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A novel mathematical model to simulate the size-structured growth of microalgae strains dividing by multiple fission

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HIGHLIGHTS

- A model to describe the growth of microalgae dividing by multiple fission is proposed.
- The model is also capable to simulate the evolution of the size structure of microalgae population.
- Model results are successfully compared with literature experimental data.
- Numerical experiments are performed to show the improvements arising from this model.
- Inferences are formulated about the effects of multiple fission on the bioreactors productivity.

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ABSTRACT

Several microalgae strains are capable to divide by multiple fission, namely they can give rise to variable number of daughter cells after cytokinesis. Such behavior may have implications on the overall growth and productivities of microalgal cultures that are difficult to infer intuitively. Consequently, a novel mathematical model to simulate the dynamics of the size-structured growth of microalgal strains characterized by multiple fission, is proposed in this work. The model relies on the use of population balance equations (PBEs) to describe the evolution of the size distribution of microalgae cells during growth and permits to decouple the single cell growth phase, which is known to take place in the light, from the division one, that on the contrary is assumed to occur under dark conditions according to well corroborated experimental observations. Moreover, the effect of light intensity, photoperiod and nutrients concentration on the continuous growth of the cells, are suitably accounted for by the model. Furthermore, in order to describe the partition of newborn cells after division, a new approach, which relies on suitable experimental observations, is developed to formulate a novel birth term related to PBEs which takes into account the possibility of multiple fission to take place. Model results and literature experimental data pertaining a strain capable to divide by multiple fission, are successfully compared in terms of biomass concentration evolution, thus highlighting a good predictive capability of the model. Subsequently, specific numerical experiments are performed in order to examine the potential improvements arising from this model with respect to the ones currently available in the literature. Finally, suitable simulation-based inferences are formulated about the potential implications of multiple fission on photobioreactor's productivity.

1. Introduction

It is well recognized that microalgae represent today one of the most promising renewable feedstocks for the production of a wide range of consumer goods such as biofuels, nutraceuticals, pharmaceuticals, bioplastics, functional food, lubricants and food for aquaculture systems. When compared to land plant crops, microalgae are characterized by higher growth rates that result in the need of less extended lands for their cultivation. Furthermore, microalgae production does not require agricultural lands and prevents the typical competitiveness concerns with the agro-food market that, on the contrary, arise from the use of land plants for producing non-food goods. These aspects, coupled with the fact that microalgae can be grown and processed in a bio-refinery

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Notations

$b(m,m')$	daughter distribution function (ng^{-1})
$B(m)$	birth rate of cells of mass m ($ng^{-1}\ mm^{-3}\ h^{-1}$)
C_j	concentration of j^{th} nutrient in the medium ($j=1 \Rightarrow NO_3^-$; $j=2 \Rightarrow H_2PO_4^-$) ($g\ m^{-3}$)
d	equivalent diameter of the cell (μm)
d_c	critical diameter at which the cell is committed to divide (μm)
$D(m)$	disappearance rate of cells of mass m ($ng^{-1}\ mm^{-3}\ h^{-1}$)
F_i	distribution in terms of frequency for the mass or diameter class i (/)
$g(I_{av})$	light dependent kinetics of continuous cell growth (/)
$G(m)$	continuous growth rate of cells of mass m ($ng^{-1}\ mm^{-3}\ h^{-1}$)
h_i	distribution in terms of frequency for the mass class I for unit mass (ng^{-1})
$H(I_{av})$	function accounting that cell division occurs only in the dark (/)
I_{av}	average photosynthetically active radiation within the culture ($\mu E\ m^{-2}\ h^{-1}$)
I_0	incident photosynthetically active radiation ($\mu E\ m^{-2}\ h^{-1}$)
K_{I1}	half saturation constant in the light-dependent term of the growth kinetics ($\mu E\ m^{-2}\ h^{-1}$)
K_{I2}	inhibition constant in the light-dependent term of the growth kinetics ($\mu E\ m^{-2}\ h^{-1}$)
m	single cell mass (ng)
m_c	critical mass at which the cell is committed to divide (ng)
m'	mass of the generic mother cell (ng)
N or n	number of cells (/)
$p_i(m,m')$	unequal partitioning function for the case of division into i^{th} daughter cells (ng^{-1})
R	radius of the cylindrical photobioreactors (m)
t	time (min)
V	photobioreactor volume (m^3)
X	biomass concentration ($g\ m^{-3}$)
$y_{X\|j}$	ratio of weight of dry biomass produced to weight of j^{th} nutrient consumed (/)

Greek letters

α_i	parameter of the Hill-Ng distribution for the case of division into i cells (/)
$\beta(\alpha_i, \delta_i)$	beta function for the case of division into i daughter cells (/)
δ_i	parameter of the Hill-Ng distribution for the case of division into i cells (/)
Γ	division intensity function (h^{-1})
$\vartheta(m, m')$	self-similar daughter distribution function (/)
Θ_i	probability of forming a number of daughter cells equal to (i) per mitotic event (/)
λ_i	distribution in terms of frequency for the mass class i for unit mass (ng^{-1})
μ_{av}	average growth rate (h^{-1})
μ_{max}	maximum specific rate of single cell growth ($ng^{1/3}\ h^{-1}$)
μ_c	mass loss rate of single cell (h^{-1})
v_m	time rate of change of cell mass m ($ng\ h^{-1}$)
ϱ	specific weight of cells ($g\ m^{-3}$)
σ_c	standard deviation of the division probability density function (ng)
τ_a	optical extinction coefficient for biomass ($m^2\ g^{-1}$)
ψ	density distribution function of the cell population ($ng^{-1}\ mm^{-3}$)
ω	Angle of incidence of light (rad)

Superscripts

0	initial conditions (/)
exp	experimental value
f	final conditions (/)

Subscripts

av	average value (/)
i	number of daughter cells or generic counter (/)
j	number of nutrients or generic counter (/)
stat	steady state value (/)

framework which might involve the capture of CO_2 from flue gases and wastewater remediation, make such technology particularly attractive both from the economic and environmental point of view. For this reason, there is today a growing interest in developing microalgae based systems for a number of applications in sectors ranging from the biotechnological to the energy one [1].

In spite of such interest, the existing microalgae-based technology is still not widespread since it is affected by economic and technical constraints that might have limited the development of industrial scale production system. Therefore, in order to be implemented at the industrial scale, the current microalgae-based technology should be properly optimized in terms of abatement of the operating costs associated to the different unit steps of the process, i.e. cultivation, harvesting and lipid extraction. In particular, the scale-up of the cultivation systems represents one of the major issues to be solved. In fact, the encouraging experimental results so far obtained at the laboratory scale have been hardly reproduced when trying to transpose the cultivation systems to the large scale.

In this regard, both the scale-up of the cultivation systems and the optimization of key operating parameters might be accomplished by exploiting suitable process engineering techniques which, in turn, rely on the use of mathematical models that are capable to predict the system behavior when changing the operating conditions. For this reason, several mathematical models of microalgae growth within different cultivation systems have been proposed in the literature in the last ten years. So far, the basic characteristics of algal kinetics have been taken into account. In particular, most mathematical models available in the literature are capable to describe quantitatively the evolution of biomass concentration as a function of light density, nutrient availability and photobioreactors operating mode. Other modeling efforts have been devoted to quantitatively describe the production of photosynthetic oxygen and the corresponding consumption of dissolved carbon dioxide within the culture as well as the pH evolution, the mass transfer phenomena and the influence of hydrodynamic regime on light availability for microalgae. In the last years, due to the growing interest in the use of microalgae for producing biofuels, the mathematical models have been focused on the quantitative description of the influence of operating conditions on the lipid biosynthesis of microalgae with particular regard to the simulation of the effect of nitrogen starvation on the accumulation of fatty acids within the cells [2–5]. Moreover, due to the interest in using microalgae as a mean to capture CO_2, mathematical model have been recently developed with the aim of quantitatively assessing the capability of microalgal cultivation to capture and convert CO_2 into useful biomass [1,6–8].

Albeit the number, the complexity and the reliability of mathematical models proposed today in the literature is still growing,

most of them are based upon the wrong assumption that individual cells constituting the population of microalgae have the same growth rate, biochemical composition and metabolism. On the other hand, it is well known that microalgae populations consist of cells having different size, morphology, age, etc., which can strongly influence the growth rate of the cultures. For instance, small cells achieve higher photosynthetic and carbon fixation rates when compared to bigger ones, and are characterized by a faster uptake of nutrients as well as an improved capability to gather light. In fact, since nutrient uptake and light gathering occur at the cell surface, it is apparent that small cells are favored with respect to bigger ones due to their larger specific surface area.

Moreover, the size structure of the population can be affected by the physiological state of microalgae and can change as a consequence of temperature and osmotic shocks or after exposure to various pollutants, UV radiations and, more in general, to stress conditions [9,10]. Therefore, the cell size distribution may also represent a critical indicator of specific phenomena occurring within the culture, thus being potentially useful to monitor and optimize the cultivation process.

Furthermore, the correct evaluation of the size distribution of cells might provide useful information to control and optimize the downstream processes involved in the production of biofuels from microalgae, i.e. harvesting and lipid extraction. In fact, the harvesting step 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 microalgal cells that need to be collected [11]. Thus, the knowledge of the cell size distribution would permit to optimize the amount of flocculants to be employed, thus reducing the costs associated to this operating step. Similar reasoning applies when algae are collected through cross-filtration since the size and the characteristics of the filter are strongly dependent from the size of algae that need to be collected [12]. Therefore, the knowledge of the size distribution of microalgae would allow one to properly design the filter and thus to optimize even this operating step.

In addition, lipid extraction processes are typically preceded by a cell disruption treatment aimed to break the cell wall of microalgae and facilitate the subsequent release of intra-cellular lipids into the extracting phase [13–15]. Since the cell disruption reaction takes place at the cell surface, it is apparent that, when compared to big cells, the smaller ones require larger amounts of reactants in order to be efficiently disrupted due to their higher specific surface. Accordingly, the knowledge of the size distribution of microalgal cells might allow one to suitably tune the amount of reactant to be used for cell disruption thus reducing the corresponding operating costs. Ultimately, the quantitative description of the size evolution of microalgae populations should not be neglected when developing suitable mathematical models of microalgae growth and processing since they might provide significant insights in view of the optimization of either cultivation systems or downstream processes.

To this aim, the most powerful mathematical tool is represented by the so-called population balance equations (PBEs). The latter ones consist of a set of partial differential equations resulting from a dynamic balance of the cells distinguished on the basis of one or more features, i.e. the so called internal coordinates which can be the size, mass, age, morphology, etc., of the cells [16]. Thus, if the size is considered as the internal variable, by using PBEs it is possible to simulate the transient dynamics of a size-structured population of cells during cultivation. This outcome would result into all the possible advantages earlier discussed. Moreover, in the PBE-based models, the growth of each cell and the division phenomena are taken into account separately. Such peculiarity may come in handy to properly simulate cultivation of microalgae since, according to Bišová and Zachleder [17], the growth and the division of cells take place separately. In particular, the former phenomenon occurs during the light period by exploiting photosynthesis while the latter one (which is related to the born of new cells) usually takes place in the dark in order to prevent DNA damage phenomena induced by UV radiation. It should be noted that such a distinction cannot be performed through the classical unsegregated models which erroneously englobe the growth and division phase in a unique step occurring during the light phase, thus leading to potential mistakes in simulations. On the contrary, by allowing this distinction, the PBE-based models would permit to effectively simulate the effect of the light–dark cycles on the growth and the division of microalgae.

So far, the PBE-based models have been widely exploited to quantitatively describe the growth of mammalian cells and bacteria [18]. Nevertheless, to the best of our knowledge, very few applications of PBEs to the simulation of the evolution of microalgae populations can be found in the literature. In particular, Concas et al. have proposed a mathematical model based on a mass structured population balance to describe the growth of Spirulina Platensis within the solar collector of a BIOCOIL photobioreactor [19]. More recently, Altimari et al. have proposed a bi-variate PBE model to describe the dynamics of the mass distribution of microalgal cells distinguished on the base of the different stage of the cell cycle (i.e. G1, S, G2/M) to which they belong during growth [20]. Along the same lines, Massie et al. proposed a stage structured PBEs mathematical model to simulate the complex transient dynamics of *Chlorella vulgaris* cells taking place in response to environmental perturbations induced within a chemostat [21].

