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Determination of Microalgae Growth in Different Temperature Condition Using a Population Balance Equation

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Microalgae are among the most promising alternative energy resources. To increase the productivity of microalgae, numerous amounts of studies were carried on in the past decades. Apart for experimental works, it is possible to find a large variety of models applied to the growth of microalgae. That becomes particularly useful if we consider the large amount of time and resources needed for experiments. This paper aims the development of a microalgae growth model utilizing the population balance equation. Other than the cell concentration over the time, the model can determine the size distribution of the microalgae population and the number of daughter cells generated through division of mother cells. The size-dependent growth rate is analysed for the proposed two cases: 22 °C and 27 °C.

1. Introduction

Because of the instability of fossil fuels price, many researchers have focused on alternative renewable energies in the past decades (Boyle, 1997). Among the numerous renewable energies, microalgae gained importance because its versatility. The possibility to utilize microalgae for biofuels, food and fine chemicals production makes of microalgae a good source in many sectors. The development of models for the prediction of microalgae growth plays an important role and has the objective to increase the productivity. Indeed, determination of the optimal growth condition may allow a higher concentration of cells. As stated by Lee et al. (2011), many models can determine the bulk growth rate of a microalgae solution as function of the environmental conditions, such as light intensity, temperature, nutrients and carbon dioxide concentration and so on. Cicci et al. (2014) studied the growth of microalgae considering light limitation. Differently, Coelho et al. (2014) made a comparison of biomass and lipid production using fed-batch and continuous reactors. However, all these models can only quantify the total amount of biomass after a certain period of growth. As underlined in other literature works (Morimura, 1959), the size of the cells as well as the number of daughter cells created when microalgae reach the mature stage is variable and depend on the actual growing conditions the cells are exposed to. The population balance equation (PBE) could give us a deeper understanding compared to other commonly used models related to microalgae growth.
The PBE is commonly used for different problems in chemical engineering and science (grinding, crystallization, biology, etc.). Several studies have concentrated on modelling the growth of bacteria (Ramkrishna, 2000), but only a very small number of works have been dedicated to microalgae (Bertucco et al., 2015). In Pahija et al. (2015) work, the breakage equation was adapted to the growth of microphytes. Differently, in Bertucco et al. (2015) work a population balance equation is used to determine the growth of microalgae over the time and the growth rate is function of light and nutrients concentration. Another recent paper (Concas et al., 2016) applied the population balance, making some consideration on how the growth rate would change with different average size distributions.
In our case, the objective is to determine possible correlations in the growth at different environmental conditions. Moreover, we want to emphasize the importance of knowing the size-dependent growth values of the cells. In order to verify the proposed model, microalgae have been grown in controlled conditions and experimental data were taken daily. Differently from previous studies, the proposed work allows the determination of the parameters with simple experimental procedures.

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On a sustainability point of view, the proposed model may play an important role since knowing the size distribution can give useful information on the cells and reduce the harvesting cost (Concas et al., 2016).

2. Population Balance Equation

The population balance equation is commonly used in many applications of chemical engineering concerning multiphase problem, but also in science for modelling growth of cells (Hjortsø, 2006).
Generally, the population balance equation can be written as shown in Eq(1):

$$\frac{\partial n(t,L)}{\partial t} + \frac{\partial}{\partial L}\big(G(t,L)n(t,L)\big) = \int_L^{\infty} X(L,w)S(t,w)B(L,w)n(t,w)dw - S(t,L)n(t,L) \quad (1)$$

where n(t,L) is the number density function and it is function of the cell size L and time t. G is the growth rate. X(L,w) represents the number of daughter cells of size L from size w. S is the probability if particles of size w to break, while B(L,w) represents the fraction of these particles that will divide from size w to size L.
Looking at the terms present in Eq(1), we can find on the left hand side the accumulation term and the term that concerns about the cell growth. On the other hand, on the right-hand side, the first term shows the cells that divide from larger cells to cells of size L. Finally, the last term counts cells that divide from size L to a smaller size.
Let's assume that cells growth rate and division rate are not time dependent and that the representative value for each interval is given by the maximum size of that interval (e.g. 3 µm is the size of particles in the interval [2 3] µm). Finally, we want to consider that only the cells which are not breaking can grow to a larger size interval. All the cells that divide, will generate particles of the smallest size
To solve this kind of equation several methodologies have been proposed in literature, such as discretized methods (LeVeque, 2002). Using an upwind scheme and considering the mentioned equations, the following system can be written:

