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to the
Department of Energy
Pittsburgh Energy Technology Center
under
Grant No. DE-FG22-93PC93204

SYSTEMS AND ECONOMIC ANALYSIS OF MICROALGAE PONDS FOR CONVERSION OF CO_2 TO BIOMASS

Submitted by
John R. Benemann
and
William J. Oswald, P.I.
Dept. of Civil Engineering
University of California Berkeley
Berkeley, CA 94720

Attention: Dr. Perry Bergman
U.S. Dept. of Energy
Pittsburgh Energy Technology Center
Pittsburgh, PA 15234
Phone 412 - 892 4890 / Fax 412 892 5917

March 21, 1996

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ABSTRACT

There is growing evidence that global warming could become a major global environmental threat during the 21st century. The precautionary principle commands preventive action, at both national and international levels, to minimize this potential threat. Many near-term, relatively inexpensive, mitigation options are available. In addition, long-term research is required to evaluate and develop advanced, possibly more expensive, countermeasures, in the eventuality that they may be required. The utilization of power plant CO_2 and its recycling into fossil fuel substitutes by microalgae cultures could be one such long-term technology.

Microalgae production is an expanding industry in the U.S., with three commercial systems (of approximately 10 hectare each) producing nutriceuticals, specifically beta-carotene, extracted from Dunaliella, and Spirulina biomass. Microalgae are also used in wastewater treatment. Currently production costs are high, about $10,000/ton of algal biomass, almost two orders of magnitude higher than acceptable for greenhouse gas mitigation. This report reviews the current state-of-the-art, including algal cultivation and harvesting-processing, and outlines a technique for achieving very high productivities. Costs of CO_2 mitigation with microalgae production of oils ("biodiesel") are estimated and future R&D needs outlined.

The concept of CO_2 utilization and fuel production by microalgae was first proposed over four decades ago, and has been the subject of several prior engineering-feasibility studies. Those analysis were based on the same type of cultivation system used by the commercial systems: open raceway ponds mixed with paddle wheels. For low cost biomass production very large individual growth ponds, about 10 ha (hectares), would be required, vs. the 0.5 ha ponds presently used in commercial systems. Costs were also reduced by increasing overall system scale to about 1,000 hectares, by dispensing with plastic liners, by using a low-cost settling process for harvesting, and by projecting high productivities. This report updates and extends these earlier cost analysis specifically for the mitigation of CO_2 emissions from coal-fired power plants. Both direct flue-gas utilization near the power plant and remote use of CO_2 captured from flue gas and piped to the algal ponds were considered. With near-term oil prices ($25/barrel) and currently achievable productivities, projected cost are $70 to 100/mt CO_2 avoided, similar to other direct flue gas mitigation options. With higher productivities and oil prices ($35/barrel), CO_2 avoidance with microalgae costs could drop below $20/mt CO_2.

Although there are no apparent engineering limitations to pond scales and designs, many important performance objectives in large-scale microalgae production remain to be demonstrated: species control, low-cost harvesting, production of biomass with a high lipid (oil) content, and, most fundamentally, the achievement of very high productivities, near the efficiency limits of photosynthesis. R&D priorities are to demonstrate CO_2 utilization in microalgal wastewater treatment and currently achievable productivities, and to develop methods to maximize productivity, both for total biomass and algal lipids. The inherent appeal of CO_2 utilization, rather than disposal, the synergisms with wastewater treatment, the production of higher value products, and the opportunity to maintain U.S. competitiveness in this industry, and the potential for reducing CO_2 emissions, all argue for an expanded R&D effort.

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1. INTRODUCTION

1.1. GLOBAL WARMING AND GREENHOUSE GAS MITIGATION

With the Framework Convention on Climate Change (FCCC), signed in Rio de Janeiro in 1992, global warming has become a major focus of the international environmental agenda. Under the FCCC, the U.S. has committed to achieve by the year 2000 total net greenhouse gas (GhG) emissions no higher than 1990 levels. To achieve this goal, the Clinton-Gore (1993) Climate Change Action Plan (CCAP) outlines fifty specific actions to reduce total U.S. greenhouse gas emissions by the equivalent of 107 MtC/y (million tons of C per year) of CO_2 emissions. Post-2000 CO_2 reduction needs are expected to be higher, and the FCCC and CCAP are subject to revision as more information is developed. At the April 1995 follow-up meeting to the FCCC ("Rio Convention"), held in Berlin, the U.S. committed to further, though as yet unspecified, actions for greenhouse gas mitigation.

Reduction in CO_2 emissions is a particular issue for electric utilities, responsible for about one quarter of total U.S. greenhouse gas emissions, and in particular for coal-fired utility boilers, which generate a larger amount of CO_2 than all other electricity sources combined. Many utilities are currently evaluating how to reduce CO_2 emissions, and relevant research is being supported by the U.S. Department of Energy (DOE) and the Electric Power Research Institute (EPRI). Over 100 utilities have joined Climate Challenge, the voluntary DOE program for greenhouse gas mitigation (DOE, 1995).

Many GhG emission reductions alternatives can be considered (Rubin et al., 1992). These can conceptually be divided into three broad categories: conservation, direct mitigation and indirect mitigation. Conservation measures reduce electricity consumption and thus GhGs emissions, direct mitigation technologies capture and remove the CO_2 emitted by specific emission sources (e.g. power plants), and indirect methods involve offsetting actions in which GhG producers (e.g. electric utility) support reductions in GhG emissions, or increases in their sinks, at another site, with the GhG producer credited with these reductions. Examples of indirect CO_2 mitigation are so-called "joint implementation" projects, which can involve forest preservation, restoration and/or biofuel projects in developing countries.

Microalgal systems belong to the direct methods, the only ones discussed further herein. Suffice it to state that all types of GhG mitigation processes will be required in the future, if global warming is to be minimized. As argued elsewhere (Benemann, 1990), even a modest, 25 to 30 %, reduction in GhG emissions would likely reduce the probability of the most damaging consequences of global warming by a large factor. Thus, even a partial reduction in GhG production is a worthwhile objective, and any technology that could contribute to this goal deserves to be evaluated and, if warranted, developed for the eventuality that it may be required.

1.2. DIRECT GREENHOUSE GAS MITIGATION TECHNOLOGIES

The simplest, at least conceptually, direct method, for GhG mitigation is the removal (capture) of CO_2 from stack gases, followed by long-term sequestration of the CO_2, through ocean disposal, injection into depleted gas and oil wells, etc. Over the past few years several major engineering and economic feasibility analysis have been completed for both the capture and disposal of CO_2 from power plants. (Brown, et al., 1993; Fluor Daniel, Inc., 1991; Intech, 1992; Johnson, et al. 1992; Herzog et al., 1993; Monenco, Inc., 1992; for examples), in particular by the IEA Greenhouse Gas Programme (IEA, 1993, 1994).

CO_2 recovery systems based on amine scrubbers are relatively well developed, but costs are high, with power production (electricity) cost increases of up to 50% (while derating the generation capacity by a quarter or more, at least for coal-fired power plants. Alternative technologies (membranes, cryogenic, etc.) are not well developed, and are expected to also be expensive. Even more critical, technologies for long-term CO_2 sequestration are relatively undeveloped or/and dependent on favorable local conditions (e.g. near-by abandoned gas or oil wells). Cost estimates for long-term CO_2 disposal are uncertain, exhibiting a large range. The combined cost of CO_2 capture and disposal is generally estimated at over $100 per metric ton of CO_2-C mitigated (emissions avoided), even at favorable sites.

The utilization of CO_2 for the synthesis of high value chemicals is either not economically viable, or the markets for such products very small, or both. Most CO_2 needs by industry are already supplied from existing sources, and anyway, commercial CO_2 is generally rapidly released back to the atmosphere. Thus, such options do not offer a significant potential for GhG mitigation.

One direct mitigation method is the co-firing of coal and biomass in utility boilers. By substituting renewable wood for coal, net CO_2 emissions are reduced at the power plant. Although limited by availability of wood fuel, and the ability of existing boilers to handle such fuels, co-firing is a low cost, near-term technology for directly reducing CO_2 emissions by utilities (Benemann et al., 1995).

Another direct CO_2 mitigation involves cultivation of algae - seaweeds and microalgae - that require an enriched source of CO_2, such as flue-gas, for their growth. The seaweeds appear to be diffusion limited for CO_2 (which diffuses 10,000 times slower in water than in air), when grown in on-shore mass cultures. Their cultivation is likely to require rather high power inputs, to break these diffusion barriers. Although that issue has not yet been satisfactorily resolved, seaweeds are not considered further in this report, which specifically addresses CO_2 mitigation from power plants by microalgae cultures. In such a process, CO_2 produced by a power plant is used to cultivate microalgae in ponds, which are converted to substitutes for fossil fuels, thereby reducing overall greenhouse gas emissions. A brief historical overview and general introduction is presented next.

1.3. MICROALGAE CULTIVATION

1.3.1. Introduction

Microalgae are microscopic plants that typically grow suspended in a liquid medium (planktonic growth), although attached growth is also common. The planktonic microalgae are the focus of this Report. The seaweeds (macroalgae) are, as stated above, not considered herein (see Ritschard, 1992, for a recent review). Planktonic microalgae range from unicells to colonies and filaments of a up to a few hundred cells. Microalgae include procaryotes (cyanobacteria or blue-green algae) and eucaryotes (green algae, diatoms, red algae, and others) (Bold and Wynne, 1985). There are thousands of species, only a fraction having been studied in any detail, and less than one handful cultured successfully in outdoors.

Microalgae mass cultures have been studied for almost 50 years, starting in the late 40's and early 50's as a potential source of human foods, research stimulated by prospective food shortages expected, at that time, within a decade or two. Concerns about water pollution in the 1960's increased interest in the use of microalgae in wastewater treatment. There was also significant interest, starting in the 1960's, in microalgae for atmosphere regeneration in space vehicles. The perception during the 1970's that fossil fuels would run out, made microalgae culture a focus of renewable fuels production R&D. Commercial interest in high value products, specifically nutriceuticals, led, during the 1980's, to the commercial development of a microalgae industry in the U.S.: three plants for microalgae production are now successfully operating, two in California and one in Hawaii. In addition many small to medium sized wastewater treatment plants use algal ponds, although in only a few cases is the algal biomass produced actually harvested.

With the 1990's the threat of global warming focused attention on microalgae as a method for CO_2 utilization. The concept is to use microalgae to convert solar energy and CO_2 to a fuel that would replace fossil fuels, thereby reducing net CO_2 emissions into the atmosphere. The interest in microalgae is essentially two-fold:

1. They are thought to have very high productivities, compared to higher plants.
2. Their cultivation requires an enriched CO_2 source, such as power plant CO_2.

Although true, neither point is fundamental. There is no inherent advantage of direct vs. indirect CO_2 mitigation. Production of renewable fuels from higher plant biomass grown with atmospheric CO_2 is just as valid a process of CO_2 mitigation as direct power plant CO_2 utilization by microalgae cultures. And, as discussed in this report, microalgae culture productivities are not inherently higher than those of higher plants, although in practice somewhat higher productivities would be achievable. Thus, arguments for microalgae culture in CO_2 utilization must be based on the relative costs of such processes, and their potential for applications.

This Section provides both a historical perspective and a general introduction to the issues in microalgae CO_2 utilization addressed in this Report.

1.3.2. History of Microalgae Mass Culture

Although based on the same basic photosynthetic mechanism as higher plants, microalgae culture has several potential advantages over vascular plant cultivation: All physiological functions are carried out in a single cell. Even colonial or filamentous species do not (with a few exceptions) differentiate into specialized cells. Compared to higher plants, with their roots, trunks, limbs, etc., relatively little of the microalgal biomass performs structural functions. Thus, more of the biomass is available for constructing more of the cell machinery responsible for photosynthesis, growth and reproduction. Typically, almost 50% of the dry weight of microalgae is protein, greater than even the seeds of higher plants.

Fast growth and high protein content suggested microalgae as a potential future food and feed source. Indeed, the first efforts to mass culture microalgae were initiated in the U.S. almost fifty years ago for that reason (Burlew, 1953). However, high growth rates are not necessarily synonymous with high productivity (still a commonly held, though mistaken, belief). And microalgae cultivation proved to be difficult and expensive, from the maintenance of cultures and high cost of the cultivation vessels (now often called "photobioreactors"), to the harvesting of these microscopic plants, to the processing of the wet harvested biomass. Thus, the optimistic title of the first book in this field, "Algal Culture: From Laboratory to Pilot Plant" (Burlew, 1953) did not fulfill for some time its implied promise of moving on to commercialization. Nevertheless, that book, with contributions by many of the pioneers in this field - Myers, Kok, Tamiya, Krauss, and others - can still today be read with profit. Most of the problems addressed at that time, from CO_2 supply to harvesting, are not yet completely resolved, as are arguments about how to increase productivities (such as by increasing culture turbulence). Most of this early work concentrated on the unicellular green alga Chlorella, which had been a favorite for laboratory studies of photosynthesis since the 1920's.

It was in Japan that the first commercial production systems for microalgae were developed: During the 1960's, several Chlorella cultivation facilities were established, using circular ponds, to produce a dried algal powder, sold as a "health food". Although during the 1950's the Japanese researchers, led by Tamiya (1958), published extensively on Chlorella culture, once commercialized, publications essentially ceased. Currently Chlorella is produced by some ten plants in Japan and Taiwan. In 1995 a large (300 t/y) plant was started-up in Indonesia, increasing world production by almost 50%. Chlorella cultivation is difficult, mainly due to contamination problems and the need for expensive centrifugation for harvesting.

The next commercial algal production system was for Spirulina, a filamentous blue-green alga that has been a traditional food source both in Mexico and Africa. Production was initiated in the 1970's near Mexico City, at a carbonate evaporation pond (these algae favor high concentrations of carbonate), and during the 1980's in the U.S., with one plant each in California and Hawaii. Spirulina is used primarily

as food supplement (nutriceutical), with some applications as a specialty aquaculture feed and food coloring. Recently the Mexican plant closed, leaving the two U.S. production facilities, in S. California and Hawaii, as the dominant world producers, with about 350 tons/year each. The U.S. systems, each with 10 hectares of ponds, use paddle wheel mixed, raceway type ponds of up to 0.5 ha each.

Most recently commercialized was the production of green alga Dunaliella salina. Some strains of D. salina, growing at high salinities, produce high levels of beta-carotene, used as a food colorant and vitamin/antioxidant food supplement. Two plants are operating in Australia, based on extensive (up to 100 ha each) ponds, and one plant of appx. 10 ha of raceway ponds each in the U.S. and Israel. Other microalgal products of commercial interest include pharmaceuticals, animal and aquaculture feeds, fine chemicals, soil conditioners, and fertilizers, among others. However, these products are currently produced either only at a very small scale or are still at a R&D stage. Also, current production costs for Spirulina, Chlorella, and Dunaliella, are rather high, at typically over $10,000/ton. A detailed discussion of the commercial production of microalgae is presented in Section 2.

Microalgae ponds are also used by many municipalities and industries for waste water treatment. However, in such ponds no effort is made to control the algal species, or productivity, and, with one exception (Sunnyvale, California), the algal biomass is not harvested. The technology for microalgae waste water treatment is reviewed further in Section 3.

Microalgae can be grown not only in light and with CO_2 (autotrophic growth), but also in dark fermenters using sugars for both energy and C sources (heterotrophic growth). Recently several U.S. companies have developed commercial products for the human and animal nutrition markets based on heterotrophic microalgae production. However, for CO_2 utilization this technology is not applicable, and, thus, here only the autotrophic, sunlight, cultivation is considered.

For the past two decades the U.S. Department of Energy (DOE), since 1979 primarily through NREL (National Renewable Energy Laboratory, formerly the Solar Energy Research Institute), supported microalgae R&D for the production of liquid (vegetable oils, biodiesel) and gaseous (methane and hydrogen) fuels. Recently, DOE - PETC (Pittsburgh Energy Technology Center) has also supported research at NREL on CO_2 mitigation with microalgae. In Japan a significant governmental and private effort to develop microalgae biotechnology for CO_2 mitigation and H_2 production was initiated about 1990. The history of these programs are briefly reviewed in Section 4. Below, a brief overview of the issues in microalgae CO_2 mitigation addressed by this report is presented, starting with a brief introduction to the basics of microalgae culture. Many reviews and proceedings cover microalgae biotechnology (Richmond, 1987; 1990, Borowitzka and Borowitzka, 1988, Cresswell, et al., 1989; Oswald, 1988, 1990; Benemann, 1987, 1989, 1995; Lembi, 1989; Barclay and McIntosh, 1986; Becker, 1994)

1.3.3. Microalgae Culture Fundamentals

Growth requirements of microalgae are similar to those of higher plants - light, water, carbon dioxide, and inorganic nutrients. Unlike higher plant cultivation, in microalgae cultures nutrients (including CO_2) and water can be relatively easily maintained at or near optimal levels, so that productivity is limited only by the amount of sunlight available, and the ultimate biochemical capabilities of the algae. Other environmental factors, such as pH, salinity, and temperature, can, within limits, also be more readily managed in algal than higher plant culture. The hydraulic nature of microalgae cultivation allows near continuous production, allowing microalgae cultures to approach the limits of photobiological solar energy conversion, limited only by the inherent mechanisms of photosynthesis (Section 5).

A central issue in microalgae cultivation are the "photobioreactors", the vessels in which the algae are cultivated. These can range from simple, unmixed (except by wind and hydraulic dilution), open ponds, to highly complex enclosed reactors. The diffuse nature of sunlight, requires that any photobioreactor must cover large surface areas. The inherent limitations of any solar energy conversion device, and solar energy itself, requires very low areal costs for any such photoconverter.

A major design parameter of photobioreactors is depth, which should be as shallow as possible. Productivity will be maximized, for given environmental conditions, at some optimal algal concentration per unit area (not volume). Thus, by minimizing depth, volume is reduced, and cell concentration maximized. This reduces overall liquid handling, and, perhaps most important, harvesting effort, whose cost primarily depends on the volume, not biomass, processed. Different algal reactors have different depths: raceway ponds: 10 - 30 cm; tubular reactors: 1 - 5 cm; thin flat plate reactors: 2 - 5 cm; and shallow cascade systems : < 2 cm. However, very shallow reactors generally suffer from a severe engineering limitations, from the outgassing of CO_2, to large temperature fluctuations, to, in particular, inability to scale-up such systems beyond very small unit sizes (typically < 100 m^2) (Benemann, in preparation). Other considerations in choosing a photobioreactor design include species control, O_2 build-up, but most important, overall capital and operating costs (See Section 4.).

As detailed in this report, the most commonly used, and potentially lowest overall cost cultivation systems are the raceway, paddle wheel mixed ponds. These optimize, to the extent possible, performance with overall costs. However, most microalgae strains cannot be easily maintained in open ponds - they are overrun by other algae, or are eliminated by grazers. Indeed, as stated above, currently only a few algal species are grown in open pond cultures, Spirulina and Dunaliella, which require a highly selective chemical environment (high bicarbonate or high salinity, respectively), and Chlorella, which is subject to frequent contamination and requires production of massive (and expensive) starter cultures (inoculations). The problem of contamination and grazing is a central one in microalgae culture.

1.3.4. Alternative Microalgae Mass Culture Systems

The relative advantages, applications and costs of various types of open and closed photobioreactors designs is a matter of active research and debate. Closed photobioreactors, have inherent problems of gas exchange (Weissman et al., 1988), in addition to high capital and operating costs. Thus, for the purposes CO_2 utilization and fuel production, only open pond reactors are plausible. Closed photobioreactors have a role in producing the inoculum for large-scale systems (Section 9), but are otherwise beyond the scope of this Report (Benemann, 1996).

Four basic open pond designs are currently used in microalgae production:
1. Deeper (30 to 100 cm), unmixed (except by wind and recirculation), ponds;
2. Shallow (1- 2 cm) "cascade" type systems;
3. Circular ponds mechanically mixed from a central pivot; and
4. Paddle wheel mixed, shallow (10 - 30 cm) raceway pond designs;

The deeper, unmixed ponds are used in wastewater treatment and in some places for Spirulina (Mexico) and Dunaliella (Australia) production. Although they can be economically viable under certain conditions, productivities in unmixed ponds are low and CO_2 addition is not practical. Cascade systems, developed in Czechoslovakia (a < 1 ha production unit is reportedly operating in Bulgaria, are very expensive, and exhibit large temperature fluctuation and high CO_2 outgassing. Circular ponds, used in Chlorella production in the Far East, exhibit poor hydraulics and their unit size is limited to about 2,000 m^2. They are also expensive, in part due to the complexity of the central pivot mixing system. Thus, in this Report, only the raceway, paddle wheel mixed ponds are considered in any detail.

As mentioned above (see also next section), current microalgae production is very expensive, at about $10,000/ton, with world-wide production being only about 3,000 tons/year for the three commercial microalgae. Greenhouse gas mitigation requires many millions of tons per year of biomass production, and a reduction in costs of almost two orders of magnitude. Of course, economics of scale will result in major, but by themselves not sufficient, cost reductions. The fundamental issue, addressed in this report, is whether through improved designs, more stable and productive cultures, development of lower cost harvesting and processing technologies, and optimal siting, in addition to increases in scale, it could be possible to reduce costs sufficiently to consider use of such systems for CO_2 mitigation.

Several prior feasibility and cost analysis (Oswald and Golueke, 1960, Benemann et al., 1978, 1982, 1993; Weissman and Goebel, 1987, Neenan et al., 1986, Regan and Garside 1983, Kadam, 1994) suggested that the answer is yes, that it would, indeed, be feasible to achieve both low costs and high productivities. Table 1.1. presents an updated summary of the two major prior studies, and Table 1.2 the land area needs for CO_2 utilization. The objective of this report is to critically review and analyze these prior efforts, to update the cost-estimates, to assess the potential of this technology in CO_2 mitigation, and to discuss the R&D needs in this field.

TABLE 1.1
SUMMARY OF MICROALGAE CAPITAL AND OPERATING COSTS
(From Benemann, 1993, based on Benemann et al., 1982, and Weissman and Goebel, 1987).

PRODUCTIVITY ASSUMED: (ash-free dry weight)	**Currently Projected**	**Maximum Theoretical**
Average Daily $g/m^2/day$	30	60
Annual mt/ha/yr	109	219
Barrels of oil/ha-year	380	760
CO_2 Fixed into Biomass mt/ha-yr	240	480
CAPITAL COSTS ($/ha):		
Ponds (earthworks, CO_2 sumps, mixing)	27,500	33,000
Harvesting (settling ponds, centrifuges)	12,500	17,000
System-wide Costs (water, CO_2 supply, etc.)	30,000	40,000
Processing (oil extraction, digestion)	10,000	20,000
Engineering, Contingencies (25% of above)	20,000	27,500
TOTAL CAPITAL COSTS ($/ha)	100,000	137,500
OPERATING COSTS ($/ha/yr):		
Direct Costs (Power, nutrients, labor, OH, etc.)	10,000	15,500
Annualized Capital Costs (0.2x Total Capital) 20,000		27,500
Credit for methane produced	- 3,000	- 6,000
Credit for oil produced ($25/barrel)	-9,500	-19,000
Net Operating Costs $/ha/yr	17,500	18,000
CO_2 Mitigation Costs ($/mt$CO_2$ fixed into oil)	**73**	**36**

TABLE 1.2. LAND REQUIREMENTS FOR ALGAE CO_2 UTILIZATION

Assumptions: 30% CO_2 average annual CO_2 utilization
1,000 MW power plant, 0.88 kgCO_2/kWh (Herzog et al., 1991).
Composition: 50% lipid, 25% carbohydrate, 25% protein.
Heat of Combustion: 7.5 Kcal/g (60% C in biomass).
Avg. Annual Solar Insolation: 500 Langleys, 45% visible.
Production: 1.05×10^6 mt/yr biomass; 3.7×10^6/yr barrels oil.

Annual Productivity mt/ha/yr	**109**	**219**
Lipid fuels barrels/ha/yr	380	760
Solar Conversion Efficiency (appx.)	5	10
Fixation C mt/ha/yr	66	131
LAND AREA REQUIREMENTS:		
,000 Ha required growth ponds area	9.6	4.8
,000 Ha total area (ponds x 1.25)	12	6

1.3.5. Prior Feasibility Analysis Cost Estimates

The above cost estimate summaries (Table 1.1, Benemann, 1993) are presented in somewhat greater detail in Table 1.3. These costs are based mainly on the two most recent feasibility studies (Benemann et al., 1982, Weissman and Goebel, 1987), of which the 1987 study presented the most detailed engineering designs and credible cost projections. (Tables 1.1 and 1.3 differ slightly, due to rounding).

Weissman and Goebel (1987) estimated substantially higher capital and operating costs in some categories than Benemann et al., 1982, and vice-versa. For example, the construction of the ponds (leveling, earthworks, walls and berms) were seven-fold more expensive in the 1987 than in the 1982 study. By contrast CO_2 supply and transfer systems were four times more expensive in the 1982 vs. the 1987 analysis. The latter differences can be explained by the high costs of transportation and use of flue gas in the 1982, vs. pure CO_2 in the 1987, study. Similarly, the rather expensive rock pond liner and concrete block construction for the walls and berms in the 1987 study, vs. simple earthworks in the 1982 report, account for those differences. In Tables 1.1 and 1.3 the costs for simple earthworks for berms was used, with some costs added for embankment protection. Some costs in Table 1.3 were interpolated between the two studies. For examples, paddle wheel costs ($4,000/ha and $6,000/ha in the 1982 and 1987 studies respectively) were averaged to $5,000/ha. Others leaned more heavily on one or the other. For example, only Benemann et al. (1982) estimated flue gas use, which, including delivery to and trasfer into the ponds, represents almost 40% of the total capital costs. Costs were twice as high as estimated in Benemann (1982), because of a two fold difference in productivity (on a C basis). However, that was a crude estimate.

The major difference with the earlier studies are the productivity assumptions. The 1987 report already assumed over a 50% higher the productivity (on a C basis) as the 1982 report (30 $g/m^2/d$, and lipid content of 50%). This estimate is used as the "current projected" case in Tables 1.1 and 1.2, with twice this productivity, labled "Maximum Theoretical", also cost estimated. With the recent publicaton by Greenbaum et al. (1995), this designation is, possibly, no longer appropriate, and an even higher efficiency could be aimed for in the future. However, it is as high a reasonably could be expected at present even from long-term research. Even with such a high productivity projection and favorable design assumptions, the costs for CO_2 mitigation and fuels production with microalgae cultures still exceed $100/t C-$CO_2$. These are very high costs, although perhaps not more so than some other direct CO_2 mitigation options, such as CO_2 recovery and ocean disposal.

However, the analysis on which these studies were based were done ten to fifteen years ago, and considerable advances have been made since. Also, prior analyses addressed fuel production, not CO_2 mitigation. Finally significant discrepancies and limitations are apparent in the prior studies. This suggests the need for an updated analysis. That is the objective of the present report.

TABLE 1.3. DETAILED SYSTEM CAPITAL AND OPERATING COSTS

	PRODUCTIVITY ASSUMPTION	
CAPITAL COSTS ($/ha)	**30 g/m^2/d**	**60 g/m^2/d**
Growth Ponds:		
1. Grading, earth works	5,000	5,000
2. Walls (perimeter, central, etc.)	4,500	4,500
3. CO_2 Sumps @ $2,400/ea, require 2/4	8,800	9,600
4. Mixing System (Paddle Wheels)	5,000	5,000
5. Carbonation System (@$2,000/sump)	4,000	8,000
6. Instrumentation (Miscellaneous)	500	500
7. Primary Harvesting(Settling Ponds)	8,000	8,000
8. Secondary Harvesting(centrifuges)	4,500	9,000
Subtotal	**40,300**	**49,600**
System-wide Costs		
9. Water Storage Reservoir	1,300	1,300
10. Water Distribution (piping)	3,300	3,300
11. CO_2 Delivery System to Module	13,300	21,600
12. CO_2 Distribution System to Ponds	4,000	6,500
13. Nutrient Supply System	750	750
14. Buildings, Roads, Drainage, etc.	1,500	1,500
15. Electrical Distribution and Supply	2,000	2,500
16. Machinery	500	500
17. Extraction Process Equipment	6,000	12,000
18. Anaerobic Digestion System	4,000	8,000
Subtotal	**36,650**	**57,950**
Other Capital Cost Factors		
19. Engineering (10% of total above)	7,700	10,750
20. Contingencies (15% of 1-22)	12,700	17,700
Subtotal	**24,100**	**28,450**
CAPITAL COSTS TOTAL	**97,350**	**136,000**
21. **Total Production t/ha/yr**	**109**	**219**
22. Capital Costs $/t-yr	890	620
23. **Barrels of Oil/y (@ 3.5 bar./t)**	**380**	**760**
24. **CAPITAL COSTS $/Barrel/y**	**255**	**180**
OPERATING COSTS ($/ha/yr)		
25. Power (mixing, harvest., misc.)	1,500	2,000
26. CO_2 (flue gas) blower power	1,600	3,200
27. Nutrients (N, P, Fe - 50% recycle)	1,250	2,500
28. Maintenance (3% of total Capital)	2,900	4,100
29. Labor	2,600	3,500
30. **Operating Costs Subtotal**	**9,850**	**15,300**
31. Credit for methane (+ CO_2 credits)	- 2,800	- 5,600
32. **NET OPERATING COSTS $/ha/yr**	**7,050**	**10,700**
COSTS $/BARREL OF OIL:		
33. Net Operating Costs	18	14
34. CO_2 Mitigation Credits ($60/tC)	-10	-10
35. Annualized Capital Costs (0.2 x Captial)	51	35
36. **TOTAL COSTS $/BARREL**	**59**	**39**

1.4. MICROALGAE CO_2 MITIGATION: ISSUES AND REPORT OUTLINE

Many aspects of large-scale microalgae production are uncertain and speculative, and require further analysis and, most important, R&D, if highly productive and low cost systems are to be achieved. The topics addressed in this report are:

- o Application of commercial systems designs to low value products (Section 2);
- o Waste water treatment systems and potential for CO_2 utilization (Section 3);
- o General design issues for low cost algal mass culture systems (Section 4);
- o Fundamental aspects of microalgal productivity (Section 5);
- o Microalgae harvesting technologies for low cost production (Section 6)
- o Conversion of algal biomass to fuels (Section 7)
- o Large-scale culture systems engineering designs and operations (Section 8);
- o Application of this process for a specific alga requiring inoculation (Section 9)
- o Future potential and research needs and recommendations (Section 10).

Here a brief introduction to these issues is presented, followed by a summary statement of the objectives of this report.

Commercial Microalgae Biotechnology (Sections 2). The three commercial raceway pond systems currently operating in the U.S. provide a foundation for the vision of large-scale, low cost, microalgae systems. The fact that such systems already exist, albeit at a small scale and with much higher costs then required for CO_2 utilization, allows for some optimism about the long-term goals. Indeed, the U.S. microalgae industry has created considerable equity appreciation, perhaps over $1 billion during this decade, a reflection of U.S. competitiveness in this field.

Microalgae Waste Water Treatment Systems (Section 3). Several municipal wastewater treatment facilities in the U.S. use the Advanced Integrated Ponding System, which incorporates a "high rate pond", similar to the raceway ponds used in commercial microalgae production and also the basis for the large-scale algal systems proposed here. Microalgae wastewater treatment represents a possible pathway towards initiating a low-cost, large-scale microalgae CO_2 utilization / fuel production technology. This would be a high priority for future R&D in this field.

Microalgae Mass Culture Systems (Section 4). Many photobioreactor designs are based on the maximizing a single parameter (depth, contamination prevention, gas exchange), while, in practice many different parameters must be optimized in the design of such systems. Also, selection of the algal species to be cultivated will depend on the reactor design. Modeling can be used for system optimization, and to determine the physiological capabilities required of the algal strains. Closed photobioreactors are of potential use for the production of inoculum. However, there is no plausible alternative to the open pond, raceway, paddle wheel pond design. One challenge is to develop strains that can be maintained in open pond cultures.

Fundamental Aspects of Microalgal Productivity (Section 5). Productivities achieved with outdoor microalgae cultivation systems have been, at best, 3 to 4% of total solar energy converted into biomass (higher heating value). A 5% efficiency could be extrapolated as being achievable in the near-term, based on reasonable (though favorable) assumptions. Such efficiencies are well below the 10% projected from theoretical models for photosynthesis and observed in laboratory cultures under low light intensities. (Indeed, a recent report by Greenbaum et al., 1995, suggests the possibility of even higher theoretical efficiencies than the approximately 10% of total solar projected in the past). A major factor that limits productivities in pond cultures is light saturation: more photons are captured by the photosynthetic apparatus under full sunlight than it can use. Light saturation could be overcome by reducing the light harvesting pigment content in algal cells, in principle doubling, or more, overall productivities. A high priority in future work is the demonstration of the feasibility of this approach to productivity maximization. Indeed, the feasibility analysis, and resulting economic projections in Tables 1.1 and 1.2, and in Section 8 of this Report, are based on the assumption that it will, indeed, be possible to overcome the light saturation effect and achieve near theoretical productivities.

Harvesting. (Section 6). The microscopic nature and dilute culture of microalgae, makes harvesting a major technical and cost issue. Microalgae harvesting has been the subject of a large number of studies. Potentially the lowest-cost process is bioflocculation, in which the algae spontaneously flocculate and settle, allowing simple gravity sedimentation to concentrate the biomass. Bioflocculation is often observed in nature, and has been demonstrated experimentally. One important observation is that nitrogen limited algal cultures exhibit bioflocculation. However, such processes are presently mainly observational, lacking theoretical foundation.

Fuels from Microalgae (Section 7). The objective of microalgae culture for CO_2 mitigation is to convert the microalgal biomass to fossil fuel substitutes. Due to their relatively high value, liquid fuels, such as ethanol or biodiesel, are preferred over gaseous or solid fuels (biogas or dried algal biomass). Production of ethanol or biodiesel requires that algal biomass high in fermentable carbohydrates or transesterifiable lipids, respectively. This report emphasizes biodiesels. How to produce algal biomass high in such components, without sacrificing overall productivity is a major research goals in this field. The feasibility of producing such storage products at high productivity has been demonstrated in the laboratory.

Systems Engineering Designs and Economics (Section 8). Even if the issues of culture stability and productivity maximization (lipid and overall) are satisfactorily resolved, the fundamental issue is whether it would be possible to design, build and operate a sufficiently low-cost process. In the engineering design of such a system many aspects must be considered, from the construction techniques of the ponds themselves to the CO_2 transfer and mass balance problems, to harvesting and infrastructures, etc. One issue not previously addressed is how to economically

recover the algal lipids. The overall economics of such systems depends on many factors, from site selection (e.g. land costs and site clearance) to financing assumptions. Indeed, some cost items (e.g. for paddle wheels, or pond construction) are perhaps not as uncertain as the general financial factors, such as contingencies, engineering fees, equipment installation multipliers, taxes, inflation, depreciation schedules, and, perhaps most important, discount rates (cost of money). One of the central points argued in this section is that prior economic studies (e.g. Table 1.1) probably over-estimated such cost-factors, derived from major construction/engineering projects, rather than agricultural engineering practice. This is particularly true for large-scale modular microalgae production systems. Also, the cost of capital used before (e.g. in Tables 1.1 and 1.2) could be lower, in analogy with other studies on biofuels (e.g. Wiltsee and Hughes, 1996) and for CO_2 mitigation (Herzog, 1993). The major conclusion from this section is that microalgae systems for CO_2 utilization, whether using flue gas near a power plant or concentrated CO_2 and remotely sited, provide a potentially cost competitive alternative to other options for direct power plant CO_2 mitigation, such as ocean disposal or storage in abandoned oil and gas wells.

Case Example: Production of Botryococcus braunii . (Section 9). This alga is naturally high in hydrocarbons. A conceptual system of how such an alga could be produced at a large-scale is presented, integrating the results of the prior sections into this specific case, which has the additional burden of requiring a large-scale inoculation system. Even a large inoculum, representing 10% of cultivation area need not increase overall costs by more than 10%. This opens the possibility of using genetically selected, even engineered, microalgae in such open systems.

Future Potential and R&D Needs and Recommendations (Section 10). Prior estimates of the potential of microalgae for CO_2 mitigation in the U.S. have ranged from very optimistic, equivalent to over 10% of current U.S. fossil fuel supplies, to modest, only a percent or two of CO_2 emissions from coal-fired power plants. The more conservative estimates only considered use of power plant flue gases by algal systems located near power plants (< 5 km), while the more expansive projections were based on estimated brackish water resources, without sufficient consideration of climatic and other limitations. Although no independent resource assessment is attempted, the resource base is clearly at neither of these extremes, but is sufficient to justify continuing development of this technology. Other justifications for continued R&D in microalgae CO_2 utilization are the relatively modest R&D efforts required, the existing research base for this technology, the relatively rapid progress that can be projected with these fast-growing organisms, and the benefit of this R&D in maintaining U.S. industrial competitiveness. Specific R&D priorities include the development of microalgae wastewater treatment processes that utilize CO_2 and produce fuels, fundamental research on increasing microalgae productivities, and demonstration of currently achievable productivities in year-round operations at a favorable site. The genetics of microalgae, in particular of lipids production, is also a priority.

2. MICROALGAE BIOTECHNOLOGY

2.1. INTRODUCTION: MICROALGAE AND AGRICULTURE

Microalgae cultivation has characteristics of both microbial production and agronomic processes. The hydraulic nature of microalgae cultures and their microscopic nature has some similarities to large microbial cultures, such as used in waste water treatment processes. The extensive use of land and sunshine has the characteristics of agriculture. The central issue is whether microalgae cultivation processes can be devised that can combine the advantages of these processes - the high productivities of microbial systems and low cost of agricultural production, or whether their respective disadvantages, the high costs of fermentations and low productivities inherent in agricultural systems, will dominate.

In principle, and practice, it is easier to control environmental conditions (water, temperature, nutrients) and maintain them at optimum values for long periods in large-scale algal culture than for higher plant crops and trees. Vascular plants are subject to water limitations, even with irrigation, air CO_2 levels can be limiting (the well-known CO_2 fertilization effect), and nutrient supplies are usually not well coupled to plant needs. Perhaps counter-intuitively, water use, per unit of biomass produced, even per unit area, is lower with microalgae than with irrigated higher plants agriculture, just as a swimming pool uses less water than a growing lawn. Microalgae production is actually an efficient method of water utilization.

In land-based crops, the time to canopy closure wastes a significant amount of sunlight, while algal cultures can absorb all incident sunlight year-round. Agricultural yields are typically 10 -20 t/ha/y of dry matter in temperate climates and 20 - 50 t/ha per year in tropical zones, under optimal conditions (irrigation, good soils, high fertilizer inputs, etc.). However these productivities are generally for total biomass, not useful harvest (seeds, tuber, etc.), which is generally less than half of total biomass. By comparison, rates of biomass production of 50 t/ha/y could be presently achieved with microalgae systems in favorable locations in the U.S. Furthermore, current agricultural productivities represent the culmination of centuries of practice and decades of intensive R&D, while microalgae systems are only in their commercial infancy. There is considerably scope for improvement in microalgal production systems, perhaps more than for agricultural productivities.

Of course, high productivities must be balanced with costs. The currently high costs of microalgae production have limited their commercial applications to very high value products. However, advances in microalgae technology could greatly reduce future costs. Whether such cost reductions could make microalgae biomass sufficiently inexpensive for CO_2 utilization and fuel production, is the central issue addressed in this report. In this section, a more detailed overview of current commercial production and utilization of microalgae is presented.

2.2. CURRENT COMMERCIAL PRODUCTION OF MICROALGAE

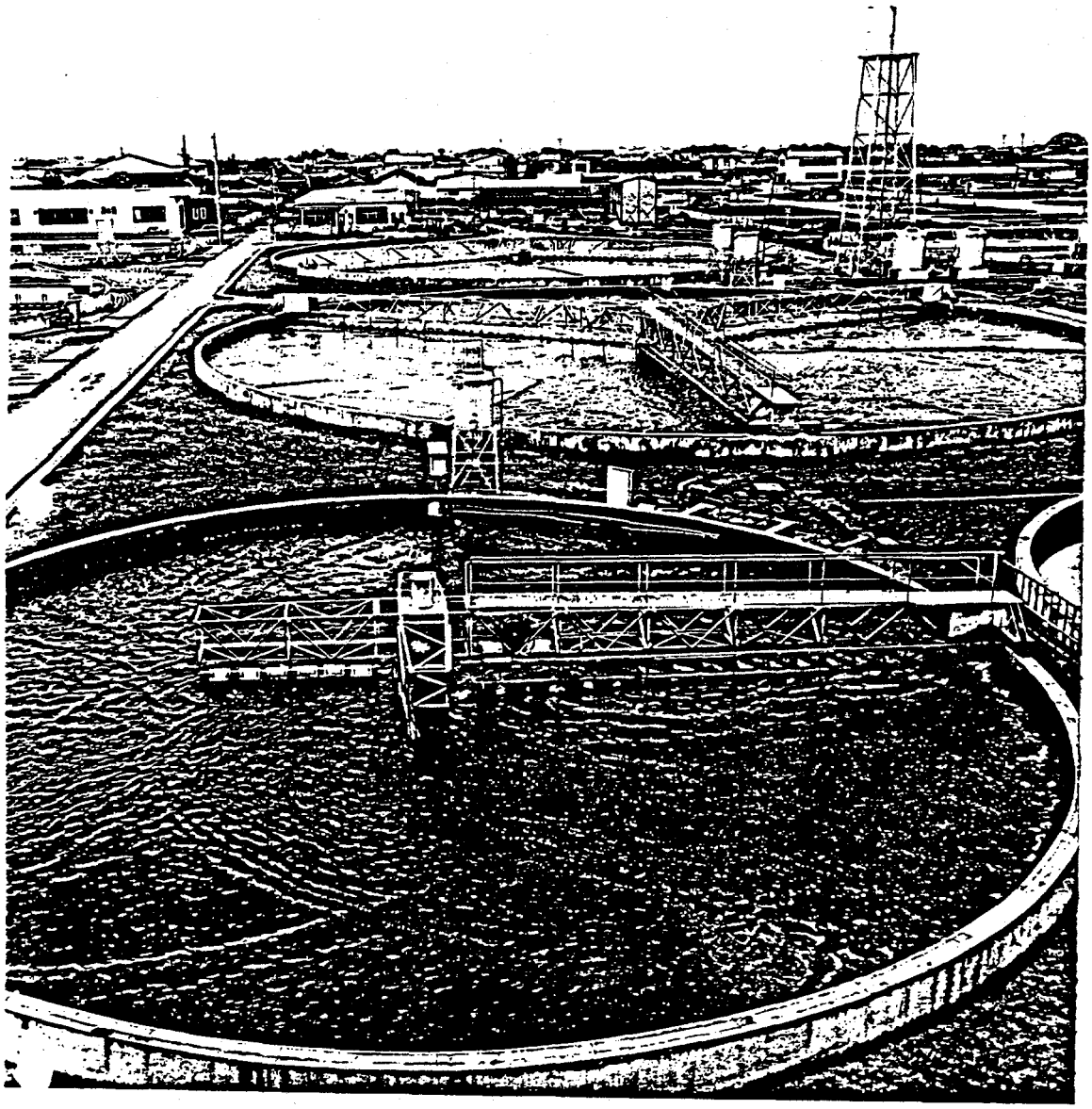
2.2.1. Chlorella Production

The first large-scale (> 1 ton/month) commercial production of microalgae was of the single cell green alga Chlorella. Starting in the early 1960's several facilities were established in Japan for production of this alga, an outgrowth of U.S. sponsored R&D after World War II (Tamiya et al., 1953; Tamiya, 1957). Since commercial production started essentially no publications have appeared on the subject of Chlorella production. Chlorella is sold, mainly in Japan, but also to a limited extent (about 100 tons/y) in the U.S. and Europe, as a food supplement (a "health food", now generally referred to as "nutriceuticals").

By the mid 1970's almost 40 plants, most located in Taiwan (where the climate is better than in Japan), were producing about 1,000 tons of dry Chlorella powder. (Tsukada et al., 1977). Most of these plants used a circular pond design, both open and covered. (Figure 2.1). Some plants used "mixotrophic" growth, in which the algae are grown with acetate (rather than CO_2) as a source of C and energy, thereby reducing sunlight requirements. (However, this also encourages bacterial growth). Production costs were high (Kawaguchi, 1980), estimated (in todays prices) to be over $10,000 for operating costs alone. Over the past two decades there has been considerable consolidation in this industry, with only about ten plants now producing about the same amount of Chlorella. This estimate includes a recently commissioned plant in Indonesia (using the traditional circular pond design), which is supposed to have an annual capacity of 300 tons, and which will be reportedly expanded in the future to 1,000 tons/year. The announcement for this plant stated that total investment, at the initial 300 t/y stage, was $30 million, a very high capital cost. Indeed, wholesale prices are high; recent quotations for delivery in the U.S. were about $30,000/ton, wholesale. At the retail level, the world market is estimated at over $200 million, with wholesale (plant-gate) markets at about $25 - 30 million. These are general estimates, there are no published reports.

Major problems with the production of Chlorella are the ready contamination of the cultures (requiring large amounts of inoculum, Figure 2.1, and short production runs) and the high cost of harvesting of these small unicellular algae with centrifuges. Also the circular ponds are very expensive and cumbersome. A few attempts have been made to develop lower costs production systems. A plant was built in Australia in 1990, using a relatively inexpensive (earthwork construction, plastic lined), shallow cascade-type pond system, with harvesting by bioflocculation (the harvesting process of choice in this report, Section 8). But it failed due to culture instability). Chlorella can also be produced in fermenters in the dark. Two U.S. companies have developed fermentation processes for Chlorella that promise significant reductions in costs, over current Chlorella price. However, commercial production in the U.S. has not yet started. Obviously, Chlorella is not a good model for the large-scale, low-cost production of microalgae.

FIGURE 2.1. CHLORELLA PRODUCTION IN CIRCULAR PONDS



2.2.2 Spirulina Production

Spirulina, a filamentous blue-green alga, is relatively resistant to contamination, when grown in a high (> 15 g/l) bicarbonate medium. Furthermore, it can be easily (cheaply) harvested by screens. Thus, it is a much easier to produce than Chlorella. Table 2.1 lists the major current production facilities. Smaller units (< 10 ton per year) are reportedly operating in India, Brazil, Cuba, and a new plant in Thailand is reportedly using farm wastes. In China over a dozen plants are reported operating, producing over one hundred tons, in aggregate, but information is very sketchy.

Table 2.1. Major Current Spirulina Production Facilities

Company	Location	Area ha	Production tons/year	System Design
Earthrise Farms	S. Calif	15	400	Raceway
Cyanotech	Hawaii	10	350	Raceway
Siam Algae	Thailand	3	100	Raceway
Sosa Texcoco	Mexico	33	300 (Closed)	Deep Pond
Blue Continent	Taiwan	10?	100?	Raceway
Nippon Spirulina	Japan	1.5	30/?	Raceway?

Most production systems use the paddle wheel mixed, plastic lined, shallow (< 30 cm) raceway pond design, which allows good control over conditions (such as CO_2 supply). Individual growth ponds are up to about 0.5 ha in size. Spirulina is grown as a continuous culture (vs. batch cultivation for Chlorella) and the media is recycled to conserve bicarbonate. The plant in Mexico, shut down since late 1993, used the high bicarbonate waters available at that site and cultivated the alga in a single, deep (appx. 1 m), unmixed, pond of 33 ha, without CO_2 addition. Spirulina harvesting involves fine mesh screens with backwash, followed by spray drying.

The main (75 - 80%) market for Spirulina is as nutraceuticals, with smaller markets in aquaculture feeds, and food coloring. In Japan the blue pigment extracted from Spirulina, phycocyanin, is used to color yoghurts and ice cream). With the closing of the plant in Mexico (which produced 300 tons) prices increased from $15,000 - 20,000/ton to over $25,000/ton recently, and led to a doubling of U.S. production in the past two years. Several companies around the world (India, Cuba, China etc.) have started production. Figure 2.2. shows a view of the Earthrise Farms plant, in S. California, which was (since the picture was taken) expanded about two-fold, to appx. 10 hectares. (Figure 2.3 shows a close-up).

