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Spirulina Production in a Controlled Environment in Niger

Naroua Koure Mamane Kabirou
Halima Oumarou Diadie
Roukaya Abdou Souley
Rabiou Abdou Abdoul Rachid
Balla Abdourahamane
Laboratoire de Recherche en Hygiène et Sciences Alimentaires et Nutritionnelles (LARHSAN), Département Production Végétale (DPV), Faculté d'Agronomie (FA), Université Abdou Moumouni de Niamey (UAM), Niger

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Abstract

Spirulina, an excellent dietary supplement, is an alga very rich in proteins, vitamins and minerals. The cultivation of spirulina requires optimum control of culture parameters (Temperature, pH, Salinity, Lighting) which are most often lower than the optimal production conditions. The objective of this study is to optimize the production of spirulina. To do this, a randomized experimental design was carried out in a greenhouse to control the effect of light and temperature. A total of nine (9) treatments repeated three (3) times were conducted in the greenhouse. The culture media of the different treatments used were composed of natron, salt, NPK fertilizer, urea, lime, iron and ash water. These media are mainly distinguished by the chemical composition linked to the quantitative variation of the inputs used. Thus, the culture parameters (pH, temperature, salinity and density of the culture medium) were recorded. The yield obtained during the experiments was also evaluated. The results from these tests show that there is a great variability in the culture parameters depending on the type of treatment used. Thus, the pH and salinity of the medium fluctuate respectively between 8.95 and 10.16 and 16g/l and 31g/l. The average temperature of the medium varies between

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28.32°C ± 0.72 (n = 189) in the morning and 34.06°C ± 1.71 (n = 189) in the evening. For the daily growth values noted in the media, T1 (control) shows a greater growth from the first (1st) day to the third (3rd) day, with a peak μ = 0.66. On the other hand, this growth becomes relatively low until the seventh (5th) day of the test. However, treatments T4, T5, T6 and T8 showed a more or less linear growth from the first (1st) to the seventh (7th) day of the test. The result indicated an optimal yield of 1.13g/l± 0.13 with treatment T6, which was significantly higher than the yields of the other treatments. However, the lowest yield (0.46g/l±0.08) was observed with treatment T3.

Keywords: Spirulina, Production parameters, controlled environment, Niger

Introduction

Spirulina is a microscopic algae, originally cultivated in the lakes of Chad (among the Kanembous) and in the Texcoco Valley in Mexico (among the Aztecs) (Branger *et al.,* 2003). Spirulina is now considered a food source of high nutritional quality, particularly due to its high digestibility and its high protein content (70%), particularly phycocyanin (Ould bellahcen et al., 2013). It has experienced renewed interest in the scientific community in recent decades (Ould bellahcen et al., 2013). Africa remains one of the continents where spirulina production is lowest despite favorable cultivation conditions (Florent, 2017). In Niger, its cultivation is very little developed and poorly controlled. Its production remains artisanal and is currently carried out in four (4) regions of the country (Niamey, Dosso, Maradi and Agadez). However, it is currently accepted that the production of microalgae, particularly Arthrospira, depends essentially on environmental conditions, which can influence their growth and cause changes in their composition (Pandey, 2011 and Hu, 2004). Thus, to evaluate the productivity of this alga according to the seasons, monitoring was carried out over a period of 12 months (Naroua Koure et al., 2022). After evaluating the yield (g/l) of production according to the different seasons in Niger, it is necessary to optimize production in order to increase the profitability of the crop. It is within the framework of improving spirulina production that this study was conducted in Niger.

Materials and Methods

Materials

Technical Materials

The technical equipment used for this study consisted of:
Twenty-seven (27) basins with a capacity of 40L each for spirulina cultivation; a pH meter to measure the pH of the culture medium; a thermometer to measure the temperature of the medium; a Secchi to measure spirulina density; a 30-micron polyester filtration cloth to intercept insects and spirulina lumps

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during harvesting; fine 0.2 mm mesh metal sieves used during spirulina filtration; a mechanical press to spin dry the spirulina; a SIKA brand manual gun with a capacity of 500 ml to extrude the spirulina in the form of spaghetti; a solar dryer for drying the spirulina.

Laboratory Equipment

The laboratory equipment used consisted of: an electronic balance to quantify inputs; an optical microscope to observe the shape of the spirulina and check for any abnormalities; a pipette to sample solutions from the culture medium for microscopic observation; pH 7 and pH 10 solutions to calibrate the pH meter; distilled water to clean the measuring devices after use; a salinometer to measure the salt content of the culture medium.

