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Spirulina endorsed by FAO, as a follow-up on a General Assembly draft resolution initiated by IIMSAM

September 8, 2013 //

During the sixtieth session of the United Nations General Assembly (Second Committee, Agenda item 52), IIMSAM initiated a revised draft resolution on the "Use of *Spirulina* to combat hunger and malnutrition and help achieve sustainable development" which was submitted by: Burundi, Cameroon, Dominican Republic, Nicaragua and Paraguay. As a follow-up on this resolution, the United Nations Food and Agriculture Organization (FAO) was requested to prepare a draft position on *Spirulina*. FAO's report was presented in 2008 and includes the following recommendations:

•
To improve technical and economic solutions to *Spirulina* production in environmentally impoverished conditions, as well as to prepare tested production packages for rapid deployment in emergency situations.
•
To develop a practical guide to small-scale *Spirulina* production that could be used for development mythologies, oriented towards:

i)
Providing nutritional supplements for use in rural and urban communities where the diet is inadequate;
ii)
Allowing diversification from traditional crops in cases where land or water resources are limited;
iii)
An integrated system for waste water treatment, small-scale aquaculture production and other livestock feed supplication;
iV)
As a short- and medium-term solution to emergency situations where a sustainable supply of high protein/high vitamin foodstuff is required. This implies the ability to rapidly install systems in a variety of environments that can be sustained by local communities to cover both the short-term food needs and to supplement longer-term nutritional requirements especially once other forms of food relief cease to be delivered.
•
To establish a better monitoring of global *Spirulina* production and product flows.
•
To develop some form of web-based resource that allows the compilation of scientifically robust information and statistics for public access.
•
To develop clear guidelines on food safety aspects of *Spirulina* so that human health risks can be managed.
Source: FAO Fisheries and Aquaculture Circular No. 1034. A review on culture, production and use of *Spirulina* as food for humans and feeds for domestic animals and fish.

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Second Committee
Agenda item 52
Sustainable development

Burundi, Cameroon, Dominican Republic, Nicaragua and Paraguay: revised draft resolution

The use of spirulina to combat hunger and malnutrition and help achieve sustainable development

The General Assembly,

Noting with concern that hunger and malnutrition are a major impediment to sustainable development, and reaffirming that reducing hunger is a primary target of the Millennium Development Goals,

Recognizing the value of new technologies to enhance food security in environmentally compatible ways, including through public-private alliances for rural development,

Noting that the nutritional benefits of spirulina (food micro-algae) have been reported in academic research and in the work of agencies of the United Nations system, including the Food and Agriculture Organization of the United Nations and the World Health Organization,

Noting in particular that the merits of spirulina have been recognized through the adoption of international agreements, namely the Free Agreement for Cooperation in Scientific Research and Humanitarian Use of Micro-alga Spirulina as Food[1] and the Convention for the Use of Food Micro-algae and the Intergovernmental Institution for the Use of Spirulina against Malnutrition,

Taking into account that an intergovernmental organization known as "Convention for the Use of Food Micro-algae and the Intergovernmental Institution for the Use of Spirulina against Malnutrition" has been established in keeping with the above agreements and has been granted observer status in the work of the Economic and Social Council, in accordance with Council decision 2003/212 of 5 March 2003,

[1] United Nations, *Treaty Series*, vol. 2151, No. 37542.

Aiming to encourage greater attention to the production and use of spirulina for the reduction of hunger and poverty and to combat the food crises,

1. *Takes note* of the potential of spirulina to reduce hunger and malnutrition and to improve the prospects for sustainable development;

2. *Calls upon* Member States, United Nations agencies and other intergovernmental organizations, as well as non-governmental organizations and the private sector, to encourage the production and use of spirulina;

3. *Emphasizes* the importance of assisting national activities for the production and use of spirulina, especially in member countries of the Convention for the Use of Food Micro-algae and the Intergovernmental Institution for the Use of Spirulina against Malnutrition;

4. *Decides* to review, at its sixty-second session, the progress made in these areas, and requests the Secretary-General to submit a report, through the Economic and Social Council, on the relevant efforts.

FAO Fisheries and Aquaculture Circular No. 1034 **FIMA/C1034 (En)**

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A REVIEW ON CULTURE, PRODUCTION AND USE OF SPIRULINA AS FOOD FOR HUMANS AND FEEDS FOR DOMESTIC ANIMALS AND FISH

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FAO Fisheries and Aquaculture Circular No. 1034 FIMA/C1034 (En)

A REVIEW ON CULTURE, PRODUCTION AND USE OF SPIRULINA AS FOOD FOR HUMANS AND FEEDS FOR DOMESTIC ANIMALS AND FISH

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PREPARATION OF THIS DOCUMENT

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 a 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's position on this. The primary objective of this review is to assess/evaluate the existing knowledge on the culture, production and use of spirulina for human consumption and animal feeds and to prepare the draft position paper on the use of spirulina.

The review is primarily a desk study based on secondary-sources of information/data derived from published literature and unpublished reports and primary-sources of data/information collected through suitable consultations with those associated with culture/production and use of spirulina.

The document was prepared under supervision of Dr Mohammad R. Hasan, Fishery Resources Officer (Aquaculture), Aquaculture Management and Conservation Service (FIMA) of the FAO Fisheries and Aquaculture Department. Mr Jiansan Jia, Chief, FIMA and Dr Lahsen Ababouch, Chief, Fish Utilization and Marketing Service (FIIU) of the FAO Fisheries and Aquaculture Department are acknowledged for their comments.

ABSTRACT

Spirulina are multicellular and filamentous blue-green microalgae belonging to two separate genera *Spirulina* and *Arthrospira* and consists of about 15 species. Of these, *Arthrospira platensis* is the most common and widely available spirulina and most of the published research and public health decision refers to this specific species. It grows in water, can be harvested and processed easily and has significantly high macro- and micronutrient contents. In many countries of Africa, it is used as human food as an important source of protein and is collected from natural water, dried and eaten. It has gained considerable popularity in the human health food industry and in many countries of Asia it is used as protein supplement and as human health food. Spirulina has been used as a complementary dietary ingredient of feed for poultry and increasingly as a protein and vitamin supplement to aquafeeds.

Spirulina appears to have considerable potential for development, especially as a small-scale crop for nutritional enhancement, livelihood development and environmental mitigation. FAO fisheries statistics (FishStat) hint at the growing importance of this product. Production in China was first recorded at 19 080 tonnes in 2003 and rose sharply to 41 570 tonnes in 2004, worth around US$7.6 millions and US$16.6 millions, respectively. However, there are no apparent figures for production in the rest of the world. This suggests that despite the widespread publicity about spirulina and its benefits, it has not yet received the serious consideration it deserves as a potentially key crop in coastal and alkaline areas where traditional agriculture struggles, especially under the increasing influence of salination and water shortages.

There is therefore a role for both national governments – as well as intergovernmental organizations – to re-evaluate the potential of spirulina to fulfill both their own food security needs as well as a tool for their overseas development and emergency response efforts. International organization(s) working with spirulina should consider preparing a practical guide to small-scale spirulina production that could be used as a basis for extension and development methodologies. This small-scale production should be orientated towards: (i) providing nutritional supplements for widespread use in rural and urban communities where the staple diet is poor or inadequate; (ii) allowing diversification from traditional crops in cases where land or water resources are limited; (iii) an integrated solution for waste water treatment, small-scale aquaculture production and other livestock feed supplement; and (iv) as a short- and medium-term solution to emergency situations where a sustainable supply of high protein/high vitamin foodstuffs is required.

A second need is a better monitoring of global spirulina production and product flows. The current FishStat entry which only includes China is obviously inadequate and the reason why other countries are not included investigated. Furthermore, it would be beneficial if production was disaggregated into different scales of development, e.g. intensive, semi-intensive and extensive. This would allow a better understanding of the different participants involved and assist efforts to combine experience and knowledge for both the further development of spirulina production technologies and their replication in the field. A third need is to develop clear guidelines on food safety aspects of spirulina so that human health risks can be managed during production and processing. Finally, it would be useful to have some form of web-based resource that allows the compilation of scientifically robust information and statistics for public access. There are already a number of spirulina-related websites (e.g. www.spirulina.com, www.spirulinasource.com) – whilst useful resources, they lack the independent scientific credibility that is required.

Habib, M.A.B.; Parvin, M.; Huntington, T.C.; Hasan, M.R.
A review on culture, production and use of spirulina as food for humans and feeds for domestic animals and fish.
FAO Fisheries and Aquaculture Circular. No. 1034. Rome, FAO. 2008. 33p.

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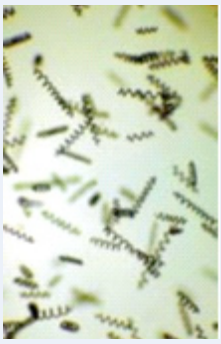
1 INTRODUCTION AND SCOPE

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 aquaculture diets. It grows in water, can be harvested and processed easily and has very high macro- and micro-nutrient contents. It has long been used as a dietary supplement by people living close to the alkaline lakes where it is naturally found – for instance those living adjacent to Lake Chad in the Kanem region have very low levels of malnutrition, despite living on a spartan millet-base diet. This traditional food, known as *dihé,* was rediscovered in Chad by a European scientific mission, and is now widely cultured throughout the world. In many countries of Africa, it is still used as human food as a major source of protein and is collected from natural water, dried and eaten. It has gained considerable popularity in the human health food industry and in many countries of Asia it is used as protein supplement and as health food.

Figure 1: **Spirulina and its sales as dried cakes in Chad**



Spirulina

Spirulina cakes (dihé) on sale in a local market in Chad

Source: www.spirulinasource.com

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 aquafeeds. 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 a 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's position on this.

The primary objective of this review is therefore to assess and evaluate the existing knowledge on the culture, production and use of spirulina for both human consumption and animal feeds.

2 HISTORICAL BACKGROUND ON THE USE OF SPIRULINA AS HUMAN FOOD AND ANIMAL FEED

Spirulina is a primitive organism originating some 3.5 billion years ago that has established the ability to utilize carbon dioxide dissolved in seawater as a nutrient source for their reproduction. Spirulina is a photosynthesizing cyanophyte (blue-green algae) that grows vigorously in strong sunshine under high temperatures and highly alkaline conditions.

2.1 Historical use

In the sixteenth century, when the Spanish invaders conquered Mexico, they discovered that the Aztecs living in the Valley of Mexico in the capital Tenochtitlan were collecting a "new food" from the lake (Sasson, 1997). Spanish chroniclers described fishermen with fine nets collecting this blue coloured "techuitlatl" from the lagoons and making a blue-green cake from it. Other legends say Aztec messenger runners took spirulina on their marathons. Techuitlatl was mentioned by naturalists until the end of the sixteenth century, but not after that, probably reflecting the loss of the lakes as they were drained for urban and agricultural development. The only remnant today, Lake Texcoco, still has a living algae spirulina population.

The Kanembu population living along the shores of Lake Chad collects the wet algae in clay pots, drain out the water through bags of cloth and spread out the algae in the sandy shore of the lake for sun drying. The semi-dried algae is then cut into small squares and taken to the villages, where the drying is completed on mats in the sun (Abdulqader, Barsanti and Tredici, 2000). When dry, women take these algae cakes for sale in the local market. Dihé is crumbled and mixed with a sauce of tomatoes and peppers, and poured over millet, beans, fish or meat and is eaten by the Kanembu in 70 percent of their meals (www.spirulinasource.com). Pregnant women eat dihé cakes directly because they believe its dark colour will screen their unborn baby from the eyes of sorcerers (Ciferri, 1983). Spirulina is also applied externally as a poultice for treating certain diseases. Abdulqader, Barsanti and Tredici (2000) further noted that the local trading value of the dihé annually harvested from Lake Kossorom in Chad (about 40 tonnes) amounts to more than US $100,000, which represents an important contribution to the economy of the area.

2.2 Rediscovery of spirulina

In 1940, a French phycologist Dangeard published a report on the consumption of dihé by the Kanembu people near Lake Chad (Dangeard, 1940). Dangeard also noted these same algae populated a number of lakes in the Rift Valley of East Africa, and was the main food for the flamingos living around those lakes. Twenty-five years later during 1964-65, a botanist on a Belgian Trans-Saharan expedition, Jean Léonard, reported finding a curious greenish, edible cakes being sold in native markets of Fort-Lamy (now N'Djamena) in Chad (Léonard, 1966). When locals said these cakes came from areas near Lake Chad, Léonard recognized the connection between the algal blooms and dried cakes sold in the market.

