2

Algae—A Special Case

WHAT AND WHY?

Algae are ubiquitous in habitat and variable in size and morphology; they have been found in the ice of polar regions and in 90°C hot springs, in both fresh water and brine lakes, and they cover a range from unicellular organisms several microns in size up to kelp 50 m in length. Their cell walls can vary from the rigid, thick silicon impregnated walls of diatoms to fragile walls susceptible to osmotic lysis; their reproduction can be sexual or asexual or a combination of both, and they can be highly mobile or totally passive. While taxonomic classification is not always obvious, algae include all photosynthesizing eucaryotic (cells with nuclear membranes) protists (unicellular or undifferentiated multicellular organisms). To this group need only be added the procaryotic (cells without nuclear membrane) algae, the blue-greens.

The two algal groups of principal interest here are the green (*Chlorophycophyta*) and blue-green (*Cyanophycophyta*) algae. Each type has much of the variety mentioned above, but the former, a eucaryote, stores energy as starch (like higher plants) while the latter, a procaryote, stores energy as glycogen.

Algae have been selected as a special case due to their unique open water habitat and a number of advantages which make them an attractive "crop" in certain areas:

- high yields are typical of the best terrestrial plants such as sugar cane;
- transport distances of nutrients and photosynthates are very small, i.e., cellular dimensions;
- nutrient diffusion from the medium, a surface phenomenon, is facilitated by algae's orders of magnitude more surface area per biomass than terrestrial plants;

- nutrients and water are held in a confined space and transpiration is eliminated;
- protein content is very high (40-60% of dry weight);
- nitrogen fixation occurs in some varieties;
- low-quality land can be used;
- low-quality water can be used and thereby improved;
- low-quality waste heat can be used to mutual advantage of e.g., power plant or diesel motors and increased photosynthesis;
- energy costs are low for biomass, protein and caloric yield;
- doubling times are low, on the order of hours;
- a wide variety of species exist which are suitable to vastly differing conditions of salinity, temperature and nutrients;
- certain species, especially blue-greens have a high temperature optimum (about 32°C). This can also be disadvantageous;
- climatic condition variations are of less importance than for terrestrial plants.

In summary, algae (grown on wastes), when compared with normal terrestrial plants, will require 1/50 the land area, 1/10 of the water, 2/3 the energy, 1/5 the capital and 1/50 the human resources for an equal amount of useful organic matter (Oswald and Golueke 1968).

Algae are, however, not preferable to terrestrial plants in all respects, and growth conditions may easily exacerbate the severity of several disadvantages:

- reflection from the water surface is about 30% of the incident light;
- evaporation of up to 1-2 m water per year can occur under arid conditions;
- low cell densities of 0.2 to 8 grams dry weight per liter makes cell concentration and drying both difficult and expensive;
- similarity of nutrient requirements and growth conditions for most algae results in great difficulty controlling the desired species, except under ideal, sterile growth conditions;
- high temperature optima (greater than 30°C) for some species, especially blue-greens;
- toxins are generated by some (especially blue-greens) algae which can be fatal for fish (see page 228);
- parasites, especially rotifers, can easily attack an algal culture;
- untreated algae are poorly digestible and require post-harvesting treatment;
- CO_2 is required in such vast amounts that nonwaste CO_2 is economically nonviable.

MASS CULTURING—GENERAL TECHNIQUES AND GROWTH LIMITATIONS

Technical details of mass algal culturing must, of course, be suited to the end use (food, fodder, fuel, fertilizer) for the algae, which also determines to a large degree the growth medium used (synthetic vs natural, fresh water or saline). Practical systems, when capital costs and technology must be minimized, invariably employ earthen ponds and differ only in the nutrient source and post-harvest processing.

Large-scale open ponds are generally earthen, dug to a depth of 30-100 cm (effective light intensity is near zero beyond 20 cm), and where the earth is porous the bottom is lined with a double polyethylene sheet. If cost allows, concrete can be used instead as plastic sheet has only a one-year lifetime. Vast improvements are needed for an inexpensive, preferably locally produced durable pond bottom. Pond size is freely variable with neither advantage nor disadvantage, and volumes in excess of 10^9 liters already exist. Common variations to the simple open pond include terraced ponds or ponds with narrow (~2m) raceways, each complication being designed to aid stirring and aeration.

Figure II.2.1 summarizes many of the components and alternatives for mass algal cultures where each variation is an attempt to meet the five basic requirements for good algal growth:

- illumination of sufficient intensity;
- CO_2 supply;
- temperature optimum;
- mineral supply;

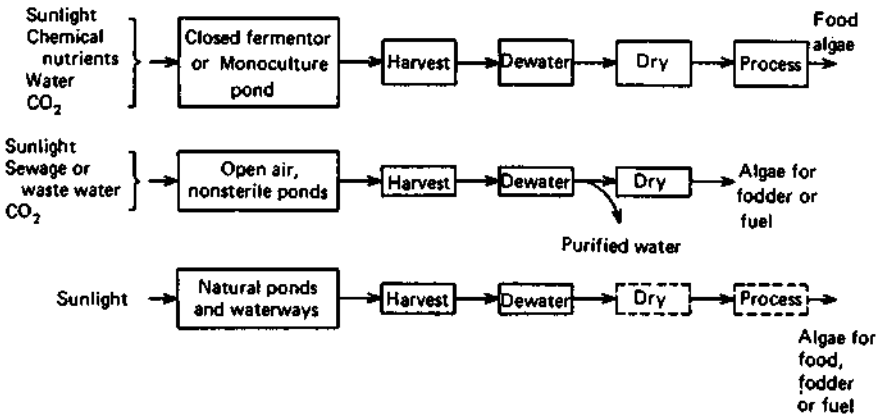

Figure II.2.1. Alternative methods of algae production.

- agitation to prevent sedimentation and promote nutrient and light accessibility.

Light

Light is the limiting factor below the first few centimeters yet is in excess in the first centimeter. This sadly paradoxical situation results from the observation discussed earlier (see page 152) of saturation of photosystem I even at intensities 5-10% of noontime sunlight; furthermore the rate-limiting step has a time constant of 10 msec which is too fast to alleviate by stirring (Lien and San Pietro 1976). That this enzymatic rate constant may well be temperature dependent might find confirmation in the observation that at 40°C the blue-green algae *Anacystis nidulans* does not saturate at high light intensity (Goedheer and Kleinen Hammans 1975). The improvement in algal yield if photosaturation were eliminated could be considerable as the top saturated layer is not only an inefficient solar converter, but filters light from lower cells. The resulting distribution of growth rate vs depth is shown in Figure II.2.2 to be about 40 cm in a natural lake (Talling 1975). For dense algal cultures, little, if any, light reaches such depths, and the maximum is shifted to less than 10 cm.

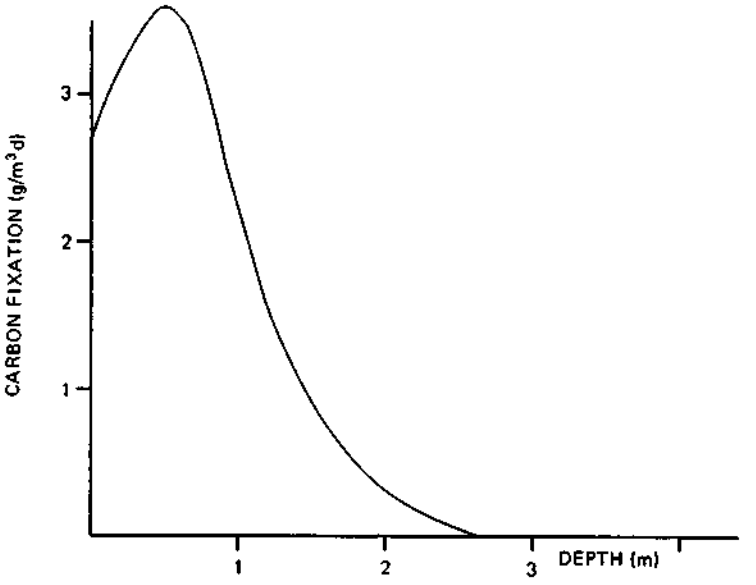

Figure II.2.2. Dependence of algal carbon fixation on depth (data from Mathiesen 1970).

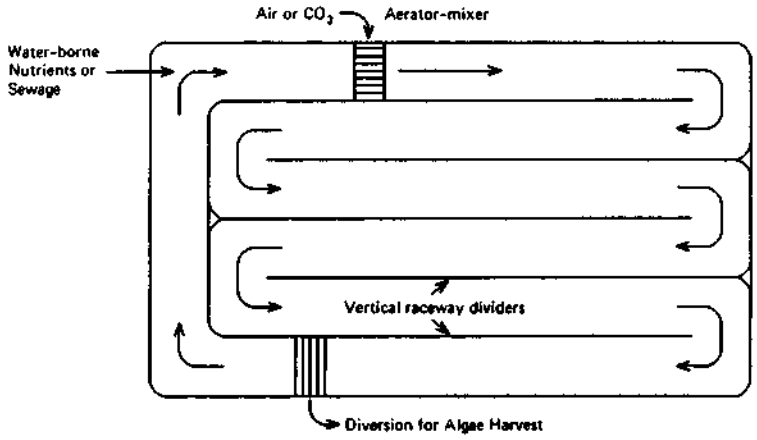

Figure II.2.3. Meandering raceway algae pond (redrawn from Shelef et al. 1976).

The uppermost scum layer, in addition to being photosaturated, tends to dry and requires agitation which also mixes nutrients and prevents sedimentation. Terracing with a slow flow of the culture down a slope is also effective, but complicates pond construction and will add greatly to energy costs if the water must be lifted again to the starting level. With a retention time of a few days and flow velocity of 30 cm/sec one can easily show that pumping requirements will far exceed—by orders of magnitude in some configurations—the solar energy stored in the algae. No terracing system known today is so advantageous as to pay for the added pumping obligations.

The meandering raceway formation shown in Figure II.2.3 provides two alternatives for mixing and aeration: a mixing and aeration pump can emit a jet of compressed air, or alternatively a paddle wheel placed in one corner can be used to provide the required 5-30 cm/sec circulation velocity (Shelef, Moraine, and Meydan 1976). Each system is amenable to windmill power with an auxiliary source for windless days. Circulation in a large, simple open pond can be provided by two pumps in opposite corners (2 × 1/3 hp for 120 liters/min for a 140,000-liter pond; Ryther 1975), but in general the methods of choice will vary with location, materials available and the type of algae (e.g., unicellular or filamentous).

Carbon Dioxide (CO_2)

Although light is the ultimate factor limiting areal yield, CO_2 concentration is the most severe, controllable limiting element. CO_2 is particu-

larly limiting at midday under bright conditions when the CO_2 fixation rate far exceeds the supply rate unless special measures are taken. While algae are not strictly C_4 plants, they do not demonstrate photorespiration; the observation of photo-oxidative death under low CO_2 may, however, account for death of algae during the summer (Abeliovich and Azov 1976). CO_2 concentrations for algae are hence mostly determined by the need to supply CO_2 at a rate matching its consumption. Gas consumption can easily be calculated from the rate of cell mass production—per year, day, or hour—using the conversion factor 1.8 grams CO_2 consumed per gram dry algae grown. A reasonable maximum hourly rate of algal CO_2 fixation is ca 20 grams/meter^2/hr or 40 mg/liter/hr (assuming 0.5 meter depth). This amount of CO_2 is contained in air (0.03% CO_2) flowing at the tremendous rate of 70 liters of air per liter of culture per hour; as not all CO_2 in air will be removed, the actual rate of flow would have to be considerably larger. These flow rates are impossible for several reasons and simply point out the need for CO_2-enriched air.

CO_2-enriched air, say 5% CO_2 instead of the normal 0.03% CO_2, decreases the required gas-flow rate both because the gas carries more CO_2 per liter and because the soluble CO_2 equilibrium value increases. The first factor decreases the rate by more than two orders of magnitude ($\times 170$), while the second factor increases both the fraction of CO_2 removed from the gas and the medium's CO_2 storage capacity. Equilibrium with air is only 0.5 mg/liter (pure water at 25°C) while with 5% CO_2 enriched air it is about 70 mg CO_2/liter water, or about two hours' CO_2 requirement.

