Production of Spirulina (Arthrospira Platentis) in Controlled Environment

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Abstract

This paper presents development of a tubular photo bioreactor for production of Spirulina (Arthrospira Platensis) as a state-of-the-art technology for the microalgae cultivation. This technology solves the problems, which faces traditional production and allows obtaining of high quality Spirulina. The implemented bioreactor has a capacity of 7 liters and a system for monitoring and management of vital growth parameters based on Arduino technology. The obtained Spirulina was microbiologically analyzed by the University reference laboratory, as well as analysis for the presence of micro toxins and results proved that it is be suitable for human consumption. Further research will be in the direction of parameters optimizing and experiments with CO2 enrichment in order to increase productivity,

Keywords: Spirulina, Arthrospira Platensis, Photo Bioreactor, Controlled Environment

1. Introduction

Spirulina (Arthrospira platensis) is filamentous blue-green microalgae that grows in water, can be harvested and processed easily and has significantly high macro - and micronutrient contents. In many countries it is used as human food as an important source of protein and is collected from natural water, dried and eaten. It has gained considerable popularity in the human health food industry and in many countries of Asia it is used as protein supplement and as human healthy food.

Spirulina platensis and many other microalgae gained attention for its highly applicable potentials for solving some of global problems. The three major applications are;

Using Spirulina platensis as a food/food supplement/ Spirulina contains high levels of proteins (50–70%), lipids (7–16%), vitamins, and omega-3 fatty acid. This nutrient composition make it often to be named as a Superfood or food of the future.

Using Spirulina for production of Biofuel. Algae contain highly oil content compared to the other feedstock. The yield of oil from algae is over 200 times the yield from the best-performing plant/vegetable oils.

Health Benefits of Spirulina based on its chemical composition, which includes proteins, carbohydrates, essential amino acids, minerals (especially iron), essential fatty acids, vitamins, and pigments. Three major bioactive components of *Spirulina*, the protein phycocyanin, sulfated polysaccharides, and *γ*-linolenic acid, seem to play significant roles in imparting improved human body functions. Furthermore, experimental evidence supports the immunomodulation and antiviral effects of *Spirulina* supplementation.

1.1 Biochemical composition of Spirulina Platensis

Spirulina is very rich in different biochemical compound and in general consist of:

Proteins:
Spirulina contains very high amounts of protein, (between 55 and 70 percent by dry weight). It is a complete protein, containing all essential amino acids.

Essential fatty acids:
Spirulina has a high amount of polyunsaturated fatty acids (PUFAs). It is rich in γ-linolenic acid (36 percent of total PUFAs), and also provides γ-linolenic acid (ALA), linoleic acid (LA, 36 percent of total), stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA).

Vitamins:
Spirulina contains vitamin B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B6 (pyridoxine), B9 (folic acid), B12 (cyanocobalamin), vitamin C, vitamin D and vitamin E.

Minerals:
Spirulina is a rich source of potassium, and also contains calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium and zinc.

1.2 Health Benefits of Spirulina Platensis

The bioactive potential of *Spirulina* is still being evaluated in preclinical studies and they seem to indicate *Spirulina*'s strong antioxidant, anticancer, and antiviral properties as well as its capacity to combat obesity, diabetes, and inflammatory allergic reactions. It also shows great immunomodulatory, hypocholesterolemic, and hypoglycemic potential.
Consistent results regarding its bioactivities have led to increasing interest in evaluating the potential of *Spirulina* as a therapeutic food. Among the others, Spirulina can be potentially used in:

Blood Pressure reduction
High blood pressure is a main driver of many serious diseases, including heart attacks, strokes and chronic kidney disease. Dose of 4.5 grams per day has been shown to reduce blood pressure in individuals with normal levels. This reduction is thought to be driven by an increased production of nitric oxide, a signaling molecule that helps blood vessels to relax and dilate.

Allergic Rhinitis
Allergic rhinitis is characterized by inflammation in your nasal passageways. It is triggered by environmental allergens, such as pollen, animal hair or even wheat dust. Spirulina is a popular alternative treatment for symptoms of allergic rhinitis, and there is evidence that it can be effective. According to a clinical study, 2 grams per day dramatically reduced symptoms like nasal discharge, sneezing, nasal congestion and itching.

