TOPICAL REPORT

on

SOURCES AND SYSTEMS FOR AQUATIC PLANT BIOMASS AS AN ENERGY RESOURCE

In partial fulfillment of the contract for
"Cost Analysis of Algae Biomass Systems"

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TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
LIST OF FIGURES	iv
LIST OF TABLES	viii
ABSTRACT	x
PREFACE AND ACKNOWLEDGEMENT	xi
1 INTRODUCTION	1
2 THE BIOLOGY OF AQUATIC PLANTS	5
2.1 Algae	5
2.1.1 Microalgae	5
2.1.2 Macroalgae	7
2.1.3 Classification of Algae	8
2.2 Aquatic Angiosperms	28
2.2.1 Freshwater Angiosperms	28
2.2.2 Marine Angiosperms	32
3 PARAMETERS AFFECTING GROWTH, PRODUCTIVITY AND YIELD OF AQUATIC PLANTS IN MASS CULTURE	35
3.1 Measurements of Growth, Productivity and Yield	35
3.2 The Theoretical Basis for the Productivity of Aquatic Plants - Light	37
3.3 Density	48
3.4 Temperature	53
3.5 Nutrients	61
3.6 Biological Limitations	70
4 HISTORICAL REVIEW OF THE MASS CULTURE OF MICROALGAE	72
4.1 Early History of Culture Systems	72
4.2 Culture After 1955	76
4.3 Summary of Microalgal Systems	103

TABLE OF CONTENTS (Concluded)

<u>Section</u>	<u>Page</u>
5	HISTORICAL REVIEW OF THE MASS CULTURE OF MACROPHYTES 106
5.1	Mass Cultivation of Macroalgae 106
5.1.1	Harvesting of Natural Populations 107
5.1.2	Seaweed Culture in Oriental Countries 108
5.1.3	Seaweed Culture in North America 114
5.1.4	Summary of Yields 137
5.2	The Mass Culture of Aquatic Angiosperms 139
5.2.1	Freshwater Angiosperms 139
5.2.2	Marine Angiosperms 143
6	DESIGN CONSIDERATIONS 149
6.1	Light Availability 149
6.2	Nutrients Availability 158
6.3	Selection of Feedstock 166
7	INFORMATION SOURCES 167
7.1	Key Research Workers, Institutions, and Specialties 167
7.2	Industries Active in Plant Aquaculture 174
7.3	Professional Societies and Conferences 175
8	REFERENCES 176

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1.1	Possible Applications of the Cultivation of Aquatic Plants	2
2.1	The Green Algae: <u>Ulva lactuca</u> , <u>Ulva taeniata</u>	10
2.2	The Green Algae: <u>Monostroma grevillei</u> , <u>Monostroma oxyspermum</u> , and <u>Monostroma zostericola</u>	11
2.3	The Green Algae: <u>Enteromorpha compressa</u> , <u>Enteromorpha intestinalis</u>	12
2.4	The Red Algae: <u>Hypnea musciformis</u>	14
2.5	The Red Algae: <u>Gracilaria foliifera</u> , <u>Gracilaria mammillaris</u>	15
2.6	Diagrammatic Representation of Life Histories of the Red Algae: <u>Gracilaria</u> , <u>Porphyra</u>	16
2.7	The Red Algae: <u>Porphyra occidentalis</u> , <u>Porphyra pulchra</u> , <u>Porphyra schizophylla</u> , <u>Porphyra perforata</u>	18
2.8	The Red Algae: <u>Gelidium pusillum</u> , <u>Gelidium sinicola</u> , <u>Gelidium caloglossoides</u> , <u>Gelidium coulteri</u> , <u>Gelidium purpurascens</u>	19
2.9	The Red Algae: <u>Eucheuma isiforme</u> , <u>Eucheuma nudum</u>	20
2.10	The Red Algae: <u>Gigartina cristata</u> , <u>Gigartina agardhii</u> , <u>Gigartina canaliculata</u>	21
2.11	The Red Algae: <u>Iridaea cordata</u> var. <u>splendens</u> , <u>Iridaea cordata</u> var. <u>cordata</u>	22
2.12	The Brown Algae: <u>Fucus distichus</u> ssp. <u>edentatus</u>	23
2.13	Diagrammatic representation of a young adult <u>Macrocystis</u> plant, 2 to 3 years old.	24
2.14	Diagrammatic Representation of Life History of the Brown Alga <u>Laminaria</u>	26
2.15	The Brown Algae: <u>Laminaria andersonii</u> , <u>Laminaria farlowii</u>	27

LIST OF FIGURES (Continued)

<u>Figure</u>		<u>Page</u>
2.16	The Freshwater Angiosperms: <u>Hydrilla verticillata</u> , <u>Myriophyllum oliganthum</u> , <u>Elodea canadensis</u> , <u>Najas</u> <u>indica</u>	29
2.17	The Freshwater Angiosperm: <u>Eichhornia crassipes</u>	30
2.18	The Freshwater Angiosperms: <u>Lemna perpusilla</u> , <u>Lemna</u> <u>trisulca</u> , <u>Lemna minor</u> , <u>Wolffia floridana</u>	31
2.19	The Marine Seagrasses: <u>Zostera marina</u> , <u>Thalassia</u> <u>testudinum</u>	33
3.1	Effect of Total Solar Radiation on Algal Yield Con- sidering Light as Sole Limiting Factor	45
3.2	Variations in Maximum Possible Solar Radiation with Time of Year for Varying Latitudes	46
3.3	Actual Solar Radiation in Woods Hole, Mass. and Ft. Pierce, Florida	47
3.4	Mean Daily Irradiance on a Horizontal Surface	49
3.5	Effect of Standing Crop (Density) of <u>Gracilaria foliifera</u> on Production and Specific Growth Rate in Mass Culture Experiments	50
3.6	Effect of Dilution (Growth Rate) on Standing Crop as Particulate Carbon and Nitrogen and on Yield as Particu- late Carbon for Marine Phytoplankton Growth in Mass Culture in Woods Hole, Massachusetts	51
3.7	Relationship between Maximum Specific Growth Rate and Absolute Temperature for Microalgae	55
3.8	Summary of data collected in outdoor ponds from January 1974 through April, 1975 in Woods Hole, Mass.	56
3.9	Concentrations of Particulate Nitrogen Produced Between 5 - 33°C for Mixed Phytoplankton Populations in Continuous Culture	58
3.10	Relationship Between Specific Growth Rate and Limiting Factor Showing Increasing Temperature-Dependent Asymptotes	59

LIST OF FIGURES (Continued)

<u>Figure</u>		<u>Page</u>
3.11	Relationship between Plant Yield and Dilution Rate During Mass Culture Experiments in Different Locations and Seasons	60
4.1	Diagrams of Early Algal Growth Chambers	74
4.2	Cycle of Controlled Photosynthesis in Organic Wastes	78
4.3	Automatic Growth Unit for Producing Continuous Cultures of Algae and Bacteria in Liquid Organic Wastes under Controlled Environmental Conditions	78
4.4	Photograph of 10 ⁶ Liter Outdoor Algal Mass Culture System at Richmond, California	79
4.5	Several Alternative Processes for Converting Radiant Energy to Useful Energy Via Bioconversion	81
4.6	Photographs of Algal Mass Culture Systems Used in Woods Hole, Massachusetts	83
4.7	Diagrams of Algal Culture Schemes Developed in Japan During 1950's	86
4.8	Photograph of Large-scale <u>Chlorella</u> Culture Units Used in Taiwan	88
4.9	Photographs of Algal Culture Units Used in Dortmund, Germany	90
4.10	First Czechoslovakian Outdoor Mass Culture Unit Built in 1960	92
4.11	Large-scale (900 m ²) Outdoor Cultivation Unit Used in Trebon, Czechoslovakia	93
4.12	Plan View of 300 m ² Algal Pond Used in Israeli Studies with Raw Wastewater	95
4.13	Photographs of Algal Culture Units Used in Israel Institute of Technology Experiments	97
4.14	Schematic View of the French Petroleum Institute Algal Pond System	99

LIST OF FIGURES (Concluded)

<u>Figure</u>		<u>Page</u>
4.15	Solar Evaporation Pond of Sosa Texcoco S.A. Where Mass Culture of <u>Spirulina</u> Occurs	102
5.1	<u>Porphyra</u> (nori) Farming in Japan	110
5.2	Air View of <u>Porphyra</u> Farms in Japan	111
5.3	Experimental Culture of <u>Gracilaria</u> on Plastic Ropes in Taiwan	115
5.4	Plywood Tanks with Sloping Bottoms for Growing Seaweeds in Suspended Culture in Seawater Enriched with Sewage Effluent at Woods Hole, Mass.	121
5.5	Concrete Raceways with Sloping Bottoms for Growing <u>Gracilaria</u> and <u>Neoagardhiella</u> in Suspended Culture on the Effluent from Bivalve Mollusc Cultures at Woods Hole, Mass.	122
5.6	Screening Tanks (50 l-Capacity) Constructed from PVC Pipe for Growing Algae in Suspended Culture in Florida	125
5.7	Plastic Mesh (Vexar) Trays with PVC Pipe Frames for Growing Seaweeds in Artificial Ponds and Natural Environments in Florida	127
5.8	PVC-Lined Ponds Used for Growing Seaweeds in Florida	128
5.9	Concrete Burial Vaults Used for Growing Seaweeds and Freshwater Plants in Florida	130
5.10	Culture of <u>Gracilaria</u> in Trays Receiving Spray Irrigation in Florida	132
6.1	Relationship Between Algal Growth Rate and Incident Radiation Showing Saturation Radiation I_s	152
6.2	Irradiance Decay in Algal Suspension as a Function of Depth Showing the Relationship Between the Efficiency of Light Utilization (E_s) and the Incident Irradiance (I_0)	153
6.3	Efficiency of Light Utilization (E_s) and Total Light Conversion Efficiency (E_T) as a Function of Incident Irradiance (I_0) at Various Levels of Light Saturation Intensity (I_s)	155

LIST OF TABLES

<u>Table</u>		<u>Page</u>
3.1	Daily and Annual Dry Weight Yields of Organic Matter and of Plants with Different Ash Contents as Functions of Mean Total Incident Solar Radiation	39
3.2	Productivity of Agricultural Crops and Seaweeds on an Annual Basis	41
3.3	Some High, Short-Term Dry Weight Yields of Crops and Seaweeds	42
3.4	Seaweed Composition	62
3.5	Contents of Trace Elements in Seaweeds Expressed as Percent of Dry Weight	66
3.6	Concentrations of Nutrient and Trace Elements in Seawater	67
3.7	Additional Publications Involving Assays of Micro-nutrients in Algae	68
3.8	Summary of Publications Describing Physiological Relationships (Uptake, Toxicity, etc.) Between Trace Substances and Algae	69
4.1	Summary of World-Wide Programs for Mass Culturing of Microalgae	104
5.1	Yields and Other Characteristics of <u>Gracilaria</u> Grown by Different Culture Methods	133
5.2	Summary of Yields from Long-term Commercial and Experimental Seaweed Cultivation	138
5.3	Productivity of Freshwater Weeds at Harbor Branch Foundation, Ft. Pierce, Florida: 1977	144
5.4	Summary of Results From Marine Seagrass Research	148
6.1	The Manipulation of Natural Energy and Materials Through Activities Involving the Use of Capital and Labor in Aquatic Plant Aquaculture Enterprise	150

LIST OF TABLES (Concluded)

<u>Table</u>		<u>Page</u>
6.2	Design Parameters Used in Recent Outdoor Mass Culture Studies	159
6.3	Potential Nutrient Sources for Mass Algal Culture	161
6.4	Compositions of Various Media Used by Algal Culturists, Compared with Elementary Concentrations in Seawater and Concentration Ranges Found in Marine Plants	163
6.5	Defined Media for Growth of Various Genera of Microalgae	164

ABSTRACT

This report documents the background information on the mass cultivation of aquatic plants and systems design that is available from the literature and through consultation with active research scientists and engineers. The biology of microalgae, macroalgae, and aquatic angiosperms is discussed in terms of morphology, life history, mode of existence, and ecological significance, as they relate to cultivation. The requirements for growth of these plants, which are outlined in the text, suggest that productivity rates are dependent primarily on the availability of light and nutrients. It is concluded that the systems should be run with an excess of nutrients and with light as the limiting factor.

A historical review of the mass cultivation of aquatic plants describes the techniques used in commercial large-scale operations throughout the world and recent small-scale research efforts. This review presents information on the biomass yields that have been attained to date in various geographical locations with different plant species and culture conditions, emphasizing the contrast between high yields in small-scale operations and lower yields in large-scale operations.

PREFACE AND ACKNOWLEDGEMENT

This Topical Report was prepared at Dynatech R/D Company under Contract EG-77-C-01-4000 entitled "Cost Analysis of Algae Biomass Systems". The report, as required in the contract, is to serve as a data/reference source for the long-term projection of the potential use of aquatic plant biomass as a fuel. While over 400 references were used in this Topical Report, thus providing the needed source information, the writing was clearly oriented towards the potential for using aquatic plant biomass as a future energy source.

Engineers and scientists at Dynatech have been privileged to call upon a number of outstanding leaders in the field to assist in the preparation of this Topical Report. Foremost has been the assistance of Drs. Joel Goldman and John Ryther of Woods Hole Oceanographic Institution. These men joined with Dynatech in responding to the RFP, which was ultimately competitively awarded to Dynatech, and are now co-Principal Investigators on a sub-contract from Dynatech to Woods Hole Oceanographic Institution to carry out supportive work on this program. Prof. Wm Oswald, Univ. of California, Berkeley, lent his name to the original Dynatech technical proposal as a consultant and, after contract award, had a portion of his on-going contract with ERDA directed to be further supportive of this program. His assistance in the area of land-based microalgal systems as well as that of his colleague, Dr. John Benemann, is acknowledged.

Following contract award, Dynatech was privileged to call upon the counsel of Prof. Wheeler North of the California Institute of Technology. Prof. North contributed data from his research on giant kelp for this report. Dr. Iain Neish, Director of Marine Science Research at Marine Colloids, Inc. contributed information on the commercial cultivation of seaweeds. Also, Prof. Robert Waaland, University of Washington, Seattle, provided invaluable service in documenting for Dynatech all worldwide activities in the broad area of aquatic plant research.

At Dynatech the editor and contributing author was Ms. Elizabeth Wilson. Supportive work was provided by Dr. Edward Ashare as well as by Dr. Donald L. Wise, Principal Investigator. Over-all administrative and technical guidance was provided by Mr. Ralph L. Wentworth, Vice President.

The engineers and scientists who worked on this Topical Report especially acknowledge the tremendous assistance and cooperation of Dr. Roscoe Ward, Branch Chief, Fuels from Biomass Program, U.S. Department of Energy.

Section 1

INTRODUCTION

E.H. Wilson

Since early in recorded history aquatic plants have been used as fertilizers, medicines and food for man and animals. In recent years, however, other possible applications of aquatic plant biomass have been proposed, such as wastewater treatment, aquaculture, the extraction of commercial chemicals and bioconversion to energy (Figure 1.1). The mass production of aquatic plant biomass as an energy resource is particularly attractive since aquatic plants have a high productivity and they can be grown in areas for which there is no projected use.

Aquatic plants are known, or believed, to be capable of exceptionally high levels of biological productivity and high organic yield per unit time and space. Optima conditions for the production of biomass are enhanced by the addition of nutrients such as nitrogen, carbon and phosphorus to the aqueous media containing the plants, and exposure to solar incident radiation (Figure 1.1). The prime goal of all mass cultivation operations, irrespective of the plant species cultivated or the type of cultivation system, is to maximize the photosynthetic conversion of radiant energy to bound chemical energy and the transformation of inorganic matter to organic chemicals. Of primary importance is the selection of a plant species with a high organic productivity (high ash-free dry weight) and minimum requirements of nutrients, water and solar energy for growth.

The species selected for mass cultivation must not only produce a high yield of biomass but also be easily cultured and maintained and easily harvested. The difficulty of culturing some aquatic plants is due to complicated life histories, such as an alternation of sexual and asexual generations which require specific physical conditions simulated in a laboratory for the propagation of the sexual generations. Regulation of physical parameters, such as

PHOTOSYNTHETIC REACTION

Figure 1.1

Possible Applications of the Cultivation of Aquatic Plants

(modified from Goldman)

salinity and temperature, and control of grazing by other organisms may be labor-intensive and cost prohibitive. A good understanding of the requirements of each stage of the life history will determine the success of the culture. Those organisms that reproduce asexually are usually easily maintained, but in the case of microalgae, may be difficult to harvest. Harvesting methods used and ease of harvesting depend on the size and mode of existence of the aquatic plants.

Many species of aquatic plants grow well naturally in existing coastal waters and wetlands and are capable of growing in simple empoundments or land areas that are not capable of supporting other crops and have no projected use. Several systems for the mass cultivation of aquatic plants have been proposed including 1) open ocean systems for culturing marine macrophytes, 2) shoreline-based systems for culturing marine microalgae or macrophytes, and 3) land-based systems for culturing freshwater or brackish water microalgae or macrophytes. Each of these systems has distinct biological and engineering advantages and disadvantages. Specifically, problems relating to culture mixing, nutrient availability and addition, species control, carbon dioxide addition, water supply, and harvesting are of major concern. It is the overall objective of all these systems to minimize total capital requirements and operating costs and to minimize total energy input, while maximizing organic production and energy output.

It is clear that to develop an economically feasible plant cultivation system, it is essential to understand the problems involved in mass cultivation both from a biological and an engineering viewpoint. This requires the integrated effort of engineers familiar with biological systems together with input from scientists actively working in the field of aquatic plant production. It is the purpose of this report to document the available background information in mass cultivation and systems design from the literature and through consultation with active research scientists and engineers. Two main objectives should be kept in mind. Firstly, it is

important to gain an appreciation for the biomass yields that have been attained to date in various geographical locations with different species and cultivation systems. Secondly, the bioengineering advances that have been made in cultivation technology will provide guidelines for the design and operation of large-scale cultivation systems, such as the 100 square mile farm recently proposed by the U.S. Department of Energy for evaluation as a potential bioconversion plant.

Section 2

THE BIOLOGY OF AQUATIC PLANTS

J.H. Ryther and E.H. Wilson

Photosynthetic plants are usually classified into four major subdivisions, the common names for which are the algae (classified together with the fungi), mosses, ferns, and the higher flowering plants. Of these, the fungi, mosses, and ferns are insignificant with respect to the total biomass and productivity of aquatic plants and therefore may be ignored for the purposes of this discussion. The algae are the major inhabitants of the aquatic biosphere while the higher plants constitute the bulk of the terrestrial flora. However, the major division of higher plants, the angiosperms, are well represented in the aquatic environment. We shall, therefore, be concerned here with the algae (microalgae and macroalgae) and the aquatic angiosperms.

2.1 Algae

The algae are among the simplest and most primitive plants, having no true roots, stems, leaves, flowers, or seeds. The entire microscopic plant may be only a single cell or it may consist of groups of cells loosely organized, but rather poorly differentiated into a plant body referred to as a thallus. Some of the larger or macroscopic algae, commonly known as seaweeds, have various parts of the thallus differentiated into organs that are roughly analogous to the roots, stems, and leaves of the higher plants (referred to respectively as holdfasts, stipes, and fronds in the seaweeds), but true vascular tissues are lacking.

2.1.1 Microalgae

The unicellular microscopic algae include some of the smallest organisms on earth, generally ranging in size from less than 2 micrometers to as much as 1 millimeter in diameter. Colonies of these unicellular organisms may form mats or slime layers on sediment surfaces or on any submerged object. Some species form chains or filaments, and the latter may aggregate to produce

large macroscopic colonies that float or in some cases rest on the bottom of fresh or marine bodies of water. The most prevalent mode of existence of the unicellular algae, however, is as single cells suspended in the water, in which form they are known as phytoplankton.

Phytoplankton are abundant in the surface layers of all natural bodies of water, both fresh and marine. Due to the passive nature of the planktonic existence, horizontal and vertical water movements are of primary importance to their existence. Prokaryotic microorganisms such as blue-green algae, however, contain gas vacuoles which regulate the plant's buoyancy, thus enabling the cell to move up and down in the water column to seek optimum light intensities. Some species of phytoplankton are equipped with flagella and are weakly motile. Others have no motility but depend on the presence of spines, which increase surface area, or mucilage envelopes and oil globules to retard sinking. Despite these modifications none of the phytoplankton are able to overcome horizontal transport by tides, winds and currents. The weakly and non-motile phytoplankton are dependent on vertical water circulation to supply nutrients from the waters below and to maintain populations in the surface illuminated layers of oceans and lakes.

Over most of the oceans and the world's larger lakes, it is the drifting, microscopic phytoplankton that are responsible for the autotrophic production of organic matter, that is, production solely from carbon dioxide and inorganic nitrogen and requiring no other metabolic substrate. It has been estimated that about one third of the organic matter produced on earth is synthesized by the phytoplankton which, in turn, serves directly or indirectly as food for virtually all other forms of life in the sea.

Normally invisible to the naked eye, phytoplankton may grow to very dense concentrations or "blooms", if light, temperature, nutrient conditions are all favorable. In freshwater, phytoplankton blooms may assume nuisance proportions, imparting a taste or odor to drinking water supplies and an esthetically unfavorable effect upon the recreational uses of ponds and lakes.

When natural waters are subjected to chronic nutrient enrichment from sewage pollution or other sources of organic wastes, a condition referred to as "eutrophication", they are often in a permanent bloom condition. Under such circumstances, the heavy load of organic matter may be not only displeasing esthetically, but also ecologically unstable and potentially damaging to the environment. Although microscopic plants produce oxygen in the process of photosynthesis when they are suspended in the surface waters exposed to sunlight, in the dark they and their remains respire and consume oxygen. Thus, when the algal blooms sink into the deep, non-illuminated waters and to the bottom, they may deplete and even exhaust these waters of their oxygen supply and thereby kill many forms of aquatic life, including the benthic fauna, through anoxia.

2.1.2 Macroalgae

With a few exceptions, the macroalgae or seaweeds are all marine. They include some of the largest plants on earth, the giant kelp Macrocystis, which may exceed 50 meters in length; although most of the seaweeds are relatively small, ranging from a few centimeters to about one meter in length.

While the phytoplankton are quantitatively the important algae in freshwaters and in the open sea, the larger seaweeds are the dominant plant form in most shallow coastal marine waters. Most seaweeds grow attached to rocks and other hard substrates by a holdfast, restricting their distribution to the very fringe of the ocean in water shallow enough for sufficient light to reach the bottom. Although some seaweeds can grow to depths of 100 meters or more in exceptionally clear waters, they are most abundant at depths of 10 meters or less. Some seaweeds are unattached and simply drift on the bottoms of estuaries and other protected marine environments. As with the phytoplankton, persistent and excessive enrichment of these coastal waters from pollution or other causes can lead to extremely heavy growth of certain intertidal species, at times to the detriment of esthetic values and ecological balance.

Seaweeds are the principal food for a variety of crustaceans, echinoderms, molluscs, microorganisms, and herbivorous fish in the coastal regions, thus contributing significantly to marine food chains. They are of use to man as mulch, fertilizers or animal feeds. A few species have considerable commercial value for their contained hydrocolloids, such as alginic acid, agar, and carrageenan, which are extracted from the dried plant material and used as emulsifiers and stabilizers in the food, drug and cosmetic industries.

2.1.3 Classification of Algae

The algae have been classified in a number of different ways by plant taxonomists, but most have divided them into five groups based primarily on their pigments and other biochemical characteristics, rather than their size, shape or mode of existence. Disregarding the scientific nomenclature, which varies with the classification used and the exact position and affinities of the algae within the plant kingdom, the common names of the five algal subdivisions, again reflecting their pigmentation, are the blue-green, green, red, brown, and golden algae. The nature of the algae in each of these subdivisions is reviewed below.

Blue-Green Algae

The blue-green algae are among the most primitive plants. In modern usage they are more often classified with bacteria than the other algae because they have no true nucleus, chloroplast, or other discrete organelles and reproduce by the most primitive form of asexual reproduction. These organisms may contain a large array of photosynthetic and accessory pigments scattered as granules throughout the cell. These include chlorophyll a, various carotenoids, phycocyanin, and sometimes phycoerythrin. Through various combinations of these pigments, the organisms themselves may be red, green, blue, yellow or any mixture thereof.

The blue-green algae are all individually microscopic. Some are truly planktonic, but the more common species, such as Oscillatoria and Spirulina, are filamentous or colonial forms which aggregate in mats or

gelatinous sheets that float on the water surface or coat submerged or damp surfaces. Although they occur in the marine environment, they are more common in freshwater and are the most common contributor to the noxious water blooms that cause problems in reservoirs and eutrophic lakes and ponds. Some species are actually toxic and have been known to kill domestic livestock through contamination of their drinking water.

Blue-green algae serve as sources of food for fish and other aquatic animals. Some blue-green algae, such as Spirulina, which is exceptionally rich in protein, are harvested and utilized as human or animal feed when they occur naturally in great abundance.

Green Algae

The green algae produce true starch as a carbohydrate storage product and contain both chlorophyll a, chlorophyll b, and carotenoid pigments in much the same proportion as the higher plants, to which they are probably more closely related than any other algae. They include both macroscopic seaweed forms in the marine environment and unicellular, planktonic species in both marine and freshwater habitats.

The macroscopic marine algae include those with a large, sheet-like thallus that is usually a brilliant yellow-green to dark green color, such as the sea lettuce Ulva (Figure 2.1a, b, c) and Monostroma (Figure 2.2a, b, c, d) and the large green filamentous colonies including Enteromorpha (Figure 2.3a, b) and Chaetomorpha. Green seaweeds, which often proliferate in the quiet waters of highly-enriched polluted estuaries and coastal embayments, are usually small and delicate. Many of the green seaweeds have complicated life cycles involving an alternation between sexual and asexual generations. A few genera such as Ulva and Enteromorpha develop large fragile fronds six feet or longer when in protected waters, but may appear as small plants of tougher consistency when they occur on exposed shores. These two genera are often responsible for ship fouling. Monostroma, Enteromorpha and Ulva are utilized as food in the Orient.

Figure 2.1

The Green Algae

- a) *Ulva lactuca* x 0.5 (from C.J. Dawes, Marine Algae of the West Coast of Florida. © 1976 University of Miami Press, Coral Gables, Florida. pg. 71)
- b) *Ulva taeniata* x 0.5 and c) *Ulva linza* x 0.5 (from G.M. Smith, Marine Algae of the Monterey Peninsula. © 1969 Stanford University Press, Stanford, California. pg. 419)

Figure 2.2

The Green Algae

- a) Monostroma grevillei x 1
- b) and c) Monostroma oxyspermum x 1
- d) Monostroma zostericola x 1

(from I.A. Abbott and G.J. Hollenberg, Marine Algae of California. © 1976 University of Stanford Press, Stanford, California. pg. 67)

Figure 2.3

The Green Algae

- a) Enteromorpha compressa x 1 (from G.M. Smith, Marine Algae of the Monterey Peninsula. © 1969 Stanford University Press, Stanford, California. pg. 423)
- b) Enteromorpha intestinalis x 1.5 (from C.J. Dawes, Marine Algae of the West Coast of Florida. © 1976 University of Miami Press, Coral Gables, Florida. pg. 68)

The unicellular green algae are characterized by simple spherical organisms, of which Chlorella is the most familiar; the highly elaborate, non-motile desmids, such as Closterium and Pleurotaenium, and a wide variety of flagellated motile organisms such as Chlamdomonas and Volvox. Except for those plants termed desmids, a freshwater group, the unicellular greens have representatives in marine, brackish, and freshwater environments. Some species are also euryhaline and can live within the complete range of salinities found in nature. Generally, however, these forms are more characteristic of freshwater and, together with the blue-green algae, are the common cause of freshwater plankton blooms in eutrophic lakes. Chlorella and Scenedesmus are also the most common constituents of the phytoplankton in sewage oxidation ponds.

Red Algae

With only a few exceptions the red algae are represented exclusively by macroscopic seaweeds in the marine environment. As their name implies, they contain the red pigment phycoerythrin as well as chlorophyll a and carotenoids, and are believed to have affinities with the blue-green algae. Characteristically, they are reddish-brown to bright red in color, but the red pigments may be masked by the abundance of other pigments and the algae may appear green or brown. They are typically branched tufted filaments, like Hypnea (Figure 2.4), or of some membranous or fleshy leaflike form, like Gracilaria (Figure 2.5a, b). In general, they are smaller and more delicate than the green and brown algae, rarely measuring longer than one meter in any dimension.