CO_2 solubility is far more complex than can be adequately dealt with here, but a few important points should be noted. First absorbed CO_2 is found in equilibrium with several other forms:

$$CO_2 \text{ (gas)} \rightleftarrows CO_2 \text{ (dissolved)}$$
$$CO_2 \text{ (dissolved)} + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^- \rightleftarrows H^+ + CO_3^{2-}$$

Equilibrium values are determined by gas CO_2 content, water medium temperature, pH, and salt content (Meynell and Meynell 1965). The first two factors for CO_2 solubility in water (mg/liter at 760 nm pressure) are summarized in Table II.2.1.

CO_2 solubility is somewhat dependent on pH and more so on salt concentration, but the amount potentially convertible to nonvolatile and highly soluble bicarbonate (HCO_3^-) is most strongly dependent on pH. Table II.2.1 and Figure II.2.4 demonstrate these equilibrium changes and emphasize the advantages of a high pH growth medium, as for *Spirulina*. Even within the more normal physiological range the variability of available CO_2 in solution (as CO_2 or bicarbonate) is large: at pH 6 (37°C) and

Table II.2.1 CO_2 Solubility in Water (mg/liter at 760 nm pressure; Meynell and Meynell 1965).

T (°C)$_2$	100% CO_2	5% CO_2	0.033% CO_2 (air)
0°C	3346 mg/liter	167 mg/liter	1.100 mg/liter
10	2318	116	.765
20	1688	84.5	.557
30	1257	63	.415
40	973	48.6	.32
50	761	38.0	.25

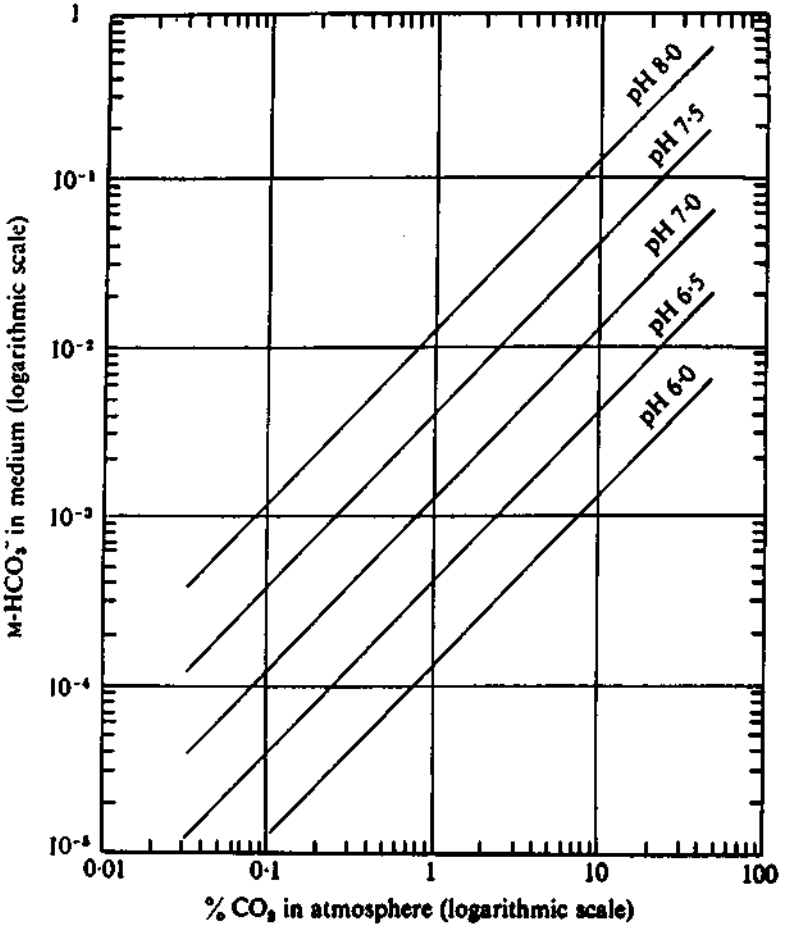

Figure II.2.4. Dependence of bicarbonate on pH and atmospheric CO_2 concentration (by permission of Meynell and Meynell 1965).

5% CO_2 gas, 90 mg CO_2/liter are potentially available (72.5% CO_2 and 27.5% as HCO_3^-), while at pH 8 nearly 3.4 grams CO_2/liter are available (97% as HCO_3^-). Any decrease in pH, often accompanying photosynthetic CO_2 absorption, can cause large amounts of CO_2 to be volatilized and points out the need to hold the CO_2—HCI_3^-—CO_3^{2-} buffering system poised at the optimum pH.

As bottled CO_2 is excessively expensive, various other schemes have been attempted:

1. In Israel (Richmond 1975) calcium carbonate is readily available from which CO_2 and hydrated lime (from calcium oxide) are the combustion products:

   $$90\% \text{ calcium carbonate} + 10\% \text{ oil} \xrightarrow{\text{combustion}} CO_2 + \text{calcium oxide}.$$

   Mixing with air for 1-2% CO_2 concentration and bubbling the gas in the medium gives near optimum conditions for *Spirulina* growth.
2. Carbon dioxide is the principal gaseous product of any combustion process and generally—perhaps after a bit of particle removal—is treated as an easily disposed of waste product. Combustion or exhaust gases are, however, ideal from another point of view: they are heated, pressurized and contain predominantly CO_2. A well integrated system for algal growth can exploit these factors to provide the required CO_2 for algal growth and extend the growth season in cool climates. On a larger scale, conventional power plant flue gases can be combined with large open algal ponds (Benemann et al. 1976), while for smaller scale ponds CO_2 from the confined combustion of biogas can be used. Cleaning of the gases may be required for food algal growth, but this has been shown feasible for diesel exhaust gas using first a (sea) water trap for water soluble gases (SO_2 and NO_2) followed by an activated charcoal filter for nonsoluble organic gases (Eisa, Zeggio, and Jensen 1971).
3. The easiest and most widely practiced large-scale production of CO_2 for algal growth is from the aerobic bacteria digesting organic wastes in waste treatment oxidation ponds. Restated from the sewage treatment point of view, the algae are used to provide O_2 for the bacteria in the oxidation pond. The happy symbiosis will be discussed in detail in the section entitled GROWTH MEDIUM on page 216 of this Chapter.

Temperature

The third growth factor, temperature, is rarely controlled, but determines in part the species which naturally bloom and where algal ponds are practicable. Oswald (1973) states that waste treatment algal ponds are

practicable wherever ponds don't freeze for more than one or two months. Uses discussed here mostly have more stringent requirements such as for the interesting blue-green algae whose temperature optima lie over 30°C.

As more than one meter water can in certain areas evaporate per year and cause evaporative cooling, a transparent plastic cover may in some cases aid in maintaining optimum temperature. If one assumes, for example, an annual water loss of one meter (10^7 liters/ha-yr), then covering the pond would save not only water but ca 23×10^6 MJ/ha in low-grade heat energy, or 35 times the energy in the algae (assuming a dry algal yield of 50 tons/ha-yr). Low-grade heat energy can be used only with low efficiency, but this waste heat (ca 95% of the incident light energy) is readily available if the temperature needs to be raised in the pond or for example in associated microbiological digesters. Clearly, conductive losses to the earth and air will become limiting in a covered pond and will increase with increased pond temperature, but depending on the application, some insulation techniques may be justified (Dickinson, Clark, and Wonters 1976). Cooling at night may not be disadvantageous as dark respiration increases with temperature.

GROWTH MEDIUM

The fourth growth factor, nutrient requirements and their method of provision, are treated separately here because they divide algal culturing into four major divisions to be discussed in detail here: "clean" algae grown on synthetic medium (for food), algae grown on wastes in conjunction with oxidizing bacteria (for food, fodder, fuel or fertilizer), algae harvested from natural or semi-natural blooms (for food or fodder), and nitrogen fixing algae grown alone or in association with other plants (as nitrogenous fertilizer). The first three of these alternatives were summarized in Figure II.2.1.

Synthetic Medium for "Clean" Algae

The cultivation of clean algae for human consumption differs from the other systems to be discussed here only in the source of CO_2 and minerals; all concepts of pond construction, temperature and harvesting can be identical with simpler systems. Economically, the justifications for these extra costs lie in possibly obtaining greater yields due to medium optimization, the greater possibility to control the algal specie, and a higher retail price (assuming algae from wastes is only suitable as fodder or would require expensive sterilization before human consumption). Presumably, the higher product value justifies a greater investment in ponds

and harvesting procedures, but evidence to date is not economically encouraging. The German effort (Soeder 1976) can produce food quality protein from *Scenedesmus* projects in India, Thailand, Peru and Israel (the latter project uses sewage effluent instead of a synthetic medium, see below). This algal protein grown on synthetic media has quality comparable with soya protein, and if the cost of the latter rises a bit, algae may become economically competitive.

Among the major algal species contending for commercial exploitation are *Chlorella vulgaris, Scenedesmus acutus, Coelastrum proboscideum* and *Spirulina maxima,* the most active work being found in Japan (*Chlorella* and *Spirulina*), France (*Spirulina*), Czechoslovakia, Bulgaria and Germany; the latter three all use *Scenedesmus.*

Scenedesmus has the advantage of being prolific, but its small size has continually plagued the economics of harvesting (see page 224). A unique alternative, one appropriate to equally unique conditions is *Spirulina,* a helicoidal blue-green (*Cyanophyte*) algae which is advantageous for several reasons (Pirie 1975a):

- its size (0.2 mm long) and helical shape causes the formation of entangled clumps which are easily removed by filtration;
- its alkaline (pH 10) growth optimum allows little competition from other microorganisms, assures abundant CO_2, and allows use of alkaline lakes common to arid regions whose waters are useful for little else:
- its protein content (64-70%) is greater than any other natural product and is of higher quality;
- it has high digestibility and mild taste;
- it has been used for centuries as a food;
- it has an annual dry weight yield of 50 tons/ha-yr under optimum conditions (35°C, pH 9, 23 grams/liter salinity) to 10 tons/ha-yr under semi-natural conditions.

One such semi-natural medium is found in Mexico, where Sosa Texcoco, S.A. (a Mexican company extracting sodium alkalies) and the French Institute of Petroleum operate a one-ton/day pilot plant using local lake water containing 30 grams solids per liter (Santillán 1974, or Pirie 1975a). The yield from their semi-natural solar evaporator is 10 dry tons/ha-yr (pH 9.8). *Spirulina* is harvested from this cultivation lagoon (normally 0.1 − 0.3 gram cells/liter) by first concentrating the algae on inclined filters to 5-10 grams/liter and then to 15-25 grams/liter using rotary filters; both stages are designed for high filtration efficiency and low energy consumption. Vacuum filtration and washing reduces the inorganic matter and provides a paste of 15-20% solids. Handling

(viscosity) and digestibility of this cell paste is improved by mechanical cell rupture (proprietory type) prior to spray or drum drying at 130°C for six seconds. If flour rather than flakes are desired, grinding can follow the drying.

Natural "Media"—Sea Farming

Natural algae and water plant densities are normally too dilute in natural waterways to justify harvesting except in some polluted waters. Cultivation of natural waterways, or sea farming, is however highly attractive in many respects:

- oceans cover 71% of the earth's surface;
- half of the ocean area lies within 30° of the equator;
- little competition exists for sea use;
- ocean plants are efficient solar-energy converters like most aquatic plants;
- ocean farming is not subject to normal climatic variations;
- terrestrial farming already employs the best arable land and fresh water.

Limitations have, however, heretofore prevented exploitation of ocean farming:

- the ocean bottom lies at depths where light intensity is nil and too deep for the growth of attached seaweeds, and
- well-illuminated surface waters have essentially no nutrients (Figure II.2.5).

This unfortunate situation has potential if a raft mesh is held 15-25 meters below the surface by cables to the sea bottom. Seaweeds are attached to this mesh, according to this proposal at three-meter intervals to provide a density of about 1000 plants per hectare (Wilcox 1976). To overcome the inadequacy of dissolved nutrients shown in Figure II.2.5, the cool sea-bottom waters, rich in phosphorus, potassium and fixed nitrogen, would be upswelled with ca 11 hp/ha pumping power derived from wind, wave or ocean thermal gradients; wave power, believed to be economically and energetically viable for electrical generation, is particularly promising. Additional nutrients can be provided from processing wastes on the shore or ship facilities.

