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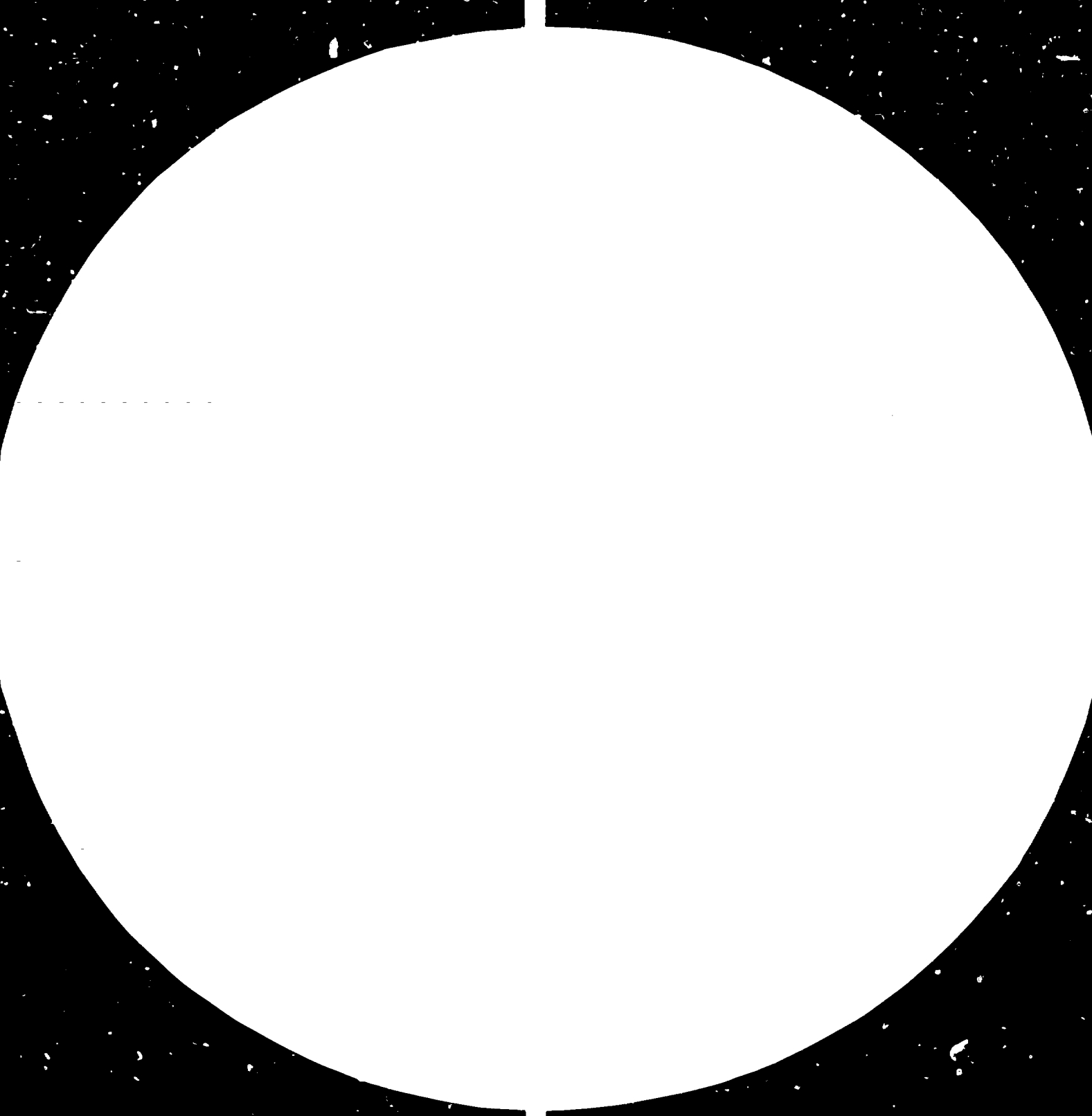

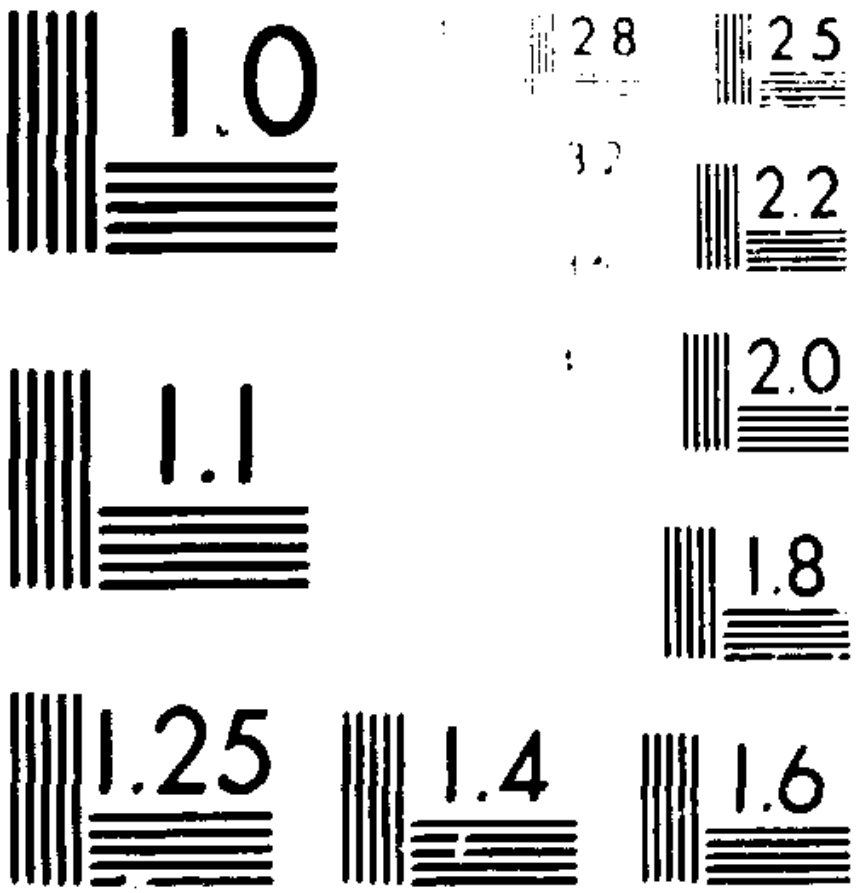
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"THE CASE OF SPIRULINA"

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# GUIDE FOR THE COMMERCIAL UTILIZATION OF SPIRULINA GEITLERI J. DE TONI OR SPIRULINA MAXIMA 1/

A review made by L. Skowronski based on the work of the Sosa Texcoco S.A., Mexico, the Research and Productivity Council, Fredericton, Canada and individual international experts.

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Acknowledgement

The author of this document wishes to transmit his appreciation to counterparts, Messrs. Torrero, Huesca, Santillan, Kamio of the Sosa Texcoco Spirulina operation, Mr. Chamorro of the University of Mexico, Mr. G. Brown and the scientific staff of the Productivity Research Council in Fredericton and Mr. Goldenberg of the Institut Français de Petrole in Paris.

He also wishes to thank the Agro-Industries Branch of the UNIDO Secretariat for its valuable guidance.

Abstract

1. The natural product successfully processed in a semi-industrial plant using modern manufacturing method, with an average daily output of 1000 kg is Spirulina Geitleri J. de Toni, synonyme Spirulina maxima known to the Aztects, and in Far Eastern and African cultures as a food source.

The periodical controls by the Holland Central Institute for Nutrition and Food Research and the Japan Food Research Laboratories reveal that this Spirulina is an exceptional natural product since only slight differences are observed in the composition when harvested in the different seasons of the year.

2. The product has a number of important uses: as a food complement, it is a valuable aid in raising mollusks, crustaceans and fish, for it stimulates their growth, sexual maturity, ovulation and early reproduction. It has also been used with great success in feeding bees as well as birds and cattle. The yellow and orange pigments contained in this product can be assimilated as natural colouring by chicken meat and egg yolks, ornamental birds and aquarium fish; it may also be used to give butter a brighter colour.

The product is now being consumed by humans. Recent Japanese work in fact shows that the product has beneficial effect on people with certain illnesses. The great variety of food products that can be used in combination allows to develop a research programme presently in progress in Sosa Texcoco, Mexico that is on the constant look out for new recipies and uses in the future.

3. UNIDO contributed in setting up the original pilot plant where simple technological equipment is used for the manufacutring process suitable for installation in other developing countries and in sponsoring research for the improvement of the inexpensive eutrophication techniques by an aimed homogeniz ion and carbonation. (Annex A).

4. The chemical analysis of the dry algae powder reveals a high content of good quality protein: linolenic acid: a moderate content of carbohydrates: a low content of nucleic acids; significant amounts of several nutritional minerals and high content of vitamins A, B, $B_{12}$ and E. Amino acid analysis shows that most of the essential amino acids fulfil the FAO requirements and surpass some cases the amino acid content of other protein sources. (Annex B ).

5. A study commissioned by UNIDO carried out by the Research and Productivity Council/RPC of Fredericton, New Brunswick, Canada developed a pilot scale process for the decolourization of dry algae, associated by-product of the cream coloured protein powder. The RPC team headed by Dr. G. D. Brown tried the published methods for decolourization of algae and other plant materials and concluded that the existing methods could not be used for complete decolourization because of the complex group of billiproteins which were not soluble in solvents.

They developed an effective way of converting these pigments into a solvent soluble stage.

The final process involves a short time extraction with a single solvent. The resulting decolourized protein product retains the protein quality of the original material and the associated pigments concentrate retains the valuable xantophylls, which may be converted into animal feed supplement with increased pigmenting value.

6. Toxicological studies were funded by UNIDO and carried out by Dr. G. Chamorro. Several aspects of possible adverse changes were studied. Short, long-term and multigeneration feeding tests on laboratory animals fed with the Spirulina product were undertaken. The results have shown no adverse reaction whatsoever.

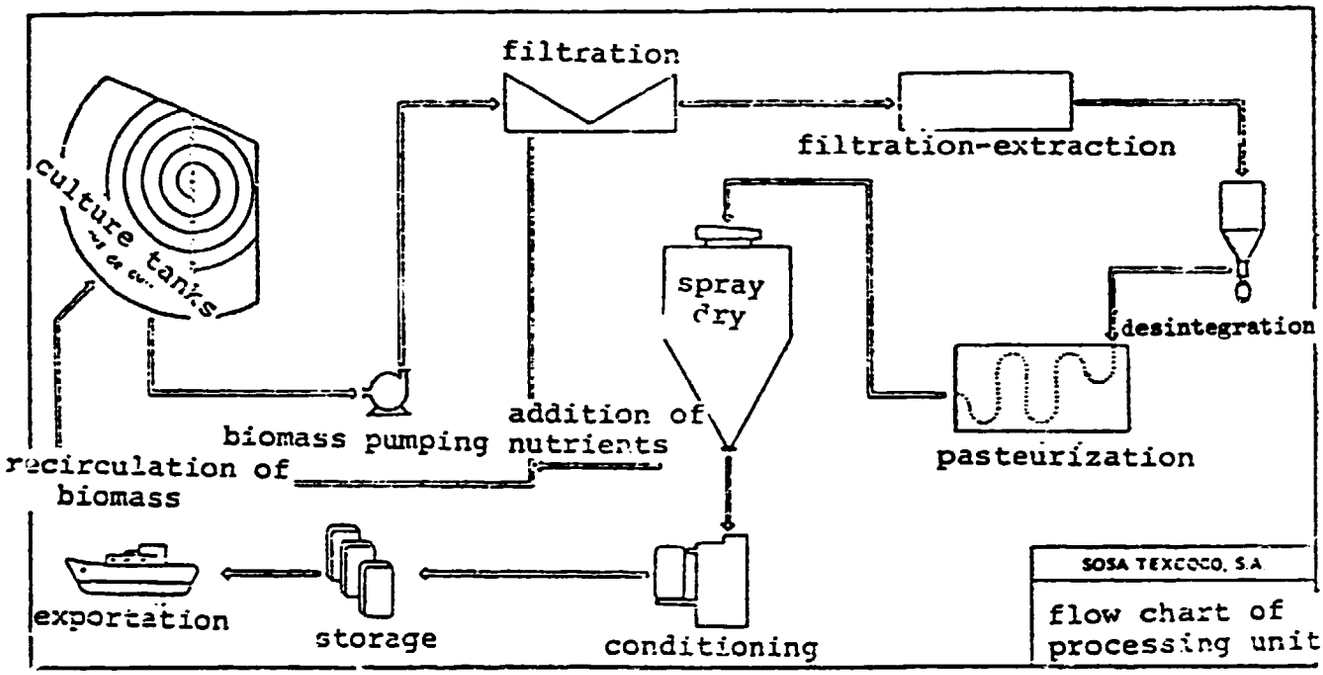
7. The marketing split reveals that 63 per cent sales in 1980 are made in human consumption. The unit price increased considerably from US$ 1,24 in 1974 to US$ 4.00 in 1980 implying an exceptionally favourable profit margin. Sales for the first half of 1980 surpassed the highest sales figures of 1979, making the generalized forecast prediction until 1985 (sales of approximately 3,500 tons annually) feasible.

8. The major problem facing the Spirulina operation in obtaining financial resources is being tied up to the balance sheet and the cash flow of the Sosa Texcoco operation aimed to exploit the sodium alkali of the lake in processing Soda Ash, Caustic Soda and Industrial Salt. This circumstance is applicable

to financial resources for fixed assets and for working capital as well and involves therefore, the on-going operation plans of Spirulina such as the construction of new modules or the expansion of the existing one. Should the Spirulina operation become an independent operation it will be subject to preferential treatment by banks, including Somex and Government. Such a project could be granted with a number of incentives such as tax reduction, import duties exemption on the importation of equipment etc.

9. The cultivation of algae can be an answer for the future global protein shortages. Combined activities of the algae in waste water treatment and as a source of protein is of utmost importance considering the world's exploding population. Manufacturing modulessimilar to the successful Sosa Texcoco plant should be established in countries possessing lakes with algae growth, thus making the developing countries exclusive producers, leading to a crossing of the trade barriers from developing to developed countries.

ANNEX A


filtration
filtration-extraction
culture tanks
spray dry
desintegration
biomass pumping
addition of nutrients
pasteurization
recirculation of biomass
exportation
storage
conditioning
SOSA TEXCOCO, S.A
flow chart of processing unit

ANNEX B

| NUTRITIONAL ANALYSIS | % |
|---|---|
| Protein | 65.0 |
| Carbohydrate | 14.8 |
| Fat | 6.5 |
| Ash (Minerals) | 7.7 |
| Fiber | 0.5 |
| Moisture | 5.5 |

| AMINO ACID PROFILE (% of Total Protein) | % |
|---|---|
| Isoleucine | 5.7 |
| Leucine | 8.7 |
| Lysine | 5.1 |
| Methionine | 2.8 |
| Phenylalanine | 5.0 |
| Threonine | 5.4 |
| Tryptopan | 1.5 |
| Valine | 7.5 |
| Analine | 7.9 |
| Arginine | 7.6 |
| Aspartic Acid | 9.1 |
| Cystine | 0.9 |
| Glutamic Acid | 12.6 |
| Glycine | 4.8 |
| Histidine | 1.5 |
| Proline | 4.1 |
| Serine | 5.3 |
| Tyrosine | 4.5 |

| | |
|---|---|
| RNA | 2.9% |
| DNA | 0.8% |
| Lysine Availability | 85% |
| Pepsin Digestibility | 84% |
| Protein Efficiency Ratio | 2.4 |
| Net Protein Utilization (% of Casein) | 89% |

| MINERALS | (mg/kg) |
|---|---|
| Calcium (Ca) | 1180 |
| Phosphorus (P) | 8280 |
| Iron (Fe) | 520 |
| Sodium (Na) | 344 |
| Chloride (Cl) | 4200 |
| Magnesium (Mg) | 1663 |
| Manganese (Mn) | 22 |
| Zinc (Zn) | 35 |
| Potassium (K) | 14353 |
| Selenium (Se) | 0.4 |

| VITAMINS | (mg/kg) |
|---|---|
| Beta Carotene (A) | 1700 |
| Biotin (H) | 0.4 |
| Cyanocobalamin ($B_{12}$) | 2 |
| d-Ca-Pantothenate | 11 |
| Folic Acid | 0.5 |
| Inositol | 350 |
| Niacin | 118 |
| Pyridoxine (B6) | 3 |
| Riboflavine (B1) | 40 |
| Thiamine (B2) | 55 |
| Tocopherol (E) | 190 |
| Chlorophyll-A | 5600 |

| FATTY ACIDS | (mg/kg) |
|---|---|
| Lauric (C12) | 204 |
| Myristic (C14) | 582 |
| Palmitic (C16) | 18,820 |
| Palmitolinoleic (C16) | 1762 |
| Heptadecanoic (C17) | 116 |
| Stearic (C18) | 177 |
| Oleic (C18) | 2490 |
| Linoleic (C18) | 12,352 |
| gamma Linolenic (C18) | 10,360 |
| alpha Linolenic (C18) | 294 |
| Others | 3850 |

## History and development of the Spirulina plant

The Great Tenochtitlan, the capital city of the Aztec Empire was settled on an island in the midst of lake Texcoco, an alkaline water basin which served as a natural military defense barrier for the city and also as a source of food for its people. Apart from fishing and other food sources, the lake provided the Aztecs with Tecuitlatl (excrement of stones in the nahuatl language) a sort of slime, "a bluish muddy substance that floats on the water of the lake which they (the Aztecs) dried in the sun and preserved, to make use of it as cheese, which it resembled in flavour and taste"(Prescott, W.H.). Many other historian's accounts of tecuitaltl are expressed in similar words (Diaz del Castillo, B., Lopez Gomara, F., Motolinia, T. de, Sahagún, B. de). The use of such a substance decreased through the centuries in parallel fashion to the size of the lake, intentionally dried by the Spaniards: the oral tradition, however, points out its consumption as recently as the beginning of this century. Today, most of lake Texcoco serves as a huge - 3.5 km diameter - solar evaporatory for the extraction of sodium hydroxide and sodium carbonate, called "el caracol" (the snail); in the more diluted waters there still exist these bluish muddy substances, Tecuitlatl, which is a film made up of agregates of a microsconic Cyanophyta (blue-green alga) named Spirulina (Clement, G. y Durand-Chastel, H.) that looks under the microscope like a 0.5 mm long Spiral filament. This alga is known to optimally grow in sodium-rich water with a pH between 8.5 and 11.0 when the temperature is 30-35°C and the illumination amounts to, at least, 25000 lux per day. (Bourges, H., Sotomayor, A., Mendoza, E., Chávez, A.)

The blue-green algae have been the ones to attract the most attention as potential, and indeed actual, sources of protein and other human and animal nutrients. Of the more general recent communications on this subject those from France (Clement, C.), Mexico (Bourges, H.) and England (Gordon, J.F.) have been noteworthy.

Algae grow naturally on the water being processed by this compnay and are now being harvested and processed for sale on a commercial basis. SOSA TEXCOCO developed the necessary harvesting concentration and dehydration processes which enabled them to achieve a maximum production rate of a few hundred pounds per day. Some of the techniques and know-how were given by Institut Francais de Petrole. Some expertise and financial help was given by UNIDO.

The market of these algae developed rapidly and the company soon realized that their pilot scale production facilities could not meet the existing marketing demand nor allow sufficient material to explore further markets.

Decisions were made to improve and enlarge the production facilities and at the same time an extensive programme of work was undertaken to find the most effective addition of natural nutrients to increase the quantity of algae growth.

It should be noted that this yield maximation does not result in production of any industrial waste product nor does it effect the main operation of the company, namely the production of solar evaporation of the brine water.

The importance of the nutritional value of the Spirulina end-product was recognized internationally. (Burghes, H. et Coll.) (Calet, C.) (Durand-Chastel, H.) (Durand-Chastel, H. and Clement, G.) (UNESCO Features) (Woodward, F.N.) (Clément, C., Giddey, C., and Menzi, R.) (Hills, C. and Nakamura, H.).

## Country profile

### Basic data: Mexico

Land area: 2,022,060 sq km, 48 per cent agricultural and 9 per cent forested.

Population: 66.9 mn

Climate: Tropical in the south: temperate in the highlands

Weather in Mexico City: (altitude 2,309 m): hottest month May, 12°-26°C (average daily minimum and maximum): coldest month January, 6 - 19°C; driest month February, 5 mm average rainfall; wettest month July, 170 mm average rainfall.

### Food crops

Maize, wheat, rice and beans are the principal food crops in Mexico. Production has been rising steadily, but has only just kept pace with the increase in population, and these basic crops must all have successful harvests for Mexico to be truly self-sufficient in foods. Poor wheat and maize harvests have recently necessitated major imports, for example 1 mn tons of maize were imported in 1978/79.

Production of principal agricultural products as protein source ('000 tons)

| | 1973/74 | 1974/75 | 1975/76 | 1976/77 | 1977/78 | 78/79 |
|---|---|---|---|---|---|---|
| Maize | 7,760 | 8,459 | 8,945 | 10,714 | 10,909 | only estimate is given |
| Wheat | 2,669 | 2,798 | 3,354 | 2,456 | 2,643 | |
| Cotton | 513 | 215 | 213 | 416 | 340 | |
| Rice | 492 | 717 | 463 | 567 | 397 | |
| Beans | 895 | 1,027 | 740 | 762 | 940 | |

Sources: Secretaría de Agricultura y Ganadería, Ministry of Planning and the Budget: Panamex.

General

The exports from the country amounted to US$ 3,030 million as against imports of US$ 6,030 million. The per capita GNP is US$ 650.

Economy

Since 1978 the Mexican economy has continued to improve. The inflation rate was reduced from 21 per cent in 1977 to 16.2 per cent in 1978. The abridged version of the 1979 - 1982 National Industrial Development Plan sets forth the basis for economic development during the next four years and on through 1990. (Mexican National Industrial Development Plan (NIDP) 1978. New implementation decree).

Due to a balance of payment problem, Mexico adopted a policy of import substitution. Raw material exports were not growing at a fast rate thus limiting the ability to import manufactured goods with the result that the country continued to depend on foreign sources for the supply of machinery, equipment and intermediate goods. The opportunities to overcome this crisis lie in the financial potential offered by the surpluses earned through exports of hydrocarbons (The Europa Year Book 1979. A World Survey).

Incentives to industry and National Industrial Development Plan

A new decree issued in 1978 provides a subsidy equal to 30 per cent of the Government authorized price for energy to be granted to new companies or new industrial installations and on purchase of basic petrochemicals provided the producer made certain export commitments. Firms benefitting from the 30 per cent discount must agree to sell their output to industrial firms located in Mexico at a ten per cent discount from the FOB price or at a price that takes account of the discounts on energy prices.

Priority sectors in industry

The priority sectors are divided into two categories. The first category comprises industries which produce food stuffs, and those which supply machinery and equipment to these sectors and industries of strategic importance. The second category covers all remaining activities which generate basic consumer goods (Mexican National Industrial Development Plan, 1979-1982, Abridged Version).

Industry

Selected products

| | | 1973 | 1974 | 1975 | 1976 |
|---|---|---|---|---|---|
| Wheat flour | '000 metric tons | 1,566 | 1,606 | 1,580 | 1,714 |
| Prepared animal feeds | '000 metric tons | 1,901 | 1,978 | 2.183 | n.a. |

Finance

Exchange rates (June 1980): US$ 1 = 22.75 pesos.

From June 1949 to April 1954 the exchange rate was US$ 1 = 8.65 pesos (1 peso = 11.56 US cents). In April 1954 the par value of the peso was fixed at 8.0 US cents ($1 = 12,50 pesos) and this remained in effect until August 1976, despite two devaluations of the U.S. dollar (in December 1971 and February 1973). Since September 1976 the peso has been allowed to "float". The average market rate (pesos per U.S. dollar) was: 15.43 in 1976; 22.57 in 1977 (The Europa Year Book 1979, A World Survey).

## Agriculture

LAND USE
(unofficial estimates, '000 hectares)

| | 1971 | 1976* |
|---|---|---|
| Arable land | 25,290 | 26,000 |
| Land under permanent crops | 1,760 | 1,790 |
| Permanent meadows and pastures | 69,200* | 66,700 |
| Forests and woodland | 74,000* | 71,100 |
| Other land and inland water | 27,005 | 31,665 |
| TOTAL AREA | 197,255 | 197,255 |

* FAO estimates.