The red algae have a complex life history consisting of an alternation between sexual and asexual generations that sometimes bear no resemblance to each other (Figure 2.6a, b). In the past the different stages were sometimes thought to belong to different species and even different genera. The complexity and until recently, lack of knowledge of the life history of these seaweeds initially led to difficulties in their cultivation. Some perennial red algae such as Eucheuma spp. and Chondrus spp., can now be cloned as vegetative propagules, thus eliminating the complex sexual stage.

Figure 2.4

The Red Algae

Hypnea musciformis x 1 (from C.J. Dawes, Marine Algae of the West Coast of Florida. © 1976 University of Miami Press, Coral Gables, Florida. pg. 139)

Figure 2.5

The Red Algae

a) Gracilaria foliifera x 1.5 b) Gracilaria mammillaris x 0.5
 (from C.J. Dawes, Marine Algae of the West Coast of Florida. © 1976
 University of Miami Press, Coral Gables, Florida. a) pg. 130 b) pg. 131)

Figure 2.6

Diagrammatic Representation of Life Histories of the Red Algae

a) Gracilaria b) Porphyra (from Dawson, 1966)

The red algae, as a group, are the most economically important seaweeds. The red alga Porphyra (Figure 2.7a, b, c, d) has been eaten by man for thousands of years in China, Japan, Hawaii, Indonesia and Malaysia and was also utilized as food by the North American Indian. As discussed in detail in Section 4.2, today the cultivation and processing of Porphyra or "nori", as it is commonly known in Japan, is a profitable, but highly labor-intensive industry. Red algae are also sources of the commercially valuable hydrocolloids, carrageenan and agar. Originally, carrageenan was obtained primarily from the Irish moss, Chondrus crispus that was harvested in New England, the Maritime Provinces of Canada and Northern Europe. The chief source of agar was Gelidium (Figure 2.8a, b, c, d, e) which was harvested in Japan. Recently, however, these sources have been depleted through over-exploitation of stocks and pollution of coastal waters. Increases in demand for these colloids has led to the development of small, but viable commercial culture of other hydrocolloid-containing red algae, such as Eucheuma (Figure 2.9a, b), a carrageenan source, in the Philippines, and Gracilaria, an agar source in Taiwan. Research is presently being conducted on the culture of other possible sources of hydrocolloids, such as Hypnea, Gigartina (Figure 2.10a, b, c) and Iridaea (Figure 2.11a, b, c).

Brown Algae

In addition to the usual chlorophyll-carotenoid complex of pigments, the brown algae contain a specific carotenoid, fucoxanthin, which gives them a rich brown color. The brown algae are the most familiar marine plants. This group consists almost solely of marine macrophytes, including only three freshwater genera and a few microscopic forms (e.g., Ectocarpus). There are no unicellular and no colonial forms of brown algae. A number of tough large marine plants belong to this group, such as the rockweed Fucus (Figure 2.12) which ranges from 0.3 - 0.6 meters in length, the knotted wrack Ascophyllum which reaches 3 meters in length, and the giant kelp Macrocystis (Figure 2.13) which may be as long as 50 meters. Characteristically, the brown algae inhabit cold water environments, but there are a few tropical species, the best known of which is the floating, drifting Sargassum natans,

Figure 2.7

The Red Algae

- a) Porphyra occidentalis x 0.5
- b) Porphyra pulchra x 0.5
- c) Porphyra schizophylla x 1
- d) Porphyra perforata x 0.5

(from G.M. Smith, Marine Algae of the Monterey Peninsula. © 1969. Stanford University Press, Stanford, California. pg. 491)

Figure 2.8

The Red Algae

- a) Gelidium pusillum x 4
- b) Gelidium sinicola x 2.5
- c) Gelidium caloglossoides x 5
- d) Gelidium coulteri x 1.5
- e) Gelidium purpurascens x 1

(from G.M. Smith, Marine Algae of the Monterey Peninsula. © 1969. Stanford University Press, Stanford, California. pg. 44)

Figure 2.9

The Red Algae

- a) Eucheuma isiforme x 0.5
- b) Eucheuma nudum x 0.5

(from C.J. Dawes, Marine Algae of the West Coast of Florida. © 1976. University of Miami Press, Coral Gables, Florida. pg. 136)

Figure 2.10

The Red Algae

- a) Gigartina cristata x 0.5
- b) Gigartina agardhii x 0.5
- c) Gigartina canaliculata x 0.75

(from G.M. Smith, Marine Algae of the Monterey Peninsula. © 1969. Stanford University Press, Stanford, California, pg. 69)

Figure 2.11

The Red Algae

- a) and c) Iridaea cordata var. splendens x 0.3
- b) Iridaea cordata var. cordata x 0.3

(from I.A. Abbott and G.J. Hollenberg, Marine Algae of California. © 1976 Stanford University Press, Stanford, California. pg. 531)

Figure 2.12

The Brown Algae

Fucus distichus ssp. edentatus x 0.4

(from I.A. Abbott and G.J. Hollenberg, Marine Algae of California. © 1976
Stanford University Press, Stanford, California. pg. 260)

Figure 2.13

Diagrammatic representation of a young adult Macrocystis plant, 2 to 3 years old: a, holdfast; b, primary stipe; c, stubs remaining from first fronds; d, sporophylls; e, developing young frond; f, deteriorating senile frond; g, bundle of intertwined stipes; h, growing point of mature frond in the canopy.

(from Dawson, 1966)

which occurs far from shore in the central gyres of the oceans and gives the Sargasso Sea its name and a highly exaggerated reputation.

Like the red algae, the browns have a complicated alternation of sexual and asexual generations, of which the latter, the sporophyte, is the typical large seaweed plant (Figure 2.14). A small kelp Laminaria (Figure 2.15a, b), which is prized as food in China, is cultivated there on ropes to which spores of the vegetative plant are attached. The principle source of the hydrocolloid alginic acid is the giant kelp Macrocystis which is harvested from the extensive kelp beds off the coast of California.

Golden Algae

The remaining group, the golden algae, are somewhat like the browns in their pigment content, which includes several brown and yellow carotenoids. In contrast to the brown algae, this catch-all group consists entirely of unicellular or colonial planktonic forms. Various organisms that seem to be taxonomically unrelated are put together in this category, apparently more for convenience than for any other reason. The golden algae include diatoms, dinoflagellates, silicoflagellates, coccolithophorids, and heterokonts.

While the group includes both freshwater and marine species, it is of importance mainly because of its planktonic marine constituents. The most prevalent phytoplanktonic organisms in the oceans are diatoms, dinoflagellates, and coccolithophorids, which together probably account for 99% of the phytoplankton in most of the marine environment. In mid-ocean the diatoms and dinoflagellates are usually the only algae present. Because diatoms are the primary food producers of the seas, forming the basic link in all marine food chains, they have been appropriately called the "grass of the sea". They play an extremely important role in that they absorb and store nutrients and in turn supply nutrients to marine animals.

Figure 2.14

Diagrammatic Representation of Life History of the Brown Alga Laminaria
(from Dawson, 1966)

a)

b)

Figure 2.15

The Brown Algae

- a) Laminaria andersonii x 0.25
- b) Laminaria farlowii x 0.25

(from G.M. Smith, Marine Algae of Monterey Peninsula. © 1969. Stanford University Press, Stanford, California. pg. 21)

While green and blue-green algae are the typical causative agents of freshwater algal blooms, their marine counterparts are usually diatoms and sometimes dinoflagellates. A phenomenon popularly known as "red tide" is caused by the blooms of the reddish-brown-colored dinoflagellates Gymnodinium and Gonyaulax. These dinoflagellate blooms usually occur in high nutrient tropical or semi-tropical inshore areas and are highly toxic, causing widespread mortality of marine life and contaminating shellfish to the extent that they become poisonous to man.

2.2 Aquatic Angiosperms

The angiosperms are distinguished from other plants by the fact that their seeds, which produce a flower, are enclosed within a well-defined ovary wall. They are recognized as the most complex form of plant life. Most terrestrial species, including cultivated crops, are members of this group. In the aquatic environment hundreds of species of angiosperms occur in freshwater habitats, but only a few occur in marine habitats.

2.2.1 Freshwater Angiosperms

Freshwater flowering plants inhabit a variety of aquatic environments, but commonly they live in quiet relatively shallow waters. Those plants which are wholly submerged, such as Hydrilla (Figure 2.16a), the water milfoil Myriophyllum (Figure 2.16b) and the pond weeds Elodea (Figure 2.16c) and Najas (Figure 2.16d), may or may not be attached to the bottom by a well-developed root system. Some plants, such as the water hyacinth Eichhornia crassipes (Figure 2.17) and the duckweeds Lemna (Figure 2.18a, b, c), and Wolffia (Figure 2.18d), are attached floating with their root systems in the water and their vegetative and flowering parts in the air. During times of drought, however, the water hyacinth may extend its roots into the muddy bottom and wait for better times. Rooted emergent plants that live at the edge of the water, such as the cattails, rushes, and reeds, can live with their roots and lower portions permanently in the water or only periodically in the water on marshy and at times dry land.

Figure 2.16

The Freshwater Angiosperms

a) *Hydrilla verticillata* x 1.7
 c) *Elodea canadensis* x 2.4

b) *Myriophyllum oliganthum* x 1.5
 d) *Najas indica* x 1.7

(from C.D.K. Cook, B.J. Gut, E.M. Rix, J. Schneller and M. Seitz. Water Plants of the World - A Manual for the Identification of the Genera of Freshwater Macrophytes. © 1974 Dr. W. Junk b.v. Publishers, The Hague, Netherlands. a) pg. 259 b) pg. 249 c) pg. 257 d) pg. 322)

Figure 2.17

The Freshwater Angiosperm

Eichhornia crassipes x 2.8

(from C.D.K. Cook, B.J. Gut, E.M. Rix, J. Schneller and M. Seitz, Water Plants of the World - A Manual for the Identification of the Genera of Freshwater Macrophytes. © 1974 Dr. W. Junk b.v. Publishers, The Hague, Netherlands. pg. 484.

Figure 2.18

The Freshwater Angiosperms

- a) Lemna perpusilla x 4
- b) Lemna trisulca x 4
- c) Lemna minor x 4
- d) Wolffia floridana x 4 side view showing water line

(from N.C. Fassett, A Manual of Aquatic Plants. © 1957 The University of Wisconsin Press, Madison, Wisconsin. pg. 167)

The freshwater angiosperms are commonly known as "freshwater weeds" because of their excessive growth in lakes, ponds, rivers and canals. Although they produce true flowers and seeds, they also propagate vegetatively and it is by this means that their most efficient, sustained organic productivity is achieved. In tropical and semi-tropical regions, particularly where nutrients from wastewater discharge and from agricultural runoff are high, they spread rapidly, choking waterways, rendering them impassable and generally assuming nuisance proportions. In Florida alone, millions of dollars are spent annually in controlling aquatic angiosperms.

2.2.2 Marine Angiosperms

The majority of the marine angiosperms, commonly known as "seagrasses", are generally tropical in distribution with only a few temperate species. They usually occur in shallow waters one to ten meters deep, but occasionally may occur at depths as great as 50 meters. It is generally considered that the higher marine plants colonize shallow areas where the bottom is unsuitable for the attachment of the holdfast of macroscopic algae.

Like the freshwater weeds, the seagrasses may be wholly submerged or only partially submerged in estuaries, embayments or sand and mud flats. Unlike the freshwater weeds, however, the seagrasses are always attached to a substrate by a root system. Those seagrasses which are wholly submerged, such as the temperate eel grass Zostera (Figure 2.19a), its tropical relative the turtle grass Thalassia (Figure 2.19b), and the ubiquitous widgeongrass Ruppia, are commonly rooted in a sand or mud bottom in nutrient-poor quiet waters. Only the "surf grasses" of the genera Phyllospadix, which are adapted to the violent wave action and foaming surge of intertidal areas, attach to rocky substrates.

Seagrass beds are common in coastal waters and are particularly important for providing shelter for a diverse assemblage of organisms and highly productive communities. The high productivity of seagrass beds is channeled

Figure 2.19

The Marine Seagrasses

- a) Zostera marina x 0.3 showing knobby rhizome, adventitious roots, and linear leaves
 b) Thalassia testudinum x 0.3 with staminate flower

(from C.J. Hillson, Seaweed - A Color-coded, Illustrated Guide to Common Marine Plants of the East Coast of the United States. © 1977. Pennsylvania State University Press, University Park, Pennsylvania. a) pg. 173 b) pg. 175.

in two major directions - to the detritus food chain and to direct consumers. In addition, there is a considerable export of organic material to other systems. The seagrass leaves act as baffles slowing water movement and increasing sedimentation of organic and inorganic material in the beds. A tangled network of roots and rhizomes hold this sediment and create an organically rich environment within the fine sediments of the beds. This detrital material is exploited by a complex fauna of detritivores. The environment created by the high standing stock of leaves in most grass beds provides a large amount of surface area for the attachment of a diverse sessile flora and fauna. The shelter and food provided by the leaves and their epiphytes is exploited by many large motile organisms, most notably echinoderms, gastropods, and fishes.

Section 3

PARAMETERS AFFECTING GROWTH, PRODUCTIVITY AND YIELD OF AQUATIC PLANTS IN MASS CULTURE

J.C. Goldman and E.H. Wilson

3.1 Measurements of Growth, Productivity and Yield

Changes in biomass of an aquatic plant are often used to define optimal conditions for growth. Growth measurements of aquatic plants have been made in a variety of ways, however, and it is often difficult and sometimes impossible to make comparisons between the results. The growth rate of an organism is defined as the rate of increase relative to some unit. The rate of increase may be expressed as one of the following:

length

area

wet weight - weight of living samples after the removal of external
adhering water

commercial dry weight - wet weight of adhering salt and residual
water after drying approximately 20%

dry weight - weight after washing and drying to a constant weight

ash-free dry weight - initial dry weight minus residual ash remaining
after combustion (indicative of organic matter
present)

organic carbon

oxygen given off

The unit may be: an individual, length, area, water surface area, wet weight, dry weight, ash-free weight, organic carbon, or protein.

A useful measure for comparing growth measurements is the specific growth rate defined as an increase per unit weight (grams/gram), or percent increase per unit time (usually reported per day for aquatic plants). The specific growth rate is independent of biomass (measured as dry weight, carbon, etc.). Since most aquatic plant tissue can photosynthesize, it can be assumed that exponential growth is directly proportional to biomass.

Disregarding those external factors which effect growth rate (discussed later in this section), there is evidence that most aquatic plants follow an exponential growth pattern in early stages. Gametophytes and young sporophytes of Macrocystis pyrifera, (Anderson and North, 1969; North, 1976), Laminaria hyperborea sporophytes (Kain, 1965), young Porphyra tenera and P. pseudalonearis (Yoshida, 1972), Gracilaria sp. and Neoagardhiella baileyi (DeBoer and Ryther, 1977) all exhibit exponential growth. High growth rates are about 20%/day under controlled conditions, but growth rates of natural populations are closer to 5%/day. At this rate it would take 0.1 kg wet weight juvenile sporophyll 645 days to become a 10 kg wet weight adult (Jackson, 1977). Specific growth rates may change with the life cycle and with age of an aquatic plant (Dawes et al., 1970; Jackson and North, 1973; Rao, 1970; Waaland, 1976; Yoshida, 1972). Consequently, control of reproductive phases and harvest timing may help maintain high growth rates. Both the numerical magnitude of growth rate and its relationship to culture density are influenced by other factors, of which solar radiation is probably the most important. Thus, values of specific growth rate alone are not meaningful.

Productivity, the product of specific growth rate and density, is expressed as growth per unit time and area. Specific growth rate and production can, therefore, be related if plant density is known; specific growth rates decrease with an increase in density (Figure 3.5). Organic production is normally measured and reported in units of dry weight that include ash or non-volatile solids. Ash content is usually small, of the order of 10%, in most terrestrial or freshwater aquatic plants, but it represents on the average 40% of the dry weight of marine plants including both microscopic and macroscopic algae (see Table 3.4). Those considering marine plants as a biomass source for energy must take the high ash content of these plants into account in evaluation of their reported yields.

Yield, the measure of material harvested, is expressed as biomass harvested per unit area per unit time. Conventionally short-term yields are given as grams/meter²/day and sustained, large-scale yields as metric tons/

hectare/year, both in units of total dry weight. Crops such as Laminaria and Porphyra may be harvested in their entirety at the end of the growing season and a new crop seeded, or they may be periodically cut leaving enough tissue intact to maintain growth as in the case of Macrocystis, Chondrus and Gelidium. It is, therefore, necessary to take into account the length of the growing season when comparing yield and productivity on a yearly basis. The annual harvest yield may be much lower than the annual production if only a portion of the crop is harvested like Macrocystis in California.

3.2 The Theoretical Basis for the Productivity of Aquatic Plants - Light

In the process of photosynthesis, which can be considered here as equivalent to organic productivity, light energy absorbed by chlorophyll causes the reduction of carbon dioxide to simple carbohydrate with the evolution of oxygen; viz,

Only the visible light spectrum (ca. 400 - 700 mμ) is effective in carrying out this process. Within that range, the number of quanta of light energy required to reduce one mole of CO₂ varies somewhat but averages about 10. Taking into account the quantum requirement of photosynthesis at each wavelength, and the normal visible spectrum of sunlight reaching the earth's surface, it is possible to calculate that sunlight can reduce CO₂ to organic matter in the photosynthetic process in nature at an efficiency of no better than about 18% of the visible solar energy or 9% of the total solar energy reaching the earth's surface, given that the heat of combustion of one mole of CH₂O is 112 kg calories.

This efficiency is obtained only at relatively low light intensities, however. Photosynthesis increases with increasing light intensity in a linear relationship up to the point where the photosynthetic mechanism becomes light saturated, after which the plant pigments continue to absorb light but no further increase in photosynthesis occurs. The saturation

intensity may be affected not only by photic energy, but also by thermal energy. It varies depending upon the plant species and its previous history, but normally ranges around 10% of the intensity of full sunlight.

There are further losses in efficiency in the conversion of incident solar energy to organic matter. A fraction of the sunlight striking the water surface and even of that entering the water is reflected back and is lost to the aquatic environment. The fraction lost depends upon the angle of the sun and other factors, but averages about 5%. Finally, in estimating the ultimate net production of organic matter, consideration must also be given to the respiratory loss of the plant in satisfying its own metabolic requirements, a process that consumes a significant portion of the organic matter it has photosynthetically produced.

None of the above processes is a constant, so it is difficult to generalize about the net conversion of solar energy to organic matter. But it appears that under the best of conditions, with no limitation of water, carbon dioxide, or essential nutrients, plants in the aquatic environment are able to produce organic matter at an efficiency of no more than about 4% of the visible or 2% of the total incident solar radiation reaching the earth's surface. Existing evidence suggests that terrestrial plants are limited to about the same conversion efficiency, though the mechanisms involved may be somewhat different.

Since incident solar radiation is normally recorded and reported as total rather than just visible energy, the conversion efficiency to be used in the following discussion will be the 2% of total solar energy. Table 3.1 shows the estimated maximum potential production of organic matter as a function of mean daily incident solar radiation. It should be kept in mind that these figures are approximations, since the actual conversion efficiency will vary systematically from 2% with varying incident radiation (somewhat higher at low intensities and lower at the higher intensities) and unpredictably with other factors. The relationship between net algal photosynthesis (productivity) and incident solar radiation, discussed in detail by Ryther (1959) and subsequently by several other investigators, will be dealt with in Section 3.3.

Table 3.1

Daily and Annual Dry Weight Yields of Organic Matter and of Plants
 With Different Ash Contents as Functions of Mean Total Incident Solar Radiation,
 Assuming 2% Conversion Efficiency and a Heat of Combustion
 of 5 kg calories/gram Ash-free Dry Weight

Mean Daily Total Incident Radiation g cal/cm ² /day	Yield			
	g ash-free dry wt/m ² /day	m tons ash-free dry wt/ha/year	m tons/total dry wt/ha/year	
			10% Ash	40% Ash
100	4	15	16	24
200	8	29	32	49
300	12	44	49	73
400	16	54	60	91
500	20	73	81	122
600	24	88*	97*	146*
700	28	102*	114*	170*
800	32	117*	130*	195*

* Since mean incident solar radiation values above approximately 550 g cal/cm²/day do not occur anywhere in the continental United States throughout the year, these yields would not be expected on an annual basis.

Since mean radiation may be considered on a daily or annual basis, values for productivity are given for both time periods in Table 3.1. Mean incident radiation values as high as $700 - 800 \text{ g cal/cm}^2/\text{day}$ occur only for a few days at a time at many mid-latitude locations during the summer. In fact, values exceeding $550 \text{ g cal/cm}^2/\text{day}$ do not occur anywhere in the United States throughout the year. For most of the continental United States, the annual mean daily incident solar radiation falls between 200 and $400 \text{ g cal/cm}^2/\text{day}$, a range that is equivalent to a potential rate of organic production of $8 - 16 \text{ g ash-free dry wt/m}^2/\text{day}$ or $29 - 54 \text{ m tons/ha/year}$.

In Table 3.1 is shown the potential total dry weight yields that can be expected for different values of incident radiation of plants that have 10% and 40% ash content respectively. It is of interest to compare the potential values of 81 and $122 \text{ m tons/ha/year}$ for terrestrial (10% ash) and marine (40% ash) plants at $500 \text{ g cal/cm}^2/\text{day}$ with the highest reported yields for such plants, $85 \text{ m tons/ha/year}$ for napier grass in Puerto Rico and San Salvador and $127 \text{ m tons/ha/year}$ for the seaweed Gracilaria foliifera in Florida (Table 3.2).

But as illustrated in Table 3.2, even the highest reported annual productivity of agricultural crops, the most productive ecosystems on earth, rarely attain the potential yields of $29 - 54 \text{ m tons/ha/year}$ that should be attainable within the latitudes of the continental United States. Section 4 will document the fact that using present culture techniques and experimentation, most aquatic plants, including both freshwater and marine microphytes and macrophytes, also fall far below the potential yield.

On the other hand, short-term yields of agricultural crops (Table 3.3) and other plant ecosystems, over periods of days to weeks, often exceed the potential values given in Table 3.1, in some cases by a factor of two or more, indicating that the calculated efficiency of 2% may indeed be conservative in

Table 3.2
Productivity of Agricultural Crops¹ and Seaweeds^{2,3} on an Annual Basis
Yields of Total Dry Matter in metric tons/hectare/year

Crop	Country	Yield
<u>Temperate</u>		
Rye Grass	U.K.	23
Kale	U.K.	21
Sorghum	U.S., Illinois	16
Maize	U.K.	17
	U.K.	5 (grain)
	Canada, Ottawa	19
	Japan	26
	U.S., Iowa	16
	U.S., Kentucky	22
Potato	U.K.	11
	Netherlands	22
Sugar Beet	U.K.	23
	U.S., Washington	32
Wheat (spring)	U.K.	5 (grain)
	U.S., Washington	12 (grain)
	U.S., Washington	30 (total)
Barley	U.K.	7 (grain)
Rice	Japan	7 (grain)
Seaweeds ²	U.S., Massachusetts	
<u>Neogardhiella baileyi</u>		63
<u>Gracilaria foliifera</u>		33
<u>Sub-Tropical</u>		
Alfalfa	U.S., California	33
Sorghum	U.S., California	47
Bermuda Grass	U.S., Georgia	27
Sugar Beet	U.S., California	42
Potato	U.S., California	22
Wheat	Mexico	18
	U.S., California	7 (grain)
Rice	Australia, NSW	14 (grain)
	U.S., California	22
Maize	Egypt	29
	U.S., California	26
Seaweeds ³	U.S., Florida	
<u>Gracilaria foliifera</u>		127
<u>Tropical</u>		
Napier Grass	El Salvador	85
	Puerto Rico	85
Sugar Cane	Hawaii	64
Oil Palm	Malaysia	40
Sugar Beet	Hawaii (2 crops)	31
Cassava	Tanzania	31
	Malaysia	38
Sorghum	Philippines	7 (grain)
Maize	Thailand	16
	Peru	26
Rice	Australia, NT	11 (grain)
	Peru	22
Rice + Sorghum (multiple cropping)	Philippines	23 (grain)

¹Cooper (1975)

²Ryther et al. (1977)

³Ryther (1977)

Table 3.3

Some High, Short-Term Dry Weight Yields of Crops¹ and Seaweeds²

Crop	Country	g/m ² /day
<u>Temperate</u>		
Tall fescue	U.K.	43
Ryegrass	U.K.	28
Cocksfoot	U.K.	40
Sugarbeet	U.K.	31
Kale	U.K.	21
Barley	U.K.	23
Maize	U.K.	24
Wheat	Netherlands	18
Peas	Netherlands	20
Red Clover	New Zealand	23
Maize	New Zealand	29
Maize	U.S., Kentucky	40
<u>Sub-Tropical</u>		
Alfalfa	U.S., California	23
Potato	U.S., California	38
Pine	Australia	41
Cotton	U.S., Georgia	27
Rice	S. Australia	23
Sugar Cane	U.S., Texas	31
Sudan grass	U.S., California	51
Maize	U.S., California	52
Algae (unicellular)	U.S., California	24
Seaweeds	U.S., Florida	
<u>Enteromorpha clathrata</u>		56
<u>Chaetomorpha linum</u>		38
<u>Gracilaria foliifera</u>		50
<u>Hypnea musciformis</u>		24
<u>Tropical</u>		
Cassava	Malaysia	18
Rice	Tanzania	17
Rice	Philippines	27
Oil Palm	Malaysia	11
Napier Grass	El Salvador	39
Bullrush Millet	Australia, NT	54
Sugar Cane	Hawaii	37
Maize	Thailand	31

¹ Anon (1976)² Ryther et al. (1976)

the short run. The failure of most plant communities to achieve a 2% efficiency on an annual basis probably means that the conditions for maximum potential photosynthesis and growth - all incident, non-reflected light absorbed by photosynthetic pigments and non-limiting concentrations and amounts of water, CO_2 , and nutrients - are seldom met on a permanent basis.

Much has been written in the past decade concerning the photosynthetic advantage of plants that reduce carbon dioxide by means of the so-called C_4 -dicarboxylic acid pathway in contrast to the more typical C_3 reductive phosphate cycle. While the advantage has been well demonstrated in the laboratory, the ecological implications are by no means clear. The fact that the most productive land plants - grasses, sugar cane, etc. - are C_4 -species may be less significant than that they live in areas of the best climate and incident solar radiation for plant production. In any event, the role of most aquatic species - algae and flowering plants alike - has not been determined in this connection.

But in other respects, the aquatic plants would appear to have distinct advantages over their terrestrial relative as producers of organic matter. At least partially suspended by the water, they do not require elaborate structural tissue and can devote a considerably larger fraction of their photosynthate to productive biomass. Water, of course, is rarely limiting as is so often the case of land, and most aquatic species can usually absorb CO_2 and nutrients through any part of their bodies. While CO_2 is often, if not usually, growth limiting to terrestrial plants, this is seldom the case at least in the marine environment with its large reservoir of dissolved carbonate and bicarbonate. And of all plants, the planktonic unicellular algae would appear to be the ideal photosynthetic machines, their small size and simple shape and structure greatly facilitating the diffusion and transport of essential substances.

In spite of these advantages, the production of aquatic plants in general is usually nutrient-limited and that of freshwater plants, CO_2 -limited as well. With rare exceptions, the growth of natural populations of aquatic plants, in unpolluted waters, ranges from one- to two orders of magnitude less

than the potential shown in Table 3.1. In the biosphere as a whole, aquatic plants occupy over three times the area of terrestrial plants, but account for less than half the annual organic production on earth.

Considering that solar radiation is the only controlling factor, and using the best available information on the quantum requirements and wave-length dependent efficiencies of photosynthesis, the spectral distribution of sunlight, the light saturation and light inhibition characteristics of microalgae and best estimates of respiratory losses, a model has been developed describing areal yields of phytoplankton as a function of total solar radiation (Figure 3.1). In comparing this curve with the maximum possible yearly variations in solar radiation striking the earth at latitudes ranging from the equator to 50° N (Figure 3.2), it is seen that the peak sunlight radiation occurring in June varies between ~630 langley/day at the equator to ~770 langley/day at 50° N. From Figure 3.1 this radiation range would indicate a possible maximum photosynthetic yield of 25 - 28 g dry wt/m²/day achieved for short durations in studies described in Section 4. Considering the many assumptions involved in the development of this model, the agreement between it and observed results is excellent.

Moreover, it is clear from Figure 3.2 that, because maximum solar radiation at the equator occurs at the autumn and spring equinoxes when the equator is directly perpendicular to the sun, the maximum possible yearly average radiation occurs somewhat north and south of 0° latitude. Hence, over a fairly wide band of latitude around the equator average yearly yields can potentially be high.

