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Ergys Pahija (Conceptualization) (Methodology) (Validation) (Formal analysis) (Investigation) (Data curation) (Software) (Writing - original draft) (Writing - review and editing) (Visualization), Chi-Wai Hui (Conceptualization) (Methodology) (Writing - review and editing) (Supervision) (Project administration)

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A Practical Approach for Modelling the Growth of Microalgae with Population Balance Equation

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Highlights

- Population balance equation is used to model the population dynamics of microalgae
- Number of daughter cells, size-specific growth and division rates are evaluated
- Prediction of number concentration and size distribution in the linear phase
- Calculation of the life-cycle time of microalgae
- Description of the methodology combining experiments and the numerical method

ABSTRACT

Microalgae are versatile microorganisms with applications in food, energy and fine chemicals, among others. Modelling the dynamics of microalgae inside a photobioreactor is a convenient and inexpensive way to determine the concentration of cells over time. Numerous models have been developed in the literature, but only a few are able to give relevant biological information. Such information can then be used to further improve the production process. The objective of this work was to develop a model for the determination of microalgal dynamics inside a photobioreactor as a function of the environmental conditions, to retrieve the size-specific growth and division rates as well as the number of daughter cells.

The results demonstrate how to evaluate the time needed for microalgae to complete a full life-cycle. Inexpensive laboratory-based procedures and mathematical modelling are combined for the determination of relevant biological parameters.

List of abbreviations: PBE, population balance equation; PBR, photo-bioreactor; OD, optical density; CT, cycle time.

Keywords: Microalgae, size-specific growth rate, size-specific division rate, number of daughter cells, life-cycle time, population balance.

INTRODUCTION

Microalgae are versatile microorganisms used in the production of biofuels, food and fine chemicals [1–3]. Mathematical models applied to microalgal growth are cost-effective tools to optimize production. Numerous models can be found in the literature. Monod's model can be considered as one of the first models applied to microalgal growth [4]. Limitation of carbon, nitrogen and phosphorus concentrations in the medium have been investigated [5,6]. Particular attention has been given to the development of models by considering inhibition caused by specific concentrations of certain elements in the medium [7]. Other environmental conditions such as temperature and light intensity may strongly affect the growth rate and the characteristics of the population [6,8]. Although temperature is a key parameter, only a limited number of reports have taken it into account [9,10]. On the other hand, light has been studied extensively, and numerous reports consider its effect on the biomass. The reduction of light intensity due to the increment of microalgal concentration have been investigated [11]. Additional studies involve the concentration of CO₂ [12,13], pH [9] and turbulence [14,15]. Finally, a combination of the above environmental parameters has been considered to obtain a complete relationship between growth and environmental parameters. For example, pH and temperature [9], nutrients and light intensity [16] and a blend of nutrients, temperature, light intensity and CO₂ [10] have been considered in research studies. A recent paper has investigated the effect of population dynamics in a mixed culture, to evaluate the change in microalgal population in a polluted environment [17].

The specific growth rate of a given algal population, which is utilized in most of the cited models, depends on a combination of cellular growth rate, division rate and the number of daughter cells. However, each specific phenomenon is often neglected and only the change in biomass concentration can be calculated [18]. Although all cited works are extremely useful in determining the change in biomass, these models do not provide any physiological information related to microalgae. Important biological parameters such as the number of daughter cells and the size distribution of microalgae cannot be calculated. Such information can be particularly relevant when trying to optimize the bioprocess. The Population Balance Equation (PBE) applied to microalgae can provide such information, but only a few studies can be found in the literature. Using some predefined growth rate expressions from the literature may be inconsistent, the

nature of the model being different from the PBE. Hence, here an expression is derived by directly fitting the population balance model to experimental data generated in different conditions. Thus, the problem is first reversed to generate a proper function for growth rate, division rate and the number of daughter cells as a function of environmental conditions. These functions are then replaced in the model to calculate the number density of microalgae over time. This approach should give a realistic relationship between the mentioned parameters, the number and the size of cells. As for all other models, this approach is reliable only for the microalgal species under consideration and only if operating within the environmental conditions defined by this work.

This work analyzes the effects of temperature and light intensity during the linear phase of growth. A similar approach can be followed for the stationary and the other phases, but the results may be different, since the mechanisms of growth and division can be affected. At the basis of this work is the fact that microalgal growth and physiology change in different environments. To understand the effects of the environmental condition on the growth, a series of experiments was performed where, in each case, one of the parameters was modified, while the remaining environmental conditions were kept constant without limiting the growth. The determination of biological parameters is relevant since it allows the development of a model for calculating the growth rate and division rate for each specific size. The goal of the following is the calculation of microalgal number density, size distribution, overall growth rate (commonly referred to as specific growth rate in other works), size-specific growth rate, size-specific division rate, the average number of daughter cells and life cycle time. The objective is to present a methodology, which allows the calculation of these parameters, which are not easily obtained from experiments. To verify the results, a detailed experimental setup and measurements procedure are proposed, as well as information on the numerical solution of the number-based PBE.

MATERIALS AND METHODS

This section includes the major information related to the experimental setup and measurements, while additional details are available in Supplementary materials (Appendix A).