*Source:* FAO, *Production Yearbook.*

## Principal Crops

| | AREA HARVESTED ('000 hectares) | | | | PRODUCTION ('000 metric tons) | | | |
|---|---|---|---|---|---|---|---|---|
| | 1975 | 1976 | 1977 | 1978 | 1975 | 1976 | 1977 | 1978 |
| Wheat | 778 | 894 | 708 | 777 | 2,798 | 3,363 | 2,454 | 2,871 |
| Rice (paddy) | 257 | 159 | 174 | 149 | 717 | 463 | 545 | 473 |
| Barley | 256 | 364 | 248 | 293 | 440 | 549 | 404 | 410 |
| Maize | 6,694 | 6,753 | 7,374 | 7,680 | 8,459 | 8,017 | 10,024 | 9,616 |
| Oats | 59 | 66 | 64 | 6[illegible] | 87 | 48 | 49 | 60 |
| Sorghum | 1,116 | 1,232 | 1,368 | 1,590 | 2,843 | 3,920 | 4,071 | 4,536 |
| Potatoes | 57 | 56 | 54 | 58 | 693 | 687 | 688 | 837 |
| Sweet potatoes | 10 | 10* | 10* | n.a. | 134 | 130* | 130* | n.a. |
| Other roots and tubers | 5 | 5* | 5* | n.a. | 45 | 45* | 45* | n.a. |
| Dry beans | 1,753 | 1,316 | 1,613 | 1,876 | 1,027 | 740 | 741 | 1,095 |
| Dry broad beans | 46 | 55 | 50 | n.a. | 38 | 44 | 40 | n.a. |
| Chick-peas | 191 | 105 | 48 | 48 | 195 | 76 | 67 | 71 |
| Soybeans | 344 | 172 | 314 | 231 | 699 | 302 | 507 | 324 |
| Groundnuts (in shell) | 62 | 43 | 43 | 55 | 69 | 56 | 56 | 72 |
| Sesame seed | 219 | 185 | 205 | 233 | 111 | 85 | 123 | 143 |
| Linseed | 14 | 8 | 12 | 8 | 27 | 13 | 18 | 11 |
| Safflower seed | 363 | 185 | 399 | n.a. | 532 | 240 | 525 | n.a. |
| Cottonseed | 227 | 235 | 386 | n.a. | 345 | 349 | 596 | 547 |
| Cotton (lint) | | | | | 197 | 224 | 325 | 296 |
| Coconuts | n.a. | n.a. | n.a. | n.a. | 960 | 960* | 980* | n.a. |
| Copra | | | | | 145 | 160 | 150 | 160 |
| Palm kernels | n.a. | n.a. | n.a. | n.a. | 15 | 30 | 30* | 30* |
| Sugar cane | 479 | 496 | 480 | n.a. | 34,366 | 31,387 | 31,407 | 30,000 |
| Sugar beet | 4 | 4* | 4* | n.a. | 99 | 100* | 100* | 100* |
| Coffee (green) | 374 | 376 | 390* | n.a. | 228 | 242 | 225 | 240 |
| Cocoa beans | 72 | 76 | 70* | n.a. | 34 | 40 | 36 | 35* |

* FAO estimate.

Source: FAO, Production Yearbook and Monthly Bulletin of Statistics.

Financial mechanism of the Spirulina plant

Bank: SOMEX, Sociedad Mexicana de Crédito Industrial, S.A.

SOMEX: 87 % Government shares
13 % Private shares

Objectives and activities

Main interest: Financing technology such as basic chemical which includes food industry. Special interest is in raw materials. The first experience outside of Mexico is in Sydney Ross Chemicals, USA: Kali Chemie, West Germany.

Net sales in 1979 18,000 million Mexican Pesos

Net profit in 1979 1,100 million Mexican Pesos.

Somex has minority shares in the following funds:

1. FOGAIN - Guarantee and Development Fund for Small and Medium Size Industry, which finances through private and public banks, credits to enterprises having a capital ranging between 50 and 40 thousand million pesos, with credit availability for the industrial project of $ 9,000.000 of the total $ 16.000.000.00 up to 4 - 7 years return.
2. FONEP - National Fund for Studies and Projects aims at facilitating financial resources and technical assistance for preparation of pre-investment studies, which may allow to know in advance the risks or benefits of investing capital before doing so.

In addition, SOMEX has majority shares in the following funds managed by the Bank of Mexico:

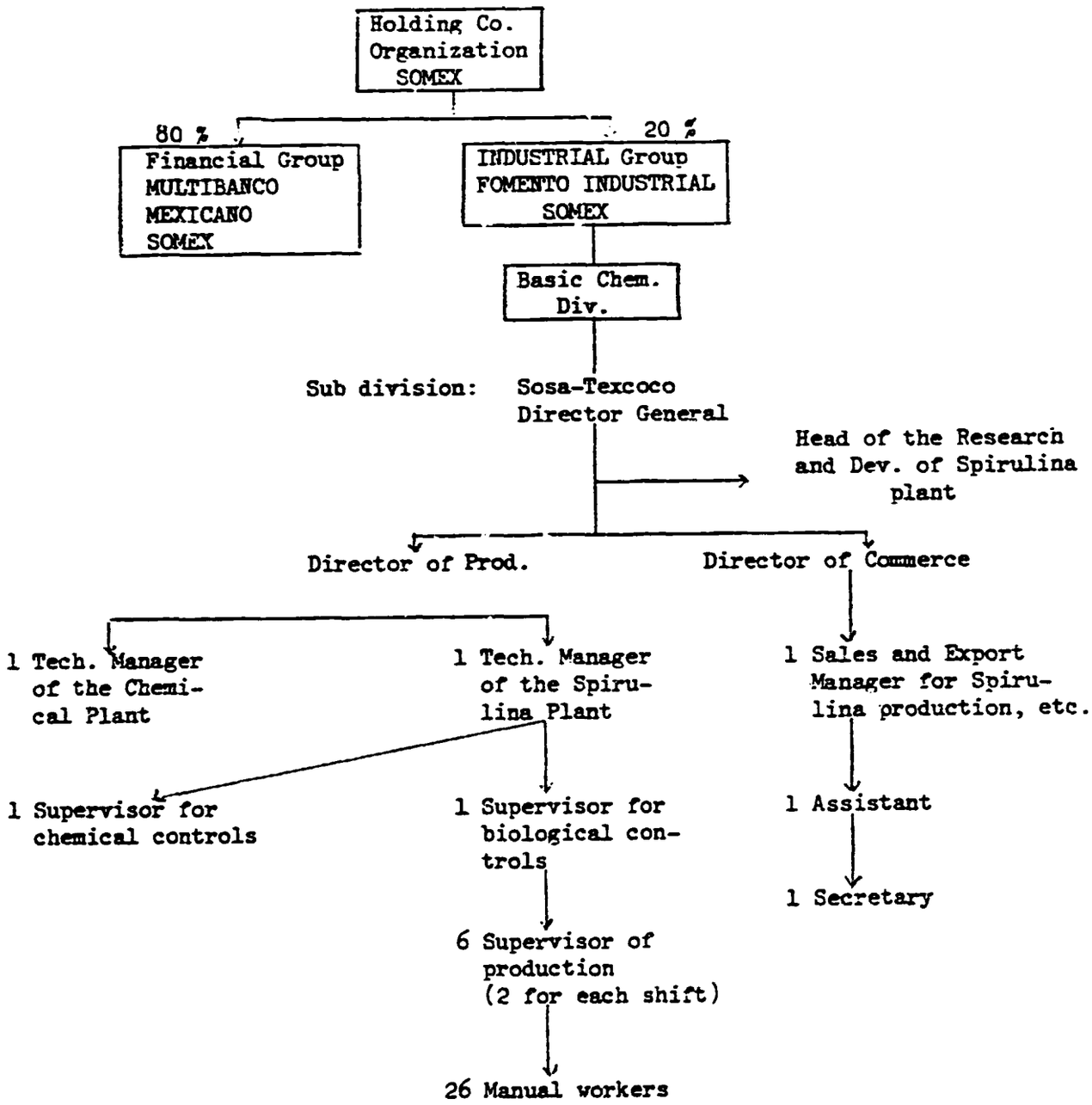
1. FOMEX - Fund for Promotion of Exports of Manufacturing Products, which promotes and encourages exports of manufactured products, as well as the substitution of imports of capital and consumption goods.

2. FONEI - Fund for Industrial Equipping, encourages the establishment and/or modernization of enterprises, which should export an important part of their production or either substitute efficiently imports of goods or services. The credits granted by the Fund vary from a minimum of 4.5 million pesos to a maximum of 100 million. The FONEI grants credits to a maximum 72 per cent of the investments in fixed assets when used for modernization or enlargement, and up to 65 per cent of fixed assets when used for establishment of new enterprises. The financial intermediate, be it a private or public bank, will grant an 11.1 per cent of the amount provided by the FONEI when used for modernization or enlargement, and 15.4 per cent for new projects.

3. FONATUR - National Fund for the Development of the Tourism Industry.

   With the help of the above mentioned funds, it is possible to borrow 3 to 6 points below the commercial level.

Resources


Holding Co.
Organization
SOMEX
80 %
20 %
Financial Group
MULTIBANCO
MEXICANO
SOMEX
INDUSTRIAL Group
FOMENTO INDUSTRIAL
SOMEX
Basic Chem.
Div.
Sub division:
Sosa-Texcoco
Director General
Head of the Research
and Dev. of Spirulina
plant
Director of Prod.
Director of Commerce
1 Tech. Manager
of the Chemi-
cal Plant
1 Tech. Manager
of the Spiru-
lina Plant
1 Sales and Export
Manager for Spiru-
lina production, etc.
1 Supervisor for
chemical controls
1 Supervisor for
biological con-
trols
1 Assistant
1 Secretary
6 Supervisor of
production
(2 for each shift)
26 Manual workers

Research on the commercial derivatives

Among others research was conducted with the Spirulina originated from the Caracol of Sosa Texcoco in obtaining important commercial products, such as ferrodoxine.

Ferrodixines are being used to an increasing extent in biochemical experiments. They belong to the group of non-haem iron proteins known as iron-sulphur proteins, and they are now known to be electron carriers involved in numerous electron transfer reactions in soluble and membrane-bound systems (Hall, D.O., Evans, M.C.W.). An ideal ferrodoxine would be stable in pure form, functional in various reactions and readily extractable from a cheap and available source of cells which can be easily stored.

The ferrodoxine originatedfrom Sosa Texcoco was assayed chemically and biologically and compared with ferrodoxines from spinach (Spinacea oleracea), lucerne (Medicago sativa), maize (Zea mays), parsley (Petroselinum cristum) and a green alga (Scenedesmus obliquus). The relative stabilities of the ferrodoxines were tested by storage for up to 7 weeks at - 196°C (liquid $N_2$), 4°C (refrigerator) and 21°C (room temperature). (Hall, D.O., Rao, K.K., and Cammack, R.).

Purified Spirulina ferrodoxine contains 2 atoms of Fe and 2 atoms of inorganic sulphur per mole, assuming a molecular weight of 12,000 and a molar extinction coefficient at 420 mm of 9,700 as is the case for spinach ferrodoxine (Tagawa, K. and Arnon, D.I.).

At liquid nitrogen temperatures all the ferrodoxines showed a small loss in activity which was probably incurred during freezing and thawing. In the refrigerator at 4°C there was appreciable loss of activity of all ferrodoxines - about 10 - 30 % loss. At this temperature Spirulina ferrodoxine was the most stable and parsley ferrodoxine the least stable. At room temperature the great stability of Spirulina ferrodoxine became apparent:

it still retained about 35 % of its original activity after 7 weeks at 21°C, whereas the others (except for maize which still retained about 15 % activity) had retained less than 5 % of their original biological activity. If, however, special precautions are taken to keep ferredoxin solutions completely anaerobic they can be stored at 4°C for longer periods (Keresztes-Nagy, S., Marpoliash, E., Rao, K.K., Fee, J.A. and Palmer, G.).

The stability of purified Spirulina ferredoxin, the ease of extraction from the dried cells, and the convenience of storage and cheapness of the cells, all seem to recommend this alga as an excellent source of a plant ferredoxin.

For identification of the lipid content of the Spirulina grown in the Sosa Texcoco Caracol, the methods employed for extraction, chromatography and detection and identification of components were those used by Hundson and Karis. Use was made of a modified procedure based on methods of Lysyj and Zaremboy (Lysyj, I. and Zarembo, J.E.) and Bertocalini and Barney (Bertocalini, R.J. and Barney, J.E.).

Tables on page 19 show the results obtained by the thin-layer chromatography, followed by densitometry, for the non-polar and polar lipid components. Assignments of lipid classes are made tentatively, on the basis of response to specific spray reagents and identification by $R_f$ values (Betram, J.F., Hudson and Ionnis G. Karis).

T.I.C. of non-polar lipids of S. Maxima*

| $R_f$ | Tentative identification | % of total |
|---|---|---|
| 0.00 | Pigments and polar lipids | 9.5 |
| 0.19 | Monoglycerides | 8.2 |
| 0.26 | Free sterols | 1.5 |
| 0.34 | Diglycerides | 3.6 |
| 0.60 | Free fatty acids | 69.3 |
| 0.89 | Triglycerides | 3.6 |
| 0.98 | Sterol esters, waxes, etc. | 4.3 |

*Solvent system: Pet. ether/ethyl ether/acetic acid (80 : 20 : 1).

T.I.C. of polar lipids of S. Maxima[a]

| $R_f$ | Tentative identification | % of total |
|---|---|---|
| 0.00 | Unresolved ploar lipids | 7.9 |
| 0.04 | Lipid A[b] | 9.1 |
| 0.08 | Lipid B[c] | 11.9 |
| 0.16 | Phospholipid (phosphatidyl inositol?) | 4.6 |
| 0.25 | Sulpholipid | 5.0 |
| 0.35 | Digalactosyl diglyceride | 23.4 |
| 0.40 | Phosphatidyl glycerol | 25.9 |
| 0.86 | Monogalactosyl diglyceride | 4.6 |
| 1.00 | Neutral lipids and pigments | 7.6 |

[a]Solvent system: chloroform/methanol/acetic acid/water (85:10:3:5).
[b]Probably tetragalactosyl diglyceride.
[c]Probably trigalactosyl diglyceride.

The presence of linoleic and $\gamma$-linolenic acid in amounts together exceeding 20 % is important in relation to the use of Spirulina as a source of human food. These two acids together are a rich source of essential fatty acids (Thomasson, H.J.). Supplementation of otherwise possibly essential fatty acid deficient diets with Spirulina could thus be beneficial quite apart form considerations of enrichment with protein or other nutrients.

Concentrated alcoholic extracts of Spirulina maxima have revealed on paper chromatograms the presence of glucose, fructose, galactose, mannose, and other low molecular weight carbohydrates. The presence of glucopeptides has also being noticed and polyalcohols such as sorbitol have been identified by chromatography. A phosphorylated cyclitol (3 - 4 %) has been reported (Quillet, M.) from alcoholic extracts three crystalline compounds which contain sugar moiety in their molecules have been isolated (Martinez Nadal, N.G.).

There is evidence that this alga contains an active principle, which has medicinal properties (Clément et al.). Antimicrobial substances have been reported in macroscopic algae by various investigators (Martinez Nadel et al). A growth inhibitery substance, "chlorollin" is produced by unicellular algae, Chicrella vulgaris and Chlorella pyrenoidosis (Prat et al.)

The evaluation of medicinal properties of Spirulina was made by studying its antimicrobial activity. Extracts of dried algae were essayed chemically and biologically and purified by chromatographic absorptions.

Three antimicrobial activities have been spotted in paper chromotographic patterns. One of the activities is mainly antifungal and is closely related to the presence of sterols.
It appears to contain a polyane antimicrobial substance.

The paper by Noemi G. Martinez Nadal "Antimicrobial Activity of Spirulina Maxima" describes isolation, separation and purification of active fractions by chromotographic methods and their selective action against microorganisms in vitro.

Concentrated extracts were bioassayed by liquid needle and the disc diffusion technique. They were analysed in vitro against the following organisms Bacillus subtilis, Stophylacoccus gumus, Proteus vulgaris, Escherichia coli, Sacharomyces pasterianus, Sacharomyces carevisiae, Candida albicans, Aspergillus niger.

Gottlieb et al reported that antifungal action of polyene antibiotics was prevented by steroid such as cholesterol. Evidence was found of presence of sterols in Spirulina maxima (Martinez Nedal et al).

Polyene antibiotics are toxic to fungi and yeast and have little or no effect on bacteria. Their selective toxicity is due to interaction with a unique component present only in the membrane of sensitive organism and the component is a sterol (Kinky). Three activities were found in Spirulina maxima. In these studies Spirulina A appears in fractions which are negative for sterols and by its selective activity on yeast and fungi, and its ultraviolet absorption packs gives evidence of containing a polyene antimicrobial. Spirulina B and C are not hindered in their action by the presence of sterols.

Hunter et al observed that tetraenes inhibited growth of higher algae but had no effect on blue-green algae. This was so, since it was prevalent that this type of algae was devoid of sterols (Carter et al). (Levin and Bloch). The presence of sterols in Spirulina maxima had been recently confirmed (Martinez Nadel).

Use of Spirulina tablets (S-Tab*) as a medication (1980 study) gave the following results

1. Treatment of diabetes (Tadaya) in 3 cases with 7 S Tab x 3 daily (Tadaya).

Results in mg/dl** of the blood sugar examination after an intake of 50 g. glucose:

| CASE | AGE | SEX | MINUTES AFTER GLUCOSE INTAKE | DAYS FOLLOWING THE S TAB INTAKE | | | |
|---|---|---|---|---|---|---|---|
| | | | | 0 | 30 | 60 | Expected Maximum Values |
| I | 48 | M | 0 | 128 | 116 | 96 | 100 |
| | | | 30 | 158 | 162 | 154 | |
| | | | 60 | 206 | 168 | 160 | 170 |
| | | | 90 | 172 | 130 | 122 | |
| | | | 120 | 134 | 104 | 94 | 120 |
| II | 55 | M | 0 | 176 | 122 | 102 | 100 |
| | | | 30 | 212 | 200 | 168 | |
| | | | 60 | 238 | 236 | 170 | 170 |
| | | | 90 | 196 | 174 | 136 | |
| | | | 120 | 136 | 120 | 98 | 120 |
| III | 56 | M | 0 | 212 | 172 | 180 | 100 |
| | | | 30 | 266 | 194 | 206 | |
| | | | 60 | 354 | 226 | 198 | 170 |
| | | | 90 | 360 | 202 | 180 | |
| | | | 120 | 380 | 178 | 146 | 120 |

*S Tab = 200 mg each originated from Spirulina platensis, of the same family Oscillatoriaceae as Spirulina Geitleri J. de Toni used in SOSA TEXCOCO.

**dl = deciliter = 1/10 liter (unit commonly used in Japan).

2. Treatment of anemia in 3 cases from total of 9 treated with 20 x S Tab x 3 daily (Tadaya)

Red blood cells, haemoglobin and haematocrit level.

| CASE | AGE | SEX | | DAYS FOLLOWING THE S TAB INTAKE | | | |
|---|---|---|---|---|---|---|---|
| | | | | 0 | 15 | 30 | 45 |
| I | 18 | F | R | 3859 | 3900 | 3880 | 3920 |
| | | | Hb | 10,6 | 11,6 | 23,0 | 13,1 |
| | | | Ht | 35,0 | 38,0 | 39,0 | 39,0 |
| IV | 22 | F | R | 3960 | 4010 | 4100 | 4060 |
| | | | Hb | 11,2 | 12,6 | 13,9 | 14,0 |
| | | | Ht | 360 | 38,0 | 39,0 | 39,0 |
| IX | 47 | M | R | 4200 | 4360 | 4260 | 4220 |
| | | | Hb | 13,0 | 14,6 | 158 | 15,6 |
| | | | Ht | 39,0 | 43,0 | 43,5 | 44,0 |

R: Number of red corpusclesx 1000
Hb: g./dl* of Haemoglobin
Ht: % of Haematocrit

3. Treatment of A and B hepatitis in 6 cases treated with 7 S Tab x 3 daily (Tadaya)

Results: All patients - 2 with hepatitis type B and 4 with hepatitis type A were cured following a 6 week treatment.

*deciliter = 1/10 liter.

4. Treatment of chronic pancreatitis in 2 cases treated with 7 S Tab x 3 daily (Minoru). Normalization of the density of amylaze in blood after 2 weeks and disappearance of other symptoms of the disease (e.g. vomiting disappeared after 5 weeks).

5. Treatment of heavy myopia in one case with 10 S Tab x 3 daily. Results: a curative effect after a 30 day period. (Hoshito).

6. Treatment of alopecia in one case with 9 S Tab x 2 daily in addition to simultaneous treatment with other medicaments. Results: dawny hair had started to grow on the bald patch following a 14 day treatment (Iwao Tanave).

There are also reports on the curative effects of S Tab in:

1. Cirrhosis of liver (Noboru)
2. Gastritis and stomach ulcer (Tadaya)
3. Glaucoma (Yoshito)
4. Gastroptosis (Tomokichi).

## Research in toxicology

### Tolerance of rats to Spirulina-rich diets

Although some Cyanophyta (Microcystis, Anabena) have toxic properties when fed to animals. Spirulina has never proven toxic after centuries of its consumption by humans. However, it was judged necessary to have at least some experimental data on the tolerance of animals to Spirulina-rich diets in short term (100 days) studies. Following the concepts expressed by Oser (Oser, L.), groups of weanling male albino rats of the Wistar strain were fed during 100 days exclusively with the following diets:

Control diet - Commercial Purina chow

Diet 1 - Composition: 73 g of Spirulina, 7.3 g of sucrose, 14.6 g of corn oil plus vitamins and minerals, with a protein concentration of 48 %.

Diet 2 - Composition: 26 g of Spirulina, 69 g of Purina chow plus vitamins and minerals with a protein concentration of 36 %.

Five groups of six rats were formed: control group fed with the control diet; experimental groups 1 and 2 fed on diets 1 and 2 respectively to test two different levels of protein concentration: experimental group 3 fed on diet 1 for 50 days and then switched to the control diet for the rest of the period, and experimental group 4 fed on the control diet for 50 days and then switched to diet 1 for the rest of the period. Experimental groups 3 and 4 intended to detect possible age differences in response.

The animals were observed for effects on growth, food intake, efficiency of protein utilization, physical appearance and behaviour and, at the end of the period, they were sacrificed and the liver, heart, guts, lungs, kidneys, thyroid and pancreas were histologically examined and no toxic symptoms were observed.

Based on literature data, similar chronic toxicity studies have been carried out by Boudene et al in 1976. No signs of toxicity were found when rats were fed during 75 weeks with 25 per cent of Spirulina product. In the same context an important study have been carried out by Jassey et al 1971. It was found that the range of nucleic acids in the alga product is 4.2 % to 4.5 %. This low value (as compared with yeasts and bacterias, in which the content varies from 10 % to 20 %) which enhances the quality of the Spirulina product puts it on the same level as e.g. fish flour derivatives.

UNIDO commissioned research in toxicology

The toxicological evaluation studies supported by UNIDO were performed in the University of Mexico in 1979/1980. Tested were, impurities at the Sosa Texcoco Spirulina plant finished product. In addition, studies in laboratory animals were conducted on toxicity, teratogenicity influence on reproduction, lactation and mutagenic effects.

The analytical studies of heavy metals, pesticides and other elements which are indicated on the table below show that its levels do not exceed the limits established for human consumption as it is proposed by international controlling agencies. Sub-chronic and chronic toxicity tests have been carried out on weanling Wistart rats fed with 10, 20 and 30 % of the Spirulina product. The results were compared with the lot fed with soya and an ordinary control. The summary of the hematological and biochemical data of these studies are presented on the table on page 31. No significant differences in the cellular constituent or on other parameters were noted.

Heavy metals and other impurities of the Spirulina product

| | |
|---|---|
| Cadmium | 0.05 ppm |
| Lead | 1.9 ppm |
| Mercury | 0.24 ppm |
| Selenium | 0.40 ppm |
| Arsen ($AS_2O_3$) | 2.4 ppm |
| Cyanogen (CN) | 1.4 ppm |
| Benzopyrene | 2.6 ppb |

Note: b = billion

Negative results were obtained

| | |
|---|---|
| BHC<br>1,2,3,4,5,6<br>Hexachlorocylohexanol | DDT<br>1,1,1 Tricloro -2 -2 bis<br>(p-Clorophenyl) Ethanol |
| BHC<br>1,2,3,4,5,6<br>Hexachlorocyclohexanol | op'DDD<br>1,1 Tricloro -2 bix<br>(p-Clorophenyl) Ethanol |
| BHC<br>1,2,3,4,5,6<br>Hexaclorocyclohexanol | pp'DDD<br>1,1 Dicloro 2- (p-Clorophenyl)<br>Ethanol |
| BHC<br>1,2,3,4,5,6<br>Hexaclorocyclohexanol | op'DDE<br>2-2 bis (O-Clorophenyl)<br>2-2 p Dichloroethylenol |

In addition no significant difference in pH, glucose, protein, ketones and in the analysis of urine sediment was noted.

