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Mathematical Modeling of the Size-Structured Growth of Microalgae Dividing by Multiple Fission

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A novel mathematical model to simulate the size-structured growth of microalgal strains dividing by multiple fission is proposed. The model is validated by comparison with literature experimental data. Then, the implications of such division mode on crucial aspects of microalgae cultivation and processing, such as time for steady state achievement, biomass productivity and flocculant dosage, were assessed through proper numerical simulations. The model well captured the experimental results and thus represents a suitable tool for the simulation of the growth of microalgae strains dividing by multiple fission.

* 1. Introduction

Microalgae are today recognized to be one of the most promising renewable feedstocks for the production of a number of consumer goods ranging from biofuels to nutraceuticals, pharmaceuticals, bioplastics and lubricants. Despite this, the current microalgae-based technology is not widespread since it is still affected by economic and technical constraints which hinder its exploitation in productive frameworks. Accordingly, such technology should be properly optimized in order to be viably implemented at the industrial scale. To this aim, suitable process engineering techniques relying on mathematical models could be exploited.

Several mathematical models of microalgae growth within different cultivation systems have been proposed in the literature during the last ten years (Concas et al., 2013; 2016a; Soru et al., 2018)**.** Though the number, complexity and reliability of mathematical models being presented is still growing, most of them are based upon the limiting assumption that all cells constituting the microalgae population have the same growth rate, biochemical composition and metabolism. On the other hand, microalgae populations consist of cells that, even if belonging to the same strain, can show different size, morphology and age which in turn result in different growth rates and metabolic properties. For example smaller cells of the population are characterized by higher photosynthetic rates and faster uptake of nutrients as well as improved capability to pick up light due to their larger specific surface. Moreover, since microalgae harvesting, cell disruption and lipid extraction are processes typically based on chemical reactions occurring at the cell surface, it is apparent that cells with small diameter might need higher amounts of reactants than larger ones. Ultimately, the knowledge of the size distribution of microalgae would allow evaluating the actual biomass productivity as well as the exact amounts of nutrients and reactants needed to perform cultivation and downstream operations. Therefore, a comprehensive mathematical model of microalgae growth and processing should properly take into account the size structure of the population. While this goal could be achieved by exploiting modelling approaches relying on population balance equations (PBEs) it is important to note that, to the best of our knowledge, very few mathematical models of microalgae growth, based on such a tool, have been so far proposed in the literature (Concas et al., 2016b). Moreover, most models using PBEs are based on the questionable hypothesis that microalgae strains divide by binary fission, i.e. by giving raise to only two daughter cells. However, it is well known that several strains are capable to generate more than two daughter cells according to a mechanism called multiple fission (Yamamoto et al., 2003). To the best of our knowledge, only one mathematical model of microalgae growth taking into account multiple cell division, has been proposed in the literature (Rading et al., 2011). However, this model neglects relevant phenomena such as the effects of light and nutrients on the growth of single cells as well as the occurrence of cell division in the dark. Furthermore, Rading et al. (2011) provided only the steady state solution of the model.

In the light of what above, there is the need for more comprehensive models able to simulate the size-structured growth of microalgae characterized by multiple fission. In this view, this work proposes a dynamic PBE-based model for the description of the size structured growth of a microalgal strain which is known to divide by multiple fission, i.e. *Pseudochloris wilhelmii.* The model was validated by comparison with literature experimental data. The effect of division mode on crucial operating aspects such as biomass productivity, transient duration and dosage of flocculants were assessed through proper numerical simulations.

