24 R.J. Matales and S.R. Tannenbaum, ed., Single-Cell Protein. M.I.T. Press, Cambridge, MA, 1968.
25 J.K. Bhattacharjee, Appl. Microbiol. *13*, 139 (1970).
26 V.R. Young and N.S. Scrimshaw, in: Single-Cell Protein II. Ed. S.R. Tannenbaum and D.J.C. Wang. M.I.T. Press, Cambridge, MA 1975.
27 R. Moraine, G. Shelef, A. Meadan and A. Levin, Biotechnol. Bioengng, in press (1981).
28 a) H. Egan, EQS Environm. Qual. Safety *2*, 78 (1973); b) Pesticides residues and radioactive substances in food: a comparative study of the problems, Environm. Qual. Safety *3*, 17 (1974).
29 A. Ben-Amotz and M. Avron, in: Algae Biomass. Ed. G. Shelef, C.J. Soeder and M. Balaban. Elsevier/North-Holland, Amsterdam 1980.
30 J. Ramus, in: Biogenesis of Plant Cell Wall Polysaccharides. Ed. F. Loewus. Academic Press, New York 1973.
31 A.L. Demain, Biotechnol. Lett. *2*, 113 (1980).
32 H.B. Woodruff, Science *208*, 1225 (1980).
33 M. Aubert, J. Auber and M. Gauthier, in: Marine Algae in Pharmaceutical Science, Ed. H.A. Hoppe, T. Levring and Y. Tanake. Walter de Gruyter, Berlin 1979.
34 K.W. Glombitza, in: Marine Algae in Pharmaceutical Science. Ed. H.A. Hoppe, T. Levring and Y. Tanake. Walter de Gruyter, Berlin 1979.
35 G. Michanek, in: Marine Algae in Pharmaceutical Science. Ed. H.A. Hoppe, T. Levring and Y. Tanake. Walter de Gruyter, Berlin 1979.
36 Z. Dubinsky and S. Aaronson, Proc. IInd int. Workshop on Biosaline Research. Plenum Press, New York, in press.
37 J.A. Hellebust, in: Algal Physiology and Biochemistry. Ed. W.D.P. Stewart. Univ. of California Press, Berkeley 1974.
38 S. Aaronson, Chemical Communication in Microorganisms. CRC Press, Boca Raton, FL, in press.
39 O. Tsukada, T. Kawahara and S. Miyachi, in: Biological Solar Energy Conversion. Ed. A. Mitsui, S. Miyachi, A. San-Pietro and S. Tamura. Academic Press, New York 1977.
40 A.S. Watson, ed., Aquaculture and Algae Culture. Noyes Data Corp., Park Ridge, NJ 1979.
41 L. Polesco-Ianasesco, Acta bot. Horti bucur. 183 (1974).
42 A.M. Muzafarov, M.I. Mavlani and T.T. Taubaev, Mikrobiologiya *47*, 179 (1978).
43 W.A. Vincent, Symp. Soc. gen. Microbiol. *21*, 47 (1971).

Mass production of *Spirulina*

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Introduction

Among the lower plants, the blue-green alga *Spirulina* (fig. 1) has been the subject of a number of basic and applied investigations[1,2]. This alga can be harvested, processed and used for food. Attention has been directed to *Spirulina platensis,* which some tribes in the Lake Chad area have been eating since ancient times[3-5], as well as *Spirulina Geitleri* J. de Toni, which was consumed by the Aztecs that lived around Lake Texcoco, near Mexico City[6-9].

Production of Spirulina

Spirulina belongs to the family of Oscillatoriaceae and grows in alkaline waters in Africa, Asia, North and South America[10], in latitudes between 35°S and 35°N, areas of incident solar irradiation from 600 to 850 $KJ/cm^2 \cdot$ year and total insolation from 3000 to 4000 h/year[11]. Like other microorganisms, *Spirulina* has a higher specific growth rate than higher plants. It has been cultivated in a semicontinuous system and harvested continuously all year round. *Spirulina* as other cyanobacteria possesses the following properties:

a) a short life cycle, approximately 1 day under optimal laboratory conditions and 3-5 days under natural conditions, depending on season and meteorological conditions[12];

b) a high specific growth rate (0.3 d^{-1}) under optimal laboratory conditions[13-15], 0.2 d^{-1} in natural conditions during the summer[16,17] and 0.1 d^{-1} in winter[17,18];

c) growth in an aquatic medium which allows growth to a dense culture of algae biomass, consequently a good efficiency of solar energy conversion is obtained (3-4.5%)[19];

d) a high yield in good quality protein (28 ton/ha · year)[12];

e) the tendency to float and stick together thus facilitating the harvesting;

f) besides the high content of protein, substantial amounts of vitamins, carotenoids, minerals and moderate quantities of lipids and carbohydrates[19,20] can be isolated.

