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REVIEW ARTICLE

Spirulina Cultivation: A Review

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ABSTRACT

Blue-green algae (Cyanobacteria) are among the most primitive life forms on Earth. Their cellular structure is a simple prokaryote. They share features with plants, as they have the ability to perform photosynthesis. They share features with primitive bacteria because they lack a plant cell wall. Interestingly, they also share characteristics of the animal kingdom as they contain on their cellular membrane complex sugars similar to glycogen. Among blue-green algae, both edible and toxic species adapted to almost any of the most extreme habitats on earth. Edible blue-green algae, *including Nostoc*, *Spirulina*, and *Aphanizomenon* species have been used for food for thousands of years. *Spirulina* are multicellular and filamentous blue green algae that has gained considerable popularity in the health food industry and increasingly as a protein and vitamin supplement to aquacultures diets. It grows in water, can be harvested and processed easily and has very high macro and micro nutrient contents.

Key words: *Spirulina platensis*, Zarrouk's medium, Mass cultivation and Protein content.

1. INTRODUCTION

The protein is an essential component of diet. The greatest single problem in the world today is Global food protein shortage. With the current system of production, agriculture cannot be relied upon to feed an ever increasing world population. Hence, there is an urgent need to find other protein sources. The best potential is seen in microbial protein or single cell protein (SCP), a new source of protein independent of agriculture. The dried cells of microorganisms such as bacteria, fungi, yeasts and algae that are grown in large scale culture systems as proteins, for human or animal consumption are collectively known as single cell protein. SCP are characterized by; fast growth rate; high protein content (43-85%) compared to field crops; require less water, land and independent of climate; grow on wastewater; can be genetically modified for desirable characters such as amino acid composition and temperature tolerance [1].

Spirulina has been used as a complementary dietary ingredient of feed for fish, shrimp and poultry and increasingly as a protein and vitamin supplement to aqua feeds [2]. China is using this micro alga as a partial substitute of imported forage to promote the growth, immunity and viability of shrimp. There has also been comprehensive research on the use of *Spirulina* as aquaculture feed additives in Japan.

During the sixtieth session of the united nations general assembly (second committee, agenda item 52), a revised draft resolution on the "Use of Spirulina to combat hunger and malnutrition and help achieve sustainable development" was submitted by Burundi, Cameroon, Dominican Republic, Nicaragua and Paraguay. As follow up of this resolution, FAO was requested to prepare a draft position paper on *Spirulina* so as to have a clearer understanding on its use and to convey FAO position on this.

Spirulina platensis has been used as food for centuries by different populations and only rediscovered in recent years. Once classified as the "blue-green algae'', it does not strictly speaking belong to the algae, even though for convenience it continues to be referred to in that way. It grows naturally in the alkaline waters of lakes in warm regions [3]. Measuring about 0.1mm across, it generally takes the form of tiny green filaments coiled in spirals of varying tightness and number, depending on the strain. Its impressive protein content and its rapid growth in entirely mineral environments have attracted the attention

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of both researchers and industrialists [4].

Spirulina are unicellular and filamentous blue-green algae that has gained considerable popularity in the health food industry and increasingly as a protein and vitamin supplement to aquaculture diets. It has long been used as a dietary supplement by people living close to the alkaline lakes where it is naturally found. *Spirulina* has been used as a complementary dietary ingredient of feed for fish, shrimp and poultry. Among the various species of *Spirulina*, the blue green alga *Spirulina platensis* has drawn more attention because it shows an high nutritional content characterized by a 70% protein content and by the presence of minerals, vitamins, amino acids, essential fatty acids etc [5].

Spirulina platensis is naturally found in tropical regions inhabiting alkaline lakes (pH 11) with high concentration of NaCl and bicarbonates. These limiting conditions for other microorganism allow cultivation of microalgae in opened reactors [6]. In Cyanobacteria, the light harvesting pigments include chlorophyll-a, carotenoids and phycobili proteins. The later are proteins with linear tetrapyrrole prosthetic groups called according to their structure: phycocyanin, phycoerythrin and allophycocyanin. Among the various microorganisms used as sources of SCP, the blue green algae, *Spirulina* is considered as the best source. The composition of the biomass, including the high protein content, low content in nucleic acids, occurrence of high concentrations of vitamins and other growth factors and the presence of cell wall that is more easily digestible than that of other microbes indicate that *Spirulina* is a promising source of food or feed.

Spirulina is commonly found in aquatic ecosystems like lakes, ponds and tanks. It is one of the nature's first photosynthetic organisms capable of converting light directly for complex metabolic processes. *Spirulina* is used for food from time immemorial by tribes living around Chad Lake in Africa. The predominant species of phytoplankton of the lake is *Spirulina platensis*. The algae *Spirulina* was eaten in Mexico under the names 'Tecuitlatl'[7]. *Spirulina* grows optimally in pH range of 9-11 and there is least chance of contamination of other microbes [8].

Spirulina can play an important role in human and animal nutrition, environmental protection through wastewater recycling and energy conservation. *Spirulina* is rich in proteins (60-70%), vitamins and minerals used as protein supplement in diets of undernourished poor children in developing countries. One gram of *Spirulina* protein is equivalent to one kilogram of assorted vegetables. The amino acid composition of *Spirulina* protein ranks among the best in the plant world, more than that of soya bean [9].