Actual long term yields are much more difficult to predict because of the many inherent variabilities involved in plant culturing and because true solar radiation levels for a particular location are as much a function of weather conditions as they are of latitude. For example, the actual sun light radiation recorded in Woods Hole, Massachusetts January 1975 - May 1977 and in Ft. Pierce, Florida during 1976 falls consistently below the maximum possible radiation levels, but within the anticipated maximum-minimum range, reflecting to a large degree the effects of cloudiness (Figure 3.3a, b).

Figure 3.1

Effect of Total Solar Radiation on Algal Yield
Considering Light as Sole Limiting Factor
(modified from Ryther, 1956)

Figure 3.2
Variations in Maximum Possible Solar Radiation
with Time of Year for Varying Latitudes

Figure 3.3

Actual Solar Radiation in a) Woods Hole, Mass. January 1975 - May 1977

and b) Ft. Pierce, Fla. January 1976 - December 1976

Shaded Area Represents Range of Maximum-Minimum Solar Radiation Possible

For the United States, yearly average solar radiation levels, based on composite data for five years (1967-71) from National Weather Service records are shown in Figure 3.4a, b. Peak yearly radiation levels above 450 langleys/day (and up to about 550 langleys/day) occur only in the southwest region and are reduced to 400 - 450 langleys/day in a belt surrounding the southwest. Once again referring to the curve in Figure 3.1, it is clear that sustained algal yields could never exceed about $20 \text{ g/m}^2/\text{day}$ in the southwest, and with the exception of Florida, increasingly lower levels north and east of this region.

3.3 Density

The productivity or yield of an organism is the product of specific growth rate and density. Yield as a function of density is described by a bell-shaped curve (Figures 3.5 and 3.6b). At low densities, plants are unable to utilize all of the incident radiation, while at high densities, self-shading begins to affect specific growth, the ratio photosynthesis : respiration becomes smaller, and net production or yield begins to decrease.

It is clear from these relationships that plant populations must be maintained at or near the density at which maximum productivity occurs if peak efficiencies are to be sustained. One reason that agricultural crops, forest plantations, and other slow growing plant populations are unable to achieve potential yield levels on a permanent basis is that their density is either too sparse or too dense during a significant fraction of their growth cycle.

Aquatic vegetation, including unicellular planktonic algae, seaweeds, and the higher plants, may be grown vegetatively for long periods of time, if not permanently, and can be harvested at will and at any desired frequency so as to maintain their populations at optimal densities. This is perhaps the greatest advantage that aquatic plants have over almost all forms of terrestrial vegetation with respect to biomass production.

Fig. 3.4a Mean Daily Irradiance on a Horizontal Surface (in g cal cm^{-2}) based on NWS(USA) Records of 5 yr, 1967-71.

Fig. 3.4b Mean Daily Irradiance on a Horizontal Surface over the U.S., with Different Shades Showing Increase in Insolation in Steps of 50 g cal cm^{-2} , based on NEWS Records of 5 yr, 1967-71.

(from Thekaekara, 1976)

Gracilaria foliifera

- production
- △ specific growth rate

Figure 3.5

Effect of Standing Crop (Density) of *Gracilaria foliifera*
on Production and Specific Growth Rate in Mass Culture Experiments
Conducted in Ft. Pierce, Florida

Figure 3.6

Effect of Dilution (Growth Rate) on a) Standing Crop as Particulate Carbon and Nitrogen (from Goldman and Ryther, 1975) and b) on Yield as Particulate Carbon for Marine Phytoplankton Growth in Mass Culture in Woods Hole, Massachusetts

However, the maintenance of a population of aquatic plants at its optimal density requires that both density and productivity be measured frequently, if not continuously. In the case of the unicellular algae, these parameters may both be monitored rather easily by cell counts or various forms of biomass measurement of relatively small aliquots of the population, assuming the algae is homogeneously distributed in the water. Dilution is accomplished easily by removing the fractional volume of the culture equivalent to incremental growth of the algae and replacing it with new culture medium. Ideally, this may be done on a continuous, flow-through mode of operation, maintaining the culture at a steady-state optimal density for maximum yield. Although diel, day-to-day, and seasonal changes in incident radiation and other factors preclude the possibility of a true steady state, experience has shown that outdoor continuous microalgal cultures can be sustained with only minor perturbations over rather long periods of time (e.g., months).

Removal of the unicellular algae from the harvested culture volume is, however, both difficult and costly, and the smaller the organisms the more difficult the problems become. Methods that have been used include centrifugation, chemical flocculation, floatation, sedimentation, and various kinds of filtration. To date, all have proved unsatisfactory from the viewpoint of cost-effectiveness and, in all likelihood, energy-effectiveness within the present context. Clearly, a new or greatly improved technology is needed for harvesting microalgae from their aqueous medium.

The density and productivity of the larger forms of aquatic vegetation, including both the seaweeds and the higher, flowering plants, are capable of being assessed only with much greater difficulty than in the case of the microalgae. The direct, and in many cases the only method available, is to remove the plants from the water periodically and weight them. On the basis of incremental weight increase, usually determined as drained wet weight, productivity or yield since the previous weighing may be determined and harvested, returning the population to its optimal starting density. As may be seen from Figures 3.5 and 3.6b, maximum yields may be sustained over a

range of densities that may be as much as twofold, in which case the doubling time would be equivalent to the frequency of harvesting, both of which are about one week in this case.

However, it is impractical to weigh entire populations of aquatic plants in any large-scale culture system, and it is difficult to select a representative aliquot. Furthermore, the removal of rooted plants, including even those that are free-floating such as water hyacinth and duckweed, is at best injurious and is often destructive to the sample. For these various reasons, it would therefore be preferable to use other indices than direct weighing to monitor productivity. The net removal of nutrients such as nitrogen and phosphorus by the plants may be used as a simple, non-destructive index of growth, if the nutrient content of the plant and its relationship to total biomass is known, if the daily mass input and outflow of the nutrient through the culture system may be determined, and if there is no other source of removal of the nutrient from solution other than plant assimilation. Unfortunately, while the first two conditions may be met by a rather rigorous monitoring program, phosphorus may be lost from solution by precipitation and ammonia-nitrogen lost to the air, both occurring at the high pH levels that accompany vigorous photosynthesis and CO_2 removal. Other criteria of plant growth such as CO_2 removal, pH change, or oxygen evolution are also subject to other influences and errors and are difficult to perform routinely and to interpret on a large-scale basis. It is perhaps for these reasons that carefully controlled and well-documented studies of the productivity of the larger aquatic plants are very few in number.

Thus it is that the microalgae may be maintained in culture with relative ease, but no suitable technique exists for their harvest from the water, while the larger aquatic plants are very easily harvested, but no simple method yet exists for monitoring their growth in culture.

3.4 Temperature

Generally, outdoors there is a direct positive correlation between solar radiation and temperature, so it is difficult to separate the individual

effects of each on aquatic plant growth. It is clear, however, that each plant species has an optimum temperature range at which high rates of growth are attained. Aquatic plants may be classified as cryophilic, mesophilic, or thermophilic depending on whether they thrive at low, medium, or high temperatures. Within that optimum range, increases in temperature increase growth rate until a critical point at which an increase in temperature either does not affect growth or decreases growth rates (Yokohana, 1973). The maximum growth rate of many marine and freshwater plants is related to temperature by the Arrhenius equation (Figure 3.7). Growth rates of phytoplankton grown in continuous culture (Goldman and Carpenter, 1974) and of the giant kelp Macrocystis pyrifera (North, 1976) have been shown to be highly temperature-dependent. Oswald (1977) explains that photosynthetic efficiency is influenced by temperature because both photosynthesis and respiration tend to accelerate with temperature increases up to a maximum after which new cell synthesis declines while respiration remains high, up to a thermal inactivation. The critical temperature is species specific and may even vary between strains of the same species. For example, warm water tolerant strains of M. pyrifera (North, 1976) and of Laminaria japonica (Cheng, 1969) have been developed to extend temperature range.

Since growth rates are temperature dependent it is not surprising that competition among plants and the resulting dominance of particular plant species are also influenced by temperature (Braarud, 1962; Mann, 1973; Patrick, 1969; Smayda, 1958). As shown in Figure 3.8, microalgal species dominance varies with season. Laboratory studies have shown that temperature is the main parameter controlling these species changes. For example, between 5 - 20°C Phaeodactylum tricornutum, a small marine pennate diatom, was dominant, at 27°C Nitzschia closterium, another marine pennate diatom, dominated and as the temperature increased further to 33°C a marine blue-green alga genus Oscillatoria became increasingly dominant (Goldman and Ryther, 1976).

Figure 3.7

Relationship between Maximum Specific Growth Rate and Absolute Temperature for Microalgae
(modified from Goldman and Carpenter, 1974)

Figure 3.8

Summary of data collected in outdoor ponds from January, 1974 through April, 1975 in Woods Hole, Mass. A, temperature data; B, particulate carbon data; C, species data (P = *Phaeodactylum tricornutum*, N = *Nitzschia closterium*, S = *Stichococcus* sp., SK = *S. costatum*) (from Goldman and Ryther, 1976)

The effects of temperature on biomass production per se appear minimal. As shown in Figure 3.9 for each microalgal species that grows (at a fixed growth rate) in a particular temperature range between 5 - 33°C, the production of biomass is essentially constant (Goldman, 1977). Therefore, biomass production is considerably more influenced by solar radiation than by temperature.

One possible explanation for this effect is that the peak aquatic plant yields occur at considerably less than the maximum growth rates, as shown in Figures 3.5 and 3.6b due to the decrease in standing crop as growth rate increases. Assuming that the response of aquatic plants to light intensity or some other limiting factor follows a saturation-type curve, a family of such curves with increasing temperature-dependent asymptotes can be constructed (Figure 3.10). What is apparent from these curves is that at low growth rates the effect of temperature is relatively small. From the few mass culture studies performed in varying geographical locations in which yield was compared with growth (or dilution) rate under optimal summer conditions (Figure 3.11), it appears that peak yields typically occur at growth rates between $\sim 0.5 - 0.75$ /day, which according to the curve in Figure 3.7, represents a small fraction of the maximum growth rate potential at typical summer temperatures ($\sim 20 - 30^\circ\text{C}$). The one curve (#2) in Figure 3.11 for winter conditions in Jerusalem, Israel, indicates a shift downward in growth rate (~ 0.25 /day) for peak production, most likely caused by the seasonal reduction in solar radiation. Hence, it is clear that even under the most optimal conditions of solar radiation, maximum yields of aquatic plants occur at relatively low growth rates, so as to greatly reduce the influence of temperature.

Little is known of the physiology of aquatic plants growing in fluctuating temperatures since most scientific studies are undertaken under stable temperature conditions. Since heat management is very expensive, this may be an important consideration for mass cultivation.

Figure 3.9

Concentrations of Particulate Nitrogen Produced Between 5 - 33°C
 for Mixed Phytoplankton Populations in Continuous Culture
 solid bars, 1:1 wastewater-seawater mixtures; shaded bars, 1:3 mixtures
 ---, influent EN concentrations (from Goldman, 1977)

SPECIFIC GROWTH RATE

LIMITING FACTOR (LIGHT OR NUTRIENT)

Figure 3.10

Relationship Between Specific Growth Rate and Limiting Factor
Showing Increasing Temperature-Dependent Asymptotes

Figure 3.11 Relationship between plant yield and dilution rate during mass culture experiments in different locations and seasons: 1) Jerusalem, Israel, June, 1969-70; 2) Jerusalem, December, 1969-70 (Jerusalem values include non-algal solids present in wastewater); 3) Ft. Pierce, Florida, spring-summer, 1974; 4) Woods Hole, Massachusetts spring-summer, 1973, (both 4 & 5 with small experimental units); 5) Woods Hole, Massachusetts, spring, 1976 with large ponds; 6) Richmond, California, summer, 1977.

3.5 Nutrients

In addition to solar energy, nutrients are required in a form utilizable by the plant to produce organic matter. The marine seagrasses and rooted freshwater macrophytes remove nutrients with their roots like terrestrial plants, but the other aquatic plants which lack root systems absorb nutrients across all exposed surfaces (Munk and Riley, 1952).

Many nutrients such as carbon, hydrogen, oxygen, chlorine, sodium, potassium, calcium, magnesium, and sulfur are generally plentiful in seawater. Those nutrients which are limited in the open-ocean and to a lesser extent in coastal waters, including nitrogen and phosphorus, may limit growth when not available in sufficient concentrations. Trace elements such as copper, zinc, cobalt, molybdenum, vanadium, iodine, etc. and organic substances such as vitamins, are also of great importance to the growth of aquatic plants.

Substances that might limit plant development may be identified by examining the degrees to which elements tend to be concentrated in tissues and compare the ratios with levels occurring in seawater. If an element is very scarce in seawater yet is strongly concentrated by a plant, additions of the substance in a medium might stimulate growth. The occurrences of carbon, nitrogen and phosphorus as a percentage of dry wt are shown in Table 3.4.

Carbon

The importance of carbon on algal growth has been reviewed by Goldman et al (1972). Inorganic carbon is available for algal growth in the form of CO_2 derived from the bicarbonate carbonate equilibrium system and atmospheric CO_2 transported into the aqueous phase. Carbon concentration is relatively high in seawater, about 25 mg/l, but may be growth limiting in freshwater systems and in mass culture marine systems when other nutrients are in abundant supply.

Carbon taken up by aquatic plants may contribute as much as 20 - 40% of the dry weight (Table 3.4). It varies, however, with the algal growth stage and availability of other nutrients, principally nitrogen (Jackson, 1977). There is a complex interrelationship between structural and storage products synthesized

Table 3.4

SEAWEED COMPOSITION (modified from Jackson, 1977)

Species	Dry wt % wet wt	Ash wt % dry wt	C-content % dry wt	N-content % dry wt	P-content % dry wt	Source
GREEN ALGAE						
<u>Codium fragile</u>	7	42				Paine and Vadas, 1969
<u>Enteromorpha compressa</u>			31.5	0.5 - 3.4		Vinogradov, 1953
<u>Ulva lactuca</u>	22	21	36	0.9 - 5.4	0.03	Paine and Vadas, 1969; Vinogradov, 1953
BROWN ALGAE						
<u>Agarum cribosum</u>	18 - 25	34	31.6 $\pm$ 1.8			Paine and Vadas, 1969; Mann, 1972
<u>Alaria esculenta</u>				1.6		Vinogradov, 1953
<u>Ascophyllum nodosum</u>	20 - 30	24	38	3.2	0.16	Vinogradov, 1953
<u>Cystoseira spp.</u>	25	16		1.2	0.24	Vinogradov, 1953
<u>Egregia laevigata</u>				2.7	0.49	Vinogradov, 1953
<u>Egregia menziesii</u>				2.6 - 2.8	0.55-0.78	Vinogradov, 1953
<u>Fucus vesiculosus</u>	18	16 - 22	35	2	0.2	Vinogradov, 1953
<u>Laminaria digitata</u>	18 - 21	18	32.7 $\pm$ 3.5	3.1	0.42	Mann, 1972; Vinogradov, 1953
<u>Laminaria japonica</u>	14 - 20					Cheng, 1969
<u>Laminaria longicruris</u>	14 - 28	33	29	1.5 - 5		Chapman and Craigie, 1977 Mann, 1972; Vinogradov, 1953
<u>Macrocystis pyrifera</u>	14 - 18	34	20 - 31	0.7 - 3.8	0.4	Paine and Vadas, 1968; North, 1971; North, 1976
<u>Nereocystis leutkeana</u>	10	52		0.81 - 3.1	0.3	Paine and Vadas, 1969; Vinogradov, 1953
<u>Saccorhiza polyschides</u>	15	45		1.8	0.23	Norton, 1970
RED ALGAE						
<u>Chondrus crispus</u>	22	25 - 28		0.7 - 3	0.035	Neish and Shacklock, 1971 Vinogradov, 1953
<u>Gigartina corymbifera</u>	20	30				Paine and Vadas, 1969
<u>Gigartina papillata</u>	38	22				Paine and Vadas, 1969
<u>Gracilaria verrucosa</u>		10		1.5	0.1	Vinogradov, 1953
<u>Gracilaria sp.</u>	10 - 15					DeBoer and Ryther, 1977
<u>Iridaea cordata</u>	20	25				Paine and Vadas, 1969
<u>Neoaghardiella baileyi</u>	10 - 15					DeBoer and Ryther, 1977
<u>Porphyra spp.</u>	14 - 38	10 - 30		5		Paine and Vadas, 1969; Vinogradov, 1953
AQUATIC ANGIOSPERMS						
<u>Phyllospadix scouleri</u>	28	14				Paine and Vadas, 1969
<u>Thalassia testudinum</u>	67 - 75			2.3	0.16	Gessner, 1971; Patriquin, 1972
<u>Zostera marina</u>	68	20	31 - 39	1.5 - 2.5	0.2 - 1	McRoy, 1970; Vinogradov, 1953

from carbon and nitrogen (DeBoer, 1977; Neish et al., 1977). In seaweeds carbon takes the form of phycocolloids such as algin in Macrocystis, carrageenan in Chondrus, laminarin in Laminaria and agar-agar in Gelidium, all of which are commercially important substances.

The carbonate system is the principal pH buffer and algal carbon usage can, therefore, affect solution pH. The pH controls the dissociation of the chemical species and the degree to which the gaseous phase exists for inorganic carbon and ammonia by the following reactions:

and

Because the pH for NH_3 is about 9.3, unless the pH goes above 9+, the ionic form NH_4^+ is the dominant species. For inorganic carbon the pH for H_2CO_3 (carbonic acid) is ~4.3 so that over most of the pH range at which algae grow (7 - 11) the ionic species HCO_3^- and CO_3^{2-} dominate. Unless the transport of CO_2 from the atmosphere can keep pace with the assimilation of CO_2 by algae, inorganic carbon as CO_2 will be derived via the dehydration of HCO_3^- , according to the reaction:

resulting in a pH rise as hydroxide ion is produced so as to maintain a charge balance and keep the alkalinity constant.

Normally, the transport of CO_2 from the atmosphere cannot keep pace with algal assimilation of CO_2 during intense algal growth and a rise in pH to over 10 is not uncommon in eutrophic natural waters and mass culture systems (Goldman et al., 1972). Such a pH rise can be detrimental to algal growth in one or more ways: 1) a carbon-limitation may exist; 2) at high pH levels sparingly soluble salts of phosphorus and many vital trace metals are precipitated out of solution and could hence become limiting; and 3) many algal species cannot tolerate high pH values, and growth rates are curtailed. A sufficient supply of CO_2 is, therefore, essential for growth of aquatic plants.

Nitrogen

Nitrogen is the element most often found to be limiting phytoplankton and algal growth in the sea (Jackson, 1976; Parsons and Takahashm, 1973; Ryther, 1963; Thomas, 1969, 1970; Thomas et al., 1974). Nitrogen may be in the form of dissolved N_2 , nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3), urea, and amino acids. Blue-green algae are the only plants that utilize dissolved N_2 . The most important sources for seaweeds are ammonia and nitrate. Ammonia is taken up by phytoplankton (Eppley et al., 1969) and seaweeds such as Neogahardiella baileyi (D'Elia et al., 1976). The giant kelp Macrocystis takes up nitrogen in the form of nitrates.

Changes in nutrient concentrations in solution lead to changes in rate of nutrient uptake, changes in internal nutrient concentrations and changes in growth rates (Jackson, 1977 for explanation). Increased nitrate and ammonia concentrations in solution lead to an increase in nitrogen concentration in plants. This relationship may be shown by using the ratio of carbon to nitrogen in the tissue. Low values of the C:N ratio indicate high relative internal nitrogen concentrations. Investigations have shown that when a phycocolloid-producing seaweed, such as Chondrus crispus and Neogahardiella baileyi are grown in nitrogen-poor solutions, there is an increase in the carbohydrate product (DeBoer et al., 1976; Neish and Shacklock, 1971). The C:N ratio may vary with age in the case of Macrocystis (North, 1976). The importance of nitrogen may also be related to its influence on pH. Inorganic carbon or ammonia exist partially in a gaseous phase and the pH controls the degree to which the gaseous phase exists. As discussed previously a rise in pH may be detrimental to plant growth.

Whatever the role of nitrogen, investigations have shown that nutrients, such as nitrogen increase growth in Ulva lactuca (Steffensen, 1976; Waite and Mitchell, 1972b), Macrocystis pyrifera (North, 1976; Seligman, 1976b). Laminaria japonica (Cheng, 1969), Laminaria longicruris (Chapman and Craigie, 1977), Fucus spiralis (Topinka and Robbins, 1976), Chondrus crispus (Prince,

1974; Neish and Shacklock, 1971), Porphyra spp. (Iwasaki, 1967; Iwasaki and Matsudaira, 1954; Noda and Horiguchi, 1971; Ogata, 1975), Hypnea muciformis, Gracilaria foliifera and Neoaghardiella baileyi (DeBoer et al., 1976).

Phosphorus

Phosphorus is not usually considered as important as nitrogen for controlling growth of phytoplankton (Parsons and Takahashi, 1973; Ryther and Dunstan, 1971). Phosphorus is believed to be more important in the case of marine macrophytes, although the reasons are not completely understood. Ryther (1977) suggested that phosphorus may limit the growth of rooted plants because it is bound to the soil. There is no doubt that phosphorus in the form of orthophosphate is needed for growth. Ratios of nitrogen to phosphorus in seaweed tissues (N:P) range from 7:1 to 30:1 (Prince, 1974).

Trace Substances

The role of trace substances is not clear because of the complexity of solution interactions between metals, solution interactions with dissolved organics and biological interactions with aquatic plants (see Jackson, 1977). The occurrences of trace substances in seaweeds as a percentage of dry weight and wet weight are shown in Tables 3.5 and 3.6, respectively. Ten elements (N, Al, P, Mn, Fe, Co, Ni, Cu, Zn, and I) are sometimes concentrated a thousand-fold or more by seaweeds. All of these occur at low concentrations in seawater. Four other elements (B, V, Mo, Sr) can display concentration factors greater than ten. Vanadium and Molybdenum occur at quite low concentrations in seawater. Bromine may be moderately concentrated among some species, but occurs at high levels in seawater compared to other elements listed in Table 3.6. Additional references in which algal species were analyzed for contents of trace metals and other micronutrient elements are presented in Table 3.7. Likewise rates at which various algae accumulate micronutrients and non-required trace substances have been determined for many elements and the references are presented in Table 3.8. Most of these studies, however, do not attempt to distinguish between absorption onto relatively inert structures such as cell walls, mucus

Table 3.5

Contents of Trace Elements in Seaweeds Expressed as Percent of Dry Weight.

Unless otherwise noted, values represent percent $\times 10^{-3}$.

Data primarily from Vinogradoff (1953) and Chapman (1970).

p means element present but concentration unknown.

Phylum Species	I	Br	Rb	Cr	Sr	Ba	Ra $\times 10^{-14}$	Fe	Cd $\times 10^{-8}$	Cu $\times 10^{-4}$	V	Co	B	Zn	Mn	As	Al	Ti	Hg $\times 10^{-8}$	Mo	Pb	Ni
GREEN ALGAE																						
<i>Codium fragile</i>	3 - 7																					
<i>Enteromorpha</i> comp.	6															1.2						
<i>E. linza</i>	22																					
<i>E. intestinalis</i>	3	3																				
<i>Ulva lactuca</i>	3 - 60		2		4	4	0.7	40				0.11	20		2.5	0.2	54					0.07
BROWN ALGAE																						
<i>Alaria esculenta</i>	50 - 100	20	2				1.1		100			0.05	16									0.04
<i>Ascophyllum</i> nod.	30 - 190	17	0.04		260		3	28	29	1 - 4	0.06	0.07	14	6	0.9	0.005		0.3		0.03	1.8	0.04
<i>Chorda filum</i>	2 - 14	20											18		2.5							
<i>Cystoseira</i> fib.	11														1.8			0.4				
<i>Egregia menziesii</i>	30 - 90												14									
<i>Fucus serratus</i>	40 - 170	190	0.05	1.5	280	6	9	38	34	5 - 17		0.13	13	7	15	6.7		0.2		0.07	1.8	0.31
<i>F. vesiculosus</i>	1 - 110	94			10		7.5	68		3 - 8		0.07	17	6	12	0.6	60	0.9	2.3	0.1	p	
<i>Laminaria digi.</i>	50 - 1700	138	2		400		10	29		1 - 5	1	0.09	18	20	0.9	4.5	52	0.2				0.83
<i>L. hyperborea</i>	480 - 730	100	19	0.1	260	5	12	10 - 22		1 - 14	0.1	0.03		12 - 20	0.5 - 2	6.3		0.5	3.7	0.05	1.6	0.2
<i>L. japonica</i>	110 - 120	70						85								2.6						
<i>L. saccharina</i>	140 - 900	50	2				10		14			0.15	23		0.6	5.6						0.17
<i>Macrocystis</i> py.	100 - 410	80	4	<5						<20		0.35	30	3	0.5	5.8				<0.1	<0.2	<0.3
<i>Nereocystis</i> le.	10 - 300	140																				
<i>Pelagophycus</i> p.	260 - 360																					
<i>Sargassum</i> bac.							14	59									79					
<i>S. siliquastrum</i>	20 - 340							93								0.5						
RED ALGAE																						
<i>Chondrus crispus</i>	76									0.6			15			1.2	51					
<i>Gigartina</i> mam.	16														13	1.8						
<i>Gracilaria</i> conf.	2													7.7								
<i>Porphyra</i> ten.	2																					
<i>Rhodryenia</i> palm.	1 - 700	11	2				1						13		1.3			0.5				
SPERMATOPHYTA																						
<i>Zostera marina</i>	90 - 420	61							2.3			3	75	28	300		14					4
<i>Z. nana</i>			0.6	p			36					1.5	91	p								1.3

Table 3.6

Concentrations of Nutrient and Trace Elements in Seawater
in mg/l According to Goldberg (1965) and Degrees to which they are Concentrated in the Living Tissues of Various Algae.
Concentration factors were computed for levels in the living plant.

Element	mg/l in Seawater	Concentration Factors										
		<u>Ulva lactuca</u>	<u>Ascophyllum nodosum</u>	<u>Fucus serratus</u>	<u>Fucus vesiculosus</u>	<u>Laminaria digitata</u>	<u>Laminaria hyperborea</u>	<u>Laminaria saccharina</u>	<u>Macrocystis pyrifera</u>	<u>Chondrus crispus</u>	<u>Rhodomenia palmata</u>	<u>Zostera marina</u>
B	4.6	9	6	7	9	6		7	9	7	5	52
N	0.5	360- 2160	450- 1280	740	500- 1450	390- 940		180- 780	280- 480	500- 1240	640- 1340	960- 1840
Al	0.01	10800			15000	7800				11200		4500
P	0.07	86	460		715	900			490- 820		770	2300
V	0.002		60			65						
Mn	0.002	2500	900	19000	15000	700	3500	400	350		1150	4800
Fe	0.01	8000	5600	9500	12000	5700	2400					
Co	0.0001	2200	1400	3000	2000	1400	4500	2000	5000			100,000
Ni	0.002	7000	40	400		600		100	<200			6500
Cu	0.003		67- 167	330- 1100	330- 670	50- 250	670		<930	43		
Zn	0.01		1200	1800		3000	1200		420			9000
Mo	0.01		6	20	30							
I	0.06	100- 2100	100- 6300	1800- 7100	50- 4600	1250- 42500	12000- 18300	3000- 19500	2300- 9500	2800	30- 21000	4830- 22500
Br	65		0.5	7	4	3		1	2		0.3	3
Sr	8	1	70	90		75						

Table 3.7
ADDITIONAL PUBLICATIONS INVOLVING ASSAYS OF MICRONUTRIENTS IN ALGAE

Reference	Genus	Element
Andrianov & Gazha, 1974	<u>Phyllophora</u>	I
Black & Mitchell, 1952	9 spp incl. <u>Pelvetia</u> , <u>Fucus</u> , <u>Laminaria</u> , <u>Ascophyllum</u>	Ni,Mo,Sr,Zn,V,Ti, etc.
Boothe & Knauer, 1972	<u>Macrocystis</u>	Co,Fe,Mn, etc.
Bryan, 1969	<u>Laminaria</u>	Zn
Bryan & Hummerstone, 1973	<u>Fucus</u>	Cu,Zn,Mn,Fe
Butler, 1931	14 spp incl. <u>Alaria</u> , <u>Fucus</u> , <u>Ascophyllum</u> , <u>Laminaria</u>	I
Dave <u>et al.</u> , 1973	6 spp incl. <u>Sargassum</u> , <u>Cystoseira</u> , <u>Hypnea</u>	I
Foster, 1975	<u>Fucus</u> , <u>Ascophyllum</u>	Fe,Cu,Mn,Zn
Foster, 1976	<u>Fucus</u> , <u>Ascophyllum</u>	Cu,Mn,Ni,Zn
Fuge & James, 1973	<u>Fucus</u>	Co,Cu,Fe,Mn,Mo,Zn
Fujikawa, 1974	<u>Porphyra</u>	Fe,Mn
Fukuda <u>et al.</u> , 1965	Many spp	I
Gryzhankova <u>et al.</u> , 1975	<u>Ahnfeltia</u> , <u>Laminaria</u> , <u>Ulva</u> , <u>Zostera</u>	Fe,Mn
Gutknecht, 1965	9 spp incl. <u>Fucus</u> , <u>Dictyota</u>	Cs,Zn
Law, 1975	<u>Fucus</u> , <u>Laminaria</u> , <u>Porphyra</u>	I
MacPherson & Young, 1949	14 spp incl. <u>Alaria</u> , <u>Fucus</u> , <u>Ascophyllum</u> , <u>Laminaria</u>	Fe
Meguro <u>et al.</u> , 1967	<u>Laminaria</u>	I
Morris & Bale, 1975	<u>Fucus</u>	Cu,Mn,Zn
Presnyakova <u>et al.</u> , 1974	<u>Chorda</u> , <u>Ulva</u> , <u>Coccophora</u> , <u>Dictyota</u> , <u>Pelvetia</u> , <u>Codium</u> , etc.	Co,Mo,Ni,V
Shimokawa <u>et al.</u> , 1971	<u>Cystophyllum</u> , <u>Undaria</u>	As
Stenner & Nickless, 1975	<u>Corallina</u> , <u>Chorda</u> , <u>Chondrus</u> , <u>Delesseria</u> , <u>Ulva</u> , <u>Zostera</u>	Cu,Zn,Cd,Pb
Styron <u>et al.</u> , 1976	<u>Achnanthes</u> , <u>Carteria</u> , <u>Chlamydomonas</u> , <u>Dunaliella</u> , <u>Nannochloris</u> , <u>Phaeodactylum</u>	Cs,Zn
Tong & Chaikoff, 1955	<u>Nereocystis</u>	I
Wort, 1955	<u>Macrocystis</u> , <u>Nereocystis</u>	Cu,Zn,Fe
Yamamoto <u>et al.</u> , 1970	Several spp	Ti,V

Table 3.8
SUMMARY OF PUBLICATIONS DESCRIBING PHYSIOLOGICAL RELATIONSHIPS (UPTAKE, TOXICITY, ETC.)
BETWEEN TRACE SUBSTANCES AND ALGAE.

