

1 **Dynamic modelling of green algae cultivation in a photobioreactor for sustainable**
2 **biodiesel production**

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19 **Running title: Kinetic Modelling of Algal Biodiesel Production**

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Abstract

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Biodiesel produced from microalgae has been extensively studied due to its potentially outstanding advantages over traditional transportation fuels. In order to facilitate its industrialisation and improve the process profitability, it is vital to construct highly accurate models capable of predicting the complex behaviour of the investigated biosystem for process optimisation and control, which forms the current research goal. Three original contributions are described in this paper. Firstly, a dynamic model is constructed to simulate the complicated effect of light intensity, nutrient supply and light attenuation on both biomass growth and biolipid production. Secondly, chlorophyll fluorescence, an instantly measurable variable and indicator of photosynthetic activity, is embedded into the model to monitor and update model accuracy especially for the purpose of future process optimal control, and its correlation between intracellular nitrogen content is quantified, which to the best of our knowledge has never been addressed so far. Thirdly, a thorough experimental verification is conducted under different scenarios including both continuous illumination and light/dark cycle conditions to testify the model predictive capability particularly for long-term operation, and it is concluded that the current model is characterised by a high level of predictive capability. Based on the model, the optimal light intensity for algal biomass growth and lipid synthesis is estimated. This work, therefore, paves the way to forward future process design and real-time optimisation.

Keywords: biodiesel production; dynamic modelling; chlorophyll fluorescence; model predictive capability; light/dark cycle; nitrogen limiting.

44 **Introduction**

45 Microalgae are considered to be a promising feedstock for the production of renewable
46 biofuels which would contribute to meeting the ever-increasing global demand for energy
47 (Mata, Martins, and Caetano 2010). Compared to plant-based biofuel precursors, including
48 both food crops such as corn or sugarcane and non-food plants, *e.g.* jatropha, microalgae
49 display superior growth rates and shorter generation time, can utilise wastewater as a nutrient
50 source, do not compete for arable land with food crops, and are expected to have low
51 environmental impacts *etc.* (Sheehan et al. 1998; Schenk et al. 2008; Brennan and Owende
52 2010). Furthermore, the metabolic reaction networks in microalgae have been extensively
53 researched over the last decades, resulting in the successful identification and genetic
54 modification of a variety of microalgae species capable of synthesising different sustainable
55 biofuels including biodiesel, bioethanol, biohydrogen, bioisoprene, and biohydrocarbons
56 (Adesanya et al. 2014; Matos et al. 2013; Eroglu and Melis 2010).

57 Amongst these, a major focus has been placed on the production of algal lipid, which can
58 contribute up to 70 wt% of dry cell weight and is readily converted into biodiesel, already
59 used as a fossil fuel substitute (Brennan and Owende 2010; Wen et al. 2016). To facilitate the
60 commercialisation of this process, comprehensive studies have been conducted with the aim
61 to enhance both the biomass growth rate and biolipid productivity. For example, the effects of
62 modifying key operating conditions *e.g.* light intensity, temperature, pH and nutrient supply,
63 have been thoroughly investigated with the conclusion that biolipid synthesis can be
64 remarkably stimulated under nitrogen limiting conditions (Converti et al. 2009; Scott et al.
65 2010). Different biomass cultivation methods (*e.g.* autotrophic, heterotrophic and
66 mixotrophic) have been widely explored and their respective advantages and limitations have
67 been discussed in detail (S. J. Yoo, Kim, and Lee 2014; Wang et al. 2016; Purkayastha et al.
68 2017). In addition, recent studies conducted life cycle assessments and process scale-up

69 experiments which revealed that the biolipid content in large scale processes is often reduced
70 by over 60% (rarely reaching 30 wt%), significantly decreasing the process profitability and
71 rendering it economically unviable at present (Wen et al. 2016; Purkayastha et al. 2017; Park
72 and Li 2015).

73 To resolve this severe challenge, it is necessary to implement rigorous process control and
74 optimisation regimes, which can achieve dense biomass concentrations as well as high
75 biolipid productivities simultaneously (Bernard, Mairet, and Chachuat 2015; del Rio-
76 Chanona, Zhang, and Vassiliadis 2016). To this end it is crucial to construct highly accurate
77 models capable of simulating the dynamic behaviour of the underlying bioprocess and to
78 identify easily measurable state variables. Meanwhile, developing robust dynamic
79 optimisation algorithms for highly nonlinear biosystems is also regarded an important
80 prerequisite for this work to be accomplished successfully. So far, different models have been
81 developed to simulate the effect of key operating conditions on both microalgae growth and
82 biofuel production (Adesanya et al. 2014; Dongda Zhang et al. 2015; Cakmak et al. 2012).
83 Specific variables including pH, dissolved oxygen, chlorophyll fluorescence (Y(II)) or light
84 irradiation have been used to monitor the process performance and design control schemes (C.
85 Yoo et al. 2015; Keymer, Pratt, and Lant 2013; S. J. Yoo, Kim, and Lee 2014; Bernard,
86 Mairet, and Chachuat 2015). We recently proposed a state-of-the-art real-time optimisation
87 strategy for long-term bioprocess optimisation which incorporates parameter re-estimation
88 into economic model predictive control and was demonstrated to be highly effective
89 compared to traditional offline optimisation methods (del Rio-Chanona, Zhang, and
90 Vassiliadis 2016).

91 Despite these achievements, it is important to note that the employed models must also have a
92 high predictive capability so that they can accurately determine the optimal operating
93 conditions for biomass growth and biofuel synthesis. In order to effectively implement real-

94 time process optimisation, it is necessary to embed variables that can be measured instantly
95 (e.g. Y(II)), allowing continuous calibration of the model and minimising deviations from
96 experimental or operational data. However, much less effort has been devoted to these areas
97 to date. For instance, whilst mathematical models specific to biolipid synthesis have been
98 proposed in the past, their predictive capabilities have rarely been evaluated. In some cases, it
99 was necessary to use different sets of parameter values when applying the models to simulate
100 different experiments, even if the experiments were conducted under similar conditions.
101 Meanwhile, instantly measurable variables that can reflect biomass growth and biolipid
102 synthesis activities, particularly chlorophyll fluorescence (Y(II)) which is widely used to
103 represent the photosynthetic activity of microalgae cells, have never been included in these
104 models. Thus, these limitations prevent their further application for process optimisation.
105 Consequently, to close this gap, the present study aims to construct a highly accurate dynamic
106 model suitable for the real-time control and optimisation of a long-term microalgal biodiesel
107 production process. In particular, the instantly measurable variable, chlorophyll fluorescence,
108 will be embedded into the current model, and the model predictive capability will be verified
109 under different operating conditions. Furthermore, the model simulation results will be used
110 to identify the primary limiting factors for biodiesel production.

111 **2. Materials and modelling methodology**

112 **2.1 Experiment setup**

113 *Nannochloropsis oceanica* IMET1 was provided by Dr. Jian Xu from the Qingdao Institute of
114 Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, and maintained in
115 seawater supplemented with modified F/2 medium. The 500 mL bubble column bioreactor (5
116 cm diameter) was supplied with 100 mL/min of filtered air, supplemented with 2% (v/v) CO₂,
117 as described by Pan et al. (2016). The pre-culture was prepared in the photobioreactor (PBR)
118 with sufficient nutrients and under continuous illumination with white fluorescent light (140

119 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for 4 days, followed by inoculation into new PBRs at an initial biomass
120 concentration of $\sim 0.18 \text{ mg mL}^{-1}$. In total, four batch experiments were carried out with
121 different initial nitrate concentrations and light intensities as shown in Table I, and a constant
122 ambient temperature of $25 \pm 1 \text{ }^\circ\text{C}$.