FIGURE 2.2.
EARTHRISE, FARMS, INC., SPIRULINA PLANT IN S. CALIFORNIA

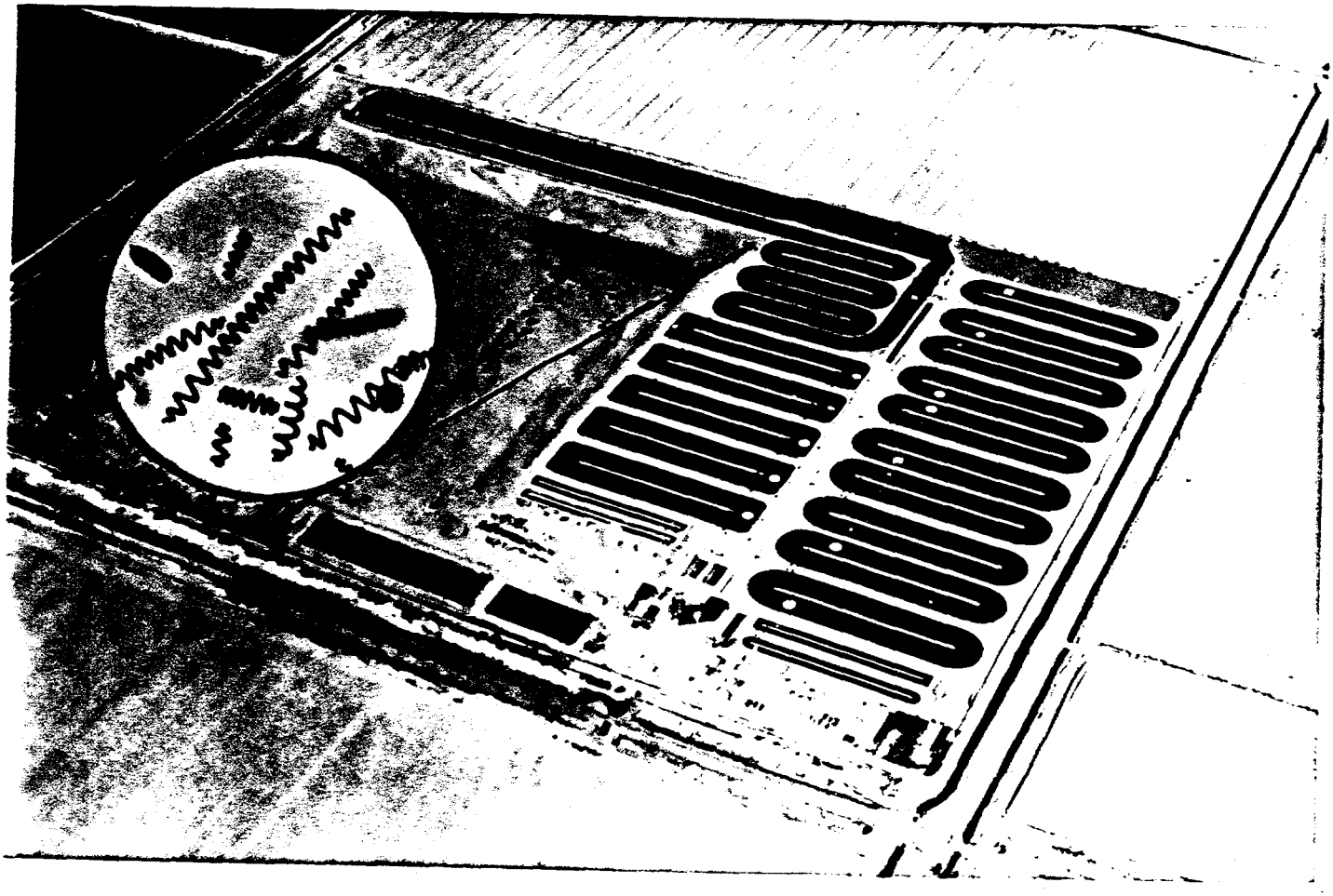
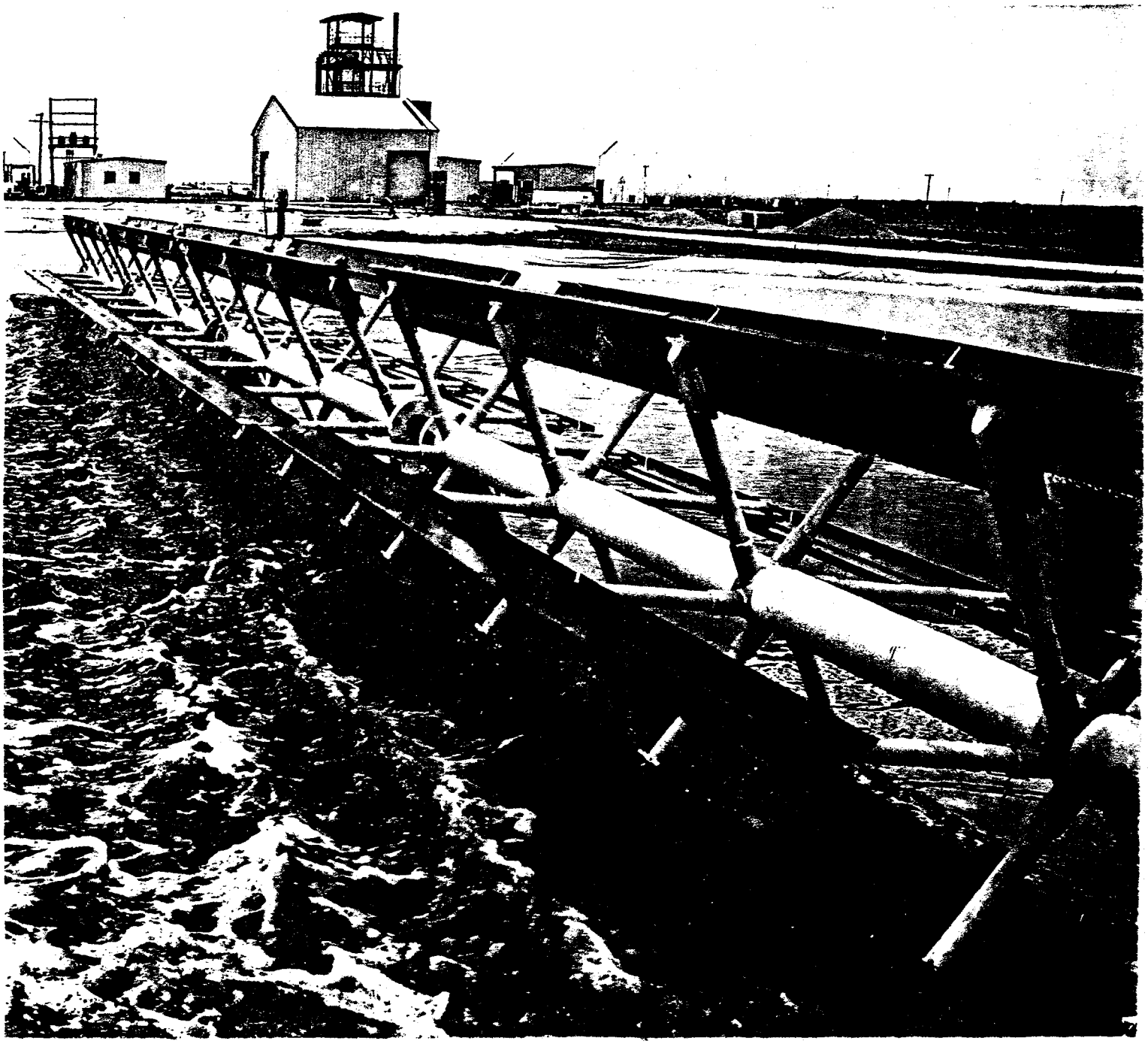


FIGURE 2.3. CLOSE-UP OF EARTHRISE FARMS, INC., SPIRULINA PLANT



2.2.3. Beta - Carotene from Dunaliella

Dunaliella is a motile green alga that lacks a cell wall and occurs naturally as essentially unialgal cultures in high salinity brines (> 150 g/l NaCl). The strains growing in such brines are deep orange in color, due to a high content of beta-carotene. Beta-carotene production from Dunaliella was first developed (at least to the pilot plant) in the Ukraine, in the 1960's, and then Israel (Ben Amotz and Avron, 1987). The first commercial production plants were established in the 1980's in Australia and the U.S. Commercially grown Dunaliella contains about 5% of dry weight natural beta-carotene, a mixture of cis and trans isomers. (Synthetic beta-carotene is only the trans isomer). The market for beta carotene is as a food coloring agent, vitamin and antioxidant supplement (nutriceutical). The synthetic, all trans, product wholesales at about $600/kg, the natural algal product sells for about twice this. Table 1 lists the major current production plants.

Table 2.2. Dunaliella Production Facilities

Company	Location	Area ha	Production tons/year of Beta-Carotene	System Design
Nutrilite	So. Calif	6	2	Raceway
Nature Beta Cart.	Israel	5	2	Raceway
Western Biotech	W. Australia	120	8	Unmixed Pond
Betatene	Australia	>300	8	Unmixed Ponds

Nutrilite, Inc., (a subsidiary of Amway, Corp., which in 1993 bought the plant from Microbio Resources, Inc.), like the (Japanese owned) Israel plant, produces the algae in raceway ponds (Figure 2.4) and sells its product through its own distribution network. Betatene (recently acquired by Henkel Corp.), the largest company, and Western Biotech, sell to third parties. The first two plants listed above use paddle wheel mixed, shallow (appx. 10 cm), raceway ponds, while the other two use large, unmixed ponds. Thus, no standard production system has developed. Overall, the raceway pond systems have operated more reliably and are more productive. However, the large, unmixed pond systems, being located at salt evaporation systems, where land costs are not an issue, have likely somewhat lower production costs (Figure 2.5.). Increasing demand is resulting in production increasing, both by these and new producers. Cyanotech Corp. in Hawaii (see Figure 2.2 above), which produced beta-carotene about five years ago at the pilot plant level, is building a new plant. In China several projects are already ongoing. Other projects, in Europe and South Africa, are likely to move toward production soon. Thus this is another example of a successful microalgae production process.

FIGURE 2.4. NUTRILITE, INC. DUNALIELLA PLANT IN S. CALIFORNIA

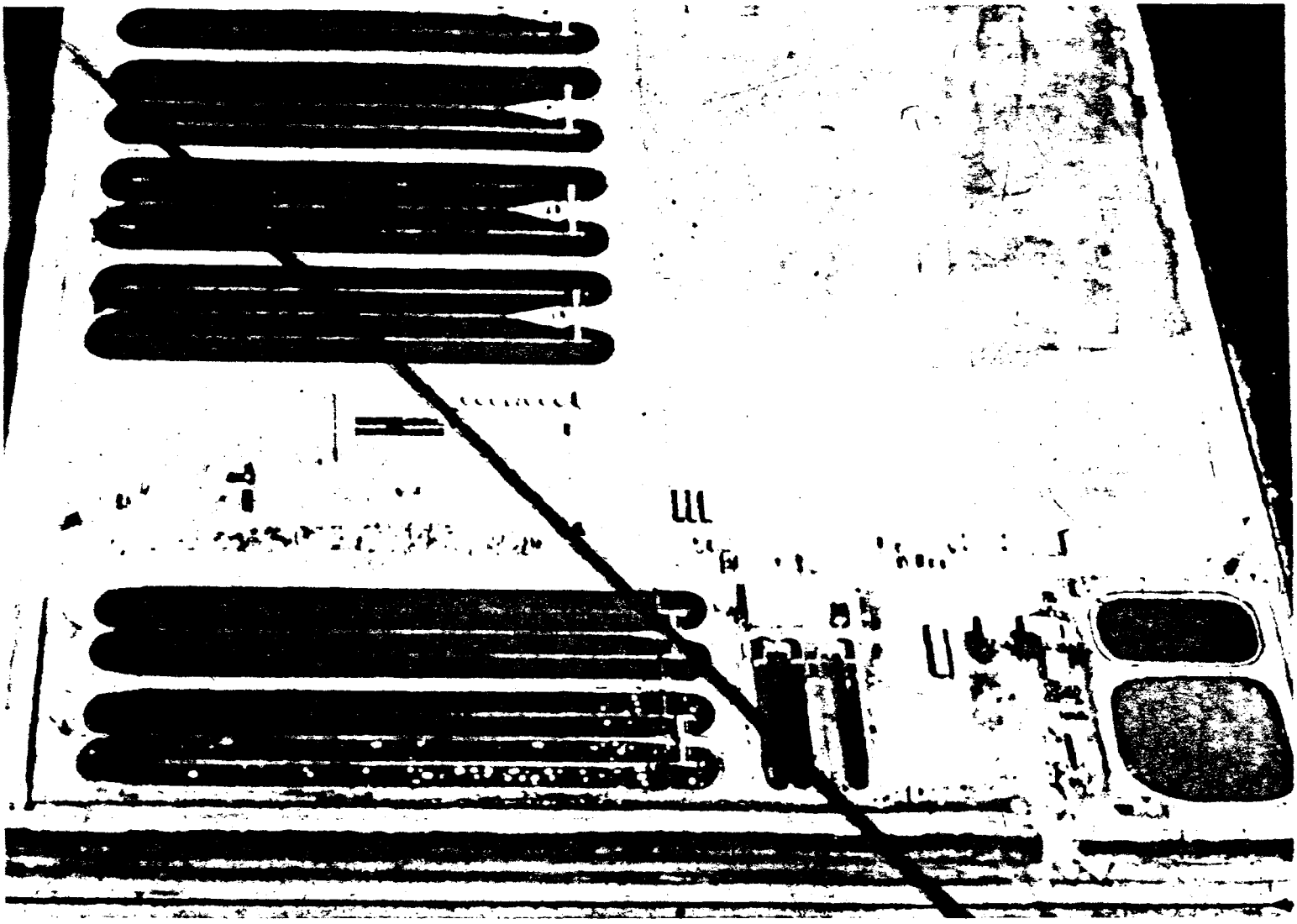


FIGURE 2.5.
WESTERN BIOTECH DUNALIELLA PLANT IN WESTERN AUSTRALIA.

2.3. POTENTIAL COMMERCIAL MICROALGAE PRODUCTS

Microalgae have been studied as a potential source of a large variety of high value products. These can be very generally defined as any product(s) that when multiplied by its fraction in the algal biomass has a value of over $5/kg (the likely current lowest plausible commercial production costs for microalgae). Here some examples of such high value products, which have not yet advanced to commercial production, are reviewed (Benemann, 1990; Cohen, 1989; Borowitzka, 1988).

2.3.1. Polysaccharides and Carbohydrates

Sulfonated polysaccharides derived from macroalgae (seaweeds) are used commercially as thickening and flocculating agents, both in the food industry and some industrial applications. The carrageenans of the red microalgae, such as Porphyridium cruentum and P. aerugineum, have been extensively investigated and they have properties (e.g. viscosity, gelling behavior) equal or even superior to those of macroalgae. The P. cruentum polysaccharide has been patented for tertiary oil recovery (Savin et al., 1973). One potential advantage of a microalgae production of these polysaccharides is that they would be more uniform, have more reliable properties, than those obtained from seaweeds, which exhibit significant batch to batch variability. As with most other cell constituents in microalgae, content and productivities depend on environmental factors, such as the light intensity, nitrogen supply, etc. However, despite considerable interest, no commercial process development has taken place. This can be attributed to the high cost of bringing new products to the market, the relatively low cost of red seaweed production, and the difficulty of developing a process for Porphyridium production. Major problems are the ease of culture contamination and the high viscosity of the culture medium when the polysaccharide is excreted. These problems have been only partially resolved (Benemann and Weissman, 1985).

Other types of polysaccharides are found in microalgae, including laminarin (gamma-1,3-glucan with 1,6 branching points); starch; inulin; fucoidin (a fucose polymer); and polymers comprised of xylose, arabinose, rhamnose, and glucuronic acid. Bioflocculants produced by algae have potential industrial applications, such as water clarification. A soluble flocculant produced by the cyanobacterium (blue-green alga) Phormidium (strain J-1) was observed to cause clay sedimentation in drainage troughs in Israel . However, for none of these has a commercial potential or production technology been demonstrated.

Carbohydrates in microalgae function not only as structural components but also as storage products (starch and glycogen) and osmoregulators (glycerol, trehalose, mannitol, sorbitol, glucosylglycerol, cyclehexanetetro, isofloridoside, etc.). These are accumulated by various microalgae in response to increases in osmotic pressure (typically high salinity) in the environment. However, the commercial value for most of these compounds remains to be established.

2.3.2. Surfactants and Fatty Acids

Microalgae are a potential source of biodegradable biosurfactants. Possible uses include acting as flocculation and emulsification agents and in the "tertiary" recovery of oil. For the most part, these compounds are glycolipids or long-chain fatty acids, although some polysaccharides also can have such properties. Algae produce a broad spectrum of surfactant-type lipids, such as phosphatydylglycerol, phosphatidylethanolamine, phosphatidylcholine, etc. The latter is also a lecithin, compounds which have a variety of applications in food and medicinal industries. As of 1988, the world consumption of lecithin was over 100,000 t/year. Neither the use of algal biosurfactants nor lecithins has been developed commercially.

Microalgae are a unique source of essential unsaturated fatty acids and prostaglandins. Indeed, much of the interest in the fermentative culture of microalgae and the applications of microalgae in aquaculture are due to the relatively high concentrations of unsaturated C18 to C22 fatty acids found in particularly marine, microalgae. Essential fatty acids present in microalgae include linonlenic acid (18:2), gamma-linolenic (18:3) acid (GLA), eicosapentaenoic acid (20:5) (EPA), arachidonic acid (20:4) (ARA), and DHA (22:6). ARA is not only an essential fatty acids, but also used as a precursors of prostaglandins, prostaxylins, thromboxanes, and leucotrienes, important products in the pharmaceutical industry. It is found in Porphyridium cruentum at a level of about 40% of total lipids (Cohen, 1990). The "omega-3" fatty acids, implicated in prevention of heart and other diseases (although a recent report contradicts this, Kolata, 1995), are currently derived from fish oils. They have potentially large markets as food supplements and in the pharmaceutical industry (including baby formula). Spirulina is considered to be good source of GLA, currently derived mainly from primrose oil. The production of high value unsaturated fatty acids from microalgae is a very active area of commercial research and development. The heterotrophic, fermentative, production of microalgal DHA has been recently commercialized in the U.S., but no autotrophic production systems have been developed.

2.3.3. Amino Acids and Vitamins

Several amino acids could be potentially produced by microalgae. Some species accumulate high concentrations of proline under conditions of high salinity, and a commercial process has been suggested (Levitt, 1985). Blue-green algae can contain high concentrations of cyanophycin bodies, a co-polymer of aspartic acid and arginine (Allen, 1994). Arginine in particular has a relatively high value.

Microalgae contain many of the vitamins needed in nutrition, in particular the antioxidant vitamins C, E, and beta-carotene. However, with the exception of the latter, discussed above, no other vitamins are currently produced commercially with microalgae, or are under present development, contrasting with the wide use of microbial fermentations in this industry.

2.3.4. Pigments

Microalgae are, essentially, light absorbing systems. Thus, not surprisingly, that among the potential products from these microbes are their pigments. Current production of pigments was already mentioned in connection with beta-carotene from Dunaliella, and phyocyanin from Spirulina. Lutein, a xanthophyll, is of commercial interest in poultry production, and has been recently commercialized by fermentation of Neospongiococcus, a process first studied in the 1960's.

Perhaps the most commercially interesting pigment is astaxanthin, with a value of about $3,000/kg, and a major feed ingredient in salmon aquaculture, with a market of over $100 million (Benemann, 1992). Haematococcus pluvialis contains relatively large (5%) amounts of astaxanthin and many groups around the world are trying to develop a commercial production process. However, it is difficult to prevent contamination during culture, suggesting closed photobioreactors. Two companies in Hawaii, Aquasearch, Inc., and Cyanotech, Inc., are currently developing two stage processes, in which plastic tubular reactors would be used to grow the (easily contaminated) actively growing (green) culture, which would then be transferred to open ponds for the nutrient limited xanthophyll induction phase.

2.3.5 Pharmaceuticals and Diagnostics.

Many algae are known to be toxic to both humans and animals (and even other plants) (Carmichael, 1994), and the compounds responsible for these toxicities are potential antifungal, antibacterial, antiviral, and even anticancer agents. Indeed, a microalgae product is likely to undergo human clinical trials in breast-cancer therapy (Patterson, personal communication, 1995). However, there is a long time (and about $250 million) between the finding of a biological activity in screening tests and a commercial product. Indeed, even if a pharmaceutical agent were to be developed based on such screening of algal products, it would be likely that the commercial production would be based on a synthetic process, or fermentative production, rather than mass culture of the microalgae in open ponds.

Already commercial is the production of diagnostic reagents from microalgae, specifically of the phycobiliproteins of the blue-green and red microalgae, used as fluorescent labels in both research and diagnostic kits. However the markets for these products are minuscule, in terms of biomass requirements (e.g. a few tens of kilograms per year). Growing microalgae in closed photobioreactors on $^{13}CO_2$, or in D_2O allows the production of isotopically labeled compounds that are used in diagnostic tests, as well as in research. Again, the amounts of such products used are small (a few grams to kilograms /year), although their value is high (several million dollars). At any rate, these are present commercial applications of closed photobioreactors.

2.3.6. Aquaculture

Microalgae are used as feed for the commercial production of molluscs, fish, and crustaceans (DePauw and Persoone, 1988; Benemann 1992). As mentioned above, Spirulina is currently used as a fish feed additive, principally for Koi, due to the desirable coloration it imparts. Astaxanthin from Haematococcus is of great interest in the salmon aquaculture industry, but the technology for production of this alga has not yet been developed. At present microalgae are mainly used during the hatchery and nursery stages of bivalves, shrimp, and some finfish cultures. After the hatching of fertilized eggs, the young animals are fed a diet of microalgae until they are large enough to be fed larger foods, or taken to the next stage in the production process. For carnivorous fish, , microalgae cultures are used to produce zooplankton, typically rotifers, which are fed to the freshly hatched fish.

Typically, in aquaculture operations small batches of algae, mainly seawater diatoms and flagellates, are produced under highly controlled conditions in translucent cylinders, plastic bags, carboys, or tanks, generally with artificial illumination and/or in greenhouses. Production seldom exceeds 100 kg (dry weight basis) per year per facility. The algae produced are used for feeding bivalve (oyster, clam, mussel, etc.) larvae and "seed", or in the hatchery operations for shrimp and planktivorous fish fingerlings. The algae are produced as needed, and the live culture used directly, without harvesting or storage. Several hundred such production system are being operated worldwide, both commercial and as part of aquaculture research facilities.

The largest operations in the U.S. are carried out by a few oyster and clam aquaculture companies (Donaldson, 1991). The larger companies produce many tens of millions of small animals ("seed") for planting in more or less protected estuarine areas, where they then grow to maturity on natural phytoplankton. The microalgae required for the raising of this seed, is produced using relatively deep tanks indoors or in greenhouses, often with artificial lights. Total algal (dry mass) production is at most one or two tons per year. Cost of production even at this scale are high, and have been estimated to be over $250/kg of algal biomass for an optimized facility (Walsh, 1987). The high cost of this feed prevents the growing of bivalves to a size larger than a few mm using cultured algae. After reaching the maximum size allowed by the algal feed supply, the animals are placed in the natural environment, where predation, mortality, and contamination with human pathogens and other pollutants are significant problems.

There is thus a significant commercial opportunity in microalgae feeds for aquaculture. Indeed, one U.S. company, SeaAg Inc., has developed outdoor pond mass culture of diatoms suitable for bivalve aquaculture applications, based in large part on technology developed under DOE funded projects (Weissman and Tillett, 1990). Another U.S. company has recently announced the development of a microalgae aquaculture feed produced in fermenters.

2.3.7. Agricultural Applications

In this application, algae are used for improving soil tilth and for producing fertilizer. Microalgae, typically of the genus Chlamydomonas, are cultivated in small indoor systems and are then applied to the soil. The algae produce extracellular polysaccharides that can bind to soil particles and control erosion (Metting, 1993). This process is being carried out commercially by one small company in Washington State. Nitrogen-fixing algal strains have also been studied in such applications, but their greatest potential is in the inoculation of rice paddies, where they can contribute to the N economy of the plants (Phady, 1985). However, the actual efficacy of any such applications remains to be established.

2.4. CONCLUSIONS.

The above survey does not exhaust the possibility of high value products from microalgae. For example, microalgae (Chlorella) are currently used by one U.S. company in immobilized form to remove heavy metals from waste waters, a process tested with, among others, a Hg contaminated waste water from a DOE facility (Feiler and Darnall, 1991). Microalgae have considerable potential in radionuclide and toxic metal removal from contaminated ground and surface waters (Wilde and Benemann, 1993). Cyanobacteria produce polyhydroxybutyrate, currently produced from bacteria for biodegradable plastics. More products from microalgae can be expected to be discovered, investigated, and developed to the commercial stage. And, clearly, the U.S. has become the established leader in the commercial applications of this new biotechnology.

However, the above does provide some reason for caution, even pessimism, for the large-scale production of microalgae. With the exception of the mass culture of a diatom in seawater for aquaculture, and that only at a scale of a few hundred square meters, no other new algal species or product has been commercialized using open pond cultures over the past decade. Indeed, the recent commercial advances have been in heterotrophic (fermentative) production of microalgae. The advantages of such a production technology is that fermenters can maintain a pure (bacterial free) algal culture, allow great control over the growth conditions and use of strains that would not be able to grow in outdoor ponds. And at about $10,000/ton, production costs are competitive with current open pond technologies.

There are, of course, other reasons for the slow development of microalgae products: many are either of very small-scale and others (e.g. food colorants) require expensive product testing before they could be used, presenting a major barrier to entry. Thus, not only the production technology, but also product characteristics have limited the development of this industry. The use of microalgae in wastewater treatment, is considered in the next section.

3. MICROALGAE IN WASTE WATER TREATMENT

3.1. INTRODUCTION

Microalgae are used for municipal and industrial wastewater treatment. In waste treatment ponds, known as "oxidation ponds", the microalgae provide the dissolved oxygen used by bacteria to break down and oxidize wastes, thereby liberating the CO_2, phosphate, ammonia, and other nutrients used by the algae. In essence the organic matter in the influent, that which does not settle and decompose in the sediments, is converted into algal biomass (Oswald, 1988, 1990). Discharge of the pond effluents, containing the algal biomass, results in a suspended solids and BOD (biological oxygen demand) load in the receiving bodies of waters, creating potential problems (oxygen deficits, eutrophication), unless the effluents can be greatly diluted. If dilution is not possible, the pond effluents must be disposed of on land, the algae settled in terminal "settling ponds", or the algae harvested and biomass disposed of. There is no effort made to grow any particular species of algae in such ponds. Species control could greatly aid in algal harvesting by favoring easily settleable or filtrable algal species (Benemann et al., 1980).

Examples of four microalgae wastewater treatment ponds in Northern California are listed in Table 3.1. Most oxidation ponds are relatively deep (about 1 to 2 m) and not mechanically mixed. However, a few use raceway, mixed, ponds, although mixing is by recirculation pumps (St. Helena) or Archimides screws (Hollister), not paddle wheels. Also these raceway ponds are deeper (about 0.5 to 1 m) than those used in the production of Spirulina (20 - 30 cm) or Dunaliella (10 -15 cm). The systems it Table 3.1 that harvest algae (Sunnyvale and Napa) use lime or alum as and polyelectrolyte flocculants. These are, however, not typical, as relatively few oxidation pond systems use either raceway ponds or harvest algal biomass. The two systems (St. Helena and Hollister) that include high rate ponds, were designed based on the "Advanced Integrated Pond Systems" technology developed at U.C. Berkeley (Oswald, 1988, 1990). Algal waste treatment systems are of interest in this Report as they could utilize CO_2 (see below). Thus they are reviewed here.

Table 3.1. Examples of Microalgae Waste Treatment Systems in N. California

Location	Area (ha)	System Design	Algae Harvest/Disposal
Napa	140	Deep Ponds	Harvest Flocculants, Digest
St. Helena	8	Raceway (2 ha)	Terminal Settling ponds
Sunnyvale	180	Deep Ponds	Harvest Flocculants, Store
Hollister	13	Raceway (5 ha)	Land Disposal

3.2. THE SUNNYVALE POND SYSTEM

This treatment plant is currently unique in that it regularly harvests the algal biomass produced in the ponds (the Napa plant, discussed below, is no longer operating) and then converts at least a portion of that biomass to energy via anaerobic digestion. Two large, somewhat irregular shaped, ponds covering a total of 175 hectares, currently receiving about 17 MGD (million gallons per day). The depth of the ponds varies from 1 to 4 meters. The ponds have a retention time of 13 to 50 days (depending on season, infiltration, etc.). The ponds receive primary treated waste water, and the effluent is treated with flocculants, allowing removal of the algal biomass in a DAF (Dissolved Air Flotation) system. From the DAF system the effluent goes through a dual media filtration (DMF) process consisting of anthracite coal, pea gravel, and sand. This is followed by chlorination and dechlorination, from where the treated wastewater is sent to the SF Bay. (Chlorine is also added prior to the DMF process, to prevent growth on the filters).

The key to the operation of the process is the flocculant and algal separation process. Initially, when algal removal was initiated in the late 1970's, alum was used as the primary flocculant. The flocculated algal biomass was disposed of by dumping it into a section of the ponds. Work carried out in the late 1970's demonstrated that alumn flocculated algae are not biologically degraded (Eisenberg et al., 1979). Thus, probably most of that algal biomass is still in the bottom of the pond.

Starting in the mid 1980's the development of cationic flocculants useful for waste treatment led to the application of these products for algae removal at this plant (as well as at Napa). These have been developed to the point where a very high degree of treatment is achieved. The polymers used are proprietary products, and have been changing over the past few years. The cost of flocculants was stated as $126/MG (million gallons). However, this must be verified. The objective is to reduce the turbidity of the effluent (to the San Francisco Bay) to 2 NTU and the suspended solids to 2 ppm, to allow water reuse for golf courses, landscaping, etc.). The polymers being currently used now are usually able to achieve these criteria, previously only about 7 to 8 NTU and 15 ppm suspended solids were achieved.

The flocculant is diluted in mix tanks and then is pumped to the DAF unit, where it injected (via perforated ring) at two sites, one below and one at the zone of decompression. In the DAF process, about 25% of the flow is compressed with air at 80 PSI and allowed to decompress during contacting with the rest of the flow.

Although there is great emphasis on the performance of the DAF process, there has been relatively little study of the ponds themselves. The ponds are provided with a set of four 62.5 MGD circulation pumps, and is also subject to wind mixing. However, there is little doubt that hydraulics are rather poor, with a rather high dispersion coefficient, and much of the area not used effectively. However, the large area of these ponds (about 175 hectares) makes this not a critical issue.

The suspended solids (mostly algae) coming into the DAF system, typically range from a low in winter of about 30 ppm to a high in summer of as much as, or over, 100 ppm. Thus there is a large variation in the effluents of these ponds. The algae present in the effluents are also very variable. A large variety of microalgae is present, with green algae and euglenoids being very dominant. Species mentioned include Closterium, Cylindrotheca, Euglena, etc. In winter Chlorella is often found (possibly selected by lower pH and higher BOD). Chlorella is difficult to harvest with the DAF system, and this is a major past, and current, problem in this system.

About half of the algal biomass harvested by the DAF system is returned in part to the ponds, with the rest being subjected to anaerobic digestion in the conventional primary digesters. Eventually most if not all the algal sludge is to be digested, rather than returned to the ponds. Thus this is the first, and thus far only, microlalgae to energy production system.

3.3. NAPA PONDS

The Napa ponds, which closed (except for effluent storage) about two years ago, consisted of four ponds in series, of approximately equal area, with a total area of about 140 hectares. The ponds receive about 15 MGD (appx. 60 x 10^6liters per day) of primary primary treated sewage, mixed with some raw sewage and wastes from near-by areas with an influent suspended solids concentration of about 300 ppm. Plant effluents (to the Napa River), after harvesting the effluent algae (see below) average less than 20 ppm. The ponds are operated in series with the first pond receiving the raw sewage, and the last pond discharging into the algae separation plant.

The ponds were operated to discharge little if any effluent in summer (when the Napa River has too low a flow to allow for sufficient dilution) and more in the winter (during the rainy season), resulting in draw-down during the Spring, and storage in summer. During the summer pond effluents are also used for irrigation of nearby fields, as a form of land disposal. These operations resulted in a very variable pond depth during the year. However, irrigation areas were limited, and thus during summer (even accounting for evaporation and infiltration) an increase in total volume, and thus pond depth, took place.

This variable, and generally low depth in summer, resulted in significant odor problems, in particular in the first pond that received the raw sewage. This was one reason for the closure of the plant. However, simple operational changes, such as recirculation from the second or third pond, and a greater operational depth in the first pond) could have prevented the odor problems. Indeed, promotion of anaerobic processes in the first pond could have resulted in a significant fraction of the overall C input to the system being degraded, and, thus, improved the overall effectiveness of the process.

From the last pond(s) the effluent is transferred to an algae harvesting system, which consists of a set of four settling chambers, to which long chain cationic polymer is added to flocculate the algae. Initially this plant used mainly lime and some alum. Like Sunnyvale, it switched to mainly synthetic cationic flocculants. The organic flocculants used during the last two years of operation were American Cyanamid polymers #515C and #1596C. These polymers were added in amounts of 100 ppm for 515C and 2 to 4 ppm for 1596C. The settled sludge is hydraulically pumped back to pond 1. (There used to be separate sludge beds when the flocculant was lime, but when they switched to organic flocculants the sludge was discharged back to the initial pond, further compounding the problems of low water levels, odors production and too little carbon destruction).

The algae ponds produce on average an influent to the algae removal system of about 100 ppm, which is reduced by the flocculants and the settling chambers to below 20 ppm. Total cost for the use of the flocculants was stated at about $150/MGD. There was little data available on algal counts and identifications. This data was collected for a couple of years during mid 1980's but not since then. According to the operators, there was a "big difference" in the performance of the algal harvesting process depending on the type of algae coming in, but there is no documentation of this (e.g. relating the algal type with process performance).

As stated above, this treatment plant was recently replaced with an activated sludge plant. There reasons for this were that the plant effluents on occasion exhibited a relatively high BOD level and also because of the odor problems already mentioned. (Pond 1 had some small surface aerators to control odors, but they were too small to do any good). The ponds are still used for storage of storm flows, and for plant outages, but they are no longer part of the treatment process.

The Napa ponds are a classical case of mismanagement of such systems. Even though rather simple operational changes would have eliminated most if not all of the problems. It can only be speculated that the reason for the abandonment of this system was that the engineering consulting companies advising the local operators found it easier (and certainly more profitable) to specify a conventional treatment system. Failure of a technology is often due to such human factors.

3.4. ST. HELENA PONDS

These ponds receive a relatively small amount of local domestic and some vinery waste. The ponds were constructed 25 years ago following a design by Prof. Oswald, and are the first (and one of the few) municipal waste treatment systems using the "advanced integrated pond" technology, which includes the raceway pond as the central working unit. Thus a description of this system is appropriate. The general layout and dimensions are shown in Figure 3.1., an aerial view in Figure 3.2.

FIGURE 3.1. SCHEMATIC ST. HELENA POND SYSTEM

POND #	SIZE Acres	VOLUME Ac-ft	DEPTH ft
1	2.9	25	10
2	5.1	15	3
3	2.5	17	8
4	4.7	52	13
5	6.3	73	13.5

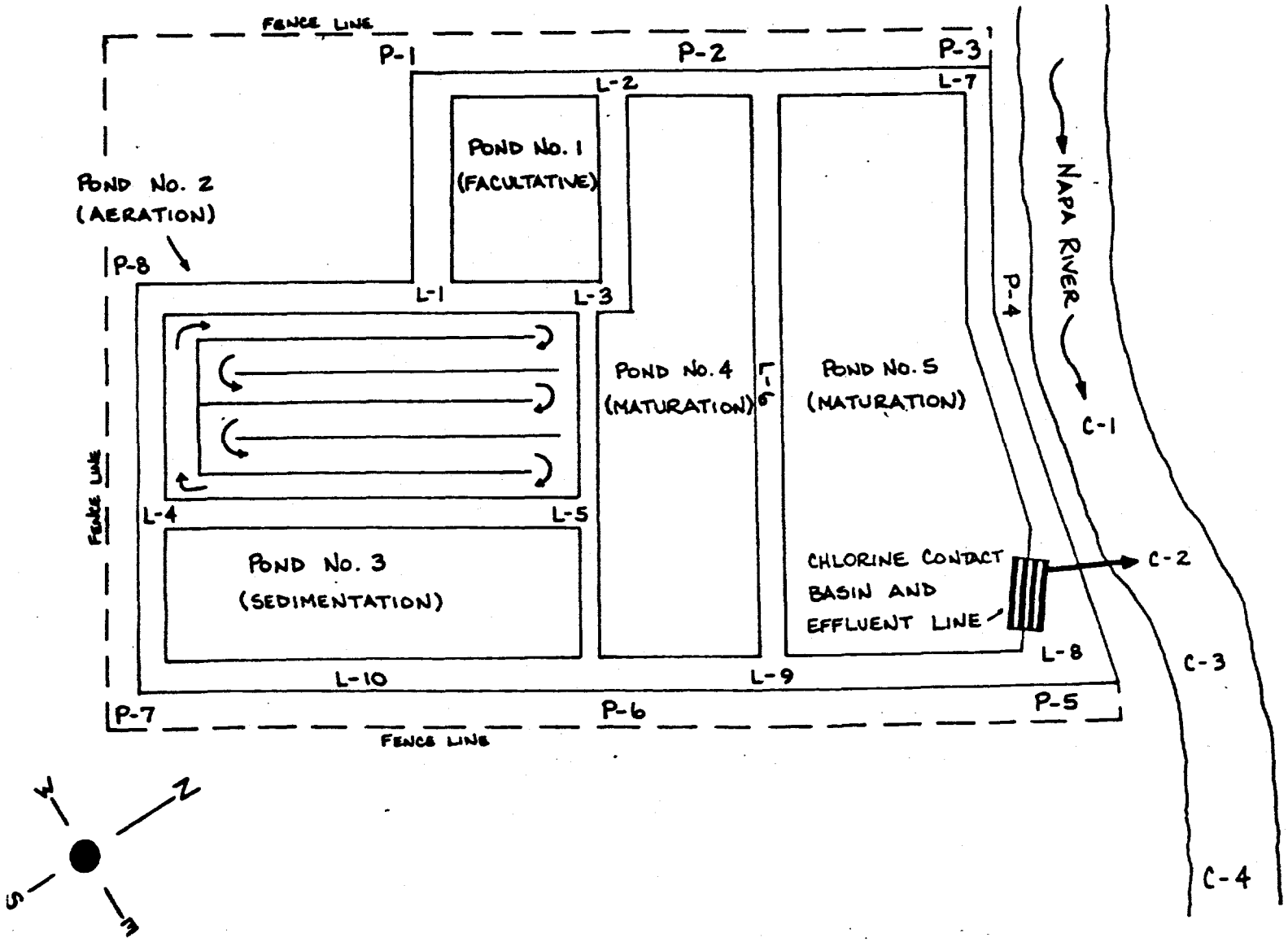
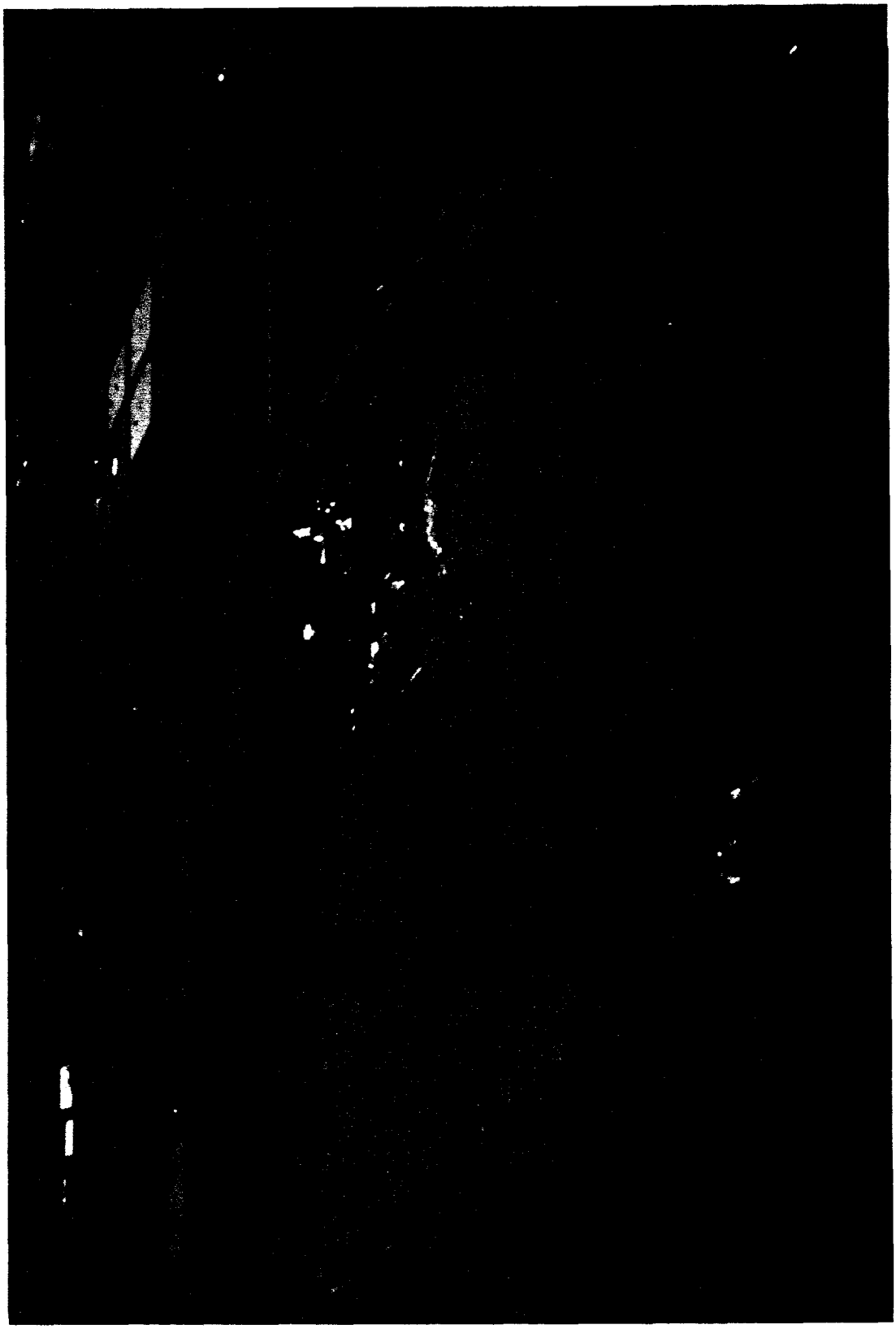


FIGURE 3.2 AERIAL VIEW OF ST. HELENA PONDS



The influent comes into Pond 1, which is a deep anaerobic pond where there is considerable destruction of solids (and even soluble) BOD. To minimize odors some effluent from Pond 2 (the raceway) is recirculated to the surface of Pond 1. This is now being carried out on the basis of the dissolved oxygen in this fraction.

The raceway pond (Pond 2) is currently mixed with pumps, which provide relatively poor hydraulics and which are to be replaced with a paddle wheel in the future.

The effluent of Pond 2 is discharged to Pond 3, which is a "maturation" pond and then to ponds 4 and 5, which are "settling" ponds. Then the effluent is discharged, after chlorination, to the Napa River. However, it should be noted that the performance of the "maturation" or "settling" ponds has not been established. Certainly some algae will settle in the latter, but there is also considerable re-growth, and some algae will not settle in this system. These latter ponds also serve to equalize flow and to provide holding capacity for periods (e.g. in the summer) when discharge limitation are in effect due to the low level of the river.

3.5. HOLLISTER PONDS

The Hollister Pond system is another "advanced integrated ponding" system, including a high rate pond. The dimensions and schematic are shown in Figure 2.3. An aerial view is presented in Figure 3.4.

The pond system consists of two primary ponds designed with two depressions in the middle that serve as settling and sludge collection sites. These ponds are classified as anaerobic, and are maintained odor free through the recirculation of an active culture of microalgae from the high rate ponds (as also practiced in St. Helena). However, as also in St. Helena, several surface oxygenators (simple floating aerators) provide additional oxygen supply to these ponds, to prevent odors. Without these aerators the odor problem could be severe on occasion.

The high rate pond consists of 16 acres of a serpentine, single channel raceway, with a large number of turns (see figures). The mixing is provided by a set of two large Archimides screws, of which one is sufficient for providing the required circulation. During the 1989 Loma Prieta earthquake, many of the dividers (baffles) in the high rate pond, were destroyed. But despite the extensive short circuiting that this obviously caused, there was no indication that this affected operations. The last pond in the series is a small holding pond for the effluent.

There is no discharge from this plant, a rather unique feature. (Napa has only a partial discharge). Instead, the effluent from the high rate ponds is directed to a series of percolation basins, which recharge the local aquifer. To prevent sealing of the percolation basins, they are periodically allowed to dry and then disked to break up the top few inches of the surface. However, this has not prevented the

FIGURE 3.3
SCHEMATIC AND SPECIFICATIONS OF THE HOLLISTER PONDS

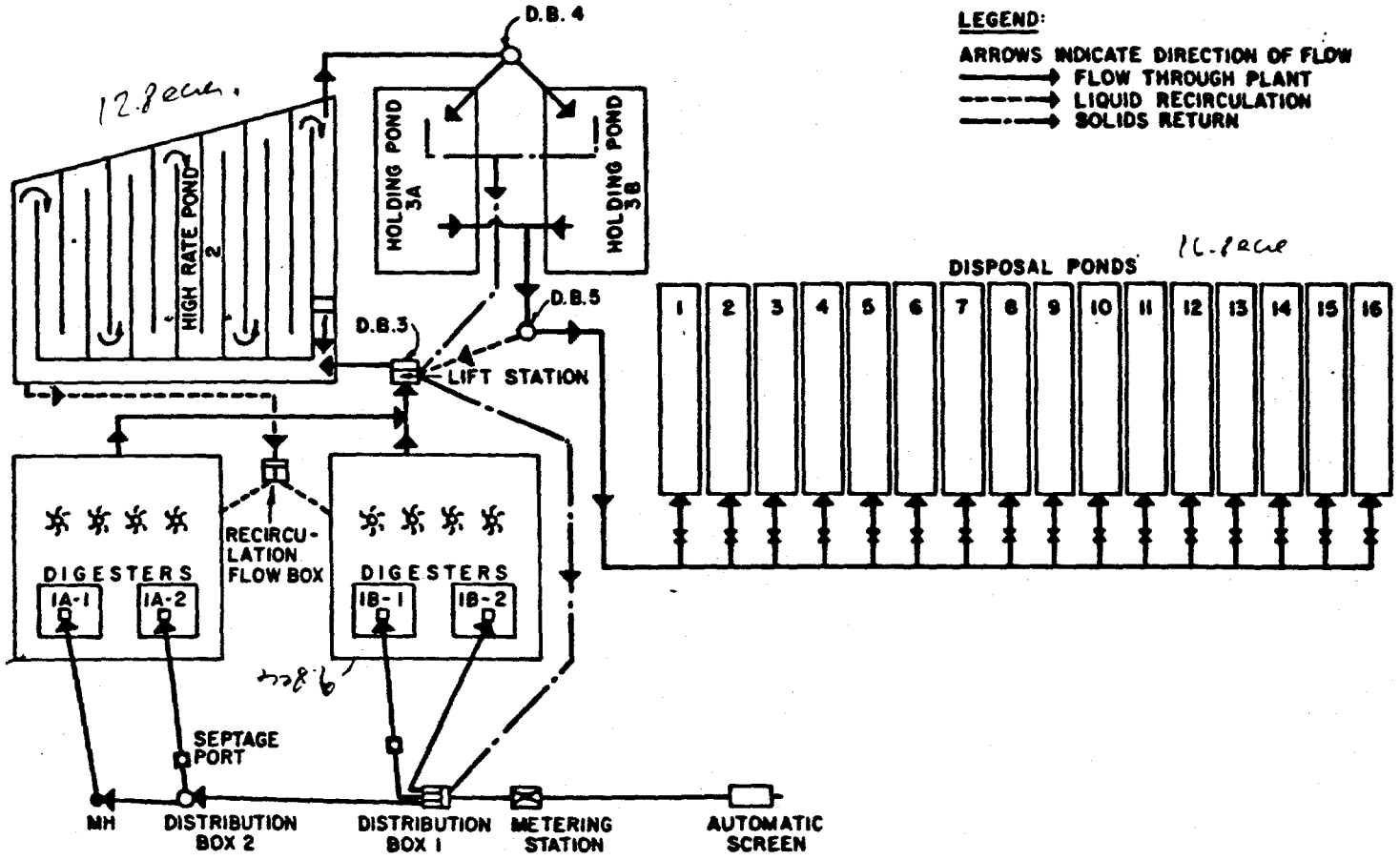
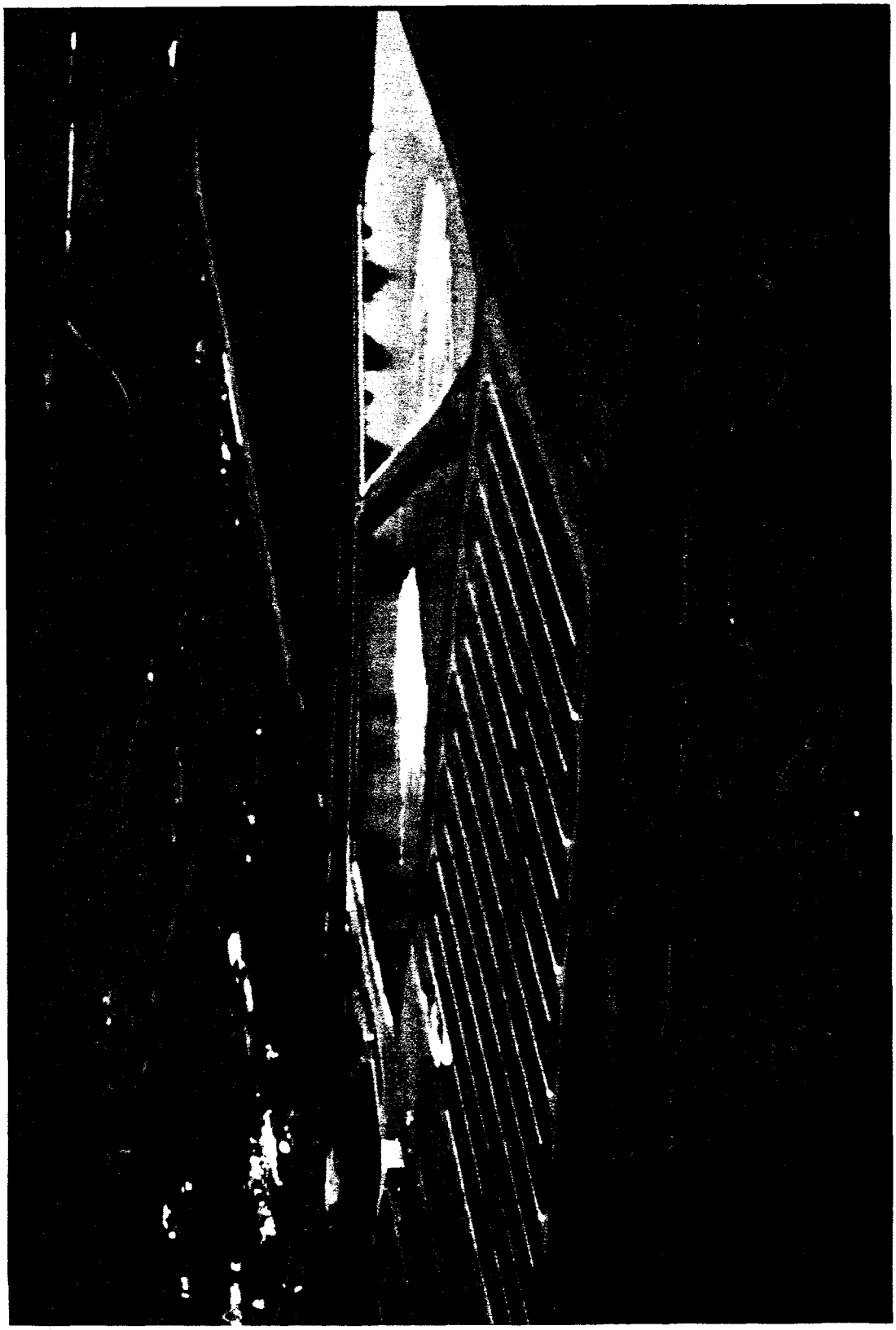


FIGURE 3.4. AERIAL PHOTOGRAPH OF HOLLISTER PONDS



percolation basins from slowly becoming clogged, resulting in lower infiltration rates. There is no data or analysis on which to base a assessment of this plant (e.g. the productivity of the high rate pond, for example). However, sufficient data appears to have been collected to possibly allow at least a preliminary assessment of the performance of this plant.

3.6. WASTEWATER TREATMENT WITH OXIDATION PONDS

The above brief examples of wastewater treatment systems does not present the full perspective of current applications and future potential of this important technology, both in the U.S. and abroad (see also Green et al., 1995 for a recent report on the potential energy savings and recovery from such plants). However, a brief overview of the potential of these systems in energy production and CO_2 mitigation is presented here. First a brief discussion of the wastewater treatment process is provided.

Waste water treatment (oxidation) ponds treat wastewaters by a combined action of:

1. Aerobic processes: aerobic bacteria break down wastes and release nutrients (CO_2, P, N, etc.) used by algae, which in turn provide the dissolved oxygen for the bacteria.

2. Anaerobic processes: the large amounts of suspended solids (sewage sludges, and also some algal biomass) that settle to the pond bottoms are broken down by anaerobic bacteria, with the release of methane, CO_2, sulfides, organic acids and other organic compounds, and various dissolved nutrients. Some of these are oxidized in the water column, and incorporated into algal biomass, others escape.

3. Photochemical and chemical processes: the relatively high oxygen tensions and (at least in the top few cm) light intensities (during daytime) result in some oxidation processes not commonly found in activated sludge systems. (The nature and extent of such reactions are, however, to a large extent speculative). One consequence of such reactions appears to be the relatively high disinfection rate of such ponds, although the pond effluents can in general not be considered disinfected under current regulations.

Design criteria for conventional oxidation ponds (e.g., the unmixed ponds that are the norm in such systems, e.g. Napa ponds, or Sunnyvale California) are based essentially on rules of thumb of so many kg of BOD (biological oxygen demand) per hectare, adjusted for local climatic conditions (e.g. latitude, temperatures, rainfall, etc.). More detailed design equations, based on fundamental principles, have been developed for the "advanced integrated ponding" systems designed by

Oswald (1990), but these have been slow to be adopted by the engineering community, in large part due to a lack of familiarity with such systems.

The unmanaged nature of the conventional facultative oxidation ponds and their generally poor hydraulics (e.g. large dispersion coefficients), make such system unpredictable in such basic performance aspects as suspended solids outputs. Algal biomass, estimated at about 80% of the total suspended solids discharged by such systems, can vary by a factor of ten within a period of days, similar to the situation found in some eutrophic lakes. Variations can be due to sudden blooms of algae, or of rotifers or other algal grazers, settling of algae, or, often, unknown causes. Essentially, conventional oxidation ponds are extremely eutrophic systems influenced by similar factors as natural ecosystems. There is no control over algal populations, or productivity, and thus specific system performance is neither predictable nor optimized.

One consequence is that the systems are designed with a large safety factor. As costs are to a large extent land dependent (e.g. economics of scale are low), such large safety factors raises costs disproportionately for such systems, compared to processes such as activated sludge which can be better predicted in terms of performance (although, in practice, their performance is also often variable, and not nearly as stable and reliable as advertised).