$$\begin{cases} N_i^{j+1} = N_i^j - \dfrac{\Delta t}{\Delta L} G_i N_i^j + \Delta t \sum_{w=2}^{L_{max}} X S_w N_w^j & for\ i = L_{min} = 1 \\ N_i^{j+1} = N_i^j + \dfrac{\Delta t}{\Delta L}\left[-G_i(1-S_i)N_i^j + G_{i-1}(1-S_{i-1})N_{i-1}^j\right] - \Delta t S_i N_i^j & for\ 2 \le i \le L_{max} \end{cases} \quad (2)$$

where i and j are the size and time discretization intervals, respectively. Eq(2) is obtained using the above-mentioned assumptions; in this case we obtain a couple of equations because the birth of cells occurs only in the first size interval and the
The model was first applied to simple case studies used to test common numerical methods applied to population balance equations. Then, the model was applied to our case study and validated experimentally (the experiments were run 4 times for each case) as it is shown in the next session.

3. Experiment

A strain of spherical shaped microalgae (Chlorococcum) supplied by a Company in Beijing was used in this experiment. The photobioreactor (PBR) consists in a glass cylinder. The PBR is then placed inside a water bath that is used to maintain the desired temperature inside the reactor. To do so, a simple temperature control system was designed: a sensor was introduced inside the reactor; then, a temperature controller could turn on/off a heater placed inside the water bath. In the case operating at 22 °C the temperature was kept at that value using a chiller unit; in this case, the water was pumped from the water bath to the chiller, cooled down and returned to its original container. The cylinder was then covered with gauze to reduce possible contamination from the surrounding environment. A schematic representation of the PBR and the water bath is shown in Figure 1.
The nutrients utilized for the experiment were introduced at the beginning of the experiment only. The medium is called BG11 and consists of the following components: $NaNO_3$, K_2HPO_4, $MgSO_4{\cdot}7H_2O$, $CaCl_2{\cdot}2H_2O$, Citric acid$\cdot H_2O$, Ferric Ammonium Citrate, $Na_2EDTA{\cdot}2H_2O$, Na_2CO_3, Trace Metals Solution and Sodium Thiosulfate Pentahydrate.
The model can be used only if the volume inside the reactor is maintained constant. Consequently, deionized water was poured inside the tank every day to keep the volume as close as possible to the one in the first day of experiment. Finally, the entire setup was entirely covered with a dark tent to avoid light contamination from the surrounding environment.

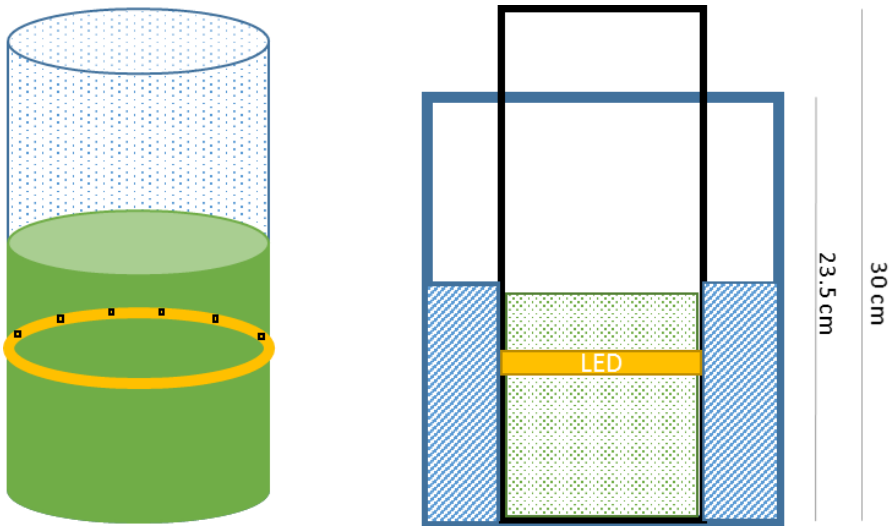

Figure 1: Schematic representation of the PBR on the left and PBR inside the water bath on the right.

