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Spirulina microalga: A Food for future

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ABSTRACT

Spirulina is a microscopic algae, known also as blue-green algae. It belongs to the family of *cyanophyceae*. These beings are original and very enigmatic: they feed themselves by photosynthesis like plants but their cells have no cellulosic membrane like bacteria (which explains their very high digestibility, about 83%). They appeared very early on Earth, more than 3 billion years ago. By their intense production of oxygen they considerably modified the atmosphere on Earth so it became possible for organic life to emerge. In this paper were emphasized the nutritional and pharmaceutical values of this algae. The economic role and the possibilities for artificial culture of *Spirulina* in a southern environment are analyzed. Then a survey of other uses of *Spirulina* is suggested. Furthermore, thermophysical studies of *Spirulina* were performed in order to understand its behavior during drying and the possibilities of riches generation by commercializing these algae for Chad. The production of *Spirulina* is very simple and it can be mastered by any peasant which is very interesting for developing countries.

Keywords: microalgae, *Spirulina*, protein, malnutrition, thermophysical characteristics, biotechnology, therapeutic properties.

1. Introduction

Developing countries face today, major problems on all domains: economic, political, financial, etc. Most countries of the African continent experience armed rebellions which imply political instability and economic stagnation.

Diseases, malnutrition and other corollaries of underdevelopment are visible everywhere. The infant mortality due to food deficiencies in the world is 33000 children from 0 to 5 who die because of malnutrition (most of them in Africa). This means that more than 12 million children die per annum (FOX R. D. & MANIN, 2000). KWASHIORKOR is the disease most frequently met. This disease is due to deficiencies in proteins, vitamins and oligo-elements.

2. Material and methods

2.1. Material

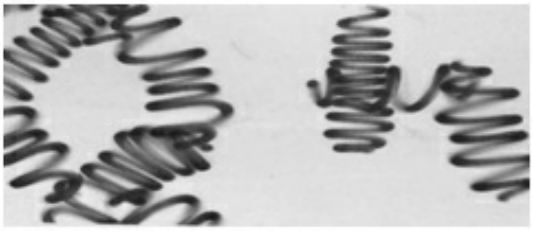

Fig. 1: Image of Spirulina trichomes seen under microscope *(x40, source Fox, 1999)*

In Fig.1 are shown Spirulina trichomes seen under microscope.

The first observations of the blue algae, known today as *Spirulina*, go up to the 16th century at the time of southern America Spanish conquest (FOX R. D., 1996) and the first scientific studies go up to the 19th century (FOX, R.D., 1999). Since then, and until our days, the interest of Man for the algae does not cease growing. Today, there is more than 3000 quotations of *Spirulina* in the specialized scientific literature and Internet. This passion for the blue-green algae is explained by the world food crisis on one hand, and the consumers mistrust towards genetically modified organisms, on the other hand. In addition, being an inexhaustible source of proteins, it constitutes an alternate source in the fight against malnutrition, the diseases due to food deficiencies, as much of other diseases (Sironval C., 1993).

Certain studies show that *Spirulina* is effective against certain cancers (cancer of thyroid for example) and many other immune system diseases (Gustafson K. R., 1989). Moreover, the microalgae produces a blue pigment-phycocyanin- which is not only an excellent substitute in the industry of the blue dye, but has remarkable therapeutic properties (Ijima et al, 1980). *Spirulina* can hide much more other not yet explored "treasures". It has been, moreover, the subject of a very significant number of works. *Spirulina* did not deliver all its secrects yet, considering the last medical applications in the treatment of various diseases, as well as the use of *Spirulina* as basic food for long duration

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space missions (MORIST A. et al, 2001). First, let us see how R. D. Fox tells us about the appearence of *Spirulina* on Earth.