Methods

Trial Site

The experimental setup was carried out on the Wright family's private farm (Simplified Cooperative Society for the Production, Processing, and Marketing of Spirulina and Dried Products (COOPROSPI)), established in 2018. It is located in the Communal District V of Niamey, more precisely in the Nogaré district, between latitude 13° 30' 20 and longitude 2° 5' 37 (Figure 1).

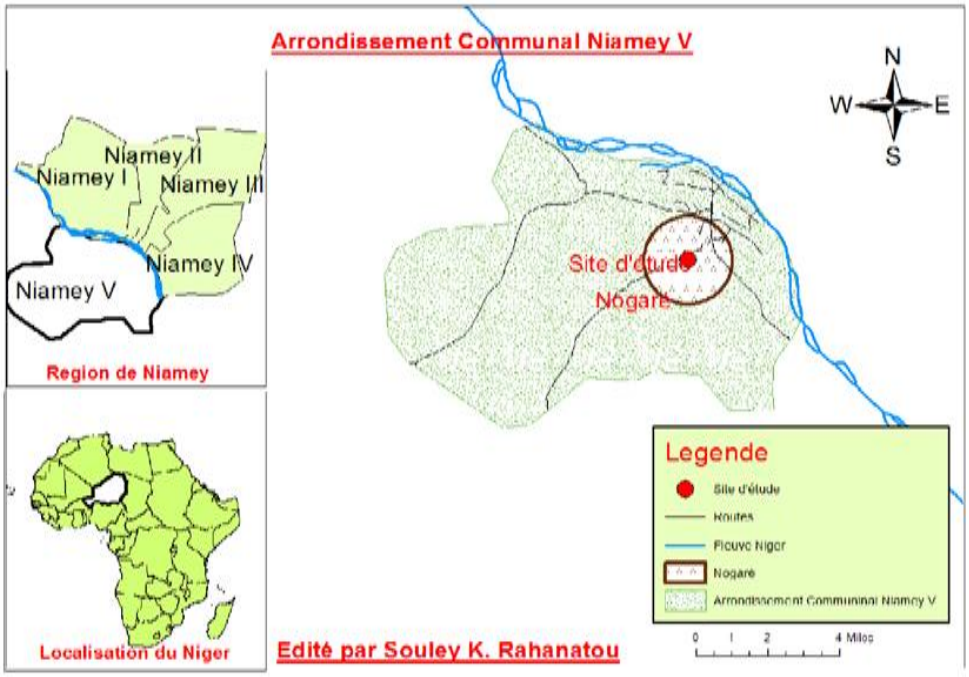

Figure 1: Location of the Wright family spirulina farm

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Experimental design for optimizing spirulina production

The experimental design consisted of a total randomization of 9 treatments repeated 3 times (Figure 2). The experiment was conducted in a greenhouse (Figure 2) to control the effects of light and ambient temperature, which can influence spirulina growth. The treatments used differed from each other in the carbon source (natron and bicarbonate) and the type of salt (table salt and Fogha salt) used (Table IX). The choice of these inputs (natron, bicarbonate, and Fogha salt) was therefore based primarily on spirulina's need for carbon for good growth and also to improve its mineral intake.