Tests on reproduction and lactation with Wistar rats were performed according to the recommendations of Fitzhugh, 1968 for multigeneration studies (Fig. I). These studies were performed for a period of two years and included 584 matings. In each generation the indexes of fertility, gestation, viability and lactation were determined as a function of intake of the Spirulina product at different concentration levels.

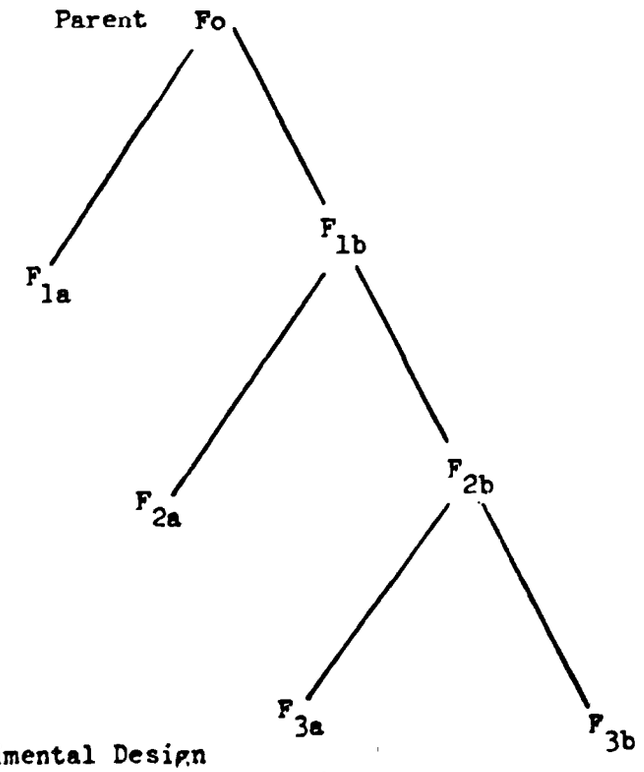



Fig. I - Experimental Design of Reproduction and Lactation

All Fa are weighed, observed, weaned and sacrificed.

All Fb are weighed, observed, weaned, selected and mated.

There were no alterations in weight of the pups as measured on the fourth and the twenty-first day after birth. The table on page 30 shows average results through the three generations. Results in the 2b generation (according to experimental design) are summarized on the table on page 32

In tables on p. 31-38 the results of the teratogenicity studies on rat, mouse and hamster are presented where the Spirulina product was given at 10, 20 and 30 per cent levels, during the organogenesis period.

For the interpretation of the results, the percentage of mothers with affected foetuses and the percentage of such foetuses were taken into consideration. (These results were adjusted using teratogenesis index according to Chamorro, 1974). From the results it is evident that the Spirulina does not cause teratogenic effects on any of the employed species. Eventual risks in genetic make-up due to intake of the Spirulina product were checked by mutagenicity studies on rat and mouse. The table on page 36 summarizes such a study. The results in rats, reveal that the male consumption of the alga product does not modify statistical range in the number of embryonic resorptions during four consecutive weeks of mating with different females. Identical results were obtai d with mice.

These results allow us to conclude that, based on the parameters listed above, the Spirulina product originated from the Sosa Texcoco plant is not toxic to laboratory animals, using the international criteria required for the controls of human foods.

Average reproduction and lactation data for three generations in rats fed Spirulina (584 matings) (Chamorro, G.A.)

| Dietary level | Matings | Pregnancies | Litters born - alive | Pups/female | | | Average weight of the pups at day | | F.I. | G.I. | V.I. | L.I. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Born | 4 days old. | weaned | 4 | 21 | | | | |
| Ordinary control | 19.8 | 18.0 | 16.7 | 9.0 | 8.1 | 7.3 | 8.3 | 40.7 | 91 | 93 | 90 | 90 |
| Soya control | 19.8 | 18.3 | 17.5 | 8.7 | 7.9 | 6.7 | 8.3 | 40.7 | 92 | 96 | 91 | 85 |
| Spirulina 10% | 19.5 | 17.5 | 16.5 | 8.8 | 7.8 | 7.1 | 8.2 | 40.1 | 90 | 94 | 89 | 91 |
| Spirulina 20% | 18.5 | 16.7 | 15.0 | 8.6 | 7.9 | 6.8 | 8.2 | 40.9 | 93 | 95 | 92 | 86 |
| Spirulina 30% | 19.3 | 18.0 | 16.5 | 8.9 | 8.2 | 7.6 | 8.2 | 40.8 | 93 | 92 | 92 | 93 |

F.I. = Fertility index
G.I. = Gestation index
V.I. = Viability index
L.I. = Lactation index

## Rats fed with the Spirulina product for 13 weeks (Chamorro. G.A.)

Hematology

| Dietary level | Hemoglobine. (g/100 ml) | Hematocryt. (%) | Erythrocytes ($10^6$/mm3) | Leucocytes total ($10^3$/mm3) | Differential count % lymph. | neut. | eos. | mono. |
|---|---|---|---|---|---|---|---|---|
| **MALES** | | | | | | | | |
| Ordinary control | 11.7 | 48.6 | 7.3 | 13.9 | 82.3 | 14.4 | 3.0 | 0.3 |
| Soya control | 14.6 | 49.9 | 7.0 | 13.7 | 81.9 | 15.8 | 2.1 | 0.2 |
| Spirulina 10% | 14.8 | 49.7 | 7.9 | 14.4 | 81.5 | 15.1 | 3.1 | 0.3 |
| Spirulina 20% | 13.9 | 50.1 | 7.2 | 14.1 | 82.2 | 15.0 | 2.6 | 0.2 |
| Spirulina 30% | 14.2 | 48.7 | 7.6 | 13.6 | 82.5 | 14.0 | 3.3 | 0.2 |
| **FEMALES** | | | | | | | | |
| Ordinary control | 14.6 | 50.2 | 7.0 | 13.8 | 84.3 | 13.1 | 2.4 | 0.2 |
| Soya control | 14.3 | 49.0 | 6.7 | 13.2 | 86.0 | 12.3 | 1.6 | 0.1 |
| Spirulina 10% | 14.0 | 48.8 | 7.0 | 14.0 | 85.3 | 12.7 | 1.8 | 0.2 |
| Spirulina 20% | 14.1 | 48.7 | 6.9 | 13.9 | 84.8 | 13.6 | 1.4 | 0.2 |
| Spirulina 30% | 14.0 | 49.2 | 6.7 | 12.8 | 83.8 | 13.9 | 2.0 | 0.3 |

Serum analysis

| Dietary level | MALES GOT (RFU) | GPT (RFU) | AP (BLU) | TSP g/100 ml | FEMALES GOT (RFU) | GPT (RFU) | AP (BLU) | TSP g/100 ml |
|---|---|---|---|---|---|---|---|---|
| Ordinary control | 158 | 32.2 | 5.3 | 6.3 | 147 | 25.3 | 5.0 | 6.6 |
| Soya control | 162 | 29.4 | 6.1 | 6.2 | 158 | 30.2 | 3.9 | 6.9 |
| Spirulina 10% | 165 | 31.7 | 5.4 | 6.5 | 143 | 27.4 | 5.2 | 7.2 |
| Spirulina 20% | 171 | 28.2 | 5.9 | 6.4 | 168 | 31.1 | 5.6 | 6.7 |
| Spirulina 30% | 159 | 33.6 | 5.8 | 6.0 | 148 | 28.6 | 5.1 | 7.1 |

GOT= Glutamic oxalacetic transaminase
GPT= Glutamic pyruvic transaminase
AP= Alcaline phosphatase
TSP= Total serum protein
RFU= Reitman-Frankel units
BLU= Bessey-Lowry units.

Reproduction and lactation data of $F_{2b}$ generation of rats fed - Spirulina. (Chamorro, G.A.)

| Dietary level | Matings | Pregnancies | Litters born - alive | Pups/female | | | Average weight of the pups at day | | F.I. | G.I. | V.I. | L.I. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Born | 4 days old. | weaned | 4 | 21 | | | | |
| Ordinary control | 20 | 18 | 16 | 9.1 | 7.9 | 7.1 | 8.2 | 41.0 | 90 | 89 | 87 | 90 |
| Soya control | 20 | 18 | 16 | 9.0 | 8.0 | 6.9 | 8.4 | 40.5 | 90 | 89 | 89 | 86 |
| Spirulina 10% | 19 | 17 | 15 | 8.8 | 7.7 | 7.0 | 7.9 | 40.2 | 89 | 88 | 88 | 91 |
| Spirulina 20% | 20 | 18 | 16 | 8.8 | 7.8 | 7.0 | 8.0 | 41.4 | 90 | 89 | 87 | 90 |
| Spirulina 30% | 20 | 17 | 15 | 9.2 | 8.4 | 7.6 | 8.3 | 40.6 | 85 | 88 | 91 | 90 |

F.I. = Fertility index
G.I. = Gestation index
V.I. = Viability index
L.I. = Lactation index
$F_{2b}$ according to the experimental design Fig.

Effects of Spirulina exposure in rats during organogenesis
(Chamorro, G.A.)

Mother data

| Females (%) | Dietary level: Ordinary control | Soya control | Spirulina 10% | Spirulina 20% | Spirulina 30% |
|---|---|---|---|---|---|
| 1. Implanted, with: | 88.9 | 86.3 | 95.2 | 91.7 | 95.4 |
| 1.1. Normal litters | 70.8 | 78.9 | 75.0 | 72.7 | 76.2 |
| 1.2. Affected litters total | 29.2 | 21.0 | 25.0 | 27.3 | 23.8 |
| with 1.2.1 Resorbed foetuses | 16.7 | 5.2 | 10.0 | 4.5 | 9.5 |
| 1.2.2 Abnormal foetuses | 4.2 | 10.5 | 10.0 | 9.1 | 4.8 |
| 1.2.3 Resorbed and abnormal foetuses. | 8.3 | 5.2 | 5.0 | 13.6 | 9.5 |
| 2. With foetuses when sacrificed | 95.8 | 100.0 | 95.0 | 90.9 | 95.2 |

Litter data

| Foetuses (%) | Dietary level: Ordinary control | Soya control | Spirulina 10% | Spirulina 20% | Spirulina 30% |
|---|---|---|---|---|---|
| 1. Normal | 85.6 | 86.7 | 85.7 | 87.1 | 85.2 |
| 2. Affected total | 14.3 | 13.3 | 14.3 | 12.8 | 14.8 |
| with 2.1. Abnormalities | 1.2 | 3.1 | 3.3 | 2.3 | 2.2 |
| 2.2. Resorptions | 13.1 | 10.2 | 10.9 | 10.5 | 12.5 |
| Average of: | | | | | |
| fetal weight (g) | 3.21 | 3.20 | 3.20 | 3.32 | 3.28 |
| implantations/female | 10.1 | 10.2 | 10.5 | 9.9 | 10.6 |
| foetuses/mother | 9.2 | 9.2 | 9.8 | 9.7 | 9.7 |

Effects of Spirulina intake in mice during organogenses (Chamorro, G.A.)

Mother data

| Females (%) | Ordinary control | Soya control | Dietary level Spirulina 10% | 20% | 30% |
|---|---|---|---|---|---|
| 1. Implanted, with | 90.0 | 95.0 | 95.6 | 87.5 | 90.9 |
| 1.1. Normal litters | 55.5 | 68.4 | 68.2 | 71.4 | 75.0 |
| 1.2. Affected litters total | 44.4 | 31.6 | 31.8 | 28.6 | 25.0 |
| 1.2.1 Resorbed foetuses | 22.2 | 21.0 | 22.7 | 14.3 | 10.0 |
| 1.2.2 Abnormal foetuses | 16.7 | 5.3 | 0.0 | 9.5 | 5.0 |
| 1.2.3 Resorbed and abnormal foetuses. | 5.5 | 5.3 | 9.1 | 4.8 | 10.0 |
| 2. With foetuses when sacrificed | 88.9 | 89.5 | 90.9 | 95.2 | 95.6 |

Litter data

| Foetuses (%) | Ordinary control | Soya control | Dietary level Spirulina 10% | 20% | 30% |
|---|---|---|---|---|---|
| 1. Normal | 73.1 | 77.2 | 79.0 | 82.0 | 83.0 |
| 2. Affected total . | 26.9 | 22.7 | 21.0 | 17.9 | 17.0 |
| with 2.1. Abnormalities | 2.7 | 3.2 | 1.3 | 2.2 | 1.9 |
| 2.2. Resorptions | 24.2 | 19.6 | 19.6 | 15.7 | 15.0 |
| Average of: | | | | | |
| fetal weight (g) | 1.40 | 1.33 | 1.38 | 1.30 | 1.36 |
| implantations/female | 10.1 | 9.9 | 10.4 | 10.6 | 10.3 |
| foetuses/mother | 8.6 | 8.9 | 9.2 | 9.9 | 9.7 |

Effects of Spirulina exposure in golden hamster (Chamorro, G.A.)

Mother data

| Females (%) | Ordinary control | Soya control | Dietary level Spirulina 10% | Spirulina 20% | Spirulina 30% |
|---|---|---|---|---|---|
| 1. Implanted, with: | 90.0 | 94.7 | 86.4 | 85.0 | 94.4 |
| 1.1. Normal litter | 77.8 | 66.7 | 73.7 | 64.7 | 82.3 |
| 1.2. Affected litters total | 22.2 | 33.3 | 26.3 | 35.3 | 17.7 |
| with 1.2.1 Resorbed foetuses | 5.5 | 16.7 | 10.5 | 23.5 | 5.9 |
| 1.2.2 Abnormal foetuses | 5.5 | 5.5 | 5.3 | 0.0 | 5.9 |
| 1.2.3 Resorbed and abnormal foetuses. | 11.1 | 11.1 | 10.5 | 11.8 | 5.9 |
| 2. With foetuses when sacrificed | 100.0 | 94.4 | 89.5 | 94.1 | 94.1 |

Litter data

| Foetuses (%) | Ordinary control | Soya control | Dietary level Spirulina 10% | Spirulina 20% | Spirulina 30% |
|---|---|---|---|---|---|
| 1. Normal | 92.3 | 87.1 | 84.8 | 85.3 | 88.3 |
| 2. Affected total | 7.7 | 12.9 | 15.2 | 14.6 | 11.6 |
| with 2.1. Abnormalities | 2.9 | 2.5 | 2.0 | 2.0 | 1.6 |
| 2.2. Resorptions | 4.8 | 10.4 | 13.2 | 12.6 | 10.0 |
| Average of: | | | | | |
| fetal weight (g) | 1.57 | 1.60 | 1.53 | 1.64 | 1.55 |
| implantations/female | 11.5 | 11.2 | 10.7 | 11.6 | 11.1 |
| foetuses/mother | 10.9 | 10.6 | 10.4 | 10.8 | 10.6 |

Results of the dominant letal test on rats fed with Spirulina at dietary level of 30%. (Chamorro, G.A.)

| Treatment | Soya control | | | | Spirulina | | | |
|---|---|---|---|---|---|---|---|---|
| | weeks | | | | weeks | | | |
| Parameter | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Females mated | 18 | 18 | 19 | 18 | 20 | 20 | 20 | 18 |
| Pregnant females | 16 | 15 | 17 | 16 | 18 | 17 | 19 | 19 |
| Total implants/pregnancy | 11.7 | 10.6 | 11.5 | 10.7 | 10.4 | 11.2 | 10.6 | 11.1 |
| Live implants/pregnancy | 10.8 | 9.6 | 10.7 | 9 8 | 9.6 | 10.5 | 10.0 | 10.2 |
| Resorptions/pregnancy | 0.9 | 1.0 | 0.8 | 0.9 | 0.8 | 0.7 | 0.6 | 0.9 |

Effects of Spirulina intake in rats during organogenesis.
Adjusted teratogenic index* (Chamorro, G.A.)

| % Spirulina | In the mothers | In the foetuses |
|---|---|---|
| 10 | 5.0 | 11 |
| 20 | 8.0 | - 5.8 |
| 30 | 3.5 | 1.7 |
| Soya control | - 11.6 | - 1.2 |

Table Effects of Spirulina intake in mice during organogenesis.
Adjusted teratogenic index*

| % Spirulina | In the mothers | In the foetuses |
|---|---|---|
| 10 | - 0.3 | - 2.2 |
| 20 | - 4.4 | - 6.2 |
| 30 | - 9.6 | - 7.3 |
| Soya control | -23.0 | - 5.7 |

*Chamorro, G., 1972. Doctoral Thesis, University of Montpellier.

Effects of Spirulina in-take in golden hamster- during organogenesis. Adjusted teratogenic index*
(Chamorro, G.A.)

| % Spirulina | In the mothers | In the foetuses |
|---|---|---|
| 10 | - 10.5 | 2.6 |
| 20 | - 0.3 | 1.9 |
| 30 | - 23.4 | - 1.5 |
| Soya control | 14.3 | 5.6 |

* Chamorro, G., 1972. Doctoral Thesis, University of Montpellier.

UNIDO commissioned research in decolourization and pigment recovery

a) Introduction

For the production of a high quality protein product for human consumption a decolourization process combined with pigment recovery was carried out by G. Brown. Selected experiments are summarized below.

b) Decolourization with intense light treatment was performed with a 0.3 % aqueous algae solution. The solution was decolourized in 24 hours under 5,000 foot candle illumination. No change in colour was apparent on illuminating a 4 % solution at 100,000 foot candles for 24 hours. These results indicate that the above 0.3 % solution is the minimal requirement for the decolourization process.

Light treatment needs a long exposure causing a destructive influence on the pigment = photoinduced oxidation. Such oxidation (Ref. Strietelmeier, D. and Koch, R.B.) is reported to oxydize the cell components (causing rancidity) and also reduce the yield of protein from 60 % to 6 %.

This process may be more effective as a means of brightening the final product from which most of the colour has been removed by other means.

c) Decolourization by chemical bleaching using hydrogen peroxide

A suspension containing 5 % peroxide with 1% Spirulina algae was stirred at 40°C for 23 hours. The colour of the suspension changed from the original deep green to yellow-brown. However, reaction of peroxide with unsaturated fatty acid substrates could lead to the formation of polymerized products, suspected of having carcinogenic properties. Further, hydroperoxide derivatives of fatty acids have been shown

to have a deleterious effect on the synthesis of lipids in the livers of rats also it was found that the bleaching can have an adverse effect on the nutritive value of the product. It was established that the bleached Spirulina end product was unfit for human consumption due to its rancidity.

d) Decolourization by enzyme treatment

At least three specific enzymes are required to achieve complete decolourization by removing biliprotein pigments, chlorophyll and carotenoids. Some of the enzymes are contained in Spirulina algae but their autolytic properties contribute to the reducing of the protein yield.

Enzyme treatments are characterized by slowness of the reaction and a lower yield (Shino, K. and Hayami, H.).

In addition the need to process in aqueus form would necessitate and add to solvent extraction and evaporation cost. For the three different classes of pigments, three different classes of enzymes are needed. Some of these enzymes are not commercially available. Furthermore, those available, are invariably contaminated with other enzyme activities such as:

1) Protein carbohydrate hydrolyzing enzymes:
2) Fatty acid oxidizing enzymes:
3) Both 1) and 2) would alter the physical state and nutritive value of the protein and would lead to losses and to the higher cost of the recovery operations.

e) Decolourization by solvent extraction

The raw material from Spirulina can be either in dry powder or dewatered paste form. Extraction conditions can be controlled to minimize loss of protein. As solvent can be recovered for re-use, there is no direct chemical consumption. Chlorphyll and carotenoid pigments can be removed by organic solvents, particularly the low boiling alcohols. A block diagram of a solvent extraction is described below.

Block-diagram - Description of the pigment extraction process (Ref. Gordon Brown, PRC, Vol. V).

The final product is fed to an in-line mixer (1) where it is blended to a weight ration of 4 parts solvent to 1 part dried algae product with recycled wash liquor tank (6). The slurry is pumped via a positive displacement pump through a single stage high temperature/short time extraction unit (2, 3 and 4). The extraction unit consits of two scraped surface heat exchangers for heating (2) and cooling (4), with an intermediate agitated slurry surge tank (5), a positive displacement pump serving to control the back pressures through the extraction unit.

The extracted slurry is pumped at a controlled rate to a screen bowl centrifuge. Miscella is pumped to a storage unit (13) for subsequent concentration while the separated solids are conveyed to a re-slurry tank (8) for washing where fresh solvent (after rectification) 45°C is added at a 3 : 1 weight ratio (solvent : solid). The re-slurried solids can be either recycled to the original centrifuge (7) for separation (in which case the centrifuge would be operating in a semi-continuous manner) or sent to a second centrifuge (9) (both centrifuges providing continuous operation). The wash liquor from the second separation process is made up with either fresh ethanol and water or with re-distilled alcohol adjusted solvent before being recycled to the in line mixer for blending with the feed solids.

The washed extracted protein solids are desolventized (10) and cooled (11) to 48°C by indirect heat exchange before being milled and bagged.

Miscella from the first separation process is fed to a flash evaporator (13) operating either under vacuum (requiring higher capital investment but lower thermal cost) or at atmospheric pressure (requiring lower capital, but higher thermal cost) where 70 % of the solvent is removed using the intrinsic heat of the hot solvent (14).

The concentrated miscella is sent to one of two pot stills (14) for further concentration to 70 % solis level or that which is found to be pumpable at the temperature necessary to minimize heat damage to the pigment. (The idle still collects miscella from the evaporator while the other concentrates).

The concentrated pigment is slowly pumped to a heated pigment blender/drier (15) previously charged with diatomaceous earth for further removal of the liquid from the tar-like pigment concentrate. The wet mass so formed after blending is vacuum dried to a flowable consistency. The dried pigment by-product is packed in cans under nitrogen to avoid oxidation.

Overhead vapours from the desolventizer (10), evaporator (13), pot still (14) and the pigment blender/drier (15) are condensed and the condensates fed to a rectifying unit (16) to remove accumulated water. Rectified solvent (93.9 % w/w alcohol) is fed to a solvent work tank and made up with fresh ethanol and water.

To avoid oxidation of the product, provision is made for nitrogen flushing of the pot stills (14) and the pigment blender drier (15), before they are charged with the concentrated miscella and the concentrated pigment respectively.

The solvent extraction was chosen because with the developed modification a single solvent ethanol effected the removal of the pigment in a simple processing operation. Unfortunately, the predicted market value of the decoloured protein powder and initial value figures for the extracted xantophyllswould not favour the decolourization process at a current US$ 4/kg (July 1980) market prize of the dried algae product.