However, most models attempting to exploit potentialities of PBEs in the microalgae sector are based on the erroneous assumption that all microalgae strains can divide only by binary fission, namely by giving raise to only two daughter cells when they reach the mitotic phase. However, it is well known that several strains are capable to generate more than two daughter cells according to a mechanism called multiple fission [17]. In particular, the same strain during each cultivation cycle can give rise to a variable number of daughter cells, which usually ranges from 3 to 32, for each division [22,23]. This multiple division is quite different from the common binary division and its effect on the size distribution of the cells, as well as on the biomass productivity, is difficult to grasp intuitively. Therefore, suitable mathematical models are needed to predict the effect of such a division mode on the size structure and biomass concentration evolution.

In spite of this, to the best of our knowledge, only one mathematical model capable to simulate the growth of microalgae characterized by multiple fission has been so far presented in the literature. Specifically, Rading et al. have proposed a mathematical model, which accounts for multiple cell division, to simulate the stationary size distribution of the algal strain *Chlamydomonas reinhardtii* [19]. While from one hand this model represents the first attempt to simulate multiple fission and is very rigorous from the mathematical point of view, on the other side, it neglects relevant biological 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. Moreover, the model is valid only for strains which are capable to generate a number of daughter cells equal to a multiple of 2, while it is well known that several microalgal strains are capable to produce an odd number of daughter cells, i.e. 3, 5, etc., [23]. Finally, Rading et al. provided only the steady state solution of the model and thus suitable information about the possible effects of multiple fission on the dynamics of the culture cannot be extrapolated from their work [24]. 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. This aspect is even more important if one considers that many promising microalgae strains used to produce biofuels are well known to divide by multiple fission. By way of example, *Neochloris oleoabundans*, *C. vulgaris*, *Chlorella sorokiniana*, *Nannochloris* sp., are just some of the many microalgae strains usually adopted to produce biofuels that are characterized by the production of multiple daughter cells [23,25].

For these reasons, in this work a novel mathematical model is proposed to describe the growth of algal strains which reproduce by multiple fission. The model is based on a mass structured population balance which takes into account growth and division phenomena separately. Specifically, single cell growth rate is considered during the light period while cell division is imposed to occur only in the dark according to the indications provided by the most recent literature about this topic. The effect of light, nutrients and cell size on the growth and division rate, respectively, is accounted for. Moreover, a novel partition function, which relies on empirical observations and is capable to simulate the effect of multiple fission on the distribution of newborn cells, is proposed. Model results have been successfully compared with experimental data concerning the growth of a microalgae strain which is known to divide by multiple fission, i.e. *Nannochloris eucaryotum*.

2. Mathematical model

The phenomena schematically shown in Fig. 1 are taken into account in the present model to the aim of simulating the size-structured growth of microalgae dividing by multiple fission. It is apparent that, during the light period the cells grow in the pre-commitment phase G1 and increase in size as a result of the building of cellular structures and the accumulation of storage molecules, such as starch and lipids, which serve as energy reserves.

At a certain point, hereafter called the commitment point (CP), the growing cells attain the minimum size that allows the triggering of a sequence of events which subsequently lead to cell division [25]. It should be noted that, according to Bišová and Zachleder [17], multiple commitment points might be achieved by microalgae. However, in this work only the case of one single commitment point is considered while leaving the case of multiple commitment points to future papers. Therefore, the model assumes that, once reached the critical size, cells are committed to divide and can subsequently enter the S, G2 and M phases and finally can divide by cytokinesis. However, when such situation is reached during the light period, the division process is postponed in order to be performed in the dark and, as a consequence, the mother cells can continue to grow even if they have already reached the critical size [17]. In fact, according to the same authors, microalgae are capable to separate the cellular growth phase, which takes place during the light period by exploiting photosynthesis, from the mitotic and cytokinetic phase that, on the contrary, occurs during the dark period in order to prevent the DNA damage phenomena potentially induced by the incident photon flux. Subsequently, at the onset of dark conditions, the cells which have attained a size greater or equal than the critical one, can actually start mitosis that, in turn, will determine the release of the newborn daughter cells. In most eukaryotic algae, the number of newborn cells produced by cytokinesis can be greater than two. For example, when the strain *N. eucaryotum* is considered, the number of daughter cells can be 2, 3, 4 or 8 according to the literature [22,23].

The mechanism on the basis of which the mother cell decides how many daughters will be generated, is still not clear. In fact, while for few specific strains the most recent studies on the matter are gradually shedding light on the concerned phenomenon [17], a universally accepted mechanism still does not exist, especially for the strains which are capable of producing an odd number of daughter cells. For this reason, a deterministic formulation of a mathematical function which allows evaluating how many cells are formed for each mitotic event cannot be still developed. However, it is possible to evaluate the probability that the mitotic event will give rise to a specific number of daughter cells by relying on

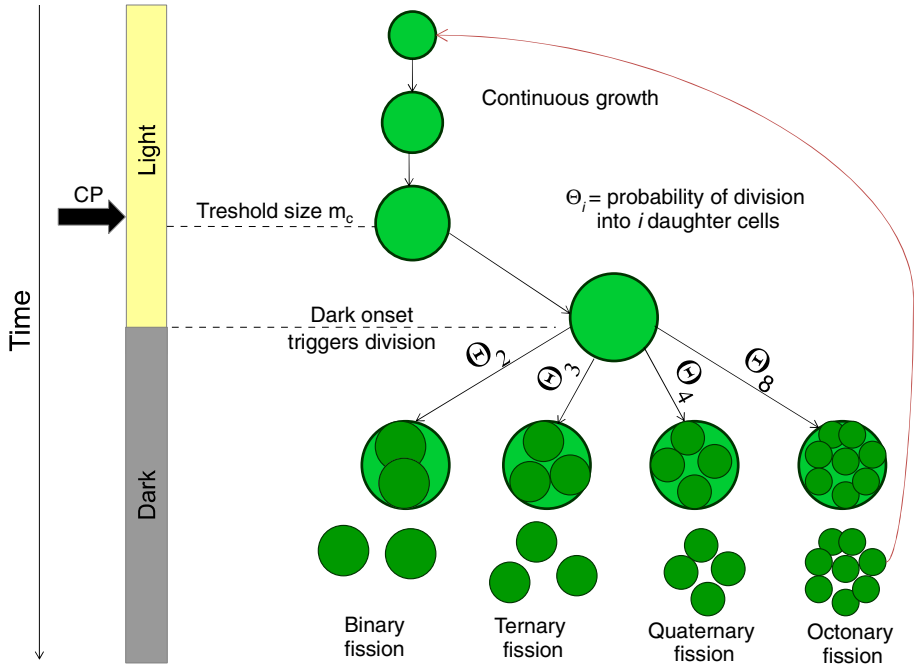

Fig. 1. Scheme of the growth and division processes of microalgal cells characterized by multiple fission. The possible numbers of daughter cells produced after division are those ones reported by Yamamoto et al. concerning the strain *Nannochloris eucaryotum* [23]. The red line indicates that the newborn cells restart a new growth-division sequence. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

empirical observations. Specifically, it can be quantitatively evaluated as the frequency with which the division into a given number of daughter cells is observed over a quite large number of division events. The symbols Θ_i reported in Fig. 1 represent just the probabilities that the mother cells produce a number of daughter cells equal to i and can be evaluated as follows. If n_{tot} is the total number of mitotic events experimentally observed and, among them a certain number equal to n_3 has produced 3 daughter cells, the corresponding probability (Θ_3) can be evaluated as the frequency n_3/n_{tot} if n_{tot} is large enough. Same reasoning applies to the case where different numbers of daughter cells are produced. When all the possible values of Θ_i are known, a mathematical model based on the PBEs can be developed in order to simulate the size structured growth of microalgae characterized by multiple cells fission.

In this work the cell mass (m) has been chosen as the internal coordinate of the PBE instead of the cell size. However, it should be noted that size and mass structured PBEs are somehow equivalent when the cell's specific weight is realistically considered to remain constant during growth. In fact, when a linear dependence between size (volume) and mass of the cells exists, the results obtained through the mass-structured population PBE can be simply expressed in terms of size-structured populations.

In the light of what above, the mass structured PBE for the batch growth of *N. eucaryotum* investigated by Lutzu et al. [26], can be written in its general form as follows:

$$\frac{\partial \psi(m)}{\partial t} + G(m) = -D(m) + B(m) \tag{1}$$

where the density distribution function $\psi(m)$ is the function adopted to describe the mass structure of the population at a certain time (t) and is evaluated as the number of cells having a certain mass (m) contained in the unit volume of culture and normalized with respect to the amplitude of the mass classes chosen to describe the size structure of the population. The term $G(m)$ in Eq. (1) accounts for the disappearance of cells having mass (m) due to continuous growth of single cells. The disappearance term $D(m)$ represents the possible instantaneous loss of cells of mass (m) provoked by their division (due to cytokinesis) into two or more daughter cells having smaller masses. Finally, the birth term $B(m)$, takes into account the possible formation of cells having mass m due to fission of larger cells. The details of symbol significance and their corresponding units are reported in the notations.

More specifically, the continuous term $G(m)$ takes into account the fact that the mass of a single cell can increase continuously with a rate equal to v_m, thus contributing to a shift over time of the density distribution function which can be mathematically described as follows:

$$G(m) = \frac{\partial(v_m \cdot \psi)}{\partial m} \tag{2}$$

where, according to Concas et al. [19], the time rate of change of cell mass (v_m) is a function of nutrient's concentration in the growth medium (C_j), light intensity within the culture (I_{av}) as well as the cell mass itself as shown in the following expression:

$$v_m = \frac{dm}{dt} = \left[\mu_{max} \cdot g(I_{av}) \cdot \prod_{j=1}^{2} \frac{C_j}{K_j + C_j}\right] \cdot m^{2/3} - \mu_c \cdot m \tag{3}$$

In Eq. (3), μ_{max} is the maximum specific growth rate, I_{av} the average light intensity and C_j the generic limiting nutrient. As it can be observed from Eq. (3), a Monod type dependence has been adopted to quantify the dependence of v_m upon nutrients concentration C_j, where the value 1 of the j index is referred to the concentration of nitrogen in the culture while the value 2 is referred to phosphorus. The latter ones are in fact the nutrients whose effect on growth limitation was investigated in the experiments [26] simulated through the present model. The function $g(I_{av})$ accounts for the effect of light on the growth kinetics which according to the literature [2] can be expressed as follows:

$$g(I_{av}) = \frac{I_{av}}{K_{I1} + \frac{I_{av}^2}{K_{I2}} + I_{av}} \tag{4}$$

It should be noted that the adopted expression for $g(I_{av})$ is capable to simulate also possible photo-inhibition phenomena affecting microalgae growth under too high levels of incident light intensities. In fact, while the kinetic parameter K_{I1} represents the half saturation constant, the symbol K_{I2} represents the inhibition constant with respect to light. The detailed explanation of the symbol significance is reported in the Notations. The average light intensity I_{av} within the culture in the batch cylindrical photobioreactor adopted in the experiments by Lutzu et al. [26] has been be evaluated as follows [1]:

$$I_{av}(t) = \frac{2I_0(t)}{\pi R \tau_a X(t)}\left[1 - \int_0^{\pi/2} \cos(\omega)\exp(-2R\tau_a X(t)\cos(\omega))d\omega\right] \tag{5}$$

where the symbol significance is reported in the Notations. The incident light intensity $I_0(t)$ appearing in Eq. (5), which in the experiments has been varied with time as a square wave having amplitude equal to 100 ($\mu E\ m^{-2}\ s^{-1}$) and a photoperiod (T_p) equal to 12 h (i.e. dark for 12 h and light for the remaining 12 h), has been simulated according to the method proposed by Concas et al. [1].