Curing Anemia
The most common type of Anemia is characterized by a reduction in hemoglobin or red blood cells in the blood. Anemia is fairly common in older adults, leading to prolonged feelings of weakness and fatigue. Spirulina supplements due to its high level of Iron inorganic form, increased the hemoglobin content of red blood cells and improved immune function.

Blood Sugar Control
Animal studies link spirulina to significantly lower blood sugar levels. In some cases, it has outperformed popular diabetes drugs, including Metformin. There is also evidence that spirulina can be effective in humans. I a clinical study on 25 people with type 2 diabetes, 2 grams of spirulina per day led to an impressive reduction in blood sugar levels. Studies estimate that this can lower the risk of diabetes-related death by 21%.

Anti-Cancer Properties
Some evidence suggests that spirulina has anti-cancer properties. Research in animals indicates that it can reduce cancer occurrence and tumor size. Spirulina’s effects on oral cancer (cancer of the mouth) have been particularly well studied. One clinical study examined people with

precancerous lesions — called oral submucous fibrosis (OSMF). Among those who took 1 gram of spirulina per day for one year, 45% saw their lesions disappear — compared to only 7% in the control group.

Lowering bad cholesterol and triglyceride Levels

Spirulina positively impacts many of the risk factors linked to an increased risk of heart disease. For example, it can lower total cholesterol, "bad" LDL cholesterol and triglycerides, while raising "good" HDL cholesterol. Clinical study on people with high cholesterol determined that 1 gram of spirulina per day lowered triglycerides by 16.3% and "bad" LDL by 10.1%

Powerful antioxidant and anti-inflammatory properties

Spirulina is a great source of antioxidants, which can protect against oxidative damage. Its main active component is called phycocyanin. This antioxidant substance also gives spirulina its unique blue-green color. Phycocyanin can fight free radicals and inhibit production of inflammatory signaling molecules, providing impressive antioxidant and anti-inflammatory effects.

1.3 Production technologies

There are two basic technologies for producing Spirulina. Outdoor pool production, which is technologically the simplest. However, this production faces many disadvantages, including:

- Control of the parameters of the growing medium including temperature, liquid medium pH and the amount of nutrition minerals.
- Liquid medium Air Pollution with PM particles and heavy metals,
- Intoxication with toxic cyanobacteria and undesirable types of microalgae,
- Intoxication of the medium with insect larvae, etc.
- Production of spirulina only in a part of the year when the weather conditions are suitable

All these problems can be solved by using of photo bioreactors. There are different designs of closed photo bioreactors and they differ according to the type of container where the algae will be propagated. The most common types of cultivation are plastic bags, flat panel reactors and tubular reactors. Flat panel containers and tubular reactors can be made of a variety of materials: either disposable polymer or glass. If algae cultivation is planned in a closed photo bioreactor, the use of flat panels initially appears to be a potentially good approach, because this method offers highest light absorption and results in rapid algae growth. In practice, a number of influencing factors diminish this advantage. The most important of them are:

- Limited shelf life of PVC
- Reduction of productivity due to degradation
- Difficulty in cleaning
- High maintenance cost

The most adequate solution is the implementation of photo bioreactors with horizontally or vertically placed glass tubes with appropriate diameter.

2. Methods and Materials

2.1 Tube construction

The system for growing Spirulina is made of a vertical glass tube with a volume of 7 litres. A strip of 20 W LEDs is placed around the tube. This arrangement allows maximum use of light. Inside the glass tube is placed a heater with a power of 100 W. This is quite enough to achieve the optimal medium temperature in the range of 32 - 35 degrees, even in adverse ambient conditions.

Fig 2.1.1 Construction of Vertical tube lighting system

Fig 2.1.2 7 L vertical tube

The glass tube is filled with 7 liters of water filtered in RO (Reverse Osmosis) filter with final TDS concentration of less than 10 ppm. Dissolved minerals are according to Zarrouk formulation (See Table 2.2.1). The glass tube is closed with a lid on which are placed sensors for measure of the medium temperature, alkalinity (pH), TDS (Total Dissolved Solids) and turbidity which actually provides information about the concentration of spirulina in the medium.

