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A new kinetics model to predict the growth of micro-algae subjected to fluctuating availability of light

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Abstract

Light is a key environmental factor for the growth of micro-algae, and optimizing the capture of light is critical for high efficiency production systems. As the density of the population of micro-algae increases, the availability of light decreases, leading to a reduction in the growth rate because of mutual shading, while other effects, such as photo-inhibition, might be especially frequent when the population density is low. Several models in the literature have been developed to take into account light phenomena and predict micro-algal growth, particularly in a mono-culture. With the help of a simple expression for the attenuation of the light, we propose and justify a new growth function that incorporates both photo-inhibition and photolimitation. In agreement with the experimental data, this new formulation describes the micro-algal response to a wide range of situations of excessive or insufficient light intensities through an explicit dependence on both the incident light and the biomass concentration. While simple, the proposed expression can be satisfactorily applied to practical cases under nutrient replete conditions in photo-bioreactors with different sizes and geometries. It extends naturally to the growth of different species, providing a dynamic model which can simulate experiments in a mono-culture as well as in poly-cultures. The investigation of the competition for light-limited growth shows that the model predicts competitive exclusion, which has also been experimentally demonstrated. This leads to new perspectives for the control and optimization of mixed micro-algal cultures.

Keywords: Micro-algae, Modeling, Light availability, Growth rate, Density dependency, Poly-culture, Interactions.

1 **1. Introduction**

2 The study of different aspects related to the behaviour of a micro-algae
3 culture growing in an intensive culture system has gained renewed interest
4 because of the wide fields of application of these photosynthetic microorgan-
5 isms. Micro-algae are viable sources of biological compounds and constitute
6 a renewable and environmental-friendly feed-stock [1]. Their intensive cul-
7 tivation is used for the production of high-value bio-products and bio-fuels
8 and also for the treatment of polluted waters. The selection of the appro-
9 priate micro-algae species and appropriate methods of culture is essential to
10 guarantee the economic feasibility of the intensive production of micro-algae.
11 *Chlorella* and *Scenedesmus* have been considered promising candidates for
12 wastewater treatment ([2, 3]) and bio-fuel production ([2, 4, 5]), thanks to
13 their maximum growth rates, biomass yields, and lipid and carbohydrate
14 contents, which can reach high levels.

15 In a controlled culture system, the growth of micro-algae may be affected
16 by a combination of environmental parameters, such as light intensity, photo-
17 period, temperature, pH, and composition of the nutrients of the culture
18 system. When nutrients are provided in sufficient quantities and the pH
19 is maintained at its optimal value, the efficient use of light is essential to
20 optimize and control the growth of an algal culture to ensure the success of
21 industrial production processes, since the light regime and photo-period are
22 critical components that directly affect the production of biomass ([6, 7, 8, 9]).

23 Several studies on the effects of light on the growth of micro-algae have
24 been carried out based on experimental as well as theoretical approaches, us-
25 ing fundamental concepts for understanding the dynamic behaviour of light-
26 limited cultures in photo-bioreactors or outdoor raceways. The proposed
27 mathematical models of micro-algae share, in general, the common objective
28 of having a growth rate as a function of the availability of the light. Accord-
29 ing to the typical photosynthesis-irradiance curve (P-I curve), describing the
30 response of the rate of photosynthesis to changes in the intensity of the light,
31 three distinct light regimes are depicted. At low intensities, the photosynthe-
32 sis rate of the algal cells is initially affected by photo-limitation and is usually
33 proportional to the intensity of the light until reaching a saturation point at
34 which the growth rate is at its maximum attainable value and the algae has

35 become light saturated. Beyond this point, the growth rate is negatively
36 affected due to photo-inhibition ([10, 11, 12]), defined as the degradation of
37 key proteins at high light intensities, which causes a loss of photosynthetic
38 yield and productivity. While photo-inhibition may appear on a short time
39 scale under high irradiance, the response to changes in the long term average
40 irradiance is usually referred to as photo-acclimation [13, 14]). This phe-
41 nomenon is linked to the ability of cells to maximize their light absorption
42 capacity under low light and to minimize energy flow under high light by
43 various changes in pigmentation, macro-molecules (e.g. enzymes associated
44 with photosynthesis and respiration), and cell morphology (e.g. cell volume,
45 thylakoids stacking, and transparency) [15, 16, 17]. These two phenomena
46 may affect the P–I curve dramatically [18, 19, 20].

47 The mathematical formulations of the effects of different light phenomena
48 on photosynthesis require more or less complex mechanistic models, depend-
49 ing on the study and the model’s application scale. Traditionally, the growth
50 rate as a function of the incident light perceived by the micro-algae is assumed
51 to follow a Monod-like function [21, 22, 23] or some other non-monotonic ex-
52 pression that accounts for photo-inhibition, such as a Haldane-like function
53 [24, 25, 26] or the Steele function [27, 28]. These formulations, considered to
54 be the simplest, do not account for the light distribution within the broth
55 (light gradients) or reactions occurring at the cell level, such as the flash
56 light effect [18], faced by individual cells moving from high-light zones to
57 near-dark zones.

58 Because the biomass and other light-absorbing substances generate a light
59 gradient in photo-bioreactors, the light intensity that micro-algae can face be-
60 comes a function of the depth and biomass concentration within the culture.
61 Light attenuation is a common phenomenon that is usually described by the
62 Beer–Lambert law [29, 30], according to which the light penetration decreases
63 exponentially with increasing biomass concentrations. When accounting for
64 the impact of light gradients, the global specific micro-algae growth rate can
65 be expressed by adding the local growth kinetics determined through a bio-
66 logical model, depending on the local light intensity faced by individual cells.
67 This approach can be described using, for example, a Monod-like function
68 coupled with the Beer–Lambert law for the light distribution. Another ap-
69 proach is to describe the average growth rate through a biological model (for
70 instance, the Monod function) that depends on the average light intensity
71 received by the micro-algae (which can be described using the Beer–Lambert
72 law). This approach assumes that the micro-algae in a well-mixed culture

73 are, on average, exposed to the same light intensity and, therefore, have the
74 same average growth rate [29].

75 Despite the fact that most photo-bioreactor models rely on the Beer–Lambert
76 law, which is based on the assumption that the light is not scattered in the
77 medium, its use increases the inaccuracy in high-density cultures where mul-
78 tiple scattering events occur ([31, 32, 33, 34]). The local light availability
79 can be calculated using complicated equations accounting for light absorp-
80 tion and scattering in the reactor. However, it is important to note that with
81 more complications (in the expressions of the light distribution or in model-
82 ing growth at the cell level), they involve additional input parameters whose
83 determination can be difficult, expensive, or time consuming. Moreover, a
84 large number of parameters can lead to over-fitting, resulting in the model’s
85 being poor at predicting the actual trends.

86 In practice, the biomass concentration and the instantaneous light inten-
87 sity available in the culture medium can both be easily monitored, allowing
88 following the light attenuation phenomenon throughout the cell cultivation
89 period. In the present study, we evaluate the accuracy of modeling the al-
90 gal growth rate as a function of the average attenuated light by cell density.
91 We used two species *C. sorokiniana* and *S. pectinatus*, as candidates for the
92 biological model, growing in one-sided illuminated photo-bioreactors under
93 nutrient replete conditions and constant temperature. The light attenuation
94 inside the culture is assumed to be non-emitting and non-fluorescing, depend-
95 ing on two independent phenomena: (i) absorption by the pigments and (ii)
96 scattering by the whole-cell mass [22]. This light phenomenon was approxi-
97 mated by the summation of the light intensity altered/shaped by the biomass
98 through a simple equation of the form of Michaelis–Menten kinetics (as sug-
99 gested by [35]), and the incident light intensity (measured perpendicularly
100 to the light source on the boundary of the reactor) modified by the photo-
101 bioreactor and its liquid content. This relationship was validated regardless
102 of the value of the initial light intensity and was an adequate approach, able
103 to cover a wide range of cell concentrations [35]. We then develop a simple
104 growth function explaining the experimental results of the response of the
105 process-rate of the micro-algae to a broad range of incident light intensities
106 and biomass concentrations. This new formulation can be considered one of
107 the simplest modeling approaches to describe the behaviour of micro-algal
108 cells in response to light phenomena.

109 This paper is organized as follows. The influence of the intensity of the
110 incident light and the biomass density on the specific growth rates of the two

111 micr-oalgae candidates (growing in batch cultures) is discussed in Sections
112 3.1 and 3.2, respectively, through comparisons of the data with classic ki-
113 netic models. The light attenuation equation is validated in Section 3.3 and
114 then incorporated in a new growth formulation in Section 3.4, allowing the
115 description of the experimental data sets obtained from the batch cultures.
116 In Section 3.5, the validation of the new kinetic function is presented for the
117 case of continuous light-limited photo-bioreactors using dynamic data for the
118 biomass obtained in mono-cultures and poly-cultures. Finally, in Section 3.6,
119 some cases of the outcome of competition for light are investigated through
120 simulations of the validated multi-species dynamic model under different op-
121 erating conditions of removal rates and periodic light supply, in continuous
122 mode photo-bioreactors.