123 **2.2 Analytical methods**

124 Biomass concentrations (mg mL^{-1}) were determined as described previously (Zhu and Lee
125 1997). Cells were harvested by centrifugation and pellets were washed twice with 0.5 M
126 NH_4HCO_3 and dried at $60 \text{ }^\circ\text{C}$ to constant weight. Nitrate concentrations in the medium were
127 measured using a UV/VIS spectrophotometer with a pre-drawn standard curve for the nitrate-
128 related light absorption (Chi et al. 2016). The fluorescence parameter Y(II), which reflects the
129 effective photosynthesis capacity of photosynthesis system II, was calculated using a
130 chlorophyll fluorometer (Water-PAM WALZ, Germany) based on the method described by
131 Yao et al. (2012). Light intensity was measured on an Optometer P9710 with a
132 photosynthetically active radiation detector (Gigahertz Optik Corporation, Germany).
133 Biomass intracellular nitrogen content was determined using an elemental analyser (Vario EL
134 cube, Elementar Analysensysteme GmbH Germany). The yields of the transesterified fatty
135 acid methyl esters (FAMES) were quantified by gas chromatography using the internal
136 standard glyceryl triheptadecanoate (Liu et al. 2015).

137 **2.2 Model construction**

138 In order to construct an accurate dynamic model, an understanding of the underlying kinetic
139 mechanisms is essential. The synthesis of the biolipid fraction is mediated by the intracellular
140 nitrogen concentration (nitrogen quota) and sufficiency in light intensity, and its production is
141 dependent on the biomass concentration which is affected by the nitrate concentration in the
142 culture (Li et al. 2008; Scott et al. 2010). Therefore, all of these variables should be included.

143 Furthermore, *chlorophyll fluorescence* ($Y(II)$) is also embedded into the dynamic model due
144 to its importance for future real-time process monitor and control.

145 **2.2.1 Algal biomass growth**

146 Eq. 1 is commonly used to estimate the algal biomass growth rate. The first term on the right
147 represents biomass growth, whilst the second term represents biomass decay. Previous
148 research concluded that the specific biomass growth rate (μ_0) depends on both light intensity
149 and nitrate concentration, whilst the biomass decay rate (μ_d) is a function of temperature
150 only (D. Zhang et al. 2015). As the temperature was fixed in this study, μ_d reduces to a
151 constant. To model the effect of nitrate concentration on biomass growth, the Droop model
152 was Eq. 2, as it is predominantly applied under nutrient limiting conditions (del Rio-Chanona
153 et al. 2017; Adesanya et al. 2014).

$$154 \quad \frac{dX}{dt} = \mu_0 \cdot X - \mu_d \cdot X \quad (1)$$

$$155 \quad \mu_0 = \mu_m(I) \cdot \left(1 - \frac{k_q}{q}\right) \quad (2)$$

156 where X is biomass concentration (g L^{-1}), μ_0 is specific growth rate (h^{-1}), μ_d is specific decay
157 rate (h^{-1}), $\mu_m(I)$ denotes the effect of light intensity (I) on biomass growth, k_q is minimum
158 nitrogen quota (mg g^{-1}), and q is nitrogen quota (mg g^{-1}).

159 **2.2.2 Nitrate consumption**

160 Whilst nitrates are essential for biomass growth, high nitrate concentrations can severely
161 suppress the accumulation of biolipid (Mata, Martins, and Caetano 2010). Consequently, the
162 nitrate consumption rate was modelled using an adopted form of the the Monod model (Eq.
163 3), commonly used to simulate nutrient consumption (Dongda Zhang et al. 2016; Fouchard et
164 al. 2009).

$$165 \quad \frac{dN}{dt} = -\mu_N \cdot \frac{N}{N + K_N} \cdot X \quad (3)$$

166 where N is culture nitrate concentration (mg L^{-1}), K_N is half-velocity coefficient (mg L^{-1}), and
 167 u_N is maximum specific nitrate uptake rate ($\text{mg g}^{-1} \text{h}^{-1}$).

168 **2.2.3 Nitrogen quota**

169 Intracellular nitrogen content, also termed nitrogen quota, is one of the key variables and
 170 predominantly determines both biomass growth and biolipid synthesis. Previous research has
 171 concluded that higher nitrogen quota can result in a higher biomass growth rates, whilst lower
 172 nitrogen quota can stimulate the synthesis of biolipid (Sharma, Schuhmann, and Schenk
 173 2012). As nitrate is only consumed by algal cells, based on a mass balance, the nitrate
 174 consumption rate must be equal to the accumulation of intracellular nitrogen (Eq. 4). This
 175 equation can then be transformed to Eq. 5, to calculate the accumulation rate of nitrogen
 176 quota.

$$177 \frac{d(X \cdot q)}{dt} = -\frac{dN}{dt} = \mu_N \cdot \frac{N}{N + K_N} \cdot X \quad (4)$$

$$178 \frac{dq}{dt} = \mu_N \cdot \frac{N}{N + K_N} - \mu_m(I) \cdot \left(1 - \frac{k_q}{q}\right) \cdot q \quad (5)$$

179 **2.2.4 Fatty acid methyl ester (FAME) production**

180 The kinetic mechanism of biolipid (fatty acids) synthesis has been illustrated in recent works
 181 (Gnansounou and Raman 2016). It is demonstrated that all the CO_2 fixed through
 182 photosynthesis is converted to sugar initially. Then, a portion of sugar is converted into fatty
 183 acids, and this reaction rate is proportional to the nitrogen quota. Meanwhile, fatty acids can
 184 also be consumed to produce functional carbon molecules (*e.g.* membranes), of which the
 185 reaction rate increases with the increasing nitrate uptake rate. Inspired from this mechanism,
 186 Eq. 6 is constructed in this study to simulate total fatty acid production ($X \cdot S$). This equation
 187 is then transformed to Eq. 7 to simulate the accumulation rate of intracellular fatty acid (S).

$$188 \frac{d(X \cdot S)}{dt} = (\theta' \cdot q) \cdot \mu_m(I) \cdot \left(1 - \frac{k_q}{q}\right) \cdot X - \gamma' \cdot \mu_N \cdot \frac{N}{N + K_N} \cdot X \quad (6)$$

$$189 \quad \frac{dS}{dt} = \mu_m(I) \cdot (\theta' \cdot q - S) \cdot \left(1 - \frac{k_q}{q}\right) - \gamma' \cdot \mu_N \cdot \frac{N}{N + K_N} \quad (7)$$

190 where θ' and γ' are kinetic constants for biolipid synthesis and consumption, respectively,
 191 and S is intracellular fatty acids content (wt%).

192 Moreover, since the current study aims to simulate biodiesel production, FAMES rather than
 193 fatty acids are chosen for model construction. The benefit of modelling FAME production
 194 instead of lipid content in cells is that FAME is the final product – *biodiesel*. Therefore, in the
 195 current study, FAME production after lipid transesterification was measured directly and
 196 described in Section 2.2. Because FAME comes from biolipid through transesterification, its
 197 synthesis rate can be approximated by modifying Eq. 7 into Eq. 8 (Gnansounou and Raman
 198 2016).

$$199 \quad \frac{df}{dt} = \mu_m(I) \cdot (\theta \cdot q - \varepsilon \cdot f) \cdot \left(1 - \frac{k_q}{q}\right) - \gamma \cdot \mu_N \cdot \frac{N}{N + K_N} \quad (8)$$

200 where θ , γ , and ε are modified parameters taking into account the complex effects of lipid
 201 synthesis and transesterification conversion, and f is FAME yield (wt%).

202 **2.2.5 Chlorophyll fluorescence (Y(II))**

203 Chlorophyll fluorescence (Y(II)) is used to estimate the efficiency of the microalgal
 204 Photosystem II (PSII), as it represents the ability of microalgae to use absorbed quanta and
 205 gives a realistic reflection of the physiological state of microalgae cells. Whilst the biolipid
 206 synthesis is not directly linked to the status of Y(II), it provides a precise reflection in the
 207 change of nitrogen quota and is highly consistent with biolipid accumulation. Therefore, it is
 208 vital to embed Y(II) into the current model, so that it can be used to monitor model deviations
 209 and calibrate the model for future real-time process optimisation using instant chlorophyll
 210 fluorescence measurements.