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

At the same time when Léonard rediscovered spirulina in Africa, a request was received from a company named Sosa-Texcoco Ltd by the "Institut français du pétrole" to study a bloom of algae occurring in the evaporation ponds of their sodium bicarbonate production facility in a lake near Mexico City. As a result, the first systematic and detailed study of the growth requirements and physiology of spirulina was performed. This study, which was a part of Ph.D. thesis by Zarrouk (1966), was the basis for establishing the first large-scale production plant of spirulina (Sasson, 1997).

While finally no micro-organism fulfilled its promise of cheap protein, spirulina continued to give rise to research and increasing production, reflecting its perceived nutritional assets (Falquet, 2000).

3 GENERAL CHARACTERISTICS OF SPIRULINA

3.1 Morphology and taxonomy

3.1.1 Morphology

Spirulina is symbiotic, multicellular and filamentous blue-green microalgae with symbiotic bacteria that fix nitrogen from air. Spirulina can be rod- or disk-shaped. Their main photosynthetic pigment is phycocyanin, which is blue in colour. These bacteria also contain chlorophyll a and carotenoids. Some contain the pigment phycoythrin, giving the bacteria a red or pink colour. Spirulina are photosynthetic and therefore autotrophic. Spirulina reproduce by binary fission.

The helical shape of the filaments (or trichomes) is characteristic of the genus and is maintained only in a liquid environment or culture medium. The presence of gas-filled vacuoles in the cells, together with the helical shape of the filaments, result in floating mats. The trichomes have a length of 50 to 500 μm and a width of 3 to 4 μm. However, cyanobacteria have a cell wall similar to that of Gram-negative bacteria: they contain peptidoglucan, a lysozyme-sensitive heteropolymer that confers shape and osmotic protection to the cell, in addition to other material not sensitive to lysozyme. In the 1970s, sphaeroplasts, produced by disintegration of their cell wall by enzymatic treatment, were isolated from *Oscillatoria formosa, Fremyella dipplosiphon, Plectonema calothricoides* and *Synecchoccus lividus*, as well as from *Anabaena ambique* and *Anacystis nidulans*. In the case of spirulina, protoplasts had been obtained by several researchers. For example, Abo-Shady *et al*. (1992) succeeded in obtaining protoplasts of *Spirulina platensis* with high efficiency by lysozyme treatment at the exponential phase of algal growth at 30 °C under illumination with 1 450 lux/m^2/second. The body surface of spirulina is smooth and without covering so it easily digestible by simple enzymatic systems.

3.1.2 Taxonomy

In 1827, P.J. Turpin isolated spirulina from a freshwater sample (Ciferri, 1983). In 1844, Wittrock and Nordstedt reported the presence near the city of Montevideo of a helical, septal and green-blue microalgae named *Spirulina jenneri* f. *platensis*. But it was not until 1852 that the first taxonomic report written by Stizenberger appeared. He gave this new genus the name *Arthrospira* based on the septa presence, helical form and multicellular structure. Gomont confirmed Stizenberger's studies in 1892. This author attributed the aseptate form to the *Spirulina* genus, and the septal form to the *Arthrospira* genus. Geitler in 1932, because of the common helical morphology, reunified the members of the two genera under the designation *Spirulina* without considering the septum presence, only morphological similarity. In 1989, these micro-organisms were separately classified into two genera *Spirulina* and *Arthrospira*; this classification is currently accepted (Tomaselli, Palandri and Tredici, 1996; Sánchez *et al*., undated). *Arthrospira maxima* and *A. platensis* are the most important species in this genus and among these existed taxonomic differences in filaments, vacuoles and external cover or capsule regularity of each filament (Tomaselli, 1997). The worldwide investigation on microalgae has been carried out under the name of "spirulina"; this common designation between scientists and consumers has proved difficult to change. The microalgae under discussion belongs to the genus *Arthrospira*, but it will probably be called *Spirulina* for some time. For the purpose of this report we refer to both collectively as "spirulina".

The systematic position of cyanobacteria has been a matter of discussion, as these photosynthetic organisms were first considered algae. In 1962, a distinction between prokaryotes and eukaryotes was clearly established. The main difference is based upon the presence of cell organelles enveloped by a phospholipidic membrane in eukaryotes. Stanier and Van Neil (1962) incorporated green-blue algae into the prokaryote kingdom and proposed to call these micro-organisms cyanobacteria. This designation was accepted and first published in 1974 in the Bergey's Manual of Determinative Bacteriology (Guglielmi, Rippka and Tandeau De Marsac, 1993).

3.2 Natural habitat, source and growth

Besides Lake Texcoco, the largest spirulina lakes are in Central Africa around Lakes Chad and Niger, and in East Africa along the Great Rift Valley. Under normal water conditions, *Spirulina* may be one of many algal species. Lakes Bodou and Rombou in Chad have a stable monoculture of spirulina dating back centuries. It is also a major species in Kenya's lakes Nakuru and Elementeita and Ethiopia's lakes Aranguadi and Kilotes. Spirulina thrives in alkaline lakes where it is difficult or impossible for other micro-

organisms to survive (Kebede and Ahlgren, 1966). In natural lakes, the limited supply of nutrients usually regulates growth cycles. New nutrients come from either an upwelling from inside the waterbodies, influxes of nutrients from rivers or from pollution. The algae population grows rapidly, reaches a maximum density, and then dies off when nutrients are exhausted. A new seasonal cycle begins when decomposed algae release their nutrients or when more nutrients flow into the lake.

Spirulina is found in soil, marshes, freshwater, brackish water, seawater and thermal springs. Alkaline, saline water (>30 g/l) with high pH (8.5–11.0) favour good production of spirulina, especially where there is a high level of solar radiation at altitude in the tropics. *Spirulina platensis* and *Spirulina maxima* thrive in highly alkaline lakes of Africa and Mexico where the cyanobacteria population is practically monospecific. The higher the pH and the conductivity of the water, the greater is the likely predominance of *Spirulina* spp. This is the case in the lakes of the lakes of the Rift Valley of eastern Africa, where pH can reach values close to 11 and sodium carbonate is abundant. *Spirulina platensis* was isolated from waters containing from 85 to 270 g of salt per litre, and optimum growth occurred between 20 and 70 g of salt per litre. A relatively high cytoplasmic pH (4.2 to 8.5) may account for the ability of this micro-organism to utilize ammonia as a source of nitrogen at high alkaline pH values (Sasson, 1997).

Spirulina is, like most cyanobacteria, an obligate photoautotroph, i.e. it cannot grow in the dark on media containing organic carbon compounds. It reduces carbon dioxide in the light and assimilates mainly nitrates. The main assimilation product of spirulina photosynthesis is glycogen. Spirulina shows an optimum growth between 35 and 37 °C under laboratory conditions. Outdoors, it seems that an increase in temperature up to 39 °C for a few hours does not harm the blue-green alga, or its photosynthetic ability. Thermophilic or thermotolerant strains of spirulina can be cultivated at temperatures between 35 and 40 °C. Such a property has the advantage of eliminating microbial mesophilic contaminants. The minimum temperature at which growth of spirulina takes place is around 15 °C during the day. At night, spirulina can tolerate relatively low temperatures. The resistance of spirulina to ultraviolet rays seems to be rather high (Richmond, 1986).

3.3 Biochemical composition

The basic biochemical composition of spirulina can be summarized as follows:

Protein: Spirulina contains unusually high amounts of protein, between 55 and 70 percent by dry weight, depending upon the source (Phang *et al.*, 2000). It is a complete protein, containing all essential amino acids, though with reduced amounts of methionine, cystine, and lysine, as compared to standard proteins such as that from meat, eggs, or milk; it is, however, superior to all standard plant protein, such as that from legumes.

Essential fatty acids: Spirulina has a high amount of polyunsaturated fatty acids (PUFAs), 1.5–2.0 percent of 5–6 percent total lipid. In particular spirulina is rich in γ-linolenic acid (36 percent of total PUFAs), and also provides γ-linolenic acid (ALA), linoleic acid (LA, 36 percent of total), stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA).

Vitamins: Spirulina contains vitamin B_1 (thiamine), B_2 (riboflavin), B_3 (nicotinamide), B_6 (pyridoxine), B_9 (folic acid), B_{12} (cyanocobalamin), vitamin C, vitamin D and vitamin E.

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

Photosynthetic pigments: Spirulina contains many pigments including chlorophyll a, xanthophyll, beta-carotene, echinenone, myxoxanthophyll, zeaxanthin, canthaxanthin, diatoxanthin, 3-hydroxyechinenone, beta-cryptoxanthin, oscillaxanthin, plus the phycobiliproteins c-phycocyanin and allophycocyanin.

Detailed biochemical composition analyses have been conducted of spirulina grown either under laboratory conditions, collected in natural condition or in mass culture system using different agro-industrial waste effluent. This was found to vary in response to the salinity of the growing medium – Vonshak *et al*. (1996) reported that salt-adapted cells had a modified biochemical composition with a reduced protein and chlorophyll content, and increased carbohydrate content. However the following provides a review of the literature on the broad composition of spirulina.

3.3.1 Proximate composition

Spirulina has high quality protein content (59–65 percent), which is more than other commonly used plant sources such as dry soybeans (35 percent), peanuts (25 percent) or grains (8–10 percent). A special value of spirulina is that it is readily digested due to the absence of cellulose in its cell walls (as it is the case for eukaryotic green microalgae such as *Chlorella*, *Ankistrodesmus*, *Selenastrum*, *Scenedesmus*): after 18 hours more than 85 percent of its protein is digested and assimilated (Sasson, 1997). The composition of commercial spirulina powder is 60 percent protein, 20 percent carbohydrate, 5 percent fats, 7 percent minerals, and 3–6 percent moisture, making it a low-fat, low calorie, cholesterol-free source of protein.

Table 1: Various proximate analysis results of spirulina (% dry matter)

Component	FOI, France	SAC, Thailand	IPGSR, Malaysia	BAU, Bangladesh
Crude protein	65	55–70	61	60
Soluble carbohydrate	19		14	
Crude lipid	4	5–7	6	7
Crude fiber	3	5–7		
Ash	3	3–6	9	11
Moisture		4–6	6	9
Nitrogen free extract (NFE)		15–20	4	17

FOI = French Oil Institute; SAC = Siam Algae Co. Ltd; IPGSR = Institute of Post-graduate Studies and Research laboratory, University of Malaya; BAU = Bangladesh Agricultural University

3.3.2 Amino acids

Spirulina protein has a balanced composition of amino acids, with concentrations of methionine, tryptophan and other amino acids almost similar to those of casein although this depends upon the culture media used.

Table 2: Amino acid composition of spirulina (g/100 g)

Source	Lysine	Phenylalanine	Tyrosine	Leucine	Methionine	Glutamic acid	Aspartic acid	Tryptophan	Cystine
Siam Algae Co. Ltd., Thailand	2.60–3.30	2.60–3.30	2.60–3.30	5.90–6.50	1.30–2.00	7.30–9.50	5.20–6.00	1.00–1.60	0.50–0.70
IPGSR, Malaysia	4.63 ± 0.07	4.10 ± 0.08	3.42 ± 0.10	8.37 ± 0.13	2.75 ± 0.05	7.04 ± 0.14	5.37 ± 0.11	1.98 ± 0.05	0.6 ± 0.03

Table 2: Continued

Source	Serine	Arginine	Histidine	Threonine	Proline	Valine	Isoleucine	Alanine	Glycine
IPGSR, Malaysia	3.84 ± 0.06	4.94 ± 0.07	2.81 ± 0.06	3.35 ± 0.06	4.11 ± 0.05	4.02 ± 0.06	3.85 ± 0.10	10.81 ± 0.14	6.66 ± 0.10

3.3.3 Unsaturated fatty acids and lipids

The essential lipids (unsaturated fatty acids) in spirulina are about 1.3–15 percent of total lipid (6–6.5 percent), mainly constituting γ-linolenic acid (30–35 percent of total lipid) (Borowitzka, 1994; Li and Qi, 1997). Some researchers found that polyunsaturated fatty acids (PUFAs) could represent 25 to 60 percent of total fatty acids in spirulina. The important fatty acids like linoleic acid and linolenic acid are also present: up to 1.0 g/100 g of dry biomass of spirulina. The predominant fatty acids are palmitic acid (44.6–54.1 percent), oleic acid (1–15.5 percent), linoleic acid (10.8–30.7 percent) and γ-linolenic acid around 8.0–31.7 percent. Gamma-linolenic acid (GLA) is an essential fatty acid rarely available in ingredients or diet. The fatty acids of spirulina cultured in Kosaric medium were also analysed in the laboratory of Aquatic Ecology, Institute of Post-graduate Studies and Research Laboratory, University of Malaya, Malaysia as: palmitic acid (14.99 percent), oleic acid (1.43 percent), linoleic acid (23.18 percent), and GLA (30.16 percent) and ecosapentaenoic acid (4.94 percent). However, *Spirulina platensis* is one of the most promising sources of PUFA as γ-linolenic acid which can be enhanced by cultivation under light-dark cycles in the laboratory or outdoors (Tanticharoen *et al.*, 1994). It can be increased from 1.2 to 1.6 percent when cultivated under light-dark cycles. In the derived mutant *S. platensis* Z19, GLA reached 2.4 percent when cultivated in outdoor conditions, made spirulina one of the highest GLA contents reported for algae (Tanticharoen *et al.*, 1994). It is a precursor of certain prostaglandins and has some influence on the amount of blood cholesterol. In addition, this and other unsaturated fatty acids seem to be essential for cell growth as structural elements of cell membranes. These PUFAs help to keep normal cell-to-cell permeability in aquatic organisms even in extreme cold conditions.