123 2. Materials and methods

124 2.1. Microalgae strains and pre-culture medium

125 The microalgae were isolated in October 2015 from samples from the
126 high rate algal pond (HRAP) located in the north of France and operated
127 for processing urban wastewater [36, 37]. The isolated species were identi-
128 fied as *C. sorokiniana* and *S. pectinatus* by the Sanger sequencing method
129 [37]. The species were systematically sub-cultured (sub-culturing of 10% of
130 the inoculum at each cycle) in flasks separately in fresh medium Z8NH₄ (Z8
131 media [38] buffered with HEPES at 20 mM, enriched with ammonium salt
132 (NH₄Cl) as the sole nitrogen source, and complemented with sodium car-
133 bonate (Na₂CO₃) to reach a C:N:P ratio of about 88:8:1), and maintained in
134 laboratory incubators under continuous light (100 $\mu\text{E m}^{-2}\text{s}^{-1}$) and temper-
135 ature 25°C.

136 2.2. Experimental procedure and cultivation conditions

137 For testing the effects of light on the growth of the biomass for each
138 species, pre-incubations were carried on for 5-day batch cultures under a
139 continuous light intensity of 100 $\mu\text{E m}^{-2}\text{s}^{-1}$ in a 100 mL flask. Then, each
140 species was diluted (by 2%, 3%, 7%, 10%, 13%, 20%, 27%, 33%, 40%, 47%,
141 53%, 60% in 40 mL flasks) with the relevant culture medium where the pH
142 was maintained constant (at a value of 7.5) in order to test the influence
143 of different biomass concentrations. The incubation of these cultures were
144 carried on for 3-day batch culture in a type 96 microwell plate (Greiner

145 CELLSTAR® 96 well plates), filled with the 12 different dilutions with 8
146 replicates (with a working volume of 250 μL per well) for each dilution. Nine
147 identical microwell plates were prepared for each algal species, and then
148 each of them was placed at a fixed position under nine fixed light intensities
149 (from 0 to 900 $\mu\text{E m}^{-2}\text{s}^{-1}$) in four identical laboratory incubators (Panasonic
150 MIR-154-PE) where the temperature was set at 25°C. The incident light
151 intensities (from cool white Luxeon Rebel LEDs, Lumileds) were measured
152 above and below each microwell plate filled with the culture medium using
153 the scalar PAR sensor ULM 500 Walz.

154 Thus, a total of 108 combinations of transmitted light intensity and pop-
155 ulation density were used, including the 12 initial dilutions (equivalent to
156 the diluted initial biomass) and 9 light intensities. The algal growth in the
157 microwell plates was evaluated for each species by fluorescence measurements
158 after 48 h of exposure to each different condition of both light and biomass
159 concentration outlined above. The specific growth rates μ (d^{-1}) were deter-
160 mined on a total of three biomass measurements (at $t = 0$ h, $t = 24$ h and
161 $t = 48$ h) using linear least-squares curve fitting on the supplied set of the
162 logarithm of the biomass $\ln(x)$ and time t . These growth rates were used for
163 identifying the growth model.

164 To visualize the changes in the shape of the light attenuation curve ac-
165 cording to the cell densities of each species when exposed to several incident
166 light intensities, we selected 9 batch cultures at different stages of growth
167 (non-diluted cultures with different biomass concentrations). Each 40 mL
168 flask reactor was placed under 8 light levels from cool white LEDs (Lux-
169 eon Rebel, Lumileds) delivered from the laboratory incubators (Panasonic
170 MIR-154-PE). The light was measured at the centres of the flasks in a water
171 solution with and without cells using the scalar PAR sensor ULM 500 Walz,
172 while the biomass concentrations of each species were determined by optical
173 density (OD) and were then converted to carbon units. Then, for each value
174 of the biomass concentration, the light attenuated by the micro-algal cells
175 can be found as the difference between the two measurements of the light
176 (with and without cells).

177 Continuous culturing was carried out in two photo-bioreactors to follow
178 the biomass of the strains over time (in mono-culture or poly-culture) under
179 the same light condition provided by one-sided lighting (using several white
180 fluorescent lamps) at $I_{in} = 165 \mu\text{E m}^{-2}\text{s}^{-1}$, and under different initial biomass
181 conditions. These experiments were used to identify the growth model and
182 for validation. Each bioreactor consisted of an Erlenmeyer glass vessel of

183 2 L with double walls. Between these walls was flowing water thermostati-
184 cally controlled at 25°C (using Thermo Scientific and VWR circulating bath)
185 allowing maintaining the inoculum temperature constant. The mineral sub-
186 strate at non/limiting concentrations (10 L of sterilized and buffered Z8NH₄
187 culture medium) was introduced continually into the glass vessel at a con-
188 stant flow by a dual Channel Precision Peristaltic Pump (Ismatec), while
189 the excess of bioreactor liquid was collected in a glass bottle using the same
190 pump, thus keeping the culture volume constant. The reactors were operated
191 at a hydraulic retention time of 4 days (corresponding to a dilution rate of
192 $D = 0.25 \text{ d}^{-1}$) maintained constant throughout the experiments. To ensure
193 a perfect mixing within the bioreactor, each reactor was agitated at 300 rpm
194 by means of a magnetic system. In addition, a bubbling aeration system was
195 designed as follows: the air is sent into a bottle of water to trap the air par-
196 ticles, an aquarium pump system sends the moisture-saturated air into the
197 culture medium, and then passes through a cannula connected to a transmit-
198 ting filter of 0.2 μm to avoid over-pressure and to limit air contamination.
199 The reactor also has a sampling cannula connected with a non-return valve
200 to minimize the risk of contamination.

201 2.3. Analytical procedures

202 *Batch cultures.* In the 3-day batch cultures, monitoring the growth of *C. sorokinii*-
203 *ana* and *S. pectinatus* in the microwell plates was carried out daily by flu-
204 orescence measurements (EX 450 nm, EM 680 nm) and optical density OD
205 at 650 nm, 730 nm, and 680 nm using a micro-plate reader (CHAMELEON,
206 Hidex).

207 *Continuous cultures.* In chemostat cultures, samples were collected for cell
208 counts and dissolved nutrient analysis. The cell counts were performed in
209 triplicate using an upright microscope (MOTIC BA310). The algal biomass
210 was also monitored by OD at 650 nm using a micro-plate reader (FLU-
211 OSTAR, BMG Labtech) at 650 nm through 48 well plates filled daily with 1
212 mL of culture sample.

213 *Carbon conversion.* The carbon content was determined as follows: 5-mL
214 samples were filtered onto pre-combusted AE filters and stored at 80°C until
215 the analysis. The filters were dried at 60°C for 24 h, pelleted, and analysed
216 using an ANCA mass spectrometer (Europa Scientific).

217 Referring to batch experiments on the same studied species for different
218 stages of growth with a working volume of 40 mL under different concentra-
219 tions of ammonia, a continual light intensity ($100 \mu\text{E m}^{-2}\text{s}^{-1}$) and a fixed
220 temperature (25°C) [37], the OD at 650 nm (measured with CHAMELEON,
221 Hidex) was found to be the best correlated with the Particulate Organic Car-
222 bon (POC) content of the cells determined for both species (POC= 496.14
223 OD_{650} , $R^2 = 0.89$).

224 For the continuous cultures, several samples were collected from both
225 the mono-culture and the poly-culture during chemostat monitoring. The
226 obtained values of the POC allowed establishing a linear correlation be-
227 tween POC and OD_{650} (measured with FLUOSTAR, BMG Labtech) (POC=
228 208.42, OD_{650} , $R^2 = 0.88$).

229 2.4. Model identification methods

230 First of all, we explored a range of nonlinear models that might be useful
231 for characterizing the growth rate μ of the studied species according to some
232 classical kinetic functions ($\mu(\cdot)$) from the literature depending on the follow-
233 ing variables: the incident light I_{in} or the biomass x . Then we proposed a
234 new kinetic function depending on both these two variables.

235 The optimal parameters of the growth functions used to explain the char-
236 acteristics of the growth rates of the algal species (determined in microwell
237 plates) were calibrated using the "fitnlm" function of Matlab, which esti-
238 mates model parameters and delivers statistics. The comparison between
239 the parameters among species for the same growth model was ensured by
240 the same function using the vector of all observations on μ (for both species)
241 as a response variable, and the matrix of the model variable along with a
242 dummy variable (which takes only the value 0 or 1 according to the species,
243 thus indicating the absence or presence of some categorical effect that may
244 be expected to shift the outcome of the parameter identification) as predic-
245 tor variables [39]. This involved the need to add to each required parameter
246 a coefficient multiplied by the dummy variable, thus constituting the new
247 model formulation (used in the "fitnlm" function). Then, one can determine
248 the significant differences between the parameters, according to the p -value
249 P of these coefficients.