The plant tentatively selected for ecological and efficiency reasons is the giant (up to 100 meters) brown or California kelp, *Macrocystis pyrifera* from the coasts of California, Mexico and New Zealand. *M. pyrifera*, one of the fastest growing plants (15 cm/day or up to 60 cm/day for young

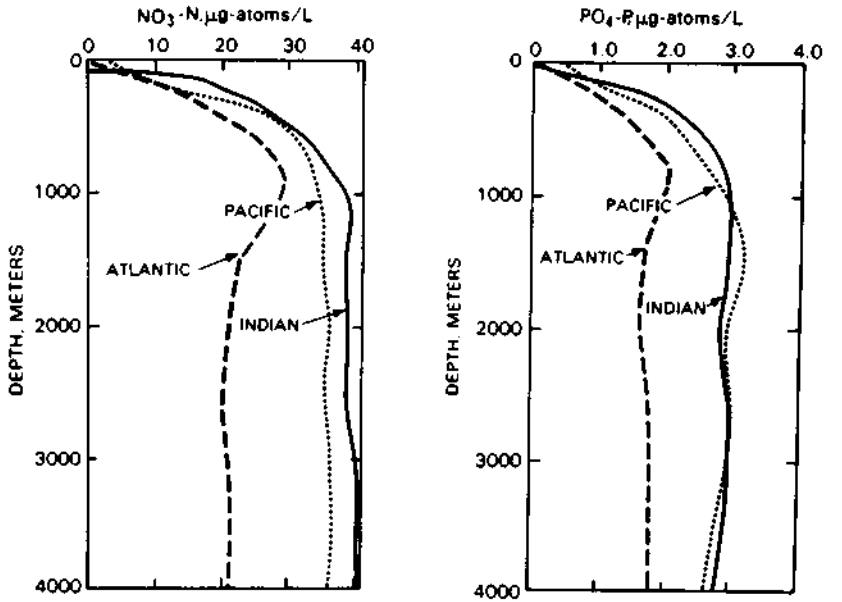

Figure II.2.5. Nutrient gradients in open seas (Sverdrup, Johnson, and Fleming, The Oceans: Their Physics, Chemistry, and General Biology, copyright 1942, renewed 1970, pp. 241 and 242. Reprinted by permission of Prentice-Hall, Inc., Englewood Cliffs, New Jersey).

fronds) has multibladed fronds lying along the sea surface and lives six months before being replaced by new growth from below. As the plant itself does not age, its doubling time of six months allows continual harvesting every three months, replacement being required only by storm, disease or fish damage.

Kelp products have long been used as thickening agents and colloid stabilizers in the food, textile, cosmetic, pharmaceutical, paper and welding industries. Years of experience have shown that kelp can be harvested and partly processed with special ships to aid in locally recycling nutrients and minimizing transport costs. Treatment of harvested kelp begins with removal of the surface coat of fucoidan by washing with warm water and then following chopping, water and salts are removed by cell rupture in weak acid. Separation technique and end-product choice is widely variable from this point.

The most ambitious kelp project, estimated to cost two billion dollars by the year 2000, is a highly integrated scheme designed to combine many product options (Wilcox 1976). While methane via anaerobic digestion is to be the main product, high-quality food in addition to fodder, fertilizers, sugar syrups and several organic chemicals will be by-products

significantly reducing the energy costs. Combined marine aquaculture with confined grazing fish is also being considered in what is ultimately hoped to be a carefully integrated, wave-powered 45,000-ha complex.

Ecological dangers must be studied as the harvested area increases, but they are predominantly avoided by the artificial feeding required for survival: the kelp require the cool nutrient-rich water provided by upwelling and will not survive nor spread if this supply is turned off. This property could allow growth in tropical and semi-tropical climates without the danger of infection in unwanted areas.

The ICES (International Council for Exploitation of the Sea), however, is not convinced of the ecological safety and refused to support a French project planned for the coast of Brittany. The viable temperatures of 2-20°C are available in large areas of Western Europe from North Africa to Norway, and ICES felt a real danger existed for navigation, salmon and lobster fishing and defence. Such problems are not expected to be relevant in California.

Water hyacinth, which though not an algae, is a prolific freshwater plant under consideration for exploitation. It commonly blocks polluted streams and waterways and produces up to 60 tons/ha-yr. Cultivation is tentatively being considered, but no projects have reached any serious dimension. An alternative viewpoint is exploiting water hyacinth and other water plants for *in situ* sewage treatment, i.e., the removal of nutrients from polluted waterways where algae size is of critical importance for harvesting (Yount and Crossman Jr. 1970, Wolverton and McDonald 1976).

ALGAE CULTURED ON WASTE

Mass algal culture on waste effluent has traditionally been viewed from the water purification perspective from which the algae's principal function is the production of oxygen for the bacterial oxidation of organic wastes. In addition, the algae functioned as a pollutant sponge for the released nutrients (P, K, and NH_3). The energy for bacterial growth is derived from the oxidation of organic wastes, whereas the algae use solar energy to reassimilate the nutrients freed by the bacteria into a more valuable high-energy form. Such treatment prevents the pollution problem of raw sewage in, for instance, rivers where the bacteria oxidizing the organic wastes deplete the oxygen and release CO_2 and NH_3 to the air and nutrients to the water. Enormous algal blooms thrive on the nutrient-rich water downstream, and the oxygen produced by the algae is in turn wasted. The confined algal oxidation pond combines algal oxygen production and bacterial CO_2 production symbiotically for maximum break-

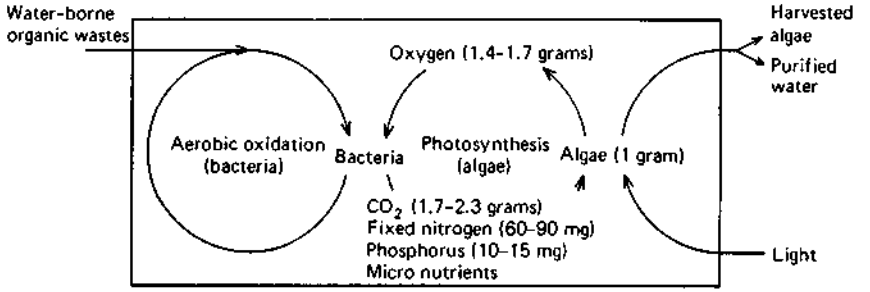

Figure II.2.6. Confined oxidation pond (redrawn from Oswald 1973).

down of organic wastes and reorganizes the nutrients into algae, leaving relatively clear water (Benemann et al. 1977). These complementary processes are best understood from Figure II.2.6.

As soon as the lack of one nutrient begins to limit algal growth, the waste water can be returned to the natural waterway while the algae can be discarded as a used up sponge or processed further. No nutrient is ever reduced to zero concentration, but Table II.2.2 shows that the limiting nutrient, phosphorus, is reduced by 90% (Shelef et al. 1976). These results are typical except that water scarcity in Israel causes nutrient concentrations to be greater than for example in the US.

A wider and more recent view of this accelerated waste water treatment system is to see algae as a valuable product, a source of fuel, food, fodder and possibly fertilizer. In arid zones, particularly, this process is a step in

Table II.2.2 Removal of Various Substances in the Technion, Haifa, Photosynthetic Pond under Favorable Operational Conditions (Shelef et al. 1976).

Substance	Raw Sewage mg/liter	Pond Effluent mg/liter	Flotator Effluent (alum dossages 80–90 mg/liter) mg/liter
Suspended matter	240	268	15
BOD_5: total	330	106	10
dissolved	—	12	5
COD: total	750	670	148
dissolved	—	64	46
Nitrogen: total	86	71	20
dissolved	—	18	12
Phosphorus: total	16	10	1.4
Coliforms/100 ml	6×10^7	3.5×10^5	8×10^3

water recycling, not just waste treatment. The technical problems for these systems always remain the same as the above more limited view, but the economics are changed dramatically: cost credits for food and fuel produced and the lowered waste disposal costs (or from the other point of view, disposal credits) reduce the net protein costs. The cost advantage over synthetic media is considerable, but in this case optimization must also include maximum algal yield and recovery, not just "pollutant" (nutrient) removal. Pressing shortages of protein and all nutrients supporting food production must make obsolete the concept of waste disposal. Which function is maximized, however, will depend on local needs, but as none is in itself sufficient for economic success, algal oxidation ponds must be viewed as a multipurpose component in a well-integrated system.

Kinetics of Algal Production

Aside from some extreme cases, the rate of algal production is limited by incident radiation rather than temperature or nutrient concentrations; in part this statement results from the ability to vary the detention time such that nutrients do not limit.

Nutrients become available for algal uptake at the rate of bacterial organic waste decomposition. As this decomposition process is an oxidation, this bacterial reaction can be measured by the rate of oxygen consumption, or what is commonly called the biological oxygen demand (BOD). Several nutrients are released simultaneously in this process but carbon dioxide is by far the most important to consume at the rate it is produced—others will accumulate, but carbon dioxide will be lost to the atmosphere due to its limited solubility. Algal growth rate can be determined from the rate of photosynthetic oxygen production, nutrient incorporation (i.e., 1.6 grams O_2 and 65.9 mg N per gram algae), or by the rate of algal harvesting. Whatever the growth rate determinant used, the rate of oxygen produced by the algae (1.7 kg O_2/kg algae) must equal the bacterial biochemical oxygen demand (BOD) to prevent O_2 or CO_2 loss to the atmosphere; in this symbiotic system, oxygen loss is the more serious as it is about fifty times less soluble than CO_2. To cite a practical example, human wastes have a BOD of ca 75 grams O_2 per day, and therefore 40 grams algae per person per day will be produced. Alternatively, the wastes of 1500-4500 persons can be treated per hectare per day (Oswald 1973).

Several parameters including pond depth, light intensity, the nature and loading rate of the organic wastes, and temperature must be considered to achieve a "well-tuned" system. Pond depth should increase with increased sunlight, and hence both seasonal and geographical variations

Table II.2.3 Probable Values of Visible Solar Energy as a Function of Latitude and Month (from Oswald 1973).

Latitude		MONTH											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
0	max	255*	266	271	266	249	236	238	252	269	265	256	253
	min	210	219	206	188	182	103	137	167	207	203	202	195
10	max	223	244	264	271	270	262	265	266	266	248	228	225
	min	179	184	193	183	192	129	158	176	196	181	176	162
20	max	183	213	246	271	284	284	282	272	252	224	190	182
	min	134	140	168	170	194	148	172	177	176	150	138	120
30	max	136	176	218	261	290	296	289	271	231	192	148	126
	min	76	96	134	151	184	163	178	166	147	113	90	70
40	max	80	130	181	181	286	298	288	258	203	152	95	66
	min	30	53	95	125	162	173	172	147	112	72	42	24
50	max	28	70	141	210	271	297	280	236	166	100	40	26
	min	10	19	58	97	144	176	155	125	73	40	15	7
60	max	7	32	107	176	249	294	268	205	126	43	10	5
	min	2	4	33	79	132	174	144	100	38	26	3	1

will occur, but depths of 30-40 cm are typically optimal for regions whose climates permit year-round operation. Table II.2.3 lists the annual sunlight variations at different latitudes and indicates that areas within 35° of the equator are ideal for algal growth, but even growth in remote regions (e.g., 60°) is possible. Light utilization efficiency is shown in Figure II.2.7 to decrease rapidly with increasing intensity above about 40 J/cm²-day) which coincides with the maximum for the product of efficiency and productivity shown in Figure II.2.8.

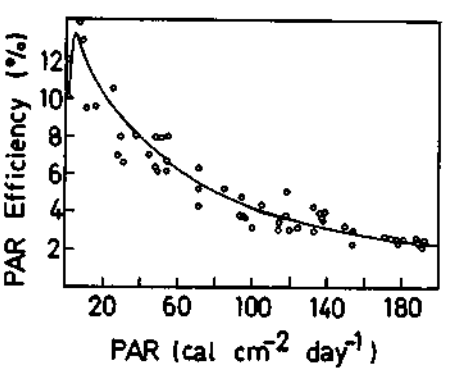

Figure II.2.7. Dependence of micro-algae's light conversion efficiency on photosynthetically active radiation (PAR) intensity (by permission of Oswald 1973).

