# Experimental Analysis of Nutrition Potential of Spirulina Cultivation In Semi-Arid Zones of India

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***Abstract-*** *Spirulina is a single cell organism. It is derived from Latin word "spiral" which means small coil. It is also called as blue green algae (or) cyan bacterium.*

*It is generally occur in seas and oceans which is rich in protein, essential and non-essential amino acids. It is generally used for manufacture of several medicines and cosmetics. It can be easily grown in water bodies. Large water tanks of 10 x 5 x 1.5 feet size are generally used and 25 $^0$c - 35 $^0$c temperature should be maintained. For fast growth of mother algae some nutrients like sodium bi-carbonate, sodium chloride, magnesium sulphate, and phosphoric acid are added in the tank.*

*The water should be agitated twice a day with a bamboo stick for a week properly. It will be harvested after 10 days of initiation of mother algae. Then the harvested spirulina shall be dried for 1 or 2 days and packed in air tight containers. It has more demand in national and international market.*

## I. INTRODUCTION

Our plunge into technology and our consumer lifestyle has upset the balance of Earth's biosphere. We transform Earth's resources into trash and pollution at a faster and faster pace. Emerging from ecosystem breakdown are super bacteria, viruses, chemical toxins, global warming and climate change that threaten our own health.

More people are becoming aware that diseases like cancer are directly related to environmental factors. A growing portion of Earth's population is seeking super health to protect against this pollution, boost their immune system, resist disease and retard the aging process.

Researchers are scouring the globe for new foods and plants rich in disease preventing substances. There's a revolution in health: We've been hearing about antioxidants, nutraceuticals, and designer foods loaded with functional nutrients Probiotics, nutraceuticals, photochemical, designer foods where do we start With the original food designed by nature – spirulina!

The first photosynthetic life form was designed by nature 3.6 billion years ago. Blue - green algae, cyanobacteria are the evolutionary bridge between bacteria and green plants. It contained within it everything life needed to evolve. This immortal plant has renewed itself for billions of years, and has presented itself to us in the last 30 years. Spirulina has 3.6 billion years of evolutionary wisdom coded in its DNA.

Each day new research brings to light the wonders (hidden) in microscopic algae. Research has shown phycocyanin and polysaccharide extracts of spirulina increase macrophage production, bone marrow reproduction, strengthen the immune system and disease resistance in fish, mice, chickens, cats and human cells. Spirulina contains sulfolipids, found to prevent viruses from either attaching to or penetrating into cells, thus preventing viral infection. An algae is in its infancy as a food, medicine and biochemical resource. Spirulina, a descendant of Earth's first photosynthetic life form, was rediscovered about 45 years ago. Just 30 years ago, it burst into public awareness as a powerful new food with a promise as a food source to help feed the world's people.

Algae are two-thirds of the Earth's biomass. Thousands of algal species covering the Earth are now being identified for food, pharmaceuticals, bio chemicals and fertilizers. Algae represent one of the solutions we need to produce food while restoring our planet.

- In the beginning were blue-green algae.
- Thousands of algal species cover the Earth.
- Algae through human history.
- Rediscovery of human use – Kanembu and Aztecs.
- A new era of ecological agriculture foods where do we start With the original food designed by nature – spirulina!

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## II. LITERATURE REVIEW

The generic name "single cell protein" (SCP) was designed in an international meeting held at Mount Lake Terrace (MLT), USA to include protein source from unicellular or multicellular microbes like bacteria, yeasts, fungi and algae. The concept of utilizing SCP is not completely new, as proteins are already being used in foods and feeds in different regions of the world at varying levels. (Dabbah, 1970; Waslien, 1975).

In 1967 Spirulina was established as a "wonderful future food source" in the International Association of Applied Microbiology. Analysis of the nutritional properties of Spirulina showed first and foremost an exceptionally high protein content, in the order of 60–70 percent of its dry weight; it also showed the excellent quality of its proteins have balanced essential amino acid content. This first data was enough to launch many research projects for industrial purposes in the 1970s, because microorganisms (yeast, Chlorella, Spirulina, some bacteria and moulds) seemed at that time to be the most direct route to inexpensive proteins the iconic "single cell proteins" (Sasson, 1997).