Experimental setup

Chlorococcum sp. (spherical-shaped single-cell microalgae) was used for the experiments and provided by ENN Co. Ltd., China. A schematic representation of the experimental setup is shown in **Figure 1**. The proposed design was chosen because when the microalgae solution is directly cooled by pumping the solution to a chiller, two major problems arise: microalgae may suffer strong shear stress and rupture when passing through the pump while some cells may tend to stack in the pipes. In contrast, direct heating of the microalgae solution may cause some significant temperature gradients inside the PBR. Hence, the reactor was placed inside a water bath to control the temperature, while the temperature sensor was inside the PBR.

BG11 medium (Qingdao Hope Bio-Technology, Qingdao, China) (see composition in Suppl. Appendix A) was used to provide the necessary nutrients to the microalgae. The nutrients were introduced into the system at the beginning of the experiment; thereafter, only deionized water was added to the reactors to maintain a constant volume. All experiments were performed in triplicate for each indicated environmental condition.

Measurements

Daily measurement of optical density (OD), size distribution and environmental parameters were required. The OD is commonly used to define the concentration of cells in the solution [19]. To avoid errors in the measurement caused by the chlorophyll content of microalgae [29], OD was measured at 750 nm. The correlation derived by [29] was used to calculate total number of microalgae [20]. Finally, the size distribution of cells was determined microscopically (Nikon Eclipse E100). Several images, accounting for several hundred cells, were taken at random and saved for later analysis. ImageJ [21] was used to define the size distribution of microalgae (e.g., **Figure 2**). Suppl. Appendix A presents the entire procedure, including a step-by-step image analysis and statistical approximation.

Given a certain population, a sample of several hundred cells was analysed to determine the size distribution. After diameter and area of particles was measured, the distribution was approximated to a lognormal function [22]. Further information is given in Suppl. Appendix A.

Effect of environmental conditions

Table 1 shows the cases utilized for defining a model able to predict the growth of microalgae using BG11 medium, with temperature and light in the range of 23-30 °C and 420-1020 lumens respectively. Two environmental parameters were considered in this study:

- Temperature effect: light flux was constant throughout the experiments at three different temperatures; light and nutrients were not limiting factors, i.e. there was no reduction in the growth rate due to lower light penetration in the solution.
- Light effect: temperature was maintained at an approximately constant value by using temperature controllers at three different light fluxes; nutrients were not a limiting factor.

This work focused on the linear phase of growth, being the one that combines an elevated increase in microalgal population as well as maintaining a high concentration of cells inside the PBR.

Microalgal modelling

Population Balance Equation to model the growth of microalgae

PBE has numerous applications, from computational fluid dynamics to biology, from multiphase reaction engineering to particulate systems. Among microalgal applications, PBE has been used in maximize microalgae harvesting [23,24] and the growth inside the PBR [25–28]. All simulation and optimization problems were performed using a machine with i7-4750HQ processor. Apart from the ImageJ software, most of the work was performed using MATLAB.

Based on the experimental system setup (Figure 1), the volume inside the batch reactor did not change. Furthermore, the number density function was not dependent on the spatial coordinates. It can be stated that:

$$\frac{\partial n}{\partial t} + \frac{\partial(Gn)}{\partial L} = B - D \quad (1)$$

n is the number density, G is the growth rate of cells, while L and t are the size and time coordinates, respectively. Equation 1 still includes the eventual source and sink terms on the right-hand side of the equation. Birth B and death D terms can include a diverse number of phenomena occurring in the system, such as agglomeration, breakage and nucleation [29].

Population Balance Equation (PBE) can be used to model the growth of cells. Several reports

have been addressed to model the growth of bacteria [30,31] and microalgae [26–28,32]. Additional work on cell growth has also been developed [33]. Cells can be seen as particles and cell division can be represented by the breakage components of the equation. The population balance can be written, as shown in Equation 2:

$$\begin{aligned} \frac{\partial n(t, L)}{\partial t} + \frac{\partial}{\partial L} (G(t, L)n(t, L)) \\ = \int_L^\infty X(L, w)S(w)b(L, w)n(t, w)dw - S(L)n(t, L) \end{aligned} \quad (2)$$

where $n(t, L)$ is the number density function, representing the number of cells in a unit volume of size included in the interval L and $L + dL$ at time t . G denotes the growth rate of the cells in terms of size change over time. The growth rate can be either a constant or a size-dependent variable. In the present case, it is considered as a function of size. On the right-hand side of the equation, the breakage function represents the reproduction of microalgae in their life cycle. The first term considers the breakage of larger particles of size w to particles of size L and depends on the number of particles of size w at time t , on the probability of larger cells to break $S(w)$ and on the possibility of these particles to break into particles of size $[L, L + dL]$. After division, a number of daughter cells are formed. $X(L, w)$ is the number of daughter cells of size L generated from the division of size w cells. The second term considers the breakage of particles of size L to smaller particles.

