



HAL
open science

Determination by response surface methodology of optimal protein and phycocyanin productivity conditions in *Arthrospira* (*Spirulina*) *platensis* under different combinations of photoperiod variation and lighting intensity

Laura Junique, Lucie Watier, Hortense Lejeune, Florence Viudes, Marie Deblieck, Denis Watier

► To cite this version:

Laura Junique, Lucie Watier, Hortense Lejeune, Florence Viudes, Marie Deblieck, et al.. Determination by response surface methodology of optimal protein and phycocyanin productivity conditions in *Arthrospira* (*Spirulina*) *platensis* under different combinations of photoperiod variation and lighting intensity. *Bioresource Technology Reports*, 2021, 15, pp.100763. 10.1016/j.biteb.2021.100763 . hal-04049494

HAL Id: hal-04049494

<https://ulco.hal.science/hal-04049494>

Submitted on 22 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 Determination by response surface methodology of optimal protein and
2 phycocyanin productivity conditions in *Arthrospira (Spirulina) platensis* under
3 different combinations of photoperiod variation and lighting intensity.

4

5 Laura Junique^a, Lucie Watier^b, Hortense Lejeune^a, Florence Viudes^a, Marie
6 Deblieck^a and Denis Watier^{*a, c}

7

8 ^aIUT du littoral Côte d'Opale, Département Génie Biologique, Université du
9 Littoral Côte d'Opale, 63327 Boulogne-sur-Mer, France

10 ^bUTC, Université de Technologie de Compiègne, Rue Roger Couttolenc, 60200
11 Compiègne, France

12 ^cUniv. Littoral Côte d'Opale, UMR 1158 BioEcoAgro, Institut Charles Viollette,
13 USC ANSES, INRAe, Univ. Artois, Univ. Lille, Univ. Picardie Jules Verne, Univ.
14 Liège, Yncréa, F-62200 Boulogne-sur-Mer, France

15

16 **Abstract**

17 The objective of this study is to determine the lighting conditions for an optimal
18 production of *Arthrospira (Spirulina) platensis* quantitatively and qualitatively for
19 the process most commonly used by producers. A design of experiments made
20 it possible to obtain surface modelling of responses which were validated under
21 pilot conditions. Spirulina growth, protein and pigment content were modelled as
22 a function of light intensity and duration of illumination. For each of them, the
23 optima have been determined: 14h (hours light exposure)/10 Klux (light
24 intensity) for the growth rate; 11.8h /7.2 Klux for phycocyanin rate;

25 6.2Klux/12.6h for chlorophyll rate; 6.6Klux/12.6h for carotene rate. The protein
26 rate is the lowest when lighting conditions are of 10.6 Klux and 14.4 hours. It is
27 then possible for the producer to partially obscure the ponds and / or increase
28 the light intensity using additional lighting.

29

30 **Keywords:** Response Surface Methodology, Phycocyanin productivity, Proteins
31 productivity, *Arthrospira (Spirulina) platensis*.

32

33

34 Corresponding author*

35 Denis Watier

36 watier@univ-littoral.fr

37 IUT du littoral Côte d'Opale, Département Génie Biologique, Université du

38 Littoral Côte d'Opale, Bassin Napoléon B.P. 120, 63327 Boulogne-sur-Mer

39 Cedex

40 Univ. Littoral Côte d'Opale, UMR 1158 BioEcoAgro, Institut Charles Violette,

41 USC ANSES, INRAe, Univ. Artois, Univ. Lille, Univ. Picardie Jules Verne, Univ.

42 Liège, Yncréa, F-62200 Boulogne-sur-Mer, France

431. Introduction

44 The market of *Arthrospira platensis* is in full development. The interest for this
45 microalga comes from its high content of high value proteins, amino acids, fatty
46 acids, polysaccharides, vitamins and pigments such as phycocyanin,
47 chlorophyll and β -carotene (Kuddus et al., 2013; Belay, 2002; Pulz and Gross,
48 2004; Raposo and Morais, 2015). This source of protein and phycocyanin used
49 as a natural blue dye in food, beverages and cosmetics makes *Arthrospira*
50 *platensis* a promising microalga (Cheah et al., 2015). It is therefore essential to
51 optimize production: composition of the medium, type of light, mixotrophy,
52 bioreactor and production process.

53 Different processes have been developed such as the fed-batch process, batch
54 culture systems or continuous cultures. The configuration of the photobioreactor
55 also has an important role on growth. Biomass production increases in parallel
56 with the area / volume ratio. Although the horizontal photobioreactor (HoP) is
57 the most efficient system (Da Silva et al., 2016), open raceway ponds remain
58 the most widely used configuration for the commercial production of *Arthrospira*
59 *platensis* due to a better compromise between economic benefits and simplicity
60 of use (Soni et al., 2017).

61 Different media were tested for the cultivation of *Arthrospira platensis*. The
62 influence of the concentrations of mineral salts (phosphates, nitrates, iron,
63 NaCl) and pH has been studied (Çelekli and Yavuzatmaca, 2008; Çelekli et al.,
64 2009). Nitrogen is a key parameter, particularly for the formation of phycocyanin
65 (Boussiba and Richmond, 1980). Chainapong et al. (2012) obtained better
66 mixotrophy production. The influence of molasses / urea concentration was

67 modelled by Setyoningrum and Nur (2015). However, the Zarrouk environment
68 (Pragya et al., 2013; Zarrouk, 1966) remains the most commonly used medium
69 at present.