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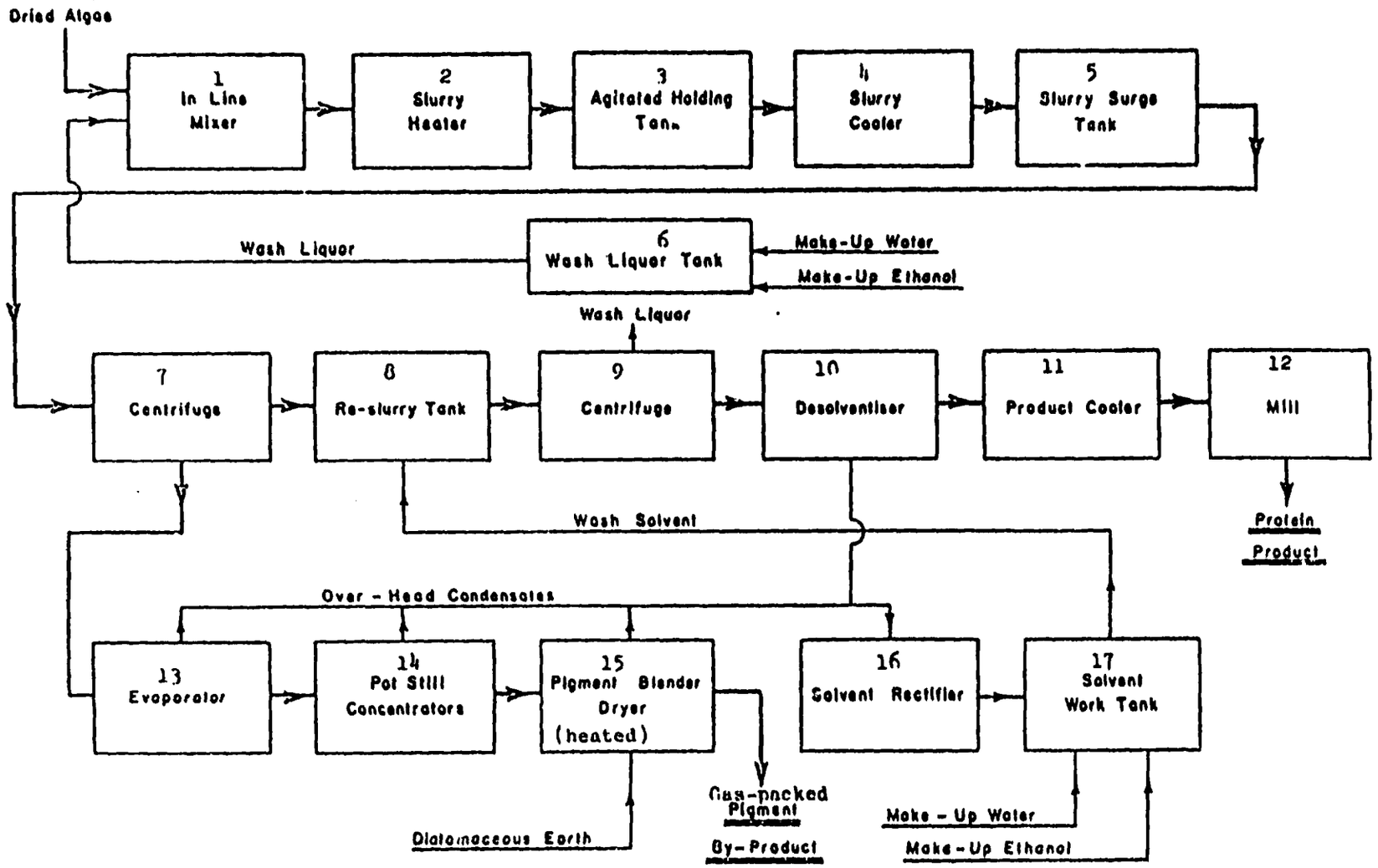
Note: No. 1 to 17 refer to the block diagram.




BLOCK DIAGRAM - PIGMENT EXTRACTION PROCESS

f) Ethanol extraction

One hundred grams of SM paste frozen for 1 month at -35°C were thawed and mixed with 500 ml of absolute ethanol (Solvent: Solids ration 25.6 w/w, blended with a high speed blender at 50°C for 5 miniutes and centrifuged at 5000 rpm/10 min.

The entire procedure was repeated twice for the 3 extractions with the following results:

| Extraction No. | 1 | 2 | 3 |
|---|---|---|---|
| Colours of supernatant | dark green | dark yellow | yellow |
| Colours of residue | light green | blue green | blue |

Final recovery of blue residue after three extractions was 9.79 g for a solids yield of 63 per cent.

The blue residue obtained by successive low temperature extractions with ethanol contains phycocyenin, the major blue pigment of blue-green algae. Phycocyanin is a biliprotein - a protein which has a bile pigment, chemically bonded to the protein chain. The pigment (or chromophore) is phycocyanobilin.

g) Methanol extraction

The good detachment capabilities of methanol for the extraction of the pigment are associated with the size of the alcohol molecule able to penetrate to the sites of the prosthetic group of the pigment attachment to the protein.

Extraction parameters can be summarized in the following table:

Soxhlet Extraction of Spray Dried SM Algae

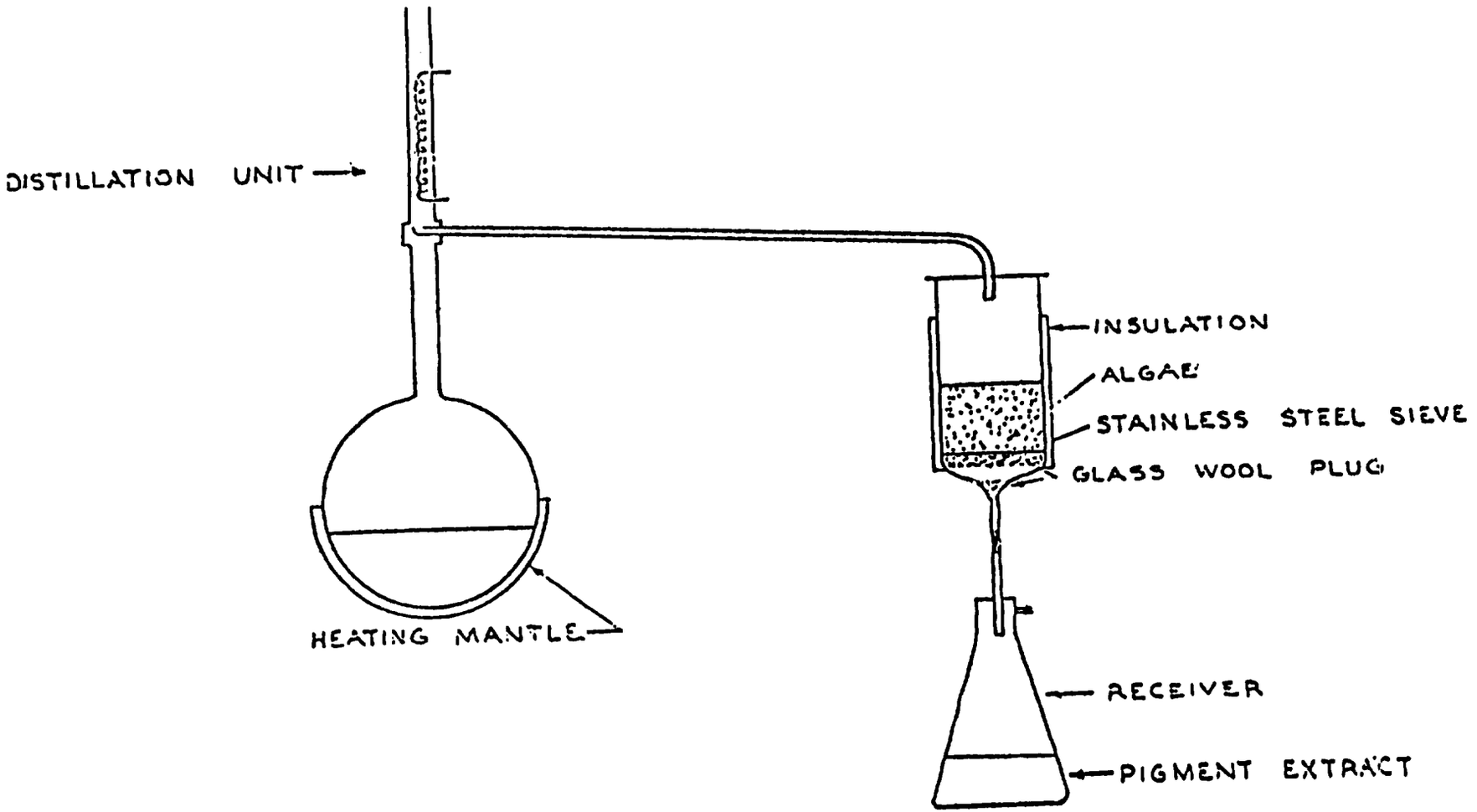
| Solvent | Extraction Time (hrs) | Final Absorbance à 436 (10 : 1) | Product Yield (%) | Product Colour |
|---|---|---|---|---|
| Methanol | 1.5 | 1.02 | 60 | blue |
| Ethanol | 2.5 | 1.05 | 72 | pale green |
| Iso-propanol | 1.5 | .88 | 82 | green |
| Acetone | 4.0 | .95 | 81 | deep green |
| Ethylene Dichloride | 3.0 | .71 | 74 | deep green |
| Hexane | 4+ | .36 | 79 | deep green |

h) Decolourization by percolation extraction

Percolation was investigated at a pilot scale operation. The unit was charged with 3.66 kg of dried algae grown in Sosa Texcoco basin. Condensed alcohol at 50°C was allowed to percolate over the algae at a rate of 75 - 100 mls/minute.

After 15 hours of percolation at 40 - 50°C and 50 litres of ethanol, pigment was still leaching out of the algae. The extraction process was stopped and the solids dried. The dried material had greenish blue colour. The yield of material on a dry solids basis was 86 %.

The initial extract was very concentrated in pigment with a carotene strength of 0.29 mg/ml and a xantophyll content of 0.28 mg/ml. Precipitation of insoluble matter appeared to have taken place as seen when the extract was decanted from its container. (Brown, G.). (Fig. shown in page 46).




PERCOLATION EXTRACTION OF DRIED SOSA TEXCOCO SPIRULINA ALGAE

Marketing

At present (July 1980) the application of the Sosa Texcoco Spirulina plant end-product is twofold: for animal and for human consumption.

Animal utilization of the Spirulina product

In the case of weanling pigs, the digestibility of Spirulina protein increases rapidly indicating that this material helps the development of digestive enzymes in the animals (Febrier, C.).

Within the range 5 - 20 % of the feed ration, Spirulina is adequately utilized by poultry. However, when high concentrationsof salts are introduced to the feeds, the portion of Spirulina which can be used is limited to a maximum of 12 per cent. It has been found that the level of pigmentation on the flesh and on egg yolks is equal or better than that obtained in comparable tests with synthetic carotenes. These effects can be obtained with 3 to 4 per cent Spirulina (Avila, E. and M. Cuca, Gutton, M.).

Five to ten per cent of Spirulina when added to the fish feed for 14 - 16 days causes a significant improvement of the red pigmentation in carp nishikigai or the gold fish kingyo. Use of the Spirulina enables to preserve the red colour for a long time and maintain its gloss.

Human utilization of the Spirulina product feeding trials

The absorption of Spirulina protein fed to adults and children at Bichat Hospital in France was found to be adequate. Although the faecal nitrogen was found to increase slightly, the gain in weight was normal. In another experiment, undernourished adults and children were fed 140, 200 and 190 g of protein daily of which 50, 50 and 100 g were provided by Spirulina. No noticeable increase in urin acid in the blood occured, indicating the absence of any effect on the metabolism of the nucleic proteins (Sautier, C.).

In clinical tests in Mexico with children suffering from third degree malnutrition, dietetic preparations of Spirulina were formulated and were alternated with reference diets of milk preparations and soya preparations. The study involved 30 periods each of 4 days per child during which the alternated diets were fed.

It was observed that the absorption of nitrogen from the ingested Spirulina was less than that from the whole soya milk; however, of that absorbed, the nitrogen retained from the Spirulina exceeded that from the soya and was equal to that for whole milk, being exceeded only by human milk.

On the basis of the ingested nitrogen it can be concluded that spirulina is better than soya, but is exceeded by whole cow's and human milk. (Galvan Mo.).

The actual (July 1980) figures of Spirulina products sales as supplied by the Sales and Export Department of the Sosa Texcoco Spirulina operation (see organigram) are presented in the Fig. on p. 49

The quantitative sales of this product, the changes in the unit price and the quantitative sales potentials for the next five years are presented in figs on p.50, 51 and 52 respectively.

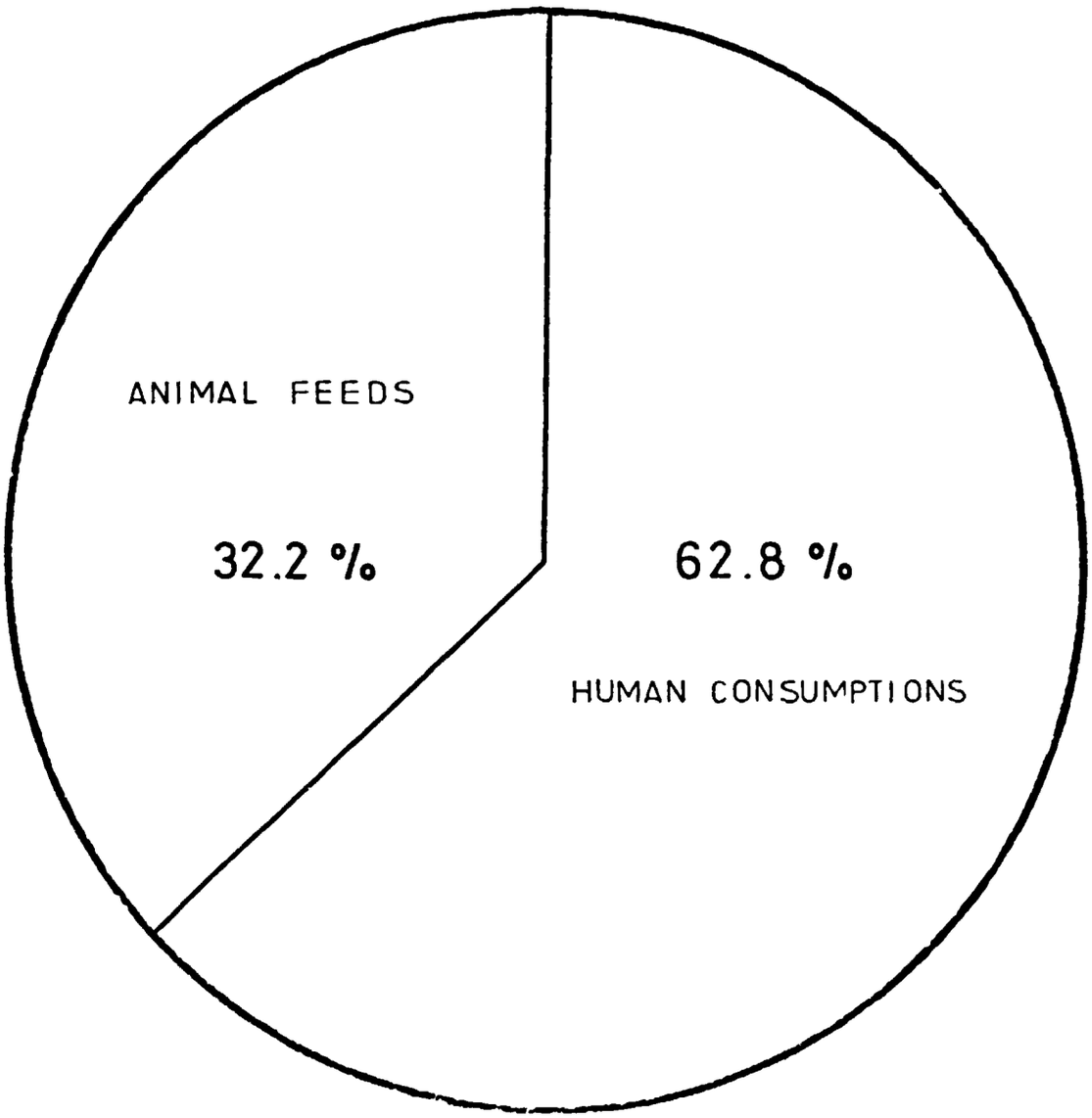
The Spirulina products as of July 1980 are sold commercially in the following varieties:

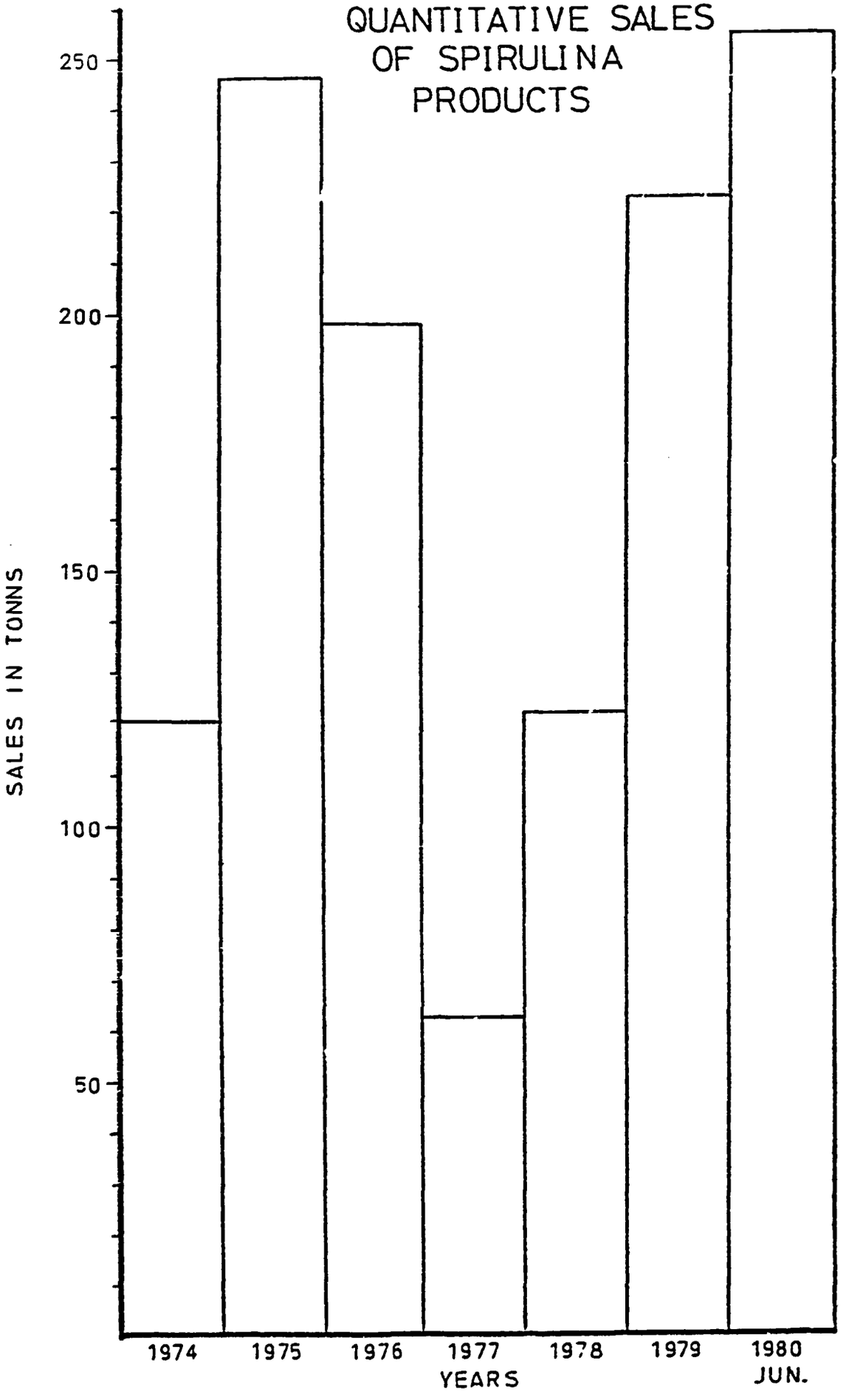
a) As tablets consisting of 100 % of Spirulina as an active ingredient. Therefore, colour sui generis resembling sea vegetables. The average weight: 500 mg, strength: 3.0 kg, desintegration time: 90 minutes in 37°C water.

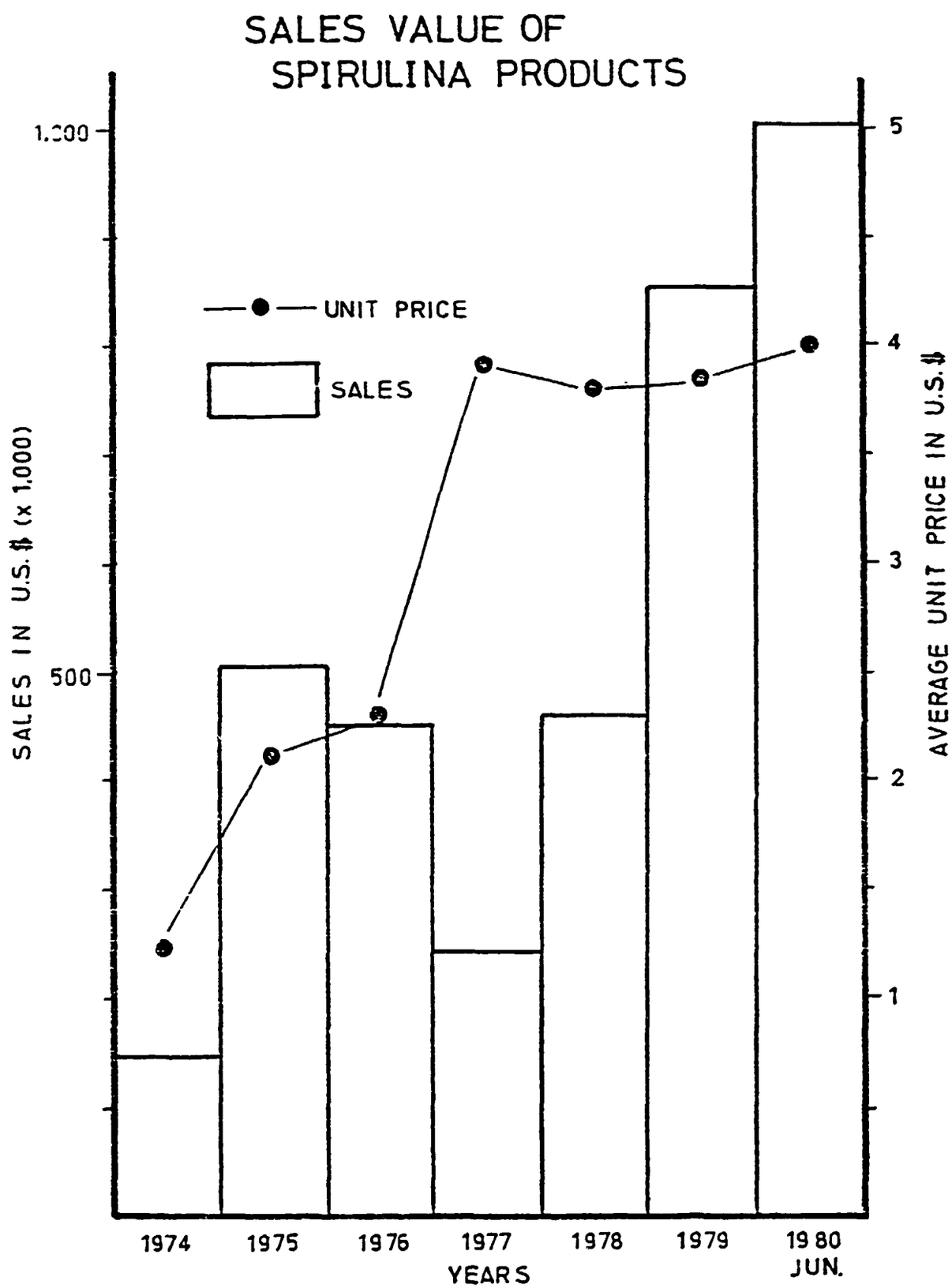
b) Cookies which are supplemented with 1 % of Spirulina;

c) Candy bars which are supplemented with 5 % Spirulina.