Numerical solution of PBE

The discretized form of the PBE that is used for the proposed problem is given by Equation 3.

$$\begin{aligned} N_i^{j+1} = N_i^j + \Delta t \left[-\frac{G_i}{\Delta L_i} S_i N_i^j + S_{i-1} \frac{G_{i-1}}{\Delta L_{i-1}} \right] - S_i N_i^j \Delta t \\ + \sum_{k=L_{cr}}^{L_{max}} (X S_k b_{ki} N_k^j \Delta t) \end{aligned} \quad (3)$$

Where N_i^j is the number of particles in the size interval i at time j , G_i is the growth rate of interval i , S_i is the size-specific division rate and X is the number of daughter cells. b_{ki} is the

probability for particles of size k to divide into particles of size i . Finally, Δt and ΔL_i are the time and size interval i , respectively. Where the size discretization is uniform, then it can be assumed that $\Delta L = \Delta L_i$. In this first example, b_{ki} is taken as equal to 1 for the first size interval ($i = 1$) and equal to 0 for all other size intervals ($i \neq 1$). This assumption will obviously influence the results since cells can be generated only at very small sizes. The discretization is done, as shown below in **Figure 3**. The right grid point of each interval in size coordinate is the representative for the interval itself. All daughter cells are generated in the first interval that has a representative size equal to L_2 .

Model adjustment for improving the first moment (total volume)

Assuming that all cells are formed in the smallest size interval, the number of daughter cells generated per division can be fixed according to the size of mother cells. The number of daughter cells from a mother cell of size i is given by $X_i = V_i/V_1 = (d_i/d_1)^3$. However, this is not the average number of cells created in the population. In Equation 3, the number of daughter cells which was indicated with X would now include a subscript k (becoming X_k), indicating that the number of new-born cells depends on the size of the mother cell. With this consideration, it is still possible to obtain the average number of daughter cells using the following equation:

$$\bar{X}^j = \sum_k X_k \frac{X_k S_k b_{k1} N_k^j \Delta t}{\sum_{k=L_{cr}}^{L_{max}} (X_k S_k b_{k1} N_k^j \Delta t)} \quad (4)$$

Equation 4 represents the ratio between particles created from size k and the total number of particles created. This means that the average number of daughter cells $\bar{X}^j$ may change at each time iteration. As shown below, the number will change only for a certain amount of time and then reach an asymptotic value. L_{cr} represents the critical size from which microalgae start dividing and, in the present case, microalgae start dividing when they are larger than 4.5 μm .

RESULTS AND DISCUSSION

After the growth rate and the division rate were retrieved from data fitting, it was observed that

the behaviour of those functions could be approximated as follows:

$$G = aL^b + c \quad (5)$$

$$S = d + \frac{1 - d}{1 + \exp(-e * (L - f))} \quad (6)$$

Based on Equations 5 and 6, the values of all parameters were calculated for each case study at different environmental conditions. These parameters enabled a decoupling of the size and environmental conditions. In this case, all introduced parameters are a function of temperature and light only. A specific parameter was calculated at a specific light flux and temperature range and repeated for each size of microalgae. This allowed the generation of a 4-dimensional matrix: three dimensions are given by the environmental conditions (light and temperature) and the size of microalgae, while the fourth dimension is given by the calculated parameter itself (in this case growth and division rate).

The supplementary materials (Appendix B) contains extended results. The dependence of parameters a , b and c on the temperature and the light are shown in Suppl. Figure B1. While it is not easy to give an exact physical interpretation of these parameters, it is possible to detect several examples of interesting behaviour, e.g. the growth rate tends to be higher at higher light intensities. From the results shown in Suppl. Figure B1, it is possible to predict that the effect of the parameter b becomes more important for larger particles and therefore their growth rate should be more dependent on parameter c compared to smaller particles. Therefore, it can be expected that the growth rate of large particles should resemble c , with higher values at average temperatures. On the other hand, small sizes are less affected by b , and therefore their growth rate will resemble a . Parameters inbetween will see a transition phase depending on the size and environmental conditions. This analysis confirms that the size of cells (or rather the stage of the life cycle in which the cell is located) may affect growth rate.

Equation 6 is inspired by the logistic function. From the fitting results of parameters d , e and f , it is possible to notice that the division rate is lower at low size ranges but increases substantially at larger sizes (Suppl. Figure B2).

Another approach that could be followed is the application of traditional growth and division terms [27,28]. There may be a drawback to this because such types of correlation derive the rate

of growth or division representative of the population and may not be ideal when dealing with individual cells (or groups of cells) of different species and operating in different conditions. Therefore, a generic quadratic function was included to create a correlation between size-specific growth rate, size-specific division rate, size of cells, temperature and light. To the best of our knowledge, such a correlation has not been proposed previously.

The change in the growth rate of microalgae can be quite significant under different conditions, depending on their size. Small cells grew faster at higher light intensities and lower temperatures (**Figure 4**). Nevertheless, when growing in size, cells grew faster at higher temperatures. Larger cells may have an increased growth rate at higher temperatures because of their cell cycle. Since the size distribution at higher temperatures favoured smaller cells, the presence of larger particles dropped by increasing the growth rate and allowing cells to grow to a critical size where division occurred. From a biological point of view, this may be caused by some internal cell reactions, which are facilitated at higher temperatures (activation temperature). The coefficient of determination, in this case, was equal to 0.9918. In contrast, the division rate of microalgae is irrelevant for small microalgae and increased at larger sizes (**Figure 5**). Small cells had a higher probability of division when the temperature and the light intensity were higher. This is feasible considering that microalgae living at high temperatures reach the mature stage earlier and therefore tend to divide sooner.