250 To readjust the parameters of the proposed growth function using the
251 data of the biomass of both species in mono-culture (in chemostat), we used
252 the function "fmincon" of Matlab to minimize the least squares criterion:

253 $\sum_{i=1}^k \sum_{j=1}^n \frac{(X_{exp_{ij}} - X_{sim_{ij}})^2}{n}$ where $k=2$ and n is the number of observations of
 254 X_{exp} , and X_{sim} results from the numerical integration of the model (describ-
 255 ing the time evolution of the biomass in continuous mode photo-bioreactors)
 256 by the "ode45" function of Matlab.

257 3. Results and discussion

258 3.1. Effects of the incident light on the specific growth rate of *C. sorokiniana* 259 and *S. pectinatus* in batch monoculture

260 At very low levels of biomass, the average light intensity received by the
 261 culture is close to that reaching the reactor surface (i.e. incident light I_{in}),
 262 particularly for reactors with a small light path. Under these experimental
 263 conditions, one can ensure that all cells are exposed to the same light inten-
 264 sity I_{in} . In order to describe accurately the relationship, for each species,
 265 of the growth rate μ with I_{in} , we will use the results obtained experimen-
 266 tally in microwell plates from the lowest concentration of biomass (1.1 ± 0.1
 267 mgC.L⁻¹). We also considered close initial biomass (1.20 mgC.L⁻¹ and 1.04
 268 mgC.L⁻¹ for *C. sorokiniana* and *S. pectinatus*, respectively) to compare the
 269 growth-light relationships of the two species.

270 The relationship between μ and I_{in} was first compared using a Monod-like
 271 kinetics, which assumes that only light limits the growth of the cells. Then we
 272 tested the Haldane- and Steele-like models, in which the light inhibition effect
 273 at high light intensities is included as well (see Figure 1). The expressions
 274 and parameters of the three kinetic functions obtained from comparison with
 275 the data are all summarized in Table 1.

276 The results show that, over the tested range of incident light intensities,
 277 the Monod-like model seems to fit the data of *S. pectinatus* far better than
 278 those of *C. sorokiniana*, whose growth appears to be inhibited at high light
 279 levels (root mean squared error RMSE= 0.159 for *S. pectinatus* < 0.195 for
 280 *C. sorokiniana*).

281 The determined values of the parameters when using the Monod function
 282 to explain the growth rate data of *S. pectinatus* are in line with the re-
 283 sults of experiments in previous work performed on the species *Scenedesmus*
 284 *caribeanus*, which was found to reach a maximum growth rate μ_m of 1.44 d⁻¹
 285 and a half-saturation constant K_{sI} of 68 $\mu\text{E m}^{-2}\text{s}^{-1}$ [40] ($\mu_m = 1.2 \pm 0.1$ d⁻¹
 286 and $K_{sI} = 95 \pm 18$ $\mu\text{E m}^{-2}\text{s}^{-1}$ in this study).

287 The reduction in the growth rates of *C. sorokiniana* observed for $I_{in} > 450$
288 $\mu\text{E m}^{-2}\text{s}^{-1}$ suggests its sensitivity to photo-inhibition. This is confirmed by
289 the smaller RMSE obtained when comparing its experimental and simulated
290 data using either the Haldane (RMSE = 0.173) or Steele (RMSE = 0.183)
291 models, both of which have non-monotonic curves which can describe the
292 photo-inhibition phenomenon. The decline in the growth rate of *C. sorokini-*
293 *ana* due to photo-inhibition at high light intensities was also reported in
294 previous studies (at a light intensity of about $250 \mu\text{E m}^{-2}\text{s}^{-1}$ and for tem-
295 peratures $\geq 22^\circ\text{C}$) [41].

296 According to the Steele model, both species reach their maximum specific
297 growth rates around an average intensity of $489 \mu\text{E m}^{-2}\text{s}^{-1}$, which is supposed
298 to be the optimal light condition under the stated conditions of biomass
299 concentration and temperature.

300 From Table 1, *C. sorokiniana* showed the higher maximum specific growth
301 rates compared to those obtained with *S. pectinatus* using either the Monod
302 or Steele kinetics. However, no significant difference was observed between
303 the two species in terms of their affinity to light intensities. This implies that
304 the species' affinities may be similar, or the experimental protocols in this
305 study did not allow determining any difference.

306 The Haldane-like model provided the lowest RMSE (RMSE = 0.173 for
307 *C. sorokiniana* and RMSE = 0.158 for *S. pectinatus*) compared with the
308 other two models, thus making it more suitable to represent the data despite
309 the sensitivity of its inhibition constant K_{iI} .

310 According to the model predictions, it appears that *C. sorokiniana* was
311 able to grow more rapidly than *S. pectinatus* when incident light intensities
312 ranged between 100 and $1400 \mu\text{E m}^{-2}\text{s}^{-1}$ (see Figure 1), but under higher
313 light intensities, the growth rate of *S. pectinatus* exceeded that of *C. sorokini-*
314 *ana*. This means that under the stated experimental conditions, *S. pectinatus*
315 was more resistant than *C. sorokiniana* to photo-inhibition. This is in agree-
316 ment with previous experiments, showing *S. quadricauda* with lower photo-
317 inhibition sensitivity than *C. sorokiniana* under light intensities of about
318 $1000 \mu\text{E m}^{-2}\text{s}^{-1}$ [42].

319 From these observations, it can be seen that the intensity of the incident
320 light can have different effects on the growth of different species of micro-
321 algae. When one species is cultivated under high light intensities and at a
322 low biomass concentration or a reduced light path, photo-inhibition is likely
323 to occur ([43]). In the case of significant photo-damage, the specific growth
324 rate can be reduced drastically, as shown by several studies ([43, 44]). In

325 poly-culture, the light intensity can favor or disadvantage the growth of one
 326 algal species compared to another, depending on its sensitivity to light. Our
 327 results suggest that in a mixed culture of the two studied species, *C. sorokiniana*
 328 *ana* may out-compete *S. pectinatus* under moderate light intensities, but may
 329 itself be out-competed by *S. pectinatus* under high light conditions. How-
 330 ever, the interactions between these two species may change according to the
 331 dynamics of their respective biomass during the algal cultivation. Therefore,
 332 the interaction between the incident light and the population density was
 333 further investigated.

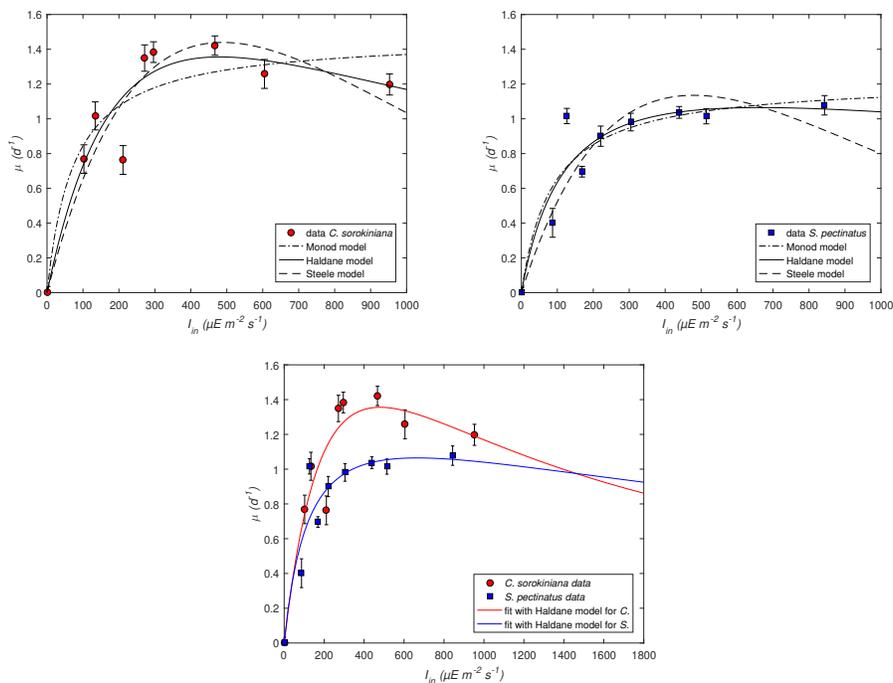


Figure 1: The growth–light relationships for *C. sorokiniana* and *S. pectinatus* compared with data obtained from batch mono-culture

334 3.2. Effects of the density of the biomass on the growth rates of *C. sorokiniana* 335 *ana* and *S. pectinatus* in batch mono-culture

