



## ***SPIRULINA PLATENSIS* – FOOD FOR FUTURE: A REVIEW**

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### **ABSTRACT**

*Spirulina* can play an important role in human and animal nutrition, environmental protection through wastewater recycling and energy conservation. The present review was focused on the various characteristics of *Spirulina platensis*. *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. 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. The present review focused the following topics: *Spirulina platensis*, Isolation and occurrence of *Spirulina platensis*, newly formulated media for *Spirulina* cultivation, Phycocyanin and Medicinal properties of *Spirulina platensis*.

**Key words:** *Spirulina platensis*, Single cell protein, Protein content and Phycocyanin.

### **INTRODUCTION**

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 [1].

The current environmental conditions deteriorations, mental and physical stress, changes in the diet have been serious risk factors for the humans, increased the death rate and civilization diseases. These are the obvious reasons why new progressive trends are being extensively developed in modern medicine, pharmacology and biotechnology and more effective harmless medicaments are being sought for to treat and prevent various diseases. One of the trends in biotechnology is associated with Blue green microalgae *Spirulina platensis* which have been widely employed as food and feed additives in agriculture, food industry, pharmaceuticals, perfume making, medicine and science

[1].

### ***Spirulina platensis***

*Spirulina* sp. 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. 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 of both researchers and industrialists alike.

*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 [2].

P. J. Turpin in the year 1827 isolated *Spirulina* from a fresh water sample. In 1884, Wittrock and Nordstedt reported the presence near the city of Montevideo of a helical, septal and green-blue microalgae called *Spirulina platensis*. In 1852, Latter Stizenberger gave this new genes the name *Arthrospira* based on the septa presence, helical form and multicellular structure. Because of the common helical morphology, reunified the members of the two genera under the designation *Spirulina* without considering the septum, only morphological similarity. In 1989, these microorganisms were separately classified into two genera *Spirulina* and *Arthrospira*; this classification is currently accepted.

According to the classification in Bergey's Manual of Determinative Bacteriology, *Spirulina* belongs to the oxygenic photosynthetic bacteria that cover the groups Cyanobacteria and Prochlorales, which are, by phylogeny, related to the sequence of the rRNA (ribosomal ribonucleic acid) sub-unit 16s. As a function of the sequence data of this sub-unit and the rRNA sub-unit 5s, these prokaryotes are classified within the Eubacteria group.

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. SCPs are characterized by; fast growth rate; high protein content (43-85%) compared to field crops; require less water and land and independent of climate; grow on wastewater; can be genetically modified for desirable characters such as amino acid composition and temperature tolerance. 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 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 [3]. In Cyanobacteria, the light harvesting pigments include chlorophyll-a, carotenoids and phycobiliproteins. The later are proteins with linear tetrapyrrole prosthetic groups called according to their structure: phycocyanin, phycoerythrin and allophycocyanin [4].

### 3. ISOLATION AND OCCURENCE OF *Spirulina platensis*

*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 [5]. In Cyanobacteria, the light harvesting pigments include chlorophyll-a, carotenoids and phycobiliproteins. The later are proteins with linear tetrapyrrole prosthetic groups called according to their structure: phycocyanin, phycoerythrin and allophycocyanin [6].

*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.

Orio Ciferri [8] studied that *Spirulina* was a ubiquitous organism. After the first isolation by Turpin in 1827 from a fresh water stream, species of *Spirulina* have been found in a variety of environments: soil, sand, marshes, brakish water, sea water, and fresh water. Species of *Spirulina* have been isolated, for instance, from tropical waters to the North sea, thermal springs, salt pans, warm waters from power plants, fish ponds, etc. Thus, the organism appears to be capable of adaptation to very different habitats and colonizes certain environments in which life for other microorganisms is, if not possible, very difficult; typical is the population by alkalophylic *Spirulina platensis* of certain alkaline lakes in Africa and by *Spirulina maxima* of lake Texcoco in Mexico. In some of these lakes *Spirulina* grows as a quasimonoculture.