As far as the dependence of v_m on the mass of the cell, it can be observed that a 2/3 power law has been considered consistently with the typical allometric scaling laws adopted in the literature to express the effect of cell size/mass on its growth kinetics [27]. In fact, according to the literature [28], the nutrient uptake rate of microalgal cells depends, in a linear fashion, from the number of nutrient ions uptake sites occurring on the cell surface. Since the surface density of these sites is kept constant during cell growth, the total number of uptake sites depends on the cell surface area and thus the nutrient uptake rate of a microalgal cell depends in a linear fashion from the area of the cell surface which, in turn, is proportional to a 2/3 power of the cell volume and thus of cell mass [29]. Furthermore, at low light intensity, the geometric cross section of the cell, which is proportional to the 2/3 power of the volume, regulates the cell capability to gather the light needed to trigger photosynthesis [30].

As already mentioned above, the term D in the right hand side of Eq. (1) accounts for the fact that a cell of mass m may disappear as a result of its division, due to mitosis and cytokinesis, into a certain number of daughter cells having smaller masses. This term can be expressed as the product of $\psi(m)$ and the so called division intensity function (Γ) which represents the tendency of the cells to divide as they approach a certain critical mass m_c, namely:

$$D(m) = \Gamma \cdot \psi(m) \tag{6}$$

The function Γ can be evaluated as the product of a division rate (v_d) which depends on the availability of nutrients, and the probability that the cell divides depending on its mass:

$$\Gamma = \Gamma(m, C_j) = v_d(m, C_j) \cdot \frac{f(m)}{1 - \int_0^m f(m')dm'} \cdot H(I_{av}) \tag{7}$$

where, according to the literature [16], the division rate can be expressed as follows:

$$v_d = \mu_{max} \cdot \prod_{j=1}^{2} \frac{C_j}{K_j + C_j} \cdot m^{2/3} \tag{8}$$

The function $H(I_{av})$ in Eq. (7) takes into account that the division can take place only during the dark phase and thus has been set as follows:

$$\begin{cases} H(I_{av}) = 0 \text{ when } I_{av} > 0 \\ H(I_{av}) = 1 \text{ when } I_{av} = 0 \end{cases} \quad (9)$$

The function $f(m)$ in Eq. (7) returns the probability density that a cell divides when its mass (m) approaches the critical one (m_c) and, according to the literature [31,32], can be expressed as a Gaussian function as follows:

$$f(m) = \frac{1}{\sqrt{2\pi\sigma_c^2}} \exp\frac{-(m-m_c)^2}{2\sigma_c^2} \quad (10)$$

where σ_c and m_c are the standard deviation and the average mass, respectively, of dividing mother cells. The birth term (B) of Eq. (1), takes into account the contribution to mass class (m) of newborn cells resulting from the division of cells having larger mass $m' > m$. The probability that a cell having mass m is formed depends on the rate $D(m') = \Gamma(m', C_j)\cdot\psi(m')$ at which the larger cells m' undergo cytokinesis as well as on the probability $b(m', m)$ that such event leads to the formation of a cell having mass m. Since each cell having mass $m' > m$ might theoretically divide to give rise at least one cell of mass m, the overall contribution of newborn cells to the mass class m is obtained by integrating this contribution over the mass domain involving all the cells having mass $m' > m$. Therefore, the birth term $B(m)$ in the population balance can be written as follows:

$$B(m) = \int_m^\infty \Gamma(m', C_j)\cdot b(m, m')\cdot\psi(m')\cdot dm' \quad (11)$$

According to Diemer and Olson [33] the so-called daughter distribution function $b(m, m')$ can be written as follows:

$$b(m, m') = \frac{1}{m'}\vartheta(m, m') \quad (12)$$

where $\vartheta(m, m')$ 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:

$$\vartheta(m, m') = \sum_{i=2,3,4,8} \frac{i\cdot\Theta_i}{\beta(\alpha_i, \delta_i)}\cdot\left(\frac{m}{m'}\right)^{\alpha_i}\left(1-\frac{m}{m'}\right)^{\delta_i} \quad (13)$$

In the equation above, i represents the number of daughter cells which can be formed by division. As far as the concerned case, namely the growth of *N. eucaryotum*, the number i can assume the values 2, 3, 4 or 8 according to the experimental observations [22,23]. The symbols Θ_i refer to term weights which must satisfy the following equation:

$$\sum_{i=2,3,4,8}\Theta_i = 1 \quad (14)$$

In this regard it should be specified that in the paper by Diemer and Olson [33], no physical meaning was attributed to the weights Θ_i. On the contrary, in this work we have assumed that these terms represent the probability with which the mitotic cells gives rise to a certain number (i) of daughter cells. Therefore, the term Θ_2 will represent the probability at which the mitotic cell divides into 2 daughter cells, while the term Θ_3 will indicate the probability with which the division leads to 3 newborn daughter cells, and so on. These probabilities have been evaluated by relying on experimental observation according to the method specified in what follows. The symbol $\beta(\alpha_i, \delta_i)$ in Eq. (13) represents the class of beta functions defined by the following equation:

$$\beta(\alpha_i, \delta_i) = \int_0^1 t^{\alpha_i - 1}(1-t)^{\delta_i - 1}dt \quad (15)$$

where the parameter values α_i and δ_i are inter-related and depend upon the considered number (i) of daughter cells through the following relationship [34]:

$$\alpha_i = \delta_i(i-1) \text{ for } i = 2, 3, 4, 8 \quad (16)$$

Moreover, the parameters α_i and δ_i must satisfy the following relationship in order to comply with the total mass conservation law [33]:

$$\sum_{i=2,3,4,8}\Theta_i\left(\frac{i\cdot\alpha_i}{\alpha_i+\delta_i}\right) = 1 \quad (17)$$

Finally, the unequal partitioning function p_i can be written as follows:

$$p_i(m, m') = \frac{1}{\beta(\alpha_i, \delta_i)}\cdot\frac{1}{m'}\left(\frac{m}{m'}\right)^{\alpha_i}\left(1-\frac{m}{m'}\right)^{\delta_i} \text{ for } i = 2, 3, 4, 8 \quad (18)$$

The overall daughter distribution function $b(m, m')$ defined in the birth term can be re-written according to the following equation:

$$b(m, m') = \sum_{i=2,3,4,8} i\cdot\Theta_i\cdot p_i(m, m') \quad (19)$$

which, once inserted into Eq. (11) and after some simple mathematical elaborations based on the properties of integrals, permits to re-formulate the birth term B as follows:

$$B(m) = \sum_{i=2,3,4,8} i\cdot\Theta_i\cdot\int_m^\infty \Gamma(m', C_j)\cdot p_i(m, m')\cdot\psi(m')\cdot dm' \quad (20)$$

It should be noted that, as far as the division of microalgal cells is concerned, the equation above represents the first formulation of the birth term available in the literature which takes into account the possibility to have multiple daughter cells as a result of the mitotic event.

The consistency of the proposed relationship is somehow confirmed by the fact that, whether applied to the case of simple binary fission where only two daughter cells are formed, i.e. when $\Theta_i = 0$ for $\forall i \neq 2$ while $\Theta_2 = 1$, it degenerates in the classical form of the birth term describing binary fission [35]:

$$2\int_m^\infty \Gamma(m', C_j)\cdot p_2(m, m')\cdot\psi(m')\cdot dm' \quad (21)$$

Ultimately, by inserting the extended formulation of the growth, death and birth terms in Eq. (1) the resulting PBE can be written as:

$$\frac{\partial\psi}{\partial t} + \frac{\partial(v_m\cdot\psi)}{\partial m} = -\Gamma(m, C_j)\cdot\psi + \sum_{i=2,3,4,8} i\cdot\Theta_i\cdot\int_m^\infty \Gamma(m', I, C_j)\cdot p_i(m, m')\cdot\psi(m')\cdot dm' \quad (22)$$

which must be solved along with the following initial and boundary conditions:

$$\psi(m, t) = \psi^0(m, 0) \text{ at } t = 0, \quad \forall m \in [0, m_{max}] \quad (23)$$

$$\psi(0, t) = 0 \text{ at } \quad \forall t \in [0, t_{final}],\ m = 0 \quad (24)$$

The PBE, once solved along with the corresponding boundary and initial conditions allows one to evaluate the evolution of the microalgae cell population over time as a function of nutrient and light availability. Subsequently, the total biomass concentration X can be evaluated as the first moment of the population through the following expression:

$$X(t) = \int_0^\infty \psi(m)\cdot m\cdot dm \quad (25)$$

While the total cell number can be evaluated as the 0th moment of the distribution ψ

$$n_{tot}(t) = \int_0^\infty \psi(m) \cdot dm \tag{26}$$

Since the rate of mass change v_m depends upon the nutrient's concentration in solution and their uptake by algae leads to their consumption in the growth medium, the following mass balance can be written to describe the time evolution of the corresponding concentrations in the bulk liquid phase of the batch system under investigation:

$$\frac{dC_j}{dt} = -\frac{1}{y_{X/j}} \int_0^\infty v_m(m, I, C_j) \cdot \psi(m) \cdot dm \quad \text{where}$$
$$j = 1, \ldots 2; \;\; 1 = NO_3^-; \;\; 2 = H_2PO_4^- \tag{27}$$

along with the following initial conditions:

$$C_j = C_j^0 \text{ at } t = 0, \text{for } j = 1, 2 \tag{28}$$

where the symbols $y_{X/j}$ refers to the yields of the nutrients *P* and *N*, namely the weight of nutrients consumed to produce the unit weight of biomass *X*.

The Eq. (22), which represents a partial differential equation in the variables *t* and *m*, has been solved by discretizing the derivative whose independent variable is the cell mass m to obtain one ordinary differential equation at the time variable for each of the mass grid point. This system of ordinary differential equations is coupled with the one resulting from the mass balances of nutrients reported in Eq. (27). The resulting system of ordinary differential equations above was numerically integrated as an initial value problem with the Gear method by means of the subroutine DIVPAG of the standard numerical libraries (IMSL). It should be noted that we typically used a number of grid points in the cell mass domain equal to N_m = 100. Finer grids did not provide significant changes in the numerical solution. The integral of Eq. (5) was solved by invoking the IMSL subroutine DQDAWO which exploits either a modified Clenshaw–Curtis procedure or a Gauss–Kronrod 7/15 rule. On the contrary, the integrals of Eqs. (22), (25), (26) and (27) were solved through the trapezoidal rule. Finally, the tuning procedure of model parameters values to fit model results to experimental data was carried out through minimizing an objective function through the Fortran subroutine BURENL, which is based on the least-squares method [36].