With current technology, 2 methods for cultivating *Spirulina* are known: the artificial culture and the seminatural culture. The 1st method, named syphogas, has been developed by the French Institute of Petroleum, which permits agitation, homogenization and supplementation with CO_2 as the carbon source through the injection of air enriched with carbon dioxide with diffusors[21]. This method has been tested on small plants with an area up to 1000 m^2 located in the Caribbean Martinique Island. It has been demonstrated that technical and economic problems limit industrial production[22].

A 2nd method, called seminatural, has been developed by the Mexican company Sosa Texcoco, SA, and consists of using the natural alkaline brines in raceway ponds supplemented with fertilizer to increase biomass production. This method has been very successful during the last 9 years, resulting in a production of approximately 3000 tons during THIS period[23,24].

Table 1. Chemical composition of *Spirulina* spray dried

Raw protein	70.0%
Essential aminoacids	
Isoleucine	4.13%
Leucine	5.80%
Lysine	4.00%
Methionine	2.17%
Phenylalanine	3.95%
Threonine	4.17%
Triptophan	1.13%
Valine	6.00%
Non essential aminoacids	
Alanine	5.82%
Arginine	5.98%
Aspartic Acid	6.43%
Cystine	0.67%
Glutamic Acid	8.94%
Histidine	1.08%
Proline	2.97%
Serine	3.18%
Available lysine	85%
Lipids	7.00%
Fatty acids	5.70%
Lauric	229 mg/kg
Myristic	644 mg/kg
Palmitic	21,141 mg/kg
Palmitoleic	2035 mg/kg
Heptadecanoic	142 mg/kg
Stearic	353 mg/kg
Oleic	3009 mg/kg
Linoleic	13,784 mg/kg
γ-Linolenic	11,970 mg/kg
α-Linolenic	427 mg/kg
Others	699 mg/kg
Ash	9.00%
Calcium	1,315 mg/kg
Phosphorus	8,942 mg/kg
Iron	580 mg/kg
Sodium	412 mg/kg
Chloride	4,400 mg/kg
Magnesium	1,915 mg/kg
Manganese	25 mg/kg
Zinc	39 mg/kg
Potassium	15,400 mg/kg
Others	57,000 mg/kg
Carotenoids	4,000 mg/kg
β-Carotene	1,700 mg/kg
Xanthophylls	1,600 mg/kg
Carbohydrates	16.50%
Ramnose	9.00%
Glucane	1.50%
Cyclitols	2.50%
Glucosamine and muramic acid	2.00%
Glycogen	0.50%
Sialic acid and others	0.50%
Nucleic acids	4.50%
Ribonucleic acid	3.50%
Deoxyribonucleic acid	1.00%
Vitamins	
Biotin	0.40 mg/kg
Cyanocobalamin	2.00 mg/kg
d-Ca-pantothenate	11.0 mg/kg
Folic acid	0.50 mg/kg
Inositol	350.00 mg/kg
Nicotinic acid	118.00 mg/kg
Pyridoxine	3.00 mg/kg
Riboflavine	40.00 mg/kg
Thiamine	55.00 mg/kg
Tocopherol	190.00 mg/kg

Large scale *Spirulina* culture requires all nutrients essential for life. The mineral content of *Spirulina* biomass and culture conditions of the alga are given in table 2.

The optimization of an algae production system draws problems such as species selection, nutrient selection, growth unit design and harvesting methods into a practical and economical overall design[23,24]. *Spirulina* has its filaments arranged in an elongated helix; this shape facilitates harvesting and permits filtration, e.g. by screens. It also allows the use of different depths of ponds and lower cell concentrations compatible with economical harvesting (see fig. 2). Zarrouk[13] and others[14,25,26] developed special nutrient solutions for the cultivation of *Spirulina*.