Suppl. Figures B1 and B2 show the correlation between the growth and the division rate with model parameters at different environmental conditions. The light is expressed in terms of luminous flux (lumen, *lm*). The constants required to calculate the parameters shown above at different light intensities and temperatures can be found in **Table 2** and **Table 3**. The physical meaning for each constant has not been given yet, and can be investigated in future studies.

In the present case, the growth rate is referred to as the increase in microalgae size. For this reason and to take into account a possible correlation between the two considered environmental conditions, a quadratic form of equation was used to calculate the parameters of the size-specific growth rate and division rate. A similar approach was followed by others when the correlation between variables was generalized [34,35]. In future, other possible functions to represent growth and division rates as functions of the size of microalgae should be further investigated. The errors presented in **Table 2** and **Table 3** are achieved by running a sensitivity analysis on

each parameter and guarantee that the model represents the experimental data within a 5% error. Future developments of the model will consider a reduction of needed parameters as well as comparison with functions such as Arrhenius type.

Effect of temperature on growth and division rates

The growth rate did not reach high values except for small-sized microalgae. Growth was favoured by higher light intensities and lower temperatures. In addition, from Suppl. Figure B3, it is observed that a stronger light favoured a faster growth for cells over a wider range of temperatures. We observed that the growth rate decreased at increasing cell size (Suppl. Figure B3), in agreement with observations from [28]. Suppl. Figure B4 shows the division rate as a function of temperature, at various constant light intensities. Almost the totality of cells divided at large sizes, and the phenomena were favoured at higher temperatures.

These results become relevant when looking at the size distribution of microalgae. Considering the experimental range of this work, when operating at lower temperatures, the size distribution of cells shifted towards larger sizes. On the other hand, microalgae were smaller at higher temperatures [20]. For example, the division at 24°C occurred mainly after the cells were larger than 8 μm , whereas microalgae growing at 30°C divided at 7 μm .

Effect of light on growth and division rates

The growth rate is a function of light intensity at some fixed temperature values. Suppl. Figure B5 shows the growth rate as a function of light intensity and microalgal size for different values of temperature. The effect of light intensities on the division rate at various temperatures is shown in Suppl. Figure B6.

Within the environmental conditions considered, the amount of light influenced the growth of microalgal cells. However, the effect was limited to those with a small size. In particular, the highest growth rate at 450 lm was approximately 0.5 $\mu\text{m}/\text{h}$ while at 1020 lm the growth rate increased to over 1 $\mu\text{m}/\text{h}$. In all cases under investigation, the growth was close to zero. These trends confirm observations from others [36,37], and are partially caused by the model assumption for which newly produced cells start growing from the first size interval. Future developments of the model will consider a relaxation of this assumption, allowing any new cells

to start growing from any size interval; in this case, a probability study needs to be carried out. The results confirm that the growth rate was higher at higher light intensities and was favoured at lower temperatures. On the other hand, the size-specific division rate was triggered at smaller sizes and at higher temperatures, but did not seem to be affected by changes in light intensity.

Additional considerations

A more intuitive representation of growth rate and division rate is shown in **Figure 6** and **Figure 7**, respectively. Once again, the same considerations performed earlier can be verified.

The difference from other observations on *Chlorella* [25] is the effect of light intensity on the number of daughter cells. It appeared that temperature affected the number of daughter cells while the light intensity did not (**Figure 7**). The trend of the size-specific growth-rate is similar to that obtained by others [28], although the approach to the problem is different, by considering an average initial distribution. The model confirms the increase in the average size of cells when the temperature is reduced. It is also in agreement with the results obtained by experiments in synchronous growth conditions, confirming that the growth rate of microalgae is higher at higher light intensities [36]. Besides modelling reports confirming the effects of environmental conditions [27,28,38], the correlation between microalgae growth and environmental conditions has been confirmed by reports in which synchronous cultures of microalgae were cultivated [36,37,39]. Among the main observations recovered from these works, it is found that:

- The growth of cells, or the increase in mass, depends on the light intensity. The average size in which the division occurs does not depend on the light intensity unless the light intensity is particularly low.
- Number of daughter cells is not affected by different temperatures, but it is highly affected by different light intensities.
- The size distribution of cells is influenced by the temperature with smaller cells at higher temperatures.

Different from what observed in [36], the number of daughter cells was not greatly influenced by light intensity since the trend of the division rate was very similar; it appears that the temperature had a greater influence on the number of daughter cells. This may be partially caused by the

assumptions in the model and partially because the species is not the same (although *Chlorella* and *Chlorococcum* belong to the same division of green algae, *Chlorophyta* [40]). Considering that the growth of the microalgal population inside a PBR depends on the growth rate of cells as well as on the division rate, the proposed methodology separates the two aspects, giving more information compared to other models.

Finally, it was observed that the nutrient concentration did not affect the growth, especially in the linear phase, when nutrients are not a limiting factor. Obviously, higher nutrient concentrations increase the maximum cell density. However, when the concentration becomes very high, the growth rate may be affected by the reduced light efficiency caused by the increasing number density (scattering effect).