3. Results and discussion

A mathematical model to simulate the effects of microalgae cells division into multiple daughter cells is proposed in this work. In order to demonstrate the reliability of the proposed model, the corresponding results are compared with the literature experimental data by Lutzu et al. [26], which investigated the effect of different cultivation conditions on the growth of the strain *N. eucaryotum*. According to the literature [22,23], the cell division of this strain occurs through a mechanism which leads to the release of a variable number of daughter cells. In particular the number of daughter cells released per mitotic event can vary from 2 to 8 according to the observed frequencies shown in Fig. 2. Since the number of observations performed to evaluate these frequencies was quite high [23], they can be considered to be equivalent to the probabilities of division into the specific number of cells reported in Fig. 2. Therefore the symbol Θ_i in Fig. 2 represents the probability at which the mother cells having a critical mass m_c divide into a number of daughter cell equal to *i*. As it can be observed, albeit reproduction of *N. eucaryotum* usually occurs via two daughter cells, it is not uncommon to find three, four or eight daughter cells per mother cell. In the category "not specified",

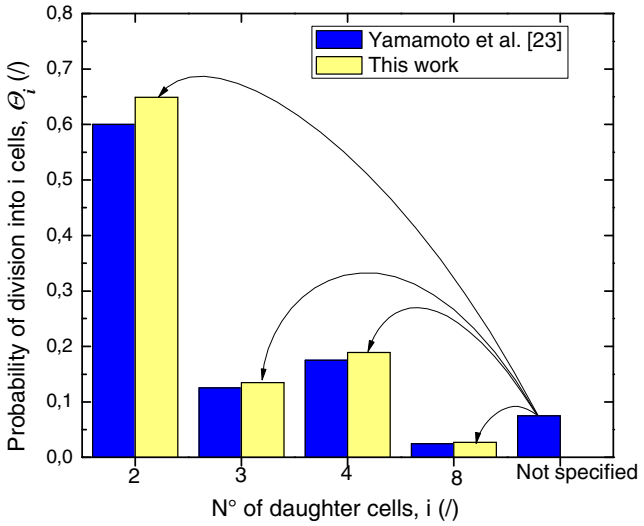

Fig. 2. Literature [23] and adopted probability of division into i daughter cells per mother cell of *N. eucaryotum*.

reported in the abscissa of Fig. 2, all the probabilities of very uncommon events leading to the production of unusual number of daughter cells have been cumulated [23]. The number of daughter cells produced in this latter case was not specified by Yamamoto et al. [23]. However, in order to ensure the mathematical consistency of the model, the terms Θ_i must satisfy the constraint $\sum_i \Theta_i = 1$ (cf. Eq. (14)) and thus all the possible numbers *i* of daughter cells being produced should be known along with the corresponding frequency Θ_i. For this reason, a slightly modified frequency distribution has been adopted in this work to the aim of obtaining the values of Θ_i needed to solve the mathematical model (cf. Fig. 2).

In particular, the probability associated to the "not specified" category has been redistributed over the four categories of well documented divisions, that is, the actual values of Θ_i have been obtained by normalizing the corresponding values available in the literature, i.e. $\Theta_{i,lit}$ [23] with respect to the cumulative probability of all the events which give rise to 2, 3, 4 and 8 daughter cell:

$$\Theta_i = \frac{\Theta_{i,lit}}{\sum_{i=2,3,4,8} \Theta_{i,lit}} \tag{29}$$

This choice allowed us to comply with the constrain $\sum_i \Theta_i = 1$ and simultaneously permits to obtain values of Θ_i very close to the ones experimentally observed by Yamamoto et al. [23] (cf. Fig. 2). The numeric values of the parameters Θ_i are reported in Table 1 along with the ones of the other model parameters.

All model parameters, except μ_{max}, have been taken from the literature. In particular, the values of the parameters appearing in the function $g(I_{av})$, which quantifies the effect of light intensity on the growth kinetics, i.e. K_{I1} and K_{I2} in Eq. (4), have been obtained by fitting the experimental data by Aleya et al. [37] obtained with the strain *Chlorella minutissima* which, according to the recent literature, is phylogenetically coincident with the *N. eucaryotum* strain [40]. As it can be observed from Fig. 3 the experimental data by Aleya et al., are quite well captured through the fitting procedure at least in the range of light intensities adopted in the experiments by Lutzu et al. [26] whose data have been used to validate the PBE model. Therefore, the obtained values of the parameters K_{I1} and K_{I2} can be considered fairly reliable.

The values of the parameters α_i and δ_i (i = 2, 3, 4, 8) reported in Table 1 have been evaluated as follows. According to the literature [32], the value of δ_i has been fixed to 40 for each value of *i*, and the

Table 1
Model parameters and initial conditions.

Symbol	Value	Units	References
C_N^0	2.77×10^{-2}	$g_N\ L^{-1}$	[26]
C_P^0	4.56×10^{-3}	$g_P\ L^{-1}$	[26]
I_0	1.00×10^{2}	$\mu E\ m^{-2}\ s^{-1}$	[26]
K_{I1}	6.00×10^{1}	$\mu E\ m^{-2}\ s^{-1}$	Evaluated from [37]
K_{I2}	1.30×10^{2}	$\mu E\ m^{-2}\ s^{-1}$	Evaluated from [37]
K_N	5.40×10^{-4}	$g_N\ L^{-1}$	[38]
K_P	2.50×10^{-5}	$g_P\ L^{-1}$	[38]
d_c	3.5×10^{0}	μm	Evaluated from [22,23,39,40]
m_c	$2.50 \pm 0.8 \times 10^{-2}$	ng	Evaluated from [22,23,39,40]
R	4.30×10^{1}	mm	Experimentally evaluated
Y_N	5.90×10^{-2}	$g_N\ g^{-1}$	[38]
Y_P	6.00×10^{-3}	$g_P\ g^{-1}$	[38]
α_2	4.00×10^{1}	–	[32]
α_3	2.00×10^{1}	–	Evaluated as reported in the text
α_4	1.34×10^{1}	–	Evaluated as reported in the text
α_8	5.71×10^{0}	–	Evaluated as reported in the text
δ_2	4.00×10^{1}	–	[32]
δ_3	4.00×10^{1}	–	[32]
δ_4	4.00×10^{1}	–	[32]
δ_8	4.00×10^{1}	–	[32]
Θ_2	6.48×10^{-1}	–	Evaluated from [23]
Θ_3	1.35×10^{-1}	–	Evaluated from [23]
Θ_4	1.89×10^{-1}	–	Evaluated from [23]
Θ_8	2.70×10^{-2}	–	Evaluated from [23]
μ_{max}	2.30×10^{-3}	$ng^{1/3}\ h^{-1}$	This work
ρ	1.10×10^{6}	$g\ m^{-3}$	[41]
σ_c	8.20×10^{-3}	ng	Evaluated from [22,23,39,40]
τ_a	6.38×10^{-1}	$m^2\ g^{-1}$	[42]

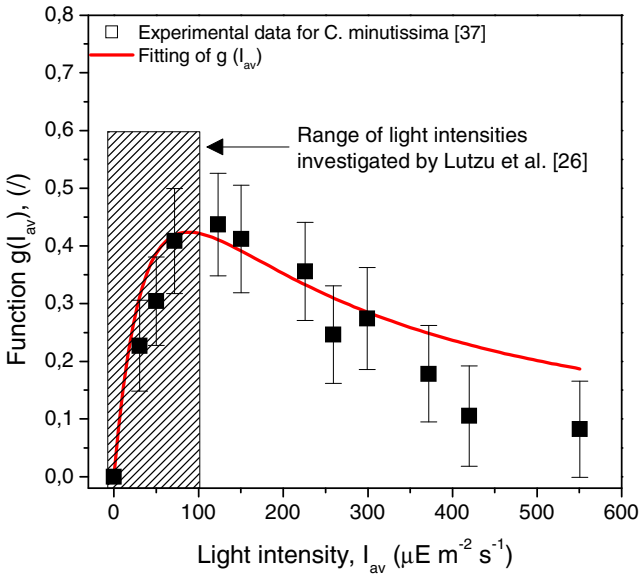

Fig. 3. Fitting of the experimental data by Aleya et al. [37] through the function g (I_{av}) for the obtainment of the values of model parameter K_{I1} and K_{I2} in Eq. (4).

corresponding value of α_i has been evaluated through the Eq. (16). The obtained values of these parameters give rise to partition functions (p_i) having a maximum where the mass ratio between the mother and the daughter cell is equal to 2, 3, 4 and 8, respectively, thus being representative of binary, ternary, quaternary and octonarian fission, respectively. It should be noted that the adopted values of α_i and δ_i satisfy the Eq. (17) and thus comply with the mass conservation law.

Furthermore, the critical cell mass (m_c) and standard deviation σ_c appearing in the function which defines the probability of cell division (cf. Eq. (10)), have been evaluated by performing a simple statistical analysis on the different corresponding values proposed in the literature by different authors for the critical mass m_c [22,23,39,40].

Finally, in order to evaluate the initial density distribution function of microalgae $\psi^0(0,m)$, the following strategy has been adopted. According to the literature, *N. eucaryotum* cells show a roughly spherical shape with an average diameter d_{av}^0 ranging from 2 to 3 μm with a most probable value of about 2.6 μm [23]. On the other hand, to the best of our knowledge, no specific data are available as far as the standard deviation of the initial distribution of this strain is concerned. For this reason, the initial distribution of the strain *Nannochloris oculata* available in the literature [43] has been considered to be representative of the one of *N. eucaryotum*. In fact, as it can be observed form Fig. 3, this strain shows an average cell diameter very similar to the one of *N. eucaryotum* (cf. Fig. 4a). Moreover, *N. oculata* is phylogenetically very close to *N. eucaryotum* [44]. Thus, by fitting the experimental data related to *N. oculata* reported in Fig. 4a, the standard deviation of the initial distribution of *N. eucaryotum* has been evaluated to be 2.5×10^{-1} μm, in terms of cell diameter. It should be noted that the initial distribution $F(d)$, in Fig. 4a, is evaluated as the number $N(d)$ of microalgae having a certain diameter d with respect of the total number N_{tot} of microalgae cells concerned. Starting from these data, the initial density distribution of the microalgae population expressed in terms of $\psi^0(0,m)$ shown in Fig. 4b, has been obtained as discussed in Appendix A.

As it can be observed from Table 1 the only tuned parameter is the maximum specific growth rate μ_{max}. Specifically, the value of this parameter has been obtained by fitting the experimental data [26] obtained when cultivating *N. eucaryotum* in batch reactors using the initial nitrogen and phosphorus concentrations, as well as the incident light flux, specified in Table 1. The comparison between experimental data and model results obtained under these operating conditions is shown in Fig. 5a. As it can be observed, the experimental behavior is well captured by the proposed model at least in terms of biomass concentration evolution over time, thus confirming that the adopted values of model parameters are quite reliable.

It should be noted that the intermittent shape of the simulated growth curve is due to the fact that the mass growth of microalgae takes place only during the light periods while it stops under dark conditions. In Fig. 5b, model extrapolations obtained under the adopted operating conditions are shown in terms of time evolution of light intensity and nutrients concentration within the culture. It can be observed that light intensity decreases as a result of the increasing biomass concentration which, in turn, determines the augmentation of solution's optical density. Moreover, nitrogen and phosphorus concentrations decrease as a result of consumption by microalgae. The simulated time evolution of the cell population distributions is shown in Fig. 5c. The initial population is indicated by the area filled by a sparse pattern. 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 after 4 days of cultivation. However, when most cells attains their critical size (m_c) they divide and generate daughter cells having lower mass. For this reason, the number of small cells starts to increase and, accordingly, a hump of the distribution appears in correspondence of smaller cell masses after 8 days of cultivation. As the time goes on, this hump increases in height as a result of the growing number of cells undergoing cytokinesis and, after 16 days, attains almost the same height of the initial peak, thus leading the distribution to become quite broadened. It is important to observe here that such broadening of the distribution is just the result of multiple fission. In fact, if only binary fission would have taken place, a more distinct peak

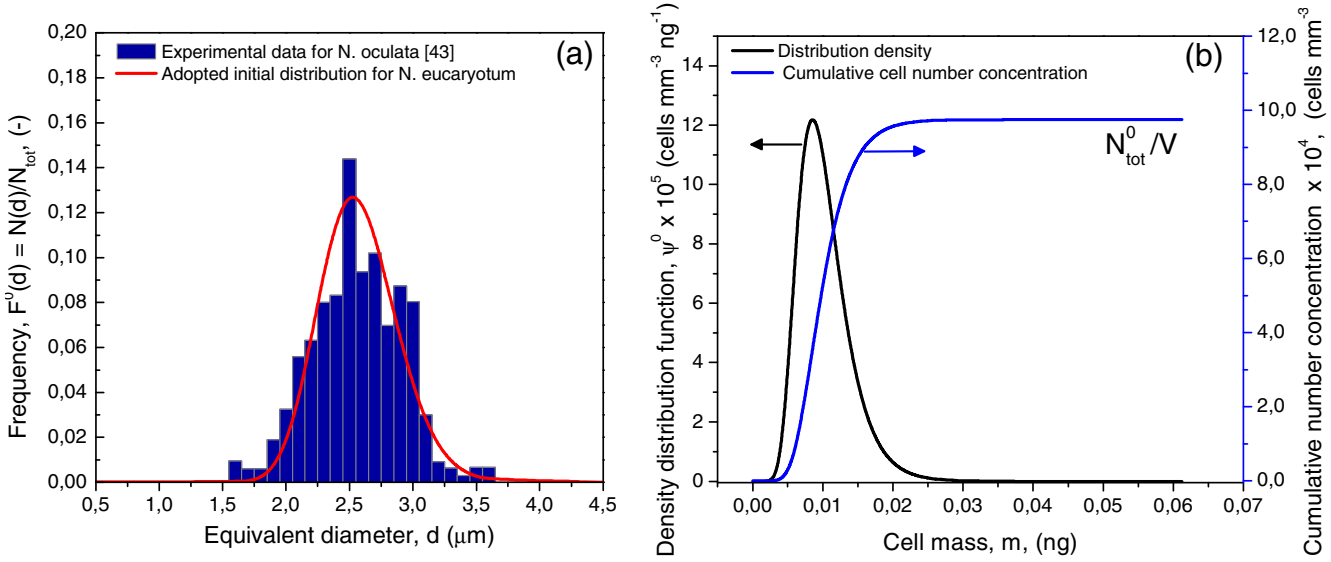

Fig. 4. Initial population distribution in terms of frequency as a function of the cell diameter (a) and in terms of density distribution as a function of the cell mass (b).