Reference	Genus	Element
Bachmann & Odum, 1960	<u>Chaetomorpha</u> , <u>Chordaria</u> , <u>Fucus</u> , <u>Laminaria</u> , <u>Ceramium</u> , <u>Porphyra</u>	Zn
Bagnyuk <u>et al.</u> , 1976	<u>Scenedesmus</u>	Fe
Bailey & Kelly, 1955	<u>Ascophyllum</u>	I
Bernhard & Zattera, 1969	<u>Phaeodactylum</u>	Zn
Cowen <u>et al.</u> , 1976	<u>Macrocystis</u>	Po
Davies, 1973	<u>Phaeodactylum</u>	Zn
Doody <u>et al.</u> , 1973	<u>Scenedesmus</u>	Cu,Cr,F,Fe,Hg,Mn,Pb
Egorov <u>et al.</u> , 1975	<u>Ulva</u>	Zn
Fries, 1966	<u>Polysiphonia</u> , <u>Neamlion</u> , <u>Goniotrichum</u> , etc.	I
Gutknecht, 1961a, b	<u>Ulva</u> , <u>Porphyra</u>	Zn, Sc
Gutknecht, 1963	<u>Ulva</u> , <u>Laminaria</u> , <u>Porphyra</u>	Zn
Gutknecht, 1965	9 spp incl. <u>Fucus</u> , <u>Dictyota</u>	Cs,Zn
Klemperer, 1957	<u>Fucus</u>	I
Knappe & Werner, 1975	<u>Asparaoopsis</u> , <u>Falkenburgia</u>	I
Levandowsky & Hutner, 1975	<u>Crypthecodinium</u>	Fe
MacLean <u>et al.</u> , 1972	<u>Anacystis</u> , <u>Chlorella</u> , <u>Chondrus</u> , <u>Crithidia</u>	Cd,Zn
Miesch & Bielig, 1975	<u>Scenedesmus</u> , <u>Chlorella</u>	V,Fe
Mills, 1976	<u>Euglena</u>	Zn
Morris & Russel, 1973	<u>Ectocarpus</u>	Cu
Nielsen, 1969	<u>Chlorella</u>	Cu
Nielsen <u>et al.</u> , 1969	<u>Chlorella</u>	Cu
Parekh <u>et al.</u> , 1969	Several spp	I
Pedersen, 1969	<u>Ectocarpus</u> , <u>Lithosiphon</u> , <u>Pylaiella</u>	I,Br
Pokrovskaya <u>et al.</u> , 1974	<u>Chlorella</u>	B,Cu,Fe,Mn,Mo,Zn
Rachlin & Farran, 1974	<u>Chlorella</u>	Zn
Rozhanskaya, 1970	<u>Cystosiera</u> , <u>Padina</u> , <u>Zostera</u>	Mn,Zn
Russell & Morris, 1970	<u>Ectocarpus</u>	Cu
Scott, 1954	Several spp	Cs
Scott & Ericson, 1955	<u>Rhodomenia</u>	Co
Shaw, 1959	<u>Laminaria</u>	I
Shaw, 1960	<u>Laminaria</u>	I
Shaw, 1962	<u>Laminaria</u> , <u>Fucus</u>	I,Cl,Br
Shimo and Nakatani, 1967	<u>Porphyra</u>	Cu,Zn
Spooner, 1949	<u>Fucus</u> , <u>Rhodomenia</u>	Sr,Y
Stillwell, 1975	<u>Cricosphaera</u>	Zn
Van Weers, 1972	<u>Fucus</u>	Zn,Co
Williams & Swanson, 1958	Several spp	Cs

layers, etc. and incorporated into metabolically active roles in the cell interior. Presumably the absolute need for a micronutrient by a plant depends on the metabolic function of the element. There is much evidence that inert absorption is a significant factor in accumulation of some elements.

3.6 Biological Limitations

Those biological parameters that affect productivity significantly include grazing, pathogens, competition with other plants, encrustaceans and senescence. In an ecologically healthy community plants are in balance with associated organisms, but under stress conditions such as an increase in temperature or density or a lack of sufficient nutrients, that balance may be upset, thus decreasing the productivity of a particular species.

A significant decline in total biomass of plants, such as Laminaria (Cheng, 1969), Macrocystis (Leighton, 1971), Thalassia (Greenway, 1974; Ogden, 1976; Randall, 1965), and Zostera (Zieman, 1975) have been reported due to grazing. Grazers known to limit the growth of macrophytes significantly include sea urchins, fishes, various gastropods (i.e., snails, albatone, sea hares) and small crustaceans. In microalgal cultures rotifiers are the primary grazers. Microscopic organisms attack early stages in the life histories of many algal species and may affect the success of reproductive processes responsible for the recruitment of juvenile plants. Leighton (1968) has shown that a particular plant may be preferred by a grazer simply because it is the most abundant species in the herbivore's natural environment. Excessive grazing can be a serious problem and if not controlled will completely destroy cultured aquatic plants. Most animal grazers can be controlled by exclusion, cropping, biological control or pesticide application.

Pathogens of aquatic plants include viruses, bacteria, fungi, nematodes and parasitic algae (Andrews, 1976). Plants are most susceptible to disease when under stressed conditions such as nutrient deficiency, high temperatures and high densities (Ogata, 1975). Mathieson (1975) has shown that disease may be minimized by limiting plant densities. Uncontrollable diseases have had profound effects on the productivity of Macrocystis (Scotten, 1971 and on Porphyra (Yoshida, 1972) and may eliminate the desired species as

in the case of Zostera, which was virtually eliminated on the east coast of the United States by the pathogen Labrinthula.

As mentioned previously in Section 3.3, other aquatic plant species may be in direct competition with the desired species. Both microalgal and macroalgal cultures have problems with phytoplankton blooms. The phytoplankton may completely replace the cultured species in a short time if not checked frequently. Macrophyte productivity is usually affected by microorganisms in the early stages of development. Macrophytes may also compete for nutrients with other aquatic plants. This problem has been reported in the culture of Laminaria in China (Cheng, 1969), the culture of Gelidium in Japan and the culture of Chondrus crispus in Canada (DeBoer and Ryther, 1977). In the case of Macrocystis, competition is rarely a problem in the adult stage, but in the gametophyte stages benthic diatoms may overgrow the culture.

A wide variety of organisms including sponges, hydroids, polychaetes, bryozoans, mollusks and tunicates and the eggs of fishes epiphytize on aquatic macrophytes. Those plants with flat blades such as Thalassia and Macrosystis are particularly susceptible to encrusting organisms. These organisms are detrimental to macrophytes because they block out light and hence, slow the photosynthetic rate. They may also be harmed by organisms feeding on the epiphytes.

Periodically cultured plants stop growing vegetatively and enter a condition of senescence which is unpredictable and unexplainable. Macrophytes fragment into small pieces and become heavily epiphytized and usually do not recover, but result in mass diebacks. Many such phenomena are simply given a name such as "aging" or "ice-ice", which serves in lieu of a description of cause, effect and cure. This occurrence is particularly important, because until an explanation can be found, there is no means of control and complete cultures will suddenly disintegrate.

Section 4

HISTORICAL REVIEW OF THE MASS CULTURE OF MICROALGAE

J.C. Goldman

Mass culture experiments with microalgae have been carried on in many countries with increasing intensity since World War II. Significant bioengineering advances have been made and high productivity rates have been attained, but problems relating to culture mixing, nutrient availability and addition, species control, carbon dioxide addition, water supply, and algal separation and harvesting have held back the commercial production of microalgae. The few commercial ventures that exist today are primarily in the Far East and deal with the production of high-priced health food products derived from single-cell algae which are sold almost exclusively in Japan.

4.1 Early History of Culture Systems

Although it is difficult to pinpoint exactly when or where the concept evolved, interest in the mass cultivation of microalgae has grown tremendously over the past 30 years. There were several successive scientific breakthroughs which set the stage for the intense mass culture research that originated in the early 1950's and which has progressed steadily over the past several decades.

Before World War II, the prime interest of plant physiologists and biochemists was to unravel the mysteries of the photosynthetic process. The hardy freshwater green algae Chlorella and Scenedesmus were the favorite specimens used in these studies, primarily because they were unicellular, they had reproducible growth characteristics and they could be produced on demand due to their fast reproductive rates. However, laboratory batch cultures were usually restricted to, at best, several hundred milliliter volumes, and frequently there was insufficient algal biomass available for performing all the desired experiments and chemical analyses.

Ketchum and Redfield (1938) devised a simple way to overcome this handicap by exploiting the concept of the "optimum catch". This concept involved the periodic harvesting of a fixed portion of a population at a crucial point in a batch growth curve. By replacing the harvested culture with new medium, the remaining population would continue to replicate and grow until the desired population was again obtained and reharvested. Hence, in this way a semi-continuous supply of algae of a common nutritional and physiological state could be maintained. Ketchum et al. (1949) used this technique to successfully grow and optimally harvest cells of several phytoplankton species.

This culture technique was the forerunner of a more automated semi-continuous cultivation system developed by Myers and Clark (1944). Although crude by today's standards, this device was one of the first systems employed for the continuous production of microalgae. Simple in design (Figure 4.1a), it consisted of five major parts: an illuminated growth chamber, a medium storage bottle, a solenoid valve on a feed line from the medium bottle, a photocell, and a clamped-off withdrawal port. Culture density was maintained at a fixed level by the photocell which controlled the opening of the solenoid valve, allowing fresh medium to enter and dilute the culture as required. Samples were withdrawn when desired by manually opening a valve on the discharge line. The system, not being fully continuous, had the main disadvantage of a varying culture volume and, consequently, it was difficult to maintain a constant cell growth rate.

The fully continuous culture chamber of Cook (1951) (Figure 4.1b), which was similar in appearance to the culture unit of Myers and Clark, solved these problems by having a withdrawal port at a fixed height in the chamber. Culture volume was thus constant (10 liters), and medium input and culture overflow were essentially continuous. Hence, for a fixed medium flow rate, the dilution rate (fractional volume displacement per unit time) equalled the specific growth rate once a steady state was reached (i.e., no change in culture biomass with respect to time).

Figure 4.1a

Figure 4.1b

Continuous Culture Apparatus
Artificial light 4-inch column

Figure 4.1

Diagrams of Early Algal Growth Chambers

a) from Myers and Clark (1944); b) from Cook (1951)

Cook's work at the Stanford Research Institute was part of the first concerted attempt to mass culture microalgae for potential commercial application. This work was an outgrowth of the earlier studies of Spoehr and Milner (1949), who showed that the chemical composition of Chlorella, particularly the fat and protein content, could be readily manipulated by varying growth conditions. Therefore, because protein was relatively expensive and in short supply throughout the world, the concept of mass culturing algae as a food source appeared particularly attractive (Spoehr and Milner, 1949).

Encouraged by the experiments with the small 10 liter cultures, Cook's group built the first pilot plant in the United States consisting of four 570 liter growth units (Cook, 1950). Unfortunately, the results were not published and mass culture work at the Research Institute ended in 1950.

Interest in mass algal culture was continued in 1951 by the Carnegie Institution of Washington through a contract with Arthur D. Little, Inc., of Cambridge, Massachusetts, where a pilot plant was constructed including culture units ranging in size from 700 to 4500 liters (Burlew, 1953). Short-term dry weight yields of Chlorella were as high as $11 \text{ g/m}^2/\text{day}$, although the average yield for 52 days of operation was only $2 \text{ g/m}^2/\text{day}$. It was concluded, however, that yields up to $20 - 25 \text{ g/m}^2/\text{day}$ were possible once culture technology improved and cultivation was performed in more suitable geographical locations. This was an interesting and prophetic projection, because almost 30 years later these estimated yields have consistently been reached for short periods, but not greatly improved upon.

Following the lead of the Carnegie Institution, interest in mass culturing algae began to blossom in numerous countries, including Germany, Japan, and Israel. These international efforts culminated in the now famous Carnegie Report on algal mass culturing (Burlew, 1953) in which the state of the art was updated through 1952. However, most of these early attempts were restricted to short-term studies in very small culture units and yields of the commonly used green alga Chlorella were invariably less than $5 \text{ g/m}^2/\text{day}$

(Mituya et al., 1953; von Witsch and Harder, 1953; Milner, 1953). Moreover, the overall goal of these early researchers was strictly to produce protein for potential human and animal consumption, as interest in growing algae for other purposes was not yet established.

4.2 Culture After 1955

During the period 1955-1970, significant advances were made in many areas of algal mass cultivation technology; small laboratory cultures were enlarged to relatively large-scale systems, the bioengineering aspects of large-scale culturing were investigated, and the overall objectives were expanded to include a variety of applications such as wastewater treatment, chemical extraction, fertilizer production, closed life-support systems, aquaculture, and more recently, bioconversion. Significant portions of these advances were made in foreign countries. It is convenient in the remainder of this section on post-1955 achievements in algal mass culture technology to review the work on a country by country basis.

United States

Following the intense research activity sponsored by the Carnegie Institution in the early 1950's, U.S. efforts diminished for a number of reasons. After the initial successes, it quickly became apparent that several important advantages of mass algal cultures (i.e., high photosynthetic efficiencies, year-round operation, and high plant protein content) were offset by major disadvantages: 1) single-cell algae such as Chlorella and Scenedesmus were difficult to separate and concentrate and had thick cell walls that made human and animal digestion difficult, 2) for commercial food production very large culture systems requiring relatively sophisticated and costly engineering design, construction, and operation were required, and 3) land-based agriculture in the U.S. was highly successful in the 1950's, and there was little interest in and virtually no need for single-cell protein.

To counter this pessimism, W.J. Oswald at the University of California, Berkeley, revolutionized the approach to mass algal culture technology in the 1950's and 60's by demonstrating that large-scale algal cultures could simultaneously be used for protein production and wastewater treatment. The basic scheme involved using domestic wastewater as the nutrient source for freshwater algal growth. The algae in turn produced oxygen for bacterial breakdown of organic matter in the wastewater and, to complete the symbiotic cycle, utilized the carbon dioxide produced by the bacteria (Figure 4.2). The algal-bacterial biomass produced represented a source of raw protein, which when harvested, not only had food value, but also resulted in a biologically treated wastewater low in BOD and dissolved nutrients such as nitrogen and phosphorus. Oswald's group at Berkeley performed numerous experiments, growing algae on wastewater from the laboratory scale (with elaborate continuous culture devices far more sophisticated than Cook's earlier system (Figure 4.3), to small-scale outdoor pilot plant systems (Oswald et al., 1953; 1957; Gotaas and Oswald, 1958). In the early 1960's a 10^6 liter capacity (2700 m^2 surface area) outdoor mass culture system was put into operation in Richmond, California. At that time this was the largest outdoor culture system in the U.S. Projected algal yields were about $12 - 18 \text{ g/m}^2/\text{day}$ (Oswald, 1969; 1970). The pond design was unique in that it consisted of a series of parallel meandering channels connected in a racetrack type configuration (Figure 4.4). Mixing was accomplished by pumping the culture to maintain a desired velocity in the channels.

During this era, Oswald's research activities represented the sole U.S. mass culturing effort, and several large-scale algal wastewater treatment ponds were put into operation in Northern California, modelled to a large extent after the Richmond system (Oswald, 1975). Algal harvesting for protein production, however, was not included in these schemes.

An outgrowth of Oswald's research activities was the demonstration that algal systems had other important functions besides wastewater treatment and protein production. During the mid-1960's interest in bio-regenerative

Figure 4.2

Cycle of Controlled Photosynthesis in Organic Wastes (from Oswald, 1970)

Figure 4.3

Automatic Growth Unit for Producing Continuous Cultures of Algae and Bacteria in Liquid Organic Wastes under Controlled Environmental Conditions (from Oswald, 1970)

Figure 4.4
Photograph of 10^6 Liter Outdoor Algal Mass Culture System at Richmond, California

life support systems for possible use in space-craft was generated and Oswald and others showed that algal systems could effectively be used in this capacity (Golueke et al., 1959; Oswald and Golueke, 1965; Miller and Ward, 1966; Shelef et al., 1968b); however, problems of reliability limited their application and scheme gave way to the more dependable physical-chemical systems used in the Apollo Moon Program during the late 1960's - early 1970's.

During this same period, Oswald and co-workers demonstrated that algae could be fermented to methane with conversion efficiencies of 50 - 70% (Golueke and Oswald, 1959; Golueke, et al., 1957). Based on these results, they conceptualized a scheme (Figure 4.5a) whereby solar bioconversion to useful energy could be achieved with algal systems (Golueke and Oswald, 1963; Oswald and Golueke, 1960). The concept of bioconversion of solar energy through algal mass culturing was not really a new idea at that time, as Burlew (1953) in the preface to the Carnegie Report suggested that energy stored in mass cultured algal cells was greater than the energy required in growing and harvesting the plants. This optimism was shared by Meier (1955), along with Oswald and Golueke, and he devised additional bioconversion schemes (Figures 4.5b and 4.5c). Their main attraction was that materials were conserved by recycling while photosynthetic conversion of radiant energy was being carried out.

Between 1967 - 1969, a major research program was conducted jointly by several State of California and U.S. Government agencies to investigate the feasibility of removing nitrate-nitrogen from agricultural tile drainage in the San Joaquin Valley of Central California by mass culturing and harvesting freshwater algae. The research facilities included a 1000 m² algal pond, similar in design to Oswald's system, and 22 small "mini" ponds, each 2.6 m² in surface area. Yields of Scenedesmus over 30 g/m²/day for short (10 day) periods and average long-term yields of 10 g/m²/day (70 days) were achieved in the large pond (Beck et al., 1969). The high yields were attained by supplementing the drainage water with phosphorus, iron, and carbon dioxide, forcing light to become growth limiting (Brown, 1971). As part of this study, a plan was envisaged whereby 7 x 10⁶ m² of land area in the San

Figure 4.5a Schematic Diagram of System for the Biological Conversion of Solar Energy to Electrical Power (from Oswald and Golueke, 1960)

Figure 4.5b A Biological Cycle for Power Production (from Meier, 1955)

Figure 4.5c Direct Process for Converting Algae to Fuel (from Meier, 1955)

Figure 4.5

Several Alternative Processes for Converting Radiant Energy to Useful Energy Via Bioconversion

Joaquin Valley, originally designated as a storage reservoir, would be converted into a modular algal pond system to treat the entire estimated 1.1×10^9 liters/day of tile drainage (Goldman et al., 1969). This system would have been the largest mass culture system in the world, but the plan was never realized.

All U.S. mass culture research on microalgae had been restricted to freshwater algae until J.H. Ryther at the Woods Hole Oceanographic Institution started work in 1971 on a marine counterpart to Oswald's freshwater system. The overall objective was to channel nutrients in wastewater through the marine food chain by growing and harvesting commercially important marine animals such as oysters, clams and lobsters (Ryther et al., 1972). The first step in the scheme involved mass culturing marine phytoplankton on wastewater-seawater mixtures and then feeding the algal cultures directly to shellfish, thus avoiding the need for the algal separation and concentration steps required with the freshwater culture systems. Experiments with continuous cultures ranged in scope from small laboratory studies to screen marine species for their ability to grow on wastewater-seawater mixtures (Goldman and Stanley, 1974), to small-scale outdoor experiments in two 2000 liter (4 m^2 surface area) circular ponds (Figure 4.6a) in both Woods Hole, Massachusetts, and Fort Pierce, Florida (Goldman and Ryther, 1975; Goldman et al., 1975), to large scale outdoor experiments in six 35,000 liter (150 m^2) ponds (Figure 4.6b) at Woods Hole (D'Elia et al., in press; Goldman and Ryther, 1976). Yields in long-term experiments (6 - 8 months) in which several marine diatom species were the dominant algae, ranged from 12 - 23 $\text{g/m}^2/\text{day}$ in the small experimental ponds that were intensively mixed to 6 - 10 $\text{g/m}^2/\text{day}$ in the larger more gently mixed units. These large ponds were each 15 m^2 square and 1 m deep, consisting of an earthen and sand base and covered with a plastic liner. Gentle mixing in each pond was accomplished by recirculation with two small pumps. The simplicity in design was a departure from the more sophisticated design of Oswald's system, and yields were less than those achieved in the smaller, more intensively mixed ponds, suggesting that in very large systems mixing problems could result in greatly reduced algal production.

Figure 4.6a Small Circular Units with Strong Mixing

Figure 4.6b Large Ponds with Gentle Mixing

Figure 4.6

Photographs of Algal Mass Culture Systems
Used in Woods Hole, Massachusetts

Similar experiments have been conducted in St. Croix, Virgin Islands by O. Roels since 1972, using deep ocean water, artificially upwelled from 870 m, to grow marine phytoplankton in two 45,000 liter and six 2,000 liter (2.4 m^2) rectangular concrete-lined ponds (Roels et al., 1976). Because the upwelled water contained only 30 - 35 $\mu\text{g-atom/liter}$ of inorganic nitrogen, productivity could never be made light limiting, hence yields in short-term experiments (<40 days) never exceeded $5 \text{ g/m}^2/\text{day}$. In addition, serious problems were encountered in trying to maintain continuous cultures for more than 1 - 2 week periods.

More recently, W.J. Oswald carried out mass culture experiments with freshwater blue-green algae, which, because of their filamentous structure, were considerably easier to separate than the unicellular green algae which typically dominate in outdoor mass cultures. The main thrust of this work has been aimed at developing the technology for species control in large systems so that the slower growing blue-greens could compete with the faster growing green algae (Benemann et al., 1977). One technique tried, the continuous recycling of a portion of easily harvested blue-green algae, was only moderately successful. To date, the problem of species control in large-scale outdoor cultures has not been adequately solved.

Japan

Japanese interest in mass algal culture dates back to the post World War II era, which more than likely stems from the Southeast Asian tradition of harvesting naturally growing and cultivated seaweeds for human consumption (Tamiya, 1959). Without question, the early Japanese effort, particularly the research conducted at the Tokugawa Institute for Biological Research and the Institute of Applied Microbiology, University of Tokyo, on the bioengineering aspects of large-scale Chlorella culturing is exemplary.

In the first outdoor experiments, conducted during the winter of 1953 in concrete-lined 15 m^2 troughs, yields were only $3.5 \text{ g/m}^2/\text{day}$ (Mituya et al., 1953). Within several years, however, average summer yields were up

to 16 - 17 g/m²/day and peak production was as high as 28 g/m²/day for several-day periods (Morimura et al., 1955). A major emphasis of the early Japanese research was to optimize pond configurations and mixing capabilities. Two basic designs evolved out of this work: the outdoor "bubbling culture" and the "open circulation" method. The outdoor "bubbling culture" consisted of long troughs either flat-bottomed (Figure 4.7a) or zig-zag-bottomed (Figure 4.7b) in which aeration pipes for bubbling in CO₂-enriched air were placed longitudinally in the troughs (Morimura et al., 1955). The "open circulation" method (Kanazawa, et al., 1958) consisted of a circular shallow tank with two radial arms on a center support by which the pond culture was circulated when the contents were pumped through small spigots in the arms, causing a jet-type rotating action (Figure 4.7c). Watanabe et al. (1959) used the "open bubbling" system to mass culture nitrogen-fixing blue-green algae with the ultimate goal of using the harvested biomass as a fertilizer in rice fields. Yields of 6.4 g/m²/day in 5 m² culture units were attained.

During the late 1950's a 3200 m² Chlorella culturing plant, probably the largest in the world at that time, was put into operation by the Japan Nutrition Association for the expressed purpose of testing the concept on an industrial scale (Tamiya, 1959). The plant was based on the design shown in Figure 4.8c, and consisted of a number of ponds, the largest being 314 m². The Microalgae Research Institute at Kunitachimachi was formed during this period with a total outdoor culture facility of over 4000 m². Peak average monthly yields were over 4 g/m²/day during summer months, but decreased to under 1 g/m²/day during the winter (Krauss, 1962).

During the 1960's the emphasis in algal mass culturing switched from the research to the commercial level as a large health food market for Chlorella was developed in Japan. Hence, much of the later Japanese research in this area became proprietary and little information is available. As of 1976, it was estimated that the yearly production of Chlorella in Japan, involving 10 commercial companies, was over 350 m tons. Yearly average

Figure 4.7a Schematic Diagram of the Flat-Bottomed Culture Pond
(from Morimura et al., 1955)

Figure 4.7b Schematic Diagram of the Zigzag-Bottomed Pond
(from Morimura et al., 1955)

Figure 4.7c Schematized Cross-Section of the Open Circulation Method
(from Kanazawa et al., 1958)

Figure 4.7

Diagrams of Algal Culture Schemes
Developed in Japan During 1950's

production values varied from 8.5 to 21 g/m²/day (Tsukada et al., 1977). However, the cost of producing this biomass is exorbitantly high, ca. \$5.4 - 10.8/kg. Although Chlorella cannot compete with more conventional sources of protein in Japan, the current retail price in health food stores is \$100/kg, so that a highly profitable, but limited, industry exists.

Taiwan

Mass outdoor cultures appear to be in extensive use in Taiwan for the commercial production of Chlorella. It was estimated that ~800 m tons were produced in 1976, a good fraction being exported to Japan for sale as a health food (Tsukada et al., 1977). Reported average yields are as high as 22 g/m²/day in culture units comprising a total of up to 16,200 m². Each pond is apparently modelled to a large extent after the Japanese open culture technique. In 1977, a massive Chlorella culturing plant, comprising a total of 180,000 m² of concrete culture ponds, each 200 - 500 m², and fashioned to some degree after Oswald's meandering channel design (Figure 4.8) was constructed and put into operation (Shurtleff, 1977). Unfortunately, as in Japan, virtually all the mass culture operations in Taiwan are proprietary and little detailed information is available.

Germany

German interest in algal mass culturing dates back to World War II, when experiments were carried out to determine if fat production from algae was possible (Harder and von Witsch, 1942a, b). Mass culture work with Chlorella was continued after the war in small-scale outdoor and greenhouse systems (Gummert et al., 1953) and with Scenedesmus grown on wastewater (Meffert, 1958).

