Algal Research 14 (2016) 28–38

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Predicting microalgae growth

Ward Blanken [a,*], P. Richard Postma [a], Lenneke de Winter [a], René H. Wijffels [a,b], Marcel Janssen [a,*]

[a] Bioprocess Engineering, AlgaePARC, Wageningen University, PO Box 16, 6700 AA, Wageningen, The Netherlands [1]
[b] Faculty of Biosciences and Aquaculture, University of Nordland, N-8049 Bodø, Norway

ARTICLE INFO

Article history:
Received 30 August 2015
Received in revised form 22 December 2015
Accepted 31 December 2015
Available online 8 January 2016

Keywords:
Photosynthesis
Microalgae
Light limited
Growth model
Productivity prediction

ABSTRACT

A generally applicable kinetic model is presented to predict light limited microalgal growth. This model combines a mathematical description for photoautotrophic sugar production with a description for aerobic chemoheterotrophic biomass growth. The model is based on five parameters which are directly measurable but were obtained from literature for the purpose of this study. The model was validated for *Chlorella sorokiniana* with 52 experiments derived from eight publications and for *Chlamydomonas reinhardtii* with 32 experiments derived from seven publications. The specific growth rate was initially predicted with a mean absolute percent error (MAPE) of 34–36%. The low accuracy is most likely caused by simplifications in the light model and inaccurate parameter estimations. When optimizing the light model per experimental dataset, a 1–2% MAPE was obtained. When optimizing input parameters separately from the light model, a 2–18% MAPE was realized. After validating this model on batch data, we conclude that this model is a reliable engineering tool to predict growth in photobioreactors provided the light field is accurately measured or calculated.

1. Introduction

Microalgae exploit photosynthesis to convert water and carbon dioxide into sugars by means of light energy. These sugars are subsequently used to support biomass growth. Microalgae growth in a photobioreactor can thus be calculated based on a model describing light-dependent sugar production by photosynthesis in combination with a model describing aerobic chemoheterotrophic growth on sugar. Ideally, the model parameters are all independently measurable in dedicated small-scale experiments in addition to the actual process to be predicted. In order to be suitable as a tool for photobioreactor engineers, the model should be as uncomplicated as possible while still including the most important reactions and providing sufficient accuracy.

Models that predict the light gradient include the Lambert–Beer Law, the radiative transfer equation (RTE), and a simplification of the two-flux model [1,2]. The Lambert–Beer Law is the simplest as it accounts only for light absorption but can be extended and improved by including light scattering [3]. The most dominant effect of light scattering is the increase in the light path travelled through the microalgae suspension increasing the probability of light absorption. This effect can be accounted for by modifying the attenuation coefficient. As such, it is possible to describe the light gradient with sufficient accuracy with the Lambert–Beer Law [4].

To describe photosynthesis, a model is required that describes the photosynthetic activity in response to light exposure. Photosynthetic activity increases linearly with light intensity under low light levels and then begins to stabilize towards a maximum photosynthetic rate at high light intensities. This trend is confirmed by the mechanistic description of photon absorption and utilization using a cumulative one-hit Poisson function [5] which results in the exponential model of Webb [6]. According to literature, the photosynthetic response, however, is best described by yet another hyperbolic function based on the hyperbolic tangent function [7]. As a result, the photosynthetic efficiency is maximal at low photon absorption rates and decreases slowly when approaching the maximal photosynthetic rate.

Sugar produced by photosynthesis in the chloroplast of the microalgae is used to support biomass growth. This growth metabolism is complex and can be described as aerobic chemoheterotrophic growth. Two general processes can be distinguished, i.e., the formation of new biomass and cellular maintenance (anabolism), which are both supported by aerobic respiration of sugars in the mitochondria (catabolism). The partitioning of sugar between anabolism and catabolism is described according to Pirt [8]. Pirt states that per biomass unit produced a fixed amount of sugar has to be respired, which is described by the biomass yield on sugar. Additionally a small amount of sugar is continuously respired providing energy for cellular maintenance.

Current light-limited microalgae growth models can be divided in photosynthesis- irradiance (PI) curve based models [3,9–12] and empirical models that are fitted to measured relations between specific growth rate and irradiance [13–15]. Although these models often include a respiratory term, Geider et al. [10] included a growth-related respiratory

* Corresponding authors.
E-mail addresses: ward.blanken@wur.nl (W. Blanken), marcel.janssen@wur.nl (M. Janssen).
[1] www.AlgaePARC.com.

http://dx.doi.org/10.1016/j.algal.2015.12.020

term. In reality, however, sugar is respired for energy to support cellular maintenance and anabolic reactions. Consequently, when neglecting this partitioning, respiration is often identified as energy loss.

What is lacking in the current models used for engineering studies is a simple microalgae growth model which takes into account compartmentalization between chloroplast and mitochondria. The proposed model, therefore, differentiates between photosynthesis and respiration by combining the Lambert–Beer Law, Jassby and Platt [7], and Pirt [8]. With this strategy, differentiation is made between photosynthetically derived sugars used for: (1) cellular maintenance, (2) growth-related respiration, and (3) cell growth. The advantage of this differentiation is that the microalgae metabolism is more accurately represented while maintaining simplicity with the model formulation as much as possible and minimizing the number of parameters required.

In this study, an engineering model for microalgae growth in photobioreactors is introduced and validated with *Chlorella sorokiniana* and *Chlamydomonas reinhardtii*. The model input parameters can be measured with dedicated experiments. For the purpose of this study, the model input parameters are acquired from literature and include: molar mass of the microalgae (M_x); specific light absorption coefficient ($a_{x,\lambda}$); sugar yield on photons ($Y_{s/ph}$); biomass yield on sugar ($Y_{x/s}$); maintenance specific sugar consumption rate (m_s); maximal specific sugar production rate ($q_{s,m}$); and maximal specific growth rate (μ_m). In this manner, a robust evaluation of the model accuracy could be constructed. This is one of the few studies where one single microalgae growth model is employed to predict growth experiments of various studies under completely different conditions.

2. Theory

2.1. Growth model

2.1.1. Photoautotrophic sugar production

All of the sugar that is used for aerobic chemoheterotrophic biomass growth is produced by photoautotrophic sugar production. In our model, the photoautotrophic sugar production is represented by coupling photosynthesis and the Calvin–Benson cycle. Hereby, it is assumed that all energy generated in the form of ATP and NADPH during photosynthesis is used in the Calvin–Benson cycle to incorporate CO_2 into triose sugars.

The rate of photoautotrophic sugar production is dependent on light intensity (Eq. (1)). This equation is equivalent to the model of Jassby and Platt which is based on a hyperbolic tangent function [7]. The original equation proposed by Jassby and Platt has been rewritten to make sugar as the end product of photosynthesis (Eq. (4)). In Eq. (1), the parameter alpha (α) describes the initial slope of the curve which levels off to the maximal specific sugar production ($q_{s,m}$). Please note that α can also be expressed as the product of the sugar yield on photons and the specific light absorption coefficient (Eq. (2)) which is in accordance with the approach of Geider [16]. Eq. (3) depicts the relation to calculate the specific photon absorption rate based on the light intensity and the specific light absorption coefficient. By incorporating Eqs. (2) and (3) into Eq. (1), the sugar production rate (Eq. (4)) becomes a function of the maximal specific sugar production ($q_{s,m}$), the specific photon absorption rate (q_{ph}), and the sugar yield on photons ($Y_{s/ph}$) which are process parameters or measurable characteristics of the microalgae. Variable q_{ph} thus replaces I_{ph} in the Jassby & Platt model, and this is practical for the integration of the light model within the growth model, which will be discussed later.

$$q_s = q_{s,m} \cdot \tanh\left(\frac{\alpha \cdot I_{ph}}{q_{s,m}}\right) \tag{1}$$

$$\alpha = Y_{s/ph} \cdot a_x \tag{2}$$

$$q_{ph} = I_{ph} \cdot a_x \tag{3}$$

$$q_s = q_{s,m} \cdot \tanh\left(\frac{q_{ph} \cdot Y_{s/ph}}{q_{s,m}}\right) \tag{4}$$

2.1.2. Aerobic chemoheterotrophic growth model

The sugar produced in the light reaction is exploited as a fundament for new biomass and is oxidized in the mitochondria to obtain extra energy that is necessary to support growth related processes and cell maintenance. This partitioning of sugar between anabolic and catabolic reactions can be described using Pirt's Law (Eq. (5)) [8] which states that a small amount of substrate (sugar) is continuously consumed for maintenance (m_s). The remaining sugar is available for growth (μ) resulting in new biomass according to a constant biomass yield on sugar ($Y_{x/s}$), which indirectly implies that a fixed amount of sugar is respired per carbon mol-x (cmol-x) produced. The validity of adopting Pirt's description for partitioning of photosynthetically derived energy has been established for several microalgae species [17,18]. Please note that the specific sugar production rate (q_s) in Eq. (5) is predicted employing Eq. (4). To summarize, a typical photosynthesis model is combined with the classical aerobic chemoheterotrophic growth model of Pirt to predict the specific growth rate of microalgae (Eq. (5)).