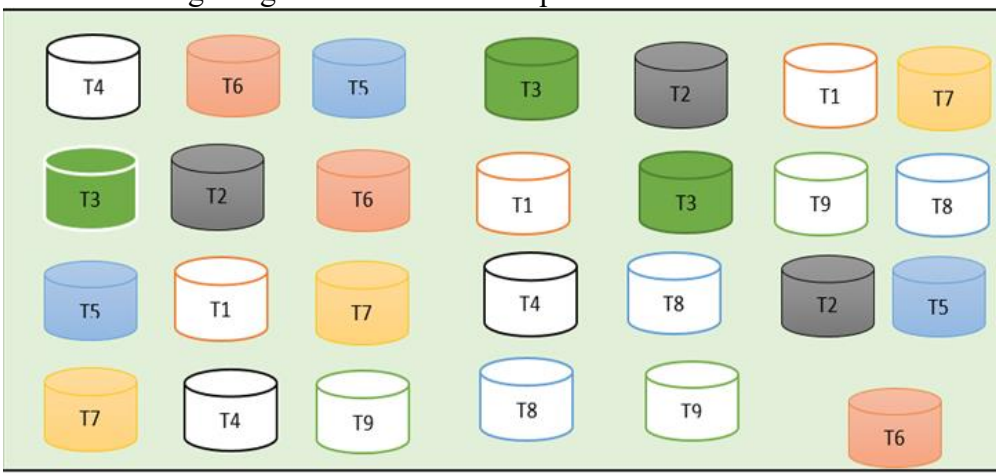

Figure 2 : Plan of experimental device

Chemical Composition of the Growing Media Used for Different Treatments

The growing media chosen for the optimization of production are composed of natron, salt, NPK fertilizer, urea, lime, iron, and ash water (Table 1). These media differ not only in their chemical composition, which is related to the variation in the inputs used, but also in their types. Table 1 summarized the chemical composition of the different treatments and the number of replicates per treatment.

Table 1: Chemical composition of the culture medium according to the treatments

Treatments	*Number of attempts*	*Inputs (g/l)*						
		Natron	White salt	NPK	Lime	Urea	Iron	Ash water
T1 (control)	3	10	7	1	0,01	0,075	0,1	0,5
T2	3	15	7	1	0,01	0,075	0,1	0,5
T3	3	20	7	1	0,01	0,075	0,1	0,5
		Bicarbonate						
T4	3	8	7	1	0,01	0,075	0,1	0,5
T5	3	13	7	1	0,01	0,075	0,1	0,5

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T6	3	18 Natron	7 Fogah Salt	1	0,01	0,075	0,1	0,5
T7	3	10	7	1	0,01	0,075	0,1	0,5
T8	3	15	7	1	0,01	0,075	0,1	0,5
T9	3	20	7	1	0,01	0,075	0,1	0,5

T1: Treatment 1 (control), T2: Treatment 2, T3: Treatment 3, T4: Treatment 4, T5: Treatment 5, T6: Treatment 6, T7: Treatment 7, T8: Treatment 8, T9: Treatment 9

Preparing the brandy for seeding the culture

- The preparation of the brandy for spirulina cultivation was done in three phases:
- The first step was to prepare a natron solution with boiling water. This step allows the culture water to be freed from all the insects hidden in the natron, which are harmful to the growth of spirulina. However, natron can be substituted by bicarbonate. In this case, the bicarbonate solution is prepared from simple water;
- The second phase consisted of filtering the natron solution using a fine 30 µm mesh fabric, this also allows the decanting of natron sludge which is generally troublesome for the culture. In fact, this operation allows obtaining a clear solution more favorable to the growth of spirulina;
- The third phase consisted of adding salt, NPK and urea to the natron and/or bicarbonate solution.

Thus, water of life can be used for spirulina cultivation. However, iron, lime, and ash water were added dropwise after spirulina seeding.

Preparation of the Iron Solution

The iron solution used was obtained by dissolving 50 g of ferfol in 1 liter of distilled water. Lemon juice is also added to facilitate iron absorption by the spirulina (Jourdan, 2012).

Preparation of the Ash Solution

The ash water solution used was prepared by dissolving 1 kg of Mangifera indica L (mango tree) ash in 5 liters of water. After preparing the mixture, the solution is allowed to settle and then filtered using a fine-mesh cloth (Jourdan, 2012).

Seeding

The cuvettes were seeded in a proportion of one-third (1/3) spirulina strain and two-thirds (2/3) brandy. Thus, for one (1) liter of strain solution, two liters of brandy were added to the cuvette.

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Monitoring Production Parameters

Spirulina production parameters, namely temperature, pH, salinity, and spirulina concentration/density, were measured three times per day: in the morning at eight (8) o'clock, at noon at twelve (12) o'clock, and in the afternoon at five (17) o'clock. Particular attention was continually paid to the color and odor of the spirulina. pH and Temperature Measurement

The pH of the culture medium was measured directly using a pH meter previously calibrated with a pH 7 reference solution and a pH 10 solution. This pH meter also allows the temperature of the medium to be determined (Figure 3).