An air diffuser was used to maintain agitation inside the PBR during the whole experiment. A LED flexible light (approximately 600 lumen) was placed around the cylinder and constantly turned on. The absorbance at 680 nm was measured daily. Finally, to measure the size distribution, a hemocytometer was utilized and some pictures were taken using a microscope (40x magnification). To make the analysis, the software ImageJ was used (Schneider, 2012).

4. Results

For this paper, it was chosen to select a case study concerning a different temperature. It is our objective to understand how this different environmental condition may affect the growth of the cells at different sizes.
The results are shown in Figure 2, Figure 3 and Figure 4 below. Figure 2 illustrates the number of cells per mL of solution over the time while Figure 3 and Figure 4 show the size distributions. The data at different times (0, 24, 48 and 72 h) and model results (M0, M24, M48 and M72) are shown.

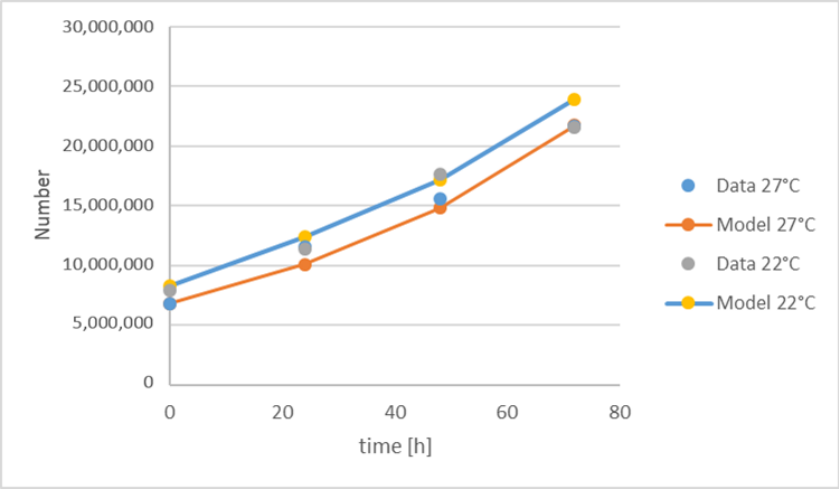

Figure 2: Number of cells per mL of solution over the time.

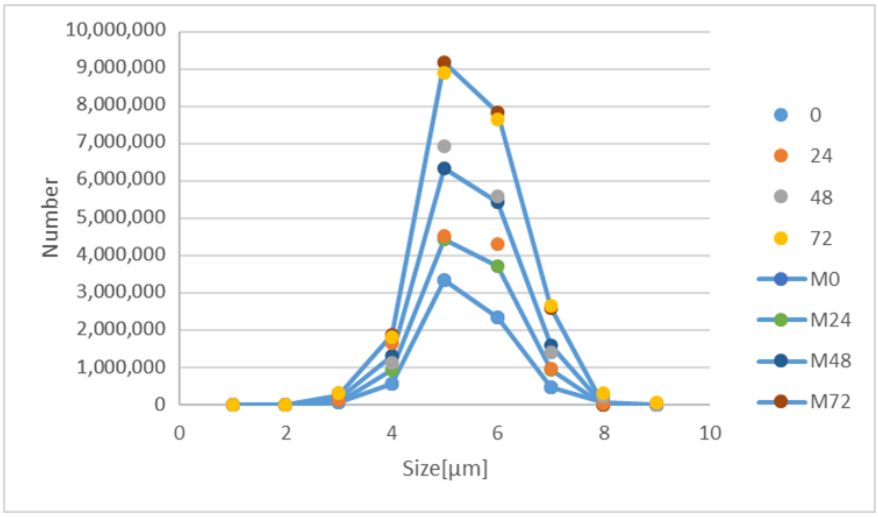

Figure 3: Size distribution in the 27 °C case.

