

## USE OF GEOTHERMAL FLUIDS FOR CULTIVATION OF THE MICROALGA SPIRULINA IN NIGRITA - SERRES

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### ABSTRACT

The geothermal field of Therma - Nigrita is the most exploited low enthalpy field in Greece. The geothermal waters have a temperature between 40-64°C and they are rich in CO<sub>2</sub> content. There are a lot of geothermally heated greenhouses producing vegetables and flowers, and subsurface heating for asparagus cultivation. This paper dwells on the results reached via a cost reduction application of geothermal fluids for commercial production of the Spirulina blue-green alga. The Spirulina biomass has an interesting composition (more than 50% of proteins, vitamins, minerals, essential fatty acids, antioxidants, phycobiliproteins, etc.). This makes it a valuable additive in terms of better nutrition, the human diet and health strengthening. It is also a rich source of biochemical components. Spirulina is cultivated autrophically in plastic-foil-protected production ponds with an area of 1,950 sq.m., and in a suitable nutrition medium. The algal suspension is cultivated with fresh water heated by geothermal waters. The geothermal energy used in this way as well as setting up the cultivation ponds in a greenhouse contribute to increasing the daily yield by 20-30% (spring and autumn), and the cultivation season is prolonged (from March until November). Using freely released geothermal CO<sub>2</sub> reduces the technological costs for the production of Spirulina biomass by over 25%. Therefore, the cultivation of Spirulina is an important ergonomic application of geothermal energy in the section of aquaculture.

### 1. INTRODUCTION

The geothermal field of Therma - Nigrita lies at the central western part of Strymon basin, at a distance of 20 km southwards of Serres city in Northern Greece (Macedonia). It is the most exploited low enthalpy field in Greece. It has been explored with 8 drillholes (in an area of 16 km<sup>2</sup>) thoroughly since 1979 [1] and during 1995-1999 an additional drilling exploration project was carried out by I.G.M.E. with the construction of 7 new wells. This exploration resulted in the increase of the area of the field by at least 3 km<sup>2</sup>. The maximum temperature recorded was 64 °C. A new area with higher temperatures (58-64 °C) was identified [2]. This exploration also resulted in the better understanding about the supply zone of geothermal fluids.

### 2. THE GEOTHERMAL FIELD OF THERMA - NIGRITA. CHARACTERISTICS AND POTENTIAL

In the field of Therma - Nigrita, the geothermal reservoir is located at depths of 100-400 m in basal conglomerates having significant thickness (some decades of meters) and

containing geothermal waters of temperatures 40-64°C. Above the conglomerates there are other sediments consisting of alternations of marls, sandstones, sands but mainly clays. These sediments form an impervious cap for the geothermal fluids. In these sediments there are aquifers with superficial waters at a temperature of 25-35 °C. The total thickness of Neogene and Quaternary age sediments doesn't exceed 500 m (usually 100-300 m) [3].

The geothermal anomaly in the area occurs mainly due to fault systems trending NE-SW and NW-SE. The intersection of these systems northwards of Therma village forms probably the main channel rising the geothermal fluids from deeper levels. The geothermal fluids supply the Neogene sediments and especially the basal conglomerates. As the horizontal distance from supply faults increase, the geothermal anomaly and the temperature of geothermal decrease because of the longer circulation of the waters and the higher participation of surface waters. The hydraulic contact among the aquifers is affected by block-faults due to neotectonic activity. The brittle tectonics during the Quaternary probably activate Miocene - Pliocene faults of the basin [3].

The geochemical study proved that the geothermal waters of the area are classified as Na-HCO<sub>3</sub> type. They are rich in Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup> and SiO<sub>2</sub> contents. They also contain large amounts of dissolved gases. The most abundant gas is CO<sub>2</sub> representing up to 99.3% by volume of the total gas content. The presence of CO<sub>2</sub> in combination with the temperature (thermo-lifting phenomenon) and hydrostatic pressure create a constantly artesian flow of geothermal aquifers.