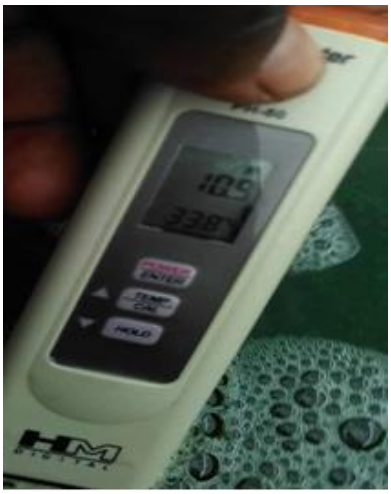

Figure 3: pH meter measurement of the medium

Spirulina Concentration/Density Measurement

Spirulina concentration was determined using a Secchi disk, which allows for rapid estimation of cell density cultured in an aquatic environment. The method involves stirring the culture medium with a wooden spatula to homogenize the medium, allowing the cells access to nutrients and light, and then allowing it to stabilize for a few minutes.

Then, the Secchi disc (figure 4) is immersed in the medium. The depth, in centimeters, from which the disc can no longer be seen once immersed in the medium is noted. This operation makes it possible to assess the concentration of spirulina in the medium.

Figure 4 : Dry Disks

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Spirulina Growth Rate

The growth rate μ was used to describe the evolution of spirulina growth over time. This rate is calculated from the equation described by Baourab et al., 2002:

$$\mu A = \frac{A_2 - A_1}{T_2 - T_1}$$

With A2 and A1 representing the density (Secchi) at times T1 and T2.

Salinity Measurement

The salinity of the culture medium was measured using a salinometer. The method involves taking one (1) to two (2) drops of the previously homogenized culture and placing them on the prism of the salinometer. The value (g/L) is directly observed through the eyepiece. However, the salinometer must be calibrated beforehand.

Morphological Monitoring of Spirulina

The shape and color of spirulina vary depending on the physical and chemical characteristics of the environment in which it grows. Therefore, to monitor its morphology, microscopic observation (Figure 5) was performed. This observation allows us to assess the shape and development of spirulina in its culture medium.

Figure 5 : Light microscope

Spirulina Harvest

Harvesting was carried out using two sieves with different mesh sizes (Figure 6): the first sieve (A) with a 300 μ mesh size allows for the interception of foreign matter such as insects, larvae, leaves, sludge, or spirulina lumps. The second sieve (B) with a finer mesh size (30 μ) is used to retain the spirulina.

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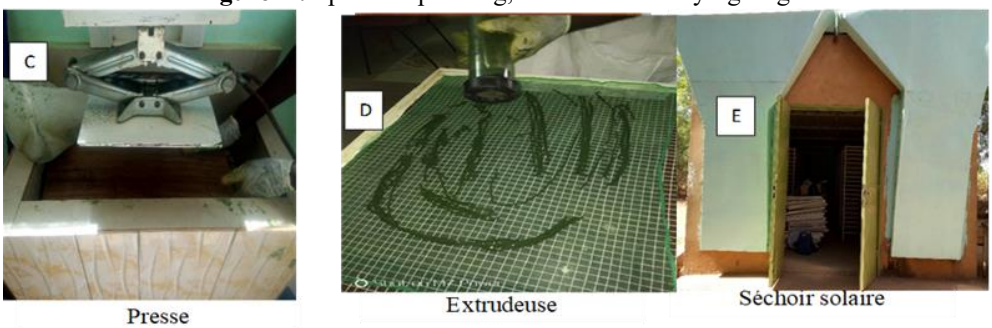
Figure 6 : Spirulina harvesting device

Pressing, Extruding, and Drying

Pressing was performed using a mechanical press (C), thus removing as much water as possible. The biomass resulting from the pressing was weighed, extruded (D) into spaghetti shapes, and distributed onto drying racks (Figure 7). The extruded spirulina was dried in a solar dryer (E). Drying time lasts a maximum of four (4 to 6) hours.

Figure 7 : Spirulina pressing, extrusion and drying stage



Source: Naroua, 2021

Data Processing and Analysis

The collected data were entered into Microsoft Excel 2019 and processed using R 3.6.0 software to determine the different average spirulina growth rates and perform correlation tests. Tables and graphs were also created using Microsoft Excel and Word 2019.