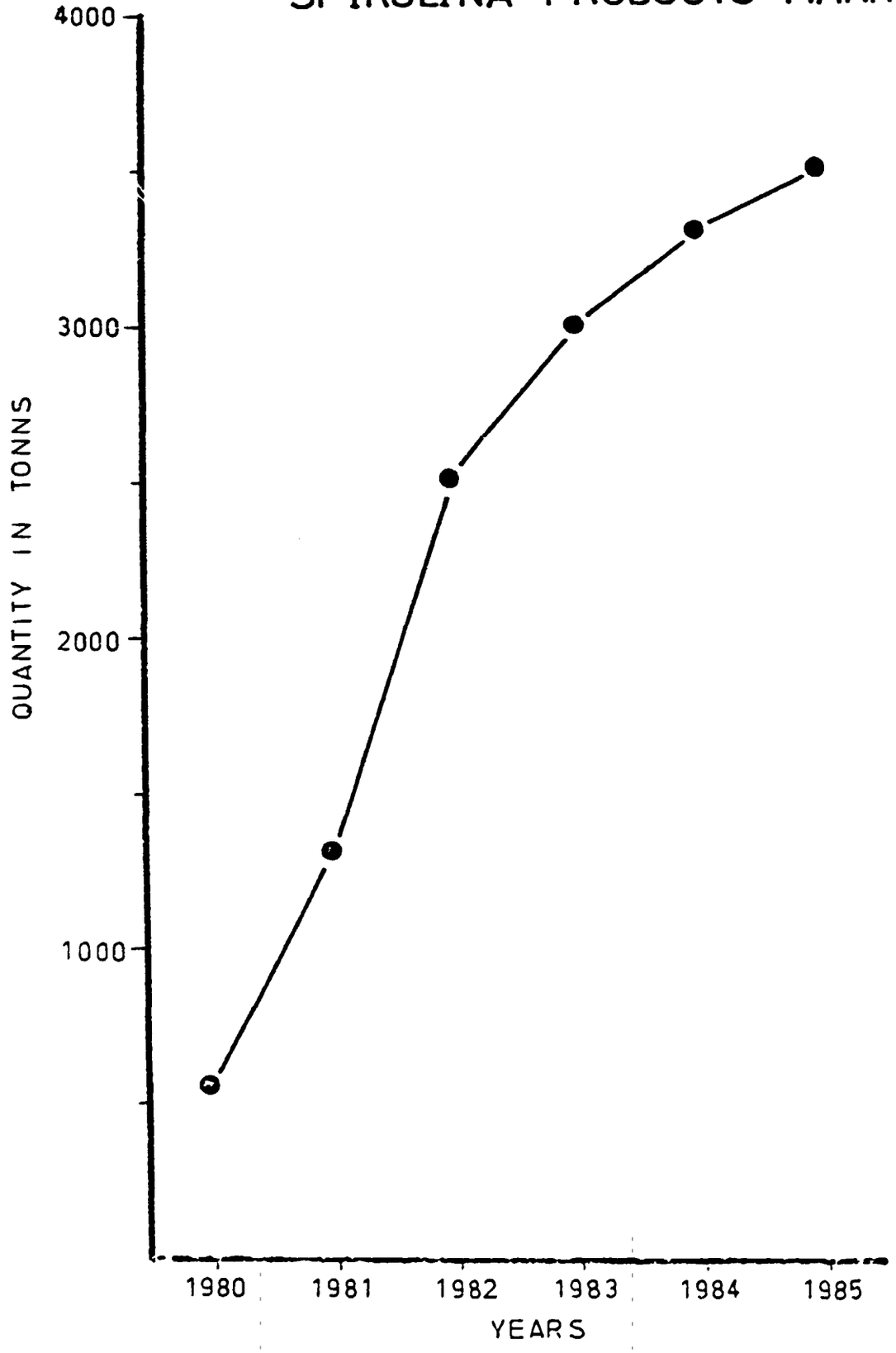
# APPLICATION OF SPIRULINA PRODUCTS -1980-




QUANTITATIVE SALES OF SPIRULINA PRODUCTS
SALES IN TONNS
250
200
150
100
50
1974
1975
1976
1977
1978
1979
1980 JUN.
YEARS


SALES VALUE OF
SPIRULINA PRODUCTS
1,000
500
UNIT PRICE
SALES
SALES IN U.S.$ (x 1,000)
AVERAGE UNIT PRICE IN U.S.$
5
4
3
2
1
1974
1975
1976
1977
1978
1979
1980
JUN.
YEARS

# FUTURE POTENTIAL OF SPIRULINA PRODUCTS MARKET



The management is considering catering the growing and promising vitamin market. Especially attractive are the future marketing plans for sales of vitamin $B_{-12}$. The content of this vitamin equals 255 mcg $B_{12}$/100 g of the edible protein (Clement, 67) the biggest as compared with fermented soy foods, other algae, dairy products, meat and fish.

The total sales figures of 1980 indicate that the plant with the present capacity, producing by the average 1 ton a day and working in 3 shifts will not suffice. New modules similar to the original one need to be constructed.

Description of the processed algae

a) Taxonomy

Spirulina geitleri I. de Toni belongs to the family Oscillatoriaceae, order Nostocales, division Cyanophyta. The following synonyms were cited in the world literature by describing the same algae:

Arthrospira máxima Setchell and Gardner (Gardner, N.L., 1917).

Spirulina máxima (Pinta M., F. Busson, 1969).

Oscillatoria pseudoplatensis nom., nov. (Bourreley, P., 1970)

Spirulina Platensis (Morty F., and F. Busson)

Arthrospira platensis (Dangeard, D., 1940)

b) Morphology

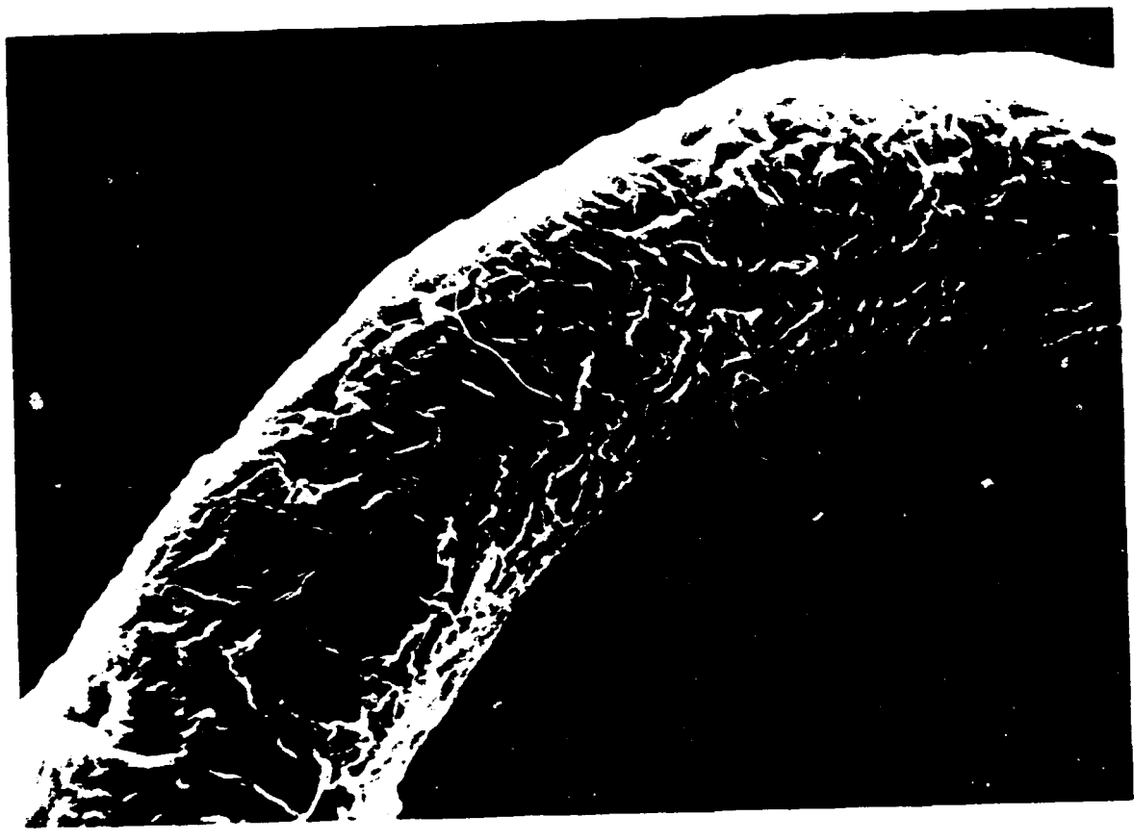
Under 350 magnifications, SG appears as a trichom 7 - 9 $\mu$ in diameter, in shape of a regular open spiral, of 3 - 8 whorls, 40 - 60 $\mu$ diameter, 70 - 8 $\mu$ long, slightly tapered at the ends, cells 5 - 7 $\mu$ long, not narrowed at the articulations, rather roughly granular protoplasm with granules often gathered alongside the cross divisions: round, slightly thickened external walls of the apical cells; verdigris colour. Living cells appearance under the microscope is presented on pages 55 and 56.

Living

750 x

Living

900 x

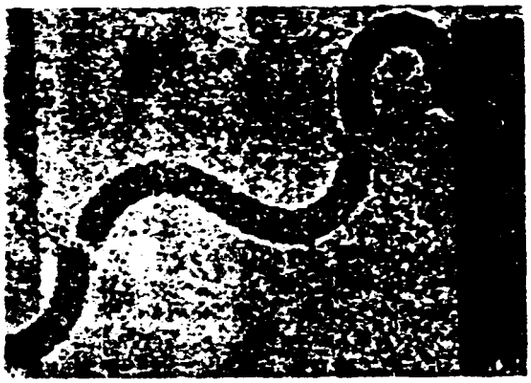


EM 7500 x

Specimen live fixed

The reproduction of the Spirulina begins by the necrosis of some cells forming transversal membranes which result in various fragments of the filament in approximately eight minutes. New filaments grow longitudinally until they reach the mature stage as illustrated below (Nakamura, H.).

In the natural environment of Sosa Texcoco Caracol it takes 2 to 4 days to duplicate the algal mass. Due to their gas vacoules, specific to the blue green algae, the Spirulina filaments are buoyant and float on the surface of the water where they often become tangled into lumps: if the photosynthesis is very intense, the turgor pressure can rise too much and the gas vesicles collapse which makes the cells lose their buoyancy and causes the algae to sink to a lower light intensity at an inferior level of the water.



Estimate of daily growth in $A_1$, $A_2$ and $A_3$ basins

An estimate of growth was worked out over a period of 66 days from 8 August 1975 at which date the basin of 10 hectares had been isolated, to 13 October 1975, in the following manner (Goldenberg, UNIDO)

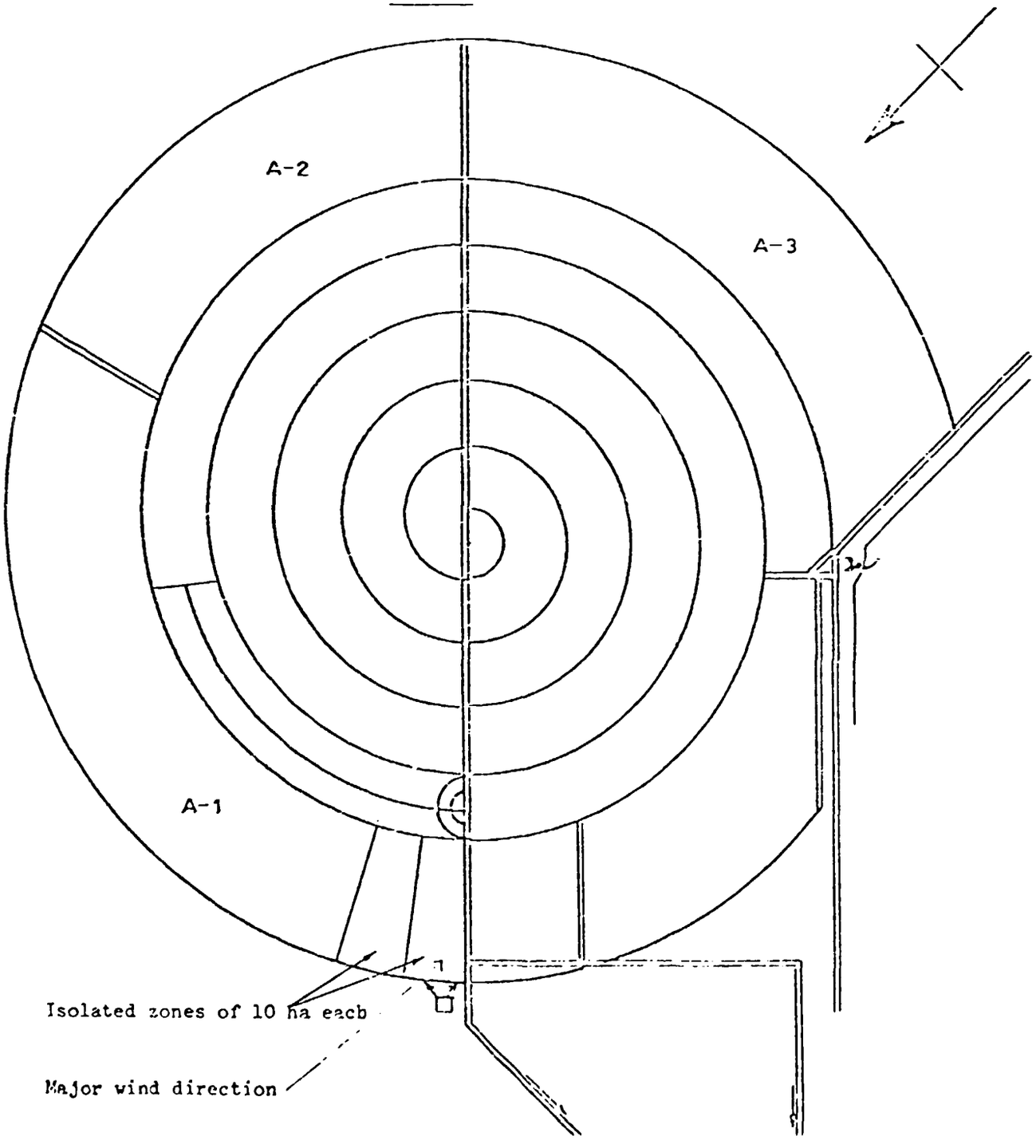
| | |
|---|---|
| Mean algae concentration on 8 August 1975 | 117 mg dry algae/l |
| Mean algae concentration on 13 Oct. 1975 | 129 mg dry algae/l |

Cultivation

The Spirulina grows in Caracol (tranlation: snail) storage basin of Sosa Texcoco used for the solar concentration of sodium salts by evaporation. The sector of the Spirulina growth has a surface area of 100 hectares volume $6.10^8$ liter. Spirulina grows in zones A-1, A-2, A-3, Fig.

For the exploitation by the Spirulina plant two zones were artificially isolated see below.

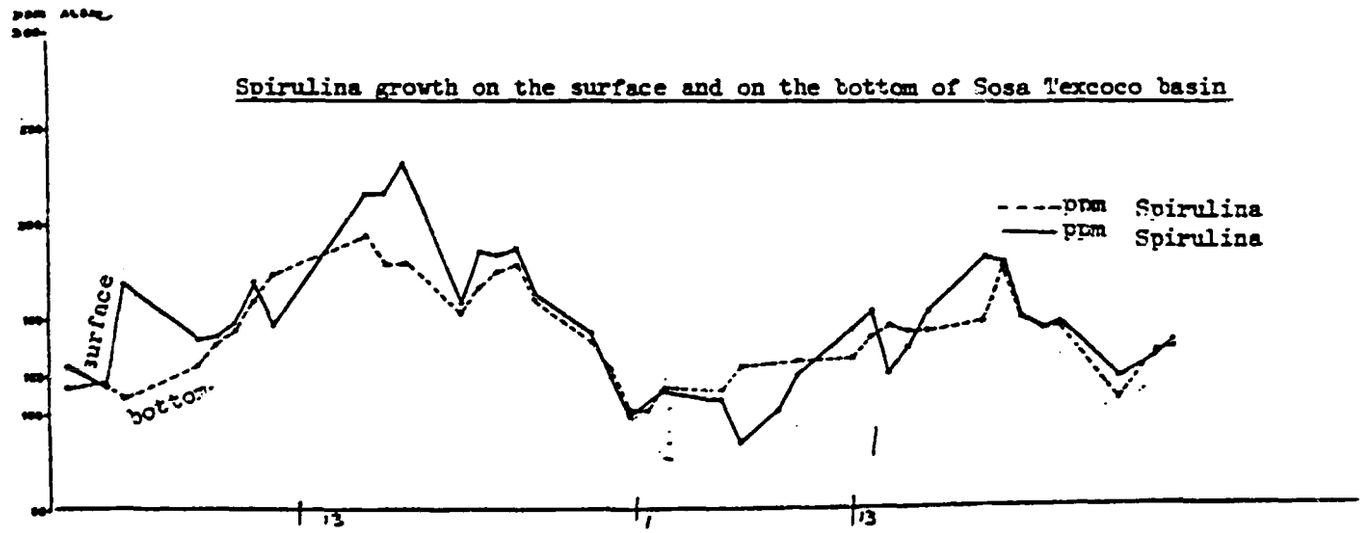
CARACOL



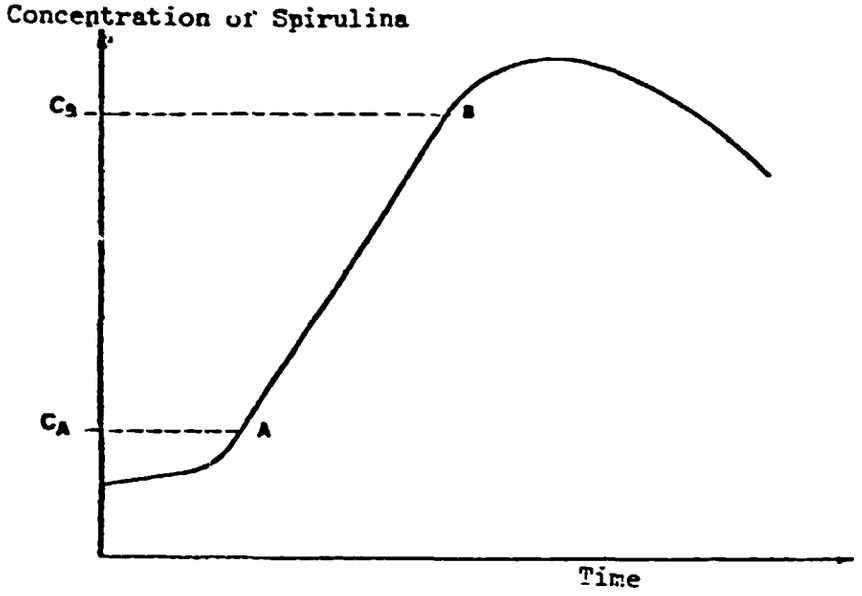
b) The concentration of Spirulina in the basin of Sosa Texcoco

The fluctuation of the algae biomasses vary between 60 to 240 ppm. This is presented below. There exists a correlation between the biomass content on the bottom and on the surface of the basin. With some insignificant exceptions the measurements taken on the surface correspond to those taken on the bottom. From the data given elsewhere (Goldenberg, UNIDO) there was no correlation between the biomass production and the nitrate nitrogen content of the medium. The nitrate nitrogen is considered as the preferred nitrogen source without detriment to the algae and with no adverse effect on outward Ph drift (while on the contrary, ammonium salt as the nitrogen source can become toxic). Experiments provide support for the idea that nitrate is reduced to ammonia before its entry into general nitrogen metabolism of the Spirulina.
(Nicholas, D.J.D.)

Spirulina growth on the surface and on the bottom of Sosa Texcoco basin



The theoretical growth curve of the Spirulina in Sosa Texcoco Basin is shown below.




The crossings of the lines $C_A$ and B depend on the conditions of the SM culture and represent the logarythmic growth phase. The constant nutritional conditions described above the lines A and B vary between 70 - 80 ppm to 270 - 300 ppm dry Spirulina/liter. In favourable season the yield of Spirulina could reach 10 $g/m^2$/day. To exceed this amount a periodical addition of specific chemical compounds is necessary. (Goldenberg, UNIDO).

The rate of growth of Spirulina culture has a maximal value and a period when the crop is in jeopardy. This could be due to inhibiting properties caused by formation of a growth inhibitory substance which could be a polyene interacting with sterols present in the membrane. There is evidence of complex formation of antibiotics and sterols (Lampen et al.). Polyenes may either inhibit synthesis of sterols, or replace the sterols as essential metabolic reaction.

Growth of algae bring many reactions into play. Crop may grow at a normal rate for several days and then be rapidly destoryed.

During this period the mean temperature of the basin was 19°C (Figure shown on page 64). The estimated yield of the basin was about 65,000 kgs which are split up as follows:

| | |
|---|---|
| - yield of dry algae | 49,630 kg |
| - losses estimated at 30 % of yield of dry algae | 14,890 kg |
| - difference between initial and final concentration of the basin of 10 hectares | 980 |
| Total yield: | 65,500 kg |

This corresponds to a daily mean growth of about 9,9 g/m2. Under these conditions - and subject to achieving the necessary investment - the potential yield of the basins $A_1$. $A_2$ and $A_3$ (500 hectares) of Sosa Texcoco would be about 50 tons per day during most of the year.

Research on lakes on which algae are growing have revealed a seasonal change in the amount of nutrient necessary. Based on the dry weight basis of the algae Mackenthun (Mackenthun, K.M.) experimenting with lake plankton with a predominant algae population found that the contents of this plankton vary as follows:

spring - 400 kg/ha summer - 140 kg/ha

autumn - 360 kg/ha winter - 110 kg/ha (dry weight).

A typical population could thus tie up about 36 kg/ha N and 3.6 kg/ha P.

Blue-green algae are 6.8 % N (approx.) and 0.69 % P. Thus an algal population could theoretically tie up about 17 kg/ha N and 1.7 kg/ha P.

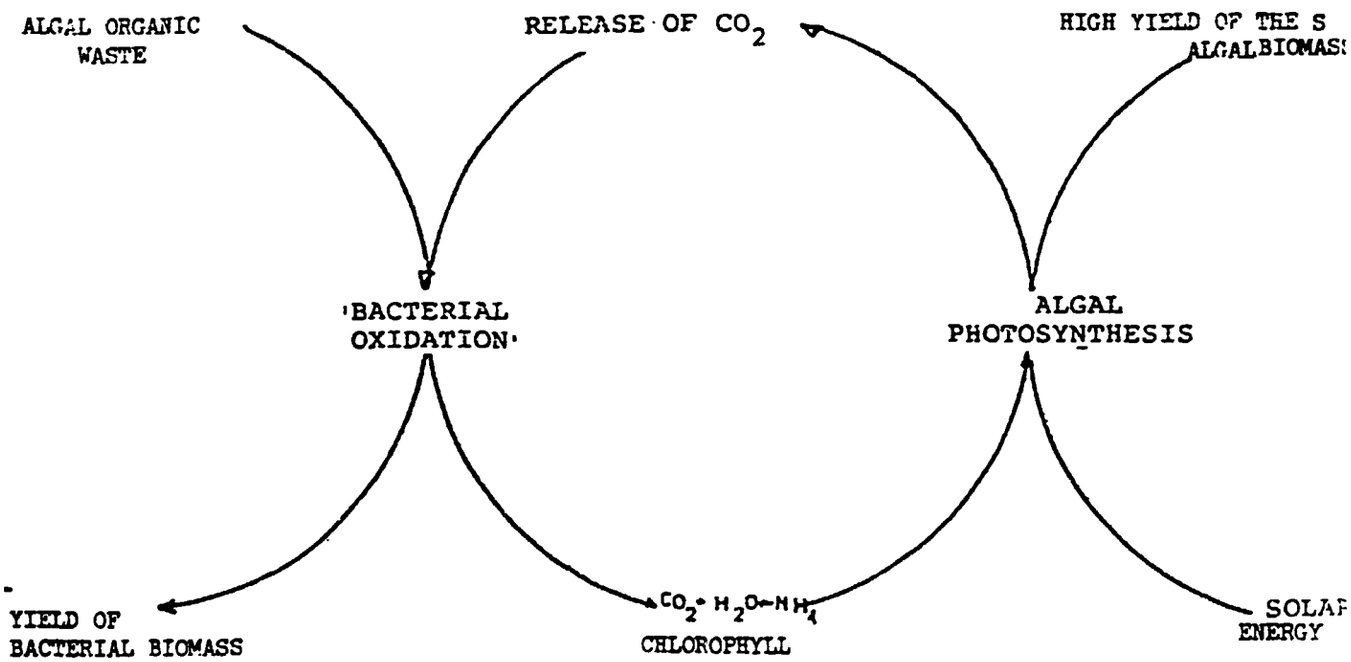
In another experimental pond with exclusive algal growth (Fekete, A., D. Riemer and H.L. Motto) the amount of N removed in the tissues of the alga was equivalent to a concentration of 23 ppm in the total pond water. Yet the maximum content determined at any time in the pond water was 4.1 ppm. Similarly, the amount of P removed by the alga was equivalent to a concentration of 1 ppm of P in the pond. Yet water analysis never revealed a P concentration of more than 0.04 ppm. Thus the N levels in the pond were apparently replenished from bottom sediments, or ammonia in rain water, or N fixation, or all three. The P levels in the water were presumed to have been restored from reserves in the bottom sediments. It can be concluded, therefore, that:

a) Large amounts of N and P can be removed from a pond by harvesting filamentous algae.

b) The effect on dissolved N and P levels in the water may be slight in relation to the amount removed, due to a continuous resupply.

c) Continual removal of the algae may alter the condition of the pond so that only a limited amount of algal regrowth will occur.