	
Fig 2.1.3 Installation of Heating system	Fig 2.1.4 Vertical tube with 7L Zarrouk's Media

2.2 Nutrition formulation

Aside of Oxygen (O), Hydrogen (H) obtained from the water and Carbon (C) obtained from the air, there are 13 elements essential for the growth of Spirulina Platensis.

Macroelements:
Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Sulfur (S), and Magnesium (Mg).

Microelements:
Iron (Fe), Chlorine (Cl), Manganese (Mn), Boron (B), Zinc (Zn), Copper (Cu), and Molybdenum (Mo)

All elements have different but essential role in successful growth of the algae.

Nitrogen is a part of a large number of necessary organic compounds, including amino acids, proteins, coenzymes, nucleic acids, and chlorophyll

Phosphorus is a part of many important organic compounds, including sugar phosphates, nucleic acids, phospholipids, and certain coenzymes

Potassium acts as a coenzyme or activator for many enzymes. Protein synthesis requires high levels of potassium. Potassium does not form a stable structural part of any molecules inside plant cells

Calcium usually precipitates as crystals of calcium oxalate in vacuoles. It is required to maintain membrane integrity and is part of the enzyme α-amylase.

Magnesium is an essential part of the chlorophyll molecule and required for activation of many enzymes, including those involved in ATP bond breakage.

Sulfur is incorporated into number of organic compounds including amino acids and proteins.

For the successful nutrient uptake it is very important to maintain correct values of pH and TDS (Total dissolved Solids). Recommendation for pH is 10 to 11 and for TDS is between 1200 and 1300 ppm. We maintained pH with highly dissolved Sodium Hydroxide. Regarding the TDS when TDS overcome 1300 ppm, we just add clear water into medium.

There is number of different formulations for medium solution. One of the most used formulation is Zarrouk's Medium composition.

Mineral	g/L
NaCl	1.00
CaCl2.2H2O	0.04
KNO3	2.50
NaNO3	2.50
FeSO4*7H2O	0.01
EDTA (Na)	0.08
K2SO4	1.00
MgSO4*7H2O	0.20
NaHCO3	16.80
K2HPO4	0.50
Trace elements	1ml/L

Table 2.2.1 Compositions of Zarrouk's media.

Trace elements formulation consist of (H3BO3, MnCl2*4H2O, ZnSO4*4H2O Na2MoO4,CuSO4*5H2O). We have used readymade product by Dutch company Mono.

Fig.2.2.1 Minerals used for preparation of Zarrouk's medium

Fig.2.2.2 Measurement of salts for nutrient solution

2.3 Growing parameters, their measurement and control

For optimal growth of the Spirulina, number of parameter need to be kept in some predefined range:

Medium temperature

The optimal growth of Spirulina is in temperature range between 32 C and 35 C. On temperature lower than 20 C and higher than 40 Spirulina dies. So it is very important to keep medium temperature in this range because it can be disturbed by different factors. To maintain the temperature, a 100 watts water heater has been installed, and self-regulation is set at 33 degrees. There is no dangerous to obtain a temperature higher than 40 degrees in our Lab.

Alkalinity

Spirulina grows in high alkaline environment (>10) and permanent measurement of pH is very important. Optimal value is between 10 and 11. Initial alkaline value of medium is obtained by high quantity of sodium bicarbonate ($NaHCO_3$). Maintaining the level is with highly dissolved Sodium hydroxide (NaOH). High alkaline medium suppress growing of other types of micro algae and number of bacteria. Spirulina itself tends to increase alkalinity and if it exceeds 11, can be decreased with the addition of filtered water.

TDS (Total Dissolved Solids)

Minerals that are dissolved in the medium over time are used in photosynthetic processes and a balance needs to be reestablished. Not all minerals are consumed with the same dynamics. Nitrate compounds (NO_3) are the most used. Accurate measurements of the amount of all minerals in the medium are very expensive, but practice has set a few simple rules that allow for efficient management.