336 The influence of different biomass levels on the growth of *C. sorokiniana*
 337 and *S. pectinatus* was studied. A set of batch tests was performed in micro-
 338 well plates exposed to 12 initial biomass concentrations between 0.5 and

Model $\mu(I)$	Param.	<i>C.</i>	<i>S.</i>	Stat. comp.
Monod	μ_m (d^{-1})	$1.47^* \pm 0.07$	$1.24^* \pm 0.06$	**
$\frac{\mu_m I}{K_{sI} + I}$	K_{sI} ($\mu E m^{-2} s^{-1}$)	$74^* \pm 15$	$95^* \pm 18$	ns
Andrews–Haldane	μ_m (d^{-1})	$3.15^* \pm 0.90$	$1.56^* \pm 0.32$	ns
$\frac{\mu_m I}{K_{sI} + I + \frac{I^2}{K_{iI}}}$	K_{sI} ($\mu E m^{-2} s^{-1}$)	$318^* \pm 136$	$151^* \pm 60$	ns
	K_{iI} ($\mu E m^{-2} s^{-1}$)	726 ± 382	2834 ± 2660	ns
Steele	μ_m (d^{-1})	$1.44^* \pm 0.03$	$1.13^* \pm 0.03$	**
$\mu_m \left(\frac{I}{I_m} e^{(1 - \frac{I}{I_m})} \right)$	I_m ($\mu E m^{-2} s^{-1}$)	$489^* \pm 20$	$489^* \pm 27$	ns

Table 1: Summary and comparison of the kinetic parameters used in the modeling of *C. sorokiniana* and *S. pectinatus* growth using Monod, Haldane, and Steele kinetics.

* significant regression parameter at $p < 0.05$

** significant difference between the parameters of the two species at $p < 0.05$

ns non-significant difference between the parameters of the two species at $p > 0.05$

339 35 mgC.L⁻¹. We here show the data obtained under a fixed incident light
340 ($467 \mu E m^{-2} s^{-1}$ and $439 \mu E m^{-2} s^{-1}$ for the cultures of *C. sorokiniana* and
341 *S. pectinatus*, respectively) for which both species showed maximal growth
342 rates, as described in Section 3.1.

343 Two classic models were adjusted to the experimental data: a generic
344 model of an exponential declining shape and a model inspired by the density-
345 dependent growth kinetic of Contois, both depending on the biomass density,
346 affecting negatively species specific growth rates. The models' expressions
347 and parameters are summarized in Table 2.

348 Figure 2 shows the kinetic data of *C. sorokiniana* against those of *S. pecti-*
349 *natus* as functions of the initial biomass concentrations. The growth rates
350 of the two cultures decreased with increasing biomass levels, reflecting the
351 cells' sensitivity to the availability of light becoming a limiting factor of the
352 growth under these conditions. A similar trend in declining growth in dense
353 algal culture has been reported for *Scenedesmus sp.* and *Chlorella sp.* due
354 to attenuation of the light [40]. Moreover, previous studies reported that the
355 growth of micro-algae *Chlorella sp.* was low under insufficient or excessive
356 light intensities ([45], [46]), which is also confirmed by our results. Table
357 2 shows that there is a significant difference between the species' specific
358 growth rates, as stated in Section 3.1.

359 The change in the species' growth performances with the culture density
 360 suggests that at non-inhibiting incident light intensities, *C. sorokiniana*
 361 growth is more efficient than *S. pectinatus* at low biomass levels ($< 5\text{mg.L}^{-1}$).
 362 At intermediate levels of biomass (between 5 and 30 mg.L^{-1}), the growth of
 363 both species was similar. However, under higher biomass densities, *S. pecti-*
 364 *natus* grew more rapidly than *C. sorokiniana* (as shown in Figure 2).

365 These observations suggest that in the case of poly-culture, *S. pectinatus*
 366 may perform well at high biomass densities despite the relatively low growth
 367 rates usually observed, because this species can out-compete light-limited
 368 species under low light. However, *C. sorokiniana* may perform better under
 369 clear waters and compete more effectively at moderate light conditions but
 370 may lose its advantage as the culture density increases over time. Conse-
 371 quently, the biomass level within a culture is a key factor that can explain
 372 the predominance of one species over another when growing together under
 373 non-inhibiting light conditions.

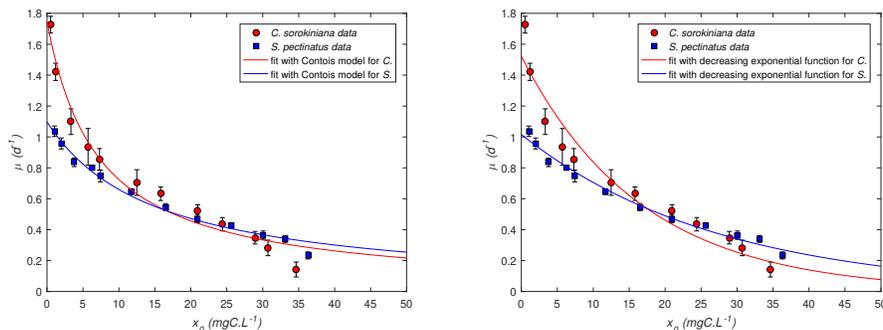


Figure 2: The effect of the initial biomass density x_0 on both *C. sorokiniana* and *S. pectinatus* specific growth rates μ using the Contois kinetics or a decreasing exponential function

374 3.3. Modeling the light attenuation within cultures

375 Light attenuation had significant effects on micro-algae growth. For a one-
 376 sided illuminated photo-bioreactor with a fixed light intensity I_{in} , the photo-
 377 synthetically active light is a maximum near the liquid boundary in front
 378 of the light supply and decreases on passing through the water column at a
 379 distance z from the light source. In addition to the effect of the depth, and the
 380 reflection and refraction at the interfaces boundaries, the absorption of the
 381 biomass when it is at high concentrations can induce light limitation within

Model $\mu(x)$	Param.	C.	S.	Stat. comp.
Exponential declining shape	a	$1.52^* \pm 0.03$	$1.02^* \pm 0.009$	**
$ae^{(-bx)}$	b	$0.06^* \pm 0.002$	$0.036^* \pm 0.001$	**
Contois [47]	$A = \mu_m$	$1.75^* \pm 0.03$	$1.1^* \pm 0.01$	**
$\frac{A}{1+Bx}$	$B = K/I$	$0.14^* \pm 0.01$	$0.07^* \pm 0.002$	**

Table 2: Summary and comparison of the kinetic parameters used in the modeling of *C. sorokiniana* and *S. pectinatus* growth depending on biomass density.

* significant regression parameter at $p < 0.05$

** significant difference between the parameters of the two species at $p < 0.05$

382 a well-mixed photo-bioreactor. Under well-mixed conditions, we assumed
383 that the individual cells are not stationed exclusively in the light or dark
384 zones of the culture but exposed, on average, to the same light intensity
385 that affects the average micro-algal growth rate. We found that the biomass
386 altered/shaped light intensity I_{attx} can be described by

$$I_{attx}(I_{in}, x) = \alpha I_{in} \frac{x}{x + K_{hsx}}; \quad (1)$$

387 where K_{hsx} is the half-saturation constant of the biomass concentration x
388 (biomass unit) and α (%) is the percentage of the maximum effective light
389 available for the growth of the micro-algae. This model was validated in
390 well-mixed batch reactors (flasks of 40 mL) illuminated at several initial
391 light intensities I_{in} for both studied strains using cultures at different stages
392 of growth. The light irradiance profiles were determined by plotting the light
393 irradiance measured at the centres of the flask reactors against the biomass
394 concentrations (measured by OD and then converted to mgC.L^{-1}). As
395 shown in Figure 3, the higher is I_{in} , the greater is I_{attx} . The light curve
396 tends towards the irradiance value αI_{in} measured at the centre of the reactor
397 when filled with only the culture medium. The shape of the obtained graphs
398 appears to be similar to that of the Monod function and was then used to
399 describe the light attenuation phenomenon.

