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**Review on Protein of blue –green alga Spirulina**

**Dr. T. Sivakumar**

Department of Microbiology, Kanchi Shri Krishna College of Arts and Science, Kilambi – 631 551.  
Kancheepuram, Tamil Nadu, India.

\*Corresponding Author: [shiva.fungi@gmail.com](mailto:shiva.fungi@gmail.com)

**Introduction**

One of the most exciting discoveries of the Belgian Sahara Expedition of 1964 – 1965 was the finding that the blue-green alga *Spirulina* which forms dense blooms in brackish warm waters is collected and regularly eaten by natives around Lake Tschad (Leonhard et Compere, 1967). Soon after this discovery the French Petroleum Institute developed methods to produce *Spirulina* at the technical scale (Clement *et al.*, 1967) which are now being tested in Egypt. The crude protein content of *Spirulina* dry matter may exceed 60% as compared to the 55% typical for *Chlorella* or *Scenedesmus* (Soeder and Pabst, 1970). Other advantages of *Spirulina* are the good digestibility of its air-dried or spray-dried biomass and the comparatively large size of the filaments which allow harvesting by simple filtration methods. Maximal yields of *Spirulina* seem, however, to be lower than those attainable with mass cultures of green algae (Soeder, 1976).

In his report on microalgae that are or have been eaten by man, Johnston (1970) not only described the fascinating discovery of *Spirulina* as a dietary component but also recalls that other blue-green algae are consumed by man as well.

Although the toxicological testing of the biomass of certain microalgae (*Chlorella* spp., *Scenedesmus obliquus*, *Spirulina maxima*) has not yet reached the same level as the experimental evaluation of some other SCP types (. Shacklady, 1975 and Stringer 1975), it is safe to state, that at least the aforementioned microalgae are free from specific toxins, i.e. they never gave any sign of acute toxicity. In addition, long-term feeding studies made it highly probably that these

materials are toxicologically safe (e.g. Pabst *et al.*, 1978) although not generally acceptable in unlimited amounts for humans (Becker, 1978) and for some animals (.Brune and Walz, 1978; Meske and Preffer, 1978), in order to avoid possible uric acid hazards or probable amino acid imbalance as in chicken.

The accumulation of environmental pollutants like toxic minerals (Payer an Runkel, 1978) or polycyclic hydrocarbons (Pyear *et al.*, 1976) by microalgae is definitely an important aspect (Bremer, 1978), however, not an intrinsic property of microalgae but rather a result of exposure to industrial emissions. This topic shall, therefore, not be considered in the present article.

It should be noted that the PAG Guidelines (PAG, 1972/1974) require a detailed analysis of the chemical composition of SCP products. For microalgae, this condition has been fulfilled for *Scenedesmus obliquus* (Becker, 1980) and *Spirulina maxima* (Durand – Chastel, 1980).

In the cell walls of blue-green algae polysaccharides dominate over murein. Amino glucosan and amino rhamnosan make up for 11 – 12 % of dry matter of *Spirulina* (Clement, 1975) but it is not yet clear, whether these compounds are exclusively located in the cell walls. In yeasts, a highly branched glucan and a mannan account for 90% of the dry weight of the cell wall. The remainder consists of some chitin, proteins. In microscopic green algae, there are a great variety of cell wall types, the composition of which has only been analyzed in very few cases.

In human adults, the total N of whole egg can be diluted with ammonia N to more than 1:1 without a loss of biological value of the resulting "Crude protein"

(Kofranyi, 1970). This shows how well nitrogen, which is neither contained in essential amino acids nor in amino acids in general, can be utilized, provided that the essential amino acid requirements is properly met.

Although the amino acids reported from cultivated microorganisms are claimed to be the common ones, it is interesting to note that the careful analysis of e.g. microfungi indicates the presence of small amounts of a number of unknown amino acids (Casslicchio *et al.*, 1975). If substances of that kind should occur in trace amounts in SCP, their correct identification appears to be imperative. It should be recalled that toxic amino acids exist, among which mimosine, a substance resembling tyrosine, is an impressive example from higher plants. Marine algae synthesize odd amino acids like kainic acid which, despite its simple structure, is an antihelminthic drug and also a potent neuromuscular inhibitor (Shinozaki and Tshidia, 1976).

As in higher plants, terpenoids, including sesquiterpenes, are found in microalgae at the per cent level (Liersch, 1976). The blue-green alga *Spirulina maxima* contains about 750 ppm of  $\alpha$ -amyrine, a pentacyclic triterpene alcohol, and about 700 ppm of cyclitol (Clement, 1975).