Susan Spiller *et al.* [9] examined the fine structure of Cyanobacteria, *Spirulina platensis* and *Spirulina subsala* as viewed by X-ray microscope, XM-1, beamline 6.1.2. in the initial stages of a project to obtain high resolution images of these spiral Cyanobacteria and the spiral chloroplasts of *Spirogyra* species. These high resolution images will allow a close comparison between morphological features of prokaryotic cells and the eukaryotic photosynthetic organelle. Cryo-technique are being developed with the cryo-stage XM-1, the X-ray microscope at beam line 6.1.2 of the advanced tight source. The bright blue-green cylindrical filaments of *Spirulina platensis* had a lazy spiral turn, filament width 5-6  $\mu\text{m}$ . Cell walls are clearly visible crossing the filament at intervals of about 2-3 $\mu\text{m}$ .

Martha *et al.* [10] studied that before Columbus, Mexicans (Aztecs) exploited this microorganism as human food; presently, African tribes (Kanembu) use it for the same purpose. Its chemical composition includes proteins (55%-70%), carbohydrates(15%-25%), essential fatty acids(18%) vitamins, minerals and pigment like chlorophyll a and phycocyanin. The last one is used in food and

cosmetic industries. *Spirulina* is considered as an excellent food, lacking toxicity and having corrective properties against viral attacks, anemia, tumor growth and malnutrition. It has been reported in literature that the use of these microalgae as animal food supplement implies enhancement of the yellow coloration of skin and eggs yolk in poultry and flamingos, growth acceleration, sexual maturation and increase of fertility in cattle.

#### NEWLY FORMULATED MEDIA FOR *SPIRULINA* CULTIVATION

Basirath Raof *et al.* [11] formulated a new medium for mass production of *Spirulina* sp. by incorporating selected nutrients of the standard Zarrouk's medium. This newly formulated medium contains single super phosphate, sodium nitrate, muriate of potash, sodium chloride, magnesium sulphate, calcium chloride and sodium bicarbonate (commercial grade). Maximum growth rate in terms of dry biomass, chlorophyll and proteins in SM was recorded between 6 and 9 days of growth and values were 0.114, 0.003, and 0.068 as compared to 0.112, 0.003 and 0.069 mg/ml significant differences were observed in the protein profiles of *Spirulina* sp. grown in both the media. From the scale up point of view, the revised medium was found to be highly economical, since it is five times cheaper than Zarrouk's medium.

Dao-Lun *et al.* [12] attempted to culture *Spirulina platensis* in human urine directly to achieve biomass production and O<sub>2</sub> evolution, for potential application to nutrient regeneration and air revitalization in life support system. The culture results have showed that *Spirulina platensis* in diluted human urine was lighter than that in Zarrouk's medium.

Raof *et al.* [13] investigated the use of a new medium formulated for mass production of *Spirulina* sp. by incorporating selected nutrients of the standard Zarrouk's medium (SM) and other cost-effective alternative chemicals. This newly formulated medium (RM6) contains single super phosphate (1.25 g/litre), sodium nitrate (2.50 g/litre), muriate of potash (0.98 g/litre), sodium chloride (0.50 g/litre), magnesium sulfate (0.15 g/litre), calcium chloride (0.04 g/litre), and sodium bicarbonate (commercial grade) 8 g/litre. The alga was grown in an illuminated (50 mmol photons/m<sup>2</sup>/s white light) growth room at 30 ± 1 °C. Maximum growth rate in terms of dry biomass, chlorophyll and proteins in SM was recorded between 6 and 9 days of growth and values were 0.114, 0.003 and 0.068 as compared to 0.112, 0.003 and 0.069 mg/ml/day in RM6. No significant differences were observed in the protein profiles of *Spirulina* sp. grown in both the media.

Harriet Volkmann *et al.* [14] cultivated the *Spirulina platensis* in laboratory under controlled conditions (30°C, photoperiod of 12 hours light/dark provided by fluorescent lamps at a light intensity of 140 μ mol photons.m<sup>-2</sup>.s<sup>-1</sup> and constant bubbling air) in three different culture media: (1) Paoletti medium (control), (2) Paoletti

supplemented with 1 g L<sup>-1</sup> NaCl (salinated water) and (3) Paoletti medium prepared with desalinator wastewater. The effects of these treatments on growth, protein content and amino acid profile were measured. Maximum cell concentrations observed in Paoletti medium, Paoletti supplemented with salinated water or with desalinator wastewater were 2.587, 3.545 and 4.954 g L<sup>-1</sup>, respectively. Biomass in medium 3 presented the highest protein content (56.17%), while biomass in medium 2 presented 48.59% protein. All essential amino acids, except lysine and tryptophan, were found in concentrations higher than those required by FAO.