Vitamins and minerals[10], Gamma-linolenic acid contained in this alga have been reported to stimulate prostaglandin synthesis and induction of the regulation of blood pressure, cholesterol synthesis, inflammation and cell proliferation [11, 12].

Spirulina provides all essential nutrients without excess calories and fats. It is recommended to control obesity and premenstrual stress. Athletes take *Spirulina* for instant energy. Many herbal cosmetics like face creams biolipstics, hair lotion have been formulated from phycocyanin pigment found in *Spirulina*. The beta carotene and other carotenoids are having a suggested role in the control of cancer in human and enhancement of pigmentation of eggs, meats and coloration of ornamental fish.

The mass cultivation of *Spirulina* is achieved both in fresh water and waste water. *Spirulina* grown in clean waters and under strictly controlled conditions could be used for human nutrition. The micro alga grown in waste water is used as animal feed and provide a source of the fine chemicals and fuels. The waste water system is highly applicable in populated countries like India where wastes are generated in high quantities and pose environmental problem. Large scale production of *Spirulina* is feasible in tropical conditions in developing countries, where land costs and labour are comparatively cheaper. The micro alga can be exploited as a potential source of food, feed and fuel.

The generic name "single cell protein (SCP) was designed in 1996 during an international meeting held at MLT, USA to include protein source from unicellular or multicellular microbes like bacteria, yeast, fungi and algae. The concept of utilizing SCP is not completely new, as protein are already being used in foods and feeds in different regions of the world at varying levels. The commercial interest of thesis protein. However, it is a new development [13, 14]. The use of which algae have been put are varied and range from being a food and feeds as a source of bioenergy [15, 16]. In the very recent years algae have been considered as a source of biochemical or pigment with possible

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therapeutic uses [17]. It will be interest to briefly trace the development in algae biotechnology and its application. *Spirulina* is a blue green microalgae probably closely linked to the first form of true plant. These organisms contained over 50 per cent good quality protein. Protein there number of full scale commercial plants under construction ranging from 50-100,000 ty^{-1} in Europe.

2.MORPHOLOGY AND TAXANOMIC STATUS

Holmgren *et al*. [18] observed cross walls in *Spirulina* major under electron microscope and suggested the transfer of *Spirulina* major into the genus *Athrospira* on the criterion of the presence of cellular septation. *Spirulina* and *Athrospira,* the non heterocyst unbranched filamentous genera of order *Nostacles* and family Osillatoriaceae wear identified. Presence of two filament in a single unit and absence of cellular septation under light microscope wear the main point which differentiated *Spirulina* from *Athrospira* [19].

Hintak [20] described a *Spirulina fusiformis* from the Kenya with a fusiform trichome construction. He mentioned that a relatively small, inconspicuous, broadly rounded calyptra was occasionally formed with or without an accompanying tapering of the trichome. *Spirulina* consisted multicellular, filamentous, unbranched and helicoidel trichomes wear formed by a single spirally twisted cell. Motile structure like flagella and heterocysts which are generally present in many blue green algae wear absent .The filaments wear called 'trichome'. The cells wear cylindrical and the spiral wear loose. The cells exhibited active rotary movements. The helical shape of the trichome was characteristic of the genus but the helical parameters varied with the species even within the same species [21].

Spirulina platensis is a filamentous, photoautotrophic, alkaliphilic cyanobacterium that belongs to the Family Oscillatoriaceae, Division Cyanophyta. This cyanobacterium blooms in bicarbonate-rich environments and has gained a significant position in recent years as a source of proteins and pigments in the food, pharmaceutical, and cosmetic industries [22].

Desikachary and Jeeji Bai [23] isolated four strains in India and described taxanomicaly. One of strains had a fusiforms structure with erect end portion. Another strain had a basic spindle shaped condition. The third type had a loosely coiled 'S' condition. The fourth type which had been described as an "I" form was essentially not a straight filament. Even through minor variations wear found between these isolates, they were conceded to belong to the same taxon and described as a new species.

Deore [24] studied the taxonomy of *Spirulina gigantea* var. *Schmidle* and *Spirulina platensis* var. *tenius*. The alga *Spirulina gigntea var schmidle* showed trichome with regular spiral, 4-6 numbers per trichome and deep blue green in colour. Trichome was 3 µm broud, 14.4 µm long and the distance between two spiral was 31 - 33 µm. *Spirulina platensis* had pale blue-green and it showed distinct separation. Trichome was 6 µm broad, 14.4 µm long, regularly coiled. The breadth of spiral was 26 µm and distance between two spiral was 31µm.

Pelizer *et al*. [25] reported that *Spirulina platensis* is generally produced in open ponds in liquid culture but there is recent production possible also In solid-state cultivation system but the estimation of cell growth is made difficult in separating cells from the cultivated medium incase of solid-state cultivation systems.

3.NUTRITIVE VALUE OF Spirulina platensis

Clement *et al*. [26] reported that *Spirulina maxima* were an alga rich in organic nitrogenous constituents, used for food in Chad Republic (Africa). Amino acids, vitamins and nutritive value were determined for a strain of the algal growth in an open air pilot production unit. It contained 62 per cent protein, high digestibility and vitamins like β-carotene, β1, β2, β6, β12 and C.

