STATISTICAL MODELLING OF MICROALGAL GROWTH

Pérez-Bibbins B.[1], Filali R.[1], Bermejo-Padilla E.[1], Kinsou H.[1], Collis R.[2] and Taidi B.[1, 3]

[1] LGPM, CntraleSupélec, Université Paris-Saclay, SFR Condorcet FR CNRS 3417, Centre Européen de Biotechnologie et de Bioéconomie (CEBB), 3 rue de Rouges Terres 51110 Pomacle, France
[2] MINITAB. 17-21 rue Saint-Fiacre, 75002 Paris, 419 061 361 R.C.S Paris, France.
[3] Université Paris Saclay, CentraleSupélec, LGPM, 3 rue Joliot-Curie, 91190 Gif-sur-Yvette, France

Abstract

Microalgae cultivation in greenhouses could become a significant method for carbon dioxide bio-fixation and biomass production (Wijffels *et al*., 2010). Domestication of microalgae is a necessary step towards the use of these micro-organisms as all-year round crops. This process will require isolation, study and adaption of wild strains to specific applications and possibly breeding to improve characteristics of the strains. An isolated microalgae consortium from Germany and a pure strain of *Chlamydomonas nivalis (Chloromonas typhlos)* were studied because of their tolerance to low temperatures and ability to grow at low light. Statistical Factorial Experimental Design 2^3 with three independent variables was used to construct growth models for the above cultures. The three variables studied at laboratory scale (20 ml) were: light intensity (20-200 µmol/m²s), CO_2 concentration (0.04-5.0 %) and temperature (5-25ºC). Based on biomass production after 14 days of culture, *C. nivalis* best growth conditions were 5ºC, 20 µmol.m^{-2}.s^{-1} and 5 % of CO_2 resulting in a final cell concentration of 0.68 g.l^{-1} after 14 days of culture with a productivity of 49.4 mg.l^{-1}.d^{-1}. The best conditions for the growth of the mixed culture were 25ºC, 20 µmol.m^{-2}.s^{-1} and 5 % of CO_2, giving 0.46 g.l^{-1} of biomass and a productivity of 33.4 mg.l^{-1}.d^{-1} over 14 days incubation. For both cultures, the main influencing variable was CO_2 in the range tested in this study. The growth models developed point out that under green-house culture conditions an adequate supply of CO_2 would allow cost savings on heating and artificial lighting.

Table 1. Factorial (2^3) Statistical Experimental Design on growth optimization for *Chlamydomonas nivalis* and an *isolated* microalgae consortium with 3 independent variables: carbon dioxide (%), light intensity (µmolm^{-2}s^{-1}) and temperature (ºC) at low, central and high coded units -1, 0, +1.

	Coded units		
Independent variable	Low (-1)	Central (0)	High (+1)
CO_2 (% v/v)	0,04	2,52	5
Temperature (ºC)	5	15	25
Light intensity (µmol photons.m^{-2}.s^{-1})	20	110	200