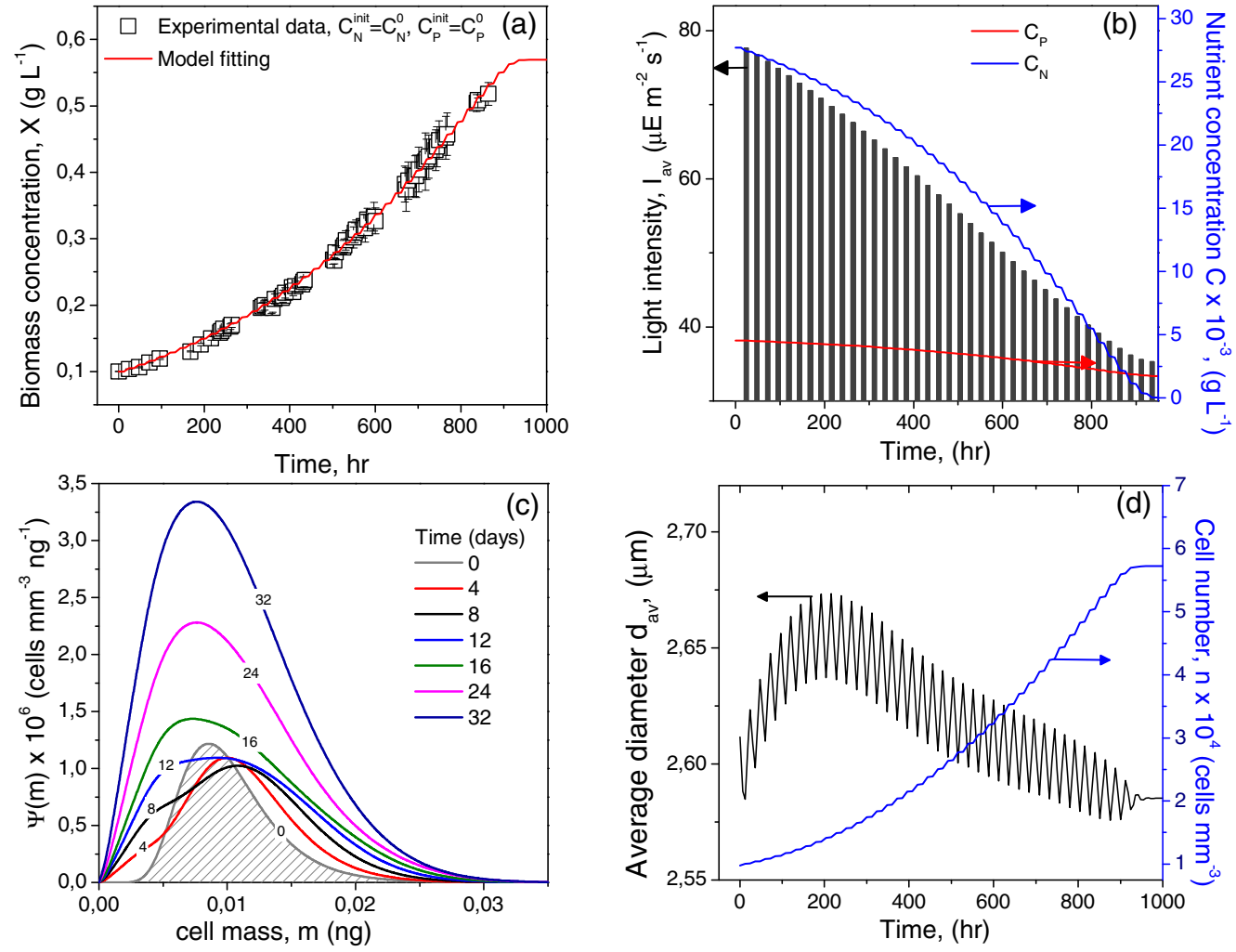

Fig. 5. Comparison of experimental data [26] and model fitting (a) and simulated evolution of light intensity and nutrients concentration within the culture (b). Simulated evolution of the size distribution of microalgae cells (c) and of the total number and average diameter of microalgae cells (d).

would have been appeared at mass values close to half the critical cell mass m_c thus leading the distribution to become markedly bi-modal. On the contrary, the multiple fission provokes the formation of daughter cells having different cell masses, i.e. $m_c/3$, $m_c/4$, $m_c/8$ and, accordingly, the resulting distribution becomes broader. Nevertheless, since the binary fissions account for about 60% (i.e. the most) of all possible divisions of *N. eucaryotum* (cf. Fig. 2), even in this case a peak at mass values close to $m_c/2$ appears more distinctly after about 16 days. From that moment on, the intensity of this peak further increases due to the accumulation of daughter cells of new generation. In Fig. 5d the evolution of the average diameter of the population is shown along with the one of the total number of cells. Aside the short term oscillations, whose origins will be better explained later, and focusing on the long term trend of the curve, it can be observed that during the first cultivation days the average diameter of the population increases as a result of the fact that continuous growth phenomena prevail over the division ones. However, when most cells approaches the critical size, bulk division takes place and several small daughter cells are formed, thus leading the average size of the population to decrease.

In Fig. 6 the evolution of average cell mass, along with the one of the cell-number, are shown in a narrow time frame in order to highlight the capability of the model to decouple single-cell growth phenomena from the division ones. In fact, it can be observed that when the light is on, the average mass of microalgae cells increases as a result of the photosynthetic growth, while the number of cells remains constant since in the presence of light, mitosis cannot take place and thus new cells cannot be generated. On the contrary, under dark conditions cells cannot increase in size but are allowed to divide. Accordingly, the cell number increases due the formation of newborn cells, while the average mass decreases because each of the newborn cells is characterized by a smaller mass with respect to the one of the parent cell from which they derive. It should be noted that, while for the strain *N. eucaryotum*, experimental data are not available to confirm the model extrapolations in Fig. 6, the simulated behavior is qualitatively consistent with the experimental data reported by Vítová et al. [45] for the strain *C. reinhardtii* and by Chisholm et al. [46] for two different phytoplankton strains. The practical usefulness of this model outcome lies in the fact that internal reserves of starch and lipid are typically used by the cells during night as source of energy to sustain the cell division process [17]. Therefore, it is apparent that suitable strategies based on the suppression of cell

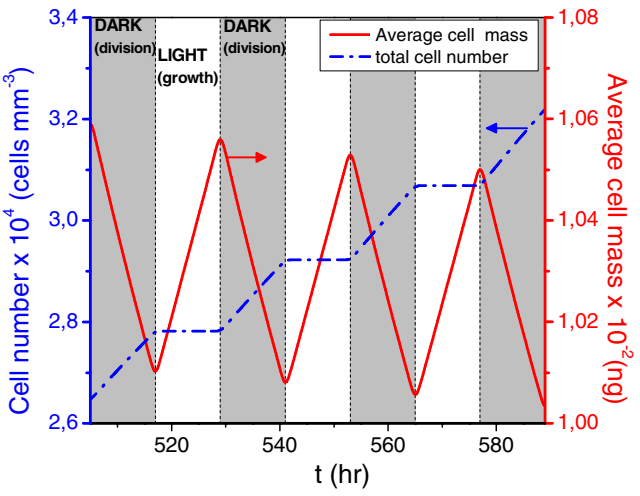

Fig. 6. Simulated evolution of the total number and the average mass of microalgae cells in a narrow time frame.

division (which in turn can be performed for example by imposing nitrogen starvation conditions or specific light dark photoperiods) might be exploited to increase the starch or the lipid content of microalgae in view of the production of biofuels. Therefore, the capability of this model to decouple the mass growth phase from the division one, might be theoretically exploited to develop strategies aimed to increase the lipid productivity of photobioreactors. Of course, to this aim, the model should be modified in order to take into account the mass balances of starch and lipids. However, even in the present form the model can provide precious information about the possibility of suitably modulating the light–dark cycles in order to favor cells division instead of their growth or vice versa.

To test the predictive capability of the proposed model, as well as the reliability of the tuned parameter, further experimental results obtained by Lutzu et al. [26], when changing the initial concentration of nitrogen and phosphorus, have been simulated. Specifically, the experiments where only the initial nitrogen concentration was halved (i.e. $C_N^{\text{init}} = 1/2C_N^0$ and $C_P^{\text{init}} = C_P^0$ at $t = 0$) and only the initial phosphorus concentration was reduced to one fourth with respect to the values reported in Table 1 (i.e. $C_N^{\text{init}} = C_N^0$ and $C_P^{\text{init}} = 1/4C_P^0$ at $t = 0$), have been simulated. Moreover, to further test the predictive capability of the model when both the initial nitrogen and phosphorus concentrations were simultaneously varied, experimental data related to the case where $C_N^{\text{init}} = 1/2C_N^0$ and $C_P^{\text{init}} = 1/2C_P^0$ at $t = 0$ have been simulated. The comparison between model predictions and experimental data are shown in Fig. 7a–c in terms of biomass concentrations as a function of time.

It is important to remark that no model parameter has been adjusted in these simulations. As it can be seen, the proposed model predicts the experimental behavior with good accuracy when the initial concentration of nitrogen and phosphorus is varied. The goodness of these results is confirmed also by Fig. 7d where all the model results are plotted against the corresponding experimental data obtained under each investigated operating condition and for each cultivation time. It should be noted that, in order to effectively validate the model, also suitable experimental data concerning the time evolution of the mass (or size) distribution of the cell should be compared with the corresponding model results. However, to the best of our knowledge, no literature data are available in this respect as far as the strain *N. eucaryotum* is concerned. A suitable experimental activity is currently under planning in order to cover this lack of information. On the other hand, the model extrapolations regarding the evolution of the cell population are qualitatively consistent with literature data concerning different green algae strains [9,47]. Therefore, the model might potentially represent a useful tool to simulate microalgae growth when cell division occurs by multiple fission. As previously mentioned, such a tool might prevent the erroneous estimation of microalgae productivity deriving from the use of unsegregated models or PBE-based models based on the assumption that cell division occurs only by binary fission.