The content of cholesterol is 32.5 mg/100 g; 10 g of spirulina powder, i.e., a soup spoon, provides only 1.3 mg of cholesterol and 36 kcal of energy, whereas the equivalent quantity of protein from egg would provide 300 mg of cholesterol and 80 kcal of energy. As a result spirulina powder is used to supplement the diet with protein and, at the same time, control extra weight. The average recommended dose is between 2 and 8.5 g in 24 hours.

3.3.4 Minerals

All the essential minerals are available in spirulina which contributes about 7 percent (average range 2.76–3.00 percent of total weight) under laboratory conditions. But in commercial spirulina production, minerals contribute about 7 percent. It bioaccumulates minerals when grown in different media, at different temperatures, pH, salinity, etc. Sharma and Azeez (1988) conducted an experiment on the bioaccumulation of copper and cobalt by spirulina at different temperatures which showed a high accumulation capacity. They also found that a negative correlation between metal accumulated and the survival ratio of this algae was observed. Gabbay, Tel and Gresshoff (1993) studied on the mechanism of salt tolerance in cyanobacteria especially on *Spirulina subsalsa*. They found that *S. subsalsa* cells in fresh sea water medium increased their sodium and chloride intracellular content and developed the capability to initiate sodium and chloride efflux in the light. In the dark, this efflux stops but the cells remain viable or active. In this situation, enhanced respiration may be driven by salt influx and triggered the events leading to salt adaptation. The biosynthesis and accumulation of organic compatible solutes in the cyanobacterium cell is a slow and secondary response at a steady state osmotic stability. Zoina, Bolsunovsky and Kalacheva (2000) found that *Spirulina platensis* shows a relatively high tolerance to salinity and a capacity of growing on the medium containing up to 70 g NaCl/litre. There is no marked inhibition of the growth rate or changes in the biomass composition usually observed in the range of 1–30 g NaCl/litre in medium.

Bolsunovskii and Kosinenko (2000) assessed the status of intracellular phosphorus (P) pool using radioactive and non-radioactive P. Results show that the stage of replenishment of the intracellular P pool may affect the P turnover estimation in aquatic environments during a short-term measurement of P uptake. Hernandez and Olguín (2002) evaluated the capacity of cells of four species of spirulina for absorption of minerals. It was found that two species contained high percentage of protein (68.95 percent) as a result of being cultivated in Zarrouk medium at two light intensities (66 μmol photon/m^2/second and 144 μmol photon/m^2/second) in batch culture. A third species of spirulina cultivated in a "Complex" medium and exposed at 66 μmol photon/m^2/second, contained a high percentage of lipids (30 percent). The fourth species of spirulina contained high percentage of polysaccharides (25.54 percent) when cultured in "Complex" medium when exposed at 144 μmol photon/m^2/second. It was found that the chemical composition of *Spirulina* sp. cells did have a strong influence on their adsorption capacities (qmax) for Pb and Cd which were highest (172.41 and 45 mg/g of cells at pH 5 and 4.5, respectively)

when cells exhibited the higher polysaccharide content. In the case of Cr VI, the highest gmax exhibited by cells cultivated in Zarrouk medium and showing the higher protein content (at pH 2.0). But pH did not affect the adsorption of Pb II in the range of 3 to 5.5, nor of Cd in the range of 4 to 7. For Cr II, adsorption observed only at a pH equal to 2.0 or lower.

3.3.5 Chelating of toxic minerals (neutralization of toxic minerals)

Spirulina has a unique quality to detoxify (neutralize) or to chelate toxic minerals, a characteristic that is not yet confirmed in any other microalgae (Maeda and Sakaguchi, 1990; Okamura and Aoyama, 1994). Spirulina can be used to detoxify arsenic from water and food. It also may be used to chelatize or detoxify the poisonous effect of heavy metals (minerals) from water, food and environment. Beijing University has extracted bioactive molecules from spirulina which could neutralize or detoxify the toxic and poisonous effect of heavy metals, and which showed anti-tumor activity. Several institutions in China are focusing on biomolecules which show anti-tumor, anti-age and anti-radiation properties (Liu, Guo and Ruan, 1991; Li and Qi, 1997).

3.3.6 β-carotene and vitamins

The β-carotene, B-group vitamin, vitamin E, iron, potassium and chlorophyll available in the spirulina can promote the metabolism of carbohydrate, fats, protein, alcohol, and the reproduction of skin, muscle and mucosa. Spirulina contains large amounts of natural β-carotene and this β-carotene is converted into vitamin A. According to the findings of the National Cancer Institute, United States of America, an intake of 6.0 mg β-carotene daily may be effective in minimizing the risk of cancer. If anybody takes 4.0 g spirulina daily, that is sufficient to get 6 mg β-carotene. At the same time, sufficient amount of B-group vitamins, iron and calcium will be obtained. However, these nutrients obtained from 4.0 g of spirulina are equivalent to or more than those obtained by eating more than 100 g of terrestrial bright-coloured vegetables.

4 CULTIVATION AND PRODUCTION OF SPIRULINA

4.1 Natural production

Most commercial production systems are based on shallow raceways in which spirulina cultures are mixed by a paddle wheel. However, there are still some examples of spirulina being harvested commercially from naturally occurring populations. In Mexico**,** following the research conducted in 1967 in collaboration with the French Oil Institute, Sosa-Texcoco Ltd has been harvesting *Spirulina maxima* from an area of Lake Texcoco, at 2 200 m above sea level, in a semi-tropical environment, in the Valley of Mexico, with an average annual temperature of 18 ºC. The same company used to extract soda from the lake deposits since 1936. This is the largest single plant for the production of spirulina biomass. The so-called "semi-natural" cultivation process consists of harvesting during day and night and the algal biomass doubles in three to four days. After filtration, the algal biomass is spray-dried after homogenization and pasteurization (Oliguín, 1986). The first pilot plant which produced 150 tonnes of dry spirulina biomass per year started production in 1973; its production capacity was thereafter raised to 300 tonnes of medium-grade product per year from 12.0 hectares of natural ponds. Sosa-Texcoco Ltd had invested some US$5 millions since 1977 to solve the engineering problems arising from the increase in production and to run the toxicological tests indispensable before marketing the product (Oliguín, 1986). The annual value of spirulina production represented a third of the company's income from the manufacture of powdered soda from the lake deposits. In 1995, Sosa-Texcoco ceased production of spirulina.

Another semi-natural lake in Myanmar has been reported to be used as a production site for spirulina. Four volcanic lakes with natural spirulina blooms were studied beginning in 1984. Production began at Twin Taung Lake in 1988, and by 1999 increased to 100 tonnes/year. About 60 percent is harvested from boats on the surface of the lake, and about 40 percent is grown in outdoor ponds alongside the lake. During the blooming season in the summer, when spirulina forms thick mats on the lake, people in boats collect a dense concentration of spirulina in buckets. Spirulina is harvested on parallel inclined filters, washed with fresh water, dewatered and pressed again. This paste is extruded into noodle-like filaments which are dried in the sun on transparent plastic sheets. Dried chips are taken to a pharmaceutical factory in Yangoon, pasteurized, and pressed into tablets.

4.2 Laboratory cultivation

Eight major environmental factors influence the productivity of spirulina: luminosity (photo-period 12/12, 4 luxes), temperature (30 °C), inoculation size, stirring speed, dissolved solids (10–60 g/litre), pH (8.5–10.5), water quality, and macro and micronutrient presence (C, N, P, K, S, Mg, Na, Cl, Ca and Fe, Zn, Cu, Ni, Co, Se) (Ciferri, 1983; Ayala, 1998).

4.2.1 Light regimes

Zeng *et al*. (2001a) observed the characteristics of light attenuation and light utilization on the growth of *Spirulina platensis* cells in culture conditions. They found two concepts useful for spirulina culture, viz. average irradiance and specific light energy utilization rate introduced to describe the relationship between cell growth and light utilization. They also recorded four basic parameters such as maximum specific growth rate (1.75 μm/day), half light-saturation constant (1.453 x 10^3 1x), light coefficient {(3.637/10^3) (OD560/cm^2)/mW}. Zeng, Cai and Ouyang (2001b) also found that introduction of 1 percent CO_2 in culture system helps to increase final cell concentration and stabilize the pH of the culture of spirulina. Pareek and Srivastava (2001) conducted an experiment to determine the optimum photoperiod for the growth and yield of the Jaipur (India) isolate of *Spirulina platensis* in different photoperiods. They found that the optimum photoperiod is 16 hours/day based on the evaluation of optical density and chlorophyll content of *S. platensis*.

Paoletti, Pushoaraj and Tomaselli (1975) studied the growth performances of *Spirulina platensis* and *S. maxima* under photo-limited conditions using Roux bottles of 1.0 litre capacity incubated in a water bath at 30 °C. It was irradiated intermittently from one side with battery of fluorescent lamps (PAR intensity equal to 65.30 J/m^2/second) in light:dark cycle (12 hours:12 hours). The initial biomass concentration of spirulina was 350 mg (dry weight)/litre which attended to a maximum dry weight of 346 and 329 mg/litre in the cases of *S. platensis* and *S. maxima*, respectively. The culture solution was the standard bicarbonate-carbonate medium at pH 9–9.5 with bubbling air and 1 percent CO_2.

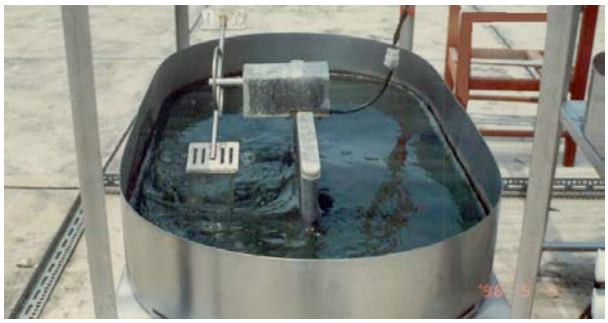
Tomaselli *et al*. (1987) examined the growth of ten strains of *Spirulina platensis* and eight strains of *S. maxima*. They obtained three strains of *S. platensis* from different laboratories (strain Cl from Leonard, strain LB 1475/4 from the Cambridge Culture Collection and strain M135 from Teronobu's collection). The remaining 15 strains were isolated in their laboratory, mostly from water samples collected from Lake Texcoco (Mexico) and Lakes Monbolo and Rombou (Chad). The properties taken into consideration include: relative photosynthetic efficiency under photo-limited conditions, protein production, rate of dark respiration, behaviour at temperatures above the optimum for growth, and tolerance of salinity.

The effect of the rate of mixing on productivity of algal mass in relation to photon flux density and algal concentration was quantitatively evaluated in cultures of *Spirulina platensis* grown in a flat-plate photobioreactor (Hu and Richmond, 1996). Maximal mixing enhanced cell concentration and productivity of biomass obtained at the highest light intensity used. At each level of incident light intensity, maximum productivity and photosynthetic efficiency could be achieved only when algal concentration and mixing rates were optimized. Hu and Richmond (1996) concluded that the higher the light intensity, the higher optimal culture density, highest algal concentrations and productivity of biomass being obtained. But too high a rate of mixing resulted in cell damage and reduced output rate. There are some changes in growth and chemical composition of two species of *Spirulina* (*S. platensis* and *S. maxima*) at different temperatures with agitation control. Again, the researcher applied mathematical models for the determination of optimal glucose concentration and light intensity for mixotrophic culture of *Spirulina platensis* (Zhang, Zhang and Chen, 1999).