$$\mu_{pre} = (q_s - m_s) \cdot Y_{x/s} \tag{5}$$

2.2. The light attenuation model

Light attenuation within a microalgae suspension in flat plate photobioreactors is described based on the Lambert–Beer Law which states that the attenuation of light over distance is proportional to the light intensity itself with the proportionality constant being the volumetric absorption coefficient. The latter is the product of the specific light absorption coefficient (a_x) and the biomass concentration (C_x).

$$\frac{dI_{ph}}{dz} = -a_x \cdot C_x \cdot I_{ph} \tag{6}$$

The Lambert–Beer Law (Eq. (6)) can be rewritten to extract the specific photon absorption rate (q_{ph}) of microalgae:

$$\frac{\frac{dI_{ph}}{dz}}{C_x} = q_{ph} = -a_x \cdot I_{ph} \tag{7}$$

Taking the integral of the Lambert–Beer from 0 to z results in:

$$I_{ph}(z) = I_{ph}(0) \cdot e^{(-a_x \cdot C_x \cdot z)} \tag{8}$$

and taking into account wavelength dependency the following expression is obtained:

$$I_{ph}(z) = \sum_{\lambda=700}^{\lambda=400} I_{ph,\lambda}(0) \cdot e^{(-a_{x,\lambda} \cdot C_x \cdot z)} \cdot \Delta\lambda \tag{9}$$

By employing Eq. (9) we calculate the light decrease per wavelength, and as such we take into account that green light penetrates deeper compared to red and blue light. The calculation of wavelength dependent incident light intensity ($I_{ph,\lambda}(0)$) is explained in Supplementary files 1. A and 2 which also provides additional detailed information on the wavelength dependency of the specific absorption coefficient. As discussed, we propose the use of the specific photon absorption rate (q_{ph}) within the photosynthesis model. Based on a microbalance of light, we can calculate a local specific photon absorption rate $q_{ph}(z)$ as follows:

$$q_{ph}(z) = \frac{I_{ph}(z) - I_{ph}(z + dz)}{C_x \cdot dz} \tag{10}$$

The variable $I_{ph}(z)$ is then calculated based on Eq. (9).

2.3. Model input parameters

The parameters required as input for the above described model to predict the specific growth rate can be divided into two categories: (1) measurable characteristics of microalgae and (2) process parameters. The measurable parameters are obtained from literature (Table 1) and include: molar mass of the microalgae (M_x); specific light absorption coefficient per wavelength ($a_{x,\lambda}$); sugar yield on photons ($Y_{s/ph}$); biomass yield on sugar ($Y_{x/s}$); maintenance-related specific sugar consumption rate (m_s); and maximal specific sugar production rate ($q_{s,m}$). Parameter $q_{s,m}$ can be calculated by substituting the maximal specific growth rate (μ_m) in Eq. (5) because μ_m values are often available in literature (Table 1). The biomass yield on sugar is divided into one value for ammonium and one value for nitrate. Cultures growing on urea are assumed to have the same biomass yield on sugar as that for ammonium. The process parameters depend on culture conditions and include: biomass concentration (C_x), wavelength specific incident light intensity ($I_{ph,\lambda}$), and reactor depth (L).

In this study, the microalgae characteristics that were required as model input were acquired or deduced from a wide range of literature studies as discussed in Supplementary 1.B. With this strategy, we obtained ranges for all of the parameters without performing any experiments ourselves. It should be noted that in some cases validation data was also used as input for the input parameter estimation. For other microalgae strains, the model parameters can either be obtained from literature or can be determined by performing dedicated experiments as discussed in Supplementary 1. B.

3. Computational methods

3.1. Computational methods

This model employs five equations to calculate the average specific growth rate within a microalgae culture inside a photobioreactor (Fig. 1). Light intensity changes along the culture depth. The specific photon absorption rate and the specific sugar production rate both depend on the light intensity and, therefore, change with the culture depth. In Fig. 1, the equations already introduced are rewritten such that they depend on culture depth. In accordance with Fig. 1, the local light intensity ($I_{ph}(z)$) is used to calculate the local specific photon absorption rate ($q_{ph}(z)$) which is subsequently coupled to the sugar production and integrated over the reactor to acquire the average specific sugar production rate (Eq. (11)). The partitioning of the produced sugar between functional biomass (anabolism), growth-related respiration (catabolism), and maintenance-related respiration is described by Eq. (5).

With the equations listed in Fig. 1, the only parameters not specified are: biomass concentration, incoming light intensity, specific growth rate, and the reactor thickness. The specific growth rate of the microalgae chemostat culture can be calculated with this model provided that the biomass concentration is known. The above equations were discretised by subdividing the photobioreactors into 199 layers along the light path and then solved with MATLAB R2012a. In case of the predictions for batch cultures Eq. (12) is solved with the MATLAB R2012a ode15s solver.

$$q_s = \int_0^L q_s(z) \cdot dz \tag{11}$$

$$\frac{dC_x}{dt} = \mu_{pre} \cdot C_x \tag{12}$$

The light limited microalgae growth model was validated for *C. sorokiniana* based on 17 chemostat experiments performed over a wide range of dilution ranges [19–21], 2 D-stat experiments including 32 data points [18] and three batch experiments [22–24]. The model was also validated for *C. reinhardtii* based on seven chemostat experiments [17], 22 turbidostat experiments [13,25–28] and three batch experiments [13,29]. All experiments utilized for validation were performed in flat plate photobioreactors or a similar design. The design details are listed in Table 2, and the chemostat and batch observations are listed in Supplementary file 3. The results from the D-stat experiment were assumed to be representative of steady state cultures according to the analysis of Hoekema et al. [30].

3.2. Monte Carlo simulations

The accuracy of the model predictions of the specific growth rate was studied with Monte Carlo simulations. The parameters $Y_{x/s}$, m_s, $a_{x,\lambda}$ and $Y_{s/ph}$ were randomly varied within the range presented in Table 1 by the MATLAB random generator. The parameter $Y_{x/s}$ makes an exception to this rule and a lower value of 0.4 cmol-x cmol-s^{-1} was selected for both microalgae and nitrogen sources. This value corresponds to the lowest reported $Y_{x/s}$ based on a stoichiometry analysis [31]. The best fit was selected based on the smallest sum of squared errors of 100,000 simulations. The Monte Carlo simulations were performed separately for *C. sorokiniana* and *C. reinhardtii* on the combined data and per set of data as presented in Table 2 (each line represents one dataset).

3.3. Light gradient fit

The light gradient might be predicted incorrectly by Lambert–Beer Law as discussed in the Introduction. To correct for this, a light

Table 1
Overview of model input parameters for *Chlorella sorokiniana* and *Chlamydomonas reinhardtii*. The specific absorption coefficient (a_x) is depicted as the spectral average over 400–700 nm. For the sugar yield on photons ($Y_{s/ph}$), an average value for microalgae and plants leafs is depicted. Parameters were obtained from literature where 'n' represents the number of experiments used to estimate their values (Supplementary file 1.B). The values reported for the maximal specific sugar production rate ($q_{s,m}$) where calculated according to Eq. (5), therefore, 'n' represents the amount of calculated values.

	μ_m	M_x	m_s	a_x	$Y_{x/s}$		$q_{s,m}$		$Y_{s/ph}$
					NH_4	NO_3	NH_4	NO_3	
	h^{-1}	g cmol-x^{-1}	cmol-s (cmol-x s)$^{-1}$	m^2 cmol-x^{-1}	cmol-x cmol-s^{-1}		cmol-x (cmol-s s)$^{-1}$		cmol-s mol-ph^{-1}
	Chlorella sorokiniana								Microalgae
used	**0.27**	**24.0**	**2.5E-06**	**7.1**	**0.59**	**0.54**	**1.3E-04**	**1.4E-04**	**0.10**
average	0.26	24.5	2.5E-06	5.8	0.59	0.54	1.3E-04	1.4E-04	0.10
high	0.27	25.0	3.7E-06	7.1	0.70	0.63	1.7E-04	1.9E-04	0.11
low	0.25	23.7	1.2E-06	4.1	0.44	0.40	1.0E-04	1.1E-04	0.08
n	3	4	18	27	5	13	270	702	8
	Chlamydomonas reinhardtii								Plants
used	**0.14**	**24.0**	**2.0E-06**	**6.2**	**0.69**	**0.58**	**6.0E-05**	**7.1E-05**	–
average	0.14	24.0	2.0E-06	4.6	0.69	0.58	6.1E-05	7.2E-05	0.10
high	0.16	24.0	3.6E-06	6.2	0.78	0.64	7.7E-05	8.9E-05	0.11
low	0.13	24.0	1.6E-07	3.0	0.61	0.52	4.9E-05	5.9E-05	0.09
n	4	2	6	15	3	2	216	144	5