The area of the field is about 20 km<sup>2</sup>, considering as exploitable the geothermal fluids having temperature T>30 °C at depths up to 500 m [4,5].

The total potential of the field seems to reach 1000 m<sup>3</sup>/h.

There are several geothermally heated greenhouses covering in total an area of 6.8 ha including 5.9 ha with various vegetables (as well as cultivation of asparagus and strawberries) and 0.9 ha with flowers. In addition there is an open-air subsurface heating for asparagus cultivation covering an area of 3.0 ha [2]. In Therma area, the known thermal springs with maximum temperature of 51.2 °C are used for balneology applications. The management of this spa is under Greek National Tourism Organization [4]. Finally, there is a plant for cultivation of the microalga Spirulina, which is the subject of this paper.

The total thermal capacity arrives at 5.23 MWt and the annual utilization and heating is estimated at 54.08 TJ/yr [5].

Table 1. Results of chemical analysis of geothermal water from the production well Θ-9.

Temperature (°C)	47.0
Conductivity (μS/cm)	3490
pH	6.6
Na <sup>+</sup> (mg/l)	620.7
K <sup>+</sup> (-/-)	78.2
Ca <sup>2+</sup> (-/-)	148.3
Mg <sup>2+</sup> (-/-)	116.7
Fe (-/-)	0.03
Mn <sup>2+</sup> (-/-)	0.042
Li <sup>+</sup> (-/-)	0.88
Sr <sup>2+</sup> (-/-)	0.79
NH <sub>4</sub> <sup>+</sup> (-/-)	0.28
Cl <sup>-</sup> (-/-)	177.2
HCO <sub>3</sub> <sup>-</sup> (-/-)	2252.6
CO <sub>3</sub> <sup>2-</sup> (-/-)	0.00
SO <sub>4</sub> <sup>2-</sup> (-/-)	129.7
F <sup>-</sup> (-/-)	0.70
NO <sub>3</sub> <sup>-</sup> (-/-)	1.5
NO <sub>2</sub> <sup>-</sup> (-/-)	0.03
SiO <sub>2</sub> (-/-)	85.0
B (-/-)	3.42
As (-/-)	0.50

### 3. PRODUCTION GEOTHERMAL WELL AND CHEMICAL COMPOSITION OF THE GEOTHERMAL FLUIDS

The plant for cultivation of the microalga *Spirulina* is located at a distance of 1.5 km northwards of Therma village. For its energy requirements, geothermal water with temperature of 47 °C is used from a neighboring production well (Θ-9) with a depth of 372 m [3].

Table 2. Chemical composition (% by volume) of the gases from geothermal well Θ-9 [6].

CO <sub>2</sub>	98.70 %
N <sub>2</sub>	1.30 %
O <sub>2</sub>	---
CH <sub>4</sub>	---
H <sub>2</sub> S	~ 3 ppm

The chemical composition of the water from this well is presented in Table 1. The geothermal water contains totally 3,529 mg salts/l and 0.5 ppm of As and it is not used directly for *Spirulina* cultivation. So, fresh water is used which is heated by the geothermal

fluids using simple heat exchangers, locally constructed.

As mentioned above, CO<sub>2</sub> is the most abundant gas in geothermal field of Therma - Nigrita. The composition of the gases from geothermal well Θ-9 is presented in Table 2.

### 4. MICROALGAE CULTIVATION. SPECIFICATIONS FOR THE SPIRULINA

Microalgae are ancient photosynthesizing organisms. Over the last 50 or 60 years, microalgae have been attracting the attention of researchers, commercial food companies and health-care practitioners alike. This interest can be explained with the following reasons: microalgae can be grown in environments normally not used for agricultural purposes; they are a rapidly renewable food source (they produce more biomass than any other food source per unit of time); microalgae can produce large amounts of biomass; they are the most nutrient-dense food currently known; they are an as yet untapped source of biochemical compounds [7, 8, 9, 10, 11, 12, 13]. Currently, the price per kilogram of biomass is high on account of the relatively large investments, costliness of pure CO<sub>2</sub>, expensive mineral nutrients, low conversion efficiency in comparison with bacteria, seasonal character of the production, etc. This necessitates the search for technology optimization.

