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Optimization of Micro Algal Biomass Production by the Method of Experimental Designs (Case of *Dunaliella salina* Teodoresco)

Koudache Fatiha[1, *], Ait Yala Abdelmadjid[2], Doukani Bachir[1]

[1]*Laboratoire d'éco Développement des Espaces, Université de Sidi Bel Abbés. Algeria;* [2]*Laboratoire LIMPAF, Université de Bouira. Algeria*

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Abstract: *Dunaliella salina* is a unique species of endemophilous microalgae. The objective of this work is to find the best conditions for the development of this microalga by optimizing four main parameters that directly influence the production of its biomass using the experimental design method. This statistical method, which results in the ordered sequence of trials of an experiment, each one acquiring new knowledge by controlling one or more input parameters to obtain results validating a robust model to produce this microalga of the *salines* in the western Algeria (salines of Arzew). For this purpose, this strain was grown under controlled conditions in a photo bioreactor. The results show that the alga *Dunaliella salina* grows and maximizes its yield for well-defined values of the four parameters.

Keywords: Dunaliella salina*, optimisation, biomasse production, parameters,*

Introduction

Algae are chlorophyll organisms that develop in water or in very humid environments. Though mostly abundant in the waters of the seas, lakes, ponds, running waters and thermal springs, they are also found on damp rocks and on land. Exceptionally, they may be endophytes of animal or plant tissues (Ilti, 1980).

Micro-algae and cyanobacteria, whose size varies from micron to hundreds of microns, are organisms that use light as an energy source to fix carbon dioxide (CO_2). Among these micro algae is *Dunaliella salina*, a halotolerant unicellular chlorophyceae that lives in saline waters (salinity close to 350 g /L) (Krinsky, 2005), because of the synthesis of a series of molecules which protect it against the extreme conditions of salinity, temperature and solar radiation.

It is a unique species of endemophilous microalgae, capable of accumulating β-carotene. This pigment of natural origin, which is ten times more active than that obtained by synthesis, is used as a food coloring agent, source of vitamin A, in the human diet and as an additive in cosmetology (Riahi, 2007) Our objective is to optimize the production of microalgal biomass. This production depends on four parameters: temperature, light intensity, salinity and nitrate concentration. For this purpose, we will use the experimental design method (Cochran, 1957).

Materials and Methods

The strain of *Dunaliella salina* used in our work comes from the *Salines* of Arzew. Figure 1. and Figure 2. We have cultivated *Dunaliella salina* for 20 days in a modified and aerated Johnson medium in a flat photo bioreactor shown in Figure.3, with a surface area of 1 m^2 and a thickness of 40 mm and therefore a volume of 40 liters. The starting concentrations are the same for each experiment since we have done a pre-cultivation of *Dunaliella salina* in a cylindrical reactor, the light is ensured by light type LED Day of last generation, in an air-conditioned hangar, all the parameters are controlled by of the probes with the aid of a specific software, while being content with atmospheric CO_2. The biomass is measured every two days in the laboratory using a glass fiber membrane filtration system.

Design of experiments (DOE) is inherently a multi-objective optimization problem (Box, 1951). It enables designers to determine simultaneously the individual and interactive effects of many factors that could affect the output results in any design (Goo, 2011). DOE also provides a full insight of interaction between design elements; therefore, it helps turn any standard design into a robust one (John**,** 1972). Simply put, DOE helps to pinpoint the sensitive parts and sensitive areas in designs that cause problems in Yield. Designers are then able to fix these problems and produce robust and higher yield designs prior going into production. In order to perform a DOE, it is necessary to define the problem and choose the

**Corresponding: E-mail: koudache@hotmail.com, Tel: 213559835291*

variables, which are allied *factors* or parameters by the experimental designer. A design space, or *region of interest*, must be defined, that is, a range of variability must be set for each variable. The number of values the variables can assume in DOE is restricted and generally small. Therefore, we can deal either with qualitative discrete variables, or quantitative discrete variables. Quantitative continuous variables are discretized within their range. The DOE technique and the number of levels are to be selected according to the number of experiments which can be performed. By the term *levels* we mean the number of different values a variable can assume according to its discretization. The number of levels usually is the same for all variables. In experimental design, the objective function and the set of the experiments to be performed are called r*esponse variable.* In this particular case we want to evaluate the effect factors on the biomass production of algae in the reactors in order to optimize the yield.