211 To date, no research has quantified the correlation between Y(II) and nitrogen quota.
 212 Nonetheless, it was found that an exponential relationship between photosynthesis rate and

213 chlorophyll content exists in algae (Béchet, Shilton, and Guieysse 2013). As Y(II) represents
 214 the efficiency of PSII which is directly related to photosynthesis rate and the nitrogen quota
 215 can have a notable effect on the intracellular chlorophyll content (Li et al. 2008), it is
 216 proposed to use Eq. 9 to simulate the change of Y(II) with respect to nitrogen quota.

$$217 \quad Y(\text{II}) = \frac{\exp[\tau \cdot q]}{\exp[\tau \cdot q] + \delta} + \varphi \quad (9)$$

218 where τ , δ and φ are kinetic parameters in this equation.

219 **2.2.6 Simulation of light intensity**

220 The effect of light intensity on biomass growth has been well studied and is commonly
 221 simulated by the Aiba model (Eq. 10) (Béchet, Shilton, and Guieysse 2013). Furthermore,
 222 photons in a PBR are either absorbed by microalgal biomass or scattered by bubbles, causing
 223 the local light intensity to diminish along the light transmission direction in the reactor. To
 224 take this light attenuation into account, a modified form of the Lambert-Beer law has been
 225 proposed and has been widely utilised in recent studies, as shown in Eq. 11 (Dongda Zhang et
 226 al. 2016).

$$227 \quad u_m(I) = u_M \cdot \frac{I}{I + k_s + \frac{I^2}{k_i}} \quad (10)$$

$$228 \quad I(z) = I_0 \cdot \exp[-(\alpha \cdot X + \beta) \cdot z] \quad (11)$$

229 where u_M is maximum specific growth rate (h^{-1}), I is light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$), k_s and k_i
 230 are light saturation term ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and light inhibition term ($\mu\text{mol m}^{-2} \text{s}^{-1}$) for cell growth,
 231 respectively, I_0 is incident light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$), α is cell absorption coefficient ($\text{m}^2 \text{g}^{-1}$),
 232 β is bubble scattering coefficient (m^{-1}), z is the distance from light source (m), and L is the
 233 width of the PBR (m).

234 However, when adding light attenuation into the current model, the model complexity is
 235 significantly increased due to the presence of both spatial and temporal dimensions. Thus, in

236 order to simplify the model complexity for future use in control and optimisation, the 10-step
 237 Trapezoidal rule (Eq. 12) is applied to eliminate the spatial dimension and the reactor is
 238 assumed to be a column with a square cross section. The area of the square is equal to that of
 239 the original circle, giving a width of 4.4 cm. This simplification was demonstrated to yield
 240 high accuracy in recent studies (del Rio-Chanona et al. 2017; del Rio-Chanona, Zhang, et al.
 241 2015)

$$242 \quad u_m(I) = \frac{u_M}{20} \cdot \sum_{n=1}^9 \left(\frac{I_{i=0}}{I_{i=0} + k_s + \frac{I_{i=0}^2}{k_i}} + 2 \cdot \frac{I_{i=\frac{n \cdot L}{10}}}{I_{i=\frac{n \cdot L}{10}} + k_s + \frac{I_{i=\frac{n \cdot L}{10}}^2}{k_i}} + \frac{I_{i=L}}{I_{i=L} + k_s + \frac{I_{i=L}^2}{k_i}} \right) \quad (12)$$

243 where I_i is local light intensity at a distance of $i = \frac{n \cdot L}{10}$ from the reactor exposure surface.

244 **2.3 Parameter estimation**

245 Due to the high complexity of the dynamic model, it is vital to employ a robust parameter
 246 estimation method to identify the model parameter values in this study. Unreliable values can
 247 severely prevent the applicability of the dynamic model for real-time bioprocess control and
 248 optimisation. Therefore, a nonlinear least-squares optimisation problem is formulated. A high
 249 order orthogonal collocation method over finite elements in time is chosen to discretise and
 250 transform the current model into a nonlinear programming problem (NLP). The optimal
 251 values of model parameters are estimated by solving the NLP using IPOPT, the state-of-the-
 252 art interior point nonlinear optimisation solver (Wächter and Biegler 2005). This parameter
 253 estimation procedure is programmed in the Python optimisation environment Pyomo (Hart et
 254 al. 2012). Once the parameters are estimated, the model's simulation results are calculated in
 255 Mathematica[®] 10.

256 **2.4 Sensitivity analysis**

257 Sensitivity analysis was developed to estimate the effect of model parameters on the system
 258 performance, and has been widely used to identify the most influential parameters that affect

259 the process dynamics (Fouchard et al. 2009). A normalised sensitivity (S_i) is presented in
260 Eq. 13. It measures the proportional change of the system's performance (c_i , e.g. FAME
261 production) with respect to the proportional change of a model parameter (p_j). A positive
262 sensitivity indicates that increasing p_j can result in an increase in c_i , whilst a negative
263 sensitivity suggests that increasing p_j will diminish the system's performance. Moreover, a
264 greater sensitivity also shows a more significant effect of the parameter on the system. In this
265 research, sensitivity analysis is carried out in Mathematica[®] 10 to explore the effects of
266 model kinetic parameters on both cells growth and FAME production.

$$267 \quad S_i = \frac{\partial c_i / c_i}{\partial p_i / p_i} \quad (13)$$

268 **3 Results and discussion**

269 **3.1 Results of parameter estimation**

270 The values of the model parameters are listed in Table II, and the model fitting results are
271 presented in Fig. 1 and Fig. 2. These figures show that our model provides a good
272 representation of the underlying dynamic behaviour of the biosystem, indicating that the
273 kinetic hypothesis and simplifications used in this study are valid. From Table II, it is
274 observed that both the specific biomass decay rate and the bubble scattering coefficient equal
275 0, suggesting that they have negligible effects on the system. This can be attributed to the fact
276 that in all the conducted experiments, biomass concentration kept increasing until the end of
277 the study, disguising the effect of cell decay. Similarly, light attenuation is predominantly
278 governed by cell absorption, and therefore the imperceptible impact of bubble scattering on
279 light transmission is estimated to be 0.

280 The fluctuation of nitrogen quota and FAME yield at the beginning of the experiments in the
281 two figures (Fig. 1(c), (d), and Fig. 2(c), (d)) can be attributed to the consumption of
282 intracellularly stored nitrogen for cell growth and its subsequent replenishment through

283 nitrate uptake. At the start of the culture, the nitrogen quota (Fig. 1(c) and Fig. 2(c)) decreases
284 significantly as it is consumed by algae biomass growth. This is followed the rapid uptake
285 and conversion of culture nitrate into intracellularly stored nitrogen, resulting in the nitrogen
286 quota to start to increase after a short period. However, as the total amount of nitrate in the
287 culture is limited, once it is exhausted, the nitrogen quota keeps decreasing with the
288 increasing algae biomass concentration. Similarly, as biolipid synthesis (hence FAME
289 production) is severely inhibited under high nitrogen quota conditions (Mata, Martins, and
290 Caetano 2010), the yield of FAME (Fig. 1(d) and Fig. 2(d)) increases when nitrogen quota
291 drops, and decreases when nitrogen quota increases.

292 Confidence intervals are computed through the parameter estimation procedure. The
293 covariance matrix for the estimated parameters is approximated by the inverse of the reduced
294 Hessian at the optimal solution. Confidence intervals are then obtained from the trace of this
295 approximated covariance matrix following standard procedures (del Rio-Chanona,
296 Dechatiwongse, et al. 2015). However, as a result of the high nonlinearity and complexity of
297 modelling metabolic kinetics, the assumption of computing the confidence intervals from the
298 above framework may not hold. For this reason, the confidence intervals presented in Table II
299 must be understood as theoretical values.