In order to assess the possible errors arising from the adoption of this assumption, 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 is simulated. For instance, in order to consider only binary fission, the following values have been set for the above parameters: $\Theta_2 = 1$ and $\Theta_i = 0$ for $\forall i \neq 2$. On the other hand, to simulate ternary fission the corresponding parameters have been set as follows: $\Theta_3 = 1$ and $\Theta_i = 0$ for $\forall i \neq 3$ and so on analogously for quaternary ($\Theta_4 = 1$ and $\Theta_i = 0$ for $\forall i \neq 4$) or octonarian ($\Theta_8 = 1$ and $\Theta_i = 0$ for $\forall i \neq 8$) division. It should be noted that the same initial population distributions have been considered during simulations. The obtained results

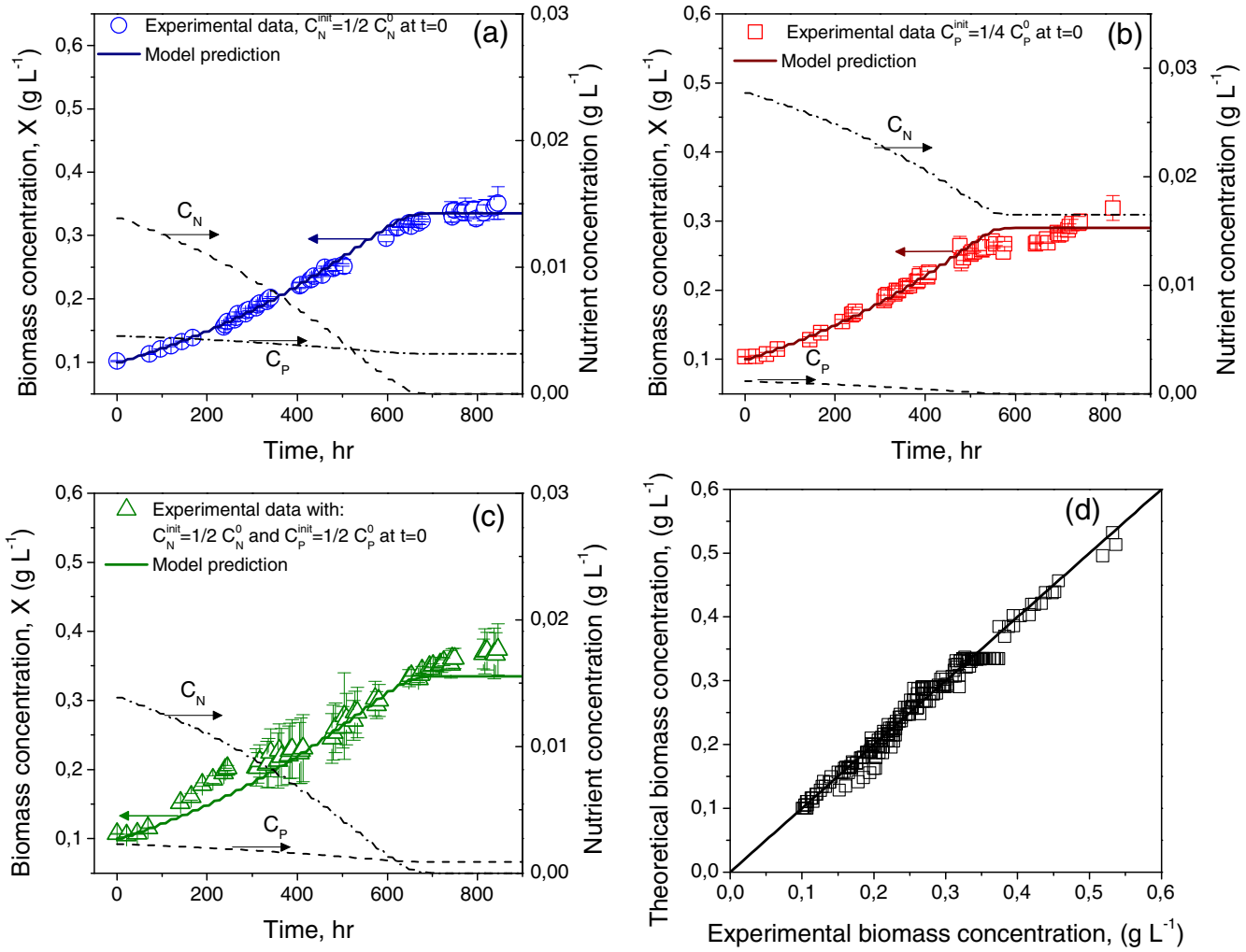

Fig. 7. Comparison of model predictions and experimental data [26] in terms of biomass concentration obtained when the concentration of was nitrogen halved (a) the phosphorus concentration was reduced to one forth (b) and both nitrogen and phosphorus concentration were halved (c) with respect to the corresponding values reported in Table 1. Overall comparison (d) of model and experimental results (the data in Fig. 4a are included).

are shown in Fig. 8 along with the ones already obtained under the assumption of multiple fission for comparison. From Fig. 8a it can be observed that growth simulated by using the values of Θ_i reported in Table 1, namely the curve tagged as "multiple", is different from the one obtained when assuming that only two daughters can be generated for each division. In particular, a slightly higher growth rate is achieved when taking into account multiple divisions for the algae. 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. In addition, it should be noted that such a low difference arises from the fact that the main division mode of *N. eucaryotum* remains the binary fission [23].

From a view inspection of Fig. 8a, the "multiple" curve is also very similar to the one obtained by assuming that only ternary cell division could take place. On the contrary, under the assumptions that each cell division could generate only 4 or 8 daughter cells, respectively, higher growth rates are achieved correspondingly 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 by division, the resulting daughter cells are very small and, according to Eq. (3), grow faster than the larger cells which would have been formed in the case where division would have produced a lower number of daughters. It should be noted that the observed similarity between the curve obtained under the assumption of multiple fission and the one related to ternary fission, is just a result of the specific combination of values adopted for the parameters Θ_i (Table 1). However, this similarity is not kept when the corresponding population distributions are compared. In fact, as shown in Fig. 8b, the simulated distributions at the end of the cultivation are significantly different for the five cases investigated. In particular, the final distribution obtained under the assumption of multiple division (the curve tagged as "multiple") is quite widened while the other ones show a relatively more pronounced peak at abscissa values slightly greater than m_c/i, being m_c the critical mass of the mother cells and i the number of daughter cells produced per division. Such result can be ascribed to the fact that, as the cultivation goes on, the number of cells having a size close to the one of daughter cells increases as a result of the division events and thus a peak near the mass value of daughter cells is formed correspondingly. On the other hand, since for the case of multiple fission the daughter cells may have different sizes, different peaks having sizes and wideness which depend from the values of Θ_i and σ_c, may be formed and thus the final resulting distribution is broader. Fig. 8c shows the evolution of the total number of cells

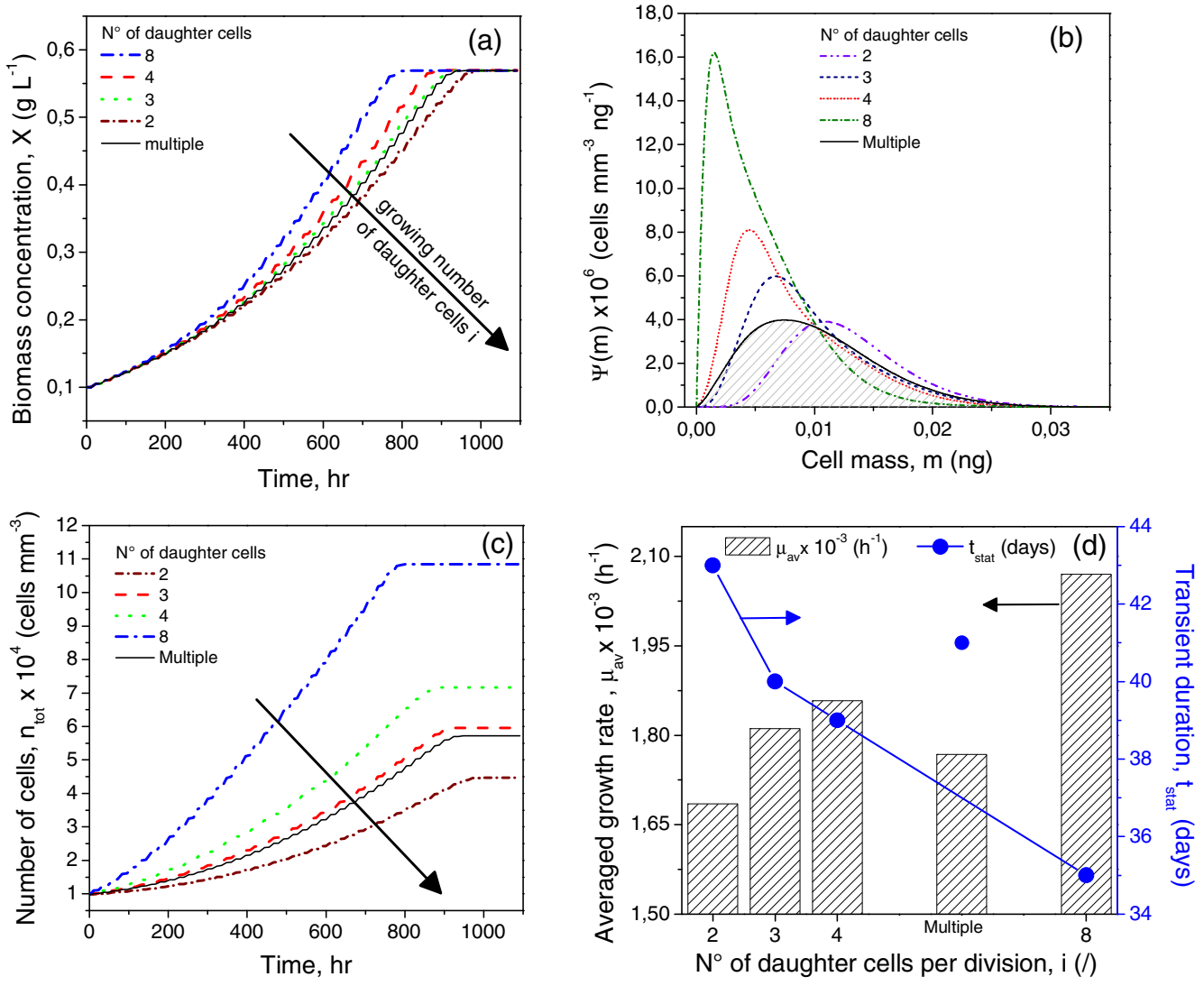

Fig. 8. Effect of the number of daughter cells produced per division on temporal evolution of biomass concentration (a); final distribution of the cell population (b); evolution of total cell number (c) and the time needed to reach the stationary phase and the averaged growth rate of the culture (d).

during cultivation. It can be observed that, the larger is the number of daughter cells generated per division the higher is the total number of cells in the culture as the cultivation time is prolonged. Moreover, it can be clearly seen that even the evolution of the total cell number is significantly different when comparing the case of binary division to the multiple fission. Finally, in Fig. 8d the time taken by the culture to achieve steady-state, i.e. the transient's duration (t_{stat}), is shown for each investigated case along with the corresponding average growth rate μ_{ave} which, in turn, has been evaluated according to the following relationship:

$$\mu_{ave} = \frac{1}{t_{stat}} \int_{X^0}^{X^f} \frac{dX}{X} = \frac{1}{t_{stat}} \ln\left(\frac{X^f}{X^0}\right) \tag{30}$$

where X^0 and X^f represent the initial and the final biomass concentration, respectively. This physical quantity provides an immediate and meaningful information about the potential biomass productivity of the strain. It can be observed from Fig. 8d that the larger is the number of daughter cells produced per division the shorter is transient duration and, accordingly, the higher is the average growth rate. This result can be ascribed to the fact that, when a large number of daughter cells are produced per division, most of the cells of the population are small (cf. Fig. 8b). However, according to Eq. (3), small cells grow faster when compared to large ones, and thus, the presence of a larger number of small cells results in a higher averaged growth rate of the culture. Since μ_{av} is directly linked to the overall productivity of the culture, it can be inferred that, "ceteris paribus", a strain characterized by the production of an high number of daughter cells should be preferred when the target of the cultivation is to maximize the biomass productivity. It should be remarked here that all the above model-based inferences should be confirmed by means of suitable experimental data. However, all these considerations are somehow corroborated by the experimental results obtained by Poyton and Branton with the strain *Prototheca* [48]. In fact they found that the growth rate and the average number of daughter cells produced per division by this strain, were linearly and positively correlated. Similar inferences were adduced by other authors for the strain *C. reinhardtii* [49].

