TIP*, a DOE** based system for microalgae physiological characterization

Bougaran G., Megrier C., Le Déan L., Kaas R., Olivo E., Cadoret J.P.

Introduction

Microalgae are widely used as feed for shellfish, shrimp and fish culture. The former relying on this production the most. One of the shellfish nurseries main expectation concern the quality and quantity of the feed provided to larvae and spat. The challenge to combine high yield with good quality when cultivating phytoplancton needs a good knowledge of the behaviour of the cells as regards to environmental conditions especially temperature, irradiance and pH. If the importance of each factor can be assessed individually, their interaction cannot be tested without the use of experimental design. To identify what factor and combination of factors significantly affect the dependant variables of interest (specific growth rate, maximum cell concentration, metabolic yield, PUFA concentrations) a set of 17 small photobioreactors has been designed and placed in a controled environment. This system meets the requirements of a full factorial central composite design with star points.

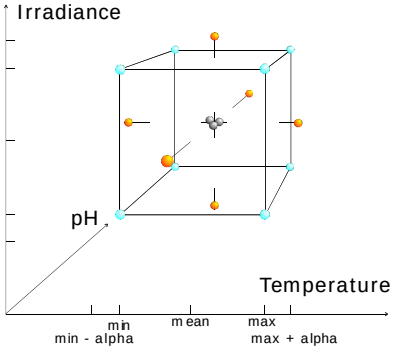


Figure 1: Full central composite design 2^{3*} with star points

Figure 2 : The TIP system

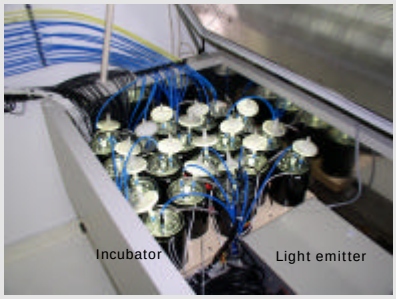


Figure 3 : The TIP system

Material and methods

The central composite factorial design for three independant variables needs at least 8 cube points, 6 star points and center points to be achieved (figure 1). With only three center points this system is not completely orthogonal and rotatable but the design revealed sufficiently robust.

This design pertain to the fitting of response surfaces following the general equation :

$y = b0 + b1 *x1 + ... + bk *xk + b12 *x1 *x2 + b13 *x1 *x3 + ... + bk\text{-}1,k *xk\text{-}1 *xk + b11 *x1^2 + ... + bkk *xk^2$

where x1,.., Xk count for the main effect for the factors, X1*X2, ...,Xk-1*Xk for the interaction and $X1^2,...,Xk^2$ for the quadractic component.

Effects are estimated by means of an ANOVA.

The physical system (figure 2-3), is based on a New Brunswik Innova 4340 shaker-incubator hosting an illuminator delivering five levels of lighting through optical fibers to the transparent bottom of 17 PMMA photobioreactors. Each is fitted with a pH electrode, a 0.22 µ filtered gas inlet and outlet, a type K thermocouple temperature probe and an electrical heating ring encompassing the reactors. A supervision progam controls the data acquisition and the different pH and temperature regulations

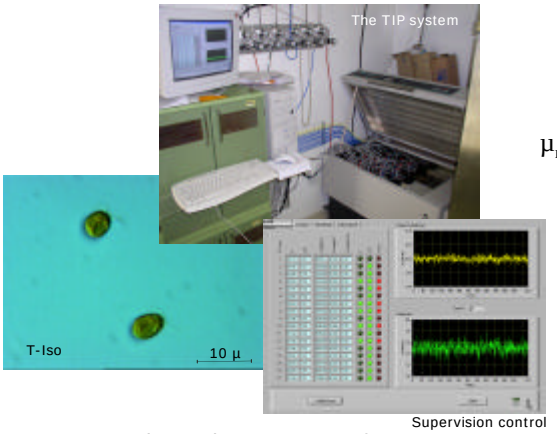
The first trials were made on the small haptophyte *Isochrysis affinis galbana* clone Tahiti, known for its high amount on DHA and widely used in shellfish nurseries and supporting good larvae and spat growth.

Temperatures were tested within the range 19 - 31°C, pH 6.5 - 8.5 and irradiance 250 -1050 $\mu mol.m^{-2}.s^{-1}$. Cell concentration and optical absorbance were followed twice a day for 5 days. Specific growth rate was determined after spline fitting using Matlab software and data analyzed with Statgraphics 5.0 plus.

Results and discussion

All dependant variables led to equal conclusions and light absorbance at 680 nm revealed the best fitting candidate with a model explaining 85% of the global variability at a 5% risk level. Significant interactions (figure 4), were pointed out by ANOVA analysis between temperature and pH as well as between pH and irradiance, meaning that optimizing growth only considering pH would likely lead to erroneous results if temperature and irradiance would not have been fixed at a proper value.

According to the optimization procedure **best growth should be obtained at pH 7, 28°C and under an irradiance of 700 $\mu mol.m^{-2}.s^{-1}$.**



Supervision control

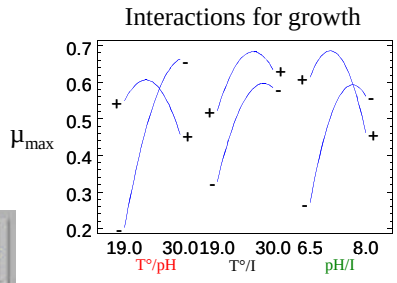
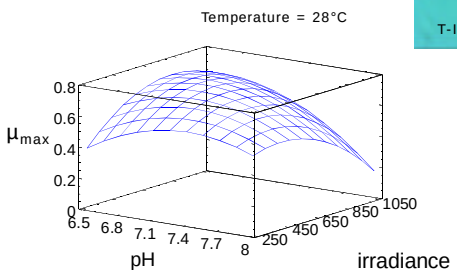
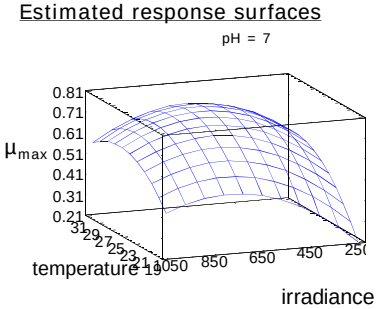
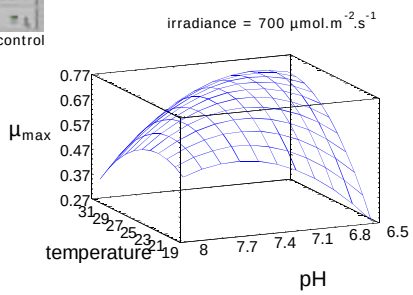


Figure 4 : Interaction plot for the three factors temperature, irradiance and pH. Temperature/pH and pH/Irradiance show significant interactions.



Estimated response surfaces





*TIP : temperature, irradiance, pH

** DOE : Design Of Experiment

Laboratory of Physiology and Biotechnology of Algae
Rue de l'île d'Yeu B.P. 21105
44311 Nantes Cedex 3
http://www.ifremer.fr/drvvppba/