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Spirulina spp. Isolation and Cultivation for Fish Feed

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Abstract

Chlorella, Nannochloropsis, Dunaliella, Schizochitrium, and *Spirulina* are just a few of the many genera of microalgae, which are unicellular and part of the phytoplankton family. They flourish in saltwater and are rich in fish-friendly nutrients. The main benefit of marine microalgae is that they are quickly and easily grown, harvested, and processed. One of the promising alternative feed protein supplements is spirulina. *Spirulina* spp. was isolated from Manyara Lake in Tanzania and cultured in a different liquid medium called Zakarrous (ZM). It has been used as a source of protein since ancient times. For ten days, pH and dry-weight biomass were measured daily in a lab setting. pH was discovered to be in the range of 9.2 to 11.4; dry weight (dw), biomass, and pH were all gradually increasing as the culture grew older, achieving 2.5 g/L. According to the findings of the current study, *Arotharon hispidus,* a marine fish, grew more rapidly when *Spirulina* spp. was isolated, with a relative gross weight increase of 70.89% as opposed to 49.73% for commercially available fish meal. Based on the results, it can be concluded that the isolate increased the weight of the fish that consumed it, and the results also imply that more testing is necessary to show the isolate's efficacy as a fish feed supplement.

***Key words*:** Zakarrous medium, *Spirulina* spp, Manyara Lake, *Arotharon hispius*, Fish feed

It has only recently become clear that there is a potential for microalgal biotechnology, despite the fact that humans have long benefited from natural populations of microalgae (Nostoc in Asia and Spirulina in Africa and North America). Technology based on microalgae possesses the potential to produce dietary supplements, biofuels, and bioremediation techniques. Natural pigments, industrial drugs, and therapeutic uses cover a wide range of goods and are all produced from a largely untapped source, with the main objective being the creation of successful business ventures [1]. Various plant-like, simple organisms known as algae use photosynthesis to synthesize energy. Algae, despite being conventionally thought of as simple plants, actually belong to both the bacteria and the eukaryota. Algae can be single-celled or multicellular organisms, some of which have fairly complex, differentiated forms. If they are marine, algae are also known as seaweeds. They are all devoid of the organ structures found in higher plants, such as leaves, roots, flowers, and flowers. They differ from other protozoa in that they are photoautotrophic; some groups are myxotrophic, obtaining energy from both photosynthesis and the uptake of organic carbon either by osmotrophy, phagotrophy, and myzotrophy, or only external energy sources are used by some unicellular species, which have altered or lost their ability to produce photosynthetic organisms [2].

Both food and nutritional supplements have been made from cyanobacteria. Cyanobacteria are single-celled prokaryotes that have characteristics that have led some biologists to question whether they should be considered bacteria or algae. In addition to typical aquatic and terrestrial habitats, cyanobacteria can also be found in harsh environments like the dry crevices of desert rocks and hot springs with temperatures as high as 71 °C. *Spirulina*, a cyanobacterium, has been a staple food in Chad for a very long time and was first consumed by the Aztecs in 16th-century Mexico. It is now grown and sold as a health food and dietary supplement in many nations, including the USA, Thailand, China, India, and Australia. Very few plants that produce high-value foods or pigments are still the only ones that can produce algae commercially.

In order to combine the production of protein with the recycling of nutrients, the elimination of inorganic pollutants, and the disposal of waste, spirulina can be grown on wastewater [3]. There are three different kinds of commercial microalgae culture systems currently in use: raceway ponds, circular ponds, and large open ponds with rotating arms to mix the cultures. Any alga can be grown, but it takes a complex system to do it, and it depends on the interaction of many internal and external variables. Most algae grow in mixed communities that contain a variety of species and genera in their natural habitats. The creation of an environment that is conducive to a desired species' growth is necessary for its isolation [4].