400 We defined the total light attenuation I_{att} within a photo-bioreactor as
401 the summation of the light attenuation by biomass I_{attx} (including both ab-
402 sorption and scattering) and the light modified by the reactor and its liquid
403 content $I_{att0} = I_{in}(1 - \alpha)$, as summarized in the following expression:

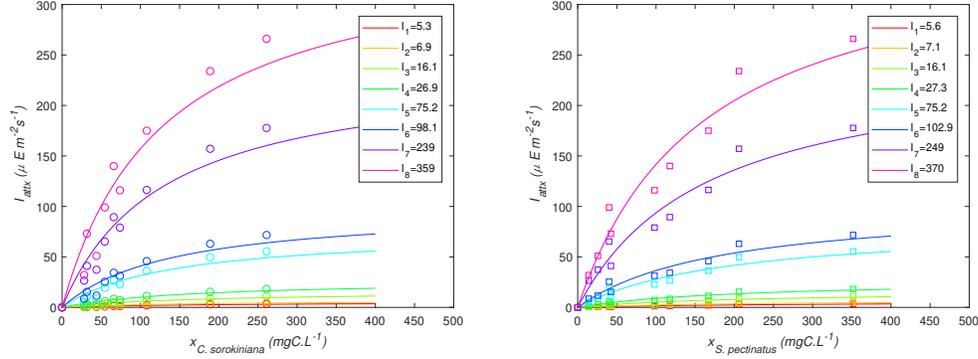


Figure 3: Simulation of the attenuated light model I_{attx} when compared to data taken on flask monocultures of *C. sorokiniana* and *S. pectinatus* at different stages of growth and different biomass concentrations

$$I_{att}(I_{in}, x) = I_{att0} + I_{attx} = I_{in} \left(1 - \alpha \left(1 - \frac{x}{x + K_{hsx}} \right) \right) \quad (2)$$

404 The parameter α can be interpreted as a characteristic of the photo-
 405 bioreactor. This parameter may be estimated with an experimental test
 406 carried out with the culture device filled with the culture medium before
 407 inoculation. Consequently, the contribution of the reactor and its liquid
 408 content to the attenuation of I_{in} can be given by the absorbed light $I_{in} - I_{out}$
 409 (both measured perpendicularly to the light source on either side of the
 410 reactor) divided by I_{in} . Then, $\beta = 1 - \alpha$ represents the percentage of the
 411 light unavailable for algal growth, and depends on the wall and depth of
 412 the reactor, the transparency of the culture medium, and also the geometry
 413 and material of the reactor (such as the reflection and refraction of the light
 414 through the walls and at the interface with the medium, which may differ).

415 For all tested values of I_{in} , the model (1) fits well the measured data for
 416 both strains (Figure 3) with different values of K_{hsx} (ANOVA test $P=0.0082 < 0.05$;
 417 $K_{hsx}=155 \pm 25 \text{ mgC.L}^{-1}$ for *C. sorokiniana* and $K_{hsx}=201 \pm 33 \text{ mgC.L}^{-1}$ for
 418 *S. pectinatus*). This suggests that *C. sorokiniana* can attenuate light more
 419 effectively than *S. pectinatus*.

420 3.4. Coupling the photo-inhibition and photo-limitation effects in micro-algal 421 growth kinetics

422 Based on the previous results, we suppose that the micro-algae growth
 423 is affected by both photo-inhibition and photo-limitation, suggesting that a

424 good kinetic model would depend on I_{in} and x . Thus we looked for one model
 425 which can represent all the experimental data, by trying to find a function
 426 that could relate μ to I_{att} . Although the curve shapes of the growth rates
 427 plotted against I_{att} resemble the classical Monod-, Haldane-, or Steele-type
 428 functions (see the experimental data for μ in Figures 4 and 5 for *C. sorokiniana*
 429 and *S. pectinatus*, respectively), there were no unique sets of parameters
 430 that could explain all the experimental data sets. However, one can com-
 431 pute the correlations between the individual parameters identified from one
 432 experiment to another.

433 The most remarkable correlation observed for any tested type of kinetics
 434 was between μ_m and the tested x condition, when taking μ_m as a decreasing
 435 function of x . Thus, we propose the following expression:

$$\mu_m(x) = \bar{\mu}\alpha\left(1 - \frac{x}{x + K_{hsx}}\right) = \bar{\mu}\left(\alpha - \frac{I_{attx}}{I_{in}}\right) \quad (3)$$

436 where $\bar{\mu}$ is the maximal value of the species' specific growth rate.

437 We built the following kinetic model using (2) and (3)

$$\mu(I_{in}, x) = \mu_m(x) \frac{I_{att}(I_{in}, x)}{K_{sI_{att}} + I_{att}(I_{in}, x)} \left(1 - \frac{I_{att}(I_{in}, x)}{I_0}\right) \quad (4)$$

438 with $K_{sI_{att}}$ the half-saturation constant of attenuated light ($\mu\text{E m}^{-2}\text{s}^{-1}$) and
 439 I_0 the light intensity ($\mu\text{E m}^{-2}\text{s}^{-1}$) for which μ takes the value of 0 for any
 440 large enough value of x (when $I_{in} = \frac{I_0}{1 - \alpha(1 - \frac{x}{x + K_{hsx}})}$).

441 As shown in Figure 4 and Figure 5, the model (4) allows describing both
 442 the light inhibition effect and the light attenuation effect, and applies to a
 443 broad range of incident light intensities ($0\text{--}1000 \mu\text{E m}^{-2}\text{s}^{-1}$) and biomass
 444 densities ($0\text{--}35 \text{ mgC.L}^{-1}$). The model parameters were identified for each
 445 species and are presented in Table 3. All the estimated parameters show
 446 that there are significant differences between the species, except for α . We
 447 recall that α is a characteristic parameter of the reactor that reflects the
 448 contribution of the culture device in the attenuation of I_{in} . Then, it is
 449 suggested that this parameter is probably the same in the microwell plates
 450 and the maximum effective light available for micro-algae growth always
 451 equals αI_{in} . For the maximal value of the species' specific growth rate, the
 452 greater $\bar{\mu}$, estimated for *C. sorokiniana*, shows its ability to grow faster than
 453 *S. pectinatus* when growing conditions are favorable, as suggested in Sections
 454 3.1 and 3.2. Moreover, the greater I_0 , found for *S. pectinatus*, demonstrates

455 its strongest resistance to high light intensities, which supports our previous
456 results in Section 3.1. We note that the half-saturation constants K_{sIatt} for
457 the two species were also different. Similarly, the significant difference of
458 K_{hsx} between the two species reflects different responses to the attenuation
459 effect, as stated above (see Section 3.3). However, we notice that the value
460 of K_{sat} identified for microwell plate cultures was not of the same order of
461 magnitude as that for flask cultures. This may be explained by the spatial
462 heterogeneity effect related to mixing. In fact, the cells initially suspended
463 in the small volume of few micro-litres (250 μL) in the microwell plates
464 tend to accumulate at the bottom of the well, which is not the case for the
465 instantaneous measurement of the light in a perfectly mixed flasks (40 mL).
466 This may result in a significant density inhibitory effect on μ_m (following
467 equation (3)) caused by the high spatial heterogeneity, thereby explaining
468 the small value obtained for K_{hsx} in micro-plates. Then, K_{hsx} will increase
469 with the degree of mixing. In addition, we observed higher values of K_{hsx} for
470 *S. pectinatus* compared to *C. sorokiniana*, whether in microplate or flasks.
471 This is probably due to the differences in shapes and sizes of the cells between
472 the two species. Having the same biomass concentration, a small number of
473 voluminous cells (such as *S. pectinatus*) would attenuate less light than small
474 cells at a much larger number (as is the case for *C. sorokiniana*). Therefore,
475 K_{hsx} would be related to both the species' bio-volumes and the mixing.

Param.	<i>C.</i>	<i>S.</i>	Stat. comp.
α	0.75* \pm 0.03	0.81* \pm 0.03	ns
$\bar{\mu}$ (d^{-1})	3.25* \pm 0.20	1.75* \pm 0.08	**
K_{sIatt} ($\mu E m^{-2} s^{-1}$)	52* \pm 6	26* \pm 3	**
K_{hsx} ($mgC.L^{-1}$)	9.89* \pm 0.31	17.07* \pm 0.53	**
I_0 ($\mu E m^{-2} s^{-1}$)	1068* \pm 41	1836* \pm 168	**

Table 3: Summary and comparison of the new model parameters used in the modeling of *C. sorokiniana* and *S. pectinatus* growth depending on both incident light intensity and biomass density (in microwell plates).