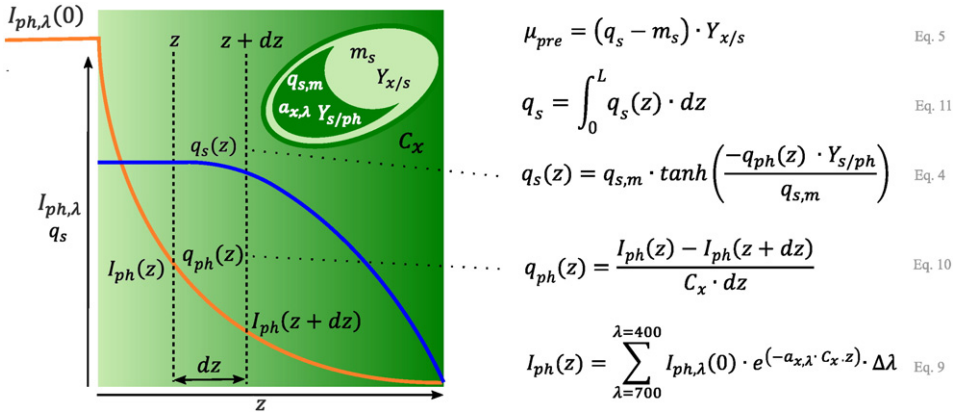

Fig. 1. Model calculation scheme, containing all equations necessary to predict the microalgae specific growth rate.

correction factor (c_l) is added to the Lambert–Beer equation (Eq. (13)). With Eq. (13), the predicted specific growth rate is fitted by changing a light correction factor with the fminsearch function of MATLAB to minimize the squared error per experimental condition. During the fminsearch, all other input parameters were as depicted in Table 1.

$$I_{ph}(z) = \sum_{\lambda=700}^{\lambda=400} I_{ph,\lambda}(0) \cdot e^{\left(-a_{x,\lambda} \cdot C_x \cdot z \cdot c_l\right)} \cdot \Delta\lambda \quad (13)$$

3.4. Calculations

The squared sum of errors (SSE) is calculated with Eq. (14).

$$SSE = \sum \left(\frac{\mu_{obs} - \mu_{pre}}{\mu_{obs}}\right)^2 \quad (14)$$

The model accuracy is measured as the mean absolute percent error (MAPE) and was used to evaluate the prediction accuracy. The MAPE is calculated according to Eq. (15) [32].

$$MAPE = \frac{100}{n} \sum \left(\frac{|\mu_{obs} - \mu_{pre}|}{|\mu_{obs}|}\right) \quad (15)$$

4. Results and discussion

4.1. Model predictions chemostat cultivation

The light-limited growth model introduced in this study was validated for *C. sorokiniana* with literature based input parameters. The datasets used for the validation data were derived from four independent studies which adopted three different photobioreactor designs (Table 2). In Fig. 2A, the predicted specific growth rate is plotted against the observed specific growth rate (MAPE of 36%). It can be determined that the predicted specific growth rate deviates from the observed specific growth rate (Fig. 2A). In Fig. 2C, the relative error between the predicted and the observed specific growth rate is depicted. From Fig. 2C, it is evident that the relative divergence is most substantial for the lower specific growth rates compared to the higher growth rates. Overall, for *C. sorokiniana*, there is a trend that low specific growth rates were overestimated while high specific growth rates were underestimated.

The light to growth model introduced in this study is validated for *C. reinhardtii* based on six independent studies (Table 2). In Fig. 2B, the predicted specific growth rate is plotted against the observed specific growth rate (MAPE 34%) and, in Fig. 2D, the relative error between the predicted and the observed specific growth rate is depicted. From Fig. 2B, it can be deduced that the predicted specific growth rate for *C. reinhardtii* tends to overestimate the measured growth rate.

Table 2
Summary of the Materials and Methods per dataset used to validate the model. FWHM stands for Full width at half maximum and gives an indication of the light beam angle from a light source.

z_r mm	$I_{ph,in}$ µmol $(m^2\ s)^{-1}$	N source	Light source	FWHM °	Reactor type	Reactor back	Operating mode	Strain number	Reference
Chlorella sorokiniana									
10	1530[a]2	Urea	High pres Na	45	Flat panel	Both sides illuminated	Chemostat	CCAP211/8 K	Tuantet et al. [21]
14	800	Urea	Red LED	68	Flat panel	Stainless steel (reflective)	Chemostat	CCAP211/8 K	Cuaresma Franco et al. [20]
14	2100	Urea	Red LED	68	Flat panel	Stainless steel (reflective)	Chemostat	CCAP211/8 K	Cuaresma et al. [19]
12.5	871	Urea	Fluorescent tube	diffuse	Flat panel	Open	d-stat	CCAP211/8 K	Zijffers et al. [18]
20.5	940	Urea	Fluorescent tube	diffuse	Flat panel	Open	d-stat	CCAP211/8 K	Zijffers et al. [18]
12	200–1500	NO_3	Halogen tungsten	27	Tube in tube	Tube (reflective)	Batch	CCAP211/8 K	Kliphuis et al. [23]
12	200–1500	NO_3	Halogen tungsten	27	Tube in tube	Tube (reflective)	Batch	CCAP211/8 K	Kliphuis et al. [22]
250	2000	Urea	White LED[a]	8	ePBR	Open (opaque)	Batch	CCAP211/8 K	van Wagenen et al. [24]
Chlamydomonas reinhardtii									
25	80	NO_3	Red LED	6	Flat panel	Open	Chemostat	CC1690	Kliphuis et al. [17]
12	620	NO_3	Halogen tungsten	27	Tube in tube	Tube (reflective)	Turbidostat	CC1690	Kliphuis et al. [25]
25	100–500	NH_4	Red-blue LED	68–55	Flat panel	Black metal	Turbidostat	CC-124	Vejrazka et al. [27]
25	110–220	NH_4	Red-blue LED	68–55	Flat panel	Black metal	Turbidostat	CC-124	Vejrazka et al. [28]
14	800–1500	Urea	Warm white LED	25	Flat panel	Open	Turbidostat	CC1690	de Mooij et al. [26]
40	110–1000	NH_4	Cold white LED	8	Flat panel	Stainless steel (reflective)	Turbidostat	137 AH	Takache et al. [13]
40	110–700	NH_4	Cold white LED	8	Flat panel	Stainless steel (reflective)	Batch	137 AH	Takache et al. [13]
20	500	NH_4	White LED	6	Flat panel	Open	Batch	WT13	Jacobi [29]

[a] The LED spectrum of the ePBR is confidential, therefore the White LED from Jacobi was used instead.