70 It has been reported that the quality, intensity and duration of light are important
71 factors in the production of microalgae (Sudhakar et al., 2012; Novoveskaa et
72 al., 2016). For *Arthrospira platensis*, the effects of light intensity and light
73 temperature have been well studied (Pandey et al., 2011). An excess of light
74 causes a phenomenon of photooxidation. The cell concentration increases with
75 the intensity of light until reaching a maximum threshold value beyond which
76 damage to the cellular photosynthetic apparatus is observed (Bezerra et al.,
77 2012). The optimal conditions seem to approach 5000 lux for the intensity. The
78 phenomenon is complicated by the fact that the depth of the culture medium to
79 be crossed limits the quantity of light absorbed by the cells (Converti et al.,
80 2006; Rodrigues et al., 2010). A turbulent mixture is therefore essential for high
81 biomass productivity (Chisti, 2016). In practice, we agree on optimal mixing
82 speeds between 5 and 60 cm / s. Chen et al. (2010) modelled the effect of light
83 temperature on the composition of *Arthrospira platensis*. They showed that red
84 light promotes growth, yellow light chlorophyll and blue phycocyanin. The effect
85 of photoperiod on growth and the composition of the biomass of *Arthrospira*
86 *platensis* are less well-known. Works on other microalgae such as *Chlorella*
87 *vulgaris* showed that photoperiod qualitatively influences growth and protein
88 compositions, chlorophyll and carotene (Seyfabadi et al., 2011; Khan and
89 Srivastava, 2003).

90 Our study aims to provide industrial producers of *Arthrospira platensis* using the
91 open raceway ponds, the tools to optimize the production of biomass, proteins
92 and pigments. The use of an experimental design accompanied by a response
93 surface modelling allows refining the optimal conditions of production with
94 greater precision, taking into account the possible interactions between the
95 luminous intensity and the duration of this luminous phase. Such a study can
96 help producers to modulate lighting conditions quantitatively according to the
97 targeted cellular constituents.

98

992. **Materials and methods**

1002.1. *Strain and culture medium*

101 *Arthrospira platensis* is one of the Lonar type and was obtained from the Hyères
102 horticultural school which provides the professional partner (Spiruline des Hauts
103 de France, Route de Pernes, 62550 Valhuon).

104 A standard Zarrouk culture medium (Zarrouk, 1966) containing (g/L): NaHCO₃:
105 8; NaCl: 5; KNO₃: 2; K₂SO₄: 1; MgSO₄: 0.2; NH₄ PO₄: 0.2; CaCl₂: 0.1;
106 CO(NH₂)₂: 0.01; FeSO₄: 0.001 was used for the cultivation of *Arthrospira*
107 *platensis*. The pH was adjusted at 10 using NaOH and then autoclaved for 20
108 minutes at 121° C.

1092.2. *Experimental design*

110 *Arthrospira platensis* was seeded at a concentration of 0.12 mg / mL of dry
111 matter in flasks (Corning 175 cm², cell flask 431080) containing 175 mL of
112 medium. The cultures were fed with CO₂ and homogenized by bubbling at 6.56
113 mL / min (99/1 air / CO₂). The temperature was regulated in a hot chamber (air

114 stirred at 30 ° C, +/- 1.2 ° C). The Central Composite Designs was established
115 using Minitab 18. The 5 levels (coded levels: - α , -1, 0, + 1, + α) correspond to
116 photoperiods (light/dark photoperiod) at 5h/19h, 7.4h/16.6h, 11h/13h,
117 14.6h/9.4h, 17h/7h and to light intensities to 530lux (10 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$),
118 3200lux (59 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$) , 7200lux (132 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$),
119 11200lux (205 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$), 13870lux (254 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$). The
120 light intensity (OSRAM luminux Cool Daylight 6500K) received by each flask
121 was measured in lux (BK lightmeter precision 615) and converted to photon in
122 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ using the conversion factor of 18.3 corresponding to Cool Daylight
123 3500K (AGi32, photosynthetic Photon Flux Density, concepts). For the sake of
124 reproducibility of the experimental conditions (inoculum, temperature and
125 location under the light source) all of the growth kinetics of the experimental
126 design were carried out simultaneously in the different flasks and the location
127 under the lamps was randomized after each sample.

1282.3. *Growth monitoring*

129 For each flask, biomass growth was monitored daily by measuring the optical
130 density (OD) at 560 nm using a spectrophotometer (Varian Cary 100 Bio) and
131 Nephelometric Turbidity Unit (NTU) measurement using a turbidimeter (Hanna
132 Instruments HI88703). The correlations between the biomass (dry weight
133 expressed in g.l^{-1}) and turbidity were established as follows: Biomass = 0.61 x
134 OD_{560} and Biomass = $4.6.10^{-4}$ NTU. The ratios used were determined by
135 centrifugation of 50mL of suspension for 5 minutes at 5000 rpm. The pellet is
136 re-suspended in 7 mL of physiological saline and centrifuged again. The pellet
137 was dried at 110 ° C to estimate the dry weight (biomass).

138 For each growth kinetics, the database Sym'Previus was used to determine the
139 growth rate with the help of the logistic model with delay and rupture (Rosso et
140 al., 1995).

$$141 \quad 1 \quad \ln N = \ln N_0 , \quad , t \leq lag$$

$$142 \quad \ln N = \ln N_{max} - \ln \left(1 + \left(\frac{N_{max}}{N_0} - 1 \right) \cdot \exp(-\mu_{max}(t - lag)) \right) , t > lag$$

1432.4. *Protein assay in biomass*

144 For each flask, a 0.4 mL sample was taken for protein analysis. The Carbon /
145 Nitrogen ratio was estimated using a CHN elemental analyser (Flash EA 1112,
146 ThermoFisher Scientific). Aspartic acid (L-Aspartic Acid Standard, ref 33840023
147 Thermo Finnigan) was used as a standard to establish the calibration curve.
148 The sample was dried and injected into the analyser. The results were
149 expressed as a C/N ratio and converted to a percentage of protein. The protein
150 rate in biomass of *Arthrospira platensis* was deduced by using the following
151 equation: Protein % = 2.75 / (C / N) x 100. The coefficient includes the
152 characteristics of spirulina platensis proteins (Harriet et al., 2008).