Bohra [15] investigated growth pattern of *Spirulina platensis* in standard and modified media based on seawater-chemicals and seawater fertilizers. During the cultivation, the cell concentrations were analyzed at 540nm along with protein and chlorophyll *a* estimation. Growth patterns of different species and strains were monitored for 25 days and specific growth rate, mean daily division rate and doubling time were calculated. *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 good growth patterns, biomass, protein content and chlorophyll content than other seawater based media, the experiment discusses the feasibility of seawater based media.

Usharani *et al.* [16] collected the water samples for the isolation of strains of alga *Spirulina platensis* from three different locations and the strain *Spirulina platensis* was isolated and it was, designated as ANS - 1 strain. The characteristics of *Spirulina platensis* ANS -1 were compared with reference CAS -10. The waste water rice mill effluent was collected and its pH was adjusted to 9-11 by using sodium bicarbonate @ 800 mg l<sup>-1</sup> and it was used as a medium. The isolated strain ANS -1 and reference strain CAS 10 were grown in substrates 1/6 diluted Zarrouk's medium (control) and rice mill effluent. The well performed strain CAS 10 under *in vitro* condition was selected as efficient one. The growth of *Spirulina platensis* was measured both in laboratory and outdoor condition by using the parameters *viz.*, optical density, population, dry weight, protein and chlorophyll content. The high growth and dry weight were recorded in 1/6 diluted Zarrouk's medium when compared to rice mill effluent medium. Maximum protein and chlorophyll content were noticed in 1/6 diluted Zarrouk's medium than rice mill effluent [17].

Lignite fly ash (LFA) is the by-product of thermal power station. In India, Neyveli Lignite Corporation (NLC) liberates tonnes of LFA as a waste product and the liberated LFA highly pollutes the soil and water bodies. The Blue Green Algae *Spirulina platensis* grows well at alkaline pH and the pH of the LFA is also alkaline. The LFA also contains an array of micronutrients and macronutrients which favours the growth of *Spirulina platensis*. In order to minimize the environmental pollution and recycle the LFA

waste, Saranraj *et al.* [18] was planned to utilize the LFA at different concentrations for the laboratory cultivation of *Spirulina platensis*. *Spirulina platensis* was cultivated in the conical flasks containing Zarrouk's medium alone [SP] and Zarrouk's medium with three different concentrations (0.5 g/l [SP - 1], 1.0 g/l [SP - 2] and 1.5 g/l [SP - 3]) of LFA supplementation. Among the four different supplementations used, SP which contained 1.5 g Lignite fly ash in one litre of Zarrouk's medium highly induced the growth and protein content of *Spirulina platensis* when compared to other supplementations. The least growth and protein content was recorded in SP which contains Zarrouk's medium alone without LFA supplementation.

## PHYCOCYANIN

Phycocyanin is a water soluble blue pigment that gives *Spirulina* its bluish tint. It is found in blue green algae like *Spirulina*. Phycocyanin is a powerful water soluble antioxidant, scientists in Spain showed that an extract of *Spirulina* containing phycocyanin is a potent free radical scavenger and inhibits microsomal lipid peroxidation [19]. Phycocyanin in *Spirulina* that is thought to help protect against renal failure caused by certain drug therapies. Phycocyanin has also shown promise in treating cancer in animals and stimulating the immune system [20]. A human clinical study showed that a hot water extract of *Spirulina* rich in phycocyanin increased interferon production and NK cytotoxicity (cancer killing)

### Physical and chemical properties of Phycocyanin

Phycobiliproteins are a small group of highly conserved chromoproteins that constitute the phycobilisome, a macromolecular protein complex whose main function is to serve as a light harvesting complex for the photosynthetic apparatus of cyanobacteria and eukaryotic groups. The most common classes of phycobiliproteins are allophycocyanin, phycocyanin and phycoerythrin all of which are formed by a and b protein subunits and carry different isomeric linear tetrapyrrole prosthetic groups (bilin chromophore) which differ in the arrangement of their double bonds.