"Just for a moment, let us return in the past at a very hot day, perhaps 3,5 billion years, certainly more than 2,8 billion years ago, when something arrived to a rather large bacterium, occupied digesting carbon dioxide of volcanic origin, sulphur, phosphorus and some metallic ions varied and tasty from the doubtful water of the puddle pool where it was. At zenith, a pale sun was shining through a yellowish atmosphere of methane, hydrogen, nitrogen and carbon dioxide. Something occurred! Most probably, a package of ultraviolet photons, after having avoided collision with gas molecules while crossing this atmosphere, strike our bacterium in a significant point of its desoxyribonucleic acid (DNA) chain; this acid commanded the cell to grow by extracting carbon, hydrogen, water oxygen and carbon dioxide with the assistance of solar energy (FOX R. D., 1999). The oxygen atoms in excess were gathered per pairs and were driven out through the membrane of the cell. Our bacterium was diverting itself seeing the small oxygen bubbles escaping, bursting the surface of water with a "plop" and an iridescent sun flash " (FOX R. D., 1996). However, the blue algae are very different from the other algae whereas they have much in common with bacteria. *Spirulina* is neither a bacterium, since it nourishes itself by photosynthesis like a plant, nor a plant since it does not have definite cytoplasm. It is to some extent an intermediate being between vegetable and animal world.

2.1.2. Biochemical composition

Proteins: The proteins form the major part of the dry mass (60% to70%) with a spectrum of acid similar to that of other micro-organisms. Phycocyanin is a chromoprotein that one meets in the algae in two forms (the c-phycocyanin and the allophycocyanin), which are characterized by their respective molecular weights. Their contents vary from 15 to 20% of the algae dry weight. Moreover, acting like a light collecting antenna, phycocyanin seems to be used as storage material for nitrogen. The ribulose 1-5 diphosphate carboxylase makes approximately 12% of the dry weight.

Carbohydrates: The carbohydrates form approximately 14% of the alga dry weight.

Lipids: when they are extracted using methanol and chloroform as a mixture, true lipids form 6% to 7% of the dry weight. The fatty acids and the insaponifiables respectively account for 83.1% and 16.9% of the total quantity of lipids. The insaponifiables fraction consists in sterols and triterpenic alcohols. Sterols represent 1% to 5% of insaponifiables, cholesterol being the essential component. triterpenic alcohols account for 5% to 10% of the insaponifiables, made up mainly of alpha-amyrin.

Nucleic acids: The nucleic acids are present within the limits from 2.83 to 4.5% of the dry weight. The ribonucleic acid represents the most significant part of the nucleic acids (2.2 to 3.5% of the dry weight). The desoxyribonucleic acid is present within the limits from 0.63 to 1% of the dry weight.

Ashes: The fraction of ash lies between 6.4 and 9% of the dry weight and its composition depend on the conditions of culture. The analysis of ashes often shows the presence of potassium, phosphorus, chlorine, magnesium, calcium, iron and other elements. It is interesting to notice that the chemical concentrations of elements in the dry biomass do not reflect at all their concentrations in the culture medium. In table 1, is represented the amino acids content of *Spirulina* obtained by chromatography, for the nitrogen based method is not exact enough.

Table 1: amino acids content of *Spirulina* from two sites in Chad.

Composition in essential amino acids	**Spirulina Mbodou (%)**	**Spirulina Touffou (%)**
Threonin	4.47	4.52
Valin	7.94	8.24
Methionin	0.08	0.08
Isoleucin	5.71	5.73
Leucin	9.92	10.17
Lysin	1.47	1.53
Phenylalin	3.54	3.65
Histidin	4.25	4.54
Arginin	5.5	5.52
Composition in nonessential amino acids		
Aspartic acid	9.42	9.91
Serine	4.55	4.66
Glutamic acid	15.14	13.77
Glycin	9.47	9.64
Alamin	12.56	12.67
Cystin	0.07	0.04
Tyrosin	1.18	1.14
Homoserin	0.4	0.8
Prolin	4.33	4.39

2.1.3. *Spirulina* as a food

Usually the algal proteins are slightly used when mono gastric organisms animals or human, are fed with intact cells. The blue-green algae in general, and *Spirulina* in particular, are unique in this situation because they are highly digestible. According to *Becker & Venkataraman*, (Becker E. W., 1982) only small differences are observed between the digestibility of fresh *Spirulina* (82%), sun dried *Spirulina* and that dried by congelation which have a digestibility of 65% and 70% respectively; differences in digestibility even smaller between various forms of Spirulina (fresh or dried) were uncovered by *Hernandez & Shimada* (Hernandez G. T., 1978).