Beyond all doubt, it is the cyanobacterium *Spirulina* (called earlier a blue-green alga), used by the Aztecs for food as early as 1,000 years ago, that ranks first among the microalgae widely used in practice for large-scale cultivation and other applications.

*Spirulina* spp. are multicellular filamentous microalgae; the size of the cells varies and ranges from 1 to 12 mm in diameter and between 3 and 20 mm in length (Fig. 1).

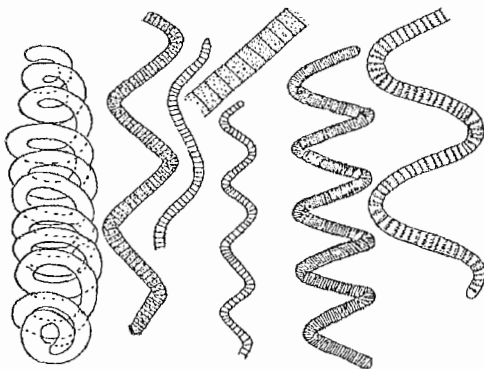


Fig.1. Various *Spirulina* species

The main reason for the leading place that *Spirulina* holds on the world market is the rich chemical composition of its biomass, the fact that it is easily assimilated and has no toxic substances.

The *Spirulina* biomass has a very high protein content (over 60%) with a well-balanced amino acid composition, rich mineral content (Fe, Se, Zn, Ca, Mg etc.), vitamins (especially B complex and B<sub>12</sub>), antioxidants (carotenoids), phycobiliproteins, essential fatty acids ( $\gamma$ -linolenic) and polysaccharides. All this accounts for the rather important spheres of application of the *Spirulina* biomass. It is used for the following: stimulation of the immune system, support for the cardiovascular system, raising the content of the "healthy" cholesterol, improvement of the gastrointestinal and digestive tracts, improvement of detoxication, reducing the risks of cancer through antioxidant protection and solving of dermatological problems [12, 14, 11, 7, 15]

All of the above has given us grounds to realize a project for large-scale cultivation of *Spirulina* in Nigrita – Serres. This paper focuses on the results obtained in terms of technology optimization and cost reduction through the use of geothermal fluids.

Microalgal cultivation is based upon the logic of the photosynthetic process: solar energy is used for the synthesis of organic compounds out of non-organic substances.

The amount of microalgae produced depends mainly on the genus/species, photoperiod and total amount of light, temperature, pH, rate of removal of cells from the medium, turbulence and nutrient composition of the medium, CO<sub>2</sub>-supply [7, 10, 8] and others.

These considerations have been taken into account in the creation and exploitation of the installations in Nigrita. To optimize the process and reduce costs, the Nigrita base was set up near a mineral spa containing freely emitted CO<sub>2</sub> (600 kg/hour). The cultivation parameters have been improved in accordance with the qualities of the geothermal source, such as capacity, temperature, water composition, CO<sub>2</sub> amount, etc.

## 5. THE INSTALLATIONS IN THERMA - NIGRITA

### 5.1. Cultivation ponds (installations)

The installations are oval and made of concrete. Their area totals 1,950 m<sup>2</sup>, split in installations of various sizes in view of their gradual inoculation (2 x 1.8 m<sup>2</sup>; 2 x 4.5 m<sup>2</sup>, 1 x 50 m<sup>2</sup>, 1 x 100 m<sup>2</sup> and 8 x 225 m<sup>2</sup>) (Fig. 2). Pedal wheels do stirring. Velocity of suspension circulation is 15 m/min. The installations are located in a greenhouse covered with French foil allowing for 80% transparency. Suspension layer thickness is 8 – 10 cm (80 – 100 l/m<sup>2</sup>).