It was not until research began at the Kohlenbiologische Forschungsstation at Dortmund in the mid-1950's, however, that German interest in mass algal culture became serious. Work has been carried on continuously since then in a very elaborate and sophisticated outdoor research facility,

Figure 4.8
Photograph of Large-scale Chlorella Culture Units Used in Taiwan

consisting of four 80 m² ponds, each formed in a raceway-type shape with paddle wheels for mixing (Figure 4.9a). In addition, two 200 m² circular ponds (Figure 4.9b), similar in design to the Japanese open circulation system shown in Figure 4.9c, were used in the earlier experiments. The main objectives of the research were to develop the technology for mass culturing algae for protein production. The cultures were grown on defined artificial media and average yields of the freshwater green algae Scenedesmus and Coelastrum were typically 10 g/m²/day and as high as 30 g/m²/day for short periods during summer months in a geographical location not particularly well-suited for mass algal culture (Soeder, 1976; Stengel, 1970; Stengel and Soeder, 1975).

Because Germany is a poor location for year-round mass culturing of algae, the Dortmund group, in cooperation with local foreign governments, set up algal research stations in Bangkok, Thailand, Peru and India. The Bangkok research station was particularly elaborate, consisting of seven 87 m² and three 25 m² raceway-type ponds similar to the Dortmund ponds. Reported maximum yields have been as high as 35 g/m²/day for Scenedesmus and 20 g/m²/day for Spirulina, although scant technical data are available (Soeder, 1976).

In 1977, the entire algal research effort at Dortmund and at the supporting foreign stations will come to a close since the German government made the decision to stop funding algal research because the results were not encouraging enough to warrant further support.

Czechoslovakia

Mass culture work was begun in 1960, when the Laboratory for Algal Culture was established in Trebon as part of the Institute of Microbiology, Czechoslovak Academy of Sciences. In a radical departure from mass culture pond design of that era (i.e., the Japanese and U.S. designs), the Czechs attempted to maximize algal yields by developing thin, extremely well mixed culture systems that were designed for efficient utilization of available solar radiation.

Figure 4.9a Raceway-type Ponds

Figure 4.9b Circular Pond

Figure 4.9

Photographs of Algal Culture Units Used in Dortmund, Germany

The first unit, built in 1960, was a tilted platform, 12 m^2 in total surface area, comprised of a series of troughs, each slightly elevated above the other. The algal culture was continually recirculated to the highest trough from the lowest by pumping so that the culture cascaded by gravity in essentially a thin sheet of liquid down the troughs (Figure 4.10). Studies with Scenedesmus were carried out during 1960-62 with these units in two locations, Trebon and Kosice, and average (summer?) yields were as high as $15 \text{ g/m}^2/\text{day}$ (Necas and Lhotsky, 1967).

By the end of 1963, two 50 m^2 units and one 900 m^2 unit were constructed employing the same concept of a tilted surface, in this case made of glass sheets, with glass baffles for strong mixing, (Figure 4.11). These units were operated during the spring-fall months between 1963 and 1970. In addition, the 50 m^2 units were operated in Tylicz, Poland in 1966, and in Rupite, Rumania in 1968. Yields varied from $16 \text{ g/m}^2/\text{day}$ (average) to $25 \text{ g/m}^2/\text{day}$ (maximum) in the 50 m^2 units in Trebon and $23 \text{ g/m}^2/\text{day}$ (average) to $30 \text{ g/m}^2/\text{day}$ in Rupite. Yields in the 900 m^2 units in Trebon were somewhat lower: $12 \text{ g/m}^2/\text{day}$ (average) to $19 \text{ g/m}^2/\text{day}$ (maximum) (Vendlova, 1969), indicating, as in the Woods Hole studies, that in a given location yields tend to decrease as the size of the culture unit increases.

Setlik et al. (1970) designed a 4500 m^2 version of the cascading system, but the unit was never built. Virtually no information beyond that found in the Laboratory's 1970 Annual Report (Necas and Lhotsky, 1973) is available and there have been unconfirmed reports (Soeder, 1977) that mass culture activity in Czechoslovakia is now at a standstill.

Israel

Israeli interest in mass algal culture began in the early 1950's when Mayer and his group at the Hebrew University conducted, first small-scale outdoor experiments with Chlorella grown in flasks (Evenari et al., 1953), and then experiments with a 2000 liter deep tank having a transparent side facing

- Cascade cultivation unit with 12 m² cultivation surface. 1- troughs made of glass fibre reinforced polyester; 2-drainage trough; 3-auxiliary tank which can store the whole volume of the suspension, when the unit is not operating; 4-suction pipeline; 5-centrifugal circulation pump, discharge 8 l.sec⁻¹ under the given conditions of operation; 6-discharge piping, main circulation line; 7-outlet overflow of the main circulation line; 8-discharge piping, by-pass line; 9-gas contactor for CO₂ absorption; 10-valve controlling flow in the night circulation² circuit; 11-night circulation pipeline; 12-supporting steel structure. Similar units of modified character were obtained using polyester troughs of different shape and other arrangements of the troughs on the supporting structure.

Figure 4.10

First Czechoslovakian Outdoor Mass Culture Unit Built in 1960
(from Necas and Lhotsky, 1967)

Figure 4.11
 Large-scale (900 m²) Outdoor Cultivation Unit Used in Trebon, Czechoslovakia
 (from Necas and Lhotsky, 1967)

the sun for better light absorption (Mayer et al., 1958, 1964). Considerable effort was directed at maximizing CO₂ addition, mixing, and light utilization, and yields varied from short-term highs of over 20 g/m²/day to average yields of 10 g/m²/day.

These studies, using artificial media, were abandoned in the mid 1960's and no further mass culture work was attempted in Israel until G. Shelef, also at the Hebrew University, built a 300 m² outdoor pond in Jerusalem, modelled after Oswald's design (Figure 4.12), and conducted experiments with wastewater-grown algae (Shelef et al., 1973). The main objective of the research was to treat raw wastewater for the removal of organics and nutrients. Summer yields of 60 g/m²/day of organic matter were reported, but no attempt was made to separate out the non-algal organics that were present in the raw wastewater. However, Shelef made the first attempt to optimize production by showing that there is a bell-shaped response in yield to varying dilution (or flow) rate, results similar to that shown in Figures 3.5 and 3.6. Hence there is a specific range of dilution rates for which yield is at a maximum.

Shelef continued his mass culture work at the Technion, Israel Institute of Technology, under a grant from the German government, and in close collaboration with the Dortmund algal laboratory. Two other mass culture projects in Israel were also funded by the German government at this time: one was conducted at the Institute for Desert Research where an attempt was made to mass culture the blue-green alga Spirulina on subsurface brackish water in the desert of southern Israel, and the other project was conducted at the Hebrew University to investigate certain microbiological and bioengineering problems.

The largest of the three projects was that of Shelef's group which focused on using wastewater as a nutrient source for mass algal culture with three concurrent objectives in mind: wastewater treatment, single-cell protein production, and water reclamation for irrigation purposes. The outdoor experimental station at the Technion consisted of thirty 110 liter "miniponds" (0.34 m² in area and 35 cm deep), six 200 liter ponds (0.9 m² in area and

Figure 4.12

Plan View of 300 m² Algal Pond Used in Israeli Studies with Raw Wastewater
(from Shelef et al., 1973)

23 cm deep), and two large ponds, one 120 m² in area and the other 150 m² in area. In addition, two 1,000 m² ponds have been in operation at Kibbutz Yagur, one of the largest collective farms in Israel. All the ponds were designed in a configuration of meandering channels, similar to Oswald's basic design, and were fed raw wastewater (Figure 4.13a,b). Algal yields up to 35 g/m²/day of ash-free dry weight were attained during summer months in both the miniponds and the larger Technion ponds for several dominating green freshwater species: Oocystis solitaria, Phytoconis sp., Scenedesmus dimorphus, and Micractinium quadrisetum (Shelef, 1976). Much research emphasis appeared to be geared towards the bioengineering aspects of algal growth such as types and degree of mixing, pond design, aeration, etc.

Two large-scale algal wastewater treatment facilities are currently in operation in Israel. The Dan Region system consists of a series of 14 conventional oxidation ponds comprising a total area of 134 hectares (1.34 x 10⁶ m²), which treats the wastewater from a large section of greater Tel Aviv. In the future, a portion of the system may be converted to high rate algal ponds, similar to the ones operated at the Technion. In addition, the City of Elat, on the Gulf of Elat is serviced by a 20 hectare high rate pond system.

Research began in the Spring of 1974 at the Beersheba campus of the Institute for Desert Research and experiments as of 1976 were restricted to six 1 m² miniponds. The research program was later moved to the Sede Boqer campus in the Negev and the six ponds were put back into operation. Two additional 100 m² ponds were built and are currently being used in outdoor experiments. The ponds have a raceway-type shape, broken at one end where the culture is pumped from one pond to the other with the aid of air lift pumps. A portion of this liquid represents the overflow and is passed over gravity-type screen filters, for algal separation. The ponds themselves are dirt filled and lined with a black polyethylene material. The best reported yields were 10 - 15 g dry wt/m²/day in the 1 m² ponds containing Chlorella, which displaced Spirulina outdoors in the autumn and winter months (Richmond, 1976).

Figure 4.13a 150 m² Pond at the Technion

Figure 4.13b 1000 m² Pond at Kibbutz Yagur

Figure 4.13

Photographs of Algal Culture Units Used In
Israel Institute of Technology Experiments

At the Hebrew University, it has been discovered that upwards of 20 - 30% of the gross algal productivity in high rate ponds fed raw wastewater results from algal heterotrophy. Wastewater-borne organic matter provides the carbon source for this growth (Weisman and Abeliovich, 1976). An entirely separate project, funded with industrial backing, involves mass culturing an unusual species of the marine unicellular green alga genus Dunaliella in salt brines up to 5 M in total salinity. At very high salt concentrations the glycerol content of the organism increases to maintain an osmotic balance and under optimal conditions glycerol constitutes up to 85% of the cells' dry weight. Laboratory and small-scale outdoor experiments were encouraging (Ben-Amotz and Avron, 1973), and two large-scale ponds similar in design to the Desert Institute's ponds were constructed in Elat to test the concept on a larger scale. Because the project is being funded by industry, the work now being carried out is proprietary in nature. The project is to continue through 1979 before a decision will be reached on the commercial potential of the process (Ben-Amotz, 1977).

France

French efforts in mass algal culture have been restricted to the work of the French Petroleum Institute, which for years has been conducting research on a blue-green alga Spirulina. This species is of special interest because it has an unusually high protein content (up to 70%), a wide array of pigments which have use as coloring agents, is easily digested by animals and humans, and does not appear to build up toxic chemicals. Currently, the Institute is collaborating with several countries on the mass culture of Spirulina (Clement, 1977). In Algeria at Oran and in southern France at Antibes small culture units between 5 and 700 m² in surface area have been constructed. The design is one developed by the Institute (Figure 4.14), and consists of two horizontal and adjacent channels, 10 - 20 cm in depth connected to deeper interconnecting troughs at each end. Carbon dioxide mixtures are introduced at diagonally opposing troughs so that the gas flow causes liquid to be lifted up to the channel where it flows by gravity to the

Figure 4.14

Schematic View of the French Petroleum Institute Algal Pond System
(from Clement, 1975a)

opposing trough and then back again in the adjacent channel. Circulation and CO₂ addition are continuous with the main energy input being the gas addition. The channels are plastic-lined and the troughs are made of concrete. No experimental data are available.

In addition to these operating systems, several other systems are being constructed or are in early stages of operation: a 2000 m² system in Cairo, Egypt, a 5000 m² unit on an island off of Taiwan (in collaboration with the Japanese), and a small 100 m² system in Martinique in the West Indies. All of these systems appear to be geared towards the production of pigments extracted from Spirulina for use as coloring agents. Clement (1975a, b) has reviewed the uses of Spirulina in detail.

Along with these studies, the French Petroleum Institute has collaborated with Sosa Texcoco, S.A. of Mexico in developing a viable Spirulina mass culture industry adjacent to Lake Texcoco, near Mexico City, where, because of a unique set of environmental conditions, a natural population of Spirulina grows. A 3200 meter diameter solar evaporation lake (850 hectares) is used to concentrate alkaline brines so that soda ash and caustic soda can be extracted and processed. In the outer ring of this lake a virtual monoculture of Spirulina grows about 10 - 11 months out of the year. In 1967 a harvesting and processing plant was built, and nitrate fertilizer was added to about a 20 hectare area to promote increased productivity. The other essential nutrients are apparently supplied with the concentrated brine. As of 1977, about 2 m tons dry wt/day of Spirulina were harvested and dried through centrifugation and drum drying. This value corresponds to an areal production of about 10 g dry wt/m²/day. By 1978 50 hectares will be harvested, leading to a 5 m ton/day production level. A new plant is under construction to handle this increased production. Currently, the entire 2 m tons/day of Spirulina are sold to the Japanese at \$5/kg, apparently to be used as a coloring agent to impart color to chickens, eggs, tropical fish and other products (Durand-Chastel, 1977).

The culture ponds themselves are primitive by current standards, being about 1 m deep with virtually no mixing (Figure 4.15). Improved pond design could probably lead to much higher productivity. It is important to stress that this system is unique because of the chemical environment developed by the solar evaporation process and could not be readily duplicated in very many other areas of the world.

USSR

Reports in English of Russian activities in algal mass culturing are very scant. However, based upon a review of Russian algal research in an American algal symposium (Gromov, 1968) and a Library of Congress literature search (Dodge, 1976), mass culturing activities in the USSR seem to be extensive, to the point where commercial applications such as the feeding of Chlorella to livestock and poultry are practiced.

Reported yields of Chlorella in outdoor culture systems (sizes unknown) were between 10 - 20 g/m²/day in the earliest studies in Leningrad during summer months (Gromov, 1968). Other mass culture facilities were located near Moscow at the Viliams Institute, in Middle Asia near Tashkent and in Tadjikistan. Average yields during April - October in a 10,000 m² pond were estimated to be ~15 - 25 g/m²/day (Dodge, 1976), although it was not clear as to whether this was a hypothetical estimate or was based on real data in an existing facility.

Gromov and Pinevich (1972) did report data on the outdoor cultivation of the blue-green algae Anabaena variabilis and Spirulina platenis in their Leningrad outdoor facility. With artificially increased temperatures (up to 35°C) yields in July varied from 4.3 - 7.0 g/m²/day for Anabaena to 9.1 - 20.0 g/m²/day for Spirulina.

Netherlands

Small-scale outdoor experiments in 1 m² concrete tanks were conducted in 1951 in Wageningen, the Netherlands, and average spring-fall yields of Chlorella were 7.2 g/m²/day (Wassink et al., 1953), although peak yields

Figure 4.15

Solar Evaporation Pond of Sosa Texcoco S.A. Where Mass Culture of Spirulina Occurs

of $13 \text{ g/m}^2/\text{day}$ were attained for short periods during June - July (Van Oorschot, 1955). Little additional work has been conducted in the Netherlands except that of Wesselius (1973), who used converted washing machines to examine the effects of external factors such as light, temperature, and mixing on the productivity of Scenedesmus.

4.3 Summary of Microalgal Systems

Advances in the technology of the mass culture of microalgae appear to be enormous, as evidenced by the great improvements in yields attained over the past 25 years (Table 4.1). From the modest beginnings in 1951 of Arthur D. Little, Inc. on the roof of a building in Cambridge, Massachusetts, the mass cultivation of microalgae has spread to virtually all corners of the world, and now in both Mexico and the Far East thriving algal culturing enterprises exist.

In Table 4.1 the maximum attainable yields, regardless of location and species seem to be about $30 - 35 \text{ g/m}^2/\text{day}$ of ash-free dry weight. However these peak yields were attained for short (<30 day) periods usually during the summer months. Average yields for longer periods were considerably lower, reflecting varying conditions of solar radiation, temperature and other environmental factors. The year-round yields attained in Taiwan and Japan during 1976 ($21 - 22 \text{ g/m}^2/\text{day}$) in commercial ventures seem to be unusually high, but because virtually no technical data is available from these operations, the validity of those yields must be questioned.

Besides the Mexican Spirulina plant ($200,000 \text{ m}^2$) and the newly constructed Chlorella facility in Taiwan ($180,000 \text{ m}^2$), there is virtually no information on the scaling-up problems that might be encountered in constructing very large algal culture systems, as might be required for energy bioconversion plants (Goldman and Ryther, 1977). The Mexican plant is a unique facility, not reproducible in other areas of the world and the Japanese

Table 4.1
SUMMARY OF WORLD-WIDE PROGRAMS FOR MASS CULTURING OF MICROALGAE

No.	Year	Location	Species	Culture System		Best Yields (Duration) g dry wt/m ² /day (days)		Reference
				Size Unit (Total)	Operation ^a	Maximum	Average	
1	1951	Cambridge, Mass.	<u>Chlorella</u>	56	N, F, SC	11 (10)	2 (52)	Burlew (1953)
2	1951	Essen, Germany	<u>Chlorella</u>	6	N, F, SC	?	4 (~30)	Gummert <i>et al.</i> (1953)
3	1953	Tokyo, Japan	<u>Chlorella</u>	15	N, F, SC	?	3.5 (10)	Mituya <i>et al.</i> (1953)
4	1954-55	Tokyo, Japan	<u>Chlorella</u>	2.8-5.5 (13.8)	N, F, SC	28 (3)	16 (27)	Morimura <i>et al.</i> (1955)
5	1957	Tokyo, Japan	<u>Chlorella</u>	7.1-78.5 (147.8)	N, F, SC	14 (25)	8.6 (365)	Kamazawa <i>et al.</i> (1958)
6	1957	Tokyo, Japan	<u>Scenedesmus</u>			14 (31)		
7	1958	Tokyo, Japan	<u>Tolypothrix</u>	5	N, F, SC	?	6.4 (?)	Watanabe <i>et al.</i> (1959)
8	1958	Tokyo, Japan	<u>Chlorella</u>	314 (3,200)	N, F, SC	?	?	Tamiya (1959)
9	1959-60	Plymouth, England	<u>Phaeodactylum</u>	2.6 (15.6)	N, M, SC	-	~10 (?)	Ansell <i>et al.</i> (1963)
10	1961	Richmond, California	Green Algae	2700	W, F, C	?	?	Oswald (1969)
11	1962	Jerusalem, Israel	<u>Chlorella</u>	4	N, F, SC	16 (27)	12 (35)	Mayer <i>et al.</i> (1964)
12	1960-77	Dortmund, Germany	<u>Scenedesmus</u>	80 (320)	N, F, SC	28 (?)	10 (?)	Soeder (1976)
13	1960-62	Trebon, Czechoslovakia	<u>Scenedesmus</u>	12	N, F, SC	?	15 (?)	Necas and Lhotsky (1967)
14	1967	Trebon, Czechoslovakia	<u>Scenedesmus</u>	50	N, F, SC	25 (10)	16 (65)	Vendlova (1969)
15	1966	Trebon, Czechoslovakia	<u>Scenedesmus</u>	900	N, F, SC	19 (10)	12 (89)	Vendlova (1969)
16	1966	Tylicz, Poland	<u>Scenedesmus</u>	50	N, F, SC	16 (10)	12 (71)	Vendlova (1969)
17	1968	Rupite, Romania	<u>Scenedesmus</u>	50	N, F, SC	30 (10)	23 (62)	Vendlova (1969)
18	1969	Firebaugh, California	<u>Scenedesmus</u>	1000	TD, F, C	35 (10)	10 (70)	Beck <i>et al.</i> (1969)
19	1970-71	Jerusalem, Israel	<u>Chlorella</u>	300	W, F, C	60 (30) ^b	27 (30) ^b	Shelif <i>et al.</i> (1973)
20	1973	Woods Hole, Mass.	Diatoms	4 (8)	WSW, M, C	13 (15)	-	Goldman <i>et al.</i> (1975)
21	1974	Fort Pierce, Florida	Diatoms	4 (8)	WSW, M, C	25 (15)	-	Goldman <i>et al.</i> (1975)
22	1975-76	Woods Hole, Mass.	Diatoms	180 (1,080)	WSW, M, C	10 (7)	-	D'Elia <i>et al.</i> (in press)
23	1967-77	Mexico City, Mexico	<u>Spirulina</u>	?	B, F, SC	20 (?)	10 (?)	Durand-Chastel (1977)
24	1976	Haifa, Israel	Green Algae	120 (270)	W, F, SC	35 (30)	15 (365)	Shelif (1976)
25	1976	Yagur, Israel	Green Algae	1000 (2,000)	W, F, SC	?	?	Shelif (1976)
26	1974	Bangkok, Thailand	<u>Scenedesmus</u>	87 (609)	N, F, SC	35 (?)	15 (?)	Soeder (1976)
27	1974	Bangkok, Thailand	<u>Spirulina</u>	87 (609)	N, F, SC	18 (?)	15 (?)	Soeder (1976)
28	1976	Japan	<u>Chlorella</u>	?	N, F, SC	?	21 (365)	Tsukaja <i>et al.</i> (1977)
29	1976	Taiwan	<u>Chlorella</u>	?	N, F, SC	?	22 (365)	Tsukada <i>et al.</i> (1977)
30	1977	Taiwan	<u>Chlorella</u>	250-500 (180,000)	N, F, SC	35 (7)	15-18(365)	Shurtleff (1977)
31	1976	Richmond, California	<u>Micractinium</u>	3 (12)	W, F, SC	12.7 (12)	-	Benemann <i>et al.</i> (1977)
32	1977	Richmond, California	<u>Micractinium</u>	12 (48)	W, F, SC	32 (30)	-	Benemann <i>et al.</i> (1978)
33	1977	Richmond, California	<u>Micractinium</u>	2700	W, F, SC	11.7 (31)	9.1 (77)	Benemann <i>et al.</i> (1978)

^aKey: N = artificial nutrients; W = wastewater; WSW = wastewater-seawater mixture; B = brine; TD = agricultural tile drainage; F = freshwater algae; M = marine algae; SC = semi-continuous harvest; C = continuous harvest.

^bIncluded non-algal solids from wastewater.

and Taiwanese commercial ventures are very elaborate and expensive systems which are geared towards a unique commercial application of limited size. Clearly, the application and value of the resulting products will dictate the size and degree of sophistication of algal culture systems.

5.1.1 Harvesting of Natural Populations

Because of the wide variation in size, type, habitat, location and abundance of red and brown macrophytes, it is not surprising that the harvesting of natural populations has not been mechanized in most areas. The red algae Gelidium, for example, is gathered by hand between the tides when it grows in the intertidal zone, by means of long-handled rakes in shallow waters, and by divers in deeper subtidal regions. (Naylor, 1976). In Japan, a major producer of Gelidium, the majority of the harvest is obtained by women snorklers, who can collect up to 300 kg dry wt/day (about 10,500 kg wet wt/day). In California, Gelidium is harvested by scuba divers who cut the fronds manually and pump them through a tube to a plastic bag anchored underwater. The bag is picked up by boats from the abalone industry for drying and baling. (Silverthorne, 1977). Similar harvesting methods and rates have been described for other species, such as Gracilaria in Chile (Cable, 1974) and in Australia (Chapman, 1970); for Chondrus crispus in the United States and in parts of western Europe (Naylor, 1976) and in the Maritime Provinces of Canada (MacFarlane, 1964); for Eucheuma (Naylor, 1976); for Hypnea (Naylor, 1976); and for Iridaea (Tseng, 1947). The red seaweed Furcellaria, which lives a pelagic existence in the subtidal waters of Denmark, is harvested by trawlers using dragnets and yields up to 91 m tons wet wt/trawler/day have been reported (Naylor, 1976). Manual methods of harvesting have proved to be profitable in developing nations where unemployment is high, but in the case of many of the major seaweed producers of the northern hemisphere, these labor-intensive methods are becoming increasingly expensive.

Mechanical harvesters have been used for about 60 years in California where the giant kelp Macrocystis grows in large beds, up to a mile wide and several miles long. Specially built, power-driven barges equipped with reciprocating underwater mowers at the stern (Booth, 1969), can harvest up to 113 m tons of kelp in four to five hours with a crew of four men (Druehl, 1972). The kelp beds are leased by the State of California and are controlled by licensing and/or royalty arrangements. In an effort to protect the natural population from over-exploitation, regulations require

that the harvester cut the kelp no deeper than 4 ft (about 1.25 m) below the water's surface, permitting about three harvests/year. (Druel, 1972; Naylor, 1976; Glube, 1977) (See Scofield, 1959 for a detailed historical review of kelp harvesting.) Similar cropping vessels are in use, or being considered for use, in harvesting the other giant kelp Nereocystis in Canada, Alaska, South America, New Zealand and Tasmania (Naylor, 1976) and the smaller brown seaweed Ascophyllum in the shallow intertidal waters of Nova Scotia (Booth, 1969). The brown seaweed Laminaria which grows only on hard, rocky ocean bottoms down to depths of 25 m, has been harvested using two mechanized models, one using reciprocating cutters mounted on dredges, and the other a system of continuous grapnels, although neither method has been particularly successful (Naylor, 1976).

5.1.2 Seaweed Culture in Oriental Countries

Porphyra Culture

The first seaweed to be cultivated was the red alga Porphyra (nori) in Japan in the seventeenth century. The culture methods used at that time involved simply placing sticks in sea water and after monospores of Porphyra had settled on them, moving the sticks to nutrient-rich estuarine waters, where the monospores gave rise to thalli. The thalli would grow for about 15 days and then be harvested by cutting. This original culture method is now obsolete and almost extinct in Japan, but still practiced in Korea.

In the seventeenth century, the primitive culturists did not understand the origin of monospores and it was not until 1949 when the English botanist, K.M. Drew (1949), discovered the missing links in the life history of Porphyra (refer to Figure 2.6b), that culture methods became more sophisticated. Today about 70% of the commercial production of nori in Japan is based on the artificial production of monospores under controlled conditions in hatcheries (Bardach et al., 1972).

Artificial seeding is carried out by placing chopped sporophyte plants into indoor concrete tanks containing oyster shells which are either loose or strung on wires. Carpogonia and spermatia are released from the adult plants. Sexual fusion between the spermatium and the contents of the carpogonia then occurs, and the latter divides to form four carpospores. The carpospores settle on the oyster shells and give rise to the conchocellis plants which grow on the shells and eventually release monospores. The entire process is highly dependent on maintaining proper temperatures in the laboratory (see Bardach et al., 1972 for details).

At the beginning of each nori culture season, the monospores are collected on nets by immersing the nets into tanks. A tank 2.4 m x 1.8 m x 0.9 m deep will accommodate 250 strings of 10 oyster shells each and will supply about 125 small 18 m collecting nets with monospores. The nets which are made of palm fiber or synthetic twine 3 - 5 mm in diameter with 15 cm openings, are generally 1.2 m wide and from 18 m to 45 m long. These nets are suspended from poles and placed in nutrient-rich estuarine waters (Figures 5.1 and 5.2) where the monospores develop into thalli. During the growing season, the nets must be continuously weeded and the level of the bed regulated to control epiphytes and to compensate for changes in salinity and temperature in the estuary. The plants on a single net may be harvested three to four times, after which the net is replaced. After harvest, nori is washed in freshwater, chopped, formed into sheets with a wooden frame, dried in the sun, and marketed as "hoshinori". One 18 m culture net produces 1000 to 3000 sheets or 35 to 105 kg wet weight of hoshinori annually, which is the equivalent of 16.2 m tons/hectare.

Although Porphyra is cultivated extensively in Japan and also in Korea, supplies are inadequate to accommodate the needs of the East Asian people. The Japanese are therefore seeking new stocks of Porphyra in New Zealand and South America and the Taiwanese are exploring the coasts of California.

Figure 5.1

Porphyra (nori) Farming in Japan

Figure 5.2

Air View of Porphyra Farms in Japan

Enteromorpha and Monostroma Culture

Enteromorpha and Monostroma (aonori), the only green macrophytes commonly utilized by man as food, are often grown in Japan as a byproduct of Porphyra culture, so the culture methods are essentially the same. Monostroma commands the highest price of any seaweed in Japan and is sometimes the object of monoculture.

Undaria Culture

The history of Undaria or wakame culture is similar to that of Porphyra. Early methods for the culture of Undaria were primitive consisting of no more than providing a substrate at suitable sites for the attachment of young plants. In the late 1950's, however, a technique for artificial spore production was developed and today the majority of wakame sold comes from cultured plants. In the laboratory, strings of synthetic fiber are immersed in concrete tanks with fertile Undaria sporophytes and in the early summer spores which are released, adhere to the fiber. The strings are lashed to rectangular plastic, wooden or metal forms roughly 1 m x 0.3 m in size and stored in tanks until late autumn when the strings are cut into short pieces and the young plants are transferred to open-ocean bamboo rafts or long-lines. The wakame grows quickly in the cold winter waters and is harvested by cutting when it has reached about 1 m in length. (See Bardach et al., 1972 for details). One bamboo raft, 36.6 m x 1.8 m in size with hanging ropes of Undaria can produce an average of 1 m ton wet weight or about 112.5 kg dry weight of Undaria (Bardach et al., 1972) in the northern waters of Japan and about half of that in warmer districts (Suto, 1974). (See also Hasegawa and Kuwatani, 1974, Okazaki, 1971; Saito, 1975, 1976).