1532.5. *Phycocyanin extraction and chemical dosing*

154 After thawing, each sample was introduced into 10 ml of 0.1M pH7 phosphate
155 buffer (Kissoudi et al. 2018). The suspension was treated by sonication for 2
156 minutes at 50% of maximum power (IKA Laboteknick U200S). After
157 centrifugation for 10 minutes at 6000 rpm (Hettlic Rotina 380), the supernatant
158 was filtered with a 0.45 µm GF / C syringe filter. The optical density was
159 measured (Cary-100 UV-visible spectrophotometer) at 612, 652 and 562 nm.
160 The total phycocyanin (phycobiliprotein) concentrations were determined using

161 the Benett and Bogorad formulas and expressed in mg.L-1 (Bennett and
162 Bogorad 1973): Total phycocyanin concentration = phycocyanin (c-PC) +
163 alloctyanin concentration (APC) + phycoerythrin (PE).

164 2 $[c - PC] = (OD^{615} - 0.474 \times OD^{652}) \div 5.34$

165 3 $[APC] = (OD^{652} - 0.208 \times OD^{615}) \div 5.09$

166 4 $[PE] = (OD^{552} - 2.41(PC) - 0.849(APC)) \div 9.62$

167 The phycocyanin level is expressed in % P = 100 * ((C * V) / Vc) / DB.

168 With C: total concentration of phycocyanin (mg / L), Vc: culture volume (ml), V:
169 volume of extraction solvent (ml) and DB: dry biomass (g).

170

171 2.6. *Extraction and chemical dosing of total chlorophylls (a and b) and total*

172 *carotenoids*

173 After extraction of phycocyanin, the samples obtained were frozen at -85° C and
174 lyophilized (lyophilizer Alpha 2-4LD Christ). The chlorophyll pigments and
175 carotenoids were extracted with 90% acetone (5 or 10mL depending on the
176 samples). The samples were centrifuged (Hettlich Rotina 380 centrifuge) for 10
177 minutes at 3000 rpm (until transparency) before reading the absorbance of the
178 filtrate with a spectrophotometer (UV-visible Cary-100) at 470, 750, 664, 647
179 and 630 nm.

180 The concentration of total chlorophylls expressed in mg.L-1 was the sum: Ca +
181 Cb (Chlorophyll c is not present in *Arthrospira platensis*)

182 With:

183 5 $Ca = 11.85 \times E^{664} - 1.54 \times E^{647} - 0.08 \times E^{630}$

184 6 $Cb = -5.43 \times E^{664} + 21.03 \times E^{647} - 2.66 \times E^{630}$

185 With $E\lambda = OD^\lambda - OD^{750}$ adjustment of the inactive biomass

186 The concentration of total carotenoids (*xanthophylls*) is given by the equation
187 (Lichtenthaler and Buschmann, 2001):

$$188 \quad C(x + c)(mg/L) = (1000 \times DO^{470} - 1.82 \times Ca - 85.02Cb)/198$$

189 2.7. *Modelling*

190 The Response Surface Methodology was used to fit the polynomial equations to
191 the experimental design using Minitab 18. For the modelling of growth rate,
192 protein content and phycocyanin content, the polynomial equations (surface
193 modelling of responses) with Box-Cox transformation were established and
194 analysed on Minitab 18. The surface modelling of responses are plotted using
195 Excel 16.

196 2.8. *Validation of model and determination of optimal productivity under pilot 197 conditions*

198 The agreement between the optimal values predicted by the model and
199 production under industrial conditions was verified using a open raceway ponds
200 (Figure 1). Optimal light intensity and photoperiod conditions were calculated
201 using polynomial equations (paragraph: Modelling). Under these conditions, the
202 kinetics of the biomass, protein and phycocyanin productivities values were
203 determined experimentally. The production pilot consists of a PVC gutter of 9
204 cm diameter with a usable volume of 7 L. Four neon lights (OSRAM Luminux
205 Cool Daylight 6500K) were placed above the gutters. The light intensity was
206 adjusted by modifying the distance between the surface of the culture medium
207 and the light source. The intensity measured in lux (BK lightmeter precision
208 615) was averaged over the entire length of the pilot. The agitation of the

209 medium was caused by a blade at the end of a shank fixed on a stirring motor
210 (Heidolph RZR 2102 Control) (40 rpm) on the model of a paddlewheel. Optical
211 density and NTU measurement were determined daily and converted in dry cell
212 mass concentration as explained in the growth monitoring. The evaporation of
213 the medium was compensated by the readjustment of the level with distilled
214 water.

2153. Results and discussion

216 Models developed in this work enabled the determination of optimal production
217 conditions. Their predictive quality has been tested and confirmed on a pilot
218 scale close to the industrial microalgae culture system.

219 The experimental values obtained from the experimental design are presented
220 graphically in Figure 2. The use of a Central Composite Design seems
221 appropriate because it underlines the center values of the experimental design
222 which correspond to the optimal values.

223 These results were obtained thanks to a judicious choice of the experimental
224 conditions. Other works showed that the protein and pigments composition in
225 *Arthrospira platensis* can vary depending on the time of sampling during the
226 light phase (Hidasi and Belay, 2018). Therefore, it was decided to analyze the
227 biomass composition in proteins and pigments 4 hours after the beginning of
228 each lighting phase (Conditions adapted to the constraints of the producer and
229 to the optimization of the microalgae metabolism). The chosen culture medium
230 (Zarrouck, 1996) is the most commonly used in industrial production of
231 microalgae (Pragya et al., 2013). Likewise, it is well established that the
232 temperature of 30 ° C corresponds to the optimal conditions and that it

233 influences the growth and the production of pigment independently of the other
234 variables (Danesi et al., 2011). As a result, the temperature was not introduced
235 as a variable in the design of experiments and was kept constant at 30 ° C.
236 The polynomial equations determined from these experimental values are
237 collected in Table 1 and the corresponding surface responses are shown in
238 Figure 3.

239 The study of the Residual Value (RVs) graphs (histogram of residual values,
240 Henry's right, and residual values according to adjusted values) shows the
241 absence of asymmetry and outliers. Despite the low R^2 for some polynomial
242 equations, it is possible to state statistically and visually that the polynomial
243 equations are superimposed satisfactorily on the experimental values.