Spirulina was tested in experiments of animals feeding. The simplest method to evaluate proteins in tests of animals food, consists in determining the proteins effectiveness ratio (PER). (Becker et al, 1976) compared the PER of *Scenedesmus* with that of *Spirulina* using various methods. As expected , the values of the PER of sun dried *Spirulina* are higher than those of sun dried *Scenedesmus*. The values of the PER of sun dried *Spirulina* are lower than those of the PER of *Scenedesmus* dried in a drum drier, thus showing the value of this method of drying. Of course, the PER of *Spirulina* dried in a drum drier is higher than that of freeze-dried *Spirulina*. The value of the PER can appreciably vary in the case of *Spirulina* produced for commercial goals. A study shows that the PER of *Spirulina* coming from Mexico is 2.20 whereas that of a sample coming from a different source is only 1.86 (Becker E. W., et al, 1976). The biological value (BV) of *Spirulina*, defined as the ratio of the quantity of absorbed nitrogen on the total quantity of provided nitrogen, is as high as 79.5% for sun dried *Spirulina*, to which methionine is added, compared with 87.7% for casein (Becker E. W. et al, 1982).

There is another method to test the nutritional value of a source of proteins: it is the cycle of depletion-repletion, in which guinea-pigs (for example of rats) are initially underfed and then provided with sufficient quantities of food, are tested. In an experiment, the regeneration of the enzymatic activity is highest by using a mode rich in casein, but the group fed by methionine enriched *Spirulina*, shows almost approximately the same results (Becker E. W. et al, 1982).

Spirulina is very well appropriate as a dietetic supplement (Nandeesha M. C. et al, 2001). In short, it was shown that *Spirulina* is a satisfactory source of proteins for much of animals. It is particularly useful as food supplement and has a lowering effect of cholesterol level. In 1963, the French Petrolium Institute had been interested in news about "*dihé*" local name of *Spirulina*, which the populations living in the area of Lake Chad in central Africa (Chad) consume. One decade later, Jean Léonard, a Belgian botanist crossing the Sahara, discovered independently *dihé* and brought back the techniques used by Kanembou for the production of this algae (Ciferri O., 1983). For Kanembou, *dihé* is frequently consumed. According to season, one finds it in almost ten different meals. *Dihé* is consumed fresh only by pregnant women who believe that its dark color protects the future baby from 'evil eye'. Generally speaking, *dihé* is consumed as seasoning with a certain number of sauces which accompany cereals paste. The dried *dihé* is crushed in a mortar and the powder obtained is pulp in water. One adds to sauce, salt, pepper, beans, meat (if available) or fish. In a meal, a person roughly consumes 10 to 12 grams of *dihé*, which makes 8% of the requirements in calories and more than 10% of the requirements in proteins (Durand-Chastel H., 1976). *Dihé* is consumed in abundance only in period of food shortage (Ciferri O., 1983). The food value of *Spirulina* is amplified by the fact that it has a rather low percentage of nucleic acids (~4%) compared to that of bacteria. This percentage is extremely high in vitamin B_{12}, the cellular wall is easy to digest, contrary to the cellulose walls met in other food algae. This cellular wall is entirely not poisonous and the lipids are made of unsaturated fatty acids which do not form bad cholesterol. This make of *Spirulina* a potential food component for people suffering coronary diseases and obesity, as it is suggested by Durand - Chastel & Santillan (Durand-Chastel et al, 1976).

The prospect to prepare fermented food like cheese, yoghourt and others, containing *Spirulina*, opens new possibilities for it, without taking into account the potentialities of various extracts (Switzer, L., 1982). The slimming effect of *Spirulina* was tested recently in Germany and the results obtained are spectacular (Richmond A., 1986). More recent studies show that *Spirulina* is very effective in feeding fish (Nandeesha M. C. et al, 2001).

It is significant to stress that the European Space Agency recently launched a draft study of the possibilities of making *Spirulina* a food of choice for space flights of long duration (the famous MELISSA project).

2.2. Methods

2.2.1. *Spirulina* culture at laboratory

Spirulina culture was carried out in the following way: a sample of algae (800 ml of a mixing of *Spirulina Lonar* and *Spirulina Paracas*) coming from a laboratory in France was plunged in a transparent plastic container. We installed in the container, an immersion heater and a bubbles producing device. Above hangs a fluorescent tube of 100 W in order to provide cultures with light even in the night. We take from time to time, a sample to observe under the microscope. At the end of 10 days, an increase in the concentration of cultures was observed.