Cyanobacteria are prokaryotic organisms that contain chlorophyll-a. The name of blue–green has been given because of the presence of phycocyanin and phycoerythrin which usually masks the chlorophyll pigmentation (Tomaselli, 2004). They are similar to bacteria in that their cells lack a nucleus, chloroplasts and also have a different gene structure (Neenan et al., 1986). Above 2,000 species belong to this group. Some cyanobacteria are known to assimilate atmospheric nitrogen (N) thus eliminating the need to provide fixed N to the cells. None of them have been found to produce significant quantities of lipid as storage (Sheehan et al., 1998).

Microalgae growth and chemical composition are mainly controlled by various environmental factors like light, temperature, available carbon dioxide, pH and nutrients (Tzovenis et al., 1997; Zhu et al., 1997; Danesi et al., 2004; Belal et al., 2012). Other factors, such as salinity, can be of vital importance to some species (Chu et al., 1996).

Spirulina maxima Geitler is a planktonic filamentous cyanobacterium that forms massive populations in tropical and subtropical bodies of water that have high levels of carbonate and bicarbonate and alkaline pH values of up to 11. Nitrogen is required for the synthesis of amino acids, which make up proteins and other cellular components (Yang et al., 2010).

The effect temperature exerts on biochemical reactions and affects the biochemical composition of algae, makes temperature one of the most important environmental factors (Hu, 2004). Spirulina, the optimal temperature for its growth falls between 35 to 38°C (Ciferri, 1983; Somasekaran, 1987).

The effect of temperature on Spirulina platensis growth rate, biomass composition and pigment production was studied. Growth kinetics of cultures showed a wide range of temperature tolerance from 20°C to 40°C. Maximum growth rate, cell production with maximum accumulation of chlorophyll and phycobilliproteins were found at temperature 35°C (Manoj kumar et al., 2011).

Vincent and Silvester (1979) reported that, the pH had a direct effect on the physiological properties of algae and the availability of nutrient. pH determined the solubility of carbon source and minerals in the culture directly or indirectly. The optimal pH of the Spirulina nutrient medium was shifted from 8.4 to 9.5 during the mass cultivation, due to the consumption of bicarbonate and sodium ions. Spirulina grew well at pH values between 9 and 11. These are limiting conditions for other microorganism allows cultivation of microalgae in open reactors (Subramaniyan and Jeeji Bai, 1992; Volkmann et al., 2008).

Spirulina was grown in presence of light (photosynthesis), full sunlight illumination may not required (30% of full sun light is enough, except that more may be required to quickly heat up the culture in the morning). During dark periods, chemical reactions take place within Spirulina, like synthesis of proteins and respiration (Jourdan, 2001).

The salt concentration (mostly carbonates and bicarbonates) plays a direct role in the growth of Spirulina. The growth of S. platensis is optimal at salt concentrations ranging from 20 to 70 g per liter, however S. platensis was found in water containing from 85 to 270 g of salt per liter. In the lakes, containing salt concentrations more than 30 g per liter, Spirulina was the only organism present in significant quantities (Ciferri, 1983).

The Spirulina production involves three major steps, viz., cultivation, harvesting and processing. Selected strains were used for cultivation of alga in specially constructed ponds. Constant agitation of water is one of the important parameters in cultivation of Spirulina. Agitation of algal culture is necessary to keep nutrients evenly dispersed and to expose all the cells to sunlight. The algal biomass was carefully harvested by using specially made filters to recover biomass. The washed biomass is dried and pulverized to get desired particle size and packed appropriately (Ciferri, 1983; Vonshak, 1997).

Bohra (2009) investigated the growth patterns of Spirulina platensis in standard and modified media based on seawater-chemicals and seawater fertilizers. Spirulina platensis was observed to have different specific growth characteristics in different media at same environmental parameters (temperature, pH and light intensity). Even though, Spirulina platensis in standard media exhibited better growth patterns, biomass, protein content and chlorophyll content than other seawater based media.