However, the fundamental problem with these systems is the large amount of suspended solids (material recovered by 0.45 um filters, mostly algal biomass) in the effluents. This has two untoward effects:

1) It makes it difficult to use conventional disinfection (e.g. chlorination) because of the large amount of organic matter represented by these solids (nominally 100 mg/l, although this can vary over a large range); and

2) When the algal biomass is discharged into a receiving body of water the algae would, at a minimum, exert a proportional oxygen demand load, and at worst could upset the ecosystem.

Overall, discharge of algal solids is not desirable (although, of course, it is much preferable over the discharge of non-algal sewage solids, the norm with conventional waste water treatment systems). Thus, harvesting of the algal biomass from oxidation ponds is the preferred solution.

Harvesting is, however, expensive (Section 7). Centrifugation costs currently probably exceed $1,000 per million gallons and chemical flocculation, the method of choice, is only about half as expensive. The experience at both Sunnyvale and Napa with chemical flocculation has not been recorded in the literature, but it must be considered generally less than optimal. This has been in large part due to the choice of flocculants, and the difficulties of harvesting what is essentially an

uncontrolled algal population. In the case of Sunnyvale, harvesting has been made difficult by the relatively small size of the dominant algae in these ponds. The literature records dozens of "solutions" to the problem of algal harvesting from oxidation ponds. None have succeeded in the marketplace, thus far.

However, the introduction in the past decade of organic flocculants (cationic polyelectrolytes) to replace, at least partially, the inorganic ones (lime, alum, ferric chloride), along with improved dissolved air floatation processes, have greatly improved overall performance, and economics, of such harvesting systems, and could revolutionize this field. The subject of microalgae harvesting is further addressed in Section 7.

Alternatives to harvesting include discharge during high flow of the receiving stream (e.g. strategic dilution), while holding the effluent in the ponds during non-discharge periods (such as practiced in part in St. Helena and Napa); overland flow or irrigation (practiced in St. Helena and Napa), ground water recharge (Hollister), and even terminal evaporation systems. One method is to discharge through a final series of ponds in which a cover of water hyacinths provides a dark, quiescent environment in which the algae will settle. Water hyacinths, however, grow only in rather warm climates and also themselves become a harvesting and disposal problem. Other solutions used are sand filters, rock filters, trickling filters, and similar systems, which, however, are only partially effective.

Although these solutions can be applied in local situations, most have technical limitations, and are not generally applicable. And in general they increase land area requirements. Perhaps most important, without recovery (harvesting) of the algal biomass neither the inherent values of this material (energy, plant nutrients) can be recovered nor the water easily re-used for most applications.

Although algae harvesting is the crux of this field, the high costs and energy inputs of activated sludge systems, compared to algae systems (Green et al., 1995), assures that even without a completely satisfactory solution to this problem, such systems could find wide-spread applications in the U.S. (and even more widely in many other countries), wherever local situations allow water re-use (irrigation, recharge) or discharge, land is not limiting, and other factors permit the application of any of the above cited harvesting alternatives. Even partial harvesting process could greatly enhance this technology if it improved the resulting effluent sufficiently to meet local needs and circumstances.

However, the ultimate goal of microalgae waste water treatment is to achieve the same, even better, performance criteria than conventional technologies, at lower costs, and with minimal land use. As discussed next, such improved performance could be best achieved through the application of the AIPS (Advanced Integrated Ponding System) technology developed at U.C. Berkeley (Oswald, 1988, 1999; Green et al., 1995), along with CO_2 supplementation and algal harvesting.

3.7. MICROALGAE WASTEWATER TREATMENT AND CO_2 UTILIZATION

The solution to the problems of conventional oxidation ponds - the large amount of land used, the uncontrolled nature of the waste treatment process, the difficulties of removing algal solids from the effluents - is to make the algal treatment system more intensive and predictable. This must involve, first, better hydraulic mixing, to achieve a more uniform and predictable pond environment. For this purpose the high rate (raceway) pond, pioneered by Oswald, is the most appropriate. The combination of this concept with a primary pond, for waste solids deposition, decomposition, and gas recovery, and final "maturation" ponds, for algal settling and effluent solids reduction, provides the equivalent of a conventional waste treatment system (primary and secondary treatment), at a fraction of the cost and energy inputs of conventional systems (Green et al., 1995). The AIPS process was applied at St. Helena and Hollister, which demonstrate the basic technology.

However, significant improvements in this technology are still possible, and desirable. The methane emissions from primary ponds, originating from the settled sewage solids, are both a lost resource and a significant GhG emission from such systems. Recent work at the University of California Berkeley (Green et al., 1995) has demonstrated an innovative concept for the capture of the methane gas from such ponds through use of a submerged plastic shroud, serving as a gas catcher. The optimization of the solids removal and digestion function, and maximization of gas recovery, would be achieved by reducing the size of these primary ponds to a minimum, lowering overall land use and costs of such systems.

The final component of the advanced integrated ponding system of Oswald, the algal settling, maturation, and effluent storage ponds (as used in St. Helena and, to a lesser extent, Hollister) can also be minimized if most of the algal solids are removed (harvested) from the effluents of the high rate ponds, prior to discharge to the maturation ponds. The issue of algal harvesting was mentioned above, and is reviewed in detail in Section 7. In essence, two harvesting processes appear attractive at present: algal flocculation (by a combination of inorganic and organic flocculants) followed by dissolved air flotation (DAF), and "bioflocculation" - the spontaneous settling of the algal biomass.

Bioflocculation is of lower cost, but, currently at least, a less reliable process, than flocculation-DAF. Thus, in recent work at Berkeley, only about half of the effluent solids from the oxidation ponds could be removed by simple settling (Bailey et al., 1995). However, in prior work (Benemann et al., 1978, 1980; Eisenberg et al., 1980) with these same ponds, operating at shorter retention times and with higher productivities, overall solids removal of nearly 90% were demonstrated on a year-round basis (albeit not in the same pond). The major parameter determining settleability of the pond culture was hydraulic retention time (which, as the ponds were held at constant depth, was equivalent to aerial loading rate). (See Table 4.1, next section).

Most effective in promoting algal settling is the growth of the algae to a point of N limitation through CO_2 supplementation (Benemann et al., 1978, Eisenberg, et al., 1980). The requirement for CO_2 supplementation is due to the fact that municipal wastewaters are deficient in C, in relation to their N and P content, and the requirements of algal growth. Thus, typically, based on composition and bioassays, the amount of C available for algal growth in wastewater treatment is less than half of that required for growth to the point of N limitation (typically about 5 to 6% of algal biomass organic dry weight).

CO_2 supplementation in high rate pond waste water treatment would thus, roughly, double overall biomass production by such systems. In principle this would require doubling the pond system size, with a proportional increase in costs. However, CO_2 supplementation would also significantly increase productivities, as it would alleviate CO_2 limitations, during sunlight hours, as a major factor in limiting productivities in wastewater treatment ponds (as evidenced by the high pH in such ponds), offsetting at least a large part of the need for more land.

Also, the reduction in nutrients in the effluents (essentially complete removal of available N sources, and at least a 50%, and likely 70 - 80%, reduction in P levels), achievable by CO_2 supplementation, would greatly improve the performance of the waste treatment ponds. This level of nutrient removal (tertiary treatment) would be much superior to the currently achieved nutrient removal levels in even advance conventional wastewater treatment processes (e.g. activated sludge). Although the removal of nutrients is not yet a general requirement, in many local conditions nutrient removal is mandated, and such mandates can be expected to increase in the future, as the effects of nutrient pollution of the environment is becoming ever more apparent and less tolerable, locally, regionally, even globally. Thus, nutrient removal is an important beneficial aspect of wastewater treatment.

Finally, in the connection of CO_2 utilization and fuel production, growth of the algae to the point of N limitation can be used to produce an algal biomass very high in lipids (Benemann and Tillett, 1987), useful as a liquid fuel precursor (for biodiesel). And, as prior work has demonstrated, high lipid production could be accomplished at high total biomass (CO_2 sequestration) productivity (Section 6). And finally, a most important effect of CO_2 supplementation, and cultivation of the algae to the point of nitrogen limitation, is the greatly improved settleability and, thus, harvestability, of the algal biomass cultivated under such conditions, as already mentioned above.

In conclusion, the use of microalgae wastewater treatment systems would be a likely initial application for CO_2 utilization, and is recommended as a near-term R&D objective (Section 10). The major advantage of such a scheme is, of course, that most of the costs of biomass production and energy recovery would be covered by the wastewater treatment function, allowing cost-effective systems at a relatively small scale, providing a starting point for commercial applications.

4. ALGAL MASS CULTURE SYSTEMS

4.1 HISTORICAL OVERVIEW

4.1. Introduction

As mentioned in Section 1, early work in microalgae production began after WW II with U.S.-sponsored work in the United States, Germany, and Japan, with the goal of producing low-cost food for a rapidly growing global population. In Algal Culture: From Laboratory to Pilot Plant, (Burlew, 1953) the researchers addressed many of the fundamental aspects of microalgae production most of which are still the subject of much R&D today, CO_2 supply, temperature and species control, algal harvesting, the inefficient use of incident light due to high intracellular pigment content, and perhaps most important, the design of the culture systems for the production of microalgae - what we today call photobioreactors. In that book the major issue that divides researchers in this field today was already clearly laid out: open and closed culture vessels. This is a major theme in this section.

However, perhaps Cook, 1949, deserves the credit for conceptualizing, and actually building and testing, the first larger (past the laboratory scale) algal culture systems, with vessels that included some of the fundamental design issues of any photobioreactor: recirculation and mixing, shallow depth, and gas exchange.

After this early effort, U.S. interest in microalgae as a human food source diminished. During the 1960s, research focused on wastewater treatment, using open raceway ponds (Oswald et al., 1956) and atmosphere regeneration for space applications, using highly controlled enclosed photobioreactors (Krauss, 1959). European work on cultivation of microalgae continued in Germany during the 1960's, leading to the introduction of paddle wheel mixed paddle wheel ponds, and continued through the 1970's (Soeder, 1978). In Czechoslovakia a unique photobioreactor was developed which involved a shallow inclined trough (Setlik, 1966, 1968). The major commercial development during this period was in Japan, where Chlorella production in circular ponds was developed into a $100 million/year retail market by the 1970's. (See discussion above).

During the 1970's microalgae cultivation in the U.S. emphasized the production of fuels from microalgae, using the raceway, paddle wheel mixed, pond designs (Benemann, 1978, 1980). In 1979 the Aquatic Species Program was established at the Solar Energy Research Institute (SERI, now NERL). The early emphasis of this program was on enclosed photobioreactors. However, the limitations of that approach (Benemann, 1982), led the program to shift to the open, paddle wheel mixed, raceway pond design. This design has became the industry standard used by all three commercial production system established in the U.S. (Section 3).

Recently, photobioreactor R&D outside of the U.S., has emphasized closed photobioreactors, including tubular and flat plate designs. Thus the field of photobioreactor design is still very much in flux. In this Section we review open photobioreactor designs and experience. Closed (tubular, flat plate, etc.) systems are addressed briefly below, Section 4.3.7, as they are of some interest in the production of inoculum for larger-scale, open pond, systems (Section 9). However, the emphasis is on open pond algal mass culture systems.

4.1.2. Early Algal Mass Culture R&D at the University of California Berkeley.

The work described in Burlew (1953) dealt mostly with closed photobioreactors. At the same time, Oswald and colleagues at the University of California Berkeley pioneered the development of large-scale algal production systems using open ponds for wastewater treatment (Gotaas et al., 1953). As discussed in the previous section, in wastewater treatment, the algae generate the O_2 needed by bacteria for the degradation of organic material, and consume the CO_2 and inorganic nutrients produced by the bacteria. By harvesting of the algae, a biomass potentially suitable for animal feed or methane production is produced, which could help defray some of the costs of wastewater treatment. After working with 50 and 200 m^2 raceway ponds in early 1950's, a 10^6L (ca. 2700-m2 surface area) outdoor raceway pond was built in Richmond, California, in 1960. That pond had four meandering channels mixed by three low head pumps. The ponds were fed settled sewage. Biomass productivities were typically 12 to 18 g m^{-2} d^{-1} (Oswald, 1969). This design formed the basis for the "high rate pond" designs used in the "Advanced Integrated Pond Systems" for municipal waste treatment discussed above (Section 3). Several techniques were investigated for harvesting the algae: centrifugation, chemical flocculation, "autoflocculation", etc. (Golueke and Oswald, 1969), however none proved to be both reliable and inexpensive. These studies provided the basis for similar work in other countries, such as in Israel (Shelef, 1980).

During the late 1960's a study was carried out to remove nitrate from agricultural drainage waters in the San Joaquin Valley in California with microalgae culture. One 100-m^2 and 22 smaller (2.6-m^2) raceway ponds, were constructed. P, Fe, and CO_2 were added to support growth of Scenedesmus. Short-term (10 d) yields of 30 g m^{-2} d^{-1} and long-term (70 d) yields of approximately 10 g m^{-2} d^{-1} were reported for the larger pond (Beck et al., 1969; Goldman et al., 1969; Brown 1971). A proposal to build a system of over 700 Ha to treat over a billion liters/day was never implemented because of the cost of algal harvesting and problems of culture management. Recent Se contamination problems with this drainage water have led to a study of microalgae treatment of this contaminant (Lundquist et al., 1994).

During the 1970's the single pond at Richmond was divided into two 1,000 m^2 paddle wheel mixed raceway ponds (Benemann et al., 1977). The major objective of the research was to develop a low-cost harvesting based on bioflocculation, the

spontaneous settling of algae after removal from the growth ponds. Considerable progress was made, with the demonstration that within a certain range of dilution rates (or loading rates), which varied seasonally, the algae exhibited very high settling. However, further research is required to support and apply these findings (See further discussion in Section 4.2., below).

4.1.3. Marine Microalgae Culture in the U.S.

Marine microalgae were first mass cultured in the U.S. at the Woods Hole Oceanographic Institute in Massachusetts (Ryther et al., 1975). The objective was to grow algae on mixtures of seawater and treated municipal wastewater and thus remove residual nutrients from the wastewaters, and use the microalgae as a food source for shellfish. This bypasses the costly and difficult harvesting processes. The photobioreactors included two intensively mixed 4 m^2 circular ponds, and six unmixed 150 m^2 ponds. Yields over a 6 to 18 months period, for several marine diatoms, varied from 12 to 23 g m^{-2} d^{-1} in the small ponds, but only half as much in larger ponds, suggesting a correlation between productivity and mixing velocities (Goldman, 1979). Another observation was that it was not possible to control algal species, at least in the larger ponds. The dominant species Phaedactylum tricornutum, was not a good food source for the bivalves. Dominance was variously attributed to temperature or CO_2 limitations (Goldman et al., 1977).

During 1972 to 1976, in St. Croix, Virgin Islands, Roels and colleagues investigated the culture of marine algae for aquaculture applications. The photobioreactors consisted of two 45,000 l and six 2,000 l 2 m deep unmixed, rectangular concrete ponds. The growth medium was ocean water pumped from a depth of about 870 m, presumed to contain no contaminating algae but sufficient N and P. However, N was limiting, and the cultures were probably not light limited. Yields in experiments of less than 40-d duration were below 10 g m^{-2} d^{-1}, and difficulty was encountered in trying to maintain continuous cultures for more than 6 weeks. (Roels et al., 1977, Goldstein et al., 1998).

4.1.4. Development of Open Culture Designs in Japan, 1950's to 1970's

In 1953 the first outdoor Chlorella mass culture experiments were carried out in Japan, using 15 m^2 concrete-lined channels. After several years of testing, yields (in summer) were reported to be 16 to 17 g m^{-2} d^{-1}, with peak productivities of 28 g m^{-2} d^{-1}. One major focus of this early work was on improving the photobioreactor designs, in particular mixing. Two major pond configurations were developed: 1. The "bubbling culture" method, with either flat or corrugated-bottomed troughs with longitudinally placed aeration tubes for bubbling CO2/air, (Morimura et al., 1955); and 2. The open circulation method, which used a circular pond equipped with a pair of radial pipes that rotated around

the center and recirculated the algal medium by pumping through small openings in the rotating arms (Kanazwwa et al., 1958).

By the late 1950's the Japan Nutrition Association operated a plant for the mass culture of Chlorella, with a total of 3200 m2 of ponds (with the largest being 314 m2). An additional 4000-m^2 facility was built by the Microalgae Research Institute at Kunitachimachi. Initially only low yields were reported about 4 g m^{-2} d^{-1}, in summer and less than 1 g m^{-2} d^{-1} winter. In the early 1960s, the focus changed from research to health food production in response to the growing market for Chlorella and several commercial plants were established, using the circular pond design. Annual average production achieved by the commercial plants ranged from 8.5 g m^{-2} d^{-1} to 21 g m^{-2} d^{-1}. The higher productivities were attributed in part to mixotropic growth from the addition of carbon sources such as acetate and molasses. But these also increased production costs. Production was expanded to Taiwan by the late 1960's (Section 2.2).

4.1.5. The German Microalgae Mass Culture Program, 1950 - 1980.

Interest in algal mass culture began in Germany even during WW II, when Harder and Witsch (1942) investigated the possibility of fat production from diatoms, and the production of algae for beta-carotene (to improve night vision of flyers) was suggested. Work with cultures of Chlorella was continued after the war at the German Kohlenbiologische Forschungsstation at Dortmund. Early photobioreactor designs used the circular systems developed in Japan, but by the early 1960's these were replaced with the raceway system of Oswald, incorporating a paddle wheel for mixing. This simple system has since been adopted by most (though not all) large-scale microalgae production facilities.

The focus of the German effort was protein production. Cultures were grown in artificial media. Due to the inability to maintain a pure culture of Chlorella, the process switched to Scenedesmus and Coelastrum, probably algal strains that become naturally dominant in the ponds. Productivities were between 10 and 30 g m^{-2} d^{-1} during the summer. Harvesting of the algae was accomplished by centrifugation and the biomass then dried. The German Government sponsored several foreign aid projects, in India, Thailand, Egypt, and Peru, as well as a collaborative project with Israel, to transfer this protein production technology to Developing Countries. In Bangkok, a facility consisting of seven 87-m2 and three 25-m2 raceway ponds was built. Short-term yields were reported to be 35 g m-2 d-1 for Scenedesmus and 20 g m^{-2} d^{-1} for Spirulina at this site. Even larger ponds were built in Peru and India (See Benemann, 1987 for a review). However, these projects were terminated when it was realized that the cost of microalgae production by this technology exceeded that of conventional foods many fold, and that a technology depended on expensive centrifuges, among others, was not appropriate for even industrialized countries, let alone developing ones.

4.1.6. The Inclined Tray Photobioreactor Developed in Czechoslovakia

Algal mass culture work in Czechoslovakia began in the early 1960's at the Laboratory for Algal Culture at Trebon. The photobioreactors developed at this site used inclined plates or trays, over which the algal culture flows. Shallow troughs in the trays catch the culture and provide storage and additional mixing. The overall result is a very thin (appx. 1 cm) and well mixed culture systems, that results in very high algal densities. Recirculation was accomplished by pumping the culture from the lowest tray to the highest tray, with the culture flowing by gravity back to the lowest tray. In Trebon and Kosice, this design was used for Scenedesmus from 1960 to 1962, giving average summer yields of 15 g m^{-2} d^{-1} (Setlik et al., 1977). Two 50-m^2 units and one 900 m^2 unit were built by the end of 1963, modifying the tilted surface design by using glass sheets with glass baffles to increase mixing. Operation of these units continued during the summer/autumn seasons from 1963 to 1970. Units with 50 m^2 surface areas also operated in Tylicz, Poland in 1966, and in Rupite, Rumania, in 1968. Yields for these 50 m^2 growth units were 16 to 25 g m^{-2} d^{-1} in Trebon and 22 to 30 g m^{-2} d-1 in Rupite. The 900-m^2 units in Trebon gave lower yields (12- 17 g m^{-2} d^{-1}). A 5,000 m^2 system was built in Bulgaria in during the 1970's. These systems were still operating recently and the system in Bulgaria is reported to now be producing Chlorella commercially for export. Thus this can be considered a commercial process.

4.1.7. Historical Development of Spirulina and Dunaliella Production Systems

During the late 1960's, the blue-green alga Spirulina started receiving attention as a potential food source, after a report from a French anthropological expedition that this alga served as a local food source near Lake Chad. With funding from the French Petroleum Institute it was quickly established that at high concentrations of bicarbonate (> 15 g/l) this alga easily dominates the cultures. The Spirulina culture system developed by this group was a raceway pond with air lift mixing. The design consists of two parallel, horizontal troughs (10 or 20 cm in depth), connected at each end to deeper channels, where CO_2 is introduced, which also promotes circulation. During the early 1970's experimental and pilot scale facilities were established in France, Algeria, Egypt and Martinique.

However, the first commercial production of Spirulina took place in Mexico City, where a large carbonate evaporation pond proved ideal site for low-cost Spirulina production (Castillan, 1975; Durant Chastel, 1980). However, all other commercial Spirulina production system use the raceway pond design with paddle wheel mixing. The second commercial Spirulina production system was established during the late 1970's in Thailand, and several of the Chlorella manufacturers in Japan and Taiwan also started to produce Spirulina, alongside Chlorella, during this period, albeit at a small scale. (In some cases they have used paddle wheel mixed ponds, but of small unit size and build of concrete, which is expensive).

However, it was in the U.S. that Spirulina production systems have achieved the greatest development, with two facilities, in S. California (Earthrise Farms and Hawaii (Cyanotech) (Section 2), each accounting for about one third of world production. The remainder is scattered over a score of plants, from Cuba and India to China and Thailand. High demand for this alga has resulted in many new plants, and the doubling in capacity over the past two years of the two U.S. plants.

The development of Dunaliella production technology, just as for Spirulina, also required almost two decades, from inception to commercial success. Work during the 1970's in Israel (Avron and Ben Amotz, 1980) was not successfully commercialized at that time, due to both the technical problem of the harvesting of the algae and organizational problems. It was in the U.S. and Australia where the production of beta-carotene from Dunaliella was first developed commercially during the 1980's. In the U.S. the process used paddle wheel mixed ponds, while in Australia unmixed ponds were used (Section 2). The harvesting and processing system, however, was essentially identical, involving chemical (alum, ferric chloride, etc.) flocculation and dissolved air flotation, followed by a hot oil extraction, with three phase centrifuges. The latter process is of interest, as it suggests a method for extracting lipids from microalgae (Section 8).

Although the U.S. (Microbio Resources, Inc.) and Australian (Western Biotech, Betatene) companies started operations by the early 1980's, none was commercially successful until relatively recently, a decade after the initial start. Indeed, one company, Microbio Resources, was forced to sell its production plant (to Nutrilite, Co, a subsidiary of Amway, which now is successfully operating the plant - Microbio Resources itself recently ceased operations), and another (Western Biotech) changed ownerships several times (including twice belonging to Hoffman La Roche, which initially started this venture). A recently established plant in Israel, NBT, proved successful rather quickly, no doubt helped by the experience developed by the other companies, in particular Microbio Resources.

The history of the development of these commercial enterprises demonstrates that a rather long time can elapse between the pilot plant and commercial success, during which large amounts of cash are consumed, and returns to investors are meager, or even negative. However, eventual rewards can also be high: the current stock market valuation of Cyanotech is over $100 million, and Betatene was recently sold by its Australian owners (a mining conglomerate) to Henkel Corp. based on a valuation of about $60 million. Thus, clearly, microalgae can now be considered a significant business opportunity, at least in the specialty markets.

However, there has been little progress towards the production of larger-scale, low cost, microalgae products. Indeed, prices for microalgae have been increasing in the recent years. However, the thesis of this report is that there are no fundamental obstacles to larger-scale lower-cost microalgae production systems and products. This thesis is also the basis for the DOE support R&D in this field, reviewed next.

4.2. R&D PROGRAMS FOR FUELS AND CO_2 UTILIZATION

4.2,1. The DOE Microalgae Biofuels R&D Programs

Research on microalgae fuel production has been funded by the Federal Government since the early 1970's, initially through the National Science Foundation, Research Applied to National Needs Program. Although most of the early projects concentrated on microalgae hydrogen production, one project was funded at the University of California Berkeley to examine the potential for microalgae as a source of methane (Uziel et al., 1975). Subsequently this program was transferred to ERDA (Energy Research & Development Administration, the predecessor agency of DOE), which during the 1970's continued to fund research at the University of California for methane production by microalgae wastewater treatment systems (Benemann et al., 1976, 1977, 1978, 1979, 1980; Eisenberg et al., 1980). This research demonstrated the ability to grow microalgae in open ponds on wastewaters with a generally stable algal culture (Micractinium), and the ability to harvest these algae by a simple settling process Table 4.1. summarizes the data collected over one year with both ponds this system, demonstrating overall high removal efficiencies by simple sedimentation.

Other projects funded by ERDA/DOE included a review of microalgae technology (Goldman, 1980a,b), as well as an economic feasibility analysis of a conceptual large-scale process for microalgae production (Benemann et al., 1978). The latter was part of a broader evaluation of both micro-and macroalgal biomass energy systems (Dynatech, 1978). Although the results of that assessment were generally unfavorable to these energy sources, in the case of microalgae most of the con arguments (Goldman and Ryther, 1977), were countered by pro-arguments (Oswald and Benemann, 1977), and the microalgae R&D continued to be supported. About that time this research along with other elements of the ERDA/DOE Fuels from Biomass Program was transferred to SERI (now NREL), where since 1979 large-scale microalgae production for fuels (and, more recently, CO_2 mitigation) has been carried out through the Aquatic Species Program (ASP).

Initially the ASP aimed to demonstrate a photobioreactor design using very shallow (< 5 cm) ponds, high mixing (> 50 cm/sec) with air lift pumps, infrared sunscreens, and other elaborations (Raymond, 1979). However, the obvious limitations of such a system (Benemann, et al., 1982) resulted in the Program quickly evolving towards the development of the standard process (e.g. open raceway, paddle wheel mixed ponds). One innovation arising from the early work were the use of "foils", placed in the ponds to provide "organized mixing" (Laws et al., 1978, 1979, 1980). Although the incremental productivities due to such foils is controversial, the work in Hawaii demonstrated relatively high productivities, over 35 g m^{-2} d^{-1}. More recently Laws and Berning (1990), in an EPRI (Electric Power Research Institute) funded project, specifically tested the potential of such systems for flue gas CO_2 mitigation (see Section 4.3.3).

TABLE 4.1.
Summary of 0.1 Hectare High Rate Pond Operations during 1978 - 1979
(The two ponds were operated at variable detention times, depths, and mixing speeds, accounting for differences in productivity and harvestability.)
(Source: Benemann et al., 1978, 1979)

Date	West Pond			East Pond		
	Total Production $g/m^2/day$	24-hr Imhoff Cone* % removal	Harvestable Production $g/m^2/day$	Total Production $g/m^2/day$	24-hr Imhoff Cone* % removal	Harvestable Production $g/m^2/day$
Sept 78	25.5	92	23.5	8.0	85	6.8
Oct	25.5	89	22.7	11.3	71	8.0
Nov	11.6	27	3.1	9.8	83	8.1
Dec	4.7	70	3.3	6.6	64	4.2
Jan 79	4.9	85	4.2	4.7	56	2.6
Feb	6.4	82	5.2	9.3	74	6.9
Mar	8.5	81	6.9	16.5	74	12.2
Apr	15.8	76	12.0	16.2	53	816
May	20.1	88	17.7	21.3	74	15.8
Jun	22.6	91	20.6	20.2	91	18.4
Jul	22.0	92	20.2	35.5	89	31.6
Aug	21.7	88	19.1	35.6	94	33.5
Sep	19.9	94	18.7	35.5	87	30.9
Oct	16.3	84	13.7	27.8	69	19.2

* Imhoff cone removals indicate the percentage of algal biomass that will spontaneously flocculate and settle.

A detailed review of the ASP is beyond the scope of this report, although such a review would, perhaps, be appropriate. (The ASP was terminated in early 1996, reflecting the priorities of DOE under current budgetary constrictions). Since 1979 the ASP carried out a wide-ranging R&D effort. In addition to the work in Hawaii, the ASP early-on also supported a project in California, to demonstrate microalgae production on irrigation waters, using small-scale (200 m^2) outdoor ponds (Benemann et al., 1981; Weissman and Goeble, 1983, 1985; Weissman et al., 1987). The ASP also supported university R&D on screening algae for high productivity, lipids, isolation of algal strains from brackish water resources, and related subjects. Staring in the early 1980's, SERI build-up an in-house R&D team that carried out a major effort in establishing a culture collection of strains, screening these for suitable properties (fast growth, high lipid content), and a genetics program with emphasis on lipid biochemistry. The ASP review meetings reports record the progress of the ASP (SERI, 1981 to 1989, NREL, 1990, 1992, 1993).

One major scale-up effort was supported by the ASP during the late 1980's: Microbial Products, Inc., designed (Weissman and Goebel, 1987), constructed (Weissman et al., 1988) and operated (Weissman et al., 1989, Weissman and Tillett 1990) several small (appx. 2 m^2) and two large (0.1 ha) raceway, paddle wheel mixed, ponds at Roswell, New Mexico. One of the large ponds was lined with plastic and the other had a dirt bottom. No significant differences were noted between the ponds in terms of productivity, which were about 35 g m^{-2} d^{-1}, with peak productivities exceeding 50 g m^{-2} d^{-1}, during the main growing season (at this site only about 200 days). A major advance was the cultivation of algae that could be potentially used in aquaculture. Indeed, this project led to the establishment of a private company, SeaAg, Inc., located in Florida, for the mass culture of microalgae for the aquacultural production of bivalves (Benemann, 1992).

Over the past several years, the ASP has emphasized the possibility of CO_2 mitigation with microalgae cultures producing biodiesel (Brown and Zeiler, 1993, Chelf et al., 1993), including a PETC funded program on direct flue gas utilization. Work included a feasibility analysis (Kadam, 1994, 1995) and resource assessment for a specific power plant (Kadam, 1994). Earlier resource assessments emphasized the saline water, land, and CO_2 resources in the arid South West (Vigon et al., 1982; Maxwell et al., 1984, Feinberg and Karpurk, 1985), concluding, perhaps somewhat optimistically, that sufficient brackish water resources were available, along with land and CO_2, to supply a large fraction (> 10%) of U.S. energy needs.

A major accomplishment of the ASP has been the ground-breaking work on the genetics of lipid biosynthesis and production by microalgae (Dunahay et al., 1995; Roessler and Ohlrogge, 1993; Schneider and Roessler, 1994; for some recent references). This research could form the basis for future applications of this technology to not only fuels (biodiesel) but also higher value products. Along with the demonstration at Roswell, New Mexico, of the feasibility of large-scale cultivation of such algae, this is likely to be the enduring legacy of this Program.

4.2.2. The Japanese Programs for Microalgae CO_2 Utilization

A large R&D program on CO_2 mitigation technologies was initiated in the early 1990's in Japan by MITI (Ministry for Trade and Industry), which has an industrial policy for Japan to become the leader in greenhouse gas mitigation technologies, including biological systems (Myers, 1992). The microalgae program is being carried out by the Research Institute for Innovative Technology (RITE), with participation of over a dozen companies, as well as universities and Government laboratories. The overall budget is estimated at probably close to $10 million per year and Myers (1992) reported that a major goal was to having a test plant running by the end of the decade within a decade at a total cost of $123 million.

However, the approach of the Japanese program is radically different from that of the U.S.: rather than open ponds, the applied R&D is concentrating on photobioreactors using optical fibers. Such reactors have the potential for overcoming the light saturation effect (see Section 5), by diffusing light throughout the culture. Indeed, Nishikawa et al. (1992) achieved high productivities at high light intensities. However, clearly, such reactors are much too expensive for most applications, particularly for low value products, such as fuels and CO_2 mitigation. suggesting that the Japanese programs are aiming to develop higher value products.

In addition to work on photobioreactors, the RITE program is supporting R&D on, among others, algae that grow at high CO_2 tensions or precipitate $CaCO_3$. Although neither is of direct relevance to CO_2 mitigation (high CO_2 in algal ponds would result in excessive outgassing, $CaCO_3$ precipitation actually releases CO_2), there is clearly a commitment to long-term fundamental research. Indeed, the emphasis seems to be on higher value products (bioplastics, for example) from microalgae. This fits with the emphasis on complex photobioreactor R&D: the aim may well be to develop production technology for high value commercial products in the near-term, to bolster Japanese industry, with CO_2 utilization a longer-term goal. However, this is not an explicitly stated strategy of this $12 million/y program.

In addition to the RITE program, the electric utility industry in Japan is also actively involved in research in this field. Indeed, a project by the Tohoku Electric Power Co., in collaboration with Mitsubishi Heavy Industries, has carried out a project at Sendai City that was based on the U.S. work, by using strains from the NREL culture collection. After studying the effects of flue gas on algal cultures in the laboratory this project tested the process in small (2 m^2) paddle wheel mixed, outdoor ponds (Negoro et al. (1991, 1992a,b, Hamasaki et al., 199). No significant effects of the flue gas were noted compared to controls grown on pure CO_2. Stable pond operations with reasonable productivities were reported over one year, with a strain that spontaneously appeared and dominated the culture.

In conclusion, the Japanese Government and industry are committed to the long-term development of this technology, contrasting with current U.S. priorities.

4.2.3. Other Programs and Projects on CO_2 Utilization with Microalgae

In contrast to the extensive R&D effort in Japan, and, until recently, the U.S., there has been essentially no work in Europe in this field. Some interest has been expressed recently by a large utility in South Africa (Eskom), which is planning to study microalgae for CO_2 fixation and lipids production.

In the U.S. a study of microalgae for CO_2 utilization was supported by EPRI (Electric Power Research Institute) in Hawaii (Laws, 1990; Laws and Berning, 1991). This project used seawater (from a deep upwelling pipe) for the cultivation of the marine alga Tetraselmis suecica in four 25 m^2 outdoor ponds. The ponds were operated at 20 cm depth and 75% of the culture was removed every other day, being replaced with fresh seawater and nutrients. Pure CO_2 was supplied as required to maintain a pH of 7.5. The study included a comparison of paddle wheels with Archimedes screws and propellers; they were all similar in power inputs. Foils placed into the pond to stimulate turbulence increased productivities (see also Laws et al., 1983, 1986, 1988). Productivities were generally lower than in prior studies by this group, about 20 g biomass ash free dry weight/m^2/day, possibly due to high respiratory losses. Laws (1990) concluded that seaweeds were favored over microalgae, as they had somewhat higher solar conversion efficiencies and are easier to harvest. However, energy inputs in seaweed culture are likely to be high and most literature data suggests that seaweeds are less productive than microalgae. Laws (1990) calculated that 20,000 ha (200 km^2 would be required to assimilate all the CO_2 from a 1,000 MW power plant. He asked "given this large requirement for land, is the idea of converting CO_2 to biomass via photosynthesis worth pursuing?". However, such negative conclusions may be premature because of the conservative assumptions (2.5% solar conversion efficiency) used. However, this dramatizes the need for very high productivities and large land areas.

At Oak Ridge National Laboratory, Woodward et al. (1992) have studied, Cyanidium caldarium, isolated from Yellowstone National Park, which is able to grow at high temperatures and very low pH (1M sulfuric acid!). They argue that such an alga would be able to withstand the very low pH that would be generated in a culture due to SOx absorption. They were able to demonstrate growth and oxygen production (evidence of CO_2 fixation) in a simulated flue gas containing 440 ppm SOx.

The Pittsburgh Energy Technology Center also has sponsored R&D in this field, through the NREL-ASP, as discussed above, through some in-house research, and by sponsoring the present Report.

However, overall, this field of research has attracted relatively little attention, when compared to other options for greenhouse gas mitigation. The present report addresses the potentials, and limitations, of this approach, and the rational for further work in this area. First the fundamentals of this technology are addressed.

4.3. PHOTOBIOREACTOR FUNDAMENTALS

4.3.1. Alternative Open Photobioreactors

The above cursory overview of the history of algal mass cultures demonstrates the variety of open photobioreactors that have been developed for commercial use over the years, from the simplest (large, unmixed ponds) to the complex, such as the Czechoslovak system. The former are inexpensive, but exhibit low productivity, the inability to control even simple parameters like CO_2 and nutrient addition, and low algal concentrations in the effluents. The latter are expensive, both capital and operating, and, have other limitations, such as low CO_2 utilization efficiency.

The objectives and applications of such systems are equally diverse, ranging from the small-scale production of very high value products, to the large-scale and very low cost culture of algae for wastewater treatment. Thus, any comparison of photobioreactors, whether open or closed, or even a comparison of different designs within each category, must specify the application intended, and even the site specific circumstances.

Indeed, even a comparison among different open algal culture systems is not easy. It is striking that in the commercial production of microalgae, reviewed above, quite different production systems are often used even for the same alga. Thus, unmixed and raceway, paddle wheel mixed, ponds are both used in wastewater treatment, Spirulina cultivation, and Dunaliella production. In the case of Chlorella both circular and paddle wheel mixed ponds, as well as fermentation systems and the "Setlik", shallow inclined, production systems are used.

The explanation for the use of such diverse production systems for the same applications can be found in both their historical developments and specific local circumstances. In the cultivation of Spirulina, where the medium provides a large reservoir of CO_2, it is possible to cultivate with only occasional addition of CO_2. In the case of the Mexican plant, which produced on average only about 3 $g/m^2/day$ of biomass in its 33 ha pond, only atmospheric CO_2 supply is required. This is also the case for Dunaliella production in large salt evaporation ponds, where very low productivities (< 1 $g/m^2/day$) are obtained, and CO_2 supplementation is not practiced. Similarly, in wastewater treatment ponds, sufficient nutrients (CO_2, N, P, etc.) are derived from the wastewaters for normal needs. (Although, as argued in Section 3, CO_2 supplementation would greatly help improve the performance of such wastewater treatment systems).

Raceway, mixed, ponds exhibit much higher productivities than unmixed ponds, and productivity is often the deciding factor where land is at a premium, bicarbonate (for Spirulina production) or salt (Dunaliella) are not readily available and thus must be reused (recycled), and algal densities need to be maximized to reduce water handling, usage, and harvesting costs.

4.3.2. Raceway Paddle Wheel Mixed Ponds.

The paddle wheel mixed raceway pond has become the design of choice for algal mass culture in open ponds. Other mixing devices are possible, in particular air lifts that combine gas exchange with mixing function. However paddle wheels exhibit a combination of flexibility in ease of operations and reliability that is hard to better, even if costs were not lower than other mixing devices (e.g. air lift pumps). Some have claimed greatly reduced costs, both capital and operating, for alternative mixing devices. For example, a mixing board (a board that moves up and down a single channel by a chain device), was claimed to increase productivity and have a ten fold lower energy requirement than paddle wheels (Haussler, 1990). The former has not been demonstrated, and the latter was an error in calculation. Air lift pumps can be relatively efficient, and could be used for degassing the culture (e.g. lowering O^2 tensions), but are relatively inflexible, and of higher capital costs (Augenstein, 1987). Mechanical mixing systems (Archimides screws, mechanical pumps) suffer from similar problems.

Thus, paddle wheel mixing is the best choice for mixing such ponds, and, indeed, has been adopted by all large commercial algal production systems in recent years. It should be noted that the design of paddle wheels for pond mixing has not yet been fully optimized, with an eight bladed design being apparently the choice for larger ponds. Indeed, the design of large paddle wheels, is perhaps not as straightforward as it may appear at first glance, as the need to operate at greatly different loads (during start-up and after steady velocities have been achieved) requires optimization of several alternative design objectives, both in the paddle wheel itself and its drive trains and speed controllers.

It should be noted, that a single paddle wheel can mix a rather large pond. Indeed, the largest pond at Earthrise farms, a 5 ha pond in a long serpentine design (without plastic liner, it should be noted), is mixed by a single paddle wheel (See Figure 2.2). In some systems, such as at the Israeli ponds for beta-carotene production, two paddle wheels were originally installed for mixing appx. 0.4 ha ponds. However, more recently the system was modified and one of the paddle wheels removed (it being clearly excessive, if not actually counter-productive).

Another advantage of the paddle wheel mixed raceway design is that it can be easily scaled-up, from $1m^2$ to many thousands. By comparison, the circular ponds favored in early Chlorella production (and still used) are limited by hydraulic consideration (e.g. the speed of the central pivot) to between one and thousand m^2. Similarly, several alternative mixing systems, such as mixing boards and air lifts, are described in the literature but have not been scaled-up beyond a few m^2, and are not further discussed here). In conclusion, of all the open pond systems designs the paddle wheel mixed pond is the lowest cost technology, at least per unit of biomass output, and will be the only one further considered in this report.

4.3.3. Mixing Velocities: Power Consumption and Silt Suspension

Within the basic paddle wheel mixed raceway pond design, many variations are possible: lined vs. unlined ponds, depth of operations, mixing speed, use of "foils", etc. Experience and engineering realities, limit some these parameters (Weissman et al. 1987). For example, power consumption increases as a cube function of mixing velocities, resulting in prohibitive energy inputs and costs at higher (> 30 cm s^{-1}) mixing speeds for any project involving fuel production or CO_2 mitigation.

The actual power consumption for paddle wheels depends not only on mixing speed but also on pond depth (non-intuitively, deeper ponds require slightly less energy than shallower ones for mixing), bottom roughness, and, perhaps most critically, the mechanical efficiency of the paddle wheel (both in the motor and drive train), which can be as low as 10% in smaller systems and prototypes, but could achieve at between 30 and 40% for optimized designs. These issues are discussed in prior reports (Benemann et al., 1978, 1982, Weissman and Goebel, 1987; see also Green et al., 1995 for another recent discussion). A mixing power requirements, for a pond mixed at a moderate velocity of 15 cm/sec and a depth of 30 cm, with a train efficiency of 40%, was calculated to require about 1 horsepower per hectare, or about 18 kWhr/day/ha (Benemann et al., 1982).

As stated, this power consumption goes up rapidly with mixing speed, by roughly a cube power factor. As the design of large ponds requires optimization of many parameters, including mixing velocity (the higher the velocity the fewer carbonation/gas exchange stations need to be provided), it would be optimal to operate the ponds with variable mixing velocities (typically between 15 and 25 cm/sec), depending on the time of day, productivity, depth, etc.

Another constraint on mixing velocity is silt and sediment suspension. For the purposes of energy production and CO_2 utilization, even for wastewater treatment, lining of ponds with plastic would be too expensive. Actually, plastic lined ponds have their own problems as their very low roughness coefficient results in too laminar a flow at low mixing velocities, which may reduce productivities. However, unlined ponds suffer from other serious problems, such as the inability to clean the ponds (e.g. remove contaminating organisms after infection, as is currently practiced with Dunaliella and Chlorella ponds, and at least in some cases Spirulina ponds. However, the feasibility of large raceway, mixed, unlined ponds has been amply demonstrated at the full scale with wastewater treatment systems and in the case of Spirulina at the Earthrise Farms plant in S. California. Also, work at the pilot scale in the U.S. (Weissman and Tillett, 1990) has demonstrated the feasibility of using unlined, paddle wheel mixed ponds. However, from data by Vavoni (1977), it would appear that suspension of small particles would become a problem at velocities above about 25 cm/sec. Thus, both power consumption and sediment suspension sets this limit to the permissible mixing speeds in such unlined raceway ponds.

4.3.4. Mixing and Productivity.

One of the most enduring controversies in microalgae production is the effect of mixing on productivity, with many experts believing that there is a positive correlation between mixing speed and productivity. Indeed, some data suggests such a correlation, and the mechanism by which this phenomenon is explained, the need to move algal cells from the light to the dark at intervals to promote their growth, is plausible. For example, devices such as foils to mix the algal cultures such that individual cells are periodically exposed to light ("organize mixing") have been studied (Laws et al., 1977). However, such systems greatly complicate both pond construction and maintenance, including cleaning, as well as increase power consumption. More importantly, as mentioned above, the actual effect of such organized mixing on productivity has not been demonstrated.

Much of the impetus for the belief in a positive effect of mixing on productivity comes from the "flashing light" effect: brief, a few millisecond duration, flashes of high intensity light, followed by a several-fold longer period of darkness, does not reduce culture productivities from those observed under constant illumination (Kok, 1953). The explanation is rather straightforward: at high light intensities more photons are absorbed by the antenna chlorophylls (and other pigments) than can be used by the dark (CO_2 fixation) reactions; providing the light in brief flashes followed by a period of darkness allows these reactions to catch up, thus resulting in the same overall rate of CO_2 fixation (productivity) as under constant high light (and thereby increasing actual light conversion efficiencies, up to five-fold). However, this "flashing light effect", is not apparent with longer light/ dark period frequencies (hundreds of milliseconds or longer) typical in ponds.

Despite the literally scores, if not hundreds, of publications on this subject, beyond the scope of this report to discuss in detail, there is no actual experimental evidence that supports a correlation between light periodicity and productivity. Where increased mixing resulted in increased productivity, other factors, such as oxygen tensions, algal settling (a major issue), or nutrient limitations, not a light frequency effects, are likely causes. Weissman et al. (1988), did not observe any effect of mixing on productivity, over a range of appx. 2 to 50 cm/sec, when other conditions (e.g. CO_2 supply, oxygen tensions) were kept constant.

High oxygen tensions in ponds, can, and likely do, reduce productivities, and are a major problem in algal mass cultures that requires more research. However, faster mixing promotes not only O_2, but also CO_2 outgassing, which is counter-productive, as O_2 and CO_2 are competitors for the CO_2 fixation enzyme (RUBISCO). For the present it suffices to suggest that the carbonation system, which supplies CO_2 to the ponds, should be designed such as to also result in increased O_2 outgassing. However, in overall conclusion, there is little, if any benefit to be realized on productivity from increasing mixing velocities from the above specified 15 to 25 cm/sec in open ponds.

4.3.5. Other Engineering Design Issues of Open Ponds.

Engineering constraints, rather than biological necessities, set limits on other parameters also. For example, pond depth: below about 15 cm, the construction and operations of large pond systems (e.g. in terms of slope accuracy) becomes limiting, and wind fetch would make operations problematic for any large pond system (> 1 ha). Above 50 cm, the cost of construction becomes prohibitive for many applications, as does the higher cost of harvesting (as density is an inverse function of depth). Thus, in general, large (> 1 ha) raceway ponds would designed to be operated at between 20 to 30 cm depth, with 10 - 20 cm freeboard.

Besides depth and mixing, the provision of the required nutrients in amounts and a times which do not constrain the productivity of the culture, nor result in waste of that nutrient, requires consideration in engineering designs. The principal constituent of algal biomass, and most important nutrient, is carbon, supplied as CO_2 (See Benemann et al., 1982, for a discussion). In brief, CO^2 supply must be carried out at intervals in the raceway (unless a covered pond is used, not considered here). The spacing of the CO_2 supply stations will be determined by the depth of the pond, the mixing velocity, the alkalinity, the outgassing from the ponds, and the productivity of the culture. These are interactive factors. The actual method of CO_2 supply (transfer) can be based on either a sump or a covered (floating) surface contactor. Sumps are preferred, being relatively efficient and cost effective. A major constraint is CO_2 outgassing from the ponds, which is a complex function of many parameters, such as mixing velocity and pond roughness. Of course, while CO_2 outgassing is to be minimized, O_2 outgassing should be encouraged. An optimization is difficult, but not impossible. Overall, the algae will be exposed to transients of pH, pO_2, and pCO_2 that will affect productivity, but in presently poorly understood ways.

Pond temperature is another critical parameter. Obviously to a large extent pond temperatures are due to external factors, not amenable to control. However, pond depth can be used as a tool to help moderate diurnal temperature swings. Greater pond depths are favored if temperature extremes are a problem. Evaporation is a primary cooling mechanisms for ponds, at low humidities. However, evaporation requires both make-up water and salt-build up management. This is particularly critical in the case of the utilization of high salinity waters (brine, seawater), which have a tendency of precipitating some salts (carbonates, etc.) during the pond operations due to the high pH generated by action of the algae.

In conclusion, the physical and chemical environment in the ponds is quite dynamic, but in general, also predictable. Thus a numerical model of the environment in the ponds could be used to predict the environment that the algae would be exposed to, and, thus, the engineering constraints in the design of such systems, for example allowable depths, dilution water, mixing velocities, carbonation stations, etc. Such a pond model is briefly discussed next.

4.3.6. Modeling of the Algal Pond Environments

The outdoor mass culture pond environment is characterized by environmental transients, some of which are predictable and recurring, and which influence both algal productivity and strain dominance. To a significant degree the actual fluctuations in these variables (e.g. temperature, oxygen, pH, light) and can be predicted, reasonably well, using pond models, from both pond design/operating and climatic data inputs (Benemann and Tillett, 1990). If, indeed, these transients are the most important in affecting relative productivity/ competitiveness of algal strains, then the study of algal strains under constant laboratory conditions would yield little insight into outdoor cultivation success, This is probably the major reason that laboratory strains of algae are not able to be maintained in outdoor ponds. And it suggests that selection for competent strains, the large-scale pond environment must be closely simulated at a smaller scale.

Table 4.2 summarizes the key pond environmental parameters, their typical values for laboratory cultures, the range of their values for the outdoor system, the time period over which changes occur, and an estimate as to the feasibility of scaling that parameter to a laboratory culture. Temperature, dissolved oxygen, pH, and water chemistry (alkalinity) can all be scaled effectively in laboratory reactors. The first three by maintaining feedback control loops using heater inputs, gas composition, and pH as controlled variables and the last one by artificial chemical adjustments which reproduces site water composition. Reproducing the light environment is, however, more difficult. The incident light intensity, cell density, and the mixing pattern combine to give the light distribution pattern experienced by individual cells in a culture. The photosynthetic system is a highly complex and adaptive process, that responds both rapidly, though not instantaneously, and over longer periods to changes. It is difficult to reproduce both the frequency and quality of the light experienced by outdoor cultures in the laboratory, suggesting that it would be best to carry out experiments, both for algal growth (productivity) and species selection, with outdoor ponds as laboratory systems can not be totally relied on to simulate the pond environment. Laboratory systems are also not subject to the invasions and contaminations to which open ponds would be exposed. In addition, features such as surface to volume ratio (important if cell attachment occurs), shear force (some strains are shear sensitive) will also scale poorly in laboratory systems.

In conclusion, pond models can be used to predict and even simulate in the laboratory the pond environment in many important parameters, such as pH/pCO_2, O_2, temperature, cell density, etc. Laboratory simulators could be used to study the effects of such parameters on productivity, species competition, lipid induction, etc. However, due to the inability to scale other important variables, including light regime, such laboratory simulations must be closely coupled with outdoor pond simulators, which would more faithfully (though not fully) reproduce the large-scale pond environment and allow more valid extrapolations to large scales.

TABLE 4.2.
ENVIRONMENTAL PARAMETERS IN LABORATORY REACTORS AND OUTDOOR PONDS.

(Source: Benemann and Tillett, 1989)

Parameter	Units	Value		Time Period (hrs)	Scale Down
		Laboratory	Outdoor		
Temperature	^oC	20-30	5-40	24	yes
Dissolved O_2	ppm	6-15	2-40	24	yes
pH	$-\log H^+$	7-9	7-10	< 1	yes
Alkalinity	mM	0-50	0-50	--	yes
Light	$uE/m^2/d$	0-500	0-2000	24	?
Cell density	mg/l	100-2000	100-500	--	?
Mixing	--	fast	gentle	--	no
Shear	--	high	low	--	no
Surface/volume	m^{-1}	50	5	--	no
Biota(invasions)	--	low	high	constant	no

4.3.7. Closed Photobioreactors.

A brief overview of closed photobioreactors is presented here, as these could have some utility in the production of inoculum for the larger production systems, as is discussed further in Section 10. Briefly, even a 0.1% inoculum production for a 1000 ha system, would require closed photobioreactors of a 1 ha scale. Unlike the situation in open photobioreactor designs, no standard or generally used closed photobioreactor design has evolved, or achieved commercial acceptance, and dozens of different designs (one or more per research group) have been studied, and many are still being developed (Benemann, 1996, in preparation).