Figure1. Localization of the *Arzew salines* (Google earth, 2016)

Figure 2. Harvesting of *Dunaliella salina*

Figure3. Photo bioreactor

These factors are; the temperature (A), the light intensity (B), the salinity (C) and the nitrate concentration (D). We assume that testing at two levels of each variable is enough. This means that the process is assumed linear with respect to continuous variables. The levels are chosen as:

Factor A: (-) level is 20 °C and (+) level is 32 °C
Factor B: (-) level is 18000 lux and (+) level is 45000lux.
Factor C: (-) level is 45 gr/l and (+) level is 250gr/l
Factor D: (-) level is 50 mg/l and (+) level is 250 mg/l.

We have thus made 16 experiments which constitute the total of the possible combinations of the four parameters mentioned above. We then apply the experimental design method to these results

Results and Discussion

The number of experimental set ups, corresponding to all the combinations of the four parameters, each of which affected with two levels are presented in table 1. Their number is equal to 4^2, hence 16.
For each set up we record the biomass produced every two days, during twenty days. The obtained results are presented in table 2. We apply the design of experiments to the obtained values of the sixth and sixteenth days. The results of the analysis are presented in table 3.

Table 1. Combinations of all parameters with their two levels

PARAMETRE					UNITE PARAMETRE			
Exp	Temp	Inten, L	Salin	Conc, Nit	°C	Lux	gr/L	mg/L
1	T1	I1	S1	C1	20	18000	45	50
2	T1	I1	S1	C2	20	18000	45	250
3	T1	I1	S2	C1	20	18000	250	50
4	T1	I1	S2	C2	20	18000	250	250
5	T1	I2	S1	C1	20	45000	45	50
6	T1	I2	S1	C2	20	45000	45	250
7	T1	I2	S2	C1	20	45000	250	50
8	T1	I2	S2	C2	20	45000	250	250
9	T2	I1	S1	C1	32	18000	45	50
10	T2	I1	S1	C2	32	18000	45	250
11	T2	I1	S2	C1	32	18000	250	50
12	T2	I1	S2	C2	32	18000	250	250
13	T2	I2	S1	C1	32	45000	45	50
14	T2	I2	S1	C2	32	45000	45	250
15	T2	I2	S2	C1	32	45000	250	50
16	T2	I2	S2	C2	32	45000	250	250

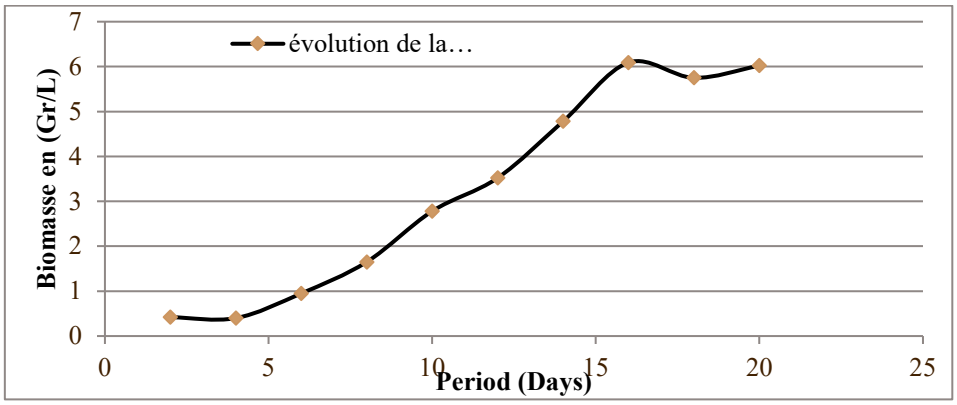

Figure 4. Evolution of the biomass (run n°1)

