Mass production of microalgae*

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The limits of the earth's arable lands[1], the continuing need for more agricultural products and/or raw materials for industry[2], and agricultural products for animal feed and human food[3], the growth of world population[4], and the increasing cost and depletion of fossil fuels[5] all point to the need for new sources of agricultural products that will not tax the earth's declining agricultural and energy resources. We suggest that microalgae may serve as a supplemental source of useful agricultural products[6-8] without making demands on land or mineral resources or requiring large amounts of scarce or depleted energy supplies[6] needed for conventional agriculture. Furthermore, the growth of microalgae in high rate domestic waste sewage oxidation ponds[9,10] can provide microalgal biomass for industrial materials[6] or biogas generation[11,12] from sunlight and CO_2 evolved during primary sewage oxidation or from industrial activity[13], and at the same time reduce the eutrophication potential of wastewater and provide reutilizable water for agriculture and/or industrial cooling[8]. Microalgae may also be grown on arid lands in the tropics or subtropics in saline or alkaline waters and at relatively high temperature to 45 °C[6]; conditions that are not useful for conventional agriculture. This type of microalgal biomass production may necessitate the addition of minerals (nitrates, ammonia and phosphate) and CO_2 unless this production is coupled to a source of these nutrients.

In terms of photosynthetic efficiency, microalgal yields are greater than those of macroalgae and similar to those of higher plants (table 1). Pirt[14] has recently estimated that up to 18% of the solar energy can be stored in algal cells in contrast to the 6% of higher plants in conventional agriculture[15]. Photosynthetic efficiencies of 36-46% (reflecting species differences) of the white light used were claimed for microalgae on continuous culture in the laboratory[16]. Unlike higher plants, the microalgal biomass has a uniform cell content and chemistry as there are no leaves, stems or roots with their different chemical composition like higher plants. Microalgal and metaphyte biomass usually have little ash content (less than 10% dry wt) in contrast to the larger amount of ash (up to 50%) of macroalgae. Microalgae may be selected for the richness of their protein, lipid or carbohydrate content (table 2) and the use to which their biomass may be put. The content of major cell biochemicals may be modified by a variety of environmental manipulations[17]. Microalgae can be grown on a large scale in a variety of outdoor ponds on different parts of the earth under varying light and temperature conditions (table 3). Goldman[10] recently reviewed outdoor mass culture of microalgae and suggested that yields of 15-25 g of dry wt m^{-2} day^{-1} could be attained for reasonably long periods of time.

Microalgae as human and animal food

Microalgae have served as human food in times of famine[18,19] and also in times of plenty[20,21]. The blue-green bacterium, *Spirulina,* is still eaten in the Lake Chad area of Africa when other foods are scarce[18,19] and the freshwater red alga *Lemanea mamillosa* is presently eaten, after frying, in India[20]. Microalgae were used as food by the Aztecs in Mexico[22,23] in the past and macroalgae, and probably microalgae as well, have served as food in Asia for millenia.

In recent years it has been proposed that microalgae, along with other microorganisms, supplement, as single-cell protein (SCP), human and animal foods[24,25]. They have proven unpleasant or even toxic for humans[26] but no attempt has been made to render algal SCP harmless or more palatable because it is

Table 1. Yields and photosynthetic efficiencies for several crops*

	Yield (+ha^{-1} year^{-1})	Total photosynthetic efficiency (%)
Theoretical maximum		
US average (annual)	224	6.6
Microalgae	17-92	0.8-2.3
Macroalgae	0.8-65	0.04-2.2
Higher plants	13-112	0.8-3.2
'Energy farm' (lumber)	25	

* See Dubinsky et al.[6].

Table 2. Range of major biomolecules in microorganisms and conventional foods*

Organism or food	Range (% cell dry wt) Protein	Carbohydrates	Lipids	Total nucleic acids
Bacteria	47-86	2-36	1-39	1-36
Blue-green bacteria	36-65	8-20	2-13	3-8
Microalgae	46-60	2-7	1-76	3-6
Fungi	13-61	25-69	1-30	5-13
Egg	49	3	45	
Meat muscle	57	2	37	1
Fish	55		38	
Milk	27	38	30	
Corn	10	85	4	
Wheat	14	84	2	
Soy flour	47	41	7	

* See Aaronson et al.[7] for details.