Methodology

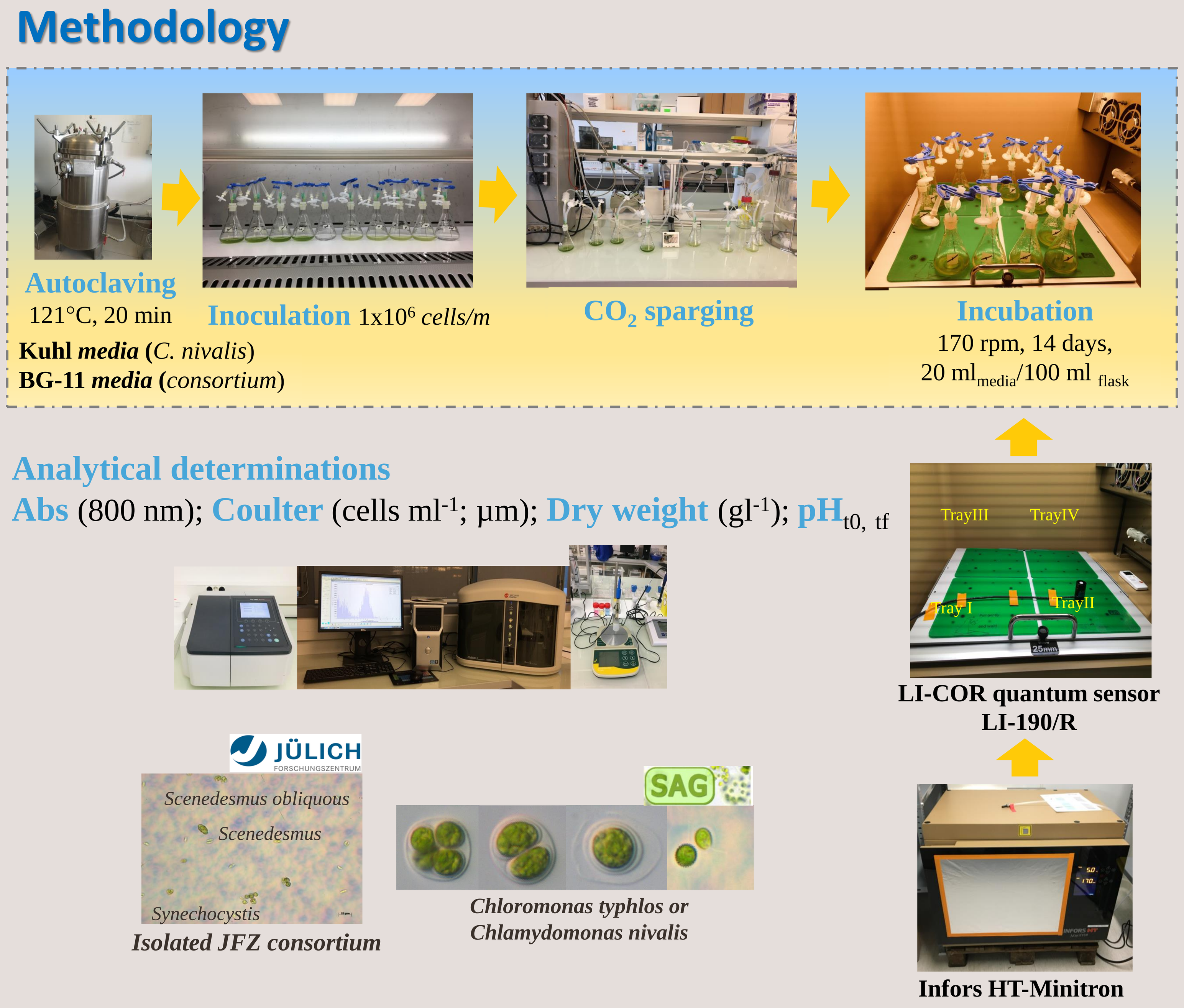


Figure 1. Methodology for *C. nivalis* and the *isolated consortium* for Factorial (2^3) Statistical Experimental Design.

Conclusion

These strains are robust and could grow under green-house conditions in the North West Europe region with minimal heating and relatively low light.

References

Wijffels RH, Barbosa MJ. (2010) An outlook on microalgal biofuels. Science 329:796–799.

Results

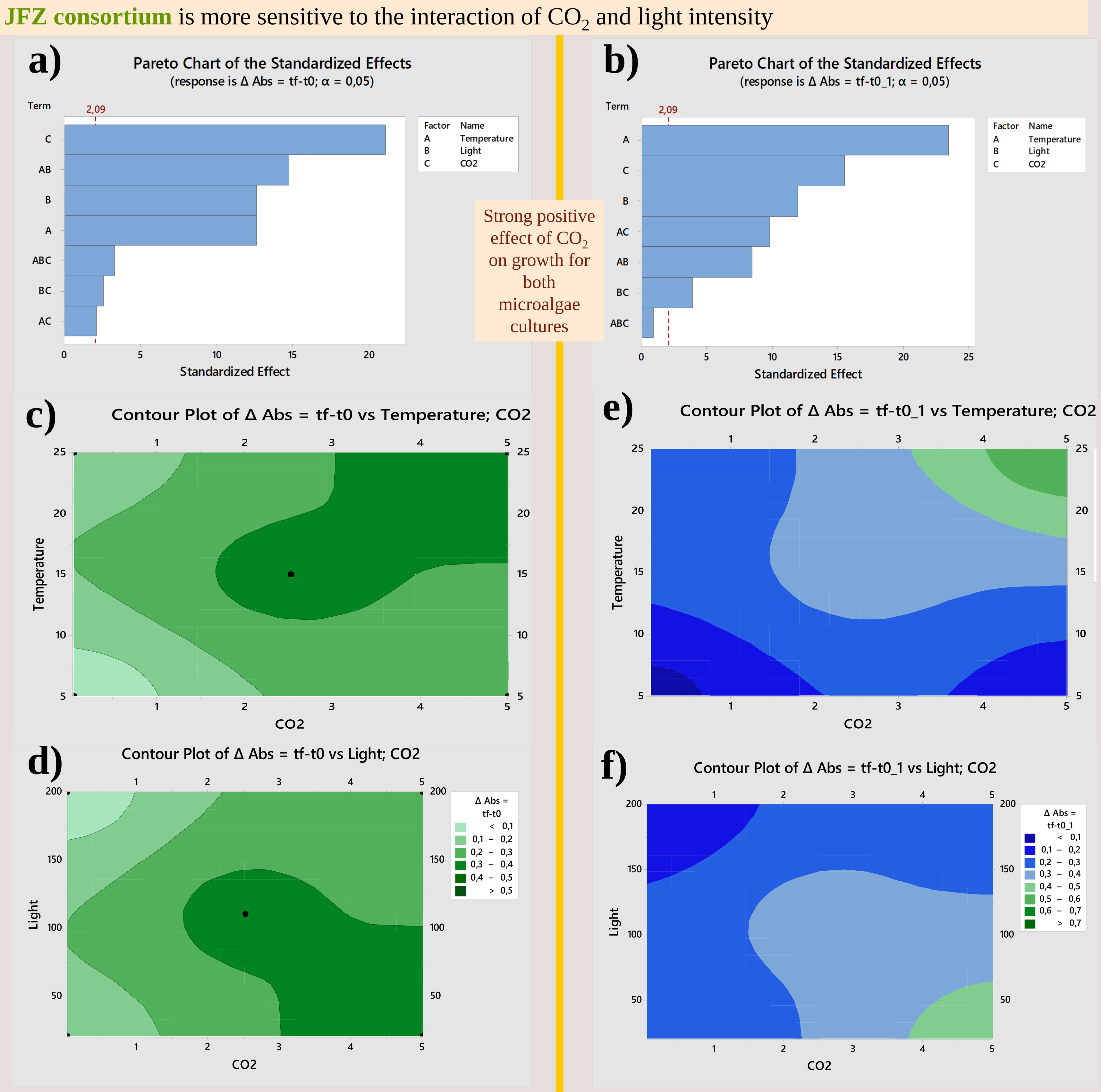
Table 2. Regression equations in uncoded units of carbon dioxide (%), light intensity (µmol.m^{-2}.s^{-1}) and temperature (ºC) with growth after 14 days for *C. nivalis* and the JFZ consortium using a Factorial 2^3 Statistical Design. CtPt= centre points; t_f=final time (14 days).