The simplest closed photobioreactor would be a covered pond. Such systems are relatively cheap, with standard, relatively low cost, greenhouse designs (e.g. plastic pipe arcs over the ponds, with a cheap plastic cover) being feasible. However, such systems have several drawbacks, including overheating in summer, and then need for frequent changes in the plastic cover. Furthermore, as already pointed out by Tamiya (1957), covers per se do not provide a sufficient barrier to contamination. Overall, covered ponds are not a major improvement over open ponds in terms of preventing contamination. However, they could be considered as a final stage in the build-up of the inoculum that would likely be required for the large-scale algal cultures envisioned herein. Other types of closed photobioreactors, scalable to at least 1,000 m^2, are of interest in the initial stages of culture build-up.

A relatively large (about 5,000 m^2) tubular photobioreactor system, based on 50 m long plastic tubes, 1.2 cm diameter, was built during the early 1990's in Spain for the production of Dunaliella. However, this project, and the company behind it (Photobioreactors, Ltd.; founded by Prof. John Pirt), failed, within days of start-up. Little information is available on this operation. Other commercial projects for microalgae production with tubular reactors were initiated in South Africa, Italy, and probably other countries, with similar results, although those failures were at smaller scales (and costs). Several relatively large (> 100 m^2) tubular photobioreactors were constructed in the Former Soviet Union, just before the collapse of that centrally planned economy (without implying thereby any cause-effect relationship). Indeed, failures of commercial projects are generally not reported, providing ample opportunities for repeating them. One project is currently underway in Portugal to produce beta-carotene from Dunaliella with "biocoils", a design of Biotechna Ltd., London.

It could be argued that the lack of commercial success of such closed photobioreactors merely reflects the limited time that these have been subject to such commercial development. After all, as discussed above, both Spirulina and Dunaliella production took well over a decade for successful commercial production. However, only the most optimistic can consider that the overall advantages of closed photobioreactors, such as higher productivities and greater control over the culture environment, would outweigh their cost disadvantages.

As for open ponds, closed photobioreactor designs are also constrained by engineering considerations. A major issue is gas exchange: build-up of oxygen in closed system would rapidly reach inhibitor levels (Weissman et al., 1988). This limits the scale of such reactors (e.g. the length of the tubes, depending on their diameter). System scale is, indeed, a major limitation: maximum unit size can range from one (for a one cm diameter serpentine tubular reactor) to at most a few hundred square meters (for parallel tubular reactors with internal gas exchange). In this context, unit scale is the minimum repeating unit that includes the gas exchange device. A more detailed analysis of the engineering issues is required, but the general constraints are relatively clear.

Comparison between the different closed photobioreactors is rather difficult, due to many factors and considerations, from costs to operating requirements. One central issue in closed photobioreactors is their cleaning. Recently several "self-cleaning" systems have been tested, using small plastic balls hydraulically pushed through the tubular reactors. Miyamoto et al. (1987) reported that vertical glass tubular photobioreactors (5 cm diameter, 2.4 m high) were self-cleaning due to the action of the air-CO_2 bubbles rising near the wall. This has also been observed by Tredici (personal communication), with near horizontal (inclined at about 2 to 3^{o}) tubular reactors with internal gas exchange. This design appears to be the most likely to be able to be scaled-up to a few hundred square meters.

Several groups have developed various versions of flat plate reactors, including systems using commercially available, two layer plastic sheets with a ribs (which can be easily converted to algal culture vessels, with a depth of about 1 to 2 cm). One company has developed a system incorporating several dozen such panels. However, the potential for scale-up would appear limited.

Another alternative would be plastic bag reactors, first studied in the early 1950's (Arthur D. Little, in Burlew, 1953) and more recently being developed for Haematococcus production by several companies. The potential is for these reactors to provide a relatively clean environment at low cost, as the bags would be disposable after relatively short-term use. However, no further information is available on such systems. It should be noted that the state-of-the-art of closed photobioreactors is still in its early stages, and only very limited data on the performance, scale-up, operational requirements, and economics of such systems is available (Benemann, 1995, in preparation).

Indeed, closed photobioreactors have many technical and engineering problems, from oxygen build, to cleaning, to temperature control. The latter has been generally solved by spraying water on their surface or immersing the cultures in a cooling bath. Despite the obvious problems of closed photobioreactors, their lack of commercial success (and their repeated failures) many, indeed perhaps most, researchers in the field of microalgae biotechnology appear to favor such systems over open ponds. This curious state of affairs is explored further below.

4.3.8. Open vs. Closed Photobioreactors

Any comparison of open vs. closed photobioreactors is currently controversial. Advocates for open ponds claim much lower costs than closed photobioreactors, both capital and operating. Champions of closed photobioreactors claim that:

1. Closed photobioreactors are more productive than open ponds

2. Capital costs of closed photobioreactors are higher than those of open ponds, but only about a factor of two fold (appx. $\$40/m^2$ vs. $\$20/m^2$.

3. Higher densities achieved in closed photobioreactors, together with higher productivities, reduce harvesting costs, balancing higher capital costs.

4. Closed photobioreactors allow cultivation of algae that can not be grown in open ponds, due to contamination or sensitivity to temperature extremes. Algal grazers and other pests can also be excluded from closed systems.

5. Closed photobioreactors allow much greater control over environmental conditions (temperature, oxygen, CO_2), providing additional advantages.

However, these arguments do not hold up to scrutiny. Closed photobioreactor productivities, were earlier projected to be double or more those of open ponds but are now stated, by proponents, as being only about 25% higher. And even that enhancement has not been demonstrated in realistic side-by-side experiments of open ponds and closed reactors. Of course, closed systems, generally operating at higher temperatures, would have an advantage under some conditions. However, overall, no advantage of closed systems on productivity has been demonstrated.

If productivities are too high, quoted economics of closed photobioreactors are too low. They only included material costs (e.g. for the tubes or the rigid panels), not fabrication and installation. When considering all the supporting systems (media supply, gas exchange, pumps, fittings, support framework, temperature control, etc.), costs would be a much larger multiple, five and ten fold ($\$100$ to $200/m^2$). And closed photobioreactors require cleaning, temperature control, etc. Indeed, the inability to commercially produce Haematococcus in closed photobioreactors, despite a value of over $\$150/kg$ of biomass based on its astaxanthin content (6%), suggests significant economic obstacles to closed photobioreactors.

As stated above, production of an inoculum in closed photobioreactors is a likely requirement for large-scale pond operations, and thus these are of interest in the long-term development of the technology being proposed here. However, there is no likelihood, despite some pronouncements by experts, and investigations in other countries of even more complex systems (e.g. optical fiber photobioreactors), that such systems could find actual applications in large-scale microalgae culture.

4.4. SPECIES CONTROL IN MICROALGAE PONDS

4.4.1. Introduction

The fundamental issue of algal mass culture is whether it is, indeed, feasible to inoculate large-scale outdoor ponds with selected strains of algae (with the inoculum grown initially in closed photobioreactors, as discussed above) and maintain these strains at high productivities for prolonged periods of time. The existing literature on this subject, which is very meager indeed, would indicate that, in general, the answer is no. Only under rather special circumstances, where the organism of interest can be cultivated under strongly selective environmental conditions has it been possible to commercially mass culture desired strains of microalgae. The two examples of such special circumstances are the commercial cultivation of Spirulina and Dunaliella, which are selected for by high alkalinities and salinities. Even in these cases it is not clear whether the cultivation process involves the cultivation of selected algal strains or whether the cultivation ponds themselves were used as the selection agent and the actual strains being cultured were not selected during the cultivation process itself (rather than a priori).

Indeed, using the actual production ponds to select for an algal species has been the most successful approach thus far in solving the problem of algal species control in mass cultures. Examples are the cultivation of Scenedesmus in Germany (and other countries) (Soeder et al., 1978), the cultivation of Microactinium in waste treatment ponds at the Richmond Field Station of the University of California Berkeley (Benemann, 1977, 1978, 1980; Eisenberg, et al., 1980, see also Green et al., 1995), and most recently, and most relevant to the present discussion, the cultivation of a strain of Tetraselmis on power plant flue gases in small-scale ponds in Japan (Negoro et al., 1994, Matsumoto et al., 1995). In all these cases the algal strains appeared spontaneously in the cultures. In the first case at least one of the strains of Scenedesmus could be isolated, maintained in laboratory culture, re-inoculated into outdoor ponds, and mass cultured. Such a procedure was not attempted in the other projects, and is not likely to be always successful. However, this does suggest using outdoor ponds for the selection of competitive algal species.

Both approaches to maintaining unialgal cultures in open ponds, the use of chemically selective media and the use of "self-selected" algal strains for mass culture suffer significant practical limitations: chemically selective environments would be too expensive or not achievable in most circumstances, and use of "wild" type algal strains that naturally dominate ponds may not yield the desired products (e.g. high lipids), productivities, or have other essential properties for practical and economic production (such as harvestability). Indeed, the mass culture of microalgal strains for practical applications has a long history of failure and disappointments. The general experience has been that desirable strains can not be cultivate, while "weeds" can grow in outdoor ponds. How to overcome these potential problems is addressed next.

4.4.2. Requirements for Species Control

The large scale, low cost production of microalgae for CO_2 mitigation and fuels (biodiesel) production requires the selection of algal species that can be easily established and maintained in open ponds; and that also exhibit high total biomass productivities of lipids suitable for conversion to biodiesel.

Conceptually it is possible to consider these problems sequentially (e.g. first develop stable algal strains, then use these to select for desirable characteristics such as high productivity and lipid contents), or in inverse order, or even in parallel, with the desired traits combined in the final process development stage. Here these issues are reviewed independently, followed by a discussion of one specific example (that of Botryococcus braunii). It must, however, be recognized that algal species that exhibit high lipid productivity (as opposed to high lipid content) are relatively rare (Benemann et al., 1986) and, similarly, relatively few algal strains exhibit dominance and stability in mass culture ponds. Even fewer species would be expected to exhibit both characteristics simultaneously. Thus, development of suitable strains will require extensive strain improvement and selection efforts. However, as an initial step, independent demonstration of these capabilities would be a major step forward.

The physiological and genetic determinants characterizing competitiveness and stability in outdoor ponds are likely to be both complex and difficult to elucidate, at least compared to those which result in high lipid productivity. Therefore the best approach would likely be to first select for algal species that can in fact be cultivated in outdoor ponds, and then to screen for high lipid producers among this set, or/and to enhance lipid productivity through selection procedures. Ultimately, genetic engineering techniques would be brought to bear on this problem. However, at this stage in this research, genetic engineering would be best applied to basic problems, such as lipid biosynthesis pathways (Roessler, et al., 1991), rather than more applied problems.

Based on prior analysis (Benemann and Tillett, 1987, 1990) the conclusion was reached that a high maximal growth rates are per se not a selective factor for outdoor pond cultivation. Although it may appear intuitive that the faster a strain is able to grow the better it competes in the pond environment, this obvious (and often repeated) statement) is false, except under exceptional circumstances. Fast growth rate is only of significance under conditions where light (or other) factors are not limiting growth. As in mass culture light must be the limiting factor, maximal productivity, not maximal growth rates, will be the determining parameter in species competition. Also, and this is a critical point, although there are significant differences in the productivity of microalgae, for many strains these are not large. This suggests that, in principle, species succession should not be as large a problem in open pond culture as it is perceived to be, and is often experienced in practice.

4.4.3. Methods for Maintaining Unialgal Cultures.

It can be concluded that the problems that most prior algal mass cultures efforts experienced, with the rapid demise of the inoculated cultures, were due to a poor choice in algal strains. Failures of maintaining algal strains in mass cultures were likely due to a failure of the desired organism to survive at the extremes (e.g. temperature, oxygen, pH) of the pond environment, compared to the uniform and mild laboratory conditions in which isolated strains are usually maintained. Also, seldom is any attempt made to select algal strains under conditions (in particular extremes of temperature, pO_2, pCO_2, light, etc.) that were actually be present in the growth ponds. Thus the general failure to be able to mass culture microalgae can be ascribed to the experimenters, rather than to any inherent lack of species/strains that could be cultivated under outdoor conditions, as seen from the rapid replacement of inoculated strains with invading species better adapted to the vagaries of the real pond world. Understanding the pond environment, and the conditions that the algal cells will, and must, confront, is an obvious requirement.

The pond model described briefly above can predict the environment the algal cell would experience, in terms of light intensities, temperatures, oxygen tensions, carbon concentrations, and other factors, as a function of time of day, month of year, operations (depth), design, water chemistry, etc., for any site for which climatic data is available. It can predict both average and extremes in these factors. It is evident that microalgae that are to be mass cultured must be able to exhibit high (if not maximal) productivities under the variable physical/chemical environment of the ponds. Most important, the algae must be able to dominate under these environmental conditions, and exclude contaminants.

There are several types of contamination, all of which could affect the performance of the system. In the above only invasions and replacement by other algal species was implied. Grazing by herbivorous zooplankton (rotifers, copepods, amoeba, etc.) or infections by fungi, lytic bacteria, and even viruses could just as well result in unacceptable culture losses, and, at least for the case of grazers, are an even more frequent cause for such losses than invasion by other algae (which tend to replace the prior dominant strain after such a grazing episode). Techniques are available to minimize, if not eliminate (which is not generally possible) grazer problems. These involve either mechanical removal methods (e.g. filters and screens, which would be practical on a large scale, if the problem is not too serious), and drastic changes in conditions (such as salinity, pH, ammonia, etc.). It should be recognized that there is almost no information published on these subjects in the literature, in part because of lack of experience with such problems at the smaller scales (where grazing is less a problem) and in part due to the proprietary nature of corrective actions used in commercial operations. In conclusion, it appears feasible to maintain selected algal species in outdoor ponds, the prerequisite for production of algal biomass at high productivity and with a high lipid content for conversion to fuels.

5. PRODUCTIVITY OF MICROALGAL CULTURES

5.1. PHOTOSYNTHESIS: EFFICIENCIES AND LIMITATIONS

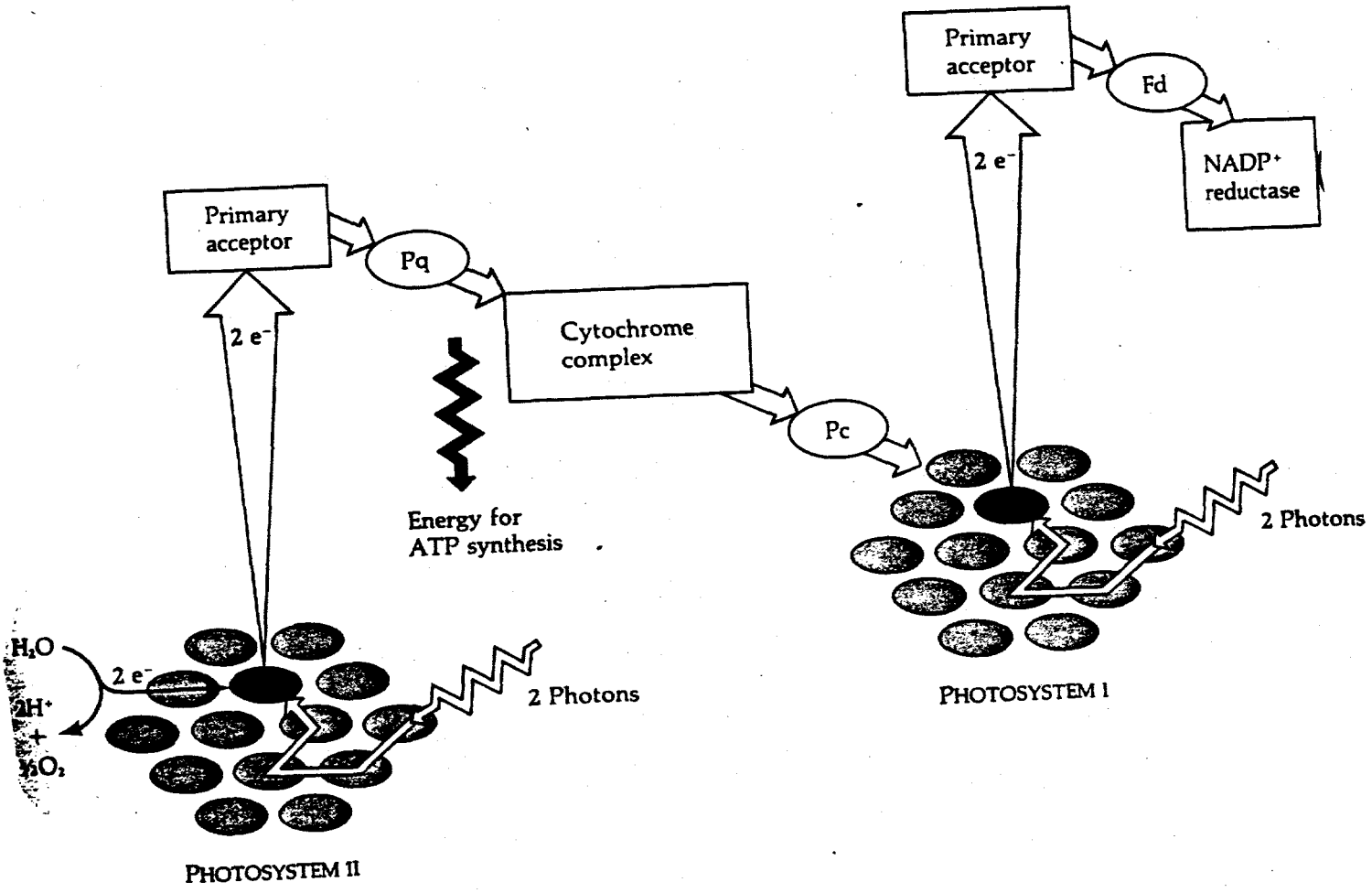
Perhaps the single dominant factor in the economics of microalgal systems is the achievable productivity, due to the high fixed capital costs, and relatively small variable (productivity dependent) costs of such systems. In Table 1.1 the theoretical maximum productivity of about a 10% solar conversion efficiency was cost-estimated. This section discusses the possibility of achieving, or at least approaching such a high efficiency, equivalent to the production of over 200 mt/ha/yr of a high lipid algal biomass, equivalent to over a 300 mt/ha/yr productivity of a conventional crop plant - a ten-fold higher yield than conventional agriculture.

The efficiency of photosynthesis is a matter of much study and continuing debate. In the laboratory, microalgal cultures grown at moderate light intensities (1/10th of age sunlight or less), exhibit maximum visible light energy conversion efficiencies (conversion of photon energy into biomass higher heating values) of about 22%, near what is was generally considered the maximum theoretical value. As visible light is about 45% of solar radiation, this extrapolates to a 10% solar conversion efficiency, if the absence of other losses (e.g. reflection, respiration, ineffective absorption, etc.). However, recently, Greenbaum et al. (1995) reported that mutants of Chlamydomonas without PSI produce H_2, fix CO_2, and evolve O_2. This suggests that, in principle, photosynthesis could be twice as efficient as assumed from the generally accepted two photosystem model (the Z Scheme, Figure 5.1).

Photosynthesis, involves two distinct processes: the light reactions (photosystems I and II - PSI and PSII), which convert water into oxygen (PSII) and a strong reducing agent (NADPH, equivalent to hydrogen) (PSI), and the dark, or CO_2 fixation, reactions, which utilize this reductant (and additional chemical energy, ATP, generated during the light reactions) to enzymatically convert CO_2 to carbohydrates, and then to other cell constituents (Figure 5.1). Billions of years of evolution has produced a photosynthetic processes of both high complexity and efficiency.

The complexity of the photosynthetic system is high, to allow the process to adapt to varying environmental conditions. Complexity is at all levels: genetic (with many hundreds of genes coding for various aspects of the process), biochemical (with many scores of proteins involved), structural (with the proteins arranged in precise three dimensional structures), and physiological (with the photosynthetic system

FIGURE 5.1.
SCHEMATIC OF THE PHOTOSYNTHETIC APPARATUS



highly regulated and adjustable to external environmental changes and internal signals) (Melis, 1991). Indeed, one of the great evolutionary achievements of the photosynthetic process is its exquisite adjustment to diverse light qualities and intensities, which permits plants and algae to attain solar conversion efficiencies near the theoretical maximum over a large range of conditions (Chow et al., 1990).

The exception to this near perfection, is the relatively low efficiencies observed at high light intensities, most particularly for algal mass cultures growing under full sunlight conditions. Typically when such cultures are moved from the low-light environment of the laboratory to the high light outdoor environment, efficiencies drop from about 10% to 2 or 3%. Of course, part of this decrease is due to the less favorable conditions in such mass culture system - variable temperature, light reflection and absorption by non-algal materials, inhibition by high oxygen concentrations in the ponds, even grazing by rotifers and others. However, even when these factors are controlled for and taken into account, efficiencies under full sunlight intensities are still much lower, as much as 80% below what could be expected from cultures operating at low light. The explanation for this phenomenon, already mentioned above, is that the typical photosynthetic system in microalgae, contains several hundred light harvesting pigments for each reaction center chlorophyll (where the photon energy is actually trapped).

When the rate of photon absorption exceeds the rate of the reaction center turnover, then the extra photons absorbed are degraded as heat or fluorescence, without generating chemical energy. (Indeed, at very high light intensities, this excess absorption of photons can actually do damage to the photosynthetic apparatus, a phenomenon known as photoinhibition, Neale, 1990). However, increasing the rate of reaction center turnover is not feasible: the dark reactions (such as the enzyme RUBISCO, responsible for CO_2 fixation), already operate at near the maximal rates and represent a large fraction of cellular protein. Further increases are not possible. Fundamentally, the light, not the dark reactions, are the limiting factor in photosynthesis.

Figure 5.2 shows the response of a dilute (optically thin, with essentially no self-shading) algal culture to increasing light intensities: at low light intensities there is a linear increase in the rate of photosynthesis per unit algae (typically expressed as CO_2 fixation or O_2 evolution per algal dry mass or chlorophyll), with the slope, alpha, a measure of the efficiency of the process. As light intensities increase, the rate slows down, to a maximum rate, P_{max}, at an extrapolated light intensity (I_S) being the saturating light intensity. At very high intensities (corresponding to actual sunlight), photoinhibition sets in. Thus three phases are evident: (i) light limitation; (ii) light saturation; and (iii) light inhibition. At least the first two phases can also be observed with dense algal cultures (Figure 5.3). In

FIGURE 5.2.
THE PHOTOSYNTHESIS VS. LIGHT INTENSITY RELATIONSHIP
(Melis, unpublished)

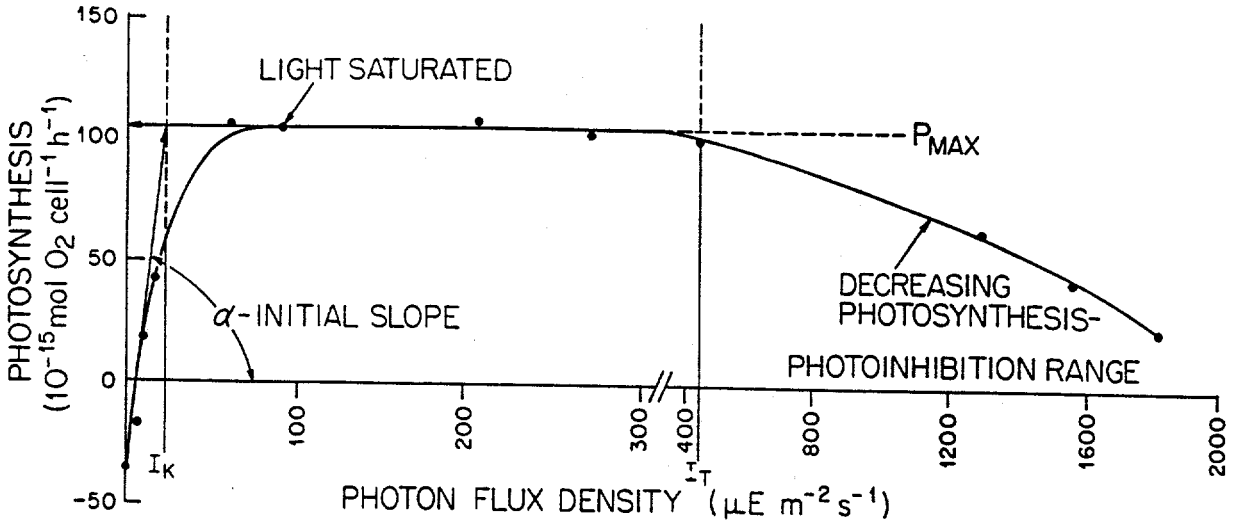
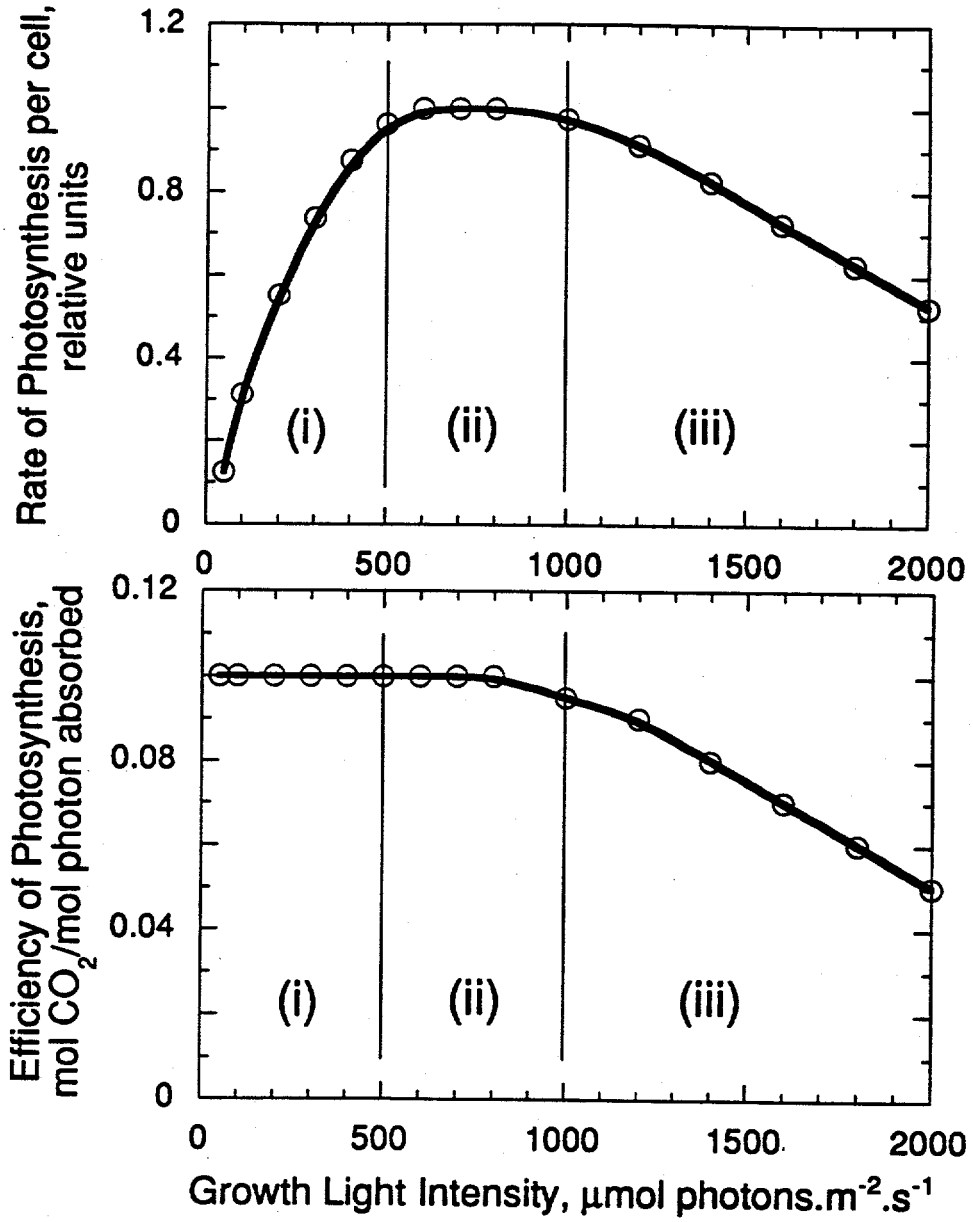


FIGURE 5.3.
MAXIMAL RATE OF PHOTOSYNTHESIS BY MICROALGAL CULTURES GROWN AT VARIOUS LIGHT INTENSITIES
(Melis, unpublished)



this case photosynthesis is expressed in terms of culture productivity (e.g. CO_2 fixation or O_2 evolution per unit culture volume), rather than per unit of cell mass, as in Figure 5.2.

The decrease in productivities with increasing light intensity is not as dramatic as with dilute cultures, and complete saturation (that is, no further increase in the rate of photosynthesis) is not observed even at high light intensities. However, the overall photosynthetic efficiency decreases at high light intensities to a fraction of that observed at low light intensities. This is the light saturation effect.

In the first phase the rate of photosynthesis by the culture is limited by photon fluency, e.g. light absorption, and the process operates at the maximal conversion efficiency that the particular algae are able to achieve under those particular environmental conditions. Unless nutrient or some other environmental factor limits this rate, or the algae exhibit some particular genetic limitation, this is generally quite high, with 8 to 12 quanta of light/mole of CO_2 fixed. In the light saturation phase, more photons are available, and absorbed, than the photosynthetic system can use, and these extra photons are wasted as heat or fluorescence. The photoinhibition phase is due to the actually damaging effect of very high light intensities. In dense, mixed, algal cultures, without other limitations (such as lack of CO_2 or high O_2), photoinhibition is not generally a major issue, as long as the mixing is sufficient to periodically remove the algae from the high light environment.

5.2. THE LIGHT SATURATION EFFECT

From the above, the key limitation of algal cultures is the decline in the rate of photosynthesis and culture productivity as light intensities increase above the saturating light intensity, typically above about 10% of full sunlight. It is this limitation, and how to overcome it, that is the subject of this section.

The decline in light conversion efficiency by dense algal cultures at increasing light intensities (Figure 5.3) is expected from the P vs. I curve of dilute algal cultures (Figure 5.2), which represent the capacities of individual cells to utilize light energy. In dense cultures at high light intensities, above the saturation level for dilute cultures, the algal cells at and near the surface receive, and absorb, more photons than they can utilize, wasting most of them, while cells further down in the water column are shaded by those above them and thus light limited.

This phenomenon is described mathematically by the "Bush Equation" (first derived by Vandevar Bush, the leading engineer of his generation, in 1949, as quoted in Burlew, 1953). This equation combines the equations of Beer Lambert

for light absorption with the equation for the "P vs. I" (Photosynthesis vs. Light Intensity) curves observed with dilute algal cultures (Figure 5.2):.

$$F = \frac{Is}{Io}(\ln \frac{Io}{Is} - 1),$$

Where F is the "Bush Efficiency" - the fraction of the rate of photosynthesis (e.g. light conversion efficiency) of a dense algal culture at high light compared to that at low light, where Io is the incident light energy and Is is the light energy at which photosynthesis saturates. Essentially the bush equation integrates the light saturation curve with the light absorption curve (modeled on a Beer-Lambert light extinction function).

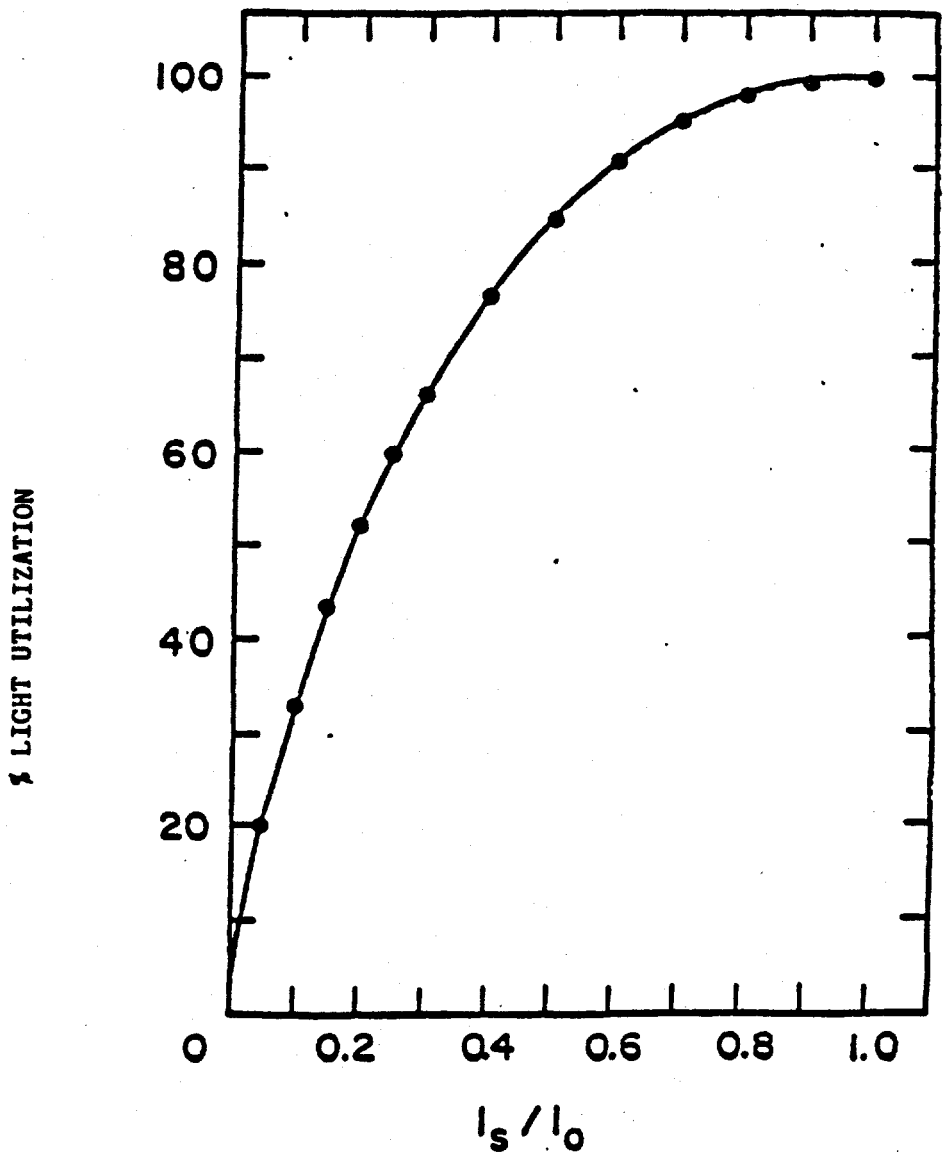
Of course, it should be pointed out that this equation, and explanation, are only gross approximations of a more complex situation: algal cells do not only absorb but also scatter light, light absorption is a function of wavelength, a minimum light input is required for zero productivity (the light compensation point, generally quite low, $< 1\%$ of light intensity, and ignored in the figures). And, furthermore, algal cultures would behave differently if grown in high or low lights, dilute or dense cultures. However, for present arguments, the simpler explanation suffices. From the Bush equation an algal culture that saturates at 5% of full sunlight intensity (a typical value), the efficiency of photosynthesis at full sunlight is only about 20% of that at or below the light saturation level (5% of sunlight). An 80% loss in potential efficiency. Or, perhaps put another way, if somehow the light saturation effect could be overcome, the productivity of such an algal culture would be increased by 400%.

When plotting the Bush Efficiency for various Is/Io ratios (Figure 5.4) it reaches 90% if the algae were to saturate at about 60% of full sunlight. Indeed, as sunlight rises and falls in intensity during the day, integrating the Bush equation over a whole day, actually improves the situation, as shown in Figure 5.5. Thus, saturating light intensities need not be at the maximal sunlight intensities, and even an increase from 10% to 50% of full sunlight would achieve most of the objective of increased productivities to near the theoretical maximum.

5.3. OVERCOMING THE LIGHT SATURATION EFFECT

Again, this problem has been recognized since the earliest days of algal mass culture research. Essentially three solutions have been proposed to overcome the light saturation effect (Myers, 1957): (a) the use of turbulence to move the algal cells into and out of the high light zone at a rate that allows the dark reactions to keep up with the light reactions (the "flashing light effect", Kok, 1953); the use of

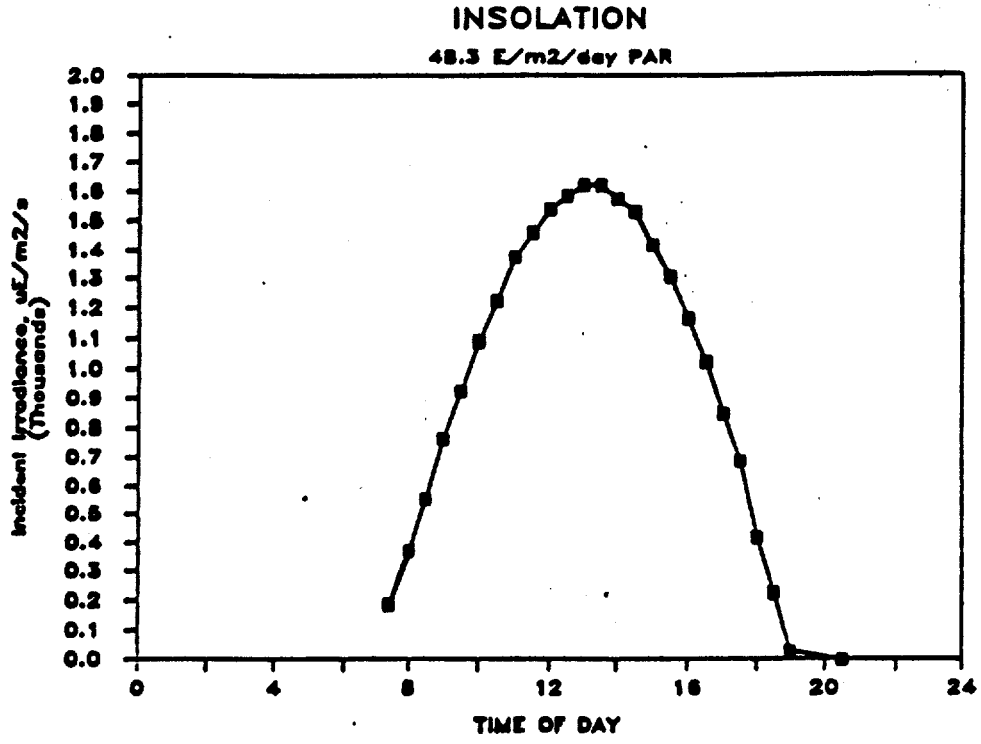
FIGURE 5.4.
THE BUSH EFFICIENCY PLOTTED AS A FUNCTION OF LIGHT SATURATION VALUES FOR PHOTOSYNTHESIS
(Weissman and Benemann, unpublished)

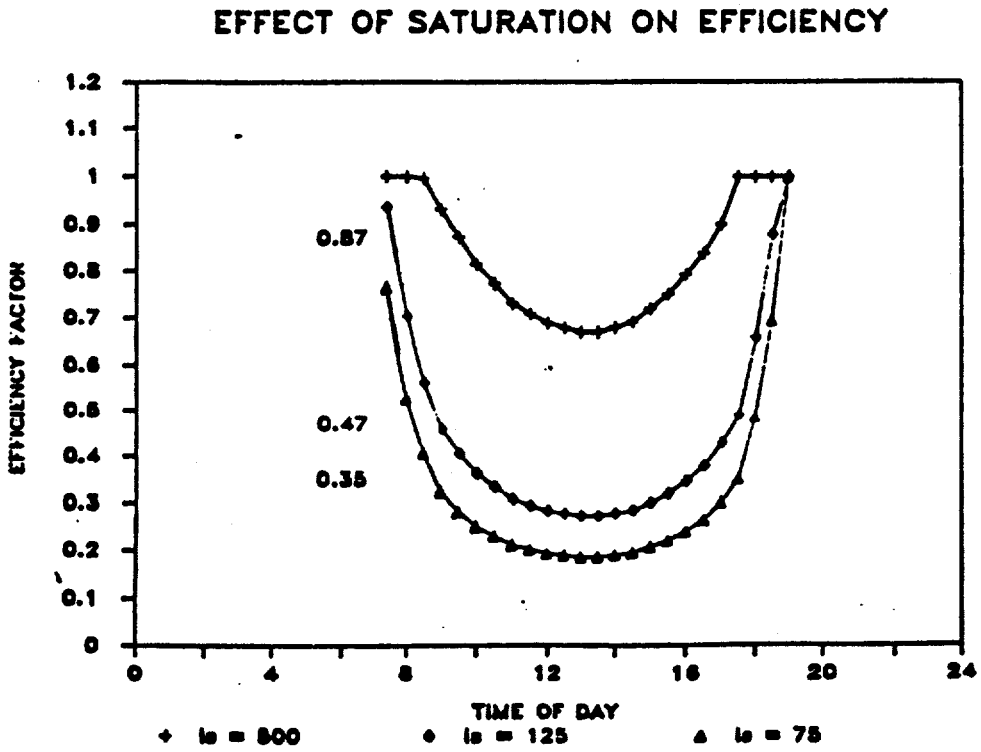


I_s = Saturating Intensity

I_o = Incident Intensity

FIGURE 5.5
THE BUSH EFFICIENCY PLOTTED AS A FUNCTION OF VARIOUS SATURATING LIGHT INTENSITIES AND A DIURNAL LIGHT CYCLE
(Weissman and Benemann, unpublished)





light diffusers that transmit the high light into the deeper areas of the cultures (Myers and Graham, 1961), and the use of algae with equal maximum efficiencies but higher values of Is (Myers, 1955). As Myers commented almost 40 years ago, none of these "has yet proved feasible for economic applications". This is still true.

The possibility of using turbulence to increase algal cultures productivities has been the subject of extensive research since the discovery of the flashing light effect by Kok (1953). Hundreds of publications on this subject have clearly demonstrated the fundamental principle, but the early view by Kok an Myers that engineering problems would likely be insurmountable in any practical applications still stands.

The major problem is the time scales involved: the light period should be only a few microseconds, with a dark period about five times longer. Although many reports on the beneficial effects of turbulence can be found in the literature on algal cultures, either these are in very small-scale laboratory systems, with high energy inputs photosynthetic efficiencies, or they are due to secondary factors, not connected to the light saturation phenomenon. (For example, increased mixing can increase CO_2 inputs and O_2 desorption, or prevent algal settling, or break down diffusion barriers, which may give the false impression that turbulence itself is responsible for increased productivities). Weissman et al. (1988) demonstrated that for a non-settling culture, with CO_2 and O_2 controlled, there was no difference in productivities if mixed between 2 and 60 cm/sec. (Higher mixing velocities are not be practical, as energy inputs increase by a cube factor).

The second approach, to use light diffusers, experimentally first demonstrated by Myers and Graham (1961) has become almost a research industry in Japan in recent years, with many groups working on the use of optical fibers in photobioreactors of various designs for microalgal CO_2 fixation and H_2 production. Actually, optical fiber photobioreactors were first studied by Manley and Pelofsky (1979) for photobiological hydrogen production, under the DOE/SERI biological hydrogen production program. Although no useable data was produced (e.g. a comparison of efficiencies with and without the fibers), the device fabricated by this group demonstrated the basic concept. Indeed, Kaneko et al. (1993) at MHI Corp., demonstrated that an optical fiber photobioreactor could overcome the light saturation effect (at least partially), resulting in a doubling of algal productivity, compared to what would have been expected in its absence. However, these are not suitable for any practical applications.

This leaves the third approach: to "search for algae .... with higher Is" (Myers, 1957). However, as earlier pointed out by Myers (1955), such algae would likely be as common as hen's teeth (our characterization). The reason for this is that algae with a high light saturation level, would not capture much light in the deeper parts

of the pond, where light intensities are low. In a dense culture, most algae will spend most of the time at low light intensities. Thus, from an evolutionary perspective, the choice between wasting light on the surface vs. being competitive in most of the water column, is an easy one: wasting light is better than being uncompetitive. Therefore, searching for such algae would not be likely rewarded. Unless some particular environmental conditions would favor this state. These are discussed later. Of course, in these days of genetic engineering and even more advanced biological technologies (protein engineering, metabolic engineering), it can be assumed that such tools could be applied to solve these problems. Although, certainly, the architectural constraints of photosynthetic systems will provide some limitations, at least in the foreseeable future, genetic engineering and tools should be able to meet this challenge.

Of course, as also already pointed out by Myers (1955), even if such algae were found (or, today, created) they would be quickly outcompeted in algal mass cultures by any contaminant or mutations that had the conventionally high antenna:reaction center ratios. Indeed, algal cultures do not maximize biomass productivity, nor even cell growth and replication, but pigment productivity and, most important, individual survival.

A biotechnology of microecological engineering will also be required to help manage such processes to favor, in open systems subject to contamination and with low levels of inoculation, the desired microorganism that maximizes productivity functions of interest to us. For now, a potentially practical solution this problem is relatively frequent inoculation combined with nutrient limitation to prevent rapid take-over of the cultures. A feasibility analysis, including the cost of inoculum production in photobioreactors, suggest that this approach could meet the economic constraints for large-scale algal cultures for CO_2 utilization and fuels production (Benemann, in preparation).

Indeed, nutrient manipulation can be used not only to maintain the desired algal cultures but also to generate the desired low antenna pigment composition. Nutrients, of course, include light itself. It is generally found that at high light intensities algal cells will reduce their antenna pigments, both by decreasing the ratio of total active photosystems and the ratio of antenna to reaction centers. Although in most cases the first effect dominates over the second. However, in the case of Dunaliella salina the second, low antenna to reaction centers, with high PS absorption dominates (see discussion below). This organism could be used during an initial demonstration of the concept discussed above. Cultures of D. salina would be grown at high light, then concentrated, and photosynthetic efficiencies (CO_2 fixation, O_2 evolution) compared to control cultures at low light. This could be the initial "proof of concept" experiment.

Further development of this system would be the use of nutrient (principally N) limitation to grow the cultures at high light intensities, to demonstrate the same principle, but under conditions more suitable for culture scale-up. Subsequent applications of this concept to microalgae capable of high lipid production (e.g. diatoms) would allow achievement of the very high productivities that underlie the economic analysis presented below.

Why were this problem, and the proposed solution, not investigated earlier. Two reasons are apparent. The early researchers found that the photosynthetic apparatus did not adapt to high light intensities by altering the antenna to reaction center ratio. Pigment levels decreased, but mainly due to a decrease in the total number of photosystems, rather than the antenna pigments alone. This apparent inflexibility of the photosynthetic apparatus discouraged further investigation.

However, other work, most clearly that of Ley and Mauzerall (1982) with Chlorella and Smith et al. (1990) with Dunaliella, did find that in Chlorella grown at high light intensities there was, indeed, a significant adjustment in the photosynthetic unit size. (Work with mutants in higher plants, also found such effects, but this is beyond the scope of this discussion). And work from the laboratory of Prof. Melis, University of California Berkeley, with Dunaliella salina (Guenther and Melis, 1990; Smith et al., 1990; Naus and Melis, 1991, 1992; Harrison et al., 1992; Kim et al., 1993) demonstrated that high light induces a large shift in photosystem II antenna size, greatly increasing the saturating light intensity.

Dunaliella salina is a green, flagellated, wall-less alga that typically grows in hypersaline environments. Some strains of this alga exhibit a rather high level of beta-carotene, up to 10% of the dry weight. This property has led to an expanding industry in the U.S. that mass cultures this alga in shallow, paddle wheel mixed, raceway ponds. Thus, this species (though not necessarily the strains to be used commercially) could be used to demonstrate the maximization of productivity by overcoming the light saturation effect. As noted above, work by Melis and coworkers demonstrated the, thus far unique, attribute of this alga: at high light intensity the PSII antenna : reaction center chlorophyll ratio is quite low, about 50:1, compared to 250:1 for the cells grown under low light. This is the lowest such ratio described up to now and demonstrates the feasibility of having low antenna:reaction center ratios in a functional photosynthetic apparatus.

It must be noted, there has been no real search for such a property. Indeed, the limited research in this field does not allow a prediction of whether this is a rare characteristic or a relatively frequent (though not dominant) property of many algal species. The Melis laboratory has also elucidated the biochemical basis for this phenomenon: the PSII antenna system consists of three separate antenna packages: of appx. 50, 130, and 250 chlorophyll molecules, which are attached to the reaction

center complex in that order. In Dunaliella salina grown at high light intensities, only the first package is present in the PSII complex, and, thus, the antenna:reaction center ratio is low. Most of the work with this alga has studied the effect of high and low light intensities on the rate of breakdown and repair of the PSII system (another important aspect, see Smith et al., 1990; Kim et al., 1993), but not yet the potential of this phenomenon in productivity enhancement.

However, in one (unpublished) experiment, comparing the rate of photosynthesis in low-light and high light adapted algal cultures, it was discovered that the high light culture performed better on a chlorophyll basis. Most important the slopes of these P vs I curves were similar, indicating no loss in efficiency. Thus, this suggests that, indeed, this alga should, under the proper conditions demonstrate a high efficiency at a high incident radiation. For this the alga would be grown as dilute cultures at high light intensity and then concentrated, and the photosynthetic efficiency measured at high light intensities. Of course, there is an important issue: it is not possible to grow algae at high light intensities in any practical system, where algal concentrations must be high enough to absorb all the light. A high algal concentration, and complete light absorption, implies that even at high light on average each cell will experience a low light environment. That would adapt the cultures to a low light regime, and, thus, high antenna:reaction center ratios.

Thus, high light intensity grown cells must, almost by definition, be grown as dilute cultures, which, implies fast growing cultures, either exponential in batch culture or near wash-out for continuous cultures. This imposes some limitations, as fast growth and high light intensities do not optimize for photosynthetic efficiency. Thus, any results obtained with such cultures (in terms of increased photosynthetic efficiencies) would be expected to be conservative. Also, and most important, the use of high light grown (e.g. dilute) cultures is not practicable on a larger-scale. Thus, the above experiment would only be a demonstration of the principle, not as an avenue towards practical applications. Indeed, in such an experiment it would be expected that after a period of time, typically one to a few hours, the algal cells would again adapt to the low light, and photosynthetic efficiency (at high light) would once again drop as the algal cells adapt to the (average) low light by increasing their antenna pigments, and the light saturation effect takes over.

In the future, a major R&D objective would be to overcome this limitation. For this nutrient manipulations are the best approach: growing algal cultures under a limiting nutrient, specifically such as N, also affects the pigment composition, with chlorophyll levels dropping dramatically. Again the question arises is this is due to a decrease in the number of photosynthetic units (with no change in antenna to reaction center chlorophyll ratio, and, thus, no change in the light saturation effect) or if the ratio of antenna to reaction centers drops. Again, this is species specific,

with, in those cases studied, the predominant effect is the first one, although, again, at least in some cases, and specifically for Dunaliella salina, the dominant adaptation is to a reduction in antenna sizes. This again suggests this organism for initial experiments utilizing nutrient limitation approaches.

Of course, the issue arises of the proper light level for such adaptation, which, perhaps, could be a compromise between the high light intensities (experienced by individual cells) and the very low light intensity in a dense nutrient sufficient culture. And, this still may require a shift from a more dilute to a more dense culture, although not as extreme as in the previous case. (Indeed, from a practical perspective, it would be possible to shift cultures from shallower to deeper ponds reducing the surface area). Although not ideal, it would be a step towards an actual process. Thus, light vs. nutrient levels (principally N) in regulating the effect reaction center chlorophylls in algae, and their effect on both short-term (1 hr) and more sustained culture photosynthetic efficiencies at high light should be the main initial subjects for research in the future, following the initial experimental approach outlined in the preceding paragraph.

The challenge is to develop culture conditions that maximize length of time over which this process can be achieved, and to maximize the overall light conversion efficiencies, which would include the initial growth and induction phase. It is not clear if satisfactory long-term (weeks) culture operations can be achieved, and, that the overall culture efficiency can be sufficiently maximized. However, the experimental approaches outlined above, should allow the devising of more sophisticated approaches using the tools of genetic engineering.

It should also be noted that in the above, the effect of nutrient limitation is to, paradoxically, increase productivity. Again, it should be recognized that productivity in terms of pigments has not really changed. Nevertheless, this is a counterintuitive result, or, more correctly at this point, prediction.