The effects of dietary *Spirulina platensis* supplementation on the development and defense mechanisms

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of *Mystus cavasius* were examined by Al-Mamun *et al*. [5]. These results imply that dietary *S. platensis* has favorable effects on the immune system and growth of *Mystus cavasius*, and maintaining a dietary replacement of 7.5–10% fish meal with *S. platensis* can be recommended for *M. cavasius* feed formulation [6]. The effects of dietary *Spirulina platensis* supplementation on the development and defense mechanisms of *Mystus cavasius* were examined by Al-Mamun *et al*. [5]. These results imply that dietary *S. platensis* has favorable effects on the immune system and growth of *Mystus cavasius*, and maintaining a dietary replacement of 7.5–10% fish meal with *S. platensis* can be recommended for *M. cavasius* feed formulation.

In order to determine the viability of producing *Spirulina* of food-grade quality in a marketable form, *Spirulina* spp. will be grown in cooling tower brine effluent, and its growth will be statistically optimized in an open tank bioreactor with brine effluent medium. 1.1.2 Purposes 1. Isolate a strain of *Spirulina spp.* that grows quickly, is not genetically altered, and has the potential to be produced on a large scale.

In order to determine whether the medium is suitable for supporting the growth of spirulina, it is necessary to evaluate the growth kinetics of the algae in laboratory-scale batch processes. 3. To ascertain, statistically, the impact of biotic and abiotic factors on spirulina growth in brine effluent. 4. To build and maintain an appropriate open bioreactor for the production of spirulina in a growth medium that is optimized. 5. To ascertain the nutritional makeup of *Spirulina spp.* and evaluate the viability of producing it on a large scale.

MATERIALS AND METHODS

Isolation and identification of Spirulina

Water samples from the lake regions were taken, and their relative abundance of BGA was assessed using a light microscope. While samples with high counts were diluted with *Spirulina* medium, those with very low counts and mixed forms were concentrated by centrifugation. The strain was discovered in Tanzania's Lake Manya. In batch culture, *Spriulina* spp. was grown in Zarrouks Medium (SM) (Appendix 1). In order to maintain viability, cultures were inoculated into a medium every month under continuous light (0.1522 Lux) and agitation at 160 rpm.

Appendix 1

Zarrouks Medium

Ingredients	gms/L
Trace metals	4 mL
EDTA Sodium salt	0.08
$FeSO_4.7\ H_20$	0.01
$CaCl_2$	0.04
$MgSO_4.7H_20$	0.2
$K2HPO_4$	0.5
$K2O_4$	1
$NaHCO_3$	16.8
NaCl	1
$NaNO_3$	2.5

Race metals

Ingredients	gms/L
Cu SO_4	0.079
Cobalt nitrate	0.049
Sodium molybdate	0.39
Zinc sulphate	0.222
Manganese chloride	1.13
Boric acid	2.86

Inoculum preparation

A rotary shaker set to 180 rpm was used to stir the inoculum into a 250-mL Erlenmeyer flask that contained 100 mL of each media type. For ten days, a constant illumination of light at 0.1522 Lux intensity and a temperature of 30 °C were used. Each culture was centrifuged at 3500 rpm for 10 minutes using 50 mL of the mixture. The pellets that remained were redissolved in distilled water after the supernatant was discarded.

Spirulina *production in batch experiments*

Spirulina spp. In order to identify the growth factor that generated the most biomass, it was studied. Twenty-four 500-mL conical flasks, each containing 250 mL of *Spriulina* spp. medium, were used in the laboratory shake culture experiments. Both of the conical flasks—one with the controls, which contained only growth media—represented the growth media for incoulums used in cultivation. About 5 mL of *Spirulina* spp. were added to one set of three flasks as an inoculant. Fluorescent lighting with a 0.1522 Lux brightness level was used to provide illumination. At room temperature, for 10 days, all flasks were inoculated in a rotating shaking incubator.