- When collecting Spirulina, $NaNO_3$ is added to the medium as much as the dry mass of the collected Spirulina.
- Once a month, 20% of the basic amount of microelements are added

Turbidity

Turbidity of the medium is another important parameter, and it is proportional of the amount of Spirulina on to the medium. When the level of turbidity exceed 80% it is time to harvest Spirulina. Avoiding of the harvesting process will lead to the eventual death of the whole colony.

2.4 Measurement and Control

In order to precisely monitor spirulina growth parameters, we developed a monitoring system based on Arduino technology. The system is based on the Arduino Mega 2560, temperature sensor pt100, pH sensor with built-in temperature compensation, conductivity sensor (TDS) with built-in temperature compensation, and turbidity sensor. The measurement results are displayed on the Oled Display.

Fig.2.4.1
Temp. Sensor

Fig.2.4.2
pH Sensor

Fig.2.4.3
TDS Sensor

Fig.2.4.4
Turbidity Sensor

The sensors are mounted on the lit of the photo bioreactor and are connected to the protoboard. From there the data is transferred to the arduino.

Fig.2.4.5
Lit with sensors attached

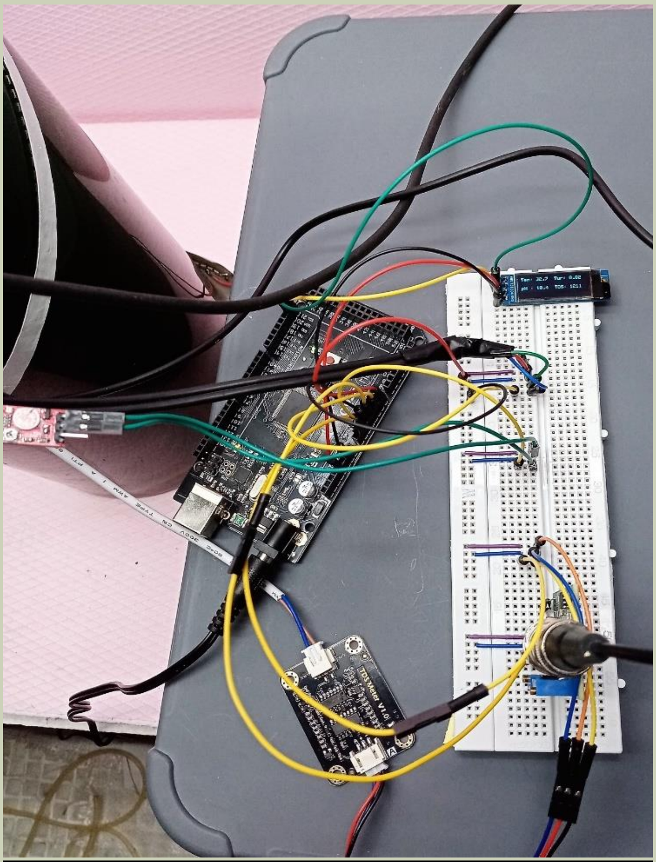


Fig.2.4.6 Connection of the sensors to Proto board and Arduino

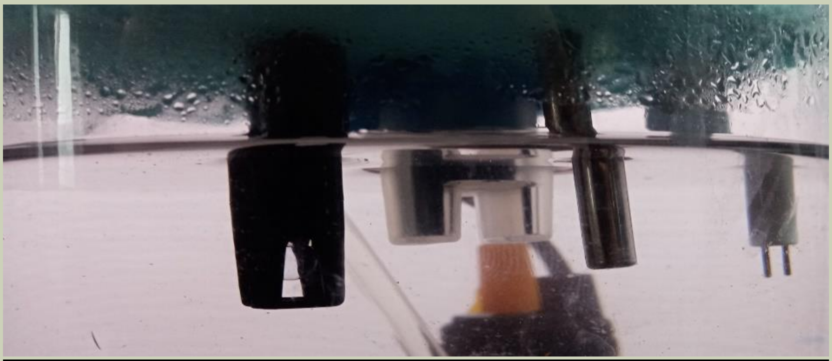


Fig. 2.4.7 Sensors set on the top of the glass tube

The software is developed in the Arduino programming language and it has four measuring functions. In each of the functions, the measurement is performed 10 times, the values are averaged and the mean value is returned. This is done in order to obtain a more accurate measurement.