300 **3.2 Sensitivity analysis results**

301 The results from the sensitivity analysis are presented in Fig. 3. These show that for all state
302 variables, a critical point exists around the 32nd hour before and after which the sensitivity of
303 variables with respect to the parameters changes dramatically. Based on the model, this point
304 is estimated to be the time when the nitrate in the culture has been fully consumed. Thus, the
305 sharp change of the parameter sensitivities indicates a rapid shift of metabolic reaction
306 mechanisms inside biomass for its growth and synthesis of metabolites. Biomass
307 concentration (Fig. 3(a)) and nitrogen quota (Fig. 3(c)) are found to be sensitive to the same

308 parameters, in particular u_M , k_q , k_s , and α , and their sensitivities are in a mild range of ± 0.8 ,
309 suggesting a greater stability compared to nitrate concentration and FAME production.

310 Initially, whilst the nitrogen quota can be replenished by culture nitrate, both biomass
311 concentration and nitrogen quota are predominantly governed by the light intensity (k_s , α)
312 and the maximum specific growth rate (u_M). As u_M represents the maximum growth rate that
313 cells can reach under nutrient sufficient conditions, it is expected that higher values of u_M
314 correspond to faster cell growth, resulting in denser biomass concentrations. Similarly, a
315 reduced algal biomass absorption coefficient (α) results in an increase in the local light
316 intensity experienced by the cells, whilst a lower light saturation term (k_s) suggests that the
317 light capacity for cells to grow is lower. Hence, biomass shows positive sensitivity to u_M and
318 negative sensitivities to α and k_s . As higher biomass growth rates correspond to higher
319 nitrogen quota consumption rates, it is unsurprising that the sensitivity of nitrogen quota with
320 respect to these parameters is opposite in sign to that of biomass concentration.

321 Furthermore, nitrogen quota is highly sensitive to u_N which reflects how rapidly the cells can
322 absorb nitrate and replenish their intracellular nitrogen storage. Consequently, once the
323 culture nitrate is exhausted, the sensitivity of this term drops significantly and its effect on the
324 nitrogen quota becomes negligible. At this point, the primary limiting factor for biomass
325 growth is switched to the availability of intracellularly stored nitrogen. Therefore, k_q
326 commences to show greater effects on both biomass concentration and nitrogen quota, whilst
327 the sensitivity of u_M , k_s , and α keeps decreasing. As k_q represents the minimum nitrogen
328 quota required by the cells to survive, a higher value of k_q suggests that cells can consume
329 less of the stored nitrogen for growth and need a higher nitrogen quota for maintenance. Thus,
330 it shows negative sensitivity to biomass concentration but positive sensitivity to nitrogen
331 quota. In addition, Fig. 3(a) shows that the biomass concentration is insensitive to the light

332 inhibition term (k_i), suggesting that the current experiments were not subject to
333 photoinhibition.

334 The sensitivity analysis reveals that both nitrate concentration and FAME production are
335 highly sensitive to the model parameters (up to ± 6.0), as a very small change (1%) of specific
336 parameters, *e.g.* θ , γ , and u_N , can cause a dramatic change (up to 6%) on these variables.
337 However, it is notable that the high sensitivities of these variables are attributed to different
338 causes. The nitrate consumption rate only depends on a few parameters (u_N and K_N , Eq. 5),
339 hence, the nitrate concentration is not substantially affected by microalgal metabolic reaction
340 kinetics. This is also proven by its weak sensitivity (except u_N which directly represents the
341 algal nitrate uptake rate) during the first 20 hours (shown in Fig. 3(b)) whilst nitrate is still
342 available in the culture. Subsequently, as the nitrate concentration approaches 0, its
343 sensitivity diverges sharply. However, this phenomenon is more probably caused by
344 mathematical noise (*i.e.* $\partial N/N \rightarrow \infty$ when $N \rightarrow 0$, based on the definition of sensitivity, Eq.
345 13) instead of a biological reason.

346 In contrast, the sensitivities of FAME can be attributed to its complicated synthesis
347 mechanisms. As biolipids constitute between 10% and 45% wt biomass, its production can be
348 affected by the same factors that influence biomass growth. Therefore, from Fig. 3(d) it is
349 found that the trends of the sensitivities of FAME with respect to both u_M and α are equal to
350 those for biomass concentration. In addition, as biolipid can be converted to other metabolites
351 and its consumption rate is proportional to the nitrate uptake rate, it is easy to see that u_N has
352 a negative impact on FAME production when the culture is nitrate available (shown in Fig.
353 3(d)). Moreover, based on Eq. 8, θ and γ can be considered as the reaction kinetic constants
354 for FAME synthesis and consumption, respectively. Thus, as presented in Fig. 3(d), these two
355 parameters possess the highest sensitivities to FAME production, and become particularly

356 influential when the culture nitrate concentration approaches 0 and biolipid starts to
357 accumulate.

358 Overall, the current sensitivity analysis demonstrates that the synthesis of FAME is more
359 sensitive to the underlying biochemical reaction kinetics and experimental operating
360 conditions than biomass growth or nitrogen quota accumulation,. Hence, in order to improve
361 FAME production, it is vital to implement advanced process optimisation strategies which
362 guarantee optimal cultivation conditions for FAME synthesis.

363 **3.3 Limiting factors for FAME synthesis**

364 Recent studies have concluded that light attenuation is one of the primary limiting factors for
365 biomass cultivation and bioproduct production (D. Zhang et al. 2015; Béchet, Shilton, and
366 Guieysse 2013). Similar results are obtained in the present work. Fig. 4(a) shows that over
367 the course of the cultivation an increase in biomass concentration causes the local light
368 intensity in the PBR to decrease rapidly, resulting in the majority of the reactor volume to be
369 immersed in the dark zone where cells cannot grow (local growth rate drops to 0, shown in
370 Fig. 4(b)). Both the local biomass growth rate and FAME production rate decrease with
371 increasing biomass concentration inside the light zone where algal cells can receive
372 illumination for their growth (Fig. 4(b) and Fig. 4(c)). This is caused by light attenuation and
373 lack of nitrogen quota.

374 As illustrated already in the model construction section (Eq. 7), the synthesis of biolipid
375 requires both illumination and nitrogen quota. During the initial experimental period when
376 nitrate is still available, local light intensity is the primary limiting factor for biolipid
377 synthesis. For example, at a biomass concentration of 0.7 g L^{-1} , the local biolipid synthesis
378 rate decreases along the light transmission direction, indicating that light attenuation limits its
379 production (Fig. 4(c)). However, after nitrate is consumed, the nitrogen quota decreases
380 significantly in order to maintain the rapid growth of biomass (Fig. 4(b), x-axis between 0

381 and 0.01). As biolipid synthesis rate is proportional to nitrogen quota, its synthesis rate is also
382 reduced dramatically (Fig. 4(c)) even when there is sufficient light for biomass growth (Fig.
383 4(a) and Fig. 4(b), x-axis in between 0 to 0.01). This clearly suggests that the primary
384 limiting factor for FAME production has been switched to nitrogen quota. Similarly, because
385 biomass growth is also related to nitrogen quota, the lack of nitrogen quota also causes a
386 lower cell growth rate when biomass concentration increases from 1.5 g L^{-1} to 2.5 g L^{-1} as
387 shown in Fig. 4(b).

388 Furthermore, based on the current simulation result, the effect of light intensity and nitrogen
389 quota on FAME production is presented in Fig. 4(d). This shows, that the FAME production
390 rate always increases with increasing nitrogen quota, whilst an optimal value exists for light
391 intensity as intense illumination can damage the essential proteins for algal photosynthesis
392 and carbon fixation. Based on the model, the optimal light intensity is identified to be
393 $96 \mu\text{mol m}^{-2} \text{ s}^{-1}$, falling within the range of optimal light intensities reported in other
394 publications (D. Zhang et al. 2015). In addition, attention should be paid to the fact that both
395 the local biomass growth rate and the FAME production rate shown in Fig. 4 represent
396 instantaneous values, as the location of individual algal cells change continuously as a result
397 of mixing. Hence, cells at different locations in the reactor share the same average growth
398 rate and biolipid synthesis rate over time.

