The present work seeks to create a methodology to estimate the biomass production in a *Nannochloropsis oceanica* production plant, as this result is required for a life cycle analysis and for an economic viability study owing to turn the production of microalgae biomass a reality. The method relied on two complementary approaches. The first one, which was referred as static optimization, maximizes the solar energy on the photobioreactor surface and minimizes the one that falls on the ground. The latter represents the lost amount, and then reduces the efficiency. In that case, tubular photobioreactors should have a horizontal distance between tubes of 1.75 times the diameter and a vertical one of 1.25 times the diameter. At the same time, the distance between two consecutive photobioreactors in a production plant must been reduced in 32% when compared to the default value. Thereupon, parameters that influence the culture growth were analyzed, and the results suggest that higher pump frequencies and the use of an air blower positively affect the productivity. Lastly, a simple model dependent on incident irradiance and culture temperature was adapted from the literature and applied to an outdoor culture of *Nannochloropsis oceanica*. Although the model must be upgraded in order to increase its accuracy, it can be used as a first order approximation when all the complexity and number of parameters on which the culture growth depends are taken into account.

**Keywords:** solar radiation; microalgae production; photobioreactors; optimization; Ecotect; *Nannochloropsis oceanica*

1. **Introduction**

The total final energy consumption has been increasing worldwide. According to the International Energy Agency’s latest Headline Energy Data, it has increased by 25% from 2003 to 2013, a rate that was also observed in the transport sector. At the same time, the growth in oil-derived products production (motor gasoline, gas oil, diesel oil, refinery gas, jet fuels etc) has increased by only 13% during the same period, which means that new energy sources will have to be developed in order to meet the future demand [1].

During the same period, an increase in 28.7% of the concentration of CO$_2$ in the atmosphere were also reported. In fact, when it comes to total greenhouse gases (GHG) emissions, their total amount increased in 70%
from 1970 to 2004. These mean that the development of new energy sources must rely on the possible social and environmental impacts consequent of their use, which have often been overlooked [1].

Under those circumstances, biofuels from microalgae are thought to be the best approach to replace the current use of fossil fuels. First, it relies on the photosynthesis process, which in brief requires a carbon source (CO\textsubscript{2}), water and an energy input to produce organic matter and oxygen. The energy input is usually the sunlight, even though artificial may also be used [2], while the organic matter refers not only to fatty acids (thereupon used to produce biofuels) but also to other valuable products [3][4]. Second, different from conventional crops used for the same propose (as sugar cane and corn), microalgae is not commonly channeled into food production and then does not lead to a competition between energy and food demands [3][5]. Moreover, it achieves a higher areal productivity (increase in biomass per square meter of production land per day), might grow in non arable lands (nutrient deprived environments) and has a huge metabolic flexibility which supports genetic improvement [6].

However, for the production of microalgae becomes a reality, its profit must overcome capital and operational expenditure [7], which has not happened yet mainly due to poor engineering development [8]. For instance, theoretical maximum photosynthesis efficient (MPE) is 12.4% [3], but the current industrial microalgae production reported in literature achieved an efficiency in the range between 0.2 and 3% [9]. This result suggest that the variables that affect the photosynthesis are limiting the culture growth, namely the available light and the temperature [10]. Moreover, other operational parameters, such as the photosynthetically generated oxygen inside the culture, may influence the microalgae growth rate [11].

Overall, the present work focused on the effect of these parameters on the microalgae growth. Light was analyzed in order to optimize its use both in an individual photobioreactor and in a production plant, which was referred as static optimization. Straightforward the main variables and phenomena associated to the microalgae behavior in a pilot scale production plant in Lisbon were analyzed and used to validate an adapted model from literature to estimate the \textit{Nannochloropsis oceanica} growth rate. In the end, it would allow the biomass production prediction coupled with the identification of the operational costs, whose comparison with the former would be the first step to an industrial microalgae production plant design life cycle assessment.