In conclusion, there is, indeed, sufficient evidence, for the validity of the proposed approach. The past lack of interest in this problem can be ascribed perhaps to the fact that most recent research in photosynthesis has focused on the genetics and biochemistry of photosynthesis. It is the resulting greatly increased knowledge in these areas that provides a new opportunity to address this issue, now with much more powerful tools and much greater insights into the workings of the photosynthetic apparatus. Thus, the prediction made here is that it would be possible to approach the "theoretical maximum" of a 10% solar conversion efficiency in algal mass cultures. Indeed, if the Greenbaum PSII type of photosynthesis is demonstrated to actually result in reduced quantum requirements, even higher efficiencies may be possible in the long-term. Of course, efficiencies must include the production of a biomass with a high content of lipids, for production of biodiesel. This is the subject of the following Section.

6. FUELS FROM MICROALGAE

6.1. INTRODUCTION

The first proposal for using microalgae for fuels was made by Meier (1955). He proposed that microalgae could be anaerobically digested to methane, and more algae grown on the effluents of the digester. This concept was experimentally demonstrated at the laboratory scale by Golueke and Oswald (1957). Oswald and Golueke (1960) also presented an early feasibility analysis of this concept of growing microalgae on power plant flue gases (as a source of CO_2), and converting the biomass to methane gas, which could be used as a fuel to operate the power plant. This concept, of coupling algae production with power plant CO_2 utilization, and thus replacing fossil fuels, received renewed attention in the 1970's with the energy crisis, and, again, in the 1990's with the concern about possible global warming due to CO_2 emissions.

There is a considerable literature with the production of methane from microalgae, both in the context of waste water treatment and as a "stand-alone" energy production concept (see also Uziel, 1975; Benemann et al., 1977, 1978, 1979; Eisenberg, et al., 1980). Between half to two-thirds of the energy content in the microalgae could be recovered from the algal biomass in the form of methane. Methane fermentations have the advantage that almost any biomass composition is suitable, and thus particular components (e.g. high lipid or starch content) is required. However, the much higher value of liquid transportation fuels justifies the relative neglect of methane fermentations in recent years. (And, indeed, the pre-treatment requirements for efficient and rapid anaerobic digestion of microalgae biomass were never clearly established). Nevertheless, methane fermentations would still be used in any microalgae fuel production process to degrade the residual biomass remaining after extraction of the lipids and hydrocarbons, or fermentation of the starches to ethanol.

Although the production of ethanol from microalgal biomass would appear to be appealing, essentially no work has been done in this area. This is in part due to past focus on methane and hydrogen, and more recently, biodiesel production, and in part to the general perception that ethanol production is an energy intensive process. Such a conclusion may be premature and this area requires further investigation. However, this subject is not further addressed in this report. Mention could also be made of the application of microalgae is in the production of hydrogen (Benemann, 1995), a subject of considerable current interest. However, again, this subject is beyond the scope of the present report, which focuses on lipids (triglycerides) and hydrocarbons production, for conversion to biodiesel type fuels.

6.2. MICROALGAE LIPIDS

Historically microalgal lipids have been of interest since World War II when von Witsch proposed them as a source of vegetable oils and beta carotene (Harder and von Witsch, 1942). Even before the war the relationship between nitrogen nutrition and lipid content of algae was already recognized (Ketchum and Redfield, 1937). After the war, as part of the initial work on microalgae culture sponsored by the Carnegie Foundation of Washington (Burlew, 1953, see Section 1.3.2) the study of microalgae lipids was pursued by groups in Germany (Von Witsch and Harder, 1953), England (Fogg and Collyer, 1953), and, the U.S. (Spoehr and Milner, 1953). The report by Sphoer and Milner (92), that, Chlorella could accumulate up to 80% of its dry weight in the form of lipids, attracted considerable attention and their procedure, involving nitrogen starvation, was even patented (Sphoer and Milner, 1956). However, since the early 1950's the study of algal lipids has been primarily associated with taxonomic or biochemical studies of metabolism (Wood, 1974; Phol, 1982).

The energy shortage of the mid 70's resulted in a renewed interest in algal lipids as a source of fuels (Shifrin, 1986), and this became the focus of the Aquatic Species Program of DOE-SERI, after 1979 (Section 4.3.1). A major focus of the DOE program has been to identify high lipid yielding strains and develop the technology for producing an algal derived liquid fuel. This chapter provides a brief review of microalgae lipids in general, and the use of nitrogen deficiency to produce algal lipids in large quantities.

Lipids are a class of molecules that, together with proteins and carbohydrates, broadly define overall cellular composition. Operationally they are defined as any cellular compound extractable in an organic solvent such as chloroform, or a mixture of chloroform and methanol. Lipids can by classified as both polar and non-polar. The polar lipids are comprised of the phospholipids and glycolipids and are functionally associated with membrane structure and fluidity. Nonpolar lipids are primarily mono-, di- and triglycerides, but also include certain pigments, vitamins, wax esters, sterols, and cyclic and acyclic hydrocarbons. Here the emphasis is on trigyclerides and some hydrocarbons.

The triglycerides have been considered an energy storage reserve due to their high specific caloric value (9.3 kcal/g) as compared with protein and carbohydrate (5.1 and 4.5 kcal/g respectively). Indeed, triglycerides are clearly an energy reserve in higher organisms, and even in algae there is evidence of triglyceride breakdown during periods of energy limitation. From the perspective of fuel production the microalgal triglycerides, because of their prevalence and similarity with the vegetable oils, are of greatest interest. Hydrocarbons, such as those produced by Botryococcus braunii would also be of interest. However they are produced in quantity by only by this one species which grows slowly and has not been mass cultured. A conceptual process for mass culturing Botryoccoccus braunii is

presented in Section 9. Thus, in this section, only the glycerides and fatty acids of microalgae are discussed.

Free fatty acids (FFA) are usually present at roughly 1-5% of the total lipid fraction. Saponifiable lipid make up roughly 30-40% of the total lipid. Fatty acid composition has been studied quite extensively since the 1950's and has been reviewed by several authors (Wood, 1974; Werner, 1977; Pohl, 1982). All classes of algae contain large proportions of saturated C16's, however the remainder are very much class specific. Green algae (Chlorophytes) have the bulk of their fatty acids as saturated and unsaturated C18's. Their composition is very similar to that of vegetable oils. Diatoms (Bacillariophyceae) contain some monounsaturated C18's and also unsaturated C20's (20:4,20:5) and saturated C14. The unsaturated C20's and C22's (omega-3 fatty acids) are of considerable current commercial interest because diets high in these have been linked to reduced heart and other diseases. However, they are of lesser interest in fuel production, and, anyway, most of the storage lipids are unsaturated triglycerides, the major focus of this review.

Factors such as temperature, light intensity, and, most markedly, nutrient supply have been shown to affect composition of microalgae overall lipid composition and content. The effect of light intensity on fatty acid and lipid composition has been studied by several authors. Opute (1974) found that the diatom Nitzschia palea changed its unsaturated to saturated fatty acid ratio from 2 to 1.3 between roughly 2% to 0.2% sunlight intensity. He also found that in low light very little triglycerides were formed and attributed this to the preferential synthesis of the necessary structural lipids in an energy limited environment. In the same study, he compared heterotrophic vs autotrophic grown cultures of Navicula muralis and found that the dark grown cells had roughly twice the amount (on a relative basis) of saturated fatty acids as compared with light grown cells. He concluded as others (Orcutt and Patterson, 1974; Nichols, 1965; Pohl and Wagner, 1972) that there was some functional coupling between photosynthetic processes and the degree of unsaturation of the fatty acids.

Fatty acids appear to increase in unsaturation as temperature decreases. It has been suggested in the literature that this is due to the need to maintain mobility of the lipoprotein complexes for proper cell function. Patterson (1970) determined the fatty acid distribution for a high temperature strain of Chlorella at seven temperatures between 14 and 38°C. He found that the degree of unsaturation did increase with decreasing temperature until 22°C after which unsaturation decreased. It may be that temperatures below 22°C was too far out of the organisms normal metabolic range and the response was of a secondary nature. In addition he noted that the total lipid content did not change as a function of temperature. Materassi et al. (1980) studying two strains of Scenedesmus, reported that saturated fatty acids increased 18 to 32% in going from 20 to 35 °C.

6.3 NITROGEN DEFICIENCY AND LIPID CONTENT

Of all environmental parameters, nutrient deficiency appears to be the most effective means for inducing changes in the lipid content and composition. Silicon limitation in diatoms (Werner, 1966) and severe P deficiency (Healy, 1973) are reported to increase lipids in microalgae. These are of minor importance, however, compared to the general increase in lipids observed upon nitrogen deficiency.

Increases of over 100% in lipid content are commonly reported in response to limiting the nitrogen supply to algal batch cultures. As already mentioned in the introduction, the first recognition that nitrogen limitation increased the lipid content of algae was made by von Witsch (1953) who observed a positive relationship between culture age (and diminished culture nitrogen) and lipid content. At the same time Spoehr and Milner (1953) demonstrated that a nitrogen starved Chlorella reached 86% lipid while containing only 5% in exponential cultures. Many other reports of lipid induction by nitrogen deficiency are found in the literature in the 1950's and 1960's. A selected overview of the literature is presented in Table 6.1.

More recently, Shiffrin and Chisholm (1981) studied 30 different species of marine and freshwater chlorophytes and diatoms and found that for log phase cultures diatoms contained on average 36% lipid as compared to 19% for chlorophytes (ash free basis). Cultures exposed to nitrogen starvation resulted in changes of - 40% to +320% in their log phase values after one week of starvation. Most notable was that while the diatoms had high log phase lipid contents they responded rather poorly to nitrogen starvation. On average there was very little change in lipid content. The greens, however, displayed a wide variety of responses, ranging from a slight reduction in cellular lipid (Dunaliella) to 2 to 3 fold increases from log phase values (Chlorella vulgaris). Methodological problems with this study limit its value: inoculum sizes varied; cultures only grew to 2-3 times their inoculum density; the nitrogen mass balances (as calculated from the reported data) show large losses or gains in many of their cultures (thus the actual nitrogen status of these cultures is uncertain); and the lipid extraction methodology resulted in the inclusion into the lipid fraction of the solvent interface, which would tend to increase lipid contents (Tornabene, personal communication). Thus their data does not allow conclusions regarding actual lipid productivity (vs lipid content per se).

Much of the current work on lipid induction in algae was carried out by the DOE Microalgae Program over the past decade. Table 6.2 summarizes some of data collected by investigators associated with that program. In general the data supports earlier studies on the importance of the nitrogen nutrition on lipid content. However the key issue of lipid productivity is still an open problem, as discussed next.

Table 6.1 SELECTED MICROALGAL LIPID CONTENTS DATA

SPECIES	LIPID CONTENT NS	ND	REFERENCE
Chlorella pyrenoidosa	20(80)	35(17)	Fogg and Collyer, 1953
" "	18(?)	65(?)	Ibid
" "	25(?)	40(?)	Ketchum & Redfield, 1949
" "	20(?)	70(?)	Guerin, et al., 1970
" "	25(?)	35(4)	Aach, 1952
" sp. strain A	20(log)	45-53(17-26)	Oorschot, 1955
" strain 10-11	19(log)	18-26(5)	Ibid
Bracteacoccus minor	25(?)	33(?)	Pohl, et al., 1971
Chlorella vulgaris	27-33(?)	54(?)	Ibid
Nitzchia palea	22(log)	39(7-9)	Opute, 1974
Chlorella pyrenoidosa	14(log)	36(7-9)	Shifrin and Chisolm, 1981
Oocystis polymorpha	13(log)	35(11)	Ibid
Monollanthus salina	41(log)	72(11)	Ibid
Nannochloris sp.	20(log)	48(11)	Ibid
Scenedesmus obliquus	26(log)	47(22)	Piorreck, et al., 1984113
Chlorella vulgaris	24(log)	64.5(28)	Ibid

NS - nitrogen sufficient, ND - nitrogen deficient.
Numbers in parenthesis represent days of batch growth.

TABLE 6.2 LIPID CONTENT OF NREL CULTURE COLLECTION STRAINS

SPECIES	GROWTH CONDITION	LIPID CONTENT NS	ND	SOURCE
Amphora sp.	B,B	4.1	14	Benemann, 1986
Ankistrodesmus	B,BW	24	40	BenAmotz, 1984
Boekilovia sp.	B,BW	28	36	Benemann, 1986
Boekilovia sp.	C(?),BW	23-29	42	SERI
Botryococcus braunii	B,FW	44	54	BenAmotz, 1984
Chaetoceros gracilis	B,SW	15	28	Benemann, 1986
Chaetoceros sp.	B,BW	--	33	SERI
Chlorella sp.	B,FW	10	34-48	Lien, 1985
Chlorella ellipsoida	B,BW	16	21-30	SERI
Cyclotella sp.	B,BW	13	42	Tadros, 1985
Isochrysis(Tahitian)	B,SW	7	26	BenAmotz, 1984
Monoraphidium sp.	B,BW	21	25	Benemann, 1984
Nannochloropsis sal.	B,SW	29	60	Tornabene, 1984
Nannochloropsis sp.	B,SW	28	53	Benemann, 1984
Navicula sp.	B,BW	32	58	SERI
Nitzschia sp.	B,SW	27	--	Thomas, 1983
Nitzschia dissipata	B,SW	26	66	Tadros, 1985
Oocystis pusilla	B,BW	10	--	SERI
Phaeodactylum tri.	C(.25),SW	20	23	Thomas, 1983
Tetraselmis ap.	SC,SW	18	15	Laws, 1981

FW, BW, SW represent fresh, brackish and seawater respectively;
B, C, SC batch, continuous and semicontinuous modes of growth.

6.4 NITROGEN DEFICIENCY AND LIPID PRODUCTIVITY

While nitrogen starvation in batch cultures (called herein nitrogen deficiency, as compared to nitrogen limitation in continuous cultures, a critical, but often misunderstood distinction) increases lipid content in many microalgal species, particularly the green algae, it is also correlated with a reduction in biomass productivity. In order to evaluate a microalgae based lipid production process, both content and productivity are important. Indeed, an optimization between the two is required: high content is desirable for efficient CO_2 utilization and processing, and high productivity is the primary concern in the economic feasibility of these systems. A detailed understanding of the factors impacting on both lipid content and productivity, in particular nutrient, and specifically nitrogen, limitation, are of central importance in the production of microalgae biodiesel fuels.

Nitrogen deficiency in algae causes a variety of responses dependent upon species, growth history, and severity of the depletion. Breakdown of chlorophyll is the most visible sign of nitrogen limitation, although this can also be observed in response to other environmental stresses. The storage of carbon is a major and universal response to nitrogen limitation. This may be partially the result of a simple overflow metabolism of carbon fixation into cellular components not containing nitrogen. A rapid reduction in protein content also occurs although there is little information concerning specific enzymology. Thurston and Rulands (1980) found protein decay of 12-40% in N starved cultures of Chlorella. Protein synthesis continues until all the nitrogen supply is exhausted. Activity of the enzymes of the nitrate assimilation pathway are all increased rapidly with onset of nitrogen depletion. Coupled with this is an increase in specific rates of uptake of nitrogen which occurs both by a reduction in cell nitrogen and an increase in the absolute rate of assimilation. The increasing enhancement in Vmax correlates well with decreasing growth rate (Collos and Slawyk, 1980). One interesting point is that nitrogen limitation results induction of enzymes (e.g. nitrate reductase, hydrogenase) which normally require an inducer without any inducer (or inducing condition) being present either before or after nitrogen limitation (Kessler, 1974).

Chlorophyll content decreases as a function of cellular nitrogen; correspondingly per cell or per weight estimates of maximum rates of photosynthesis also decrease (Eppley and Renger, 1974; Everest et al., 1986). On a per weight chlorophyll basis however, maximal rates of photosynthesis are unchanged. Thus the observed reduction in photosynthesis rates does not appear to be due to a specific loss of the proteins involved in carbon fixation. And the light intensity at which photosynthesis is saturated generally remains unchanged (see Section 5).

In some strains carbohydrate levels can reach above 70% without reduction in culture productivity (Weissman and Benemann, 1986). This occurs relatively rapidly(< 1day). More slowly(> 1day), lipid content increases anywhere from 0 to 200 % of healthy cultures. Thus, there appear to be two phases following

nitrogen limitation: an early phase of carbohydrate accumulation, followed by conversion of the carbohydrate into lipids in those strains that accumulate lipids.

The issue of lipid productivity was already clearly stated over forty years ago, in the Burlew (1953) book, where it was pointed out that the production of lipids would likely be most economical with nitrogen sufficient cells having a low lipid content rather then by nitrogen deficient cells which, despite their much higher lipid content, had such a low productivity that total lipid yields were negligible. This particular issue seems to have been essential overlooked since then. For example, Shiffrin (1984) stated that Monalanthus salina would be"...a worthy prospect for further consideration..."(as a lipid producer). However his data (1981) reveals that while M. salina achieved a lipid content of over 70% its lipid productivity was practically zero after nitrogen depletion. On the other hand a Scenedesmus sp. had the highest lipid productivity for all of the species studied before and after nitrogen deficiency, however this organism was not mentioned as a good lipid producer.

At SERI, Lien and Roessler (unpublished manuscript, ca. 1986) measured biomass and lipid productivities as well as photosynthetic efficiencies for various cellular components in five species undergoing nitrogen starvation in batch culture. In two of the five strains, Boekilovia sp. and Cyclotella, lipid productivity decreased after nitrogen limitation. In Cyclotella, biomass productivity dropped from 400 to 20 mg/l/day. The other organisms studied showed moderate (Chlorella 25%; and Ankistrodesmus 17%) and substantial (Nannochloropsis sp., "Nanno Q", 90%) increases in lipid productivity. Photosynthetic efficiency and biomass and lipid productivity all appeared to be closely coupled. Significantly, photosynthetic efficiency actually increased for Nanno Q for the early stages of nitrogen deficiency, while decreasing between 20 and 90% for the other strains. Independent studies by Benemann et al. (1986), screening eight strains from the same collection, also demonstrated the relative uniqueness of Nanno Q in lipid production and again illustrated the variability which exists between species in their response to nitrogen deficiency. This variability exists not only between species but also within a species, as Opute's (1974) study of 40 species of Chlorella demonstrated: some exhibited only carbohydrate storage and others lipid storage in response to nitrogen limitation.

The biochemical basis for lipid storage is poorly understood. However, it must have an evolutionary and selective basis. One suggestion has been that a high lipid content would allow algae to regulate their buoyancy. However this can be dismissed, (perhaps Botryococcus is an exception) on the basis of hydrodynamic arguments. Lipids do not sufficiently alter cell buoyancy to affect their settling behavior in most cases. Thus the best explanation is that lipids indeed serve as an energy reserve. Lipids contain almost nine times more energy than carbohydrates (on a hydrated weight basis), thus there is an advantage to store lipids for long term utilization. However this has not yet been demonstrated experimentally, by, for example, comparing long-term survival of lipid vs. carbohydrate storing strains.

One must, in this context, differentiate between nutrient limited (continuous, e.g., chemostat) and nitrogen deficient (batch, starved) cultures as there are significant differences between them. A nutrient limited culture is growing in an external environment of constant, but insufficient inputs of the limiting nutrient. The resulting intracellular levels of that nutrient determine the growth rate at which the organism grows. Operationally this is actually achieved by setting the hydraulic dilution rate of the continuous culture, which imposes a growth rate on the organism, since slower growth leads to wash out and faster growth to population (cell density) increases until growth is balanced with dilution rate. Extracellular nutrient levels in such continuous cultures are often so low as to be below detection by any but the most sophisticated analytical methods. This is particularly true for algae growing on phosphate or nitrate as limiting nutrients. In contrast a deficient (starved batch) culture can not adjust its cell density to cope with declining nutrient supply nor does it receive a continuous supply of the limiting nutrient. Therefore cell growth in batch cultures, once the external supply is exhausted, occurs at the expense of the limiting nutrient stored in the cells. The metabolic consequences become increasingly more severe as a function of time after nutrient depletion. Growth rate continuously declines as intracellular nutrient concentrations, becoming zero at a certain minimum intracellular concentration of the nutrient.

Due to the economic limitations inherent in any fuel production process the key issue in the production of lipids by microalgae is the overall productivity of the process, suitably optimized. To a first approximation it can be assumed that lipid productivity should be maximized for a biomass containing about half of fixed energy in the form of extractable and utilizable lipids. Allowing for inevitable losses, about 35 to 40% of the dry weight of the algae should be total lipids. This is higher than the lipid content found in algae grown under nitrogen sufficient (light limiting) conditions.

From the above, rather abbreviated discussion, the best approach is to separate the main biomass production phase from the lipid production phase, using nitrogen deficiency (not limitation, defined above) as the environmental factor that controls algal lipid biosynthesis and content. How to best optimize such a two stage process in which lipid induction takes place in a light limited growth phase is the central objective of future R&D in this field.

As already mentioned above, the best results demonstrating such a two stage process, and the achievement of high overall lipid content and productivity, have been obtained, in the laboratory, with the organism called "Nanno Q", a species of Nannochloropsis. In further work, Benemann and Tillett (1990) demonstrated that light supply (total area exposed), or the severity of the light limitation (cell density at the onset of nitrogen limitation) affected the requirement for nitrogen and thus the growth rate-nitrogen quota relationship. This a logical consequence of the energetic requirements for nitrogen metabolism. That is, a high enough cell density must first be established, prior to nitrogen deficiency, such that biomass

productivity is maximized. The implication for mass cultures, where cell density must be maximized to reduce costs for the harvesting process, nitrogen limited cultures could operate at as high a cell density as possible (though below where productivity losses due to respiration become significant). Most important, it was demonstrated that an increase in the light supply after the onset of nitrogen limitation results in overall lipid productivities significantly greater than those obtained for cultures kept at constant high light levels. It is reasoned that this may be due to an increased biosynthesis of chlorophyll in the culture shifted to higher light intensity, thus enabling a higher relative rate of photon capture and supply of reductant. This result suggests that an optimum process design should include an initial period of light limitation followed by, after nitrogen deficiency has begun, an increase in light supply. In a pond environment this could be done by decreasing the culture depth or density (by dilution) in a second stage. This implies a larger pond area for the second (lipid induction) stage, but, overall, lipid productivity, as would be maximized. Table 6.3 summarizes the laboratory results:

TABLE 6.3. MAXIMUM LIPID PRODUCTIVITIES FOR NANNO Q
(mg/l/day, for a Variety of Growth Conditions.
(Benemann and Tillett, 1990)

Condition	Batch	Continuous
Nitrogen Sufficient	100	90
Nitrogen Deficient	150	50
Light Shift	180	--
Continuous to Batch	--	20-30

The data demonstrates the differences between continuous and batch cultures, and the effect of light shifting (from low to twice as much light). It should be noted that the comparisons are for cultures that receive the same amount of total light, thus reflecting photosynthetic efficiencies. The challenge for the future is to demonstrate that such techniques can be applied in outdoor pond cultures.

Another challenge will be to apply the tools of genetic engineering to this problem, to develop the high lipid productivity strains that will be required in practical applications for low-cost production systems. The work of Roessler et al., at NREL, over the past decade (see Dunahay et al., 1995; Roessler and Ohlrogge, 1993; Schneider and Roessler, 1994; for some recent references), has provided a strong foundation for future practical applications of genetic engineering technology to this problem.

7. MICROALGAE HARVESTING

7.1. INTRODUCTION

In addition to high solar conversion efficiencies and lipid productivities, the cultivation of microalgae for fuel production coupled with CO_2 mitigation requires a low cost harvesting process that produces an algal biomass concentrate suitable for further processing (e.g. extraction of lipids). Although the actual solids concentrations required will vary depending on processing needs, in general solids concentrations must be several percent, and typically above 10%, on an organic dry weight basis.

The harvesting of microalgae is one of the most difficult, and thus far least satisfactorily resolved, problems in algal mass culture. The problem is simple to define: Microalgae mass cultures are dilute, typically less than 500 mg/l (0.05% w/w) on a dry weight organic basis. Microalgae cells are small - usually less than 20, and sometimes under 5 um in diameter, for unicellular species. Thus, the recovery of one ton of dry weight biomass requires the extraction of these small particles from over 2,000 tons of medium.

This compares to a microbial (yeast or bacterial) fermentation processes, where biomass concentrations are usually at least 25 g/l (2.5%), and often over 50 g/l, or 100 fold higher than microalgae cultures. Although harvesting costs are not necessarily a linear function of concentration, to a first approximation microalgae harvesting is well over one order of magnitude more difficult than yeast or bacteria harvesting.

It is generally simple and not too expensive to increase algal biomass concentrations from a few % solids slurry to a high (> 10%) solids, suitable for processing to fuels and other products or drying. In some cases, as for the fermentation of the algal biomass to methane gas for example, a cell concentration of a few (3 - 4) percent solids is already suitable. Thus, the microalgae harvesting problem is how to increase algal biomass concentrations from a few hundred mg/l to a few tens of g/l, or about a one hundred-fold concentration. It is this aspect of algal harvesting that is emphasized in this report. Upgrading even a 1 - 2% slurry to a high solids (10-20%) algal paste, for extraction of lipids, alcohol fermentations or drying, can be carried out by centrifugation at a generally acceptable cost.

For microalgae cultivation on seawater, the high ionic strength will affect many of the harvesting processes (e.g. chemical flocculation), compared to freshwater systems. Also, different types of algae would be cultivated in such systems. However. It is difficult at present to specify harvesting systems simply on the basis of media salinity, and, both fresh water and seawater systems are considered.

The literature on microalgae harvesting is extensive, starting with the first studies of algal mass cultures (Burlew, 1953). There is no lack of methods for harvesting algae. However, none are universally satisfactory on both an economic and performance basis, and relatively little experience exists with low cost harvesting from seawater systems. Indeed most experience is with harvesting waste grown microalgae, where there was little control over cultivation conditions or algal species. Thus, the data base is restricted. It must be stated at the outset, that no simple solution to this problem is at hand and harvesting must be a R&D focus of any microalgae project. However, sufficient information is available to suggest promising approaches and processes that can meet the economic and performance goals of a microalgae-commodities production process.

As stated, for CO_2 mitigation and energy production, only very low-cost harvesting processes can be considered. The cost goals for producing algae for CO_2 mitigation and energy production are, prior to processing, about \$150-200 /ton dry weight organic matter. Thus, methods such as centrifugation of the pond medium can be dismissed almost out-of-hand, as being too expensive. Although high cost methods are reviewed, this report emphasizes low-cost harvesting. However, algae harvesting methods that do not produce an actual biomass product (as preferred in waste treatment) are not included in this review: filter-feeding organisms (bivalves) or food chains (rotifers -fish); land application (percolation and over land flow); in pond settling (with chemicals, water hyacinth covers, etc.); rock and sand filtration; etc. (see Harris, 1968, Russel et al., 1980).

Another approach to reducing algae harvesting costs is to increase the algal biomass concentrations thus reducing the amount of liquid processing required. This can be achieved by decreasing culture depth, increasing illumination per unit area (with optical fibers and solar concentrators), recycling biomass ("activated algae"), using tubular reactors, cascade reactors, etc. Another approach would be to grow algal mats or biofilms, which would could be easily recovered. A more subtle approach is to increase the optimal standing biomass for maximal productivity. This would require, basically, decreasing maintenance energy, a fundamental problem. These approaches are not reviewed here, but should be addressed in a separate report at a later date. Ultimately, some (only a few) of these approaches could be used in combination with a low cost harvesting method to meet the exacting economic constraints of a low cost microalgae process.

First a brief review of the current experience in algae harvesting in large-scale commercial operations is presented. This is followed by a general discussion of various algae harvesting processes, and then a comparative evaluation of the various processes and discussion of future prospects. The next section provides a short glossary of terms used in this section.

7.2. GLOSSARY

This Section uses a number of terms that may have different meanings in other publications or are not standard usage. The following definitions are used:

Activated algae: A process described by McKinney and coworkers which involved the recycle of algae, as in an activated sludge, to increase algal concentrations and process effectiveness, and produce a flocculant culture that easily sediments (see McGriff and McKinney, 1972; Regan and McKineey, 1979).

Autoconcentration. Harvesting process which involves the swimming capabilities of some algae, usually towards the light (phototaxis) to effectuate concentration. Autoconcentration can also be applied to algae that regulate their buoyancy with gas vacuoles or lipid content.

Autoflotation. O_2 supersaturated pond waters release small O_2 bubbles, which attach to flocs, making them float.

Autoflocculation. The high pH resulting in ponds due to the action of photosynthesis can result in the precipitation of Ca, Mg and phosphate ions, which form a chemical flocculating agent that results in algal settling.

Bioflocculation. The spontaneous floccuation of algal cells, either in the ponds or after removal from the culture system, that results in algal sedimentation. Does not involve chemical flocculating agents, including any that may be present in the medium.

Discrete Sedimentation. Settling of algae upon removal from the mixed ponds due to their growth as large enough colonies to exhibit sufficient (for harvesting purposes) sedimentation rates. Does not include bioflocculation.

Foam fractionation. A process by which algae are concentrated in the foam generated by fine bubblers in the presence of surfactants, which result in the algae becoming attached to the bubbles. In some cases foam fractionation may be observed even in the absence of added surfactants, due to the presence of algal surfactants.

Froth flotation. The process flotation of algal flocs, generated by chemical flocculation or bioflocculation, by small air bubbles generated by porous diffusers.

Microstraining. Rotating screens with backwash. Term can also apply to shaking screens or inclined screens, with relatively large openings (e.g. > 10 um).

Sedimentation and Settling. Used as synonyms.

7.3. CURRENTLY USED COMMERCIAL ALGAE HARVESTING METHODS

Microalgae harvesting processes used in large-scale commercial installations are generally proprietary. Relatively little information is available, with the exception of waste water treatment facilities. Even with these there is a lack of specific data. Thus, this discussion is general in nature. As the commercial plants produce algae worth many thousands of dollars per ton, they are not constrained by the economics of fuel or commodities production and their harvesting technologies would not likely be useful in this context.

Chlorella production has been the oldest commercial application of microalgae. Chlorella are small (< 5 um), spherical cells. Centrifugation has been the only commercial methods used and Chlorella manufacturing plants typically have a battery of centrifuges, a major capital investment and operating cost factor. No information is available on actual equipment used, through-put rates, etc. Centrifugation is a standard technology and off-the-shelf equipment is available. A recent project in Australia attempted to harvest Chlorella by bioflocculation. However, after a few weeks of operation the process failed (for the rather obvious reason that the culture liquid was recycled, thereby selecting for non-settleable algae, see Weissman and Benemann, 1978).

Spirulina production relies on the filamentous nature of these algae for harvesting. The process used at the largest microalgae production plant, Sosa Texcoco near Mexico City consists of:

1. Pre-screens to remove large fragments (e.g. insects, straw, debris); followed by
2. Inclined screens with a moveable spray that hydraulically pushes the algae retained to a collecting trough; followed by
3. Rotating, backwashed filters (microstrainers); followed by
4. Moving belt vacuum filters.

Details (screen sizes, flux rates, concentration factors, etc.) are proprietary. Screen mesh openings are estimated at between 25 and 35 um; inclined screen sizes are probably between 50 and 100 m^2 and about 10 m^2 for the rotating drum screens, for a daily harvest rate of about one ton of algae biomass, representing several probably about 1,000 m^3 per day of harvest.

Harvest efficiency is low, probably less than 50%, but this is not a major issue as the underflow is returned to the production pond. Similar systems are known to operate at other production facilities (Earthrise Farms, California; Cyanotech Hawaii), but they are likely much more efficient. Some of the smaller production systems use shaking screens which achieve higher concentrations with a single stage, but have higher operating costs. No actual capital and operating costs for the harvesting process for Spirulina are available for a commercial plant, although Benemann (1987) provided an estimate, which suggests relatively low costs (< $100/mt) (See further discussion in Section 7.4.7). The low costs of microstraining has made the cultivation of filamentous algae of great interest in microalgae,

including wastewater treatment systems (Benemann et al., 1975), but, asides from Spirulina production, this has not been successful.

Dunaliella production has been commercialized in the past decade with harvesting being a major problem. The initial development of Dunaliella production in Israel during the 1970's failed due to lack of a suitable harvesting process. The initial approach, use of centrifugation, failed due to the sizing of the centrifuges based on yeast harvesting specifications. Although Dunaliella is similar in size to a typical yeast cell, the lack of a cell wall by this algal species makes it very susceptible to shear breakage. For this reason the initial operation of the centrifuges resulted in unacceptable losses, and a centrate (clarified supernatant) with a large amount of cell debris.

Although centrifugation can be adapted to the harvesting of Dunaliella by operating the centrifuges at relatively low throughputs (e.g. low shear rates), this was viewed as being unacceptably expensive at the time. Also, the high salinity of the Dunaliella medium resulted in the corrosion of the centrifuges, which required more expensive, ceramic lined centrifuges. During the 1970's and early 80's several groups worked on this problem, and several patents were issued (e.g. Bloch et al., 1982, Curtain and Snook, 1983, Kessler, 1982). One method tested was a lamellar settler, but it did not prove practical. The current Dunaliella production plant in Israel (owned by a Japanese company) is thought to have reverted to centrifugation as the harvesting method.

At the Western Biotech Plant in Australia, now owned by the Hoffman La Roche Co., considerable work was carried out on chemical flocculation, see Moulton et al., 1990, Borowitzka and Borowitzka, 1988). However, a recent visitor reports that they use a large number of lined centrifuges representing a large capital and operating costs. Harvesting is a major problem because of the low concentration of the algae in the deep pond culture systems used (typically < 100 mg/l). Another plant in Australia, Betatene, which also harvests "natural" (e.g. low density) cultures has developed a proprietary methods for adsorbing the algal cells on a hydrophobic absorbant (Curtain and Snook, 1983). By contrast, the Nutrilite (formerly Microbio Resources) facility in California, harvests Dunaliella by means of ferrric chloride and alum flocculation. The flocculated biomass is extracted into vegetable oils to recover the beta-carotene oil (see Section 8).

The other major harvesting of microalgae takes place at few microalgae oxidation ponds, where the algae are harvested to prevent excess suspended solids in the effluents of these plants (see Section 3 for a detailed discussion).

A conclusion of this review of the state-of-the-art is that none of the processes used commercially at the present time are suitable for low-cost harvesting of microalgae. Nevertheless all potential harvesting methods are reviewed in the next sections, to evaluate which may have potential for future development of a low cost process.

7.4. ALTERNATIVE MICROALGAE HARVESTING METHODS

7.4.1. Overview

Algae harvesting processes can be classified as:

1. **Centrifugation** for initial and final concentration;
2. **Chemical flocculation** and algal settling or flotation with lime, alum, chitosan, polyelectrolytes;
3. **Filtration** of unicellular algal cultures with or without pre-coats or algal cell absorbers;
4. **Microstraining** of filamentous or colonial algae;
5. Use of **high gradient magnetic fields** by adsorbing magnetic particles to the algal cells or vice versa;
6. Control of growth medium chemistry to result in spontaneous chemical flocculation (**"autoflocculation"**);
7. **Natural settling** of large colonial algae or species that aggregate (**"discrete sedimentation"**) or flocculate (**"bioflocculation"**) without chemical floccculants.
8. Cultivation of motile or buoyant algal species allowing **"autoconcentration"** by controlling motility;

The above classification is somewhat arbitrary. Some harvesting systems combine two or more processes. Some processes are broadly applicable broadly to all or most algal species, others depend on the cultivation of specific algae or even strains and a few require control over the pond chemistry and environment to achieve acceptable performance criteria. Performance criteria include the concentration factor, harvest efficiency and, most important, costs. The ability to control algal species and cultivation conditions is at the heart of the development of low cost harvesting processes.

In the next subsections these processes are briefly discussed, with some estimation of costs and recommendations for any further need for investigation. The processes that best meet the goals of a commodities production process are discussed in greater detail.

This literature review is not comprehensive; for reviews see Mohn (1978, 1980, 1988) and Shelef et al. (1984). For a Russian review see Kominiskaya (1984). The earliest comprehensive study of algae harvesting was that of Golueke and Oswald (1965). Twenty years later, Shelef (1984) concluded that things had not changed much, suggesting a lack in progress. Although that may still be true, it can be concluded from what follows that very low cost harvesting processes could indeed be developed, based on existing experience from microalgae wastewater treatment ponds and small-scale experiments.

7.4.2. Centrifugation

Centrifugation is a well established process in microbial biotechnology (See Axelsson, 1985; Brunner and Hemfort, 1988; and Belter et al., 1988, for reviews). Several equipment manufacturers sell industrial units. (In the U.S. the major vendors are Alfa Laval and Westphalia).

Centrifugation depends on the achievement of relatively high centrifugal forces in rotating chambers. Centrifugation costs are primarily dependent on the density difference of the particles and their size. Typically, in large industrial centrifuges centrifugal forces of several hundred to several thousand times gravity are achieved. Rotor size and rotational speed determine both the centrifugal force and costs of the system. Another major cost factor is the amount of clarification and concentration required, which, along with algal size and density, determine residence time in the centrifuge and, thus, capacity and cost. Finally, for industrial operations, continuous centrifugation is desirable, which entails continuous discharge of not only the clarified supernantant but also continuous or intermittent discharge of the concentrated biomass (underflow).

In the case of microalgae the concentration of the cultures is much lower than in conventional fermentations. Thus much larger volumes have to be handled, which makes the overall process more expensive, as costs are strongly dependent on volume handled, but only relatively weakly on solids concentration. Thus, centrifugation options favored by conventional fermentation processes may not be optimal for microalgae harvesting. Obviously only the largest machines available will be suitable for large-scale projects.

Table 7.1 summarizes the basic types of centrifugal separators based on solids content in the feed (0.02 - 0.05% for algae). It can be seen that for largest throughputs a nozzle separator with intermittent discharge is the best. The solids outputs from such a system are typically a few %, so that a second stage centrifugation should be considered. Decanter type centrifuges appear to be best as they achieve very high solids (up to 20% with algae, Mohn, 1980), reliability is high and operating costs relatively low.

Centrifugation was used for algae harvesting since the early studies of algal mass culture of Chlorella (Burlew, 1953). Golueke and Oswald (1965) studied centrifugation of sewage grown microalgae with a field-scale nozzle centrifuge which could be operated with various disc angles in the rotor. From a process perspective, as the algal media can be recycled, the harvest efficiency is a secondary consideration to the total amount of algae recovered per unit time, which is also proportional to unit power requirement (the major operating cost). Optimal harvest was recorded with a 45o angle rotor operating at 3,000 rpm at a throughput of

TABLE 7.1 CENTRIFUGAL SEPARATORS

Basic Types of Centrifugal Separators[a]

	Transport of Sediment	Solids Content in Feed, (vol%)	Maximum Throughput (m^3/hr)
Bowl separators	Stays in bowl	0–1	150
Solid ejecting: nozzle separator	Intermittent discharge through axial channels	0.01–10	200
Solid ejecting: slot separator	Intermittent discharge through radial slot	0.2–20	100
Nozzle separator	Continuous discharge through nozzles near bowl edge	1–30	300
Decanter	Internal screw conveyor	5–80	200

[a] These are general ranges only, and should be used to focus discussions of alternatives.

somewhat above 1,000 l/min for an algal input of about 200 mg/l and a harvest efficiency of about 70 %, requiring about 3,000 Kwhr/ton of algae, a major cost factor. The concentrate in these experiments was only about 1%. This same group (Dugan et al., 1970) also used this centrifuge in further studies, finding that, as would be expected, the larger Scendesmus was recovered much more effectively than small Chlorella cells.

Many others tested various centrifuges for algae harvesting, but the only detailed studies where by Mohn (1978, 1980). He tested various centrifuges - all had some drawbacks and high costs associated. As above, the nozzle disc type centrifuge with intermittent discharge was concluded to be the best option. One requirement is that all feed be carefully pre-screened to remove larger materials, debris etc., which can clogg the nozzle, abrade the centrifuge, etc. He also estimated relative capital costs and operating power costs for various harvesting methods with centrifugation being the most expensive on both accounts.

From this data it would require (at 333 mg/l) 3,000 Kwh/ton of microalgae, or the same as estimated by Golueke and Oswald (1965). This is, clearly, excessive for any CO_2 mitigation project, as energy inputs (and, thus, CO_2 emissions) would exceed the fuel value of the recovered algal biomass.

Barclay et al. (1987) estimated the cost of centrifugation, compared to cross flow filtration and polyelectrolyte flocculation, and concluded that for a 4 ha facility producing 370 mt/y of algal biomass, total biomass cost were about $2,500/ton, of which about 40% was for harvesting (if all overhead, labor, and other costs are apportioned). Thus, harvesting alone was estimated at about $1,000/ton. About half the capital costs of $2.2 million were due to the centrifuges. (These costs would need to be updated to current $ by multiplying by about 1.25). No economies of scale are likely above this size. Benemann et al. (1977) estimated similar cost, adjusting for inflation.

In conclusions, centrifugation is clearly impractical for primary harvesting method. However, it can be considered as a secondary harvest method, to concentrate an initial slurry, from a few (1 - 5%) solids to produce an algal slurry or paste (15 to 20% solids), such as would be required for lipid extraction or otherwise processing the biomass.

This was the process recommended in the prior feasibility studies (e.g. Table 1.3, see also Section 8) for thickening the algal biomass after the primary harvesting (settling) process, prior to extraction of the biomass for lipids. Second stage centrifugation costs are estimated at between $15 and 20/ton of biomass produced. This should be verified by more detailed cost estimates and some laboratory centrifugation experiments from which scale-up could then be projected with greater accuracy.

7.4.3. Chemical Flocculation: Inorganic Flocculants

Chemical flocculation using alum, ferric chloride or ferrous sulfate, and lime are effective methods for algae removal. The processes involve charge neutralization (algae have negative charges (Ives, 1953) by the positive cations, followed by agglomeration (flocculation) of the thus destabilized algal suspension. (A colloidal suspension, such as algae, that exhibits no spontaneous tendency to flocculate is termed stabilized). Chemical flocculation is widely practiced in water treatment. Extracellular products of microalgae can interfere in the flocculation process (Hutson et al., 1987; Berhardt et al., 1985; Hoyer et al., 1985). The possibility of interference in chemical floccculation of algae by their extracellular products does not seem to have been considered in any of the studies discussed next.

Again, the ground-breaking study in this field was that Golueke and Oswald (1965), who studied lime and alum flocculation. As expected, removal efficiencies dependent on pH and dosage, as well as (although this was not investigated during these studies) the algal culture themselves. The major problem were the very high amounts of chemicals required. Lime flocculation was optimal when 120 mg $Ca(OH)_2$ were combined with 40 mg $FeSO_4$. Dosages over 100 mg/l of alum were required to achieve a 90% clarification of the waste pond effluent. Recovery of the alum by acidification was studied, but proved to be relatively unsuccessful. (Others have tried to recover alum, with no greater success). An unpublished study from the same period (Hicks, 1958), found that alum flocculation of algae from the Auckland, N.Z., oxidation ponds was feasible, though expensive. However, cost chemical flocculation was estimated at roughly 40% lower than those of centrifugation (Golueke and Oswald, 1965). A contemporary project in South Africa, also concluded that chemical flocculants, followed by sedimentation or (as recommended) flotation, were effective in algae harvesting (Van Vurren et al., 1965, 1972).

These results, and the familiarity of the waste treatment professionals with chemical flocculation, encouraged its application for algae removal, an urgent matter as increasingly strict water quality standards restricted the discharge of algae containing effluents from oxidation ponds after 1977 (See Benemann et al., 1980). The algae harvesting from an oxidation pond, built in 1970 in Lancaster, California, with a capacity of 2 million liters per day, was actually designed for water reclamation. Alum flocculation was followed by sedimentation and filtration (Figure 7.1). Alum dosages were rather high (300 mg/l) as phosphate removal was also desired. This experience and other ongoing research on chemical flocculation (McGarry, 1970, McGarry and Tongkasame, 1971; Ort, 1972; Folkman and Wachs, 1973; Al-Layala and Middlebrooks, 1975) encouraged the use of this technology (Parker, 1977; EPA, 1973) at other California sites: Modesto, Napa, Stockton, and Sunnyvale.

TABLE 7.2. RESULTS OF CHEMICAL FLOCCULATION - SEDIMENTATION
(Source: EPA, 1973)

–Summary of coagulation-flocculation-sedimentation performance

Investigator and location	Coagulant	Dose, mg/l	Overflow rate, gal/min/ft²	Detention time, min	BOD			SS		
					Influent, mg/l	Effluent, mg/l	Percent removed	Influent, mg/l	Effluent, mg/l	Percent removed
van Vuuren et al.,[9] ..... Windhoek, South Africa	Alum[a]	216-300	0.27	200	27.3	9.5	95	85	17	80
	Lime[b]	300-[c]400	.27	200	27.3	3.5	87	85	8	92
Goleuke et al.,[2] ..... Richmond, Calif.	Alum	100	.78	150	23.0	1.0	96	199	13	93
Goodwin,[17] Napa, Calif. ...	Lime	[d]200	(e)	(e)	30.0	3.6	88	102	23	79
	Alum	45								

[a]As $Al_2(SO_4)_3 \cdot 14.3\ H_2O$ (molecular weight = 600).
[b]As CaO.
[c]pH 10.7.
[d]pH 10.8.
[e]Not available.

-Summary of typical coagulation-flotation performance

Investigator and location	Coagulant	Dose	Overflow rate, gal/min/ft²	Detention time, min	BOD			SS		
					Influent, mg/l	Effluent, mg/l	Percent removed	Influent, mg/l	Effluent, mg/l	Percent removed
Autoflotation:										
van Vuuren et al.;[23]	Alum	220 mg/l	3.5	8	12.1	2.8	77	(a)	(a)	(a)
Windhoek, South Africa	CO_2	to pH 6.5	1.8	8	12.1	4.4	64	(a)	(a)	(a)
Parker et al.;[14] Stockton, Calif.	Alum	200 mg/l	2.0	22	(a)	(a)	(a)	70	11	85
	CO_2	to pH 6.3								
	Alum	200 mg/l	2.0	22	(a)	(a)	(a)	156	75	44
	Acid	to pH 6.5								
Dissolved-air flotation:										
Parker et al.;[14] Stockton, Calif.	Alum	225 mg/l	[b]2.7	[b]17	46	5	89	104	20	81
	Acid	to pH 6.4								
Ort;[16] Lubbock, Tex.	Lime	150 mg/l	(a)	[d]12	280-450	0.3	>99	240-360	0-50	>79
Komline-Sanderson;[24] El Dorado, Ark. .	Alum	200 mg/l	[c]4.0	[c]8	93	<3	>97	450	36	92
Bare et al.;[25] Logan, Utah	Alum	300 mg/l	[e]1.3-2.4	(a)	(a)	(a)	(a)	100	4	96
Stone et al.;[26] Sunnyvale, Calif.	Alum	175 mg/l	[f]2.0	[f]11	(a)	(a)	(a)	150	30	80
	Acid	to pH 6.0-6.3								
Snider[27]	Alum	125 mg/l	3.3	(a)	65	7.7	88	90	10	89

[a]Not available.
[b]Including 33-percent pressurized (35-60 psig) recycle.
[c]Including 100-percent pressurized recycle.
[d]Including 30-percent pressurized (50 psig) recycle.
[e]Including 25-percent pressurized (45 psig) recycle.
[f]Including 27-percent pressurized (55-70 psig) influent.

However, rather than simple sedimentation, the latter two chose to use dissolved air flotation (Van Vuuren et al., 1965a,b; Bare et al., 1975; Stone et al., 1975) for the separation step. In dissolved air flotation, a small amount of the liquid phase is pressurized to dissolve additional air, and then this liquid flow is mixed in with the forming flocs, preferably using alum as flocculant (Cassell, et al., 1971). The dissolved air is released as microscopic air bubbles that attach or are trapped by the flocs and impart to them buoyancy. In general it was observed that dissolved air flotation was more effective in terms of solids concentration (5 to 8% solids vs. 2 to 5% for sedimentation), reduced hold-up in the separation tank (a major cost factor) and increases removal efficiency (See Table 7.2). The disadvantage is the complex nature of such systems. Indeed, some portion of the flocs fail to float, and must be recovered from the bottom.

Although relatively little information has been published over the past decade on the operation of these plants, they all suffered from severe start-up problems, continuing operational difficulties, and much higher costs than anticipated, due in part to increases in the costs of the chemicals after the oil crisis. Benemann et al. (1977) estimated costs to be near (90%) those of centrifugation, not the appx. 60% estimated earlier by Golueke and Oswald (1964). Indeed, only one - Sunnyvale - is currently operating, and this one (along with Napa earlier) has switched to organic polyelectrolytes as principal flocculating agents. (See Section 3).

Also, the algae-chemical sludge obtained is a major disposal problem, as it can not be used as animal (e.g. fish) feed (e.g. Dickson, 1987), or for methane production (Eisenberg, et al., 1979). In conclusion, flocculation with inorganic chemicals is not a promising technology for harvesting microalgae for CO_2 fixation and fuel production.

Other techniques can also generate the gas bubbles needed in flocculation-flotation process. In "electroflotation" electrolysis is used to produce small H_2 bubbles, but power consumptions are high (see Shelef et al., 1984). Flotation can even be effected by "froth flotation", simple sparging with fine bubbles (see Smith, 1988, for review). The algal culture itself can generate fine gas bubbles after mechanical pumping, as it is supersaturated with O_2 during day time. In that case the process is termed "autoflotation" and has been described repeatedly (Koopman and Lincoln, 1983; also see literature reviewed therein). Of course, the process can only be carried out in daytime on sunny days. Nevertheless, it presents a potentially attractive option to assist bioflocculation harvesting, as very high tank overflow rates (< 1m/hr) may be possible. The high dissolved oxygen in ponds does present a significant problem in pond operations, perhaps it could provide an opportunity to help in low cost algal harvesting. However, the complexities introduced by such a process seem to be rather severe, and this concept is not recommended for further research at the present time.

7.4.5 Chemical Flocculation: Polyelectrolytes

Organic cationic polyelectrolytes work in a manner similar to that of inorganic flocculants by neutralizing the (negative) surface charges on the algal cells. However, their effectiveness in promoting floc formation is enhanced by the potential for forming "bridges" between the algal cells thereby producing more stable flocs with lower amount of chemicals used. However, it must be cautioned that polyelectrolyte flocculation is a very complex process, and no simple models should be considered to apply in all cases. For comprehensive reviews of the fundamentals see articles in Attia (1987), for applications of polyelectrolytes in waste treatment in general see articles in Schwoyer (1981).

Organic polyelectrolytes have been tested for algae harvesting for over two decades (Golueke and Oswald, 1964; Tenney et al., 1968; Tenney, 1969; McGarry and Tongkasame, 1971; Tilton et al., 1972) and are still an active area of R&D (Bilanovic et al., 1988; Buelna et al., 1990, for examples). The general conclusion are that, as would be expected, anionic and neutral polyelectrolytes and polymers were not effective in flocculating microalgae - only cationic polymers can be used. The major advantages of cationic polymers are the relatively small amount required, typically less than 10 mg/l, as well as the ability to use the biomass in further applications (e.g. anaerobic digestion, animal feeds, etc.). The major disadvantages were the relatively high cost of the polyelectrolytes, which made such processes somewhat more expensive than inorganic flocculants.

The improvements in commercial polyelectrolytes flocculants over the past decade, which allows their use at even lower concentrations (typically < 5 ppm), suggests that the economics of flocculation with organic polyelectrolytes have improved, making them a preferred option over chemical flocculation process. This is evidenced by the switching to these chemicals by algal separation processes at the waste treatment plants in Sunnyvale and Napa, California (Section 3).

Weissman (personal communication) carried out a number of harvesting studies (bench-scale) of brackish and saltwater grown microalgae with a variety of novel polyelectrolytes supplied by one of the vendors with good effect, including for the harvesting of Dunaliella from a very high salinity medium. This is an important observation, as the experience with conventional cationic polyelectrolytes is that salinities above 5g/l will severely reduce flocculation effectiveness by a number of the polyelectrolytes tested. Barclay et al., (1987) estimated that the cost of flocculants themselves at about $50/ton of algal biomass, but total harvesting costs were then estimated (1985$) at about $450/ton (about $600/ton in current $). Recent estimates (Tryg Lindquist, personal communication) are for similar costs in current $ suggesting a signficant decrease in costs due to equipment, process, and flocculant improvements. These polyelectrolyte flocculants could also assisting bioflocculation, as discussed in Section 7.4.7.