* significant regression parameter at $p < 0.05$

** significant difference between the parameters of the two species at $p < 0.05$

ns non-significant difference between the parameters of the two species at $p > 0.05$

476 The new kinetic function (4) highlights the interactions between the inci-
477 dent light and the population density. It reflects the effect of the availability
478 of light, and describes the different phenomena that may occur during algal

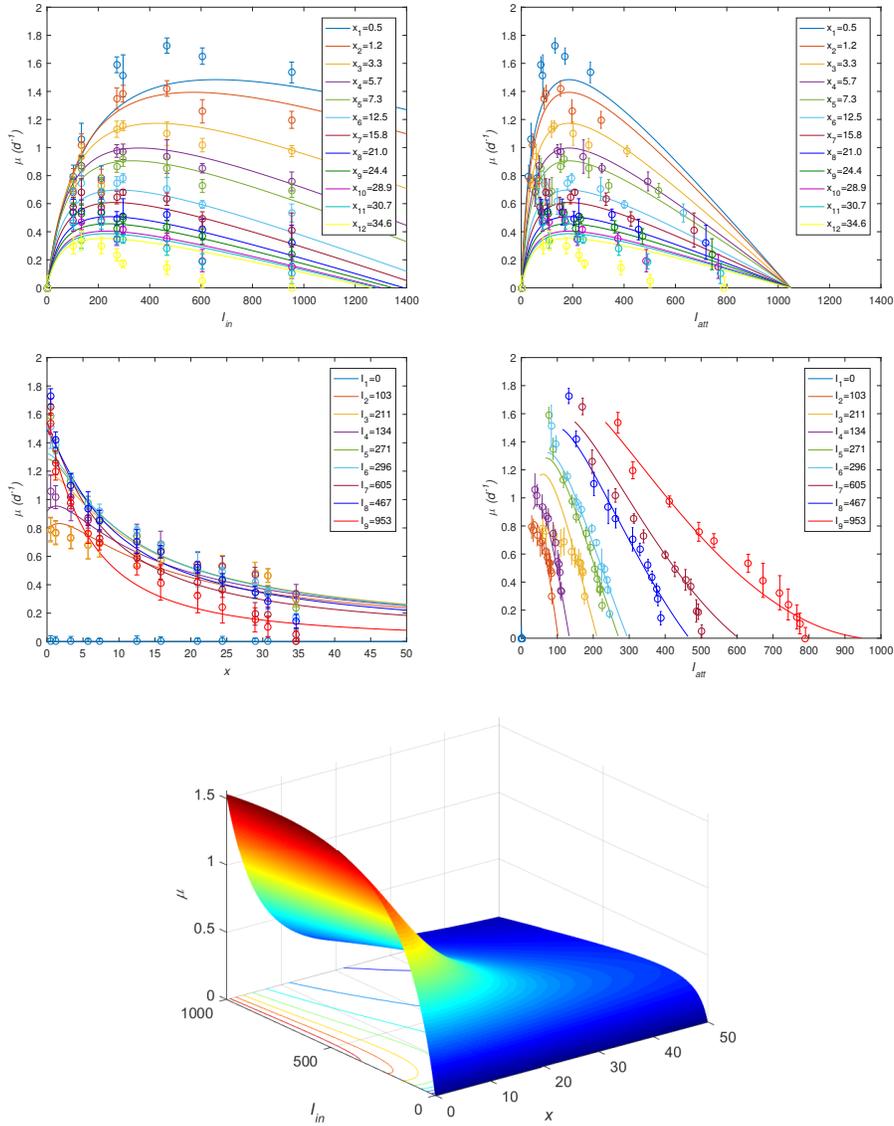


Figure 4: The effect of incident light intensities I_{in} ($\mu Em^{-2} s^{-1}$) and the biomass densities x ($mgC.L^{-1}$) on the growth of *C. sorokiniana*

479 cultivation, such as photo-inhibition (following exposure to high light intensi-
 480 ties at low biomass concentrations), photo-limitation (under insufficient light
 481 conditions) or, more likely, photo-acclimation, which occurs in a time scale

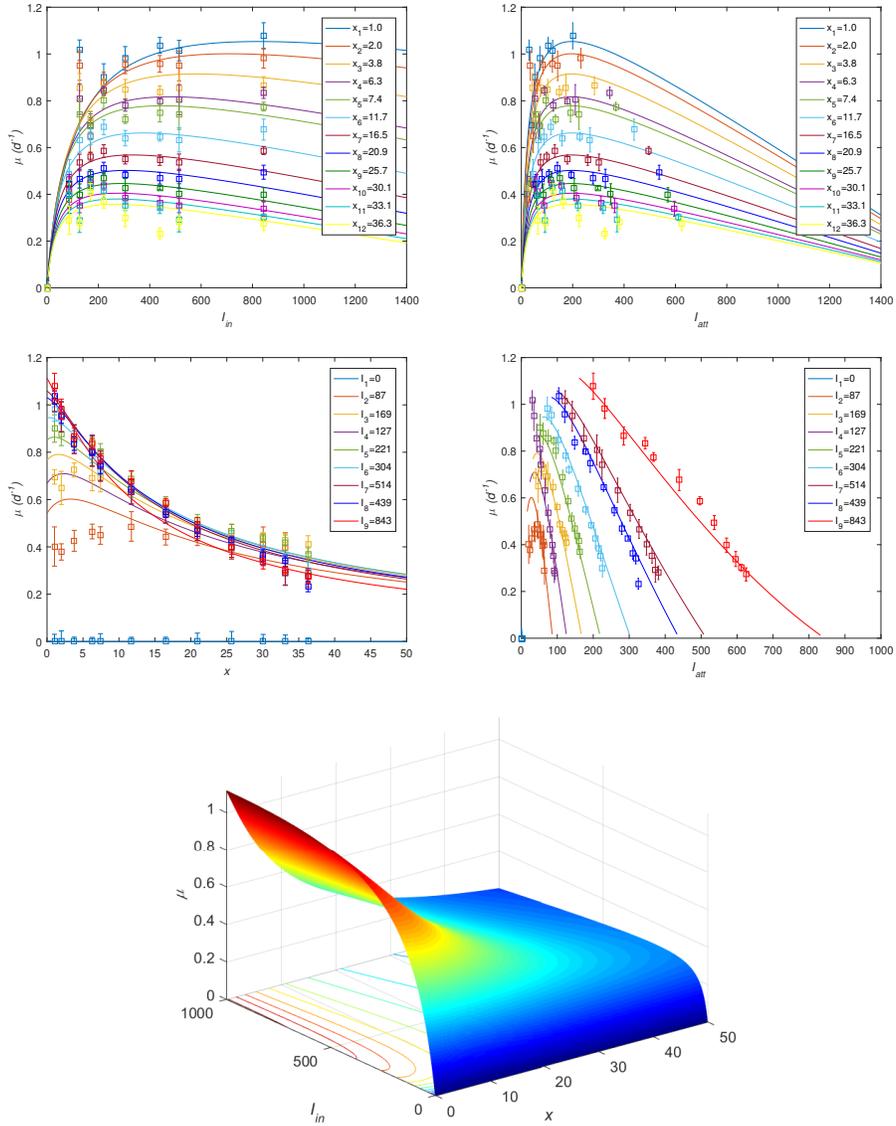


Figure 5: The effect of incident light intensities I_{in} ($\mu Em^{-2} s^{-1}$) and the biomass densities x ($mgC.L^{-1}$) on the growth of *S. pectinatus*

482 of days (given that the model was established based on experimental data
 483 obtained on the scale of three days). This model requires a limited number
 484 of strain-specific parameters and allows comparisons of species growth per-

485 performances and optimization of the operational parameters of algal cultures.
 486 Its simplicity makes it a valuable tool that can be integrated into any type
 487 of photo-bioreactor geometry and can apply to a microwell plate (as shown
 488 here) or to Erlenmeyer flasks (as shown below). Such a growth function also
 489 offers a tool for simulating and predicting the potential production rate in
 490 poly-culture of different species in algal mass culture systems under light
 491 fluctuations (as further explored).

492 *3.5. Model validation and extension for poly-culture predictions in continu-*
 493 *ous mode photo-bioreactors*

494 We considered the data of species growing in mono-culture (in an Erlen-
 495 meyer photo-bioreactor exposed to continual I_{in}) to compare them to the
 496 data generated by the growth kinetics derived by the proposed growth func-
 497 tion (4) for growth limited by light. We first need the usual mass balanced
 498 model to describe the time evolution of the biomass concentration [48] using
 499 the proposed kinetic function $\mu(\cdot)$ from (4) for a fixed intensity of incident
 500 light I_{in} .

$$\dot{x} = (\mu(I_{in}, x) - D)x \quad (5)$$

501 The simulations of this model for each species grown in mono-culture are
 502 presented in Figure 6 against the data of biomass obtained under continuous
 503 mode cultures, using the same coefficients represented in Table 3 except for
 504 α and K_{hsx} . These two parameters are likely to vary considerably depending
 505 on the operating conditions. Then, they were both re-identified. α which
 506 depends on the culture device, was found to be equal to 0.4, while K_{hsx} , ap-
 507 parently sensitive with regard to mixing, was equal to 21 and 61 mgC.L⁻¹ for
 508 *C. sorokiniana* and *S. pectinatus*, respectively. The parameters $\bar{\mu}$, $K_{sI_{att}}$ and
 509 I_0 , considered as characteristic parameters of the species, were held constant.

510 In the second step, we sought to validate our growth function (4) on
 511 another data set. So, we used the experimental data of biomass tracked over
 512 time in the same Erlenmeyer photo-bioreactor but inoculated with a culture
 513 of a mixture of the species. This required an extension of the model to multi-
 514 species growths. The same parameters (applied in mono-culture) were used
 515 to simulate the following system of differential equations (6), considering both
 516 species growing together (let us underline that these kinetics are coupled here,
 517 but differently than the usual interaction terms, such as in the generalized
 518 Lotka–Volterra models), and taking into account the nonlinear functions μ_i .