2. Background

Physical background

Light is the energy input to photosynthesis and, as a consequence, to the microalgae production in photobioreactors. Each cell inside the latter must be exposed to light time enough to receive the required amount of electrons to initiate the photosynthesis reaction.

In fact, due to attenuation and scattering of light phenomena done by the cells, there is an energy gradient inside the geometry [12]. Indeed, the culture closer to the photobioreactor surface absorbs part of the available light, and then its amount reduces as
closer to the centre it gets. This is referred as the self-shading effect [13].

Although the light gradient contributes to a reduction in efficiency, it can be countered by coupling the photosynthesis kinetics to the turbulence inside the photobioreactor. In fact, the elements responsible to absorb the light inside the microalgae cells, which are known as pigments, have limit in photon supply. It means that the increase in radiance does not completely follow the increase in the photosynthesis rate, as after some value the pigments are saturated and the excessive photons that achieve the cell are dissipated as heat [14].

It means that the relevance relies on the exposure time of the individual cell to the light inside the photobioreactor, because as long as this time is synchronized to the photosynthesis reaction one, light would not be limiting the culture growth. This exposure time is translated into an exposure frequency, which is referred as light-dark cycles [15]. This parameter is a function of the mixing promoted by the turbulence inside the culture.

Nevertheless, outdoor microalgae culture depends on the maximization of the solar radiation on the photobioreactor surface through the geometry optimization. Provided that the Sun position can be calculated over the year, the geometry that maximizes the amount of sunrays that falls onto its surface turns to be a function of the same angles that define the former. Moreover, the solar positions allows the calculation of the shadow area created by one geometry, which not only estimates the direct energy incident on its surface but also identifies the amount of energy that falls onto the ground [16].

Moreover, part of the incident energy of the photobioreactor surface is also lost because the microalgae cells are not able to use the photon of that specific wavelength. For instance, only photon between 400 nm and 700 nm are absorbed by microalgae pigments, and this interval is defined as photosynthetically active radiation (PAR) [3].

The available PAR inside the culture may be estimated by using the Lambert-Beer Law, which assumes that the light is monochromatic, the scattering is negligible when compared to the absorption and the direction of the incident radiation does not change as it crosses the culture [17]. It is a first order approximation to the radiative transfer equation.

Under those circumstances, the average light available inside the culture on the radial direction of a cylinder geometry is calculated by the Equation 1,

\[
I_{\text{inside}} = \frac{1}{D} \int_0^D I(r)dr \Rightarrow I_{\text{inside}} = \frac{I_0}{D \cdot Ka \cdot DW} \left[ 1 - \exp \left( -D \cdot Ka \cdot DW \right) \right] 
\]

**Biological background**

When microalgae cells receive light under non-limiting conditions, they grow both in size and in number. As the cells have enough energy available for photosynthesis, an accumulation of organic matter inside them takes place. The culture is thus able to reproduce by mitosis and exponentially increase the number of cells per volume (cells density) [13].

As the culture gets denser (i.e. the number of cells per volume increase), the less energy, carbon source and other nutrients
required for their metabolism are available for all microalgae cells. Then, rate in which the population has been growing starts to decrease up to a limit at which the growth rate is equal to the death rate. This stage is defined as stationary phase and further increase in cellular density may lead to culture death [13]. On the other hand, culture too diluted (i.e. small amount of cells per unit volume) may be photodamaged due to the excessive light intensity for the cells. This phenomenon is called photoinhibition and may also reduce the culture growth rate [3].

In a microalgae photobioreactor, usually the culture is diluted up to a photoinhibition condition. However, the cells are able to adapt themselves to the detriment of its growth through pigment production. This refers to the lag phase of the culture life, in which the increase in biomass is reduced even with the necessary available energy. Hereafter, both pigment production and cellular division overcome the photodamage problem, and then the available light becomes the limiting factor the culture growth. At this stage, which is know as exponential or log phase, the growth rate is maximum [13].