Laminaria and Eucheuma Culture

The brown alga Laminaria is cultured in China as "haidai" and in Japan as "kombu". Like the other brown algae, the life history of Laminaria (Fig. 2.14) involves an alternation of generations, and hence complicates the

culturing process. The "dark dry" technique is often employed by the Chinese to stimulate discharge of large numbers of zoospores from mature sporangia. Sori-bearing plants are placed in open baskets which are covered with cotton cloth or rush mats and then the baskets are hung in a dark room at a constant temperature for 3 - 6 hours. The plants are returned to the seawater where excessive absorption of water by the partially dehydrated sporangia rupture their outer coverings and release zoospores into the surrounding water.

For artificial propagation the spores are lodged in short bamboo "ladders" hanging from rafts in the sea in autumn. In January the sporophytes appear and within 4 - 5 months they may reach 3 m or more in length and are ready to harvest. The Chinese have had a great deal of success fertilizing nutrient-deficient waters with porous earthenware containers filled with sodium nitrate and ammonium nitrate and suspended on the rafts. Rates of seepage of water and of fertilizer solution from various earthenware containers have been carefully calibrated. (Cheng, 1969).

Sea floor culture is also popular in China because it requires less labor, material, and capital investment and achieves greater cost-effectiveness. Kelp is grown on rocks or other hard substrates in the same area as the rafts and fertilized water, or in the intertidal zone surrounded by tiers of dams to contain the water, or in baskets in coastal areas. A detailed review of Laminaria culture in China has been published by Cheng (1969).

In Japan, Laminaria is cultivated using stone-planting and rope-culture techniques similar to those used to grow Undaria. Dynamite is sometimes used to improve the substrate and to control harmful weeds (Wildman, 1974). A detailed account of Laminaria culture in Japan has been published by MacFarlane (1968).

Besides that in Japan, Korea, and China, the most successful seaweed culture in recent years has been that of Eucheuma culture in the Phillipines. There Eucheuma farming is based on methods developed at the University of Hawaii and supported by the principal producer of carrageenan in the

United States, Marine Colloids, Inc. (Wildman, 1971). The carrageenanophyte is farmed on the flats immediately behind coral reefs where there is usually a rapid flow of seawater as a result of tides or currents. Individual farms are small, usually one hectare or less in area, and consist of series of 2.5 m x 5 m nets that are pegged horizontally a short distance above the bottom. Vegetative cuttings of Eucheuma are tied to the intersections of the netting. Yields are about 13 tons dry wt/ha/yr, but growth of the plant is seasonal and populations of the algae die from senescence periodically (Parker, 1974). Recently, net culture has given way to monoline culture (Neish, 1978). Details on the methods used, productivities obtained, costs and returns, and the socio-economic impact of Eucheuma farming on local communities have been published (Alvarez, 1975; Caces-Borja, 1974; Deveau and Castle, 1976; Doty, 1973; Parker, 1974).

Gracilaria Culture

Gracilaria is farmed in Taiwan primarily in shallow ponds that were formerly used for the culture of fish, such as the milkfish Chanos chanos. As with Eucheuma, Gracilaria farms are started by seeding the ponds with vegetative fragments of algae. Except in some experimental projects (Figure 5.3), the Gracilaria plants are not normally tied to netting, but they are usually held down by bamboo stakes or covered with netting to prevent movement towards one end of the pond by wind action. The algae grow at depths ranging from 20 to 80 cm in water ranging in salinity from 8‰ to 30‰ and at temperatures of 10 to 30°C. Gracilaria does not require the same rapid exchange of water as Eucheuma, but is grown in Taiwan pond farms with only one water change every two to three days. (See Shang, 1976 for details.) Average yields of 4.17 g dry wt/m²/day have been reported (Shang, 1976).

5.1.3 Seaweed Culture in North America

The labor intensive cultivation methods used in Oriental countries have been profitable because of cheap labor, but in North America there is a need for the development of efficient mechanical techniques to lower the cost of cultivation and harvesting operations. Efforts have concentrated on the cultivation of macroalgae in land-based systems and based on the experience of Oriental growers in coastal waters and in the open ocean.

Figure 5.3

Experimental Culture of Gracilaria on Plastic Ropes in Taiwan

Canada

In the late 1960's a group at the Canadian NRC Atlantic Regional Laboratory, Halifax, N.S., under the direction of A.C. Neish, initiated research on the culture of Chondrus crispus. Investigations by this group and subsequent authors in the field (Allen et al., 1971; Prince and Kingsbury, 1973b) and in the laboratory (Neish and Fox, 1971; Neish and Shacklock, 1971; Prince and Kingsbury, 1973a; Shacklock et al., 1973, 1974, 1975) have provided extensive information on the growth requirements of C. crispus. The work of Neish and his colleagues made two major contributions to the art of culturing macroalgae: 1) They demonstrated the value of selecting strains by isolating the T-4 strain C. crispus which had a rapid growth rate and a high carrageenan content; 2) They developed a technique for growing unattached plants in suspended culture. Not only does the suspended culture method result in generally more rapid growth of seaweeds, but it maintains vegetative plants permanently in a non-reproductive, non-fruiting stage so that continuous growth and organic production are possible. A preliminary engineering and economic assessment of the culture method has been made by Huguenin (1976).

Following Neish's lead, two commercial seaweed companies, Marine Colloids, Inc. of Rockland, Maine and its Canadian subsidiary and Genu Products Canada, Ltd. (a subsidiary of a Danish firm now owned by Hercules Corp., Wilmington, Del.) have started pilot Chondrus culture projects in Nova Scotia, with partial support from the Canadian government (DeBoer, 1977). These companies grow unattached plants in suspended culture using modifications of Neish's basic technique. Both of these pilot projects are in their very early stages (1 - 2 years) and only the most preliminary results are available. One of the major objectives of the Marine Colloids projects is to screen samples collected from a large number of natural Chondrus beds in an attempt to select improved strains with respect to growth, carrageenan content, and other favorable characteristics.

Marine Colloids

Since 1970 the seaweed industry has partially funded studies on other commercially valuable seaweeds with the objective of developing techniques for the cultivation of carrageenan-containing species other than Chondrus crispus, such as Eucheuma, Hypnea, Iridaea and Gigartina (Dawes et al., 1974a, 1974b; Fralick and Mathieson, 1975; Mathieson and Dawes, 1974; Waaland, 1973). As an outgrowth of that research, Marine Colloids, Inc. initiated a modest pilot experiment in the Florida Keys to study the potential cultivation of Eucheuma, Hypnea musciformis and other tropical and semi-tropical red algae. A study by Dawes (1974) on the potential and economic feasibilities of culturing Eucheuma isiforme in Florida 1) in outdoor tanks and 2) in natural sites (as in the Central Pacific), indicated that tank culture would yield a much higher crop per unit area than mariculture in natural embayments. Dawes postulated that under controlled conditions in outdoor tanks, a potential yield the equivalent of 220 m tons dry wt/ha/yr could be attained; but operating costs would be high. In the case of field culture, yields in the Florida Keys might average about 18 m tons wet wt/ha or 3.6 m tons dry wt/ha/yr, but problems could arise from predators, harsh weather conditions or contamination by other seaweeds; conversely, operating costs would be low. (Naylor, 1976). Further studies have been funded by the Sea Grant Program at the Universities of New Hampshire and South Florida to determine the basic biology and environmental requirements of Eucheuma and at the University of Hawaii to develop techniques for the culture of Eucheuma. These studies have led to the successful cultivation of Eucheuma in the Phillipines as discussed in Section 5.1.2.

Washington

The potential, through cultivation activities, for the development of a major red seaweed industry in the State of Washington was first recognized in 1970 by D. Jamison of the Department of Natural Resources (Jamison and Beswick, 1972). His research on the growth of seaweeds on ropes generated interest which led to a number of studies on the biology and ecology of Pacific red algae, particularly Iridaea cordata and Gigartina exasperata,

and the potential for cultivation of these species. Studies have been conducted on the life history in laboratory culture (Kim, 1976; Norris and Kim, 1972), on physical parameters for optimal growth (Waaland, 1973; 1974) and on techniques for growth on artificial substrates (Waaland, 1976; 1977). The ecology of natural populations has been examined in Washington (Hruby, 1974; 1975; 1976), in British Columbia (Austin and Adams, 1975) and in California (Hansen and Doyle, 1976) and the effects and potential of harvesting natural populations have been outlined (Fralick, 1971; Austin and Adams, 1975). In 1970-71 an attempt by the Lummi Indian Tribe to harvest natural beds of Iridaea failed due to low plant density and high harvesting costs. Since then research funded by the Washington Department of Natural Resources and the Washington Sea Grant Program has focused on two methods for cultivation: cultivation using artificial substrates in the natural environment and sustained semi-closed cultivation.

Building on the experiments of D. Jamison and techniques used in Japan, C. Kemp of DNR successfully artificially seeded and grew Iridaea on nets in Puget Sound. Since T. Mumford took over the project in 1976, he has compared growth at different locations and using different mesh sizes of nets, and he has improved seeding methods and harvesting techniques (Mumford, 1977a; 1977b). However major problems, particularly with grazers still exist. Mumford (1977c) has shown that timing of harvests before plants senesce is critical. Yields of 6 - 8 kg dry wt/m² were obtained in 1975 and 3.3 kg dry wt/m² in 1976. Currently experiments are being conducted on 25 - 0.25 m² nets in a test bed in Puget Sound.

The system used by J.R. Waaland at the University of Washington for semi-closed cultivation involves pumping unsupplemented sea water from Puget Sound into tanks of various sizes: 1400 liter polyester resin-coated or plastic-lined plywood tanks with 1.2 m² surface area and 1.1 m water depth, 350 liter painted or plastic-lined plywood tanks with 2.8 m² surface area and 1.1 m depth, and 73 liter plastic tanks with 0.26 m² surface area and 0.3 m water depth. The plants and sea water in the tanks are continuously moved by aeration supplied through a perforated plastic pipe located at the middle or

to one side of the tank bottom. Mean daily growth rates of numerous seaweeds have been measured for several months in the system, but the most successful species were Iridaea cordata (11.9 g dry wt/m²/day) and the M-11 strain Gigartina exasperata (9.0 g dry wt/m²/day). Yields are 6 times those of net cultured plants in the natural environment. Studies have shown that because Puget Sound waters are sufficiently rich in nutrients, artificial fertilizers do not increase growth. The M-11 strain G. exasperata, which was developed by Waaland and his colleagues, has several advantages over other species: 1) The mean daily growth rate of the M-11 strain is twice as high as that of the wild type, 2) This species can be propagated vegetatively by means of cuttings from thalli or outgrowths from thalli; in fact, this strain has never been observed to reproduce by means of spores or gametes so no energy is expended on synthesis of such reproductive bodies. Currently a method is being tested on a small scale to grow plants in the semi-closed system on circulating artificial substrates. If successful, such a method would provide a means of retaining the perennial holdfasts, so that after the large blades have been harvested, the holdfasts on their substrates can be returned to a culture tank where they will sprout new blades. (Waaland, 1976, 1977)

Woods Hole, Mass. and Ft. Pierce, Florida

At the Woods Hole Oceanographic Institution in Woods Hole, Massachusetts and at the Harbor Branch Foundation in Ft. Pierce, Florida, extensive research on the growth of seaweeds has been conducted under the direction of J. Ryther. Seaweeds were grown initially as the final component or "polishing step" of a waste recycling-mariculture system (Ryther et al., 1975), and more recently under contract with the U.S. Energy Research and Development Agency (ERDA) as a potential biomass source for fermentation to a fuel or petroleum sparing product.

In the early Woods Hole studies various species such as Chondrus crispus, including the T-4 strain obtained from Halifax, and the green alga Ulva lactuca were employed to assimilate nutrients regenerated by shellfish

in a controlled food chain, so that high nutrient removal efficiencies could be attained (Goldman et al., 1974; Ryther et al., 1975). The seaweeds were particularly attractive for this purpose because of their large size, and hence ease of separation. However, Ulva frequently went into a reproductive stage, leading to complete fragmentation of the plant tissue and loss of the culture. Similarly, Chondrus grew slowly and became heavily overgrown with other species. Both species were subsequently replaced by other warm-water species that appear as summer annuals in the Woods Hole region, including Neoagardhiella baileyi, Gracilaria foliifera, and Hypnea musciformis. In these early studies yields were not recorded, although the biotechnology for growing the macrophytes as free-floating plants was being perfected.

So successful was the growth of seaweeds, especially Neoagardhiella and Gracilaria, in the Woods Hole experiment, that a separate project was initiated in which the seaweeds alone were grown in mixtures of sewage effluent and seawater as a one-step waste recycling-aquaculture system. In addition, seaweed culture experiments were started in 1973 at the Harbor Branch Foundation, Ft. Pierce, Florida. During the initial Ft. Pierce experiments average yields of Hypnea grown in small experimental tanks, varying in size from 350 - 600 liters (Figure 5.4), were $17.6 \text{ g/m}^2/\text{day}$ for five months and yields of Gracilaria were $16.9 \text{ g/m}^2/\text{day}$ for nine months (Lapointe et al., 1976). Subsequently, Gracilaria has grown in culture tanks throughout the year, although the plants become heavily epiphytized at times and twice during the year Gracilaria was discarded for that reason and replaced with new material collected from the wild. Yields ranged from 0 to $31.5 \text{ g dry wt/m}^2/\text{day}$ and averaged $20 \text{ g dry wt/m}^2/\text{day}$ over the entire year.

Larger-scale experiments were conducted in Woods Hole (DeBoer and Ryther, 1977). The unattached, free-floating seaweeds were grown in concrete raceways $12.2 \text{ m (L)} \times 1.2 \text{ m (W)} \times 1.5 \text{ m (D)}$, which had sloping plywood bottoms with a depth ranging from 0.6 to 1.5 m (Figure 5.5). The seaweeds were kept in suspension by aeration from an airline located at the bottom of the raceway and extending its entire length. The aeration provided for mixing, gas exchange

Figure 5.4

Plywood Tanks with Sloping Bottoms for Growing Seaweeds
in Suspended Culture in Seawater Enriched with Sewage Effluent at Woods Hole, Mass.

Figure 5.5

Concrete Raceways with Sloping Bottoms for Growing Gracilaria and Neoagardhiella
in Suspended Culture on the Effluent from Bivalve Mollusc Cultures at Woods Hole, Mass.

and uniform exposure of the plants to sunlight. The seaweed cultures received the effluent from similar raceways containing various species of bivalve molluscs. Neoagardhiella had surprisingly high rates of production in the spring (22 - 41 g dry wt/m²/day) and summer (20 - 36 g/m²/day). Gracilaria production rates were quite variable, increasing from 4 g/m²/day in April to 43 g/m²/day in early June and decreasing again during the latter part of the summer to 7 - 18 g/m²/day. Production rates of both species declined during the fall and early winter. By mid December the production rate for Gracilaria had fallen to zero and the plants had deteriorated, while Neoagardhiella remained viable (although stunted) with a production rate of 6 g/m²/day.

The mean annual dry weight production rate was 17.3 g/m²/day or 63.2 m tons/ha/yr for Neoagardhiella and 8.9 g/m²/day or 32.9 m tons/ha/yr for Gracilaria. These rates may be higher than would be realized in a commercial enterprise because the raceways were maintained at elevated temperatures for approximately six months of the year. Annual production rates based on a 5-1/2 month growth season when the raceways were not heated (May 8, 1975 to October 20, 1975) were 46.1 m tons/ha/yr and 28.5 m ton/ha/yr for Neoagardhiella and Gracilaria, respectively.

In 1976, research supported by ERDA on the mass culture of seaweeds as a biomass source for conversion to energy was initiated both at the Woods Hole, Mass. and Harbor Branch Foundation, Florida facilities. Since the objective in this research was to produce organic matter in toto, and not simply the commercially-valuable hydrocolloids from red macrophytes, the species of seaweed produced in culture was no longer of importance. Rather, the immediate goal was to select the species and develop a culture technique that would provide the highest possible yield of organic matter per unit of time and space and with the smallest possible input of energy.

For screening different species of algae for their growth potential in artificial culture, a standard assay system was developed consisting of four 6-m long, longitudinally-sectioned, 0.4 m diameter PVC pipes divided into 0.75 m long (50-liter capacity) compartments by means of plywood sections.

Each section was provided with a calibrated flow of enriched seawater and a screened overflow drain. Thirty-two individual assay chambers were provided in this way (Figure 5.6).

As of 1977, 42 species including representatives of the red, green, and brown algae, had been tested (Ryther et al., 1977). Of these only six showed significant growth potential in short-term (1-2 week) culture experiments. These included the green seaweeds Enteromorpha clathrata, Chaetomorpha linum, and Ulva lactuca and the red seaweeds Hypnea musciformis, Gracilaria foliifera, and Gracilariopsis sjoestedtii. Of the six, the three species of greens and the red H. musciformis, could not be maintained over long periods of time in culture, but fragmented or disintegrated into spores after one or two weeks. G. sjoestedtii appeared promising, but had not been cultured for more than three months at the time of this writing. The same clone of G. foliifera was maintained for a full year in the smaller (50 liter) culture mode. Individual clones became overgrown with epiphytes twice during the one-year period, however, and had to be replaced with new material. Yields of that culture from July, 1976 to July, 1977 ranged from a high of 50 g dry wt/m²/day during July-September to lows of 7 - 15 g dry wt/m²/day in December-January.

The time-weighted mean production of Gracilaria during that year was 35 g dry wt/m²/day, which is equivalent to an annual dry weight production of 127 tons/ha/yr, a figure that is more than twice the annual yields of G. foliifera and N. baileyi at Woods Hole and greater than the highest sustained agricultural yields reported in the literature. (See Tables 3.2 and 3.3).

One major problem with growing algae in suspended culture in tanks using aeration is that large volumes of water and compressed air are necessary to sustain high growth rates. Such practices are highly energy intensive and possibly would not be effective on a large commercial scale, either economically or in terms of the net energy balance. For these reasons, efforts have been made at Fort Pierce to develop other, non energy-intensive culture methods and to determine long-term yields of seaweeds grown under those conditions,

Figure 5.6

Screening Tanks (50 ℓ -Capacity) Constructed from PVC Pipe
for Growing Algae in Suspended Culture in Florida

using primarily the same strain of G. foliifera. To date, the four non-intensive methods for growing Gracilaria described below have been assessed.

1. Plastic mesh trays made from ca. 2 cm Vexar on 2.5 cm diameter PVC pipe frames measuring 1 m (L) x 1 m (W) x 25 cm (D) (Figure 5.7) were either floated at the surface or anchored to the bottom in about 1 m of water in the Indian River (a large, shallow embayment behind the barrier beach along Florida's central East Coast). Gracilaria was stocked in the trays at a density of approximately 2.0 kg wet wt/m². The seaweed was weighed and its incremental growth removed at one-week intervals. Trays anchored to the bottom were covered with a layer of Vexar mesh to prevent the algae from washing out. Trays were placed in several locations in the Indian River, at distances ranging from 0.8 km to about 16 km from the nearest inlet to the Atlantic Ocean and in areas receiving various amounts of tidal current flow.

Growth of Gracilaria was variable and unpredictable and appeared to be independent of depth (surface or bottom) or location in the Indian River. Yields ranged from 0 to 6 g dry wt/m²/day and averaged about 2.5 g/m²/day. Cultures gradually became covered with silt and epiphytes and died after 4-8 weeks.

2. The same plastic mesh trays were floated at the surface or sunk to the bottoms of 20,000 liter 13 m (L) x 5 m (W) x 1 m (D) PVC-lined ponds (Figure 5.8), through which seawater was passed at rates of either 0.5 or 5.0 pond volumes per day. The seawater was enriched with treated sewage effluent or with sodium phosphate and sodium nitrate to give N and P concentrations of 1000 and 100 μ moles/l respectively at the slow (0.5 vol/day) turnover rate and 100 and 10 μ moles/l respectively at the fast (5 vol/day) exchange rate. When the trays were

Figure 5.7

Plastic Mesh (Vexar) Trays with PVC Pipe Frames for Growing Seaweeds
in Artificial Ponds and Natural Environments in Florida

Figure 5.8

PVC-Lined Ponds Used for Growing Seaweeds in Florida

floated, water was pumped from the bottom of the pond and sprayed down on the algae, which was barely submerged beneath the water surface. Otherwise, water circulated through the ponds passed beneath the trays and not through the algae. Gracilaria was stocked in the trays at the same density and harvested in the same routine as in (1).

Growth was highly variable and unpredictable and was apparently independent of culture depth, flow rate of enriched seawater, or nutrient concentration. Yields ranged from 0 to 12 g dry wt/m²/day and averaged about 5.0 g dry wt/m²/day. Culture became heavily epiphytized and growth ceased after about 8 weeks.

3. Concrete burial vaults measuring 2.2 m (L) x 0.8 m (W) x 0.6 m (D) were made into 325 liter culture chambers using PVC piping for input and overflow drains (Figure 5.9). Seawater was enriched with treated sewage effluent adjusted with sodium phosphate and sodium nitrate to give final concentrations of 50 μ moles/l N and 5 μ moles/l P. The enriched seawater was passed through the vaults at an exchange rate of 21 volumes per day. G. foliifera was stocked in the vaults at the same density and harvested in the same way as in (1).

Growth in the vault cultures of G. foliifera collected from the wild or taken from stock cultures was initially high, reaching maximum values of 25 g dry wt/m²/day. However, yields gradually decreased with the age of the culture to levels of 5 - 10 g/m²/day and some cultures ceased growing entirely. For the first 8 weeks the mean yield was approximately 10 g/m²/day.

4. The technique for growing Gracilaria that has been most recently developed at the Florida research facility involves growing seaweed in trays completely out of water, but continuously

Figure 5.9

Concrete Burial Vaults Used for Growing Seaweeds and Freshwater Plants in Florida
The Vault in the Foreground Contains a Culture of Water Hyacinth.

irrigated by enriched seawater sprayed down on the algae from above (Figure 5.10). This method is based upon a technique described by A.R.O. Chapman and attributed by him to L. Hanic (Stein, 1973). Seawater enriched with 100 μ moles N and 15 μ moles/l P as sodium nitrate and sodium phosphate respectively is pumped through a series of household shower heads upon plastic 0.6 m (L) x 0.6 m (W) x 0.08 m (D) trays (constructed as shellfish grow-out trays by Nestier Corp., Cincinnati, Ohio), using four shower heads per tray, at a rate of irrigation that is equivalent to approximately 50 liters per minute per m^2 of tray surface. The enriched seawater used for the spray is held in a 400 liter reservoir which is continuously recirculated (i.e., every eight minutes). In addition the reservoir is continuously replaced by new enriched seawater at a rate of 20 volume exchanges per day, or a little more than 5 liters per minute. The choice of the exchange rate of the reservoir, the nutrient content of the enriched seawater, and the rate of spraying the seaweed were all arbitrary and will be the subjects of designed experimental variation in future studies.

Gracilaria is stocked at different densities ranging from less than 1 kg wet wt/ m^2 to 12 kg wet wt/ m^2 of tray surface to determine optimum densities for best yields. After approximately eight weeks of experimentation, yields from weekly weighings varied with stocking densities, but averaged about 20 g dry wt/ m^2 /day. The cultures were only mildly epiphytized at their upper surface, so that the epiphytes could be removed with the harvest. The algae grew vertically upward in the trays, and high yields were sustained at densities as high as 12 kg/ m^2 .

Figure 5.10

Culture of Gracilaria in Trays Receiving Spray Irrigation in Florida

The yields and other characteristics of Gracilaria obtained by the six culture methods described above are shown in Table 5.1, which also includes that from the commercial pond culture of Gracilaria in Taiwan as described above and by Shang (1976).

Table 5.1
Yields and Other Characteristics of Gracilaria
Grown by Different Culture Methods

Method	g/m ² /day *		m tons/ha/yr *	Exchange Rate (volumes/day)
	Mean	Range		
Ponds, Taiwan (Shang, 1976)	2.7		10	0.3 - 0.5
Indian River cages	2.5	0 - 10	9	not known
20,000 liter ponds	5.0	0 - 10	18	0.5 - 5.0
325 liter cement vaults	10.0	0 - 25	37	21
600-l plywood tanks, aerated (suspended culture)	20.0	0 - 31.5	73	5
50-l PVC tanks, aerated (suspended culture)	35	10 - 50	127	22
Spray cultures	20		73	20

*Total dry weight (ash included).

California

Commercial interest in the kelp beds of California initiated in 1910 when the Department of Agriculture, Bureau of Soils conducted a survey of possible sources of potash within the United States. The occurrence of three kelp genera which are high in potassium - Alaria, Macrocystis and Nereocystis - were mapped and plant densities were qualitatively assessed. Realizing the potential of kelp as a marketable item, companies formed to harvest and process the brown algae. With the advent of the first world war German potash sources were completely eliminated, and the annual harvest of kelp in California increased from about 2,500 tons in 1913 to 130,000 in 1916. At the end of the war in 1918, the demand for kelp decreased dramatically, and did not begin to recover until the early 1930's. Since that time the annual yields of kelp from California beds has steadily increased (for details see North, 1971) and is now a highly profitable enterprise. At present a polysaccharide, algin, is the major chemical product extracted from California kelp by Kelco Company.

Early methods for harvesting giant kelp plants were rather crude and often destructive. A large number of plants were "lassoed" with a cable and drawn into a tight bundle for cutting. The cut kelp fronds were then either pulled aboard a boat by hand or allowed to drift ashore for collection. As the need for larger quantities of kelp developed more efficient methods of harvesting were employed, giving rise to a moving barge with a trapper bin for bringing cut material on board and storing it. Present day harvest vessels (see Section 4.2.1) are equipped with reciprocating underwater mowers at the stern (Booth, 1969) and a conveyor belt for moving the cut kelp aboard.

As mentioned in Section 5.1.1, the kelp beds are presently leased by the State of California and controlled by licensing and/or royalty arrangements. Regulation of the kelp beds began in 1916 when representatives from the kelp farms, the U.S. Department of Agriculture, the University of California and the California Fish and Game Commission set forth a foundation for controlled utilization and conservation of kelp bed resources. The resulting

laws and regulations have proved successful in accurately monitoring and controlling kelp harvests and consequently preserving kelp beds.

In the mid-1950's, concerned biologists, government and industrial leaders began to notice a decline in the standing crop of the nearly 100 square miles of kelp forest along the coast of California. Wheeler North of the California Institute of Technology initiated a study to determine whether the decline was due to overharvesting or the result of a biological occurrence, such as grazing by sea urchins, or physical parameters, such as water pollution or a temperature change. These studies were the beginning of a series of research projects which have continued for over 20 years. Numerous research projects on the life history (Neushul and Haxo, 1968) growth and reproduction (Neushul, 1957, 1959, 1963; Neushul and Haxo, 1963; Sargent and Lantrip, 1952), and transplantation (North, 1964; North and Neushul, 1968; Sargent and Lantrip, 1952) of Macrocystis led to the development of techniques for re-establishing kelp beds. Further research was carried out on the ecology (North, 1957, 1958a, 1958b, 1959, 1960, 1971), the biochemistry and physiology (Clendenning, 1957, 1968c, 1971), the nutritional requirements (North and Wheeler, 1977), the life span (North, 1971), and the effects of canopy cutting (North, 1968, 1977c) on Macrocystis and on the ecology of organisms directly associated with kelp (Clendenning and Wing, n.d.; Davies, 1968a, b; Limbaugh, 1955; Quast, 1968a, 1968b, 1968c, 1968d, 1968e, 1968f). Based on the results of these studies a program of transplanting, monitoring, and controlling predation and competition was developed which led to the restoration of the kelp forests. These studies also provided scientific data which was critical to an understanding of the role of Macrocystis to maintain an ecological balance in Pacific coastal waters. Research continues under the direction of W. J. North at the California Institute of Technology and is co-sponsored by the National Science Foundation (NSF), the Kelco Company, Phillip R. Park Research Foundation and three southern Californian counties.

Due to the overwhelming success of restoration efforts, the potential for cultivating Macrocystis in new areas was recognized. In the mid-1970's an ambitious Ocean Food and Energy Farm (OFEF) Program funded jointly by ERDA,

NSF, the American Gas Association (AGA), the U.S. Navy and various organizations in public and private sectors was begun. The objective of the farm is to cultivate kelp as a source of energy. An ocean farm system was designed under the management of H.O. Wilcox of the Naval Undersea Center, San Diego and production costs and performance of the system were estimated. The ocean farm systems design, published in seven volumes (Anderson, 1976a, 1976b; Budhraj, 1976a, 1976b; Budhraj et al., 1976; Nickels, 1976; Walsh, 1976; Walsh and Schneider, 1975; Wilcox, 1975, 1976a, 1976b) was divided into five major subsystems each performing unique and independent functions - cultivation, harvesting, processing, support, and mariculture.