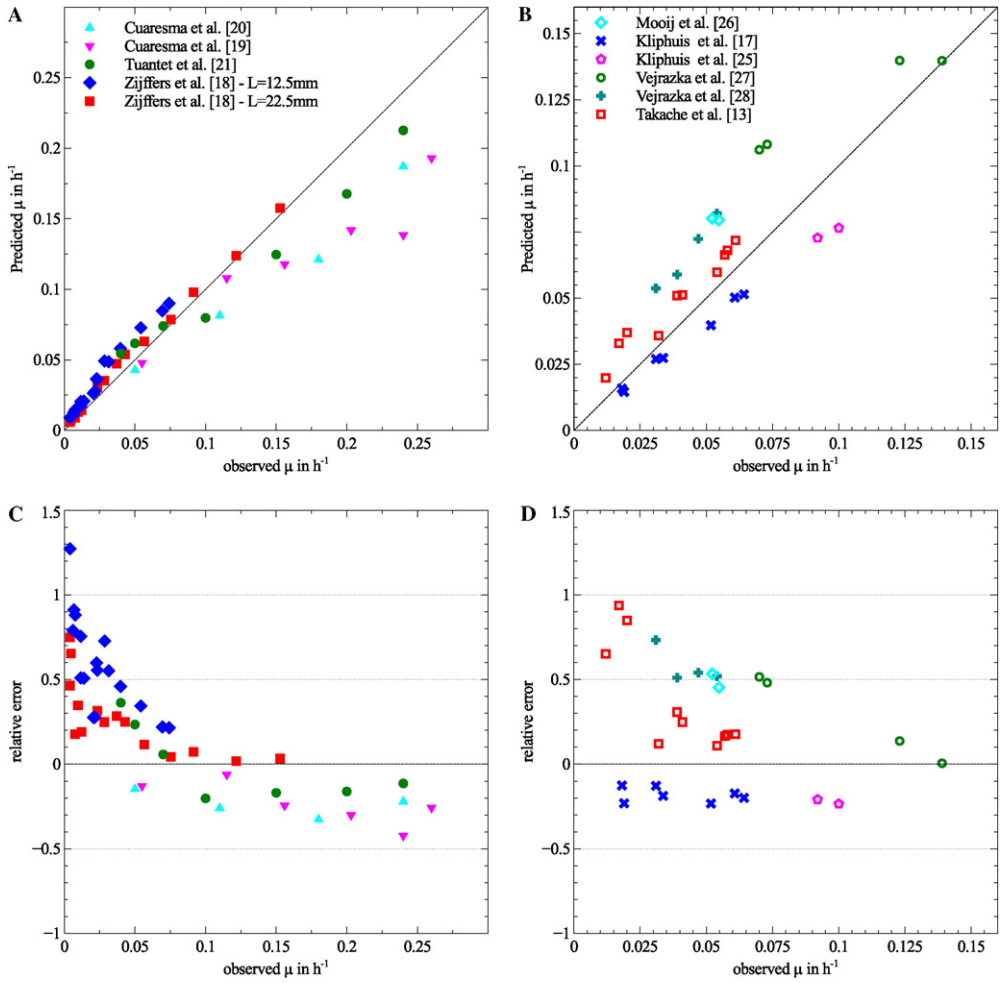

Fig. 2. Predicted specific growth rate plotted against the measured specific growth rate (A & B) and the relative error of the prediction (C & D). The dashed line represents a relative error of zero. A and C. Data for *C. sorokiniana*. B and D. Data for *C. reinhardtii*.

For both, the microalgae accuracy of the prediction based on the literature based parameters was low. Most likely, the low accuracy originates from: (1) inaccuracy in the light gradient prediction as Lambert–Beer Law neglects photoacclimation, light scattering, and incident light angle; and (2) inaccuracy in the literature based estimation of the model input parameters. In order to illustrate that our proposed simple engineering model is able to accurately predict the microalgae specific growth rate, both possibilities were more extensively evaluated by computational experiments.

4.2. Light gradient description and model prediction

The Lambert–Beer Law was used to predict the light gradient through the culture suspension. The accuracy of the Lambert–Beer Law can be increased by introducing a light correction factor in the exponent of Eq. (9). Included in such a light correction factor are: (1) differences in the incident light angle on the photobioreactor surface in the different studies included (Table 2); (2) scattering of light by microalgae leading to a change in light direction within the reactor; and (3) changes in specific light absorption due to photoacclimation (Fig. 3). Changes in the light direction can result in a longer light path through the reactor. In literature, similar strategies to improve the Lambert–Beer Law were reported and include: introducing a scattering correction factor for microalgae [3], including scattering by gas bubbles [33], including a backscattering coefficient [34], or including an extinction coefficient determined for the actual photobioreactor and microalgae suspension that is used which thus includes both light absorption and scattering [12]. In all three examples, the light gradient correction factor is included in the exponent of the Lambert–Beer Law equation.

In our model, the specific absorption coefficient is assumed to be constant, however, it varies because of photoacclimation. Based on the data reported in Table 2, the minimal specific absorption coefficient is approximately half of the maximal value which clearly indicates the impact of photoacclimation. The actual value, however, was often not reported for the studies used for the model validation. For the initial model predictions the measured higher values were utilized which represent low light acclimated microalgae. In some situations the actual absorption coefficients would be closer to high light acclimated microalgae. This would imply that they will employ a reduced absorption coefficient which will be reflected by a light correction factor between 1 and 0.5. As previously discussed, both scattering and a decreasing angle of the incident light will increase the light path which will be reflected in a correction factor greater than 1. In literature, the highest measured light correction factor correlated to scattering is 2.5 [3]. Therefore, realistic values for the light correction factor should fall within the range of 0.5 to 2.5.

The overall accuracy of the model was maximized with the light gradient fit, with a MAPE of only 1% for *C. sorokiniana*, and a MAPE of 2% for *C. reinhardtii*. In Fig. 4A and B, it can be observed that the fit reached 100% accuracy for most experimental points, however, a few predictions still deviate. In this simulation experiment, we fitted the predicted specific growth rate to the measured specific growth rate by changing the light gradient. Due to the design of the simulation experiment, a high prediction accuracy was logically obtained. The value of the correction

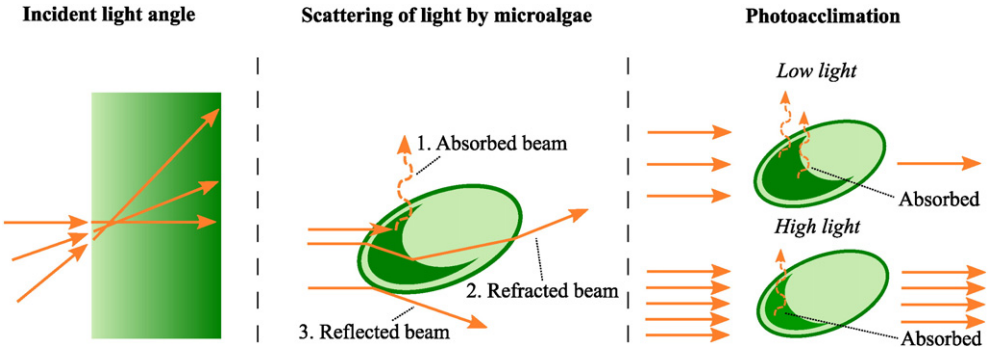

Fig. 3. Illustration of the effect of incident light angle, scattering of light by microalgae, and photoacclimation on the light path travelled within a microalgae culture.

factor for the different experiments, however, then provides information on the extent errors in the light gradient estimation and can explain the deviation between model predictions and experimental results.

The light gradient correction factor is plotted against the observed specific growth rate (μ) in Fig. 4C and D. For *C. sorokiniana*, it can be observed that the correction factor is larger at low μ, which was expected. This correlation appears to be similar for the light correction factor of *C. reinhardtii* plotted against observed specific growth rates. The light gradient correction factors predicted for *C. sorokiniana* were close to, or within, the realistic range of 0.5 to 2.5, although there were a number of outliers.

The light gradient correction factors for *C. reinhardtii* included many outliers beyond the maximal value of 2.5 and almost no correction factors under the minimal value of 0.5. The primary outlier is from the dataset of Vejrazka et al. [27] with experiments performed at low biomass concentrations and 500 μmol $(m^2\ s)^{-1}$ incident light. Under these light saturating conditions, the influence of the correction factor on the predicted growth rate is very low and result in a substantial

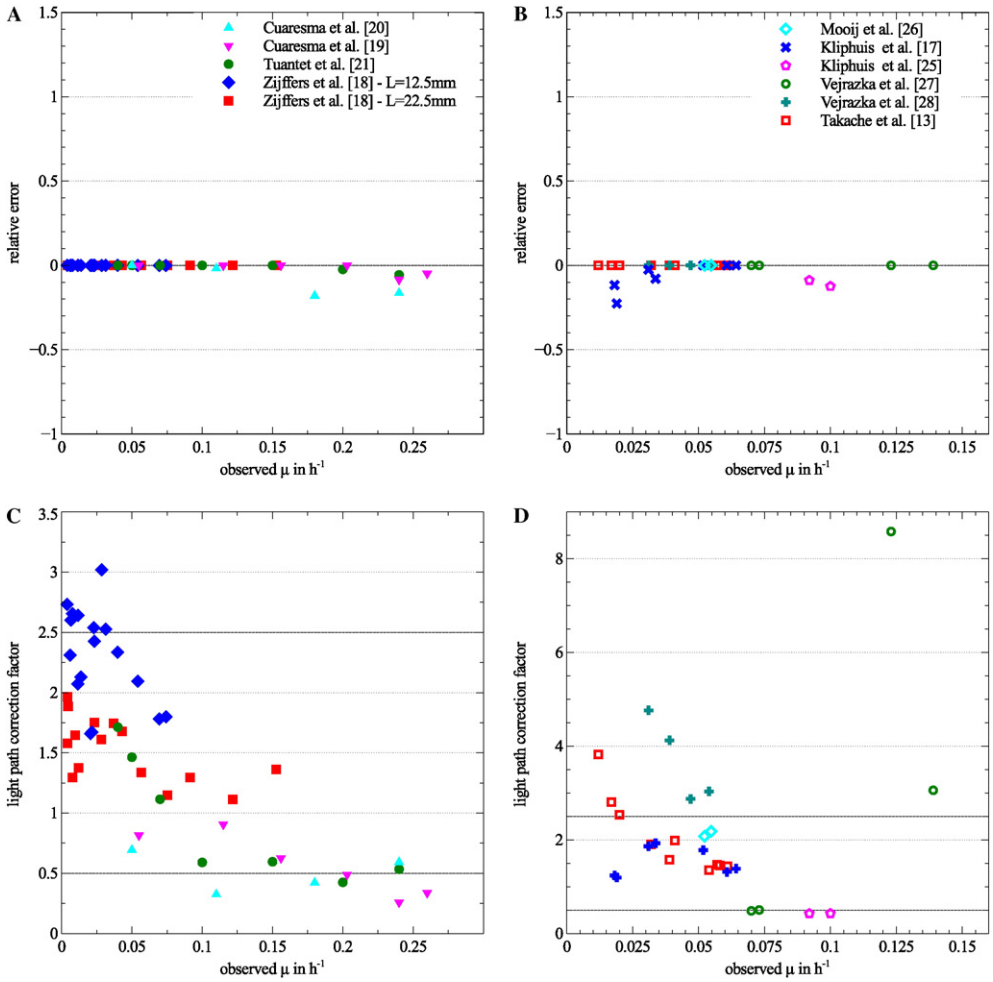

Fig. 4. Results of the specific growth rate prediction employing light gradient correction factors per data point. On the left, results are depicted for *C. sorokiniana* and on the right for *C. reinhardtii*. A and B show the relative error for the prediction. C and D depict the light gradient correction factor plotted against the specific growth rate. The dotted lines in C and D represent the range for realistic light gradient correction factors (0.5 to 2.5).