399 **3.4 Model predictive capability validation**

400 To estimate the optimal operating conditions for long-term bioprocess optimisation, besides
401 accurately representing a known experiment, the model must possess great predictive
402 capability when simulating unknown processes. For this reason, the predictive capability of
403 the constructed model is investigated through two scenarios. In the first scenario, the model is
404 used to predict the dynamic performance of a continuous illumination batch experiment
405 lasting for 11 days (252 hours). In the second scenario, the model is applied to predict a

406 light/dark cycle batch experiment lasting for one week (168 hours). It is worth emphasising
407 that due to the frequent change of light intensity, the second system becomes more complex
408 and has a higher uncertainty compared to the first scenario. Both light intensity and initial
409 nitrate concentration in these two experiments are different from those used for model
410 construction. The detailed operating conditions of these experiments are listed in Table I.
411 Fig. 5 and Fig. 6 present the model prediction results. Specific to the light/dark (14h: 10h)
412 cycle experiment, biomass specific growth rate is slightly modified due to the significant
413 impact of cell respiration on biomass growth in this case. The average specific biomass
414 growth rate is assumed to be 85% of that under continuous illumination conditions
415 (Edmundson and Huesemann 2015). The figures demonstrate that the current model is
416 capable of accurately predicting the complex behaviour of long-term microalgal FAME
417 production processes under different operating conditions, which indicates its great potential
418 for future process control and optimisation applications. More importantly, as microalgae
419 based bioprocesses are generally carried out under outdoor conditions for large scale
420 production, it is impossible to provide continuous illumination for FAME production when
421 scaling up this process.

422 During future research, we will implement an online optimal control strategy which measures
423 experimental parameters (*e.g.* nutrients and biomass concentration) in real-time, whilst the
424 model is adjusted to best represent the system under consideration. Through this framework,
425 optimal inputs (*e.g.* nutrient supply) can be computed and implemented in an ongoing process
426 (*e.g.* economic model predictive control). However, for this strategy to be possible, the model
427 must be able to display solid predictive capabilities and robustness to model parameters.
428 These have been clearly shown in the work above, particularly regarding to the second
429 scenario, demonstrating its applicability for future process real-time optimisation and scale-
430 up design.

431 **Conclusions**

432 In the current research, a mathematical model was constructed to simulate the growth and
433 biodiesel production from *Nannochloropsis oceanica*. By conducting a sensitivity analysis, it
434 was found that biolipid synthesis is more sensitive to the operating parameters of the system
435 than cell growth. Therefore, in order to maintain high biomass concentrations as well as high
436 biolipid productivities in long-term processes, it is vital to precisely estimate the nitrogen
437 dosing requirements and implement advanced process optimisation strategies. This
438 emphasises the importance of constructing a highly accurate dynamic model characterised by
439 good predictive capability as presented in this study. During future work, this model will be
440 incorporated into a state-of-the-art process real-time control framework, such as economic
441 model predictive control, to optimise the operating conditions for semi-continuous (fed-batch)
442 and continuous biodiesel production processes, in particularly under light/dark cycle
443 circumstances.

444 **Acknowledgement**

445 This project is granted by EPSRC project (EP/P016650/1, P65332) and Natural Science
446 Foundation of China (No. 21576253).

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Table I: Operating conditions of current experiments

	Experiment 1	Experiment 2
Incident light intensity, $\mu\text{mol m}^{-2} \text{s}^{-1}$	80	160
Initial nitrate concentration, mg L^{-1}	35.0	24.6
Initial biomass concentration, g L^{-1}	0.18	0.17
Initial FAME yield, wt%	12.0	11.2
Initial nitrogen quota, wt%	8.0	7.9
Initial chlorophyll fluorescence	0.561	0.555
Operation time, day	11	11
	Experiment 3	Experiment 4
Incident light intensity, $\mu\text{mol m}^{-2} \text{s}^{-1}$	120	140, (light/dark (14h:10h))
Initial nitrate concentration, mg L^{-1}	46.8	15.2
Initial biomass concentration, g L^{-1}	0.18	0.18
Initial FAME yield, wt%	12.0	11.7
Initial nitrogen quota, wt%	8.0	8.2
Initial chlorophyll fluorescence	0.561	0.571
Operation time, day	11	7

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Table II: Model parameter estimation result

Parameter	Value	Parameter	Value
u_M, h^{-1}	0.359 ± 0.014	θ	6.691 ± 2.247
u_d, h^{-1}	0.0 ± 0.000	γ	$(7.53 \pm 2.25) \times 10^{-3}$
$k_q, \text{mg g}^{-1}$	1.963 ± 0.283	ε	0.010 ± 0.0004
$u_N, \text{mg g}^{-1} \text{h}^{-1}$	2.692 ± 0.641	τ	1.376 ± 0.139
$K_N, \text{mg L}^{-1}$	0.80 ± 0.029	δ	9.904 ± 3.013
$k_s, \mu\text{mol m}^{-2} \text{s}^{-1}$	91.2 ± 1.727	φ	-0.456 ± 0.011
$k_i, \mu\text{mol m}^{-2} \text{s}^{-1}$	100.0 ± 0.290	β, m^{-1}	0.0 ± 0.009
$\alpha, \text{m}^2 \text{g}^{-1}$	196.4 ± 21.6		

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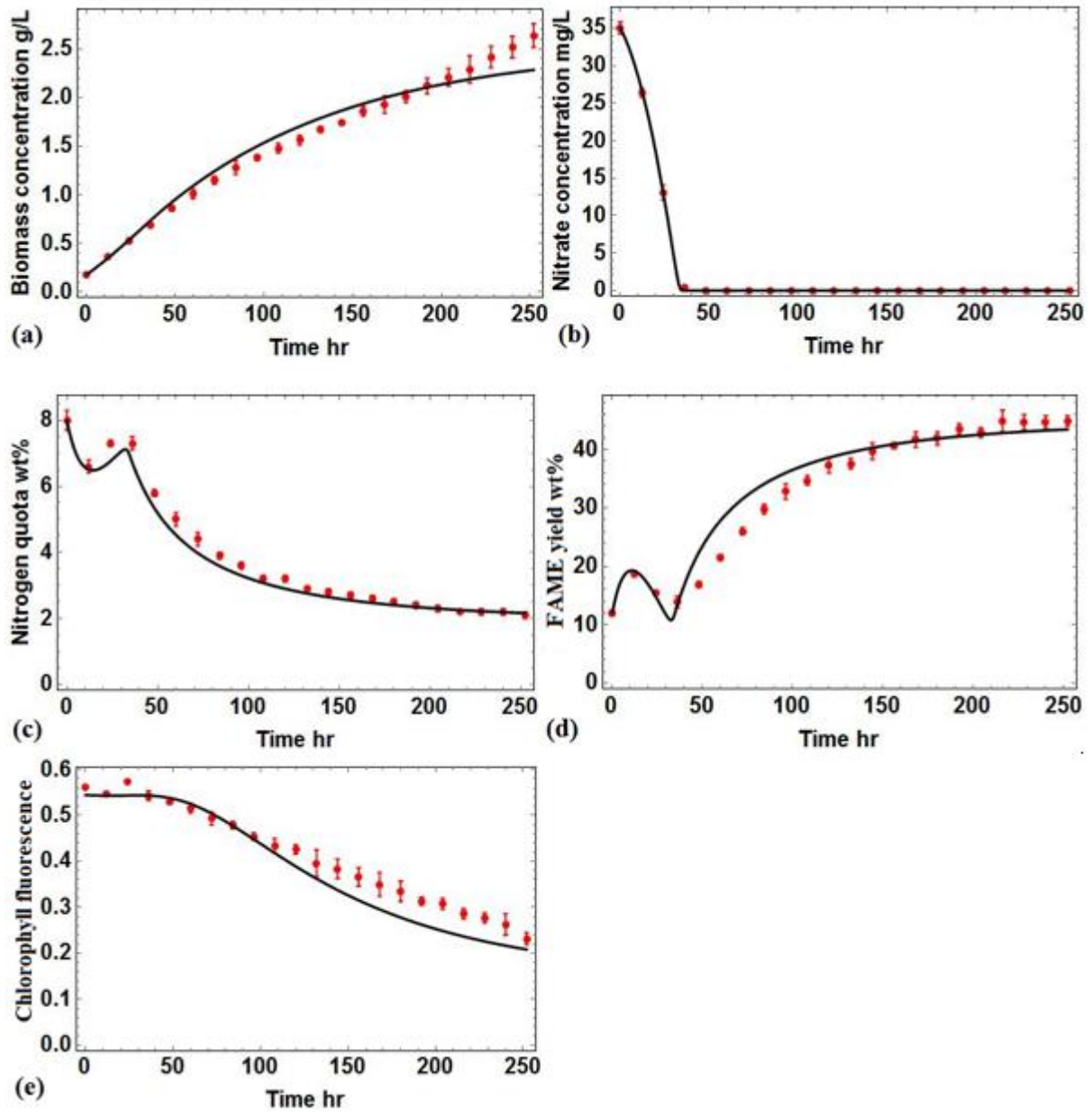
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606 Figure 1: Comparison of model simulation results and real experimental data (Experiment 1).