244 For each polynomial equation, a prior modelling (not presented here) showed
245 for the term $\text{Int} * \text{Photo}$ the p values ($\alpha = 0.05$) were greater than 0.9. Moreover,
246 the Pareto coefficients (significance level of 0.05) showed that the term $\text{Int} * \text{Photo}$
247 could be neglected without statistical risk. The luminous intensity (Int)
248 and duration of the light phase (Photo) have a significant and independent
249 effect on growth and spirulina composition. For these reasons and for the sake
250 of simplicity, the equations presented in this work do not integrate this
251 interaction.

252 Equation A (Table1) is used to plot the response surface (Figure 3A). This
253 equation gives the growth rate of *Arthrospira platensis* as a function of
254 photoperiod and light intensity. Growth rates were calculated using the logistic
255 model, R^2 are all greater than 0.98. The growth was slower for lower and higher
256 intensities of light. The growth was maximum at 10 Klux (183 $\mu\text{mol photons.m}^{-2}$

257 $^2.s^{-1}$). For the photoperiod, the growth increased with the duration of the light
258 phase with a maximum growth for 14h of light exposure. For these optimal
259 conditions, the growth rate was $0.0218 h^{-1}$. Equation B (Table 1) characterized
260 the protein composition of *Arthrospira platensis* (percent protein proportion).
261 Figure 3B shows the corresponding response surface. For a variability which
262 remained however limited (range of 10%), the production of protein of the
263 microalgae was noticeably lower for the central values of conditions of
264 illumination. The protein production was in inverse proportion to the growth. The
265 optimal conditions were close to the limits of the experimental design for the
266 extreme values of the two factors. It was therefore difficult to determine the
267 optimal conditions, on the other hand, it was possible to determine the worst
268 conditions. The protein rate was lowest for lighting conditions of 10.6 Klux (194
269 $\mu\text{mol photons.m}^{-2}.s^{-1}$) and 14.4 hours of light exposure. Under these conditions,
270 the protein rate was only 35.6%. Equation C (Table 1) corresponds to the
271 proportion of phycocyanin in percent of the dry weight of *Arthrospira platensis*.
272 The surface response graph is shown in Figure 3C. The amount of pigment is
273 strongly influenced by the light conditions. The pigments concentration of the
274 biomass for the extreme values of the experimental design was very low, that is
275 to say photoperiod lower than 5h or higher than 15h and intensity lower than
276 2Klux ($37 \mu\text{mol photons.m}^{-2}.s^{-1}$) or higher than 14 Klux ($256 \mu\text{mol photons.m}^{-2}.s^{-1}$).
277 The optimal lighting conditions were 11.8 hours of light exposure and 7.2
278 Klux ($132 \mu\text{mol photons.m}^{-2}.s^{-1}$) for a proportion of phycocyanin higher than
279 4.2%. For the other two pigments, chlorophyll and carotene (Equations D and
280 E), the response surfaces were similar (Figure 3D and 3E). Reciprocally, the

281 following optimal conditions were obtained 6.2Klux (114 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$),
282 12.6h for 0.32% for chlorophyll and 6.6Klux (121 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$), 12.6h
283 for 0.1% of carotene. Results showed that growth, proteins and pigments
284 production have all different optimal conditions. This work shows that if the
285 biomass increases with the duration of illumination it is not the same for the
286 production of proteins and pigments. Pigments (phycocyanin, chlorophyll and
287 carotenoid) production is optimal when lighting conditions correspond to the
288 central values of the experimental design contrary to protein production which
289 requires lower values. Also it is possible to boost a rapid growth by extending
290 the duration of the light phase at limited intensities. Several authors have
291 determined the optimal light intensities promoting growth and the production of
292 pigments. The proposed values differ according to the studies: 2 KLux for
293 Kumar et al. (2011), 24-60 $\mu\text{mol photons m}^{-2}.\text{s}^{-1}$ for Danesi et al. (2011), 5 KLux
294 for Pandey et al. (2011), 1.6 KLux for Singh et al. (2010) and 60 $\mu\text{mol photons}$
295 $\text{m}^{-2}.\text{s}^{-1}$ for Markou et al. (2012). These differences can partly be explained by the
296 fact that the majority of studies use only a limited number of intensity levels
297 without modelling and also by the fact that the color temperatures (in Kelvin) of
298 lamps used in each study are not similar. In the case of *Arthrospira platensis*,
299 no study simultaneously deals with the influence of the intensity of light, of the
300 photo period on the growth and the composition (protein and pigments) of the
301 microalga (Niangoran, 2017).
302 These different models made it possible to determine the optimal production
303 conditions for each constituent and to bring them together on the same graph
304 (figure 4) allowing a producer to choose the best compromise according to

305 these objectives. This shows the areas for which productivity was higher than
306 95% of the optimum. The protein and phycocyanin productivity percentages
307 were determined from equations A, B and C.

308 Optimums were shown by stars. It was clearly visible in the figure that the three
309 productivities (biomass, proteins and phycocyanin) were distinctly influenced by
310 the lighting conditions. It should be noticed that protein productivity was less
311 depend on the illumination conditions than phycocyanin productivity. The
312 lighting conditions influence in an acquired way the productivity of proteins, of
313 chlorophyll, of carotene and phycocyanin. The best production was obtained
314 when the lighting conditions were 8.3 Klux ($152 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and 12.6
315 hours of exposure. Under these optimal conditions, a pilot production enabled
316 us to determine the theoretical values of productivity that could be obtained in
317 industrial production. Three growth kinetics of *Arthrospira platensis* (carried out
318 in triplicate) were performed and the growth rates were determined using the
319 logistic model with delay and rupture. The average growth rate obtained (0.026
320 $\text{h}^{-1} \pm 0.0013$) was slightly higher than the growth rate obtained under the
321 conditions of the model.