Previous applications of PBE to microalgae

A modified breakage equation has been applied to predict cellular growth [9]. However, the results achieved were unsatisfactory and it was not possible to define any correlation between the particle size and growth rate. Following the modified breakage equation model applied to microalgae [8], the classical form of PBE was employed to model the microalgal growth [27]. The equation contained in separate terms the cell growth and division rate, and it consisted of simulating the dynamic growth of microalgae as a function of nutrient concentration and light intensity [27]. Most of the parameters were obtained from literature data and the number of daughter cells was assumed to be equal to 2, while other environmental conditions were neglected [27]. The approximation of cell division as binary breakage is a strong assumption since the number of daughter cells may easily differ from 2, depending on the environmental conditions. Size distribution was generated using a cellometer, while the cell density was regarded as a constant. No discussion was provided on the linear correlation between the growth rate and the mass of microalgae. Others took account of a variable number of daughter cells [38]. Yet, the growth rate was retrieved from literature. It was shown that the size distribution of microalgae might change over time along the algal life cycle (for example the size distribution in the first day could be different from that after several days in a specific environmental condition); it was considered that division may occur from different average sizes of mother cells. Although temperature is a relevant factor in the population dynamics, which may strongly

affect the growth of microalgae [38], the authors operated under isothermal conditions. A more complex (yet similar to [27]) growth rate calculation was utilized; as explained elsewhere by the same research group, the growth rate can be assumed linearly proportional to the mass of cells [41], but still not much discussion is given. The work is a very relevant analysis since it can tell how the initial average size affects growth and performance of the population in different average mother cell sizes. However, this does not explain how cells behave in the system if they are smaller or larger than the average, and the approach used in this paper is different.

Life-cycle time

The dependence on environmental condition affects the parameters under investigation and, therefore, the average time needed by cells to complete a life cycle. The cycle time (CT) depends on the growth rate of each size interval, as shown in Equation 7.

$$CT = \sum_i \frac{\Delta L_i}{G_i} \quad (7)$$

The parameters obtained above can be used to retrieve the cycle time of microalgae. For example, at $T = 30^\circ\text{C}$ and $L_t = 420\text{ lm}$, the life-cycle time is equal to $CT_{420}^{30} = 20.4 \pm 7.8\text{ h}$. At 30°C and 600 lm , $CT_{600}^{30} = 16.8 \pm 1.4\text{ h}$, while at 30°C and 1020 lm , $CT_{1020}^{30} = 22.5 \pm 7.7\text{ h}$. Although no value related to *Chlorococcum* was found in literature, the results can be compared with those achieved experimentally by synchronous culture of *Chlorella* [36]. In that case, microalgae completed a life cycle in approximately 28 h, which is in the same range as here. A minimum of three experiments was performed to achieve the indicated values while the standard error was calculated using the theory from [42]. The indicated values express the minimum amount of time needed for a cell to grow from the smallest size interval to the first size interval in which microalgae can divide. Different values (either smaller or larger) will be obtained if cells start growing from a different starting interval and if the division occurs at larger sizes. A statistical study needs to be addressed for a better estimation of the life-cycle time in future studies.

Conclusions and future perspectives

This work illustrates a methodology to retrieve important biological information from microalgal cells. The proposed approach allows the determination of size-specific parameters which are not

commonly found in the literature. Although the proposed methodology is applied to *Chlorococcum sp.*, a similar approach can be followed for other microalgal species or, generally, to other types of microorganisms. It has two major advantages: first, it allows the calculation of biological parameters from scratch, without requiring data from literature, and secondly, it requires a basic instrument that can be found in almost any laboratory.

This work can be extended by including the uncertainty analysis to reduce the number of parameters. Additionally, different correlations for growth, division and environmental conditions can be tested. Application to other types of microorganisms to test the model and retrieve biological parameters could be an additional investigation. Finally, some research can be suggested to develop a correlation with internal substances of interest, e.g. by forcing growth to larger cell sizes, if the desired substance is more concentrated in larger cells to optimize the production.

CRedit authorship contribution statement

Ergys Pahija: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Software, Writing - original draft, Writing - review & editing, Visualization. **Chi-Wai Hui:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] Li J, Liu Y, Cheng JJ, Mos M, Daroch M. Biological potential of microalgae in China for biorefinery-based production of biofuels and high value compounds. *N Biotechnol* 2015;32:588–96. <https://doi.org/10.1016/j.nbt.2015.02.001>.
- [2] Guo M, Song W. The growing U.S. bioeconomy: Drivers, development and constraints. *N Biotechnol* 2019;49:48–57. <https://doi.org/10.1016/j.nbt.2018.08.005>.
- [3] Chang JS. Microalgae-based wastewater treatment and circular economy. *N Biotechnol* 2018;44:S162. <https://doi.org/10.1016/j.nbt.2018.05.1178>.
- [4] Lee E, Cumberbatch J, Wang M, Zhang Q. Kinetic parameter estimation model for anaerobic co-digestion of waste activated sludge and microalgae. *Bioresour Technol* 2017;228:9–17. <https://doi.org/10.1016/j.biortech.2016.12.072>.
- [5] Novak JT, Brune DE. Inorganic carbon limited growth kinetics of some freshwater algae. *Water Res* 1985;19:215–25. [https://doi.org/10.1016/0043-1354\(85\)90203-9](https://doi.org/10.1016/0043-1354(85)90203-9).
- [6] Grima EM, Camacho FG, Pérez JAS, Sevilla JMF, Fernández FGA, Gómez AC. A mathematical model of microalgal growth in light-limited chemostat culture. *J Chem Technol Biotechnol* 1994;61:167–73. <https://doi.org/10.1002/jctb.280610212>.
- [7] Santos FM, Mazur LP, Mayer DA, Vilar VJP, Pires JCM. Inhibition effect of zinc, cadmium, and nickel ions in microalgal growth and nutrient uptake from water: An experimental approach. *Chem Eng J* 2019;366:358–67. <https://doi.org/10.1016/j.cej.2019.02.080>.
- [8] Pahija E, Zhang Y, Wang M, Zhu Y, Hui CW. Microalgae growth determination using modified breakage equation model. vol. 37. 2015. <https://doi.org/10.1016/B978-0-444-63578-5.50060-8>.
- [9] Mayo AW. Effects of temperature and pH on the kinetic growth of unialga *Chlorella vulgaris* cultures containing bacteria. *Water Environ Res* 1997;69:64–72. <https://doi.org/10.2175/106143097X125191>.
- [10] Franz A, Lehr F, Posten C, Schaub G. Modeling microalgae cultivation productivities in different geographic locations - estimation method for idealized photobioreactors. *Biotechnol J* 2012;7:546–57. <https://doi.org/10.1002/biot.201000379>.