Phycocyanin is composed of two dissimilar a and b protein subunits of 17 000 and 19 500 Da, respectively, with one bilin chromophore attached to the a subunit (a 84) and two to the b subunit (b 84, b 155). Pc exists as a complex interacting mixture of trimer, hexamer and decamer aggregates. When the secondary, tertiary and quaternary structures of the protein are denatured, the visible absorption band as well as the fluorescence will drop in intensity. The chemical structure of the bilin chromophores in Pc is very similar to bilirubin, a heme degradative product. Bilirubin is considered to be a physiologically important antioxidant against reactive species [22].

### Biochemistry of Phycocyanin

The phycocyanins, biliproteins involved in the

light-harvesting reactions, have been resolved by gel electrophoresis in *Spirulina platensis* and *Spirulina maxima* and isolated from the former. Both c-phycocyanin and allophycocyanin appear to be oligomeric complexes composed of at least two different subunits that may be resolved by electrophoresis under denaturing conditions. The  $\alpha$ - and  $\beta$ -subunits of c-phycocyanin showed mobilities corresponding to molecular weights of 20,500 and 23,500, respectively, resulting in an oligomer with a minimum molecular weight of ca. 44,000. Allophycocyanin was found to be composed of subunits with molecular weights of ca. 18,000 and 20,000 to give an oligomer with a minimum molecular weight of ca. 38,000. Absorption and fluorescence spectra were similar to those reported for c-phycocyanins and allophycocyanins isolated from other cyanobacteria. A study of the denaturation and renaturation of c-phycocyanin indicated the possibility that more than one chromophore exists in this biliprotein [23].

### Extraction and purification of Phycocyanin from *Spirulina platensis*

Phycocyanin is a water soluble blue pigment that gives *Spirulina* its bluish tint. It is found in blue green algae like *Spirulina*. Phycocyanin is a powerful water soluble antioxidant, scientists in Spain showed that an extract of *Spirulina* containing phycocyanin is a potent free radical scavenger and inhibits microsomal lipid peroxidation [24]. Phycocyanin in *Spirulina* that is thought to help protect against renal failure caused by certain drug therapies. Phycocyanin has also shown promise in treating cancer in animals and stimulating the immune system [25].

Phycocyanin is the bluish pigment used by blue-green algae to photosynthesize. It accounts for as much as 20% of the protein in cyanobacteria, and attaches itself to photosynthesizing membranes. C-phycocyanin is fluorescent, with an extremely high absorptivity, high quantum efficiency, a large Stokes shift and excitation and emission bands at visible wavelengths. It is a stable protein which can be linked to antibody and other proteins by conventional protein cross-linking techniques without altering its spectral characteristics.

In Japan, phycocyanin from *Spirulina* is used as a natural blue-pigment for food coloring. In addition, owing to its fluorescence property, pure phycocyanin is used as labeling substance in immunoassays, microscopy and cytometry. Moreover, it has been demonstrated that *Spirulina* has therapeutic effects against hyperlipidemia. Therefore, there is a potential for future pharmaceutical use of *Spirulina* to produce many health related products. It may be possible to further enhance the production of certain compounds such as  $\beta$ -carotene in *Spirulina* through genetic engineering. A human clinical study showed that a hot water extract of *Spirulina* rich in phycocyanin increased interferon production and Nk cytotoxicity (cancer killing cells) when taken orally [26].

Lorenz [27] reported that *Spirulina* and other blue-green algae contain c-phycoyanin, which acts as an accessory pigment when light energy is captured and transferred to chlorophyll a. This is a spectrophotometry method adapted to extract and quantify a relatively pure c-phycoyanin fraction from *Spirulina* Pacifica.

Cheyla Romay and Ricardo Gonzalez [28] experimented the antioxidative property of the phycocyanin. They used the human erythrocytes for examine the antioxidant property of phycocyanin. Their results provided protective effect of phycocyanin against hemolysis induced by peroxy radicals in human erythrocytes, which seems to be due to the scavenging action of the radicals in the aqueous phase. Lipid peroxidation is inhibited similar to trolox and ascorbic acid.