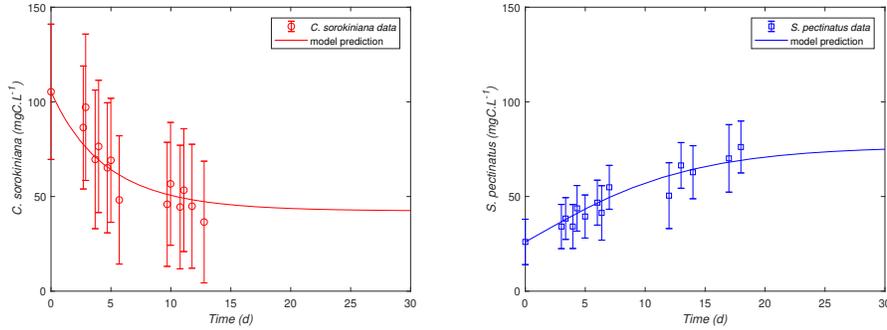


Figure 6: Simulation of chemostat model using the new kinetic function compared to biomass data (from OD and cell count converted to $mgC.L^{-1}$) tracked in mono-cultures of *C. sorokiniana* and *S. pectinatus* under similar conditions of incident light intensity $I_{in} = 165 \pm 5$ and dilution rate $D = 0.25 \pm 0.02$ (in Erlenmeyer photo-bioreactors)

$$\begin{cases} \dot{x}_1 = (\mu_1(I_{in}, x_1 + x_2) - D)x_1 \\ \dot{x}_2 = (\mu_2(I_{in}, x_1 + x_2) - D)x_2 \end{cases} \quad (6)$$

519 The superimposition of the data on the predictions of model (6) in Figure
 520 7 allows a satisfactory description of the dynamics of the different concentra-
 521 tions of the two species, which validates the proposed model (6) in co-culture.

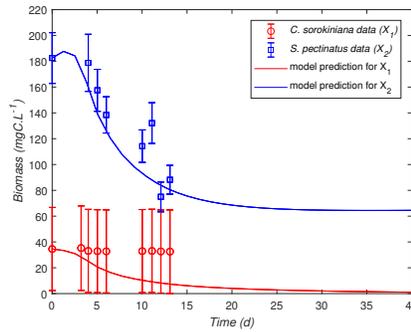


Figure 7: Validation of the chemostat model using the new kinetic function on biomass data (from cell count converted to $mgC.L^{-1}$) tracked in species assemblage of *C. sorokiniana* and *S. pectinatus* under a fixed incident light intensity $I_{in} = 165 \pm 5$ and a piece-wise constant dilution rate $D = 0.11$ for $t < 2.74$; then $D = 0.25 \pm 0.02$ (in Erlenmeyer photo-bioreactors)

522 3.6. Prediction of the possible outcomes of the competition for light availabil-
523 ity in continuous mode photo-bioreactors under periodic light conditions

524 While competitive exclusion is more likely to occur at the laboratory
525 scale [49], the coexistence of species is observed in both natural and artificial
526 ecosystems and may play an important role in the resilience of cultivation sys-
527 tems or even in reducing the risk of extinction under particular conditions
528 [50, 51]. In this section, we discuss three possible outcomes of the multi-
529 species model, including the possibility of species coexistence, through theo-
530 retical prediction under periodic light, as a more realistic growth condition.
531 The different situations were corroborated by some simulations (presented
532 in Figure 8) obtained using the growth characteristics previously validated
533 for *C. sorokiniana* (species 1) and *S. pectinatus* (species 2) in an Erlenmeyer
534 photo-bioreactor (see Section 3.5), but under different operating conditions
535 (as stated in Table 4).

536 We recall that the specific growth rate of each species in the multi-species
537 model (6) is influenced by the total biomass density of both species contribut-
538 ing together to attenuate the available light within the photo-bioreactor.
539 Thus, the expressions for μ_1 and μ_2 in the assemblage depend on the total
540 biomass $x_1 + x_2$ instead of x_i only, leading to the model (6) that couples
541 the growth of each species. However, for constant incident light I_{in} , one can
542 easily see that coexistence at steady state is generically impossible, because
543 it would need to have very particular values of D such that the graphs of
544 μ_1 and μ_2 intersect with a common value exactly equal to D . Indeed, this
545 model satisfies the Competitive Exclusion Principle in a very similar way to
546 the classical multi-species chemostat model, for which the common resource
547 is a limiting substrate [48] (to be replaced here by the total biomass). Con-
548 sidering the biomass at steady state in mono-culture, denoted by x_i^* , which
549 satisfies the equation $\mu_i(x_i^*) = D$ (recall that μ_i is a decreasing function pro-
550 viding a unique positive solution when $D < \mu_i(0)$, and no positive solution
551 for $D \geq \mu_i(0)$), the winner of the competition is the species with the largest
552 x_i^* . This competitive exclusion was observed experimentally under constant
553 light in Section 3.5 (see Figure 7). We note that *S. pectinatus* won the com-
554 petition, reaching a value at steady state x_2^* which verifies $\mu(x_2^*) = D$, as
555 predicted by the competitive exclusion principle.

556 Let us now consider a periodic $I_{in}(\cdot)$ as a time-varying function. The
557 competitive exclusion principle no longer applies. When the input nutrient
558 fluctuates with time (with variable input concentration or variable input flow
559 rate), it is known that species coexistence is possible [52, 53, 54]. Let us see

560 that a similar phenomenon can occur when the incident light is fluctuating
 561 (even though the dependency in I_{in} is non-linear, unlike D).

562 We consider first mono-cultures under periodic light:

$$\dot{x}_i = (\mu_i(I_{in}(t), x_i) - D)x_i, \quad i = 1, 2 \quad (7)$$

563 It is easy to see that when the condition

$$C_i := \int_t^{t+T} (\mu_i(I_{in}(\tau), 0) - D)d\tau > 0$$

564 is fulfilled, the washout solution $x_i = 0$ is repelling, and that the scalar
 565 dynamics (7) admits an unique positive periodic solution $\tilde{x}_i(\cdot)$ (see, for ex-
 566 ample, the simulations in Figures 8(A), 8(C), 8(E) and 8G, corresponding to
 567 mono-cultures obtained under different operating conditions given in Table
 568 4), which is asymptotically attractive for any initial condition with $x_i(0) > 0$
 569 (as μ_i is decreasing with respect to x_i).

570 Now, consider the co-culture under periodic light:

$$\begin{cases} \dot{x}_1 = (\mu_1(I_{in}(t), x_1 + x_2) - D)x_1 \\ \dot{x}_2 = (\mu_2(I_{in}(t), x_1 + x_2) - D)x_2 \end{cases} \quad (8)$$

571 the asymptotic solutions with the absence of one species, which are $(\tilde{x}_1(\cdot), 0)$
 572 and $(0, \tilde{x}_2(\cdot))$, are both repelling for the dynamics (8) when conditions

$$C_{21} := \int_t^{t+T} (\mu_2(I_{in}(\tau), \tilde{x}_1(\tau)) - D)d\tau > 0 \quad (9)$$

573

$$C_{12} := \int_t^{t+T} (\mu_1(I_{in}(\tau), \tilde{x}_2(\tau)) - D)d\tau > 0 \quad (10)$$

574 are both fulfilled. Let us give some insight into these quantities. When
 575 a single species i settles, its concentrations converge with time towards an
 576 unique periodic solution $\tilde{x}_i(\cdot)$ as previously recalled. When this periodic
 577 solution is reached (or almost reached), consider at time t an invasion by the
 578 other species $j \neq i$ with a small concentration $x_j(t)$. From equations (8), one
 579 can see that the time derivative \dot{x}_j is small when x_j is small. Therefore, if the
 580 invasion is such that $x_j(t)$ is sufficiently small, x_j remains small during the
 581 time period T , and consequently, the concentration x_i is very little impacted
 582 while x_j remains small. Then, one can assume that $x_i(\cdot)$ remains close to the

583 periodic solution $\tilde{x}_i(\cdot)$ on the time interval $[t, t + T]$, and the dynamics of x_j
 584 can be approximated by

$$\dot{x}_j(\tau) = (\mu_j(I_{in}(\tau), \tilde{x}_i(\tau)) - D)x_j(\tau), \quad \tau \in [t, t + T]$$

585 whose solution is given by the expression

$$x_j(t + T) = x_j(t) \exp\left(\int_t^{t+T} (\mu_j(I_{in}(\tau), \tilde{x}_i(\tau)) - D)d\tau\right) = x_j(t) \exp(C_{ji})$$

586 If $C_{ji} < 0$, one has thus $x_j(t + T) < x_j(t)$ and one can iterate this calculation
 587 on the next time interval $[t + T, t + 2T]$ and so on. We conclude that the
 588 species j cannot grow. In contrast, when $C_{ji} > 0$, species j grows, and its
 589 concentration cannot remain close to 0. We conclude that species j settles
 590 in the ecosystem. If the symmetric condition $C_{ij} > 0$ is fulfilled for species
 591 i , we conclude that neither concentration x_i , x_j can approach 0. Then,
 592 there is necessarily the coexistence of species. This case was illustrated by
 593 the example E_1 in Table 4 and the corresponding simulation presented in
 594 Figure 8 (B). We thus demonstrate that coexistence is possible, although not
 595 systematic.