- [11] Edwards KF, Thomas MK, Klausmeier CA, Litchman E. Phytoplankton growth and the interaction of light and temperature: A synthesis at the species and community level. *Limnol Oceanogr* 2016;61:1232–44. <https://doi.org/10.1002/lno.10282>.
- [12] Chinnasamy S, Ramakrishnan B, Bhatnagar A, Das KC. Biomass production potential of a wastewater alga *Chlorella vulgaris* ARC 1 under elevated levels of CO₂ and temperature. *Int J Mol Sci* 2009;10:518–32. <https://doi.org/10.3390/ijms10020518>.
- [13] Ben-Amotz A. Bio-fuel and CO₂ capture by micro-algae. *Wind. Sea Algae Conf. Present.*, Maribo, Denmark: 2009, p. 20–2.
- [14] García-Portela M, Reguera B, Ribera d'Alcalà M, Rodríguez F, Montresor M. Effects of small-scale turbulence on two species of *Dinophysis*. *Harmful Algae* 2019;89:101654. <https://doi.org/10.1016/j.hal.2019.101654>.
- [15] Thomas WH, Gibson CH. Effects of small-scale turbulence on microalgae. *J Appl Phycol* 1990;2:71–7. <https://doi.org/10.1007/BF02179771>.
- [16] Jeon YC, Cho CW, Yun YS. Combined effects of light intensity and acetate concentration on the growth of unicellular microalga *Haematococcus pluvialis*. *Enzyme Microb Technol* 2006;39:490–5. <https://doi.org/10.1016/j.enzmictec.2005.12.021>.
- [17] Olguín EJ, Mendoza A, González-Portela RE, Novelo E. Population dynamics in mixed cultures of *Neochloris oleoabundans* and native microalgae from water of a polluted river and isolation of a diatom consortium for the production of lipid rich biomass. *N Biotechnol* 2013;30:705–15. <https://doi.org/10.1016/j.nbt.2013.03.003>.
- [18] Lee E, Jalalizadeh M, Zhang Q. Growth kinetic models for microalgae cultivation: A review. *Algal Res* 2015;12:497–512. <https://doi.org/10.1016/J.ALGAL.2015.10.004>.
- [19] Griffiths MJ, Garcin C, van Hille RP, Harrison STL. Interference by pigment in the estimation of microalgal biomass concentration by optical density. *J Microbiol Methods* 2011;85:119–23. <https://doi.org/10.1016/j.mimet.2011.02.005>.
- [20] Pahija E, Hui C-W. A systematic study on the effects of dynamic environments on microalgae concentration. *Algal Res* 2019;42:101599. <https://doi.org/10.1016/J.ALGAL.2019.101599>.
- [21] Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 2012;9:671–5.
- [22] Limpert E, Stahel WA, Abbt M. Log-normal distributions across the sciences: Keys and

