FINAL REPORT

on

RESOURCE ASSESSMENT FOR MICROALGAL/ EMERGENT AQUATIC BIOMASS SYSTEMS IN THE ARID SOUTHWEST

to

SOLAR ENERGY RESEARCH INSTITUTE

December 23, 1982

by

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TABLE OF CONTENTS

Page

I. INTRODUCTION 1

Emergent Aquatic Plants in the U.S. Southwest . . 3
Production System Assumptions 4

II. HYDROLOGY/WATER QUALITY 6

Criteria Development 6

Water Supply and Demand 6
Water Quality 15

Data Sources 23

Arizona 23
California 24
Permit Information 25
Colorado 25
Nevada 26
New Mexico 26
Texas 27
Permit Information 27
Utah 27

References 28

III. LAND AND CLIMATE RELATED FACTORS 29

Soil Characteristics 29

Slope Topography 30
Temperature and Moisture Regime 30
Soil Depth 31
Soil Permeability 32

Land Ownership/Availability 33

Category of Land Ownership 34
Land Sale and Leasing Policies 35
Barriers to Availability 35
Evaluation of Relative Availability 35

Land Use/Cover 36

Land Use/Cover Category 36

Solar Radiation 37

TABLE OF CONTENTS
(Continued)

	Page
Temperature	38
Annual Normal Daily Minimum Temperature	39
Annual Normal Daily Average Temperature	39
Annual Normal Daily Maximum Temperatures	39
Annual Average Daily Evaporation Pan Temperature	40
Severe Weather	40
Annual Average Total Snowfall	40
Annual Average Total Precipitation	41
Mean Annual Number of Days With Thunderstorms	41
Mean Annual Numbers of Days With Hail	41
Mean Annual Numbers of Days With Tornadoes	42
Mean Average Daily Wind Velocity	42
Fastest Mile Wind Speed	42
10-Year Maximum 6- and 24-Hour Precipitation Events	43
References	43
IV. CLASSIFICATION OF AVAILABLE AQUATIC SPECIES	44
Species Description	45
Marine and Halophilic Species	45
Freshwater Species	55
Other Species	59
Criteria for Selecting Microalgae	61
Culture Media	61
Carbon Source	62
Illumination	62
Temperature	63
pH	63
Salinity	64
Physiological Responses of Algae	65
Summary	65
Information Needs	66
References	68
V. TECHNO-ECONOMIC CONSTRAINTS	71
Carbon Sources for Algal Biomass Systems	71
Gaseous CO_2	71
Carbonate Minerals	72
Problems and Limitations	73

TABLE OF CONTENTS
(Continued)

	Page
Water Supply Costs	74
Seawater Systems	76
Groundwater Systems	78
References	81
VI. RESEARCH NEEDS	84
Fatal Flaw Criteria	85
Species Selection	85
Other Limiting Factors	88
Carbon Dioxide Supply	89
Water Supply	90
Temperature	92
Discretionary Criteria	92
Algal Species	92
Land and Climate Resources	93
Water Resources	95
Fundamental Research Needs	96
Carbon Supply	96
Control of Medium Composition	98
Effect of Nutrient Salt Addition	99
System Models	100
Field Test Sites	101
Integrated System R&D Opportunities	102
Summary of Criteria and Research Needs	105
References	105

TABLE OF CONTENTS
(Continued)

LIST OF TABLES

		Page
TABLE II-1.	Hazardous and Minimum Risk Criteria Concentrations of Toxins for Protection of Marine Biota	22
Table II-2.	Macronutrient Concentration Criteria	23
Table IV-1.	Physico-Chemical Conditions of the Great Salt Lake	47
Table IV-2.	Selected Parameters of Pyramid Lake, Nevada	48
Table IV-3.	Selected Parameters of Borax Lake	48
Table IV-4.	Trace Elements of More than 1 mg l^{-1} in the Great Salt Lake	49
Table IV-5.	Trace Elements of Less Than 1 mgl^{-1} in the Great Salt Lake	49
Table IV-6.	Selected Parameters of the Dead Sea	51
Table IV-7.	Chemical Components of *Phaeodactylum Tricornutum*	53
Table IV-8.	Chemical Composition of *Spirulina Maxima* Meal	55
Table IV-9.	Information Needs for Microalgae Species Potentially Useful for Aquatic Biomass Systems	67
Table V-1.	CO_2 Requirements and Cost for Microalgal Systems	75
Table V-2.	Water Supply Cost Analysis - Seawater Systems	79
Table V-3.	Parametric Analysis - Well Field (Saline Groundwater) Systems	82
Table VI-1.	Summary of Research Needs on Biomass Systems	107

TABLE OF CONTENTS
(Continued)

LIST OF FIGURES

		Page
Figure I-1.	Study Area for Microalgal Biomass Systems	2
Figure I-2.	Components of a Large-Scale Microalgal Culture System	5
Figure II-1.	Salt Concentrations in Microalgal Systems as a Function of Time	11
Figure V-1.	Water Supply Cost Analysis for Seawater Systems . .	80
Figure VI-1.	Integration System R&D Opportunities	103
Figure VI-2.	Criteria for Ideal Culture System/Location	106

Final Report

on

Resource Assessment for Microalgal/ Emergent Aquatic Biomass Systems in the Arid Southwest

to

Solar Energy Research Institute

Battelle
Columbus Division

December 23, 1982

I. INTRODUCTION

This research project has been designed to facilitate the eventual selection of biomass production systems using aquatic species (microalgal and emergent aquatic plant species [MEAP]) which effectively exploit the potentially available resources of the Southwest. In cooperation with RRAIB (SERI's Renewable Resources and Instrumentation Branch), five specific subobjectives were addressed:

- Criteria development for identification of available water resources, their relative importance to siting, and use constraints
- Criteria development for identification of available land resources, their relative importance to siting, and use constraints
- Compilation of microalgal growth and compositional characteristics
- Identification of techno-economic constraints to commercialization
- Identification of and prioritization of research needs.

Attention was limited to applications in the southwestern desert areas, as shown in Figure I-1.

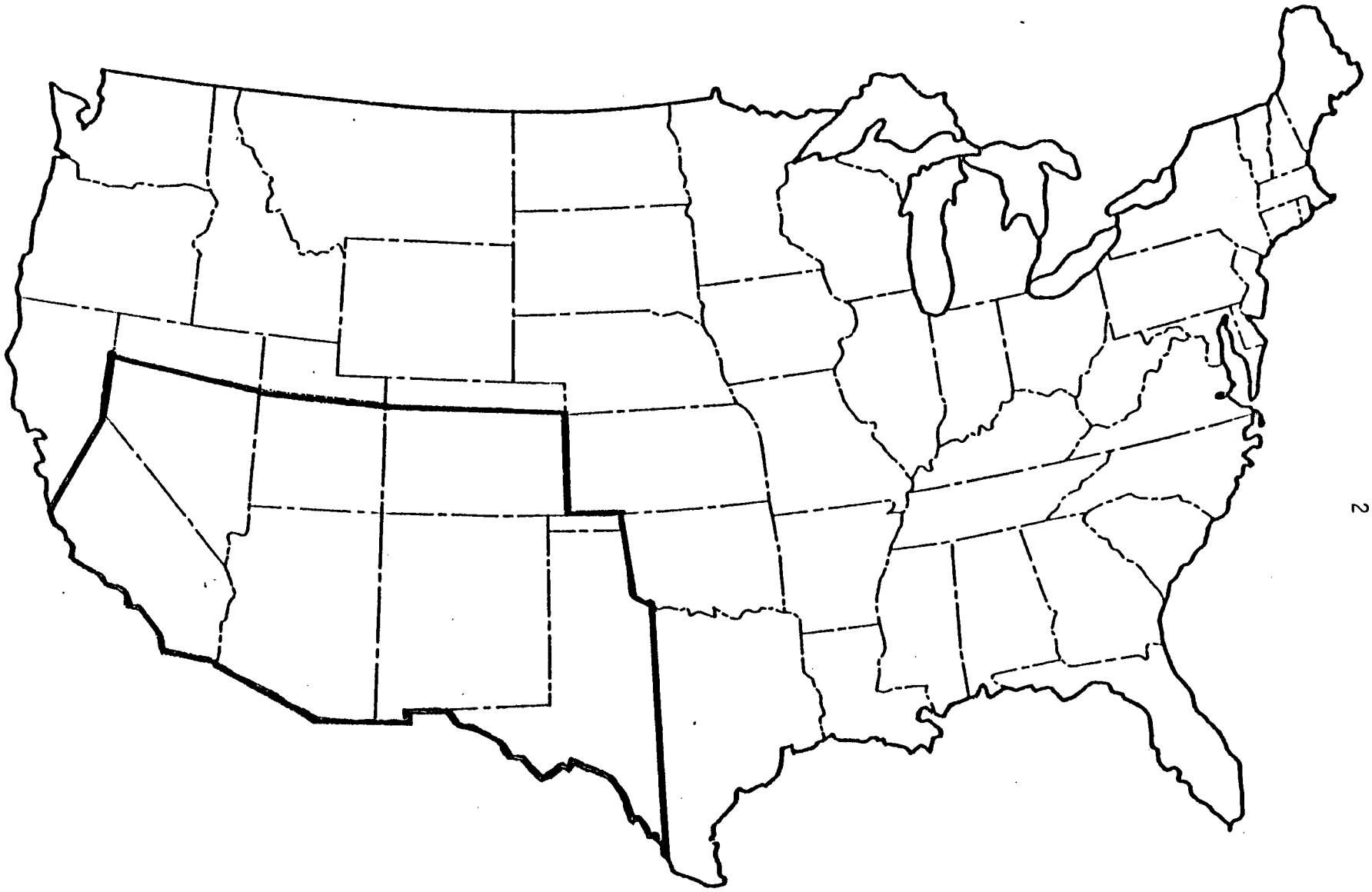

FIGURE I-1. STUDY AREA FOR MICROALGAL BIOMASS SYSTEMS

(S of 40° latitude, W of 100° longitude (U.S. only).

Emergent Aquatic Plants in the U.S. Southwest

The scope of this research program originally included both microalgae and emergent aquatic plants. Early in the program, the relative merits of these alternative biomass resources were evaluated by the project team. The considerations presented below render emergent aquatic species so unpromising for production in the U.S. Southwest that further research on emergent aquatic species in this program was terminated.

Emergent aquatic species biosynthesize such carbohydrates as cellulose, hemicellulose, and starch. These carbohydrates are already abundant in other biomass resources that are plentiful in the more humid areas of the United States. Cellulose and hemicellulose are abundant in trees and agricultural residues. Starch is plentiful in corn, wheat, and other cereals. The economic attractiveness of growing emergent aquatics as a source of such readily available, cheap products is exceedingly low.

Emergent aquatic plants do not biosynthesize much lipid. Typical lipid contents are in the range of 1 percent to 3 percent, far below soybeans (20 percent), sunflowers (44 percent), or peanuts (50 percent). It is also far below the lipid contents of the microalgae species reported elsewhere in this document.

Emergent aquatic plants are not noted for other chemical constituents that would be attractive for their potential chemical or fuel applications. Emergent aquatic plants are not known for their content of terpenes or alkaloids, for example.

With this pattern of chemical composition, there is not a strong motivation for growing emergent aquatic plants in the U.S. Southwest. The types of products that microalgae biosynthesize that have commercial potential, such as glycerin, beta-carotene, novel triglycerides, and glycolic acid, are notably absent from emergent aquatic plants. Instead, they represent a redundant resource containing almost exclusively cellulose, hemicellulose, and some starch.

In addition to the chemical composition problems, emergent aquatic plants also grow in environments that are relatively unusual in the American

Southwest. These plants are best adapted for growth in wetlands, such as are prevalent in Minnesota and Louisiana. These environmental conditions are rare to nonexistent in the American Southwest.

Given these two major problems with emergent aquatics (chemical composition and lack of growth sites), both the project team and the SERI program managers concurred that the research effort should be directed toward the microalgae.

Production System Assumptions

No specific production system designs were identified for consideration. Therefore, to define construction and operating characteristics, assumptions were made concerning the nature of microalgal production systems identified in the literature (Hollaender, 1979). The characteristics exhibited by these systems span the range from

- Open uncovered to enclosed and rigidly covered
- Pond-type to channelized-type
- Agitated or non-agitated
- Construction from native soil materials to engineered plastics or concrete
- Operating depths of 1 meter or less.

Based on an analysis of the economies of scale of processing of harvested materials for chemical feedstocks, it was determined that an appropriate size for commercial development would be 1000 acres (405 hectares) (Blakesley, 1982). Biomass produced for purposes other than chemical extraction could involve systems smaller than that used here and, under favorable resource conditions, it may be economical to exceed 1000 acres in size. Components of a generic microalgae growth and processing system are illustrated in Figure I-2.

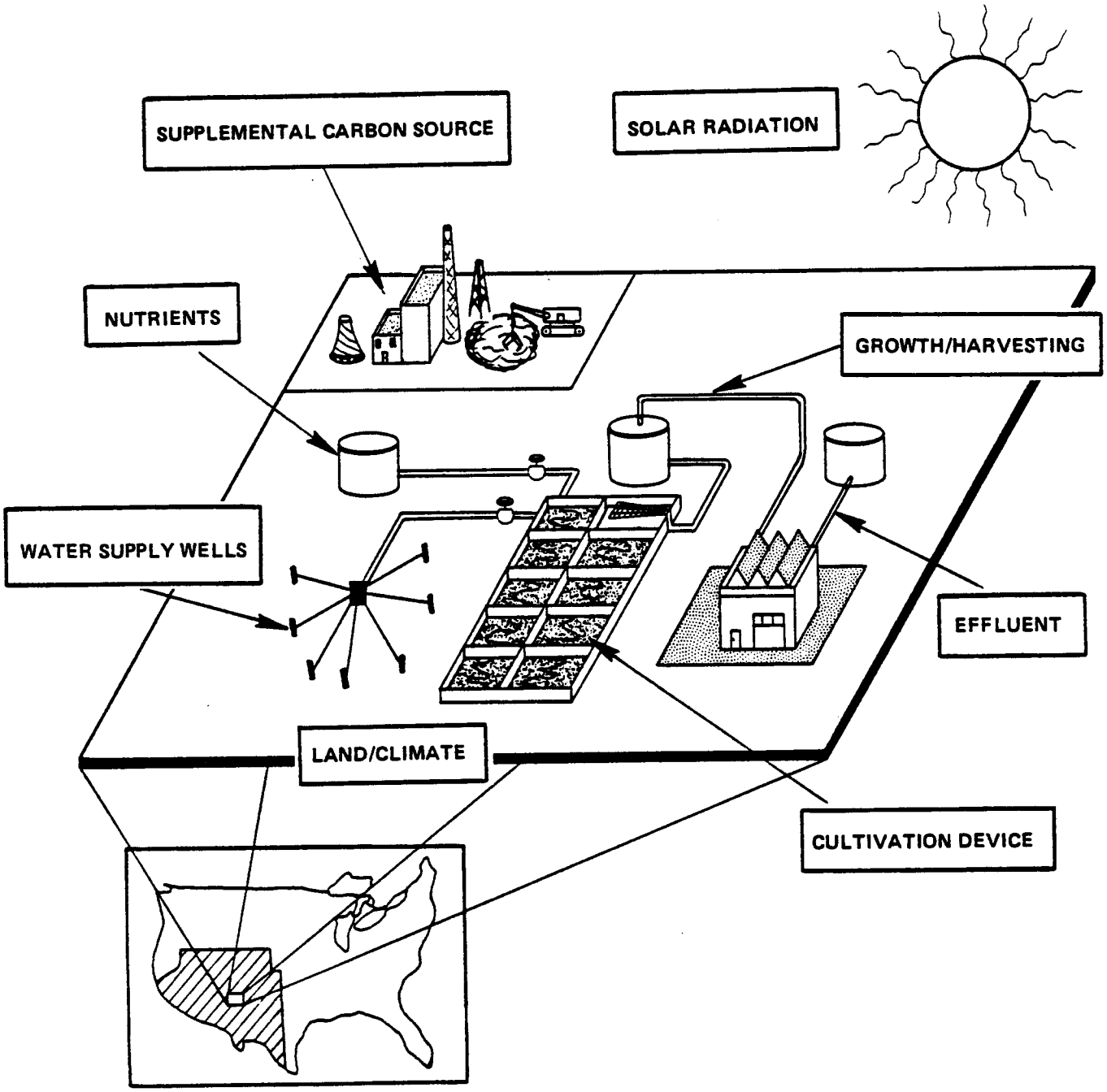

FIGURE I-2. **COMPONENTS OF A LARGE-SCALE MICROALGAL CULTURE SYSTEM**

II. HYDROLOGY/WATER QUALITY

Criteria Development

Water Supply and Demand

Water in sufficient quantity is needed to supply the metabolic and non-metabolic needs of microalgae systems. The principal water demands will be the initial filling of the system and the utilization of water by the crop species.

Water demands to offset evaporation or seepage could also be significant, depending on the manner in which the systems are engineered. In the arid southwestern U.S., evaporation greatly exceeds precipitation and, water losses will be significant over the course of a year, unless the systems are totally enclosed.

Three potential sources of water for microalgal systems have been identified--coastal seawater, irrigation return flow and groundwater. Since the available species span a considerable range of tolerance in salinity and other water quality characteristics, any of the three sources of water could be matched with an appropriate species of algae.

In coastal areas seawater could be pumped ashore and used in the systems. Concerns which would have to be addressed for this water source include:

- Economics of water supply as a function of distance and elevation changes
- Prevention of contamination of near-surface or surface fresh water resources due to brine disposal (see below).

Water supply economics for this source are described in Section V as a barrier to commercialization of the technology.

In areas where irrigated agriculture is prevalent, return flows may be marginally too saline for effective use on terrestrial crops. In theory, this water is ideally suited for reuse in algal culture systems. However, in practice, serious barriers to using this water may arise because of the prior appropriations doctrine of water rights which exists in most of the states

comprising the study area. Under this doctrine legal rights to defined quantities of water are held by individuals or business entities. Any use of this water by microalgal systems would require negotiation and/or payment to the holders of the water rights. Furthermore, the water may not be increased in salinity beyond the point where downstream users can put it to beneficial use. Even assuming that salt-tolerant crops will be grown, it seems unlikely that evaporative concentration of salts due to consumptive use can be reduced to a point where the downstream users are unaffected. However, use of this water source should not be entirely ruled out at present pending the acquisition of information on several salinity control projects now being planned.

(Consumptive use is a term used to delineate that portion of a quantity of water withdrawn from a water body and not returned at substantially the same location. The reduction is most often defined to be limited to evaporative losses, but more generally may be defined to include water removed from the system in the harvested crop.)

The most promising source of water for microalgal growth systems appears to be saline groundwaters. It is likely that saline groundwaters will be encountered at varying depths throughout the study area. Provided that adverse effects on freshwater aquifers can be prevented, these deeper groundwaters are potentially attractive sources for several reasons:

- Given the water rights situation in the western U.S., it would be difficult if not impossible to obtain adequate surface water (as for example, from the Central Arizona Project) or shallow groundwater for the anticipated project scale.
- These resources are presently considered to be a nuisance when compared to less saline waters and often constitute disposal problems during exploratory well drilling.
- Depending on the geologic formation, nutrients required for plant growth may be already present in significant quantities.

Concerns regarding the use of this source of water include the following:

- Economics of water supply as a function of depth and hydrogeologic conditions.

- Identification of data indicating where saline groundwater may be found, its depth and the quantities available.
- Obtaining a consistent set of data using the same definition of salinity.
- Potential for downward movement of freshwater due to withdrawal of more dense saline waters.
- Environmental protection of potable water aquifers from contamination due to water seeping through the bottom of the culture system or due to the need to discharge a waste stream (known as blowdown) to maintain the salt levels within an acceptable range.

Computations regarding the relationship between the system design and operating rules and the resulting salt concentrations have been made and are presented below.

A water balance for the system might be as shown below:

Net Make Up	=	Evaporation	+	Turbulent Mass Transfer Losses	+	Product Moisture	+	Seepage -
		Precipitation	-	Recycle of Water in Product	+	Blowdown for Salt Control.		

For the purpose of this computation, a unit area of 1 hectare was chosen. Since scale effects for some of the processes, such as evaporation, are not explicitly accounted for, there is a linear relationship between a one hectare system and any other size system.

For computational convenience, an evaporation rate of 1200 mm/yr was chosen. Lake evaporation rates vary across the study area from less than 1016 mm to more than 2032 mm. Therefore, the value chosen tends to be somewhat below average study area evaporative water losses. An additional loss term was included to account for the effects of turbulence due to agitation transfer on evaporation rates. An additional loss of 250 mm/yr from this source may be experienced, for a total loss of 1450 mm/yr. Also, as the salinity increases, the water in the system will experience a small vapor pressure lowering, which will decrease the evaporation rate. For example, the effect of sodium chloride at concentrations of 29,000 and 58,000 mg/l is a reduction of 1.6 and 3.3 percent, respectively.

Some water losses will also be experienced as a result of product harvesting. Since this is an unavoidable activity, it appears desirable to use this activity as the principal means of removing excess salt from the system. It was assumed that cell densities could be pushed as high as 10 percent, although current levels of 2.5 to 5.0 percent (approximately 2.5 - 5 g/l) are more probable for the initially commercialized systems.

A productivity of 65 metric tonnes/hectare - yr was assumed based on a value previously used for literature cost estimates (Benemann, 1979). This results in 603 m^3/ha-hr (0.300 gal/min-ha) of water losses. Since this is near the middle of the possible range, the salt concentrations projected below are close to a typical case estimate. In theory, most of this water could be recovered from the dewatering operation, but discharge for salt control appears to be the preferable option. Prior to the achievement of steady-state conditions, it may be economical to recycle this water in lieu of additional make-up to reduce the need for additional nutrient salts. This cost savings would be realized at the risk of inhibiting growth due to the build up of extra-cellular toxins.