Spirulina contains unusually high amounts of protein, between 55 and 70 percent by dry weight, depending upon the source. It is a complete protein, containing all essential amino acids, though with reduced amounts of methionine, cystine, and lysine, as compared to standard proteins such as that from meat, eggs or milk; it is, however, superior to all standard plant protein, such as that from legumes (Ansuya Devi et al., 1981; Phang et al., 2000). These facts are making this Spirulina interesting in terms of fishmeal replacement. Spirulina species have been already tested as a substitute protein source for Cyprinus carpio, where equal or even higher growth rates were obtained by diets containing 25% algae meal, replacing 80% of the dietary fishmeal (Paoletti et al., 1980).

Shekharam et al, (1987) have found that S. platensis contain about 13.6% carbohydrates; some of these are glucose, rhamnose, mannose, xylose and galactose. Spirulina does not have cellulose in its cell wall, a feature that makes it appropriate and important food stuff for people with problems of poor intestinal absorption, and geriatric patients (Richmond, 1992).

Seoet al, (2013) reported the method for stably purifying a functional dye, phycocyanin from Spirulina platensis developed by a hexane extraction process combined low temperature with high pressure. The purification yield of this method was estimated as 10.2%, whose value is 3–5% higher than in the case from another conventional separation method using phosphate buffer. The results were achieved because the low temperature with higher pressure extraction effectively disrupted the cell membrane of Spirulina platensis and degraded less the polypeptide subunits of phycocyanin as well as increasing the extraction yield.

The United Nations world food conference declared Spirulina as “the best for tomorrow”, and it is gaining popularity in recent years as a food supplement (Kapoor and Mehta, 1993). The Spirulina ability as a potent anti-viral (Hayashi et al., 1993; Patterson, 1993), anti–cancer (Schwartz, 1988), hypocholesterolemic (Iwate et al., 1990) and health improvement agent is gaining attention as a nutraceutical and a source of potential pharmaceutical (Annapurna et al., 1991).

Hanel et al, (2007) conducted experiment on the growth effect of Pacific white shrimp Litopenaeus vannamei determined through the replacement of fishmeal by the microalgae Spirulina platensis in the diet. Growth rates of a Spirulina-fed group differed highly significantly ($p < 0.001$) compared to two groups other fed to less suitable diets and even though not significantly superior to that based on commercial reference fish diet. The side effect of Spirulina fed shrimps showed measurable differences in pigmentation. The results clearly indicated that S. platensis constitutes an effective food ingredient for shrimp

## III. METHODOLOGY

The spirulina is grown in different types of ponds. They are:

1) Commercially small size ponds.
2) Commercially big size ponds.

### 1. Commercially Small Size Ponds:

The commercially small size ponds having capacity of 4000 lts. Generally these are of two types. They are

a) Iron frame tanks.
b) Cement tanks.

### a) Iron Frame Tanks:

Manufacturing of Iron Frame:

The iron tanks are made up of square shaped iron bars whose length is 20ft, breadth is 10ft and height is 1.5ft. It is welded together in the shape of cuboids. We can observed in the figure

### Preparation of land:

The land should be dried and not be loose soil. After selection of land then marking the land of length is 21ft and breadth is 11ft then dig the land for depth of 10 inches, from the ground surface. Here we can observe the labour digging the land in the figure.

### Filling the land Surface with Sand:

After completion of digging land the digged portion is filled with sand up to 10cm height. So the surface will be smoothen by filling the sand the temperature difference should

be there between the surface of land and surface of tarpaulin. We can observe the labor filling the sand in the figure.

**Fixation of Tarpaulin, Nuts and Bolts:**

The iron frame is inserted in the digged portion then the tarpaulin is fixed to iron frame. The tarpaulin is made up of nylon material which is of 600 Gsm thicknesses, length is 25 ft and breadth is 15 ft. By arranging tarpaulin over the iron frame then by making holes to the tarpaulin and fixing the nuts and bolts of 2mm dia and length 21/2 inches. We can observe that in figure.

**b) Cement tanks:**

In the preparation of cement tanks is simple after selection of land by marking of length is 21 ft, breadth is 11 ft and in the shape of rectangle and dig the land up to 10 inches depth. Here by using bricks and cement constructing the tank of length 20ft, breadth is 10ft and height is 1.5ft. After completion the ground surface is filled with concrete. Then dried for two days by applying water proof seal all over inside the tank for no water leakage.