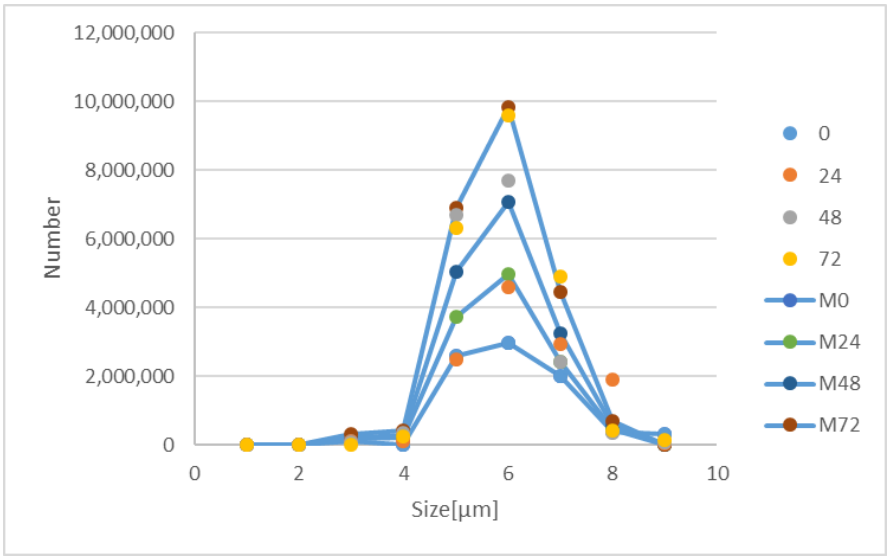

Figure 4: Size distribution in the 22 °C case.

Looking at Figure 2, it is possible to realize that the overall growth rate of microalgae population is higher in the 27 °C case. The number of cells in the low temperature case is higher, but the increment in population is lower compared to the other case. According to Figure 3 and Figure 4, it is possible to notice that the cells are slightly larger in the case with lower temperature. This is an indication about the size of the mother cells, meaning that cells tend to divide earlier in high temperature conditions.
Therefore, the model was validated experimentally and the resulted R^2 was found greater than 0.98 for both the cases.
Finally, the growth and breakage parameters are illustrated in Table 1 and Table 2.

Table 1: Growth parameters.

Temperature [°C]	2	3	4	5	6	7	8	9
22	0	1.71	1.66	0.08	0.04	0.05	0	0
27	0	1.98	0.27	0.04	0.01	0	0	0

Table 2: Division parameters.

Temperature [°C]	2	3	4	5	6	7	8	9
22	0	0	0	0	0	0	0.22	0.8
27	0	0	0	0	0.024	0	0.56	1

As expected the growth is higher for smaller cells, while it becomes less important when cells get ready for division; also, large cells will tend to divide more easily compared to smaller cells.
The first table shows the size specific growth rate. It is possible to notice that cells will stop growing at large sizes. This means that only a small number of cells grows from the first size step to large sizes. On the other hand, the high value obtained in the first interval may be caused by the assumption in which all the new-born cells get generated in the first interval; for that reason, the growth value in this interval should be high in order to compensate the fact that many cells are born in each time step and the actual number of cells in this size interval is quite limited as shown by the experimental data.
In the second table presented above, cells in the first size interval are not able to break. The maximum value of division rate is equal to one; this constraint is necessary to guarantee the zeroth moment, which represents the total number of microalgae cells (if greater than one, the division would occur in a number of cells larger than the one present in the interval of interest).
The number of daughter cells is equal to 3.5 and 2.8 for the low and high temperature and it is an average value (see microscope picture in Figure 5). In other words, cells will break into 2, 3, 4, etc. and that is in accordance with Concas et al. (2016).
Looking at overall picture depicted by the mentioned parameters, the number of daughter cells in the low light case is higher, it has a lower growth rate in the first step and a higher growth rate in the intermediate steps. This result is necessary in order to achieve a higher peak of cells in the [5 6] µm range. In other words, cells at lower temperatures tend to divide less, but they generate a larger number of daughter cells. Differently, in the case with higher temperature, the number of daughter cells is lower and, after the first size step, there is a clear reduction in the growth rate probably due to the fact that cells tend to have a population peak in the [4-5] µm interval range.