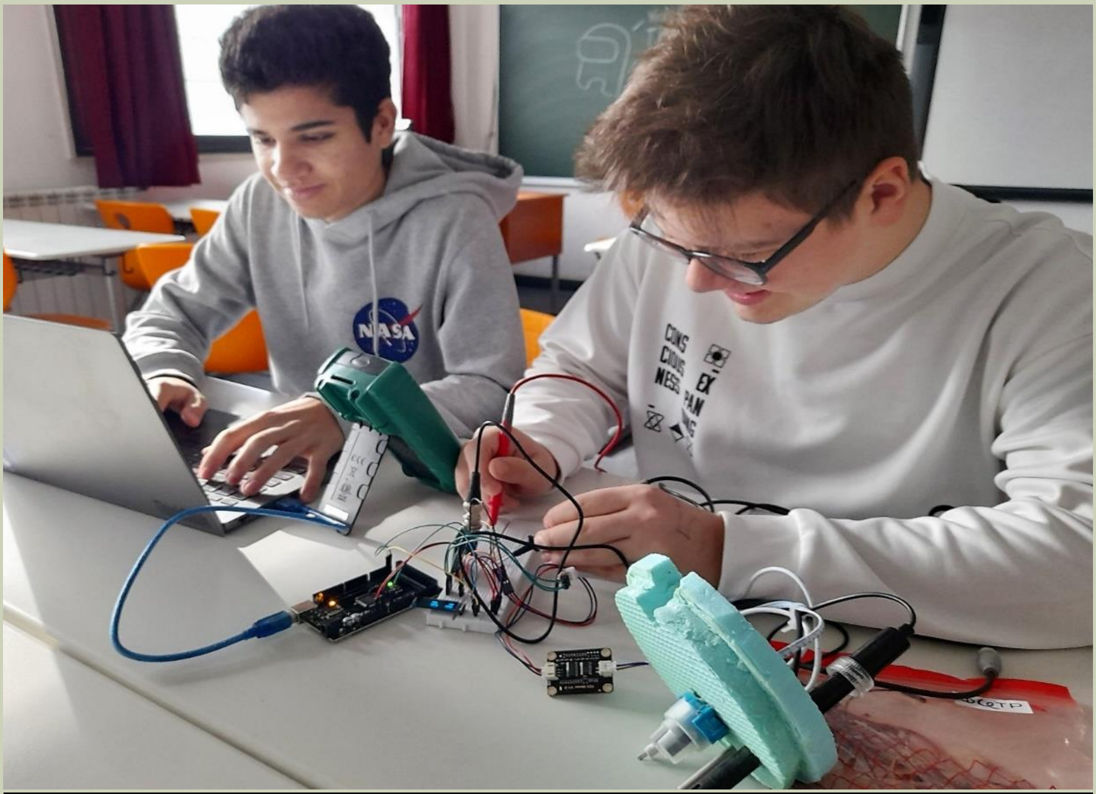


Fig.2.4.8 Development of a software for measure and control of bioreactor

Initial sequence when program starts is shown on Fig. 2.4.9

Fig.2.4.9 Initial Sequence of Arduino based monitoring system

3. Experiment

Spirulina living culture was procured from the company Health Algae from Sweden. According to the recommendations, the culture was placed on a bright place in ambient temperature in order to adapts to the environment in period of 24 hours. After this period, the medium, previously heated on 32 degrees Celsius, was inoculated with 250 ml f living culture.