The nutritive value of processed *Spirulina maxima* by *in vitro* in rat skeletal muscle. Rats were fed six consecutive days on a diet containing 20 per cent protein level. Lyophilized *Spirulina platensis* had a nutritional quality between that of wheat gluten and casein supplemented with methionine. Drum dried preparations of *Spirulina platensis* were fairly similar in quality to that of casein sample with methionine was reported by Omstedt *et al*. [27].

Wahal *et al*. [28] reported that water-soluble sugars constituted the major carbohydrates of *Spirulina*. The low amount of starch was due to the high activity of α and β amylase in *Spirulina*. The *in vitro* digestibility of *Spirulina* had been reported using an amylase enzyme. A sufficient amount of protease activity indicated that the enzyme was

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mainly involved in protein turn over rather than in storage hydrolysis.

Po Chung *et al*. [29] studied that the production and nutritive value of *Spirulina platensis* on swine wastes. The alga contained 55 to 61 per cent crude protein. Three indoor culture ponds 0.65m^2 were designed and built under the light intensity of 500 foot candles. *Spirulina* yielded about 5 g m^2 d^{-1} A sample containing 2.038 of NH_3-N produced 16.25 mg of dry algae and contained 9.75 mg of protein. The protein showed a protein efficiency ratio of 2.25 and no toxic effects were noted.

Natives of the chad region still supplements their diet with *Spirulina* harvested from lakes. They fed *Spirulina* to nourished adults. It showed positive nitrogen balance with no harmful effects. Ansuya Devi *et al*. [30] reported the characteristics of the protein of fresh water mass cultures *Spirulina platensis*. The total protein was 50-65 per cent of which nearly 9.9 per cent was non-protein nitrogen. Total protein was extracted by three successive extractions with water. The isoelectric point was found to be 3.0. The polyacrylamide gel electrophoretic pattern showed seven bands. The *in vitro* digestibility was found to be 85 per cent when assayed with a pepsin pancreatin system.

Krishna Kumari *et al*. [31] studied the protein of *Scendes musacutus* and *Spirulina platensis*. These were fed to the rats upto the dosage of 800 mg kg^{-1} of body weight. The absence of gastrointestinal disorders such as diarrhea indicated that these algae were tolerated by animals even at 800 mg kg^{-1}. Application of these algae into albino rats did not elicit any skin allergy. Weight gains, PER and nitrogen balance studies with *Spirulina fusiformis* supplementation to poor rice diets showed significantly higher values of all parameters over casein supplementation [32].

Balasubramanya and Sampath [33] investigated the PER of *Spirulina* diet at 10 per cent protein level. Thirty six albino rates of 21 days old each were used as animal models for the experiment. Concentrated milk, casein and *Spirulina* diets were prepared at 10 per cent protein level. The PER of *Spirulina* alga diet was found to be as high as (2.28) than that of casein (2.45) or milk (2.46).

OrioCiferri and Tinoni [34] studied the biochemistry and industrial potential of *Spirulina*. The composition of the biomass recorded, high protein content, low nucleic acids content and the presence of cell wall that was more easily digestible than that of yeasts or eukaryotic algae. It's consumption by human populations indicated a lack of toxic activities. It could be used as a source of chemical and other basic commodities like enzymes, vitamins, lubricants, pigments, etc. Experiments on humans over an eight week period of *Spirulina* intake (4.2 g d^{-1}) showed greater reduction in serum cholesterol level, especially in subjects with higher initial levels and improved the atherosclerosis index [35].

Bonotto [36] reported that many species of microalgae and blue green bacteria under suitable conditions grew fastly and produced large biomass with high protein content. They constituted a source of single cell protein and the algal biomass supplemented or even replaced animal proteins, thus short circuiting the rather inefficient animal food chain.

Mitchell *et al*. [37] demonstrated that *Spirulina maxima* significantly altered the storage and utilization of vitamins A and E. These were investigated by feeding diets containing 0, 2.7, 10.7, 18.7 and 26.7 per cent protein or by a mixture of them. Growth results indicated that rats did not utilize the diets containing *Spirulina maxima* as well as the case in control diet. The ingestion of *Spirulina maxima* caused a significant increase in dry matter and chloroform extractable crude fat in the faeces.

The carotenoid composition of the blue green alga *Spirulina* was estimated using HPLC. Freeze-dried *Spirulina* had a total xanthophylls concentration of 5787 mg kg^{-1}. Adult Japanese Quail were fed a pigment free basal diet for four weeks and with diets containing 0, 0.25, 0.5, 1.0, 2.0 and 4.0 *Spirulina* for 21 days. Yolk colour was determined using the Roche colour fan. Optimum pigmentation was obtained with 1 per cent *Spirulina* in a diet [38].