Growth estimation of Spirulina

Concentration of Spirulina

Every twenty-four hours, five mL aliquots from each set of flasks were taken, and the turbidity was measured at 670 nm with a spectrophotometer to determine the cell density. This was used to calculate the amounts of biomass produced and compare them to standard curves. A digital pH meter equipped with an ATCCL 120 Chemi line was also used to calculate the pH.

Research instruments used Spectrophotometer A VIS spectrophotometer was used to measure the turbidity of the culture at 670 nm in order to calculate the cell density. The pH meter ATCCL 120 was used for this purpose.

Mechanical shaker

As an inoculum for transfer to the photobioreactor, overnight culture was used. The mechanical shaker used in this study (Rivotek Rivera Galss Pvt. Ltd. in Mumbai, India).

Microscope

Spirulina was confirmed using a binocular light microscope.

Construction of a photobioreactor

For the purpose of microalgal culture, a raceway algal photobioreactor was built (Fig 2), and a 60 W fluorescent tube (4.28 Lux) is providing even artificial lighting. The reactor was simply a paddle that was powered by electricity. Consisting of an AC/DC output and a rheostat to regulate the voltage supplied (Fig 3). The electrical output device was in charge of regulating the paddle's rotational speed. The bioreactor's overall volume was 2 liters, and its depth and diameter were 150 mm and 130 mm, respectively. For adequate light exposure, the working volume was 1 L and the depth was 40 mm (Fig 4). From the light source to the bioreactor, the light transmission distance was 300 mm, and it was focused.

Measurement of *Spirulina spp.*

Spriulina spp. was used as the inoculum. 250 mL in total were used to culture the organism in the lab. Every twenty hours, five mL aliquots from the bioreactor were removed, and the optical density at 670 nm was measured with a

spectrophotometer to calculate the growth. To keep the culture's volume constant, a quantity of fresh medium was added.

Drying of Spirulina powder using a hot air oven

The remaining volume of media was used to recover the algae after a suitable cultivation period of 20 days had passed. The *Spirulina spp.* cell mass was transferred to Whatman filter paper after it was centrifuged. The wet slurry was measured and dried for 16 hours at 100 °C in a hot air oven (Sigma, Chennai). Following desiccation, it was cooled to room temperature. The cell mass was weighed using an analytical balance, and the following calculation was used to determine it [7].

$$\text{Dry weight \%} = \frac{[\text{Wet weight} - \text{dry weight}]}{\text{Dry weight}} \times 100$$

Experimental design for fish feeding

Setting fish of the *Arotharon hipidus* T0 (5% fish meal) and T_1 and T_2 (5% *Spirulina* spp.) strains were fed a diet. Throughout the duration of the experiment, the physiochemical parameters of water were kept within the ideal range (pH 7.3–8.2 and temperature 18–20 °C). Feeding took place at a rate of 5% of body weight. The daily ration was divided into two split doses; roughly two-thirds of the total ration was administered at 9:00 and the remaining third at 18:00. To maintain the best possible concentration of dissolved oxygen throughout the experiment, the feces were removed by siphoning and water was continuously replaced.

Growth study

Spriulina spp. growth was monitored using a pH meter and spectrophotometer. Spirulina's dry weight was calculated using a digital electronic balance. The following equations were used to calculate the growth rate of fish in terms of weight gain / relative growth rate%.

$$\text{Relative growth rate} = \frac{\text{Final weight minus initial weight}}{\text{Initial weight}} \times 100$$

RESULTS AND DISCUSSION

Microscopic examination of Spirulina

The strains of *Spriulina sp.* We were isolated, prepared as wet mounts, and viewed at 10 X magnification under a light microscope (Fig 1). *Spirulina* are free-floating filamentous cyanobacteria characterized by cylindrical, multicellular trichomes in an open, left-handed helix. In liquid media, the cells are helical in shape, and this changes to a complete spiral in solid media. The cells appeared green due to the predominant presence of the pigment chlorophyll. The microorganisms that were used in this present research were the cultures of *Spirulina* isolated from a nearby lake.