Under those circumstances, in biological engineering the culture is commonly maintained at the exponential growth rate. This is achieved through harvesting (culture renewals): when the culture density has become significantly large, part of the biomass is harvested and the culture is diluted in a new culture medium (or in a recycled culture medium, with reutilization of the same medium after separation of the microalgae cells). Then, density decreases and the culture remains at optimal conditions, while the collected amount can be post-processed to obtain derived valuable products [13]. In fact, this harvesting process can be done daily (turbidostat method), periodically (semi-continuous method) or at the end of the cultivation (batch method) [13].

The culture cultivation is done in structures referred as photobioreactors. Although there are different kinds depending on the culture exposure level to the atmosphere [18], all photobioreactors aim for the maximization of the volume of culture exposed per unit area. This fact would reduce the amount of dark zones inside the culture due to the self-shading effect, and as a result increase the reactor efficiency concerning the light supply.

When it comes to the exposure level, the photobioreactors are classified as open or closed as shown in Figure 1. In brief, the first ones are simpler and cheaper, as they are similar to pool in which the culture flows as shown. However, as they are in directly contact to the atmosphere, the number of contaminants inside the culture is much higher and only a limited number of microalgae species can be cultivated under these conditions [19].

Figure 1 - Different kinds of photobioreactors for microalgal production, namely: open cascade raceways (a), closed tubular (b) and green walls (c) (courtesy A4F)
On the other hand, closed photobioreactors allow a more controlled cultivation concerns the number of contaminants or other parameters such as temperature, and then a wider sample of microalgae species may be produced in these geometries. However, their benefits are strictly followed by an increase in the operational costs. Moreover, they require a degassing system to remove the photosynthetically generated oxygen from the culture, as its concentration may reduce the culture growth through photooxidation phenomena [7].

The applied methodologies of optimization and the culture growth analysis done in this work rely on the closed photobioreactors, namely the tubular photobioreactor (TPBR) and the green wall (GW).

3. Materials and methods

Computational methods

Two complementary optimization methodologies were proposed in order to efficiently use the solar irradiation in a microalgae industrial production context. The first one was restricted to the individual tubular photobioreactor and it was based on the yearly calculation of the incident solar energy on the geometry surface. At the same time, as the TPBR is a discontinuous geometry, the amount of energy that travels through it and falls onto the ground was also considered, which is known as shutter effect.

This first methodology, which was named static optimization for a tubular photobioreactor, used the Autodesk Ecotect software to perform the calculations of the incident light for different geometries in Lisbon. It allows the definition of the geometry and also of the finite elements used in each simulation done. Here, the default option of thirty six elements per tube was used after a sensibility analysis between the increase in the result accuracy and the computational time required.

The vertical and the horizontal distance between the tubes in one TPBR were gradually increased as a function of the tube diameter, and then the incident energy on their surface and the one lost in the shutter effect were both numerically estimated. The methodology proposed that the one that maximizes the former and minimizes the latter is the optimal one.

For instance, the first simulation was the one whose vertical and horizontal distance between tube's axis were equal to the diameter, which defined the geometry (D; D). Then, the vertical distance was maintained constant and the horizontal varied, which defined the geometries (1.25D; D), (1.5D; D) etc. The last geometry simulated was the (2.5D; 2.5D), whose vertical and horizontal distance between tube's axis was both equal to 2.5 times the tube diameter.

A second optimization methodology was done to optimize the light use in a production plant. Differently from the first one, it takes into account the direct energy incident on the photobioreactor and the one that falls on the ground due to the distance between two consecutive geometries in one production plant. It did not consider the light that travels throughout the photobioreactor because this method was developed for a green wall geometry.

In order to calculate both incident direct solar energy on the vertical photobioreactor and on the ground, daily calculations of the shadow area were performed based on the solar angles and on the photobioreactor's characteristics in
Matlab. These values were coupled with the direct horizontal irradiation from Ecotect, as the product between them results in the incident direct energy on the vertical geometry [20]. Moreover, the difference between the shadow area and the area delimited by two consecutive photobioreactors would lead to the lost energy in a production plant. In fact, the result of this method proposed a different industrial production plant design that reduces these illuminated areas on the ground.