322 The present study has the singularity to determine accurate optimal conditions
323 of *Arthrospira platensis* cultivation by simultaneously taking into account
324 potential interactions and a larger number of levels allowing better
325 representativeness than other authors do. Another singularity of this work was
326 to show that it is justified to take into account the different constituents of
327 *Arthrospira platensis* and to make a compromise to determine the optimal
328 conditions of production. Proteins proportion which composed *Arthrospira*

329 *platensis* biomass is inverse in proportion to the growth speed which confirms
330 that a microalga with a fast growth will be less rich in proteins. For producers,
331 the nutritional quality of their product is really important and this is why it is
332 necessary to take the different optimums into consideration to determine the
333 best yield with the highest quality of the product as possible.

3344. **Conclusion**

335 The results of this work on spirulina production optimization stress on the
336 importance of considering quantitative and qualitative aspects and
337 simultaneously studying the various influencing parameters using a response
338 surface methodology.

339 The compilation of models proposed in this study enables producers to find their
340 own optimal conditions adapted to their production system. From a practical
341 point of view, a producer will be able to control the lighting of his ponds by
342 shading or supplementing natural lighting with artificial light if necessary in order
343 to boost qualitatively and quantitatively his production.

344 **Conflict of Interest Statement**

345 The authors declare that the research was conducted in the absence of any
346 commercial or financial relationships that could be construed as a potential
347 conflict of interest.

3485. **Acknowledgements**

349 We thank the producer Loïc Anselin (Spiruline des Hauts-de-France) for
350 providing us with the spirulina strain and for his technical information. We also
351 thank Thierry Gard for proof reading the article and ANSES proofreading
352 service for language help.

3536. Bibliography

- 354 Belay, A., 2002. The potential application of *Spirulina (Arthrospira)* as a
355 nutritional and therapeutic supplement in health management. J. Am. Nutraceut.
356 Assoc. 5, 26-49.
- 357 Bennett, A., Bogorad, L., 1973. Complementary chromatic adaptation in a:
358 filamentous blue-green alga. J. Cell Biology. 58, 419-435.
359 <https://doi.org/10.1083/jcb.58.2.419>
- 360 Bezerra, RP, Matsudo, MC, Sato, S., Perego, P., Converti, A., Carvalho JCM,
361 2012. Effects of photobioreactor configuration, nitrogen source and light
362 intensity on the fed-batch cultivation of *Arthrospira (Spirulina) platensis*.
363 Bioenergetic aspects. Biomass Bioenerg. 37, 309-317.
364 <https://doi.org/10.1016/j.biombioe.2011.11.007>
- 365 Boussiba, S., Richmond, AE, 1980. C-phycocyanin as a storage protein in the
366 blue-green alga *Spirulina platensis*. Arch. Microbiol. 125, 143–147.
367 <https://doi.org/10.1007/BF00403211>
- 368 Çelekli, A., Yavuzatmaca, M., 2008. Predictive modeling of biomass by
369 *Spirulina platensis* as function of nitrate and NaCl concentrations. Bioresour.
370 Technol. 100, 1847-51. <https://doi.org/10.1016/j.biortech.2008.09.042>
- 371 Çelekli, A., Yavuzatmaca M., Bozkurt, H., 2009. Modeling of biomass
372 production by *Spirulina platensis* as function of phosphate concentrations and
373 pH regimes. Bioresour. Technol. 100, 3625-3629.
374 <https://doi.org/10.1016/j.biortech.2009.02.055>

375 Chainapong, T., Traichaiyaporn, S., Deming, RI, 2012. Effect of light quality on
376 biomass and pigment production in photoautotrophic and mixotrophic cultures
377 of *Spirulina platensis*. J. Agric. Technol. 8, 1593-1604.

378 Cheah WY, Show, PL, Chang, JS, Ling, TC, Juan, JC, 2015. Biosequestration
379 of atmospheric CO₂ and flue gas-containing CO₂ by microalgae. Review.
380 Bioresour. Technol. 184, 190-201.
381 <https://doi.org/10.1016/j.biortech.2014.11.026>

382 Chen, HB, Wu, JY, Wang, CF, Fu, CC, Shieh, CJ, Chen, CI, Wang, CY, Liu,
383 YC, 2010. Modeling on chlorophyll a and phycocyanin production by *Spirulina*
384 *platensis* under various light-emitting diodes. Biochem. Eng. J. 53, 52-56.
385 <https://doi.org/10.1016/j.bej.2010.09.004>

386 Chisti, Y., 2016. Large-scale production of algal biomass: Raceway ponds.
387 algae biotechnol. 21-40.

388 Converti A., Lodi, A., Del Borghi, A., Solisio, C., 2006. Cultivation of *Spirulina*
389 *platensis* in a combined airlift-tubular reactor system. Biochem. Eng. J. 32, 13-
390 18. <https://doi.org/10.1016/j.bej.2006.08.013>

391 Da Silva, MF, Casazza, AA, Ferrari, PF, Perego, P., Bezerra, RP, Converti, A.,
392 Porto, AL, 2016. A new bioenergetic and thermodynamic approach to batch
393 photoautotrophic growth of *Arthrospira (Spirulina) platensis* in different
394 photobioreactors and under different light conditions. Bioresour. Technol. 207,
395 220-228. <https://doi.org/10.1016/j.biortech.2016.01.128>

396 Danesi, EDG, Rangel-Yagui, CO, Sato S., Monteiro de Carvalho, GC, 2011.
397 Growth and content of spirulina platensis biomass chlorophyll cultivated at
398 different values of light intensity and temperature using different nitrogen

399 sources. Braz. J. Microbiol. 42, 362-73. <https://doi.org/10.1590/S1517->
400 83822011000100046

401 Harriet, V., Imianovsky, U., Oliveira, JL, Sant'Anna, ES, 2008. Cultivation of
402 *Arthrospira (spirulina) platensis* in desalinator wastewater and salinated
403 synthetic medium: protein content and amino-acid profile. Braz. J. Microbiol. 39,
404 98–101. <https://doi.org/10.1590/S1517-83822008000100022>

405 Hidası, N., Belay, A., 2018. Diurnal variation of various culture and biochemical
406 parameters of *Arthrospira platensis* in large-scale outdoor raceway ponds. Algal
407 Res. 29, 121-129. <https://doi.org/10.1016/j.algal.2017.08.027>

408 Khan, MA, Srivastava, P., 2003. Determination of photoperiod for highest
409 content of protein and comparative biochemistry of 2-strains of *Spirulina*
410 *platensis*. Indian J. Environ. Sci. 7, 155-158.