In the growth ponds, depth is usually less than 0.5 m for the algae production, with a baffle system and recirculation and mixing equipment. The mixing by flow is usually more practical and economical than the paddle agitator. The former also induces the water mass to circulate through the raceways at a speed of 0.03–0.06 m/sec, thus preventing thermic stratification. The maximal length of the channels depends upon the depth and the surface properties of these channels.

Processing and use of the harvested biomass

Spirulina is ruptured by homogenization or sonication of the cells[20,27,36]. The fluid is pasteurized to eliminate microbiological contamination and spray-dried to the final product.

So far, industrial plants have produced such a dry product from which its chemical composition, its nutritional value and its toxicity have been investigated. The material has been shown to have an exceptionally high content of vitamins, especially of the B group, E and H (Biotin) (see table 1). A total of 4.2–4.4% of nucleic acids[28], as well as of 6.2–7.0% of unsaturated lipids have been found. Fatty acids represent 83% of this fraction and the rest is made up of the insaponifiable fraction[29]. Among the pigments, chlorophyll is present in 0.8%, β-carotene in 0.23% and xanthophylls in 0.12–0.15%. Phycobillins amount up to 12–15%[30,31].

Table 2. Mineral content of *Spirulina* and culture conditions

Minerals (to be supplied in growth medium)	Dry weight (g/kg)	Growth conditions	
Carbon (from CO_2)	550	pH	9–11
Nitrogen (from NO_3)	100	Salinity	3–8%
Sulfur	30	Average temperature in ponds	7–30 °C
Potassium	30		
Phosphorus	16	Average sunlight	8–60 klx
Magnesium	2		
Calcium	1		
Micronutrients	–		

The product is nontoxic and has no side effects in animals or humans[32]. Nutritional tests have demonstrated that the *Spirulina* algae has a protein efficiency ratio (PER) of 2.2–2.6 (74–87% that of casein), a net protein utilization (NPU) of 53–61% (85–92% that of casein) and a digestibility of 83–84%.
In animal feeding experiments *Spirulina* protein is perfectly digested in weanling pigs[37] and in broilers[39]. In hens it has a good pigmentation effect on the skin and on egg yolks[38,40]. For fish it has an excellent effect in cripinids[41].
In human feeding experiments, *Spirulina* protein is adequately taken up when fed to adults and results in a low level of uric acid of the serum and a moderate increase in fecal nitrogen[42]. With children suffering from third degree malnutrition, *Spirulina* proved to be better than soya, but not as good as whole cow's milk and human milk[43], with regard to nitrogen which is retained.
More than 50 food products made with *Spirulina* or complemented by it have been explored in commercial systems. The most successful ones are health foods (capsules, tablets and powder); especially promising are the ones mixed with cereals and peanuts, e.g. cookies and nutritious candies where it has been possible to increase the protein content up to 12 and 18%, respectively[20]. Actually, in the USA it is widely used as a health food to help people lose weight without being hungry or suffering malnutrition.

Figure 1. Alga *Spirulina*.

Conclusions

A large industrial production of *Spirulina* and its products is possible already now, thanks to technological development. This production capacity and its excellent properties as a food have justified the effort to overcome the barriers of sanitary regulations in countries such as Mexico, Japan, Canada, the United States of America, France, Great Britain, the Federal Republic of Germany, New Zealand, Australia, Korea etc., where its consumption is expanding.
In the forecoming years, the production of *Spirulina* is expected to reach several thousands of tons yearly, which will initiate a massive production and consumption program. *Spirulina*, which Farrar[9] calls 'glimpse of the Aztec food technology', will undergo a broad distribution and its alimentary benefits will be within everyone's reach.
At present, Sosa Texcoco is ready to market 500 tons more in 1982, and is working on another expansion of 2000 tons per year which should be realized by 1983.

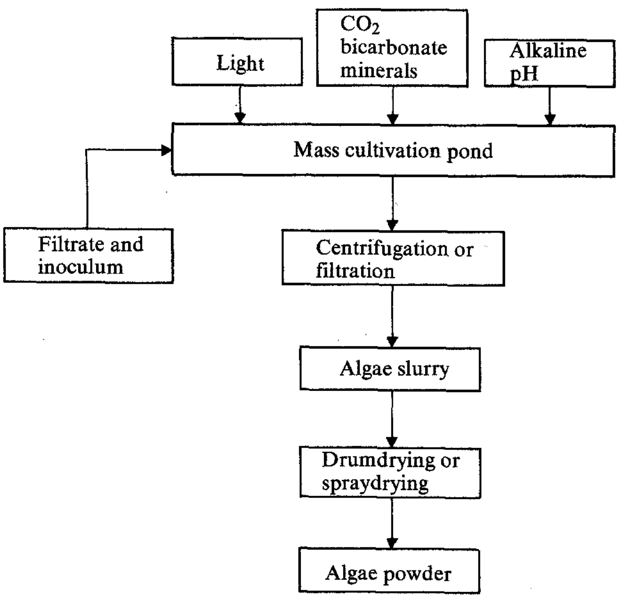

Figure 2. Flow sheet of the industrial cultivation and processing of *Spirulina*.