The overall cultivation process includes the following stages:

- I. Museum algal culture of *Spirulina* (extensive conditions)
- II. Inoculation (intensification of the cultivation process)
- III. Cultivation in production ponds
- IV. Separation
- V. Drying and packaging

The laboratory collection of different kinds of *Spirulina* strains is maintained as liquid cultures under suitable extensive conditions (illumination of approximately 2,000 lx, temperature 20-22°C).

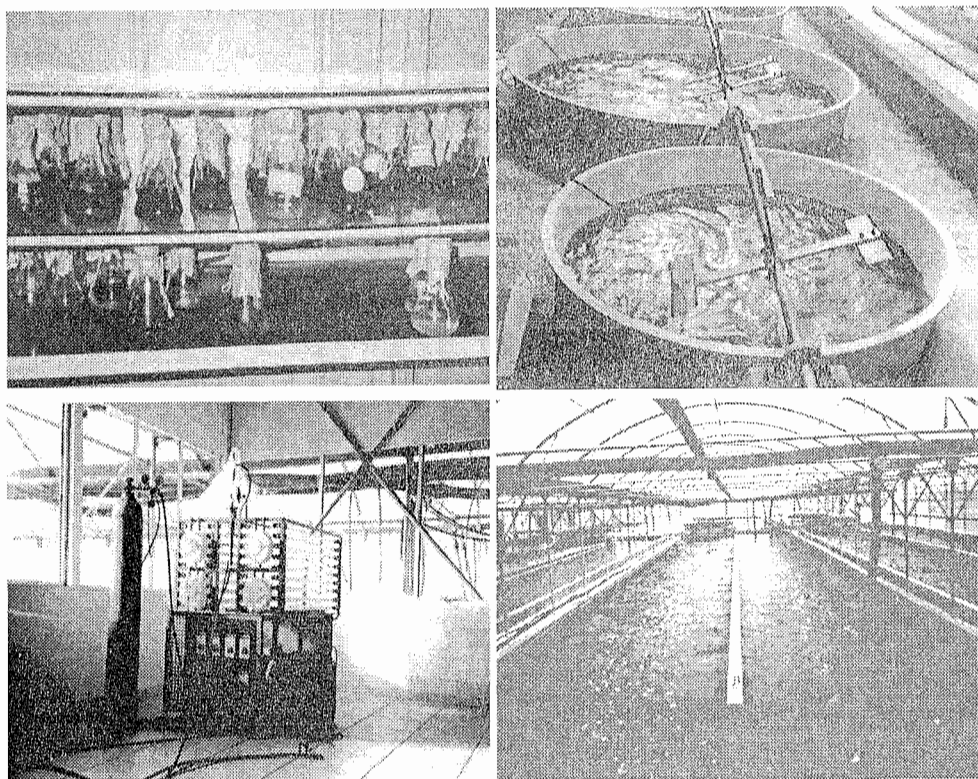


Fig.2. Stages of the cultivation process

The algal culture is transferred from the collection to an intensive cultivation installation (bubbling with air containing 2% of  $\text{CO}_2$ ,  $t \cong 34\text{-}36^\circ\text{C}$ , illumination of 8,000 lx). As a result of its growth and dilution with nutrition medium, suspension volume continually increases up to reaching the amount necessary for inoculation at semi production installations ( $50$  and  $100 \text{ m}^2$ ). It is then transferred to the production ponds ( $225 \text{ m}^2$ ).

## 5.2. Geothermal water for nutrition medium

The nutrition medium for the cultivation of *Spirulina* in Nigrta is based on the classical recipe [16] but has been modified, depending on mineral salts' prices and the results of our laboratory research on the growth rate of *Spirulina* (Fournadzhieva, unpublished data). The quality of the water used to prepare the nutrition medium is extremely important for achieving optimum growth rate [17].