presently not competitive with conventional human foods or food supplements. Microalgal SCP has, however, proven useful as an animal feed supplement and microalgae are used extensively as part of the food chain of invertebrates larvae and adults in aquaculture (table 4). As the price of fish and soybean meal currently used as a protein supplement in domestic animal feed continues to rise, microalgal SCP may become economically useful especially as it is a cost saving by-product of a necessary wastewater process[8,27]. Microalgal SCP is sufficient for proper nutrition for it contains adequate to rich amounts of the essential and non-essential amino acids as well as most fat- and water-soluble vitamins needed by animals[7]. Microalgae can be grown in large quantity in outdoor ponds or tanks in many climates and environments (table 3) and the annual protein yield is better than any other source (table 5). It may be argued that microalgae accumulate toxic materials such as pesticides and heavy metal ions which may render them toxic; these same toxic materials accumulate in widely accepted food crops and animal feed materials if they are exposed to air or water containing them[28a,b].

Lipids

Microalgae contain large quantities of fats and oils (table 6) for the manufacture of surfactants, fatty nitrogen compounds, rubber, surface coatings, grease, textiles, plasticizers, food additives, cosmetics, and pharmaceuticals. In the United States alone, industry used over 10^9 lbs in 1 year and the amount appears to be increasing. Algal lipids for industrial use could reduce the use of petroleum products for energy purposes and plant and animal fats for human consumption. (See Dubinsky et al.[6,8] and Aaronson et al.[8]

Table 3. Yields of microalgae in mass culture*

Alga	Place	Yield range (g dry wt · m^{-2} · day^{-1})
Chlorella	Cambridge, Mass. USA	2-11
Chlorella	Essen, Fed. Rep. of Germany	4
Chlorella	Tokyo, Japan	4
Chlorella	Tokyo, Japan	16-28
Chlorella	Tokyo, Japan	14
Chlorella	Jerusalem, Israel	12-16
Chlorella	Jerusalem, Israel	27-60
Chlorella	Japan	21
Chlorella	Taiwan	22
Chlorella	Taiwan	18-35
Chlorella	Rumania	22-36[41]
Diatoms	Woods Hole, Mass., USA	13
Diatoms	Fort Pierce, Fla., USA	25
Diatoms	Woods Hole, Mass., USA	10
Microactinum	Richmond, Cal., USA	13
Microactinum	Richmond, Cal., USA	32
Microactinum	Richmond, Cal., USA	12
Phaeodactylum	Plymouth, England	10
Scenedesmus	Tokyo, Japan	14
Scenedesmus	Dortmund, Fed. Rep. of Germany	28
Scenedesmus	Trebon, Czechoslovakia	12-25
Scenedesmus	Tylitz, Poland	12-16
Scenedesmus	Rupite, Rumania	23-30
Scenedesmus	Firebaugh, Cal., USA	10-35
Scenedesmus	Bangkok, Thailand	15-35
Spirulina	Bangkok, Thailand	15-18
Spirulina	Mexico City, Mexico	10-20
Selenastrum bibrajanum	Rumania	20-40[41]

* Adapted from data cited in Goldman[10] except as shown.

Table 4. Use of microalgae single-cell protein for animal feed*

Vertebrates	Invertebrates
Fish	Molluscs
Poultry	Silkworm larvae[42]
Swine	Bees[42]
Rabbits	

* See Aaronson et al.[7] for references except as shown.

Table 5. Protein productivity of microalgae compared with other protein sources*

Protein source	Protein yield (kg wt ha^{-1} $year^{-1}$)	Reference
Microalgae		
Chlorella (54% protein)	37,449	1
Diatoms (33% protein)	22,886	1
Scenedesmus (43% protein)	29,821	1
Spirulina (57% protein)	39,530	1
Clover leaf	1,680	43
Grass	670	43
Peanuts	470	43
Peas	395	43
Wheat	300	43
Milk from cattle on grassland	100	43
Meat from cattle on grassland	60	43

* Data compiled from mean yield 19 g dry wt m^{-2} day^{-1} of microalgae in table 2 of this paper, ha = 10,000 m^2, and mean protein for these algae in table 3 in Aaronson et al.[7].