Based on the need to prevent contamination of near surface aquifers, it is assumed that the seepage rate through the pond or channel bottom will be reduced to essentially zero due to either the low permeability of the native soils or to the use of a synthetic liner.

The final water loss would be the need to make up water discharged for salinity control. By varying the amount of water purged, the salinity could be controlled at any level in the range between the inlet concentration and the steady-state (no additional discharges beyond harvesting losses) concentration value. This computation assumes that the need to minimize water losses is more important than maintaining the salinity at some point below the steady-state concentration. The assumption of no incremental blowdown may have some implications for the selection of algal species as discussed below.

The water loss via evaporation will be replenished to some extent by rainfall and possibly snowfall. For computational convenience a precipitation rate of 200 mm/yr was chosen. Average annual precipitation varies across the study area from less than 100 mm to more than 1000 mm. However, a much

greater portion of the area is below 400 mm, and 200 mm is not too far from an average for the area.

Covered systems will require a continuous average make up of 4.6 gal/min-acre (7.07 l/min-ha) of which 3.9 gal/min-acre (5.94 l/min-ha) leaves the system as water vapor. Due to the covers, seasonal consumption of water, while variable, would be less so than with uncovered systems. It was assumed that the covers would be 75 percent effective in reducing water losses due to evaporation and turbulence and that precipitation falling on the covers is collected in channels and stored until needed to reduce water usage to a minimum.

Under steady-state conditions, salts entering the production system will be concentrated by an ultimate factor of 6.23 under steady-state conditions. However, the realization of this steady-state factor will require a considerable length of time under natural conditions as shown in Figure II-1. The reason for the long lag time is that the volume of the system is rather large in relation to the make-up requirements. Operated in this fashion the system would tend to support a progression of algal species as the salinity increases beyond the limits of tolerance of a given species.

Inasmuch as the design and operation of the system components will likely be optimized for a single species, it appears more desirable to artificially increase the salinity initially to a point within the tolerance range of the species selected for growth at or near the steady-state salt concentration. For example, if the system inlet salt concentration is 1 gram per liter and the algal species grows optimally at 5 to 7 grams per liter, it would be preferable to initially set a level of 5 grams per liter and begin the system operation from that concentration. Even assuming a minimum dissolved solids concentration of 1g/l, the blowdown stream would be brackish to saline in quality. Disposal would require either evaporation or possibly reinjection to meet environmental constraints.

Some available data indicate a potentially lethal temperature problem with closed systems. If this proves to be a legitimate limiting factor, then the additional water requirements of an open system may be unavoidable.

Uncovered systems will require a continuous average make-up of 16.3 gpm/ac (24.19/min-ha) of which 15.5 gpm/ha (23.8 l/min-ha) is lost to

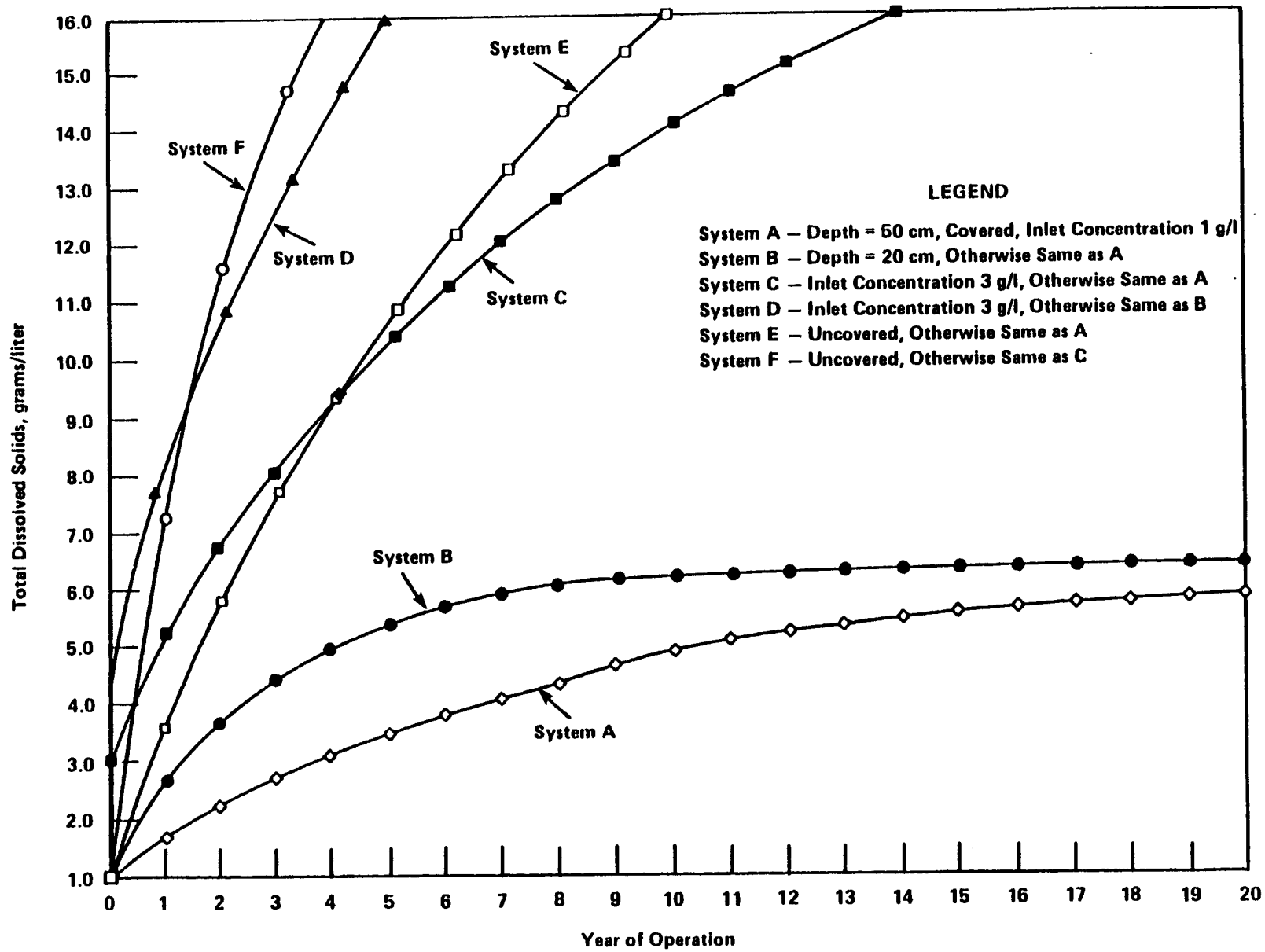

FIGURE II-1. SALT CONCENTRATIONS IN MICROALGAL SYSTEMS AS A FUNCTION OF TIME

vaporization. Even more so than with covered systems, seasonal water usage would be expected to be higher, though the amount of the increase would be area specific. Salts in these systems will be ultimately concentrated by a factor of 21.9. Again, the option to initiate growth at a salt level higher than the inlet concentration must be weighed against possible required transitions in species as tolerance levels are exceeded.

In this type of system, inlet TDS concentrations above 3000 mg/l almost force the use of an additional blowdown stream to maintain the salt levels at tolerable concentrations. This could have the effect of more than doubling the amount of effluent requiring disposal or recycling.

Gross Water Yield. For groundwater sources this criterion refers to the amount of water available without regard to depth, quality, or institutional barriers. Ideally, this information should be presented on an areal basis, for example, cubic meters per minute per hectare, to facilitate an analysis which recognizes the large scale of microalgal systems. The reason for this manner of displaying the information is to assure that a sufficient number of wells can be drilled to supply the system without having to pump water over long distances. While no particular difficulty may be encountered in finding an area where a well may supply adequate water for a few hundred acres, obtaining a supply for perhaps ten thousand acres may be quite a different matter.

When water is pumped from wells, a cone of depression in the surface of the groundwater usually occurs, because the water cannot flow to the well fast enough initially to balance the rate of withdrawal. Pumping rates are usually limited to prevent the extent of this effect, particularly when more than one well is involved. The overlap between the cones of depression creates an interference and reduces the total water supply available from a multiple well situation to below the quantity determined from use of single well pumping test data, unless the wells are spaced widely enough to avoid interference. Under certain geologic circumstances, the cone of depression may extend over large areas requiring widely spaced wells. Thus, yield per unit area is a critical parameter which recognizes that multiple wells will be

needed and that the overall system size will be dictated by the area needed to obtain the water.

Unfortunately, information at this level of specificity will not be available except in isolated areas. Typically, three types of information are reported: (1) subjective impressions of flows obtained without a pumping test, (2) capacity (gal/min) data for a single isolated well, and (3) capacity (gal/min) and drawdown (reduction in water surface elevation) in feet from which specific capacity (gal/min-ft) can be computed. Data should be spatially plotted as gallons per minute isoquants. The economics of groundwater supply are addressed in Section V. Spacing of the isoquants will be dependent on the data, but 100 to 200 gpm increments seem reasonable at this time. No general statements are possible regarding the appropriate number, spacing, and flow of wells, except as related to actual pumping data.

Depth to Water. This criterion addresses the concern that suitable quantities of water may be found only at considerable depth. A significant fraction of the operating cost associated with delivering water to the site will be attributable to bringing the water from the aquifer depth up to the surface.

Depending on the geologic configuration of the aquifer, the physical distance between the ground surface and the level of the aquifer may not be the controlling factor. Aquifers are broadly characterized as unconfined or confined. An unconfined aquifer is one in which the water in the pores of the medium is exposed to atmospheric pressure and which is not overlain by strata which prevent downward movement of precipitation. Depth to water in these situations refers to the physical depth in the aquifer where water is encountered.

A confined aquifer, on the other hand, is one overlain and underlain by impermeable strata such that the water at a particular location may be at a pressure other than atmospheric. In these situations a well drilled to tap the waters can exhibit a water level in the well considerably higher than the physical upper limit of the water-bearing strata. In extreme cases this level may actually be at or above the ground surface, creating an artesian

condition. Various degrees of confinement intermediate to the two extremes are typically encountered.

In many cases determining the depth to water is difficult because the water levels are a function of the pumping rate and the rate of recharge. The recharge rate is an important concept for continued long-term use of wells for algal culture systems. Aquifers are subjected to a state of dynamic equilibrium when water is withdrawn. In arid regions this water may have been in groundwater storage for long periods of time and withdrawal may require very close control to avoid continually declining levels over time. The situation where the pumping rate consistently exceeds the recharge rate leads to a condition known as groundwater "mining". Often this practice leads to ultimate depletion of the resource and subsidence of the land surface above the aquifer.

As in the case of groundwater quantity, the manner in which the data are recorded varies greatly. When water is found incidentally to other purposes, for example, oil exploration, the supervising geologist may note only the drilling depth at which the water was first noted. On the other hand, exploration for geothermal sources may provide a very detailed evaluation of water levels as a function of pumping rate.

Data should be mapped for the most productive aquifer(s). A data resolution of 100 feet may be acceptable, but the number and spacing of the isopleths should consider the available information.

Current and Potentially Competing Uses. This criterion addresses the very significant question of whether institutional constraints exist to use of physically available water. Most of the western states have permitting programs which appropriate to potential users the right to a certain amount of water obtained from a particular source.

When a potential user acquires a claim, his priority to use the water is based on the date of the claim and subsequent claims are lower in priority. Thus, a distinction should be made between currently permitted users and potential future users who are projected but not currently permitted. Provided that a claim for a microalgal system water permit is filed prior to such users, no conflict exists.

In the case where more than one permit application is pending, most western states have established an order of preference among appropriations based on the beneficial use for which the permit is sought. Generally, domestic supply is preferred to irrigation and irrigation is preferred over manufacturing.

A further distinction must be made between the case where a permit holder has not yet made use of his right and which is termed "inchoate" and the case where the water is actually in use and thus "perfected" or "vested". In the former situation a right may be challenged on the basis of disuse.

Data on the status of water rights to saline aquifers should be mapped to indicate the degree of restriction which exists. Three to four levels should be considered:

Level 1: No current usage, no reasonable potential usage (unrestricted)

Level 2: Potential future usage based on projected activities in the near term (5 years) but no current usage

Level 3: Inchoate permit holders and/or projected water using activity in the near term time frame

Level 4: Current significant usage (most restricted).

Data should be available for most of the study area on current status of allocation permits. Less reliable or detailed information may exist for projected activities such as mineral resource extraction or geothermal systems.

Evaporation. This criterion is discussed under climatic criteria in Section III.

Precipitation. This criterion is discussed under climatic criteria in Section III.

Water Quality

Water of adequate quality must be provided to microalgae systems to allow maximum growth rates and environmentally acceptable residuals disposal.

In relative importance, nutrient characteristics are of lower importance than the other water quality criteria.

From the standpoint of salt tolerance, microalgal species can be found which span the salinity range from freshwater to highly saline water. Therefore, if the salinity is maintained in a given range, it becomes a matter of matching the species to the system. For a given system, the salt concentration in the channels (or ponds) is a function of the evaporation rate, the salt concentration in the make-up water and the salt losses from the system. The two major salt removal mechanisms are seepage through the channel (or pond) bottom and moisture removal in the harvested crop.

If no other means of removing salt is provided, the concentration of dissolved solids is then fixed. Assuming that the seepage is reduced to negligible amounts to prevent contamination of surface water supplies, the salt concentration at the outlet of the system will increase by about a factor of 22 or so in open systems and by about a factor of 6 in enclosed systems.

This means that a species tolerant of the resulting concentrations must be selected. Alternatively, the system could be set up to provide varying growth conditions (increasing in saltiness) in successive channels (or ponds) allowing growth of different species. Most practical from an engineering standpoint is to withdraw a continuous blowdown stream to maintain salt content at the desired level.

The latter approach, however, creates a continuous waste stream which must be disposed of in an environmentally sound manner. The most economical manner of disposal would be to simply pump the brine to evaporation ponds, but solar-assisted evaporators or reverse osmosis (for example) may be cost effective if the need for make-up water can be reduced significantly by recycling. Reinjection may be required by some states to minimize land subsidence.

Along with ions such as sodium and chloride present in brines, hardness cations such as calcium and magnesium will be present. In some geologic formations these ions may be in equilibrium with solid phase minerals. Withdrawal of groundwaters from the geologic setting, addition of CO_2 via aeration or sparging, and algal growth (use of bicarbonate ion and carbonic acid) may alter the pH of the system and may cause the formation of calcium

carbonate. The most pronounced effect of precipitation of significant amounts of calcium carbonate would most probably be to reduce the effectiveness of the incident solar radiation by increasing the shading of the culture. Other undesirable side effects, such as inhibition of nutrient uptake and increased processing cost, may also occur. Beneficial effects may be the removal of some toxins.

Water obtained from deeper aquifers may contain phytotoxic concentrations of some trace elements. These waters would either require treatment to reduce the concentrations or, more likely, would have to be avoided altogether.

The addition of fertilizer elements may be necessary in order to achieve the required yields and growth rates. The macro-nutrients--carbon, nitrogen, phosphorus and potassium--may need to be added. However, waters may vary significantly in the concentrations of these elements, with those having the higher concentrations being more desirable as make up sources (other factors being equal).

The nutritional requirements of the algae and the relationship between the carbon source and the pH in the system are discussed below. Based on the Redfield-Ketchum model for algal biochemistry, algal protoplasm is of the approximate composition: $C_{106}H_{263}O_{110}N_{16}P_1$ (Stumm and Morgan, 1970). From this stoichiometric model, it is possible to estimate the minimum quantities of nutrients necessary to produce a given amount of biomass. On a weight basis the elemental composition is 36.8 percent carbon, 6.3 percent nitrogen and 0.9 percent phosphorus. Due to use efficiencies below 100 percent, the amount of carbon required to be supplied is about 67 percent greater than the stoichiometric amount, while the nitrogen requirement is about 60 percent greater, and the phosphorus requirement about 10 percent greater. Carbon requirements in actual systems have shown considerable variation (Lipinsky and Litchfield, 1970).

If an annual yield per unit area of 65 MT/ha-year (30t/ac-yr) is assumed, then the carbon requirements are 6590 kg, the nitrogen requirements are 1098 kg and the phosphorus requirements are 106 kg, accounting for the use efficiencies. Some perspective on the relative potential carbon supply from make-up water sources can be gotten by computing for typical water sources the

carbon input flux and the chemical species distribution. For ocean water supply, total alkalinities average 2.3 milli-equivalents/l. At pH 8, 40 C and chlorinity of 19 0/00, about 85 percent of the total dissolved CO_2 in the system is present as bicarbonate, and only 0.61 percent is present as dissolved carbonic acid ($CO_2 + H_2CO_3$). In terms of the mass of carbon available, the direct supply to algae capable of assimilating only CO_2 is insignificant. If bicarbonate can also be used, then the seawater can supply about 5 percent of the carbon requirements in an open culture system. As CO_2 or bicarbonate is withdrawn from the system by photosynthesis, a diffusion gradient for atmospheric CO_2 is created which will increase the rate of supply of CO_2. The concentration and mass transfer characteristics are not sufficient to achieve the needed supply using atmospheric CO_2.

The supply potential for terrestrial water appears somewhat more favorable from both a mass flux and carbonate species distribution standpoint. Data compiled for various terrestrial sources, not necessarily groundwater, indicate that the median bicarbonate concentration is 236 mg/l and that 10 percent of the waters exceeded 405 mg/l (Davies and DeWiest, 1966). Since western groundwaters typical of those in the study area tend to have high alkalinity, they probably fall in the upper portion of the distribution.

At pH 7, about 6 percent of the total CO_2 is carbonic acid, while 92 percent is bicarbonate. At pH 8 the distribution will not be very different from that in seawater, particularly if the dissolved salts are above 3 g/l.

For the median bicarbonate concentration, terrestrial water could supply less than 1 percent of the carbon to CO_2 users and 10 percent to algae capable of using bicarbonate as well. At the 90 percentile level the percentages increase to about 1.3 percent and 17 percent, respectively. Therefore, carbon augmentation, regardless of the water source, is still required, although some waters are more favorable than others.

The question then becomes one of determining how to best augment the carbon supply in view of the need to maintain the pH within an acceptable growth range. Several methods of doing this can be identified:

- Addition of atmospheric CO_2 plus bicarbonate
- Addition of "concentrated" CO_2 alone

- Addition of "concentrated" CO_2 plus bicarbonate
- Addition of bicarbonate alone
- Addition of bicarbonate and mineral acid.

The first method could conceivably be cost-effective if the rate of CO_2 dissolution is high enough to offset the pH increase due to CO_2 removal and HCO_3 addition. The second method is that proposed by Benemann et al. (no date) and depends only on the cost of CO_2 delivery to the site. The third method could be employed if the cost of CO_2 is high and bicarbonate mineral salts are available near the site. The fourth method will be effective only if the algae are capable of using considerable amounts of bicarbonate as a carbon source. Otherwise, the rate of input of alkalinity, together with the removal of CO_2 will exceed the pH limits for tolerable growth conditions in a short time. The fifth method uses the fact that alkalinity can be titrated by a mineral acid, and, thereby, the pH can be maintained at a low enough level to permit growth. Supply options are discussed further in Section V.

With regard to nitrogen requirements, seawater contains relatively small concentrations of nitrate, nitrite and ammonia. Total nitrogen concentrations range from 0.004 - 0.192 mgN/l. From a mass loading standpoint this corresponds to 0.05-2.51 kg/yr, which is insignificant in satisfying the nutritional needs.

Terrestrial water, on the other hand, can contain very high concentrations of nitrogen compounds. Median concentrations of nitrate are 1.22 mg/l and 5 percent of these waters exceed 10.00 mg/l. However, analyses as high as 86 mg/l have been reported (Hem, 1970). Ammonia concentrations will be distributed in a similar manner, however, concentrations as high as 476 mg/l have been measured in some brines. It is unlikely that both ammonia and nitrate will be present in the same water.

For nitrate or ammonia concentrations of 10.00 mgN/l, the corresponding supply potential is 131 kg/yr, or approximately 12 percent of the required amount.

Phosphorus concentrations rarely exceed 1 mgP/l due to solubility limitations and biological uptake. Waters of this concentration could supply about 13 percent of the algal needs.

Potassium requirements are not defined in terms of mass, but a defined algal culture medium recommends a concentration of 1.65 mg/l. This concentration is exceeded by seawater and about 75 percent of terrestrial waters (Stumm and Morgan, 1970).

Salinity or Total Dissolved Solids Concentration. This criterion refers to the amount of dissolved constituents present in the make-up water to the biomass system. High concentrations of salinity will limit the flexibility to operate the system and in extreme cases will require a blowdown stream to make the system feasible at all.

Salinity, while having a precise chemical meaning, is used rather loosely in practice. Typically, a distinction is made between fresh and saline groundwaters at a dissolved solids concentration of 1000 mg/l. Frequently, this is the only distinction which is possible given the available information.

Ideally, chemical analysis data will allow a further delineation of the range above 1,000 mg/l into the following subranges as defined by the U.S. Geological Survey:

Slightly saline	1,000-3,000 mg/l
Moderately saline	3,000-10,000 mg/l
Very saline	10,000-35,000 mg/l*
Briny	more than 35,000 mg/l*.

Further resolution of the isopleths would have to be justified by the data. If actual analysis data are available, the salinity tolerance of those algal species exhibiting desirable characteristics should be used in lieu of these alternative subranges. Mapping of dissolved solids data should be limited to the most productive aquifers as defined in the water quantity criteria description.

Soil Textures (Permeability). The ability of the native soils to prevent the transmission of water is an important economic consideration in determining the feasibility of a microalgae system. If the water losses due

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* Waters in this range will require a blowdown stream to control salt concentrations.

to leakage are significant, not only will the overall water usage increase, but a potential exists for environmental degradation.