Biological, laboratory and pilot scale materials and methods

The microalgae analyzed was the Nannochloropsis oceanica, a green unicellular microalgae from salt-water habitats, eukaryote, with a globose to oval shape whose dimension may vary between 2-4 x 3-5 µm [21]. The choice for this species was based on its commercial value, and also on the fact that some research has already been performed on its mathematical model for growth rate estimation [2][22].

A culture started on June 23th close to the summer solstice, and ended on August 5th, which defines the first analysis done. The second analysis was done in the same photobioreactor and started on August 23th and ended on September 16th. Overall, it is important to realize that although there was no analysis done in the interval between them, the culture were still being cultivated and it was renewed with recirculation four times.

During the experiments, three main parameters were chosen to describe the microalgae culture stage and its evolution over time, namely: the dry weight, the optical density and the turbidity. They were all measured at 11h, as it has proved to be approximately the time when the culture is at the same stage. The first is a measurement of the biomass per unit volume (g/L) done by sampling 30 mL of the culture directly from the TPBR and drying it at a moisture analyzer. The second is the measurement of the light intensity before and after it travels throughout a specific path length (defined in the spectrophotometer used), which means that both absorption and scattering phenomena at one or more wavelengths are accounted in this parameter. The last one is similar to the optical density, but it is done at a wavelength outside the PAR and then only the scattering phenomenon is taken into account [23].

The parameters volumetric productivity \( P_{volumetric} \) and specific growth rate \( \mu \) were used to analyze the culture behavior under different environmental and operational conditions, namely: incident solar irradiance, dry weight and pump frequency. As shown in Equations 1 and 2, both depend on the dry weight \( DW_i \) at different times \( t_i \). However, the specific growth rate is an adaption of the proposed equation in literature [13]. Other parameters were measured over the essay in order to guarantee that their were not limiting the culture growth.

\[
P_{volumetric} = \frac{DW_{i+1} - DW_i}{t_{i+1} - t_i} \tag{2}
\]

\[
\mu = \frac{\ln \left( \frac{DW_{i+1}}{DW_i} \right)}{t_{i+1} - t_i} \tag{3}
\]

4. Results and discussion

Static optimization

The static optimization applied to a tubular photobioreactor geometry resulted in a configuration whose vertical distance between tube’s axes is 1.25 times the diameter and the horizontal is 1.75 times the diameter. Under those circumstances, the annual energy lost in the shutter effect is less than 5% of the total
solar energy that falls onto the tubes’ surface. Figure 2 shows the total monthly incident energy on the optimized geometry surface per meter of the photobioreactor’s length.

Figure 2 - Incident monthly energy on the optimized geometry

When it comes to the static optimization applied to a production plant, the photobioreactor chosen was the green wall. Its characteristics are shown in Table 1. The direct incident energy on its surface and on the area between two consecutive ones in an industrial plant were monthly calculated and the results are shown in Table 2 for the whole year.

Table 1 - Green wall characteristics

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (L)</td>
<td>1 m</td>
</tr>
<tr>
<td>Height (hPBR)</td>
<td>1.143 m</td>
</tr>
<tr>
<td>Thickness (e)</td>
<td>0.250 m</td>
</tr>
<tr>
<td>Orientation</td>
<td>East-West</td>
</tr>
</tbody>
</table>

First, a geometry defined as default was simulated as a control, in which an arbitrary distance between the green walls was used. The methodology resulted in a more compact design, whose the area between two consecutive photobioreactors reduced in 32%.

Table 2 – Direct incident energy on green wall production plants

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct incident energy (kWh/year)</td>
<td></td>
</tr>
<tr>
<td>Default</td>
<td>Optimized</td>
</tr>
</tbody>
</table>

An efficiency in light utilization is defined for the production plant designed based on both scenarios. This parameters is defined as the used energy deducted from the lost on the implanted ground per square meter of implanted area. For instance, it takes into account the incident on the 2nd green wall panel and the amount incident on the ground. These parameters are divided by the area that this photobioreactor occupies up to the adjacent one.