Berg *et al*. [39] studied the bioavailability of vitamin B_{12} in rats using *Spirulina*. Two different seafood products nori and *Spirulina* were evaluated with synthetic cyanocobalamine as control, in 30 male weaning wistar rats, given a diet deficient in vitamin B_{12} for six weeks, followed by a four week repletion period in which the rats were given supplements of equal doses of vitamin B_{12}. After repletion, cobalamine contents of serum and kidney were significantly lower and liver cobalamine content was higher, for both the nori and *Spirulina* fed rats than for the cyanocobalamine supplemented controls.

Lactation experiments with different levels of dietary supplementation of *Spirulina platensis* showed six fold increases in milk content to litter size with 500 ppm level and only a threefold increase at a lower level [40].

Kumar and Singh [41] studied two strains of *Spirulina platensis* by enriching of cobalt and iodine, the repeated sub-culturing resulted in increased resistance to these trace metals. The cobalt tolerant strain which grew at 55mg $CoCl_2$ $6H_2O$ l^{-1} showed maximum uptake of 158.43 n mol Co ion mg^{-1}proteins which was 3.98 times higher than its parent. The iodine tolerant strain which grew at 7.0 g Kl l^{-1} showed maximum uptake of 0.65 m mol mg^{-1} of protein which was 1.25 times higher than its parent. Both the strains had the potential in relieving the deficiency of vitamin B_{12} and iodine in vegetarians.

Manjit Kaur and Ahluwalia [42] carried out the biochemical studies of *Spirulina* sugars and enzymes. Total water soluble sugars were more (7.31 per cent) than acid soluble sugars (6.19 per cent). The activities of α amylase (8.5 per cent), β amylase (5.33 per cent) and protease (1.07) were recorded. The activity of acid phosphatase was insignificant.

Powdered *Spirulina* contained 480 mg of iron Kg^{-1}. In order to test the chelated form of iron, the hudrated $FeSO_4$ without EDTA. The sample grown in chelated iron showed a higher percentage of available iron in the wet algae (15-20 per cent) than the dried algae (8-10 per cent) as compared to the control which had traces of iron in the available form Manoharan [43].

Manoj *et al*. [44] reported the application of *Spirulina* for cancer chemo-prevention. Water extract of *Spirulina* was found to inhibit the lipid peroxidation to 76 per cent and the alcohol extract to 65 per cent. The chemical antioxidants like α tocopherol, Butylated hydroxyanisole and β carotene gave 35, 45 and 48 per cent respectively.

Rushmikapoor and Mehta [45] studied the lactation performance of dams fed *Spirulina platensis* supplemented diets. The composition of milk was estimated in terms of protein, fat and lactose of days 7, 14 and 21 of lactation. The rats were fed five different kinds of diets (Casein, *Spirulina*, wheat gluten, *Spirulina* + wheat gluten, *Spirulina*, devoid of vitamins and minerals) each providing 22 per cent proteins during the period of pregnancy and lactation. Casein and *Spirulina* containing dietary regiments were able to maintain fat levels even at later stage of lactation.

Hayashi *et al*. [46] reported the enhancement of antibody production in mice by dietary *Spirulina platensis*. Mice fed on a *Spirulina platensis* diet showed increased numbers of splenic antibody producing cells in the primary immune response to sheep red blood cells. *Spirulina* enhanced the immune response by stimulating macrophage functions, phagocytes and immunoglobin production.

Sundararaman *et al*. [47] studied the bioactive potential of marine Cyanobacteria in the animal based systems. Twelve different strains were administered to male albino wistar rats. The strains of *Spirulina subsalsa*, Oscillatoriasalina, *Phormidium valderianum* were appeared as highly promising in their nutrition.

Spirulina platensis, a cyanobacterium was evaluated as a ameliorant of hexachloro cyclohexane (HCH), an organochlorine insecticide, widely used in India, HCH induced dietary toxicity in retinal deficient male albino rats by feeding HCH mixed with diets free of vitamin. Growth rate was considerably reduced in rats fed with vitamin A free diet with and without HCH. The body weight gain increased in rats fed with alga supplemented diets with or without HCH [48].

Lactococcus lactis, *Streptococcus thermophilus*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* and *Lactobacillus bulgaricus* were grown in rich media as well as in minimal saline medium with and without addition of extracellular products from cultures of *Spirulina platensis*. The stimulatory effect was observed in media with pH adjusted to 5.3, 6.3 and 7.0. The results showed significantly increments of bacterial growth in all media enriched with extracellular algal filtrates [49].

Phycocyanin, a blue colourant from the cyanobacterium *Spirulina platensis* was evaluated for its natural food colour by toxicity evaluation studies in albino rats. Acute and subchronic studies on albino rats did not include any symptoms of toxicity. Feeding of low concentration of phycocyanin at 0.5 to 4.0 g kg^{-1} in the diet for 14 weeks did not affect food intake and body weight gain of phycocyanin fed rats [50].

Hirajashi *et al*. [51] studied 12 adult males were administered an oral hot water extract of *Spirulina* and the number and activity of their natural killer (NK) cells was measured before and after treatment (NK cells destroy tumor cells by binding to them and delivering lethal chemicals that kill on contact). At the end of the study, there was a significant increase in the production and cancer killing ability to these NK cells. When their NK cells were exposed to a bacterial product after treatment, production of interleukin-12 (IL-12), a measure of immune strength, was significantly increased in comparison to IL-12 production in NK cells without pre-exposure to *Spirulina*.