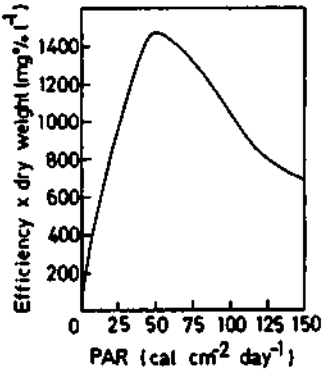

Figure II.2.8. Variation of the product of efficiency and dry weight production with PAR (by permission of Oswald 1973).

Daily oxygen and algal production yields can be calculated using the following simplified relations and the data in Table II.2.3 and Figure II.2.7 (Oswald 1973):

$$\text{Cell yield (kg/ha-day)} = 0.17\ FS$$
$$\text{Oxygen yield (kg/ha-day)} = 0.28\ FS$$

where,

F = the conversion efficiency (Figure II.2.7)
S = the incident solar energy (Table II.2.3)

Figure II.2.8 shows that 4000 cal/liter-day (16.7 kJ/liter-day) gives the optimal algal productivity. This optimum can be attained by adjusting the pond depth to compensate for the light intensity from Table II.2.3; this depth is typically less than 0.5 meters. The optimal productivities for various latitudes and assuming different solar-conversion efficiencies were calculated for Table I.6.1. An annual efficiency of 3% is the typical present maximum.

The third controllable factor, the dilution rate (or its inverse, the retention time), also determines the concentration of algal cells. Figure II.2.9 shows that a clear optimum exists for maximum production efficiency: the decrease at low retention times, less than a day, is due to culture washout while at too long retention times (greater than 4 days) higher cell density prevents adequate light penetration. Optimum detention period varies with light intensity and depth, but three to four days is a typical average (Shelef et al. 1973).

The fourth component determining the rate of algae production is of course the sewage or waste which provides the nutrients for algal growth.

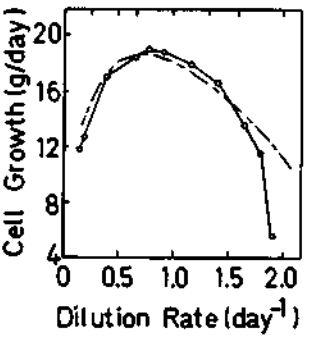

Figure II.2.9. Variation of *Chlorella pyrenoidosa*'s net production rate with dilution rate (detention period^{-1}; solid curve—experimental data, broken curve—theoretical calculation) (redrawn from Shelef, Schwartz, and Schechter 1973).

As each gram of algae requires 0.5 gram carbon, 0.1 gram nitrogen and 0.01 gram phosphorus, the relative nutrient balance in the sewage is of prime importance. If the system is optimized for algal *production* rather than sewage treatment, supplements of the limiting nutrient may be justified for total nutrient usage, particularly ammonia. Ammonia is the nutrient required for protein synthesis and is the most costly energetically, but in excess of 2mM or at pH values over 8.0 it can severely inhibit algal growth (Abeliovich and Azov 1976). Potential nutrient losses include phosphate precipitation under some conditions (flocculation) and ammonia evolution due to the high surface pH on sunny days.

The rather well-balance nutrient characteristics of domestic (US) sewage are shown in Table II.2.4. Table II.2.5 summarizes the additives required for maximal use of other wastes. Vegetable wastes, for example, are typically limiting in nitrogen and phosphorus, while animal wastes are limiting in carbon (carbon dioxide); if CO_2 is provided, 5-10 kg algae can be grown from the nutrients in each kilogram of human or other animal wastes.

Depending on the end use of the effluent water, a marine system may be of interest. At Woods Hole Oceanographic Institution in the US (Ryther 1975), secondary treated urban effluent was mixed with equal amounts of sea water to produce a near monoculture of a marine diatom with yields comparable to fresh water systems (see page 218). If the effluent is to be disposed of, this scheme is highly advantageous for water conservation. More commonly the treated water should be kept salt-free for reuse.

An alternative "waste nutrient" source, the sludge from an anaerobic digester, is neither as energy-rich nor does it have as high a BOD as raw

Table II.2.4 Domestic Sewage Characteristics (Oswald 1973).

pH	9.4
Total solids	553 mg/liter
Volatile solids	212 mg/liter
Fixed solids	342 mg/liter
OH^-	1.6
CO_3^{2-}	48
HCO_3^-	141
Total N	44.5
NH_3	25.5
NO_3	0.1
Organic N	18.9
Total C	80.7
PO_4^{3-}	38.8
H	27.2
MG	11.6
Ca	7.4
K	25.5
S	7.1
Fe	0.5
C-BOD (20°C 5 day)	140
Ultimate BOD	218

Table II.2.5 Potential Nutrient Sources for Mass Algal Culture (Oswald 1976a).

Sources of Water and Nutrients	Required Supplementary Nutrients	Potential Use (subject to toxicological and epidemiological evaluation)
Man-made algal nutrient solutions	None	Human food
Potato-processing wastes	None	Human food
Sugar, tomato, winery wastes	N and P	Human food after heat drying
Natural bodies of water, brackish or fresh	N and P	Human food after pasteurization
Spring and wells, including geothermal waters	N, P, C	Human and animal food
Domestic sewage	None	Animal feed after pasteurization
Animal manure	None	Animal feed after pasteurization
Meat-processing wastes	None	Animal feed after pasteurization
Reduction-plant wastes	C	Animal feed after pasteurization
Petroleum-refinery wastes	P	Animal feed
Irrigation and land drainage	N, P, Fe	Animal feed after pasteurization
Storm waters	C, N, P, K	Animal feed

sewage and hence will not support as much bacterial growth. All the nutrients are present in a simpler, soluble form, but the anaerobic bacteria have transformed most of the energy from fats, protein and carbohydrates to methane (CH_4). Some CO_2 will evolve, however, from aerobic bacteria in an algal pond fed anaerobic-digester sludge as the former break down the more inert organic particles, but optimal growth required additional CO_2; some low-energy CO_2 sources were discussed earlier in this chapter of which CO_2 from the digester gas (ca 40% of the gas) is the most readily available.

SPECIES CONTROL

All open-pond algal growth systems with the exception of the proposed kelp plantations accept whatever species spontaneously dominate with little (successful) effort to alter Nature's choice. Two motivations exist for desiring a given specie: first, if the algae is intended for human food, toxological tests for each specie are required. *Spirulina* is favorable in this case as its optimum medium is suitable for little else. A second case involves selecting filamentous algae because of their ease of harvesting and the resulting tenfold reduction in cost (see following section on "Harvesting").

Filamentous algae rarely dominate except in instances where nitrogen is limiting, and then naturally nitrogen-fixing blue-green species have a selective advantage; this is, of course, one method of assuring their dominance and exploiting their ammonia-producing capacity, but for the purpose of waste-water treatment the advantages of simple harvesting make filamentous algae—in the presence of ammonia—attractive.

Pilot experiments have been conducted using a rotary microstrainer (discussed in the following section on "Harvesting") to harvest filamentous algae grown in large open ponds (Benemann et al. 1976). A fraction of the harvested algae are used to reinoculate the pond to displace the specie(s) which normally would dominate. Recycling favors the selected specie by effectively lengthening its residence time in relation to the faster-growing species.

Such techniques have been applied to encourage the growth of algae on waste water. *Oscillatoria,* a filamentous blue-green algae, was inoculated in an open pond, but the filtering and recycling scheme also favored a colonial green algae (*Microactinium*) which later dominated even without continued recycling. Although the inoculated specie was not established, the goal of obtaining a filterable algal specie was attained. If this technique proves successful, the economically and energetically costly harvesting methods for unicellular algae can be avoided. Similar techniques

are to be tried to sustain nitrogen-fixing blue-green algae, the energy requirements and growth rates of which rarely allow their natural dominance except when nitrogen is limiting (Benemann et al. 1976).

HARVESTING

For microalgae, harvesting is the major economic constraint and prevents the use of naturally occurring algal blooms whose densities rarely exceed 3 mg/liter. The minimum density which can be economically harvested is approximately 250 mg/liter, and artificial algal ponds can create densities up to 1 gram/liter. Several stages are generally required for harvesting:

1. The initial concentration step has in principle several alternatives, but in practice none is yet entirely satisfactory:
   a. Centrifugation is the technically ideal method, removing 84% of the algae at 1500 liters/min. Power costs are, however, prohibitive: 2.7×10^3 KWh/ton or $60 per ton algae (at 300 mg/liter; Oswald and Golueke 1968). Capital costs are even more prohibitive: $50,000 (1969 prices!) for a 1600 liter/min capacity unit. Centrifugation is a practical laboratory concept, but holds no applicability on mass scale.
   b. Auto-flocculation is a natural process which occurs on sunny afternoons when the increase in pond temperature, depleted CO_2, and increased pH (up to pH 9.8) causes algae to clump together and sink to the bottom. To exploit this method would require a separate flocculation pond of 8-15 cm depth where the supernatant could be decanted and returned to the growth pond. Economically this is an excellent method, but its dependability remains to be established.
   c. Chemical flocculation or coagulation of microalgae can be induced with the addition of aluminum sulfate (or alum, $Al_2(SO_4)_3$), lime ($Ca(OH)_2$), ferric chloride ($FeCl_3$) or a few other chemicals. The addition of, for example, 70-120 mg/liter alum at pH 5.5-6.0 is followed first by a sequence of rapid and gentle stirring and then by air or electroflotation (Shelef et al. 1976). As shown in Figure II.2.10, the flocculated algal froth (ca 5-10% solids) is skimmed off after a few minutes in the flotator, and after the addition of acid the now soluble alum is separated in a low-power centrifuge from the algae. At least 50% of the alum and acid can be removed for recycling, while the 15-25% solid algal paste is dried or processed further. While flocculation is the harvesting method most widely practiced today, one re-

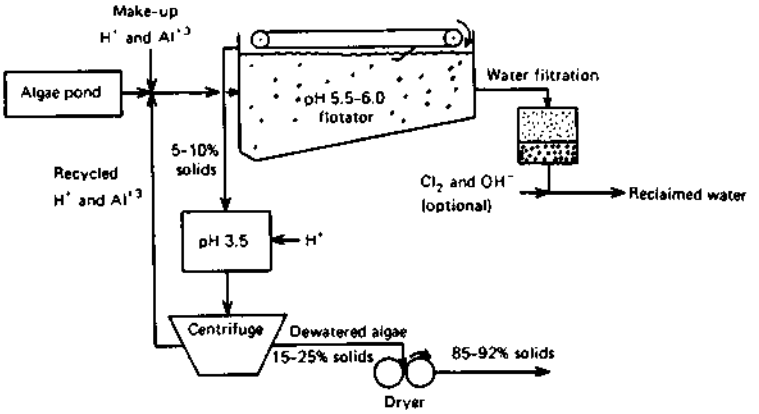

Figure II.2.10. Harvesting by recycled alum flocculation (redrawn from Shelef et al. 1976).

maining problem is the presence of about 4% alum in the algae. This alum greatly limits the algae's end uses, but is not a problem when used as fish fodder due to the near neutral pH of the fish digestive system; a 30% algal diet has been shown to replace 85% of the fish meal supplement (15% of the fish diet; Shelef et al. 1976).

d. Magnetic harvesting of algae which itself is nonmagnetic is possible via the chemical attachment of algal cells to waste magnetite from mining operations (Kolm, Oberteuffer, and Kelland 1975). The complex is then passed through a magnetic pole gap filled with a three-dimensional stainless steel grid and there trapped. The algae can then be removed from the magnet-bound magnetite by caustic soda (or sonication) which hydrolyzes the cells and allows recovery of the protein. Nearly 99% of the algae is removed from the medium of which 84% is hydrolyzed and recovered. Magnetite can also be recovered, but at $15/ton is not yet economically justified. The overall cost for a magnetic separator is approximately $50,000 giving an optimistic separation cost estimate of about $0.004/kg protein, including chemical additions (Mitchell 1977). Smaller-scale units employing permanent magnets are expected to reduce costs substantially, but this will remain a high-capital system.
e. Harvesting by raking partially submerged or floating algae is the least expensive method of harvesting, but unfortunately this is applicable only to filamentous algae. Filamentous algae dominate an algal population only under special conditions or when special efforts are made for its dominance (see page 223 of this

chapter). In the latter case the more sophisticated "raking" method or rotary microstrainer shown in Figure II.2.11 is used. The microstrainer is a rotating drum supporting a 25-100 micromesh screen which collects the filamentous algae introduced at the bottom and releases them into a trough after 180° rotation through the help of pressurized water. Costs are estimated to be a tenth that of chemical flocculation (Benemann et al. 1976).

f. Harvesting of algae or aquatic weeds with herbivorous fish, especially bivalves with their built-in filter system, is both effective and economically attractive, but of course assumes that fish is the desired product. This important and traditional algal use, commonly called aquaculture, is discussed separately in the following section "Uses for Harvested Algae."