## IV. PROCEDURE

1. Commercially Big Size Ponds:

In the commercially big size tanks only cement tanks are there because of its capacity is of 60,000 lts. These are of very big in size whose length is 100ft, breadth is 30 ft and height is 1.5 ft. By constructing a wall in centre of length is 80 ft, breadth is 1 ft and height is 1.5 ft. It is in the shape of semi-oval for free circulation of water. In this arranging the collecting frames at a side of the tank whose length is 10 ft and breadth is 14.5 ft. A pedal wheel is arranging in a tank of low rpm motor for agitating the water for certain duration of time.

**Beginning of a New Pond:**

After fixation of tank the tank should be cleaned with water and sea salt. Firstly 10 buckets of water is added to the tank and wash it thoroughly with brushes as shown in the fig. and remove the water. Then add 5 kg of sea salt and brush it properly and again add 10 buckets of water in it rub it with a sponge smoothly. After completion dry the tank and expose to the sun light for 1 day completely.

**Culturing of a new pond:**

**Testing the water:**

The water that we are using should be tested in the laboratory. The value should laid between

P^HValues - 7.5- 9
Total hardness – below 400 ppm.
Fluorine content – below 1.5gm/lit.

**Filling the tank with water:**

The water is filled in the tank up to 25cm height from the ground surface of the tank that is of 4000 lts because the sun light is only enters up to 25 cm in water. So the algae can be grown in the tank by using photosynthesis process.

**2. Day to day process:**

**Day 1 (Adding of chemicals to the tank):**

Adding of chemicals to the tank by using zarrouk formula1. Here adding of sodium bicarbonate to the tank with the help of poonam cloth as shown in the fig. and agitated thoroughly. After 1 hour add Nacl (sea salt) to the tank by using same process and again agitated thoroughly for every 1 hour for a day properly as shown in fig.

**Day 2 (Adding of mother culture to the tank):**

The next day, take a container of 20lit capacity adding urea, potassium sulphate, magnesium sulphate, phosphoric acid, ferrous sulphate acid and agitated thoroughly for every half an hour up to 7 hours continuously. After completion of agitation adding mother culture (spirulina) by using the poonam cloth for spreading of about 4 kg spirulina.

**Zarrouk formula 1:**

Table 1. Application of zarrouk formula 1.

| S.NO | Chemicals | 1 litre | 4000 litre | 60000 litre |
|---|---|---|---|---|
| 1. | Sodium bicarbonate | 8 gm | 32 kg | 480 kg |
| 2. | Sodium chloride | 5 gm | 20 kg | 300 kg |
| 3. | Urea | 0.2 gm | 800 gm | 12 kg |
| 4. | Potassium sulphate | 0.5 gm | 2 kg | 30 kg |
| 5. | Magnesium sulphate | 0.16 gm | 640 gm | 9.6 kg |
| 6. | Phosphoric acid | 0.052 ml | 208 ml | 3120 ml |
| 7. | Ferrous sulphate acid | 0.05 ml | 200 ml | 3000 ml |

### 3. Step by Step Procedure of Adding Chemicals for a New tank:

Step-1:First add 32 kgs of sodium bicarbonate powder in to tank and agitate the tank thoroughly.
Step-2:Add 20kgs of sea salt in the tank with using of poonam cloth to dissolve it.
Step-3:Take a 20 lts capacity bucket and fill 10 lts water in it. Then add 800 gm of urea, 2kgs of potassium sulphate, and 640 gm of magnesium sulphate dissolved all of them properly.
Step-4:Add 208 ml of phosphoric acid in to the same solution of the bucket.
Step-5:After add 200 ml of ferrous sulphate acid in bucket.
Step-6:Dissolve all the chemicals in the bucket for 2 minutes.
Step-7:After dissolving the solution is added to the tank.
Step-8:Then agitate the tank for an every ½ hour continuously up to 7 hours.
Step-9:After agitating the tank put 4 kgs of mother culture with the help of white poonam cloth.

**Day-3 to Day-8:**

Until 8th day only agitate the tank for every ½ hour continuously up to sun light is coming.