411 Kissoudi, M., Sarakatsianos, I., Samanidou, V., 2018. Isolation and purification
412 of food- grade c-phycoyanin from *Arthrospira platensis* and its determination in
413 confectionery by HPLC with diode array detection. J. Sep. Sci. 41, 4-18.
414 <https://doi.org/10.1002/jssc.201701151>

415 Kuddus, M., Singh, P., Thomas, G., Al-Hazimi, A., 2013. Recent developments
416 in production and biotechnological applications of c-phycoyanin. BiomMed.
417 Res. Int. 2013, 1-9. <https://doi.org/10.1155/2013/742859>

418 Kumar, M., Kulshreshtha, J., Singh GP, 2011. Growth and biopigment
419 accumulation of cyanobacterium *Spirulina platensis* at different light intensities
420 and temperature. Braz. J. Microbiol. 42, 1128-1135.
421 <https://doi.org/10.1590/S1517-83822011000300034>

422 Lichtenthaler, H., Buschmann, C., 2001. Chlorophylls and carotenoids:
423 measurement and characterization by UV-vis spectroscopy. *Curr. Protoc. Food*
424 *Anal. Chem. supplement* 1 F4.3.1-8 (2001).
425 <https://doi.org/10.1002/0471142913.faf0403s01>

426 Markou, G., Chatzipavlidis, L., Georgakakis, D., 2012. Effects of phosphorus
427 concentration and light intensity on the biomass composition of *Arthrospira*
428 (*Spirulina*) *platensis*. *World J. Microbiol. Biotechnol.* 28, 2661-2670.
429 <https://doi.org/10.1007/s11274-012-1076-4>

430 Niangoran NUF, 2017. Optimisation de la culture de la spiruline en milieu
431 contrôlé : éclairage et estimation de la biomasse. Doctoral thesis in Photonique
432 et systèmes optoélectroniques. Under the direction of Zissis G. and Haba C.T.
433 University of Toulouse 3, Toulouse, France (2017) PhD Thesis.

434 Novoveskáa, L., Zapataa, AKM, Zabolotney, JB, Atwood, MC, Sundstrom, ER,
435 2016. Optimizing microalgae cultivation and wastewater treatment in large-scale
436 offshore photobioreactors. *Algal Res.* 18, 86-94.
437 <https://doi.org/10.1016/j.algal.2016.05.033>

438 Pandey, JP, Tiwari, A., Singhand, S., Tiwari, D., 2011. Potential of Different
439 Light Intensities on the Productivity of *Spirulina maxima*. *J. Algal Biomass Util.*
440 2, 9–14.

441 Pragya, N., Pandey, KK, Sahoo. PK, 2013. A review on harvesting, oil
442 extraction and biofuels production technologies from microalgae. *Renew. Sust.*
443 *Energ. Rev.* 24, 159-171. <https://doi.org/10.1016/j.rser.2013.03.034>

444 Pulz, O., Gross, W., 2004. Valuable products from biotechnology of microalgae.
445 Appl. Microbiol. Biotechnol., 65, 635-648. <https://doi.org/10.1007/s00253-004->
446 1647-x

447 Raposo, MFJ, Morais, AMMB, 2015. Microalgae for the prevention of
448 cardiovascular disease and stroke. Life Sci. 125, 32-41.
449 <https://doi.org/10.1016/j.lfs.2014.09.018>

450 Rodrigues, MS, Ferreira, LS, Converti, A., Sato, S., Carvalho, JCM, 2010. Fed-
451 batch cultivation of *Arthrospira (Spirulina) platensis*: potassium nitrate and
452 ammonium chloride as simultaneous nitrogen sources. Bioresour. Technol. 101,
453 4491-4498. <https://doi.org/10.1016/j.biortech.2010.01.054>

454 Rosso, L., Lobry, JR, Bajard, S., Flandrois, JP, 1995. Convenient model to
455 describe the combined effects of temperature and pH on microbial growth. Appl.
456 Environ. Microbiol. 61, 610-616. <https://doi.org/10.1128/aem.61.2.610-616.1995>

457 Setyoningrum, TM, Nur, MMA, 2015. Optimization of C-phycoerythrin production
458 from *S. platensis* cultivated on mixotrophic condition by using response surface
459 methodology. Biocatalysis Agric. Biotechnol. 4, 603-607.
460 <https://doi.org/10.1016/j.bcab.2015.09.008>

461 Seyfabadi, J., Ramezanpour, Z., Khoeyi, ZA, 2011. Protein, fatty acid, and
462 pigment content of *Chlorella vulgaris* under different light regimes. J. Appl.
463 Phycol. 23, 721–726 <https://doi.org/10.1007/s10811-010-9569-8>

464 Singh, SG, Sofi, MY, Masih, S., 2010. Potential of Different Light Intensities on
465 the Productivity of *Spirulina platensis* under Agra Conditions. Res. J. Agric. Sci.
466 1, 468-469.

467 Soni, RA, Sudhakar, K., Rana, RS, 2017. Spirulina - From growth to nutritional
468 product: A review. Trends Food Sci. Technol. 69, 157-171.
469 <https://doi.org/10.1016/j.tifs.2017.09.010>

470 Sudhakar, M., Rajesh, MA, Premalatha, M., 2012. Mathematical model to
471 assess the potential of algal bio-fuels in India. Energy Sources, Part A:
472 Recovery, Utilization, and Environmental Effects, 34, 1114-1120.
473 <https://doi.org/10.1080/15567036.2011.645121>

474 Zarrouk, C. 1966. Contribution à l'étude d'une cyanophycée influencée de
475 divers facteurs physiques et chimiques sur la croissance et la photosynthèse de
476 *Spirulina maxima* (Setch. et Gardner) Geitler. University of Paris, Paris, France
477 (1966) PhD Thesis

Figure Captions

Figure 1: Sketch of the production pilot (open raceway ponds) at scale 1/40 used to determine productivity in optimal conditions. **A:** front section. **B:** top view. **C:** side section. The culture pond consists of a PVC gutter of 9 cm diameter with a usable volume of 7 L.