4 GROWTH FACTORS

Spirulina production plants for mass cultivation are to be done in areas with suitable climatic conditions, particularly with the sunshine throughout the year. It is difficult to have an ideal growth due to different environmental factors like solar radiation, rain, wind, temperature fluctuation, etc.

4.1. Nutrient medium

Spirulina required high nutrient inputs and salt concentrations compared to *Scenedesmus* and *Chlorella*. This might be the reason for *Spirulina* was naturally grown in salt lakes exclusively [52]. The difficult media currently in use in various centers of production were small alternations of the medium first developed by Zarrouk's for *Spirulina* culture. *Spirulina* required a medium of high alkalinity and a steady supply of bicarbonates ions [53].

4.2. Carbon source

Ogawa and Terui [54] reported that *Spirulina* did not grow in organic compounds as the sole carbon source. To increase the productions of cyanobacteria, during mass culture, the minimal medium without sodium chloride fertilized with extracts of different organic manures were used. The dry matter production of *Spirulina subsalsa* and *Spirulina platensis* was increased by 5 to 30 per cent [55].

Nasima *et al*. [56] reported that the Rice Husk Ash (RHA) and $NaHCO_3$ l^{-1} were used as a source of carbon in *Spirulina* culture. Addition of 2g of $NaHCO_3$ l^{-1} every other day supported better growth than 1 g RHA l^{-1} everyday. However, from an economic point of view RHA was preferred.

Gardillo *et al*. [57] studied the effect of increased atmospheric CO_2 on photosynthesis and growth of *Spirulina*. The increase of CO_2 did not cause any change in maximum growth rate while it decreased maximum biomass yield as it affected the pigment content of the algae.

Vieira Costa *et al*. [58] reported that, the influence of nutrient for biomass production of *Spirulina platensis*, in open raceway ponds in addition of (carbon as sodium bicarbonate, nitrogen as urea, phosphate, sulfate, ferric iron, magnesium and potassium) on the growth rate of the cyanobacteria *Spirulina platensis*. In unsupplemented lagoon water production of *S. platensis* was 0.78 ± 0.01 g/l (dry weight basis) while the addition of 2.88 g/l of sodium bicarbonate resulted in 0.82 ± 0.01 g/l after 40 hours of culture.

4.3. Temperature

Temperature in the range of 30-35°C even if the outside temperature as 38°C was most ideally suited for getting maximum yield of *Spirulina*. Temperature above 35°C leads to bleaching of cultures. Partial shading provided a culture temperature of about 30°C even if the outside temperature was 38°C [59].

Rafiqul Islam *et al*. [60] reported that, the maximum specific growth rate of 0.141 was found at 32^0C for *Spirulina platensis* and that of 0.144 was found at 37^0C for *Spirulina fusiformis*. Maximum biomass production of 2.4 g l-1 and chlorophyll *a* production of 16.6 mg l^{-1} were observed at 32^0C for *Spirulina platensis*. Maximum biomass production of 2.3 g l^{-1} and chlorophyll - *a* production of 14.2 mg l^{-1} were observed at 37^0C for *Spirulina fusiformis*.

Luciane Maria Colla *et al*. [61] found that, temperature was the most important factor and that the greatest amount of gamma-linolenic acid (GLA) was obtained at 30 ºC, the fatty acid profile of the *Spirulina* cultivated showing that (in order of abundance) palmitic, linolenic and linoleic acids were most prevalent.

4.4. Water quality

The characteristics of water quality contributed in the algal mass production. It had dual influence, firstly by affecting the solubility of nutrients added in the medium and also selective accumulation of certain heavy metals by algae during the growth phase [62].

4.5. Light

Spirulina required light intensities during its growth phase. The optimal light intensity was between 20 and 30 K lux [63]. Subramanian and Jeejibai [64] reported on the effect of different light quality on growth, protein and pigment synthesis of *Spirulina fusiformis*. The light was provided for 10 hours at 2 K lux intensity, with coloured cellophane paper covering the fluorescent bulbs. Blue light yielded highest protein content followed by yellow, white, red and green light. It was evident that white fluorescent light with a red-orange component provided higher energy for better protein and pigment synthesis.

Watanable and Hall [65] investigated the photosynthetic productivity of the filamentous cyanobacaterium *Spirulina platensis* in a cone-shaped helical tubular photobioreactor. It was constructed with 0.255 m^2 basal area and conical shape. The inner surface of the photostage was illuminated with compact fluorescent cool white lamps and the input radiation energy was 1249 kj d^{-1}. The maximum photosynthetic efficiency obtained was 6.83 per cent which is corresponded to a production rate of 15.9 g dry biomass $m^{-2}d^{-1}$.

Dubey [66] had reported moderate light intensity in the cultivation of *Spirulina*, suggesting low light intensity at the beginning to avoid photolysis. He also noted that exposing *Spirulina* to high light intensity photolysis them.

Pandey *et al*. [67] found that, the influence of light intensity for *Spirulina platensis* growth at 5 Klux light intensity the dry weight of *Spirulina platensis* was 0.85g/500ml while protein content and Chlorophyll *a* were 64.3% and 9.8mg/gm respectively.