Some types of soils, particularly those containing large percentages of clay and silt, are well suited to minimize seepage. At the other extreme, soils comprised predominantly of sand- or gravel-sized materials tend to be quite permeable.

The data used for mapping should be relatively uniformly available. Given the study area size, a generalized soils map of each state should provide adequate resolution for purposes of this analysis. Areas should be shaded based on soil associations as follows:

Level 1: Associations consisting predominantly of impervious soils or subsoils, e.g., homogeneous clays and silty clays

Level 2: Associations consisting predominantly of poorly drained soils or subsoils, e.g., very fine sands, organic and inorganic silts, and loams

Level 3: Associations consisting predominantly of well drained soils or subsoils, e.g., clean sands, clean sand and gravel mixtures, and clean gravels.

This criterion, designed to be applied on a statewide basis, is more general than that proposed under the land-related criteria.

Chemical Characteristics - Toxic Substances. Maximum growth rates for microalgae systems can only be obtained if the concentrations of algal toxins are below a threshold level. Considering the nature of groundwater, most of these substances are likely to be trace metals or non-metals (for example, arsenic or boron). In coastal areas other types of toxic substances such as industrial pollutants could be important. Phenolic compounds and free cyanides are two additional constituents relevant to toxicity evaluation. Although there is evidence in the literature that synergisms or antagonisms may exist in the presence of multiple toxins, these effects have been quantified only for a limited number of toxins.

The data may be overly conservative in that, to date, few toxicity thresholds specific to algae have been identified. The concentrations should be modified when specific data can be obtained. Hazardous and minimum risk

TABLE II-1. HAZARDOUS AND MINIMUM RISK CRITERIA CONCENTRATIONS OF TOXINS FOR PROTECTION OF MARINE BIOTA (μg/l)[a]

Parameters	Level 1 (Least Desirable)	Level 2 (Intermediate)	Level 3 (Most Desirable)
Aluminum	>1500	200-1500	<200
Arsenic	>50	10-50	<10
Barium	>1000	500-1000	<500
Beryllium	>1500	100-1500	<100
Boron	>5000	750-5000	<750[b]
Cadmium[c][f]	>2000	500-2000	<500
Chromium	>100	50-100	<50
Copper	>50	10-50	<10
Cyanide	>10	5-10	<5
Lead[f]	>1000	500-1000	<500
Manganese	>1200	600-1200	<600[d]
Mercury	>100[e]	10-100	<10
Nickel[f]	>2000	1000-2000	<1000
Selenium	>10	5-10	<5
Siver	>5	1-5	<1
Zinc[g]	>400	200-400	<200[d]

(a) Source: National Academy of Sciences, Water Quality Criteria, 1972, U.S. Gov. Printing Office (1974).

(b) Source: U.S. EPA, Quality Criteria for Water (1976).

(c) A reduction of an order of magnitude is recommended when copper and/or zinc are present at 1000 μg/l or more.

(d) Minimum concentration based on nutritional requirements, not on NAS criteria.

(e) Based on algal growth in medium containing this level of mercury, not of NAS criteria.

(f) Based on algal toxicity data, not NAS criteria.

(g) A reduction of any order of magnitude is recommended when other heavy metals are present at Level 1 concentrations.

criteria concentrations of toxins for protection of marine biota are shown in Table II-1.

Chemical Characteristics - Nutrients. This criterion is similar in approach to that described for toxins, requiring the acquisition and mapping of data on concentrations of individual chemical constituents. Data ranges should be mapped as shown in Table II-2. In addition, a map of pH isopleths should be prepared at 0.5 pH unit intervals.

TABLE II-2. MACRONUTRIENT CONCENTRATION CRITERIA

	Bicarbonate,[a] $mgHCO_3/l$	Nitrogen mgN/l	Phosphorus mgP/l	Potassium mgK/l
Level 1 (Most Desirable)	>400	>10	>1.0	>2
Level 2 (Intermediate)	100-400	5-10	0.5-1.0	1-2
Level 3 (Least Desirable)	<100	<5	<0.5	<1

(a) Alternatively, data on alkalinity could be converted from the normal units of $mgCaCO_3/l$ to $mgHCO_3/l$ by multiplying the value by 0.61.

Data Sources

Arizona

Groundwater Data Base (GDB)

* Dept. of Water Resources - Basic Data Unit
- The information display format consists of maps with accompanying booklets.
- Telephone *602-255-1560

Permit Information

* Permit Applications to drill or divert water are filed with the State Department of Water Resources.

- Permit applications to divert surface water must contain:
  1. A description of the project
  2. An exact definition of the point of diversion
  3. The site of use
  4. A statement of the intent of use
  5. A consumption estimate
  6. And USGS quad maps defining the above specifications.
- Permit applications to drill water wells must contain:
  1. A notice of intent to drill
  2. A site layout on a township-range scale map
  3. Name of the drilling company
  4. The name of the owners of the well
  5. The physical specifications of the well.

The application process requires approximately 1-1/2 weeks to complete. A three dollar processing fee must be submitted with the application. New legislation has been recently passed in Arizona concerning groundwater allocation.

Information concerning the quantity and quality of return flow irrigation water can be found at the University of Arizona Agricultural Engineering Department or the Agricultural Extension Service. Dr. Herman Bauer, of an unspecified Federal Resource Research Lab, is highly recommended as an expert in this area.

California

GDB

* California Division of the U.S.G.S.

- Contact Ed Richie of the Management Data System (916-445-0469).
- Water quality data is recorded in epm and ppm units.
- Information is displayed in microfiche forms (20¢/fiche, 268pp/fiche, 10 well records/page).
- Records are available for 40,000 wells.
- Selective retrieval costs - manpower + 2¢/rec + computer time.

Permit Information

* Contact Joyce Peters of the State Water Resources Div. (916-445-6582).
- No groundwater laws encompassing the entire state exist in California.
- Seven groundwater basins in California do not permit drilling. (Most occur in Southern California near metropolitan areas.)
  1. Certain parts of the state require the submission of a drilling notification and a periodic log.
  2. New laws have been recently passed concerning drilling in coastal zones.

Colorado

GDB

* Contact Dick Pearl of the State Geologic Survey (303-866-2611) or Ted Hurr (USGS) (303-234-3815).
- The State Geologic Survey publishes a groundwater quality report for selected aquifer systems.
- A series of reports by the USGS contains relevant data. No single office or publication is totally comprehensive.
- The Colorado District Water Resources Division publishes miscellaneous water quality data covering "county size" or larger geographic regions.

Permit Information

* Permit applications are filed through the State Engineers Office. The application must contain:
- The physical specification of the well
- The well location
- The intent of use.

Nevada

* Contact Darrell Rasner of the State Consumer Health Division (702-855-4750)
- Water quality is untested in a large portion of the state.
- A chemical analysis of public water supplies is available through his office.
* Suggestions
- Contact the Southern Nevada Water District (702-565-9763). The Las Vegas area has an abundance of water of the quality sought.
- Also contact Ben Bartlett, City Engineer of Falon, Nevada. Mr. Bartlett is most knowledgeable about saline groundwater. (702-423-5107). Falon, Nevada, could be a good source of saline and geothermal water.
* Permit Information
- The requirements for the application are flexible.
- Most applications require the following data:
  1. A map showing the proposed well location, diversion site, and place of use
  2. The name of the water rights surveyor (who must be registered with the Office of Water Resources)
  3. Totals of water consumption modes and a breakdown of usage.

Drilling in designated areas without the proper permits is illegal. Drilling in undesignated areas without a permit is allowed, however, water diversion is illegal without a permit. The permit processing time is six to nine months. Permits cost $100/cfs.

New Mexico

GDB

* Contact Perry Pearce (505-827-2741) of the Oil Conservation Division of the Energy and Minerals Department.
- Water quality maps based on salinity tests taken before 6/82 have recently been prepared.

* Permit Information
- Permit applications must be filed with the Water Rights Division (505-827-2423).

Texas

GDB

* Contact Robert Organ of the Texas Natural Resources Information System (512-475-3321).
- 45,000 separate chemical analyses are recorded on tape or card format
- Selective retrieval cost = $15 + computer time
- The NRIS office also publishes a 4 or 5 volume report on saline water resources
- Also the Texas Health Department publishes a list of cities and counties using water which contains 1000 ppm TDS.
* Suggestions

Gerald Baum of the Texas Department of Water Resources stated that an abundance of water of the quality specified exists at shallow depths on the Gulf Cost (512-475-3606).

Permit Information

- Groundwater is privately owned in Texas.
- Licensed drillers must submit a well log.
- Many groundwater districts (especially the high plains and Houston districts) regulate pumping.

Utah

GDB

* Contact Earl Staker of the Utah Division of Water Rights. This division publishes a document which contains an inventory of the state's groundwater resources. The cost is $5.

- The Utah Water Pollution and Environmental Health Board is a viable source of information concerning groundwater quality. (801-533-6121). To obtain the groundwater inventory write to:

  Utah Department of Health
  c/o Calvin Sudweeks
  Attention: Terry Riding
  Director of the Bureau of Water
  Pollution Control
  150 W.N. Temple
  P.O. Box 2500
  Salt Lake City, UT 84110

* Permit Information

- Important information for permit applications includes:
  1. The size and depth of wells
  2. The proposed use of the water.

Processing of permit applications requires a time period lasting from six months to one year. The maximum filing fee is $150. The review procedure is critical and done on an individual basis.

References

(1) Benemann, J. 1979. Chemicals and Fuels from Semi-Arid Zone Biomass in The Biosaline Concept, Hollaender, A. editor, New York, Plenum Press, pp. 309.

(2) Stumm, W., and G. G. Morgan, 1970. Aquatic Chemistry. New York. John Wiley and Sons, pp. 429 and 384.

(3) Benemann, J. R., R. P. Goebel, J. C. Weissmann, and D. C. Augenstein. undated. Microalgae as a Source of Liquid Fuels.

(4) Lipinsky, E. and Litchfield, G., 1970, "Algae Bacteria and Yeasts as Food or Feed" CRC Critical Reviews in Food Technology, 581-617.

(5) Davies, S. N. and DeWiest, R.C.M., 1966, Hydrogeology, New York: J. Wiley.

(6) Hem, J. G., 1970, "Study and Interpretation of the Chemical Characteristics of Natural Water", U.S. Geological Survey, Water Supply Paper No. 1473.

III. LAND AND CLIMATE RELATED FACTORS

In order to develop a data base from which to assess the land and climatic resources of the southwestern United States for the development of microalgal biomass production systems, it is necessary to (1) identify the pertinent land and climatic factors which are important to the siting of biomass production systems and (2) develop screening criteria for each factor. The recommended set of land and climate-related factors is as follows:

Land-related factors

- Soil characteristics
- Land ownership/availability
- Land use/cover

Climate-related factors

- Solar radiation
- Temperature
- Severe weather.

Each of these factors is discussed below in relation to its use as a screening criterion for providing guidance to the development of information to permit the statewide differentiation of areas of varying potential for microalgae biomass production system development.

Soil Characteristics

There are a number of soil characteristics which are potentially important considerations in identifying areas of the Southwest which could be utilized for biomass production systems. The important soil characteristics which have been identified are (1) slope or topography, (2) temperature and moisture regime, (3) soil depth, and (4) soil permeability. The screening criteria related to soil and land characteristics have been categorized as to major criteria and minor criteria. Major criteria are those which will be useful in differentiating areas of varying potential for siting of microalgal production systems at a statewide level. Minor criteria are those which, although important as siting considerations, will not be particularly useful in statewide differentiation. These minor criteria will be useful in

differentiating areas of varying potential for system development on a regional, subregional or site-specific basis. The major screening criteria for soil/land characteristics are (1) slope/topography and (2) temperature and moisture regime. The minor criteria are (1) soil depth, (2) engineering/technical classifications and (3) soil permeability.

Slope Topography

It is technically and economically preferable to site microalgal systems on level or nearly level land so as to preclude or minimize the necessity of terracing and deep excavations. Terracing would require a greater capital expenditure and would likely require a greater soil depth.

The ideal site would be limited to level or nearly level land where land leveling, earth moving, and excavation costs would be at a minimum. Slope is a major criterion used to characterize soils at both the state and site-specific level. A soil association map of each state should be available which assigns a generalized slope description to each mapping unit. Effort will be required to convert generalized soil association maps to soil slope/topography maps having slope categorizations reflecting site development costs. Another possible data base for slope/topography information at the state scale are the 1:500,000 or 1:1,000,000 USGS topographic maps. The smaller scale USGS maps as well as the county/area soil survey reports would be particularly useful in differentiating areas of varying potential for biomass production system development on a regional or site specific basis.

Temperature and Moisture Regime

Soils commonly are categorized into groups or soil associations of similar temperature and moisture regimes. Soil temperature and precipitation, which dictate soil temperature and moisture regime, affect the technical and economic feasibility of these systems at a given location. Also, soil temperature and moisture regime generally determine natural vegetation type and, to a large degree, land use. Hyperthermic, arid soils, which are soils having a mean annual soil temperature (MAST) of greater than 22 C (72 F),

generally are desert areas, which typically are underutilized. Mesic subhumid soils typically are utilized for agricultural production as cropland. An appropriate set of screening criteria for use in differentiating areas of varying potential for biomass production system development recognizes both the need to minimize competitive land use and avoid lethal temperatures:

Temperature and Moisture Regime		Potential Category
Hypothermic Arid	MAST greater than 22 C (72 F)	Most acceptable
Thermic Arid Semiarid	MAST 15-22 C (59-72 F)	Most acceptable
Mesic Arid Semiarid Subhumid	MAST 8-15 C (47-59 F)	Moderately acceptable
Frigid Subhumid	MAST less than 8 C (47 F)	Least acceptable

The soil resource information available from each state should contain information allowing the development of statewide soil maps assigning soil associations to the above temperature and moisture regime categories. This information is currently available for several of the states in the study area. Effort will be required to convert soil association maps to temperature and moisture regime maps depicting the above temperature and moisture categories.

Soil Depth

Adequate soil depth is desirable to permit the shallow (5-6 feet) excavations necessary for the construction of the physical facilities involved in biomass production systems. Consideration should be given to favoring areas of adequate soil depth in order to provide stable support for foundations of concrete and steel structures without the necessity of rock

excavation. In areas with rainfall adequate to facilitate weathering, the upper few feet of most bedrock formations is rippable and can be removed with ordinary construction equipment. However, in areas of low rainfall or with bedrock types resistant to weathering, explosive fracturing and subsequent rock excavation may be required. Rock excavation, if required over much of the project area, could adversely affect the economics of site construction. Given the scale of the project and the fact that the most economical type of construction would be the type where the top surface of the culture media would be at or slightly above the existing ground elevation, a reasonable set of screening criteria for soil depth would be based on the effect on site development costs.

The ideal site would, therefore, be limited to locations where soil depth is in excess of 60 inches. In sloping terrain, because the entire project site would have to be smoothed and leveled to ideally a minimum number of elevations (terracing), greater minimum soil depths would be required to permit necessary cut and fill operations.

Generalized information on soil depth up to 6 feet is readily available at the statewide soil association level from the soil resource information available from each state in the 8-state study area.

Generalized information on soil depth (depth to bedrock) extending below 5 to 6 feet should be available from the geological information sources in each state. In the absence of depth to bedrock maps, oil and gas well records, if available, would be a good source of this type of information once specific areas have been identified. In any event, preconstruction design considerations would likely require that a subsurface soil/rock investigation be completed as part of any site-specific evaluation.

Soil Permeability

It may be technically and economically preferable to site certain types of biomass production systems in areas where soil permeability is low. The type of system most sensitive to this criterion is the open-ponded, unlined system constructed of native soil materials. With this type of system, soils with minimum permeabilities are required to assure minimum

seepage and maximum efficiency of water utilization. At the other extreme, the siting of raceway-type systems constructed of concrete and steel would be rather insensitive to this criterion, since they would not depend upon soil material to control the downward movement of water.

This level of detail in permeability is intended to be used for screening of areas previously found to be suitable with respect to fatal flaw criteria.

Land Ownership/Availability

Land ownership is a critical parameter in the siting of any project of this scope and magnitude and must be taken into consideration in identifying general areas of the Southwest which could be utilized for the siting and development of biomass systems. Not only must land areas having adequate soil characteristics be identified, such land must be available for developing microalgal biomass production systems. There are many categories of land ownership depicted on the various "land ownership" maps for each state. The basic set of categories is (1) federal government lands controlled by various federal agencies including the Department of Interior, the Department of Defense, the Department of Agriculture and the Department of Energy; (2) state lands; (3) Indian land; and (4) private land controlled by corporate or individual entities. Land of adequate character exists in each of the above ownership categories, and without regard to political, institutional and economic considerations, is potentially available.

Ideally, the information screening criteria developed for the land ownership/availability factor should provide for the collection of all information necessary to allow the assignment of all major categories of land ownership present in each study-area state to an availability category rating system. In general, the assignment of land ownership categories to the availability rating system should involve at least the following steps:

- Identification of all categories and subcategories of land ownership for each state which could conceivably have differential availability.

- Description, history, and summary of the land sale and leasing policies relative to each ownership subcategory
- Identification of barriers to the availability of land in each subcategory of land ownership
- Evaluation of land availability barriers and the assignment of each subcategory to an availability category rating.

Category of Land Ownership

The various categories of land ownership within each state are likely to have different degrees of availability for use in the development of biomass production systems. Land ownership maps for all states except Oklahoma and Texas have been acquired from the Bureau of Land Management (BLM) and the following general land ownership categories have been identified for the various states in the study area:

Federal Land
- Military Reservations
- Public Lands (BLM Lands, Public Domain, National Resource Lands)
- National Parks, Monuments and Recreation Areas
- National Wildlife Ranges, Refuges and Management Areas
- National Forest and Grasslands
- Department of Energy Land
- Miscellaneous Federal Land

State Land

Indian Land

Private Land

Miscellaneous Land.

State-level land ownership/availability maps for each state identifying the above categories of land ownership should be developed. The BLM land ownership maps are a major source of this information. Other sources may include USGS state scale (1:500,000) topographic maps, USGS federal land ownership maps, maps available from the various state and federal agencies controlling land, and Indian reservation maps available from the Bureau of Indian Affairs.

Land Sale and Leasing Policies

Information on the land sale and leasing policies of all entities controlling land should be obtained in order to ascertain the relative availability of land in the various categories. Descriptive and historical information on the land sale and leasing policies of the controlling entities should be compiled.

A very important part of this information collection effort is the identification of the individual/organizational unit with decision-making authority and the mechanism by which land sale and leasing decisions are made.

Barriers to Availability

Barriers to the availability of each of the land ownership categories must be determined in order to ascertain the relative availability of land in the various land ownership categories. SERI should identify the barriers to availability of land in the aforementioned ownership categories.

One of the major barriers to the use of federal land is the apparent necessity to conduct both a programmatic and site-specific environmental impact statement as part of the siting process (CEQ regulations 40 CFR 1500-1508).

Evaluation of Relative Availability

The degree to which land availability barriers can be overcome must be determined in order to place lands of the various ownership classifications into categories of relative availability.

The development of state-level land availability maps would allow the differentiation of areas of varying potential for biomass production system development based on the land ownership factor.

Land Use/Cover

Present land use and land cover are major factors in determining the competition for and thus the availability of land for production system development. Land currently utilized in a manner which provides good economic return is likely to be less available than land used in a manner or covered with vegetation which provides little or no economic return. The most available land for development of biomass production systems is likely to be the under-utilized desert lands of the study area. For this reason, it is necessary to compile information on land use/cover and to differentiate areas of the various states as to their varying potential for biomass development.

Land Use/Cover Category

The land use/cover category of a given land area is related to its potential availability for use in these biomass production systems. The set of screening criteria developed for use in guiding the data collection effort for the land use/cover factor is as follows:

Land Use/Cover Category	Availability Category
Desert or barren land	Most available
Range land	Moderately available
Agricultural crop land	Minimally available
Forest land	Least available
Urban land	Least available

The U.S. Geological Survey (USGS) is a primary source of land use and land cover information. LANDSAT imagery is available for the states in the study area at an appropriate state-level scale. In addition, USGS is in the process of compiling standardized land use and land cover maps at a state-level scale for the entire United States. The status of map compilation for the states in the study area should be determined. The above categories of

land use and cover were selected to coincide with the USGS land use/cover classification system.*

Solar Radiation

The intensive outdoor production of algal biomass is limited by several major factors including minerals, carbon source, temperature and light. In cultures kept under optimal conditions with respect to the first three factors, growth is generally considered to be limited by the amount of available sunlight energy for the cells in the culture. The amount or quantity of solar radiation (flux density) reaching a horizontal unit of the earth's surface depends upon a number of factors including (1) the intensity of radiation emitted by the sun, (2) astronomical considerations determining the position of the sun and (3) the transparency of the atmosphere (cloudiness). Common units of radiant flux density are cal/cm^2/minute or langleys/minute (1 langley equals 1 cal/cm^2). The two components of radiant flux density which are measured directly are direct solar radiation and diffuse sky radiation. The total of these two measurements is referred to as total global radiation. Common units of total global radiation are KJ/m^2, Btu/ft^2, and langleys which are expressed on a daily, monthly and annual basis. Other data which attempt to quantitate solar radiation include: (1) mean total hours of sunshine (monthly and annual), (2) mean percentage of possible sunshine (monthly and annual), (3) mean annual number of clear days, (4) mean daily sky cover, sunrise to sunset (monthly and annual) and (5) global KT cloudiness index.