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Results and Discussion

Results

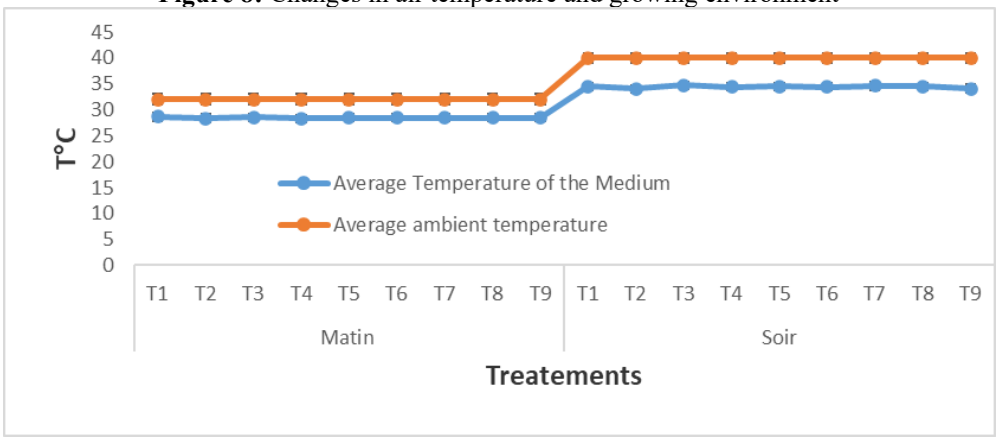
Physical and Chemical Parameters

Spirulina is an algae that thrives in an alkaline environment. Hence, the need to measure the parameters that can influence the growth spirulina. Therefore, for this study, temperature, pH, salinity, and density were measured throughout the experiment.

Temperature as a Function of Treatment

Analysis of Figure 10 indicated that the outside temperature determines the pond water temperature. Thus, the average daily environmental temperatures recorded according to the different treatments range from 28.32°C ± 0.72 (n=189) in the morning to 34.06°C ± 1.71 (n=189) in the evening. Ambient air averages recorded during the same test period ranged from 32.01°C ±1.78 (n=189) to 40°C ±0.12 (n=189).

Figure 8: Changes in air temperature and growing environment



T_1: Treatment 1(witness), T2: Treatment 2, T3: Treatment 3, T4: Treatment 4, T5: Treatment 5, T6: Treatment 6, T7: Treatment 7, T8: Treatment 8, T9: Treatment 9

pH as a function of treatment

The evolution of pH during spirulina growth in the different media tested (Figure 11) is manifested by its progressive increase from day 1 to day 5 for all treatments. However, from day 5, the pH remains stable until day 7 for all media tested.

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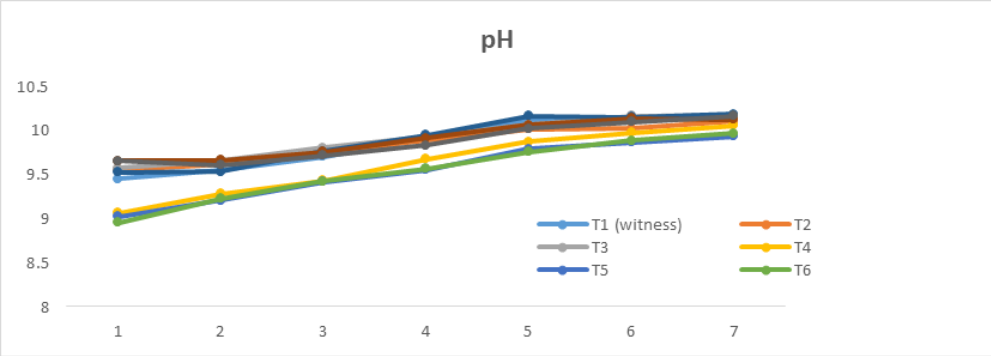

Figure 9: Evolution of pH depending on treatments

Salinity as a Function of Treatment

Figure 12 shows the variation in salinity as a function of treatment. Thus, the result showed differences in level of salinity according to treatments; it varies from 16g/L to 31g/L. This salt content remains stable from day 1 to day 2 of culture for all treatments.

Thus, a gradual increase in the salinity of the media (T1, T2, T3, T4, T6, T7, T8, T9) is observed from day 3 to day 4, with the exception of treatment T5 where this increase continues until day 5. A decrease in salinity level is observed from day 4 (day 5 for T5) until day 7 of monitoring.