4.2.2 Nutritional media

The culture of spirulina is practised in different media, especially inorganic and decomposed organic nutrients. Different types of spirulina were cultured to evaluate growth and biochemistry under similar controlled conditions (Bhattacharya and Shivaprakash, 2005). They cultured three species of *Spirulina* viz. *Spirulina platensis*, *S. laxissima* and *S. lonar*. Of the three species *S. platensis* showed highest growth rate, biomass, pigment concentration and low intracellular phenolics. The results indicate that *S. platensis* reached highest growth in shortest doubling time and the importance of strain selection for large-scale cultivation. Sanchez-Luna *et al*. (2004) found that the intermittent addition of urea in the autotrophic culture of *Spirulina platensis* yielded similar results to those obtained by the continuous feeding. They further concluded that the operation mode of using urea intermittently would be preferable to reduce the production costs of this cyanobacterium in large-scale facilities.

Figure 2: *Spirulina platensis* culture in digested sago waste in tank



Mini high rate algal Tank, Institute of Post-graduate Studies & Research,
University of Malaya, Kuala Lumpur, Malaysia (photo courtesy: S.M. Phang).

Faintuch, Sato and Aguarone (1991) also studied the influence of the nutritional sources on the growth rate of cyanobacteria. They reported that there is very significant influence of mixtures of defined proportions of KNO_3, urea and ammonia-N on the growth of *Spirulina maxima*. The most favourable growth rates of *S. platensis* occurred in the presence of 2.57 g/litre KNO_3 with growth rate of 0.3–0.4/day. Chang *et al*. (1999) studied the possibility of using nitrifying bacteria for the fulfillment of nitrogen fertilizer in spirulina mass culture. They first adapted the nitrifying bacteria with pH 8–10, 0.6–2.2 percent salt and 6–12 mg/litre of $NaHCO_3$ in the culture solution. They found that the concentration of NO_3-N reached over 20 mg/litre after the nitrifying bacteria was inoculated in the spirulina culture solution and then incubated for 6 days at 25–35 °C.

In Bangladesh, spirulina is cultured in different agro-industrial wastes such as sugar mill waste effluent, poultry industry waste, fertilizer factory waste, and urban waste and organic matter. The growth parameters of *Spirulina platensis* grown in the supernatant of digested 4.0 g/litre poultry waste were higher than other cultures on the supernatant of 2.0 and 6.0 g/litre digested poultry waste which might be due to appropriate nutrient content and other environment parameters (Parvin, 2006). Growth performance of *Spirulina platensis* was studied in three different concentrations of banana leaf ash added with 0.4 g/litre jackfruit seed powder and 0.2 g/litre with urea in the laboratory (Toyub *et al.*, 2005).

Figure 3: *Spirulina platensis* culture in supernatant of 4.0 g/litre digested poultry waste

Live Food Culture Laboratory, Bangladesh Agricultural University, Mymensingh, Bangladesh (photo courtesy: M.A.B. Habib)

4.3 Small-scale commercial production of spirulina

Spirulina cultivation has a number of advantages over traditional agriculture:

High yield: With around 60 percent protein content, spirulina's rapid growth means it yields 20 times more protein per unit area than soybeans, 40 times more than corn, and over 200 times more than beef.

Soil requirements: Spirulina culture does not require fertile land and can actually benefit from saline conditions.

Efficient water use: Spirulina uses less water per kilo of protein (approximately 2 100 litre/kg protein) than other crops. Water can be recycled and the only significant water loss is through evaporation. Spirulina culture uses 25 percent of the water of soy, 17 percent of corn and 2 percent the water required for beef protein. As mentioned above, brackish or saline water can be utilized.

Efficient energy use: Spirulina requires less energy input per kilo than soy, corn or beef, including solar and generated energy. Its energy efficiency (food energy output/kg/energy input/kg) is 5 times higher than soy, 2 times higher than corn, and over 100 times higher than grain-fed beef.

The small-scale production of spirulina is considered as a potential income-generating activity for households or village collectives. Spirulina might be also dried and processed for local consumption, especially where poor dietary regimes need to be supplemented. In addition, the extensive or semi-intensive production of spirulina for animal or aquatic feeds might be conducted for small-scale farming and aquaculture.

As early as 1949, Spoehr and Milner (1949) suggested that the mass culture of algae would help to overcome global protein shortages. Ironically, in spite of the lamentably low per capita protein supplies in many parts of the world, mass cultivation of algae has received only casual interest. The United Nations Environmental Programme (UNEP) is emphasizing nitrogen fixation and nutrient recycling through a programme that will establish microbiological centers (MIRCENS), and it is hoped that this will stimulate interest in micro-algae technology as a component of an integrated recycling system for rural communities.

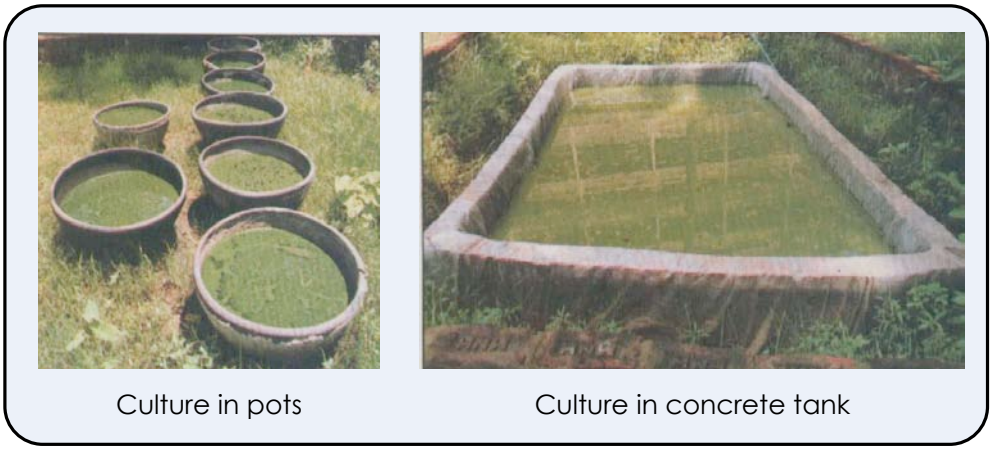
Spirulina indeed lends itself to simple technology: cultivation may be carried out in unlined ditches through which flow is low (e.g. 10 cm/second). Stirring may be provided by a simple device driven by wind energy or harnessed to humans. Harvesting mat be readily performed using some suitable cloth, and

the biomass dehydrates in the sun. The quality of the spirulina product obtained in this fashion would not be as high as what is attained in "clean cultures", but product could serve well as animal feed.

The system proposed by Fox (1985) integrating village-level waste management, biogas generation, spirulina production; composting and fish culture was designed for developing countries. A digester processes sewage and other wastes, producing biogas for running community cooking facilities and a liquid effluent that is treated in a solar heater and then used to fertilize the algal ponds. The algal biomass is harvested with a woven cloth and dewatered in a solar drier. The system is designed to provide supplementary amounts of spirulina – several grams per person each day – to the village inhabitants. Problems regarding technology transfer, public health hazards and obstacles to recycling waste hindered progress. A somewhat simpler approach was provided by other researchers.

In Bangladesh, spirulina was produced through a pilot project using paddle-wheel under transparent shade in the campus of BCSIR (Bangladesh Council for Scientific and Industrial Research) in 1980s. Later BCSIR established a system for the rural culture of spirulina.

Figure 4: Forms of small-scale spirulina culture in Bangladesh (photo courtesy: F. Majid)



In India, the Murugappa Chettiar Research Centre in Chennai has developed the technology and this has been successfully propagated on a large scale in the rural areas of Pudukottai district of Tamil Nadu. For instance, "mud pot" spirulina production uses a medium consisting of a biogas slurry, 2–3 g of sea salt or chemical medium (potassium dihydrogen phosphate, cooking soda and sodium chloride) and pure spirulina culture. The mud pots are buried up to their necks in the ground, filled with water mixed with the medium and a small quantity of pure spirulina added. The medium has to be stirred 3 to 4 times a day as the spirulina cannot grow in stagnant conditions. The pots have to be exposed to sunlight as the spirulina takes 3 to 4 days to mature. The mature spirulina (when the pale medium turns into dark green) can be harvested by a simple cloth-filtration. After washing the spirulina in fresh water (to remove the adhering chemicals), it can be directly mixed with chapatti dough, chutneys, noodles, pulses, vegetables etc. Spirulina can also be preserved by drying, which must be conducted immediately in order to preserve its quality and value.

The major cost involved in such small-scale production is the growth medium. Raoof, Kaushika and Prasanna (2006) 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^2/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. Cost calculations indicate that the preparation of 1 000 litres of SM (Zarrouk's medium) would cost Rs. 3 659 (US$79.5) as against Rs. 736 (US$16.0) for revised medium (RM6). Therefore, the merits of the revised medium are clearly emphasized, not only as a low-cost

alternative but also as a highly productive input, which can be used profitably by the rural population for large-scale biomass production of protein-rich spirulina.

4.3.1 Production of spirulina in organic nutrients including waste effluents

One of the main barriers to the small-scale culture of spirulina is the cost and availability of inorganic nutrients. However, there is the alternative of using organic nutrient sources, especially from waste effluents, that may be available in rural locations (Laliberte, Olguín and de la NoÜe, 1997).

Waste effluent from a fertilizer company in Nigeria was used for cultivation of *Chlorella* and *Spirulina* (Anaga and Abu, 1996). The fertilizer factory waste on an average contained phosphate-P (107–187 ppm), nitrate-N (3.0–4.0 ppm), sulfate SO_4^{-2} (146–150 ppm), had a pH of 7.4–8.5 and electric conductivity of 700–2457 μmhos/cm. This physico-chemical status of fertilizer factory waste is suitable for the growth of *Chlorella* and *Spirulina*. Approximately, 11.0 percent (w/w dry matter) as N of spirulina was obtained when cultured in 50:50 mixture of effluent and filtered sea water (pH 8.30) after 21 days. Anaga and Abu concluded that this non-sewage effluent could be used for the production of microalgal biomass and value-added biochemicals.

The response of *Spirulina maxima* growth to different concentrations of aeration stabilized swine waste was compared as a way to determine the treatment efficiency of suspension and immobilized systems (Canizares *et al*., 1993). They found that the best results were obtained with the suspended growth for biomass concentration and nitrogen removal with aeration at a dilution of 50 percent swine waste. In the immobilized system, at dilutions of 25 and 50 percent swine waste, more than 90 percent ammonium nitrogen removal was obtained. Canizares and Dominquez (1993) studied the ability of *Spirulina maxima* to grow on aeration-stabilized swine waste, nutrient removal (total P, Phosphate-P and ammonium-N) and biomass production. The best results for biomass production (36 percent protein, 6 percent lipid and 0.02 percent crude fiber) as well as nutrient removal were obtained using 50 percent dilution of the waste.

Rice husk ash (RHA) and $NaHCO_3$ were used as a source of carbon in *Spirulina* culture (Akter *et al*., 1996). 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 grounds.

The fermented wastewater of Thai rice noodle factories has the potentiality as the source of nutrients for cultivation of *Spirulina platensis* (Vetayasuporn, 2004). 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 *S. platensis* where the growth of this microalgae was very favourable. The chlorophyll (2.36 mg/g), biomass (1000 mg/litre), crude protein (59 percent) and phycocyanin (14 percent) were detected which was almost similar to those of spirulina when cultured in Zarrouk medium.

Lagoon water can be used with some nutrient supplementation to grow *Spirulina platensis* (Costa, Colla and Filho, 2004). They used Mangueira lagoon (Rio Grande do Sul State, Brazil) water rich with carbonates and a high pH, and in addition of 1.125 or 2.250 mg/litre of urea and 21 or 42 mg/litre of $NaHCO_3$ during fed-batch culture of *S. platensis*, respectively using a 32 factorial design. They found that lagoon water in addition of 1.125 mg/litre of urea resulted in a 2.67 fold increase in the final biomass of spirulina.