Each day the external conditions are measured (temperature, air relative humidity), as well as the density of the cultures using a Secchi disc.

In fig.2 is represented schematically the experimental *Spirulina* culture system.

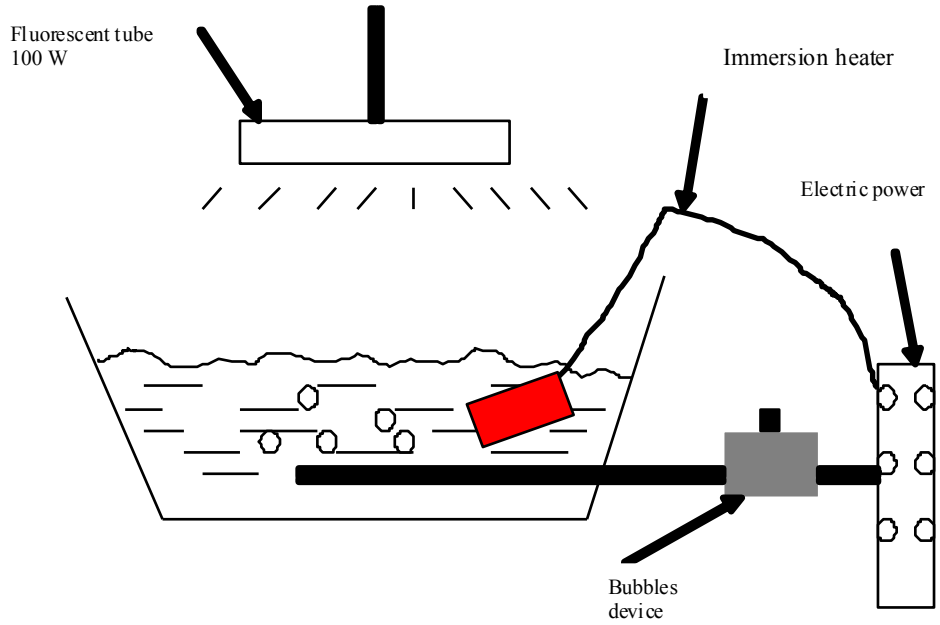

Fig.2: *Spirulina* culture experimental device

2.2.2. Measurements of water activity

To determine the isotherms of sorption, a sample of dried *Spirulina* paste, was spread out in a sufficiently homogeneous thin film over an aluminum foil. The prepared sample is placed in a drying oven at adjustable temperatures. The mass of the product is measured all the ten or fifteen minutes. Then a second sample is subjected to the same treatment for a longer time (from 30 to 40 minutes). A third sample is handled the same manner as previously but for a longer period of time going from one hour and half to two hours. The water content is thus determined according to equation:

$$X_{eq} = \frac{m_H - m_S}{m_S} \quad (1)$$

Where, m_H is the wet mass of the product m_S is its dry mass.