Figure 2: Experimental values of design of experiments (DOE) obtained for the growth rate in hours⁻¹ (A), the protein rate in biomass percentage (B), the phycocyanin rate in biomass percentage (C), the chlorophyll rate in biomass percentage (D) and the carotene rate in biomass percentage (E) of *Arthrospira platensis* as a function of the luminous intensity in Klux and the duration of the light phase in hours on a period of 24h.

Figure 3: Response-Surface model representing the growth rate in hours⁻¹ (A), the protein rate in biomass percentage (B), the phycocyanin rate in biomass percentage (C), the chlorophyll rate in biomass percentage (D) and the carotene rate in biomass percentage (E) of *Arthrospira platensis* as a function of the luminous intensity in Klux and the duration of the light phase in hours on a period of 24h. The graphs A, B, C D and E are respectively corresponding to the equations A, B, C D and E.

Figure 4: Graphical representation of conditions (light intensity and duration of light phase) for which growth rates, protein levels and phycocyanin levels are

higher than 95 percent of the maximum value. Stars represent the optimal conditions of productivity.

Figures

Figure 1

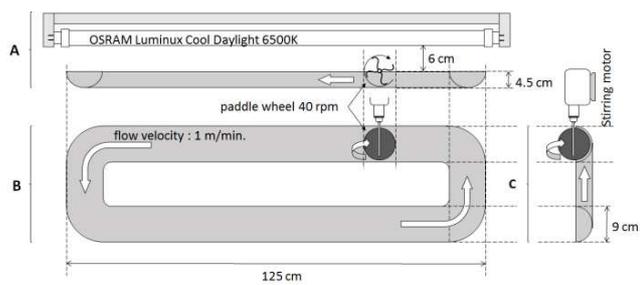


Figure 2

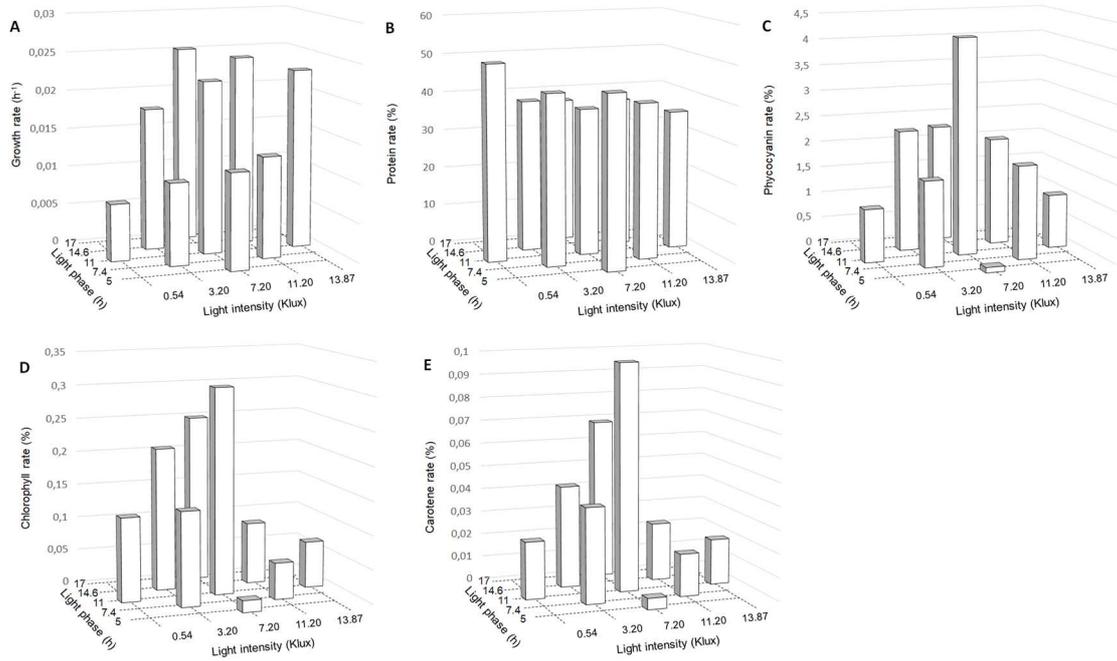


Figure 3

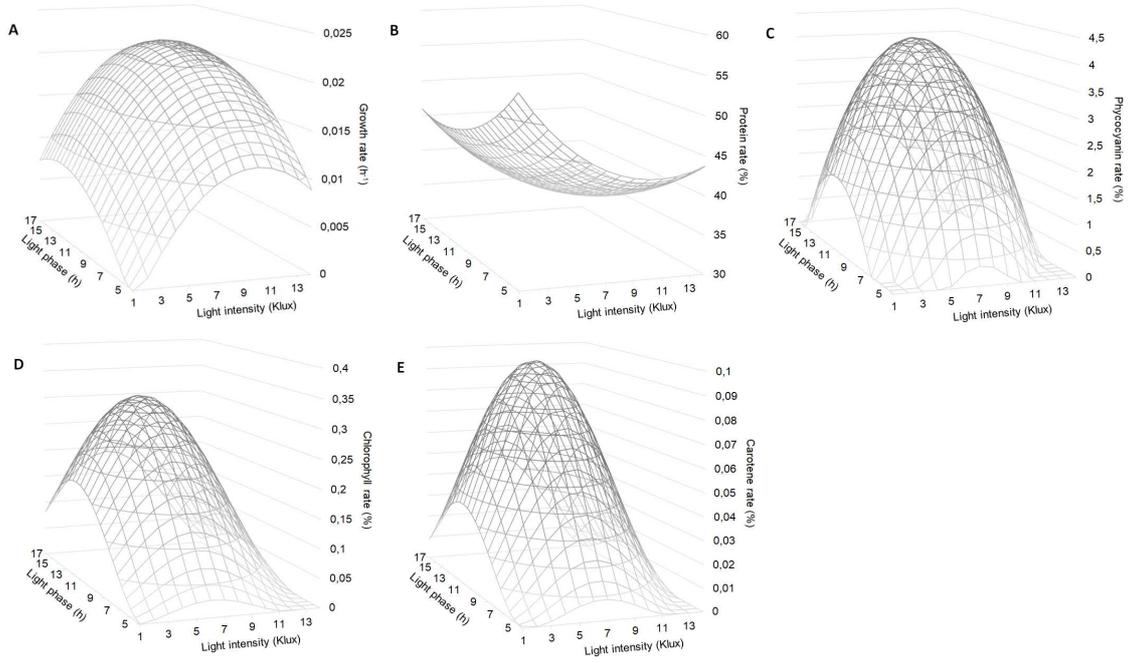
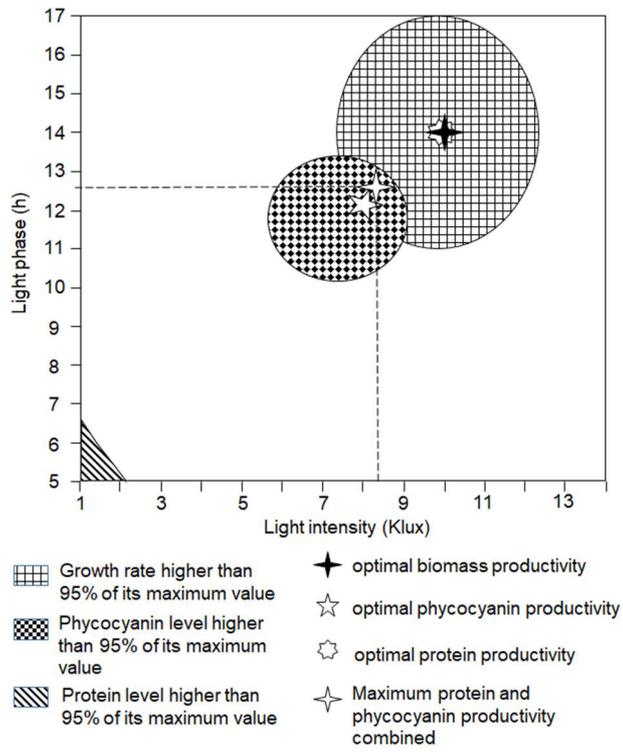


Figure 4



Table

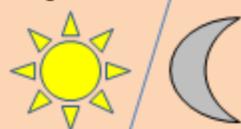
Table 1:

Polynomial equations										
Equation A : Growth rate (h^{-1}) = $-0.01926 + 0.003303Int + 0.00352Photo - 0.000166Int^2 - 0.000107Photo^2$										
Equation B : Ln(Protein rate)(% of biomasse)= $4.284 - 0.0552Int - 0.0584Photo + 0.00259Int^2 + 0.00204 Photo^2$										
Equation C : Phycocyanin rate (% of biomasse) = $-10.97 + 1.013Int + 1.957Photo - 0.0705Int^2 - 0.0830 Photo^2$										