Table 6. Total lipids of microalgae in vitro, in nature, and in sewage oxidation ponds

Algae	Range of total lipids (% dry wt)
Blue-green bacteria	2-13
Bacillariophyceae	1-39
Chlorophyceae	1-53
Chrysophyceae	12-39
Cryptophyceae	13
Dinophyceae	5-36
Euglenophyce	17
Haptophyceae	5-48
Phaeophyceae	1-9
Prasinophyceae	3-18
Rhodophyceae	tr-14
Xanthophyceae	6-16
Sewage oxidation (high rate oxidation ponds)	
Chlorella-Euglena (Israel)	23
Euglena (Israel)	11
Microactinum (Israel)	17
Oocystis (Israel)	20
Scenedesmus (Israel)	22
Scenedesmus, *Microactinum*, *Selenastrum* (Richmond, CA., USA)	24

for details on algal lipids, their value, and the economics of their production.)

Carbohydrates

Many microalgae accumulate large quantities of polysaccharides as reserve materials or to compensate for higher external osmotic pressures. A green microalga, *Dunaliella*, is currently being exploited for the production of glycerol[29]. Seaweed (macroalgae) currently supplies phycocolloids (polysaccharides such as agar, carrageenan, etc.) for food additives. If the world's supply of seaweed diminishes as the result of overexploitation and/or pollution, it may become necessary to look to the mass culture of microalgae such as the red alga, *Porphyridium,* which produces a sulfated galactan[30]. Polysaccharides are also used in the petroleum industry; microalgae may provide the long chain polymers with flocculating properties that are needed for oil drilling.

Pharmaceuticals

Microorganisms such as bacteria or fungi have been exploited for almost a century to provide useful drugs, antibiotics, and other pharmacologically active compounds[31,32]. Microalgae like macroalgae may produce a wide variety of pharmacologically-active compounds. Antibiotics, active against bacteria, fungi and even viruses, have been isolated from marine algae, especially macroalgae[33,34]. Antibacterial and antifungal agents have also been found in microalgae (table 7). Microalgae produce phycocolloids like macroalgae and these were reported to have hypocholesteremic properties[35]. Folk medicine contains several microalgal prescriptions to alleviate the symptoms of gall and other stones, gout, cancer, fistula, piles, and vaginitis[36]. Microalgae contain acetylcholine and similar molecules, amines and several alkaloids[36].

Miscellaneous substances

Microalgae may contain volatile compounds. Among these are organic acids, aldehydes, essential oils[7]. All microalgae contain significant amounts of carotenes and xanthophylls which could satisfy the needs for these pigments for coloring poultry, eggs, human food, animal feed, and carp and goldfish[7]. Microalgae contain plant growth factors and they have also been used in small quantity to prepare radioactive biochemicals for research from labeled CO_2, water, etc.

Microalgae as a source of useful molecules on a continuous or discontinuous basis

Microorganisms have proven useful for the production by secretion or excretion of a variety of large and small organic molecules for the food and pharmaceutical industries (Demain[31] and Woodruff[32], for reviews). No published work is available that might indicate the usefulness of microalgae in this area. We suggest, however, that microalgae may be harnessed to produce useful molecules. Microalgae may excrete large quantities of organic molecules (see Aaronson et al.[7], table XIV for details). Among these molecules are small molecules: sugars, nucleic acid derivatives, cAMP, amino acids, amines, fatty acids, volatiles and macromolecules: polysaccharides, nucleic acids, peptides, proteins (including enzymes) (see Hellebust[37] and Aaronson[38] for reviews). Microalgae may be induced to produce large quantities of extracellular molecules in the same way as other microorganisms but without the expenditure of expensive natural raw

Table 7. *Microalgae producing antibiotics**

Algae	Compound
Prokaryota	
Blue-green bacteria	
Hydrocoleus sp.	Terpene, carbohydrate
Lyngbya majuscula	Terpene, carbohydrate
Trichodesmium erythraeum	Terpene, carbohydrate
Eukaryota	
Bacillariophyceae	
Asterionella notata	Unidentified
Asterionella japonica	Nucleosides, fatty acids
Bacillaria paradoxa	Unidentified
Bacteriastrum elegans	Fatty acids
Chaetoceros lauderi	Polysaccharides
Chaetoceros lauderi	Fatty acids
Chaetoceros lauderi	Acid polysaccharide
Chaetoceros peruvianus	Fatty acids
Chaetoceros pseudocurvisetus	Unidentified
Chaetoceros socialis	Fatty acids
Cyclotella nana	Unidentified
Fragillaria prinata	Peptides
Gyrosigma spenceri	Unidentified
Liomophora abbreviata	Unidentified
Lithodesmium undulatum	Unidentified
Navicula incerta	Unidentified
Nitzschia longissima	Unidentified
Nitzschia ascicularis	Unidentified
Nitzschia seriata	Unidentified
Rhizosolenia alata	Unidentified
Skeletonema costatum	Fatty acids
Thalassiosira decipiens	Fatty acids
Thalassiosira nana	Fatty acids
Thalassiothrix frauenfeldi	Unidentified
Chlorophyceae	
Dunaliella sp.	Unidentified
Spirogyra sp.	Unidentified
Chrysophyceae	
Stichochrysis immobilis	Unidentified
Cryptophyceae	
Hemiselmis	Unidentified
Rhodomonas	Unidentified
Dinophyceae	
Gonyaulax tamarensis	Terpene, carbohydrates
Prorocentrum micans	Terpene, carbohydrates
Goniodoma sp.	Unidentified
Prymnesiophyceae	
Coccolithus sp.	Terpene, carbohydrates
Isochrysis sp.	Terpene, carbohydrates
Monochrysis (= Pavlova) sp.	Terpene, carbohydrates
Phaeocystis pouchetti	Acrylic acid
Prymnesium parvum	Terpene, carbohydrate