1 E. Guérin-Dumartrait and A. Moyse, Ann. Nutr. Alim. *30,* 489 (1976).
2 P. Chanthavong, Ultrastructure de Spirulina platensis. Diplôme d'Etudes aprofondies. Université de Dijon, France, 1974.
3 P. Dangeard, Act. Soc. linn. Bordeaux *91,* 39 (1940).
4 P. Compere, Bull Jard. bot. Nat. Belg. *37,* 109 (1967).
5 M.Y. Brandily, Sci. Aven. *152,* 516 (1967).
6 F. López de Gomara, The Conquest of the West Indies, New York 1940, Scholars' Facsimiles & Repr., XXI+231 408; Historia de la Conquista de México, con una introducción y notas por D. Joaquín Ramírez Cabañas. Pedro Robredo, Mexico 1943; 2 vols.
7 F. Toribio Motolinía, Memoriales (Historic Documents of Mexico 1541), vol. 1, p. 327, Mexico, edn 1903.
8 F.B. Sahagun, Historia General de las Cosas de la Nueva España 1580, Ed. Robredo. Mexico, edn 1938.
9 W. Fárrar, Nature *211,* p. 341 (1966).
10 F. Busson, Spirulina platensis (Gom.) Geitler et Spirulina Geitleri J. de Toni, Cyanophycees Alimentaires. Service de Santé. Parc du Pharo, Marseille 1971.
11 H.E. Landsberg, Global Distribution of Solar and Sky Radiation in World Maps of Climatology, 3rd edn. Ed. E. Rondenwalt and H.J. Jusatz. Springer, Berlin 1966.
12 H. Durand-Chastel, El Tecuitlatl (Spirulina). II Coloquio Franco-Mexicano de Alga Spirulina, México, D.F., 1975.
13 C. Zarrouk (1966), Contribution à l'Etude d'une Cyanophycée sur la Croissance de la Photosynthèse de Spirulina maxima. (Stech et Gardner) Geitler. Thèse, Paris 1966.