- clues. *Bioscience* 2001;51:341–52. [https://doi.org/10.1641/0006-3568\(2001\)051\[0341:LNDATS\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2001)051[0341:LNDATS]2.0.CO;2).
- [23] Pahija E, Hui CW, Woodley JM, Sin G. Effect of Selective Size Extraction of Microalgae from a Photobioreactor. *Comput. Aided Chem. Eng.*, vol. 48, Elsevier B.V.; 2020, p. 331–6. <https://doi.org/10.1016/B978-0-12-823377-1.50056-2>.
- [24] Lee P-Y, Pahija E, Liang Y-Z, Yeoh K-P, Hui C-W. Population Balance Equation Applied to Microalgae Harvesting. *Comput. Aided Chem. Eng.*, vol. 43, 2018, p. 1299–304. <https://doi.org/10.1016/B978-0-444-64235-6.50228-X>.
- [25] Pahija E, Liang Y, Hui CW. Population Balance Applied to Microalgae Growth. *Comput. Aided Chem. Eng.*, vol. 38. 2016. <https://doi.org/10.1016/B978-0-444-63428-3.50236-8>.
- [26] Pahija E, Lee PY, Hui C-W. A Revision of Population Balance Equation Applied to Microalgae with Birth, Growth, and Death. *Process Integr Optim Sustain* 2019;3:125–41. <https://doi.org/10.1007/s41660-018-0059-9>.
- [27] Bertuccio A, Sforza E, Fiorenzato V, Strumendo M. Population balance modeling of a microalgal culture in photobioreactors: Comparison between experiments and simulations. *AIChE J* 2015;61:2702–10. <https://doi.org/10.1002/aic.14893>.
- [28] Concas A, Pisu M, Cao G. A novel mathematical model to simulate the size-structured growth of microalgae strains dividing by multiple fission. *Chem Eng J* 2016;287:252–68. <https://doi.org/10.1016/j.cej.2015.11.021>.
- [29] Kumar J. Numerical approximations of population balance equations in particulate systems. 2006.
- [30] Liou JJ, Srien F, Fredrickson AG. Solutions of population balance models based on a successive generations approach. *Chem Eng Sci* 1997;52:1529–40. [https://doi.org/10.1016/S0009-2509\(96\)00510-6](https://doi.org/10.1016/S0009-2509(96)00510-6).
- [31] Jędrak J, Kwiatkowski M, Ochab-Marcinek A. Exactly solvable model of gene expression in a proliferating bacterial cell population with stochastic protein bursts and protein partitioning. *Phys Rev E* 2019;99:042416. <https://doi.org/10.1103/PhysRevE.99.042416>.
- [32] Sadegh-Vaziri R. Biomass to Biofuel: Investigating Syngas Cleaning and Biomass Feedstock. 2017. <https://www.diva-portal.org/smash/record.jsf?pid=diva2%3A1085282&dswid=-9797>.
- [33] Hjortsø MA. Population balances in biomedical engineering : segregation through the

- distribution of cell states. McGraw-Hill; 2006.
<https://www.accessengineeringlibrary.com/content/book/9780071447683>.
- [34] Van Rijssel M, Gieskes WWC. Temperature, light, and the dimethylsulfoniopropionate (DMSP) content of *Emiliania huxleyi* (Prymnesiophyceae). *J Sea Res* 2002;48:17–27.
[https://doi.org/10.1016/S1385-1101\(02\)00134-X](https://doi.org/10.1016/S1385-1101(02)00134-X).
- [35] Jo JH, Lee DS, Park JM. Modeling and optimization of photosynthetic hydrogen gas production by green alga *Chlamydomonas reinhardtii* in sulfur-deprived circumstance. *Biotechnol Prog* 2006;22:431–7. <https://doi.org/10.1021/bp050258z>.
- [36] Morimura Y. Synchronous Culture of *Chlorella* I. Kinetic Analysis of the Life Cycle of *Chlorella* Ellipsoidea as Affected by Changes of Temperature and Light Intensity. *Plant Cell Physiol* 1959;62. <https://doi.org/10.1093/oxfordjournals.pcp.a075750>.
- [37] Morimura Y. Synchronous Culture of *Chlorella* II. Changes in Content of Various Vitamins During the Course of the Algal Life Cycle. *Plant Cell Physiol* 1959. <https://doi.org/10.1093/oxfordjournals.pcp.a075751>.
- [38] Pahija E, Liang Y, Hui C-W. Determination of microalgae growth in different temperature condition using a population balance equation. *Chem Eng Trans* 2017;61.
<https://doi.org/10.3303/CET1761118>.
- [39] Harvey JD. Synchronous Growth of Cells and the Generation Time Distribution. *J Gen Microbiol* 1972;70:99–107. <https://doi.org/10.1099/00221287-70-1-99>.
- [40] Guiry, M.D. & Guiry G. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway 2020. <https://www.algaebase.org> (accessed December 6, 2020).
- [41] Concas A, Pisu M, Cao G. Novel simulation model of the solar collector of BIOCOIL photobioreactors for CO₂ sequestration with microalgae. *Chem Eng J* 2010;157:297–303.
<https://doi.org/10.1016/j.cej.2009.10.059>.
- [42] Altman DG, Bland JM. Standard deviations and standard errors. *BMJ* 2005;331:903.
<https://doi.org/10.1136/bmj.331.7521.903>.

List of Figures

Figure 1. Schematic representation of the experimental setup. The PBR was placed inside a water bath to regulate the temperature with a temperature controller. LED lights represented the light source, while air was introduced into the system through an air pump.

Figure 2. Raw microscope image (a) and image after analysis with ImageJ (b).

Figure 3. Example of a uniform size discretization to solve the PBE.

Figure 4. Growth rate [$\mu\text{m/h}$] of microalgae as a function of temperature and light at four assigned sizes. Generally, high light intensities and lower temperatures favoured the growth.

Figure 5. Division rate [-] of microalgae as a function of temperature and light at four assigned sizes. Division occurred when cells were mature (large-sized cells).

Figure 6. Change in microalgal growth rate as a function of size in different environmental conditions. Growth was favoured at small sizes, high light intensities and low temperatures.

Figure 7. Change in microalgal division rate under different environmental conditions. Division occurred when cells were mature, at smaller sizes with a higher temperature.

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Table 1 Experiments conducted using BG11 medium.