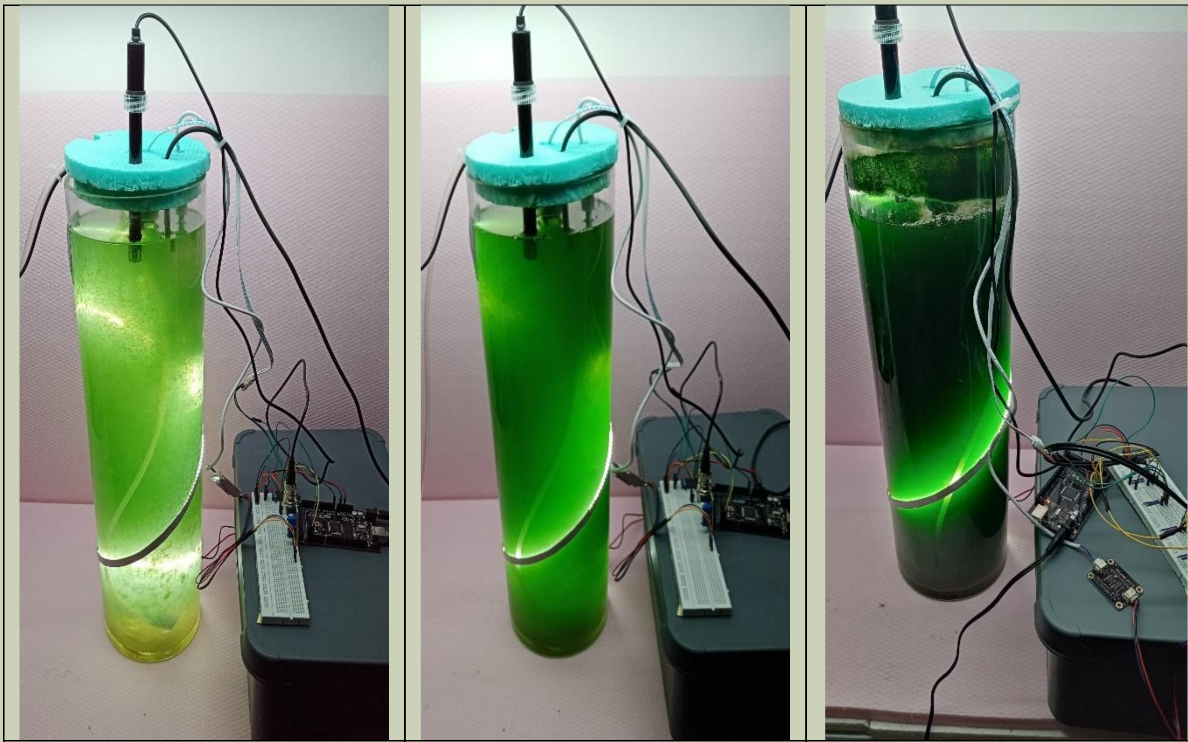
Fig.3.1
Spirulina living Culture

Fig.3.2 Inoculation
of Spirulina in photo bioreactor

Growth parameters were set to:

- Day/night mode 12/12 hours
- Medium temperature 32 +/- 1 degree Celsius
- Aeration 3 min every hour 24/7
- Ph 10.5 +/- 0.5
- TDS 1200 ppm +/- 150 ppm

Figure 3.3 shows the growth process of spirulina over a period of 15 days. Visually it is represented by the color of the medium which gradually turns to dark green. Theoretically, under optimal conditions, the doubling period of the amount of spirulina in the medium is about 8 hours. But it's very hard to obtain and include active enrichment with concentrated CO_2.In suboptimal conditions, this period is about 18 to 24 hours.



Day 3	Day 8	Day 15

Fig. 3.3 Spirulina growing process

After 15 days, turbidity of 0.82 was achieved, when the first extraction of spirulina was performed.

Fig. 3.4 OLED Display with measured values day 15

The process of spirulina extraction involves several stages. In the first stage, the medium is filtered through a 40 micron filter. The resulting sludge is then diluted two to three times in a 1g/L NaCl solution. Plain water should not be used to flush the spirulina as a sudden change of pH from 11 to 7 causes the rupture of the spirulina membranes. The third stage id drying spirulina sludge. There are different techniques and most efficient is so called spray drier, but it is relatively expensive. We coated spirulina in a thin layer and allowed it to dry under an infrared bulb. The dry spirulina was then scraped off and spirulina flakes were obtained.

Fig.3.5 Extraction of Spirulina from photo bioreactor

Fig.3.6 Washing Spirulina with 1g/l solution of NaCL

4. Results

The aim of this project was design and implementation of a closed photo bioreactor for cultivation of Spirulina (Arthospira platensis) in controlled environment. After intensive literature study, mainly available on the Internet, we proceeded to the construction of a closed photo bioreactor with a capacity of 7 liters which we equipped with LED lighting, heating and monitoring system for measuring the main parameters important for spirulina cultivation, based on Arduino Mega 2560. Spirulina Living culture was sourced from Sweden.