* 1. Mathematical model

The model takes into account the following characteristics of the microalgae cell cycle. When exposed to light the cells exploit photosynthesis to grow in the phase G1 and increase in size. At a certain instant, hereafter called the commitment point (CP), the growing cells attain the minimum size that allows triggering of a sequence of events leading to cell division. This way, the cells are allowed to enter the S, G2 and M phases and finally divide by cytokinesis. Nevertheless, when the CP is reached during the light period, the division process is postponed in order to prevent possible DNA damage phenomena induced by the photon flux (Bišová and Zachleder, 2014). As a consequence, if the CP is achieved when light intensity is high, the cell continues to grow rather than dividing. Only at the onset of dark (or very low light) conditions, the cells which had attained a size greater or equal than the critical one, can actually start mitosis to then release the newborn daughter cells. In most algae, the number of newborn cells produced by cytokinesis can vary and can be greater than two. In particular, when the strain *Pseudochloris wihlelmii* is considered, the number of daughter cells can be 2, 3, 4 or 8 (Yamamoto et al., 2003). The mechanism on the basis of which the mother cell decides how many daughters will be generated, is still not clear. For this reason, a deterministic formulation of a mathematical function which allows evaluating how many cells are formed at each mitotic event cannot be still developed. However, it is possible evaluating the probability that the mitotic event will give rise to a specific number of daughter cells by relying on empirical observations. In fact, such a probability, hereafter called *i,* can be evaluated as the frequency with which the division into a given number (*i*) of daughter cells is observed over a quite large number of division events. In this work the division probability was evaluated for *Pseudochloris wihlelmii* while normalizing the observations by Yamamoto et al. (2003) so that only the events producing a well determined number of daughters are taken into account (cf. Figure 1a).

 

Figure 1. *Probability of division into i daughter cells (a) and, initial distribution used for simulations (b)*

In Figure 1b, the initial size distribution adopted to perform simulations is shown. Since no experimental data were available for *Pseudochloris wilhelmi*, the initial distribution of *N. oculata*, phylogenetically close to the former strain, was used*.* While in Figure 1b the size distribution is reported as a function of cell diameter, in this work the cell mass (m) was chosen as the internal coordinate of the PBE. On the other hand, it should be noted that size and mass structured PBEs are equivalent when the cell's specific weight is realistically considered to remain constant during growth. The general form of the PBE here adopted is reported in Eq. (1).

 (1)

where, according to the literature (Concas et al., 2016b), the time rate of change of cell mass (m) is a function of nutrient’s concentration in the growth medium (*Cj*), light intensity within the culture (*Iav*) as well as the cell mass itself as shown in the following expression:

 (2)

The function *g*(*Iav*) was evaluated according to Concas et al. (2016a). The death term *D*(*m*) of Eq. 1 accounts for the disappearance of a cell of mass *m* as a result of its division and can be expressed as follows:

 (3)

In Eq. (3), *f*(*m*) is the normal having parameters mc and σc which represent the mean and the standard deviation of the critical mass at which cell is committed to divide, respectively. Moreover, the term within square brackets, wherein *H*(*Iav*) is the Heaviside function of light intensity, takes into account that cell division may occur only during the dark phase, i.e. when *Iav=0*.

The birth term *B*(*m*) of Eq. 1 takes into account the contribution to mass class (m) of newborn cells resulting from the division of cells having larger mass m’ > m, and can be expressed as follows:

 (4)

The symbol $ϑ\left(m,m'\right) $represents the self-similar daughter distribution and obeys the extended Hill-Ng power law which, in its generalized form contemplating the possibility that a variable number (*i*) of daughters cells may be generated (i.e. multiple fission), can be written as follows:

 (5)

where the symbol *pi* (*m,m’*) is the partition function and is clearly defined by equation (5) while **(*i,i*) represents the classical beta function with parameters *i* and *i.*

By substituting expressions in Eqs (3), (4) and (5) in Eq. (1) and after some mathematical manipulations based on the properties of integrals, the following particular form of the population balance can be obtained:

 (6)

The latter one can be solved along with the initial condition $ψ\left(m,t\right)=ψ^{0}\left(m,0\right)$ at $t=0, ∀ m $and the boundary condition $ψ\left(0,t\right)=0$ for $∀t, m=0$. In particular the adopted initial distribution $ψ^{0}$ was obtained starting from the one already shown in Figure 1b. In order to validate the model reliability, the corresponding results were compared with the literature experimental data regarding the growth of *Pseudochloris wihlelmii* in batch photobioreactors where the controlling nutrients were nitrates and phosphates (Concas et al., 2016b). The PBE model should be coupled with the mass balances for nutrients which could be written as follows:

 (7)

which was solved along with the initial conditions $C\_{j}=C\_{j}^{0}$ at $t=0.$ The evolution of the total number of microalgal cells *n*(*t*)[#*cells L-1*] and the total biomass *X(t)* [*g L-1*] in solution have been evaluated as the *0th* and 1th moment of the distribution $ψ\left(m,t\right), $respectively, at each integration time.

* 1. Results and discussion

In order to demonstrate the reliability of the proposed model, the corresponding results are compared with the literature experimental data (Concas et al., 2016b) which investigated the effect of different cultivation conditions on the growth of the strain *Pseudochloris wilhelmii,* formerly known as *Nannohcloris eucaryotum,* which is well known to divide by multiple fission. The comparison of model and experimental data shown in Figure 2a and 2b was made in terms of first moments of the distributions, i.e. in terms of total biomass concentration, since no data were available in terms of size distribution evolution.

 

Figure 2*: Comparison of model results and experimental data in terms of biomass concentration.*

It should be noted that only the maximum growth rate *max*was suitably tuned to fit the experimental data obtained under the base condition, i.e. initial nitrogen and phosphorus concentration equal to N0 and P0, respectively. The remaining model parameters where taken from the literature (Concas et al., 2016b). As shown in Figure 2a, the predictive capability of the model was then evaluated by simulating the growth under different initial concentration of nutrients. It can be seen that the model well captures the experimental behaviour of the strain *Pseudochloris wilhelmii*. While further experimental data related to size distribution evolution are needed to corroborate model results, it should be noted that, for the general purpose here pursued, the comparison shown in Figure 2 represents a good starting point to assess model reliability. The output of model results obtained under the base case conditions (N0, P0) are reported in terms of simulated cell size evolution in Figure 3a as well as in terms of average diameter and total number of cells in Figure 3b.

 

Figure 3*. Simulated evolution of cell size distribution (a) and average diameter or total number of cells (b)*

From Figure 3a, it can be observed that, as the cultivation begins, the cells start to gain mass as a result of the continuous growth and consequently the mode of the distribution is shifted to the right. However, when most cells attains their critical size (mc) they divide and generate daughter cells having lower mass. For this reason, the number of small cells starts to increase (cf. Figure 2b) and, accordingly, a hump of the distribution appears in correspondence of smaller masses of daughter cells after 8 days of cultivation. As the time goes on, this peak increases as a result of the growing number of cells undergoing cytokinesis. This behaviour is confirmed by the evolution of the average cell diameter shown in Figure 3b that achieves its maximum value after 200 hours (8 days) of cultivation and then keeps on decreasing until the culture achieves its steady state. The oscillating behavior of the cell diameter is due to the fact that during the day, cells size increases as a result of photosynthesis while at night cells can divide thus reducing their average size. The continuous occurrence of mitotic events as cultivation proceeds, leads then to the increase of cell number (cf. Figure 3b).

In order to assess how the division mode can affect the growth of microalgal cultures further model simulations have been performed by setting the values of parameters $Θ\_{i} $so that the division into a fixed number of daughter cells j is simulated, i.e. $Θ\_{j}=1$ and $Θ\_{i}=0$ for $∀i\ne j$. Specifically, the following situations might occur: $Θ\_{3}=1$ and $Θ\_{i}=0$ for $∀i\ne 3$ for ternary fission and, $Θ\_{4}=1$ and $Θ\_{i}=0$ for $∀i\ne 4$ for quaternary fission, etc. The obtained results are shown in Figure 4 along with the ones related to multiple fission.