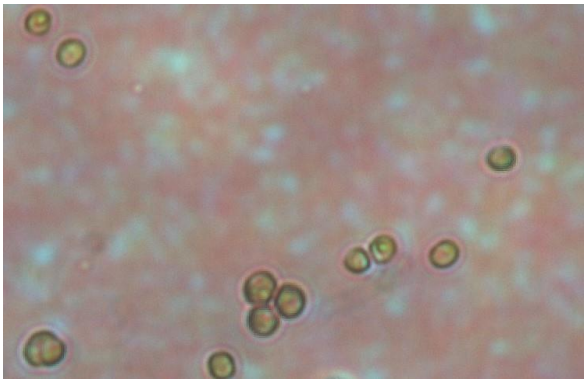

Figure 5: Ternary division (27 °C).

The bulk growth rate was found to be 0.28 day^{-1} and 0.32 day^{-1} for the 22 °C and 27 °C case, suggesting that the temperature is not affecting too much the growth (unless the optimum temperature is located among the two chosen values) in this phase. This is a typical optimal temperature range for this kind of microalgae (Coelho et al., 2014).
In the warmer case, the cells tend to break earlier and with a higher frequency. However, the number of daughter cells generated at 27 °C is a bit lower than the one at 22 °C This explains quite clearly why the bulk growth in the higher temperature is slightly higher.
In this example, variations in the growth rate, division rate and number of daughter cells during the time were not considered. However, in reality, all of the mentioned parameters may be influenced by factors which are changing over the time (for example the change in population number as well as the change in nutrients concentration could affect the growth rate, while the change in light can cause a different number of daughter cells at each division).

5. Conclusions

It has been shown that the population balance is a useful tool to better understand the growth of microalgae and possibly operate on the limiting growth factors. Differently from other models, the model developed in this work can determine the number of microalgae over the time as well as the size-dependent growth parameters. These parameters are particularly difficult to be determined experimentally. To do so, a single cell should be taken under observation for the whole life cycle. For that reason, this model can be very useful to avoid time-consuming experimental procedures.

According to the obtained results, a higher temperature would enhance the growth of microalgae. However, it's worth to mention that the model is applicable only to the mentioned species. A different kind of microalgae would lead to different results. Obviously, also changing few environmental conditions may strongly affect the obtained results. Additional investigation will be necessary to understand whether the behaviour at other temperatures is different from the one observed in this work. Other than that, different environmental conditions may be applied to verify how they affect the growth behaviour.

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References

Bertucco A., Sforza E., Fiorenzato V., Strumendo M., 2015, Population balance modeling of a microalgal culture in photobioreactors: Comparison between experiments and simulations. AIChE Journal, 61(9), 2702-2710.

Boyle G., 1997, Renewable energy: power for a sustainable future. Oxford University Press, UK.

Cicci A., Stoller M., Bravi M., 2014, Analysis of microalgae growth in residual light. Chemical Engineering Transactions, 38, 79-84.

Coelho R.S., Vidotti A.D., Reis É.M., Franco T.T., 2014, High cell density cultures of microalgae under fed-batch and continuous growth. Chem Eng Trans, 38, 313-318.

Concas A., Pisu M., Cao G., 2016, A novel mathematical model to simulate the size-structured growth of microalgae strains dividing by multiple fission. Chemical Engineering Journal, 287, 252-268.

Hjortsø M.A., 2006, Population balances in biomedical engineering. McGraw-Hill, Ney York, USA.

Lee E., Jalalizadeh M., Zhang Q.,2015, "Growth kinetic models for microalgae cultivation: a review," Algal Research, 12, 497-512.

LeVeque R.J., 2002, Finite volume methods for hyperbolic problems (Vol. 31). Cambridge university press.

Morimura Y., 1959, Synchronous culture of Chlorella I. Kinetic analysis of the life cycle of Chlorella ellipsoidea as affected by changes of temperature and light intensity, Plant and Cell Physiology, 1, 49-62.

Pahija E., Zhang Y., Wang M., Zhu Y., Hui C.W., 2015, Microalgae growth determination using modified breakage equation model, Computer Aided Chemical Engineering, 37, 389-394.

Ramkrishna D., 2000, Population balances: Theory and applications to particulate systems in engineering. Academic Press, USA

Schneider C.A., Rasband W.S., Eliceiri K. W., 2012, NIH Image to ImageJ: 25 years of image analysis, Nature Methods, 9, 671.