Rijn and Shilo (1986) conducted an experiment on nitrogen limitation in natural populations of cyanobacteria (*Spirulina* spp. and *Oscillatoria* spp.) in Israeli fish ponds in summer. They found that carbohydrates synthesized at the lighted surface partially utilized for protein synthesis at the bottom of these ponds when cells labeled by ^{14}C under simulated pond conditions.

4.4 Commercial and mass cultivation

The main commercial large-scale culture of microalgae started in the early 1960s in Japan with the culture of *Chlorella*, followed by *Spirulina* in the early 1970s at Lake Texcoco, Mexico. Spirulina is produced in at least 22 countries: Benin, Brazil, Burkina Faso, Chad, Chile, China, Costa Rica, Côte d'Ivoire, Cuba, Ecuador, France, India, Madagascar, Mexico, Myanmar, Peru, Israel, Spain, Thailand, Togo, United States of America and Viet Nam. Shimamatsu (2004) reports that the total industrial production of spirulina is about 3 000 tonnes a year. FAO FishStat data (FAO, 2006) suggests this is a substantial underestimate – although the current dataset (up to and including 2004) only shows production in China, it indicates a production of 19 080 tonnes in 2003 rising sharply to 41 570 in 2004, worth around US$7.6 millions and

US$16.6 millions, respectively. China started to produce spirulina through factories in 1990 and there were more than 80 factories by 1997 (Li and Qi, 1997). Other countries with significant spirulina production not accounted for in the FishStat reports include the United States of America (California and Hawaii), Taiwan Province of China and Thailand (see Section 4.5).

Mass cultivation of spirulina is usually carried out in shallow ponds, equipped with paddle wheels to mix the culture. Two types of open raceway ponds are typically used: the first is lined by concrete and is therefore expensive, the second is a shallow earthen tunnel lined with polyvinyl-chloride (PVC) or some other durable plastic material. Lining of the raceways increases the cost of production of algal biomass, hence the search for cheaper material and processes, such as low-cost clay sealing (Vonshak and Richmond, 1988). The surface of commercial raceways varies from 0.1 to 0.5 hectares and culture depth is usually maintained at 15–18 cm. The paddle wheel, large (with a diameter up to 2.0 m and a speed of 10 rpm) or small (with a diameter of 0.7 m and a speed 2 to 3 times faster than 2.0 m diameter paddle wheel), is the most common stirring device. One difficulty of this paddle stirring is that the flow is not sufficiently turbulent to produce an optimum light pattern for single-cell algae. Thus, other means were used to increase turbulence in shallow ponds or raceways, and consequently photosynthetic efficiency (Vonshak and Richmond, 1988). The following section looks at the main variables involved in production.

Oxygen concentrations: Assuming that the rate of photosynthesis can be used as an indication of the metabolic activity of outdoor algal cultures, the day-time changes in oxygen concentration in the pond are correlated with diurnal changes in light and temperature. In summer, the main limiting factor for growth of spirulina in outdoor culture is light; the daily peak in oxygen concentration is reached at the same time as light intensity is maximum. In winter, the main limiting factor is temperature because of a shift in the peak of oxygen which follows the peak in the pond temperature rather than light intensity (Vonshak, 1997).

Light: When growing algae at a depth of 12–15 cm in open raceway ponds, self-shading governs the light availability to the single cell in the culture. Unless one uses a much diluted culture which allows penetration of light throughout the water column, a certain part of the culture will always fail to receive enough light to fulfill photosynthesis needs. Thus, almost by definition this kind of culture will be light-limited. It was demonstrated in the early 1980s that augmenting cell concentration of the culture which increases self-shading resulted in a decrease of the growth rate. This kind of experiment was carried out during the summer, winter and spring, and findings indicated that the highest response of growth rate to cell concentration, i.e. self-shading, was observed in the summer. The initial interpretation was that in summer temperatures were high enough, so that main limitation for growth of spirulina outdoors was light. In winter and spring, however, when the temperature in the outdoor cultures was lower, the effect of self-shading was less pronounced (Vonshak, 1997).

In 1996, at the Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, *Spirulina platensis* was grown in a normal Zarrouk medium and the effect of various environmental factors on the β-carotene content evaluated although the growth of the cells was retarded at pH 10.5 and 11.0. A temperature higher than 35 ºC caused a drop in β-carotene content. Increased light intensity up to 10 000 lux resulted in an increased β-carotene content. When the cells were grown under red light illumination, β-carotene content was highest followed by that under blue light and white light, respectively. Nitrate deficiency in the growth medium did not appear to cause an increase in β-carotene content. It was also found that β-carotene content was increased with increasing NaCl in the medium. However, a drastic rise in β-carotene content could be obtained by either growing the cells initially in the medium containing 10 g/litre NaCl and then transferring the cells to the new medium with 30 g/litre NaCl or initially acclimation the cells in the medium with 40 g/litre NaCl and then transferring them to the new medium containing the same content of NaCl (40 g/litre).

Growing *Spirulina maxima* in polyethylene tubes in Italy helped to extend the growing and production season during the winter but caused overheating in summer. Torzillo, Sacchi and Materassi (1991) showed that the productivity and biochemical composition of *Spirulina platensis* can be influenced by biomass synthesis in the culture grown at the optimum temperature of 35 ºC, during the daylight period, which was superior by 14 percent to that in the culture grown at 25 ºC. It was also observed that biomass losses due to night respiration were significantly higher in the culture grown at 25 ºC (6.8 percent of the dry biomass on average) than in the culture grown at 35 ºC (4.4 percent of the dry biomass on average) so that more pronounced differences appeared between the two cultures in the net productivity

(26.6 percent on average). The photosynthetic activity of the culture grown at 25 ºC was directed more towards carbohydrate synthesis than toward protein synthesis. On average, in the morning, protein and carbohydrates represented 67.5 and 18.7 percent of dry biomass, respectively; in the evening, these values changed to 59.9 and 26.3 percent. *Vice versa*, in the culture grown at 35 ºC, biomass composition was scarcely modified throughout the day. Night respiration led to higher carbohydrate losses in the culture grown at 25 ºC; the amount of carbohydrates lost during the night was slightly higher than the biomass losses; the difference was due to a significant protein synthesis during the night.

Photoinhibition: Vonshak and Guy (1988) were the first to describe the phenomenon of photo-inhibition in outdoor-grown spirulina cultures. By following the *in-situ* photosynthetic activity of outdoor cultures grown at full solar radiation or under shaded conditions, they observed that shading could increase photosynthetic activity. It was also observed that shading resulted in an increase in productivity. When the pond was shaded to reduce light intensity by 25 percent, the degree of inhibition was also significantly reduced. It is worth noting that once solar radiation lowers in the afternoon, recovery in photosystem-II activity occurs. Light availability in outdoor culture is highly dependent on cell concentration (Vonshak, 1997). After 15 years of study related to the role of light in productivity of outdoor algal cultures, spirulina, in particular, Israeli researchers reached a better understanding of the complicated light environment to which algal cells are exposed. It was demonstrated that due to extreme shifts in the level of light intensity, at least in spirulina cultures, photoinhibition may take place. The fact that photoinhibited spirulina cultures have a lower photosynthetic efficiency means that they require more light to reach the same level of activity as non-photoinhibited cells, thus, making photo-inhibited cultures actually light-limited. This finally leads to what may be seen as the paradox of light in outdoor spirulina cultures: during a significant part of the day, the outdoor cultures are photoinhibited and light-limited at the same time (Vonshak, 1997). Vonshak, Torzillo and Tomaselli (1994) demonstrated that fluorescence measurements can be used as a fast reliable indication for photoinhibition in outdoor cultures of *Spirulina platensis*.

Temperature: In many regions of the world, temperature may represent the main limitation for high-biomass production rates in outdoor open ponds of spirulina cultures. An outdoor algal culture undergoes a diurnal cycle which in areas out of the tropics may show a difference of 20 ºC. In the morning, the pond temperature may only be in the range of 15–20 ºC, an optimal temperature, in the range of 35–38 ºC is reached only in the early afternoon. Even in the tropics where the culture reaches the optimal temperature, during a significant part of the day the temperature will still be much below the optimum. It has been demonstrated that relatively high temperatures at night could increase respiration rate which may result from the phenomenon described as night loss of biomass. The degree of loss varies as a function of the biomass composition and may reach values of 30 percent of the previous daily productivity (Vonshak, 1997).

Contamination: Contamination by different algal species may present a severe problem for microalgal cultures grown in outdoor open ponds. In most cases, the steps that proved effective in prevention of *Chlorella* contamination were maintaining a high bicarbonate concentration (e.g. 0.2 M), taking precautions to maintain the dissolved organic load in the culture medium as low as possible, and increasing winter temperature by greenhouse heating. Development of grazers in the culture, mainly the amoebae type, was prevented by the addition of ammonia (2mM) (Vonshak and Richmond, 1988). Experience indicates that contaminating organisms do not present a serious difficulty as long as good growth is maintained in a monoalgal culture. It is worth noting that no cyanophages attacking spirulina have been observed so far (Vonshak, 1997).

Culture in seawater: The first successful culture of spirulina (*Spirulina maxima*) using untreated seawater in laboratory condition was reported in Italy in 1984 by Materassi, Tredici and Balloni (1984). The culture technique developed in the laboratory has been successfully applied to outdoor mass culture of *S. maxima*. The climatic condition is very suitable for spirulina culture especially in south of Italy throughout the year (Tredici, Papuzzo and Tomaselli, 1986). The mean annual yield of biomass on sea-water plus urea was 7.35 g (dry weight)/m^2/day, which was slightly lower value than that obtained on the standard sodium bicarbonate medium with sea-water (8.14 g/m^2/day) under controlled pH, ranged from 8.0 to 8.3.

Production systems: In the United States of America, China, India, Thailand, Viet Nam and Taiwan Province of China, two types of open raceway ponds are used; the first, which is more capital-intensive, is lined by concrete (Thailand, India); the second is a shallow earthen tunnel lined with PVC or some other durable plastic. The cost and durability of the lining significantly influences the capital costs and thus the

economic feasibility of this biotechnology. Any durable liner will add up to US$0.5 to the cost of production of each kg of algal biomass produced, demonstrating the need for cheaper lining such as low-cost clay sealing. Such lining has to be tested for durability under turbulent flow and periodic cleaning of the pond (Vonshak, 1997).

Mixing devices: Although the paddle wheel is the most common stirring device for spirulina in commercial plants, other mixing devices are being tested. One difficulty with paddle stirring lies in the nature of the flow. It is usually not sufficiently turbulent to affect an optimal light regime for the single cell. When introducing into the raceway array of foils, a design similar to airplane wing, which affected systematic mixing through the vortices created by the foils, a more than two-fold increase in photosynthetic efficiency was reported. Another device of mixing shallow algal ponds consists of a board which closes the pond cross-section, except for silt above the bottom of the pond. The board is moved back and forth, creating a turbulent back whirl as the culture is forced through the silt. This method of inducing turbulence in shallow raceways had not yet been scaled up, and a comprehensive evaluation of the system has yet to be carried out (Vonshak, 1997).

Monitoring: Gitelson *et al*. (1995) of the Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev in Israel, have studied the optical properties of dense algal cultures outdoors and their application to the remote estimation of biomass and pigment concentration in *Spirulina platensis*. They have investigated the spectral properties of the reflectance and vertical attenuation coefficient of high-density productive algal ponds in the visible and near infrared region of the spectrum in as wide a range of biomass and pigment concentrations as possible. The objective of that research was to create the indices sensitive to pigment and biomass concentration which may serve as indicators for the physiological state of outdoor algal cultures. The Israeli researchers' findings may serve as a basis for remote real-time monitoring of phytoplankton quality in high-density productive algal ponds.

4.5 Examples of production around the world

Commercial production of spirulina takes two broad approaches. That of industrialized countries who are interested in producing the blue-green alga for the natural food and health food market, as well as for the extraction of high-value biochemicals. And that of developing countries which are in search of a rich source of protein, produced under local conditions and using marginal land and saline water not suitable for agriculture, as well as the opportunity for treating animal and human waste.