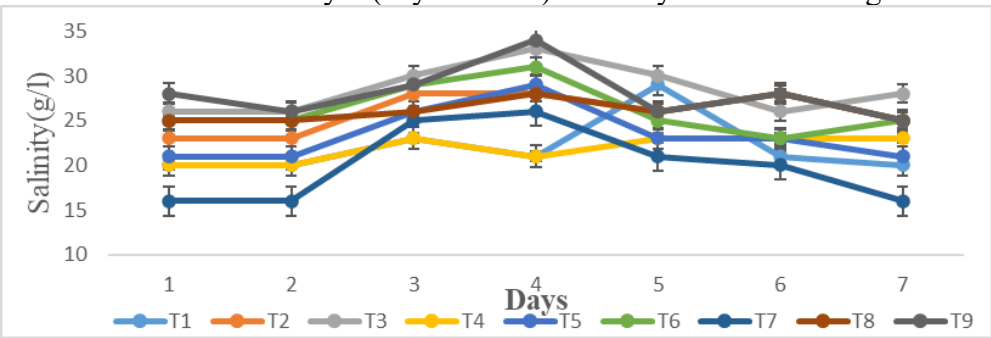

Figure 10: Variation of salinity as a function of treatments

Spirulina Growth as a Function of Treatment

Figure 13, relating to the different treatments, highlights greater growth between the 1st and 3rd days in the control medium (T1) compared to the other treatments, but from the fourth (4th) day, this growth becomes relatively low until the seventh (7th) day of the trial. However, treatments T2 and T3 exhibit the lowest growth rate throughout the trial period (from day 1 to day 7).

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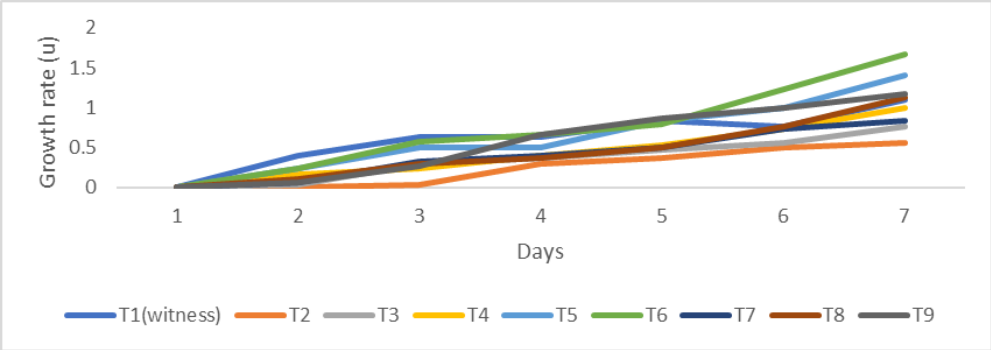

Figure 11: Evolution of spirulina growth rate depending on treatment

Nevertheless, treatments T4, T5, T6, and T8 generally exhibit the same growth trends, although variability is observed depending on the growth rate. Furthermore, treatment T6 exhibits the highest growth peak (μ=1.66), recorded on days 5 and 7.

Furthermore, the variation of treatments has a significant influence on the average daily growth of spirulina (P-value=0.00). Thus, treatment T6 presents the best average growth (0.84) recorded during the experiment (Table II). Indeed, the average growth of treatments T4 (0.56), T8 (0.71) and T3 (0.49) does not present a significant difference, while they are statistically different from all other treatments. Furthermore, the result, observed with treatment T2 presents the lowest average growth (0.3).

Table 2: Influence of treatments, time (in days) and time of day

Treatements	*Average Growth ± SE*	*N*	*Min*	*Max*	*P-value*
T6	0.84 ± 0.09 [a]	42	0.00	2.20	0,000 ***
T5	0.72 ± 0.07 [ab]	42	0.00	1.60	
T1(witness)	0.71 ± 0.05 [a]	42	0.00	1.50	
T9	0.66 ± 0.07 [ab]	42	0.00	1.70	
T4	0.56 ± 0.06 [abc]	42	0.00	1.60	
T8	0.53 ± 0.06 [abc]	42	0.00	1.50	
T3	0.49 ± 0.05 [abc]	42	0.00	1.20	
T7	0.43 ± 0.05[bc]	42	0.00	1.10	
T2	0.30 ± 0.04[c]	42	0.00	1.00	

Values not having the same superscript letter in the same column are significantly different (P < 0.05).