2. Dewatering is the second harvesting step, and while a centrifuge can be employed, it is economically uninteresting. Filters for the larger microalgae tend to clog easily, but are highly effective and inexpensive for filamentous algae. Microalgae are best dewatered and dried in one step.
3. Drying to less than 12% moisture content is required if long-term storage is desired. Several methods are available (Oswald and Golueke 1968):
   a. Mechanical methods are best, but are energy- and capital-intensive. Fast drum drying (5-7 sec at 135°C) in very thin layers costs about $20/ton dry algae, while spray-drying produces a better-quality product due to shorter drying time and lower temperature.

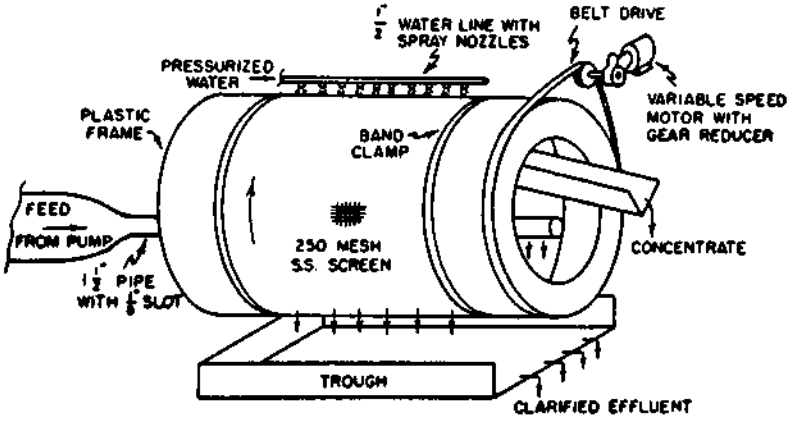

Figure II.2.11. Continuous rotary separator (by permission of Benemann et al. 1976).

b. Sun-drying on a lightly oiled surface is effective beginning with a 10% solids slurry, ca 0.25 cm thick. After a day the moisture content is below 15%, and the flaked solids are easily collected; an area equal to about 5% of the growth area is required.
c. Sand-bed drying can combine dewatering and drying, beginning with 8-12 cm thick slurry. The water quickly drains away while full drying takes three to five days, a rather long period leading to the loss of some vitamins. As in the sun-drying method, the algae curls and flakes and can be separated from most of the sand, but the residual sand content remains a problem for this economically attractive method.

None of these methods, however, simultaneously meets the criteria of high effectiveness and both low capital and energy costs. The awaited development is a simple and effective solar dryer or oven whose effectiveness as far as loss and contamination are concerned, could be greatly improved at little cost. Many schemes at varying levels of complexity are conceivable, and adaptations could possibly permit application to other crop drying problems.

PROCESSING

Processing can range from simple mixing of dried algae in normal food or fodder, grinding to flour, to a complete solvent extraction of the protein. The minimum processing required is the breaking of cell walls, not only to increase digestibility but also to release some of the 10-20% nitrogen content found in cell walls. Cell-wall thickness and hence digestibility varies from *Dunaliella,* which has no actual cell wall and is thus very osmotically fragile, to *Chlorella,* which has a very thick, difficult to break cell wall. Nonruminants cannot even digest the relatively fragile *Scenedesmus* unless the cellulose-like cell wall is ruptured mechanically (homogenization or sonification), chemically (urea soaking or solvent extraction), or by heating (dry steam or cooking). Chemical extractions have the important advantage of removing most pigments and, more importantly, nucleic acids.

USES FOR HARVESTED ALGAE

Algae for Food

The use of algae as food, with protein content as high as 70% (e.g., *Spirulina*) is extremely attractive, but certain risks and feed trial results

must first be discussed. The most obvious risk involves direct contamination in the culture medium, a microbiological contaminant, or the normal production by certain algae of exo- or endo-toxins (Schwimmer and Schwimmer 1968). The latter are particularly a problem with blue-green algae: massive mammal poisoning due to *Microcystis toxica* has been reported in addition possibly to *M. aeruginosa, M. flos-aquae, Anabaena flos-aquae, A. lemmermannii* and *Aphanizomenon flos-aquae*. Other possible intoxicating culprits include *Nodularia spumigena, Coelosphaerium kutzingianum* and *Gloeotrichia echinulata*. A minimum lethal dose of an extract from *A. flos-aquae* killed mice in one to two minutes while a small (2600 molecular weight) cyclic oligopeptide from cells of a contaminated *M. aeruginosa* culture killed in 30-60 minutes. Toxins from nonaxenic *Aphanizomenon flos-aquae* have been shown to be structurally similar to a shellfish toxin from *Gonyaulax calenella,* and a dermatitic factor has been isolated from an impure *Lyngbya majuscula* culture. Whether these toxins are avoidable in pure or axenic algal cultures, or if such cultures are possible, remains unknown.

The above toxic possibilities are supplemented by a suspicion of the algal cell-wall material, particularly the common presence of odd-numbered fatty acids; none are found in *Spirulina.*

For human consumption of algae, there exists no doubt of a dietary limitation due to high nucleic acid content in all single-cell proteins: as high as 25 grams nucleic acids per 100 grams protein is known to occur (Scrimshaw 1975). Nucleic-acid content is roughly proportional to the rate at which the organism's cells multiply; this so-called doubling rate tends to decrease with organism size, and hence nucleic-acid levels are much lower in algae than bacteria but still serious. *Spirulina* has, for example, 7 grams nucleic acids per 100 grams protein vs 4 grams per 100 in liver, the latter being unusually high in nucleic acids for animal tissue.

The difficulty with dietary nucleic acids are their depolymerization by nucleases in pancreatic juice and the following conversion to nucleosides by intestinal enzymes prior to absorption. The metabolism of the nucleotides guanine and adenine to uric acid is a problem only for humans who have suffered an evolutionary loss of the enzyme urease, whose function is to oxidize uric acid to a soluble and excretable form, allantoin. Low uric-acid solubility may result in its accumulation as uriate in tissues and joints with gout-type results. Methods for nucleic-acid removal from single-cell protein do exist, but in the absence of such procedures, human nucleic-acid intake should be limited to about 2 grams per day or about 50 grams *Spirulina* (i.e., 30 grams protein or about 40% of the daily requirement). This is not a severe constraint as rarely would consumption of

single-cell protein constitute more than half of the daily protein diet either on the basis of need or taste.

The few limited algal-dietary trials indicate ready acceptance of algae as a food additive but not as a staple food (Scrimshaw 1975, Oswald 1976a). *Spirulina* has been eaten for centuries by Africans on Lake Chad, and athletes in Mexico took 20-40 grams *Spirulina* daily for 30-45 days with good results, whereas two men in the USSR who ate 150 grams *Spirulina* daily developed edema of the face and hands, petechial hemorrhages, cyanosis of the nail beds and peeling of the fingers; little problem was however encountered after alcohol extraction of the color. Long-term (500 days) feeding (25% of the diet was *Spirulina*) revealed no toxicity in (100) rats, however, and in limited amounts (5-15%) provides a good food additive to pigs, poultry and fish; even as a limited additive, algae can replace up to 40% of the soybean and fishmeal proteins for chickens and fish (Shelef et al. 1976).

On the basis of absorbed nitrogen, *Spirulina* is surpassed only by cow and human milk. In general, the food quality of algae varies greatly with growth medium and age of culture. Extremes of protein content are 8-75% of dry weight, lipids are from less than 1% to 86%, carbohydrates from 4-40% and ash from 4-45%. (Algae grown on glucose medium can have high (30-47%) intracellular carbohydrate while old cultures or algae grown on low oxygen or fixed-nitrogen contents can store lipid (28-86%). These extremes are rarely relevant, and more typical compositions are shown in Table II.2.6 together with cow's milk and hen's eggs for comparison (Oswald 1976a).

Vitamin and mineral contents for algae, eggs and cow's milk are also shown in Table II.2.6. Soluble vitamin content in algae is high while vitamins A, D, E and K are negligible or nonexistent. Mineral contents, like vitamins, are highly variable with growth media and conditions, but normally compare favorably with both eggs and milk.

High protein content, 50-75%, is not the only criteria for protein's nutritional value. Of the twenty-odd amino acids naturally occurring in proteins, half must be supplied in the correct chemical form while the other half can be synthesized from these so-called "essential" amino acids. Algal protein, while abundant, is not as good as animal protein, e.g., eggs or milk, due to an unbalanced amino-acid content; low contents of sulfur-containing amino acids (methionine and cystine) are the most significant deficit.

Even with all reservations stated, algae has an extraordinary potential nutritional role, most likely as an additive. As a 10% by weight additive, *Spirulina* can still provide half a person's protein requirement. In times of

Table II.2.6 Summary of Values for the Proximate Composition, Vitamin and Mineral Contents of Milk, Eggs and Microalgae (from Oswald 1976a).

	Cow's Milk	Hen's Eggs	Algae
Proximate Composition			
Protein, gram	28	49	51
Carbohydrate, gram	39	3	27
Fat, gram	28	44	7
Fiber, gram	0	0	6
Ash, gram	6	4	9
Kcal/gram	5.2	6.2	3.6
Vitamins			
Thiamine, mg	.24	.42	1.2
Riboflavin, mg	1.25	1.14	3.0
Niacin, mg	.8	.4	10.0
Pyridoxine, mg	.4	.4	0.2
Folacin, mg	4.7	16.3	3.4
Vitamin B-12, mg	3.2	7.6	25
Ascorbic acid, mg	8	0	40
Pantothenic acid, mg	2.4	.4	0.7
Biotin, mg			26
Carotenoids, IU	1110	4500	52
Vitamin E, mg			2.6
Minerals			
Calcium, gram	.93	.21	0.2
Phosphorus, gram	.74	.78	1.8
Calcium/Phosphorus	1.27	.37	0.2
Magnesium, gram	.10	.42	0.6
Sodium, gram	.40	.46	0.1
Potassium, gram	1.14	.49	0.8
Sodium, mg			187
Iron, mg	trace	9	31

acute food shortages, areal productivity will become one of many crucial criteria; one hundred times more algal protein than wheat protein can annually be produced per unit area, while for animal proteins that figure can rise to 500 or 1000. However used, algae represent an enormous potential.

Aquaculture

As was mentioned earlier (see page 224) harvesting is at present the cost-limiting stage in algae production. Aquaculture, the cultivation of fish to harvest algae and water plants, was there listed as an alternative, special-purpose harvesting method.

Aquaculture, like agriculture, implies an active role by Man in not just harvesting but in cultivating the growth of food. Whereas food gathering

developed into agriculture due to the press of increasing population densities, open sea or lake fishing has not evolved into aquaculture on the same scale. The chief reason is not difficult to guess: lack of water. For this reason aquaculture has often been linked to areas with well-developed irrigation, as in Hawaii where aquaculture dates back to the 14th century (Kikuchi 1976). Irrigated fields in some of the Hawaiian Islands were often managed in a manner intermediate between aqua- and agri-culture.

In Asia aquaculture has had an impact and history comparable to no other region (Bardach, Ryther, and McLarney 1972). Fish ponds have formed the major link for recycling plant and animal wastes in these regions—human wastes were cycled by placing the latrines directly over the ponds. Cultivation of algae and higher aquatic plants, plus the stocking of primarily herbivorous fish, thus provided not only waste treatment but also high-quality fish protein. Estimated yields vary widely with the amount of cultivation and feeding involved, but harvests range from ca 350 kg/ha-yr in Hawaii to highs of 18 tons/ha-yr in China. Great care is required in estimating yields as fish, like all animals, are rather inefficient converters of protein. High fish yields attained by the supply of high-quality protein (e.g., soya) is just as extravagant as beef feedlots where only a tenth of the feed is recovered as meat. Similar feeding techniques can yield phenomenal quantities of fish; for example, 29 kg/meter^2-yr (290 tons/ha-yr) harvests were obtained in Japan. Intensive fish farming may, however, prove preferable to the energy intensity of open-sea fishing. Energy ratios (energy in the fish caught divided by the energy input for the catch) and the energy input per weight protein harvested are listed in Table II.2.7 to summarize the problem (Leach 1976):

Table II.2.7 Energy Efficiency of Fish Production (Leach 1976).