A geographic location with the highest radiant flux density coupled with the lowest KT cloudiness index should also be a site with the least

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* The system and the categories are explained in Geological Survey Professional Paper 964 which, along with "Index to Land Use and Land Cover" and associated maps, is available from Branch of Distribution, U.S. Geological Survey, 1200 South Eads Street, Arlington, Virginia 22202. Technical information about land use and land cover and associated maps may be obtained from Geography Program, Land Information and Analyses Office, 710 National Center, Reston, Virginia 22092, (703) 860-6256.

limitation due to available sunlight energy. The range of data over the study area is on the order of 17 to 21 MJ/m^2. A geographic display of the data with 1.0 MJ/m^2 isolines and cloudiness index in tenths would indicate areas of the greatest flux density and minimal radiant attenuation and, therefore, areas with the greatest potential for development of biomass production systems.

State-level maps of annual average daily global radiation and cloudiness should be developed on the basis of a functional relationship between individual species growth and incident radiant flux.

Temperature

Although solar radiation generally is considered to be the primary limiting factor at maximum rates of algal biomass production (i.e., other factors supplied in excess), temperature under outdoor, uncontrolled conditions has been shown to be a limiting factor during winter months at several locations (Richmond et. al., 1980). The influence of temperature on algal biomass productivity generally has been related to its effect on the photosynthetic apparatus of the algae. Microalgae, like plants in general, can be considered to have a minimum temperature below which survival of the culture does not occur, an optimum temperature at which maximum productivity can occur, and a maximum temperature above which the production of biomass declines. Although generalizations can be made regarding minimum, optimum and maximum temperatures required for microalgae, each species is considered to have its own characteristic temperature response curve established by varying temperature while holding other factors constant. The shape of the temperature response curve, where productivity is plotted against temperature, is generally considered to be proportional to temperature at low temperatures and independent of temperature at high temperatures. The temperature range over which most algae grow has been cited as between 10 and 40 C (Goldman, 1979). Measurement of culture temperatures in outdoor production systems indicates that culture temperatures can exceed air temperature by as much as 13 C, resulting in values up to 45 C on sunny days. The principal climatic factors which determine the temperature of algal cultures are (1) the absorption of available sunlight and (2) the

humidity of the air. Light energy absorbed by the system is transformed into heat at an efficiency of about 90 percent. The humidity of the air influences culture temperature by permitting cooling of the culture by more or less evaporation of the water from the culture (Goldman, 1979).

Annual Normal Daily Minimum Temperature

The normal minimum air temperature of a given location may be used as an indicator of its potential for algal production system development. The temperature over the study area varies from 0 to 16 C. The National Oceanic and Atmospheric Administration (NOAA) data base is a primary source of these data. The various state climatic agencies are an additional information source which should be investigated.

Annual Normal Daily Average Temperature

The normal average air temperature of any given location may be used as an indicator of algal system development potential. Presently available information indicates that the normal daily average temperature ranges from 8 to 24 C over the study area.

Annual Normal Daily Maximum Temperatures

The normal daily maximum air temperature at any given location may be used as an indicator of the relative potential of the location for development of biomass production systems. Depending upon the design configuration of the particular biomass production system, maximum daily air temperature may or may not be a limiting factor to achieving maximum levels of productivity. Closed systems would be particularly sensitive to this criterion. Normal daily maximum air temperatures which occur over the study area range from 12 to 32 C.

Annual Average Daily Evaporation Pan Temperature

The ambient water temperature of a shallow water body at any given location should be related to the ambient culture media temperature if a microalgae production facility were sited at that location. Evaporation pan temperature data are collected at all class A evaporation stations in the United States. A class A evaporation pan is a shallow metal pan about 10 inches deep and 48 inches in diameter. Consequently, it may simulate limitations due to culture media temperature more closely than does air temperature data. The actual range of evaporation pan temperature data occurring over the study area has not been determined, so specific quantitative screening criteria have not been developed. However, it is likely that evaporation pan temperature maps should have a resolution of about 2 C.

Severe Weather

Severe weather is an important consideration in the siting of biomass production systems because such severe weather elements as heavy precipitation and high wind could adversely affect the structural integrity and performance of microalgae systems. Heavy precipitation in the form of rain, snow or hail could overload and damage the support structure and plastic covers of enclosed systems or cause flood damage and detrimental fluctuations in chemical and physical characteristics of the microalgae system culture media. High winds from tornadoes, thunderstorms and other sources also could damage physical facilities and adversely affect the performance of microalgae systems. The severe weather factors selected as screening criteria for severe weather are identified and discussed below.

Annual Average Total Snowfall

A build-up of snow on the plastic covers of enclosed systems could overload and cause damage to the plastic covers and their support structures. Heavy snowfall could adversely affect the performance of open microalgae systems through its effect on culture media temperature. Snow falling onto

the surface of open systems would temporarily reduce culture media temperature, and this could reduce production efficiencies during the winter months. Presently available information on annual snowfall indicates that yearly snowfall for the study area ranges from less than 1 inch in southwestern Arizona to over 400 inches in the mountainous areas in south-central Colorado.

Annual Average Total Precipitation

Yearly total rainfall at a particular geographic location could influence the feasibility of a microalgae system in that area. Rainfall, if occurring during the summer months, could be a significant source of water to offset evaporation losses, however, the cloudiness usually associated with precipitation events could adversely affect long-term productivity. The U.S. scale map of average annual precipitation indicates that precipitation in the study area ranges from less than 4 inches (10 cm) in the desert areas of southern California and Arizona to over 64 inches (163 cm) in eastern California.

Mean Annual Number of Days With Thunderstorms

Rainfall and particularly high winds associated with thunderstorms, could adversely affect the structural integrity and performance of microalgae systems. Closed systems would probably be more susceptible to wind damage than open systems. The national scale map of thunderstorm days indicates that data for the study area ranges from less than 10 days for the extreme southern border area of Arizona and California to over 70 days for areas of central Colorado.

Mean Annual Numbers of Days with Hail

Hail events could damage the plastic covers of microalgae systems and, therefore, statewide areas should be differentiated on this basis to reflect varying potential for microalgae system development. National-scale maps of this data indicate that the mean annual number of days with hail

varies from less than 1 in the border areas of California, Nevada and Arizona to over 8 in north-central Colorado.

Mean Annual Numbers of Days With Tornadoes

The high winds associated with tornado conditions could cause severe damage to lightweight cover systems of enclosed microalgae systems. Therefore, it should be used as a basis for differentiating statewide areas as to varying potential for development of algal production systems. The above tornado data should be available from the NOAA data base from which the required state-level geographic displays could be developed.

Annual Average Daily Wind Velocity

Moderate to high winds could dislodge and suspend particulate matter from barren areas and abrade the plastic covers of enclosed systems or contaminant open systems with particulate matter and, possibly, native algal species. Consequently, this criterion should be used as a basis for differentiating statewide areas of the Southwest as to varying potential for development of microalgae production systems. National-scale maps of average daily wind velocity should be available from the NOAA data base from which the general range of wind velocity for the study area could be determined. These data ranges should be categorized and state-level maps developed to differentiate areas as to varying potential for microalgae system development on the basis of average daily wind velocity.

Fastest Mile Wind Speed

Peak gusts of wind could cause severe damage to lightweight cover structures of enclosed microalgae systems. Therefore, it may have utility as a basis for differentiating statewide areas as to varying potential for development of biomass production systems. Information is available from the NOAA data base from which state-wide geographical displays of fastest mile wind speed could be developed.

10-year Maximum 6- and 24-Hour Precipitation Events

Short, intense precipitation events could cause structural and flood damage to microalgae systems. Flood damage could be particularly significant in that it could contaminate the culture media and containment structure with sediment and native algal species. Accordingly, this criterion should be used as a basis for differentiating areas as to varying potential for the siting of microalgal systems on the state level. Information should be available from the NOAA data base, from which state-level maps could be developed depicting categories of 10-year maximum 6- and 24-hour precipitation events.

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IV. CLASSIFICATION OF AVAILABLE AQUATIC SPECIES

This section has three major objectives:

- To describe candidate microalgae species which may be appropriate as producers of biomass and/or chemicals in systems to be located in the arid Southwest
- To discuss specific criteria for the future selection of candidate microalgae species for southwestern biomass systems
- To identify data gaps and suggest methods which may be useful in future programs to fill the data gaps.

During the search for available species and the development of criteria, several assumptions were made, including the following:

- Although both freshwater and marine species were considered, it appears from the previous tasks that aquatic biomass systems in the Southwest would best utilize saline water. Therefore, special emphasis was devoted toward describing the salt tolerance of the various species, when such information was available.
- The water to be utilized in the Southwest will probably be alkaline. Thus, unhydrated carbon dioxide probably will not be the major form of carbon supply, and carbonate or bicarbonate may serve as alternative carbon sources. Not all microalgae species are capable of utilizing hydrated forms of carbon dioxide.
- It generally was assumed that carbon supply will be the factor limiting microalgae production potential in the Southwest. Thus, carbon use efficiency is presented when possible.
- Southwestern water supplies may contain significant levels of potentially toxic compounds, such as arsenic or boron. Whereas most phycological studies have been conducted under laboratory conditions utilizing controlled media, an effort was made to find information on the response of microalgae to potentially toxic compounds.
- Temperature control may be an important concern for many species. Thus, temperature tolerance data are presented if possible.

- This effort was not to be a selection of species per se. Species selection efforts are being undertaken by other subcontractors for SERI. Our efforts were to be descriptive in nature.
- An attempt was made to describe the current knowledge concerning end-product formation (e.g. glycerin, lipids, etc.) by the various species, as well as biomass production.
- Finally, our approach to completing the objectives of this task included a literature analysis of what is and is not available regarding individual species. As this was a relatively short-term, low-level effort, the analysis is not comprehensive with respect to each species identified. In addition, the species described may or may not be potential candidate species; but their inclusion serves some specific purpose--such as showing metals toxicity data--which might be extrapolated to other species and thus are potentially useful data for researchers in species selection.

Species Descriptions

The various microalgae species are presented in three basic groups. The first group represents an alphabetical presentation of those marine and halophilic species about which substantial information was found. The second group is an alphabetical presentation of those freshwater species fairly well described, and the third group presents alphabetically the remaining species identified in this search.

Marine and Halophilic Species

Aphanothece halophytica. This species has been found in the south arm of the Great Salt Lake (Borowitzka, 1981). It is a unicellular, blue-green species 6 to 9 x 5 um in dimension but forms clumps of cells 10 to 50 cm in diameter. A. halophytica carries out oxygenic photosynthesis, has an optimum CO_2 concentration range between 0.033 and 3 percent, and an optimum temperature of 43 C at 5300 lux light and NaCl content of 6-12 percent (w/v).

The salt tolerance range is 3 to 35 percent, with an optimum range of 16 to 23 percent. In response to increasing salt concentration, this species accumulates betaine and glutamate.

Chlorella saccharophila. This marine, green algae has a cell diameter of 5 to 7 μm, a temperature range of from 16 to 25 C, and an optimum temperature of 23 C (De Pauw et al., 1980). The salinity tolerance range is 1 to 4 percent, with an optimum of 3 percent and an optimum pH range of 7.5 to 8.0. Summertime growth rates (1300 Joules cm^{-2} day^{-1} irradiation) are 5 to 6 g m^{-2} day^{-1}. Greater than 200 Joules cm^{-2} day^{-1} irradiation are required for growth. The mean light utilization efficiency is 1.14 percent of total irradiation.

Dunaliella spp. Several species of Dunaliella have been described and the ecology of the different species has been described largely as a result of studies in the Great Salt Lake (Post, 1981). Characteristics general to several species or to undescribed species will be presented first, followed by descriptions of individual species.

The genus respresents halophilic green algae which lack a rigid cell wall and produce glycerol at a level up to 85 percent of cell weight (Brock, 1975). Various species tolerate salt concentrations ranging from 0.9 to 35 percent (w/v). Species of Dunaliella are often the only organisms found in very saline lakes (Post, 1977), and are capable of growing at very low water potentials. Borowitzka (1981) showed that the doubling time of Dunaliella increased with increasing salt concentration; at 19.2, 28.0, and 35.0 percent salt (w/v), the doubling times were 30, 42, and 78 hours, respectively. Van Auken and McNulty (1973) studied Dunaliella spp. in order to define optimum conditions with respect to CO_2 concentration, temperature, salt concentration, irradiation. The optimum CO_2 concentration was 1 to 2 percent (v/w) in air, supplied at a rate of 2.18 ml min^{-1} ml^{-1} culture medium at 8 psi. With such a CO_2 supply, the doubling time for Dunaliella cultures was 10 hours. Dunaliella spp. was shown to have an optimum temperature of 32 C, which gave a doubling time of 23.8 hours under the other conditions employed. At temperatures of 5 C and 40 C, the doubling times were 550 and 415 hours,

respectively. The optimum NaCl concentration for Dunaliella spp. was shown to be 19.2 percent (w/v); at less than 3.15 percent (w/v) NaCl, no growth occurred. Finally, the optimum light intensity was shown to be 25 to 35 Klux.

Dunaliella bardawil was shown to accumulate B-carotene up to 8 percent of its dry weight and glycerin up to 50 percent of its cell weight (Ben-Amotz and Avron, 1980). Glycerin accumulation was calculated to be approximately 8 g m^{-2} day^{-1}, based on a yield of 20 g m^{-2} day^{-1}.

Dunaliella parva was shown by Ben-Amotz and Avron (1973) to produce approximately 60 u moles of glycerol mg^{-1} chlorophyll. In the presence of 5 percent CO_2 the intracellular content of glycerol more than doubled, and the glycerol content increased linearly with increasing salt concentration from 0.6 to 2.1 M. Glycerol synthesis was shown to be ATP-dependent.

Dunaliella salina is a very salt-tolerant species which has been isolated from saline lakes such as the Great Salt Lake, especially the north arm (Post, 1977), Pyramid Lake, Nevada, and Borax Lake, Oregon (La Rivers, 1978). Physicochemical conditions of these lakes are shown in Tables IV-1, IV-2, and IV-3, and illustrate the diversity of habitats occupied by D. salina. Ben-Amotz and Avron indicated a growth rate for D. salina of 0.3g (dry weight)l^{-1} day^{-1}, with production of glycerol and B-carotene. The organisms are motile and average 17 x 9 µm in size (La Rivers, 1978). They are motile at temperatures below 0 C and are active to at least -6 C (Post, 1977). D. salina is the principal organism in the north arm of the Great Salt Lake and can be found in concentration ranging from 200 to 10,000 organisms ml^{-1}. Tables IV-4 and IV-5 show trace element concentrations of the Great Salt Lake, and thus indicate acceptable levels of trace elements for culturing D. salina.

Borowitzka (1981) and Post (1981) described several physiological parameters of D. salina. Although the salt tolerance range is from very low to saturated, at NaCl concentrations less than 10 percent (w/v) Dunaliella cells lose their flagella and settle out as 6 µm round, green cells. It was not discussed whether this might be a potential method to effect harvesting. Although D. salina has a very broad temperature tolerance range (i.e. less than 0 C to greater than 40 C), the highest photosynthetic rate occurs at 35-40 C. The optimum irradiation conditions were reported as 10,000 lux

TABLE IV-1. PHYSICO-CHEMICAL CONDITIONS OF THE GREAT SALT LAKE. VALUES ARE IN g l^{-1} UNLESS NOTED (POST, 1977)

	North Arm	South Arm
Mg^{2+}	11.1	4.2
Na^{+}	105.4	35.9
Ca^{2+}	0.3	0.2
K^{+}	6.7	3.0
Li^{+}	0.07	0.02
Cl^{-}	181.0	65.3
Br^{-}	0.2	0.09
SO_4^{2-}	27.0	8.7
HCO^{3-}	0.45	Not available (NA)
CO_3^{2-}	0.27	NA
B	0.04	0.02
o-PO_4 (μg l^{-1})	290-1600	NA
Total P (μg l^{-1})	630-4000	NA
NH_3 (ug l^{-1})	0-1080	NA
Soluble-N (mg l^{-1})	3.0-6.1	NA
Total-N (mg l^{-1})	4.3-7.2	NA
Oxygen (mg l^{-1})	0-1.70	NA
pH	7.7	8.2

TABLE IV-2. SELECTED PARAMETERS OF PYRAMID LAKE, NEVADA (LA RIVERS, 1978)

Dissolved salts	4700 ppm
Bicarbonates	800 ppm
Carbonates	225 ppm
Salinity	0.35 percent
pH	9.1

TABLE IV-3. SELECTED PARAMETERS OF BORAX LAKE, OREGON (LA RIVERS, 1978)

Bicarbonates	390 ppm
Carbonates	0
pH	7.8
Temperature	82°F

TABLE IV-4. TRACE ELEMENTS OF MORE THAN 1 mg l^{-1} IN THE GREAT SALT LAKE (POST, 1977)

Element	Concentration (mg l^{-1})
Al	4.00
Ba	4.25
F	15.90
Fe	22.51
Mn	1.33
Si	6.45
V	2.35

TABLE IV-5. TRACE ELEMENTS OF LESS THAN 1 mg l^{-1} IN THE GREAT SALT LAKE (POST, 1977)

Element	Concentration (μg l^{-1})	Element	Concentration (μg l^{-1})
Ag	34	Ni	10
As	220	Pb	530
Be	<10	Sb	10
Cd	167	Se	350
Co	10	Tl	20
Cr	119	Ti	50
Cu	226	W	<10
Hg	10	Zn	500
I	0		
Mo	50		

continuous white light; with CO_2 enrichment the optimum irradiation is 25,000 to 35,000 lux. Finally, the photosynthetic rate of D. salina is 600 umole O_2 mg^{-1} chlorophyll hr^{-1}, and in green species occurs at 4000 uEinstein m^{-2} sec^{-1} and in red species (those which have accumulated B-carotene in response to high salt concentration) occurs at 7000 μ Einstein $m^{-2}sec^{-1}$.

Dunaliella tertiolecta, grown with 3000 Joules m^{-2} min^{-1} of continuous light at 20 C and a dilution rate of 0.5 day^{-1}, was examined by Azov and Goldman (1982) for response to increasing ammonia. Maximum growth occurred at free NH_3 concentrations less than approximately 0.5 mM; the growth curve dropped rapidly at greater concentrations of free NH_3, and 1.2 mM NH_3 resulted in a 50 percent reduction in photoassimilation of ^{14}C. These data suggest that the pH at which D. tertiolecta should be cultured may need to be slightly alkaline as opposed to strongly alkaline, if ammonium salts are prevalent in the culture medium. This conclusion arises from the following equilibrium:

$$NH_4^+ + OH^- \underset{pK_a = 9.25\ (25°C)}{\rightleftharpoons} NH_3 + H_2O$$

Obviously, as the pH increases, the concentration of free ammonia increases.

While little information is available on the effects of trace metals on D. tertiolecta, Devi Prasad and Devi Prasad (1982) pointed out that lead at a concentration of 3 mg l^{-1} is lethal to this organism.

Dunaliella viridis reportedly is the only algae present in the Dead Sea (Post, 1977). Some physicochemical characteristics of the Dead Sea are shown in Table IV-6. It is also the principal planktonic species in the southern arm of the Great Salt Lake, reaching concentrations of 200,000 organisms ml^{-1} in late spring. D. viridis, unlike D. salina, prefers nitrate-nitrogen as opposed to ammonium-nitrogen. The organism is motile at temperatures below 0 C and is active to at least -6 C (Post, 1977). The temperature tolerance range is less than 0 C to greater than 40 C (Borowitzka, 1981). In vitro, the optimum generation time is 10 hours at 32 C (Post, 1977).

TABLE IV-6. SELECTED PARAMETERS OF THE DEAD SEA (POST, 1977)

Parameter	Concentration (g l^{-1})	Parameter	Concentration (μg l^{-1})
Mg	42.4	Cd	8
Na	39.7	Co	8
Ca	17.2	Cu	500
K	7.6	Fe	1
Li	0.02	Mn	7100
Cl	219.3	Ni	20
Br	5.3	Pb	120
SO_4	0.4	Zn	500
HCO_3	0.2		
pH	5.9		

Phaeodactylum tricornutum. This marine species was reported by Persoone and Clark (1980) to have a temperature range of 10 to 20 C. Mann and Myers (1968) stated that the optimum temperature for P. tricornutum is 18 C. However, Laws (1981) reported that a temperature of 24.5 C is near optimum and 26.5 C is nearly lethal for P. tricornutum. The cellular morphology of the species varies from oval cells 8 x 3 μm in size to fusiform cells approximately 25 to 35 um long; in liquid culture, naked protoplasts 6 to 12 μm in diameter have sometimes been observed (Lewin et al., 1958).

According to Mann and Myers (1968) the minimum doubling time is 18 hours and the optimum aeration is 1 to 2 percent CO_2 in air. No rate of CO_2 addition was given. The maximum photosynthetic rate reported by Mann and Myers (1968) was 33 to 35 μl O_2 evolved mm^{-3} hr^{-1}, at an irradiation intensity of approximately 4000 uwatt cm^{-2}. Laws (1981) reported that peak carbon production occurred at approximately 35 μEinsteins m^{-2} sec^{-1}, and that P. tricornutum Thomas showed 63 percent greater productivity than P. tricornutum Woods Hole at the optimum irradiation. Laws (1981) showed further

that peak lipid-carbon production of 15.8 and 22.5 tons ha^{-1} yr^{-1} for the Woods Hole and Thomas strains, respectively, could be expected at a light intensity of 35 μEinsteins m^{-2} sec^{-1}. Protein production also occurs maximally at 35 μEinsteins m^{-2}; the Thomas and Woods Hole strains produced 27.6 and 16.2 tons protein (ash-free dry weight) $acre^{-1}$ yr^{-1}, respectively. Thus, protein and lipid production by P. tricornutum are maximized in light-limited systems. In addition, Laws (1981) demonstrated that carbon, protein, and lipid production are negatively correlated with increasing temperature.