Figure 5: A standard design for industrial production of spirulina

Source: Sun Chlorella Corporation, Shimogyo-ku, Kyoto, Japan

The United States of America has a number of the largest intensive farms in the world, mainly based in Hawaii and California. Earthrise has a large 43 hectares (108 acres) site located in the Sonoran Desert of south-eastern California utilizing water from the mineral-rich Colorado river (see figure below) that produced some 450 tonnes in 2002. Cyanotech cultures 350 tonnes/annum of *Spirulina pacifica* (specially bred strain of the *Spirulina platensis*) in 32 hectares (80 acres) of shallow, open ponds (approximately 20 cm deep) adjacent to the Pacific Ocean. These use a combination of fresh water and supplemental deep ocean water to fill the ponds. The main inputs are sodium bicarbonate and carbon dioxide, as well as inorganic fertilizers.

Figure 6: Intensive production of spirulina facilities in the United States of America

Source: www.Earthrise.com and cyanotech.com

In Thailand an integrated system makes use of effluent from a starch production factory using cassava, at the King Mongkut's Institute of Technology Thonburi (KMITT). In this plant, a 160-cubic meter digester is operated for producing biogas with 4 x 200 m^2 algal ponds that produces *Spirulina* biomass which is tested for nutritional value. The system has been scaled up to 14 ponds 1 000 m^2 each and an annual production of 30–50 tonnes. In other work carried out at the Thai National Inland Fisheries Institute *Spirulina* strains, mainly from the north-eastern part of Thailand, were isolated. Those strains capable of growing in brackish water were mixed with cassava or fishmeal and served as a locally-produced protein source for fish feed (Vonshak, 1997).

In China, spirulina culture has been sponsored by the State Science and Technology Commission since 1987. There are four main geographic areas of production: (i) a plateau alkaline salt lake farm, Chenhai Lake in Yunnan, the largest grower; (ii) the southern coastal outdoor farms in Guandong, Hainan, Fujian, Jiangshu (the DIC farm on Hainan Island may be the largest, with a capacity of 300 tonnes/year); (iii) inland semi-closed systems in Hubei and Shandong and (iv) high latitude saline-alkaline water farms in the Yellow River Valley and Hubei.

In the middle of the Atacama Desert in Chile, the "Solarium Appropriate Biotechnology Group for Desert Development" has developed a culture and processing system for producing spirulina. Spirulina is cultured in polyvinyl chloride-lined raceway ponds with agitation by a paddle-wheel giving a flow rate of 20–25 cm/second. Ponds are covered with 0.15 mm-thick translucent UV-resistant polyethylene film. Such protection maintains adequate temperatures in the culture medium most of the year resulting in an optimal growth of spirulina. Simple and easily-operated harvested gravity filtration systems allow to filtrate 15–20 m^3/hour and obtain a pre-concentrated biomass with about 5 percent total solids. Production reached nearly 3 tonnes of dry spirulina per year from 2.4 hectares (6 acres) of intensive ponds (Sánchez *et al*, undated). Part of that production is commercialized in bulk powder and encapsulated in gelatin capsules for the local and national health-food market, as well as for export to neighbouring countries and Europe. Another part of the production is delivered to humanitarian agencies which use spirulina as a nutritional supplement for children in Africa and Latin America.

Spirulina can also be cultured in cooler climates – in 1967 an experimental centre for mass cultivation of microalgae was founded in Roupite, Bulgaria (41° Northern latitude). The total cultivation area is 2 600 m^2 and the cultivation process has been optimized through the:

- isolation and culture of highly productive strains of the species;
- creation of an appropriate culture medium with a minimized, but not limiting, salt concentration, allowing for a long cultivation period without changing the medium, and use of cheap mineral fertilizers; and
- adaptation of the cell density to daily and seasonal changes in temperature and irradiance conditions.

An average daily productivity of 25 ± 3 g *Scenedesmus*/m^2 for the cultivation season has been achieved, whereas for spirulina the average daily productivity in experimental plots of 7 m^2 is about 18 ± 4 g/m^2. As

the cost of nutrients is a major component of the final production cost of spirulina biomass (especially carbon nutrients which account for 15–25 percent of total operation costs), attempts were made to grow spirulina in a medium made from wastes, bone meal and effluents from biogas digesters.

4.6 Harvesting and processing

Spirulina harvesting, processing and packing has eight principle stages:

1. Filtration and cleaning (see also Box 1 below): A nylon filter at the entrance of the water pond is needed;
2. Pre-concentration: To obtain algal biomass which is washed to reduce salts content;
3. Concentration: To remove the highest possible amount of interstitial water (located among the filaments);
4. Neutralization: To neutralize the biomass with the addition of acid solution;
5. Disintegration: To break down trichomes by a grinder;
6. Dehydration by spray-drying: This operation has great economic importance since it involves about 20–30 percent of the production cost;
7. Packing: It is usually in sealed plastic bags to avoid hygroscopic action on the dry spirulina; and
8. Storage: Stored in fresh, dry, unlit, pest-free and hygienic storerooms to prevent spirulina pigments from deteriorating (Ayala, 1998).

Box 1: Filtration of spirulina production

In all commercial production processes, filtration devices used for harvesting are basically two types of screens: inclining or vibrating. Inclined screens are 380–500 mesh with a filtration area of 2–4 m^2/unit and are capable of harvesting 10–18 m^3 of spirulina culture per hour. Biomass removal efficiency is high, up to 95 percent and two consecutive units are used for harvesting up to 20 m^3/hour from which slurry (8–10 percent of biomass) is produced. Vibrating screens can be arranged in double or triple decks of screens up to 183 cm in diameter. Vibrating screens filter the same volume per unit time as the inclining screens, but require only one-third of the area. Their harvesting efficiencies are often very high. At one commercial site a combination of an inclining filter and a vibrating screen has been used. In the process of pumping the algal culture to be filtrated, the filaments of spirulina may become physically damaged, and repeated harvesting leads to an increasingly enriched culture with unicellular microalgae or short filaments of spirulina that pass through the screen readily (Vonshak and Richmond, 1988; Vonshak, 1997). The slurry (8–10 percent of dry biomass) obtained after filtration is further concentrated by filtration using vacuum tables or vacuum belts, depending on the production capacity.

Quality control for food-standard spirulina includes microbiological standard tests, chemical composition test, and test for heavy metals, pesticides and extraneous materials (insect fragments, rodent hair and feather fragments) (Belay *et al.*, 1993).

5 PRODUCTS, USES AND BENEFITS FOR HUMANS, FISH AND OTHER ANIMALS

5.1 Spirulina and its use by humans

Clinical trials have shown that spirulina can serve as a supplementary cure for many diseases. Spirulina capsules have proved effective in lowering blood lipid level, and in decreasing white blood corpuscles after radiotherapy and chemotherapy (Ruan, Long and Guo, 1988; Ruan, Guo and Shu, 1990), as well as improving immunological function. Spirulina also is used for health food, feed and for the biochemical products since 1980s (Becker, 1988; Borowitzka, 1988; Richmond, 1988).

Immune system enhancement: The Academy of Chinese Military Medical Sciences showed that spirulina could effectively improve the survival rate of mice after exposure to a lethal dose of radiation, prolong their survival time, and improve their immunity and activity of superoxide dismutase (SOD). Some hospitals in Kunming city, Yunan Province, have adopted spirulina as an auxiliary medicine, which proved to be effective in lowering blood lipid, combating fatigue and increasing the level of immunoglobulin A (IgA) and immunoglobulin M (IgM). Phycocyanin of *Spirulina platensis* inhibits the growth of human leukemia K562 cells when supplemented with diet (Liu *et al*., 2000).

Nutritional supplement: Spirulina is rich in high quality protein, vitamins, minerals and many biologically active substances (Becker, 1994). Its cell wall consists of polysaccharide which has a digestibility of 86 percent, and could be easily absorbed by the human body. There are different categories of spirulina food where pills and capsules made from dry spirulina are important. The Wuhan Botanical Institute, China has collaborated investigating the effect of oral intake of spirulina pill on the physical status of athletes (Li, 1995). The results showed that female athletes showing an increase in their haemochrome level, whereas the male athletes did not show any apparent increase after taking 10 g spirulina pills per day for four weeks. The lung capacity of juvenile weight-lifting and *Jujutsu* athletes was improved. The spirulina pill had no effect on blood pressure.

In Viet Nam, first culture of spirulina was conducted in 1980s (Nguyen *et al*., 1980). Mass culture of spirulina was started in 1990s (Kim, 1990). *Spirulina platensis* powder is used as a health food tablet under the brand name "Linavina" and "Pirulamin" in Viet Nam. Another canned product named as "Lactogil" is used to enhance milk secretion in mothers showing a decrease in lactation. Good results have been obtained by treating children suffering from serious malnutrition diseases with spirulina powder at Thuanhai Hospital, Viet Nam.

Spirulina contains high concentrations of essential PUFAs: *Spirulina platensis* contains up to 2 percent of dry biomass of γ-linolenic acid (GLA), which is synthesized through direct desaturation of linoleic acid. Numerous studies showed that GLA and subsequent PGE1 deficiency may figure in many degenerative diseases. The few known sources of GLA include human milk and spirulina, and oil extracts of the evening primrose plant, blackcurrant and borage seeds; 10 g of spirulina provide over 100 mg of GLA (more than two capsules of evening primrose oil) which can help cure arthritis, heart disease, obesity and zinc deficiency (Henrikson, 1989).

Furthermore, a study conducted by the Department of Internal Medicine of Tokai University, Japan, on 30 male employees with high cholesterol, mild hypertension and hyperlipidemia concluded that spirulina lowered serum cholesterol, triglyceride and low-density lipoprotein particles (LDL). Group A consumed 4.20 g of spirulina daily for eight weeks, and total cholesterol dropped within four weeks from 244 to 233 mg. Group B consumed spirulina for four weeks, then stopped; serum cholesterol decreased, but then returned to the initial level. The Japanese clinicians concluded that a diet enriched with spirulina could alleviate disease since the atherosclerosis index improved. Researchers in Germany had previously discovered that cholesterol reduction occurred during a weight loss study with spirulina. The Japanese research team showed lower cholesterol without weight loss. Spirulina was tested because it previously lowered serum cholesterol; reduction may be partially due to the action of γ-linolenic acid (Henrikson, 1989).

Because spirulina is well known to have a very high iron content, it was tested against a typical iron supplement, iron sulfate. Spirulina-fed rats absorbed 60 percent more iron that rats fed the iron supplement. This study suggested that there is a highly assimilable form of iron in spirulina. An earlier study also showed that it was effective in correcting anaemia in rats. Another study was conducted in Japan with eight young women, who had been limiting their meals to stay thin, and showed hypochronic

anaemia; they took 4 g of spirulina each after meal and in 30 days, blood hemoglobin content increased from 10.9 to 13.2 ($\pm$21 percent), a satisfactory level no longer considered anaemic (Henrikson, 1989). Spirulina significantly reduced the blood glucose level of both male and female aged between 40 to 60 years. An experiment was conducted on 20 males and 20 females in Coimbatore, India, and found significant reduction in blood glucose levels due to supplementation in diet with spirulina (Anuradha and Vidhya, 2001).

Food source: when the algal cells or filaments of spirulina are transformed into powder it can provide the basis for a variety of food products, such as soups, sauces, pasta, snack foods, instant drinks and other recipes. Attempts have been made by Proteus, a marketing company mainly associated with Earthrise Farms in the United States of America, to incorporate spirulina into a variety of food products such as granola bars and various kinds of pasta (Vonshak, 1990). Spirulina powder is also an ingredient of an orange-flavoured chewable wafer and other types of candy, of protein flours (10 percent spirulina added to soybean or to milk-egg powders), and of Pastalina, a green soy-whole wheat noodle. The preparation of fermented foods such as cheese, yogurt and tofu, offered many new possibilities to the use of spirulina. Furthermore, extraction methods could provide a decoloured spirulina powder (yellow-white) which is odourless and tasteless, and thus suitable for widespread use.

Spirulina is also used to prepare food with other ingredients. For example, instant noodles, stylish noodles, nutritious blocks, beverages and cookies. The first three food items are recommended as luncheon food for middle-grade students. Diets in which spirulina provides up to 100 percent of the protein produced growth rates comparable with those obtained with standard diets in several animal species. As little as 10 g a day of spirulina brings rapid recovery from malnutrition, especially in infants. This was achieved in Mexico for undernourished children and adults. In Togo, rapid recovery of malnourished infants was reported in a remote village health clinic; children were given 10 to 15 g/day as a dietary supplement mixed with millet, water and spices, and they recovered in several weeks. In China, spirulina was prescribed at Nanjing Children's Hospital as part of a "baby nourishing formula" with baked barley sprouts: 27 out of 30 children aged 2 to 5 years recovered in a short period from bad appetite, night sweat, diarrhea and constipation (Henrikson, 1989).