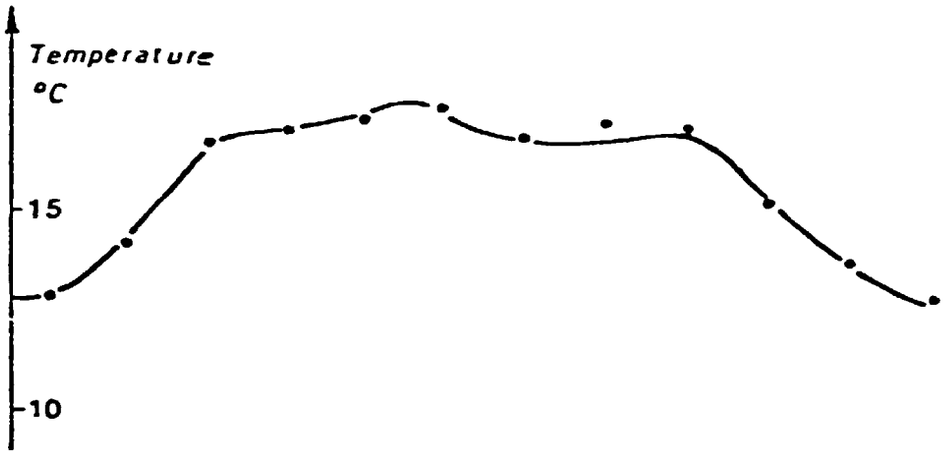
The constant reuse of the same culture tank is possible since the reflux brings to the 2 tanks (in p.58) small filament which were not used in the production process. Mixed with bacteria in this natural habitat their biomass increases due to the autopurifications system presented below.



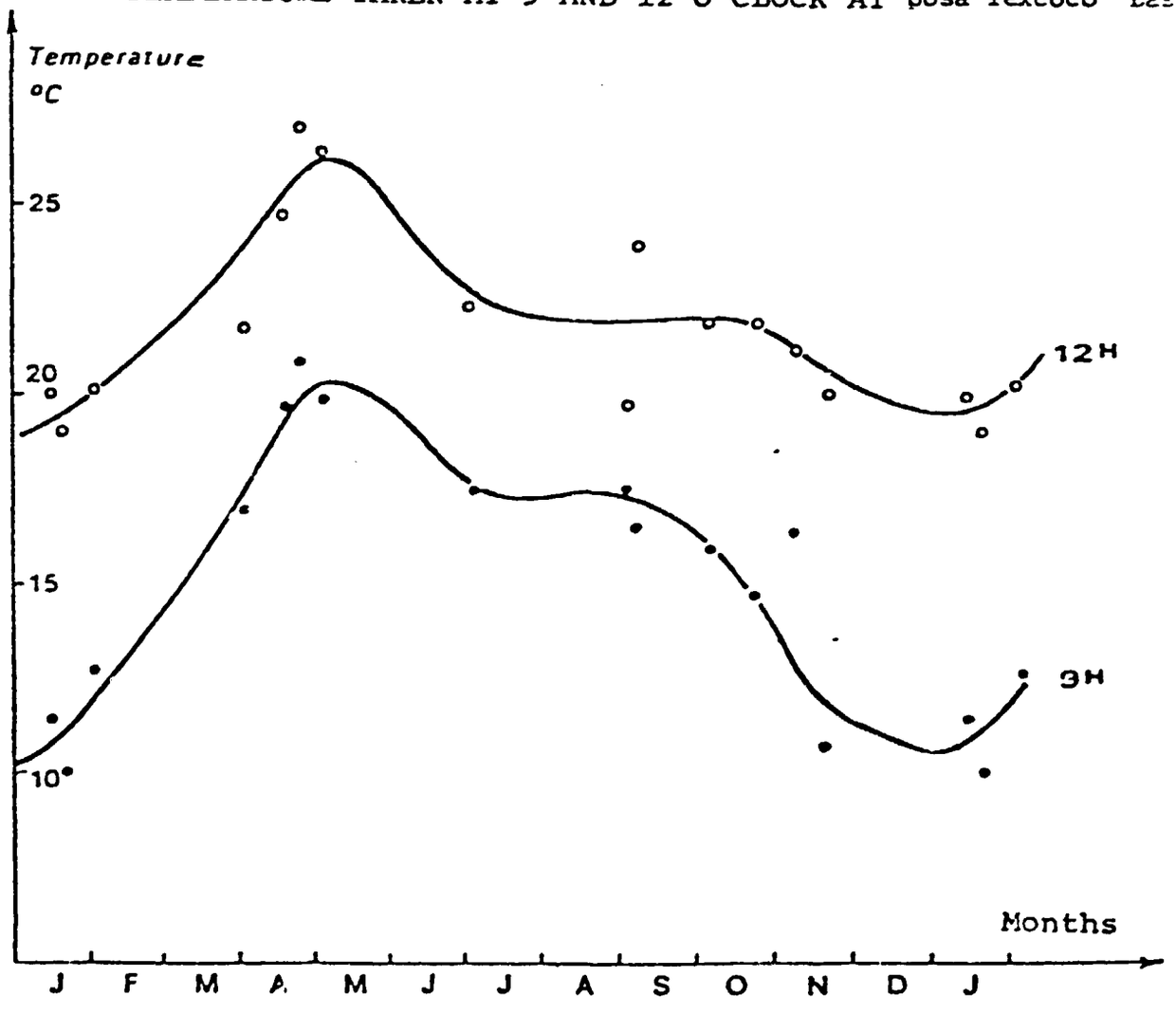

SYMBIOSIS OF ALGAE AND BACTERIA

IN THE CYCLE OF $CO_2$ AND $O_2$

AVERAGE MONTHLY TEMPERATURE OF Sosa Texcoco Basin




TEMPERATURE TAKEN AT 9 AND 12 O'CLOCK AT Sosa Texcoco Bas



d) Aeration with $CO_2$

Carbon source in form of $CO_2$ is indispensable for a good growth of the Spirulina according to the chemical reation:

$$CO_3^{--} + H_2O + CO_2 \rightleftharpoons 2\ HCO_3^-$$

$$HCO_3^- + 2H_2O \xrightarrow[SM]{} (CH_2O) + H_2O + O_2 + OH^- \text{ (Syret, P.J.)}$$

The formation of $(CH_2O)$ is important in light metabolic pathways of nitrate reduction and assimilation according to:

$$2\ (CH_2O) + NO_3^- + 2H^+ \longrightarrow NH_4^+ + 2\ (CO_2) + H_2O \text{ (Bongers, L.H.J.)}$$

(Reduction of nitrate ion to the level of ammonium). The ion $OH^-$ take part in the assemilation pathway of $NO_3^-$ (which is also accompanied by the release of $OH^-$). (Nicholas, D.J.D.)

$CO_2$ plays also an important role in lowering the pH of the medium beyond 11.3. Nutritive elements like carbonate phosphates, calcium and magnesium, important for the growth of Spirulina diminish quantitatively when pH of the medium increases. Since high growth rates are dependent on efficient exchange of gases, a large gas-liquid interface is important. (Lewin, R.A.).

e) Temperature and the illumination of Sosa Texcoco basin

The range of temperature suitable for the growth of Spirulina is significantly wider than that of other algae species. In the Sosa Texcoco basin, the range of the temperatures during a period of 9 day-light hours is 10°C (Fig. on page 64).

f) Solar illumination of the Sosa Texcoco basin and its influence on the algal growth was investigated by Luna, J.L. based on the intensitivity of the solar illumination.

A calculation method for the productivity of the algal biomass on illuminated basis can be extrapolated as follows:

$$1 \text{ mg } O_2 \text{ ---------- } 3.68 \text{ cal.}$$

$$\bar{S} \text{ ---------- } X \qquad X = \frac{\bar{S} \text{ mg } O_2}{3.68 \text{ cal.}}$$

Introducing the photosynthetic efficiency we have

$$Es = \frac{Is}{Io} \left(1 + \ln \frac{Io}{Is}\right)$$

Es = photosynthetic efficiency

Is = luminization at the saturation point

Io = solar irradiation at the culture tanks

Is is calculated from the graph of the solar irradiation at different depths of the culture tanks.

we obtain: $$X = \frac{Es \cdot \bar{S} \cdot mg \cdot O_2}{3.68 \text{ cal.}}$$

where:

$\bar{S}$ = Solar irradiation (cal/cm$^2$/ day)

$O_2$= Dissolved oxygen in the culture tanks (mg/l)

Es= Photosynthetic efficiency

X = Production of cellular biomass (g/m$^2$/day)

Trace elements in water are responsible for massive increases in numbers of algae - the so-called "bloom". (Telitchenko, M.M., G.V. Tsttsarin and Ye. L. Shirokova) It was established that blue-green algae concentrate 18 trace elements from water, one of which is copper. When copper stocks are exhausted the "bloom" of Cyanophycea (blue-green algae) in the water ceases. Repeated blooms are noted only when the water is enriched with copper by the death of the previous generation of algae.

The chemical composition of Sosa Texcoco basin zone $A_1$ is as follows

| Actual stand | ppm | Microelements (Arnon, D.) | ppm |
|---|---|---|---|
| $Cl^-$ | 5200 | | |
| *$HCO3^-$ | 1500 | | |
| $CO_3^{--}$ | 2250 | B | ** |
| *$N_{(NO_3^-)}$ | 18,5 | Mn | 0,1 |
| N org. and as ammonia | 22,1 | Jn | 0,41 |
| $SO4^{--}$ | 460 | Cu | 0,064 |
| *P $O_4^{--}$ | 70,7 | Mo | ** |
| *$Fe^{++}$ | 0,30 | V | ** |
| *$Ca^{++}$ | 10,90 | Cr | ** |
| *$Mg^{++}$ | 8,35 | Ni | 0,009 |
| Na | 5150 | Co | 0,016 |
| K | 520 | Ti | ** |
| pH = 10 à 22°C | | W | ** |

* Ions regularly adjusted

** Planned to be added

The actual variation of the nutrients contained in the basin over a quarter of a year period is presented on page 69 from where the Spirulina is harvested and as a comparison from the other zone of the Spirulina basin = A, page 70. (Goldenberg, UNIDO).

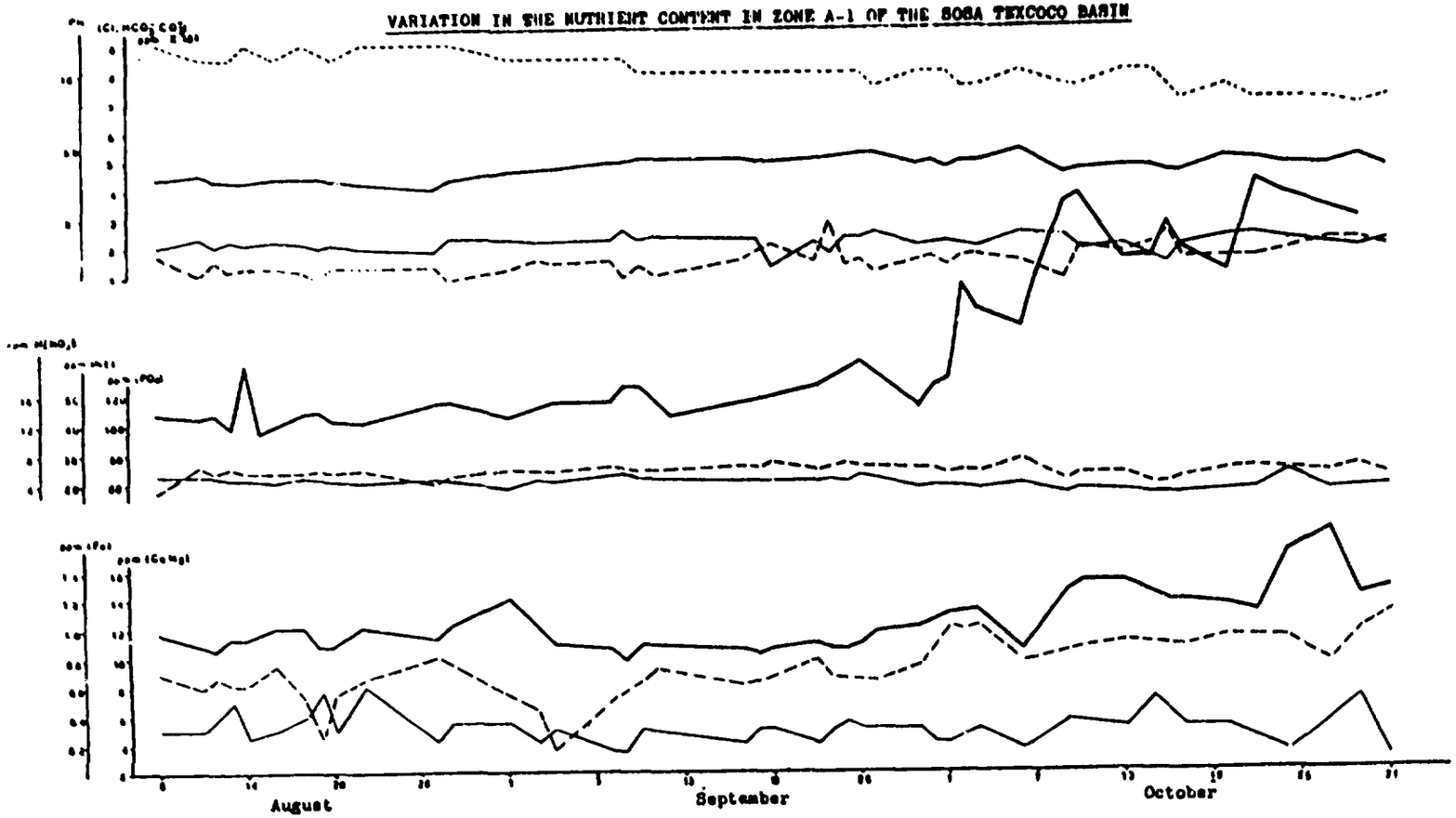
Eutrophication of the Sosa Texcoco basin (Zone $A_1$)

Ninety per cent of macroand micronutrients supplied are utilized (Luna) It was also calculated (Luna) that for the production of 1 kg of dehydrated end-product of the Spirulina plant are needed:

| | | |
|---|---|---|
| Macronutrients: | 550 Kg | C |
| | 100 Kg | N |
| | 30 g | S |
| | 15 g | Na |
| | 16 g | P |
| | 2 g | Mg |
| | 1 g | Ca |

Trace amounts and micronutrients

VARIATION IN THE NUTRIENT CONTENT IN ZONE A-1 OF THE SOSA TEXCOCO BASIN

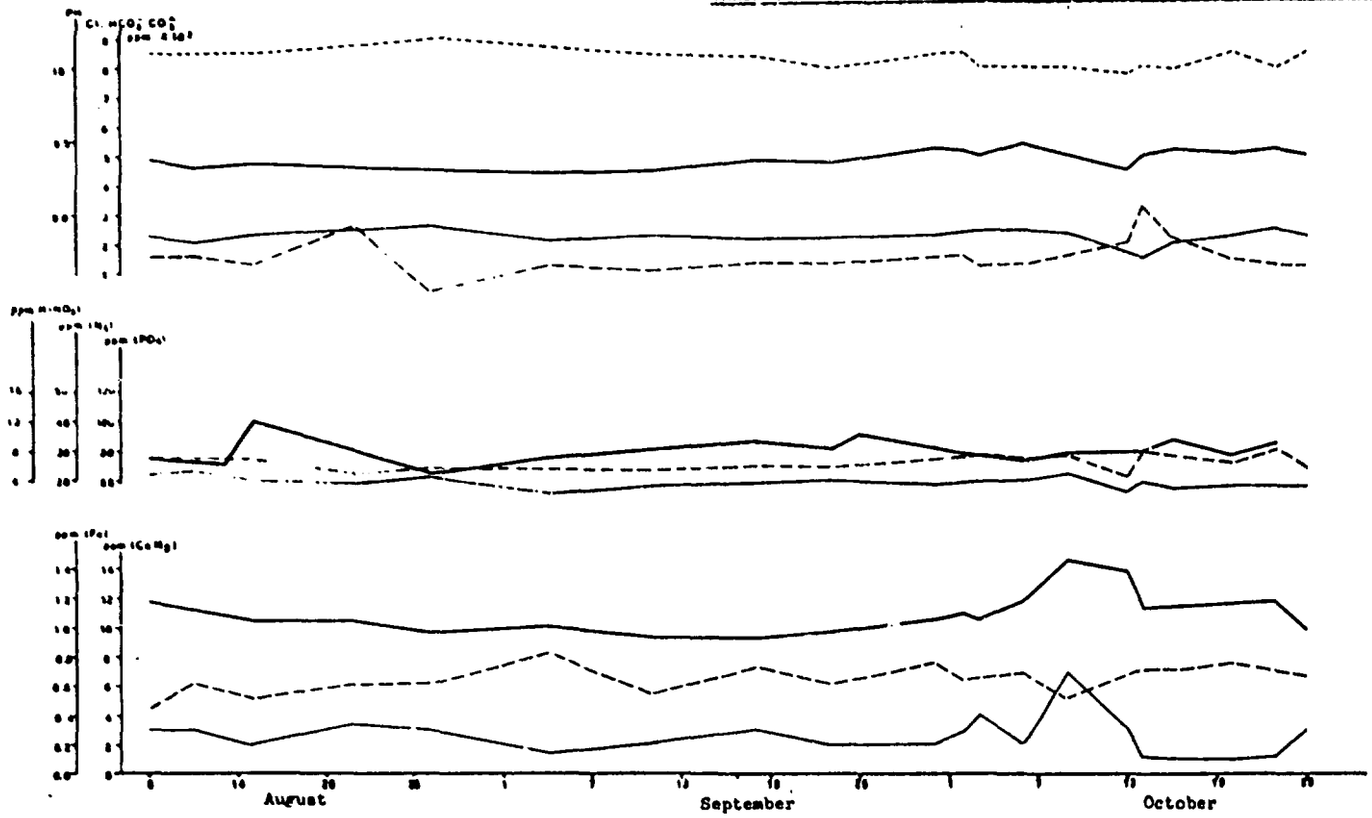



PM
chloride
bicarbonate
carbonate

phosphate
nitrogen (N.Org. + N of the Ammoni
nitrate-nitrogen

calcium
iron
magnesium

VARIATION OF NUTRIENTS IN THE ST BASINZONE USED FOR CULTIVATION OF SPIRULINA OF SOSA TEXCOCO PLANT



UNIDO sponsored experimentation in Eutrophication

The experiments aimed to increase the carbonation of the zones used by the Spirulina plant were conducted (Goldenberg, UNIDO) both on the level of filtration unit and the reflux (replenishment tower) with the results summarized in the Table shown below and in the Table on page 72.

- Carbonation of the filter level

| | $CO_2$ fed in % volume | | | |
|---|---|---|---|---|
| | 100 | 38 | 38 | 10 |
| L = liquid 1/mn | 200 | 200 | 200 | 200 |
| L' = liquid g/mn | 203000 | 204522 | 204522 | 204522 |
| G = gas 1/mn | 14,2 | 76 | 46,7 | 165,6 |
| G' = gas g/mn | 21,5 | 89,6 | 55,1 | 171,6 |
| ($Na_2CO_3$) initial in g mol/l | 0,0415 | 0,0480 | 0,0480 | 0,0480 |
| ($NaHCO_3$) initial in g mol/l | 0,0192 | 0,0345 | 0,0345 | 0,0345 |
| L' / G' | 9442 | 2283 | 3712 | 1192 |
| $CO_2$ fed in g/mn | 21,5 | 47,5 | 26,5 | 23,6 |
| $CO_2$ absorbed in % | 98 | 77,7 | 84 | 31 |
| $CO_2$ absorbed in g/mn | 21,1 | 36,9 | 22,3 | 7,31 |
| $CO_2$ absorbed in Kg/15000 $m^3$ liq. | 1583 | 2767,5 | 1657 | 548 |
| pH initial | - | 9,81 | 9,81 | 9,81 |
| pH final | - | 9,76 | 9,77 | 9,79 |
| T ° of carbonated water | 19,5 | 17,5 | 17,5 | 17,5 |
| Time of reaction in sec. | 4-5 | 4-5 | 4-5 | 4-5 |

Feeding pressure with $CO_2$/air = atmospheric pressure

Pressure on the exit of the reactor = atmospheric pressure

- Carbonation in a replenishment tower

| | $CO_2$ fed in % volume | | |
|---|---|---|---|
| | 100 | 12,3 | 10 |
| L = liquid l/mn | 18,6 | 16,7 | 16,7 |
| L' = liquid g/mn | 18879 | 16950 | 16950 |
| G = gas l/mn | 5,6 | 39,5 | 38,5 |
| G' = gas g/mn | 8,7 | 41,6 | 40,05 |
| ($NaCO_3$) initial en g mol/l | 0,0412 | 0,0393 | 0,0390 |
| ($NaHCO_3$) initial en g mol/l | 0,0254 | 0,0295 | 0,0296 |
| L' / G' | 2170 | 407,5 | 423 |
| $CO_2$ supplied in g/mn | 8,7 | 7,3 | 5,77 |
| $CO_2$ absorbed in % | 90,4 | 26,5 | 37,7 |
| $CO_2$ absorbed in g/mn | 7,87 | 1,94 | 2,17 |
| $CO_2$ absorbed in Kg/15000 $m^3$ liq. | 6350 | 1742 | 1950 |
| pH initial | - | 9,80 | 9,80 |
| pH final | - | 9,73 | 9,71 |
| T ° of carbonated water | 20,5 | 19,5 | 19,5 |
| Time of reaction in sec. | 72 | 80 | 80 |

Feeding pressure with $CO_2$/air

Pressure on the exit = atmospheric pressure

It is important to make the culture basin homogenous.

At the present time, there is no real homogenization, since the basin is subject to the action of the wind and of a weak current caused by filter rejection (500 to 750 m3/hour), a current which furthermore follows preferential ways.

Considering the mean depth of the basin (80 to 85 cm) and its extension (10 hectares, length 500 m), the choice of methods capable of making it homogenous is very limited.

Indeed, if the method used is to be effective, it must produce not only a strong agitation, but also a strong and deep horizontal current that is sufficientto put into motion large amount of the liquid body.

Nevertheless, homogenization may be a double-edged weapon and its indiscriminate use could have grave consequences since in winter the water at surface is colder, homogenization cools the basin's water. Indeed, the temperature of the culture medium is a limiting factor of growth: at temperatures below 17 to 18°C, growth diminishes strongly.

The mean depth of the basin being important, the absorbed solar energy creates a certain stratification (= layer structure) of temperatures. Depending on the conditions, the difference of temperatures between 5 and 30 cm from the surface may reach 2 to 7°C, and between the surface and 30 cm, 10 to 15°C.

It follows that the basin, which constitutes a very large thermic supply, will have to be made homogenous in such a way as not to lower the temperature of the upper layers inconsiderately. This will be done in accordance with the atmospheric temperature, with the formation of the plaque culture of algae and at least during those periods of the day when the surrounding temperature and the one of the culture medium are equal. The plaque formation require a careful mixing technique otherwise the algae will be destroyed.

For a proper re-circulation (See page 76 ) homogenization will have to be done on 24 hours basis.

One of the aims of this recirculation is to increase the algal concentration in the zone of the filtration unit which is located close to the reflux water without creating conditions of latent growth (See figure on page 76).

Among the means practicable to achieve homogenization may be listed a marine type propeller with horizontal axis, paddle wheels and the use of various kinds of pumps.

With such devices the following can be avoided (Goldenberg, UNIDO):

1. The slow down of the diffusion of the nutritive elements;
2. The formation of algal plaques which results in its decay and a consequent pollution of the basin.

Speed obtained in an experimental basin 85 cm deep, surface 100 $m^2$ after 1 hour and 40 minutes of operation

| Distance of the engines | 90 m | | 120 m | | 150 m | | 175 m | |
|---|---|---|---|---|---|---|---|---|
| | F | S | F | S | F | S | F | S |
| Distance from partition | | | | | | | | |
| 10 m** | 122 | 145 | 52 | 37 | 52 | 24 | 39 | * |
| 20 m | 159 | 145 | 49 | 69 | 33 | 33 | 20 | * |
| 30 m | 83 | 145 | 63 | 26 | 18 | 54* | 18 | * |

Speed obtained in an experimental basin 85 cm deep, surface 100 $m^2$ after 1 hour and 40 minutes of operation

| Distance of the engines | 75 m | | 90 m | | 150 m | | 175 m | |
|---|---|---|---|---|---|---|---|---|
| | F | S | F | S | F* | S | F | S |
| Distance from partition | | | | | | | | |
| 5 m | 76 | 103 | 54 | 95 | 50 | 86 | 36 | 86 |
| 10 m | 88 | 70 | 54 | 93 | 69 | 86 | 43 | 88 |

F = speed obtained at the 15 cm distance from the bottom expressed in mm/sec.
S = Speed obtained at the 15 cm distance from the surface expressed in mm/sec.
F*= Speed obtained at 30 cm distance from the bottom because of the wind.