correction factor. Most likely, the discrepancy between measured and predicted specific growth rates is then related to other factors such as the different strains and nitrogen sources used for *C. reinhardtii* (Table 2) or differences in reactor operation related to pH, temperature, and mixing intensity (i.e. shear stress).

The light gradient correction factor includes the change in specific absorption coefficient due to photoacclimation and, therefore, the results can be compared to the measured specific absorption coefficient in the studies that were used. Only Zijffers et al. [18], Mooij et al. [26], Takache et al. [13], and Vejrazka [27,28] measured the real specific absorption coefficients (Supplementary file 1.B). When evaluating the datasets separately, they all indicate photo acclimation, however, when combined, no trend was observed. Furthermore, comparing the predicted light gradient correction factors to the measured specific absorption coefficient did not reveal a trend (data not shown). This would indicate that differences in the incident light angle and scattering of light within the microalgae suspension are also important factors in the light gradient prediction.

The light source will determine the incident light angle. An indication of the incident light angle can be obtained by looking at the full width at half maximum (FWHM) (Table 2). Where, the larger FWHM indicate that a large part of the incident light is falling on the reactor surface at an angle and the smaller the FWHM indicate that incident light is collimated into a beam. Although a trend can be observed that increasing FWHM results in increased light correction factors, it is evident that the incident light angle is not the only factor influencing the light correction factor.

For the majority of datasets, only the average incident light intensity is reported, however, light intensities often vary over the illuminated surface [12,26,27]. In the case of Vejrazka et al. [27,28], the light intensity ranges from 30% of the average in the corners to 140% of the average in the center of the reactor (FMT150, PSI, Czech Republic). All other datasets do not report the light distribution over the illuminated surface. The light distribution over the reactor surface will influence the growth rate as high light will result in increased photosaturation as reflected in the hyperbolic trend of photosynthesis versus irradiance (Eq. (4)). This effect will also be included in the light correction factors fitted. To eliminate this effect, a reactor surface should be subdivided into sufficiently small zones with their corresponding incoming light intensity which should all be measured [11,12]. Alternatively, indoor research reactors should be designed such that illumination is actually homogenous across the surface.

Apart from light absorption characteristics of the microalgae and the angular distribution of the incoming light, light scattering within the microalgae suspension also influences the light gradient in a photobioreactor. When light hits a microalga but is not absorbed, the direction of light propagation will change due to reflection or refraction events. The scattering of light, therefore, will change the light path through the reactor. The effect of scattering can be accommodated for by using the two-flux model which includes scattering but neglects the angle of incident light [1,2]. As an alternative approach the radiation field can be simulated based on a Monte Carlo approach [35] which includes scattering as well as the angular distribution of incident light. However, with increasing accuracy, the complexity and number of parameters of the model also increases.

To summarize, in order to model microalgae light limited growth, an accurate predictive light model is essential in combination with sufficient measurements of light distribution across the reactor surface and the angular distribution of incident light. Nevertheless, this analysis demonstrates that a more significant part of the deviation between predicted and measured growth can be accounted for by a better light description. This conclusion is based on the observation that the light gradient correction factor falls within the realistic rage of 0.5 to 2.5. Our simple model for microalgae growth, therefore, could continue to provide sufficient accuracy for engineering purposes provided the light field is better characterized.

4.3. Improving estimation model parameters

Stepping back from the accuracy of the light field prediction, part of the variation observed in the initial model predictions of the specific growth rate can be related to remaining errors in the estimation of the model parameters. For this reason, Monte Carlo simulations were performed, varying the parameters $Y_{x/s}$, m_s, $a_{x,\lambda}$ and $Y_{s/ph}$ randomly within the range presented in Table 1. Please note that, also in this approach, we take into account the possible effect of photoacclimation since parameter $a_{x,\lambda}$ is allowed to vary within the range reported in literature. For $a_{x,\lambda}$, it was assumed that the relative spectral distribution of the specific absorption coefficient remained constant (Supplementary file 1.A). The maximal specific growth rate (μ_m) was fixed and, because of its simple and reliable measurement, the accuracy of this parameter is high.

Monte Carlo simulations were performed on all datasets of either *C. sorokiniana* or *C. reinhardtii* to identify characteristics of microalgae species that were not correctly estimated. Furthermore, simulations per dataset were performed to identify variances between cultivation conditions which can include dissimilarities in: oxygen and carbon dioxide levels, temperature, and shear stress as well as variation between isolates of the *C. reinhardtii* that was employed in the different studies. Datasets were specified as presented in Table 2. The combinations of parameters that resulted in the lowest SSE are presented in Table 3, and the corresponding predictions are depicted in Fig. 5A to E.

The growth predictions for *C. sorokiniana* were clearly improving with the parameter estimation based on the Monte Carlo simulation (Fig. 5). The predictions range from 36% MAPE to 18% MAPE with the overall fit, and 9–17% MAPE for the fit per dataset. The new parameters presented in Table 3 demonstrate an obvious deviation between the datasets of Zijffers et al. [18] and the other datasets. It appears that the datasets from Zijffers et al. [18] are characterized by less efficiency of photosynthesis and growth on sugar compared to the other datasets. This is visible from the low $Y_{x/s}$ and $Y_{s/ph}$ fitted for Zijffers et al. [18] in combination with a high $a_{x,\lambda}$. Due to the substantial number of points derived from Zijffers et al. [18], the overall fit is also close to the values of Zijffers et al. [18]. This could be an indication that the experiments from Zijffers et al. [18] were performed under suboptimal conditions compared to the studies of Tuantet et al. [21] and Cuaresma et al. [19,20]. The medium recipe, pH, and gas flow rate were similar for all studies. Hydrodynamic forces were plausibly different within the various reactors resulting in variable shear stress between the studies [36]. It should be noted that, although trends can be observed from the results, errors in the light gradient prediction (see previous section) will also affect the outcome of the Monte Carlo simulations.

The growth predictions for *C. reinhardtii* also improved by adjusting the model parameter based on Monte Carlo simulations. Compared to *C. sorokiniana*, the increase in accuracy is similar; from a 34% MAPE, the MAPE decreased to 15% with the overall fit and 2–18% with the fit per dataset. For *C. reinhardtii*, all datasets were predicted accurately except for the dataset from Takache et al. [13]. The dataset of Takache et al. [13] might be difficult to predict due to the significant variation in the observed specific absorption coefficient. A clear photoacclimation response was thus observed by Takache et al. [13], and this effect cannot be described with our approach based on a constant specific absorption coefficient [13]. Compared to *C. sorokiniana*, there is much more variation in the m_s parameter which fluctuates between the low and high boundary (Table 1). For *C. reinhardtii*, the $Y_{x/s}$ varies over the complete range and, against expectations, the $Y_{x/s}$ for nitrate is predicted to be higher than the $Y_{x/s}$ for ammonium.