High  $\text{NaHCO}_3$  content is of vital importance for *Spirulina* to maintain a pH between 9 and 10.5 as well as limit possible contamination from other algae and microorganisms.

Geothermal water in Nigrta contains a multitude of microelements and  $1.5 \text{ g NaHCO}_3$  per liter. Our laboratory experiments have proven that it stimulates the growth of *Spirulina* by 5 to 10%. However, the geothermal water in Nigrta contains also As and Sr, which accumulate in the algal biomass. This is in breach with health standards. Due to this reason the water has never been used for mass cultivation.

### 5.3. Use of geothermal energy and CO<sub>2</sub> to optimize photosynthesis

This aim was achieved by supply of the inorganic carbon substrate necessary for the photosynthesis (geothermal CO<sub>2</sub>) and optimizing of the temperature-radiation regime by heating with thermal energy.

The mass algal cultivation is accompanied by daily and seasonal changes in the temperature and radiation.

With optimum levels of nutrients, it is temperature and light that determine photosynthetic intensity. Convincing data exist that the effect of temperature and light on algal growth are in correlation [18]. Literature data show that optimum daily conditions for *Spirulina* are temperature of approximately 40°C (night temperature ~ 25°C) and light intensity of 20-30 lx [19]. Light intensity can be corrected by the thickness of the suspension layer, culture density or by placement of the installations in green houses. Photoinhibition of photosynthesis has been proven for blue green algae. Due to this reason we decided to cover the installations in Greece with plastic foil allowing 80% transparency. The results show that even though reduced the light is sufficient even at 8 p.m. and further reduction of the light intensity during the afternoon hours satisfies the physiological needs of *Spirulina*. There is no doubt that covering the cultivation installations is of crucial importance for the protection of algal cultures from harmful weather conditions (rain, dust or others) and, in particular, contributes for optimum heating effectiveness.

Heating the algal suspension is very important for maintaining an optimum night temperature and an effective start of the cultivation day. The morning hours provide good light intensity for the photosynthetic activity but temperatures are low. Therefore, a heating device was built for the installations. As soon as it was introduced, temperatures of the suspension were optimum for algal growth even in March and April (average data, Fig. 3). Elevation of morning temperatures by 5°C (from 20-22 to 25-26°C) at the same light intensity results in an increase of photosynthetic activity as measured by oxygen production (Fig. 3). These results are confirmed by the data obtained regarding the average daily yield in installations with and without heating of the cultures, i.e. 0.2 g/l/day and 0.14 g/l/day, respectively. That is the reason for the fast inoculation of the installations – 20 days at a total suspension volume of 195 m<sup>3</sup>.

The heating of the suspension is extremely important for the growth of *Spirulina* and the entire cultivation process, especially in spring and autumn. Our experiments in Nigrita and the data about the climatic conditions (sunny days and radiation, temperature) show that *Spirulina* can be cultivated from March to November with satisfactory results.

According to data of Becker and Venkataraman obtained in 1980, the CO<sub>2</sub> supply accounts for 27.4% of the algal cultivation expenses [20]. Comparing this number with the low cost when using geothermal CO<sub>2</sub> supply clearly emphasizes the economic advantage of using the geothermal carbon dioxide source. The carbon dioxide emitted from the mineral spa in Nigrita does not need any compression because its amount is very high.

Every endeavour was made to find an optimum solution for the daily separation of the algal yield. A centrifuge of the Alpha – Laval type proved to be the best solution.

Until 2001, drying was done in natural conditions (in greenhouses an air temperatures reaching 80°C). The biomass was then milled.

A spray dryer is about to be implemented.

The biomass produced by Algae A.C. Therma Company, Nigrita has an ISO9002 standard, which is a guarantee for the quality of this product.