In order to deepen the effect of the cell size on the growth of the culture, further numerical simulations have been carried out by using different initial size distributions, while keeping fixed all the model parameters in Table 1. Specifically, new simulations have been performed by using the different initial distributions (ψ^0) shown in Fig. 9a. The latter ones have been obtained by

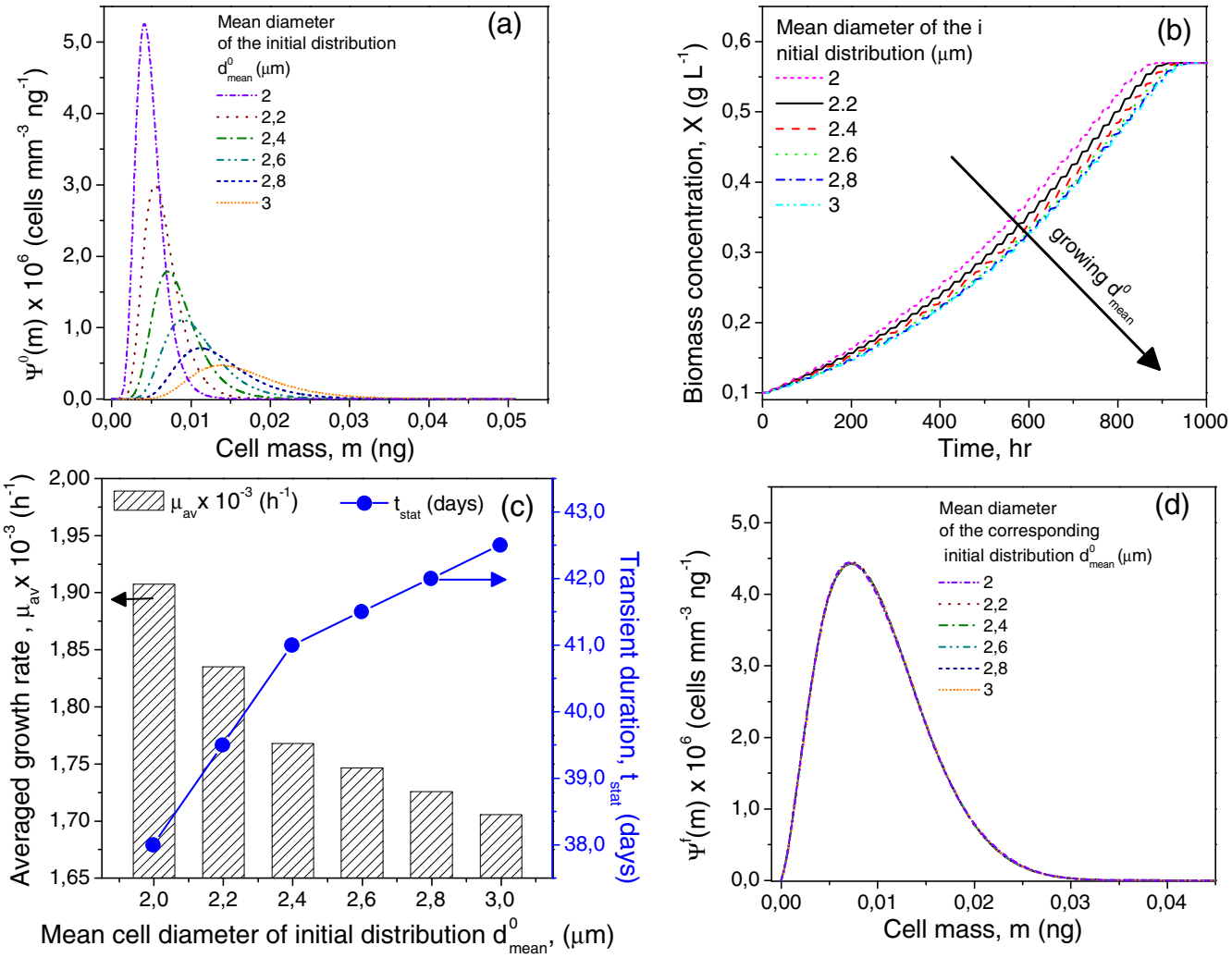

Fig. 9. Effect of the mean cell size of the initial distribution on the biomass concentration evolution (a), the final distribution after 40 days of cultivation (b), the final distribution of the population (c) and the transient duration and the average growth rate of the culture (d).

considering initial distributions similar to the one reported in Fig. 3a while considering different mean diameters. In particular, the corresponding distributions in terms of $\psi^0(m)$ have been obtained according to the method illustrated in Appendix A while setting the initial total number of cells per unit volume, i.e. N^0/V in Eq. (A8), so that the initial biomass concentration X^0 is kept constant for each simulation run. In this regard, it should be noted that the initial distributions characterized by a lower mean diameter in Fig. 9a shows larger peaks since, in order to have the same biomass concentration X^0, the populations with smaller cells must have more cells.

From Fig. 9b it can be observed that, albeit X^0 is kept fixed, the different initial distributions give rise to a different evolution of the biomass concentration. In particular, as confirmed also by Fig. 9c, the cultures having smaller cells at the start of cultivation, achieve the steady state more quickly and thus are characterized by higher average growth rates. This modeling outcome is consistent with the ones reported in the literature [27,50] and confirms that, to the aim of maximizing biomass productivity, microalgae strains characterized by a smaller cell size should be preferred, all else being equal. In Fig. 9d the final distributions (after 40 days of cultivation) are shown. It can be observed that, while starting from very different populations (cf. Fig. 9a), after about 40 days of cultivation, the same size-distribution is obtained for all simulated cases.

This behavior depends on the fact that the occurrence of several mitotic events leads the parent cells of the initial population to progressively disappear while the daughter cells of new generations accumulate in the culture. Therefore after a prolonged period of cultivation, the size of the daughter cells released at the cytokinesis events rules the shape of the size spectrum of the entire population of cells. However, the distribution of the daughter cells remains almost the same irrespective of the initial distribution since it depends from the critical cell mass m_c, from the partition functions p_i and from the probabilities Θ_i which have been kept constant for each simulation run. In the last analysis, even if through different dynamics, the cultures tend to the same steady-state distribution. This is due to the fact that the size-homeostatic mechanism (i.e. the coordination of cell growth and division) imposed by the model has been kept equal for all the simulations irrespective of the initial distribution of the cells considered. In Fig. 10a and b the different transient leading to the same final distribution is shown for the two extremes cases considered in Fig. 9.

The same information are reported in Fig. 10c in terms of the time evolution of the mode of the distributions related to the remaining cases investigated and reported in Fig. 9. Distributions are not shown in this case for the sake of Figure's clarity. It can be clearly seen that, while starting from different modes, all the distributions tend to the same point indicated with an asterisk

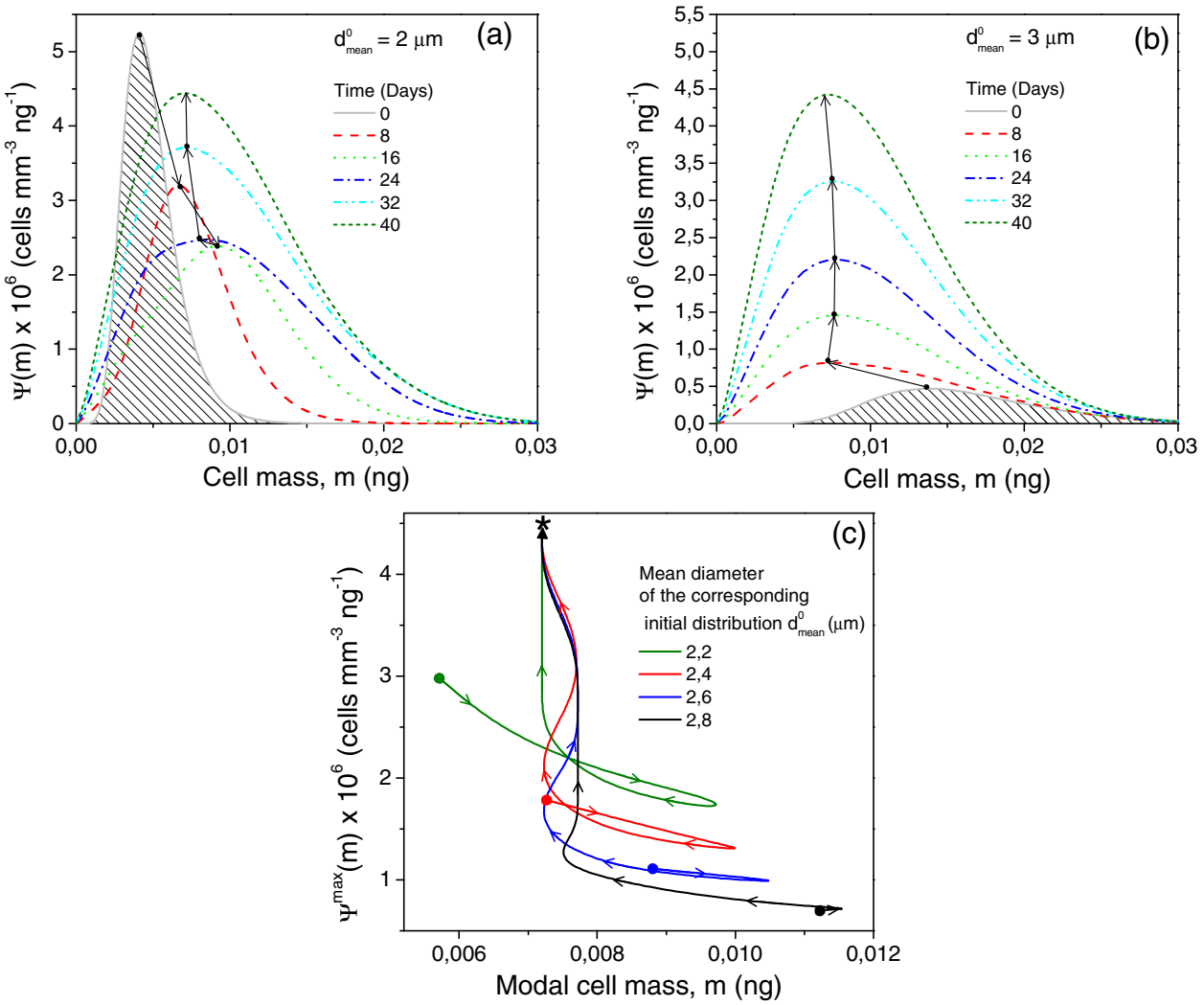

Fig. 10. Dynamics of the size distribution of *N. eucaryotum* in the case where the initial size distribution have a mean diameter equal to 2 μm (a) and 3 μm (b). Time evolution of the mode of the distributions (c) related to the different investigated mean diameters (arrows indicate the direction of increasing time).

(which acts as a kind of attractor) through different dynamics. These modeling outcomes, although need to be quantitatively confirmed through dedicated experiments, are somehow coherent with the information reported by relevant papers dealing with cell size-homeostasis and cell size control of different kind of cells [51–53].

Finally, since according to some authors [54], the critical size at which cells commit to divide is not static but may depend on the specific environmental conditions, it is useful to verify how a change in the value of the critical size (d_c or m_c) can influence the model results. Moreover, it is reported in the literature that, the number of daughter cells produced per division is dependent from the mass of the mother cells and that, more than one commitment point can be attained by the cells [17]. For this reason, further numerical experiments have been performed by changing the value of the threshold size d_c and thus of the corresponding mass m_c. It should be noted these simulations may serve also as a sensitivity analysis with respect to the model parameter d_c (or m_c).

In Fig. 11a the effect on the growth dynamics of *N. eucaryotum* deriving from changing the value of d_c of about ±11% with respect to the base case value reported in Table 1, is shown. It can be observed that, as the critical cell size is augmented, the growth rate of the culture decreases correspondingly. Therefore, the steady state is achieved later with respect to the case where a larger d_c was considered (cf. Fig. 11d). This result might be ascribed to the fact that, when division occurs at a larger size of a mother cell, due to mass conservation, the resulting daughter cells are larger than those ones which can be produced by smaller mother cells. Accordingly, after a prolonged period of time the populations of cells having a larger d_c will show a size spectrum shifted toward right, i.e. characterized by a larger average size of the cells (cf. Fig. 11b) and thus, in the light of what above discussed, will grow slower than the cultures characterized by a smaller critical size.