Noam Adir *et al.* [29] studied the crystal structure of the light-harvesting phycobiliprotein, c-phycoyanin from the thermophilic cyanobacterium *Synechococcus vulcanus* has been determined by molecular replacement to 2.5 Å resolution. The crystal belongs to space group R32 with cell parameters  $a = b = 188.43 \text{ \AA}$ ,  $c = 61.28 \text{ \AA}$ ,  $\alpha = \beta = 90^\circ$ ,  $\gamma = 120^\circ$ , with one (ab) monomer in the asymmetric unit. The structure has been refined to a crystallographic R factor of 20.2% (R-free factor is 24.4 %), for all data to 2.5 Å. The crystals were grown from phycocyanin ( $\alpha\beta$ )<sub>3</sub> trimers that form ( $\alpha\beta$ )<sub>6</sub> hexamers in the crystals, in a fashion similar to other phycocyanins.

Minkova *et al.* [30] purified C-phycoyanin from *Spirulina (Arthrospira) fusiformis* by a multi-step treatment of the crude extract with rivanol in a ratio (v/v), followed by 40% saturation with ammonium sulphate. After removal of rivanol by gel filtration on Sephadex G-25, the pigment solution was saturated to 70% with ammonium sulphate. After the last step of purification, C-phycoyanin had an emission and absorption maxima at 620 and 650 nm, respectively and absorbance ratio A<sub>620</sub>/A<sub>280</sub> of 4.3, which are specific for the pure biliprotein. Its homogeneity was demonstrated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, yielding two bands of molecular masses 19 500 and 21 500 kDa, corresponding to a and b subunits of the pigment, respectively. The yield of C-phycoyanin was: 46% from its content in the crude extract.

Jose *et al.* [31] extracted astaxanthin and phycocyanin from microalgae with supercritical extraction and using carbon dioxide. The samples of microalgae were crushed by cutting mills and the manually ground with dry ice (solid carbon dioxide). The *Haematococcus* extracts were analysed by liquid chromatography, using astaxanthin (purity of 98%) as a standard. Phycocyanin being insoluble in carbon dioxide was indirectly separated. Thus, lipid-soluble substances from *Spirulina* were extracted and analysed by liquid chromatography. The maximum total recovery of astaxanthin, calculated from its initial and residual content in the alga (0.0147 and 0.0004, respectively) exceeds 97%. For phycocyanin extraction, the addition of cosolvent (10 mass % of ethanol) has a strong effect on the extraction yield of

lipidic substance. The total extraction yield of about 3 mass%.

Noam Adir *et al.* [32] The Crystal Structure of a Novel Unmethylated Form of C-phycoyanin, a Possible Connector Between Cores and Rods in Phycobilisomes A novel fraction of c-phycoyanin from the thermophilic cyanobacterium *Thermosynechococcus vulcanus*, with an absorption maxima blue-shifted to 612 nm (PC612), has been purified from allophycocyanin and crystallized. The crystals belong to the P63 space group with cell dimensions of with a single monomer in the asymmetric unit, resulting in a solvent content of 65 %, and diffract to 2.7 Å. The PC612 crystal structure has been determined by molecular replacement and refined to a crystallographic R-factor of 20.9% (R free - 27.8%).

Zhang *et al.* [33] C-phycoyanin and allophycocyanin were separated and purified from *Spirulina platensis* by precipitation with ammonium sulphate, Ion exchange chromatography and gel filtration chromatography. Pure C-phycoyanin and allophycocyanin were finally obtained with an A<sub>260</sub>/A<sub>280</sub> value of 5.06 and an A<sub>655</sub>/A<sub>280</sub> value of 5.34, respectively.

Ying Zhang *et al.* [34] studied the spectral properties of the glutaraldehyde-treated phycobilisomes. The results showed that glutaraldehyde was effective in preventing phycobilisomes from dilution induced dissociation and preserving the intra-phycobilisomes energy transfer.

Badrish *et al.* [35] reported that the phycocyanin is a major phycobiliprotein produced by cyanobacteria, but only few strains for its efficient purification have been reported. The phycocyanin was extracted by repeated freeze-thaw cycles and purity by a three-step process: ammonium sulphate fractionation, sephadex G-150 size exclusion chromatography and DEAE cellulose ion exchange chromatography.