A fairly optimistic outlook to the future of microalgae production is given by reports, which appeared in 1978, in international trade journals about the plans of the Italian company Montedison to install large scale *Spirulina* production plants along the shores of lake Tschad. The commercialization of this project is expected for 1985. According to Montedison, microalgal protein will have gained a 5 % share of the world protein market by 1990. This forecast is definitely stimulating, although it may presently bear a touch of science fiction.

In recent years Japanese companies began to produce also *Spirulina* on Okinawa and in Thailand for manufacturing algae pills, too, and also pet fish feed, pigments or other products.

So far we have stressed the use of microalgal proteins for food and feed purposes. Proteins are the most conspicuous but by no means the only component of algal cells with an economic interest. A Japanese company for instance is extracting a blue pigment (Phycocyanin) from the alga *Spirulina* and is offering the material as natural food colouring agent. The economy of the pigment extraction is beyond any doubt.

In 1976 Blum and Calet (1976) reported about broiler experiments with *Spirulina* algae. From one day old chicken to 8 weeks, body weight gain was less when *Spirulina* meal replaced the usual protein sources. This effect was not significant for diets with 5% or less of algae meal. Everywhere we find the necessary restriction of the percentage of algae meal in chicken's ration to have a good growth performance. Blum and

Calet (1976) thought this effect was to account for low metabolizable energy content of algae. He tried to raise the digestibility adding glycolytic enzyme to diet. There was no effect. In case of metabolizable energy, we have not to expect a very different result because between algae meal, extracted soybean meal and low fat fish meal less than 3%, only a caloric difference about 2% .

Fovrier and save (1975) reported in 1975 about *Spirulina* meal and early weaned pigs. To have no problems, they emphasize a restriction of algae meal to 25% of total dietary protein that means 8 to 12% algae meal in average.

The ever-increasing population in the world, coupled with the shortage of food supplies, has been one of the major problems of the last decade, and is expected to become more important in their future.

The present storage in conventional protein production of the world (meat, fish, soya beans etc.) is between 10-15 million tons annually, and is predicted to reach 25 – 30 million tons by 1980.

Two promising sources for the production of proteins for enrichment or replacement of conventional proteins, are the single cell proteins produced from the petroparaffins and three proteins obtained from algae such as *Spirulina* etc. There is a list of relevant parameter, which determines the nutritional value of a substance, and its application for man or animals.

The first parameter is the chemical composition. In the white column give the values alga *Scenedesume* (Venkataram *et al.*, 1977), the black columns for soybean meal are the figures of German foodstuff regulations for soybean, peeled and extracted. Both foodstuffs, algae and soybean have a high content of protein, about 50% and a small content of ether extract, Carbo-hydrates and fibre. Therefore, algae biomass is a high concentrated protein source. Protein will determine its nutritional value at first. The lipids may have a specific nutritive value, we know little. The low content of fibre gives a chance for algae to be a foodstuff for monogasters. There are high differences in the ash content of algae, but here this is not relevant for the review.

Algae as protein food or feedstuff range with other high concentrated protein sources, e.g. soybean products, very young green plant materials as alfalfa meal dehydrated, but algae products are less comparable to material of animal origin, e.g. fish meal, casein, which have a higher protein concentration and digestibility.

Based on its high protein concentration, algae biomass is to respect first a feed component for poultry, fish, swine and perhaps man. In these species we expect the best utilization in respect of nutrient transformation. Here the energy content is not relevant.

The basic requirement of the quality of protein (biological value) is the digestibility of the substance. In the fifties and sixties, there was observed that the cell walls of microalgae resist the enzymatic digestion of the cell content in the gut of animals. Processing of algae biomass by different methods improved the apparent digestibility of algae.

Processing of algae biomass has to be the first step for a good biological utilization. Necessary for a high concentrate protein is an apparent digestibility of the organic matter about 80%. Similar data should be expected for protein digestibility. E.g. digestibility of protein was found with pigs  $\phi$  82.3% (Koch, 1974) with fish  $\phi$  75.8% (Sendbank and Hopher, 1978). The protein quality of algae can be best pre-examined in experiments with laboratory animals.

Pabst (1978), using nitrogen balance method, described by the Food and Nutrition Board of USA (FNB, 1963) operates with rats and a 10% protein diet. In Fig 2 the data of these experiments were registered as N-utilization difference ingested total nitrogen minus nitrogen out of urine and feces, that is tantamount to nitrogen retention. This N-utilization of *Scenedesmus* and *Spirulina* (about 60%) is similar to that of soybean meal, but smaller compared to fishmeal protein (about 65%).