On the contrary, as shown in Fig. 11c, when the division occurs at smaller mother cell sizes, the total number of cells produced over time is higher. In fact, the critical size can be achieved more quickly by the cells growing in the G1 phase with respect to the case where the parameter d_c is large, and thus a larger number of cells division events can take place in the unit time so that a larger number of cells can be produced accordingly. All these aspects are reflected in the higher average growth rate of the cultures characterized by a smaller d_c (cf. Fig. 11d).

Finally, while it is important to remark that all the modeling-based inferences here discussed should be confirmed by a specific experimental activity, on the other hand several modeling outcomes are qualitatively consistent with literature experimental data. For this reason the model proposed in this work might

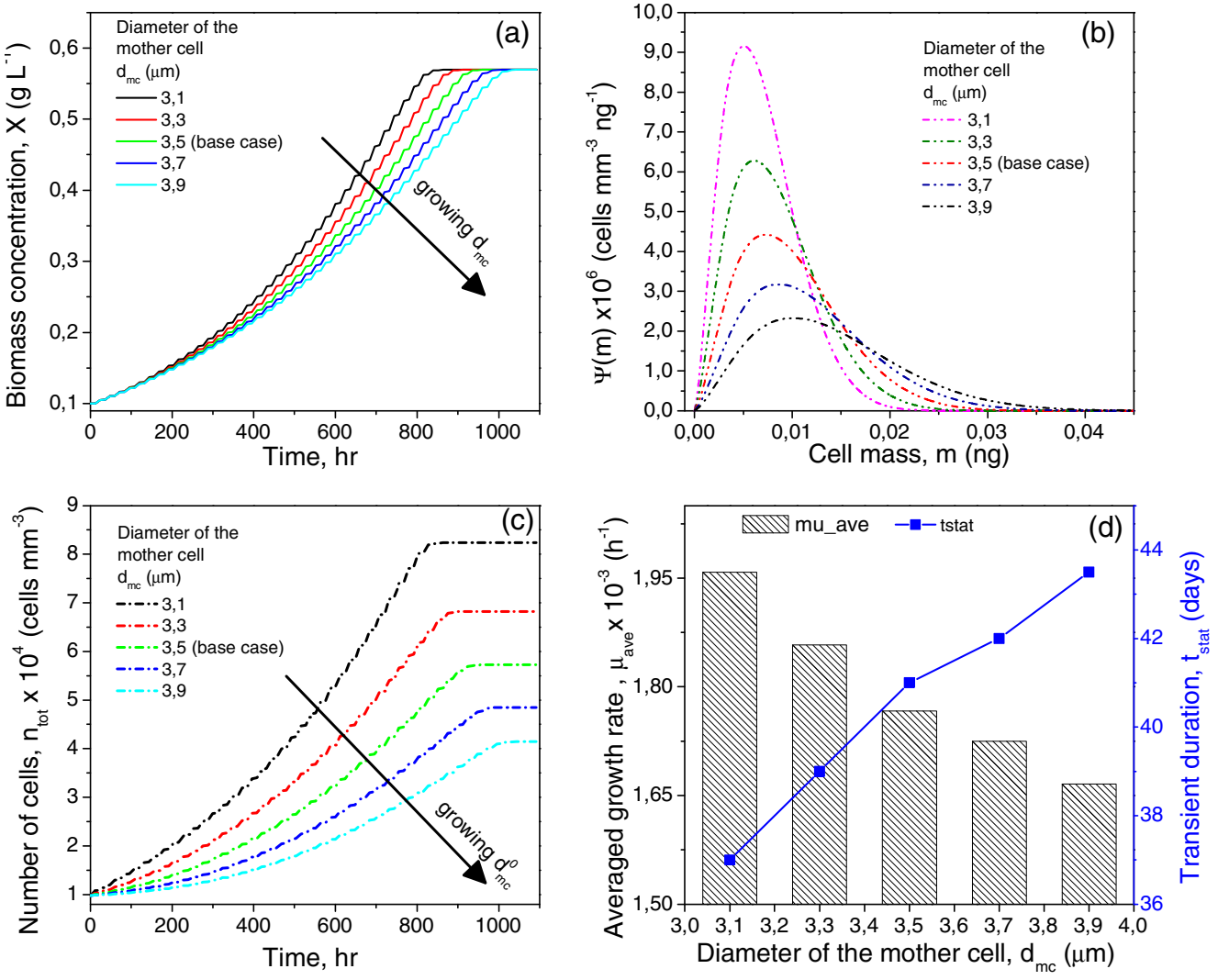

Fig. 11. Effect of the variation of the size at which mother cell divides on the biomass concentration evolution (a), the final (steady state) size distributions (b), the number concentration evolution (c) and the transient duration and the average growth rate of the cultures (d).

represent a first step toward the development of a more detailed mathematical tool aimed to simulate the growth of microalgae dividing by multiple fission and to identify the potential implications of such division mode on the performance of photobioreactors. In fact, as discussed above, the present model can be exploited to evaluate the overall growth rate as well as the time needed by the culture to achieve the steady state when changing the number of daughter cells produced at each mitotic event. The latter ones are features affecting the design and the performance of photobioreactors which hence might be optimized by using the proposed model.

4. Conclusions

Almost all the mathematical models adopted in the literature to simulate the growth of microalgae do not allow to separate the cell growth and division phases, thus preventing to suitably interpret the effect of the light–dark cycles on the growth of the culture. Moreover, in the best of cases, i.e. when segregated models are used, the erroneous assumption that microalgae divide only by binary fission is considered. However, it is well known that most of eukaryotic algae can divide by multiple fission. In this work, a mathematical model for the size-structured simulation of the growth of microalgae dividing by multiple fission has been proposed. The model is based on a population balance equation wherein a new formulation of the birth term has been proposed in order to account for the possibility of mother cells to give rise to different number of daughter cells. Model results have been successfully compared with literature experimental data expressed in terms of biomass concentration evolution of a microalgae strain characterized by multiple fission, i.e. *N. eucaryotum*. However, in order to be fully validated in all its aspects the model requires specific and dedicated experiments whose results should be compared with the theoretical ones. On the other hand, most of the non-trivial model results are qualitatively consistent with most recent literature on the matter. Beyond enabling to simulate the dynamics of size distribution evolution, the model permits to decouple the single cell growth phase from the division. This modeling outcome might be potentially useful to develop suitable strategies aimed to inhibit cell division and accumulate lipids in the cells. Moreover, the knowledge of the size distribution at each cultivation time might suitably exploited to optimize microalgae cultivation and downstream processing. Finally, it has been demonstrated through specific numerical simulations that, if multiple fission is accounted for, the erroneous evaluation of biomass productivity which might arise from the assumption that the concerned strain divide only by binary fission, can be avoided.

In particular, the improvements of the present model are more pronounced as the higher is the tendency of the simulated strain to produce a number of daughter cells greater than two. Ultimately, it should be remarked that, while further dedicated experiments should be performed in order to validate the model results, the simulation approach presented here might represent a first step toward the development of a tool which might facilitate the identification of better cultivation and processing strategies in view of the transposition of the microalgae technology to the industrial scale.

Acknowledgement

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Appendix A

In order to evaluate the initial density distribution $\psi^0(0, m)$ in Eq. (23) of the text, the following strategy has been adopted. Firstly, the experimental histogram by Kandilian et al. [43] reported in Fig. 4a and referred to the size distribution of *N. oculata*, has been considered.

In such histogram the height of the bins $F_i^{0,exp}(d)$ represents the number $N_i^{0,exp}(d)$ of microalgae having a certain diameter $d \ni [d_i - \Delta d/2, d_i + \Delta d/2]$ with respect of the total number $N_{tot}^{0,exp}$ of microalgae cells concerned (cf. Fig. 4a). The symbol Δd refers to amplitude of the bins of the histogram of Fig. 4a.

The corresponding histogram expressed in terms of numbers $N_i^{0,exp}(m)$ of microalgae having a certain mass $m \ni [m_i - \Delta m/2, m_i + \Delta m/2]$, with respect of the total number $N_{tot}^{0,exp}$ of microalgae cells, has been then evaluated by considering that, for a roughly spherical cell, the following relationship holds true between cell mass and cell diameter:

$$m = \rho \frac{\pi d^3}{6} \tag{A1}$$

This way, the experimental histogram by Kandilian et al. [43] has been converted in a mass-structured histogram. Subsequently, in order to obtain the corresponding distribution $h_i^{0,exp}(m)$, the experimental values of $F_i^{0,exp}(m)$, have been normalized with respect to the experimental mass step size Δm^{exp} of the bins according to the following equation:

$$h_i^{0,exp}(m) = \frac{F_i^{0,exp}(m)}{\Delta m^{exp}} = \frac{N_i^{0,exp}(m)}{N_{tot}^{exp} \Delta m^{exp}} \tag{A2}$$

The experimental distribution $h_i^{0,exp}(m)$ has been then fitted through a log-normal function using the fitting tool of the software Origin 8. According to the considerations made in the text, the so obtained log-normal distribution $\lambda^0(m)$ has been then considered to be representative of the one related to *N. eucaryotum*. Subsequently, the distribution $\lambda^0(m)$ has been evaluated for all the mass values m_j ($j = 1, ..., n_{class}$) adopted to discretize the population balance. It should be remarked that $\lambda^0(m)$ is the distribution of *N. eucaryotum* and thus, the discretized values $\lambda_j^0(m)$ have the meaning reported in the following equation:

$$\lambda_j^0(m) = \frac{F_j^0(m)}{\Delta m} = \frac{N_j^0(m)}{N_{tot}^0 \Delta m} \tag{A3}$$

Starting from the values of λ_j, the corresponding discrete form of the density distribution function may be evaluated as follows:

$$\psi_j^0 = \frac{1}{V} \frac{N_j^0}{\Delta m} = \frac{N_{tot}^0}{V} \lambda_j \tag{A4}$$

where the term N_{tot}^0/V is the initial total number of cells contained in the unit liquid volume V, namely the initial number concentration, or number density, of cells. Since the initial number concentration of algae was unknown, it has been evaluated starting from the initial mass concentration of algae X^0, whose value was reported in the work by Lutzu et al. [26]. In fact, according to Eq. (25) in the text, the initial mass concentration can be evaluated as:

$$X^0 = \int_0^\infty \psi^0(m) \cdot m \cdot dm \tag{A5}$$

which, by adopting the trapezoidal rule to numerically evaluate the integral, can be re-written as follows:

$$X^0 = \left(\frac{1}{2} \psi_1^0 m_1 + \sum_{j=2}^{n_{class}-1} \psi_j^0 m_i + \frac{1}{2} \psi_{n_{class}}^0 m_{n_{class}} \right) \Delta m \tag{A6}$$

Subsequently, substituting the expression of ψ_j^0 reported in Eq. (A4) into Eq. (A6), the following equation could be obtained:

$$X^0 = \frac{N_{tot}^0}{V} \left(\frac{1}{2} \lambda_1^0 m_1 + \sum_{j=2}^{n_{class}-1} \lambda_j^0 m_j + \frac{1}{2} \lambda_{n_{class}}^0 m_{n_{class}} \right) \tag{A7}$$

which can be rearranged to evaluate the initial number concentration of cells as:

$$\frac{N_{tot}^0}{V} = \frac{X^0}{\left(\frac{1}{2} \lambda_1^0 m_1 + \sum_{j=2}^{n_{class}-1} \lambda_j^0 m_j + \frac{1}{2} \lambda_{n_{class}}^0 m_{n_{class}} \right)} \tag{A8}$$

Finally, by inserting the obtained value of N_{tot}^0/V into Eq. (A4), the discretized version of the initial density distribution ψ_j^0 could be obtained for each value of $j = 1, \ldots, n_{class}$.

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