Silveira *et al.* [36] experimented c-Phycocyanin extraction from cyanobacteria *Spirulina platensis* was optimized using factorial design and response surface techniques. The effects of temperature and biomass-solvent ratio on phycocyanin concentration and extract purity were evaluated to determine the optimum conditions for phycocyanin extractions. The optimum conditions for the extraction of phycocyanin from *Spirulina platensis* were the highest biomass-solvent ratio, 0.08g/mL/1, and 25°C. Under these conditions it's possible to obtain an extract of phycocyanin with a concentration of 3.68mg/mL/1 and purity ratio (A<sub>615</sub>, A<sub>280</sub>) of 0.46.

Silvana *et al.* [37] presented the evaluation of some important parameters for the purification of phycocyanin using ion exchange chromatography. The influences of pH and temperature on the equilibrium partition coefficient were investigated to establish the best conditions for phycocyanin adsorption. The separation of phycocyanin using the Q-Sepharose ion exchange resin was evaluated in terms of the pH and elution volume. The highest partition

coefficients were obtained in the pH range from 7.5 to 8.0 at 25°C.

Narayan and Raghavarao [38] experiments aqueous two phase extraction was employed directly to the cell homogenate of *Spirulina platensis* for the downstream processing of C-phycoerythrin. This enables integrating the process steps of cell removal, extraction and concentration into a single unit operation of aqueous two phase extraction. The effect of different parameters such as molecular weight, tie line length, volume ratio and neutral salt (NaCl) were studied employing PEG 4000/potassium phosphate system and PEG 4000/sodium sulphate system to select the best system among the two.

Zhu *et al.* [39] performed the extraction of C-phycoerythrin from fresh *Spirulina platensis* by deploying a species of non-pathogenic nitrogen-fixing bacteria, namely, *Klebsiella pneumoniae*. The algal slurry was neither washed nor centrifuged; the bacterial culture was poured into the slurry, the vessel sealed, and crude C-PC extracted after about 24 hrs. The extraction was clean and efficient, and the purity and concentration of C-PC proved to be of adequate quality.

Asha Parmer *et al.* [40] extracted, purified and characterized the C-phycoerythrin using novel method based on filtration and single step chromatography. The protein was extracted by repeated freeze-thaw cycles and the crude extract was filtered and concentrated in stirred ultra filtration cell (UFC). The UFC concentration was then subjected to a single Ion exchange chromatographic step. A purity ratio of 4.15 was achieved from a starting value of 1.05. The recovery efficiency of C-phycoerythrin from crude extract was 63.50%. The purity was checked by electrophoresis and UV spectroscopy.

Zhang *et al.* [33] obtained C-phycoerythrin under the best operational conditions for high C-phycoerythrin recovery and purity using the precipitation technique. Crude

C-phycoerythrin from *Spirulina platensis* was used. The best purification condition was ammonium sulfate fractionation at 0-20%/20-50%, in relation to a resuspension volume/initial volume of 0.52 in a 7.0 pH buffer. Under these conditions, in an one-step purification only, the purity increased 70% compared to the initial extract, with an 83.8% recovery.

#### MEDICINAL USES OF *Spirulina platensis*

Studies have shown that *Spirulina* consumption during 4 weeks reduces serum cholesterol levels in human beings by 4.5% [41] and significantly reduces body weight. *Spirulina* extract induces the tumor necrosis factor in macrophages, suggesting a possible tumor destruction mechanism (Shklar and Schwartz, 1988) [42]. An extract of sulfated polysaccharides, called Calcium-*Spirulina* (Ca-SP), made up of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galacturonic acid, and calcium sulfate, obtained from *Spirulina*, showed activity against HIV, Herpes Simplex Virus, Human Cytomegalovirus, Influenza A Virus, Mumps Virus and Measles Virus. Cell extract of *Spirulina maxima* has shown antimicrobial activity against *Bacillus subtilis*, *Streptococcus aureus*, *Saccharomyces cerevisiae*, and *Candida albicans*. *Spirulina* reduces: hepatic damage due to drug abuse and heavy metal exposure, inflammatory response, cells degeneration, anaphylactic reaction. *Spirulina* contains vitamin A, important in preventing eye diseases; iron and vitamin B12, useful in treating hypoferric anemia and pernicious anemia, respectively;  $\gamma$ -linolenic acid, appropriate in treatment of atopic child eczema therapy; to alleviate premenstrual syndrome, and in immune system stimulation [16]. *Spirulina* has been studied as an animal cell-growth stimulant and in the treatment of residual waters using alginate [18].

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