Japan, the United States of America and the European countries have been the main importers of Mexican spirulina powder. Sosa-Texcoco Ltd. manufactured lozenges and capsules from such powder and added to it vitamins A and C, following the regulations of the importing countries. Crude powder was also exported to the United States of America. In Mexico, Sosa-Texcoco Ltd has marketed lozenges and capsules similar to those being exported; they were sold almost exclusively in dietetic shops.

5.2 Food safety aspects related to human consumption of spirulina

Since many of the existing blue-green algae species are known to produce toxin (microcystins, in particular MCYST-LR), it is very important to clarify the specific species used for human consumption as in all likelihood there is a danger of species substitution and/or contamination of spirulina with other cyanobacteria. It is particularly important in countries where no such regulation exists on this type of products.

The studies and the risk management decisions about blue-green algae products are relatively recent. Although there has been no conclusive evidence on the presence or absence of microcystins in spirulina, only products from *Arthrospira platensis* have so far been cleared for consumption (United States of America, Australia, Canada and probably EC), under specific conditions, by public health authorities. A Canadian study found that no microcystins was detected in blue-green algal products containing only spirulina (Health Canada, 1999), while a study conducted for the Oregon Department of Agriculture (ODA) published in 2000, found MCYST-LR in all the 15 spirulina samples (dietary supplements) analysed (Gilroy, Kauffman, Hall, Huang and Chu, 2000). Nevertheless, MCYST-LR content in spirulina samples were below the regulatory level established by the ODA for microcystins in blue-green algae products (1 μg/g). Spirulina has been recognized as GRAS (generally recognized as safe) under the "indented conditions of use" implying that it is "for use as an ingredient in foods, at levels ranging from 0.5 to 3.0 grams per serving". This means in relatively small amounts. Nevertheless, considering that safety level and possible hazards for consumption of spirulina and spirulina-related products have not been established beyond doubt, special precautionary measures would be necessary on the consumption of spirulina products to some segments of the population at risk to include pregnant women, nursing

mothers, and people in dialysis and immune-compromised. Additional references to related food safety aspects of spirulina are listed in Appendix A and readers may consult those for further information.

5.3 Spirulina and agriculture

5.3.1 Use as fertilizer

In 1981 the FAO documented the possibilities of blue-green algae replacing chemical fertilizers and rebuilding the structure of depleted soils (FAO, 1981). In India, blue-green algae are grown in shallow earthen ponds. When the water evaporates, the dried algae are scooped up and sold to rice farmers. This natural nitrogen source is only one-third the cost of chemical fertilizer and it increases annual rice yield in India by an average of 22 percent. Where chemical fertilizers are not used, algae give the same benefit as 25 to 30 kg of chemical nitrogen fertilizer per acre. Where chemicals are used, algae use allows the reduction of an equivalent amount of inorganic fertilizer. The use of spirulina-based fertilizers is impeded by the low cost, ready availability and preferred use of inorganic fertilizers.

Spirulina used in combination with other fertilizers gave good yield of tomato (Zeenat, Sharma and Rizvi, 1990). During the study, the N_2-fixing cyanobacterium, *Aulosira fertilissima*, the non N_2-fixing cyanobacterium, *Spirulina platensis*, and the chemical fertilizer, diammonium phosphate (DAP) were applied in various combinations to the tomato seedlings in pots four times at seven day interval. Highest plant fresh weight (290 g/plant), number of leaves (127/plant), number of flowers (29/plant), number of fruits (37/plant) and fresh weight of fruits (71 g/plant) were achieved with the application of 2.25 g *Aulosira* + 2.25 g *Spirulina* + 0.50 g DAP in each pot. This result represented a 522 percent increase in number of fruits and a 977 percent increase in yield over the control. Cyanobacteria and DAP did not show any significant increase in yield when applied alone. The use of biological nitrogen is more beneficial than inorganic nitrogen as, apart from supplying the much needed nitrogen, and they release carbon components and other nutrients, which enhance plant growth (Banerjee and Deb, 1996). Spirulina contains 10 percent N w/w (high percentage), and other macro- and micro-nutrients which are slowly released under normal soil conditions, and increases fertility.

5.3.2 Use as a protein supplement in poultry and livestock feeds

Fishmeal, groundnut meal and soybean meal can be partially replaced by spirulina in the preparation of diets of fish, poultry, cattle and domestic animals (Venkataraman, Somasekaran and Becker, 1994; El-Sayed, 1994; Britz, 1996). Fishmeal and groundnut cake in a commercial diet containing both protein sources may be replaced on an isonitrogenous basis with dried spirulina 140 and 170 g/kg (starter), and 120 and 128 g/kg (finisher) for broiler chicks (Venkataraman, Somasekaran and Becker, 1994). A vitamin and mineral supplement was not added to the two algal diets because spirulina is rich in these nutrients. All the growth parameters of chicks were similar fed diets with spirulina. Meat colour was not affected by diet except for a more intensely coloured meat in broilers fed on spirulina containing diets.

5.3.3 Use as a colourant in poultry, livestock and food products

The blue-green colour of spirulina is due to two pigments: phycocyanin (blue) and chlorophyll (green). These two pigments are combined with another group of pigments known as carotenoids (red, orange and yellow). This phycocyanin extracted from spirulina was first marketed in 1980 by the Dainippon Ink & Chemicals Inc. under the brand name "Lina Blue-A". This was mainly used as a food colourant, as an edible dye in ice creams and as a natural dye in the cosmetics industry. However, as the pigment was light sensitive, special care must be taken in protecting it from bleaching (Vonshak, 1990). The phycocyanin market is considered small, estimated at 6 tonnes/year. According to some estimates, 100 kg of dry spirulina biomass per month extracted from a pond area of 200 m^3 could give 60 kg of crude protein and 1–3 kg of phycocyanin. Following water extraction, as well as small amounts of enzymes and cytochromes; 1–2 kg of chlorophyll, 100–200 g of xanthophylls and 100–200 g of β-carotene, following solvents extraction (Olguín, 1986).

The redness of *pectoralis superficialis*, *profundus* and *sartorius* muscles of broiler chickens reaches maximum when fed 40 g spirulina/kg diet, while the yellowness of all fillets including the *semitendinosus* muscle increases in a sub-linear fashion with increased spirulina in the diet (Toyomizu *et al.*, 2001). The overall relationship between the yellowness and zeaxanthin content in the *pectoralis* muscle usually shows directly significant. Therefore, it is evident that dietary spirulina influences both the yellowness and redness of broiler flesh, and that the increments in yellowness with dietary spirulina content may possibly

be reflected in the common yellow pigment related to the accumulation of zeaxanthin within the flesh (Toyomizu *et al*., 2001).

5.4 Spirulina and aquaculture

5.4.1 Spirulina as a nutritional supplement

Spirulina can be used as a partial supplementation or complete replacement for protein in aquafeeds. It was found that the growth of silver seabream (*Rhabdosargus sarba*) fed on spirulina meal at up to 50 percent level was usually not different from, and feed conversion efficiency not superior to, those given control diets with solely fishmeal (El-Sayed, 1994). At 75 percent inclusion level, growth reduces significantly, but feed conversion efficiency still comparable to the control diet, which sharply reduces at 100 percent inclusion level. It is better to replace 50 percent fishmeal by spirulina meal so that there will be no adverse effect on growth.

In the shrimp farming industry many feed additives have been utilized, but *Spirulina* is the only microalgae additive which demonstrates benefits to growers that offset the initial cost and provide a significant cost/performance ratio. Spirulina was studied as a feed supplement for the giant freshwater prawn (*Macrobrachium rosenbergii*), and found to significantly improve growth, survival, and feed utilization. The supplementation range was 5–20 percent and results were similar at any of the ranges added to the feed (Nakagawa and Gomez-Diaz, 1975).

Spirulina is a cheaper feed ingredient than others of animal origin. China is using spirulina as a partial substitute of imported feed to promote the growth, immunity and viability of prawns (example *Penaeus monodon*). Spirulina-containing feed was found to reduce the cultivation time and mortality, and increase shell thickness of scallop. The survival rate of abalone (*Haliotis midae*) was improved by 37.4 percent. Feeding on spirulina helped to improve disease resistance of high value fish resulting in an improvement in their survival rate from 15 to 30 percent.

Abalone (*Haliotis midae*) shows good growth when fed a diet containing spirulina meal (Britz, 1996). Abalone shows a significantly higher growth when fed diets based on fishmeal and spirulina than that fed diets prepared with soybean meal, torula yeast, casein and dried *Ecklonia maxima*. Protein efficiency ratios of abalone fed formulated diets ranged from 3.3 for torula yeast to 6.5 for spirulina based diet. It is found that fishmeal and spirulina are the most suitable proteins for inclusion in practical diets for Abalone. The replacement of artificial diet for post-larvae of abalone, *Haliotis discus discus* (Reeve) using spirulina gives good growth performance (Stott, Tokeuchi and Koike, 2004). The metamorphosis rate of abalone post-larvae increases by using spirulina.

The reproductive performance of Nile tilapia (*Oreochromis niloticus*) was tested using freshly harvested *Spirulina platensis* in comparison with control parent fish and progeny fed on commercial diets (Lu and Takeuchi, 2004). There were no significant differences in the relative fecundity, spawning intervals and egg size among the various size groups. The fertilization rate and the hatching rate of the fertilized eggs, as well as the survival time of larvae from the parents fed the two types (spirulina and commercial diet) were similar. There was an increase in the synthesis of essential lipids when fed solely on spirulina. Significant differences were found in the fatty acid profile of fish eggs fed spirulina supplements containing more linoleic acid, γ-linolenic acid, eicosatrienoic acid, eicosatetraenoic acid, docosapentaenoic acid than those of fish fed solely on spirulina.

Mozambique tilapia (*Oreochromis mossambicus*) was cultivated in artificial ponds with relatively high stocking density and fed with a mixture of solar-dried spirulina that had been cultivated and processed using low-cost technology and added to groundnut cake. The resulting average food conversion ratio was lower than that observed using control fish fed with the usual fishmeal-based ration. Furthermore, the yield of tilapia fed on spirulina mixed with groundnut cake was 4–5 higher than that of fish fed on groundnut cake alone (Vonshak,1997).

5.4.2 Spirulina as a colourant

Carotenoids and carotenoproteins are responsible for the various colours of crustaceans (Britton *et al*., 1981). Astaxanthin has been shown to be the predominant carotenoid associated with the red body colour of the black tiger prawn *Penaeus monodon* (Howell and Matthews, 1991). Although animals do not have the biosynthetic pathways to synthesize carotenoids, certain crustacean and koi species are unique in that they have the ability to convert dietary β-carotene and zeaxanthin directly into astaxanthin.

Spirulina platensis strain *pacifica* contains the highest levels of β-carotene and zeaxanthin of any natural source, both of which are converted to astaxanthin through an oxidative process, the red pigment desired by consumers. The effectiveness of spirulina as a pigment for *P. monodon* is especially attributable to zeaxanthin, which can be converted into astaxanthin via 4-ketozeaxanthin. Analysis of carapace carotenoids of prawns that received the spirulina-supplemented diet revealed the absence of zeaxanthin, and only a small amount of 4-ketozeaxanthin was found. This finding suggests that dietary zeaxanthin is rapidly metabolized to astaxanthin in *P. monodon*. That may be the principal reason why spirulina is such an efficient pigmentation source. A marked increase in carotenoid content of the carapace of black tiger shrimp (*Penaeus monodon*) occurred when spirulina-supplemented diets were given. The maximum effect was found when the diet was supplemented with 3 percent spirulina (Liao *et al*., 1993). A practical strategy for the improved pigmentation of cultured *P. monodon* is the incorporation of 3 percent spirulina for one month before harvest.

5.5 Recent development and future outlook

5.5.1 Gene manipulation

Studies on the recipient system for transgenic manipulation in *Spirulina* (*Arthrospira*) *platensis* is now practised in different countries in the world, especially China (Gaoge *et al*., 2004). With a high protein content and growth rates, *S. platensis* is a potential host for foreign gene expression. To date a replicable and stable gene transfer system has not been established for *S. platensis* (Vonshak, 1997) and this has hindered foreign DNA introduction and influenced genetic modification in microalgae. As it is difficult to obtain axenic *S. platensis* strains; high endonuclease activity interfered with foreign gene behaviour in most species of the *Spirulina* (Vonshak, 1997); endogenous plasmids used for construction of shuttle vectors are not detected in *S. platensis*; and no regenerated spheroplasts and single cells are applied as transformation recipients.