From the experimental data and model predictions for *C. reinhardtii*, it appears that it employs light more efficiently cultivated on nitrate compared to urea or ammonium. This was an unexpected result as the reduction of nitrate to ammonium expends energy [17] which is in accordance with the lower $Y_{x/s}$ for nitrate obtained from literature compared to the $Y_{x/s}$ for ammonium (Table 1). Based on the experimental design, the only clear difference between the experiments performed

Table 3
Estimated model parameters based on Monte Carlo simulations (Fig. 5). Results are shown for the overall fit and for every dataset separately which are both compared to the literature based estimates.

	m_s	$a_{x,\lambda}$	$Y_{x/s}$		$Y_{s/ph}$	MAPE
			NH_4	NO_3		
	cmol-s (cmol-x s)$^{-1}$	m^2 cmol-x^{-1}	cmol-x cmol-s^{-1}		cmol-s mol-ph^{-1}	%
	Chlorella sorokiniana					
Literature based estimates	**2.5E-06**	**7.1**	**0.59**	**0.54**	**0.10**	**36**
Results Monte Carlo simulations						
Overall fit	3.7E-06	4.5	0.50	–	0.08	18
Tuantet et al. [21]	3.7E-06	6.9	0.69	–	0.08	17
Cuaresma Franco et al. [20]	3.6E-06	5.1	0.70	–	0.11	8
Cuaresma et al. [19]	3.7E-06	5.6	0.70	–	0.11	14
Zijffers et al. [18]	3.6E-06	6.9	0.46	–	0.08	10
Zijffers et al. [18]	3.7E-06	7.1	0.50	–	0.10	9
	Chlamydomonas reinhardtii					
Literature based estimates	**2.0E-06**	**6.2**	**0.69**	**0.58**	**0.10**	**34**
Results Monte Carlo simulations						
Overall fit	1.8E-06	6.1	0.41	0.64	0.10	15
Kliphuis et al. [17]	1.6E-06	6.1	–	0.64	0.11	5
Kliphuis et al. [25]	2.1E-07	3.2	–	0.64	0.11	2
Vejrazka et al. [27]	4.0E-07	6.2	0.43	–	0.08	4
Vejrazka et al. [28]	4.0E-07	6.2	0.40	–	0.08	5
de Mooij et al. [26]	3.0E-06	6.1	0.40	–	0.08	6
Takache et al. [13]	3.6E-06	6.2	0.66	–	0.08	17

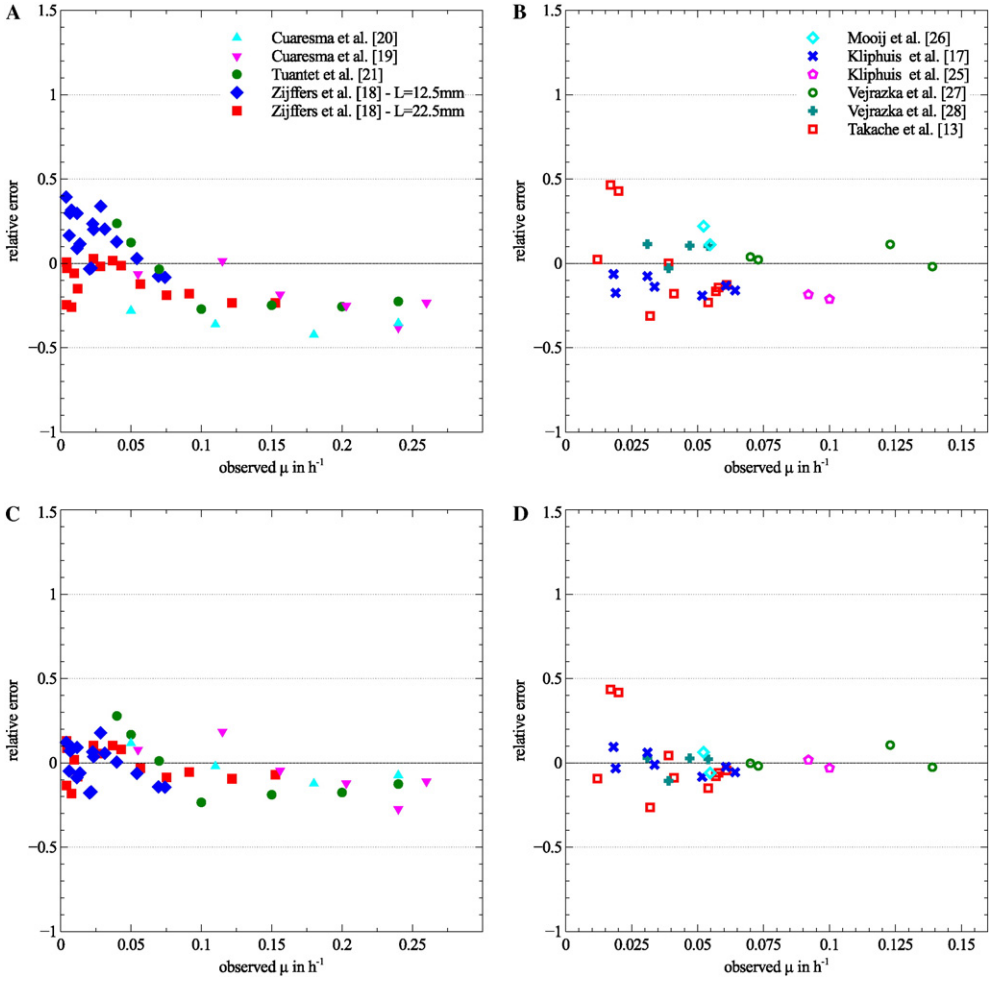

Fig. 5. Results of Monte Carlo simulations to improve estimation of model parameters. The results are plotted as the relative error for predicted specific growth rate against the measured specific growth rate. For the Monte Carlo simulations, the model input parameters were varied (Table 3). On the left, *C. sorokiniana* is depicted and, on the right, *C. reinhardtii*. A and B show the results with the smallest SSE of the Monte Carlo simulation per microalgae species. C and D depict the results with the smallest SSE of the Monte Carlo simulation per dataset (every line in Table 2 represents one dataset). The initial literature based prediction are depicted in Fig. 2C and D.

with nitrate and ammonium is this nitrogen source (Table 2). This observation is strengthened by the fact that the high $Y_{x/s}$ for nitrate is predicted for two different reactors and light sources. The significant divergence between nitrate and ammonium cultivated *C. reinhardtii* is also observed in the overall fit where the $Y_{x/s}$ for nitrate remains at its maximum while the ammonium $Y_{x/s}$ decreases to its minimum. The decrease in $Y_{x/s}$ for ammonium is to counteract the increase in $Y_{s/ph}$ which is necessary for accurate prediction of the data of Kliphuis et al. [17,25].

The datasets of *C. reinhardtii* are likely more difficult to predict as the various studies employed different species of *C. reinhardtii*. It is possible that these species exhibit different growth characteristics. The strain used by Vejrazka et al. [27,28], for example, is a wild type which carries mutations in the nitrate reducing genes and can only grow on ammonium. Furthermore, there is a more extensive variation in incident light intensities between the datasets used for *C. reinhardtii* compared to *C. sorokiniana*. *C. reinhardtii* cultures grown at high incident light intensities are expected to grow at reduced efficiency due to negative effects of high light, e.g. photoinhibition. Photoinhibition is not included in this model because of its complexity and time dependence.

4.4. Model predictions batch cultivation

The light limited growth model was validated on published studies on microalgal batch cultivation. The light limited growth model is able to describe the exponential growth phase at low biomass concentrations and the transition to slower growth with increasing biomass concentrations. The light limited growth model is, however, unable to predict the lag phase in some cases observed at the start of a batch. For this reason, the start of the prediction is in some cases not equivalent to the start of the batch. All data used is presented in Supplementary file 3, where also the start of the simulation is indicated.

For *C. sorokiniana* the model was validated with batch data from three studies [22–24]. The study of Kliphuis et al. [22] was also used to obtain a value for the maintenance sugar consumption. The biomass increase of *C. sorokiniana* was slightly over estimated for two studies [22,24] when employing the literature based estimated parameters (Fig. 6). For both of these studies the prediction accuracy increases with the parameters obtained with the overall fit for *C. sorokiniana*. In case of Kliphuis et al. [23], however, the data is actually predicted accurately with the literature based parameters and is underestimated with the parameters obtained with the overall fit. The predictions starting at low biomass concentration, however, are sensitive to the starting concentration and will in reality feature a lower specific absorption coefficient resulting in less over-saturation and a more rapid increase in biomass concentration. This in combination with the observation that dry weight measurement on dilute cultures often feature a lower accuracy we believe that the model predicts all cases accurately. Furthermore, the experiment of Kliphuis et al. [22] is predicted accurately and those experiments are the continuation of the experiments in Kliphuis et al. [23]. Finally it should be noted that Kliphuis et al. [22] observed biofilm formation and that therefore the observed biomass concentrations are underestimations.