Fig 1 Shows *Spirulina* under 10 X objective lens

The study used Zarrouks culture medium as an economic, growth medium for the cultivation of *Spirulina* spp. This type of study is relevant because lower production costs derived from the use of a low-cost effluent could lead to a completive process.

Laboratory scale bioreactor

In the lab, *Spirulina* spp. was cultured using a 2-liter bench-top bioreactor with an agitator under steady-state conditions (Fig 2). An exponential growth rate was seen on a typical sigmoid growth curve. After the first two days, growth slowed down, but biomass continued to grow, reaching a maximum of 2.5 g per liter after seven days. At the end of the 28th day, similar research by Nagle *et al.* [8] revealed 2.48 gm (dw) per L.

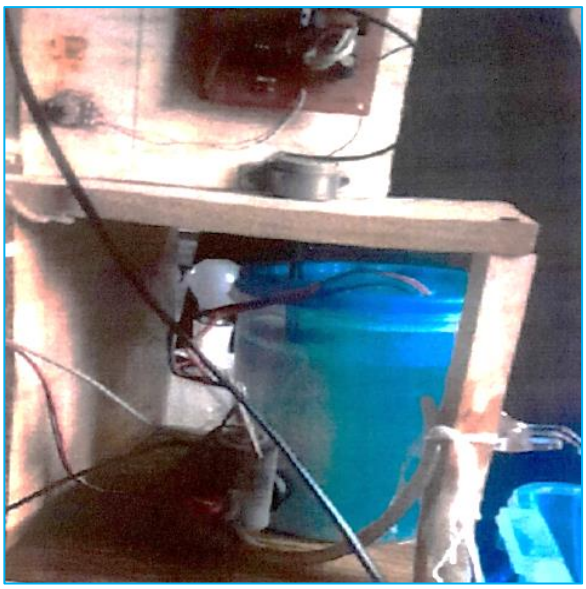

Fig 2 Lab made bioreactor

According to research by Pelizer *et al.* [9], both the age and concentration of the inoculums had an impact on cell growth. According to their research, an inoculum that was three days old grew better than one that was 10 or 14 days old. However, a high volume of inoculums would be needed for cultivation. They concluded from additional research that a 6-day inoculum with a starter biomass concentration of 50 mg/L was preferable.

Table 2 Growth performance of fishes fed with fish meal and *Spriulina*

Day	pH
0	7
1	9.5
2	9.2
3	9.8
4	10.2
5	10.5
6	10.8
7	11.2
8	11.2
9	10.8
10	10

After three days of growth for both cultures, it was determined that six days of cultivation weren't necessary in order to obtain a 50 mg/L (wet biomass) concentration of *Spirulina* for the preparation of an inoculum for this study.

Spirulina maxima 1.16 × 10^7 cells per mL were inoculated into three media, including Zarrouks Medium (ZM), over the course of three days. The lag, exponential, and stationary phases of growth could be distinguished when an organism was grown in a homogenous batch culture, for instance, in mixed liquid media in an Erlenmeyer flask [10]. These stages show the patterns of growth for microbes. At a temperature of 25 °C, *S. maxima* (Fig 3) were cultured, and they revealed typical growth in ZM. The *S. maxima* in SSM had shown a 20-hour lag phase, after which the culture very well adapted to the environment, and a steady increase in concentration was observed until 172 hours, when the stationary phase started.

Data in (Table 1) depicts how the media's pH has changed. After 216 hours, there is a drop in the pH for *S. maxima* grown in ZM, which may be caused by a product of extra-celluar substance release that lowers the pH. However, growth in ZM continues to raise the pH until day 9.

