x) \) and yield \( (Y_{\text{x/s}}) \). It is noted that the \( \text{NO}_3^- \) consumption 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 microalgae 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% \( \text{CO}_2 \) 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 m² 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 m² 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 m² 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.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C^i ] (g L⁻¹)</td>
<td>1.78 ± 0.18</td>
</tr>
<tr>
<td>[C^i_\text{U} ] (g L⁻¹)</td>
<td>0.38 ± 0.07</td>
</tr>
<tr>
<td>[C^x ] (g L⁻¹)</td>
<td>5.19 ± 0.47</td>
</tr>
<tr>
<td>(k ) (day⁻¹)</td>
<td>0.49</td>
</tr>
<tr>
<td>(Y_{x/i} = \frac{C^x}{C^i - C^i_\text{U}} )</td>
<td>3.86</td>
</tr>
</tbody>
</table>

**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 \(k_d = 0 \).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F_w ) (m³ day⁻¹)</td>
<td>0.1 0.2 0.3</td>
</tr>
<tr>
<td>(R_{\text{min}} )</td>
<td>0.10 0.25 0.50</td>
</tr>
<tr>
<td>(\theta_r ) (day)</td>
<td>9.0 5.0 3.6</td>
</tr>
<tr>
<td>(C^i ) (g/L)</td>
<td>32.0 14 6.0</td>
</tr>
<tr>
<td>(C^x ) (g/L)</td>
<td>58.0 22.5 9.0</td>
</tr>
<tr>
<td>(\rho^\text{w} ) (day)</td>
<td>2.97</td>
</tr>
</tbody>
</table>

*Maximum values.

**Table 5: Simulation results for \(k_d = 0.05 \text{ day}^{-1}\).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F_w ) (m³ day⁻¹)</td>
<td>0.1 0.2 0.3</td>
</tr>
<tr>
<td>(R_{\text{min}} )</td>
<td>0.14 0.38 0.90</td>
</tr>
<tr>
<td>(\theta_r ) (day)</td>
<td>9 5 3.6</td>
</tr>
<tr>
<td>(C^i ) (g/L)</td>
<td>19.0 8.0 0.9</td>
</tr>
<tr>
<td>(C^x ) (g/L)</td>
<td>35 14 1.5</td>
</tr>
<tr>
<td>(\rho^\text{w} ) (day)</td>
<td>3.49</td>
</tr>
</tbody>
</table>

*Maximum values.

The values of \(R_{\text{min}}\) are satisfactory for \(k_d = 0\) (absence of cell death) when cell purge flow rates (\(F_w\)) of 0.1 and 0.2 m³ day⁻¹ were used. The values of \(C^x\) and \(C^x\) 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. \(R_{\text{min}}\) 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 con-
centration 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 \(R_{\text{mil}}\) increases and \(C_{\text{U}}\) 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.
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 $R_m$ and $R_{min}$ were changed, not only in terms of kinetic data, for instance the celluular 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-Maceió-Alagoas-Brazil.

**LIST OF SYMBOLS**

- $C$: Concentration of component i (g L$^{-1}$)
- $\theta$: Residence time or hydraulic retention time (HRT) (day)
Rate of production or consumption of component $i$ (g L$^{-1}$ day$^{-1}$)

$K_M$ Monod saturation constant for substrate (g L$^{-1}$)

$k$ Maximum 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$^{-1}$)

$F_L$ Inlet flow rate (m$^3$ day$^{-1}$)

$\theta_c$ Solid retention time (SRT) (day)

$\theta_{cw}$ Wash-out time (day)

$Y_{xs}$ 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 (-)

**REFERENCES**


Wu, C., Wang, W., Yue, L., Yang, Z., Fu, Q., Ye, Q., Enhancement effect of ethanol on lipid and fatty acid accumulation and composition of *Scenedesmus sp*. Bioresource Technology, 140, 120-125 (2013).