	23°C	27°C	30°C
420 lm	✓		✓
600 lm	✓	✓	✓
1020 lm	✓		✓

Table 2 Constants needed to calculate the growth rate parameters a, b, c . All the parameters and their absolute errors are calculated using the same type of equation. Following is an example for a : $a = k_{G,1}^a Lt^2 + k_{G,2}^a T^2 + k_{G,3}^a Lt^2 T + k_{G,4}^a T^2 Lt + k_{G,5}^a Lt \cdot T + k_{G,6}^a Lt + k_{G,7}^a T + k_{G,8}^a$

	$k_{G,1}^{par}$	$k_{G,2}^{par}$	$k_{G,3}^{par}$	$k_{G,4}^{par}$	$k_{G,5}^{par}$	$k_{G,6}^{par}$	$k_{G,7}^{par}$	$k_{G,8}^{par}$
a	-8.721 (± 0.010)	1808.0 (± 3.3)	0.3156 (± 0.0004)	-3.198 (± 0.008)	-321.9 (± 0.2)	$1.18 \cdot 10^4$ (± 4.1)	$5.39 \cdot 10^4$ (± 75)	$-3.04 \cdot 10^6$ (± 1721)
b	$1.49 \cdot 10^{-5}$ ($\pm 2.2 \cdot 10^{-7}$)	0.09907 ($\pm 7.4 \cdot 10^{-5}$)	$-2.38 \cdot 10^{-7}$ ($\pm 9.6 \cdot 10^{-9}$)	$-1.1 \cdot 10^{-4}$ ($\pm 1.8 \cdot 10^{-7}$)	0.00609 ($\pm 4 \cdot 10^{-6}$)	-0.0974 ($\pm 9.3 \cdot 10^{-5}$)	-5.303 ($\pm 1.7 \cdot 10^{-3}$)	65.45 (± 0.04)
c	$1.81 \cdot 10^{-7}$ ($\pm 4.4 \cdot 10^{-9}$)	$-3.8 \cdot 10^{-4}$ ($\pm 1.5 \cdot 10^{-6}$)	$-4.57 \cdot 10^{-9}$ ($\pm 1.9 \cdot 10^{-10}$)	$-4.18 \cdot 10^{-8}$ ($\pm 3.5 \cdot 10^{-9}$)	$9.38 \cdot 10^{-6}$ ($\pm 8.1 \cdot 10^{-8}$)	$-2.97 \cdot 10^{-4}$ ($\pm 1.9 \cdot 10^{-6}$)	0.0173 (± 0.030)	-0.1545 ($\pm 7.8 \cdot 10^{-4}$)

Table 3 Constants for the calculation of the division rate parameters d, e, f . Absolute errors are indicated between brackets. Following is an example for d : $d = k_{S,1}^d Lt^2 + k_{S,2}^d T^2 + k_{S,3}^d Lt^2 T + k_{S,4}^d T^2 Lt + k_{S,5}^d Lt \cdot T + k_{S,6}^d Lt + k_{S,7}^d T + k_{S,8}^d$

	$k_{S,1}^{par}$	$k_{S,2}^{par}$	$k_{S,3}^{par}$	$k_{S,4}^{par}$	$k_{S,5}^{par}$	$k_{S,6}^{par}$	$k_{S,7}^{par}$	$k_{S,8}^{par}$
d	$-3.33 \cdot 10^{-7}$ ($\pm 3.6 \cdot 10^{-10}$)	$1.54 \cdot 10^{-4}$ ($\pm 1.2 \cdot 10^{-7}$)	$1.33 \cdot 10^{-8}$ ($\pm 1.6 \cdot 10^{-11}$)	$1.53 \cdot 10^{-7}$ ($\pm 2.8 \cdot 10^{-10}$)	$-2.47 \cdot 10^{-5}$ ($\pm 6.5 \cdot 10^{-9}$)	$5.28 \cdot 10^{-4}$ ($\pm 1.5 \cdot 10^{-7}$)	-0.00261 ($\pm 2.7 \cdot 10^{-6}$)	-0.0325 ($\pm 6.3 \cdot 10^{-5}$)
e	0.000202 ($\pm 4 \cdot 10^{-6}$)	0.1206 ($\pm 1.6 \cdot 10^{-3}$)	$-5.93 \cdot 10^{-6}$ ($\pm 1.6 \cdot 10^{-7}$)	$-6.01 \cdot 10^{-5}$ ($\pm 3.1 \cdot 10^{-6}$)	0.01245 ($\pm 7.2 \cdot 10^{-5}$)	-0.3481 ($\pm 1.7 \cdot 10^{-3}$)	-9.583 (± 0.030)	217.4 (± 0.7)
f	$2.12 \cdot 10^{-6}$ ($\pm 2.7 \cdot 10^{-8}$)	0.07068 ($\pm 9 \cdot 10^{-6}$)	$-7.66 \cdot 10^{-8}$ ($\pm 2.1 \cdot 10^{-10}$)	$5.38 \cdot 10^{-6}$ ($\pm 2.2 \cdot 10^{-8}$)	$-1.6 \cdot 10^{-4}$ ($\pm 5 \cdot 10^{-7}$)	0.000358 ($\pm 1.2 \cdot 10^{-5}$)	-4.078 ($\pm 2 \cdot 10^{-4}$)	65.27 ($\pm 5 \cdot 10^{-3}$)