607 Line: model simulation results, point: real experimental data. (a): biomass concentration; (b):

608 nitrate concentration; (c): nitrogen quota; (d): FAME yield; (e): chlorophyll fluorescence.



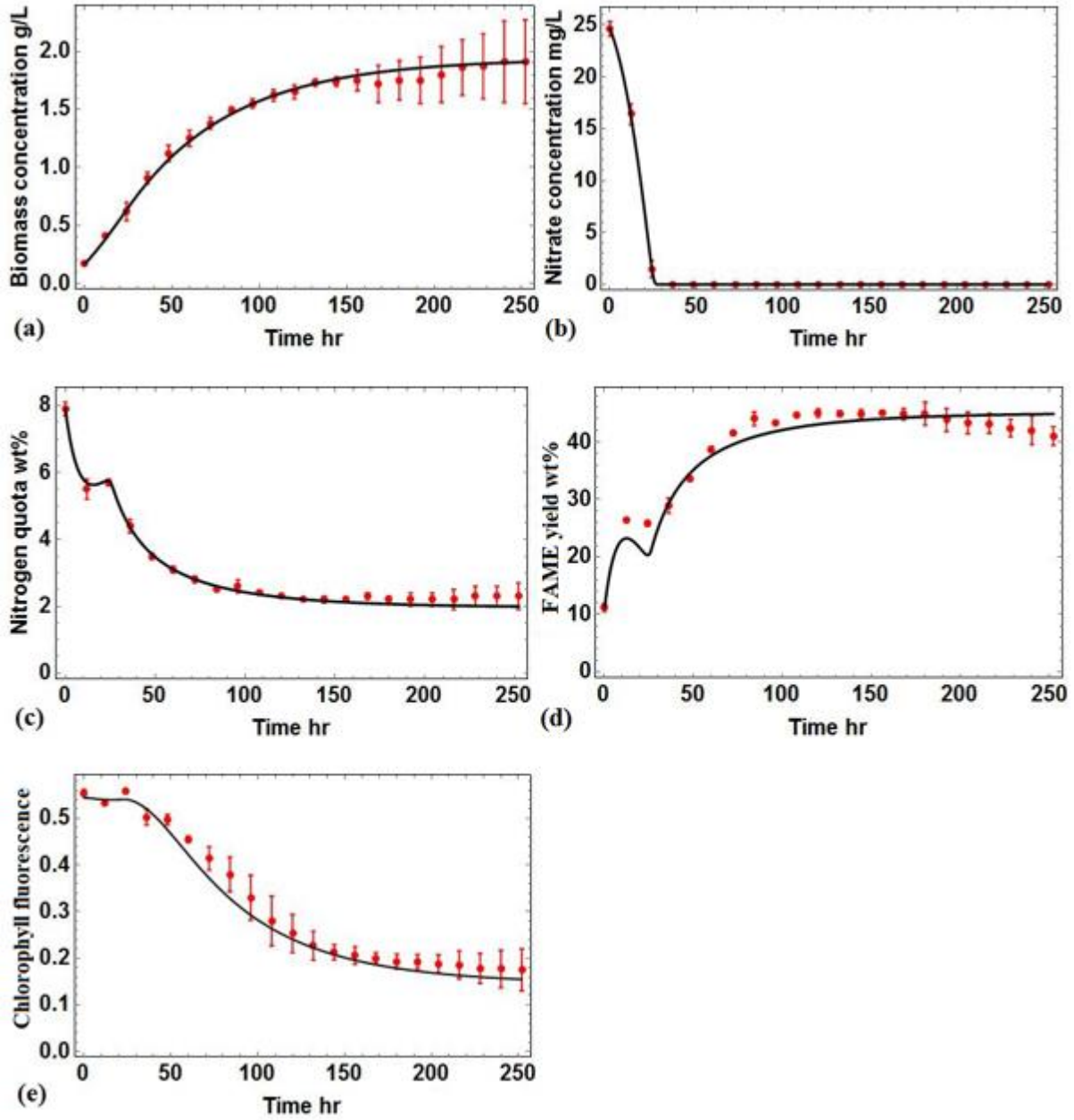
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611 Figure 2: Comparison of model simulation results and real experimental data (Experiment 2).

612 Line: model simulation results, point: real experimental data. (a): biomass concentration; (b):

613 nitrate concentration; (c): nitrogen quota; (d): FAME yield; (e): chlorophyll fluorescence.



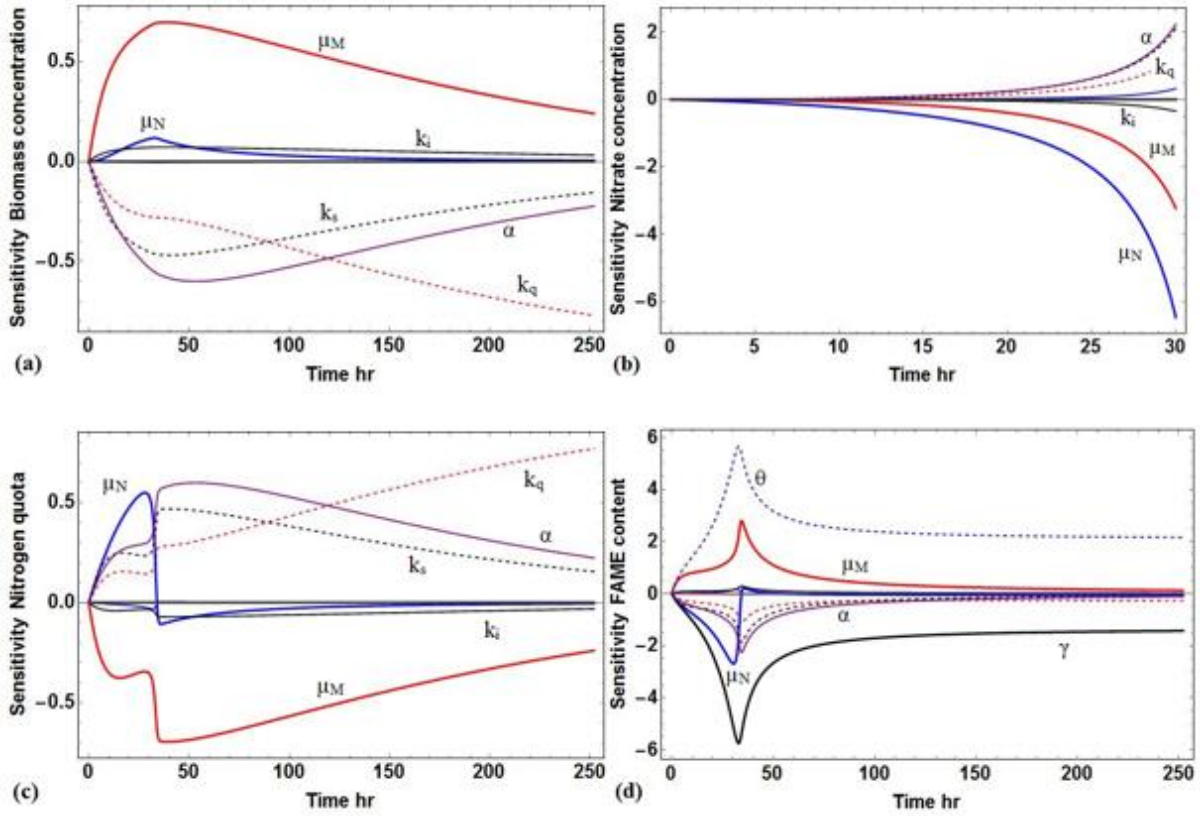
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618 Figure 3: Sensitivity analysis of different variables on model parameters. (a): sensitivity of
 619 biomass concentration; (b): sensitivity of nitrate concentration; (c): sensitivity of nitrogen
 620 quota; (d): sensitivity of FAME yield.