A well-documented method for gene transfer in filamentous cyanobacteria is conjugation. No replicable transformation procedure for filamentous cyanobacteria has been developed, as there are for a number of strains of unicellular cyanobacteria (Porter, 1987; Shestakov and Reaston, 1987). Electroporation, which is well-known for its independence of the cell's ability to take up DNA, can be applied to different genera. It is a simple procedure with high transformation efficiency and has been successfully used to introduce DNA into unicellular cyanobacterium *Synechococcus* sp. (Matsunaga, Takeyama and Nakamura, 1990) and filamentous cyanobacterium *Anabaena* sp. M131 (Thiel and Poo, 1989). Through a recipient system for genetic transformation in spirulina with electroporation, it is recorded that single cells isolated by lysozyme-free method could regenerate with a rate of 28.6 percent. The electroporated cells are recoverable within seven days which might be confirmed by the ultrastructural observations for normal and electroporated cells.

During electroporation, the ultrastructure of electroporated single cells with short filaments exhibited thick cell wall, and a disappearance of thylakoids and phycibilisomes inside the cell. There was some breakage along the cross-wall. Fresh regeneration saw tooth sheath and thylakoids are observed in seven days. No breakage was observed on the cross-wall. Many gas vacuoles are found in the single cell with short filament recipient. This suggests that electroporation seemed to be a valid method for transferring foreign genes into to *S. platensis*.

Robinson *et al*. (1982) obtained spheroplasts by washing filaments in the solution of 1.0 mol/litre potassium chloride and 5 mmol/litre EDTA. Lanfaloni *et al*. (1989) used 1.5 mol/litre sodium chloride to obtain spheroplasts for regeneration. Their results showed that many single cells could be separated after rinsing in hypertonic solution. Single cells had a regeneration rate of 28.6 percent prepared by 0.75 mol/litre sodium chloride, and proved that partial or complete removal of the external sheath exposed under the highly concentrated sodium chloride solution condition was essential for the preparation of single cells. Compared to the normal cell, thylakoids and phycobilisomes disappeared, and a vague ultra-structure of thylakoid was observed in the electroporated cells. The density of the electroporated cells decreased due to the damage of thylakoids and phycobilisomes and even led to the destruction of chlorophyll a, phycocyanin, a-allophycocyanin and phycoerythrin. However, cell density increased with the normalization of thylakoids and phycobilisomes and no breakages appeared on the cross-walls after the regeneration.

5.5.2 Plant growth regulators

Plant growth regulators may be used to enhance spirulina growth. Among the plant growth regulators, the combination of 6-BA (6-benzyladenine) and NAA is suitable for the growth of *Spirulina platensis* A9 compared to other growth regulators and/or combination of other regulators (Shi *et al*., 2004). There are some other growth regulators such as GA (Gibberellic acid), IBA, 2,4-D and IAA used to enhance the growth of microplant like spirulina and other microalgae but not effective like the combination of 6-BA and NAA.

5.5.3 Strain development and improvement

The species diversity of spirulina is limited, with not more than 15 species (Tompkins *et al*., 1995; Phang and Chu, 1999; Bhattacharya and Shivaprakash, 2005). The available species are as follows:

a) *Spirulina platensis* (Gomont) (*Arthrospira fusiformis*) (Voronichin)
b) *S. platensis* NIES-39
c) *S. platensis* Geitler
d) *S. platensis* (Nordstedt) Geitler
e) S. subsalsa fo. *versicolor* (Cohn) Koster
f) S. subsalsa Oersted
g) S. maxima (as *S. geitleri*) (Setch. et Gardner)
h) S. subsalsa Oersted ex Gomont
i) S. major Kützing
j) Arthrospira fusiformis (Voronichin)
k) A. maxima
l) A. jenneri (Kützing) Stitz
m) S. labyrinthiformis
n) S. laxissima
o) S. lonar
p) S. nodosa
q) S. princeps West & West
r) S. laxa G.M. Smith

New strains of *Spirulina platensis* with improved nutritional quality and high essential micronutrient levels can be developed through replicable and stable gene transfer system (Torzillo, Pushparaj and Florenzano, 1985). This transfer system has not been successfully established since 1997 (Vonshak, 1997). A recipient system for genetic transformation in spirulina with electroporation, isolated single cells by lysozyme-free method was found to be regenerated with a rate of 28.6 percent (Gaoge *et al*., 2004). Ultrastructural observations for electroporated cells indicated that electroporated cells recovered in seven days. Electroporation is a valid transforming method for transgenic manipulation of foreign genes into *S. platensis*. Thus, strain development and improvement of *S. platensis* through transformation of foreign gene could be conducted using this method.

5.5.4 Expanding production to new countries

At present, spirulina production is restricted to either countries with a high demand for high value, processed products that allows commercially viable intensive production (i.e. the United States of America and China) or to a few countries that have specifically focused on small-scale production to supplement human diets or to integrate animal and fish production. It is suggested that there could be a considerable expansion of the latter category, especially in the following areas:

a) High altitude alkaline ponds or lakes

Spirulina can be grown in ponds or lakes at high altitudes if the water is sufficiently alkaline. If spirulina is not found in these habitats, then it can be imported from strains developed for similar environments. A note of caution should be made, in that a detailed investigation should be conducted before any new organism is introduced into a watershed where it is not naturally present in order to ensure that it will not have any adverse ecological impacts in the probable circumstance of its loss into external watercourses.

b) Coastal area with high temperature

Tropical coastal belt areas with a high ambient water temperature may be suitable for spirulina production. For instance, much of China's spirulina expansion has taken place in the southern China Sea coastal areas of Hainan Province, Guangdong Province and Shenzhen city.

c) Saline-alkaline water

When the groundwater is rich in calcium and other minerals, conventional crop production can be poor, especially in areas with high summer irradiation levels, the culture of low-temperature-resistant spirulina may be an option, especially for small-scale rural development. For example, groundwater of Huanghe river (Yellow river) valley, China and Guchen city in Hebei Province, China.

5.6 Potential use as a nutritional supplement in humanitarian emergencies

In the aftermath of humanitarian emergencies, it is important that the relief food supplies are culturally acceptable, rich in proteins and vitamins, digestible, mixable with cereals and able to be stored in ambient conditions over long periods. As demonstrated earlier in this report, spirulina can provide many of these attributes.

One major additional advantage of spirulina over most other foodstuffs is that it can often be grown *in situ* and therefore provide a longer-term solution to nutritional strategy development. The various small-scale production systems reviewed in Section 4 can be adapted to many post-emergency situations. Indeed many of the more vulnerable communities – e.g. coastal communities prone to storms, flooding and tsunami damage, as well as arid areas vulnerable to crop-failure and overgrazing show particular potential for small-scale spirulina production development.

This potential has been recognized by a number of organizations and international bodies. Antenna Technologies has developed a spirulina production system based on field trials, consisting of 4 m² growing basins. Shallow (20 cm) ponds are built using simple materials (plastic sheeting, planks, earth) using a fast-growing strain of spirulina. A 4 m² basin produces around 40 g of dry spirulina per day and requires 10 times less water than growing the equivalent weight of soybean.

In Viet Nam, a spirulina-producing centre distributed food to thousands of marasmic (suffering from severe protein deficiency) children each year, and several projects were under way in India, Senegal, Togo, Burkina Faso, Benin, Central African Republic, Brazil, Cuba and Thailand.

At the international level, the *Convention/Intergovernmental Institution for the Use of Food Microalgae Spirulina against Malnutrition* (CISRI-ISP, see Box 2) was established with the goal of diffusing the humanitarian use of spirulina and have emphasized how microalgal foods could be used to prevent malnutrition and alleviate extreme hunger during emergency humanitarian relief efforts. In November 2005, they submitted a draft resolution entitled the "use of spirulina to combat hunger and malnutrition to help achieve sustainable development" that would encourage Member States to take up the production and use of spirulina, emphasize the need for assistance in national activities for spirulina production and requested a report to the UN Economic and Social Council on efforts and progress in this regards. CISRI-ISP intends to build 10 spirulina-producing centres - five in Africa, two in Asia, two in Latin America and a Central Coordination Centre (Irini Sarlis, ECOSOC/6120, 1 July 2004).

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Spirulina appears to have considerable potential for development, especially as a small-scale crop for nutritional enhancement, livelihood development and environmental mitigation. In particular, the production and use of spirulina has the following advantages:

- it provides an easily digestible high (c. 60 percent) protein product with high levels of β-carotene, vitamin B_{12}, iron and trace minerals and the rare essential fatty acid γ-linolenic acid (GLA). In addition, it has no obvious negative cultural or religious issues associated with its consumption;
- its production occupies only a small environmental footprint, with considerable efficiencies in terms of water use, land occupation and energy consumption when compared to traditional terrestrial crops;
- its production is especially suitable to saline and alkaline conditions that are often unfavourable to traditional crops and are frequently occupied by disadvantaged people suffering from, or vulnerable to, natural disasters;
- its production can be conducted at a number of different scales, from household "pot culture" to intensive commercial development over large areas;
- it has the potential for integration with rural organic waste treatment processes to improve both environmental conditions and improve energy transfer efficiencies;

FAO fisheries statistics show a clear increase in production of spirulina over the recent years. For example, production in China was first recorded at 19 080 tonnes in 2003 and rose sharply to 41 570 tonnes in 2004, worth around US$7.6 millions and US$16.6 millions, respectively. However, there are no apparent figures for production in the rest of the world. This suggests that, despite the widespread publicity about spirulina and its benefits, it has not yet received the serious consideration it deserves as a potentially key crop in coastal and alkaline areas where traditional agriculture struggles, especially under the increasing influence of salination and water shortages.

An examination of the literature and research on spirulina also suggests that interest in its development has waned over recent years. Much of the work was conducted over the 1980s and 1990s with relatively little over the last decade. Most modern writing available on the internet appears to be "grey" literature, with relatively little internationally recognized scientific material. Given the increasingly widely recognized impacts of global climate change in terms of both desertification and the more prevalent extreme weather conditions that impact poor communities in particular, this loss of focus is regrettable.

6.2 Recommendations

There is a role for both national governments – as well as inter-governmental organizations – to re-evaluate the potential of spirulina to fulfill both their own food security needs as well as a tool for their overseas development and emergency response efforts. Other international organizations that have developed both an interest and capacity for developing small-scale spirulina production would be key potential facilitators in demonstrating available technologies for local uptake. They could also develop improved technical and economic solutions to spirulina production in environmentally impoverished conditions, as well as prepare tested production packages for rapid deployment in emergency situations.

International organization(s) working with spirulina should consider preparing a practical guide to small-scale spirulina production that could be used as a basis for extension and development methodologies. This small-scale production should be orientated towards:

(i) providing nutritional supplements for widespread use in rural and urban communities where the staple diet is poor or inadequate;

(ii) allowing diversification from traditional crops in cases where land or water resources are limited;

(iii) an integrated solution for waste water treatment, small-scale aquaculture production and other livestock feed supplication;

(iv) as a short- and medium-term solution to emergency situations where a sustainable supply of high protein/high vitamin foodstuffs is required. This implies the ability to rapidly install systems in a variety of environments that can be sustained by local communities to cover both the short-term food needs and to supplement longer-term nutritional requirements, especially once other forms of food relief cease to be delivered.

A second need is a better monitoring of global spirulina production and product flows. The current FishStat entry which only includes China is obviously inadequate and the reason why other countries are not included investigated. Furthermore, it would be beneficial if production was disaggregated into different scales of development, e.g. intensive, semi-intensive and extensive. This would allow a better understanding of the different participants involved and assist efforts to combine experience and knowledge for both the further development of spirulina production technologies and their replication in the field.

A third need is to develop clear guidelines on food safety aspects of spirulina so that human health risks can be managed during production and processing. Production of spirulina for human consumption should specifically take into account that there are potential risks of contamination of spirulina with toxin producing blue-green algae harvested from open pond or such water body, culture should be encouraged only in controlled ponds or in other media where single cell culture is achievable.

Finally, it would be useful to have some form of web-based resource that allows the compilation of scientifically robust information and statistics for public access. There are already a number of spirulina-related websites (e.g. www.spirulina.com, www.spirulinasource.com) – whilst useful resources, they lack the independent scientific credibility that is required.

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8 APPENDIX A: ADDITIONAL READINGS

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