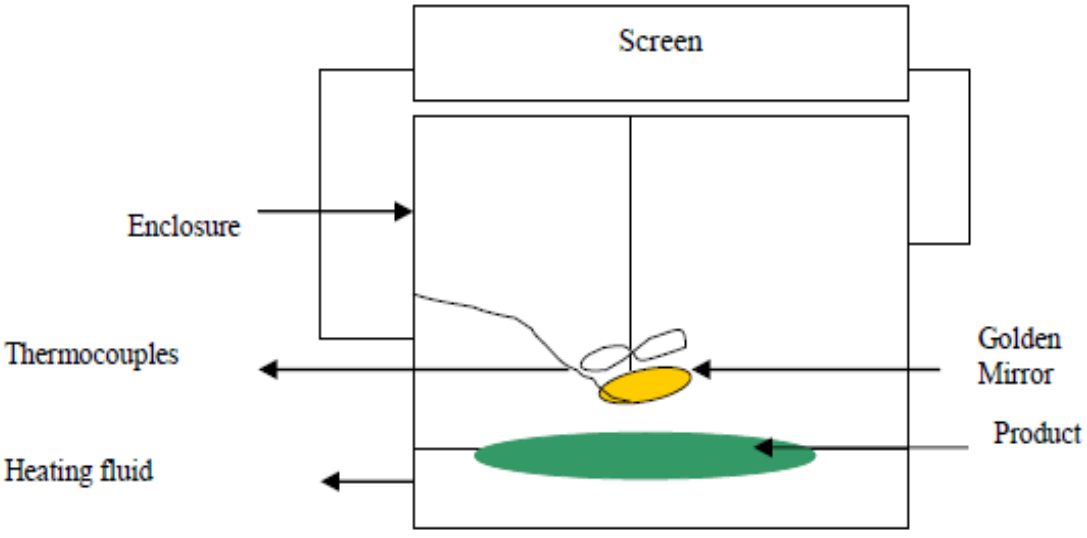

Fig. 3: General diagram of the Activimeter

Activimeter FAST (Food Analysis-Science and Technology) is used to measure AW (the water activity) of the product. This apparatus is used to measure the water activity of agrofood, chemical, medicinal and cosmetic products.

Its technology is based on the measure of air relative humidity above the product, by measurement of the dew point.

A golden mirror is heated then cooled, so a mist is formed on its surface. An optical sensor, located above the mirror measures the temperature of this mist T_m. This temperature is equal to the temperature of dew point.

An IR thermometer measures the temperature of the sample surface T_S, activity is obtained according to formula:

$$A_w = f(T_m/T_S) \quad (2)$$

3. Results and discussions

In this section are represented plots of sorption isotherms, relative ambient air humidity evolution, ambient air temperature evolution, culture's medium temperature evolution, culture's density versus time in days.

Spirulina is not appreciably affected by rather significant variations of temperature (Yacoub I. H., 2010).

Let us note however, that beyond certain temperatures (higher than 70°C for example), the trichomes open and enter in hibernation; what could lead them to death if the temperature continues to increase.

In addition, with increase in water content, thermal conductivity grows and reaches a limit value close to that of water's thermal conductivity (Yacoub I. H., 2010). In our opinion this is explained by the fact that at a certain limit value of the water content, the properties of the algae are inhibited with regard to its "microscopic" character and the "macroscopic" character of measurements.

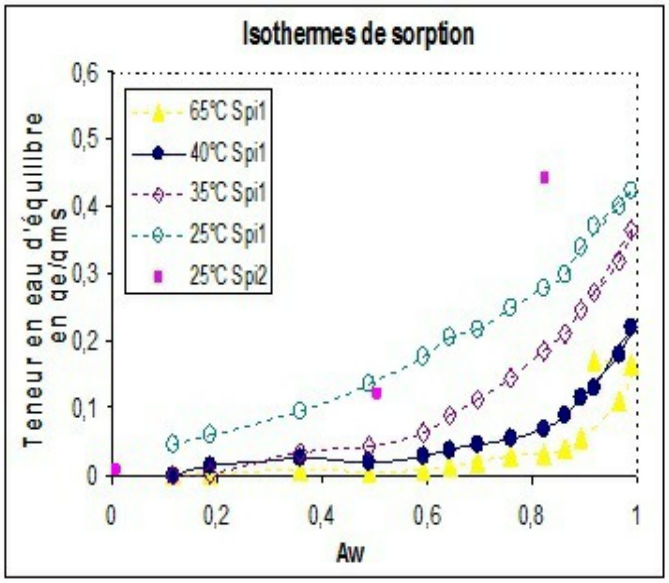

Fig.4: Sorption isotherms at different temperatures

On Fig. 4, are plotted the experimental sorption isotherms obtained in N'Djamena.

The experimental points corresponding to spi2, were carried out at the University College of *Bourg En Bresse* (situated about 100 km eastern of Lyon (France)), with the same method. Apart the points obtained with water activity (A_w) close to 1, the values agree with expectations. The sorption isotherms are close to that of tropical products dried in sahelian zone, such as onions or mangos. During the drying of *Spirulina* in Chad, the average moisture of the air can vary from 10% in February to 55% in August. If the air temperature is higher than 35°C, these values correspond to equilibrium water content lower than **0.05 g water per gram of dry matter, i.e. 5%**.