Even though all energies and the area between the green walls reduced in the optimized scenario (see Table 2), the direct light utilization was 50% bigger when compared to the default one.

Dynamic analysis

On the assumption that all parameters which affect the microalgae growth are controlled (temperature, pH and nutrients), the only limiting factor is light intensity throughout the culture. For this reason, not only is the culture concentration relevant but also the flow mixing. A more efficient mixing for higher cell concentrations would lead to an optimized cell exposition to light, and therefore the mutual shading phenomena would not limit the culture growth.

For this reason, the experiment was based on volumetric productivity and specific growth rate (see Equations 1 and 2) calculations over a period during which the pump frequency was maintained constant. Three different frequency values were tested, namely: 15 Hz, 19 Hz and 38 Hz.

From June 23th to September 16th the culture experienced different environmental and operational conditions. The latter are shown in Table 3. Different pump frequencies were set.
and in one period the air blower was turned on in order to drag the photosynthetically generated oxygen accumulated inside the tubes.

Figure 3 shows the volumetric productivity and available light for the cells for each operational condition.

Table 3 - Operational conditions over the essay

<table>
<thead>
<tr>
<th>Operational conditions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>23/06 - 27/06</td>
<td>19</td>
</tr>
<tr>
<td>28/06 - 05/07</td>
<td>19</td>
</tr>
<tr>
<td>06/06 - 10/07</td>
<td>38</td>
</tr>
<tr>
<td>21/07 - 27/07</td>
<td>15</td>
</tr>
<tr>
<td>28/07 - 04/08</td>
<td>15 + Air blower</td>
</tr>
<tr>
<td>23/08 - 31/08</td>
<td>38</td>
</tr>
<tr>
<td>01/09 - 08/09</td>
<td>38</td>
</tr>
<tr>
<td>09/09 - 15/09</td>
<td>19</td>
</tr>
</tbody>
</table>

Figure 3 shows the volumetric productivity and attenuated energy for each interval in Table 3. Above all, the influence of the pump frequency is evident every time it changed from 19 Hz to 38 Hz. The second and third columns show that the volumetric productivity was the same even with the reduction in the available amount of energy per cell, supporting the conclusion that the mixing was enough to guarantee a high frequency of light-dark cycles. On the contrary, the reduction in pump frequency from 38 Hz to 19 Hz seen at the last two columns also implied a reduction in the volumetric productivity even that the amount of available energy had been bigger.

Likewise, the increase in volumetric productivity was straightforward to the air blower use. First, the pump frequency was the same from July 21st to August 4th, which means that also was the mixing. Second, the average available energy when the air blower was on was smaller, and then a reduction in volumetric productivity would be expected under the same operational conditions.

5. Conclusion

The present work resulted in two complementary methodologies to optimize the...
solar irradiance used in a microalgae production context. Moreover, it analysed operational and environmental conditions in an outdoor pilot scale production plant in Lisbon in order to identify the parameters that affect the culture growth. Together, these approaches would allow further research on microalgae production field in order to optimize its photobioreactors and to step closer to the future of this technology worldwide.

It has first optimized the use of the solar irradiance on an individual tubular photobioreactor geometry in Lisbon, which resulted in a horizontal distance between tubes of 1.75 times the diameter and a vertical one of 1.25 times the diameter. Then, the design of an industrial production plant was proposed accounting for the lost energy on the area between two consecutive green walls. Instead of the default value used, the final design would have a distance between photobioreactors 32% lower than the default case, and an increase in approximately 50% on the light utilization in a production plant.

When it comes to the operational parameter in one pilot scale plant in Lisbon, the analyse proposed that both higher pump frequencies and the use of a degassing system improve the culture growth. Furthermore, it was observed that a semi-continuous cultivation approach based on periodic renewals with recirculation media reduces the volumetric productivity in the medium range.

References