 

Figure 4. *Simulated biomass evolution (a) and final distribution of cells (b) under different division conditions.*

From Figure 4a it can be observed that growth simulated under multiple fission assumption is different from the one obtained when considering that only 2 daughters can be generated at each division. In particular, a slightly higher growth rate is achieved when taking into account multiple divisions. While at a first view such a difference might seem not relevant, when translated to the massive scale production that is typical of microalgal production systems, even these differences might lead to significant underestimation of the overall biomass productivity. Even from Figure 4a it can be seen that, under the assumption of 4 or 8 daughter cells per division, respectively, higher growth rates are achieved with respect to the case of multiple division. In general, it can be inferred that the greater is the number of daughter cells formed by division, the higher is the rate at which the whole culture grows. This is due to the fact that, when a great number of cells is generated per division, the resulting daughter cells are very small and, according to Eq. 3, grow faster than the larger ones that are formed when fewer daughters are generated per division. This is confirmed by Figure 4b where it can be seen that “faster” cultures are those displaying a size distribution shifted towards the smaller cells as a result of the high number of daughter cells produced at each division.

To verify how the number of daughter cells produced for division might affect industrially relevant parameters such as biomass productivity and the dosage of flocculant (FeCl3 or AlSO4) for microalgae harvesting, further simulations where performed. The biomass productivity was evaluated according to the following equation:

 (8)

where ttrans represents the transient time, i.e. the time needed for the culture to achieve steady state. Harvesting of microalgae is typically preceded by a flocculation treatment, aimed to promote gravity settling of microalgae in a realistic time frame. This operating step involves the use of different amounts of flocculants depending on the size distribution of microagal cells that need to be collected. In particular, according to the literature (Chatsungnoen and Chisti, 2016), the flocculant/coagulant dose (*DFCL*) can be evaluated through specific function of the cell diameter that, once declined in our mass-structured PBE- model, can be written as follows:

 (9)

Where**, ’ and ’ are empirical coefficients whose values were obtained from the ones proposed by Chatsungnoen and Chisti (2016). In Figure 5 the simulated effect of division mode on these two paramters is shown. It can be observed that the division affects both the biomass productivty and the dosage of flocculants. In particular, the higher is the number of cells produced per division, the higher is the final productivity. The multiple fission mode (variable n° of daughters) leads to a biomass productivity close to the one achieved when the number of daughter cells is 2 or 3. This is because the frequency of division into 2 or 3 daughters is higher with respect to that one into 4 or 8 cells (cf. Figure 1a). As shown in Figure 5b, the dosage of flocculants/coagulants (AlSO4 or FeCl3) increases with the number of daughter cells. This outcome is due to the fact that flocculants react at the cell surface. Thus, since the average cell size of populations characterized by a higher division number is lower (cf. Figure 4b), the total corresponding specific surface is higher.

 

Figure 5. *Effect of division mode on the biomass productivity (a) and dosage of flocculants (b)*

Accordingly, larger amounts of reactants are needed to destabilize the electric double-layer of cells and permit coagulation/sedimentation. When multiple fission is considered, the amount of flocculants needed is close to the one obtained in the case of division into 2 o 3 cells due to the reasons above explained which are related to the division probability as reported in Figure 1a.

* 1. Concluding remarks

A novel mathematical model for the size-structured simulation of the growth of microalgae dividing by multiple fission has been proposed. Model results have been successfully compared with literature experimental data. Beyond enabling to simulate the dynamics of size distribution evolution, the model permits to decouple the single cell growth phase from the division. Moreover, the knowledge of the size distribution at each cultivation time might be suitably exploited to optimize microalgae cultivation and downstream processing. Finally, it has been demonstrated that the values of industrially relevant parameters such as productivity and flocculant doses might be strongly affected by the number of daughter cells produced per division as properly shown by the model simulations. Therefore, the proposed model might represent a useful tool to gather relevant information regarding the cultivation and processing of strains dividing by multiple fission. The only constraint consists in the need of knowing a priori the probability of division into a given number of daughters for the specific strain being considered time by time. For this reason, a dedicated experimental activity should be performed before applying this model.

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