- Speed obtained as a function of operational time

| Duration of operation | 5' | | 10' | | 15' | | 30' | |
|---|---|---|---|---|---|---|---|---|
| | F | S | F | S | F | S | F | S |
| Marine motor | 186 | 199 | 184 | 198 | 188 | 202 | 186 | 202 |
| Banres* pump | 155 | 213 | 169 | 207 | 175 | 198 | 176 | 202 |

F = Speed obtained at the 15 cm distance from the bottom expressed in mm/sec.
S = Speed obtained at the 15 cm distance from the surface expressed in mm/sec.
* = Homogenization stopped after 2 hours because of the rupture of 30 % of algae.

The following marine type propellers were tested

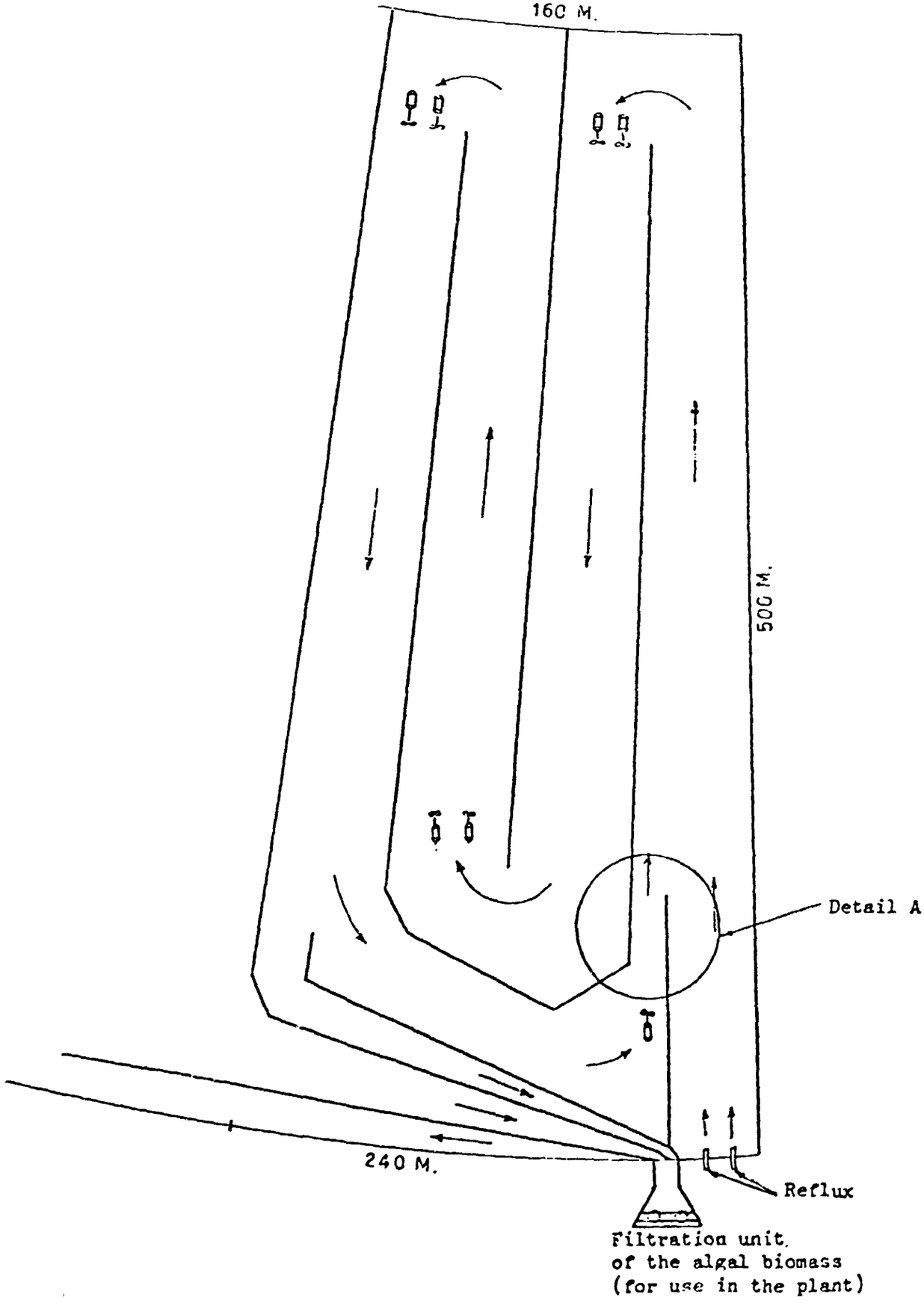
| | |
|---|---|
| Trade name | British Sea Gull |
| Power | 6,5 cv |
| Engine velocity | 4000 |
| Gearing ratio | 12/48 |
| Diameter of the blades | 28 cm |
| Number of blades | 5 |

| | |
|---|---|
| Trade name | Johnson |
| Power | 4 cv |
| Engine velocity | 4500 |
| Gearing ratio | 12/25 |
| Diameter of the blades | 7 1/2 |
| | 6 |
| Number of blades | 3 |

Centrifugal pump used

| | |
|---|---|
| Manufactured | Barnes |
| Flow | 88 $m^3/h$ |
| Electrical engine | 15 cv |
| Rotational speed | 1459 |
| Diameter | 3 |
| Length of input | 6 m |
| Length of output | 10 m |

Isolated zone of the A, sector of Sosa Texcoco coracol (basin) for the experimental homogenisation (Goldenberg, UNIDO)



Manufacturing steps

1. Preconcentration (Filtration)

The concentration of the algae which grows in the outer ring of Caracol in the culture tanks normally lies between 100 - 300 mg/l. In view of this, it is necessary to preconcentrate the suspension. This is achieved by passing the dilute suspension first over parallel inclined filters which concentrate the biomass from 0.1 to 5-10 g/l. The screens are washed continuously. This wash water flows by gravity to rotary filter. The screening surfaces are of nylon mesh. Wash water from the rotary screens contains 15 to 20 g/l algae.

Both inclined and rotary filters were especially designed and constructed to have a high filtration efficiency with low energy consumption.

2. Filtration-extraction

This step removes the remaining cultivation medium. The algae suspension is dewatered to a cake containing 15 to 20 % solids on a vacuum filter.

3. Desintegration

A mechanical rupture of the cells results in a liquid with a specific viscosity. This step is also beneficial for improvement of the digestability of the end-product. The proprietory type of desintegrator both fluidizes the product and pumps it to the next production stage, pasteurization.

4. Pasteurization

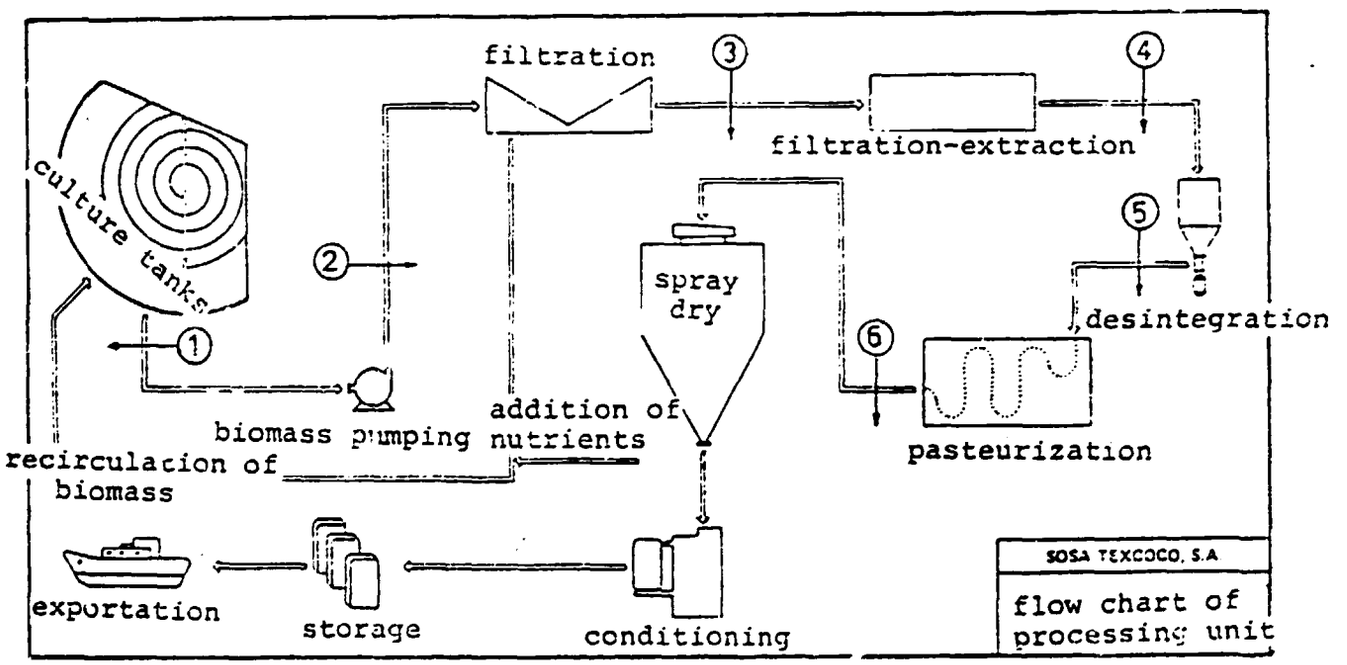
Pasteurization is necessary since the next step, namely spray-drying is not a guarantee for destruction of certain bacteria, able to survive the short time of the high temperature, followed by rapid cooling afterwards.

5. Spray drying

Spray drying is done through exposure to high temperature for a few seconds following rapid cooling stage. With this method, the destruction of important aminoacids as lysine, typtophan vitamins and pigment is minimal.

6. Conditioning

Conditioning involves grinding where the flakes originate from the spray drying are converted to a flour ready for a filling system for package and storage. The shelf-life of such a powder, as long as it is protected from the influence of light and heat, is unlimited.




**Flow chart as presented by officials of Sosa Texcoco. It represents a schema of a commercial scale operation which is being developed to harvest and process a specific natural resource - Spirulina.**

Routine and periodical quality control

a) Samples for the routine daily quality control are taken from the average of ten drums (100 kg each) which represent the daily production output.

| | Expected value |
|---|---|
| Humidity | 4 - 7 % |
| Ash | 5 - 8 |
| Protein | 60 - 70 % |
| Density | 0.43 - 0.55 g/cm$^3$ |
| Carotene | 0.19 % (1.9 g/kg of product) |
| Xantophyll | 0.14 % (1.4 g/kg product) |
| Standard count of colonies 2 days 35°C | Maximum 20000/g |
| Count on blood agar 2 days 35°C | 0 |
| Count on malt agar 4 days 25°C | |
| Fungi | 20 colonies/g |
| Yeast | 20 colonies/g |
| MPN on coliforms 2 days 35°C | 20/g maximum* |
| Esch. Coli, Eosyn Methylene Blue 1 day 25°C | 0/g maximum* |

*If the maximum is exceeded, then the following tests are to be carried out:

for E.Coli.

for Salmonella

for Shigella

b) Routine daily control of the cultivation unit.

1) Determination of physical factors (temperature, light, water level, precipitation, evaporation)

2) Determination of chemical factors

The expected chemical composition of the ST basin is as follows:

| | ppm |
|---|---|
| $CI^-$ | 5200 |
| $*HCO3^-$ | 1500 |
| $CO_3^{--}$ | 2250 |
| $*N_{(NO_3^-}$ | 18.5 |
| N org. and as ammonia | 22.1 |
| $SO4^{--}$ | 460 |
| $*P\ O_4^{---}$ | 70.7 |
| $*Fe^{++}$ | 0.30 |
| $*Ca^{++}$ | 10.90 |
| $*Mg^{++}$ | 8.35 |
| Na | 5150 |
| K | 520 |
| pH = 10 a 22°C | |

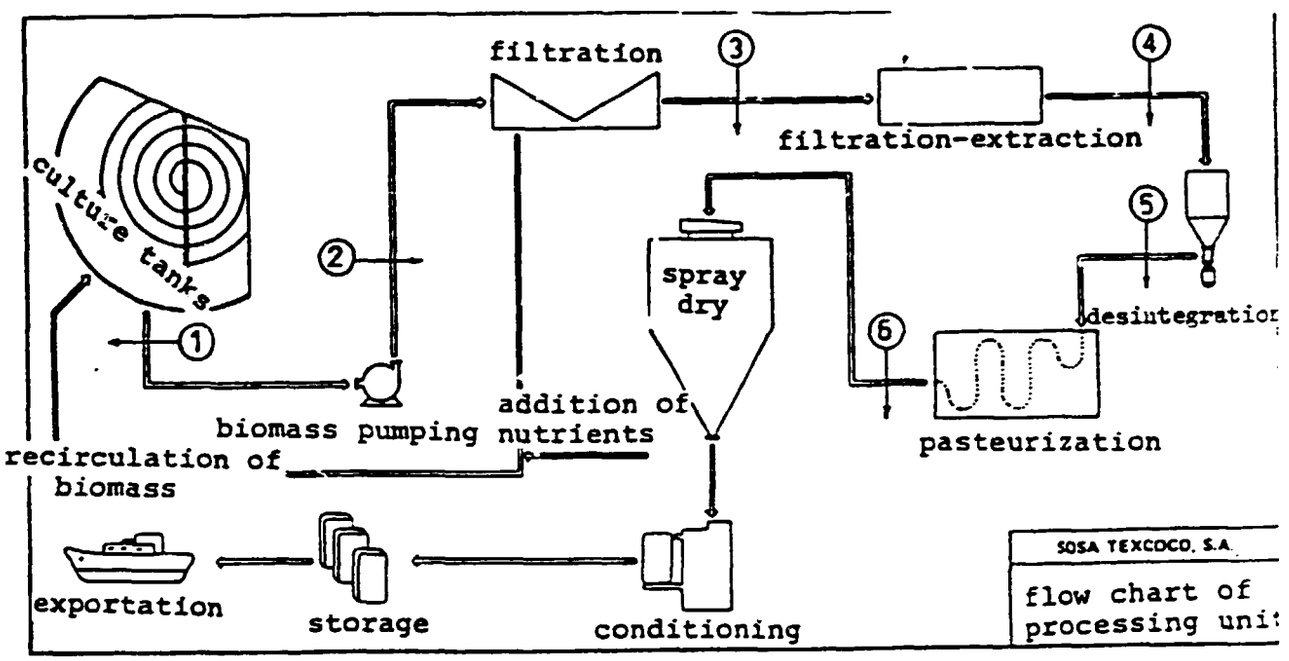
*Ions regularly adjusted by daily addition to the reflux.

The actual variation of the nutrients contained in the basin over a quarter of a year period is presented on page 69 from where the Spirulina is harvested and as a comparison from the other zone of the SOSATEXCOCO basin shown on pg.70.

3) Biological, microscopical examination of the morphology, colour, size of the Spirulina. Also determination of eventual contaminants, e.g. other algal species, protozoa, bacteria, etc.

c) Routine weekly control of the production plant:

points 2 to 6 of the flow chart. Microbiological check-up and sanitary condition of the equipment and working personnel.




d) Periodical quality control

These controls are done every three months for the following components of the finished product:

Water

Protein

Fat

Fiber

Ash

Non-fibrous carbohydrate

Tocopherol

Thiamin

Riboflavin

Biotine

Niacine

Vitamin $B_6$

Vitamin $B_{12}$

Folic acid

Amino Nitrogen

0 Linolenic acid

Cyanide

Cadmium

Lead

Arsenic

Selenium

Mercury

Zinc

Amino acid Composition

- Arginine
- Lysine
- Histidine
- Phenylalanine
- Tyrosine
- Leucine
- Isoleucine
- Methionine
- Valine
- Alanine
- Glycine
- Proline
- Glutamic acid
- Serine
- Threonine
- Aspartic acid
- Tryptophane
- Cystine

Summary of the properties of the spray-dried natural product of the Sosa Texcoco Spirulina plant

## PHYSICAL PROPERTIES

| | |
|---|---|
| Appearance: | Fine powder |
| Color: | Dark green |
| Odor and Taste: | Mild, resembling sea vegetables |
| Bulk Density: | 0.5 g/ml |
| Particle Size: | 9-25 microns |

## CHEMICAL COMPOSITION

| | MINIMUM | MAXIMUM |
|---|---|---|
| Moisture | | 7.0% |
| Ash | | 9.0% |
| Proteins | 60.0% | |
| Xanthophylls | 1.40g/kg of product | 1.80g/kg of product |
| Carotene | 1.50g/kg of product | 1.90g/kg of product |
| Chlorophyll a | 6.10g/kg of product | 7.60g/kg of product |

## CHEMICAL ANALYSIS

| | MINIMUM | | MAXIMUM | |
|---|---|---|---|---|
| CRUDE FIBER | | | 0.9% | |
| MOISTURE | | | 7.0% | |
| ASH | | | 9.0% | |
| Calcium (Ca) | 1,045 | mg/kg | 1,315 | mg/kg |
| Phosphorus (P) | 7,617 | mg/kg | 8,942 | mg/kg |
| Iron (Fe) | 475 | mg/kg | 580 | mg/kg |
| Sodium (Na) | 275 | mg/kg | 412 | mg/kg |
| Chloride (Cl) | 4,000 | mg/kg | 4,400 | mg/kg |
| Magnesium (Mg) | 1,410 | mg/kg | 1,915 | mg/kg |
| Manganese (Mn) | 18 | mg/kg | 25 | mg/kg |
| Zinc (Zn) | 27 | mg/kg | 39 | mg/kg |
| Potassium (K) | 13,305 | mg/kg | 15,400 | mg/kg |
| Others | 36,000 | mg/kg | 57,000 | mg/kg |
| TOTAL CARBOHYDRATES | 13.0% | | 16.5% | |
| Ramnose | average | | 9.0% | |
| Glucane | average | | 1.5% | |
| Phosphoryled cyclitols | average | | 2.5% | |
| Glucosamine and muramic acid | average | | 2.0% | |
| Glycogen | average | | 0.5% | |
| Sialic acid and others | average | | 0.5% | |

| | MINIMUM | MAXIMUM |
|---|---|---|
| TOTAL ORGANIC NITROGEN | 10.85% | 13.35% |
| NITROGEN FROM PROTEINS | 9.60% | 11.36% |
| CRUDE PROTEIN ( % N x 6.25) | 60.0% | 71.0% |
| ESSENTIAL AMINOACIDS | | |
| Isoleucine | 3.69% | 4.13% |
| Leucine | 5.56% | 5.80% |
| Lysine | 2.96% | 4.00% |
| Methionine | 1.59% | 2.17% |
| Phenylalanine | 2.77% | 3.95% |
| Threonine | 3.18% | 4.17% |
| Tryptophan | 0.82% | 1.13% |
| Valine | 4.20% | 6.00% |
| NON-ESSENTIAL AMINOACIDS | | |
| Alanine | 4.97% | 5.82% |
| Arginine | 4.46% | 5.98% |
| Aspartic Acid | 5.97% | 6.43% |
| Cystine | 0.56% | 0.67% |
| Glutamic Acid | 8,29% | 8.94% |
| Glycine | 3.17% | 3.46% |
| Histidine | 0.89% | 1.08% |
| Proline | 2.68% | 2.97% |
| Serine | 3.18% | 4.00% |
| AVAILABLE LYSINE average | 85% | |
| NITROGEN FROM NUCLEIC ACIDS | 1.25% | 1.99% |
| RIBONUCLEIC ACID (RNA) | | |
| RNA= % N x 2.18 | 2.20% | 3.50% |
| DEOXYRIBONUCLEIC ACID (DNA) | | |
| DNA= % x 2.63 | 0.63% | 1.00% |

| | MINIMUM | | MAXIMUM | |
|---|---|---|---|---|
| TOTAL LIPIDS | 6.0% | | 7.0% | |
| FATTY ACIDS | 4.9% | | 5.7% | |
| Lauric ($C_{12}$) | 180 | mg/kg | 229 | mg/kg |
| Myristic ($C_{14}$) | 520 | mg/kg | 644 | mg/kg |
| Palmitic ($C_{16}$) | 16,500 | mg/kg | 21,141 | mg/kg |
| Palmitoleic ($C_{16}$) | 1,490 | mg/kg | 2,035 | mg/kg |
| Palmitolinoleic ($C_{16}$) | 1,750 | mg/kg | 2,565 | mg/kg |
| Heptadecanoic ($C_{17}$) | 90 | mg/kg | 142 | mg/kg |
| Stearic ($C_{18}$) | traces | | 353 | mg/kg |
| Oleic ($C_{18}$) | 1,970 | mg/kg | 3,009 | mg/kg |
| Linoleic ($C_{18}$) | 10,920 | mg/kg | 13,784 | mg/kg |
| $\gamma$ Linolenic ($C_{18}$) | 8,750 | mg/kg | 11,970 | mg/kg |
| $\alpha$ Linolenic ($C_{18}$) | 160 | mg/kg | 427 | mg/kg |
| Others | 7,000 | mg/kg | 699 | mg/kg |
| INSAPONIFIABLE | 1.1% | | 1.3% | |
| Sterols | 100 | mg/kg | 325 | mg/kg |
| Triterpen alcohols | 500 | mg/kg | 800 | mg/kg |
| Carotenoids | 2,900 | mg/kg | 4,000 | mg/kg |
| Chlorophyll a | 6,100 | mg/kg | 7,600 | mg/kg |
| Others | 1,400 | mg/kg | 150 | mg/kg |
| 3-4 Benzypyrene | 2.6 | $\mu$g/kg | 3.6 | $\mu$g/kg |

| | MINIMUM | | MAXIMUM | |
|---|---|---|---|---|
| STEROLS | 100 | mg/kg | 325 | mg/kg |
| Cholesterol | 60 | mg/kg | 196 | mg/kg |
| β Sitosterol | 30 | mg/kg | 97 | mg/kg |
| Dihidro 7 Cholesterol<br>Cholesten 7 ol 3<br>Stigmasterol<br>others | 10 | mg/kg | 32 | mg/kg |
| CAROTENOIDS | 2,900 | mg/kg | 4,000 | mg/kg |
| ∝ Carotene | | | traces | |
| β Carotene | average | | 1,700 | mg/kg |
| XANTHOPHYLLS | average | | 1,600 | mg/kg |
| Cryptoxanthin | average | | 556 | mg/kg |
| Echinenone | average | | 439 | mg/kg |
| Zeaxanthin | average | | 316 | mg/kg |
| Lutein and Euglenanone | average | | 289 | mg/kg |

VITAMINS

| | | | |
|---|---|---|---|
| Biotin (H) | average | 0.4 | mg/kg |
| Cyanocobalamin ($B_{12}$) | average | 2 | mg/kg |
| d-Ca-Pantothenate | average | 11 | mg/kg |
| Folic Acid | average | 0.5 | mg/kg |
| Inositol | average | 350 | mg/kg |
| Nicotinic Acid (PP) | average | 118 | mg/kg |
| Pyridoxine ($B_6$) | average | 3 | mg/kg |
| Riboflavine ($B_2$) | average | 40 | mg/kg |
| Thiamine ($B_1$) | average | 55 | mg/kg |
| Tocopherol (E) | average | 190 | mg/kg |

A-SPRAY-DRIED AND PASTEURIZED NATURAL PRODUCT
TYPICAL MICROBIOLOGICAL ANALYSIS

| | SPIRULINA | | POWDER MILK | |
|---|---|---|---|---|
| | MAXIMUM VALUE | MINIMUM VALUE | STANDARDS IN MEXICO | STANDARDS IN USA |
| STANDARD PLATE COUNT | 20,000/g | 4,000/g | 50,000/g | 50,000/g |
| FUNGI | 10/g | 3/g | 20/g | 11/g |
| YEASTS | 10/g | 3/g | 20/g | 11/g |
| COLIFORMS | 20/g | 3/g | 20/g | 11/g |
| SALMONELLA | None | None | None | None |
| SHIGELLA | None | None | None | None |
| E. COLI ENTEROPATHOGENE | None | None | None | None |

5. Nutritive value

PROTEIN EFFICIENCY RATIO
(PER) of 2.2 to 2.6 (74 - 87% that of casein)