The design consisted of an ocean farm covering 40,000 ha (a square, approximately 20 km on a side), located 160 km off the coast of Southern California. Following site survey studies, 3 sites in Southern California were recommended (Seligmann, 1976b). The farm substrate, maintained at a depth of 34 m, was made up of flexible triangular modules 312.5 m on a side each covering about 4 ha. Each module was held in place by diesel-powered propulsors. Nutrient-rich water was upwelled from a depth of about 91 m by wave-powered pumps. Those upwelling pumps considered for the project included the Isaacs buoy propeller pump, a wave turbine propeller pump, a windmill propeller pump, a wave vane propeller pump, a modified Isaacs pump and the Wilcox bellows pump (Hoffmann et al., 1976). Studies on the growth of Macrocystis in upwelled water (North, 1976) and on the dynamics of the dispersion and sinking of upwelled water (Kirchhoff, 1976; North, 1975; Seligman, 1976a) provided background information in designing the system.

Kelp plants attached to the substrate at a density of one plant per 34 square meters would take about 4 years to mature and then the standing crop would be harvested by a harvesting ship six times per year. The estimated yield of the farm was about 136 m ton wet wt/ha/yr composed of about 38.5 m tons of solids of which 21.6 m tons were volatile and the remaining 17.0 m tons were ash. In comparison, the productivity of natural kelp beds is about 4.5 m tons/ha/yr (North, 1977c).

A barge-type concrete platform was provided for living and work space to operate and maintain the farm. The kelp would be processed to produce methane and by-products. Along with the kelp cultivation, the system also included the mariculture of noncompetitive organisms such as kelp bass and oysters.

Wilcox's design should be a good building-block for future open-ocean aquaculture, but recent unpublished data suggests that there may be problems keeping the farms in tension and with upwelling water using wave-powered pumps (Vandiver and Burke, 1977).

To test the technical and economic feasibility of the commercial-sized production of seaweeds is one of the goals of the Energy from Marine Biomass Program. This program is sponsored by the American Gas Association and managed by the General Electric Company. Scientific and engineering support is provided by the Institute of Gas Technology, the U.S. Department of Agriculture and Global Marine Development, Inc. In addition, the marine biology portion of the program is sponsored by ERDA. Under this program a quarter acre (.1 ha) module (QAM) of a sea farm is being constructed at a site off Laguna Beach, Ca. The QAM consists of a 2.5 m diameter buoy which stands upright in the water and is attached by a swivel joint to an umbrella-shaped set of radial arms to which kelp plants are attached. Nutrient-rich water is pumped up through .6 m diameter fiberglass pipe using 3 pumps of 3,300 GPM driven by a 35 HP diesel. The test farm is scheduled for deployment at sea by April 1978. The rationale for such a system is documented by North (1977a, 1977b).

5.1.4 Summary of Yields

Average daily yields for large-scale cultivation (Table 5.2) are generally much lower than those attained in experimental studies for a number of possible reasons. A research scientist gives more attention to biological and physical parameters affecting growth of experimental cultures, than does the aquaculturist on a large farm. In small-scale studies noticeable decreases

Table 5.2

Summary of Yields from Long-term Commercial and Experimental Seaweed Cultivation

Species	Location ^a	Average Yield (g. dry wt./m ² /day) ^b	Duration (days)	References
<u>Commercial Cultivation (large scale, 1-10⁵ ha)</u>				
<u>Porphyra</u> spp.	Japan (C)	0.23	~150	Parker (1974)
<u>Porphyra</u> spp.	Japan (C)	0.51	~150	Bardach, <u>et al.</u> (1972)
<u>Porphyra tenera</u>	Japan (C)	1.2-2.1	~150	Tamura (1970)
<u>Undaria pinnatifida</u>	Japan (C)	3.2	~150	Ryther & Bardach (1968)
<u>Gelidium</u> spp.	Japan (C)	1.6-3.4	730	Tamura (1970)
<u>Gelidium</u> spp.	Japan (C)	1.4-2.6	1095	Tamura (1970)
<u>Gracilaria</u> spp.	Taiwan (C)	4.17	~240	Shang (1976)
<u>Eucheuma striatum</u>	Philippines (C)	3.64	365	Parker (1974)
<u>Macrocystis pyrifera</u>	California (N)	1.46 ^c	365	Neushul (1978)
<u>Experimental Cultivation (small scale, 0.3-15 m²)</u>				
<u>Neogoidhiella baileyi</u>	Massachusetts (L)	27.7	166	DeBoer & Ryther (1977)
<u>Gracilaria foliifera</u>	Massachusetts (L)	16.9	166	DeBoer & Ryther (1977)
<u>Hypnea musciformis</u>	Florida (L)	17.6	150	Lapointe, <u>et al.</u> (1976)
<u>Gracilaria foliifera</u>	Florida (L)	16.9	150	Lapointe, <u>et al.</u> (1976)
<u>Gracilaria foliifera</u>	Florida (L)	20.0	365	Ryther, <u>et al.</u> (1976)
<u>Gracilaria foliifera</u>	Florida (L)	35.0	365	Lapointe, <u>et al.</u> (1977)

^a Key: L - land-based system; N - natural population in coastal waters; C - cultivation in coastal waters.

^b Total dry weight including ash: Ash-free dry weight = 0.3 - 0.5 x total dry wt.

^c Original data converted to dry weight assuming dry wt = 0.20 x wet wt.

in growth of the culture can be corrected by changing physical conditions, eliminating grazers or competitive species or replacing the diseased or "senescening" seaweeds with new plant material. Yields are probably higher in land-based systems than in natural waters because nutrients have a longer residence time and, therefore, can be utilized more efficiently by the plant. Seaweeds grown in land-based systems also do not incur losses due to harsh weather conditions and unharvested plant material, as is the case of open-ocean farms.

5.2 The Mass Culture of Aquatic Angiosperms

5.2.1 Freshwater Angiosperms

For nearly 100 years agriculturists have tried to fight off freshwater angiosperms, which obstruct waterways in the southeastern United States, with toxic chemicals, mechanical removers and natural predators. Only over the past decade have scientists recognized the beneficial aspects of these plants and now attention is focussing on utilization of their high nutrient content and ability to absorb substances directly from the water. Of the many common freshwater aquatic plants, several are particularly notorious for their abundance and presumed rapid growth rates. These include emergent species such as the water hyacinth Eichhornia crassipes, and the duckweeds Lemna and Wolffia, of which only the roots are in the water, and completely submerged species such as Hydrilla, Egeria, the coonail Ceratophyllum and the southern naiad Najas.

Culturing of freshwater aquatic plants is in its infancy, but progress is being made growing primarily water hyacinths as part of wastewater treatment. Conventional treatment of raw sewage involves, among other things, the removal of nitrogen and phosphorus and, since the water hyacinth can directly absorb and incorporate these nutrients into new cell masses, scientists have investigated and applied the hyacinth in sewage treatment (Wunderlich, 1964). Dymond (1948) in a study on the use of water hyacinths in domestic sewage treatment, found that dry-weight hyacinth contains an average of 2.23% nitrogen and 2.34% phosphorus. Dymond hypothesized that one acre of water hyacinths

would yield about 3,075 pounds (1,398 kg) of nitrogen per year, which is the equivalent of the wastes of 220 persons in one year. The efficiency of this absorption is maximized under anaerobic conditions. Therefore, the process is best applied in a tank tightly packed with water hyacinths so that anaerobic conditions exist below the plants.

A NASA laboratory and test plant in Bay St. Louis and Orange Grove, Mississippi, has a sewage treatment plant based on this principle and, in addition, produces methane and fertilizer as by-products. The water hyacinth is harvested and fed into tanks where the anaerobic treatment of secondary sewage effluent takes place. After the phosphorus and nitrogen are absorbed, the bacteria present break down cellulose into carbon dioxide and methane. 450 g dry wt hyacinth will produce 1.86 cubic meters of methane (Smith, 1977). The rich residue left behind may be used for fertilizer, provided that no other noxious chemicals or heavy metals were originally present in the effluent. Whenever the water hyacinth is used for fertilizer or as a feed additive, the chemical content of the water must be considered, since the plant absorbs industrial pollutants, heavy metals, pesticides, and herbicides along with nutrients. (Sheffield, 1966).

A closed environmental system (Solar Aquacell System) for growing water hyacinths as part of a sewage treatment process using 1,500 acres of ponds in the Tijuana River Valley has been proposed for 1990. The water hyacinths would be grown in 3 - 6 ft (1 - 2 m) deep ponds under inflatable plastic domes. The domes would act as a greenhouse increasing the temperature and hence, doubling the growth rate for each 10° increase. After the gross solids are settled out of the sewage, the waste water would flow into the first series of Solar Aquacell cells containing hyacinths, which would remove most of the toxic materials, like metals and organic pollutants. In between the first and second cells the waste water would be treated with ozone to kill any viruses. In the second and third cells, more ponds encompassing hundreds of acres would be planted with hyacinths, which would progressively absorb and metabolize pollutants. The hyacinths would provide a dietary staple for animals like prawns and fishes in the carp family cultured in these ponds.

The water would finally pass through a sand filter for clarification and be used for farm irrigation (Smith, 1977). Bob Wolverton of NASA has estimated that one acre of water will produce about 145 kg dry wt hyacinths/ha in sewage lagoon effluent. In the Solar Aquacell System this rate could be maintained at approximately 50.8 m tons dry wt/ha/yr (Alsten, 1977). A pilot plant is being constructed in the upper Mission Valley and will operate for two years, complete with prawns, fishes and hyacinths (Smith, 1977).

Development of efficient methods to harvest, chop, press and dry or ensile hyacinths has been the task of L.O. Bagnall at the University of Florida. He has designed a mechanical harvesting and processing system which he believes is highly efficient. An air boat begins the job by plowing into a mass of matted hyacinths, breaking apart large sections, and pushing them to a system of conveyor belts on shore. Once the plants reach the conveyor belts, the basic operation of harvesting is complete. This method allows for the collection of about 26 m tons of hyacinth per hour. Bagnall is designing an onshore chopping and pressing system which is incorporated into the conveyor mechanism. With this system, up to 75% of the water can be removed. Thus, 900 kg of hyacinths can be reduced to 225 kg after pressing, resulting in reduced costs of subsequent transportation. In order to be economically feasible, such a system would have to be mobile, since the hyacinth mats are so extensive. The chopping machine consists of a rolling cylinder of angled blades, similar to an old-fashioned lawnmower. The hyacinth emerges from the rollers in a muddy brown paste, and a conveyor belt then transports this mulch to a screw press. The press squeezes out most of the remaining water which is collected and filtered and may be used to feed pigs and poultry.

In the last stage of processing, the hyacinths are either ensiled through fermentation to be used as feed for cattle, or dried. Methods using anaerobic digestion fermentation discussed previously have been proven at the NASA laboratories. There are two major ways of drying the crushed hyacinth. The fastest method involves a rotary drum and heater which dehydrates the mulch but requires 1,500 BTU for every pound (453 g) of water evaporated. This amounts to about \$35 per ton (0.9 m ton) of hyacinth, not an economical figure. The second method of drying is inexpensive, simple, and utilizes a

nearly unlimited energy source - the sun. Bagnall's system of solar drying consists of a box with a screen on top and a fan inside. Crushed hyacinth is placed on top of the screen, where it is exposed to sunlight. The fan constantly pulls the heated air through the crushed hyacinth, into the box, and out an exhaust hole. In this way, sun-heated air is constantly rushing across a maximum amount of surface area on the hyacinth mulch, drying it faster than if it were heated on the top surface only. The final product is a light yellow-brown mass of fine fibers (Bagnall et al., 1973). The estimated cost of processing one ton (0.9 m ton) of hyacinth by solar drying is about \$24.

Although high levels of productivity are attributed to all of the aquatic "weed" species, in fact their yields have seldomly been measured and reliable data are lacking. Claims have been made that under ideal conditions a water hyacinth plant will normally double in weight in seven to ten days and that in only eight months 10 water hyacinth plants can hypothetically multiply to 600,000 plants which would cover an acre (0.4 ha) of water (Vietmeyer, 1975). Detailed economic analyses have been carried out of hypothetical water hyacinth farms for municipal wastewater treatment (Robinson et al., 1976) and for energy conversion (Lecuyer et al., 1976), but the authors of the first of these reports admit to an uncertainty in the potential yield of these plants between 8 and 151 m tons/ha/yr. The latter figure which is most frequently quoted in projections concerning the potential productivity of water hyacinths, has its origin in Westlake (1963). However, that author actually states: "It seems possible that about 150 m tons/ha/year might be obtained in a good climate if Eichhornia crassipes could be grown so that young plants always predominated and the water surface was always completely covered . . . etc." Earlier in the same article, Westlake states "However, reliable data on its (hyacinth's) annual productivity has not been found." The latter fact, unfortunately, holds true up to the present.

Experiments were initiated in the Spring of 1977 at the Harbor Branch Foundation (Ft. Pierce, Florida) for measurement of the productivity of three species of freshwater weeds, Eichhornia crassipes, Lemna sp. and

Hydrilla verticillata. Studies were made with the water hyacinths and duckweed in 12 m (L) x 2.4 m (W) x 1.2 m (D) PVC-lined earthen ponds (15,000 - 20,000 liter volume, ca. 30 m² area) (Figure 4.24) through which well water enriched with treated sewage effluent was passed at varying residence times (0.5 to 16.5 days) and varying concentrations of N (50 - 1500 μ moles/l) and P (5 - 150 μ moles/l).

Experiments with Hydrilla were delayed because of the problem of how to measure productivity (i.e., weight increase per unit time and area) in a plant that grows well only when rooted to the bottom, the culture of which must be virtually destroyed to obtain the measurement. This problem was resolved when it was found that Hydrilla would grow if the root ends were tied to a piece of Vexar mesh suspended a short distance off the bottom of the pond. In this way, the entire section of Vexar mesh with plants attached could be removed and weighed without harm to the plants or their root systems.

Hydrilla has been grown in the above manner in PVC-lined ponds (Figure 5.8) and also in concrete culture chambers measuring 2.2 m (L) x 0.8 m (W) x 0.6 m (D), using a rapid exchange of enriched well water (residence time 0.05 days) (Figure 5.9). Only one value for the yield of Hydrilla had been obtained at the time of this writing.

Data that have been obtained to date for the yields of the three freshwater weeds are shown in Table 5.3.

5.2.2 Marine Angiosperms

Techniques for the mass culture of marine angiosperms are less advanced than for any other group of aquatic plants, due basically to a lack of background information. In recent years a concerted effort has been made by the International Decade of Ocean Exploration-Seagrass Ecosystem Study to understand the importance of seagrasses and to develop techniques to culture them from seed and to transplant seagrasses.

Table 5.3

Productivity of Freshwater Weeds
at Harbor Branch Foundation, Ft. Pierce, Florida: 1977

	Dates	Average Yield g dry wt/m ² /day
I. <u>Water Hyacinth (Eichhornia crassipes)</u>	5/16 - 5/20	20.4
a) Residence time = 5 days	5/20 - 6/3	23.1
Influent N = 1000 μ moles/l	6/3 - 6/17	15.9
Influent P = 100 μ moles/l	6/17 - 6/30	18.4
	6/30 - 7/14	18.0
	7/14 - 7/27	14.8
b) Residence time = 0.5 days	5/16 - 5/24	16.6
Influent N = 100 μ moles/l	5/24 - 6/3	20.6
Influent P = 10 μ moles/l	6/3 - 6/17	16.6
c) Residence time = 16.5 days	6/17 - 6/30	12.8
Influent N = 1500 μ moles/l	6/30 - 7/14	11.3
Influent P = 150 μ moles/l	7/14 - 7/27	12.3
II. <u>Duckweed (Lemna sp.)</u>	5/18 - 6/6	3.1
a) Residence time = 5 days	6/6 - 6/14	3.9
Influent N = 500 μ moles/l	6/14 - 6/21	3.0
Influent P = 50 μ moles/l	6/21 - 7/1	4.5
	7/1 - 7/8	4.6
	7/8 - 7/15	5.6
	7/15 - 7/22	4.9
	7/22 - 7/29	4.8
b) Residence time = 0.5 days	5/16 - 6/6	3.2
Influent N = 50 μ moles/l		
Influent P = 5 μ moles/l		
c) Residence time = 16.5 days	6/6 - 6/14	2.3
Influent N = 1500 μ moles/l	6/14 - 6/21	2.6
Influent P = 150 μ moles/l	6/21 - 6/30	4.8
	6/30 - 7/8	3.9
	7/8 - 7/15	5.0
	7/15 - 7/22	4.9
	7/22 - 7/29	4.2
III. <u>Hydrilla verticillata</u>	7/7 - 7/21	6.2
Residence time = 0.05 days		
Influent N = 50 μ moles/l		
Influent P = 5 μ moles/l		

The seed of the eelgrass Zostera marina was once used by the Seri Indians, living along the shores of the Gulf of California in Mexico's Sonoro desert, as a basis for a gruel (Felger and Moser, 1973). In that area the Zostera population consists of 100% seed plants which produce 19,000 seeds/m²; the equivalent of about 20% of the production of commercial rice crops. Under the proper management Zostera could be cultivated as a food resource. Dried eelgrass leaves have been used as fuel, packing, upholstering, insulation, fodder, and fertilizer, but the manufacture and use of eelgrass products has been local and intermittent in nature. (Canadian Dept. of Interior, 1926; Cottam, 1933; Felger and McRoy, 1975; Phillips, 1975; Tressler and Lemon, 1951; Uphof, 1959; Van Breedveld, 1966).

The culture of seagrasses is being studied at Michigan State University. To date, however, the culture of seagrasses axenically from seeds has not been successful.

Interest in transplanting seagrasses was generated by Phillips (1960) and Strawn (1961) who emphasized the desirability of developing techniques to re-establish seagrasses in locations where coastal dredging and filling had destroyed seagrass beds (Hutton et al., 1956; Phillips, 1960; Sykes, 1967). Early attempts to transplant and grow marine seagrasses for long periods of time under laboratory conditions were unsuccessful. Setchell (1942) was able to grow Ruppia maritima and Marmelstein (1966) grew the turtle grass Thalassia testudinum under artificial conditions, but only for a short time. It was not until 1969 that Fuss and Kelly (1969) successfully transplanted Thalassia and the shoal grass Diplanthera wrightii into aquariums and flow-through sea water tanks. In the aquariums Thalassia survived 7 months and shoal grass 3-1/2 months. In tanks, the turtle grass lived for 12 months and produced new leaves, roots and rhizomes, but only a few shoal grass plants survived. This study showed that seagrasses could be vegetatively propagated from fragments, but that unless fragments with active rhizomatic tips, or sprigs with well-developed short-shoots were selected, rhizomatic growth would be slow. In subsequent studies (Druel, 1973; Kelly et al., 1971;

Phillips, 1974; Ranwell et al., 1974) transplantation techniques have been perfected so that seagrasses can be maintained under controlled conditions almost indefinitely. A giant flume has been constructed at the University of Virginia for that purpose and for other seagrass studies (Zieman, 1977).

In the field, fragments of seagrasses have been successfully transplanted along the coast of Washington (Phillips, 1975, 1974), and in Great Britain (Ranwell et al., 1974). Intensive transplantation studies are being conducted in Izembeck Lagoon, Alaska; Puget Sound, Washington; and Port Aransas, Texas.

The productivity of seagrasses has been widely studied, especially for Zostera marina and Thalassia testudinum, but the results are difficult to compare with those of other aquatic plants because seagrass productivity is usually expressed as grams of carbon and hence, does not include other constituents of dry weight, which could as much as double the value. There is no doubt that seagrass productivity is high, however. Values for the annual production of Thalassia range from 200 gC/m² (Jones, 1968) to 3000 gC/m² (Buesa, 1972) and annual values for Zostera range from 5 gC/m² to 600 gC/m² (Phillips, 1974). Representative annual dry weight production values for eelgrass are 581 g dry wt/m² for Puget Sound, Washington (Phillips, 1974) and 10 - 58 g dry wt/m² for Great Pond, Massachusetts (Conover, 1958). Recent studies at Beauford, North Carolina (near the southern edge of Zostera's range on the Atlantic Coast of North America) indicate mean annual production of about 340 gC or about 690 g dry wt/m² (Dillon, 1971). Associated with the Zostera in these grass beds are Halodule and Ectocarpus which together contribute an additional 300 g C/m²/yr (Dillon, 1971).

Thus, on an areal basis, Thalassia and Zostera beds are more productive than the world averages for cultivated corn (412 g C/m²), rice (497 g C/m²), or the U.S. average for hay fields (420 g C/m²) or tall prairie grass (466 g C/m²) (Odum, 1959). These seagrass production rates are higher, on an area basis, than phytoplankton production in the upwelling area of Peru (Ryther, 1969). Seagrass production per se is supplemented in these communities,

or in estuaries in general, by the production of benthic microalgae, macroalgae, epiphytes, phytoplankton, and shore-based vegetation such as salt marsh.

Productivity rates from the literature are summarized in Table 5.4.

Table 5.4

Summary of Results From Marine Seagrass Research

SPECIES	LOCALITY	STANDING STOCK g dry/m ²		DENSITY No/m ²	PRODUCTIVITY			REFERENCE
		Range	Mean		gC/m ² /day	mgC/g dry/h	gC/m ² /yr	
1 <u>Cymodocea nodosa</u>	Mediterranean	13 - 342	-	-	5.4 - 18.7	0.82 - 2.9		Gessner and Hammer, 1960
2 <u>Halodule beaudettei</u>	Florida	-	-	-	-	1.81		Brylinski, 1971
	N. Carolina	105 - 200	200	-	0.48 - 2.0	-		Dillon, 1971
	Texas	22 - 250	93	15,118	-	-		McMahan, 1968
3 <u>Posidonia oceanica</u>	Malta	543 - 1072	742		2 - 5	0.21 - 0.52		Drew, 1971
4 <u>Syringodium isoetifolia</u> <u>Thalassia hemprichii</u>	Laccadives	-	-		5.81	-		Qasim and Bhattathiri, 1971
5 <u>Syringodium filiforme</u>	Texas	29 - 67	45	3,099	-	-		McMahan, 1968
6 <u>Thalassia testudinum</u>	Venezuela	-	1200		-	-		Gessner, 1971
	Cuba	-	400		-	-		Murina et al., 1969
	Cuba	30 - 690	390		-	-		Buesa and Olacchia, 1970
	Cuba	200 - 800	340		9.3 - 12.5	1.7 - 2.3	3000	Buesa, 1972
	Puerto Rico	-	-		2.4 - 4.5	-		Odum, et al., 1959
	Puerto Rico	538 - 7376	-		-	-		Burkholder et al., 1959
	Florida	-	-		3.13	-		Odum, 1956
	Florida	-	-		10.2	-		Odum, 1957
	Florida	-	325		-	-		Phillips, 1960
	Florida	20 - 400	-		5.7 - 16	2.3 - 6.3		Odum, 1963
	Florida	74 - 89	81		0.35 - 1.14	0.36 - 1.3		Pomeroy, 1960
	Florida	300 - 1800	835		0.9 - 2.5	0.13 - 0.25	200	Jones, 1968
	Florida	-	-		1.7 - 2.3	-		Zieman, 1968
	Florida	500 - 8100	3100		-	-		Bauersfield, et al., 1969
	Florida	-	-		-	1 - 3		Brylinsky, 1971
	Florida	-	-		2.8 - 14	-		Thorhaug and Stearns, 1973
	Texas	-	-		0.9 - 9.0	-		Odum and Hoskin, 1958
	Texas	-	3000		-	-		Parker, et al., 1963
	Bahamas	-	-		0.5 - 3	-		Patriquin, 1972
7 <u>Zostera marina</u>	Puget Sound				1.59		581	Phillips, 1974
	Massachusetts				0.16		58	Conover, 1958
	N. Carolina				0.93		340	Dillon, 1971

Section 6

DESIGN CONSIDERATIONS

J.C. Goldman and E.H. Wilson

Although all systems for the cultivation of aquatic plants have the same basic requirements (as discussed in Section 3), there are an infinite number of design alternatives depending on location and the species cultivated. A final system will ultimately be designed to minimize the input of monetary and energy costs while maximizing biomass output. An outline of those activities which involve the use of energy and materials is shown in Table 6.1 and some of the design considerations are discussed in the following sections. A more thorough review of design considerations will be presented in a final report prepared by Dynatech R/D Company on the cost analysis of open ocean and land-based systems for cultivating aquatic plants.

6.1 Light Availability

Whether the system is in the open ocean or inland the major objective in mass producing aquatic plants is to force light to become the limiting factor. Although light may be supplied artificially in land-based systems (Table 6.1), this option may be cost prohibitive on a large-scale and the systems would be limited to locations with sufficient incident solar radiation. In the open ocean, the plants would be totally dependent on natural sunlight for radiant energy.

The concepts discussed in this section are based on a model developed by Shelef et al (1968a) to describe the conditions under which yield optimization could be achieved in land-based outdoor mass algal cultures employing continuous flow. The two engineering parameters influencing the availability of sunlight were determined to be 1) depth of the photosynthesizing plant tissue and 2) the growth rate (or dilution rate) when it affects self-shading. Although this model applies equally well for microalgae and most seaweeds, its application to the kelp, which translocate photosynthetic products from the canopy to lower tissues, floating seaweeds and freshwater and marine angiosperms has not yet been established.

Table 6.1

The Manipulation of Natural Energy and Materials Through Activities
Involving the Use of Capital and Labor in an Aquatic Plant Aquaculture Enterprise
(modified from Neish, 1977)

ACTIVITIES INVOLVING THE USE OF CAPITAL AND LABOR	ORGANIC ENERGY AND MATERIALS			INORGANIC ENERGY AND MATERIALS	
	SEED STOCK	CONTAMINANTS DISEASES AND PESTS	WATER DISSOLVED NUTRIENTS AND GASES	SOLAR RADIATION	
				PHOTIC ENERGY	THERMAL ENERGY
1. Invest in capital equipment which transports and modifies natural energy and materials.	Enclosures, ponds, buildings, substrate, rafts and other containment materials.	Filters, sterilizers, and distribution of chemicals.	Water distribution system and equipment for fertilizer provision.	Artificial lights, reflectors and shades (to control weed growth).	Heaters, coolers, thermal effluent.
2. Purchase and apply consumable materials; purchase energy, materials and labor for maintenance and operation of equipment	Materials, energy and labor to maintain enclosures, substrate and suspension materials.	Filtration materials, pest control agents, water treatment materials, energy and labor for pest and containment control.	Energy to pump water; materials and labor to maintain distribution system.	Light bulbs, maintenance materials and energy to operate and maintain lights or shades.	Materials and energy to operate and maintain heat exchangers valves heating plants or refrigeration units.
3. Conduct applied biological research to develop crop control and management techniques.	Genetics, clone selection; population biology, ecology, phytosociology.	Pathology, microbiology, mycology, biochemistry, herbivore control.	Nutritional physiology	Physiology of photosynthesis and respiration.	Physiology.
4. Monitor crop and culture conditions control use of energy and materials to achieve optimal benefit:cost ratio	Control population structure by monitoring and regulating harvest rates, density distribution pattern, etc.	Monitor and control levels of pests and contaminants.	Regulate water flow and fertilizer rates to get optimal cost: benefit.	Control light intensity, quality and duration if possible.	Monitor and control water temperature.
CULTURED ALGAE BIOSYNTHESIZE THE DESIRED END PRODUCTS					
5. Harvest, process and market the yield; maximize efficiency or profitability by regulating aquaculture farm production to suit market structure to as great an extent as possible	Regulate harvest rate to maintain optimal population structure and density.	Integrate harvest regime with pest control levels to suit market demands.	Integrate harvest techniques and water management.	Manipulate access to light to "finish" the product before harvesting.	Manipulate temperature as an aid to "finishing" or storing end product.

As pointed out earlier, high photosynthetic efficiencies occur only with relatively low light intensities. This is because algal growth (or photosynthetic) rates are a function of light intensity up to a saturation intensity (I_s), and then remain constant at a maximum level ($\hat{\mu}$) as light intensity increases further (Figures 3.10 and 6.1). With high enough intensities inhibition can occur and growth rate actually begin to decline. Saturation light intensities have been shown to be species specific and somewhat temperature dependent. Typical I_s values for algae at 20 - 25°C vary from ~0.03 langley/min for freshwater and marine green algae (Ryther, 1956; Shelef *et al.*, 1968a) to ~0.07 and 0.16 langley/min for marine diatoms and flagellates, respectively (Ryther, 1956). In relationship to typical levels of peak visible solar radiation (0.6 - 0.8 langley/min), saturation levels are low, and, as a result, much of the light striking a water body is not used.