C. nivalis	Regression equation in uncoded units	% R2
Δ Abs (t_f –t_0)	= -0,0115 + 0,01168 Temperature + 0,000379 Light + 0,02909 CO_2 - 0,000061 Temperature*Light + 0,001172 Temperature*CO_2 + 0,000050 Light*CO_2 - 0,000007 Temperature*Light*CO_2 + 0,1244 Ct Pt	98,3
Dry weight, t_f (gl^{-1})	= 0,3824 - 0,00200 Temperature + 0,000272 Light +0,0607 CO_2 - 0,000025 Temperature*Light + 0,000264 Temperature*CO_2 - 0,000054 Light*CO_2 - 0,000005 Temperature*Light*CO_2 + 0,0607 Ct Pt	93,8
JZF consortium		
Δ Abs (t_f –t_0)	=0,0178 + 0,01378 Temperature + 0,000221 Light +0,00951 CO_2 -0,000051Temperature*Light +0,002670Temperature*CO_2 - 0,000068 Light*CO_2 - 0,000003 Temperature*Light*CO_2 + 0,0660 Ct Pt	98,4
Dry weight, t_f (gl^{-1})	= 0,3171 -0,001738 Temperature – 0,000703 Light + 0,03105 CO_2 – 0,000011 Temperature * Light + 0,000391 Temperature *CO_2 – 0,000180 Light *CO_2 + 0,000008 Temperature * Light * CO_2 + 0,0513 CtPt	98,2

For both *C. nivalis* and the JFZ consortium, there was a:

- ✓ positive effect on growth by CO_2,
- ✓ a slightly negative effect of temperature and light intensity interaction

JFZ consortium is more sensitive to the interaction of CO_2 and light intensity



0,68 gl^{-1} of biomass after 14 days and a productivity of 0,05 gl^{-1}d^{-1} at 5 ºC, 20 µmol.m^{-2}.s^{-1} and 5 % of CO_2

0,46 gl^{-1} of biomass and a productivity of 0,03 mgl^{-1}d^{-1} at 25 ºC, 20 µmol.m^{-2}.s^{-1} and 5 % of CO_2

C. nivalis ***JFZ consortium***

Figure 2. Pareto charts for **a)** *C. nivalis* and **b)** JFZ consortium cultures. **c)** *C. nivalis* Δ Abs *vs* Temperature and CO_2 Contour Plots; **d)** *C. nivalis* ΔAbs *vs* Light intensity and CO_2 Contour Plots; **e)** JFZ consortium Δ Abs *vs* Temperature and CO_2 Contour Plots; **f)** JFZ consortium Δ Abs *vs* Light intensity and CO_2 Contour Plots.
All data was analyzed by the Minitab 19 IOS Software.

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