Somewhat similar results were reported by Thomas et al. (1981). With P. tricornutum cultures irradiated with 39.5, 59.6, and 69.8 percent of the maximum radiation at La Jolla, the highest yield--21.74 g m^{-2} day^{-1} --occurred with the lowest light intensity. In terms of photosynthetically active light (400 to 700 nm), a light-use efficiency of 12.3 percent was obtained at the lowest level of irradiation. Thomas et al. (1981) grew their cultures with 1 percent CO_2 in air supplied at 2000 ml min^{-1}. They also demonstrated that although nitrogen deficiency increased the total lipid content (up to 30 percent of the cell dry weight), the resulting reduction in total cell yield offset the increased lipid production. Thus, no advantage was obtained, in terms of total lipid production for unit volume of culture, by limiting nitrogen in the culture media.

Lewin et al. (1958) cultured P. tricornutum in either enriched sea water or in artificial seawater. These media illustrate the nutritional requirements of the organism. Enriched sea water contained 9 volumes of sea water and 1 volume of mineral supplement, to arrive at a final concentration as follows: $Ca(NO_3)_2 \cdot 4H_2O$, 0.01 percent; K_2HPO_4, 0.002 percent; Fe, 0.5 ppm; Zn, 0.3 ppm; B, Co, Cu, Mn, and Mo, 0.1 ppm each; enough $Na_2SiO_3 \cdot 9H_2O$, to give 5 ppm Si; and plus or minus tryptone at 0.1 percent. Artificial sea water contained the following: NaCl, 2 percent; $Ca(NO_3)_2 \cdot 4H_2O$, 0.05 percent; K_2HPO_4, 0.01 percent; $MgSO_4 \cdot 4H_2O$, 0.10 percent; Fe, 0.05 ppm Zn, 0.3 ppm; B, Co, Cu, Mn, and Mo, 0.1 ppm each; and $Na_2SiO_3 \cdot 9H_2O$, to give 5 ppm Si. As a result of their investigations, Lewin et al. (1958) reported the chemical composition data for oval and fusiform cells shown in Table IV-7.

TABLE IV-7. CHEMICAL COMPONENTS OF PHAEODACTYLUM TRICORNUTUM

	Percent Dry Weight	
	Oval	Fusiform
Lipid	24	84
Protein	84	41
Carbohydrate		
Soluble in cold dilute acid	8	2
Soluble in hot water, precipitated with ethanol (capsular material)	16	0
Miscellaneous		
Soluble in cold dilute acid (not carbohydrate, not ash)	10	10
Soluble in hot water, not precipitated with ethanol	8	2
Ash	13	12
Total	103*	101*

* Averages of several determinations.

Similar to their work with Dunaliella tertiolecta, Azov and Goldman (1982) showed a 50 percent reduction in cell growth of P. tricornutum in the presence of 1.2 mM free ammonia. The control of pH may be important in the large scale culturing of P. tricornutum if ammonium salts are added as nitrogen sources.

Skeletonema costatum. This cold-tolerant marine species was reported by De Pauw et al. (1980) to have a temperature range of 0 to 15 C, while Persoone and Claus (1980) reported a temperature range of 0 to 5 C. Shifrin et al. (1980) examined the effect of nitrogen starvation on lipid production and produced the following data:

	Log Cells	N-starved Cells
Cell nitrogen content (percent)	10.2	5.8
Lipid content (percent)	23.8	30.3

Thus, as the cell nitrogen content decreased, the lipid content increased. However, whether the increased lipid yield was great enough to offset a reduction in total cell yields, as studied by Thomas et al. (1981) with Phaeodactylum, was not reported. Shifrin et al. (1980) also reported that S. costatum which had been acclimated to copper produced more lipid in response to nitrogen starvation in a copper-containing medium than did copper-intolerant cells. These results suggest some ability of various microalgae species to adapt to increased concentrations of trace metals.

Spirulina spp. Various species of Spirulina tolerate from freshwater to brackish waters. They have been included in this section on marine species simply for the sake of comparison with other halotolerant species.

Spirulina spp. are blue-green, filamentous algae which prefer alkaline waters. Goldman (1980) stated that 2 g l^{-1} bicarbonate is a growth requirement. Individual species vary in their product accumulation. Tel-or et al. (1980) reported that a Spirulina spp. contained approximately 1 percent (dry cell weight) chlorophyll a, 0.2 percent B-carotene content, and 0.2 percent xanthophylls. Materassi et al. (1980) presented the following information for a Spirulina spp.: total lipid content, 8 to 12 percent (cell dry weight); unsaponifiable fraction, 32.7 to 40.6, 39.3 to 52.2, and 5.3 to 8.2 percent hydrocarbons, alcohols, and sterols, respectively.

Spirulina maxima has a cell size of 200 to 300 x 5 to 10 μm, but is filamentous and forms multicellular, 1000 μm-long spirals (Durand-Chastel, 1980). It grows well at 30 C (Shelef et al., 1980). The total cholesterol content was reported by Materassi et al. (1980) as 149 mg kg^{-1} dry weight.

In another study, Durand-Chastel (1980) analyzed the composition of meal made from Spirulina maxima (Table IV-8). These data show that S. maxima, in addition to lipid production, produces a substantial amount of protein.

TABLE IV-8. CHEMICAL COMPOSITION OF SPIRULINA MAXIMA MEAL (Durand-Chastel, 1980)

Component	Fraction of Meal
Protein	60 to 70 percent
Fiber	0.1 to 0.9 percent
Xanthophylls	1.4 to 1.8 g kg^{-1}
B-carotene	1.5 to 1.9 g kg^{-1}
Chlorophyll a	6.1 to 7.6 g kg^{-1}
Total lipids	6 to 7 percent

Spirulina platensis has a temperature tolerance range of 20 to 40 C, with the optimum range being 25 to 35 C (Soong, 1980). The pH tolerance range is 7.0 to 10.5, with an optimum range of 8.5 to 9.5. An indication of the nutritional requirements of S. platensis is shown in the medium used by Venkataraman et al. (1980) for culturing the organism: $NaHCO_3$, 4.50 g l^{-1}, $NaNO_3$, 1.50 g l^{-1}, NaCl (crude sea salt), 1.00 g l^{-1}, K_2SO_4, 1.00 g l^{-1}; K_2HPO_4, 0.50 g l^{-1}; $MgSO_4$, 0.20 g l^{-1}; $CaCl_2$, 0.04 g l^{-1}; Fe_3SO_4, 0.01 g l^{-1}; and pH, 9.5 to 10.5.

Freshwater Species

Ankistrodesmus falcatus. This freshwater, green algae has been examined by Devi Prasad and Devi Prasad (1982) with respect to effects of cadmium, nickel, and lead. It is included here because very little information on the response of microalgae to trace metals was located. Even though this particluar species has not been examined in terms of biomass systems, this information may prove useful with respect to other species.

No effect on the growth of A. falcatus was exhibited in the presence of Cd at 0.1 to 1.5 ppm; of Ni at 0.1 to 1.5 ppm; or of Pb at 0.1 to 0.5 ppm. Growth inhibition was observed in the presence of Cd at 2.0 to 3.0 ppm; of Ni at greater than or equal to 2.0 ppm; and of Pb at 1.0 to 5.0 ppm. Finally, Cd at 5 ppm or Pb at 10 ppm were lethal to A. falcatus. Nickel was not lethal at

the concentrations examined. In addition, Cd and Ni, and Cd and Pb, exhibited synergistic interactions of increased toxicity at low doses when presented together.

Chlorella spp. Most species of Chlorella are freshwater species. Chlorella saccharophila, a marine species, was discussed under the previous section. Considerable information exists on the growth of Chlorella spp., largely due to early investigation on the potential for using various microalgae as food for humans or animals (Carnegie Institution, 1953). Chlorella spp. are green algae with a cell diameter of 5 to 10 μm. The lipid content may vary from species to species, but Materassi et al. (1980) reported the following data for Chlorella spp.: lipid content, 15 to 27 percent (cell dry weight); unsaponifiable fraction of the biomass, 1.34 percent; and the composition of the unsaponifiables, 27.3, 44.4, and 21.8 percent for hydrocarbons, alcohols, and sterols, respectively. Soong (1980) states that the growth rate of Chlorella spp. varies from 15 to 30 g m^{-2} day under summer conditions.

Chlorella ellipsoida was shown by Kawaguchi (1980) to exhibit differing growth rates depending on whether growth was autotrophic, autotrophic with pH control, or mixotrophic. Mixotrophy resulted in a growth rate (over approximately 4 days) of 27 g m^{-2} day^{-1}. Autotrophy resulted in a growth rate of 18.7 g m^{-2} day^{-1}. Autotrophy plus pH control (presumably to enhance carbon availability) gave the highest growth rate: 27.5 g m^{-2} day^{-1}. Thus, under appropriate conditions, autotrophic growth may be as great as under mixotrophic conditions. The effect of Cd on the growth of C. ellipsoida was reported by Devi Prasad and Devi Prasad (1982): Cd greater than 50 uM interrupted cell division and inhibited chlorophyll synthesis.

Chlorella fusca var vacuolata was shown by Rodriguez-Lopez et al. (1980) to produce varying levels of protein depending on the nitrogen source in the medium, as shown below:

N source	Protein (percent cell weight)
KNO_3 (1.011 g l^{-1})	79.5
$(NH_4)_2SO_4$ (1.32 g l^{-1})	52.5
no N	48

These workers also examined the soluble and total amino acid contents of cells as influenced by nitrogen source.

Chlorella pyrenoidosa exhibited 4-day growth rates of 30.0 g m^{-2} day^{-1}, 31.9 g m^{-2} day^{-1}, and 19.3 g m^{-2} day^{-1} under conditions of mixotrophy, autotrophy plus pH control, and autotrophy, respectively (Kawaguchi, 1980). Under conditions of light saturation, the growth rate of C. pyrenoidosa was 39 ul of oxygen evolution mm^{-3} hr^{-1} (Mann and Myers, 1968). Shifrin et al. (1980) reported that log phase cells had cell nitrogen contents of 7.4 percent and lipid contents of 14.4 percent. On the other hand, nitrogen-starved cells had cell nitrogen and lipid contents of 3.1 and 35.8 percent, respectively. It was not reported whether the decreased total cell yield was compensated by the increased cellular lipid content. Finally, Taub (1980) reported that cells of C. pyrenoidosa contained up to 63 percent protein on a dry weight basis. Such high protein content was reached using adequate nitrate and short exposures to light.

Chlorella vulgaris exhibited a growth rate of 10.47 to 14.03 g m^{-2} day^{-1} (Shifrin et al., 1980). Under copper stress (0.01 μM to 4 μM), lipid synthesis in C. vulgaris was depressed: from 40.6 percent of total cell weight in Cu-tolerant cells to 28.8 percent in Cu-stressed cells.

Chlorococcum spp. Devi Prasad and Devi Prasad (1982) have accumulated some metals toxicity data on this freshwater green algae. Cadmium at 0.1 to 1.5 ppm, Ni at 0.1 to 1.5 ppm, or Pb at 0.1 to 1.0 ppm showed little effect on growth of Chlorococcum spp. Increasing inhibition of growth occurred with increasing concentrations of Cd up to 5.0 ppm, by Ni up to 5.0 ppm, and by Pb up to 10 ppm. At greater than 5 ppm, Cd was lethal to this organism.

Oscillatoria spp. Several species of the blue-green algae Oscillatoria have been characterized with respect to their fatty acid composition (Materassi et al., 1980). The total lipids content of Oscillatoria spp. was 13 to 18 percent.

Scenedesmus spp. Like Chlorella, substantial work has been done with Scenedesmus with the intention of using this green, freshwater species as a food source (Carnegie Institution, 1953).

Materassi et al. (1980) reported the total lipid content of a Scenedesmus spp. to be from 17 to 26 percent of the cell dry weight. Payer et al. (1980) listed the chemical composition of Scenedesmus as follows (in g $100g^{-1}$ dry matter):

Crude protein	50 to 56
Carbohydrates	10 to 17
Fat	12 to 14
Fiber	3 to 10
Moisture	4 to 8
Nucleic acids	3 to 6.

Payer et al. (1980) also listed the vitamin content and the minerals content of Scenedsmus spp. Scenedesmus acutus exhibited a growth rate of 25 g m^{-2} (Castillo et al., 1980). It showed an upward temperature tolerance of approximately 38 C and an optimum temperature range of 30 to 32 C.

The optimum culture density reported by Castillo et al. (1980) was 30 to 50 g m^{-2} and an optimum pH of less than 7.4. Finally, the water use rate of S. acutus reportedly is 43,000 m^3 ha^{-1}, or 1000 m^3 ton protein $^{-1}$, and a light requirement of at least 800 cal cm^{-2} day $^{-1}$. Venkataraman et al. (1980) utilized the following medium to culture S. acutus, and is thus indicative of the nutritional requirements of this species (concentrations are in mg l^{-1}): urea, 60; K_2CO_3, 25; Na_3PO_4, 25; crude sea salt, 25; NH_4SO_4, 20; $MgSO_4$, 10; $FeSO_4$, 2; and pH, 7.0 to 8.0.

The fatty acid composition of S. acutus, and the influence of varying irradiation intensity on lipid synthesis, were studied by Materassi et al. (1980). Fatty acids of 16 carbons with 0 or 4 double bonds, and fatty acids of 18 carbons with 2 or 3 double bonds, made up the majority of the lipids in S. acutus. In response to increased irradiation, a large decrease occurred in the synthesis of fatty acids of 18 carbons with 2 double bonds.

Scenedesmus obliquus, according to Abelovich (1980), is inhibited by a free ammonia concentration of greater than 2 mM at a pH greater than 8.1. Azov and Goldman (1982) refined the observation and showed a 50 percent reduction in the photoassimilation of ^{14}C in the presence of 1.2 mM free ammonia. The optimum pH in order to prevent NH_3 toxicity was 8.0; at higher pH values growth was decreased, due to increased free NH_3. Exposure of S. obliquus to 0.5 mM or less of free NH_3 had inhibitory effects on growth. Devi Prasad and Devi Prasad (1982) showed that Cd between 0.1 to 1.0 ppm had no effect on S. obliquus, while greater than 5.0 ppm were lethal. Between 0.1 to 1.5 ppm, Ni had no effect on this algae, and showed inhibition, but not lethality, at greater than 2.0 ppm. Lead at greater than 1.0 ppm caused growth inhibition, but lesser concentrations had no effect.

Materassi et al. (1980) examined the influence of growth temperature on the fatty acid composition of S. obliquus and S. obtusiusculus. When the temperature was changed from 20 C to 30 C, substantial changes in the fatty acid profile were apparent.

Other Species

Limited information on the algae species listed below was obtained. These species were identified during the search for information on the previously described species and serve to illustrate an important point: many potentially useful species are yet to be thoroughly characterized or identified. As mentioned previously, species selection efforts are currently ongoing by other SERI subcontractors.

Amphiprora spp: A marine algae with an optimum temperature range of 20 to 25 C (Persoone and Claus, 1980).

Amphiprora alata: Isolated from Pyramid Lake, Nevada (La Rivers, 1978).

Amphiprora lepidoptera: Isolated from Pyramid Lake, Nevada (La Rivers, 1978).

Amphora spp: A marine, green algae with an optimum temperature range of 20 to 25 C (De Pauw et al., 1980).

Calothrix spp: A blue-green algae with a percentage unsaponifiable content (based on total biomass) of 1.19 percent, and this fraction is made up of hydrocarbons (48 percent), alcohols (39.6 percent), and sterols (7.8 percent). (Materrassi et al., 1980).

Monallanthus saline: Lipid content was increased from 40.8 percent of the cell dry weight to 72.2 percent of the cell dry weight by nitrogen starvation; and 4 μM Cu reduced lipid synthesis in Cu-intolerant cells (Shifrin et al., 1980).

Neochloris oleoabundans: Potential high lipid content producer being studied by Lien (1981).

Neochloris pseudostigmata: Potential high lipid content producer being studied by Lien (1981).

Neochloris texensis: Potential high lipid content producer being studied by Lien (1981).

Nitzchia closterium: A marine species which grows well between 20 and 25 C (Persoone and Claus, 1980).

Nitzschia longissima: Marine species with a temperature range of 15 to 22 C (De Pauw, 1980).

Nitzschia palea: Marine species in which lipid content (percent of biomass) increased from 22.9 to 39.5 percent in response to nitrogen starvation (Shifrin et al., 1980).

Nostoc spp: Blue-green algae with a lipid content of 5 to 8 percent. (Materassi et al., 1980).

Nostoc communa: The unsaponifiable portion of the biomass is 0.49 percent, and is composed of hydrocarbons (41.8 percent), alcohols (40.4 percent), and sterols (14.5 percent) (Materassi et al., 1980)

Ochromonas danica: Lipid content increased from 39.9 to 53.2 percent of the dry weight when the temperature was increased from 15 to 30 C.

Selenastrum gracile: A green algae with a total lipids content of 22 to 27 percent; the unsaponifiable percent of the biomass is 4.34 percent and contains hydrocarbon (23.1 percent), alcohols (35.5 percent), and sterols (28.1 percent). (Materassi et al., 1980)

Synechococcus spp: A unicellular blue-green algae which is capable of anoxygenic photosynthesis; may be similar to or identical to Aphanothece halophytica (Borowitzka, 1981).

Criteria for Selecting Microalgae

In order for microalgae biomass systems to be successful, a number of useful microalgae species must be identified and characterized. A useful species is one which produces a desirable end-product and can be grown under the specific conditions employed in the biomass system. The specific conditions to consider include, in addition to selecting species which produce a useful product, the culture media including the chemical elements present and their concentrations; the source, amount, and purity of carbon; illumination; temperature; pH; salt concentration; and the physiological response of the algae to these parameters.

Culture Media

The culture media for microalgae biomass systems will be saline water of variable quality. Ideally, species would be found which not only tolerate the quality of the water to be used (trace metals, pH, salinity, etc.) but can extract their nutrient requirements without the need for additional supplementation. Laboratory maintained strains, although certainly essential for basic studies, may not reflect the water quality requirements of microalgae *in situ*. For this reason, indigeneous strains may be desirable, assuming species can be isolated which produce useful products. Intuitively, the ideal species would be one that utilizes nutrients most efficiently, i.e., produces the greatest biomass or end-product while using the least amount of a nutrient.

Little information exists with respect to the responses of various species to potentially toxic trace elements. A specific criterion is to select useful species resistant to the quality and quantity of trace elements likely to occur in southwestern saline water. An alternative approach may be to acclimate useful species to the level of toxic elements likely to be encountered.

Carbon Source

Because CO_2 is the form of carbon which interacts with the major carboxylase, ribulose diphosphate carboxylase, unhydrated CO_2 is the major source of carbon for algal growth. Carbon dioxide enters the cell by diffusion, and for maximum growth rates, CO_2 concentrations should be as high as possible. In the Southwest, however, saline groundwater is alkaline and thus contains more carbonates and bicarbonates than unhydrated CO_2. Not all microalgae species can absorb bicarbonate and those that do must convert the ion to unhydrated CO_2 for fixation via ribulose diphosphate carboxylase. Uptake of bicarbonate is an energy-requiring process and bicarbonate is converted intracellularly to CO_2 and OH^- by carbonic anhydrase. This enzyme does not occur in all microalgae but is prevalent in marine species; however, it is inducible in many species and may serve as an adaptation to low CO_2, high bicarbonate, high pH.

For southwestern microalgae biomass systems, the status of carbon in the water will dictate which species will grow. The ideal species, and thus a criterion for selection, would possess carbonic anhydrase constitutively or inducibly, and thus could employ both hydrated and unhydrated forms of CO_2. Laboratory studies in which CO_2 is bubbled through water as the source of carbon supplementation should employ high enough pH's to insure that bicarbonate is readily available.

Illumination

The ideal microalage species for biomass sytems would exhibit a very high photosynthetic efficiency, yet have an optimum growth rate when light is somewhat limiting. Several species described earlier produced the greatest cell yield when light was limiting; the optimum growth rate, as opposed to the maximum growth rate, has been discussed by Goldman (1980). The advantage to such a characteristic is that in a large pond system, irradiation intensity will be attenuated rapidly with increasing depth. This is often compensated for by agitation in order to expose as many individual cells as possible to the most irradiation. Species which grow optimally over the long term at less

than saturating irradiation may be adaptable to a pond in which the need for (energy intensive) agitation is greatly reduced.

Temperature

Southwestern saline pond systems will be subjected to extreme fluctuations in temperature, from less than 0 C to greater than 40 C. In lieu of some form of temperature control, which may be very costly on the scale proposed for pond systems, species should be selected which exhibit extreme tolerance to temperature fluctuations. The ideal species would remain metabolically active at temperatures below freezing and up to 40 C or greater. A species which produces a desirable product yet is killed at moderately warm temperatures (e.g., 30 C) may find limited use in southwestern biomass systems.

pH

Groundwater supplies in the Southwest are likely to be alkaline due to the calcareous nature of the soils and the salinity of the water itself. Microalgae may tolerate extremes in pH as high as 13.5; *Chlamydomonas acidophila* can be cultured at pH 2.0 in vitro and may survive pH 1.0 in situ. Some species of Chlorella have a lower tolerance limit of pH 2.0, while other *Chlorella* have a lower limit of pH 5.5. Thus, it is not easy to generalize optimum conditions with respect to pH, and candidate microalgae must be delineated individually with respect to hydrogen ion tolerance. An important consideration in biomass programs, in which CO_2 may be the limiting factor, may be the use of inorganic carbon other than CO_2, e.g., bicarbonate. As discussed previously, bicarbonate concentrations are increased at high pH, and carbonic anhydrase is active also at these higher pH's. Marine species are adapted to these higher pH levels, and the maintenance of alkaline pH may be the best option for maximizing carbon use efficiency.