Mohammad-Taghi Golmakani *et al*. [68] studied that, *Spirulina platensis* is an important source of pharmaceuticals and nutraceuticals such as g-linolenic acid (GLnA). GLnA yield of the culture medium (32 mg/L) were obtained at the highest light intensity of 5.0 klx.

4.6. pH

Vincent and Silvester [69] 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. *Spirulina* grew well at pH values between 9 and 11. 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.

Pandey *et al*. [70] found that, the influence of pH for *Spirulina platensis* growth, protein and Chlorophyll a content were examined and the dry weight of *Spirulina platensis* was 0.91g/500ml and protein and Chlorophyll *a* content were 64.3% and 13.2mg/gm respectively at pH 9.

4.7. Inoculum size

Tasneem Fatma [71] reported that synchronous growth of *Spirulina platensis* was failed to grow both in liquid and solid media at its higher dilution. It was observed that minimum cell population is necessary to initiate and sustain *Spirulina* cultures. The culture filtrate had an absorbance of 0.96 on ninth day increased 50 per cent growth against control (0.63 absorbance).

4.8. Agitation

Agitation of algal cultures had the advantages of uniform distribution of CO_2 and prevention of thermal stratification. Many agitational devices had been reported which range from motor driven paddles, pumps, gravity flow, air light systems and manual agitation [72].

Dubey [73] found that, aeration, which could be achieved by rotators, and which provides agitation of growing cells to maintain the cells in suspension, has been described as very necessary in getting good quality and better yields of *Spirulina* species

4.9. Contamination

Venkatraman and Sindhukanya [74] reported the insect contamination in mass culture of *Spirulina platensis*. The mosquito larvae fed on the algal biomass for 2-3 days before entering into purpal stage and the decrease in algal yield was upto 10 per cent. The use of fine wire mesh frame removed all extraneous materials.

Mahadevaswamy and Venkatraman [75] reported the presence of bacterial contaminants in outdoor cultivation of *Spirulina platensis*. The bacterial forms occurred in cultures were identified as aerobic spore formers. No the pathogenic forms affecting the products safety were identified.

4.10. Other growth factors

Hofner *et al*. [76] reported that the hexavalent chromium was most toxic and the zinc had little effect on the growth of *Chlorella fusce*. All elements tested arrest growth of *Spirulina maxima* at 10^{-4} M and smaller doses had no effect. Ahluwalia and Kochar [77] studied the effect of mercuric chloride, cadmium chloride, nickel sulpahte and zinc chloride on the growth of *Spirulina platensis*. Among the metals, mercury had been most toxic and the algal growth was reduced even at 0.01 ppm. It was followed by cadmium (0.1 ppm). Higher doses resulted in fragmentation, lysis and death of the algae.

A variety of *Spirulina* products for human consumption were analysed for mercury and lead content, using cold vapour atomic absorption and

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graphite furnace atomic absorption detection. No *Spirulina* product tested contained Hg or Pb concentrations high enough to approach the WHO/FAO caution guidelines for daily intake of those elements [78].

Nanda and Padhi [79] studied the effect of sodium salt to 2,4-Dichlorophenoxyacetic acid (2,4-D), on the growth and pigmentation of *Spirulina platensis*. It was found to be very sensitive towards 2,4-D and the growth of algae inhibited at 5 mg ml^{-1}.

Ken Sasaki *et al*. [80] reported that the growth of *Spirulina platensis* was stimulated by adding 5-Aminolevulinic acid (ALA, 500 mg l^{-1}). The photosynthetic activity was enhanced by rapid stimulation and accumulation of phycocyanin and chlorophyll II.

5. SUBSTRATES USED FOR MASS PRODUCTION OF *Spirulina platensis*

Algal cultivation on wastewaters aims at producing biomass and, at the same time, removing organic and inorganic pollutants. The use of microalgae can offer a valuable alternative to the conventional purification treatments and provides several advantages, among which:

(a) It is not environmentally dangerous, because it rests on the principles of natural ecosystems;
(b) Biomass can be re-cycled reducing the causes of secondary pollution;
(c) Algal growth on effluent removes heavy metals and xenobiotic substances and allows, under photosynthetic conditions, oxygen to be released, thus enhancing the auto-depuration potential of water bodies.

Phang *et al.* [81] reported that, the waste water is usually discharged into the rivers, each factory producing about 10- 22 tons waste water per day which contains a very high carbon to nitrogen ratio (105:0.12), but it has been made more suitable for fermentation by anaerobic fermentation in an up-flow packed bed digestor. The digested effluent with an average C: N: P ratio of 24:0.14:1 supports growth of *Spirulina platensis*. The highest crude protein, carbohydrate and lipid content of the biomass were 68, 23 and 11%, respectively. The reduction in COD, ammoniacal-nitrogen and phosphate levels of the digested effluent reached levels of 98.0, 99.9 and 99.4%, respectively.