7.4.5. Filtration of Unicellular Algae

There are three major problems with the filtration of unicellular microalgae: the very small size of the cells, their near (for practical purposes) spherical shape, and the gelatinous and other extracellular material normally present on the algal cells, which result in deformation on the filter surface. These properties result in generally poor filtration due to the plugging of the filters and/or breakthrough of algal cells. Extensive studies have been carried out on algal filtration, most without much success. Filtration is best if carried out with filamentous or large colonial algae, which can be readily handled by large mesh screens. These are discussed in subsection 7.4.6. Here the filtration of other algal types, alone or in combination with chemical flocculation, are briefly considered. Methods which do not recover the algal biomass (slow sand filtration, rock filtration) are not discussed in his review.

There are many variations on the basic filtration process, involving a large variety of filter materials, including regenerable precoats; use of pressure, vacuum, centrifugal forces (basket centrifuges); and methods to keep the filter surface from clogging (blinding) by hydrodynamic means (shear forces). Shelef et al. (1984) describe some of the basic filtration systems. Mohn (1978, 1980, 1988) carried out research on various filtration systems, particularly for recovery of the alga of Coelastrum, a relatively large algal colony of open structure, and would be expected to exhibit good filtration. Several filtration techniques proved effective for this alga but not for smaller Scenedesmus colonies.

Dodd developed (and patented) a continuous belt vacuum filter with a cellulosic pre-coat. The pre-coat could be an animal feed, which would be enriched by the algal biomass or could be washed (to remove algae) and recycled. This process was tested in Australia (Dodd and Anderson, 1976) with some success. This system was studied as part as an animal waste recycle project in Singapore, but variations in algal populations resulted in poor reliability (Dodd, 1980).

Tangential or cross flow filtration (CFF, see Shilocah et al., 1988 for a review) was tested for microalgae about 1976 by using novel membranes with relatively large pores (see McGregor and McGregor, 1978, for a review). However, the membrane performances were difficult to reproduce and this was not pursued. Shelef et al. (1984) mention testing CFF, but power consumptions were high (probably due to using a fine pore membrane). Barclay et al. (1987) estimated the costs of such a process for seawater grown microalgae as being essentially the same as polyelectrolyte flocculation, at about $500/ton of algae, vs. $1,000 for centrifugation and $450 for polyelectrolyte flocculation. These estimates were based on assumed flux rates of 2.5 l/min/m^2; actual experimental data is lacking. In conclusion, the use of filtration devices for the purposes of large-scale algae production can not be recommended.

7.4.6. Microstraining

Microstrainers are rotating, backwashed screens, which act as relatively coarse filtration units. The major design and cost factor is flux rate, which is determined by the mesh size and head. Microstrainers are used in water purification, to remove relatively low concentrations (typically < 10 mg/l, but occasionally > 100 mg/l) of planktonic blue-green algae blooms that grow in water supplies. These algae grow as long filaments (like Spirulina) or large colonies, and are easily removed by 20-30 um opening screens. The algae are removed because they produce odors and tastes, and, as recently recognized, toxic materials that are potential health problems. Thus there is considerable experience with such systems, and large units are commercially available.

Microstrainers were studied by several groups during the 1960's and early 1970's for the removal of algae from oxidation ponds. As the algae in oxidation ponds are dominantly small unicellular or colonial green algae, and not filamentous and mat forming blue green algae, results were poor (Table 7.3). Very small mesh microstrainers with openings of 1 to 5 um were tested in the 1970's (Kormarik and Cravens, 1979, see also Shelef et al., 1984) and favorable results and economics were reported. However flux rates were low and costs would be relatively high (although probably not higher than chemical flocculation). In general, microstrainers should be operated with screens openings of above 20 um. The larger the mesh the faster the flux and lower the costs.

This restricts microstrainers to larger colonial or filamentous microalgae. The ability to more easily harvest such algae was recognized already by Tamiya (1957) who suggested the cultivation of Cladophora, and by Bogan (1958) who grew Stigleolcolonium on sewage, although this alga could also be harvested by settling. Benemann et al. (1980) argued that filamentous algae could be purposefully cultivated in waste ponds by a process involving selective biomass recycle (as is practiced, inadvertently, in the activated sludge process). Some success was obtained, but this approach was abandoned when if was noted that the algae would spontaneously flocculate and settle, an even simpler process (see below).

Microstraining is relatively inexpensive. Benemann et al. (1977a) estimated costs at about 1/10 of centrifugation. More recently microstrainer harvesting was proposed for a large (10^6 m^3/day), low algal concentration (25 mg/l), system for removing blue-green algae (and, thus, nutrients) from a cooling reservoir (Wilde and Benemann, 1988, Wilde et al., 1990). Extrapolation of costs estimates (for 35um screens) gives $100-150/t dry weight for this very low initial cell concentration. Using 62 um screens, e.g. if relatively long algal filaments were grown, cells are used, reduces costs by about 50%. Thus, microstrainer harvesting may be considered, if filamentous algae are cultivated. However, that is not considered likely in the near-term, at least in the context of CO_2 mitigation.

7.4.7. Natural Settling

Natural settling is the spontaneous gravity sedimentation of algal cells after removal from the cultivation system. As most algal cells, or even colonies, are rather small, they do not exhibit sufficiently high settling rates to allow their practical (low cost) recovery by simple gravity sedimentation. From the well known Stokes law, only relatively large (> 100 um diameter) colonies or aggregates of cells (flocs) will allow gravity sedimentation, over a reasonable time period, to produce a biomass concentrate (typically 1.5 to 4%).

The term "natural settling" encompasses all phenomena by which algae, after removal from the cultivation system exhibit acceptable settling rates, without addition of flocculating chemicals. Acceptable settling rates are herein defined as at a minimum of 10 cm/hr (Benemann et al., 1982), but preferably several fold higher.

If settling rates are high enough (> 1m/h) continuous processes (clarifiers) could be used. At the lower settling ranges, the preferred process would be batch settling using deep (e.g. 2-3 m) ponds (Benemann et al., 1978, 1982). Natural settling in this context can be either positive or negative (e.g. natural flotation). Natural flotation could be achieved by means of gas vacuoles (blue-green algae) or because of high lipid contents, (Botryococcus).

One issue that should be reviewed, and which involves rather straight-forward engineering analysis, is the cost sensitivity of the settling method to sedimentation velocities. This could give a better fix at the desired, and acceptable, performance of any settling process developed. Various settling options should be considered in such a review, including continuous clarification, batch processes, and lamellar settlers (see Mohn, 1980, among several others).

Natural settling does not specify a mechanism, only a (desired) phenomenon. We can define two basic mechanisms for natural settling: 1. The settling of large algal colonies cultivated in the ponds ("discrete sedimentation"); and 2. The settling of algal flocs formed by a natural process flocculation that algae as all microbes) exhibit as part of their survival strategies.

The latter process has been termed "bioflocculation" in prior work (Benemann et al., 1980), a usage that is in agreement with the general microbiological literature. In most prior work and discussions, the bioflocculation process was assumed to occur after the algae were removed from the cultivation system and transferred to the sedimentation tank or pond. However, general usage and logic would extend this term to any natural flocculation process, not involving external chemical additions, whether it occurs in the pond or outside. Thus, in this discussion,

"bioflocculation" involves an aggregation of cells and colonies into sufficiently large flocs to allow gravity sedimentation.

Although the emphasis and focus in this discussion will be on the bioflocculation processes (in or ex pond), discrete sedimentation, involving the cultivation of algal species or strains that grow as large colonies may be just as good, or even a better, approach to algal harvesting. The species Coelastrum has been discussed repeatedly in connection with the work of Mohn (1980, 1988). This alga grows as a large ball of cells, with rather loose structure (as would be expected to minimize diffusion limitations). Its settling characteristics are not reported, and may not be sufficient. However cultivation of even larger colonial algal strains may be the best method to achieving a very low cost harvesting process.

Indeed, there is some experience with such a process: in a campaign to produce several kg of N_2 fixing microalgae biomass (Benemann, 1986), a Nostoc species was grown in a 100 m^2 outdoor pond which grew as macroscopically visible balls, typically one to several mm in diameter. As would be expected, after removing from the ponds these cultures exhibited very fast sedimentation rates, estimated at about 1 m/h, producing a sludge of several % solids (appx. 2 to 4). This experience suggests that there is no inherent limitation to the growth of such large colonial algae in high rate ponds - the mechanical mixing keeps the colonies suspended until removed from the ponds, when they settle rapidly.

There are, however, some fundamental issues that need to be addressed: large colonies are self-shading, have nutrient uptake limitations (due to diffusion barriers), and there may be trade-offs between algal productivity and colony size. However, these trade-offs are likely to be overall favorable. Even though the remainder of this discussion and report addresses the bioflocculation alternative, development of cultivation technologies of algal species that grow as large colonies should be given further attention in the future. In particular, strategies should be developed to selectively encourage the growth of such algae and consideration given to potential drawbacks to such an approach. Of course, this also applies, to the development of bioflocculation processes.

Bioflocculation as used herein refers to the spontaneous flocculation (agglomeration) of dispersed microbial cells, colonies, or small flocs. This process is, when it occurs, usually observed after the removal of the algal culture from the growth pond, and sequestration for a time in a quiescent container, preferably kept in the dark. However, as stated above, bioflocculation may also occur in the cultivation ponds themselves, with the production of a culture that grows as small flocs, which further agglomerate and settle as soon as removed from the mixed ponds.

Bioflocculation is essentially a biological process, although influenced by the chemical environment in which the algae find themselves and which may be under some control by the pond operator (e.g. nutrient levels, pH, pO_2, etc.). By definition, no chemical additions are involved in the bioflocculation process, although, no doubt, small dosages of flocculants may well be used in practice to facilitate or accelerate the overall settling. This is an issue that needs to be addressed from a practical perspective during the process development effort.

If the flocculation process is dependent on the participation (e.g. flocculation by) the major ions in the culture medium (e.g. Ca, phosphates, etc.) then the process is termed autoflocculation, which is discussed in the next section. However, it should be noted that from a practical perspective the result, and not the mechanism, are what is important.

The general observation is that microalgae cultivated in conventional (unmixed) oxidation ponds remain suspended for long periods after removal from the cultivation system, exhibiting negligible or very modest sedimentation rates. Thus, tests (most, of course, never reported) for settling of microalgae cultures from such ponds, were generally unsuccessful.

The reason for this is rather easy to understand. The pond environment, particularly unmixed oxidation ponds, selects against any algae that settle, and thereby are removed from the photic zone. Even high rate ponds, were continuous mixing prevents ready settling of algae, does not provide any selective advantage to an alga that exhibits bioflocculation. Indeed, even in high rate ponds, the algae may settle out, particularly in larger ones with poor hydraulics and "dead zones". Thus these ponds do not provide any reason for the algae to settle, and settling (bioflocculation) can actually be a detriment.

Nevertheless, algal settling is a well known phenomenon. Algae settle in lakes and oceans, often through a process of bioflocculation, and this is a major factor in their ecology and life cycle (Hutchinson, 1957; Harris, 1978). Even in oxidation ponds settling is a well known phenomenon. Indeed, oxidation ponds are normally designed with additional ponds, above and beyond those strictly necessary for photosynthetic oxygenation, based on the observation that algal concentrations actually decrease in these final "polishing" lagoons. Obviously, even without being observed, algae settle, possibly after having exhausted available nutrients.

Indeed, settling appears correlated with nutritional deficiencies. Thus, N limited algal cultures often clump and settle, although this phenomenon has neither been widely studied nor reported. However, these phenomena were neither clear cut nor reproducible. Due to nutrient regeneration, the polishing lagoons could

exhibit large secondary blooms of algae, or little or no settling would take place for long periods. Nevertheless, there was considerable interest, and some fundamental and applied work (see Pavoni et al., 1971, 1974) in bioflocculation for algae harvesting and removal.

The first application of natural or spontaneous settling ("bioflocculation" in this terminology) as a method to actually remove microalgae from the effluents of conventional oxidation ponds was carried out at the Woodland, California, ponds (Koopman et al., 1979, 1980). Basically the method involved a long (typically 20 days) fill and draw cycle of a terminal settling pond. The process worked remarkably well, in that removal efficiencies for chlorophyll of 80% or higher were achieved in 10 out of 15 runs, and in 4 cases removal efficiencies were over 95%. However, the process could not meet the exacting requirements of waste water treatment, due to lack of sufficient reliability and wind suspension of silt materials (which also count as suspended solids). Of course, such long cycle times would be impractical in any actual algae recovery process for biomass production.

That relatively rapid settling could remove some of the algae some of the time from the effluents of high rate oxidation ponds had also been observed by others. Thus, Golueke et al., 1957 observed significant amounts of "settleable solids" (mostly sewage, but also some algae, and Dugan et al. (1970) reported that a significant fraction of the algae from an experimental pond fed chicken wastes could be settled; however this data was not easily interpretable.

Based on personal communications over the years, settling of algae cultures removed from high rate ponds has been observed quite frequently, but is seldom reported or investigated. A major reason is the irreproducible nature of the phenomenon, it does not occur with any apparent regularity.

The most extensive study of bioflocculation was that of Benemann et al. (1976, 1977b, 1978, 1980), which started with the accidental observation that Micractinum cultures would settle in the sampling containers removed from these cultures. This Micractinium, a rather common microalga in oxidation ponds, originally appeared in small ponds and then also dominated the larger (0.1 ha) pilot scale ponds at this facility (Richmond, Calif.), and persisted there as essentially unialgal culture for over a decade of intermittent operations. Settling characteristics of the cultures were studied as a function of several pond operating variables.

These studies established that there were operating conditions which favored the settling of these algae, and that these operating conditions also allowed maximizing productivities. In over one year of operations of a 0.1 Ha pilot plant demonstrated high (appx. 90%), but not totally reliable, removal efficiencies. However,

relatively little was learned about the fundamental mechanisms involved. More recently Nurdogan (1986) studied various alternative methods for Micractinium settling from the same ponds, including tube and lamellar settlers, which can harvest algae that have relatively low settling velocities. He reported that EPA standards for suspended solid effluents could be consistently met with acceptable overflow rates.

Benemann and Weissman (unpublished) carried out laboratory experiments with several fresh-water and saline microalgae, including Porphyridium and Dunaliella in which the settling of algal cells was studied as a function of various growth conditions. The most important was N limitation, which induced the cells to flocculate and settle. However, other factors were also involved, and N limitation itself was not essential for settling, although it was the strongest single factor.

The obvious advantage of bioflocculation for algal harvesting is that it has low capital and operating costs, if the algae exhibit sufficiently high sedimentation rates (> 20 cm/h) (Benemann et al., 1978, 1982). However, up to now only modest successes have been achieved in controlling this phenomenon, which in most cases is not observed, and when observed can not be reproduced, and when reproduced can not be scaled-up, and when scaled-up is not sufficiently reliable to allow year-round, high (> 95%) removal efficiencies, producing a 50 to 100 fold concentrate (1.5 - 3%).

However, there is sufficient information to clearly demonstrate that algal bioflocculation is a widespread and potentially controllable phenomenon. Economic considerations make the development of such a process of high priority for any microalgae CO_2 mitigation/energy production process.

Indeed, one of the strongest arguments in favor of bioflocculation as the preferred algal harvesting approach, comes not from the limited experience with (discussed above), or the extensive basic algal physiology and applied ecological literature relevant to this phenomenon, but from the wealth of information related to microbial flocculation in various waste water treatment, fermentation and other biotechnological processes. Microbial flocculation is key to the operation of many of such systems, with the activated sludge system being only one of many examples.

In conclusion, this review of bioflocculation concurs with prior studies and analysis that the spontaneous flocculation and settling of algae once removed from the mixed growth pond environment, is both potentially feasible, and sufficiently low cost to be considered for fuel production and CO_2 mitigation. However, two additional processes, autoflocculation and autoconcentration also merit consideration, and are reviewed next.

7.4.8. Autoflocculation

Autoflocculation is phenomenologically similar to bioflocculation: after removal from the ponds the algal culture flocculates and settles. However, mechanistically the processes differ in that autoflocculation depends on the chemical flocculation of the algae by precipitation of calcium and phosphate ions in the pond medium. The term "autoflocculation" was first used by Golueke and Oswald (1965) who observed that during active photosynthesis in shallow ponds the entire algal culture would settle to the bottom. Moellmer (1970) found that autoflocculation was caused by the chemical flocculation of the algae due to the precipitation of calcium, magnesium, carbonate, and phosphate as a result of the high pH in the ponds. Indeed, high phosphate removals from municipal wastes due to algal photosynthesis mediated increases in pH was already noted by Bogan (1960) in laboratory experiments.

A study at the University of California Berkeley, by Nurdogan (1986), studied the effect of lime addition on the settling of Micractinium cultivated in the same ponds studied earlier by Benemann et al. (1980). He found that a concentration of 60 mg/l of CaO added to the pond increased phosphate removal from about 46% (without lime) to essentially 100%, due to the precipitation of various phosphate salts. Addition of 60 mg/l CaO also increased the size of the Micractinium flocs and their settleability, from 70% to 95%+ in a 24 hr settling test.

Flocculation of sewage grown algae by lime addition was studied extensively over a decade earlier (Folkman and Wachs, 1972, and references therein), concluding that the main effect of lime addition was to generate positively charged magnesium hydroxide particles which neutralized the algal surface charges, resulting in flocculation. Ca and Mg ions themselves were not effective. Sukenik and Shelef (1983, Sukenik et al., 1985) studied autoflocculation in some detail in small outdoor ponds in a 2.25 mM $CaCl_2$ and 0.6mM $MgSO_4$ enriched medium. Flocculation was induced by stopping CO_2 supply, which raised pH (from about 7 to 9), and mixing, which resulted in a rapid clearing (95%+) of the culture, with loss of almost all the phosphate and some of the Ca and alkalinity. This was a light mediated pH effect, dependent on the presence of both Ca and PO_4 ions. Laboratory experiments demonstrated that Ca (in the absence of Mg) was effective in autoflocculation at pH above 8.5, but only in the presence of PO_4. Mg mediated autoflocculation required a higher pH but no phosphate. They proposed that positively charged $CaPO_4$ particles neutralized the algal charges, causing flocculation.

These above studies were limited to sewage grown algae. Balloni et al. (1981) reported that seawater grown algae could be harvested by simple sedimentation. Although details are lacking, this may have been due to high pH due to CO_2 limitation.

7.4.9. Autoconcentration

Autoconcentration are processes by which motile algal cells self-concentrate due to their motility and tendency to swim toward the light (phototaxis) or some other chemical attractants (or away from repellants).

Apparently, the first to study of such a process were Miller and Wilke (1972) who used Pandorina, a colonial (16 cell) motile alga. The coordinated beating of the flagella in these algae allows the achievement of considerable swimming speeds, over one cm/min. In principle such a swimming speed would be sufficient to achieve rapid autoconcentration. Laboratory-scale experiments were promising, with 99.9% of the motile cells being removed from the bulk solution, but operational problems, such as the settling of non-motile cells, hydraulic problems, low concentration factors, etc., do not allow a clear interpretation of the results. The conceptual scale-up. suggested, after correction for calculation errors, an overflow rate for a separator of 100 m/d. This is highly optimistic and premature.

Kessler carried out studies of the harvesting of Dunaliella, another flaggelate, through phototaxis, patenting a process involving the entrapment of the motile Dunaliella in a fibrous material (from which it could be washed out) (Kessler, 1982, see also Kessler, 1985). However, his patent does not provide a basis for an engineering scale-up design.

More recently, Nakajima and Takahashi (1991) studied phototaxis by Euglena as a method of algal separation from waste waters. The effluent from an Euglena culture was passed through a "photoclarifier", a darkened vessel with a small illuminated area where the cells congregated. The medium near the light was recycled to the culture vessel and that at the far end (in the dark) was discharged. In one configuration, essentially no cells were observed in the effluent after stabilization of the system (appx. 20 days). They speculated that this was due to selection for highly phototactic cells. Of course, such complete recycling resulted in a very high density in the growth vessel and thus the increase in recycle efficiency could be equally well be due to increasing light limitation. This paper also does not present any specific data useful in process design.

Mohn (1988) in his review of algae harvesting briefly mentions phototactic separations, dismissing it by stating that a 1 m^3/min process would "probably involve hundreds of square meters of controlled illumination and consequently to be economical, applications would require a most exotic and valuable product". However, such a negative attitude is not warranted by the (few) facts and autoconcentration is worth future investigation. In the context of CO_2 mitigation and fuel production motile algae may be of some interest, in particular Botryococcus braunii (Section 11).

7.5. COMPARATIVE EVALUATIONS AND RECOMMENDATIONS

The above review is not exhaustive. For example, low cost natural polyelectrolytes, such as chitosan, extensively studied for algae harvesting (Nigam, 1980; Buelna et al., 1990, and references therein), were not discussed. Neither was the "activated algae" process, nor the effects of oxidants (e.g. O_3) on enhancing algal flocculation (Sukenik, 1987; Betzer and Argaman, 1980). Another ignored process, which deserves some attention, is "foam fractionation" (or "foam floatation"), in which algae are concentrated in the foam generated by bubblers by the attachment of the algae cells to the gas bubbles in the presence of surfactants (Levin et al., 1962; Honeycutt et al., 1983, see Smith, 1988 for a review). However, harvesting efficiencies are low and cost likely high. A microalgae production process proposed by Raymond (1979) and later patented (1982) included foam fractionation. However, this part of the process (like others) was not very successful when process development was attempted by the SERI Aquatic Species Program (Section 4), headed at the time by the inventor. Sometimes the algae produce their own surfactants. In that case, an autofloccuation process could be considered. Surfactants are produced by many blue-green algae and possibly others. Finally, "froth fractionation" could be considered in conjunction with bioflocculation. At any rate, this technique requires further evaluation.

Paradoxically, many, if not most, microalgae harvesting studies have ignored the subjects of the investigation: the algae. They are often treated as homogeneous, uniform and unvarying colloidal particles when in reality they are complex and highly variable, not only between species but even with the same strain exhibiting differing surface charges (a major determinant in harvesting responses), depending on culture condition. The development of a universal harvesting technologies applicable to all algae is unrealistic, except with crude and expensive methods such as centrifugation and chemical flocculation. Even then there are large variations in the amount of, for examples, centrifugal forces or chemical dosage requirements, and, thus, the economics of such processes.

Thus, the first conclusion of this review is that the harvesting process must address the very specific, and often unique, attributes of the production system under consideration. Most fundamentally, no algal harvesting, or production, system can be successful that does not rigidly control algal strains and cultivation conditions. Also, the cultivation process must take into consideration harvesting. The cultivation process may need to operate at somewhat lower than maximal productivities to improve harvesting and optimize overall production economics.

Some of the costs of various algal harvesting methods were discussed above. A more detailed cost comparison of the various harvesting methods would require a major effort and has not been attempted for this report. Indeed, it is almost impossible to compare various processes because of the large differences in their

performance and operating conditions depending on the algal strain under consideration, cultivation conditions, scale, etc. Thus only for specific algal species and production processes could such comparisons be actually made. For some harvesting systems even basic performance standards and equipment specifications on which an economic analysis must be based are unavailable. However, for a preliminary evaluation and ranking of the processes, only a relative, not absolute, economic and performance comparison is required. Table 7.3. presents such a relative comparison based on the above review.

The obvious conclusion is that most processes currently used (centrifugation, chemical flocculation) are too expensive to be considered in a low cost process. Cross-flow filtration is not likely to be operated at sufficiently high flux rates to allow economical operations. Membrane fouling is a major problem. Although very porous, non-fouling membranes were discussed almost two decades ago (Gregor and Gregor, 1978), they appear not to have been further developed.

For high gradient magnetic separation (HGMS) costs were not located during this review, but are not likely to be sufficiently low, except possibly if magnetotactic algae, which are reported (although they have not been documented further), could be cultivated. However, this does not appear promising.

Microstraining may be economically feasible, but probably only with large (> 40 um) mesh screens. A more detailed cost estimate of such a process should be carried out and the feasibility of growing very long filamentous algae (e.g. chain forming diatoms, Cladophora, Spyrogyra, Spirulina etc.) should be evaluated.

Autoconcentration, depending on phototaxis, has not been sufficiently studied, and conceptual design and analysis still requires to be carried out (based on some basic assumptions about swimming rates, eye spot sensitivity responses (algae can theoretically detect single photons!, see Foster and Smith, 1980, for a detailed review) and other factors. Such a conceptual development should be carried out, as autoconcentration could indeed be a very low cost process in the case of the cultivation of marine flagellates.

Autoflocculation may be applicable to seawater systems. This should be able to be determined from a closer examination of seawater chemistry. Seawater is supersaturated for Ca, even after considering chemical activities (which are much lower than concentrations), and considerable $CaCO_3$ precipitation takes place in seawater as a result of microalgal photosynthesis (both discretely on the algal cells and as a general precipitation phenomenon, known as "whiting"). A high pH can be induced in seawater ponds in a matter of minutes after cessation of CO_2 supply. Whether this will result autoflocculation or assist in the bioflocculation of algae is something that could be determined both from a closer examination of seawater characteristics and rather simple experiments.

TABLE 7.3. COMPARATIVE EVALUATION OF HARVESTING PROCESSES
Sources: This report, Benemann, et al. (1977)

Process	Main Mechanism	Major Inputs	Dependence on algae	Relative Cost	Concent. Solids	Energy Inputs
Centrifugation	Accelerated discrete settling	Power Equipment	minor	10	>10%	high
Chem. Flocculation						
Inorganic lime	floc enmeshment	Lime + Mix.	minor	6 - 8	8 - 10%	high
alumn	" + destabilize	Alumn + Mix.	minor	6 - 8	8 - 10%	high
Polyelectrolytes	" + " + bridging	PE + Mixing	minor	4 - 6	8 - 10%	medium
Cross Flow Filtration	Membrane self cleaning	Power Equipment	minor	4 - 6	2 - 6%	high
Microstraining	fabric straining "Schmutzdecke"	Power Equipment	high	0.5 - 1.5	2 - 4%	medium
High Grad. Mag. Sep.	Adsorption of Magnetic Particle	Power Equipment	Unk.	Unk.	Unk.	Unk.
Discrete Sedimentation	Gravity Discrete Settling	Pumping, Clarifier	high	0.5 - 1	1 - 3%	low
Bioflocculation	Spontaneous Flocculation	Pumping, Clarifier	high	0.5 - 1	1 - 3%	low
Autoflocculation	Ca/Mg ppt. induced flocc	Pumping, Clarifier	minor	0.5 - 1	1 - 3%	low
Autoconcentration	Phototaxis	Pumping, Clarifier	high	Unk.	Unk.	low?

NOTES: All figures are approximate and meant to allow relative, not absolute, comparisons and rank.
Dependence on algae: ability to cultivate specific algae suitable for harvesting
Relative Costs: to centrifugation at 10 (arbitrary scale). Actual costs estimated at $1,000 - $1,500/t.
Solids Concentrations: best estimate, cost depend in part on concentration factor.
Unk.: Unknown, not sufficient data for even a guess

Natural settling - bioflocculation and discrete sedimentation - are the methods of choice for developing a low cost harvesting system. The cultivation of large colonial algae and the process of bioflocculation have the greater potential. Froth flotation, and autoflocculation, may be able to assist bioflocculation processes, particularly as, in general, flotation processes produce a higher solids product with higher overflow velocities. However, this is of relatively lower priority at present.

Based on earlier analysis and research (Benemann et al., 1978, 1980, 1982), it was concluded that the only realistic possibility for a low cost harvesting process was to achieve a self-settling culture based on some type of spontaneous settling process. The above review of the alternative harvesting methods and relative economics, summarized in Table 7.3, does not suggest any new alternatives. However, as pointed out above, there are several mechanisms that could result in such a process, in addition to bioflocculation: growing large colonial algae that readily settle ("discrete settling"), autoflocculation and, possibly, autoconcentration, are equally acceptable alternatives. Indeed, methods based on autoflocculation, autoconcentration, and autoflotation could also be of very low cost. However, they require very specific situations, medium chemistry, algal species, and provide significant operating limitations. Thus, the basic conclusion of this review is that the cultivation of large colonial or bioflocculating algae has the greatest potential for developing a low cost harvesting process.

Developments in polyelectrolytes flocculation over the past decade have considerably improved overall performance and costs, making them the preferred option for harvesting algae in any chemical flocculation process. They also seem to be suitable for marine water algae. It appears to be economically feasible to add some polyelectrolytes as flocculation aids to a bioflocculation process, greatly improving the overall performance of the bioflocculation process. This may be cost-effective, and, indeed, this is the method recommended in the next section as an aid for increasing solids concentration in the bioflocculation harvesting. It may, indeed, be economically feasible to use such flocculants in combination with a dissolved air flotation (DAF) unit, to achieve a sufficiently high solids concentration for further processing. This is discussed in the next section.

In the case of microalgae production and CO_2 utilization in conjunction with wastewater treatment, the severe economic constraints faced by stand-alone systems do not apply. The recent advances in DAF systems in combination with polyelectrolyte flocculants, suggests that this process is low-cost enough to be applicable at even relatively small (e.g. 1 MGD) facilities for direct harvesting of microalgae (e.g. without prior bioflocculation). This needs to be confirmed through process demonstration, but both capital and operating costs appear to be acceptable. Indeed, if combined with some modicum of process (e.g. species) control (such as avoidance of very small unicells like Chlorella), through utilization of high rate ponds, such harvesting methods may be represent a viable technology in wastewater treatement. This is a future high priority R&D objective.

8. MICROALGAE SYSTEM DESIGN AND ECONOMICS

8.1. INTRODUCTION

This section presents an overall systems design and economic analysis for a large-scale microalgae production system for CO_2 mitigation. As discussed in Section 1.3.5, such analysis have been carried out previously by the authors and colleagues (Benemann et al., 1978, 1982; and Weissman and Goebel, 1987). Other studies, specifically those of Regan and Garside (1985) in Australia and Neenan et al. (1987) at NREL, were derivative of the Benemann et al., 1978 and 1982 studies, respectively. However, the Australian study presented independent cost estimates and thus is further discussed here. The Neenan et al. (1986) study essentially used the Benemann et al. (1982) designs and cost estimates as a basis for a computer model. Other recent publications (Benemann, 1993, 1994, see Table 1.1, and Ikuta, 1994) are also derivative. The very early analysis (e.g. Oswald and Golueke, 1960, and Benemann et al., 1976) were quite preliminary, although, certainly, Oswald and Golueke (1960) provided the overall concept and many of the specifics on which the prior studies were based (e.g. harvesting by sedimentation, very large unlined earthen ponds, integration with CO_2 obtained from power plants, etc.).

Here the three reports first listed above (Benemann et al., 1978, 1982, and Weissman and Goebel, 1987) (referred to as the "1978", "1982" and "1987" studies) and the "Australian" study (Regan and Garside, 1985) are reviewed, discussed, compared and updated for each major pond system design elements (e.g. pond construction, paddle wheels, carbonation, etc.). Updates and modifications of these designs and new cost estimates are then provided for selected cost elements. This section is not meant to provide as much detail as the earlier studies, but, in the main, has for its objective to verify, where possible, the earlier cost data, select from among alternative design choices, and develop, where required, new cost estimates on which to base the overall economic analysis presented in the conclusion to this section.

Tables 8.1 comparatively summarizes the cost estimates of these four studies, and updates these to 1994$ with suitable inflation factors. The original reports contain many details, designs, and information that are not repeated here. In particular the Weissman and Goebel (1987) report presents a wealth of engineering designs and detailed cost estimates for most major system components, and sensitivity analysis for the major assumptions (e.g. productivity, CO_2 costs, etc.). Thus, the present report does not stand on its own, but builds on these prior efforts. This section concludes with a revised capital cost estimate for the overall pond culture system, including all infrastructures and support systems, for two options for CO_2 mitigation with microalgae: direct flue gas utilization with a microalgal system closely-linked to a power plant, and a remotely sited pond system, utilizing CO_2 scrubbed from the power plant and delivered via pipeline.

TABLE 8.1. SUMMARY AND UPDATES OF PRIOR COST ESTIMATES

Study	Benemann et. al		AUSTRALIAN STUDY		Beneman, et. al		Wiesman & Goebel	
year of study and update	1978	1994	1983	1994	1982	1994	1987	1994
unit area for calculations	100 mi2		100 ha module	100 ha module	809 ha		192 ha module	
cost update factor		1.95 x $1978		x 1.3281		1.41 x $1982		x 1.22
CAPITAL COSTS ($/ha)								
Growth Ponds:								
1. Grading, earth works	1,817	3,543	1,996	2,759	2,270	3,205	9,885	12,060
2. Walls (perimeter, central, etc.)	2,026	3,951	4,118	5,692			7,176	8,755
3. CO2 Sumps	NA		not given		2,780	3,925	1,378	1,681
4. Mixing System	371	723	969	1,339	2,471	3,489	4,919	6,001
5. Carbonation System	403	786	2,083	2,879	see 11 & 12		1,830	2,233
6. Instrumentation (Miscellaneous)	see note		not given		not given		500	610
7. Primary Harvesting (Settling Ponds)	see note		4,361	6,027	4,942	6,977	7,406	9,035
8. Secondary Harvesting	see note		included in primary		6,919	9,768	3,906	4,765
Subtotal	**4,617**	**9,003**	**13,527**	**18,696**	**19,382**	**27,364**	**37,000**	**45,140**
System-wide Costs								
9. Water Supply System	not given		455	629	618	872	3,473	4,237
10. Water Distribution (piping &/or channels)	1,586	3,093	2,985	4,125	see line 9		984	1,200
11. CO2 Delivery System to Module	not given		155	214	4,510	6,367	not given	
12. CO2 Distribution System to Ponds	321	626	5,620	7,768	1,587	2,241	260	317
13. Nutrient Supply to System	not given		not given		371	524	781	953
14. Building, Roads, Drainage, etc.	25	49	184	254	3,460	4,885	1,094	1,335
15. Electrical Distribution and Supply	350	683	640	884	3,364	4,749	1,922	2,345
16. Machinery	not given		10,562	14,598	not given		417	509
17. Extraction Process Equipment	not given		included in 16		3,707	5,234	not given	
18. Anaerobic Digestion System	not given		not given		not given		10,455	12,755
19. Blow Down Disposal System	not given		not given		not given		833	1,016
Subtotal	**2,282**	**4,450**	**20,601**	**28,473**	**17,617**	**24,871**	**20,219**	**24,667**
Other Capital Cost Factors								
20. Engineering (10% of total above)	690	1,345	3,413	4,717	3,700	5,223	5,722	6,981
21. Startup costs (5% of PFI)	213	415	1,706	2,358	1,850	2,612	2,861	3,490
22. Contingencies (15% of 1-21)	1,138	2,220	5,887	8,137	6,382	9,010	9,441	11,518
Subtotal	**903**	**1,761**	**11,006**	**15,212**	**11,932**	**16,846**	**18,024**	**21,989**

Table 8.1. (Continued)

CAPITAL COSTS TOTAL	**7,802**	**15,214**	**45,135**	**62,380**	**48,931**	**69,080**	**75,243**	**91,796**
OPERATING COSTS ($/ha/yr)								
23. Power (mixing, harvest, misc.)	309	603	159	220	513	724	1,850	2,257
24. CO2 (flue gas) blower power	not given		62	86	1,976	2,790	not given	
25. Pure CO2 Supply	not given		not given				8,650	10,553
26. Nutrients (N, P, Fe - 50% recycle)	69	135	269	372	1,560	2,202	2,260	2,757
27. Maintenance (3% of total Capital)	234	456	1,354	1,871	1,468	2,072	2,257	2,754
28. Labor	253	493	345	477	1,347	1,902	1,390	1,696
29. Flocculent	not given		not given		not given		1,120	1,366
30. Other Primary Harvesting Costs	not given		9,661	13,352	not given			
31. Secondary Harvest Costs	not given		1,278	1,766	712	1,005		
32. Salt disposal	not given		not given		not given		1,130	1,379
33. Extraction cost of fuel	not given		6,351	8,778	335	473	not given	
34. NET OPERATING COSTS $/ha/yr	**865**	**1,687**	**19,479**	**26,922**	**7,911**	**11,169**	**18,175**	**22,173**
COSTS $/BARREL OF OIL:								
35. Total Production (mt/ha/year)	**45**	**45**	**73**	**73**	**67**	**67**	**112**	**112**
36. Productivity assumption	12 g C/m2/d		20 g C/m2/d		18.5 g C/m2/d		30 g C/m2/d	
37. Barrels of Oil/ha-y (@ 3.5 Bar./t)	**158**	**158**	**256**	**256**	**235**	**235**	**393**	**393**
38. Annualized Capital Costs (0.2 x Cap./bar.)	10	19	35	49	42	59	38	47
39. Net Operating Costs/barrel	5	11	76	105	34	47	46	56
40. Depreciation (apportioned per barrel)	2	4	11	15	15	21	10	12
41. Taxes and Insurance per barrel	2	3	8	11	9	13	8	12
42. TOTAL COSTS $/ BARREL	**19**	**28**	**130**	**180**	**99**	**140**	**103**	**146**

8.2. REVIEW OF PRIOR FEASIBILITY ANALYSIS

8.2.1. Introduction.

Table 8.1 demonstrates order of magnitude differences between the first (1978) and latter (1982, 1987, Australian) studies, with updated costs ranging from $25 to $231/barrel of oil produced. A closer look reveals that in many subsystems similar almost order of magnitude differences in capital and operating costs exist. And the fundamental assumptions about productivities also differ, by over a factor of two. And there are also differences in the studies in what was included or not. Thus, for example, the 1987 study did not address the question of biomass processing (e.g. for lipid extraction), stopping, rather, at the "biomass precursor" stage. Clearly, a more detailed comparative review is appropriate.

8.2.2. Growth Ponds.

This category include site clearing, grading and leveling (with laser graders), and berm or channel dividers (also called walls, levees, etc.) construction. All of the reports assumed unlined (with plastics), earthen ponds. This is because it is prohibitive to consider any type of plastic liner, which would essentially double capital costs. The 1982 study was of much lower cost than the first two in this regards, while the 1987 study was almost seven fold higher. That was because the 1987 study incorporated a crushed rock liner of about 1.5 to 2" (4 to 5 cm) thickness, and concrete wall construction, both of which are very expensive. There is no evidence that either will be required, unless much higher mixing velocities (> 30 cm/sec) than anticipated, and allowable from a power consumption perspective, are designed for. The 1982 study used earthen berms, constructed according to rice field construction, without any erosion prevention. The 1978 study included gunnite protection of the levees. These items exemplify the differing, design options selected by these various conceptual studies. It also calls into question which option, if any, should be used in the present analysis.

Perhaps one reason for the high cost design choices in the 1987 study was that this team was at the time proposing to construct a small system at Roswell New Mexico and wanted to use concrete wall construction in their pilot plant design. By contrast the 1982 study chose only earthen berms as the lowest cost option, even though, clearly, without protection at the water line, there would be a significant risk of erosion and weed growth, resulting in added upkeep costs. In rice cultivation the berms and walls are reconstructed every few years. The assumption was made that the larger walls and berms specified in the 1982 design would last for longer times. This is, however, in the absence of specific information, probably too optimistic. Several other issues in pond construction differed between the studies, from initial field clearance to laser leveling and percolation control.

8.2.3. CO_2 Supply and Transfer Systems

This category includes several components: the CO_2 supply system (e.g. pipes to the ponds from a power plant, in case of flue gas, or a storage system, for pure CO_2); the internal distribution system to the ponds, and CO_2 transfer systems (a sump or cover) and the carbonation system (the diffusers, or spargers, with pH controllers, etc.). (Items 3, 5, 11, and 12 in Table 8.1). How to supply CO_2, from what source, and at what concentration, are all issues that differ among the studies.

The 1978 study assumed that CO_2 could be transferred into the ponds by simply covering the paddle wheels or a small part of the pond (the latter system actually used commercially until recently at the Spirulina production facility of Earthrise, Inc., in S. California). However, this design, and the low cost estimated for the entire carbonation system are probably unrealistic.

The 1982 study considered various options, and selected sumps (with the CO_2 injected counter-currently), as the best option. Although the report does not specify the depth for such sumps, being uncertain at that time) the cost estimates were based on 10 ft deep sumps. The 1987 study, used 1.5 m deep sumps for transfer of pure CO_2. More recent information (J. Weissman, unpublished) derived from operation of 0.1 ha ponds and pure CO_2 transfer systems in 1 m deep sumps, suggests that for countercurrent flow a 1.5 m depth would suffice for flue gas CO_2 transfer. At any rate, the use of pure CO_2 assumed in the 1987 study is much cheaper to transport to and transfer into the ponds than the flue gas CO_2 only considered in the 1982 cost estimate. In particular as that design used a 3 mile distance for the ponds from the power plant, a major capital and operating (power for blowers) cost.

Recently, Kadam (1995) at NREL analyzed the cost of scrubbing (MEA process) flue gas to produce a concentrated CO_2 that would be piped at 1,500 psi over a 100 km pipeline to the algal ponds, and compared this with compressing and piping flue gas directly. His analysis concluded the cost of CO_2 delivery from a 500 MW coal-fired power plant was \$40.5/mt CO_2 for purified CO_2 and \$57.2 for direct flue-gas (increasing to \$57.2 and \$88.0 for a 50 MW power plant scale). Clearly, for any significant pipeline distance, the purification option is preferred. However, as discussed in Benemann et al. (1982), there is a tradeoff between distance, pipe diameter, and flue gas compression, which would favor relatively little compression and large pipe diameters for short distances. Indeed, the amount operating costs in the Kadam (1995) study for the flue gas compression option suggest that the CO_2 emissions consequent to the electrical power consumption would exceed the CO_2 fixed in the algal biomass. Clearly, use of flue gas is only an option close to the power plant, with purified CO_2 being feasible at a longer distance. In the case of purified CO_2 utilization, the algal process would compete with other disposal options, such as storage of CO_2 in depleted oil and gas wells, or ocean disposal.

8.2.4. Mixing

In all cases paddle wheels were the mixing devices, with estimated costs increasing with the date of the studies. The 1982 study provided no specific information to back up the cost estimates, referencing only internal studies. These were updated and extended in the 1987 study, which presents detailed designs and cost estimates. Air lift pumps were also considered, but rejected, as providing no advantage in terms of energy efficiency, and having higher capital costs and less flexibility.

8.2.5. Harvesting and Processing.

Again the 1982 and 1987 reports are the most applicable, and have essentially the same design: a primary harvesting pond followed by centrifuges. However, the 1987 report added a secondary thickening (with added chemical flocculant) stage between the primary settling and centrifugation stages, which saves on subsequent centrifugation costs. The Australian study assumed use of sand beds for secondary harvesting and drying the algae, but that is unlikely to be workable. The 1978 study did not include a secondary harvesting step for further concentrating the algae after settling. Although questionable, the 1978 study only considered methane fermentation of the algae, not requiring as high a solids concentration as processing for lipid extraction.

Cost estimates for processing of the algal biomass to extract the lipids for use as fuels, is one of the major deficiencies of all these prior studies. The Australian study lumped these costs into "machinery", which were not clearly specified. The 1982 study based costs on three times the costs of a soybean extraction plant, a dubious proposition, considering the moisture difference ($< 10\%$, vs. $> 80\%$, a 40 fold difference in actual water to lipid ratio!). The 1987 study sidestepped this issue, by only addressing "lipid precursor" (e.g. biomass) production.

The Neenan et al. (1986) report, which emphasized the algal biomass to fuel conversion processes, also specified a soybean process, without discussion of its applicability. They called for evaluation of alternative solvents, but that approach is not credible, as from laboratory experiments the solvent to biomass ratios required are very high, for any solvent tested (and all likely ones have been), and the extraction conditions, required to recover lipids from microalgae would be difficult to scale-up (Benemann, et al., 1984). Thus, in this most critical area, no credible technical approach has been developed previously. In this regards, it Neenan et al. (1986) study used a fixed composition biomass (30% lipid, 20% carbohydrate, 32% protein) for all the conversion processes, a composition neither optimal for lipid production nor realistic for ethanol recovery, the two major products of interest. Also, the actual costs for processing lipid triglycerides to higher value fuels (e.g. methyl or ethyl ester biodiesels, gasoline) were not provided.

More fundamentally, as input process and cost parameters in Neenan et al., 1987, were embedded in a computer program, with results reported as single parameter sensitivities, or as pie-charts giving only % costs, that analysis is difficult to evaluate. Model inputs and cost routines, and many outputs, were not specified and in the sensitivity analysis, interactive effects may not have been accounted for. Further, this report was based, in essentially all design and cost inputs, on the earlier analysis of Benemann et al. (1982), which has since in large part superseded by Weissman and Goebel (1987). Therefore the Neenan et al. (1986) analysis, and recent derivatives thereof (e.g. Kadam, 1994), are not evaluated below.

8.2.6. Other Capital Cost Factors.

There is large variability between the studies in what components were included. For example, only the 1987 study reports on waste disposal options ("blow down system"). Indeed that report also used a rather high cost for water supply, though probably realistic for brackish ground water drilling and pumping.

There was also variability in terms of indirect capital costs, such as contingencies and engineering. Table 8.1. used standardized indirect capital costs (engineering at 10%, contingencies at 15%, and start-up, or working capital, at 5% of capital costs), to minimize variability due to different assumptions in the original studies. However, these factors can, reasonably, be questioned, as being too high for a project of this nature.

8.2.7. Operating Costs.

Considering the diversity of studies, the overall operating costs are fairly uniform (except for the 1978 study, which is not complete) (Table 8.1). However, the overall agreement is coincidental, with major differences in details. Also, some of these costs need to be recalculated. For example, power costs, assumed in the 1982 study at \$0.10 /kWhr, should be deflated, not inflated. In that study, a major cost factor is for flue gas transfer by a 3 mile (appx. 5 km) pipeline, both in capital and operating (power). Power costs for the flue gas transfer amount to over half of the power cost.

The cost of 100% CO_2 used in these studies was the largest operating cost, over half of the total in the 1987 study. If this could be avoided or minimized, or, even, a credit obtained for CO_2 utilization, then costs would be greatly reduced. A major objectives of this report is to determine not the cost of algal oil (which would be set by competitive fossil fuel prices) but the necessary CO_2 mitigation credit required to make this technology cost competitive with present fossil fuel prices.

8.2.8. Conclusions

The 1982 study provided a preliminary comparison (albeit imperfect) between the use of flue-gas and pure CO_2 utilization. The two options had similar costs, depending on the actual cost of pure CO_2 and other assumptions, most critically the alkalinity of the medium. In recent reviews, Benemann (1974, 1975) favored the direct flue gas utilization approach, as being lower cost. Kadam (1995) at NREL recently reported on the second option, the capture, concentration, and delivery (over 100 km, via pipeline) of CO_2 from a coal-fired power plant to an algal pond system. The cost of the CO_2 was estimated at $40/mt for a 500 MW power plant, and almost 50% higher if flue gas were compressed and transported over this distance (and a further 50% increase in both costs for a 50 MW power plant scale). Clearly, the flue gas option is favored only at short transportation distances, and requires suitable land near the power plant. It is, however, not clear how near this must be. The 3 miles (5 km) assumed in Benemann et al. (1992), may already at, or even beyond, the limit of cost-effectiveness. Also, the Kadam (1995) estimates were not CO_2 mitigation (avoidance) costs, which would have been considerably higher, due to the CO_2 emissions resulting from these processes.

Even though the concentrated CO_2 -remote location case would, indeed, be more expensive than the flue gas-nearby sited option, remote siting (up to a few hundred km) would increase the potential applicability of this technology by well over an order of magnitude. In that case the comparative economics would be the cost of alternative CO_2 disposal options: ocean dumping, permanent sequestration underground in oil or gas wells, or even aquifers, for examples. This issue is addressed again, in the conclusions to this section. However, clearly, the costs of growing microalgae on both pure CO_2 and flue gas must be evaluated.

Final costs per barrel of oil in Table 8.1 reflect differences in not only process design and engineering, but also productivity assumptions, and do not reflect either methane or CO_2 credits (with the latter, of course, not an input but an output of the present study). Tables 1.1 and 1.2 presented two versions, the first with a CO_2 credit yielding a cost for the oil, the second with an oil cost (e.g. $25/barrel, to give the necessary CO_2 credit. One potential problem with the comparative assessment presented in Table 8.1. is that all the studies could be considered derivative of the first, and even earlier ones (see Benemann et al., 1982, for a review of those cost analysis). However, even a casual inspection of the original reports would reveal even if so, this did not influence the designs or conclusions of those studies, which can, indeed, be considered independent efforts. The 1987 study by Weissman and Goebel is clearly the most complete and detailed, and perhaps the least optimistic. It is used here as the major source of data. The next section reviews some of the critical design issues, presents new design and cost estimates for some subsystems, as well as for the various cost estimating factors (e.g. contingencies, engineering, etc.), and incorporates information on the use of flue-gas, to arrive at a final cost of CO_2 mitigation with microalgae.

8.3. PROCESS DESIGN AND CAPITAL COST UPDATES

8.3.1. Introduction

From the above, considerable progress has been made since the first cost estimates of producing microalgae for commodity foods and feeds were published four decades ago (Tamiya, 1955, and Fisher, 1955).

Any generic and conceptual cost estimates must be based on engineering and biological assumptions that must be validated by future R&D and site specific designs. Indeed, these feasibility and cost analysis serve most importantly to target future engineering and biological R&D priorities. For example, the 1987 study served as a basis for the design of the Roswell Test Facility of NREL (Weissman et al., 1989), and subsequent studies at that site (Weissman and Tillett, 1990). There several of the assumptions were tested and confirmed, or, in some cases, actually improved upon:

- Unlined ponds were not significantly different productivities than lined ones;
- The projection of a 30 g/m^2/day average productivity is reasonable (at least for a more favorable climate where ponds do not freeze for several months a year);
- The efficiency of CO_2 transfer can be practically 100% in shallow (1 m) sumps;
- Demonstration of stable cultivation of particular strains in these ponds.
- Confirmation that power inputs for mixing the algae is indeed very low;
- Initial work on producing a high lipid algal biomass in algal mass cultures.

The limited funding of that project did not allow a more comprehensive effort. For example, the New Mexico project did not examine the harvesting of the algae, which would be required for studies on water use efficiency (e.g. recycle), and carbon and nutrient recycle. However, certainly, this work provided a major foundation stone to the technology of microalgae mass culture for commodity products, greatly increasing the confidence in the present effort.

There is a scale-up by a factor of one hundred, from the about 0.1 hectare pilot ponds used at Roswell, New Mexico, to the 10 hectare ponds specified in the present study. Such a scale-up is speculative. Major uncertainties are wind fetch and other hydraulic effects. These will need to be studied in the future in an actual pond, as hydraulics can not be easily modeled. However, there are a few examples of very large raceway ponds, of over 5 hectares, such as the Hollister pond and, perhaps most important, one of the ponds at Earthrise Farms in S. California (Figure 2.3), constructed in the early 1990's. Thus, the pond scales used in the prior (and current) designs are not beyond current experience.

With this background, an updated systems design and capital cost estimate is presented here, using as basic input the productivities assumptions in Table 1.1. Operating costs are considered separately in the next subsection. It should be noted that all costs quoted below have been updated to 1994$ from the originals.