The experiment was conducted in our chemistry laboratory, under the supervision of our mentor. In the period of 15 days after the inoculation of living culture, the first results were obtained.

Fig 4.1 Washed Spirulina Sludge

Fig 4.2 Dry Spirulina flakes

Fig. 4.1 shows three times washed spirulina sludge, ready for drying, while Fig. 4.2 obtained dry spirulina. In the first extraction, 6 grams of dry Spirulina were obtained, i.e. slightly less than 0.9 g/L. Then, the spirulina was collected every three days, and the collected quantities ranged from 15 to 20 grams (Depending on what percentage of the total mass of the medium was filtered).

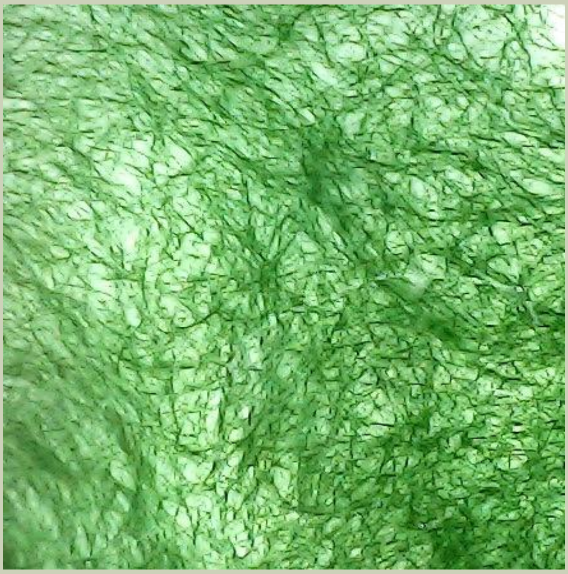


Fig.4.3 Live Spirulina microscope image (100x)

Fig.4.4 Live Spirulina microscope image (1000x)

The obtained spirulina was examined at the food institute of the University of Ss. Cyril and Methodius in Skopje. Both Microbiological and presence of micro toxins examination tests were negative.

5. Discussion

The application of dietary supplements based on natural basis, is a globally upward trend in the recent period. This was especially evident with the outbreak of the Corona virus pandemic. Spirulina ranks very high in the nutritional supplement market due to its exceptional nutritional and pharmacological properties. On the local market, spirulina can be found in the form of tablets or as a powder. Spirulina's global market for 2027 is estimated nearly at $ 900 million with estimated average annual growth of 10.5%. Most of this production is in open outdoor pools and production technologies in tubular photoreactors is in early stage. Due to the controlled environment, Spirulina that we produced is with high quality and it is appropriate for human use. Another possibility is use of fresh Spirulina. According to number of scientific research, fresh Spirulina has 40% higher nutritional value, compared to the dry. It can be produced in the form of sludge, for use in cosmetics and as a raw material for the pharmaceutical industry, as well as the production of fresh spirulina paste, which will be used in healthy food shops and restaurants. We believe that the ability to produce high quality Spirulina is a challenge that will provide a product with high commercial value.

6. Conclusion

The main goal of the project was to develop a system for production of Spirulina in controlled environment. After initial phase, our system produces about 6 gr. of dry Spirulina per day, which is almost 0.9 gr/L of Medium. According to the leading professional growers, production can go up to 4 gr/L/day and we will continue our research in order to increase our production. Number of technical problems need to be resolved in order to optimize production.

7. Further Research

The next step of our research will be including the CO_2 Enrichment system. According to the professional growers, CO_2 enrichment can double the production. Another issue is development of alternative nutrition formulations due to the relatively high price of Zaroouk's formulation. In addition, we are planning to implement a bigger system (consisting of 3 glass tubes with total volume of 105 Liter). Collected Spirulina will be used in our School Restaurant for preparation of Spirulina Smoothie and other healthy beverages.

8. Acknowledgment

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