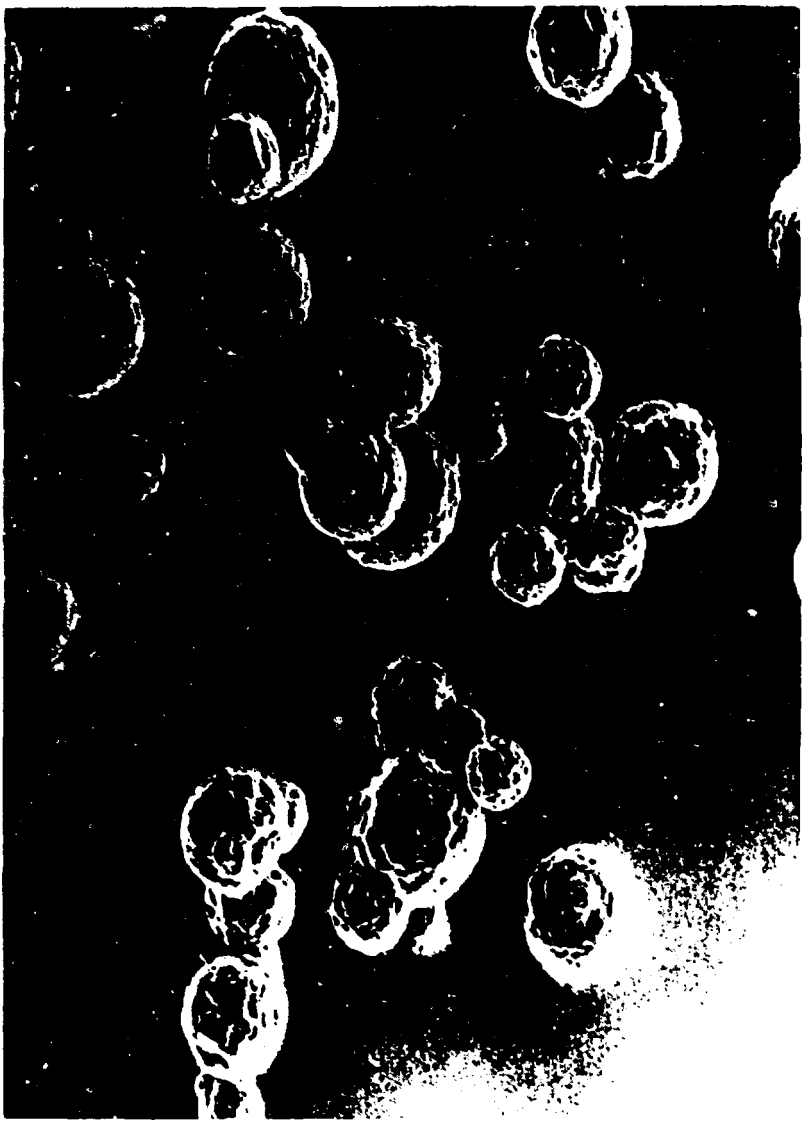
NET PROTEIN UTILIZATION
(NPU) of 53 to 61% (85 - 92% that of casein)

DIGESTIBILITY
of 83 to 84%

Biological value
72

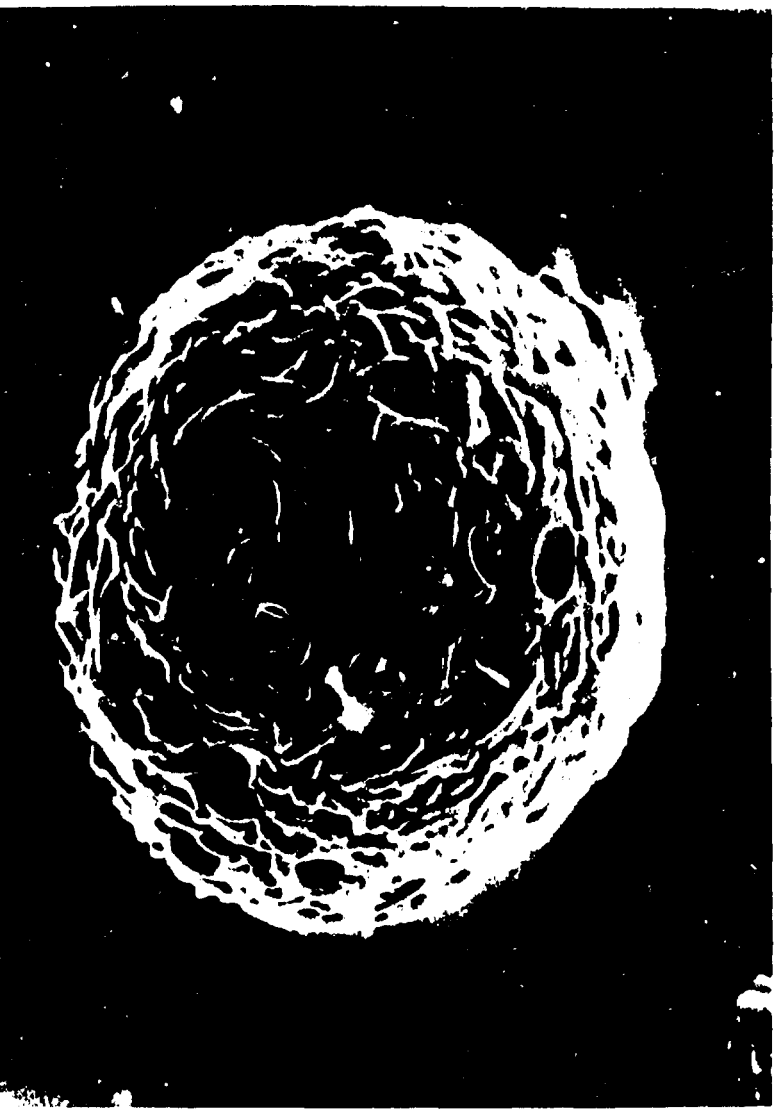
EM 54000 x POWDER PARTICLE

105 x

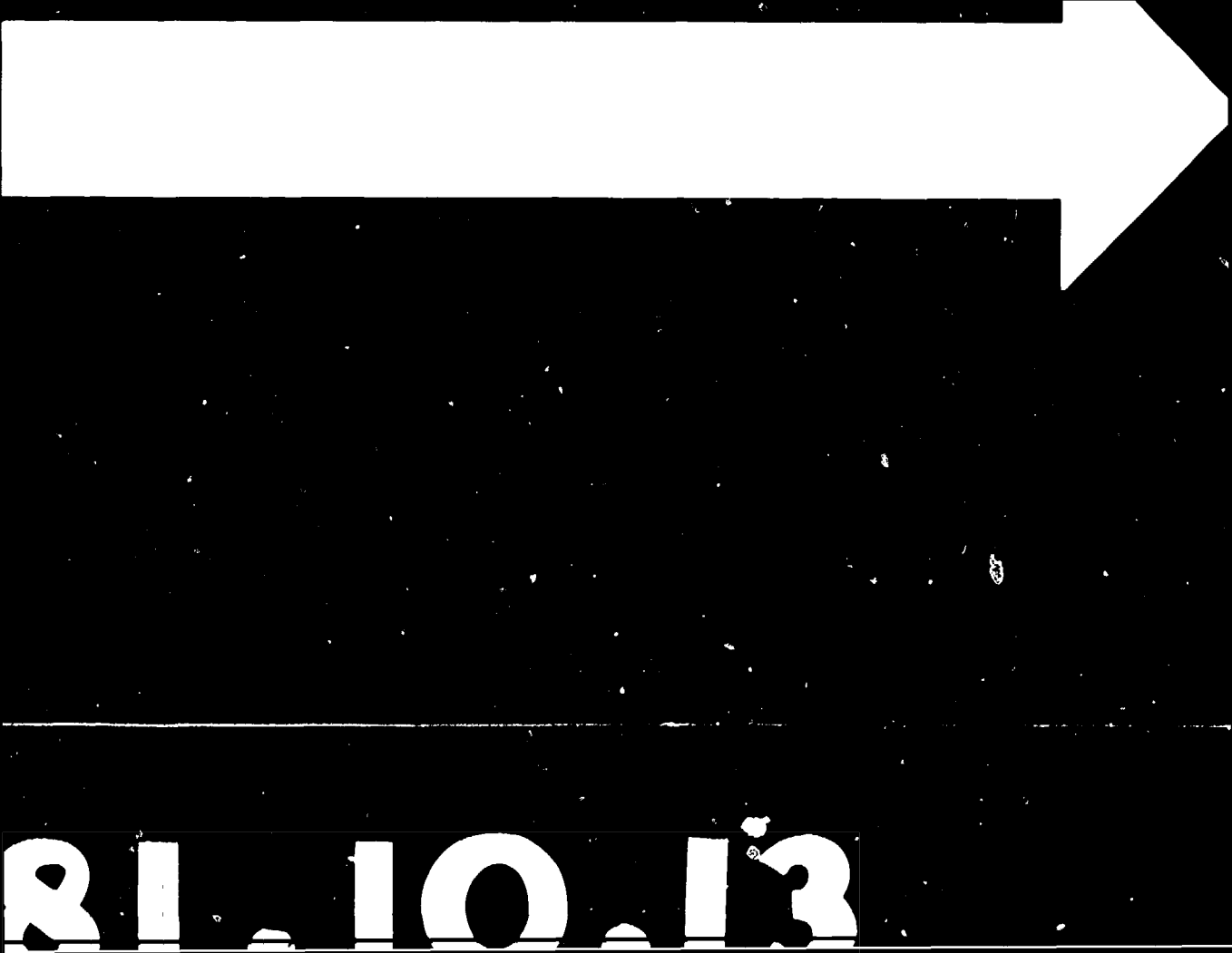


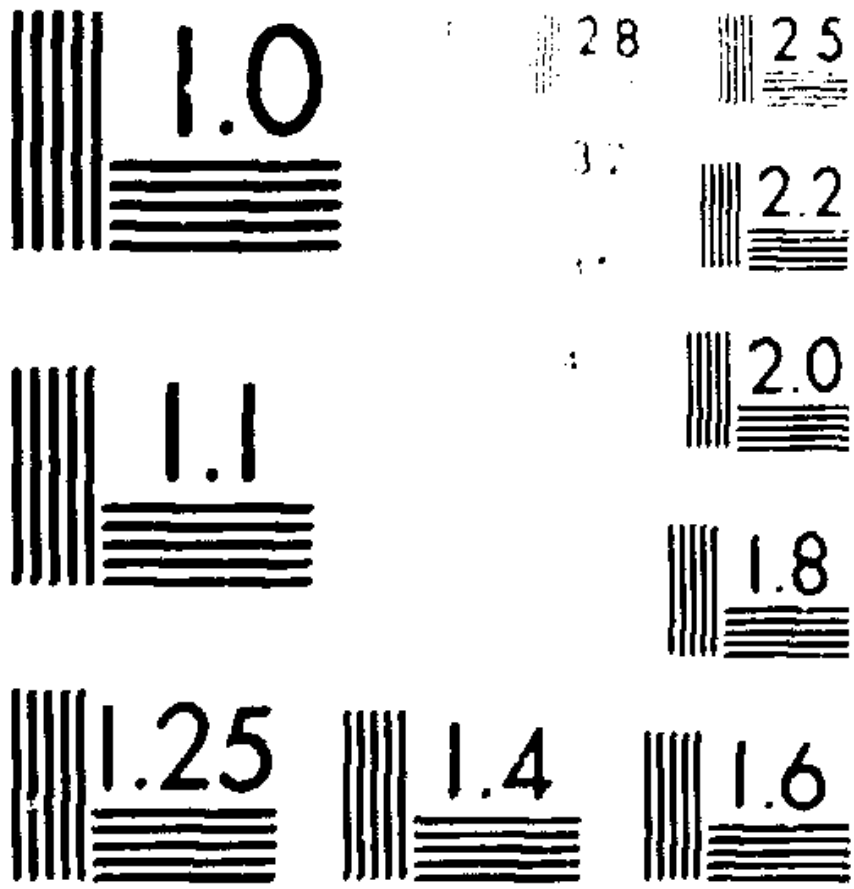
Powder particle

510 x



Powder particle


81-10-13


2.8
2.5
1.0
3.2
2.2
2.0
1.1
1.8
1.25
1.4
1.6

Global implication - General recommendations

It became clear long ago that conventional agriculture, even though its yields may be improved applying modern technology, has limitations to provide all the protein needed for the world's fast growing population, and that non conventional protein sources must be used. Among non conventional protein sources, microgranisms have received special attention: in 1968 a meeting was held at the Massachusetts Institute of Technology ( Mateles, R.J., Tannenbaum, S.R.) and most of the experience with microganisms was discussed under the name of " "single cell protein" (SCP).

The Institut Français du Petrole developed a method for intensive mass-culture of Spirulina whereby the yield may reach 14g dry alga/m2/day, i.e. 50,000kg/hectare/year, equivalent to some 32,000 kg dry protein per hectare per year. This is an impressive yield indeed, specially when compared to that of some agricultural products; for example, the yield of corn in many developing countries is equivalent to 50 to 400 kg of protein/hectare/cron. It has been stated that "half the average daily human protein requirement for the total world's population, could be obtained from an area of the size of the Essex County" using algal protein (Gordon, J.F.).

Combined activities of the algae in waste water treatment and as a source of protein is of utmost importance considering the world's exploding population, 6.6 billion by the year 2000 according to UN's Fund for Population Activities and the fact that at the present 26 cities have 5 million or more residents each.

A source of high-quality protein for animal feed, based upon algae recovered in the process of upgrading waste oxidation pond effluents is promising to be particularly economical. Unlike other types of single cell protein (SCP), the algal protein does not have to return the full production cost value of waste disposal and the reclaimed water. Whereas such systems as activated sludge require considerable mechanical energy to supply the oxygen needed for aerobically degrading organics in waste water oxidation ponds utilize solar energy for that purpose. (Moraine, R., Shelef, G., Meydan, A. and Levi, A.).

Harvesting and processing of algae grown in sewage is worth while if there is a profitable market for the product and/or for the reclaimed water (Gcluke, C.G. and Oswald, W.J.). If there is, then communities forced by rigid water pollution control requirements to undertake tertiary treatment should consider algae production as an early and ultimate step in their waste disposal systems.

The following are the theoretical calculation of the algal requirements in relation to the P concentration (Mackenthun, K.M.) "Growth of algae in sewage has been reported in the laboratory at 1 - 2 g/l (dry weight), and in sewage ponds at 0.5 g/litre. Thus, assuming optimal growth conditions, and maximum phosphate utilization, the maximum algal crop that could be grown from 1 kg of P would be 1 ton of wet algae under laboratory conditions or 250 kg in the field. If a cellular content of P in algae is 0.7 % then 1 kg of P could be distributed among 1 450 kg of algae (wet weight). This suggests that to prevent biological nuisances total P in flowing water should not exceed 100 g/l, and in standing water not more than 50 g/litre."

The relationship of nutrients in water to algae and aquatic plants can be related to a sewage plant (Mackenthun, K.M.). The annual contribution of nitrogen and phosphorus from domestic sewage per head of population is about 1.9 kg of N and 0.5 kg of P. This is sufficient N to fertilize 0.4 ha of lake water to a depth of 1.6 m in respect of

N, and 2.8 ha of lake to the same depth in respect of P to such an extent that algal blooms might occur during summer months.

On the global scale, the Spirulinas of the Nostocales family similar to Spirulina Geitleri J. de Toni, which is utilized in the successful Mexican venture of Sosa Texcoco can also be used in the following areas where Spirulina platensis is indigenous. It is generally appreciated that for example Spirulina platensis grows predominantly in strongly alkaline waters and seems to prefer tropical and sub-tropical climates, where pHs of 9 to 11 are often encountered. The few data brought together on page 98 merely confirm this. However, in view of the dense growth of Spirulina which occurs in Lake Aranguadi which is poor in sulphate compared with for example Lake Yoan, it may be possible to modify the belief implied (Stewart W.D.P.) that high concentrations of sulphate are needed for dense growth.

Spirulina platensis is indigenous in the following areas:

| Country | Region | Described and/or discovered by in year | Reference |
|---|---|---|---|
| Ethiopia | Province of Choa Lake Aranguadi | A. Haggblom, Nov. 1954 | Thomasson K. |
| | Lake Chiltu 7 23' N 38 27' E | R. B. Wood, July | Wood, R.B. |
| Egypt | Low Egypt, Nazlet el Arab (Sand Island) | D. Simpson, June 1924 | Rich, F. |
| Dem.Rep. of the Congo | Kivu, Lake Mougounga (immediately North of Lake Kivu) | J. Lebrun, November 1937 | Leonard J. and Compere P. |
| Rep. of South Africa | East-Witwatersrand Brakpan | Allanson, 1960 | Welsh, H. |
| Kenya | Central Island Crater Lake B | Worthington April 1931 | Rich, F. |
| Kenya | Central Island Crater Lake C | Worthington April 1931 | Rich. F. |
| Kenya | Lake Elmenteita | Jenkin, P. April 1929<br>Ross, R. 1953 | Ross, R.<br>Ross, R. |
| Kenya | Lake Losougouta | Gregory, May 1983 | West, W. and West, G.S. |
| Kenya | Lake Naivasha | P. Jenkin April 1929 | Ross, R. |
| Kenya | Lake Nakuru | P. Jenkin April 1929 | Ross, R. |
| Kenya | Lake Rodorhe Ferguson spit | Worthington Dec. 1931<br>Worthington March 1931<br>P. Ross | Rich. F.<br>Rich. F.<br>Ross, R. |

| Country | Region | Described and/or discovered by in year | Reference |
|---|---|---|---|
| Tanzania | Very likely Lake Natron | | |
| Rep.of Chad | Massakori (market) | P. Cronc'h, 1939 | Dangeard, P. |
| Rep.of Chad | Fort-Lamy (market) | J. Leonard, Dec. 1964<br>G. Le Guedes, Dec. 1963 | Leonard, J. and Compere, P. |
| | Bol.rond with carbonated waters | J. Leonard, Aug. and Sept. 1964 May 1965 | Leonard, J. and Compere, P. |
| | Lake Guna, sub-prefecture of N'gouri | G. le Guedes, July 1968 | Busson |
| | Borkou, Fava Largeau | Leonard J., Dec. 1964 | Leonard J. and Compere, P. |
| | Guniansa kebir Lake Djobo | J. Leonard, Dec. 1964 | Leonard J. and Compere, P. |
| | Lake Katan | J. Leonard, Dec. 1964 | Leonard J. and Compere, P. |
| Zambia | Lake Bangweoulou Foualya Niponda | D.Harding, Nov.1928 | Thomasson, K. |
| U.S.A. | Del Mar (California) in the coastal waters of the Pacific Ocean | Ralph A. Lewin, Nov. 1969 | Lewin, R.A. |
| Peru | Huancavelica Lake Huacachina near Ica | K. Thomasson. 1960 | Thomasson, K. |
| Uruguay | Montevideo | J. Arechavaleta, March 1884 | |
| Ceylon | Lake Beira | Holsinger, 1955 | Holsinger, E.C. |
| India | Calcutta | K. Biswas, 1927 | Biwas, K. |
| Pakistan | Lahore | S. Ghose, 1924<br>M. Randhava, 1936 | Ghose, S.L.<br>Randhava, M.S. |
| Hungary | Adasztevel Oroshaz | J. Kiss, 1957 | Kiss, J. |
| U.S.S.R. | Azerbaidjan Transcaucasus Koumbasha | Woronichin, 1924 | Woronichin |

Spirulina Geitleri J. de Toni - Synoyms: Arthrospira maxima, Spirulina maxima Geitleri.

Areas where Spirulina geitleri is indigenous

They are very little known and studied. Only two sites are known to date: U.S.A., California, Oakland, Key Route Power House;

Mexico, Mexico City, Caracol of Sosa Texcoco, S.A.

The study of the Sosa Texcoco Spirulina operation have shown that there are existing major marketing areas each capable of considerable expansion:

1. Human food as a high protein and vitamin supplement;
2. Animal feed additive for nutritive and pigmenting value:
3. Health food market:
4. Further development of decolourized product and the commercial utilization of the pigment by-product which would assist the market penetrution and give added value to marketing of algae:
5. The development of what must be regarded as almost infinite potential, the growing "medical possibilities (pharma sector)", may well be encouraged by recent Japanese research, showing beneficial results when fed to people with certain illnesses.
6. The development of the algae product based on processes of waste disposal and water reclamation by saving considerable amount of mechanical energy using solar energy only.

One of the most common pitfalls incurred when trying to develop products intended to help in the solution of nutrition problems in developing countries, is the excess of technology. Often, costly processes are used to effect fancy and usually unnecessary changes in the products according to western food habits and preferences, which result in high prices and low unselective sales.

Major Chemical Constituents of Some "Spirulina" Lakes

Concentrations in mg/l except where otherwise stated.

| Lake | Na | K | Ca | $CO_3 + HCO_3$ (m.eq/l) | Cl |
|---|---|---|---|---|---|
| Elementeita (Kenya) | 9,450 | 381 | 10 | 289 | 5,200 |
| Rudolf (Kenya) | 810 | 21 | 6 | 25 | 475 |
| Nakuru (Kenya) | 38,000 | 1,312 | 10 | 1,440 | 13,000 |
| Huacachina (Peru) | 38,000 | | | 106 | 3,430 |
| Ycan (Chad) | 24,650 | | | 484 | 9,660 |
| Chiltu (Ethiopia) | 12,400 | 670 | 10 | 400 | 846 |
| Aranguedi (Ethiopia) | 1,540 | 316 | 14 | 51 | 770 |

| $SO_4$ | $PO_4$-P (µg/l) | Total -P | References |
|---|---|---|---|
| 2,200 | | 2,000 | (Jenkin, P.M.) and (Talling, J.F., Talling, I.B.) |
| 67 | | 2,600 | (Talling, J.F.) (Talling I.B., Beadle, L.C.) |
| 4,270 | | 12,200 | (Jenkin, P.M.) (Talling, J.F., and Talling, I.B.,) (Beadle, L.C.) |
| | | 550 | (Loeffler, H.) (Thomasson, K.) |
| 151,100 | | | (Leonard, J. and Compere, P.) |
| 4,290 | 1700 | | (Wood, R.B.) |
| 34 | 3200 | | (Baxter, R.M., Prosser, M.V., Talling J.F. and Wood, R.B.) (Baxter R.M., Wood, R.B.) (Prosser M.V., Wood, R.B. and Baxter, R.M.). |

The simplest technology is sufficient to supress real disadvantages, gives the best results, since then it is easier to reach the optimal point of low-price and good quality. Weakly supported presumptions about the target population's likes and dislikes should be avoided.

A strategy must be created in order to provide a permanent flow of information among the countries with potential for the manufacture of algal feed so that they could be placed in a position to establish a close co-operation at regional level. The countries should be informed of the advantages of establishing a system which will reduce their traditional dependence from foreign sources in the supply of their requirements of nutritional proteins for human and animal consumption.

UNIDO would be the logical catalyst to create this system of regional co-operation in developing countries to assist these countries in the establishment of priorities and to create a framework to set up basic production modules as per the local requirements of each and everyone of these countries. Said framework for regional co-operation should not be confined to the countries listed in this report, but should include also countries on different developmental stage.

The reason for an investment decision to establish an algae plant operation does not need to be based solely on a national profitability analysis, since the Sosa Texcoco venture has proven a secure commercial profitability. This report is in accordance with UNIDO Task Force incentives for Agro-based Industries as it exemplifies a food processing industry. It can serve as a background in providing a world-wide picture on a particular sector and identifies potential development. It is also an example where the algae in most cases can be exclusively produced in developing countries and the trade barrier can be crossed from developing to developed countries.

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3. Mr. F. J.Garfias y Ayala, Director of Investigation, Banca Somex, Division of Basic Chemsitry, Mexico
4. Mr. R. Huesca V., Export Manager, Sosa Texcoco. S.A., Mexico
5. Mr. H. Kamio Yamamoto, Assistant, Sosa Texcoco, S.A., Mexico
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8. Mr. C. Santillan S., Research and Development, Sosa Texcoco, Mexico
9. Mr. D. Jimenez, Resident Representative, UNDP, Mexico
10. Mr. F. Fajnzilber, SIDFA, UNDP, Mexico
11. Mr. A. Faust, UNIDO/JPO, UNDP, Mexico
12. Mr. C. Bursill, Executive Director. N.B. Research and Productivity Council, Fredericton , Canada
13. Mr. D.Abbot, Head of Applied Science. N. B. Research and Prod. Council
14. Mr. P. J. Silk, Head of Chemistry, N.B. Research and Prod. Council.
15. Mr. H. L. Drummond, Head of Engineering, N.B. Research and Prod. Council.
16. Mr. G. D. Brown, Head of Industrial Technology, N.B. Research and Prod. Council
17. Ms. M. J. Gould, Microbiologist N.B. Research and Prod. Council
18. Mr. S. M. Lalla, Chemical Engineer. N.B. Research and Prod. Council
19. Mr. P. A. Lewel, Management Services, N.B. Research and Prod. Council
20. Mr. L. Millin, Secretary of the Council, Science Council of Canada.