596 The values of C_{21} and C_{12} can be interpreted as the ‘specific invasion
 597 speed over one period’ and their sign reflects the ability of one species to
 598 invade the ecosystem (with small initial density) when the other species is
 599 already settled in the periodic regime. Moreover, the magnitudes of C_{12} and
 600 C_{21} provide information about the reactivity of the ecosystem to an invasion:
 601 the more positive C_{ji} is, the faster is the invasion by the species j , and
 602 conversely the more negative C_{ji} is, the faster species j is eradicated by the
 603 system.

604 Let us underline the necessity to have the growth functions μ_i alternating
 605 its dominance depending on the light to have these two conditions verified.
 606 If not, one has for instance $\mu_1(I_{in}(t) - x) > \mu_2(I_{in}(t) - x)$ for any t and any
 607 $x > 0$, which implies

$$\int_t^{t+T} (\mu_1(I_{in}(\tau), \tilde{x}_1(\tau)) - D)d\tau = 0 > \int_t^{t+T} (\mu_2(I_{in}(\tau), \tilde{x}_1(\tau)) - D)d\tau = C_{21}$$

608 and then $C_{21} > 0$ cannot be fulfilled and species 2 cannot invade the system
 609 when species 1 is present (see example E_2 in Table 4 and the corresponding
 610 simulation in Figure 8 (D)). Conversely, species 1 cannot invade a culture

Ex.	Parameter				Test condition				Outcome	Fig.
	T (d)	D (d^{-1})	I_{min} ($\mu\text{E m}^{-2}\text{s}^{-1}$)	I_{max}	C_1	C_2	C_{21}	C_{12}		
E1	8	0.427	340	1510	0.68	0.16	0.001	0.02	coexist.	8(A,B)
E2	12	0.45	52	280	2.43	0.13	-0.62	1.97	1 wins	8(C,D)
E3	10	0.25	400	900	4.57	2.60	0.89	-1.05	2 wins	8(E,F)
E4	1	0.16	0	700	0.19	0.09	0.03	-0.05	2 wins	8(G,H)

Table 4: Some illustrative examples of the possible outcomes of the multispecies model using different operational conditions of dilution rate D and periodic illumination (taking I_{min} and I_{max} over the period T). The test conditions C_1 and C_2 are computed on species 1 (*C. sorokiniana*) and species 2 (*S. pectinatus*) in monoculture, while C_{12} and C_{21} are given for assemblages.

611 with species 2 when $C_{12} < 0$ (see examples E_3 and E_4 in Table 4 and the
612 corresponding simulations for the assemblages in Figures 8 (F) and 8 (H)).

613 These results show that the coexistence or exclusion of one species or the
614 other are possible and depend on the operating conditions $I_{in}(\cdot)$ and D . We
615 note that the chosen values of the parameters in examples E_1 , E_2 and E_3
616 in Table 4 are easy to implement at the laboratory scale for operating in-
617 door photo-bioreactors. We propose that the model can also apply to outdoor
618 cultures. For such a case, we considered in E_4 (in Table 4) more appropri-
619 ate conditions for the light for simulating the light–dark cycles, which may
620 be given with an illumination fluctuating between $I_{min} = 0$ and an average
621 value I_{max} (at about $700 \mu\text{E m}^{-2}\text{s}^{-1}$ [36, 55]) over a period T of one day.
622 Under these latter conditions, the model (8) theoretically predicted a com-
623 petitive exclusion in favor of *S. pectinatus*, as shown in Figure 8(H). The
624 predominance of *Scenedesmus* predicted by the simulation reproduces the
625 experimental observations of several studies [56, 57, 36, 37, 55].

626 One can notice in Figure 8 that during the transients, the densities of both
627 species increase (or decrease) simultaneously before one of them reaches a
628 stage from which it declines. This is qualitatively different from the transients
629 of the exclusion obtained with the classical model of competition on an abiotic
630 resource (such as limited substrate) described by the usual growth functions
631 [54, 48, 37]. This feature could be a matter for future research to discriminate
632 which kind of exclusion (due to light or substrate) is dominant, and when.

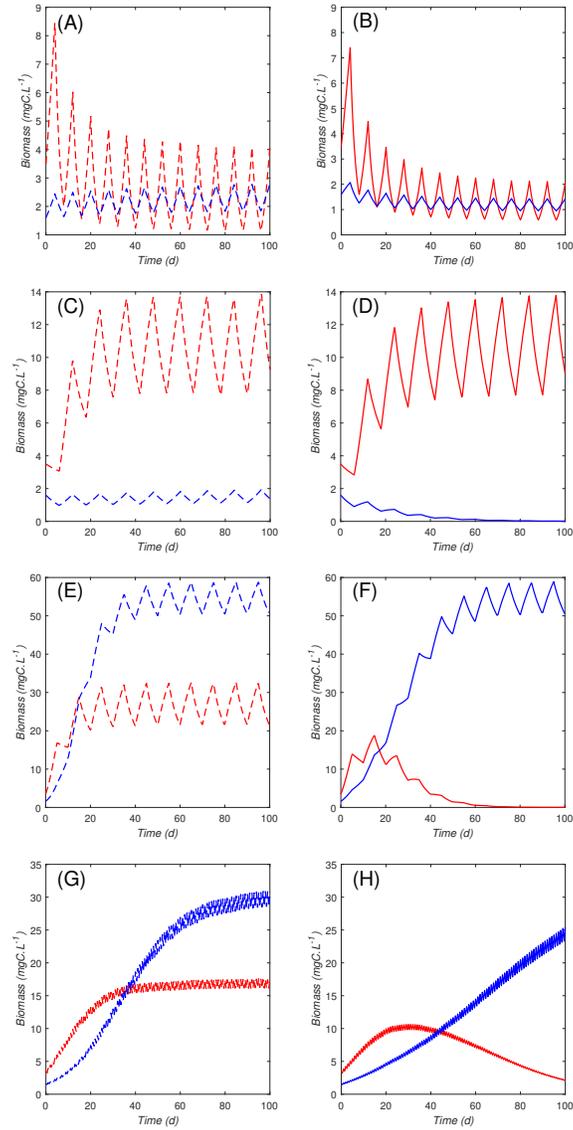


Figure 8: Some illustrative simulations obtained in continuous mode photo-bioreactors exposed to periodic illumination in mono-culture (first column A, C, E and G) and in assemblage (second column B, D, F and H) for species 1 (*C. sorokiniana*) (in red) and species 2 (*S. pectinatus*) (in blue) according to the examples of operational conditions stated in Table 4

633 4. Conclusion

634 Light inhibition and attenuation appear to have significant effects on the
635 growth of micro-algae. The presented results show that the reduction of
636 species growth rates was mainly attributed to high cell densities, which re-
637 duce the penetration of light into the culture, but may protect cells from
638 photo-inhibition when exposed to high light levels. *S. pectinatus* demon-
639 strated better performances than *C. sorokiniana* at insufficient or excessive
640 light availability, while *C. sorokiniana* was able to achieve faster growth un-
641 der non-inhibiting light levels in clearer waters. We have shown that the
642 newly developed kinetic model, depending on both the incident light and
643 the biomass densities through the attenuated light model, can describe the
644 simultaneous effects of photo-inhibition and photo-limitation and predict the
645 biomass production in mono-culture and species assemblage. The use of mod-
646 eling and experimental approaches allows the characterization of the species
647 and the proper model identification for estimating the biomass production
648 under different operating conditions and assessing the optimal operational
649 parameters, which is of great benefit for the evaluation of a small or large
650 scale algal mass culture, particularly in poly-culture systems.

651 This new model offers various possible future applications, such as its use
652 for automatic monitoring of the instantaneous biomass concentration through
653 light measurements within the reactor, or even the effective optimization of
654 the incident light intensities, in addition to possible control (playing with
655 the light availability in indoor cultures or shadowing in outdoor culture).
656 The control of the incident light, the dilution rate, and the choice of initial
657 biomass for the optimization of productivity in poly-culture will need further
658 investigation.

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