In addition, the analysis of the evolution of spirulina growth over a week was presented in Table 3

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Table 3: Average growth of spirulina in the 7 days of the week

Days	*Average Growth* ± SE	N	Min	Max	P-value
j7	1.19 ± 0.05^{a}	54	0.50	2.20	
j6	0.91 ± 0.04^{b}	54	0.40	1.50	
j5	0.72 ± 0.03^{c}	54	0.20	1.20	
j4	0.54 ± 0.03^{d}	54	0.10	1.10	0.00 ***
j3	0.41 ± 0.03^{e}	54	0.00	1.00	
j2	0.25 ± 0.03^{f}	54	0.00	1.00	
j1	0.06 ± 0.02^{g}	54	0.00	0.50	

Values not having the same superscript letter in the same column are significantly different ($P < 0.05$).

The analysis of Table 3 shows spirulina growth from the first day (0.06) to the seventh day (1.19) of the experiment.

In general, time has an effect on the average growth of spirulina (P-value = 0.00). The results obtained revealed that all average growth rates were statistically different from one day to the next.

Also, the analysis of spirulina growth by time of day (morning and evening) did not show any significant difference (Table 4) regardless of the treatment. However, in general, the observed spirulina growth is significantly higher in the evening, with an average growth rate of 0.66.

Table 4: Spirulina growth according to time of day (morning and evening)

	Croissance de la Spiruline selon le moment du jour		
Treatements	Morning	Evening	P-value
T1(witness)	0.62 ± 0.35^{a}	0.81 ± 0.33^{a}	0.08 ns
T2	0.25 ± 0.24^{a}	0.35 ± 0.28^{a}	0.22 ns
T3	0.43 ± 0.36^{a}	0.55 ± 0.35^{a}	0.33 ns
T4	0.47 ± 0.36^{a}	0.65 ± 0.42^{a}	0.22 ns
T5	0.63 ± 0.46^{a}	0.81 ± 0.45^{a}	0.20 ns
T6	0.73 ± 0.58^{a}	0.94 ± 0.58^{a}	0.24 ns
T7	0.40 ± 0.34^{a}	0.45 ± 0.31^{a}	0.67 ns
T8	0.45 ± 0.40^{a}	0.61 ± 0.47^{a}	0.27 ns
T9	0.57 ± 0.47^{a}	0.74 ± 0.49^{a}	0.25 ns
Mean	0.51 ± 0.42^{a}	0.66 ± 0.45^{b}	0.001 **

Values not having the same superscript letter in the same column are significantly different ($P < 0.05$).

Normality (T1 : P = 0,107, T2 : P = 0,000, T3 : P = 0,001, T4 : P = 0,017, T5 : P = 0,007, T6 : P = 0,049, T7 : P = 0,008, T8 : P = 0,001, T9 : P = 0,001 ; T1-T9 : P = 0,000)

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Table 5 : Influence of physicochemical parameters (pH, environmental and ambient temperature and salinity) on growth

Parameters	*Coefficients*	*T-statistic*	*P-value*
Constant	-13.11	-3.41	0.000 ***
pH	0.46	7.00	0.000 ***
Midium temperature	0.07	7.87	0.000 ***
Ambient temperature	-0.05	-7.08	0.000 ***
Salinity	8.41	2.24	0.02 *

pH, temperature of the medium, ambient temperature and salinity have an influence on the growth of spirulina (P-value = 0.000) (Table 5). This influence is less strong with salinity (P-value = 0.02) than with the other parameters (pH, temperature).

The analysis of Table 6 shows that treatment T6 has the best yield (1.13g/l± 0.13) and is statistically different from the other treatments. The treatments T8, T9 and T1, T7 are not statistically different. However, the lowest yield (0.44± 0.08) was observed with treatment T3.

Table 6 : Dry biomass as a function of treatments

Treatments	*Dry biomass (g/l) ± SE*	*Min*	*Max*	*P-value*
T6	1.13 ± 0.13^{a}	1.05	1.23	0.000 ***
T5	$0.93 \pm 0,01^{b}$	0.90	0.97	
T8	$0.84\pm 0,02^{bc}$	0.73	0.93	
T9	$0.80 \pm 0,08^{bc}$	0.72	0.88	
T4	$0.76\pm0,03^{bcd}$	0.67	0.84	
T1(witness)	$0.72 \pm 0,09^{cd}$	0.63	0.88	
T7	$0.69 \pm 0,01^{cd}$	0.68	0.71	
T2	$0.59\pm 0,1^{de}$	0.58	0.61	
T3	$0.44\pm 0,08^{e}$	0.43	0.47	

Values not having the same superscript letter in the same column are significantly different ($P < 0.05$).