It is worthy to remark that all these experiments are done with laboratory animals and a low amount of protein in the diet, namely 10% protein. The protein utilization is also a question of the protein concentration in the ration. The general roots of the protein quality are connected with the amino acid pattern of the source, out of the chemical data of the essential amino acids; we can predict a standard of biological protein value with caution.

The data are determined for the metabolism trials. Which will be handled later (Brune and Walz, 1978). Though the total amino acids pattern are known, only the above demonstrated figures are relevant for the supplement purpose of algae protein in diets for human and animal nutrition. The concentration of lysine is obvious in the same range for exchangeable feedstuffs as alfalfa and soybean. The difference of the data of methionine have little weight with supplementation effects, and there is a possibility to have cheap synthetic methionine for animal feeding. The reference protein in this case is whole egg protein. The total nutritional effect of protein quality is normally resumed in the EAA – Index, as postulated by Poser.

The lack of low sulfuric amino acids-content in algae are often overrated in literature. We find this lack in most vegetable protein sources for animal feeding in practice. More expensive than the supplementation with sulfuric acids is the supplementation with lysine. Therefore, we

are interested in the utilization of algae lysine first. Backer (1978) gives some pattern for available lysine in the alga *Scenedesmus*. By different methods of processing of *Scenedesmus* (total lysine 4.6 g/100g protein) Backer specified the availability of lysine as follows: drum dried 79.6% freeze dried 80.4% and cooked and sun dried 76.5% these findings are comparable with the lysine pattern of soybean protein.

Koch (1974) estimated in our institute the protein utilization of the alga *Scenedesmus* with growing pigs (18-20 kg body weight). Growing pigs give a good response to different protein sources.

*Scenedesmus* protein compared in this way with alfalfa protein was three times better. The maintenance N-requirement of alfalfa protein was 41.25 g protein equal to 6.6 g N daily. This method gives a good differentiation for various protein sources.

Kofrenyi (1978) formerly Dortmund, tested the protein source for human adults. *Scenedesmus* is considered to be a valuable supplementary protein source for adult persons. The findings of Kofrenyi show a relative biological value of algae protein between 81.5 and 96 of the whole egg standard protein. That is the same order we found with pigs. Namely about 15% less than a high valuable standard protein. But in human nutrition there is a fact, which limited the amount of algae to prevent health disorder.

The FAO / WHO protein Advisory Group (PAG, 1978) recommended, that nucleonic acid intake should not exceed 2.2 g per head, thus setting a safety limit. Kofrenyi (1978) emphasized a daily intake of 20-30 g algae for healthy adults to prevent health hazards. He found, that persons receiving up to 45 g *Scenedesmus* per day had a uric acid excretion within their normal range.

In experiment with broiler we found (Walz *et al.*, 1975) that it is necessary to limit the *Scenedesmus* algae dose. On a 24% protein level in the diet total, two third or one half substitution of the protein need by algae protein caused a significant growth depression. In 1976 Blum and Calet (1976) reported about broiler experiments with *Spirulina* algae, from one-day-old chicken to 8 weeks, body weight gain was less when *Spirulina* meal replaced the usual protein sources.

### Antibacterial activity of *Spirulina*

The extracts of *Spirulina* inhibited the growth of bacteria, yeasts and fungi (Martinez-Nadal, 1970; Jorjani and Amirani, 1978). Antibacterial activity from *Calothrix brevissima*, *Lyngbya majuscula*, *Androcoleus* sp., and antifungal activity from *L. majuscula*, and antifungal activity from *Scytonema hofmanii* have been reported

(Metting and Pyne, 1986), Scopytin from *S.pseudohofmanii* (Ishibasi *et al.*,1986).Tijpanazoles from *Tolypothrix tijpansensis* (Bonjounklian *et al.* ,1991) and laxaphycins from *Anabaena laxa* (Frankmolle *et al.*, 1992) have been reported to possess antifungal activity.

Interestingly, activity was seen against both Gram – positive and a spectrum of Gram- negative organisms, which compared with activity shown by different algae (Caccamese *et al.*, 1989 and Rao *et al.*, 1988), while a review of literature shows antibacterial activity mostly against gram- positive (Allen and Dawson, 1960). It is noteworthy that disc of 100µg/disc((Caccamese *et al.*, 1989; Pesando and Caram,1984 and Rinehart *et al.*, 1981).

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