Equation D : Chlorophyll rate (% of biomasse) = $\sqrt{(-0.734 + 0.0699Int + 0.1720Photo - 0.00567 Int^2 - 0.00681 Photo^2)}$										
Equation E : Carotene rate (% of biomasse) = $\sqrt{(-0.486 + 0.0509Int + 0.1030Photo - 0.0038 Int^2 - 0.00422 Photo^2)}$										
Term	Equation A		Equation B		Equation C		Equation D		Equation E	
	Box-Cox	$\lambda = 0.5$	Box-Cox	$\lambda = 0$	Box-Cox	$\lambda = 1$	Box-Cox	$\lambda = 0,5$	Box-Cox	$\lambda = 0.5$
	Coefficient	P	Coefficient	P	Coefficient	P	Coefficient	P	Coefficient	P
Constant	-0.01926	0.000	4.284	0.000	-10.97	0.000	-0.734	0.000	-0.486	0.000
Int	0.003303	0.003	-0.0552	0.005	1.013	0.965	0.0699	0.048	0.0509	0.297
Photo	0.00352	0.001	-0.0584	0.024	1.957	0.046	0.1720	0.006	0.1030	0.034
Int ²	-0.000166	0.012	0.00259	0.045	-0.0705	0.001	-0.00567	0.003	-0.0038	0.004
Photo ²	-0.000107	0.116	0.00204	0.159	-0.0830	0.001	-0.00681	0.004	-0.00422	0.007
R ² (%)	91.93		85.31		90.33		90.19		85.56	

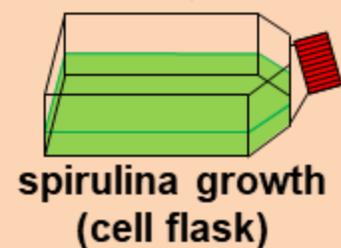
Table 1: Polynomial equations determined by using MiniTab 18 software simulating growth rate (equation A), protein rate (equation B), phycocyanin rate (equation C), chlorophyll levels (equation D) carotene rate (equation E) of *Arthrospira platensis* as a function of the light intensity and the duration of the light phase.

Response Surface Methodology

Experimental design



variable photoperiod

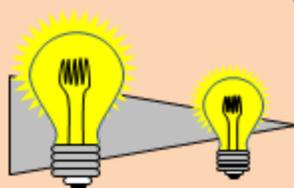


spirulina growth
(cell flask)



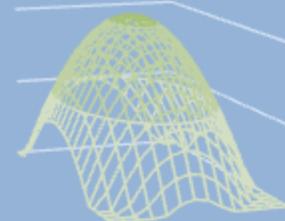
- Growth rate
- Protein rate
- Phycocyanin rate
- Chlorophyll rate
- Carotene rate

variable intensity



Polynomial equations

Phycocyanin



Chlorophyll



Carotene



Protein
Growth rate

Compilation of equations to determine the best productivity compromise

Validation under pilot conditions (open raceway ponds)