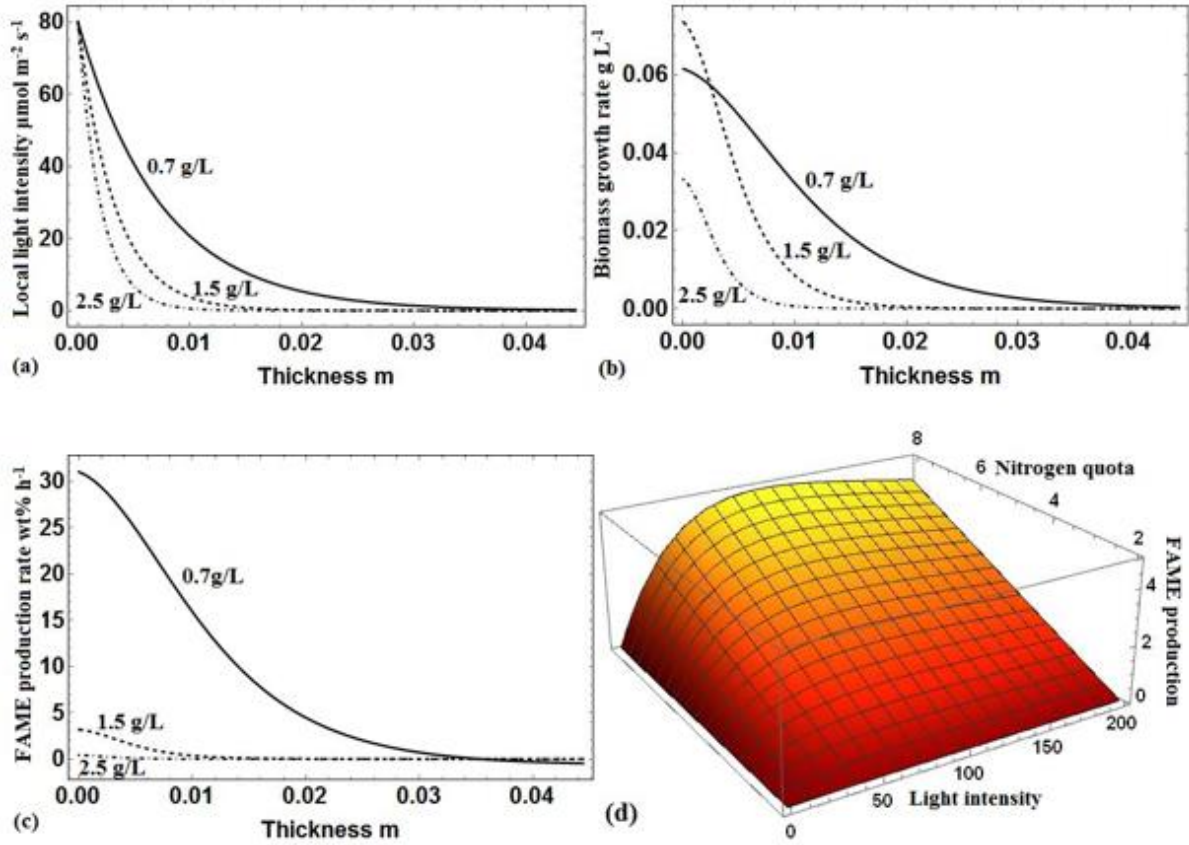


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624 Figure 4: Effects of light attenuation and nitrogen quota on biomass growth and FAME
 625 production. (a): local light intensity; (b): local biomass growth rate; (c): local FAME
 626 production rate; (d): effect of light intensity and nitrogen quota on FAME production. Fig.
 627 4(d) is obtained by Eq. 5, 8, 10, and 11, instead of the entire dynamic model.

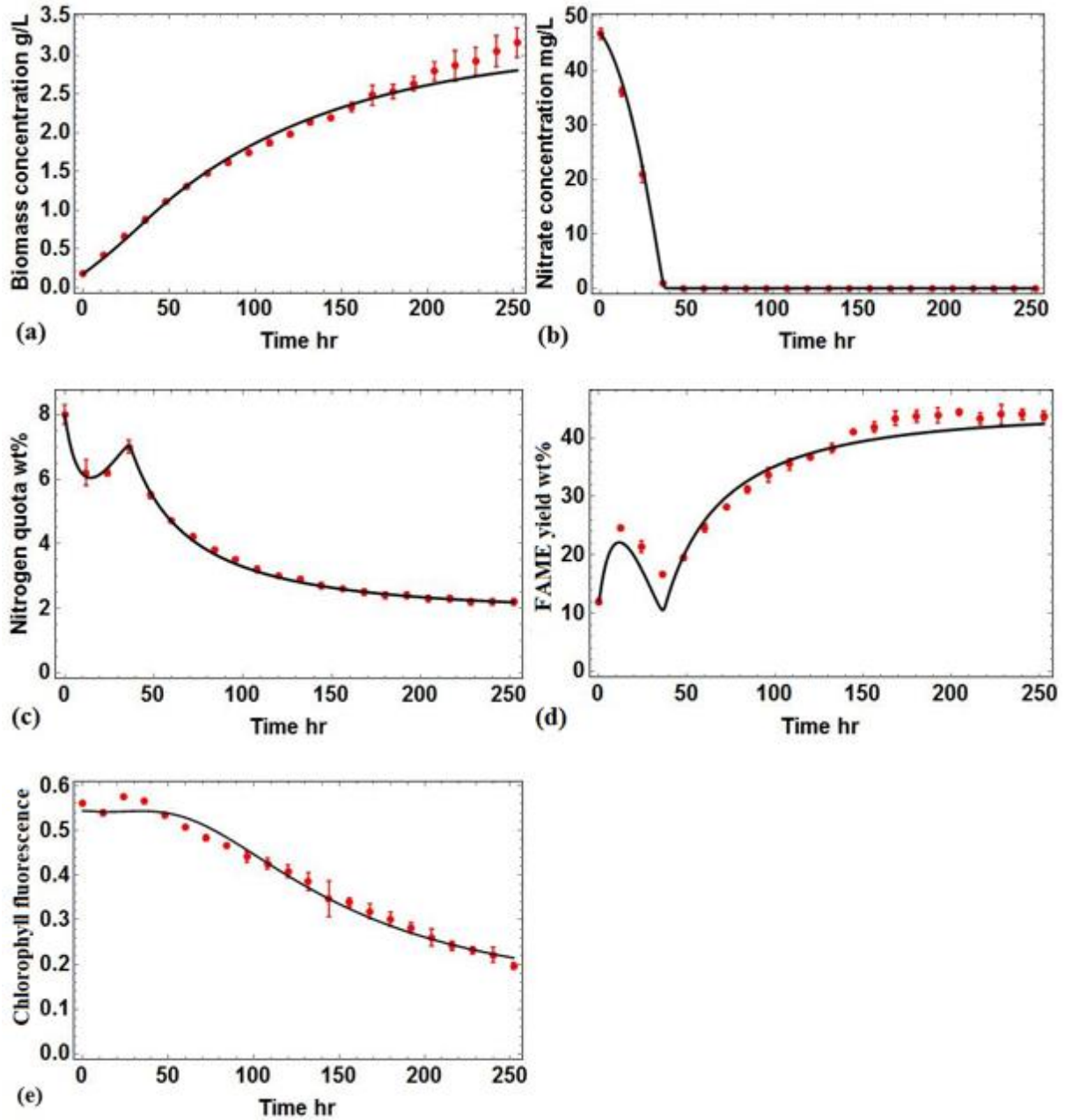


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631 Figure 5: Comparison of model prediction results and real experimental data (Experiment 3).