14 T. Ogawa and G. Terui, J. Ferment. Technol. *50*, p.? (1971).
15 R.E. Martínez and C. Santillán, Estudio del Efecto de Hormonas Vegetales en el Alargamiento y División Celular del Alga Cianoficea Spirulina, II Coloquio Franco-Mexicano de Alga Spirulina, México 1975.
16 V.J. Luna, Tesis Prof., E.N.C.B.-I.P.N., México 1979.
17 M. Craules, Tesis Prof., E.N.C.B.-I.P.N., México 1978.
18 A. Richmond and A. Vonshak, Spirulina Culture in Israel. Applied Algology Institute for Desert Research Ben-Gurion, University of the Negev Sede Boqer, Israel 1976.
19 H. Durand-Chastel and M. David, The Spirulina Algae. European Seminar on Biological Solar Energy Conversion Systems. Grenoble-Autrans, France 1977.
20 S.C. Santillán, Progresos con el Alga Spirulina en la Alimentación de Animales y Humanos. VIII Congreso Interamericano de Ingeniería Química. Bogotá, Colombia 1979.
21 A. Buisson, P. Trambouze, H. Van Landeghem and M. Rebeller, Process for the Culture of Algal and Apparatus Therefore, U.S. Patent No. 3468057, 1969.
22 Anonymous, Culture Expérimentale d'Algues Spirulines. France-Antilles Quotidien d'Information, 13 October 1976.
23 H. Durand-Chastel, Procedimiento para Acelerar el Desarrollo de Algas en Medios Naturales. Mexican Patent No. 22619, 1970.
24 S.C. Santillán, El Desarrollo y Proyección del Alga Spirulina, Un Alimento del Mañana. Semin. de Alim. Proteinicos no Tradic. en México, Centr. Investig. y Estud. Avanz., México, 1974.
25 H. Nakamura, Spirulina, Ed. Keimo Sha, Tokyo, Japan, 1978.
26 N.H. Kosaric, H.T. Nguyen and M.A. Bergougnou, Biotechn. Bioengng *16*, 881 (1974).
27 L. Enebo, Conference on Preparing Nutritional Protein from Spirulina. Swedish Council for appl. Research, Stockholm 1968.
28 Y. Jassey, C.r. Acad. Sci., Paris 1356 (1971).
29 B.J.F. Hudson and I.G. Karis, J. Sci. Fd Agric. *23*, 759 (1974).
30 C. Paoletti, G. Florenzano and W. Balloni, Ann. Microbiol. 71 (1971).
31 S. Boussiba and A.E. Richmond, Arch. Microbiol. *120*, 155 (1979).
32 C.G. Chamorro, Estudios Toxicológicos de Alga Spirulina, Proyecto VC/MEX/76/090, DP/MEX/77/009, UF/MEX/78/048, UNIDO, 1980.
33 H. Burges, Nutr. Report Int. *4*, 32 (1971).
34 S.K. Kim, Protein Value of Spirulina Maxima Growing Rats, XI Int. Congr. Nutr., Rio de Janeiro, Brazil, 1978.
35 TNO, Nr. R3193 Report, Zeist, Holland, 1970.
36 E. Bujard, V. Bracco, J. Maurow, R. Mottu, A. Nabholz and J. Wuhrmann, Nestle Res. News 59 (1971).
37 C. Février and B. Seve, Ann. Nutr. Alim. *29*, 625 (1976).
38 A. Bezares, C. Arteaga and E. Avila, Valor Pigmentante y Nutritivo de Alga Spirulina en Dietas para Gallinas en Postura, 1976.
39 J.C. Blum and C. Calet, Valeur Alimentaire des Algues Spirulines pour la Croissance du Poulet de Chair. Ann. Nutr. Alim. *29*, 651 (1975).
40 J.C. Blum, S. Guillaumin and C. Calet, Valeur Alimentaire des Algues Spirulines pour la Poule Pondeuse. Ann. Nutr. Alim. *30*, 675 (1976).
41 J. Stanley, Utilization of Algae by Fish. Final Report US Department of the Interior, US Fish and Wildlife Service. Stuttgart, Arkansas, USA 1975.
42 C. Sautier and A. Trémolieres, Valeur Alimentaire des Algues Spirulines chez l'Homme. Ann. Nutr. Alim. *30*, 517 (1976).
43 R.R. Galvan, Experimentación Clínica con Spirulina Colloque sur la Valeur Nutritionnelle des Algues Spirulines. Rueil Malmaison, France, 1973.

Microorganisms as hydrocarbon producers

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Multifaceted and diverse energy sources will replace our once massive accumulations of energy reserves. One of these energy sources will be biomass and its natural products; in fact, it will most certainly be one of the essential elements in the complex of the future energy structure.

Solar and chemical energy conversion, through biology as a practical energy conversion mechanism, has been extensively documented and reviewed; therefore, this discussion will be restricted to microbial fermentations with specific evaluations of the potentials for microorganisms to synthesize oily hydrocarbons as fermentation products. In biosynthesis, the acyclic hydrocarbons are referred to as fermentation products on the basis of the strict definition of fermentation as being those chemical energy yielding reactions that require organic components as electron acceptors. A generalized fermentation scheme is given in the figure. The scheme is purposely restrictive to emphasize products that are potential fuels. Each of the fermentation products represents a valuable energy form. The most efficient of these fermentation products, in terms of cost of production, cannot be fairly evaluated at this time because of the differences in cell cultivation requirements, product recovery, and most importantly, since many of these products via microbial fermentations are not yet sufficiently developed for commercial consideration. With increasing awareness of microorganisms which grow well or adapt to marginal, extreme or waste environments (taking into account the benefit value of these environments and the rising expenses of waste treatment) the distinct probability exists that the production costs in developing fermentation systems for fuel will become increasingly feasible and attractive. Although the compounds listed in the figure are acceptable fuels and are accessible through microbial processes, the obvious selection of a biochemical fuel for development cannot be determined at this time because not all systems have been adequately investigated. The competitive readiness of the different fermentation systems and the economics of producing each product as they become developed will automatically map out our course of action in years to come.