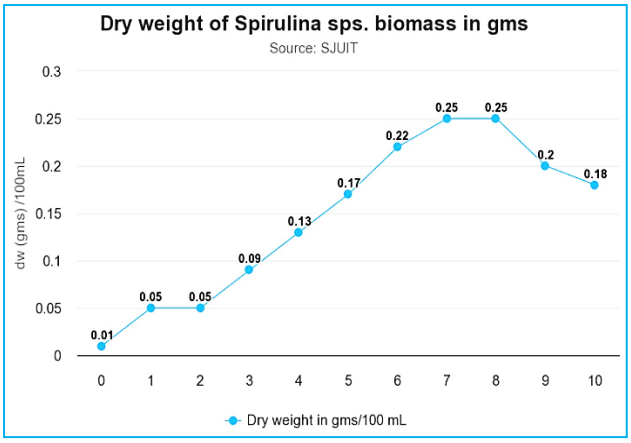

Fig 3 Dry weight of *Spirulina spp*. biomass in gms

The (Fig 3) depicts how the media's culture has changed. After 216 hours, there is a drop in the DW for *S. maxima* grown in ZM, which may be caused by a depletion of contents in the medium that lowers the dry weight of the culture. However, growth in ZM continues to raise the DW until the 9th day.

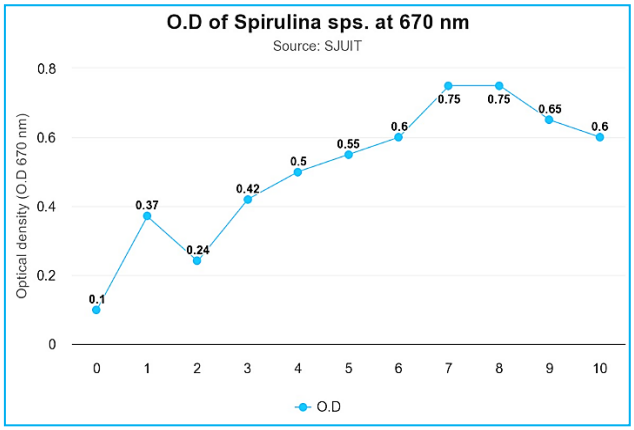

Fig 4 Growth curve of *Spriulina spp*.

Data in (Fig 4) shows how the growth curve has been obtained. After 192 hours, there is a drop in the growth curve for *S. maxima* grown in ZM, which may be caused by a depletion of contents in the medium that lowers the dry weight of the culture. However, growth in ZM continues to increase the O.D. until the 8th day.

Growth of fish

For the experimental fish and tested diets, the initial average weight and final weight were calculated and recorded in (Table 2).

Table 2 Growth performance of fishes fed with fish meal and *Spriulina*

Treatment	Initial weight	Final weight	RGR (g)
T_0	3.78	4.53	19.84
T_1	3.78	5.66	49.73
T_2	3.78	6.46	70.89

The fish fed the fish meal diet had the lowest RGR (19.84), while the fish fed the *Spirulina* spp. diet had the highest RGR (70.89%). In the current study, a diet containing *Spriulina* spp. led to improved growth. These results are consistent with those of Jha *et al.* [11], who found that a diet fortified with *Spirulina* spp. has a greater impact on growth and nutrient profile but less of an impact on pigmentation than a diet fortified with marigold, which has a greater impact on pigmentation but less of an impact on fish growth and nutrient profile.

Fish survival rates and meat quality were unaffected by spirulina supplementation, according to Mulokozi *et al.* [12]. When used as a feed additive in Rufiji tilapia mariculture, it appears that Momella Lake spirulina may be a suitable plant protein that stimulates growth.

CONCLUSION

Many research projects have been carried out on spirulina. This research has therefore been the focus of the current study in order to improve the yield of spirulina for the fish. The primary goal of the current investigation was to separate the strain spirulina and characterize it using growth-related metrics. The outcomes are motivating to continue working on enhancing fish feed. On the basis of the findings, it can be said that the isolate improved the weight of the fish that were fed it, and the findings further suggest that additional criteria are needed to demonstrate the isolate's effectiveness as a fish feed supplement.

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