	Energy Ratio	Energy Input
Wheat (UK)	3.35	42 MJ/kg protein
Average for UK fisheries	0.05	489
Shrimps—Australia	0.058	366
Gulf of Mexico	0.0061	3450

Ten to one-hundredth times as much energy is required for (caught) fish protein as grain protein! Aquaculture will be attractive only if its energy investments can be reduced signficantly below these levels.

The goal of aquaculture is to lower the energy and feed supplements substantially and yet provide high-quality fish protein. Criteria for success include, but are not restricted to, the yield per hectare. Of at least equal importance is the ability to produce fish protein without competing

for resources required for normal agriculture:

- *Land:* Aquaculture is possible on any type of land holding or capable of holding water. This includes sea-water ponds on coastal sand dunes, cleared swamps, bogs, sewage purification ponds, old salt pans, and virtually every river, lake or pond.
- *Feed:* Any type of organic matter, human, animal, or plant waste can be used as fish feedstock. Low-quality agricultural wastes such as rice polishings, grain-mill sweepings, raw-coffee wastes or oilseed residues, are suitable directly, or together with animal wastes as an algal-growth medium. Alternatively all these wastes can be digested anaerobically first (producing methane) and the sludge alone used as a nutrient-rich medium for algal growth. In this latter sense, herbivorous fish, especially shell fish, can be viewed as a natural harvesting method for unicellular green algae. Harvesting and drying are (see page 224 of this Chapter) the energy- and capital-intensive stages of any system which exploits the highly efficient green algae.
- *Water:* Water in rather generous supply, but of only reasonable quality, is required for aquaculture. Water is, of course, not consumed but rather is lost only via evaporation: typically a meter of water (10^7 liters) is lost per hectare per year from standing water and a half to a third that for terrestrial crops. Relative yields are then the deciding factor, and, on a per-protein-yield basis, aquaculture far exceeds traditional agriculture-based animal production. Algae consumes 33-70% as much water per dry weight product as agriculture (500 liters/kg vs 700-1500 liters/kg) but only 5-60% as much produced protein (700-100 liters/kg vs 1600-15,000 liters/kg protein). This advantage is retained in fish production, of course, only if animals are fed grains and fish fed algae. In most cases multipurpose water can be used for aquaculture, such as by growing fish confined in cages in irrigation ditches. Such integration will reduce both attributable water and land costs.

Whatever aquaculture system is used, the specie of fish must be carefully chosen. For the most part, fish common to Western diets are flesh eaters, they eat primarily smaller fish, worms and insects, and therefore are not suitable to aquaculture. Two herbivorous species are the most common choices for both modern and historic fishponds: carp (grass carp—*Ctenopharyngodon idellus,* bighead—*Aristichtys nobilis,* silver carp—*Hypophthalmichtys molitrix,* mud carp—*Cirrhinus molitoretta,* common carp—*Cyprinus carpio* and black carp—*Mylopharyngodon piceus*) and *Tilapia* or St. Peter's fish (Bardach, Ryther, and McLarney 1972 and USNAS 1976). The latter variety, while tropical in origin and

hence more sensitive to temperature variations than carp, is advantageous for its superior taste. Both are effective herbivores and hence allow harvesting of the primary product in the food chain, algae.

Alternatively, bivalve mollusks, such as oysters, mussels, octopus, or clams, are uniquely well suited to harvesting unicellular algae. Their filter-feeding mechanism is far more efficient for the small algal cells than even herbivorous fish, and they can double their weight in one week. They should normally not be cultivated in the algal pond directly as the algal cell concentration in the fish pond should be lower (about 10^5 cells/ml), and both the flow and aeration of the water should be greater (Ryther 1975). Accumulated metabolites can also easily reach toxic levels.

While not normally considered as aquaculture, fowl and certain other herbivorous animals are potential algae and water-weed harvesters (NAS 1976b). Ducks, geese and swans commonly forage on water weeds, but if extensive cultivation is considered some grain supplement is normally required (NAS 1976b). The manatee or sea cow (ICMR 1974), water buffalo, pigs and certain species of rodents (capybara and nutria; NAS 1976b) also forage on water weeds and can be of extreme local importance for weed control. None of these animals, however, are of such value nor display such voracious herbivorous appetites that water plants are cultivated for their feed.

Typical Systems. Aquaculture as a system is sketched in the same input-output manner as normal agriculture in Figure II.2.12. Note that the waste water from the fish pond will contain algae and excrement from the fish and would suggest that the fish and algae should be grown in the same pond. Such a system is possible, but it will be optimal for neither and will more resemble the low productivities of a normal healthy lake. Care must be taken not to try for too high productivity by heavy fertilization as eutrophication can occur.

A more logical solution is to still keep the system closed but to return the output "waste" water to the algal pond. Depending on the type of fish or bivalve used some dilution of the algal output will be required, but if

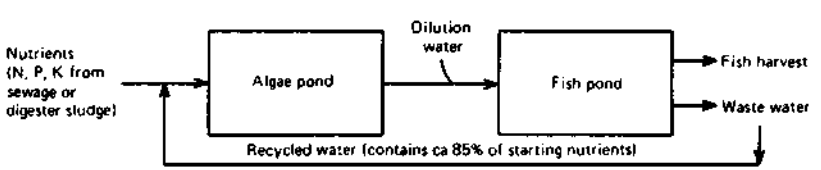

Figure II.2.12. Two-stage aquaculture system.

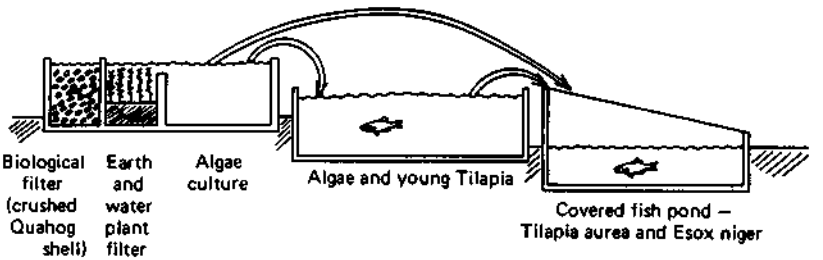

Figure II.2.13. Three-stage aquaculture system (redrawn from McLarney and Todd, 1974).

the water added is greater than that needed to compensate for evaporation, it can be used elsewhere in a complete integrated system.

A variation on this theme is shown in Figure II.2.13 (McLarney and Todd 1974) where a series of three-terraced ponds are used. The first is basically an aerobic biological waste-treatment plant (and could be eliminated profitably if an anaerobic digester existed anyway) while the second pond mainly produces the algae. In addition the second pond may produce some small algae-eating animals, such as the crustacea, daphnia, and higher water plants (e.g., water sprite) which will be eaten in this case by tilapia. The final pond, covered to raise its temperature in temperate climates for the tropical tilapia, is for cultivating the fish whose wastes will be recycled back to the first pond.

Yet another variant is to employ aquaculture as a multicomponent water treatment system. Figure II.2.14 shows one variant which employs sea water. This system is designed to produce economically valuable seafoods and water which is clean enough for irrigation or for returning to open waterways. As developed by the Woods Hole Oceanographic Institution in the US (Ryther 1975) the system consists of:

- Waste water received after secondary sewage treatment diluted with sea water (e.g., 1:3 for sewage effluent of 20-25 mg/liter nitrogen and 10-15 mg/liter phosphorus). Urban waste water typically is imbalanced with excess phosphate (N:P=5−7:1) due to detergents, whereas the algae would equally remove nutrients if the N:P ratio

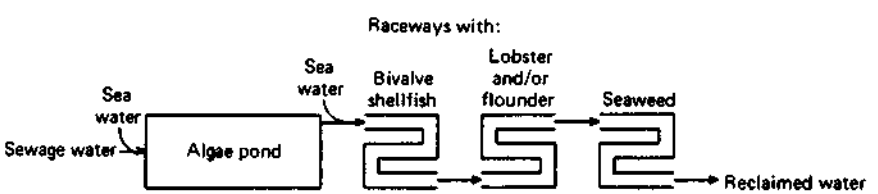

Figure II.2.14. Salt-water aquaculture system for waste-water treatment.

were 15:1. In the former case all phosphate will not be removed, and nitrogen will limit growth.

- Open algal pond (1 meter deep × 16 meters × 16 meters) for mass culturing of phytoplankton, unicellular marine algae; the diatom *Skeletonema costatum* (winter) and *Phaeodactylum tricornutum* were dominant species. Both circulation and aeration are provided by two one-third HP (40 gal/min) pumps in opposite corners whose return jets are just above the surface to aid aeration. Maximum algal production was 12 grams dry weight/meter^2-day while 9 grams was typical for summer and 6 grams for spring and fall periods.
- Bivalve mollusks confined to vertically stacked trays which are fed algae diluted with seawater (1:1-5 for cell concentration of 10^5/ml) flowing rapidly to improve feeding, aeration and removal of wastes.
- Lobster (*Homarus americanus*) and flounder (*Pseudopleuronectes americus*) could also be grown in trays in the raceways (the same or in one following the bivalves even though they are carnivores/omnivores. Feed is provided by small invertebrate detritovores (amphipods, polychaetres, bryozoans, tunicates and mussels) which in turn feed on the shellfishes' solid wastes.
- Seaweed (red algae, *Gracilaria* and *Agardhiella*) confined in raceways (12 meters × 1.3 meters × 1.6 meters deep) served as a final polishing step to remove any remaining nutrients, especially those returned to soluble form by the fish cultures. Seaweed is harvested once a week with nets, dried and potentially salable for commercial extraction of agar or carragenan. Red-algal yields surpass the initial open green-algal yields: maximum dry weight yields were 16 grams/meter^2-day with 13 grams typical for summer and 5 grams for spring and fall. Nitrogen removal is so effective that the final portions of the raceway contain red algae which are pale yellow, due to nitrogen deficiency.
- Water leaving the final red-algal raceway contains little nitrogen due to the 90% removal efficiency, including nitrogen added to the system by the sea water. The flow of nitrogen at the various stages is shown in Table II.2.8. Note that the phytoplankton removed 98% of the sewage effluent plus seawater nitrogen, but the shellfish convert a third of the algal nitrogen to inorganic nitrogen as waste material. Two-thirds of this nitrogen is removed by the red algae, but more complete removal could be achieved by a one-third increase in raceway length.

Fish Yields. Yields of edible fish from the above systems can vary by orders of magnitude, but the desire for increased yields must be bal-

Table II.2.8 Mass Flow of Inorganic Nitrogen (Ammonia, Nitrite and Nitrate) through the Phytoplankton-Oyster-Seaweed System (Ryther 1975).

		Grams of Nitrogen per Day
Phytoplankton pond input		85
Sewage effluent	84	
Seawater	1	
Phytoplankton pond output		1.5
Shellfish raceway input		4.5
Phytoplankton pond harvest	1.5	
Seawater	3.0	
Shellfish raceway output (= seaweed raceway input)		27
Seaweed raceway output (Final effluent from system)		9.4
Total N removal efficiency (including seawater)		89.3%
Effluent N removal efficiency		93.6%

anced against the capital and energy costs incurred. Feed is a major cost determinant, but, with care conversion efficiencies (net weight fish/net weight food) of 10-20% are attainable. Protein conversion efficiencies are somewhat better, 18-25%, and are comparable to milk (21%) and egg (25%) production efficiencies; beef has a predictably low protein conversion efficiency (12%; Windsor and Cooper 1977). As presently practiced in the West, fish farms produced food-quality fish at a cost over three times that of milk (per unit protein), twice that of eggs and broiler chickens, and about comparable to beef.

Feed partly determines yields, but energy intensity also plays a role. Aeration of experimental high intensity ponds in Israel, for example, increased yields by more than threefold (6.6-9.5 tons/ha-yr to 20-25 tons/ha-yr). A more typical yield for relatively inefficient grass carp and fresh water shrimp fed on the green macroscopic algae, *Chara sp.*, was about 4 tons/ha-yr, with a conversion efficiency of only 2.6%.