* See Aubert et al.[33] and Glombitza[34] for references to antibiotics in microalgae.

materials and energy. This production might become continuous with the continuous efflux of useful molecules and biomass at the expense of inorganic salts and solar energy. Furthermore, some of this production might be coupled to domestic wastewater treatment or smokestack efflux of CO_2 where the saleable end products might be useful biomass, products for industry, and reuseable water for agriculture in arid lands and/or cooling water for industry. Microalgae, as is true of other microorganisms, may be induced to excrete desired molecules under a variety of environmental or life cycle manipulations such as stage in life cycle, senescence, nutrient deprivation, chemical or physical stress. The production of useful molecules, as in other microorganisms, may be enhanced by the selection of deregulated mutants.

Microalgae as traps for toxic or polluting compounds

Microalgae, like other microorganisms, may prove useful in the uptake of heavy metals in industrial waste outfalls by accumulating the toxic metals in their cell bodies in a waste trap and then being harvested to remove the toxic compound(s) from the fresh or salt water. Among the metal ions that accumulate as much as several thousand-fold in microalgae are zinc, mercury, cadmium, copper, uranium, and lead. Microalgae also accumulate pesticides and other polluting hydrocarbons (Dubinsky and Aaronson[36]). This concentrating capability of microalgae may be useful in 'scrubbing' waste waters of industry or possibly smokestack effluent to remove and concentrate toxic materials. The algal product, however, may have no further economic use unless it concentrates useful amounts of toxic molecules or can be used as biomass for biogas production. Microalgae may also remove excess nitrate and/or phosphate or sulfite from domestic industrial or feed lot or paper mill waste water. This type of microalgal 'scrubbing' of organic pollutants is coupled with bacterial oxidation in the high rate sewage oxidation pond which has proven useful for the sewage treatment of domestic or feed lot wastes[40]. The resulting algal biomass may be used for any of the products mentioned in earlier sections of this review.

Economics of microalgal biomass and products

The products of microalgal biomass must compete in quality and price with conventional material. Because of our lack of experience with algal products, they must offer significant economic and/or quality benefits to induce the consumer to use them. At present, we think that there is not enough economic return from the production of microalgal biomass for a single product i.e., protein, lipid, etc. to warrant exploitation at current costs of the product unless that product commands unusually high prices, as for example *Chlorella* which is consumed a health food in Japan[39]. However, the production of microalgal biomass becomes economically, advantageous when all of its products such as lipids, defatted algal meal and reutilizable water, and its services (domestic or feedlot wastewater treatment) are viewed together. Based on 1978 prices, Dubinsky and co-workers[8] calculated that microalgal biomass would yield a profit only if algal oil and algal meal were sold separately and the value of sewage treatment and reutilizable water was factored into the calculation. In 1980 the price of soybean oil and meal (suggested as price references) increased 40% and 53%, respectively, while costs have probably increased about 30% in 2 years making the net yield from microalgal production more profitable. Thus it appears economically feasible at present to couple the use of microalgae in domestic and feedlot wastewater treatment with the production of useful compounds for industry and animal feeds. This should not, however, be construed to indicate that these are the only uses for microalgae. If the cost of production and the value of the product warrant it, other microalgal products may become competitive on the world market.

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Mass production of *Spirulina*

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Introduction

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

Production of Spirulina

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

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

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

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

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

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

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

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

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