For *C. reinhardtii* the model was validated with batch data from two studies [13,29], from which Takache et al. [13] also was used to identify the specific absorption coefficient and the molar mass for *C. reinhardtii*. The observed biomass increase during batch growth of *C. reinhardtii* is overestimated when using the literature based parameter estimation (Fig. 6). Because of the big difference in the parameter fit between *C. reinhardtii* cultivated on ammonia or nitrate (Table 3) the fit parameters for the four studies cultivated on ammonia where averaged instead of including the nitrate-derived data as well. In all cases this approach improved the prediction although still a discrepancy between observed

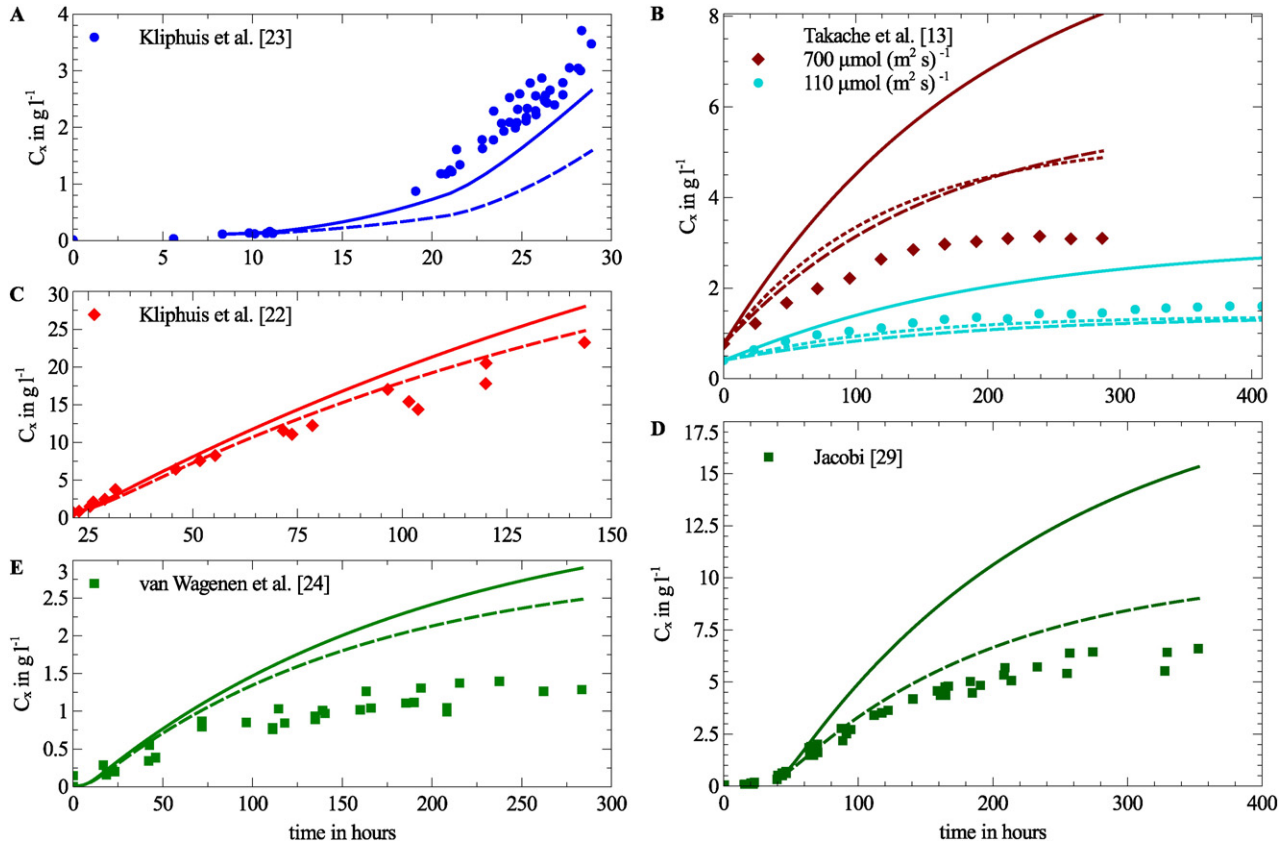

Fig. 6. Light to growth model validation on batch data for *C. sorokiniana* (A,C,E) and *C. reinhardtii* (B, D). The data represented by symbols is observed data from the corresponding study in the legend. The solid line represents the model prediction with the literature based estimations. The dashed line represents the model prediction with for *C. sorokiniana* the overall fit parameters and for *C. reinhardtii* the averaged parameters from the Monte Carlo fit for *C. reinhardtii* cultivated with NH4.

and predicted values remains for *C. reinhardtii*. Additionally the batch data of Takache et al. [13] is also predicted by employing the parameters obtained with the fit performed on the chemostat data reported in Takache et al. [13] (Table 3), which obtained very similar results to the averaged parameters. The batch validation validates the lower values obtained with the parameter fit compared to the literature based estimates in case of *C. reinhardtii*.

In summary the light limited growth model is able to predict batch cultivations for *C. sorokiniana* accurately. For *C. reinhardtii* it seems that at high light intensities the growth model seems to overestimate the productivity while at lower light intensities the model is accurate. These results are an indication that *C. reinhardtii* features reduced photosynthetic capacity or higher maintenance at high light intensities, which could imply photo damage. Furthermore, the light limited growth model is able to predict the exponential, linear and stationary growth phase.

4.5. General discussion

This model was able to predict the specific growth rate for a wide range of chemostat conditions with a MAPE of 36% for *C. sorokiniana* and 34% for *C. reinhardtii*. This is lower compared to light limited growth models reported in literature [12,14]. With *Chlorella vulgaris*, Bechet obtained an overall accuracy of 15% in one lab scale system [12] and a 8% overall accuracy for one outdoor system [37]. Cornet et al. [14] obtained a 15% overall accuracy for eight different reactor configurations with *Arthrospira platensis*. The model proposed, however, increased the accuracy to similar accuracies reported in literature after fitting per dataset, however, a perfect prediction was not obtained. The advantage of the proposed model over previously reported models is that this model introduces a simple but clear mathematical distinction between processes related to photosynthesis and processes related to growth.

Another advantage of this model is that the parameters necessary for the model predictions are measurable characteristics of the microalgae. By using parameters that are measurable characteristics, it is possible to modify this model for other microalgae utilizing the enormous amount of information already present in literature and/or performing a limited number of dedicated experiments to derive those parameters. The most convenient experiments to determine the model parameters include: (1) The maximal specific growth rate being measured by performing a light limited turbidostat where the average light intensity is close to the light saturation point; (2) The specific absorption coefficient being measured with a dedicated spectrophotometer featuring an integrated sphere and a wavelength scan; (3) The molecular weight being derived from the ash weight and an elemental analysis of the microalgae biomass; (4) The biomass yield on sugar being measured with a dark sugar limited growth experiment or with an experiment at subsaturating light from which the biomass yield on sugar can be derived based on the linearity of photosynthesis versus light intensity [17]; and (5) Measuring the sugar yield on photons is experimentally challenging, therefore, we estimated it based on theoretical considerations. A detailed overview and additional detailed experimental designs can be found in Supplementary file 1.B.

To increase the accuracy of microalgae growth models, experimental work should be further standardized. To properly compare various reactor set ups and validate biological growth models, the dry biomass concentration and biomass specific absorption coefficient should be measured. Furthermore, based on the presented results, an accurate description of the light field is important but difficult to model. Modelling light would be facilitated if the spatial distribution of the incident light, the spectral distribution of the incident light, the incident light angle, and light intensity at the back of the photobioreactor were all measured and reported. For research purposes, flat photobioreactors are preferably used where light is homogeneously distributed over the surface and the incident light angle is well-defined.

5. Conclusions

This paper has introduced and validated a model to describe microalgae growth under light-limited conditions. The model is based on only five measurable characteristics of the microalgae, and photosynthetic sugar production is separated from other growth-related processes. With this compartmentalization, the model is able to distinguish between sugar used for growth related respiration, maintenance related respiration, and precursors for biomass. Validation with different datasets obtained from literature was successful. Furthermore, input parameters where accurately identified from literature and improved with Monte Carlo simulations. This approach can be easily modified for other microalgae species. Due to its simplicity and acceptable accuracy, this model represents a beneficial engineering tool for the design and operation of microalgae based production processes.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.algal.2015.12.020.

Nomenclature

Parameters

a	specific (light) absorption coefficient in m^2 $cmol^{-1}$
A	area in m^2
c	correction factor
C	concentration in mol m^{-3}
E	energy of a photon
FWHM	full width at half maximum in degrees
I	light in mol-ph $(m^2\ s)^{-1}$
L	reactor depth in m
M	molar mass in g mol^{-1}
m	cell maintenance in mol $(cmol\text{-}x\ s)^{-1}$
MAPE	mean absolute percent error
N	number of steps
n	number of experimental points
PAR	photosynthetic active region
PI	Photosynthetic irradiance
q	rate in mol $(cmol\text{-}x\ s)^{-1}$
r	rate in g $(m^3\ s)^{-1}$
SSE	squared sum of errors
z	distance in m
Y	yield in (mol/mol)
α	Initial slope of PI curve

Subscripts

in	incident light
m	maximal
n	normalized
obs	observed
ph	photons in mol-ph
pre	predicted
s	sugar in cmol-s
x	dry biomass in cmol-x
λ	wavelength in nm^{-1}
I	light

Acknowledgements

The authors would like to thank the Algadisk consortium for their participation in this manuscript. The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007–2013) – managed by REA Research Executive Agency – under grant agreement no. 286887.