Therefore, an important criterion for screening potentially useful microalgae species is that they should be able to tolerate alkaline pH. Those species screened on the basis of carbon use (bicarbonate) should be adaptable

to alkalinity. However, as shown in the section on species descriptions, alkaline pH in the presence of high ammonium salts may result in high concentrations of free ammonia, and thus toxicity. It may be necessary to screen species also on the basis of ammonia toxicity, or control the ammonium salts in microalgae ponds.

Salinity

Water ranging from fresh to salt-saturated will probably be exploitable for microalgae aquatic biomass sytems. This conclusion is based in part on the ecology of halophilic and halotolerant microalgae, such as species of Dunaliella. This genera reportedly is the only eukaryotic algae which grows in extremely saline lakes.

The Great Salt Lake in Utah has been studied fairly extensively in terms of microbial ecology and may offer an ideal site from which to gain perspective on the saline culturing of microalgae. This lake is approximately 10 m deep, 116 km long, and 50 km wide, and is divided approximately in half by an east-west traversing causeway. Fresh water enters only in the southern half of the lake and migrates slowly to the north half. The result is a salinity gradient ranging from less than 10 percent (w/v) NaCl in the southern half to greater than 30 percent (w/v) NaCl (saturated) in the northern half. The elevation of the lake is 1280 m and so it is subject to annual air temperature variations of from -30 C to 40 C. The water temperature, however, varies from 5 C to 30 C for the most part; in isolated shallow areas of little slope, the water temperature can reach 50 C. In addition, the water and sediments reportedly contain Li, B, Cu, Zn, Pb, Cd, and As in unusually high concentrations (Brock, 1975).

Interestingly, Dunaliella spp. are found throughout the salinity and temperature ranges of the Great Salt Lake. Whereas some other prokaryotic and eukaryotic algae may tolerate some degree of salinity, only Dunaliella can tolerate salt-saturated conditions. The optimum salinity for Dunaliella, however, is about 15 percent (v/v) NaCl, much lower than its tolerance limit. Thus, Dunaliella dominates at higher salinities not because it thrives optimally under such conditions, but because it out-competes all other species.

It should be apparent that there are naturally occurring species capable of producing useful products and which can tolerate various levels of salinity. Microalgae species need to be selected which will span the salinity range expected. A feasible system may be a series of ponds, each of a slightly greater salinity and each with a differently adapted algae.

Physiological Responses of Algae

The ideal microalgal species would produce a valuable product at a rate wihch can be controlled by external variables such as temperature, salinity, illumination, or nutrient level. Progress has been accomplished with a few species (discussed earlier), but considerable work needs to be done on other species. Microalgae species need to be screened on the basis of their responses to environmental parameters so that their production can be easily controlled in large-scale systems.

Summary

To summarize the above discussion, the ideal microalgae species for biomass systems in the Southwest would exhibit the following characteristics:

- It would produce a highly desired product which, ideally, would be easily recoverable.
- It would utilize nutrients and water efficiently and would show some tolerance to trace metals.
- It would be able to utilize alternative sources of carbon, such as bicarbonate and low quality CO_2.
- It would show optimum growth and/or production of the desired product at less than saturated irradiation.
- It would have a broad temperature tolerance range, possibly from 0 to 40 C.
- It would prefer a neutral to slightly alkaline pH.
- It would possess a high tolerance to saline water.

- It would exhibit predictable responses to changes in growth conditions with the intent to alter the rate of formation or quality of end product.

These ideal characteristics also illustrate some of the criteria which should be employed for screening potentially useful species of microalgae.

Information Needs

It should be quite apparent that there are very few species which have been characterized well enough to be employed in a microalgae biomass system. Data are sorely lacking with respect to those species which produce desirable products and which tolerate the environmental and cultural conditions of pond systems in the Southwest. A systematic search for potential species should be performed, based in part on the criteria established in the previous section. Basic data such as carbon use efficiency, water use efficiency, photosynthetic efficiency, tolerance to pH, salinity, temperature, and trace elements, and response to irradiation are necessary for each species. The most important criterion, however, is to determine whether a given species produces a potentially useful product. Based on this initial screening, the other criteria can be applied systematically.

Programs are currently underway, sponsored by SERI, to identify candidate species. A part of these efforts will examine species indigenous to the Southwest, and then characterize them with respect to chemical composition, growth requirements, etc. The current efforts for SERI might benefit if the previous list of criteria, or an expanded version of the criteria, were applied to each species.

In Section VI, more specific research needs have been identified. To summarize the data gaps, the information which should be compiled on each potentially useful species is shown in Table IV-9.

TABLE IV-9. INFORMATION NEEDS FOR MICROALGAE SPECIES POTENTIALLY USEFUL FOR AQUATIC BIOMASS SYSTEMS

- Useful product obtained
- Carbon use data
  - use efficiency
  - chemical form(s) required
- pH data
  - tolerable range
  - optimum
- Temperature data
  - tolerable range
  - optimum
  - effect of changes on end-product composition
- Salinity data
  - tolerable range
  - optimum
  - effect of changes on end-product accumulation
- Irradiation data
  - maximum response
  - optimum response
  - long-term growth rate
  - use efficiency
  - effects of changes on end-product accumulation
- Other data
  - water use efficiency
  - ease of maintenance
  - response to potentially toxic trace elements

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V. TECHNO-ECONOMIC CONSTRAINTS

Carbon Sources for Algal Biomass Systems

Because algal biomass contains approximately 50 percent carbon by weight, large quantities of CO_2 must be provided to obtain high yields of algae continuously on a long-term basis. Due to the low concentration of atmospheric CO_2 and the slow transfer of CO_2 from air to water to the algal cell, a supplemental source of highly concentrated CO_2 is required.

Several potential sources of CO_2 have been identified. These include natural deposits of CO_2, byproducts of ammonia or syngas production, power plant flue gases, spent rock from oil shale processing, and naturally occurring deposits of carbonate minerals.

Gaseous CO_2

Gaseous CO_2 occurs naturally in several western states. Enhanced oil recovery (EOR) has become common in the southwest, and many projects are utilizing CO_2 as an injectant to mobilize underground reserves. In several instances, naturally occurring CO_2 is being drilled at its origin and then piped to the EOR field. For example, the SACROC pipeline, operating since the early 1970s, transports a 90 percent CO_2, 10 percent CH_4 gas to and from various points in southwest Texas. ARCO, EXXON, and Amerada Hess have plans to construct a pipeline from natural CO_2 fields in southeast Colorado and northeast New Mexico to oil fields in west Texas (Oil and Gas J., 4/26/82, p. 117). Shell Oil Company will be undertaking a similar project in Mississippi. Shell also has announced plans to build a pipeline from Montezuma County, New Mexico, to West Texas.

CO_2 may be recovered in the gaseous state from the production and processing of natural gas. Reservoirs currently being developed contain higher CO_2 concentrations (15 to 66 percent CO_2) than those which were profitable to drill in the past.

When hydrocarbons are steam reformed in anhydrous ammonia plants to produce hydrogen for ammonia synthesis, CO_2 is one of the byproducts. The

CO_2 is currently considered a waste, but may be made available for a beneficial use, such as biomass production (Sittig, 1968).

Production of other industrial gases may also result in CO_2 as a byproduct, which may be scrubbed out of exhausts. Therefore, other types of industrial plants may be sources of CO_2 gas (Sittig, 1968).

In the production of synfuels, coal and steam are reacted with a catalyst to produce methane and carbon dioxide. A similar reaction is used to make methanol. CO_2 associated with synfuels is not destined to be utilized for any purpose and may be available for distribution. Stationary synfuels plants would usually locate near large coal deposits. Recently, a portable unit has been developed which can perform the catalytic conversion in the field near sources of small amounts of coal (Oil and Gas J., 6/28/82, p. 116). Thus, in suitable areas, CO_2 may be produced on site.

Carbonate Minerals

Oil shale processing produces both shale oil and solid waste material. The spent rock remaining after extraction of the oil may contain carbonates which could be used in some algae systems. The first commercial oil shale project is scheduled to begin operation in early summer of 1983. The plant, owned by Union Oil Company of California, is located in Parachute Creek, Colorado, near the northern border of the SERI study area. At the present time, plans call for the waste rock to be disposed on the canyon floor. As of June 1982, 10 to 15 other oil shale projects were in the planning stages. Many of them are within the SERI study area. The earliest is expected to begin construction in early 1983 (Oil and Gas J., 6/28/82, p. 71).

Naturally occurring deposits of carbonate minerals are plentiful but not necessarily useful sources of carbon for algae production. Deposits of nahcolite (sodium bicarbonate, $NaHCO_3$) occur in northwest Colorado and the Green River Basin in Wyoming. Wyoming deposits produced 250,000 tons in 1979. The nahcolite in Colorado has not yet been mined. Common impurities in the mineral include arsenic and heavy metals. Nahcolite also occurs with oil

shale in the Piceance Creek Basin in Colorado, Utah, and Wyoming and may be recovered from spent rock left over after processing, as discussed above.

Problems and Limitations

Several of the CO_2 sources discussed above have limitations which may restrict or prohibit their use for microalgal biomass systems.

Since enhanced oil recovery is very common in the southwest, large amounts of high purity, high pressure CO_2 will be required in that region. Competition between biomass and EOR projects will be keen for CO_2 from such sources as natural deposits of gas and coproduction with natural gas.

Because EOR produces more energy per unit of CO_2 supplied than can algal biomass fuels projects, EOR can pay higher prices for CO_2 and will obtain sources of pure CO_2 more readily than biomass fuel producers. Therefore, biomass projects will be forced to utilize less pure sources or produce higher valued chemical products.

The types and levels of impurities that can be tolerated by microalgal species in the proposed type of system is not well understood. The types of impurities expected from power plant flue gases include H_2S, SO_2, SO_3, CO, and CH_4. Impurities from other sources may include heavy metals and bacteria. Pretreatment of impure sources may improve their suitability for algal biomass use, but the cost for treatment is an important consideration.

Geographic location of sources is an important consideration. Fossil fuel burning power plants, ammonia synthesis plants, and other process plants, are typically located in urban or industrialized areas, whereas the biomass project will probably be located in a more underdeveloped setting. Costs of transporting CO_2 from these relatively inexpensive distant sources must be included to determine a realistic price estimate.

The use of spent rock from oil shale processing also depends upon the geographic location of oil shale facilities. Use of bicarbonate minerals would require rail or truck transport, which, over long distances, could become quite expensive and could exceed the potential profits from a biomass facility. Therefore, minemouth location probably would be required.

Use of solid bicarbonates may be unacceptable due to the rise in system pH, which occurs upon application. A culture dilution rate higher than that required for maximum productivity may be required and may considerably reduce economic efficiency of the project.

An additional disadvantage of the fossil fuel power plant source is that its utilization depends on the burning of fossil fuels in future years. If the particular plant being utilized should cease energy production for any reason, a different source for CO_2 would be necessary.

If a 1,000 hectare system were to be developed, a yield of 40 tons per hectare per year would require the amount of CO_2 shown in Table V-1, assuming 2 lbs. CO_2 required for every pound biomass produced.

Costs of CO_2 will vary depending on cost to purchase from the source, treatment necessary to remove contaminants or to pressurize, distance of travel and mode of transportation, and methods of application.

Water Supply Costs

One of the major barriers to full-scale commercialization of microalgal pond systems may be the cost of water supply. Although direct competition with supplies of freshwater has been avoided for institutional reasons, this has the effect of requiring supply from less accessible sources. Prior analysis that mandates microalgal system costs of $0.50 to $3.00 a square foot places limits on the cost of water supply (Hill, 1982). It would be difficult to profitably operate a system whose water supply costs exceeded 30 percent of the total annualized capital and operating costs. Therefore, this analysis was undertaken to determine whether water could be supplied from marine or saline groundwater sources for between $0.15 and $0.90 per square foot.

As with any conceptual analysis not based on site specific data, a number of generalizations were made. Furthermore, assumptions were required concerning the economic atmosphere prevailing between now and 1987, when the first full scale systems could be operating. To maintain consistency with other economic analyses of microalgal systems, a design life time of 20 years and a return rate of 12 percent on the investment were used.

TABLE V-1. CO_2 REQUIREMENTS AND COST FOR MICROALGAL SYSTEMS

$$1{,}000 \text{ ha } \frac{40 \text{ tons algae}}{\text{hectare}} = \frac{2 \text{ lbs } CO_2}{1 \text{ lbs algae}} = 80{,}000 \text{ tons } CO_2/\text{year}$$

$$80{,}000 \text{ tons } \frac{1.63 \times 10^4 \text{ SCF}}{\text{Ton}} = 1.304 \times 10^9 \text{ SCF} = 1.3 \text{ million MSCF } CO_2/\text{year}$$

Where

SCF = standard cubic feet
MSCF = thousand standard cubic feet

Since costs range from \$1.25 - \$3.00/MSCF, (Chemical Week, 1982), the cost for CO_2 for the project will range from:

1.3 million CO_2/year x \$1.25/MSCF = \$1.6 million/year for CO_2 to
1.3 million MSCF CO_2/year x \$3.00/MSCF = \$3.9 million/year.
This is equivalent to about \$50 per oven dried ton of algae.

Other economic data necessary for use of the costing model included the following:

- Engineering News Record construction cost index = 3845 (July, 1982);
- Operation and Maintenance labor cost = $12/hr.
- Electric cost = $0.06/kwh
- Inflation = 8 percent/yr.

The principal analysis tool was the Methodology for Areawide Planning Studies (MAPS) model developed by the U.S. Army Corps of Engineers (1982). It provides the user with an estimate of annualized system costs based on a modular definition of linked system components. Although considerable flexibility in the specification of the system is possible, some standardization in the materials and/or configuration is required. Where these assumptions may have affected the outcome is pointed out in the discussion of the results.

Seawater Systems

Water supply from seawater for microalgal systems has the advantage over groundwater systems in that a virtually unlimited supply is available.

This system was conceptualized as a pressure conduit-pumping station arrangement discharging to an equalization pond of 3 million gallon capacity. Minor head losses were accounted for by estimating the number of valves, bends and exit orifices that would be typical of each system.

A number of fixed specifications were maintained across all nine systems evaluated to reflect likely study area conditions and favorable costs. Each of these specifications is explained below:

(1) Flow - Both average and peak system flows were provided for uncovered systems. Therefore, costs for each of the systems is conservative if covers are used, since the average flows are reduced and peak to average flow ratios will be close to unity. Average and peak values were 23.04 and 28.80 million gallons per day, respectively.

(2) Land Use Type - Pipelines were to be constructed across undeveloped areas. Construction in some coastal areas could require

portions of the line to pass through built up areas, increasing the cost.

(3) Dewatering - Construction in wet soils would necessitate special consideration of conditions for laying and operation of pipeline, which would increase the cost.

(4) Rock Excavation - Extra costs associated with excavation into rock would have to be developed on a site specific basis from soil depth maps and were not included for this analysis.

(5) Trench Dimensions and Pipe Supports - The trench was specified as three feet in depth, 1.5 feet in width and with vertical sides. Specified soil conditions where these conditions would yield unstable trenches or unacceptable loads would have to be developed for an individual site.

(6) Although these systems will be constructed in relatively remote areas, no allowance was made for incremental labor or material costs associated with the excavation or pipe laying work.

(7) Pipe Type - Reinforced concrete pipe was specified for reasons of ease of construction and durability under study area conditions. Other pipe types are also unavailable in the diameter required.

(8) Roughness - Friction head losses were computed based on new pipe.

(9) Delivery Pressure - End-of-line pressure was specified as equivalent to 200 feet of head except for System 9. Alternately, the given system could deliver water to a 200 foot higher final devation at zero head.

(10) Initial Elevation - All systems withdraw water at sea level.

(11) Pump Station - All systems were cost minimized with no wet well, switchyard, simple protective structures and a 60 percent efficiency.

Two principal variables determined system costs--final elevation and length. Land costs, although computed for a 10 foot easement at 1000/acre and $1000 per pumping station, were basically insignificant in their effect on unit area costs.

Table V-2 and Figure V-1 summarize the analysis of seawater systems. Unit costs range from about $0.13 for a 2000 ft. long line with a 25 foot elevation differential to $5.14 for a 38 mile line and a 2500 foot differential. Therefore, seawater systems below $0.15/ft^2 must be essentially coastal systems. Allowing for a reduction in pumping head to 10 feet could permit a pipeline length of perhaps 3.5 miles over level terrain. At the other extreme, 0.90/ft^2 could be met by 3.8 mile pipelines at up to 600 foot elevation or longer lines with lower elevation differentials.

Any conclusions regarding water supplying costs for covered systems based on these data would have to be qualified due to the uncertainties at this time, but could and should be explicitly accounted for in future work. Nevertheless, the factors cited earlier as differences between open and closed systems guarantee that the water supply costs will be significantly lower for covered systems.

Groundwater Systems

The parametric cost analysis for groundwater supply systems utilized many of the same design assumptions as the seawater system. One artifact of the analysis which had some effect on cost was the need to assume a radial well configuration. Depending on the potential interference between wells, which could be evaluated only if actual data were available, a rectangular well field grid would reduce the amount of connecting pipe required.

Fixed specifications for these systems were as follows:

(1) Flows - Same as seawater systems.

(2) Pump Efficiency - Same as seawater systems.

(3) Delivery Pressure - 10 feet

(4) Test Wells - Often exploratory wells are drilled to determine capacity and interference data. It was assumed that this would be a research need not associated with the commercial scale system at least for the first few systems built.

(5) Special Material and Labor Costs - As with the seawater systems, standard costs were assumed even though the systems may be

TABLE V-2. WATER SUPPLY COST ANALYSIS - SEAWATER SYSTEMS

	MOD 1	2	3	4	5	6	7	8	9[c]
Title	Seawater 1	Seawater 2	Seawater 3	Seawater 4	Seawater 5	Seawater 6	Seawater 7	Seawater 8	Seawater 9
Length, ft.	2000	20,000	200,000	200,000	20,000	200,000	20,000	2,000	20,000
Final Elevation, ft.	25	250	250	1,000	1,000	2,500	25	250	250
Diameter, in.	42	42	42	42	42	42	42	42	42
Gate Valves, No.	2	2	4	4	4	4	2	2	2
Std. Elbow, No.	0	0	0	0	0	0	0	0	0
Med. Elbow, No.	0	0	0	2	2	2	0	0	0
Long Elbow, No.	0	0	0	0	0	0	0	0	0
Exit Losses, No.	1	1	1	1	1	1	1	1	1
Land Cost, $[a]	460	4,600	46,000	46,000	4,600	46,000	4,600	460	4,600
No. Stations	1	1	1	2	2	3	1	1	1
Total Cost (mill. 1982$)	5.7	30.9	84.8	132.6	73.6	224.0	11.9	29.6	25.6
$/ft2[b]	0.133	0.710	1.949	3.046	1.692	5.144	0.273	0.450	0.588

(a) 10' easement @ $1,000/acre.

(b) Unit cost includes lined equalization basin at $94,000 (1982 dollars).

(c) System costed for MOD 9 is identical with system in MOD 2 except for reduction of delivery head from 200 ft. to 10. ft.

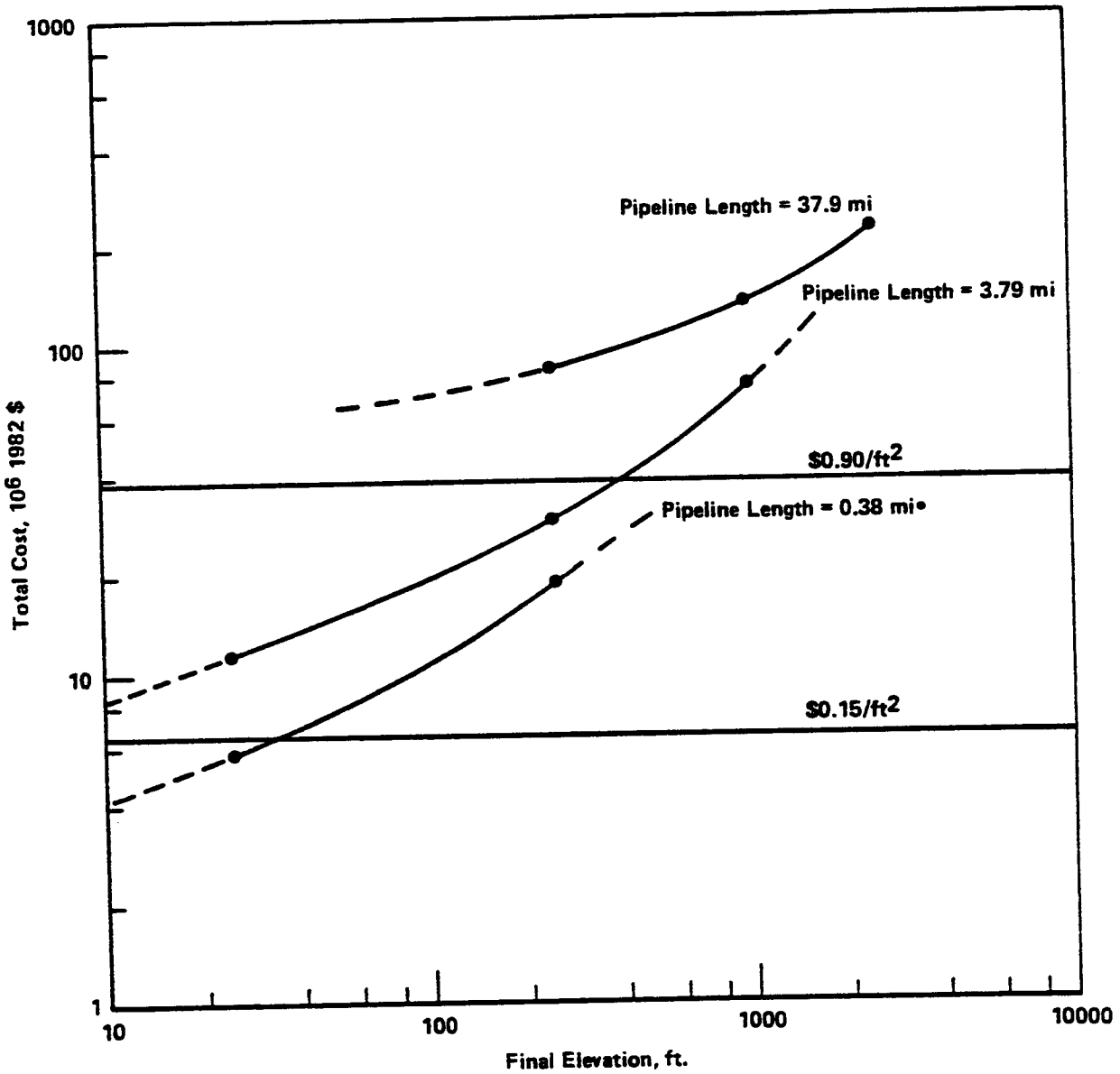

FIGURE V-1. WATER SUPPLY COST ANALYSIS FOR SEAWATER SYSTEMS

located in relatively isolated areas since the incremental cost would be site specific.