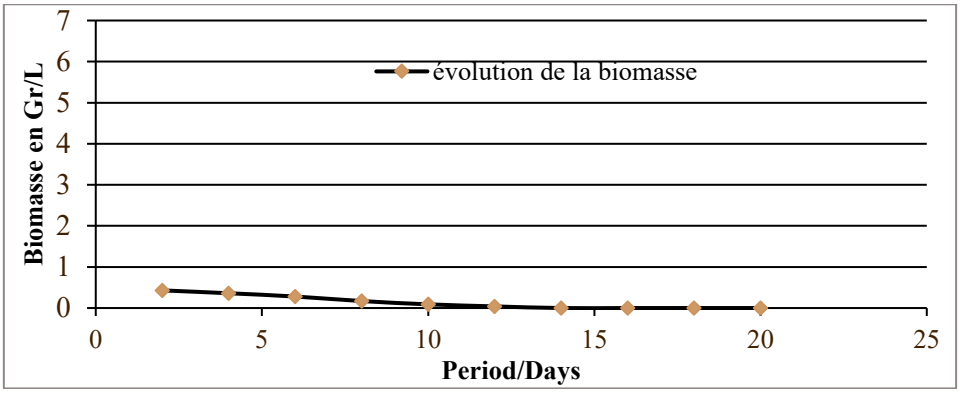

Figure 5. Evolution of biomass (run n 16)

Table2. Results obtained with all the combinations.

Temps/jour	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**
Biomasse en Gr/L For T1 I1 S1 C1	0.42	0.4	0.95	1.65	2.78	3.52	4.78	6.09	5.75	6.02
Biomasse en Gr/L For T1 I1 S1 C2	0.42	0.38	0.55	0.78	1.13	1.42	2.03	2.32	1.98	2.27
Biomasse en Gr/L For T1 I1 S1 C2	0.42	0.38	0.55	0.78	1.13	1.42	2.03	2.32	1.98	2.27
Biomasse en Gr/L For T1 I1 S2 C1	0.42	0.41	0.48	0.51	0.58	0.67	0.69	0.72	0.7	0.69
Biomasse en Gr/L For T1 I1 S2 C2	0.42	0.4	0.42	0.45	0.49	0.53	0.58	0.61	0.68	0.67
Biomasse en Gr/L For T1 I2 S1 C1	0.39	0.4	0.75	1.25	1.65	1.95	2.28	2.63	3.12	3.15
Biomasse en Gr/L For T1 I2 S1 C2	0.39	0.42	0.56	0.79	0.99	1.41	1.78	2.01	1.96	2.01
Biomasse en Gr/L For T1 I2 S2 C1	0.39	0.38	0.41	0.48	0.62	0.58	0.54	0.49	0.42	0.35
Biomasse en Gr/L For T1 IL2 S2 C1	0.39	0.39	0.38	0.36	0.34	0.28	0.26	0.24	0.23	0.19
Biomasse en Gr/L For T2 I1 S1 C1	0.41	0.65	0.92	1.21	1.78	2.5	3.13	3.67	3.82	3.99
Biomasse en Gr/L For T2 I1 S1 C2	0.41	0.43	0.51	0.65	1.01	1.18	1.42	1.68	1.73	1.78
Biomasse en Gr/L For T2 I1 S2 C1	0.41	0.41	0.43	0.46	0.49	0.53	0.57	0.59	0.61	0.58
Biomasse en Gr/L For T2 I1 S2 C2	0.41	0.41	0.42	0.39	0.36	0.32	0.28	0.28	0.27	0.28
Biomasse en Gr/L For T2 I2 S1 C1	0.43	0.41	0.4	0.38	0.38	0.36	0.36	0.37	0.36	0.36
Biomasse en Gr/L For T2 I2 S1 C2	0.43	0.4	0.36	0.34	0.32	0.28	0.28	0.24	0.26	0.25
Biomasse en Gr/L For T2 I2 S2 C1	0.43	0.4	0.4	0.38	0.36	0.31	0.28	0.25	0.25	0.26
Biomasse en Gr/L For T2 I2 S2 C2	0.43	0.36	0.28	0.17	0.09	0.04	0	0	0	0

Table 3. Effets of parameters and their interactions.