References

[1] L. Pilon, H. Berberoğlu, R. Kandilian, Radiation transfer in photobiological carbon dioxide fixation and fuel production by microalgae, J. Quant. Spectrosc. Radiat. Transf. 112 (2011) 2639–2660.

[2] J.F. Cornet, C.G. Dussap, J.B. Gros, C. Binois, C. Lasseur, A simplified monodimensional approach for modeling coupling between radiant light transfer and growth kinetics in photobioreactors, Chem. Eng. Sci. 50 (1995) 1489–1500.

[3] A.J. Klok, J.A. Verbaanderd, P.P. Lamers, D.E. Martens, A. Rinzema, R.H. Wijffels, A model for customising biomass composition in continuous microalgae production, Bioresour. Technol. 146 (2013) 89–100.

[4] H.P. Luo, M.H. Al-Dahhan, Airlift column photobioreactors for Porphyridium sp. culturing: part II. Verification of dynamic growth rate model for reactor performance evaluation, Biotechnol. Bioeng. 109 (2012) 942–949.

[5] Z. Dubinsky, P.G. Falkowski, K. Wyman, Light harvesting and utilization by phytoplankton, Plant Cell Physiol. 27 (1986) 1335–1349.

[6] W. Webb, M. Newton, D. Starr, Carbon dioxide exchange of *Alnus rubra*, Oecologia 17 (1974) 281–291.

[7] A.D. Jassby, T. Platt, Mathematical formulation of the relationship between photosynthesis and light for phytoplankton, Limnol. Oceanogr. 21 (1976) 540–547.

[8] S.J. Pirt, The maintenance energy of bacteria in growing cultures, Proc. R. Soc. Lond. Ser. B Biol. Sci. 163 (1965) 224–231.

[9] J. Quinn, L. de Winter, T. Bradley, Microalgae bulk growth model with application to industrial scale systems, Bioresour. Technol. 102 (2011) 5083–5092.

[10] R.J. Geider, H.L. MacIntyre, T.M. Kana, Dynamic model of phytoplankton growth and acclimation: responses of the balanced growth rate and the chlorophyll a: carbon ratio to light, nutrient-limitation and temperature, Mar. Ecol. Prog. Ser. 148 (1997) 187–200.

[11] P.M. Slegers, R.H. Wijffels, G. van Straten, A.J.B. van Boxtel, Design scenarios for flat panel photobioreactors, Appl. Energy 88 (2011) 3342–3353.

[12] Q. Bechet, P. Chambonniere, A. Shilton, G. Guizard, B. Guieysse, Algal productivity modeling: a step toward accurate assessments of full-scale algal cultivation, Biotechnol. Bioeng. (2014).

[13] H. Takache, J. Pruvost, J.F. Cornet, Kinetic modeling of the photosynthetic growth of *Chlamydomonas reinhardtii* in a photobioreactor, Biotechnol. Prog. 28 (2012) 681–692.

[14] J.-F. Cornet, C.-G. Dussap, A simple and reliable formula for assessment of maximum volumetric productivities in photobioreactors, Biotechnol. Prog. 25 (2009) 424–435.

[15] E. Lee, J. Pruvost, X. He, R. Munipalli, L. Pilon, Design tool and guidelines for outdoor photobioreactors, Chem. Eng. Sci. 106 (2014) 18–29.

[16] R.J. Geider, H.L. Macintyre, T.M. Kana, A dynamic model of photoadaptation in phytoplankton, Limnol. Oceanogr. 41 (1996) 1–15.

[17] A. Kliphuis, A. Klok, D. Martens, P. Lamers, M. Janssen, R. Wijffels, Metabolic modeling of *Chlamydomonas reinhardtii*: energy requirements for photoautotrophic growth and maintenance, J. Appl. Phycol. (2011) 1–14.

[18] J.W. Zijffers, K.J. Schippers, K. Zheng, M. Janssen, J. Tramper, R.H. Wijffels, Maximum photosynthetic yield of green microalgae in photobioreactors, Mar. Biotechnol. 12 (2010).

[19] M. Cuaresma, M. Janssen, C. Vílchez, R.H. Wijffels, Productivity of *Chlorella sorokiniana* in a short light-path (SLP) panel photobioreactor under high irradiance, Biotechnol. Bioeng. 104 (2009) 352–359.

[20] M. Cuaresma Franco, M. Buffing, M. Janssen, C. Vílchez Lobato, R. Wijffels, Performance of *Chlorella sorokiniana* under simulated extreme winter conditions, 24 (2012) 693–699.

[21] K. Tuantet, H. Temmink, G. Zeeman, M. Janssen, R.H. Wijffels, C.J.N. Buisman, Nutrient removal and microalgal biomass production on urine in a short light-path photobioreactor, Water Res. 55 (2014) 162–174.

[22] A.J. Kliphuis, M. Janssen, E. van den End, D. Martens, R. Wijffels, Light respiration in *Chlorella sorokiniana*, 23 (2011) 935–947.

[23] A.M. Kliphuis, L. de Winter, C. Vejrazka, D.E. Martens, M. Janssen, R.H. Wijffels, Photosynthetic efficiency of *Chlorella sorokiniana* in a turbulently mixed short light-path photobioreactor, Biotechnol. Prog. 26 (2010) 687–696.

[24] J. Van Wagenen, M.L. Pape, I. Angelidaki, Characterization of nutrient removal and microalgal biomass production on an industrial waste-stream by application of the deceleration-stat technique, Water Res. 75 (2015) 301–311.

[25] A.M.J. Kliphuis, D.E. Martens, M. Janssen, R.H. Wijffels, Effect of O2:CO2 ratio on the primary metabolism of *Chlamydomonas reinhardtii*, Biotechnol. Bioeng. 108 (2011) 2390–2402.

[26] T. de Mooij, M. Janssen, O. Cerezo-Chinarro, J.H. Mussgnug, O. Kruse, M. Ballottari, R. Bassi, S. Bujaldon, F.-A. Wollman, R.H. Wijffels, Antenna size reduction as a strategy to increase biomass productivity: a great potential not yet realized, J. Appl. Phycol. (2014).

[27] C. Vejrazka, M. Janssen, M. Streefland, R.H. Wijffels, Photosynthetic efficiency of *Chlamydomonas reinhardtii* in flashing light, Biotechnol. Bioeng. 108 (2011) 2905–2913.

[28] C. Vejrazka, M. Janssen, M. Streefland, R.H. Wijffels, Photosynthetic efficiency of *Chlamydomonas reinhardtii* in attenuated, flashing light, Biotechnol. Bioeng. 109 (2012) 2567–2574.

[29] A.J.C. Jacobi, Optimierung der Lichtverteilung in Photobioreaktoren, der Fakultät für Chemieingenieurwesen und Verfahrenstechnik Karlsruher Institut für Technologie, Deutsche national bibliothek, 2013.

[30] S. Hoekema, A. Rinzema, J. Tramper, R.H. Wijffels, M. Janssen, Deceleration-stats save much time during phototrophic culture optimization, Biotechnol. Bioeng. 111 (2014) 792–802.

[31] U. von Stockar, J.S. Liu, Does microbial life always feed on negative entropy? Thermodynamic analysis of microbial growth, Biochim. Biophys. Acta Bioenerg. 1412 (1999) 20.

[32] D.G. Mayer, D.G. Butler, Statistical validation, Ecol. Model. 68 (1993) 21–32.

[33] D. Zhang, P. Dechatiwongse, K. Hellgardt, Modelling light transmission, cyanobacterial growth kinetics and fluid dynamics in a laboratory scale multiphase photo-bioreactor for biological hydrogen production, Algal Res. 8 (2015) 99–107.

[34] M. Fachet, R.J. Flassig, L. Rihko-Struckmann, K. Sundmacher, A dynamic growth model of *Dunaliella salina*: parameter identification and profile likelihood analysis, Bioresour. Technol. 173 (2014) 21–31.

[35] J.M. Heinrich, I. Niizawa, F.A. Botta, A.R. Trombert, H.A. Irazoqui, Analysis and design of photobioreactors for microalgae production I: method and parameters for radiation field simulation, Photochem. Photobiol. 88 (2012) 938–951.

[36] P.L. Walls, J.C. Bird, L. Bourouiba, Moving with bubbles: a review of the interactions between bubbles and the microorganisms that surround them, Integr. Comp. Biol. 54 (2014) 1014–1025.

[37] Q. Bechet, A. Shilton, B. Guieysse, Full-scale validation of a model of algal productivity, Environ. Sci. Technol. (2014).