(6) Pump Type - A vertical turbine type pump was specified.

(7) Well Cover - Wells would have no added cost for protective structures.

The results shown in Table V-3 for the ten different systems examined show a smaller range than do the seawater systems. However, it is difficult to draw any other conclusions about the comparison between the two water sources because of the different types of variables involved. Nevertheless, if the wells can be tightly spaced and have very high capacity, it should be possible to use wells of considerable depth. If conditions of hydrogeology are less favorable, requiring more wells spaced farther apart, costs per unit area of pond surface could force production of high value products.

Again, any extrapolation of these data to covered systems would have to be highly qualified, but a larger range of hydrogeologic conditions could be accommodated. Since the individual well capacity assumed for the 10 well case is 2000 gallons per minute, a very favorable supply condition would have to exist.

Further work should be based on more accurate study area conditions. For example, yields may be only 100 gallons per minute or less over a large portion of the area, necessitating either a very considerable number of wells or a reduction in water demand, i.e. a covered or partially covered system.

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TABLE V-3. PARAMETRIC ANALYSIS - WELL FIELD (SALINE GROUNDWATER) SYSTEMS

	MOD 1	2	3	4	5	6	7	8	9	10
Title	GND1	GND2	GND3	GND4	GND5	GND6	GND7	GND8	GND9	GND10
No. of wells	10	10	10	10	20	20	20	20	20	20
Drilled depth, ft.	100	250	250	2,500	100	250	250	2,500	2,500	2,500
Gw. depth, ft.	10	50	50	50	10	50	50	250	250	1,000
Drawdown rate, ft/mgd	20	20	20	20	20	20	20	20	20	40
Land cost (.1 ac/well)	1,000	1,000	1,000	1,000	2,000	2,000	2,000	2,000	2,000	2,000
Radius, mi	1	1	1	1	5	5	5	5	5	5
Material,[a]	U	U	S	U	U	U	S	U	D	D
Total Cost, (mill. $ 1982)	6.180	7.160	7.120	7.700	20.200	21.800	21.200	29.200	37.000	61.200
$/ft^2	0.142	0.164	0.163	0.177	0.463	0.500	0.486	0.670	0.849	1.405

(a) U = unconsolidated material.

S = shallow bedrock (<500 ft.)

D = deep bedrock (>500 ft.)

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VI. RESEARCH NEEDS

Many unanswered questions remain regarding the technical and economic requirements for large commercial-scale production of microalgae. This section identifies the nature and general magnitude of research needed to answer those questions.

The development of biomass systems for production of microalgae has not yet progressed to the point that all of the scale-up issues have been addressed or even identifed. As described in previous chapters, a reasonable argument can be made about the kinds of resources, both physical and biological, needed to transform what is presently a small-scale research program into a commercial enterprise. As with most attempts to explore a new concept, data gaps have been identified. The philosophy in this section has been dictated by two major factors which surfaced during the course of this research.

The first of these factors is concerned with a distinction perceived to exist between the practical documentation of the existing resource base and the fundamental research leading to a more complete understanding of the engineering and biogeochemical requirements of the biomass system. Furthermore, it is apparent that the sheer size of the study area and the nature of the evaluation criteria themselves makes it reasonable to prioritize the resource assessment activities into those applicable to fatal flaw criteria mapping and those associated with discretionary criteria.

The rationale for this prioritization, while technically defensible, is at the same time a very real economic concern. After reviewing the criteria in their entirety, it seemed there was a dilemma between the desire to use a multiple factor evaluation technique and the difficulty in assembling a uniform data base for all of the factors across the entire study area. This is particularly true if additional field data collection is necessary.

A way to resolve this situation was developed which would recommend that three groups of criteria be mapped for the entire study area. These criteria groups are species growth characteristics, carbon supply and water supply. Regions which proved to be acceptable from this standpoint would then be further evaluated for their suitability when judged against the remaining criteria. In this way data gaps present in the discretionary criteria would

not have to be addressed for the entire study area, but only for a limited subarea.

Another advantage of this approach is that a more desirable timetable could be adopted for balancing the need to derive a preliminary picture of area availability against the benefits obtainable from additional research on the fundamental questions. In other words, the near-term goal would be to map the fatal flaw criteria, filling those data gaps as needed. Then, basic research and a directed effort at filling discretionary criteria data gaps would follow.

In summary, data base information on the assessment criteria has not, as yet, been developed by SERI-RRAIB. Consequently, assumptions have been made regarding the availability of this information. In instances where the data supporting the screening criteria is judged to be available in a utilizable form, no data gap is believed to exist. Instances where the screening criteria data are unavailable or only available in a form that is not readily utilizable are designated as practical research needs. Other, more fundamental research needs are also described.

Fatal Flaw Criteria

The four groups of criteria considered to be necessary to the siting of any microalgae system--species characteristics, carbon, water and temperature--reflect the belief that system biochemical economics will determine where the systems can or cannot be sited versus other considerations suggesting where and why some subareas are more desirable than others.

Species Selection

In order to bring microalge biomass systems to fruition, information on individual microalgal species needs to be accumulated in the following areas:

1. Salinity and temperature tolerance versus end-product accumulation
2. Potential or flexibility to use alternative carbon sources

3. The response of microalgae to limiting factors other than carbon dioxide.

The critical point is that individual species vary in their responses to external stimuli and in their metabolic potential to produce useful products. Not enough is currently known at the species level to adequately select a number of useful organisms likely to survive under the varied environmental conditions to which they will be exposed in a system of ponds. That this is the case should become clear in the following discussion.

Salinity and Temperature Tolerance versus End-product Accumulation. For microalgae biomass systems to be feasible, species must be selected which will synthesize a useful product. The obvious examples are species like *Dunaliella* and *Oscillatoria*, which produce relatively large quantities of glycerin and lipids, respectively (Ben-Amotz and Avron, 1980; Materassi et al., 1980). These products are produced as osmoregulators in response to varying salinity levels. Not enough basic information exists concerning product accumulation in response to environmental changes in most microalgae species. It is imperative to develop a "catalog" of many species, listing their specific growth requirements (temperature, nutrients, pH, salinity, etc.) end-product accumulation, and response to changes in photoperiod, presence of toxic compounds, or presence of other algal species. Such a compilation, which necessitates considerable basic research, will lend flexibility to a microalgae biomass system. For example, if the demand for a particular end-product changes, a species currently being cultivated could be replaced by another species which not only produces the product desired, but also is adaptable to the range of cultural conditions employed at that particular moment.

Our compilation of species and their characteristics, described in Section IV, illustrates the paucity of information on most recognized species. In addition, there undoubtedly are many species yet to be identified or characterized, many of which may tolerate some extreme environmental condition and/or produce a highly desired product. Finding and describing such species and determining ways to manipulate end-product accumulation are of utmost importance. Otherwise, microalgae systems may be limited to a very few

species and, as a result, may not offer enough flexibility for responding to changing demands for end-products.

Flexibility for Alternative Carbon Sources. It has been assumed in this report that carbon will be the limiting nutrient for most microalgae systems. Since the potential to supply adequate carbon to microalgae systems in the Southwest may be limited, it is important to know the carbon use efficiency for different microalgae species, as well as the form of carbon employed by a particular species.

Unhydrated CO_2 is the major source of carbon for algal growth; CO_2 is the form of carbon which interacts with the major carboxylase, ribulose diphosphate carboxylase. Since CO_2 enters the cell by diffusion, the greater the CO_2 concentration, the greater the assimilation rate. Thus, for maximum growth, CO_2 concentrations should be as high as possible. The specific effective concentration range should be known for each species, but this information does not appear to be available readily. The minimum half-saturated growth rate concentration for the binding of CO_2 to ribulose diphosphate carboxylase is 11-18 μM. Molecular CO_2 in solution in water has a concentration of about 12 μM, depending on factors such as solubility, atmospheric partial pressure, temperature, pH, ionic strength, etc. Other forms of inorganic carbon in water include H_2HCO_3, and HCO_3, and CO_3. The percentage of bicarbonate and carbonate increases with increasing pH.

An important consideration is that not all species can absorb bicarbonate, and those that do must convert the ion to unhydrated CO_2 for fixation via ribulose diphosphate carboxylase. Uptake of HCO_3 is an active (energy-requiring) process. Bicarbonate is converted intracellularly to CO_2 and OH^- by carbonic anhydrase, an enzyme undispersed among the algae but which is especially prevalent in marine species. Carbonic anhydrase is inducible in many species and thus serves as an adaptation to low CO_2, high bicarbonate, high pH conditions.

Thus, in order to gain as much flexibility as possible with respect to species selection, those microalgae which can efficiently utilize forms of carbon other than unhydrated CO_2 and still produce useful end-products require further identification. For example, a species which produces a

highly valued product but which does not produce carbonic anhydrase for bicarbonate utilization may be of little or no value in a microalgae biomass system in the Southwest. Thus, in the cataloging process mentioned above, carbon use information is essential, including the ability of individual species to adapt to various concentrations of carbon.

Other Limiting Factors

Our analysis shows that factors other than salinity, temperature, or carbon may be limiting in some situations. For example, the presence of algal toxins such as arsenic or boron may be of concern in southwestern waters. Unfortunately, little information is available for most species on their response to potential toxicants.

Another important consideration concerns the effect of pH on the potential toxicity of ammonia. As pointed out previously, southwestern waters are alkaline and as a result should have relatively high quantities of bicarbonate. This is desirable for using alternative carbon sources. However, Azov and Goldman (1982) have shown recently for species of *Dunaliella*, *Phaeodactylum*, and *Scenedesmus*, that at pH greater than 8.0 ammonia toxicity may become a problem in algal culture. For *Scenedesmus obliquus*, a 50 percent reduction in photoassimilation of ^{14}C occurred with an ammonia concentration of 1.2 mM. It was suggested that, because both freshwater and marine species behaved similarly to increasing pH and concomitant increasing ammonia toxicity, that a pH of approximately 8.0 may be the upper limit for most species when ammonium salts may be prevalent. Similarly, the effect of high pH on the availability of other potential toxicants requires investigation.

A desirable candidate species (i.e., one which produces a desirable product) would utilize light at a very high efficiency, ideally close to the theoretical maximum efficiency discussed by Goldman (1979). Unfortunately, little information is available on light-use efficiency for the individual species. Considerable research should be conducted on determining photosynthetic efficiency in desirable species, as well as in ways to maximize light-use efficiency by altering culture conditions. Also, the validity of

extrapolating results obtained in vitro to a larger scale, such as a pilot plant, needs to be examined.

Carbon Dioxide Supply

Production of microalgae on a scale that is significant to the United States in terms of energy substitution either in fuels or chemical feedstocks and in terms of land area devoted to this means of biomass production requires supplementation of atmospheric carbon dioxide with additional carbon resources. This additional carbon might be supplied by gaseous carbon dioxide from wells, carbon dioxide contained in natural gas, gaseous carbon dioxide contained in flue gases, bicarbonate minerals, and carbonate minerals. Other soruces of organic carbon include sewage, food processing waste, and similar materials.

Many areas of the southwestern United States that have desirable solar insolation and water will not have an adequate supply of inexpensive carbon dioxide. Therefore, carbon supply is an excellent screening tool in mapping the specific locations at which it may be practical to produce algal-biomass on a significant scale.

Massive supplementation of atmospheric carbon dioxide with fossil carbon dioxide (such as the CO_2 that is contemplated for use in enhanced oil recovery) implies a limitation to the size of the algae biomass production units. Because CO_2 injection into oil fields produces such a rich reward of energy through enhanced oil recovery, CO_2 that can be used for this purpose is relatively valuable in energy terms and in dollar terms. If algae production is to compete with enhanced oil recovery, the end products must be sufficiently valuable to justify the algae use. This consideration tends to tie algae to chemicals or fuels with unusually high selling prices. The economics also can be improved by making this supply of carbon to algae a part of an overall system that can result in a sharing of carbon supply costs. Typical examples would include scrubbing CO_2 from natural gas that would otherwise be too contaminated with CO_2 to be worth a high selling price and then using the CO_2 thus recovered to grow algae. In this scheme, the cost of CO_2

could be shared between the algae venture and the associated methane production venture. For this type of venture, the algae might even be anaerobically digested to methane and CO_2 so that the only product that had to be sold was methane.

Another example of sharing costs would be flue gas utilization in which stack gas cleanup could be combined with algae production. Stack gases are not suitable for enhanced oil recovery, thus this approach has less competition.

The development of new aluminum oxide resources in the United States is a matter of considerable concern, because bauxite is available from a limited number of suppliers. The mineral dawsonite contains both alumina and sodium bicarbonate. The cost of isolating the alumina could be shared with the production of carbon dioxide from this resource.

Water Supply

As stated previously, water supply is a major issue from both a resource supply and an economic viewpoint. The criteria have been developed from the premise that there is an ideal data base which could be employed for the purpose of determining water supply constraints to siting. However, it is unrealistic to anticipate that such a data base exists at present or could even be assembled given any reasonable level of research funding. Hence, these expectations were incorporated into the criteria as proposed.

The information reviewed to date, together with past experience, suggests that even this set of criteria will fail to be adequately supported by the available data. In particular, there will probably remain two areas of research needs. The first of these is the reconciliation of differing ranges and levels of detail in reporting the gross water yield (pumping capacity) and salinity data. Much of this reconciliation can probably be accomplished by accessing some of the original raw data and revising the available maps collected by SERI-RRAIB. A more difficult data gap to fill will be the criteria where information is not routinely collected, for example, amounts of drawdown per unit pumping capacity or where policy issues make it difficult to map the information, as, for example, water rights approprations.

Filling of the former type of data gap will necessitate either a field data collection effort and/or a judgment made by an expert on the water resources in a particular region. As discussed in the water supply cost analysis, there are some clear indications that a multi-well system is required if groundwater is used. Potential interference between wells is a serious question at this time.

Further research on policy issues should indicate how the water rights record-keeping system in each state could be used to portray availability geographically.

The cost analysis performed during this study assumed that an enterprise of this nature would be responsible for providing its own water at current production costs. This is in direct contrast to most irrigated agriculture which is supplied with water by separate if not necessarily independent entities. Many critics have contended that the prices charged for this resource are well below the true value and that this situation should be changed in the future. Thus, the approach used is more consistent with future expectations than with current practice.

Nevertheless, this approach should be verified. The engineering definition of the water supply system also requires more refinement to provide a more accurate basis for costing. In particular, a better design for the well field configuration should be developed based on more realistic hydrogeologic information.

It has been the philosophy of this study to examine water resources which are presently underutilized in order to avoid competition for scarce freshwater sources. However, several areas in the West have experienced such an increase in the salinity of irrigation return flows that the discharge to surface streams is being curtailed to avoid degrading the quality of the entire resource. Such return flows are being handled essentially as "wastes" and current Bureau of Reclamation plans are to collect and discharge these waters to evaporation basins. While the salinity is marginally too great for conventional crops, it would be well within the tolerance range of a number of microalgal species. Therefore, additional effort should be expended to pinpoint the locations of these salinity control projects and the quality and quantity of water expected to be available. The availability issue analysis

should also consider the legal status of these waters. Conceivably, some appropriative rights "strings" may still be attached to the use of these resources, which may increase the cost to an algal system developer or, at a minimum, requires a clearer definition of their status in the water balance of an area.

Temperature

Although it is recognized that fatal temperatures must be avoided in the culture systems, there is not yet a comprehensive analysis of the relationship between temperature, duration and heat transfer characteristics of a number of specific microalgal systems to allow for a rational thermal criterion to be specified.

Additional information is required on:

1) Relationships between duration-temperature (degree-days or degree hours) indices and net heat loss from microalgal systems, and
2) Allowable heat loss versus recovery or non-recovery of economic growth rates.

Discretionary Criteria

Discretionary criteria, can be viewed as secondary or lower level screening criteria and can be used to differentiate various subregions (states or parts of states) as to potential for microalgal production facility development.

Algal Species

With the exception of a few microalgae species, surprisingly little information is available concerning end-product accumulation in response to growth conditions, carbon use efficiency, photosynthetic efficiency, or effects on growth of pH, temperature, salinity, or potential toxicants. Considerable information concerning the growth requirements of species such as *Chlorella* and *Scenedesmus* is available, largely as a result of early interests

in microalgae as a source of food. However, in order to insure enough flexibility with respect to species selection and various cultural parameters, such a water quality and temperature or changing end-product demands, desirable species need to be identified and thoroughly characterized. Research needs include basic information such as optimal growth characteristics, response to various forms of carbon, and responses to alterations in salinity, pH, temperature, and radiant energy. Applied research needs include species performance in scaling up from bench-level to pilot plant conditions, and maintenance of the desired species over a long term without the invasion of competitor species. As mentioned earlier in this section, the ideal situation would be an extensive catalog of desirable species, whose individual growth responses, end-product accumulation, response to toxicants, etc., are well characterized. From our analysis in Section IV, it is obvious that considerable research is necessary to identify and characterize new species and to further characterize known species.

Land and Climate Resources

Land Related Factors. The important land-related factors are soil characteristics, land ownership/availability and land use/cover. The important soil characteristics are slope/topography, temperature and moisture rege, soil depth, engineering permeability. The county/area soil survey reports of the USDA and state level soil resource information developed by individual state agencies are the primary infonmation sources for the soil characteristics data base. Much of the area in the study area is without a modern published soil survey report and the soil resource information available from the various states is highly variable. Consequently, it will be difficult to determine if research needs exist for the soil characteristics data base until the specific geographic location under consideration has been identified. If the area under consideration is the entire eight-state study area, the effort required to address these research needs would be prohibitive. If the specific area under consideration is narrowed to portions of counties and soil survey reports were not available to characterize the soils in the specific area, the effort required to address the research needs were

met for such a large area, a site specific investigation would probably still be required to aquire sufficient soil data to design the facility. It is conceivable that site specific investigations would be required to select one specific site over another.

The important land ownership/availability factors are category of land ownership, land sale and leasing policies, barriers to availability and evaluation of relative availability. The primary sources of information for this data base is the Bureau of Land Management, its state and county counterparts and the various Federal and state agencies controlling specific segments of land under consideration. Again, if the area under consideration is not narrowed to specific sites, the effort required to meaningfully address the research needs for this data base will be prohibitive.

The important land use/cover factor is the land use/cover category of the area under consideration. The degree to which this factor represents a research need is dependent upon the status of the USGS effort to develop land use and land cover maps for each state. If such maps are available, a research need may not exist on the state-wide level of consideration. If such maps are not available or do not provide sufficient resolution for evaluating specific sites, research needs may exist to define the desert/barren and range lands within specific sites. This effort would appear to be quite manageable.

Climate-Related Factors. The important climate related factors are solar radiation, and severe weather. The important solar radiation criteria are daily global solar radiation and cloudiness index. The important severe weather criteria are average total snowfall, average total precipitation, number of days with snowstorms, number of days with hail, number of days with tornadoes, average daily wind velocity, fastest mile wind speed, and 10-year maximum 6-and 24-hour precipitation events. Because of the general availability of such climatic information, research needs probably do not exist for the climatic data base if the area under consideration is defined as the study area or each particular state. Study area and state level maps of the solar radiation and severe weather criteria data are available, however, some effort will be required to produce geographical displays of the data with the proper categorizations. If these climatic criteria are used to screen site specific

areas, the degree of resolution for some of the individual criteria may not be adequate in certain cases. As a result, some research needs for the climatic data base may develop as the site selection process becomes more site specific.

Water Resources

From a discretionary screening standpoint there are several remaining water resources criteria for which research is needed to allow the development of an adequate data base. These criteria are classified as discretionary principally because areas with less desirable characteristics can either be avoided or ameliorative techniques are available. These criteria include soil permeability, toxic substances and nutrients.

With respect to research needs, it is by no means certain that soil permeability data will be available for all areas which appear favorable after application of the fatal flaw criteria. Therefore, it may be necessary to augment the data to produce the criteria maps. A similar situation may exist with regard to nutrient availability in the water supply, although the analysis described in Section II makes it clear that a supplemental nutrient source will be required regardless of the water source. Still, the nutrient cost for microalgal systems could be more accurately determined if this data were available.

Finally, the algal toxin criterion presents two research needs. One is similar to those discussed above in that it is unlikely that data are available at present for all parameters and areas of interest. The second is a more fundamental need to know the effects of toxins on inhibition of algal growth. Inasmuch as the economic viability of these systems depends on achieving high yields, any significant inhibition of growth or product formation by toxins, either singly or in concert, will likely have serious consequences. This concern is addressed below in the section of this chapter on fundamental research needs.

Fundamental Research Needs

Information presented in this section is the result of research team discussions concerning basic knowledge gaps that make it difficult or impossible to develop resource assessment criteria.