However, to ensure its conservation, water content must be a little lower than 8% in dried *Spirulina* (according to standards). These figures thus show that under the conditions of N'Djamena's hygroscopy and average temperature, the equilibrium water content reached is sufficient to preserve the product. Moreover, as the theory envisages it, the curves corresponding to high temperatures are lower than those of low temperatures. The experimental sorption isotherms obtained better fit the Henderson model. In a forthcoming article, we will compute the parameters of this model using experimental data.

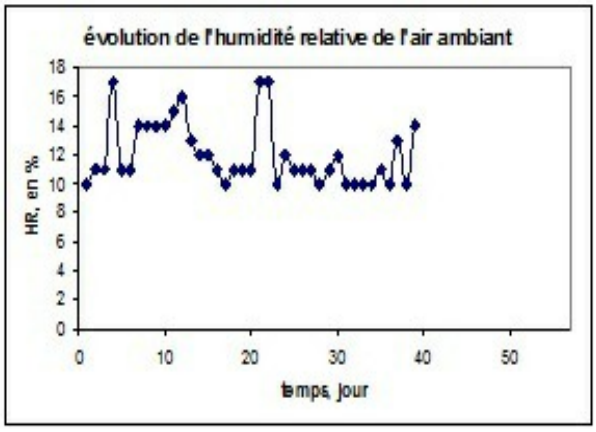

Fig. 5: evolution of ambient air relative humidity

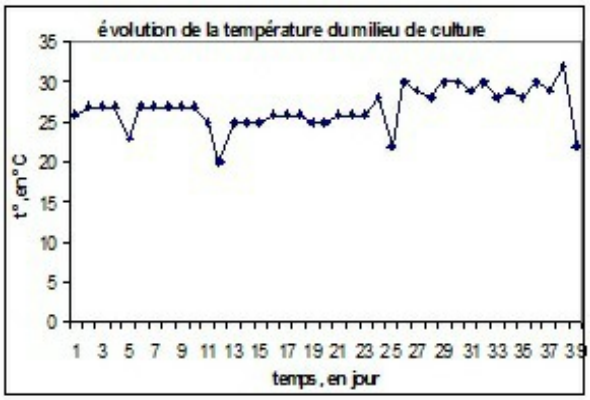

Fig. 6: Evolution of the culture's temperature

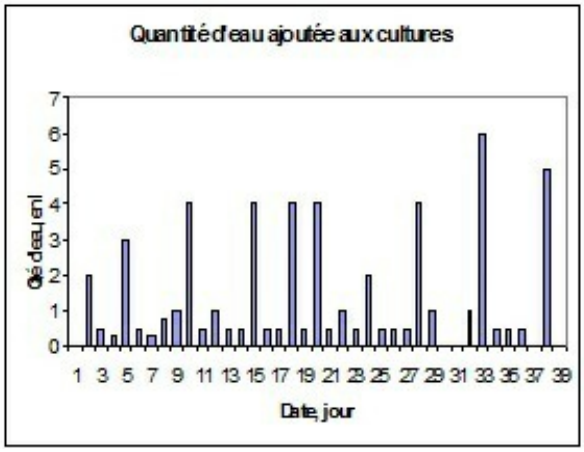

Fig. 7: Quantities of water added to culture

Fig. 8: culture's density evolution in time

Fig. 9: ambient air temperature's evolution

Fig. 5 gives the evolution of ambient air relative humidity with respect to time.

Temperature is measured in Celsius and time in days. In Fig. 6 is represented the evolution of culture's temperature in time. Fig. 7 account for the quantities of water added to culture medium during 40 days. In The same units as in Fig. 6 are used.

In Fig. 8 is represented the evolution of the culture's density in time. The density is measured using a Secchi disc and time is measurement in days. Fig. 9 shows the evolution of ambient air temperature in time measured in days.

It interesting to notice that, the "Bell" shaped curve (Bonin G., 1992) giving the evolution of *Spirulina* density, could be seen in our results.

4. Conclusion

We see then that *Spirulina* has no viable concurrent as source of food supply. It doesn't need special skills to perform *Spirulina* culture at the semi-industrial level. Furthermore the production of *Spirulina* is more economic than that of conventional food as meat, corn, maize or sorghum (it necessitates less water, less space and less time for harvest).

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