The system at the New Alchemy Institute (Woods Hole, Mass., USA) consistently strives for lower limits on the energy and capital consumption spectrum. By employing windmill-driven water pumps and the scheme sketched in Figure II.2.13, they have attained highly respectable yields with minimal energy intensity and feed supplements. The level of energy and capital intensity cannot be decided in general, but the overall feasibility increases dramatically if waste treatment, water recycling and algal and fish production are combined.

Potential Research Developments

Algal Photoproduction of Hydrogen. Many of the same arguments used elsewhere (see Part I, Chapter 3, page 272) discussing bacterial photoproduction of hydrogen apply here. Of principal importance is the realization that the release of energy and reducing capacity is normally self-defeating especially for a photosynthetic organism. Hydrogen transfer and activation systems ($H_2 \rightarrow 2H^+$ via hydrogenase) in algae have undoubtedly evolved from the absorption and metabolism of hydrogen gas an an energy and electron source. No well-accepted theories as yet exist for normal hydrogen evolution nor does any known ecological niche exist for such organisms, but possible functions include (Kok 1973):

- Under severe anaerobiosis all components of the photosynthetic electron transport chain become reduced.
- Bleeding off H_2—an insoluble gas—converts primary acceptor X to the oxidized state. This allows operation of photosystem I (see Part II, Chapter 1).
- P^+_{700}—a strong oxidant—oxidizes additional intermediates and as a result turns on Photosystem II and O_2 evolution.
- Algae frequently encounter anaerobiosis, especially in mixotrophic growth.
- Leaves never do, need no "priming," and contain no hydrogenase.

Persistent interest in the improbable photoproduction of hydrogen by algae is principally motivated by its attractiveness. If a photosynthetic organism is convinced to evolve hydrogen instead of growing at normal rates, the following process (biophotolysis) would result:

$$H_2O \xrightarrow[\text{algae}]{\text{light}} H_2 + 1/2O_2$$

Storage of solar energy as hydrogen gas at the expense of only water would be a nearly ideal process, the reverse of which releases 285 kJ (68 kcal) per mole hydrogen via combustion to heat or via a fuel cell to electricity. Biophotolysis would ideally employ the two photosystems (see Part II, Chapter 1) of the algal chloroplast to split water (with the evolution of oxygen) and lift the resulting electron about one volt for the immediate reduction of protons to hydrogen gas; some minimal amount of energy would be needed for plant survival, but overall efficiencies of a few percent could be hoped for.

Photoproduction of hydrogen by algae does occur naturally and was first observed 35 years ago (Bishop, Frick, and Jones 1977). Laboratory

conditions for sustained algal hydrogen evolution are not exactly natural, however: carbon dioxide and oxygen must first be excluded during a 10-20-hour dark-incubation period. Following this adaptation period, the reactions listed in Table II.2.9 can be catalyzed by hydrogenase containing green algae (e.g., *Scenedesmus*). Note in the reactions in Table II.2.9 that sugars, i.e., cell material, can be synthesized via normal photosynthesis reaction (1), or by metabolizing hydrogen gas with (2), or without (7) the help of light. Sugar synthesis, in the light or dark, requires energy (ATP) processed in a reaction (phosphorylation) which, when chemically inhibited, allows the photoproduction of hydrogen (3).

Green algae are the principal group of organisms known to be capable of hydrogen evolution, but blue-green algae and a few fresh and marine red algae are also among the hydrogen evolvers; blue-green algae are a special case which will be discussed later. The green algae *Scenedesmus* was the first observed hydrogen evolver, but others include *Chlorella (vulgaris, fusca, pyrenoidosa,* and *autotrophica), Chlamydomonas (dysosmos, moewusii,* and *reinhardii*), *Ulva, Enteromorpha, Codiotum, Acetabularia* and *Dunaliella*. Typical rates of hydrogen production are, however, extremely low: the peak rate is 1% of the theoretical maximum and normally 100 times more oxygen is evolved than hydrogen (Lien and San Pietro 1976). More recently, dark-adapted anaerobically (He) grown *Chlamydomonas reinhardii* produced 1.9 times as much hydrogen as oxygen, very near the stoichiometrically expected ratio of two (Greenbaum 1977). These experiments, which employed single light flashes intense enough to excite all pigments but of short enough duration to

Table II.2.9 Reactions Catalyzed by H_2-adapted Algae (from Stuart and Gaffron (1972).

Light-dependent reactions:	
1. Photosynthesis:	$CO_2 + 2H_2O + \text{light} \longrightarrow (CH_2O) + O_2 + H_2O$
2. Photoreduction:	$CO_2 + 2H_2 + \text{light} \xrightarrow{H_2\text{ase}} (CH_2O) + H_2O$
3. H_2 photoproduction:	$RH_2 + \text{light} \xrightarrow{H_2\text{ase}} R + H_2$
Dark reactions:	
4. Respiration:	$1/2O_2 + RH_2 \xrightarrow{H_2\text{ase}} H_2O + R + \text{energy}$
5. Dark H_2 production:	$RH_2 \xrightarrow{H_2\text{ase}} R + H_2$
6. H_2 absorption:	$H_2 + R \xrightarrow{H_2\text{ase}} RH_2$
7. Oxy-hydrogen reaction:	$O_2 + 2H_2 \xrightarrow{H_2\text{ase}} 2H_2O + \text{energy}$
	$CO_2 + 2H_2 + \text{energy} \xrightarrow{H_2\text{ase}} (CH_2O) + H_2O$

allow only a single photoact, showed that hydrogen evolution has neither the delays nor intermediate formation characteristic of oxygen evolution (see Part II, Chapter 1).

Although the biochemical mechanisms of algal hydrogen production are not known, comparisons with the nitrogenase mediated hydrogen evolution from photosynthetic bacteria show that an ATP independent hydrogenase is involved (Lien and San Pietro 1976). The absence of an energy requirement for the actual proton reduction promises a potentially higher efficiency for the algal system. Studies with mutants deficient in one photosystem (II) showed decreased hydrogen production in the dark while stimulation of hydrogen evolution by glucose indicates that the photosystems are *not* necessarily directly involved. Photosynthetically produced sugars may be metabolized in both the light and dark, but some argue that the photosystems reduce hydrogenase directly by demonstrating that no phosphorylation (ATP synthesis) is involved, i.e., no generation of reducing power via sugar metabolism is required (Lien and San Pietro 1976).

Much of the uncertainty, low average hydrogen production rates, and the rapid decrease in the initial rate can however be due to oxygen inhibition of the hydrogenase system (see Part II, Chapter 1, page 144). The anaerobically dark-adapted algae produce oxygen immediately when the light-driven photosynthesis begins. Hydrogen is also evolved immediately, but when the oxygen concentration exceeds 0.2% it inhibits the hydrogenase and decreases the rate of production (Stuart and Gaffron 1972). The only potential solutions to the dilemma of hydrogenase inhibition by photosynthetically evolved oxygen are:

- select for oxygen insensitive hydrogenases;
- provide algae with organic substrates;
- separate oxygen and hydrogen evolution, physically or temporally.

Breeding experiments have been started by looking for hydrogen evolving cells at osygen tension slightly higher than normally permissible (2%). *Chlamydomonas reinhardii* was chosen for study due to its constitutively synthesized hydrogenase, but one inspection of 43 algal strains yielded no differences in oxygen sensitivity (Bishop 1975).

The second alternative above, metabolizing sugars to produce hydrogen, is simply not practical; if organic materials (wastes) are to be used, photosynthetic bacteria provide a much simpler conversion system (see Part II, Chapter 3, page 272).

The third alternative above, i.e., the temporal or physical separation of oxygen (photosynthesis) and hydrogen (light or dark reaction) production is exemplified naturally in certain blue-green algae. Physical separation

of the two functions occurs in some filamentous blue-green algal species (e.g., *Anabaena cylindrica* and *Nostoc muscorum,* see also Part II, Chapter 1) when some vegetative cells eliminate the oxygen evolving system (Mn^{+2} for biophotolysis is missing), develop thick walls to exclude extracellular oxygen and hence become nonvegetative "heterocysts." Elimination of both extra- and intra-cellular oxygen in these heterocyst cells allows the synthesis and functioning of two oxygen-sensitive enzyme systems: hydrogenase and nitrogenase.

Heterocysts in blue-green filamentous algae normally function by receiving photosynthates (a sugar, maltose) from the vegetative cells which presumably provide all the fixed carbon and reducing power required. CO_2 fixation does not occur in heterocysts due to a missing enzyme system (ribulose diphosphate carboxylase). Only photosystem I is believed to be present, and its function is mostly for photophosphorylation (ATP production), but in principle could also directly reduce ferredoxin which in turn reduces nitrogenase/hydrogenase. Heterocysts will normally fix nitrogen and exchange the sugar from vegetative cells for glycine, the fixed-nitrogen form produced.

Under unnatural conditions, this "symbiotic" cell specialization in blue-green algae can use photosynthetic energy and reducing power to evolve oxygen and hydrogen simultaneously:

$$H_2O + \text{light} \xrightarrow[\text{algae}]{\text{blue-green}} H_2 + 1/2O_2$$

Both oxygen and hydrogen can be produced simultaneously because:

- the enzyme system involved (hydrogenase or nitrogenase) is protected from extracellular oxygen;
- no oxygen is evolved in the heterocyst;
- nitrogen, the normal nitrogenase substrate, is eliminated by a purge gas, e.g., argon.

This final point, of course, requires that the cells are first grown normally on nitrogen (fixed or gaseous) for protein synthesis, and then the system is closed and nitrogen removed. Oxygen at normal tensions (18%) is only mildly inhibitory (ca 25%), while nitrogen gas nearly eliminates all hydrogen evolution (Benemann and Weare 1974). Carbon monoxide selectively inhibits the nitrogen-fixing function of nitrogenase but *not* hydrogen evolution, not even in the presence of nitrogen. In principle, 2% carbon monoxide is as effective for hydrogen production as growth under argon.

Most laboratory attempts to produce hydrogen begin with a normal growth period (with 0.3% CO_2) and then replace all nitrogen with argon (plus 3% CO_2). Hydrogen/oxygen production from a two-liter culture has

continued for several weeks with conversion efficiencies of ca 0.4% (Benemann and Weissman 1976). The decrease in hydrogen production shown in Figure II.2.15 is believed to be due to filament fragmentation. While a pure photolytic process would produce a hydrogen-oxygen ratio of 2:1, observed ratios vary from 1.7-4.0:1. Such variations are not at all surprising as the link between the two processes is long and complex: hydrogen is evolved only after the CO_2 fixed as sugars in the vegetative cells is transported to heterocysts where the sugars are metabolized and oxygen is released. The rate of reductant supply from vegetative cells limits heterocyst hydrogen production, but efficiencies of a few percent should be possible.

The enzymatic mechanism of hydrogen production in blue-green algae is not known, but both hydrogenase and nitrogenase are known to be present. Either enzyme can evolve hydrogen, but nitrogenase requires ATP while hydrogenase does not. This energy requirement for hydrogen production from nitrogenase is a key factor in the low energy-conversion efficiency and is thermodynamically a totally unnecessary waste. One recent experiment with purified heterocyst cells from *Nostoc muscorum* showed that hydrogen could be evolved in the light (with dithiothreitol) or in the dark (with dithionite), but in neither case was ATP added (Tel-Or

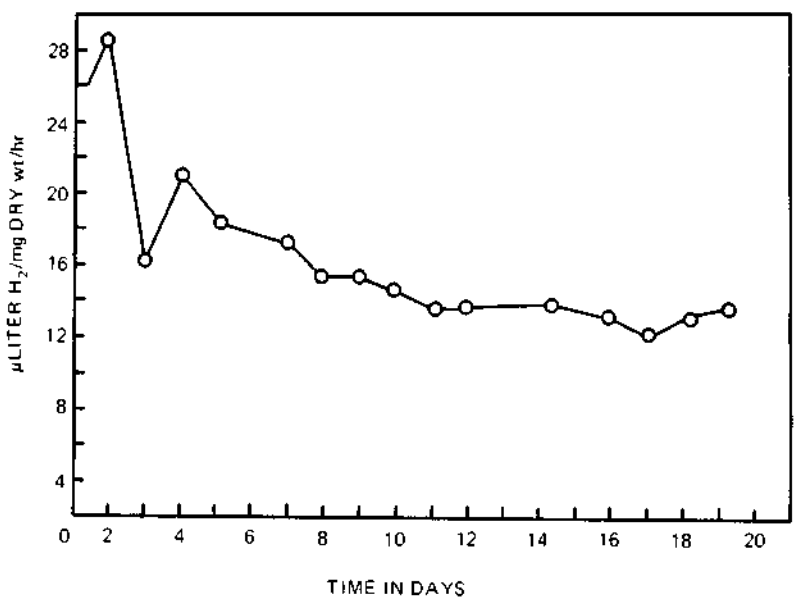

Figure II.2.15. Hydrogen evolution from a two-liter culture of *Anabaena cylindrica* under low light (by permission of Weissman and Benemann, 1977).

and Packer). If substantiated, the ATP-requiring nitrogenase cannot be involved. Further evidence against nitrogenase having a role in hydrogen evolution is that nitrogen was slightly stimulatory, while carbon monoxide was inhibitory. Hydrogenase *is* known to have a role for hydrogen uptake, a competitive reaction which can become serious if the gas is not continually removed.