8.3.2. Growth Ponds: Site Preparation, Grading, Percolation Control.

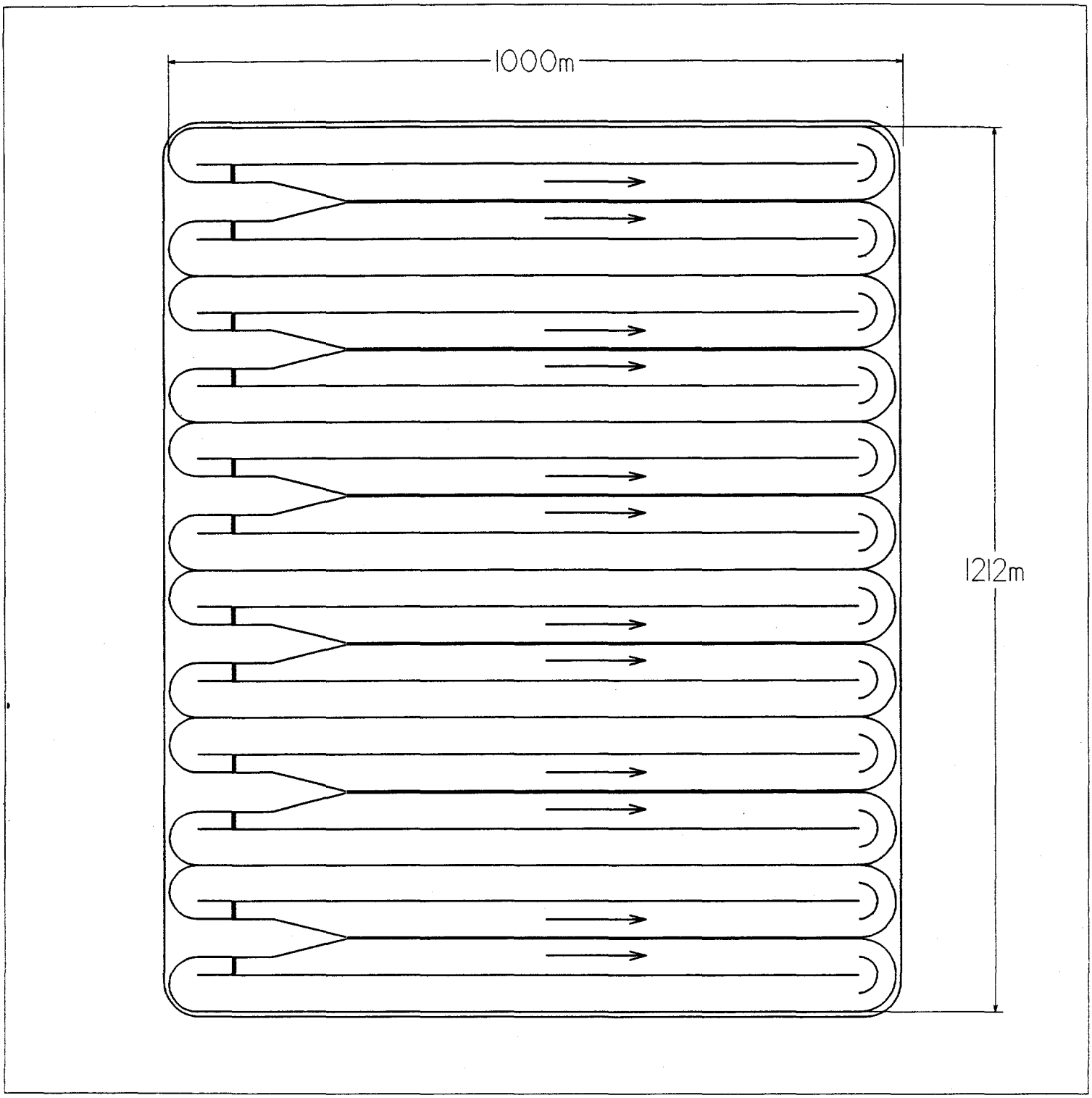
The algal growth ponds, 10 ha each, in modules of 10 such ponds (Figure 8.1), by size alone, dominate the overall system design. Four such modules are grouped together in a unit with harvesting and other common facilities, similar to the scale used in the 1982 and 1987 reports. The major assumption made in all prior studies is that for low cost production the ponds would not be lined with plastic (as is practiced for most commercial microalgae culture), and, at most, could only be provided with a minimal clay layer to reduce percolation rates, and/or possibly an overburden to reduce silt suspension. The cost of plastic liners, at a minimum, even for very large scale systems, of about $\$5/m^2$, installed, exceed the maximum allowable capital costs for a CO_2 utilization/fuel production process. Thus, in this, as in all prior studies, plastic liners are dispensed with. However, percolation and silt suspension remain central issues in the design of the ponds.

Percolation depends on the soil type (e.g. clay to sand ratios) and structure. Experience with both conventional and high rate waste treatment ponds, both in the U.S. and Israel, suggests that such systems self-seal, if the sand content is not too high. Self-sealing also was observed in the unlined pond operated in New Mexico by Weissman and Tillett (1990, personal communications). It should be possible to assist such a self-sealing process by application of a thin layer of clay applied to the surface, or by suspension of clay in water, applied to the ponds until percolation decreases. This latter was suggested in Benemann et al. (1978), but it is uncertain how widely applicable this technique would be.

Silt suspension is another problem. From the literature (Vavoni, 1977), mixing velocities of about 10 to 25 cm/sec would be an acceptable range for ponds, where silt suspension would be avoided while sedimentation of organic solids would be prevented. However, again, there is little experimental support. The 1987 study specified a crushed rock liner for their ponds to prevent silt suspension. However, this factor accounted for most of the four-fold increased costs for the basic earthworks in this study, compared to the earlier studies (see discussion below). And the actual need, or effectiveness, of this technique is at best uncertain. Thus, herein, the only method for sealing ponds is the compaction of the pond bottom after laser leveling.

The first step in pond construction is site preparation: removal of vegetation (trees, shrubs), large (and small) rocks, and other impediments, and rough cut and fill to level the land. Such costs can be quite variable. In Hawaii, where algae ponds are build on lava fields, this initial leveling is quite expensive, at a reported cost of about $100,000/hectare. By contrast, in the California Central Valley, where most land has little slope, and much is already leveled for agriculture (but often not used due to limited water allocations), site preparation and rough grading would be of minimal cost, only a few hundred dollars per hectare (for a $< 0.1\%$ slope land).

FIGURE 8.1. 100 HECTARE POND MODULE LAYOUT



The 1987 study estimated a rough grading cost range of from $0 to $5,000/ha, and used a figure of $1,400/ha. By contrast, the 1982 study assumed only $175/ha (plus twice this for site surveying, a cost not mentioned in the 1987 study). Clearly, any figure from near zero to many thousands of dollars a hectare would be plausible, depending on site characteristics. For purposes of this study a total site preparation cost, including surveying, of $1,000/ha is assumed. The large uncertainties about costs on this item indicate the difficulties of such a generic analysis.

The next issue is the cost of laser grading. The 1978 report ignored this cost, the 1982 report provided (always in updated figures to $1994) a cost of about $600/hectare. The 1987 study used a figure of $1,100/ha for laser leveling of rice fields and then assumed (without stating a basis for this assumption) that for pond construction a higher cost, of about $3,000/ha, would apply, due to the more rigorous tolerances in slope that would have to be achieved. But an independent private contractor (Dennis Hennigan, Dixon, California, 1994, personal communication) provided an estimate for the present study of $1,000/ha for a large job, such as herein contemplated, without the need for higher costs for the design features (e.g. channelized leveling with build-up of berms in between channels). An even lower cost would be likely if this job were carried out with purchased or leased equipment (as any large system would be build in modules over a number of years), but this is not considered here.

As already mentioned above, the 1987 study estimated about $5,000/ha for the cost of providing a 3 to 5 cm crushed rock layer, specified to reduce the suspension of silt from the pond bottom. There is, however, little basis for this. Certainly in some areas of the pond bottom, such as near the paddle wheels and carbonation sumps, or possibly at the bends, some provision of erosion control must be provided, in the form of plastic liners or gunnite. And some clay liners may be necessary to help seal the ponds. However, a crushed rock layer over the entire surface of the ponds is probably unnecessary, and, anyway, unlikely to be economically or even logistically feasible. (The amount of crushed rock required would be indeed large). For the present study, a total cost of $500/ha is assumed for pond bottom erosion and percolation control. Pond bottom compaction would cost about $100/ha, based on U.S. Soil Conservation worksheets that a 200 HP diesel tracklayer, costing $66/hr (including labor @ $6/hr) can compact 1.75 ha/hr, and assuming three passes for final compaction (John Sinkovits, Soil Conservation Office, Colusa, CA, personal communication, 1994). Thus, $500/ha would leave some room for additional percolation minimization measures.

Total costs for site preparation and rough leveling ($1,000/ha), laser grading ($1,000/ha) and percolation and erosion control ($500/ha), are, thus, estimated at $2,500/ha. Of course, these estimates could vary significantly depending on site specific conditions, but appear reasonable for many favorable sites.

8.3.3. Pond Walls and Levees

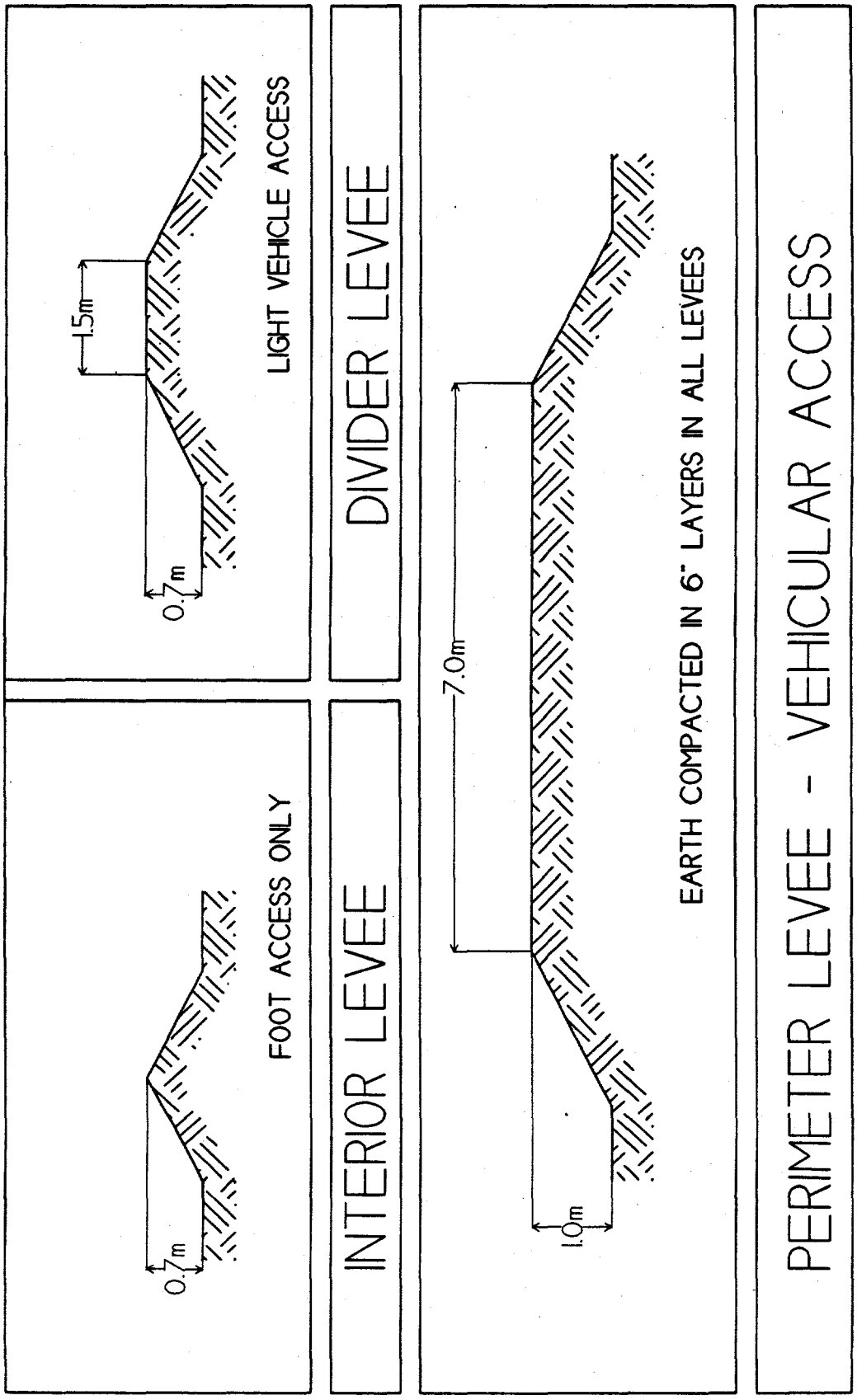
The next major issue is pond walls and levees. The 1978 and 1982 reports used earthwork berms, similar to those used in rice cultivation (from where much of the technology for large pond construction has been adapted). The assumption in the 1982 report was that the cost of the earthworks (walls and levees, also called berms) would be included in the cost of the rough and fine (laser) grading, which would move the excess dirt from the cut and fill operations to form the pond walls (side and central berms). The 1978 report had used a similar approach, but used gunnite (a thin layer of concrete with chicken wire to hold it in place) to protect the berms and walls from erosion. This increased earthwork costs considerably.

The 1987 report, based on an evaluation of various alternatives, selected slipform poured concrete walls and dividers (baffles) as the design choice, because these were only about 10% more expensive than earthen berms with gunnite protection, in these estimates. For the curved portions of the walls and berms they specified corrugated walls, with an average cost of about $25/meter. This resulted, for their pond layouts, of almost $9,000/ha for the walls (perimeter central, etc.). Overall, the cost differentials between the 1982 and 1987 studies is about 7 fold for the actual pond construction (earthworks and walls).

An independent cost estimate was carried out for the present study. It was assumed that levees (perimeter around the ponds, dividers between ponds, and interior baffles), of various crown width (to accommodate access by equipment, as needed) and with 1:2 slopes were build up from the soil, in 15 cm layers, compacted with a 200 HP tracklayer. Crown widths varied from 7 m for the perimeter levees that access the paddle wheels (to accommodate a road), to 1.5 m for the divider levees, to allow light vehicle access, to no crown (allowing only foot access) for the interior dividers (baffles). (Figure 8.2) (It should be noted that as the ponds are very shallow, foot access to any part of the pond is possible with waders). The major innovation in the present design is a geotextile cover over the slopes, to prevent erosion, including due to burrowing animals and plant growth.

Costs were estimated based on a simplistic layout (Figure 8.1) of ten 10 ha ponds in a overall unit, which then provided the length of the different levees (5,000 m perimeter, 4,000 m divider, 12,600 m interior). The tracklayer would pull a levee forming attachment. A bulldozer would also be required to help build-up the levees. Based on estimates on the speed with which this equipment can be operated (0.3 km of levee/hr), and hourly costs ($60/hr tracklayer, $6/hr labor), and the number of runs required to build up a levee (5, 6, and 7, for interior, divider, and perimeter levees, respectively), the total estimated cost to build the levees would be about $300/ha, plus $100/ha for compaction runs (assuming a 2.5 km/hr rate for the compaction runs). However, these costs are a bit soft, and a $500/ha cost is assigned to the building of the earthen levees.

FIGURE 8.2. CROSS SECTION OF POND LEVEES



The geotextile support was selected based on vendor contacts (Chad Weigmann, Gundle Liners, 1994, personal communication), and was based on a cost of $\$2.2/m^2$ ($\$0.20/ft^2$) for a "Polyfelt" material. This material can be purchased in 14 foot wide rolls and would be placed along and over all pond perimeter, divider, and interior levees, as well as along the pond bottom near the paddle wheels, to prevent erosion. The total amount of geotextile required for a 100 ha module was estimated at 1.25 million square feet, giving a cost of $2,500/ha.

Installation of the geotextile would include trenching to secure (bury) it (Figure 8.4). This requires a trenching machine (at $25/hr, including labor) operating at about 1.5 km per hour. About 43.2 km of trenches are required for a 100 ha module, or 38.6 hr, or giving a total of $725 for 100 ha. A tracklayer and three laborers (total of $84/hr) were estimated to be required to lay the geotextile, at a rate of 0.5 km/hr, for a total of 21.6 km/100 ha, at a cost of $3,629 for the entire system (100 ha). Backfilling the trench after laying the geotextile would be slower, estimated to require 3 times longer than trenching time, to prevent damage to the geotextile. Total cost for liner installation thus are about $150/ha. These costs were based on the estimated efficiency of the use of the machinery, from estimates provided by the Soil Conservation service. However, these costs are uncertain, a large contingency is added, for a total geotextile installation costs to $500/ha.

In conclusion, the costs of walls and berms, with geotextile protection, are estimated from the above at $3,500, which allows for some safety margin (such as the need for flow deflectors, not included). The expected lifetime of the geotextile material is over ten years, so no replacement is included in the estimate. For comparison, these costs are similar to the 1977 estimate (with gunnite protection of the berms), over twice of the 1982 estimate (no protection), and less than half of the 1987 estimate (which used slip-form concrete walls). The use of geotextiles is considered adequate and realistic for present purposes.

8.3.4. Mixing

A six bladed paddle wheel is specified (Figure 8.4), spanning a 26 m narrowed section of the ponds. The 1987 study has relatively detailed cost estimates for such paddle wheels (based on the 1982 study, which did not report the details), slightly wider (32 m) and for a somewhat smaller pond (8 ha), with a cost (as always herein updated to 1994$) of $6,000/ha. A cost reduction adjusting for pond area, would reduce the cost to about $5,000, used here. A further reduction to adjust for width is not considered here, as it would be minor. The design of paddle wheels is still advancing. For example, the paddle wheels of the Cyanotech ponds meet the water at an angle. Eight bladed paddle wheels may have an advantage in terms of efficiency of transmitting mechanical power to hydraulic water movement. The motor controllers could be improved. However, overall, no great reduction in costs, capital or operating, are anticipated, and this issue is not further addressed.

FIGURE 8.3. CROSS SECTION OF GROWTH POND WITH LINER

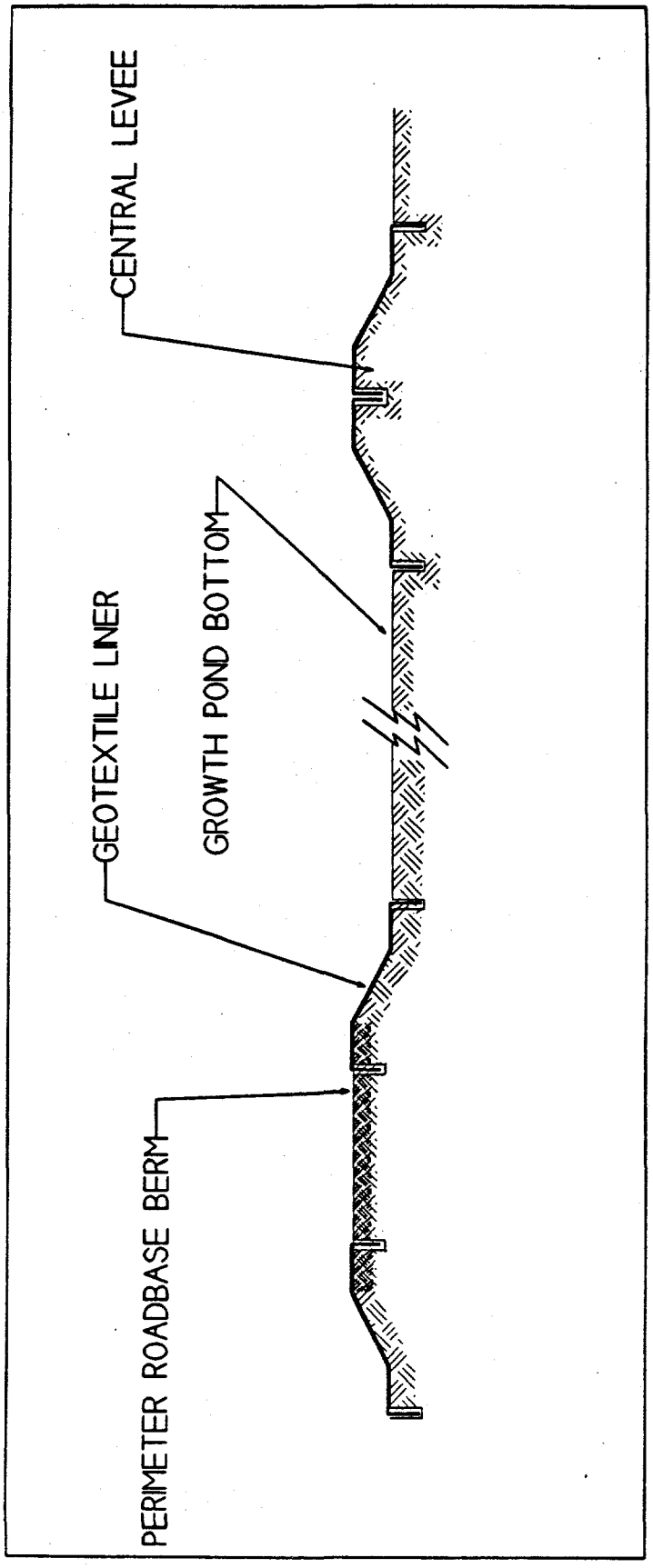
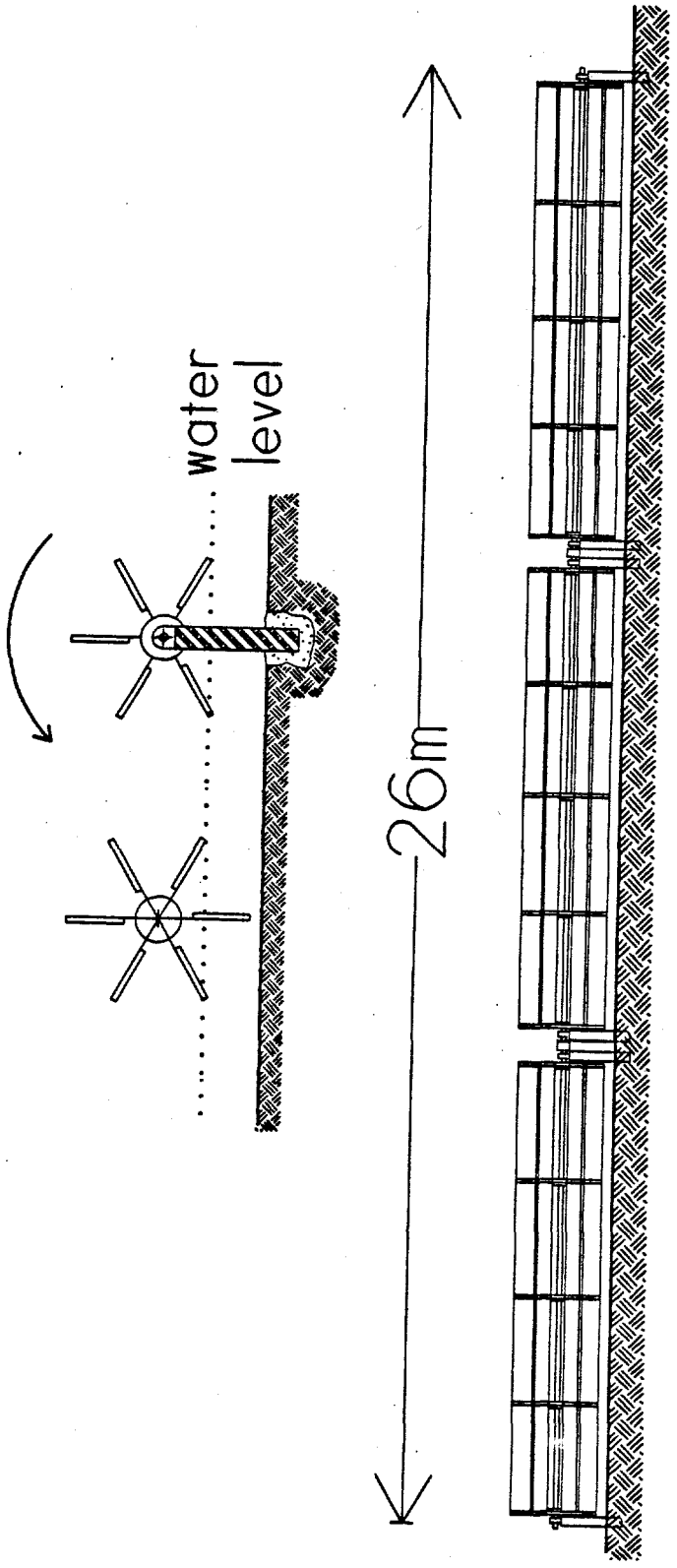


FIGURE 8.4. GENERAL SCHEMATIC OF PADDLE WHEELS



8.3.5. CO_2 Transfer Sumps and Carbonation

The transfer of CO_2 into the ponds has been the subject of much analysis and development. In small-scale systems, where efficiency and costs are not major concerns, simply laying a bubble diffuser tube in the bottom of the pond, to supply pure CO_2 when a selenoid is actuated by a pH meter, is sufficient and satisfactory. Indeed, even such a rather small depth (20 - 25 cm) of liquid, results in remarkable efficiencies for CO_2 transfer (over 85%, with a fine bubble diffuser). However, for large pond systems, where large amounts of CO_2 must be supplied at a fixed point, and, in particular, for the use of flue-gas CO_2, which contains at most 15% CO_2 (v/v, about 20% w/v), and thus exhibits a proportionally smaller driving force, such simple systems are not adequate. (See Weissman and Goebel, 1987, for a analysis).

A number of different carbonation systems were suggested in the 1982 study, including sparging under a surface cover (as was practiced for several years at Earthrise, although this has, apparently, recently been discontinued), sparging under a submerged inclined baffle (allowing for counter-current flow), gas transfer under covered paddle wheels, gas transfer by means of airlifts (which could also double as mixing devices), and sparging in sumps, including sumps with a baffle that directs the flow into one side of the sump, and out of the other, achieving a counter-current effect. The 1987 study reviewed some of these options in some detail. The options selected was a sump without counter-current flow, also the method of choice, as also was the case in the 1982 study. (The 1978 and Australian studies do not discuss these costs).

In contrast to the earthworks and walls, where the 1987 study had three times the costs of the 1982 study, in the case of the CO_2 supply (carbonation) system, the earlier study presents three times higher costs than the latter one. The reason was the much greater sump depth in the earlier study, about 3 meters, vs. only 1.5 m in the 1987 study. Of course, the latter used only pure CO_2, vs. flue gas in the 1982 report. However, experimental data from the Roswell plant (Weissman and Tillett, 1992), demonstrated an essentially 100% CO_2 uptake efficiency by 1 m deep sumps. The 1.5 m sump depth of the 1987 study should be sufficient for a 95 + % transfer efficiency for flue gas CO_2 (Weissman, personal communication), even without a counter-current flow arrangement.

To be conservative, the basic system for CO_2 transfer into the ponds, for both flue gas and pure CO_2 specified herein is a 1.5 m deep sump, with a baffle, with the CO_2 sparger at the downflowing side, for counter-current contacting. This maximizes transfer efficiency, as the rise velocity of the bubbles would be counterbalanced, at least partially, by the flow down the sump. Of course, this would also create an impediment to flow, and would increase mixing energy inputs. This is ignored here, although it should be addressed in the future. The sumps also serve as a harvesting point, for location of intake pipes for the settling ponds.

The costs of the sumps were estimated in the 1987 study at $1,680/ha, for one sump per pond (8 ha), which would be able to supply all the CO_2 for maximum periods of demand. This included excavation, sump walls, and sump bottoms. An additional cost of about $1,400 was specified for a solids removal system, but this is not likely to be required as any sludge could be drawn off during harvest, or by occasional cleaning. Anyway, little sludge accumulation was noted during the pilot plant work (Weissman, personal communication). For the present purposes, this device is not included.

The transfer of CO_2 in the sumps requires a sparging system. This was neglected in the 1982 study, but a significant cost in the 1987 study, $2,870/ha, which included the instrumentation ($600/ha) pH controllers, valves, flow meters and associated piping). This was considered a "conservative approach", based on vendor quotes. It should be noted that this design is sufficient for the average productivity of 30 g/m^2/day, assuming maximum demand of about 7 g CO_2/m^2/hr (maximal 45 g/m^2/day in summer). For the higher (near theoretical) productivities, two sumps, and carbonation units would be required per pond.

For the case of pure CO_2 supply, these costs could be reduced as the above estimate, of $4,550/ha, was based on a generic analysis, for either flue gas or pure CO_2. In practice flue gas would be more expensive than pure CO_2, due to the larger pipe sizes and diffusers. Thus, for present purposes, the cost is increased somewhat to $5,000/ha for flue gas, and reduced to $4,000/ha for pure CO_2. Adjustments could also be made for strategies for carbon supply (for example, the digester effluents could be supplied during peak demand periods), but this is not further considered. For the high productivity case, twice these costs are estimated, as two, rather than one, CO_2 transfer sumps would be required per pond, and there are, thus, no economies of scale.

8.3.6. CO_2 Supply

Of the two options, clearly the pure CO_2 supply is the lower cost, as long as the cost of the CO_2 itself is not considered. Pure CO_2 would be delivered under pressure from a pipeline or a storage system, and the piping and valving system would be relatively simple. In the 1982 and 1987 studies this cost was estimated at only about $250 and $300/ha respectively, neither of which included storage. By contrast, in the 1982 study, the delivery of the flue gas CO_2 from the power plant to the pond system (3 miles) was $6,300/ha (for the pipe and blowers) and another $3,650/ha for the internal distribution piping to the individual growth ponds and blowers, for a total of almost $10,000/ha. Forty fold higher than the CO_2 case! Together with the much higher operating costs, flue gas supply is a major cost.

However, the above cost estimate was, as stated earlier, for a 2 meter diameter, 5 km long pipe (3 miles), delivering flue gas at low pressure to the center of the

module, and, perhaps most important, also for the case where low alkalinity did not allow sufficient CO_2 storage in the pond, requiring a higher hourly supply rate. And no C recycle was assumed. For a higher pH, C recycle (from the anaerobic digestion unit, see below), and a shorter 2.5 km (1.5. miles) pipeline, overall costs were reduced for the 1982 report to $5,000/ha, almost exactly half. Of course, this was for only a 32.5 g/m^2/day maximal productivity, with 30% lipids, and 50% C in the biomass dry weight. For the base case productivities and C content assumed in the present analysis, a 66% higher maximal daily CO_2 supply rate would be required, twice this for the higher (60 g/m^2/day) productivity case. (Of course, this included only capital costs, not operating costs for the blowers, see below).

It also should be noted that this supply system was for "only" a 400 ha plant, corresponding to about a 40 to 80 MW power plant (see Table 1.1) for the two productivity assumptions used here. Note that, from Table 1.1., the annual production of CO_2 from a 1000 MW power plant would be about 7.6 million tons of CO_2, sufficient in principle to produce about 3.5 million tons of algal biomass. However, adjusting for daytime only use (half of the CO_2 is "wasted"), a 1.5 summer:average productivity ratio, and another 0.9 factor for CO_2 losses (transfer, outgassing), this reduces to a total of 1 million tons of biomass produced, which corresponds to roughly 10 ha ponds/MW power plant). If C recycle were used, in which one third of the C was re-used, this area would increase by 50%. The higher productivity cases decrease the required area by half.

Kadam (1995) provided a cost estimate for a 100 km compressed flue gas pipeline from a 500 MW power plant which had capital costs of $430 million, and which would supply 2.83 million tons of CO_2 per year. He assumed that the pipeline could store the CO_2 for night time, but, clearly, it could not adjust for seasonal variations. Thus, this would be sufficient to produce about 0.77 million tons of biomass or for a 7,000 ha pond system in the present analysis (half the size calculated by Kadam, 1995, due to differing, not explicitly stated, assumptions). For a $430 million capital cost this would amount to $60,000/ha, clearly an excessive cost. For the pure CO_2 case capital costs were about half as much, still very high. Further, as mentioned above, for the flue gas case, the high compression power would result in more CO_2 emissions than would be captured by the algae.

Thus, clearly, the preferred option is a relatively large diameter pipe, with a minimal distance to the ponds, with relatively low compression. (The 1982 system was explicitly designed to use only 1% of energy outputs). No attempt has been made to carry out a new design effort. Thus, the cost estimate used here is the $5,000/ha for the CO_2 supply system estimated in the 1982 study. This includes the main pipe from the power plant, distribution piping, blowers, and miscellaneous items. For the higher productivity case, where CO_2 supply would double, this cost is increased by 60%, to $8,000/ha, to account for expected economies of scale. (However, it is not clear if in this case bigger pipes, which may need to be field constructed at higher unit cost, or more blower power should be specified).

There is, of course, a trade-off between direct flue-gas utilization and CO_2 recovery from flue-gas (e.g. an amine scrubbing process) and delivery to a remotely sited pond. As already mentioned, the trade-off is very favorable when considering the overall resource base. Economically, however, the issue is the cost of delivered concentrated CO_2 vs. flue gas. Kadam (1994) estimated a $40.5/mt cost for CO_2, which corresponds fairly closely to the commercial cost of CO_2 in both the 1982 ($45/mt) and 1987 report ($35/mt) (original, not updated, costs). Here, for simplicity, a cost of $40/mt CO_2 is assumed for the pure CO_2 case. This correspond also to other estimates for CO_2 scrubbing and delivery (e.g. Herzog, at $37/mt CO_2, personal communication, 1996). Still, in all these examples, it must be remembered that average utilization of CO_2 is only a fraction of peak power plant CO_2 outputs, about one third for flue gas utilization (due to diurnal and seasonal variations in demand) and about two thirds for the concentrated CO_2 case (seasonal variations only, with diurnal storage in the pipeline, as per Kadam, 1995).

8.3.7. Harvesting and Processing.

It is generally agreed, since the earliest days of the examination of such systems (e.g. Oswald and Golueke, 1960), that for low cost commodities, only a settling process would be low-cost enough for a primary harvesting (concentration) process. However, many details of such systems remain to be specified. And, most important, the secondary concentrating process, after settling achieves a cell density of a few percent solids (typically 1 to 3%) is the critical issue.

More contentious is the cost of the secondary process. The 1982 report assumed centrifugation, using the largest commercially available centrifuges. These were quite expensive, at almost $10,000/ha. The 1987 study used a three stage process: primary settling, secondary settling in which flocculant was added to increase the solids contents of the algal biomass fed to the centrifuges, thus reducing the volume to be processed. This greatly reduced the centrifugation costs.

For the primary harvesting, the settling, system, the both the 1982 and 1987 studies give almost exactly $7,000/ha, based on reasonably detailed engineering designs and cost estimates. Thus this is also used here. This cost is productivity independent.

The 1987 study also projected costs of $2,000/ha for a chemical flocculation steps, followed by gravity settling. The latter was added to increase the concentration of solids going into the centrifuges, to about 3 - 4 %, from about 1 to 2% coming from the primary settling ponds, thereby saving overall on capital and operating costs. For a 100 ha module, assuming a 20 fold concentration in the primary (settling) harvest system, a depth of 20 cm at harvest, a two day retention time (e.g. half the pond is harvested each day), and 45 g/m^2/day maximal productivity, this

gives a final concentration of 0.9% solids, and 5 million liters/day, going into the flocculation stage. The flocculation stage consisted of a gravity settling system, with chemical addition, producing a 3 to 4% solids output.

Here, a somewhat higher solids (8%) is desired. This could be obtained with improved polymers and a DAF (dissolved air flotation) system. From discussions with vendors (Mr. Jerry Walden, Krofta Co., personal communication, 1994), such a process appear to be cost-competitive with gravity settling, while producing a higher concentrate. A 1 MGD DAF system was stated to cost $215,000, with a 15 MGD unit costing $1.2 million, a typical economics of scale for such systems.

One issue is the flow rate that would need to be handled by this system, which depends on the period of time per day for the algal concentrate from the primary settling pond. The primary pond is filled once a day, and drained once a day, and the concentrate would need to be recovered over a relatively small time period each day (one to two hours). However, this primary concentrate could be stored in a holding tank until processed further. Although some bacterial breakdown may take place, over the period of interest (24 hrs, maximum) this is not considered a major problem. Thus, to a first analysis, the flow to the DAF unit, and subsequent steps, could be equalized over the day.

Of course, DAF unit size would depend on influent and effluent concentrations, polymer addition, etc. However, for the present system, and assuming a 400 ha system (about 5 MGD flow to the DAF), it would appear that a capital cost estimate $600,000, or $1,500/ha, would apply from the above, with some contingency (in the absence of a detailed analysis) of $2,000/ha. This is similar to the flocculation-settling process designed in the 1987 report, reflecting the advances in this technology over the past decade. For twice the productivities a 50% increase to $3,000/ha is estimated, due to economics of scale.

The final step is centrifugation. Centrifugation can be used not only to concentrate the biomass but also to extract the algal lipids into an oil phase in a simultaneous operation. This is because of the relatively large difference in the densities of the water, algal lipids, and other biomass constituents. This concept is based on the commercial process for the extraction of beta-carotene from flocculated algal biomass by a hot oil extraction process. Thus, the harvesting and processing steps overlap, with the flocculation and centrifugation steps being compatible with the oil extraction process. This is a major innovation proposed in the present report. It should be pointed out that this is based on well known technology, although the present application obviously represents a large scale-up.

The key to this is the emulsification of the biomass as it leaves the clarifiers after flocculation, into a hot oil, and then phase separation with a large three phase self-cleaning centrifuge, that continuously expels three fractions: oil, biomass

cake, and water. The largest capacity centrifuge of this type on the market is a Clarifier Model HSA 200-06-777, able to handle up to 20 m^3/hr, would cost $600,000 (Wicklund, Westphalia, Personal communication, 1994). (Note that the centrifuges used for secondary harvesting in the 1987 report have half these costs, while handling twice the flows, which is reasonable considering the differences in centrifuge type). Assuming a 22 hour/day operation (during maximum season), this would allow processing about 440 m^3/day. Assuming that the algal biomass harvested from the ponds is concentrated to a 8% solids level by the combined settling and flocculation process, this allows processing of about 35 tons/day of biomass organic solids (ash and flocculant content is ignored here). Assuming, for current design purposes, for a maximum of 45 g/m2/day, this amounts, 45 tons biomass/day per module (100 ha). For a four hectare module 6 such centrifuges would be required, providing one spare.

Before the centrifugation, the biomass must be converted to an oil emulsion. This would involve heating the sludge coming from the DAF unit, to about 60 to 70 ^oC, adding some (hot) oil (recycled from the oil extraction step), and emulsification, which could involve a cell breakage step, to increase oil extractability and recovery. In brief the extraction process consists of the following steps, after the cells are flocculated and concentrated to near 8% solids with a DAF system:

1. **A cell homogenizer** to disrupt the cells. Maton Gaulin is the standard equipment in the industry, used to disrupt yeast and other microbes. High throughput rates (e.g. no recycle) are considered appropriate, reducing costs.
2. **A heated paddle mixed emulsification tank**, in which the recycled algal oil would be contacted with the homogenized biomass, heated (to 60-70^oC). Heating would use reject heat from the biogas generator sets (see below);
3. **A jacketed holding tank**, with cooling water to educe the temperature as required before centrifugation (probably to 40 - 50 ^oC.
4. **A 3 phase, solid bowl decanter (centrifuge)**, produces three streams: waste water (containing most of the glycerol), solids, and the oil/beta-carotene.
5. **A heated, mixed, holding tank** for the lipid extracted, which would then be fed to a refinery to produce biodiesel. The other fractions, water and non-lipid solids, would go to the blow-down system and digesters, respectively.

Overall, the largest cost item is $600,000 for each centrifuge, or $3.6 million for six, for a 400 ha system. To this must be added the cost of installation, electrical, piping, and the above items for homogenization, emulsification, and heating steps. In prior studies, centrifuge installation costs (concrete pad, housing, pumps, piping) were estimated at 10 to 15% of purchased costs (see 1987 study). The tankage for the lipid extraction, heating, etc., would also not be very expensive. No specific cost estimate has been carried out, but a rough overall cost of $5 million can be estimated. This is the largest single capital costn can be
estimated. This is the largest single capital cost item yet, at $12,500/ha. For twice the productivity, twice this cost is assumed, as there would be no economic of scale at this point (just more centrifuges would be required).

This compares to only about $5,250/ha for soybean-type hexane extraction system used in the 1982 study, which, if adjusted for productivity, would probably be a little over half the cost estimated here. Of course, the extraction process proposed above is able to dispense with the $10,000/ha (1982 study, half this in the 1987 study) for centrifugation (even without adjusting for productivity). Thus, overall, the present estimate is overall significantly below the earlier cost estimates.

It should be noted that this is, obviously a somewhat soft estimate. However, unlike prior studies (e.g. 1982) this process is based on known principles, and even commercial experience with algal biomass (though at a much smaller scale). Indeed, all other studies in this field have ignored this step, as it was not clear how it would be achieved. The major factor favoring a hot oil extraction is that the algal lipid is, indeed, an oil, and thus soluble in such oil. This should assure a high efficiency of extraction, with the major R&D issues being the requirement for cell homogenization (e.g. a relatively inexpensive step, if shear forces are not excessively high), the temperature of the extraction, the amount of oil added for emulsification, and the shear forces required for emulsification (which could be combined with the cell homogenization process, to help break down cell walls at the higher temperature). Although these parameters are uncertain, they are not expected to be major cost factors. The major cost are the capital and operating costs for the centrifugation step, as noted above.

One important issue is the efficiency of extraction. Based on commercial experience with beta-carotene, where an almost quantitative recovery (98%+) is obtained, a 90 to 95% extraction efficiency can probably reasonable assumed. Here a 100% extraction is assumed, with any losses made up by somewhat higher oil content in the algae. (Thus, the 50% oil content thus refers to recoverable oil).

In conclusion, the final harvesting step would also result in the extraction of the algal oil, ready for further processing to biodiesel. Conversion to biodiesel was not specifically considered here. It is estimated to add about $0.35/gal in processing costs for the methanol/KOH process (John Sheehan, private communication, 1996). It is assumed here that algal lipids would compete with crude oil, prior to refining into higher value fuels and products. The concept would be to ship the algal oil to a refinery, where it would be transesterified.

In summary, harvesting and processing costs (to give a pure algal oil stage) are estimated to be $21,500/ha, of which $7,000 is for the primary harvesting system, $2,000/ha for the DAF system, and $12,500 is for the lipid extraction step. Actually this is similar to the $22,000/ha estimated in the 1982 study. The main differences are a doubling of the extraction equipment costs, but a significant decrease in the secondary harvesting costs, which now doubles with the extraction process. For the higher productivity case, costs increase to $35,000/ha, due to lack of economies of scale in the centrifugation-processing step.

8.3.8. Anaerobic Digestion and Nutrient Recycling.

Another major subsystem is the anaerobic digestion of the microalgae, to allow recycling of C and nutrients, as well as recovery of the fuel value of the algal residues. Figure 8.5. shows an estimate of the C flows in the overall system. These are not exact, but exemplify the process: of the C in the algae about 60% is recovered in the lipids, the remainder is in the algal solids, with some minor amount in the solids blowdown. Of the C in the residues about half is recovered in the methane, the remainder in the digester effluents. The latter, also containing essentially all of the N and P (as well as other nutrients) contained in the algal biomass. It would be fed back to the pond, bacteria present in the ponds, would use the oxygen generated by the microalgae to decompose the sludge. Overall, about 85% of the C supplied from the power plant would be recovered in the algal lipids, with the remainder being lost by outgassing from the ponds and through blow-down from the system.

The anaerobic digestion system is based on a covered lagoon. Such systems are not conventional technology, but one such covered lagoon, for piggery wastes, was designed and installed in the late 1970's in California (Benemann, et al., 1980) and has been operating ever since. Several other covered lagoons have been used for animal wastes, food processing, and other wastes with success. The 1982 report mentions, and uses, C recycle, but does not provide any cost estimates. The 1987 report contains a detailed estimate of the covered pond for biogas production, on which the following analysis is based.

Assuming an average (maximal) productivity of 30 (45) $g/m^2/day$, this gives a daily production of 120 (180) mt/day for a 400 ha facility. Of this half is extracted as lipids and the remainder, 60 (90) mt/day is used, at about a 7% solids level, as substrate for anaerobic digestion in a covered lagoon. Assuming a 65% destruction of this material and conversion to biogas (60% methane, 40% CO_2), with a yield of 304 m^3 per mt of residual biomass added (4.83 SCF methane /lb VS solids). Thus, the 400 ha facility would produce 18,240 (27,360) m^3 methane per day, or, for 35,000 BTU/m^3 of gas (@ 1,000 BTU/ft^3), 640 (960) MMBTU/day of biogas.

The covered lagoon was estimated in the 1987 report to cost $1.3 million for a 400 ha facility (same as here), with, actually, somewhat higher solids inputs (due to somewhat lower lipid production in that design). (The cost estimate added a total of 35% for contingencies and engineering, which is excessive, see below, and not included here). Thus, the digestion system, cost $3,250/ha. For the higher productivity assumption this cost is doubled, as two digesters, rather than one larger one, are specified here.

Generally the best use for biogas is in electricity production (Augenstein et al., 1993), and this is also true in this case. Skid mounted generators (such as those fabricated by Carterpillar) can use biogas and are available at various sizes to about 1.5 MW. Costs at the larger sizes are about $1,000/kW capacity, including all necessary items (Augenstein, personal communication, 1995). In the 1987 report the cost for three 1 MW trailer mounted, turbocharged gen-sets (for a 192 ha system) were estimated at $2.7 million, or $900/kWhr. However, the efficiencies of the two systems differ considerably, from about 24% for the gen-sets in the 1987 study, to 31 - 32% for the units recommended by Augenstein (reflecting the technological advances in this field). For the high productivity case, power generation costs double, there are no economies of scale. (Note that these cost estimates are based on firm prices, for off-the shelf equipment that requires no significant installation. Thus no engineering and contingency costs were included in the 1987 report, or used herein, for the power plant).

Thus, for 640 (960, max.) MMBTU/day of methane, the system would produce about 61,000 (91,000) kWhr/day (average of 152 kWhr/ha/day, or 55,000 kWhr/ha/yr). Assuming 24 hour operations (base load), a maximum daily 3.8 MWe capacity would be produced. Thus, about 4 MW capacity, at a cost of $4 million (@ $1,000/kWhr) would be required for peak production. This comes to $10,000/ha, another major cost factor. Annual output would be 21 million kWhr. Assuming O&M cost of $0.018/kWhr (1987 report, also Augenstein, personal communication, about half each for O and M), and a sales prices of $0.065/kWhr, this would yield a net cash flow of $1.0 million, or $2,500/ha/yr. This is a significant value. Of course, this does not consider the capital charge (cost of capital, depreciation) which at a combined 15% of capital investment (see next section) for an investment of $4.0 million gives a cost of $600,000 ($1,500/ha/yr) (see next section), leaving only $1,000/ha in net receipts from power sales. And this is just for the capital of the gen-sets, not including the digester.

For the digester, assuming a 20% annual cost for the digester (15% capital and depreciation, 5% operating and maintenance), for an investment of $3,750/ha (after adding 15% for engineering and contingencies to the above), amounts to about $560/ha-yr, reducing the above net cash-flow by over half. Thus, power production Indeed, even selling power at $0.065/kWhr is probably optimistic under present conditions. Thus, power generation is not a major profit center. (Not too surprising a conclusion - otherwise there would be many operating waste to methane plants around the country).

In the case flue gas utilization, where a coal-fired power plant is readily available, another option would be to send the methane to the power plant, thereby reducing coal consumption (and CO_2 production) and saving on costs (in particular capital). However, in this case the question becomes of the value of the methane fuel value.

Assuming (optimistically) a fuel replacement value of $2/MMBTU for the biogas, this would yield about $460,000/year, or about $1,150/ha/yr. This is a slightly higher return than for the gen-set case (the digester costs would be the same in each case).

The alternative would be to just recycle the waste biomass to the ponds for carbon and nutrient recycle, without bothering with the digestion and power generation. Of course, this ignores the value of the CO_2 mitigation (due to avoidance of coal combustion, when biogas is converted to electricity). At 0.88 kg CO_2/kWhr produced by burning coal (Table 1.1), and assuming a value of $16/mt CO_2 (based on $60/mt CO_2-C), this would amount to another $0.014/kWhr CO_2. This issue is considered in Section 8.5 below.

In conclusion, the methane recovery part of this system is a better, but not by much, than break-even proposition (for either fuel replacement or on-site power generation). Most important, perhaps, the power generated from the methane more than offsets the CO_2 emissions from the power used to operate the system (mixing, centrifuges, blowers, etc.). This is perhaps its greatest importance for the present analysis.

8.3.9. Other Capital Costs.

All other system costs used here are based on the 1987 study, which is the most detailed, and generally, most conservative. However, the water supply and distribution, estimated at $5,500/ha, appear somewhat high, and, anyway, only $4,200 is documented in the 1987 report. Thus, that value is used here. Nutrient supply adds another $1,000/ha. Waste treatment (blow-down) is estimated at $1000/ha, and general machinery $ 500/ha. (Instrumentation was included under the CO_2 supply system). These are all productivity independent costs. Note that the water supply system is quite expensive, five times the cost of the 1982 study. The difference is due to the use of relatively deep brackish groundwater, that needs to be pumped to the surface (contributing also to power consumption, see below).

Also important are the various system-wide capital costs, for infrastructures, support systems, etc. These include buildings, roads, drainage, and electrical. These are quite disparate in the various reports, with, surprisingly, the 1982 study at almost $10,000/ha while the 1987 study allocated less than $4000/ha for these combined items. Review of the two studies demonstrate that the 1987 report specified size of buildings required, etc., while the 1982 study used a factor, based on other capital costs, which is less realistic. Here the 1987 example is followed, and $2,000/ha is allocated to the buildings/ roads/drainage and a similar amount for the electrical systems (estimated in the 1987 study as 3% of other capital items). for total infrastructure costs of $4,000/ha, increasing to $5,000/ha for the higher productivity case.

The other major capital items are the engineering, contingencies, and working capital ("start-up costs" in Table 8.1). In the 1987 engineering costs have been given as 10%, and contingencies as 15%. The latter was justified by the uncertainties involved in these analysis. The former as being a standard cost factor in general studies. However, a more conventional 10% contingency, as used in the 1982 study, is appropriate. Also, engineering (and contracting, etc.) fees are too high for such modular systems, which require little engineering work past the initial modules. Thus a 5% factor is used here. Finally, the working capital should be set as a fraction of operating costs, not capital costs. A total factor of 25% of annual operating costs (without depreciation or ROI) should be allocated.

Two final issues are land costs and working capital. The type of land used for such systems would not be expected to be high value or quality land. Of course, in addition to growth ponds additional land is needed, from 25% to 100%. A cost of $2,000/ha of growth pond area is used here, which should cover most likely suitable land, at least for land rents. Working capital, which includes facilities start-up, are estimated here at 25% of annual operating costs (excluding capital related charges), as derived in Section 8.4 below.

8.3.10. Summary of Capital Costs.

Table 8.2 summarizes the above updated costs, both for a flue-gas and a pure CO_2 system. The overall costs are somewhat higher than the 1987 and 1982 studies, mainly because of the processing (extraction) subsystems.

The 1987 study estimated over $20,000 /ha for the earthworks (site preparation, leveling, grading, percolation control, levees), compared to only about $8,000 in the present analysis. As discussed above, this was subjected to an independent design and the 1987 study was found to be overdesigned and too expensive.

Another major issue was the pipeline for the flue gas. Although a significant cost, overall it turned out not to overwhelm the cost analysis, at least not for the assumptions made here (e.g. 2.5 km distance), and using the 1982 capital cost data.

Indeed, the largest single item was the extraction process, which is novel to the present effort. Insufficient information exits about the design basis for such a system, and costs for centrifuges can only be considered approximate, as it is not clear what their operating specifications (solids inputs, flow rates) would be. This could only be determined with actual bench-scale experimentation. This is also true for the DAF process. Indeed, the harvesting/processing system, at between 35 to 40% of total costs, is the single largest capital cost input, and the most uncertain.