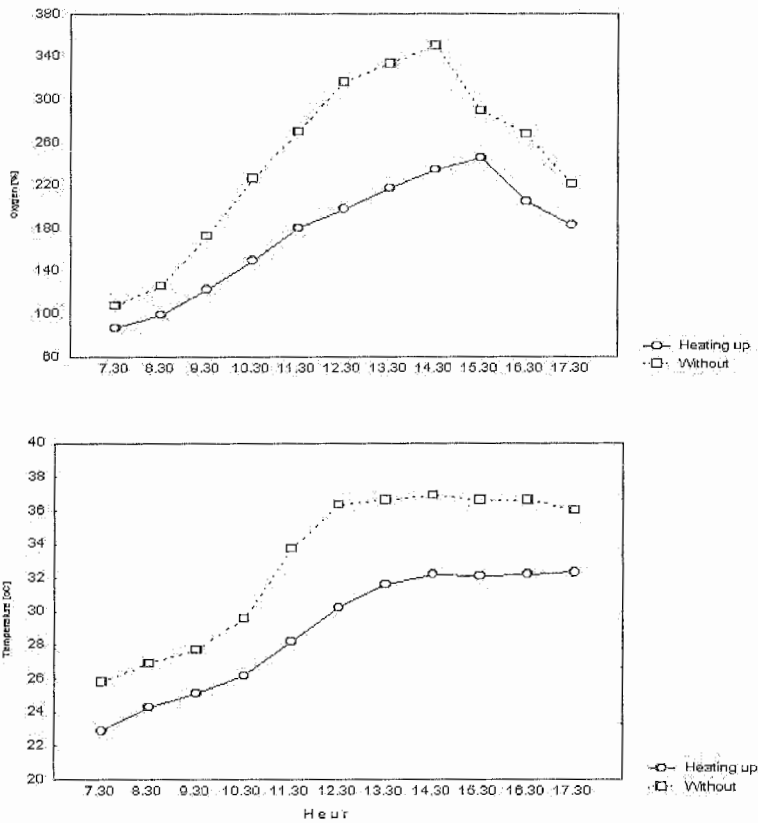


Fig. 3. Correlation between photosynthetic activity of Spirulina and temperature of algal suspension

## 6. CONCLUSIONS

Using geothermal CO<sub>2</sub> reduces technological costs for the production of Spirulina biomass by more than 25%.

Using geothermal energy as well as setting up installations for Spirulina cultivation in a greenhouse contributes to optimizing the temperature and radiation technological regime. Thus the cultivation season is prolonged (from March until November) and the daily yield increases by 20 to 30%. The natural and climatic information about the region make it possible to forecast a reachable annual output of approximately 2,000 kg/decare (1 decare = 0.2471 acres).

Positive results were obtained in overcoming two of the production deficiencies, namely, costs, hence biomass price, have been reduced, and the technology and product have been standardized.

More efforts should be made to automate and complete the last two stages of the cultivation process (separation and drying) and improve pond construction (increase stirring velocity and smooth out the surface).

Literature references focus widely on the opportunities to use geothermal energy for aquacultures. We have not found data for the carrying out of similar projects, that use geothermal energy in algology, elsewhere except in Roupite - Bulgaria [17] and Nigrita - Greece. Regardless of that, the results obtained give us enough reasons to draw the conclusion that Spirulina cultivation is an important ergonomic application of geothermal energy in the section of aquacultures.