632 Line: model prediction results, point: real experimental data. (a): biomass concentration; (b):

633 nitrate concentration; (c): nitrogen quota; (d): FAME yield; (e): chlorophyll fluorescence.

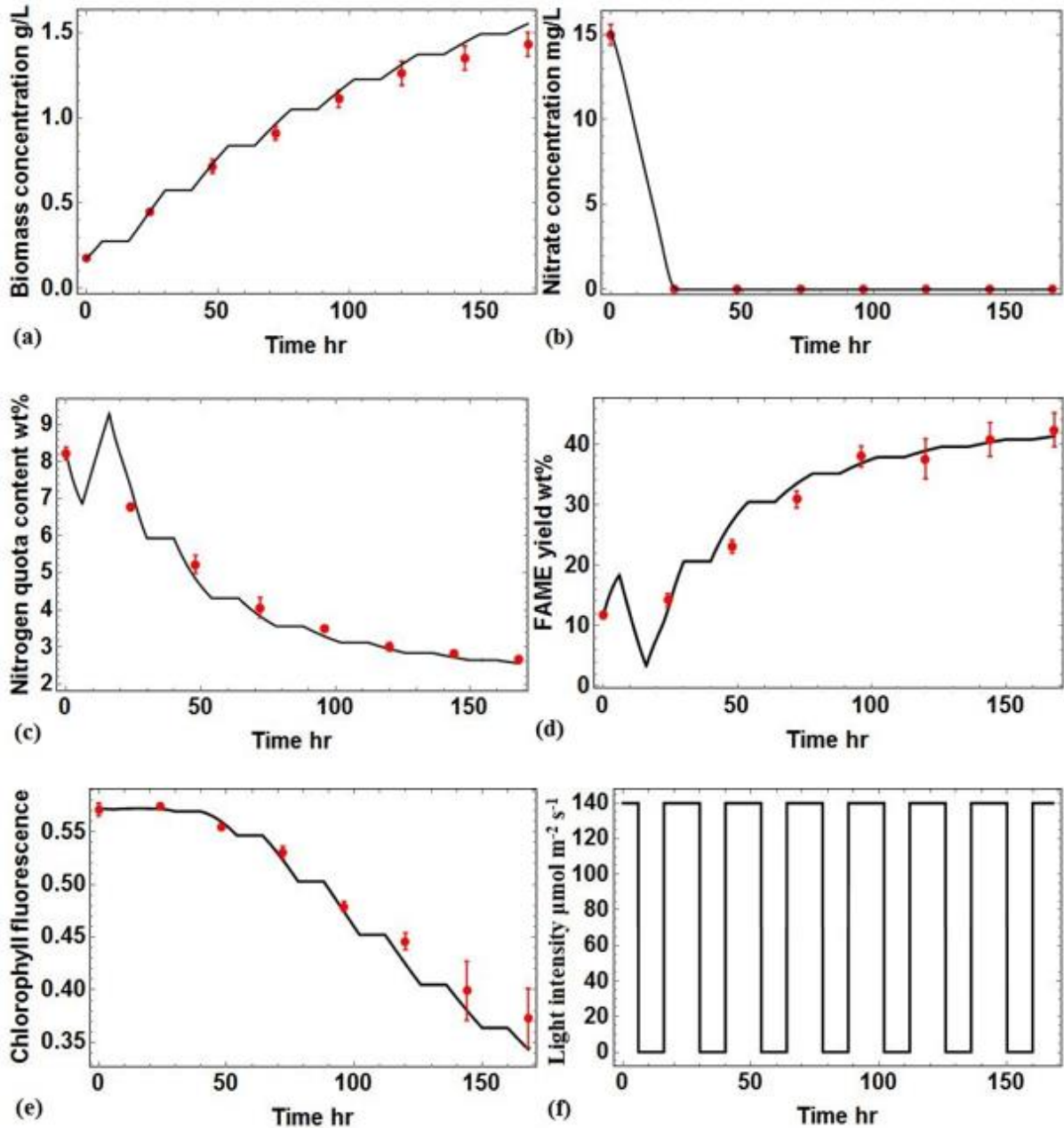


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637 Figure 6: Comparison of model prediction results and real experimental data (Experiment 4).
 638 Line: model predication results, point: real experimental data. (a): biomass concentration; (b):
 639 nitrate concentration; (c): nitrogen quota; (d): FAME yield; (e): chlorophyll fluorescence; (f):
 640 incident light intensity.



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644 Graphical Table of Contents: A robust kinetic model was constructed to simulate the dynamic
 645 behaviour of green microalgae biomass growth and biolipid (precursor of biodiesel)
 646 production; correlation between chlorophyll fluorescence, an instantly measurable variable
 647 and indicator of photosynthetic activity, and intracellular nitrogen content, which directly
 648 affects biolipid synthesis rate, is quantified for the first time; through experimental
 649 verification, the current model is characterised by a high level of predictive capability, and
 650 the optimal light intensity for algal biomass growth and lipid synthesis is estimated.

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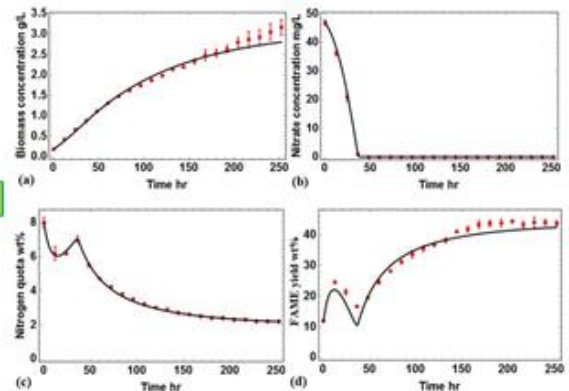
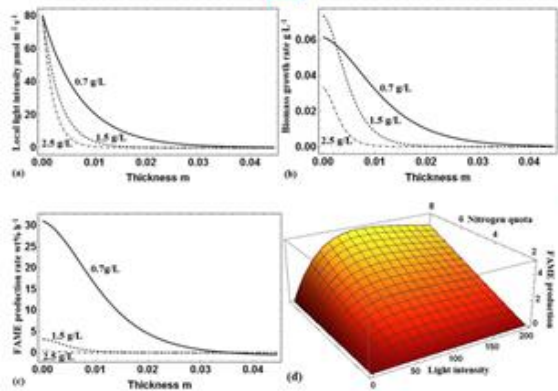
$$\frac{dX}{dt} = u_M \cdot \frac{I}{I + k_s + \frac{I^2}{k_i}} \cdot \left(1 - \frac{k_q}{q}\right) \cdot X - \mu_d \cdot X$$

$$\frac{dN}{dt} = -\mu_N \cdot \frac{N}{N + K_N} \cdot X$$

$$\frac{dq}{dt} = \mu_N \cdot \frac{N}{N + K_N} - \mu_m(I) \cdot \left(1 - \frac{k_q}{q}\right) \cdot q$$

$$\frac{df}{dt} = \mu_m(I) \cdot (\theta \cdot q - \varepsilon \cdot f) \cdot \left(1 - \frac{k_q}{q}\right) - \gamma \cdot \mu_N \cdot \frac{N}{N + K_N}$$

$$Y(II) = \frac{\exp[\tau \cdot q]}{\exp[\tau \cdot q] + \delta} + \varphi$$



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