Phang *et al.* [82] the production of wastewater arising from sago starch has a high carbon to nitrogen ratio, which is improved with anaerobic fermentation. The effluent supported growth of *Spirulina platensis* with an average specific growth rate (μ) of 0.51 day^{-1} compared with the average μ of 0.54 day^{-1} in the inorganic Kosaric Medium in a high rate algal pond. Supplementation with 6 mM urea and 2.1 mM K_2HPO_4 produced gross biomass productivity of 14.4 g m^{-2} day^{-1}. A flow-rate of 24 cm s^{-1} increased the μ and gross biomass productivity (18 g m^{-2} day^{-1}).

Eugenia Olguin *et al.* [83] evaluated the effect of low light and nitrogen deficiency on growth and chemical composition of *Spirulina* sp. (straight elements strain, SF) in batch cultures utilizing a complex medium containing sea-water supplemented with anaerobic digested pig waste, was undertaken. Cultivation was carried out either at a light of 66 (lower) or 144 l mol photon my^2 sy1 (higher), utilizing bench raceways. Biomass concentration (as dry weight) after 12 days of cultivation in the complex medium was similar. P < 0:05 to the one observed in a chemically defined medium (Zarrouk's), regardless of the light intensity. Protein content of the biomass in the complex medium was significantly lower P < 0:05, compared to the Zarrouk's medium, regardless of the light.

The fermented wastewater of Thai rice noodle factories has the potentiality as the source of nutrients for cultivation of *Spirulina platensis* [84]. The fermented noodle factory wastewater as modified medium (1:11 dilution ratio of wastewater) supplemented with 90 mg/litre nitrate-N, 590 mg/litre phosphate-P, 180 mg/litre potassium and 3 000 mg/litre Na_2CO_3 shows high potential for cultivating *Spirulina platensis* where the growth of these microalgae was very favourable. The chlorophyll (2.36 mg/g), biomass (1000 mg/litre), crude protein (59 per cent) and phycocyanin (14 per cent) were detected which was almost similar to those of *Spirulina* when cultured in Zarrouk's medium.

Promya and Traichaiyaporn [85] studied the mass culture of *Spirulina platensis* in kitchen wastewater and fermented solution of oil-extracted soybean. The statistical experimental designed was of Completely Randomize Design (CRD) by having 5 treatments with 3 replications: Zarrouk's medium (Zm), Kitchen wastewater (Kw 90%), (Kw100%) and fermented solution of oil-extracted Soybean (Sb 5%), (Sb 10%) were tested for 30 days. Primary production and water quality

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from culture of *Spirulina platensis* were monitored every 5 days. As the results, the highest primary production of *Spirulina platensis* was achieved in Sb 5 % effluent (0.83 g/l). All experimental units had decreased removal percentage of NH_3-N, NO_3-N and NO_2-N, where they had met the laws and standards of pollution control.

According to Rajeev Kaushik *et al*. [86], anaerobically digested distillery effluent (ADE) of molasses based distilleries with high BOD and COD cause deterioration of land and ground water quality when discharged in the environment *Spirulina platensis* (ARM 730) was grown in different dilutions and used for treatment and safe disposal of ADE. It was observed that growth and other parameters like protein, carbohydrate, lipids were significantly higher in 50% ADE over standard Zarrouk's medium and other ADE dilutions. After growing *Spirulina* for 14 days in all the different dilutions of ADE, a significant decrease was observed in the following: BOD, 51-94.6; total C, 27.2-71.8; and total N, 35.4- 58.0 per cent.

6. MASS CULTIVATION OF *Spirulina* sp.

Spirulina is an economically important filamentous cyanobacterium. The annual production of the algae is about 10,000 tons which makes it the largest microalgal cultivation industry in the world [87]. Due to its richness in protein, phycocyanin, essential amino acids, polysaccharides, carotenoids, minerals, vitamins and essential fatty acids has been regarded as an ideal bio-resource and has drawn increasing attention in recent decades [88, 89, 90].

Spirulina platensis is a cyanobacterium that has been largely studied due to its commercial importance as a source of protein, vitamins, essential amino acids, and fatty acids [91]. More recently, special attention has been given to *Spirulina platensis* as a potential source of pharmaceuticals, and other high value products such as chlorophyll [92]. The utilization of chlorophyll from *Spirulina platensis* is an attractive alternative that should be considered due to its high content of this pigment, and ease of cultivation. The Cyanobacteria *Spirulina platensis* possesses a high tolerance to alkaline pH, for ease of cultivation; a large size for its cell aggregates for ease of harvest; and an easily digestible cell wall.

Stanca and Popovici [93] demonstrated that the utilization of urea as a nitrogen source in *Spirulina platensis* cultivation leads to an increase in both the total biomass and the biomass chlorophyll content. Urea is easily assimilated by *Spirulina platensis*, probably due to its spontaneous hydrolysis to ammonia under alkaline cultivation.

Rice husk ash (RHA) and $NaHCO_3$ were used as a source of carbon in *Spirulina* culture [94]. They reported that the addition of 2.0 g $NaHCO_3$/litre every two days supported better growth of *Spirulina* than 1.0 g RHA/litre every day, although this might not be supported on economic ground

Zhang *et al*. [95] investigated the effects of initial glucose concentration and light intensity on specific growth rate, phycocyanin concentration and cell dry weight concentration in mixotrophic batch cultivation of *Spirulina platensis* using both shake flask and fermentor. Based on experimental results in shake flask culture, a number of mathematical models were constructed, and the optimal initial glucose concentration and the optimal light intensity were calculated. Finally, a time-dependent kinetic model for mixotrophic batch cultivation of *Spirulina platensis* in fermentor was also proposed. This was in good agreement with the experimental results and could be employed to predict the production of biomass and phycocyanin, and the consumption of glucose in fermentor culture.