## REFERENCES

- [1] Karydakis G., "Report - Study of low enthalpy geothermal field in Therma - Nigrita area", I.G.M.E., Athens, 1983.
- [2] Karydakis G., Andritsos N., Fytikas M., "Geothermal energy developments in the Prefecture of Serres", Proceedings of 6<sup>th</sup> National Conference on Renewable Energy Sources, Institute of Solar Technology, Vol. A, pp. 427-434, 1999
- [3] Arvanitis A., Fytikas M., Dotsika E., "Geothermal conditions in Therma - Nigrita area (Strymon basin, Northern Greece)", Bulletin of the Geological Society of Greece, Vol. XXXII/4 (Proceedings of 8<sup>th</sup> International Congress of the Geological Society of Greece, Patras, May 1998), pp. 229-242, 1998.
- [4] Fytikas M., Arvanitis A., "Exploitation problems in Therma - Nigrita geothermal field. Impacts to the environment", Proceedings of 5<sup>th</sup> National Conference on Renewable Energy Sources, Institute of Solar Technology, Vol. B, p. 211-222, 1996.
- [5] Fytikas M., Andritsos N., Karydakis G., Kolios N., Mendrinis D. and Papachristou M., "Geothermal exploration and development activities in Greece during 1995-1999", Proceedings of World Geothermal Congress 2000 (Kyushu - Tohoku, Japan, May 28 - June 10, 2000), Vol. 1, pp. 199-208, 2000.
- [6] Karabelas A. J., Andritsos N., Karydakis G., "a) Preliminary estimation for CO<sub>2</sub> exploitation, b) Study about the choice of piping diameter for the increase of yield in artesian wells", Report to the Region of Central Macedonia, CPERI / IGME, Thessaloniki, 47 pp., 1993.
- [7] Richmond A. Ed., "Handbook of Microalgal Mass Cultures", CRC Press, Boca Raton, FL., 1986.
- [8] Shelef G. and Soeder C.J. Eds., "Algae Biomass Production and Use", Elsevier/North-Holland Biochemical Press, New York, 1988.
- [9] Stadler T., Mollion J., Verdus M.C., Karamanos Y., Morvan H., Christaen D., "Algal Biotechnology", Elsevier Applied Science, New York, 1988.
- [10] Borowitzka M.A., Borowitzka L.J. Eds., "Micro-Algal Biotechnology", Cambridge University Press, Cambridge, 1988.
- [11] Becker E.W. 1994, "Microalgae: Biotechnology and Microbiology" ed. J. Baddiley, N.H. Carey, J.J. Higgins, W.C. Potter. Cambridge University Press, Cambridge, 1994.
- [12] Cifferi O. "Spirulina, the edible microorganism", Microbiol. Rev. 47, pp.551-578, 1983.
- [13] Kay K.A. "Microalgae as Food and supplement. Critical reviews in Food Science and Nutrition", 30 (6), pp.551-573, 1991.
- [14] Cifferi O., Tiboni O.. "The biochemistry and industrial potential of Spirulina", Ann. Rev. Microbiol. Vol. 39, pp.503-526, 1985.
- [15] Jassby A. "Spirulina: a model for microalgae as human food. In: Algae and Human Affairs", Ed. Carole A. Lembi, J. Robert Waaland, Cambridge University Press, Cambridge, 1988.
- [16] Aiba S., T. Ogawa, "Assessment of Growth Yield of a Blue-Green Alga, Spirulina Platensis, in Axenic and Continuous Culture", J. gen. Microbiol. 102, pp. 179-182, 1977.
- [17] Fumadzhieva S., Georgiev D., Bozkova M., "Geothermal fluids technology of microalgal cultivation in Bulgaria", In: Proceedings of the international Workshop, Bansko, Bulgaria, Chapter 24, 1983.
- [18] Eppley R.W., P.R. Sloan, "Growth Rate of Marine Phytoplankton: Correlation with Light Absorption by Cell Chlorophyll." Physiologia Plantarum, 19, 44-55, 1966.
- [19] Ogawa Terui. "Studies on the Growth of Spirulina", Platensis I On the pure culture of Spirulina Platensis. J. Ferment. Technol. 48, 361-367, 1970
- [20] Becker E.W., Z.V. Venkataraman. "Production and Processing of Algae in Pilot Plant Scale Experiences of Indo-German Project". In: Algal Biomass, Eds. G. Shelef, C.J. Soeder. Elsevier North - Holland Biomed. Press, pp.35-50, 1980.