	Coefficient		
Factor	**Estimate**	**Low**	**High**
Intercept	-0.65	-0.78	-0.52
A-Temperature	-0.24	-0.37	-0.11
B-Intensité.lumi	-0.26	-0.39	-0.14
C-Salinité	-0.38	-0.51	-0.26
D-Nitrat.Constrat	-0.18	-0.31	-0.056
E-Temps Séjour	0.25	0.13	0.38
AB	-0.14	-0.27	-0.013
AC	0.027	-0.100	0.15
AD	-0.060	-0.19	0.066
AE	-0.25	-0.38	-0.13
BC	0.016	-0.11	0.14
BD	-0.023	-0.15	0.10
BE	-0.26	-0.39	-0.13
CD	-0.048	-0.17	0.078
CE	-0.37	-0.50	-0.25
DE	-0.17	-0.30	-0.048

As we can see the highest yield is obtained with the lowest values of the four parameters. And the lowest yield is obtained with the highest levels of these parameters. Figure 4 and Figure 5 show the evolution of biomass production with respect to time for these two cases.

Let us now analyse the results obtained with the design of experiments procedure. Results presented in Table.2 show that salinity is the most influential parameter (0.38) followed by light intensity (0.26), residence time (0.25), temperature (0.24) and last concentration (0.18). It should be noted that the minus sign (-) indicates that the maximum is reached with the low level of the parameters and vis-versa. At the level of the interactions, the weight of influences is the following in descending order: salinity-residence time (0.37), luminous intensity-residence time (0.26), residence time-temperature (0.25), nitrate-residence time (0.17), and temperature-light intensity. The other interactions have rather a negligible role. It is interesting to note that the interactions that have a significant influence are all related to the period of time. These results are confirmed by the overlay graphs (Khuri, 1987) which clearly indicate that the best combinations correspond to the overlay presented in Figure 6-a and Figure 6-b.