Tri-Panji *et al.* [96] attempted to grow *Spirulina platensis* using optimization media from low-cost nutrient sources. Optimum medium composition consisting of mineral salt and organic complex derived from low-cost nutrient sources. *Spirulina platensis* grown on complex media containing latex serum from concentrated latex factory, supplemented with salt minerals might produce high yielding carotenoids. Among eleven media composition containing latex serum examined, best growth on a formulated medium with a ratio of C: N: P: Mg = 1:3:0.3:0.2 yielding 0.350 g biomass/L This amount was slightly lesser than those on synthetic Aiba & Ogawa medium that yields 0.407 g biomass/L, after eight-week incubation.

Elias *et al.* [97] documented improvement for the mass-scale culture of microalgae with the use of sophisticated closed systems. The proposed photo-bioreactor in this study is a combination of helical

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parts receiving strong light with a main culture vessel mixed and degassed by airlift. A first experimental trial was carried out with the filamentous cyanobacterium *Spirulina platensis* achieving maximal volumetric productivity of 1.2 $gl^{-1}d^{-1}$ comparable with the maximal reported values by other closed culture designs for the same organism. The proposed system is under optimization of its engineering elements in order to comprise a sound modular solution for commercial production of microalgae of economic interest.

Dansei *et al*. [98] cultivated the microalgae using urea as the nitrogen source by a fed-batch process. The addition of urea was done in four different modes: intermittent addition every 24 or 48 h, continuous addition by exponentially increasing the added mass, and continuous addition by using a constant mass rate. The experiments were carried out at three different temperatures: 27°C; 30°C and 33°C and at a constant light intensity. The results showed a positive influence of urea in the growth of *Spirulina* but no effect on the chlorophyll content of the cultures. Best results were obtained by continuous urea addition in exponentially increasing amount, at 30°C.

Jorge *et al.* [99] studied the influence of nutrient on the growth rate of the Cyanobacteria *Spirulina platensis* using a 22 factorial design. In unsupplemented lagoon water production of *Spirulina platensis* was 0.78 ± 0.01 g/l (dry weight basis) while the addition of 2.88 g/l of sodium bicarbonate (without added urea, phosphate, sulfate or metal ions) resulted in 0.82 ± 0.01 g/l after 400 hours of culture. The further addition of phosphate and metal ions resulted in growth for up to 750 h and a final *Spirulina platensis* biomass of 1.23 ± 0.04 to 1.34 ± 0.03 g/l.

Hidenore [100] studied the mass production of *Spirulina* in the open pond system. They used modified aeration technique produce high mass in ambient temperature and decrease some metals in the medium to obtain more yield, production cost was minimized cost was minimized by improved harvesting and filtration methods.

Reichert *et al.* [101] studies the specific growth rate and productivity of two *Spirulina platensis* strains. *Spirulina platensis* strain used was found that low concentration (0.50g/L) and high renewal rates (50% v/v) resulted in high specific growth rate and productivity. These values are two to four times higher than those obtained in simple batch cultivation and indicate that the semi continuous cultivation of *Spirulina platensis* is viable.

Kemka Ogbonda *et al*. [102] studied the influence of temperature and pH on biomass production and protein biosynthesis in a *Spirulina* sp. isolated from an oil-polluted brackish water environment in the Niger Delta. The isolated organism was identified on the basis of its phenotypic characteristics such as nature and direction of helix, temperature, pH and salt tolerance ranges. Biomass concentration in the culture media was calculated as cell dry weight. The combination of 30°C and pH 9.0 gave the highest values of 4.9mg/ml and 48.2 g/100 g for biomass and total crude protein, respectively. The effect of pH was modulated by temperature and vice versa during biomass production. This native isolate of *Spirulina* sp. act as a good source of natural protein that could be easily accepted by rural communities as single cell protein in the form of feed, food and health supplement when properly processed.

7. BIOCHEMICAL COMPOSITION OF *Spirulina platensis*

The basic biochemical composition of *Spirulina* can be summarized as follows,

Protein: *Spirulina* contains unusually high amounts of protein between 55 and 70 per cent by dry weight. It is a complete protein, containing all essential amino acids.

Essential fatty acids:

Ishikawa *et al.* [103] studied that, GLA has mainly respect to its therapeutic properties such as its ability to decrease blood cholesterol levels.

Spirulina has a high amount of polyunsaturated fatty acids, 1.5-2.0 per cent of 5-6 per cent total lipid. *Spirulina* is rich in linolenic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid.

Vitamins: *Spirulina* contains vitamin B1, B2, B3, B6, B12, vitamin C, vitamin D and vitamin E.

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

Photosynthetic pigments: *Spirulina* contains many pigments including chlorophyll-a, xanthophylls, β-carotene, echinenone, myxoxanthophyll, etc. and the phycobiliproteins C-phycocyanin and allophycocyanin.

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