As light penetrates a water body it is absorbed and decreases in intensity exponentially with depth. The Beer-Lambert law,

$$\frac{I_x}{I_o} = e^{-\alpha Cx} \quad (1)$$

in which I_x is the light intensity at depth x , I_o is the incident light intensity at the surface of a water body, C is the concentration of material (in this case, plant material), and α is an extinction coefficient, all in appropriate units, is a good approximation of light extinction with depth. However, the value of the extinction coefficient is wave length - dependent and for natural sunlight the equation must be integrated over the visible wave length spectrum 400 - 700 mμ.

The actual efficiency of light absorption and utilization in photosynthesis at a given depth is dependent on both I_x (equal to $f(I_o)$) and I_s . As seen in the example of Figure 6.2, for two levels of visible incident radiation, 0.2 and 0.7 langley/min, and for a given I_s value of 0.05 langley/min, the actual solar energy that can be converted to plant material is represented by

Figure 6.1
Relationship Between Algal Growth Rate and Incident radiation
showing Saturation Radiation I_s

. Figure 6.2

Irradiance Decay in Algal Suspension as a Function of Depth

Showing the Relationship Between the Efficiency of Light

Utilization (E_s) and the Incident Irradiance (I_0)

(from Shelef et al., 1968a)

the hatched areas, ABEG for $I_o = 0.02$ langleys/min and ABFH for $I_o = 0.07$ langleys/min whereas the total energy available is respectively shown by the areas ACG and ADH. The actual efficiency of light utilization, E_s , at a particular depth is then:

$$E_s = \frac{I_s}{I_x} \left[1 + \ln \frac{I_o}{I_d} \right] \quad (2)$$

for $I_x > I_s$ and 1.0 when $I_s > I_x$.

Equation (2) is known as the Bush equation, and, although first presented in its complete form in the Carnegie Report (Burlew, 1953), it was formally derived by Shelef et al. (1968a). As seen in Figure 6.3, the value of E_s at a particular depth is very dependent on I_s at low light intensities and becomes increasingly less dependent as I increases. Hence, in very shallow cultures, diatoms should utilize light more efficiently because they have relatively higher I_s levels than green algae.

The actual productivity of light-limited algal cultures over a vertical water column can be expressed as:

$$P = C \int_0^d \mu_x dx = C \int_0^d f(I_x) dx \quad (3)$$

in which P is the areal production per unit time, C is the concentration of algae, μ_x is the specific growth rate at depth x , $f(I_x)$ is the relationship between specific growth rate and light intensity I_x and d is the full depth. Then:

$$P = C \int_0^d f(I_o e^{-\alpha C x}) dx \quad (4)$$

A number of different expressions for $\mu_x = f(I_x)$ (to describe the shape of the curves in Figures 3.10 and 5.1) exist, which were analyzed in detail by Van Oorschot (1955) and Shelef et al. (1968a). After lengthy mathematical deviations, Shelef et al. (1968a) presented the integrated models of

Figure 6.3

Efficiency of Light Utilization (E_s) and Total Light Conversion Efficiency (E_T)
as a Function of Incident Irradiance (I_0) at Various Levels
of Light Saturation Intensity (I_s)
(from Shelef et al., 1968a)

several expressions to account for wave-length dependent variations in both the quantum efficiency of photosynthesis and the extinction coefficient, and the variation of I_x with depth. For illustrative purposes the final form of one of the models, for monochromatic light, based upon a rectangular hyperbola expression:

$$\mu_x = \hat{\mu} \frac{I_x}{I_s + I_x} \quad (5)$$

is:

$$P = \frac{k}{J} E_m I_s \ln \left[\frac{I_s + I_o}{I_s + I_o e^{-\alpha Cd}} \right] \quad (6)$$

in which k is a time dimension constant (1440) to convert minutes to days, J is the heat of combustion of algae, usually taken as 5.5 kcal/g of ash-free weight, and E_m is the thermodynamic efficiency. The value of E_m is a function of the quantum number (usually taken as 8), the quantum efficiency, the heat content of algae, and the wave length. For a given wave length E_m is a constant.

A simple example will demonstrate the importance of the dilution (growth) rate μ and the depth d in controlling algal standing crop, and resulting yield P . Assume that k , J , E_m , I_s , and α are all known and that for a given level of I_o a combination of μ and d is desired to give a maximum P . Then P becomes a function of the product of Cd , the only two unknowns in the left side of the equation. A plot of P vs (Cd) using Equation (6) will have a parabolic-type shape with a maximum P at a unique value of Cd . Using this information in the equation:

$$P = \mu Cd \quad (7)$$

or,

$$\mu = \frac{P}{Cd} \quad (8)$$

the value of μ corresponding to the maximum P can be determined. Because in a continuous culture μ is equal to the dilution rate, which in turn, is equal to the flow rate/culture volume, it is clear from this example that the growth rate giving the maximum P is the major determinant of the overall dimensions of the system. The depth d , as seen by Equation (6), can vary and be used as an independent variable, as long as μ is chosen to give a maximum P ; the only effect will be on C , the concentration of algae (which will then be a dependent variable), so that the product of Cd remains a constant. Hence, once μ is chosen (thus also an independent variable) for a particular incident radiation I_0 , and the total quantity of algae required is known, the dimensions (area and depth) of an algal system can be chosen primarily on an economic basis.

In real engineering practice the use of P vs I_0 equations such as Equation (6) may be impractical for design purposes because of 1) the tremendous uncertainties in choosing proper numerical values for the many coefficients involved, 2) the need to integrate the equations over the visible portion of the light spectrum, and 3) the daily and seasonal variations in sunlight, which tremendously complicate the equations. They do have value, however, in providing some estimate of potential yields for given solar radiation levels, similar to Ryther's model (Figure 3.1), and they demonstrate which variables are important in attaining such yields. As seen in Figures 3.5, 3.6 and 3.11, the bell shaped curves of P vs μ attained in real systems, are precisely as predicted by Equation (6), and give some indication of the dilution rates required to produce peak yields.

The concepts applied in this section hold equally well for microalgae and seaweeds, as seen by the similar shapes of the curves in Figures 3.5 and 3.6. The only major difference is, whereas microalgal systems are complete flow-through systems with continuous harvesting, macrophyte systems, because of the large size of the plants, must be harvested at regular intervals. In this case C , the standing crop, becomes the independent variable and is chosen along with d to give a μ (then the dependent variable) corresponding to a maximum P . The interval of harvesting then must be chosen to keep the variation from the desired μ small, but within the economic constraints of the harvesting scheme employed.

From a practical standpoint, Ryther's P vs I curve (Figure 3.1) appears to predict reasonably well the attainable yield for a given daily solar radiation intensity as evidenced by the results of previous studies (Sections 4 and 5). With respect to the choice of growth rate, the rather narrow range of dilution rates ($\sim 0.5 - 1.0/\text{day}$) for which peak yields have been attained during summer months in widely scattered locations (Jerusalem, Israel, Woods Hole, Massachusetts, Ft. Pierce, Florida and Richmond, California) (Figure 3.11) is not coincidental, probably having occurred because during summer months in these latitudes (range $\sim 27 - 42^\circ\text{N}$) maximum possible solar radiation levels, as seen in Figure 3.2, reach a common peak value of ~ 770 langleys/day. Actual levels as seen in Figure 3.3, are lower, but probably of about the same magnitude for the different locations. In addition, these high radiation levels represent the flat asymptotic portion of P vs I curves and variations of even $100 \pm$ langleys/day solar radiation would only change theoretical peak yields by $1 - 2 \text{ g/m}^2/\text{day}$ (Figure 3.1).

Hence, assuming that any large-scale plant system were to be located in a fairly low latitude to take advantage of high yearly solar radiation levels, Ryther's curve together with the solar radiation information shown in Figure 3.4, would provide a reasonable estimate of the maximum attainable yield for a given location in the United States. The dilution rate to achieve this yield would probably be between $0.5 - 1.0/\text{day}$, based on available experimental data. Thus, the only remaining design parameter would be the depth. Although the depth can vary without altering yield, typical values for depth vary between $15 - 70 \text{ cm}$ (see Table 6.2). A median value of $40 - 50 \text{ cm}$ would probably represent a reasonable depth for design purposes. Hence, with I_0 , P, μ , and d estimated or established the only dimension to be calculated is the total area, which is solely a function of the total production of biomass desired.

6.2 Nutrients Availability

Because algae require, in addition to light, carbon dioxide and other nutrients, primarily nitrogen and phosphorus, these materials must always

Table 6.2

Design Parameters Used in Recent Outdoor Mass Culture Studies

Location	Species	Culture Size m ²	Depth cm	Mixing Speed cm/sec	Reference
Woods Hole, Mass.	Marine diatoms	180	70	? (intensive)	Goldman and Ryther (1976)
Ft. Pierce, Florida	Marine diatoms		50	? (gentle)	Goldman <u>et al.</u> (1975)
Ft. Pierce, Florida	Seaweeds	0.3	20	? (bubbling)	Ryther <u>et al</u> (1977)
Haita, Israel	Freshwater greens	150	45	5 - 10	Shelef (1976)
Beersheva, Israel	Freshwater greens	1	25	15 - 40	Richmond (1976)
Dortmund, Germany	Freshwater greens	80	12 - 15	50	Stengel (1970)
Richmond, California	Freshwater greens	12	30	5 - 20	Weissman <u>et al.</u> (1977)
Taiwan	Freshwater greens	500	15	4	Shurtleff (1977)

be supplied in excess. The requirements for these nutrients occur in a stoichiometric fashion, based to a large extent upon the growth rate of the cells (Goldman, 1977). Typically, the chemical composition of healthy cells is ~50% carbon, 5 - 10% nitrogen and <1% phosphorus. In addition, trace quantities of heavy metals and, for some algal species, vitamins are required. For diatoms, in addition to the above nutrients, silica at about the same concentration as nitrogen is necessary. Other macro nutrients such as oxygen, hydrogen, potassium, sodium, calcium, sulfur, and chloride are also required, but, because they are usually plentiful in fresh and marine waters relative to the requirements of aquatic plants, are not usually limiting factors.

The potential sources of nutrients are outlined in Table 6.3. Numerous research studies have shown that nutrients may be supplied by coastal waters (Ryther and Dunstan, 1971), by natural and artificially upwelled deep water (Barber and Ryther, 1969, 1971; Haines, 1975; Langton et al., 1977; North and Wheeler, 1977), by wastewater (Benemann et al., 1977a, 1977b; Dunstan and Tenore, 1972; Goldman and Ryther, 1975; Oswald, 1970; Prince, 1974; Smith, 1977; Waite and Mitchell, 1972a, 1972b; Weissman et al., 1977), and by artificial media (Provasoli et al., 1957; Cheng, 1969; Tamura, 1970).

Coastal waters are usually not sufficiently high in nutrients, particularly nitrogen and phosphorus, to support high yields (Ryther, 1977) and may limit seaweed production at least part of the year (Dawes et al., 1974a; Topinka and Robbin, 1976; Jackson, 1975). Extra nutrients in coastal waters may be the result of direct wastewater discharges, agricultural run-off, or a natural upwelling. In Puget Sound the nutrients are sufficiently high to support good growth (Mumford, 1977a, 1977b) and adding nutrients does not increase growth (Waaland, 1976, 1977). Wastewater is sufficiently high in nutrients to sustain good growth rates, but the removal of viruses and detrimental heavy metals presents a major problem.

The concentration of nitrates and phosphates increases significantly from low surface concentrations to maximal values at depths of 800 to 1000 meters deep. Although nitrate concentrations in the San Diego Point, California, kelp

Table 6.3

Potential Nutrient Sources for Mass Algal Culture
(from Oswald, 1977)

Sources of Water & Nutrients	Required Supplementary Nutrients	Potential Use*
Man-made defined algal nutrient solutions	none	human food, pigments, pharmaceuticals
Potato processing wastes	none	human food, pigments, pharmaceuticals
Sugar, tomato, winery wastes	N & P	human food, pigments, pharmaceuticals -- after heat drying and pasteurization
Natural bodies of water, brackish or fresh	N & P	human food, pigments -- after pasteurization
Springs & wells, including geothermal waters	N, P, C	human and animal food -- may not require pasteurization
Irrigation & land drainage	N,P,Fe	animal feed -- after pasteurization
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
Storm waters	C,N,P,K	animal feed -- after pasteurization

*subject to toxicological and epidemiological evaluation

beds are generally low (less than $1 \mu\text{g-at/l NO}_3\text{-N}$) (Jackson, 1976). during periods of natural upwelling the concentration can be as high as $5.8 \mu\text{g-at}$ near the surface (Lindner et al., 1976). The occurrence and dynamics of natural upwelling has been reviewed by Smith (1968).

The development of a precisely defined medium that supplies exact amounts of various substances required by a species is a major undertaking. Culturists find that a medium suitable for a given organism frequently suffices for closely related organisms or even for distantly related species. Such media may contain excess amounts of certain elements for the particular organism being cultured, but the excesses are usually not harmful. It is far simpler to use general formulae that yield satisfactory results than to develop laboriously a medium tailored precisely to the needs of a given species. Table 6.4 lists media that are generally used for growing seaweeds and are often identified by the name of the investigator who first prepared them. Table 6.5 lists media that are used for microalgae.

In land-based systems control of the pH problem discussed in Section 3.5 is usually accomplished by adding CO_2 -enriched air to the culture. In marine systems seawater is well-buffered with inorganic carbon (typically 25 mg/l) so that aeration with air alone can usually suffice, as has been demonstrated in the Woods Hole and Ft. Pierce seaweed studies previously described.

The addition of CO_2 is normally carried by sparging CO_2 -air mixtures into solution. Various techniques have been employed, but no standard method exists. The use of pH-stat systems to establish a range of desired pH levels works well in small-scale experimental cultures, but have the disadvantages of being expensive, prone to problems of probe-fouling, and may not be effective in very large-scale cultures in which long lag periods can exist before CO_2 is distributed to the entire culture, resulting in spatial variations in pH.

Table 6.4

Compositions of Various Media Used by Algal Culturists, Compared with Elementary Concentrations
in Seawater and Concentration Ranges Found in Marine Plants

Chelating and buffering agents were omitted as is SiO_2 (added to culture diatoms).

Values represent mg/l.

Substance	Conc. in Seawater	Range in Seaweeds	Miller & Fogg	Provasoli ASP2	Provasoli ES	Lewin	Hoagland AZ	IMR	Gullard f2
B	4.6	30 - 240	0.2	6.0	6.9	0.11	0.08		
N	0.5	90 - 1100	280	8.2	2.1	126		7	12
Al	0.01	45 - 150					0.07		
P	0.07	6 - 160	37	0.9	0.8	31		1.5	1.1
Mn	0.002	0.7 - 960	0.2	1.2	0.013	0.14	0.16		0.05
Fe	0.01	24 - 120	1.1	0.8	0.5	0.19		1	0.65
Co	0.0001	0.1 - 10	0.02	0.003	0.0003		0.12	0.61	0.0025
Ni	0.002	0.1 - 14					0.13		
Cu	0.003	0.2 - 4	0.02	0.0012		0.13	0.07	0.03	0.0025
Zn	0.01	4 - 90	0.02	0.15	0.0017	0.33	0.23	0.11	0.005
Mo	0.01	0.06 - 0.3	0.2			0.1		0.63	0.0025
I	0.06	6 - 2600					0.39		
Br	65	20 - 480					0.37		
Li	0.17	present					0.09		
Sn	0.0008	0.21					0.27		
Ti	0.001	0.3 - 4.5					0.42		
Vit. B ₁₂				0.002	0.002			0.001	0.5
Thiamine				0.5	0.1			0.001	0.1
Biotin				0.001	0.001			0.001	0.05
Nicotinic acid				0.1					
Ca-pantothenate				0.1					
Para-amino benzoic acid				0.01					
Inositol				5					
Folic acid				0.002					
Thymine				3					

Table 6.5
Defined Media for Growth of Various Genera of Microalgae
(from Oswald, 1977)

Chemical Required in Milligrams per liter of Pure Water	1 <i>Chlorella</i> and <i>Scenedesmus</i>	2 <i>Scenedesmus</i>	3 <i>Euglena</i>	4 <i>Anabaena</i>	5 <i>Oscillatoria</i>	6 <i>Spirulina</i>
H ₃ BO ₃		6.2		0.5	0.25	5.72
CaCl ₂ · 2H ₂ O	0.58	14.6		36.0	18.0	
CaSO ₄ · 5H ₂ O						53.0
Ca(NO ₃) ₂			40			
CO(NH ₂) ₂		600.0				
Co(NO ₃) ₂ · 6H ₂ O				.01	0.005	
Co(SO ₄) ₂ · 7H ₂ O		2.8				
Cr ₂ K ₂ (SO ₄) ₄ · 24H ₂ O				.01	0.005	
CuSO ₄ · 5H ₂ O		2.5		.02	.01	.098
FeCl ₃ · 6H ₂ O	3.00		.08			
Fe(NH ₄) ₂ SO ₄ · 6H ₂ O						9.5
FeSO ₄ · 7H ₂ O				4.98	2.49	
Fe ₂ (SO ₄) ₃ · 9H ₂ O		28.1				
K ₂ HPO ₄	250.0	680	10.0	39.0	20.0	
K ₂ SO ₄						1000.0
MgSO ₄ · 7H ₂ O		1976.0	25.0	75.0	37.0	200.0
MnCl ₂ · 4H ₂ O	513					3.62
MnSO ₄ · 4H ₂ O		2.0		0.5	.25	
MoO ₃				0.1	.05	
NaCl						1000.0
Na ₂ CO ₃			20.0	20.0	10.0	
Na ₂ EDTA				6.34	3.17	8.85
NaHCO ₃						16800.0
Na ₂ HPO ₄						200.0
Na ₂ MoO ₄ · 2H ₂ O						.78
NaNO ₃	1000				200.0	2500.
NaSiO ₃ · 9H ₂ O			25.0			
(NH ₄) ₂ MoO ₄		1.96				
NH ₄ NO ₃		800.0				
ZnSO ₄ · 7H ₂ O		2.9		.05	.025	.44
Soil Extract			Req.			
Reference	Chu ²⁵	Jarka ²⁶	Chu ²⁵	Burman ²⁷	Burman ²⁷	Soeder ²⁸

The media describe for *Chlorella* and *Scenedesmus* (column 1) may also be initially include .05 gram NH₄Cl, 1.0 gram Na acetate, 2.0 gram tryptone, and 1.0 gram yeast extract per liter if accelerated growth is desired.

For *Scenedesmus* (column 2 above) the micro-elements Mn, Cu, Zn, Mo, Co, and Ca are chelated with EDTA. Iron must be chelated separately as an Fe-EDTA complex. The pH value is to be made to 6.5-7.0 by means of NH₃. Excess EDTA is required for water with high Ca and/or Mg.

All algae exhibit satisfactory growth on the described media, with the exception of young *Euglena* (*gracilis*) (column 3 above) which should be grown initially in an acetate-tryptone-yeast broth for algal flagellates prepared by dissolving 1 gram of sodium acetate · 3H₂O; 2.0 grams of tryptone (Difco) and 1 gram of yeast extract in a liter of glass distilled water. After cultures are growing vigorously, the media in column 3 above can be slowly substituted.

Mixing can, to some degree, enhance CO₂ transport from the atmosphere, but, because of the very low concentration of CO₂ in the atmosphere (0.03%), the transport gradient is always small and CO₂ transport is ineffective unless very turbulent mixing is employed.

However, mixing is required for several other reasons: to prevent settling and subsequent decay of organic matter, to prevent thermal stratification, to break down diffusion gradients of essential nutrients which could develop at the cell surface in intense mass cultures (this is particularly true with seaweeds), to prevent epiphyte buildup on the surface of the plants, and to provide uniform cell exposure to light. With respect to the latter factor, claims have often been made that mixing in mass algal cultures enhances the "flashing light effect", a biochemical phenomenon by which photosynthetic rates of an algal cell are increased when the cell is transferred back and forth from light to dark in a time sequence corresponding to the light and dark biochemical reactions. Such effects have been produced in the laboratory with corresponding increases in photosynthetic rates (Miller *et al.*, 1964; Shelef *et al.*, 1968b). However, Powell *et al.* (1965) have shown that the random dispersion of cells due to mixing alone (even turbulent mixing) prevents the "light-dark" sequence to be continuous for an individual cell in the time periods required; hence, mixing alone cannot result in increased photosynthetic rates through the "flashing light effect."

The actual degree of mixing required in outdoor cultures to avoid the aforementioned problems is not well-defined. Oswald (1976) suggested that flow velocities of 5 cm/sec. with a periodic increase for short periods to 30 cm/sec. in his meandering channel design was adequate to control thermal stratification and settling with microalgae. Yet in recent studies it was shown by his group that increased mixing up to 20 cm/sec led to increased productivity. As seen in Table 6.2, mixing speeds varying from 5 - 50 cm/sec have been used. Unfortunately, scant experimental data is available on mixing effects in large scale cultures, and considerable future research in this area is required.

6.3 Selection of Feedstock

The selection of a fast-growing high-yielding species of aquatic plant is one of the most significant steps that can be made toward establishing economically useful mass cultivation systems. If the biology of the species is well-understood strains of that species with desirable characteristics can be genetically developed.

Mass culture technology of marine macroalgae has arisen around two distinctly different culture strategies: 1) systems in which perennial, sterile clones are propagated vegetatively and 2) systems in which plants are propagated from spores produced by various life stages of the cultivated species. The development of clones results from simple selective screening programs in which the most desirable plants are propagated and observed until they provide sufficient biomass for seeding. Unfortunately, a potentially good clone can be lost if it fruits, as in the case of some Eucheuma strains. Since unanticipated reproduction can be potentially disastrous, extensive research is needed to understand the processes which induce fruiting (Neish, 1977).

Selective breeding and manipulation of physical growth parameters have been particularly advantageous to the phycocolloid industry for developing species which have a high carrageenan content. If aquatic plants are to be used for the production of methane, those species which have a great methane production potential should be identified and strains with desirable characteristics developed. The cultivation system could then be designed to meet the requirements of the plants.

Section 7

INFORMATION SOURCES

R. Waaland and E.H. Wilson

The following list of key research workers, industrial concerns and professional societies and conferences provides a guide to recent activity in aquatic plant aquaculture both domestic and foreign. Research activity, which is primarily centered in academic laboratories, involves studies on the ecology and management of aquatic plant species and techniques for the cultivation and processing of freshwater and marine microalgae and macrophytes. Industrial concerns deal with the cultivation of aquatic plants for the production of food (in Oriental countries), phycocolloids, fertilizers or biomass for fuel.

7.1 Key Research Workers, Institutions, and Specialties

Abeliovich, Aharon, The Institute for Desert Research, Ben-Gurion University of the Megev, P.O. Box 2053, Ein Gedi Bldg., Sede Boqer Campus, Beersheva 84120, Israel. Culture of Spirulina and Chlorella.

Adams, R.W. Dept. of Biology, University of Victoria, Victoria, B.C., Canada V8W 2Y2. Cultivation and ecology of Iridaea cordata.

Austin, A. Dept. of Biology, University of Victoria, Victoria, B.C. Canada V8W 2Y2. Cultivation and ecology of Iridaea cordata.

Barilotti, C. Kelp Ecology Project, San Diego State University, P.O. Box 908, Solano Beach, California 92075. Kelp and Gelidium ecology and management.

Ben-Amotz, Ami, Israel Oceanographic and Limnological Research Ltd., Marine Botany, P.O. Box 1793, Haifa, Israel. Culture of Dunaliella.

Benemann, John, Sanitary Engineering Research Laboratory, College of Engineering and School of Public Health, University of California, Berkeley, California. Mass culture of microalgae.

Braud, J.P. I.S.T.P.M., B.P. 1049, 44037 Nantes, France. Farming Eucheuma spinosum in Djibouti.

Bridges, Kent, Department of Botany, University of Hawaii, Honolulu, Hawaii 96822. Seagrass ecology.

- Bryce, Armond J. General Electric Co., Re-entry and Environmental Systems Division, 751 Vandenburg Drive, King of Prussia, Pennsylvania 19406. Kelp biomass production and conversion.
- Chapman, A.R.O. Dept. of Biology, Dalhousie University, Halifax, N.S., Canada Laminaria genetics.
- Chapman, D.J. Dept. of Biology, University of California, Los Angeles, California 90024. Chemostat culture of Scenedesmus.
- Cheney, D.P. Jackson Estuarine Laboratory, University of New Hampshire, Durham, N.H. 03824. Chondrus biology, ecology and genetics.
- Clement, Genevieve, Institut Francais du Petrole, B.P. 311, 1 et 4 avenue du Bois Breau, 92506 Rueil Malmaison Cedex, France. Mass culture of Spirulina in southern France, Algeria, Egypt, Taiwan, West Indies, and Mexico.
- Coleman, Victor, Sandia Laboratories, Albuquerque, New Mexico. Fresh-water algal production.
- Cosson, J. Laboratoire d'Algologie Fundamentale et Appliquee, University de Caen, 39 rue Desmoueux, 14000-Caen, France. Kelp gametophyte culture.
- Craigie, James S. Atlantic Regional Laboratory, National Research Council of Canada, Halifax, N.S., Canada. All aspects of marine algal cultivation, biology, ecology, and biochemistry.
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6640 N.W. Marine Drive, Vancouver, B.C., Canada V6T 1X2. Kelp
and Gracilaria growth and chemistry.

Wilson, Kenneth C. California Dept. of Fish and Game, State Fisheries
Laboratory, 350 Golden Shore, Long Beach, California 90802. Kelp
ecology and management.

Zieman, Jay C. Dept. of Environmental Science, University of Virginia,
Charlottesville, Virginia 22093. Seagrass ecology.

7.2 Industries Active in Plant Aquaculture

Alginate Industries, Ltd. (U.K.). Gwan-Ayrshire, London, England.
(Dr. K. Clare, Chief Chemist).

American Gas Association, 1515 Wilson Blvd., Arlington, Virginia 22209.
(Dr. Ab B. Flowers). Culture of kelp for producing methane.

CECA, 11 Avenue Morane Saulnier, Velizy-Villacoublay, Yvelines 78140.
Subsidiary of Pierrefitte Aubry, Nevilly-sur-Senine, Haut-Sen,
France. (Mr. Louis Marvier, President). Cultivation of Eucheuma
for phycocolloids.

Copenhagen Pectin Factory, Ltd., D.K. 4623 Lille Skenud, Denmark.
(Mr. Keld Nielsen).

General Electric Co. Re-entry and Environmental Systems Division, 751
Vandenburg Drive, King of Prussia, Pennsylvania 19406. (Dr. Armond
J. Bryce, Technical Director, Marine Biomass Program). Kelp culti-
vation and methane production.

Global Marine Development, Inc. P.O. Box 3010, Newport Beach, Califor-
nia 92660. (Mr. W. Wallerstedt). Selection of site for quarter-
acre module kelp farm.

Kelco Co., 8355 Aero Drive, San Diego, California 92123. Cultivation
of kelp for phycocolloids.

Marine Colloids, Inc., P.O. Box 308, Rockland, Maine 04841. (Dr. Harris
J. "Pete" Bixler, President). Cultivation of seaweeds for phycocolloids.

Stauffer Chemical Co. (Mr. Bela Szelenyi and Dr. H. Reisman). Commercial
kelp harvesting, uniform raw kelp supply, kelp process residue
supply.

Oriental Firms. Much algal cultivation in Asia is performed on small
family farms. The harvest is usually sold to buyers from larger
trading companies.

7.3 Professional Societies and Conferences

International Phycological Society. Secretary: Dr. Sylvia Earle, California Academy of Sciences, Golden Gate Park, San Francisco, California 94118. Annual meetings.

International Seaweed Symposium. Held every three years. Publishes a proceedings. North American members of the advisory committee are:

Dr. M.S. Doty. Dept. of Botany, University of Hawaii, Honolulu, Hawaii.

Dr. J. McLachlan. Atlantic Regional Labs, National Research Council of Canada, Halifax, Nova Scotia, Canada.

Phycological Society of America. Secretary: Dr. Elenor R. Cox, Dept. of Biology, Texas A & M University, College Station, Texas 77843. Annual meetings.

World Mariculture Society. Holds annual meetings. Publishes a proceedings. Membership list includes many academic and business personnel active in aquaculture. Emphasis is on animal aquaculture. Proceedings available from: Louisiana State University, Division of Continuing Education, Baton Rouge, Louisiana 70803.

Section 8

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