A second remaining question is the role of the remaining photosystem I in the heterocyst. In theory it could directly reduce ferredoxin which in turn can reduce nitrogenase or hydrogenase, but an electron donor at about zero potential must be added. Alternatively, the photosystem can be used entirely for photophosphorylation with the ferredoxin reduced by sugars metabolized via pyruvate to NADP. The latter reaction certainly occurs in the dark.

A practical system clearly needs to be completely enclosed with constant removal of the photoproduced hydrogen to minimize the competitive uptake reaction, oxygen to prevent inhibition, and nitrogen to prevent nitrogen fixation. In blue-green algae carbon monoxide can profitably be added to inhibit nitrogen fixation selectively. Care should be taken in choosing the gases used to assure their easy removal or suitability for end use. For fuel-cell use the purity requirements for the gas are much more stringent, but for combustion carbon dioxide added in large quantities for photosynthetic carbon fixation (sugar production) will only lower the heating value of the produced gas. Carbon dioxide can of course be removed, and any inert gases used must be removed in any case for reuse. Carbon monoxide added to inhibit nitrogen fixation need not be removed for combustion, as it is also a good fuel.

Ammonia Production by Blue-Green Algae. As discussed earlier, heterocystous blue-green algae can fix nitrogen for direct fertilizing into rice paddies, production of a dried organic fertilizer, or fed to an anaerobic digester to increase the total amount of fixed nitrogen in a completely recycled system.

Research directed toward increased ammonia yield from blue-green algae takes one of two alternative directions:

1. Increase the relative number of heterocysts. One method, successful on a laboratory scale, uses the natural response to persistently low ammonia levels which causes a fivefold increase in heterocysts if fixed nitrogen is occasionally provided in small amounts for required protein synthesis (Benemann and Weissman 1976). A conceivable alternative is genetic selection of mutants which overproduce heterocysts.

2. Impair self-regulation. This more promising approach deals directly with the limits to nitrogen-fixation rates irrespective of heterocyst frequency. These regulatory mechanisms can either halt nitrogenase synthesis or perhaps cause the reversion of heterocysts to vegetative cells. Temporary regulation, that involving ADP inhibition of nitrogenase activity is probably not alterable. Inhibition due to excess nitrogen is believed to be effected by the amino acid glutamine, synthesized from ammonia via glutamine synthetase, and not due to ammonia directly. Two approaches for deactivating this control are conceivable: ideally a mutant could be found which is not responsive to ammonia concentration, i.e., one which most probably lacked glutamine synthetase. No efforts have yet been successfully directed toward this goal, nor is it known which of the poorly understood regulatory steps is most susceptible to uncoupling. The alternative of chemically poisoning the regulatory system has successfully caused massive excretion of ammonia into the medium (Stewart and Rowell 1975). Apparently, ammonia conversion to glutamine is blocked, and fixation is stimulated by apparent nitrogen starvation. Heterocyst formation is likewise freed from regulation. Practical applications of these recent developments have not yet been considered, but would require removal of the ammonia analogue to prevent ammonia-uptake problems in the crop to be fertilized.

Potential fixation rates can only be estimated by maximum natural rates and a theoretical maximum. The theoretical maximum can only be a crude guess, as the energetics of the fixation process are poorly understood. If one assumes three electrons and 15 ATP molecules per fixed ammonia molecule, then a 10% conversion efficiency produces several tons of ammonia per hectare per year. This figure, taken as a very crude theoretical estimate, can be compared with the maximum observed fixation rates discussed in Part II, Chapter 1, for natural systems. Estimates there extended to 500 kg/ha-yr, but more reasonable extreme maxima are 300 kg. *Azolla* in symbiosis with the blue-green algae *Anabaena* gives a particularly useful figure in the present context of ca 160 kg/ha-yr (Stewart 1977). Of course a great deal of the solar energy is used for plant growth.

A free-living *Anabaena* can produce a biomass of 50 tons/ha-yr which could contain (assuming 40% protein and 16% nitrogen content in protein) 3.8 tons fixed nitrogen, but most of this nitrogen is taken from the medium and not fixed by the organism. How much nitrogen would be fixed by an ammonia-free medium rich in all other nutrients remains to be

seen. A natural organism has of course the regulatory mechanisms discussed above intact and does not represent the full potential; *Anabaena* fixation in symbiosis with *Azolla* is only mildly inhibited by fixed nitrogen (Talley, Talley, and Rains 1977). Ideally the biomass would be semi-permanent and tricked by some of the means discussed above into devoting nearly all its efforts toward fixing nitrogen. Potential ammonia yields should exceed a ton, but a more exact estimate is premature.

Algal Production of Alcohol. Nature's adaptability is exemplified in an unusual unicellular algae: *Dunaliella parva* which thrives in high-salinity (from 1.4% salt to saturated solutions) waters typical of arid regions. Osmotic pressure, resulting from the enormous and variable salt gradient across the cell membrane, is balanced by rapid synthesis of glycerol. The glycerol content doubles within one hour after the salt concentration doubles.

Glycerol, an energy-rich derivative of propyl alcohol, has an energy content of (4.3 kcal/gram) and constitutes up to 85% of the cell's dry weight. Glycerol is also an important industrial chemical for lubrication, pharmaceuticals, cosmetics, soaps, and foods.

The *Dunaliella* cell itself lacks the characteristic indigestible cell wall and hence is easily breakable. It can be grown on simple salts at temperatures up to 40°C (its optimum is 33°C) with a density of ca 5×10^{12} cells/meter^3, a doubling time of 30 hours and a production rate of about 50 grams/meter^2-day (Avron 1976). Assuming a daytime insolation of 500 cal/cm^2-day, the efficiency can be calculated to be an optimistic 4%.

Dunaliella can be cultivated in semi-arid regions in natural or artificial ponds using brackish water which supports few other organisms. Whereas harvesting of unicellular algae is normally difficult, *Dunaliella* can be separated using its property of settling in the cold, or alternatively it can be centrifuged at 100 grams for ten minutes. A sudden decrease in salinity of the harvested cells causes the thin membrane to rupture and allows the glycerol to be separated. The cell membranes, containing 40% protein plus some glycerol, make an easily digested energy- and protein-rich fodder without further treatment.

ECONOMIC AND ENERGETIC COSTS FOR ALGAL PRODUCTION

At the present stage of development, analysis of both the economic and energetic costs of mass algal production point to the major burden: harvesting of unicellular algae. Drying and CO_2 costs, if the latter is not provided as a waste by-product, are the next greatest burdens.

Two points must be remembered when discussing algal system efficiency, whether economic or energetic. Firstly, mass algal culture in its

modern perspective is in its infancy, nearly all efforts in the past, present and near future are rather scientific and technological in nature, with little effort or motivation to reach any economic or energetic optimum. An exception in this context is the Sosa Texcoco *Spirulina* project in Mexico which is a commercial effort, although one of a rather special character. No major attempts at low or intermediate technology (capital) mass algal culture have yet been made. Secondly, the type of algal culture on which the analysis is based must be clearly stated. Reasonable economic estimates have been made for only three systems:

1. *Spirulina* grown by Sosa Texcoco in brine lakes;
2. *Scenedesmus* grown on artificial media by the various German-(Dortmund)-inspired projects plus one proposed by the Proteus Corporation of Concord, California;
3. Mixed unicellular green algae grown on waste water, predominantly in California, USA.

Spirulina from Sosa Texcoco presently costs about $2.50/kg (in one-ton lots), but while its being of food quality is a distinct advantage, this price still cannot compete with conventional protein sources at one-tenth the cost. Present uses are confined to health foods or as an industrial chemical source.

Figure II.2.16 summarizes calculated costs for large-scale algal growth on inorganic media (for human consumption) and on waste waters (fodder or industrial uses). At 10 tons per day, food-quality algae is estimated to cost about $0.33/kg, while fodder would cost about $0.20/kg (Oswald 1976a). The former begins to compete quite well with competitive conventional foods especially in light of impending food shortages.

Waste-grown algae is the most advanced technology on a large scale and provides an interesting comparison with alternative foods. Soybean-oil meal (40% protein) sells for $200 per metric ton compared with an equivalent cost for algae. As algal protein content is somewhat higher, it may command a higher selling price and thereby become economically viable. On the other hand, algae presently suffers from being an unconventional food, but in the long term its competitive position should improve. Prospective decreases in algae costs are exemplified by a crude breakdown for large-scale waste grown algae in Table II.2.10 (from Oswald 1976a, and Lewis 1976).

Separation and drying are the greatest operational costs and are economically the costs most sensitive to energy costs and shortages. Here lies the greatest hope and need for improvement and re-emphasizes the importance of recent successes with species control (see page 223 of this chapter). Growth of filamentous algae permits an estimated tenfold decrease in the separation costs (Benemann et al. 1976). Drying costs are expected to

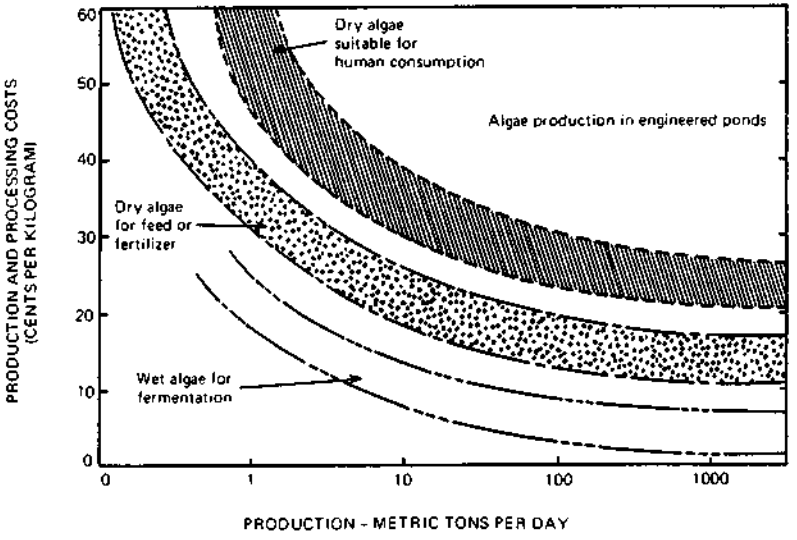

Figure II.2.16. Estimated costs of producing and processing algae of various grades (37° North Latitude; from Oswald 1976a).

remain unchanged, but these are not relevant for algae used in methane production.

Simplified separation techniques with filamentous algae has the additional advantage of not only decreasing the capital costs, but also makes possible practical operation on much smaller scales. Little advanced work has been done on low- or intermediate-scale algal growth, and hence estimates for such installations have been neglected here. This latter area is one deserving highest priority.

Table II.2.10 Estimated Economic and Energy Costs for Algal Production (Oswald 1976a; Lewis 1976).

	Economic Cost (5000 tons/year)	Energy Cost (500 tons/year)
Amortized capital	$110/ton	2.8 GJ/ton algae
Labor	18	—
Mixing costs	7.30	2.8
Separation	80	42
Drying		14
Misc. (taxes, insurance, etc.)	20	—
Sewage medium (credit)	—	−4.3
	$235/ton	57.6 GJ/ton