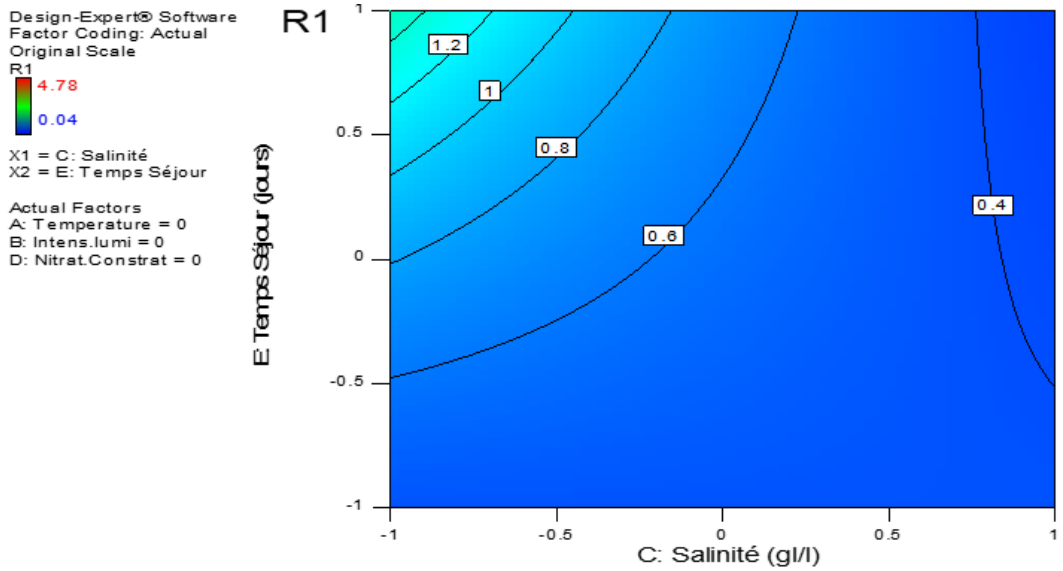

Figure.6-a. Projected yields overlay

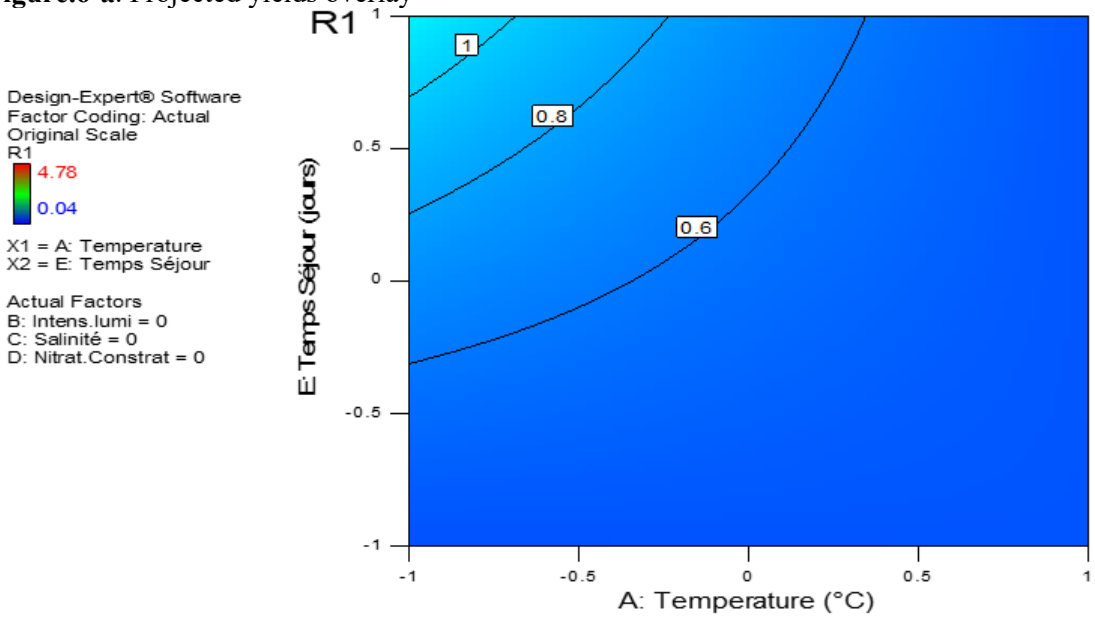

Figure 6-b. Projected yields overlay

These representations constitute the most important tool for determining the best conditions that allow the highest yields. By considering Figure 6-a, we can see that the yield will exceed 1.2 gr/L with the lowest degree of salinity and the medium values (between the lowest and highest values) of the temperature, the light luminosity and the nitrate concentration. However, if keeping the temperature at a lower level is not a big constraint, we can reach almost the same yield with the lowest temperature and medium salinity. This shows how this method allows us to adapt our parameters to existing constraints in order to obtain the desired result.

Conclusion

The optimal growth conditions deduced from the analysis of these experiments are: 20 °C for the temperature, 18000 lux for the luminous intensity, 45 gr / l for the salinity and 50 mg / l for the concentration of nitrate The culture of the microalgae with the optimized conditions confirmed that the maximum responses were reached for the minimum values of the four factors mentioned above. the maximum was recorded at the end of the 16th day for a production of 6.02 gr /L. This also allowed us to determine the factors acting directly on the response (biomass production), their interactions and their actions on the productivity of this alga. The production of biomass is a dynamic operation. We were able to determine the importance of each factor as well as the interactions between them. To improve this study, we will have to carry out a dynamic study that considers the rate of growth, using real-time control of this phenomenon.

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