Discussion

The results from the optimization of spirulina production show that the pH values of the medium measured according to the treatment fluctuate between 8.95 and 10.16. Furthermore, they are relatively low in all culture media tested with bicarbonate (8.95 to 10.12) compared to the other treatments (9.65 to 10.16). This difference can be explained not only by the specific chemical composition of each treatment but also by the slow solubility of bicarbonate compared to natron. Furthermore, an increase in pH was observed from the 1st to the 5th day, regardless of the treatment. This change may be due to a reduction in carbon dioxide associated with photosynthetic activity related to spirulina growth. These same findings were also reported by Bellahcen *et al*. (2013) and Kanon et al. (2016). According to Jourdan (2012),

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a good growth test for a spirulina culture is an increase in its pH. According to Doumandji *et al*. (2012), an increase in pH represents a positive indicator of photosynthetic efficiency in Arthrospira platensis.

Salinity also plays an important role in reducing the risk of contamination. The salinity obtained for this culture varies between 16 g/l and 31 g/l depending on the treatment. This difference can be explained by the variable chemical composition of each treatment. According to Saggai (2008), salinity is a limiting abiotic factor in spirulina culture. It promotes growth, provided that it does not exceed the optimal development range, which is 30 grams of salt per liter, with a minimum salinity of 13 grams of salt per liter. According to Fox (1999), the average is between 22 and 60 g/l.

The average temperature of the medium recorded for the different treatments ranged from 28.32°C ± 0.72 (n = 189) in the morning to 34.06°C ± 1.71 (n = 189) in the evening, the latter being close to the optimal temperature (37°C) for spirulina production. According to Jourdan, 2012, the first reference temperature is roughly the same as in humans, 37°C: the ideal growth temperature. It is also higher than the highest average temperature (30.46°C) recorded during production trials Naroua Koure et al. (2022). This result confirms that the greenhouse has an effect on the variation of the average temperature of the environment. The lowest average recorded during these tests was 28.32 ± 0.7 (n = 189), well above the lower tolerance limit of 20°C (Jordan, 2012).

Regarding spirulina proliferation according to the different treatments, the control medium (T1) showed greater growth from the first (1st) day to the third (3rd) day, with a peak $\mu = 0.66$. However, it became relatively low until the seventh (5th) day of the test. This result could be explained not only by the faster depletion of mineral elements in this medium, but also by the fact that natron dissolves more quickly in water, which facilitates easier access to the mineral elements essential for spirulina growth. This study shows that treatments T4, T5, T6 and T8 showed more or less linear growth from the first (1st) to the seventh (7th) day of the test. These results allow us to say that in these environments, the carbon source necessary for the growth of spirulina is sufficient throughout the experimentation period.

According to Zarrouk (1966), cyanobacteria use the mineral elements present in these environments to ensure their growth. In addition, this study showed that the treatments have a significant effect on the average growth of spirulina (P value = 0.000). This is not statistically different depending on the time of day (morning and evening) and regardless of the treatment. But overall, the observed growth of spirulina is significantly higher in the evening, with an average of 0.66. The statistical analysis result shows that the optimal yield obtained is 1.13g/l± 0.13 (treatment T6) and it is statistically superior to the other treatments. Also, this result is also superior to those obtained during

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spirulina production trials according to the different seasons in Niger, whose average yield obtained is 0.46g/l±0.08 with a maximum 0.53g/l±0.03 during the hot dry season Naroua Koure et al. (2022).

Conclusion

The results of this study show that spirulina growth varies depending on the type of treatment applied to the brandy. Thus, spirulina growth is greater in the control medium during the first three (3) days of cultivation, but gradually decreases until the seventh (7th) day of the trial.

Furthermore, statical analyses showed that overall spirulina growth varies from the first to the seventh day of production. However, no significant difference was recorded between growth rates over time (in days). The results of this study reveal that treatment T6 has the best yield (g/l) compared to all other treatments.

However, it is necessary to continue production trials with the T6 treatment and possibly carry out all the additional chemical and biochemical analyses in order to establish a scientific basis for the nutritional quality of the product resulting from this treatment.

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