Basic questions remain in the areas of carbon supply sources and the means by which each source influences site development economics and system pH stability. These questions can only be answered by site directed research which accounts for the needs of each species and pond type.

Carbon Supply

The previous discussion has pointed out that CO_2 reservoirs exist in the southwestern United States and that use of this CO_2 supply for algal production will have to compete with its use in enhanced oil recovery. The quantity of CO_2 present in the atmosphere (about 0.03 percent) is too low to support large-scale algal production, given the present state of knowledge on carbon source requirements for algal growth and the means to contact the algae with gases. Consequently, additional CO_2 must be supplied.

Theoretical values for carbon requirements for algal growth can be calculated based on the chemical composition of the cells (Goldman, 1980). However, the available data on CO_2 requirements for actual operating algal systems vary widely. For example, for Chlorella production in Taiwan, 4.8 Kg CO_2 were required per Kg dry weight of algal cells while in the Indian-West German project on cultivation of Scenedesmus, the minimum requirement was 1.6 Kg CO_2 per Kg (dry weight) of cells (Kawaguchi, 1980; Becker and Venkataraman, 1980).

Further research is needed on the factors affecting the efficiency of CO_2 utilization by species of algae selected for use in large-scale culture systems. Also, operating data are needed on CO_2 conversion by algal cultures to cell products.

Research is also needed on factors that limit gaseous CO_2 availability to and uptake by cells of dependable algal species in mass open culture systems at high biomass densities [above 1-2 g/l (dry weight)]. These

factors include investigations of (1) diffusional boundary layers around algal cells that limit CO_2 diffusion, (2) effects of ionic strength, (3) effects of ionic strength and pH, and (4) the effects of improved mixing systems under given light intensities. The Brune and Novak model of the carbon-limited growth response of algae in continuous culture systems needs to be verified experimentally (Brune and Novak, 1981).

In Israel, flue gases have been used as a form of CO_2 for growing Spirulina platensis in channel cultures (Berend, Timovitch and Allian, 1980). However, there is a lack of published information on actual efficiency of CO_2 utilization from flue gases by algae.

Experimental studies should be conducted to determine the conditions giving the best utilization of CO_2 from flue gases. The extent to which flue gas constituents such as H_2S, SO_2, SO_3, CO and CH_4 are inhibitory to various algal species is not well understood. There is a need for an investigation of the levels of these impurities that can be tolerated by algae when flue gas is used as a CO_2 source. Also, methods and costs for reducing the concentrations of these impurities to acceptable levels, e.g., scrubbing should be evaluated.

The possibility of integrating natural gas sweetening processes with algal production is an interesting possibility. The removal of CO_2 from CH_4/CO_2 mixtures with amines, followed by steam dissociation of the amine should be investigated. The CO_2 produced should be used in experimental algal culture systems. The results of these studies should provide data to determine if an integrated natural gas sweetening-algal production process merits further attention.

CO_2 availability to and utilization by algae might be enhanced by novel methods for contacting the gas with the algal cells. The application of mineral flotation methods should be investigated to determine if improved cell surface gas contact and improved CO_2 utilization can be achieved.

The use of carbonate-bicarbonate sources of carbon has been investigated for the production of Spirulina maxima biomass in naturally alkaline waters in Mexico (Durand-Chastel, 1980). However, there is no indication in the published literature that a $NaHCO_3$ carbon source has been evaluated for growing algae that produce high-valued chemicals, such as glycerol or

carotenoids. Experimental studies should be conducted to determine if algae, such as those in the genus Dunaliella that produce glycerol and B-carotene could utilize $NaHCO_3$-Na_2CO_3 from mineral sources in such a process. These organisms are salt-tolerant and produce glycerol under brackish water conditions (Ben-Amotz and Avron, 1980). Spirulina platensis can be grown in alkaline waters. Studies in Israel have shown that this organism can produce B-carotene, xanthophylls, chlorophyll, and phytocyanin and enzymes or co-factors such as ferridoxin, cytochromes, etc. (Tel-Or et al., 1980) The possiblility of improving algal cultures known to produce high valued chemicals by strain selection or genetic modification should be investigated.

Control of Medium Composition

The selection of appropriate nitrogen sources is an important factor in algal production since nitrogen utilization is known to lag the photosynthetic utilization of carbon dioxide and pH control is affected by carbon and nitrogen source utilization (Lincoln and Hill, 1980). Experimental studies should be conducted on the utility of various nitrogen sources such as ammonia, ammonium salts and nitrates from the standpoint of algal growth and productivity and pH control when CO_2 or $NaHCO_3$ - Na_2CO_3 is used as a carbon source.

Optimal biosynthesis of lipid materials in algae occurs under conditions of nitrogen limitation. A two-stage culture system consisting of a growth stage and a fattening or lipid synthesis stage should be investigated. In the first stage, pond conditions including pH, temperature, light intensity and spectral quality, and nitrogen supply should be optimized for total biomass production. In the second stage pond, the biomass produced in the first pond would be supplied with a carbon source under nitrogen limiting conditions to optimize the production of all storage products such as lipids. It has been pointed out previously that Spirulina platensis and Dunaliella sp. are capable of accumulating lipid products, such as carotenoids. Phaeodactylum tricornutum also is capable of synthesizing lipid substances. These species should be evaluated for the production of lipids and higher-valued

cell storage substances such as carotenoids in an experimental two-stage culture system.

It has been mentioned previously that pH control is intimately associated with carbon source and nitrogen source utilization. Experimental studies are needed to determine best methods for controlling the pH for optimum algal growth and lipid production in both single-stage and two-stage culture systems.

Salinity is an important consideration in large scale algal culture. Algae vary widely in their tolerance to saline environments. For example, Dunaliella sp. grow well in brackish waters and under controlled conditions can produce significant qualities of intracellular glycerol when grown in saline environments. Other microalgae of marine origin such as P. tricornutum are also of interest in this connection.

The possibility of using several algal species sequentially, each having various degrees of saline tolerance should be investigated. For example, less saline tolerant cultures might be used in ponds having lower salinities, followed by the use of more salt-tolerant strains as salinity increases through evaporation.

The results of research conducted at the University of Hawaii with P. tricornutum indicate that cupric sulfate ($CuSO_4$) solutions used to absorb infrared light to retard overheating of the culture, also might stimulate lipid production. This phenomenon should be explored further. Also, possible effects of blue light on lipid synthesis by algae should be investigated experimentally using cultures that produce desirable end products.

Effect of Nutrient Salt Addition

The addition of carbon salts such as sodium bicarbonate or sodium carbonate could introduce concentrations of dissolved solids which may affect growth of the desired species. Use of gaseous CO_2 and salts could be required to alleviate the effects of this potentially undesirable situation.

Whether there is a serious problem could be easily determined for each carbon source individually, but combinations of sources which would

increase the operational flexibility of the site would have to be evaluated on the basis of economics, optimum pH control, and other interrelated factors.

Systems Models

One of the largely unresearched areas for large-scale systems is the unknown response of the system to interaction of a large number of variable factors. As with other biomass systems now under development, the formulation of a dynamic growth model via mathematical simulation would provide a valuable tool to predict areas of weakness in the concept and test a wide range of conditions without some of the extensive experimental testing necessitated by non-simulation alternative approaches.

Although the model's computational structure has not been worked out in detail, it would be similar to other simulations of biological systems. Namely, there would be a set of coupled differential equations describing the time rate of movement of materials in the system. This set of equations could be further subdivided into those describing the purely abiotic components of the system and those describing the mass or heat transfers between the abiotic system and the algae or between the algae and their predators.

Abiotic Systems. This portion of the model would consist of a hydrodynamic routine describing the inputs and outputs of water to the system via make-up, seepage, evaporation, processing and so on. To model the concentrations of dissolved materials in the systems, kinetic equations representative of the transformation of chemical species in the system would be included. These processes would represent the behavior of a sterile system when coupled to a heat transfer analysis which determines the temperature coefficients of the reaction rates and the mixing of the water mass through convection.

Combined Biogeochemical System. This level of development provides as near a mathematical representation of the actual system as is currently possible with state-of-the-art models. At least two important processes would be added by this treatment. First, the uptake and excretion of nutrients and other materials by the algae as well as the removal of biomass by harvesting

are incorporated. The growth rate equations would be structured in such a way that the effects of growth inhibition by light, toxins or other factors could be included based on laboratory or small-scale system data.

Second, any predation or competition by other organisms with the algae should be included to the extent feasible. In most models the ability to do this is limited by the fact that the complexity of the system equations increases rapidly and stable solutions are not always possible.

Field Test Sites

As noted by several speakers in the recent SERI Biomass Program principal investigators meeting, much of the data for biomass systems has been collected from laboratory scale systems or small field scale systems not necessarily located in arid areas. Therefore, an outgrowth of the mapping and evaluation of the fatal flaw criteria would be the selection of two or three field test stations. These stations would consist of parallel ponds of one-half to three-quarters acre in total surface area which could be operated in a variety of ways to obtain pilot scale biological and engineering data. Locations would be selected with some sensitivity to the geographic variation in optimum growth conditions for several different species.

These stations would provide a wealth of data on the following aspects of arid biomass systems:

- Relationships between algal species, product yields, and arid zone growth conditions
- Engineering data such as light extinction coefficients, water balances, salinity variation and so on
- Heat transfer calibration data and effects of covers on reducing water usage
- Other calibration and validation data for the systems' model.

Quite possibly, one or more of these test systems could be scaled up to a full-scale commercial system provided the recommendations for the location of the test facilities are made with the ultimate site development in mind.

Integrated System R&D Opportunities

Although critical needs in specific areas can be identified for algae production, many of the research and development opportunities involve the entire integrated system. As shown in Figure VI-1, the algae system consists of growth, product biosynthesis, product recovery, and effluent treatment stages. Product biosynthesis may be integrated with growth or may be a separate activity taking place in a separate vessel under conditions of nitrogen limitation and/or darkness. Control of radiation with respect to intensity and wave length mix can have a significant impact both on growth rate and product biosynthesis. For example, the copper sulfate filter in the Hawaiian algal system stimulates the production of lipids.

The selection of algal species involves two selection criteria:

(1) Ability to synthesize product that have desirable selling prices and markets

(2) Ability to make use of media that are available in sufficient quantities and at appropriate prices. It is not within the scope of this project to specify the desired product mix. Examples of desired products include triglycerides, glycerin, beta-carotene, methane, glycolic acid, and polysaccharides can be produced by algae, either as constituents or as metabolic products.

Considering the cost (in biological and financial terms) of producing new algal cells, there are benefits to employing cells as factories to produce chemicals that can be obtained from the algae by nondestructive means. However, the alternative strategy of building up a relatively high concentration of a desired chemical or chemicals within the cell and isolating the product from the disrupted cells also can be beneficial. For example, it may be easier to isolate cells from a dilute medium than to isolate low molecular weight chemical products dissolved in a dilute medium.

Resource availability studies should provide information on the composition of alternative media for algal production systems. Typical media parameters include the level of salinity and the ions constituting the salinity, pH, nitrogen concentration, and carbon source. These media need to be

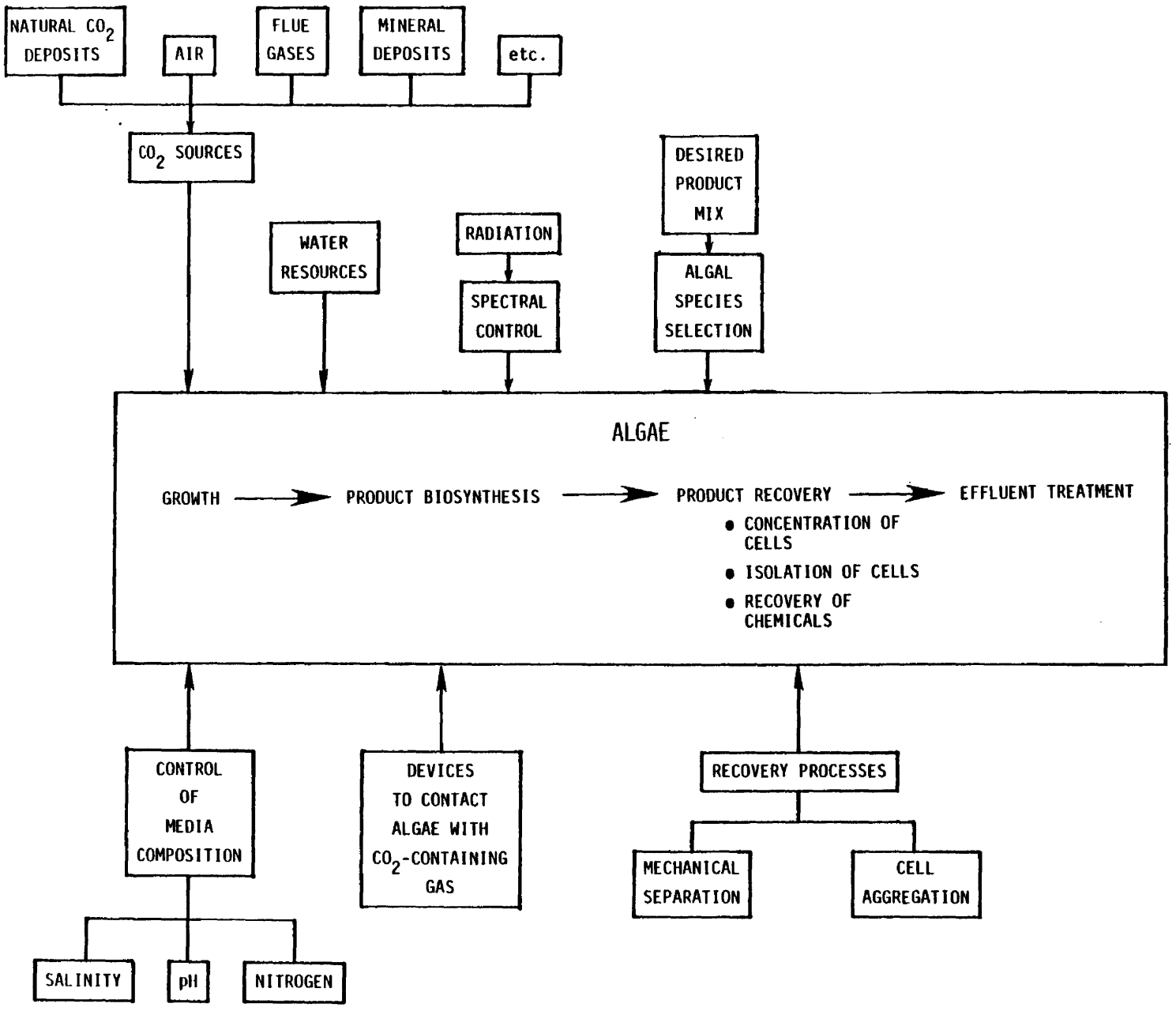

FIGURE VI-1. INTEGRATED SYSTEM R&D OPPORTUNITIES

evaluated with respect to their ability to sustain growth of desirable algal species.

Comparison of the list of algae that make desirable products with the list of algae that grow under attractive environmental conditions is expected to show many information gaps that can be the basis for research studies. for example, there may be many freshwater algae that yield desirable products but the readily available media in the Southwest will be highly saline. Thus, there will be a need to search for corresponding saline tolerant micro-organisms or to undertake breeding programs to build up salt tolerance without losing the ability to manufacture the desired product. The breeding programs could be either of a conventional type or make use of genetic engineering techniques.

Under commercial conditions, water will evaporate from algae ponds, leading to more highly saline conditions. This salinity increase can be controlled by adding water that is less saline or employing a series of ponds in which algae with increasing salt tolerance are used. This type of integrated system would require special attention to product mix and environmental conditions.

Integrated systems will depend greatly on the means by which carbon is supplied to the algae. If gaseous CO_2 is employed, species selection, gas-contacting devices, and product recovery need to be geared to the low solubility of CO_2 in water. pH control also plays a significant role. One type of integrated system is one in which the algae is used as a scavenger for CO_2 that causes an initially mildly alkaline solution to become highly alkaline. This solution then can be used in mining or chemical applications, instead of expensive alkali that is purchased directly for these uses.

Product recovery depends primarily on the species of algae that is selected and the technology for obtaining the salable products. For example, a species of algae may manufacture glycerin and hold it tightly within the cell while another may manufacture glycerin and release it through the cell membrane under certain conditions. Some algae form large aggregations that make it easy to collect them while others elude the best micro-strainers. Sometimes the cells can be recycled after products have been isolated and in other instances the metabolite production cannot be resumed, once it is

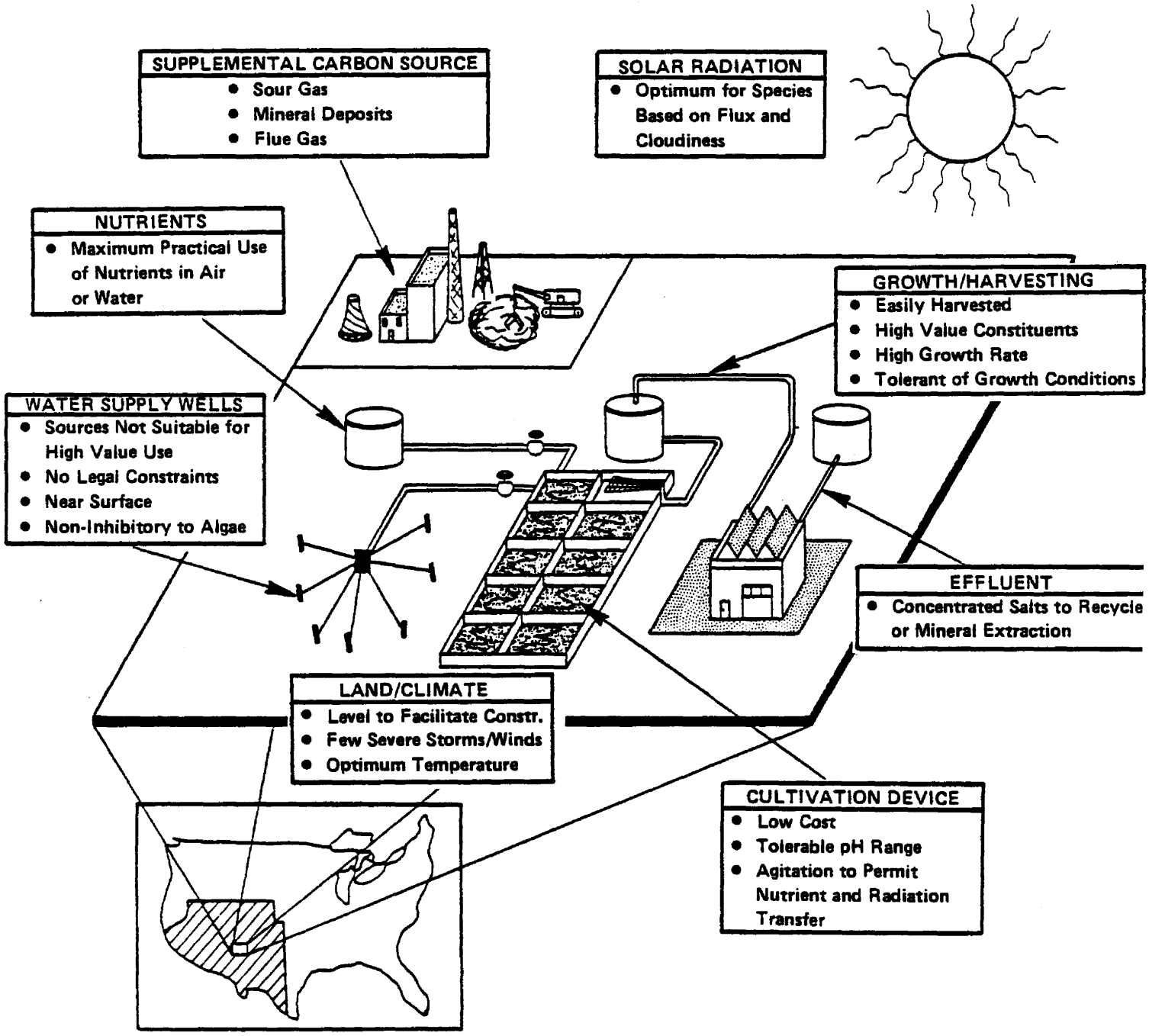

FIGURE VI-2. CRITERIA FOR IDEAL CULTURE SYSTEM/LOCATION

TABLE VI-1. SUMMARY OF RESEARCH NEEDS ON BIOMASS SYSTEMS

Research Need	Level of Effort Needed	Time Frame	Comments
(1) Product accumulation in response to environmental conditions	High	Intermediate-term	Could be obtained from lab or field scale systems
(2) Carbon use efficiency versus form	High	Intermediate-term	Could be obtained from lab or field scale systems
(3) Microtoxin dose-response data	High	Intermediate-term	Could be obtained from lab or field scale systems
(4) Reconciliation of ranges and detail of water supply data	Moderate	Near-term	
(5) Non-routine water supply data acquisition	High	Should be expanded and refined as site-selection proceeds	May also require judgmental criteria
(6) Non-routine water supply cost analysis	Moderate		Should follow supply data mapping exercise
(7) Salinity control project cooperative agreements	Low	Near-term	May require judgmental criteria to be applied
(8) Species considerations related to scale up	Moderate	Intermediate to long-term	Relates also to Items 1-3
(9) Soils data	Moderate to High	Intermediate (general) to long-term (site specific)	
(10) Land ownership	Moderate	Should be refined and updated as site-selection proceeds	
(11) Land-use/land cover maps	Low to Moderate	Intermediate to Long-term	
(12) Nutrient salt addition	Low	Near-term	
(13) Development of systems model	High	Intermediate to long-term	
(14) Selection and operation of field test sites	High	Intermediate to long-term	

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