Day-9:From 9th day onwards the harvesting will start.

### 4. Harvesting the culture:

Until 8th day the algae will be grown slowly. The harvesting is usually done at morning hours before sun lighting starts because the mother algae is at top layer and it is 1mm thickness after sun light is falling it will split in to baby algae is of 0.5mm thickness. So it is a difficult task to collect that baby algae.

**Preparation of filters:**

The filters are made up of wood which is in the shape of square whose length is 3ft and breadth is 3ft. One filter is having a poonam cloth as a screen to filter the phosphate and any dust particles. Another filter is having plastic screen for supporting the cloth.

**Filtering the algae:**

The algae was collected in the gaadha cloth is placed between the two filters. By using buckets the algae water was poured on the filter cloths as shown in fig. finally the algae was collected in gaadha cloth. It was collected in a bowl by using a scoop very gently. Filter the algae twice or thrice as well collected in the cloths because only the gaadha cloth is having below 1mm sieve.

**Cleaning the Algae:**

After collecting the algae it should be cleaned with fresh water because some salts present in it. By using screen filter cloth of 500mesh thickness the algae was taken in the screen filter cloth and pour fresh water for cleaning the algae by using scoop can be observed

**Adding of Chemicals by using Zarrouk Formula 2:**

After cleaning the algae it was weighed by getting of wet algae the chemicals were added to the tank using zarrouk formula 2 and agitate the tank properly foe every ½ hour continuously up to sunlight is present.

### 5. Storing the Algae:

After drying the algae it was stored in a air tight container and keep at cool and dry place.

### 6. Making the Dried Algae in to Powder:

The dried algae collected in some amount it was making in fine powder with the help of pulveriser. Then again collected in air tight containers and kept in cool and dry places.

### 7. Maintenance of the Tank:

Table 2. Daily work: Table 3.3: daily work

| S.N | Timings: | Work: |
|---|---|---|
| 1. | 06.00 am to 10.30 am | Filtering the culture |
| 2. | 10.30 am to 11.00 am | Cleaning and draining the culture |
| 3. | 11.00 am to 11.30 am | Noodling the culture |
| 4. | 11.30 am to 12.30 pm | Bottom and upper layer cleaning |
| 5. | 12.30 pm to 01.00 pm | Application of zarrouk formula 2. |
| 6. | 01.00 pm to 04.30 pm | Agitation of water for every ½ hour |

**Upper layer cleaning of the tank**:

Cleaning the upper layer daily by using the poonam cloth to collect the phosphate wastage and once in a week brush the sides of tarpaulin to remove the dust particles.

**Bottom layer cleaning:**

Once in a month remove the bicarbonate wastage which is collected at bottom of the tank.

**Maintaining the water level:**

The water level should be maintained between 25cm – 20cm. Check the water level daily by using a scale is there any deficiency add some fresh water to the tank.

**Maintaining the P^H value:**

The P^H value is maintained between 9 to 11. Check the P^H value by using the digital P^Hmeter.

**Maintaining the water temperature:**

The water temperature should be maintained between 25˚c – 35˚c. Check the water temperature by using the thermometer. Agitating the tank: Agitating the tank for every ½ hour continuously for better production.

## V. CONCLUSION

1. For the project Analysis purpose, three types of algae are used those are i) Spirulina (Blue – Green Algae) ii) Green Algae iii) Brown Algae
2. Spirulina (Blue – Green Algae) grown in the pond of size
3. 20x10x1.5 feet of capacity 4000 liters
4. Spirulina (Blue – Green Algae) started producing from 9th day of its addition in the pond.
5. After 9th day onwards, 300gm of wet Spirulina produced from the pond.
6. Regularly Production is harvesting day by day after 9th day, and getting 500gm of wet Spirulina.
7. The harvested Spirulina can be used as a high Protein content food for human beings and Aqua Feed as well.
8. Protein content of harvested Spirulina was tested in the VIMTA Food Testing Laboratory, 68.6% protein formed in 1 gm.
9. The harvested Spirulina dried up, making in to fine powder and produce in the form of Capsules for Human Being consumption.
10. The Spirulina can be consumed daily in the form of capsules and Tablets.

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