TABLE 8.2. SUMMARY OF CAPITAL COST ESTIMATES
$/ha for a 400 hectare system

Section	Capital Cost Items	Productivity Assumptions 30 g/m^2/day		60 g/m^2/day	
		CO_2	Flue Gas	CO_2	Flue Gas
8.3.2.	Site prep., grading, compaction	2,500		2,500	
8.3.3.	Pond levees, geotextiles	3,500		3,500	
8.3.4.	Mixing (paddle wheels)	5,000		5,000	
8.3.5.	CO_2 sumps, diffusers	4,000	5,000	4,000	5,000
8.3.6.	CO_2 supply, distribution	300	5,000	300	8,000
8.3.7.	1^o Harvesting (settling)	7,000		7,000	
8.3.7.	Flocculation, DAF	2,000		3,000	
8.3.7.	Centrifugation, Extraction	12,500		25,000	
8.3.8.	Anaerobic digestion lagoon	3,250		6,500	
8.3.8.	Gen-set	8,700	----	17,400	----
8.3.9.	Water & nutrient supply	5,200		5,200	
8.3.9.	Waste treatement (blow down)	1,000		1,000	
8.3.9.	Buildings, roads, drainage	2,000		2,500	
8.3.9.	Electrical supply and distribution	2,000		2,500	
8.3.9.	Instrumentation and machinery	500		500	
	Subtotals of above	59,450	56,450	85,400	76,700
8.3.9.	Eng. & Conting. (15% above)	8,900	8,500	12,800	11,500
	Total Direct Capital	68,350	64,950	93,900	88,200
8.3.9.	Land Costs	2,000		2,000	
8.3.10.	Working Capital (25% op. cost)	3,800	2,700	4,200	3,800
	Total Capital Investment	74,150	69,650	104,400	94,000
	$/mt-yr biomass	680	640	480	430

8.4. OPERATING COSTS

8.4.1. Power

Table 8.3 lists operating costs. The first item is power. As for other costs order of magnitude differences exists between the various studies. One immediate issue is the unit cost for electricity. In the earlier studies \$0.1/kWhr was assumed, which in Table 8.1 was escalated with the present cost factors. However, currently power costs are actually much lower, and are projected to remain low far into the future, in particular with the deregulation of the electric power industry. Thus, here, a power cost of \$0.065/kWhr is used, the same as in the 1987 study.

Current power costs do not carry with them the externality costs, in particular of greenhouse gases. An energy analysis of the overall system is required, but prior analysis have demonstrated that only a relatively small amount of parasitic energy is required. Here this power is subtracted from the power generated from the methane gas by-product, with net power outputs used to calculate CO_2 mitigation costs (Section 8.5).

The 1987 study estimated a total of 28,500 kWhr/ha-yr, for a cost of \$1,850/ha-yr (@ \$0.065/kWhr). This included mixing (10,750 kWhr/ha-yr), harvesting (1,770 kWhr/ha-yr for settling; 5,730 kWhr/ha-yr for centrifugation), water supply (5,730 kWhr/ha-yr, mainly for pumping), nutrient supply, and buildings. The 1982 study used (adjusting for \$0.065/kWhr) \$1,420/ha for the pure CO_2 case, but did not break down the contributions of the individual items. However, these estimates were consistent with the 1987 study, as there was a major difference in power use: \$570/ha-yr in the 1987 study for water pumping, which had been nominal (e.g. \$100/ha-yr, if memory serves) in the earlier study. Here the 1987 study is used, as it provides detailed breakdowns and is the more conservative.

The 1982 estimated \$1,220/ha-yr for power required to supply flue gas, without recycle with a 5 km pipe. This reduced to \$680/ha-yr for the recycle case, with half the distance from the power plant. (Again, these were adjusted to \$0.065/kWhr). For the present case, the amount of flue gas required is similar to the first case, and the distance similar to the second. As the 1982 report does not provide a breakdown of the components (pipeline, distribution, transfer), these costs are interpolated to \$1,000/ha-yr. This should be evaluated further in the future.

In this regard, the power consumption for the DAF screen and the centrifuge are not known at present. They are expected to be similar to the centrifuge power costs estimated in the 1987 study, of about \$500/ha-yr, also used here. (In that study the centrifuges processed a larger volume of liquid, which should make up for power use of the DAF units). For the higher productivity case, power inputs into flue gas supply and harvesting are doubled, for pond mixing (\$700/ha-yr), water supply (\$570/ha-yr) and nutrient and buildings (\$100/ha) they are held constant.

TABLE 8.3. SUMMARY OF OPERATING COST ESTIMATES
$/ha-yr for a 400 hectare system, @ $0.065/kWhr

Item #	Operations Items	Productivity Assumptions 30 g/m^2/day		60 g/m^2/day	
		CO_2	Flue Gas	CO_2	Flue Gas
1.	Power, mixing	700		700	
2.	Power, harvesting, processing	500		1,000	
3.	Power, water supply	570		570	
4.	Power, flue gas supply	---	1,000	---	2,000
5.	Power, other	100		100	
6.	Nutrients, N,P, Fe	900		1,800	
7.	CO_2 (@ $40/mt)	7,400	----	7,400	----
8.	Flocculant	1,000		2,000	
9.	Labor + Overheads	3,000		4,000	
10.	Waste Disposal	1,000		1,000	
11.	Maint., Tax, Ins. (@ 5% Cap.)	3,400	3,250	4,900	4,400
12.	Credit for Power or fuel	(3,400)	(1,150)	(6,800)	(2,300)
13.	Total Net Operating Costs	15,170	10,870	16,670	15,270
14.	Capital Charge (15%)	11,100	10,500	15,650	14,100
15.	Total Annual Costs.	26,270	21,370	32,320	29,370
16.	$/mt biomass	241	196	148	135
17.	$/barrel algal oil	69	56	42	39

Against power consumption can be balanced power production. For the flue gas case, the methane is provided to the power plant, which, for simplicity here is assumed to be used at the same efficiency, thus providing the same amount of net power as for the pure CO_2 case. From the above, a generation potential of 55,000 kWhr/ha-yr was estimated. Thus, for the pure CO_2 case, where 28,500 kWhr/ha-yr is used, a net of a net of 26,500 kWhr/ha-yr is projected. (Note that this was higher than in the 1987 report, mainly due to the higher efficiencies of power generation in the present design). Much of this, 15,400 kWhr/ha-yr would be used for flue-gas supply, leaving a net of only a net of 10,100 kWhr/ha-yr for the flue gas case. For the higher productivity case, twice as much power, 110,000 kWhr/ha-yr, would be produced, but power consumption would only increase for the flue gas case, leaving a net of 83,500 and 50,700 for the pure CO_2 and flue gas cases, respectively. These net outputs are important when considering the net CO_2 balances of such a system.

8.4.2. Fertilizers and Chemicals

Fertilizer unit costs have varied considerably over the years, with, for examples, N fertilizer prices going up and then down since the 1978 study, as did phosphates. Here, the costs used in the 1987 study are used, without an inflation factor. They correspond closely to recent data by Kadam (1994). Total fertilizer costs (with 75% recycle of N and 50% for P, 0% for Fe) were $900/ha-yr in the 1987 report. . This is much higher than in the 1982 study, which (without adjusting for inflation, but adjusting for higher productivity) estimated only half these costs. Here, as in most cases, the 1987 study cost estimate is used. Flocculant costs were $1,120/ha-yr in the 1987 study, or $10/mt algal biomass. This was somewhat speculative, and a round $1,000/ha-yr is used here (twice as much for the higher productivity case).

For the pure CO_2 case a CO_2 cost of $40/mt is used, an average (without inflation adjustment) of the 1982 and 1987 studies, and similar to other estimates for the costs of concentrating and transporting CO_2 from a power plant. Note that this does not reflect in any way the CO_2 credit (or mitigation cost), which is discussed later. CO_2 is assumed to be used with an overall efficiency of 85%. For a 60% C content, this amounts to 2.6 mt CO_2 supplied/mt of biomass harvested. However, by recycling about one third of the C from the wastes, this reduces to a ratio of 1.7 mt CO_2/t of algae. For a productivity of 109 mt/ha/yr, and a cost of $40/mt, this becomes a cost of $7,400/ha-yr, twice this for the higher productivity case.

8.4.3. Labor and Overheads.

Hourly rates of labor have not significantly increased since 1978, despite an inflation of over 50%. Indeed, estimated hourly rates actually decreased slightly from 1982 to 1987, in actual dollars, and the latter are still valid, reflecting the economic reality of U.S. labor markets. The 1982 and 1987 studies had similar

labor costs ($1,350 and $1,390/ha-yr, without inflation adjustment). These labor costs are used here. However, labor was somewhat skimpy in these estimates. In particular as the above design now include DAF screens and extraction operations, anaerobic digesters, and power generation (for which no labor costs were apparently allocated in the 1987 study). Thus labor costs are increased by 50% and 100% for the two productivity cases, respectively, from the 1987 study.

The prior studies also did not have allowances for corporate overheads, indirect costs, etc. These are added here as a % of labor costs, at 50%. The justification is that in general such costs are sensitive to the employee base, more so than the capital base. This should cover most corporate overheads, and related miscellany.

8.4.4. Capital Related and other Costs.

The 1987 study estimated maintenance material costs as a % of investment, using an appropriate factor for each item, for example 1% of electrical and earthworks, and 3% for most other items (except the centrifuges, at 5%). Maintenance labor was assumed to be included in general labor. Here we use an average of 3% for all items, including any special maintenance labor that would not be included in the general labor category. To these 3% annual maintenance are added another 2% for insurance and property taxes. The total of 5% applied to all capital investments, except land costs and working capital.

Capital related costs ("carrying" or "fixed charge rate") are generally the largest single cost factor. The 1982 study assumed a depreciation cost of 10%/yr, and an overall capital cost of 15%/yr. This is appropriate for a speculative commercial investment, but does not reflect long-term investment costs in mature technologies, which this report attempts to project. Neenan, et al. (1986) used an economic model, also used in the 1987 study (and by Kadam, 1994), which contains many assumptions (such as common to preferred stock, debt to equity ratios, tax rates, escalation factors, etc.). That model appeared to have an overall capital charge of 20% (including depreciation). A recent analysis by EPRI of short rotation trees (Wiltsee and Hughes, 1995), used EPRI's Technical Assessment Guide to arrive at an overall capital charge of (roughly) 15% (somewhat lower for renewable energy projects, reflecting favorable tax treatment). Herzog et al. (1993) used a 15% capital charge for ocean disposal of CO_2, which included maintenance, insurance and taxes. Clearly, there is little agreement on this point.

A 15% capital charge factor is used here. Essentially, this can be considered the annual charge which would be required to pay off the facility within a 20 year period assuming current cost of money without inflation (assumed to be neutral, in that any increase in costs would be offset by higher revenues). This is single largest cost element in this analysis (Table 8.3).

8.5. CO_2 MITIGATION ANALYSIS

8.5.1. Energy Analysis

The objective of this report is the overall amount and cost of CO_2 mitigation with microalgal systems (Table 8.4). Such an analysis has not been carried out previously. Even the recent NREL studies, dealing with CO_2 mitigation, neglected to address the net CO_2 mitigation achieved by such systems, considering only the gross CO_2 utilization. Indeed, prior studies did not even present an energy analysis of such systems - the input and output of fuels and power required/produced. Such an analysis would allow a rather direct estimation of net greenhouse gas emissions. However, it should be noted, that energy analysis is different from greenhouse gas mitigation analysis, as discussed further below.

The first net energy analysis of microalgae production was that of Odum (1969), who argued, in connection with the microalgae food production systems developed by the Carnegie Institute of Washington (see Burlew, 1953; Fisher, 1955), that microalgae processes would require more fossil energy inputs than outputs. He accused algal researchers of perpetrating a "cruel illusion" on the unsuspecting public and gullible granting agencies with such schemes. However, his analysis was flawed methodologically (he, for example, assumed that the personal energy consumption of the work-force should be charged against the process), and the technology (closed plastic tubes with refrigeration for temperature control).

Odum's caustic remarks about microalgae systems (and their perpetrators) were reflected by others. For example, Goldman and Ryther (1977), argued that energy inputs into fertilizer, harvesting, mixing, water pumping, etc. would exceed outputs. They projected that to produce 10% of the fuels required by the U.S. economy, such systems would use water supplies exceeding the flow of the Mississippi, and fertilizer inputs equal to all the U.S. production. As Oswald and Benemann (1977) pointed out, that analysis was flawed because, among other reasons, it failed to recognize that most water and fertilizer supplies would be recycled

Dynatech (1978), based on the 1978 report, presented the first net energy analysis. Assuming N and P recycle of 90% efficiency, inputs due fertilizer production were only about 1% of gross outputs (e.g. in the higher energy content of the biomass). Mixing power was (assuming a 33% conversion efficiency of algal heating value of 5.5. Kcal/g, to electricity, and a productivity of 68 mt/ha/yr) were 2%. Embedded energy in the system (materials construction), when amortized over 20 years, also had an energy input of only about 1%. Total inputs were thus about 4% of outputs.

The 1982 and 1987 studies did not address energy efficiency directly. The 1982 study stated that for the flue gas case with C recycle, energy inputs would be about 12,350 kWhr/ac/yr, about one third of outputs (based on a heat rate for electricity production of 10,000 BUT/kWhr) and that most of his power could be generated by

the biogas produced from anaerobic digestion. However, no further details were provided. The 1987 study also did not address this directly, although it provided all the necessary data for such an energy analysis.

The energy inputs for the two studies are compared in Table 8.4, both in terms of energy inputs (kWhr and Kcal, based on a heat rate of 2150 Kcal/kWhr. Note the large differences in the two studies, in most of the components, such as water supply, processing, and, most important, CO_2 supply. These inputs were used in the above analysis. Next, we review the actual CO_2 mitigation issues.

8.5.2. CO_2 Analysis

Any production of a replacement for fossil fuel is a CO_2 mitigation process. By using the actual CO_2 generated from a specific source, a power plant in this case, this becomes a direct CO_2 mitigation process. The actual CO_2 savings realized depend on the overall system inputs and outputs, including the utilization of the fuels. In the algal process two types of fossil fuel substitutes are produced, biogas (methane), used for power production, and "biodiesel", for vehicular fuels.

For biogas, only net power outputs result in CO_2 mitigation. Here we assume that for each net kWhr of power produced by the system, 0.88 kg CO_2 are emitted, assuming a coal-fired power plant (Herzog et al., 1991). From Table 8.5, there was a net power production of 26,500 kWhr/ha-yr for the pure CO_2 and 10,100 kWhr/ha-yr for the flue gas case. For the higher productivity case, these increase to 83,500 and 50,700, respectively.

The inputs due to fertilizers are somewhat more difficult to estimate. Based on a 75% recycle, and 5% content of N in the biomass, the amount of net N required is 1.25%. The energy used in nitrogenous fertilizer production, using natural gas is about 30 MMBTU/mt, based on relatively efficient modern conversion processes (a decrease of about 25% from 1970's values). The emission factor for natural gas is about 52 kg CO_2/MMBTU (DOE, 1994). Thus, for a 110 mt/ha/yr productivity, about 1.4 mt/ha/yr of N fertilizer would be required, resulting in an emission of 2.2 mt CO_2/ha-yr. The other fertilizers (P, Fe) and chemicals (flocculants), would add a small amount to this, to about 2.5 mt CO_2/ha-yr.

Other inputs would be the fuels used for the operation and maintenance (such as vehicles on site), as well as an amortized energy inputs for the capital items, including working capital (and start-up). Generally such analysis have found that these are rather negligible, although a detailed analysis would require some significant effort (complicated by the fact that there are not standardized methodologies for such analysis). Here it is estimated that the total would be about equal to that of fertilizer and chemical inputs, or 2.5 mt/ha-yr of CO_2 emissions. for a total of 5 mt CO_2 emissions/ha-yr, twice this for the higher productivities.

TABLE 8.4. ENERGY ANALYSIS OF THE 1982 AND 1987 REPORTS

Kcal of fuel inputs (Kwhr = 2150 Kcal of fuel)

Component		**Weissman & Goebel** 30 g/m^2/d, 365 d/y	**Benemann et al.** 22.5 g/m^2/d, 300 d/y
Mixing Energy:	/ha/d	60,300	36,567
	/g Algae	0.201	0.162
1o Harvesting:	/ha/d	10,275	23,660
	/g Algae	0.034	0.105
2o Harvesting:	/ha/d	33,763	51,624
	/g Algae	0.112	0.230
Water Supply:	/ha/d	51,565	1,344
	/g Algae	0.172	0.006
Nutrient Supply:	/ha/d	3,070	2,420
	/g Algae	0.011	0.011
Carbonation:	/ha/d	none	143,400
(flue gas only)	/g Algae	none	0.637
Buildings,etc.:	/ha/d	6,140	14,346
	/g Algae	0.022	0.064
Processing:	/ha/d	none	26,890
	/g Algae	none	0.120
TOTALS Pure CO_2	**/g Algae**	**0.552**	**0.718**
Flue Gas	**/g Algae**	**not designed**	**1.355**

TABLE 8.5. SUMMARY OF CO_2 MITIGATION ESTIMATES
for a 400 hectare system, @ $0.065/kWhr

Item #	Productivity Assumptions 30 g/m^2/day		60 g/m^2/day	
	CO_2	Flue Gas	CO_2	Flue Gas
1. Gross Power Produced kWhr/ha-yr	52,000		104,000	
2. Net Power Exported kWhr/ha-yr	26,500	10,100	83,500	50,700
3. CO_2 mitigated from #2, mt/ha-yr	23	9	73	45
4. CO_2 due to fertilizers, etc. mt/ha/yr	5		10	
5. CO_2 mitigated before oil mt/ha/yr	18	4	63	35
5. Algal oil outputs barrel/ha-yr	380		760	
6. CO_2 mitigated from oil, mt/ha-yr	150		300	
7. Net CO_2 avoided, mt/ha-yr	168	154	363	335
8. Net CO_2 avoided, mt/barrel oil	0.44	0.40	0.48	0.44
9. $/barrel algal oil (from Table 8.3)	69	56	42	39
10. Net cost of CO_2 avoided $/mt				
for $25/barrel oil	100	77.5	35	32
for $35/barrel oil	77	52.5	14.5	9

Next are the oil outputs. Here we assume that one barrel of algal oil contains 5.7 MMBTU, and displaces and equivalent amount of petroleum derived oil, with an emission rate of about 70 kg CO_2/MMBTU (DOE, 1994). This results in a CO_2 avoidance of 300 and 600 mt/ha-yr of CO_2, for the two productivities.

Table 8.5 summarizes this preliminary analysis. Most of the CO_2 mitigation is due to the algal oil produced, with the methane outputs taking care of the CO_2 emissions due to system operations, maintenance, and construction, with significant (10 - 20%) additional CO_2 mitigation from the net power generation only at the higher productivities. Note that the actual amount of CO_2 fixed is only about 60 to 70 % (for flue gas and pure CO_2 cases, somewhat greater for higher productivities) of the CO_2 actually fixed into biomass. The last two lines of Table 8.5 calculate the CO_2 mitigation costs at two levels of algal oil prices, \$25 and \$35/barrel. Clearly, net CO_2 mitigation costs are very sensitive both to the value of the oil and productivity. At about \$40/barrel oil CO_2 mitigation and for the highest productivity cases, CO_2 mitigation costs would tend to zero. This is perhaps one of the major advantages of microalgae CO_2 mitigation systems, compared to CO_2 disposal: as energy prices increase, CO_2 mitigation costs decrease with microalgae processes, while they increase with the disposal options.

Finally, the CO_2 balance for purified CO_2 recovery process must be considered. In the above a cost of \$40/mt purified CO_2, delivered by pipeline, was assumed. This was based on, among others, Karam (1995) and Herzog (personal communication, 1996), who indicated a cost of \$37/mt CO_2 purified, and other sources. However, such a cost does not consider the additional CO_2 that is emitted due to the purification process itself. Indeed, this would increase the actual cost of CO_2 avoided by about 50%, to about \$60/mt CO_2. However, a number of studies in the literature (Hendrycks, 1993, IEA, 1994) suggest that the cost for CO_2 capture can be reduced considerably and that \$40/mt CO_2 actually reflects costs of avoidance, rather than just production. (The difference, of course due to the fact that there is currently no cost associated for the additional CO_2 emitted during CO_2 capture, due to operation of the amine process). Also, based on the above analysis of 1.7 mt CO_2 required/mt algal biomass produced, the actual CO_2 avoided is only about 90% of that supplied in the form of pure CO_2 (98% for the higher productivity case) of that supplied in the form of pure CO_2. This is not a large correction, but should be kept in mind in case of any changes in assumptions. Overall, no change in the cost of CO_2 avoidance is proposed, although, clearly, this is an issue that could have major impacts on the overall analysis. Indeed, a more detailed review of this issue (including pipeline costs) appears warranted.

Finally, many other scenarios could be considered. For example, converting all the algal biomass to methane and using it for power production would result in higher CO_2 mitigation than oil production, but economics would not be as favorable, due to the lower value of fuels in electricity generation compared to transportation fuels. In conclusion, the above analysis should suffice for present purposes.

8.6. SENSITIVITY ANALYSIS

Typically sensitivity analysis vary one key parameter at the same time, over a reasonable range, and determine the resulting changes in costs (e.g. the 1987 study). Although valuable insights can be gleaned, single parameter changes generally result in relatively small overall changes that make little difference. An alternative is to use two extreme cases, one in which all the parameters are as favorable as plausible, and another more conservative one (e.g. the 1982 study).

For the present effort no further sensitivity analysis is presented other than the consideration of the two productivities, the use of flue gas vs. pure CO_2 and two prices (values) for the (unrefined) algal oil, as per Table 8.5. The reason is that most other design concepts and assumptions (operational, engineering, costs), already represent favorable cases, as favorable as can be reasonably considered at present. Although in some cases significantly higher costs than in prior studies were used (e.g. labor costs), and although in a few cases more favorable assumptions are plausible for particular sites, resulting in some further cost reductions, such exercises would not contribute usefully to the present effort.

The present study is the most optimistic, in most parameters, of any of the prior efforts. Indeed, the likely higher productivities, greater lipid contents, and simpler harvesting-processing technology were assumed than in prior studies. The justification for such favorable assumptions, compared to earlier analysis, is that new information and theory, developed over the past decade, allows for greater optimism about this technology. Indeed this was also the the case for the 1982 report (compared with the 1978 study) and the 1987 report (compared to the 1982 one), each being more favorable than the previous one in these or other aspects. However, clearly, a limit is being reached, in terms of productivities and algal composition. Indeed, the highest values used herein are unlikely to be achieved in practice, and represent more a limit than a plausible possibility.

Even if the work of Greenbaum and colleagues (Greenbaum, 1995) is shown to potentially lift the "theoretical" limit of solar energy conversion, to over 10%, even achieving a 10% solar conversion efficiency in practical applications would be rather problematic. Anyway, at present a higher quantum efficiency for the Greenbaum type of photosynthesis has not yet been demonstrated.

In conclusion, reductions in costs are as unlikely, at least in aggregate, as further increases in productivities. For most systems the lowest plausible engineering and construction options were selected and rather optimistic assumptions were made (e.g. harvesting-processing). Overall, estimated costs are as low a can be reasonably extrapolated at present. Thus, the cost projections in Tables 8.2 to 8.5 are unlikely to be greatly reduced. In brief, from Table 8.5 the cost of CO_2 mitigation is a function of many components, of which productivity and competitive energy prices are the most important.

8.7. DISCUSSION AND CONCLUSIONS

The concept of microalgal fuels production by using power plant CO_2, is already over forty years old has been the subject of considerable research over the years, including a number of feasibility analyses, starting with Oswald and Golueke (1960). That report provided the basic design concepts, a schematic of the system the cost elements, performance parameters and R&D issues. Since then considerable progress has been made, both in the development of microalgae biotechnology for waste water treatment and specialty products generally, and in the research in development of dedicated microalgal fuel production systems. In regards to the latter, the technological development work culminated in the U.S. earlier this decade, with the demonstration of microalgae culture for lipids at the Roswell Test Facility (Weissman and Tillett, 1989, 1990).

The last feasibility analysis in this field (Weissman and Goebel, 1987) was carried out a decade ago, before the Roswell work, the commercial success of the U.S. microalgae industry, or the emphasis on the issues of CO_2 mitigation. The major objective of the present analysis was to update and extend the prior studies, specifically for the objectives of CO_2 mitigation from coal-fired power plants. As before, no major technological limitation to an microalgal fuel production process that could utilize power plant derived CO_2 was identified. Some conceptual advances were made in the present effort, compared to previous ones, in particular in the extraction of the algal lipids. However, clearly, many unknowns remain, and achieving the performance objectives will require considerable R&D.

In this analysis, direct utilization of flue gas was compared with remote supply of pure CO_2. The flue gas delivery pipe in the former case and use of gen-sets in the latter were the major capital cost differences, with the gen-set capital costs outweighing the flue gas utilization, making pure CO_2 utilization more capital intensive. However, this extra capital cost was more then outweigh by the additional income from power production (assuming it is sold for \$0.065/kWhr, a rather optimistic assumption in the current utility environment). The major operating cost difference between the two options was the high cost of purified CO_2. However, overall, the difference in costs between these options were not as large as anticipated, particularly at the higher productivities. Indeed, productivity was the most important parameter in determining overall costs. Thus this must be a major emphasis in any future R&D.

The present results are comparable to prior efforts. Indeed, remarkably, the actual (e.g. not adjusted for inflation) costs estimated in the 1987 report are almost identical to the present ones, for similar process configurations (C recycle, use of pure CO_2) and productivities (30 and 60 $g/m^2/day$). Of course, this may have been expected, as much of the 1987 report cost data was also used here. However, significant differences exists, such as the incorporation of a lipid extraction process.

It should be noted that in the present effort all cost data was approximated and rounded. This was deliberate, as none of the cost inputs have a high precision, and reporting costs with many significant numbers may create a misleading impression of accuracy. Also, such rounded numbers are easier for simple visual comparisons, within and between cost estimates.

Perhaps the most important issue is how microalgae CO_2 mitigation costs compare to other CO_2 direct CO_2 utilization and disposal technologies. In that regards, it should be noted that the pure CO_2 case already incorporates the cost for CO_2 capture and transportation. Thus, we can compare these costs with the overall costs of both capture and disposal. For consistency, capture and pipeline transportation costs should be set, as above, at \$40/mt CO_2 avoided. Disposal costs for CO_2, after capture, are highly uncertain. The lowest cost for ocean disposal, based on a pipeline from shore, is estimated, very roughly, at about \$10/mt CO_2 (MIT, 1993; Herzog, private communication, 1996). The MIT study also estimated CO_2 disposal costs of about \$15 to 50/mt for other options, in particular aquifer storage and depleted oil and gas well storage. The IEA report on CO_2 disposal (IEA, 1994) estimates costs of about \$15 to 30/mt for disposal of CO_2 in the ocean (similar to MIT study), aquifers and gas and oil wells. Although these are "soft" estimates, these combined cost of CO_2 capture and disposal are similar to the microalgal processes described above, even for the lower productivities and oil prices assumed.

Considering the potential for achieving the higher productivities (through control of the light saturation effect), and the advantages of algal systems at higher energy prices, at a minimum algal systems can be considered competitive with, and potentially of much lower cost, than the alternative options. Thus, from Table 8.5, at the higher productivities and for the lower oil prices, algal CO_2 fixation is already competitive with CO_2 capture, without even considering ultimate disposal. As oil prices approach \$40/mt, CO_2 mitigation costs decrease to near zero at the highest productivities. Even for the lower productivities and oil prices, costs are still comparable to those of alternative direct CO_2 mitigation processes. A review of the cost estimates for alternative processes (MIT, 1993, IEA, 1994), reveals that the present effort is much more detailed in experimental data and engineering design and analysis than the alternative concepts.

Of course, indirect CO_2 mitigation processes (tree-planting, energy conservation, etc.) are generally less expensive than the direct processes (Rubin, 1993). Indeed, there are many opportunities for the mitigation of CO_2 (and other greenhouse gases) at relatively low costs ($<$ \$5/mt CO_2). However, as such "easy" opportunities are exhausted, the cost curve incre ases rapidly, and more difficult methods, such as capture/disposal of CO_2, alternative energy sources, or combinations thereof (e.g. microalgae systems), may need to be introduced, if the problem of global warming turns out to be severe enough. Timely R&D to develop these alternatives is required, in case such options need to be implemented in the future.

9. CASE EXAMPLE: BOTRYOCOCCUS BRAUNII

9.1. INTRODUCTION

One, and only one, microalgae, indeed even microbe, is known to produce hydrocarbons in copious amounts: Botryococcus braunii. The hydrocarbons produced by this alga are visible under the microscope as large oil droplets oozing out of the colonies, with contents of over 80% (although typical values are 20% to 40%) of dry organic matter reported. These are long chain (generally C23 to C40, depending on strain) unsaturated hydrocarbons that can be easily cracked into diesel fuel or even used directly as fuel. This section presents a conceptual process for the production of hydrocarbon fuels by B. braunii that integrates the various issues discusses in the previous sections.

In most (but not all, see Regan and Garside, 1983) prior analysis of microalgae energy production, Botryococcus braunii has been essentially ignored. A major reason is that it grows notoriously slowly, with doubling times in the order of several days. Two problems are apparent: the potential for contamination of the culture by faster growing algae and the low productivity of the overall process. Although slow growth rate and low productivity are not synonymous, the very slow growth of this alga might, indeed, likely result in lowered productivities. Productivity could be improved through both strain selection (for faster growing strains) and operation at higher densities. Contamination is the more critical issue, and one essentially ignored in the above sections. It must be overcome by the production of a large inoculum, under controlled conditions, and relatively frequent culture start-up. Although any system will require inoculum production, something ignored in the above discussions, this could well be the limiting factor in the case of Botryococcus braunii, as well as other algae.

The feasible limits, and conceptual design of an inoculum production system are explored in this section. The conclusion is that although the production of even a very large inoculum, representing some 5 to 10% of the total biomass used, would be a significant overall cost, it would not be prohibitive. Of course, considerable more work is required to verify and narrow the range of these cost estimates. But it does suggest that even relatively non-competitive strains of algae could, in principle, be produced at a very large scale and low cost.

The objective of R&D for B. braunii production, as for other algae systems, are to minimize inoculum requirements and maximize productivities. This requires a directed genetic selection program coupled to a process development effort. Overall, it would appear that production of B. braunii is a viable option, perhaps not that much more difficult than other algal species.

The uniqueness of Botryococcus braunii, and its association with various natural oil deposits, led to the proposal in the 1970's that this alga could be produced to supply fuels (Casadevall, 1978; Wake and Hillen, 1980). Over a hundred publications on this alga have been published since then (Section 9.3). However, most of these publications were on the chemistry and biosynthesis of the hydrocarbons, only a few on laboratory cultivation of this alga, and none on mass culture. One discovery was that there are several "races" of B. braunii, differing in the amounts and types of hydrocarbons produced.

There is thus little data to extrapolate the mass culture of this organism. One reason is the low growth rate of this alga: shortest doubling times range from about two days to over a week, depending on strain and condition. Comparative maximal growth rates for most other microalgae would be about one order of magnitude faster (a few hours). Slow growth, low productivity, and culture contamination have discouraged all but the most dedicated researchers. Indeed, over a decade ago, Wolf (1983) concluded that "available data do not point favorably to the widespread use of Botryococcus derived fuel anytime in the near future."

A similar problem is that of the alga Haematococcus. This alga produces a very valuable pigment, astaxanthin, a xanthophyll used in salmon feeding and valued at about $3,000/kg. Despite the fact that this algae can contain up to 5%, even more, of this pigment, commercial production has not been achieved. Although such a high value product (corresponding to a value of over $150/kg of algal biomass), should allow for truly heroic efforts at keeping a clean culture, no commercial success has been achieved. Obviously this is a key problem, not only for high value, and, even more critically, for low value algal products.

Even if it should be possible to cultivate, through a combination of strategies, such as the production of large amounts of inoculum, a slow growing algal species such as Botroyococcus braunii, this would only be practical if high productivities were achieved by such a culture. Although slow growth is often equated with low productivity, that is not necessarily the case. (See Section 4 for a discussion). In any event, the general strategy advocated in this section is to combine inoculation with a selective environment in the production pond, such as nutrient limitation. As reviewed below, the data base on B. braunii is scant enough to allow for optimism that such an approach would be successful even in this case. Indeed, it should be possible to genetically select this alga for higher productivity, while still maintaining its unique biosynthetic pathways for hydrocarbon production.

From the prior sections, only the raceway, paddle wheel mixed, pond design is a plausible method for large-scale, low -cost microalgae production, and that would also apply to B. braunii. Thus, in this section, only the unique attributes of this alga, and its cultivation are discussed. The most unique of which is, of course, its hydrocarbon content.

9.2. HYDROCARBONS OF BOTRYOCOCCUS BRAUNII

9.2.1. Ecophysiology of B. braunii.

Botryococcus braunii is a colonial alga classified among the chlorophyceae (green algae). As stated, its claim to fame is the ability to produce large amounts of pure hydrocarbons, which appear as oil droplets excreted by the cells. Its other claim to fame is that hydrocarbons characteristic of this alga are found in association with oils from a variety of geological sources with its characteristic hydrocarbons (for example botryococcene) found as major (> 1%) components in many oil deposits, both ancient and modern, making this the largest of any biomarker in petroleum (see McKirdy et al., 1986, and references therein).

B. braunii has been the subject of well over 100 scientific reports and reviews over the past dozen years, almost half from one group in France (Prof. Casadevall). However, the available information is still very limited, with most research on hydrocarbon chemistry, some on their biosynthesis, and relatively little on laboratory cultivation, most of which is of limited relevance to mass culture anyway. This paucity of data is not unusual in the microalgae field, where few algae are subjected to detailed physiological studies under both laboratory and outdoor conditions (Spirulina being an exception). Lack of data limits our ability to predict the behavior of the alga in mass culture (besides the rather obvious fact that it is slow growing, due to the metabolic energy diversion to hydrocarbon production).

Thus, at present, no useful information is available that could be extrapolated any to mass culture situations. On the other hand, data limitation also imposes fewer constraints on speculation regarding the possibility of genetic selection and physiological adaptations to harness the unique biosynthetic machinery of this alga to the production of low cost fuels.

B. braunii has a rather interesting ecological niche. It appears to be a relatively universal alga, found mainly in fresh and brackish waters, although marine strains are also known. It is found sometimes to dominate in large blooms, of various appearances, from orange to green, but mainly characterized by an oily appearance and a rather high hydrocarbon content, ranging typically about 20 to 40%, but also as high as 86% and as low as 1% or less (see next section).

Despite their widespread occurrence, little is known of the factors responsible for its dominance in blooms. Indeed, it is not clear even if this is a true planktonic algae. It is possible that it spends much of its time in sediments, and the blooms represent only a temporary floating stage, triggered by certain environmental conditions. This may account for the low growth rate of the alga, and its relatively low light requirements. However, no data on photosynthetic rates as a function of light intensity is available and, anyway, the strains (clones) maintained in culture are not likely representative of the planktonic forms.

Indeed, one strain, the "Showa" strain (isolated by Dr. Nonomura and patented by the Univ. of Calif. Berkeley), had, at least after initial isolation (from a birdbath) tolerance to rather high, even full sunlight, light intensities (A. Nonomura, personal communication). Indeed, the strains of B. braunii isolated thus far are not likely to represent the typical alga found in nature, as strong selection is imposed during the laboratory transfer of such strains. However, as the requirements of mass culture are rather unique, predictable, and narrow (Section 4), this is not a major issue: in the future strains must be isolated, and if possible adapted, to these conditions.

In any event, the conditions favoring bloom formation of this alga are not certain (e.g. Wake and Hillen, 1981), as is the case for most bloom producing alga, even those of economic interest, such as toxic algal blooms . For example, different authors found blooms of B. braunii (a bloom being defined as a relatively dense assemblage of algae in which the biomass is dominated by a single species) under rather diverse conditions, with no particular factor or combination thereof exhibiting high correlation with bloom formation. This lack of correlation may signify either that there are no unique parameters or that the factors are rather subtle. The first could be the case if the alga had indeed a sediment phase, with the planktonic bloom incidences thus reflecting more the results of that compartment and history than the near surface conditions where the bloom appears.

Whatever the reasons, at present we do not know if there are indeed any nutritional or environmental factors that favor B. braunii blooms, and which could be used as tools in mass culture. Indeed, it may be that physiological differences between different populations of this algae would make such a search of common factors rather difficult, if such factors indeed existed.

Each bloom is likely composed of several original clones, and mutation rates are likely to be high (a phenomenon commonly observed among organisms that exploit relatively extreme environments). In clones of other species of algae, phenotypic variability is high in relation to physiological parameters such as nutritional capabilities, responses to light and temperature, etc. Morphology is the dominant characteristic that defines algal species, along with some basic biochemical properties (principally pigments). Thus, variability in B. braunii in relation to characteristics useful in mass culture systems would be expected.

This argument is fundamental in the proposition that selection of suitable strains would be feasible. Of course, the central issue is the ability to mass culture this alga without loosing the capability of high hydrocarbon production. Preservation and enhancement of this capability must not be compromised during the genetic selection process for a highly productive and competitive algal strain suitable for mass cultures.

9.2.2. Functions of the Hydrocarbons of B. braunii.

The above raises the issue of the function of the hydrocarbons in the algal life cycle. Several theories have been proposed or could be considered. That the hydrocarbons could be a form of energy storage (as are the triglyceride lipids produced by green algae and diatoms, particularly under N limitations, see Section 5) is unlikely, as the extracellular stored hydrocarbons (>95% of total) are not metabolized (Largeau et al., 1980; Templier et al., 1992).

The concept that the lipid bodies could provide a flotation aid is appealing. However, the advantage of this flotation is not clear. As with the gas vacuoles of blue-green algae, it results in large surface blooms being created which are blown on shore where the algal cells perish (leaving behind their hydrocarbons, the source of future oil deposits). Perhaps under more normal conditions such a process does aid in dispersal, to a near shore sediment, which could be of interest to the alga if it is adapted to grow associated with sediments (as suggested by A. Nonomura, personal communication). Near shore sediments would have more light and receive more nutrients that the deeper areas where the alga may find itself. It should be noted that such adaptations in which algal species exists in both as sediment growing forms and planktonic blooms known for other cases, particularly diatoms. Thus this would not be unique to B. braunii.

Another theory is that the hydrocarbons protect the alga from zooplankton grazing. This has some appeal, as grazing is a common reason for the crashes of blooms, and a major problem in algal mass cultures (which requires much further study). However, it does not appear that any experiments have been done (e.g. the use of similar size colonial green algae in various competitive feeding experiments).

Finally, we could speculate that hydrocarbon production is somehow correlated with protecting the algae against high light, as the highest hydrocarbon concentrations are found in the "orange" form, colored due to the dissolution of beta-carotene in the hydrocarbons, a pigmentation often associated with high light intensity. However, there is no evidence in support of this theory.

We do not yet know why B. braunii makes such large amounts of hydrocarbons, and no other algae (or even microbe) comes near in the amount of hydrocarbons produced both in nature and the laboratory (e.g. 20 to 40% for pure hydrocarbons, vs. well below 1%, typically 0.1%, for other algae. If, indeed, B. braunii were less susceptible to zooplankton grazing, it may be possible to use this as a factor in maintaining a unialgal culture. Of course, this is not likely to be a panacea as any sustained cultivation would rather quickly select for zooplankton specializing in the dominant alga. This is an area of future study.

The flotation capabilities of this alga could also be used in a harvesting process based on flotation. Such a capability also needs to be considered in relation to the

mixing in the ponds, as it would not be desirable to have the culture exhibit flotation (surface scum formation) in the growth ponds. Experimental work is required to test both of these issues.

Hydrocarbon production has significant metabolic costs for the algae. In an ideal environment most of the resources of the algal cell should be devoted to production of biosynthetic machinery, to allow rapid reproduction. Diversion of over half of metabolism to hydrocarbon production, which has no immediate advantage gives this alga a severe handicap in the competition with other strains.

Of course, the same problem is faced in the production of "lipids" (vegetable oils) by microalgae, where lipids are to be 60% of the dry weight of the biomass produced. In that case the concept is to produce algal biomass of low lipid content at a high productivity, followed by a phase of nutrient (typically N limitation) that would divert the biosynthetic products to triglycerides. The algae would be harvested at a point that optimizes productivity and lipid content. Laboratory experiments suggest that it is possible, to manipulate light and nutrients to induce high lipid content (over 50%) with no loss (and actually some gain) in photon conversion efficiency (See Section 6).

In the case of B. braunii, hydrocarbon production appears to be at least in large part constitutive, and not controlled by nutrient limitations. However, a 60% hydrocarbon content, a likely objective for fuel production, would result in a large metabolic drag, reducing growth rates to such an extent that it may not be feasible to prevent contamination. Development of a hydrocarbon inducible strain should be within the capabilities of a genetic program for this organism, although perhaps that may require significant effort. In this report a compromise case is assumed: a 20% constitutive hydrocarbon production (corresponding to current data) which is increased to 60% on nitrogen limitation. This would result in a significant, but likely manageable, decrease in growth rate in the production phase, and no significant loss in productivity in the induction phase. This is a basic process assumption of this feasibility analysis.

9.2.3. Botryococcus Hydrocarbons: Structure and Content

Botryococcus braunii is defined morphologically and by the high hydrocarbon content, but the hydrocarbons differ significantly in both amounts and chemistry. At present three "races" are recognized: A, B, and L. These are characterized as:

Race A. (Type strain is the Austin Algal Culture Collection Strain of B. braunii). Produces odd carbon numbered C23 to C31 dienic and trienic unbranched hydrocarbons.

Race B. Produces isoprenoid C30 to C37 hydrocarbons of general formula CnH2n-10 with the major component usually a C34 tertramethylated

triterpene given the familiar name botryococcene. This race includes the "Showa" strain isolated in Berkeley in which a number of novel structures were identified (Huang and Pouler, 1989).

Race L. Two strains, morphologically similar but somewhat smaller, isolated from the Ivory Coast and Thailand, were classified as belonging to the L race, produce a single C40 tetraterpene hydrocarbon, a lycopadiene (Metzger and Casadevall, 1987).

In addition to the major hydrocarbons, B. braunii strains also produce small amounts of a variety of chemically related aldehydes, long chain phenols, epoxides , ethers, and other lipids (Metzger and Casadevall, 1989), with the ethers sometimes constituting a relatively large amount of chemically "resistant" biopolymers (Derenne et al., 1989; Metzger and Casadevall, 1991).

The biosynthesis of the Botryococcus hydrocarbons has been the subject of recent studies by the French group (e.g. Templier et al., 1991; Metzger and Casadevall, 1992), but the available information provides little of current applicability to the requirements of mass culture. However, for the future, development of improved strains such knowledge is important.

Of course of greatest interest is the content of hydrocarbons in this alga, which is often quoted to be up to 86% of dry weight (Brown et al., 1968, Maxwell et al., 1968) a truly astonishing content. Brown et al. (1969), stated that the hydrocarbon content of the "brown resting state colonies ... may be up to 86%", while for the green active state colonies (from which the brown resting stage is derived) contain only "up to 17%" hydrocarbons. There is the suspicion that the high hydrocarbon content could have been due to the disintegration of the algal cells at the end of the growth phase (no data is provided of how long the cultures were kept), leaving the hydrocarbons behind. Similar suspicions exists regarding the Maxwell et al. (1968) data. No other data on absolute amounts of hydrocarbons was provided by these authors.

Brown et al., 1968, also found a difference in hydrocarbon chemistry between the laboratory cultures (making botryoccocene) and a wild culture (containing mainly linear chain hydrocarbons. Similar differences were also observed for Australian blooms of B. braunii, where "red" blooms contained up to 40% of branched botryococcene type hydrocarbons, and "green blooms" a somewhat lower contents (27 - 32 %) of straight chain hydrocarbons (Wake and Hillen, 1981). Although this was explained by these authors as being due to differences in the growth conditions and stage of the culture, this could not be confirmed by others (e.g. Wolf and Cox, 1981), and we now know that different hydrocarbons are produced by the different races of alga discussed above.

By contrast Gelpi et al. (1968) reported that "these hydrocarbons [C23 to C33] are present in high concentration in the cells (0.1 to 0.3 percent of dry cell weight)".

Perhaps they considered such content "high" in relation to other algae, but it is almost insignificant in the current context. However, again, no further details on quantitative extractions were presented (with the objective of this study being to relate the chemistry of the hydrocarbons to oil deposits). Murrey and Thompson (1977) took up this issue and found that hydrocarbon fraction in B. braunii were about 1%, still a rather low amount. The differences in results are probably due to both growth conditions (not well characterized in the reports) and strains used.

Following up on this early work, others reported relatively high concentrations of hydrocarbons with laboratory cultures, typically in the 15 to 30% range: Knight et al. (1970) found that for laboratory cultures 20% of the algal dry weight were hydrocarbons (again without detailed data). The French group (Casadevall, et al., 1980) found hydrocarbons of up to 36% in laboratory cultures of a linear hydrocarbon producing strain, which had doubling times of about 60 hours.

Wolf et al. (1985), using a newly isolated strain (the "Showa" strain) that produced botryococcene, found that best growth was obtained with a CO_2 enriched, stirred culture, which exhibited a doubling time of about 40 hours. Hydrocarbon content in these culture was 24 to 29%, but increased to 39% in a CO_2 limited (air only) culture. Neither N nor P deficiency increased hydrocarbon content. This strain, which was orange in color, demonstrated that the differences in hydrocarbon contents between various cultures were not due to a "resting stage" phenomenon, but to genetic differences, and led to the "race" classification discussed above.

9.2.4. Growth Physiology of Botryococcus

All of the laboratory cultivation efforts of Botryococcus braunii have been in the laboratory using essentially three methods: static cultures (in which the algae are growing in flasks which are typically shaken by hand once a day), shaker or mixed (by magnetic mixing), or airlift cultures, the latter being the method used by the French group in most of their studies (Casadevall, et al., 1985).

The airlift cultures basically provided good mixing and CO_2 supply, and allowed for faster growth than cultures not supplemented by CO_2, as also observed by Wolf et al (1985, see prior paragraph). Hydrocarbon content was rather constant during the growth phase, at about 40% of total dry weight, actually decreasing in the stationary phase (to about 25%). (This is a race A strain, producing linear hydrocarbons). Continuous cultures in airlift fermenters (at 0.25 and 0.3/day dilution rate) gave somewhat lower (26 - 27%) hydrocarbon content as the batch growth phase. Neither P nor N limitation appeared to have a major influence on hydrocarbon content. A major conclusion of this, and other (e.g. Wolf et al., 1985, see above) work was that hydrocarbon biosynthesis is essentially constitutive, and not associated with stationary culture metabolism. Thus, B. braunii is fundamentally different to the lipid producing microalgae being considered for "biodiesel" production.

Of course, the major issue in algal mass culture is not only hydrocarbon content but, more important, hydrocarbon productivity. There is very little data on this point that would allow extrapolation to mass culture. One major question is the efficiency (photon conversion) of such production at low light. There appears to have been no such studies. Another is the effect of high light. All of the work has been carried out under relatively low light conditions, typically under 5% of full sunlight and apparently not above 10% of full sunlight and no data on photosynthetic activity vs. light intensity appears to have been collected. Thus in terms of fundamental physiological parameters we have essentially no information.

A number of studies have determined the effect of nutrient availability on hydrocarbon production. For example, Ohmori et al. (1984) found that addition of ammonia to cultures of this alga resulted in rapid shifts in metabolism, measured by $14CO_2$ incorporation, from botryococcene production to protein biosynthesis, particularly in cases of prior N limitation. However, these were very short term experiments, in the order of minutes, a small fraction of one generation time. The effects of such manipulations on actual hydrocarbon contents were not determined. Aaronson et al. (1983) reported that in laboratory cultures there was an increased in hydrocarbons as the culture aged, and became nitrogen limited, but the experimental conditions were not reported.

One issue in microalgae mass culture is the effect of associated bacterial flora. It is not feasible to produce axenic (bacteria-free) algal cultures outside the laboratory (at most a few hundred liters). However, the effect of associated bacteria on culture growth and productivity has not been much studied. In the case of B. braunii, Chirac et al. (1985), who found that bacterial associations could, depending on conditions and algal strain used, either stimulate or inhibit both growth and hydrocarbon production. Positive effects were likely due in some cases to the CO_2 produced by bacterial metabolism of extracellular materials in CO_2 limited (e.g. air sparged cultures). Other positive effects, of more uncertain nature, were also noted, and the authors concluded that: "Association with selected microorganisms could therefore solve, in part, some of the major problems encountered in renewable hydrocarbon production in large scale cultures of B. braunii".

Of course, the associated bacteria grow at the expense of the extracellular metabolites produced by the algal cultures, which can vary from the insignificant ($< 0.1\%$ of photosynthate) to a major fraction ($> 10\%$) of total CO_2 fixation products, depending on algal species, strain, and experimental conditions. In the case of B. braunii, Casadevall et al., (1985) noted a great increase in culture viscosity, suggesting the production of extracellular polysaccharides, which was confirmed by Allard and Casadevall (1990) for five strains of B. braunii (from the three races), finding that in stationary phase cultures extracellular polysaccharides released into the culture medium ranged from 0.25 (A and B strains) to 1 g/l (L strain), with galactose as the main sugar components. Although such large extracellular polysaccharide production would suggest this as another factor in

reducing growth rates and productivity, it would not be reasonable to extrapolate data from stationary cultures to actively growing ones. The production of extracellular metabolites is an issue that needs to be addressed further.

The French group has also carried out considerable work on the effect of immobilization on algal growth (Bailliez et al., 1985, 1986, 1988), reporting in some cases some improvements in algal and hydrocarbon production with immobilized systems. Although artificially immobilized algal systems are of practical interest in some applications, such as heavy metal removal for example, they are not practical in any concept for mass cultivation. It has been suggested that immobilized systems may be more resistant to contamination. However that has not been established, and the complications introduced by the immobilization process far outweigh any reported (minimal) or potential (modest) advantage over liquid cultures and thus need not be considered further in the present context.

Another issue in algal mass cultures is the quality of water required for the process. B. braunii is an ubiquitous alga found in fresh, brackish, saline and even hypersaline (3M NaCl) waters (Aaronson et al., 1983; Bauld, 1981). At high salinity the alga (race A) produces a unique (for algae) osmoregulator, alpha-laminoribiose (Vazquez-Duralt and Arredondo-Vega, 1991). Thus, it appear that there should not be a major problem in growing this alga on brackish waters.

Although information on B. braunii mass culture is lacking, Sawayama et al., (1992) have recently investigated the potential of this alga (Showa strain) in waste water treatment, apparently assuming that outdoor mass culture would be feasible. They found that B. braunii would grow in (filter sterilized) secondary domestic sewage effluent (e.g. containing little residual BOD, and appx. 5 to 10 mg/l nitrate), reducing nitrate and phosphate concentrations to essentially undetectable levels. This information is interesting in that it demonstrates that this alga utilizes nutrients very efficiently, as would be expected from a bloom forming species.

One interesting observation reported recently is that the addition of methanol to Botryococcus braunii Showa will double the yield of a batch culture. This observation led to the application of methanol to higher plants, also demonstrating a large increase in productivity (Nonomura and Benson, 1992), a finding which is thought to have significant agronomic potential in horticulture of high value plants (Bishop, 1992). However, application of this fundamental discovery in B. braunii mass culture appears to be unlikely.

In conclusion, although some work has been carried out on the laboratory growth of B. braunii, existing data is very limited. The only continuous culture work reported (Casadevall et al., 1985) appears to be rather favorable, as the dilution rate (0.25 to 0.3/day) is sufficiently fast for outdoor algal cultures and the hydrocarbon content relatively high (about 25%). Thus, there is at least some basis for extrapolation to mass culture conditions.

9.3. CONCEPTUAL BOTRYOCOCCUS MASS CULTURE PROCESS

9.3.1. Introduction

As discussed in previous sections, to be economically competitive for fuels and CO_2 utilization, microalgae cultures must achieve very high productivities, exceeding 5% total solar energy conversion efficiency (more than the productivities reported for sugar cane, the most productive higher plant), and approaching 10%, near the (currently) theoretical limit. Further, the lipid (in this case hydrocarbon) content of the final algal biomass harvested must be relatively high, at least 50% on a dry weight basis. And the microalgae must carry out the overall process of hydrocarbon production under high light intensities and fluctuating temperatures in open ponds subject to contamination by other algae and predation (protozoa, etc.). The conditions under which hydrocarbon production takes place must allow for relatively easy operation of the process, such as minimal mixing or other energy intensive operating requirements. Next, the algae must be able to be harvested with a low cost process and the water recycled, with minimal blow-down and the hydrocarbons must be extracted and converted to a useful fuel at relatively low cost. And finally, the residual biomass able to be subjected to anaerobic digestion, and the nutrients and water recycled.

Although development of such a process would be difficult, no single requirement presents an insurmountable obstacle. The fundamental genetic, biochemical and physiological processes are well enough understood to provide at least a general guide on how to achieve the above goals. Of course, combining all these attributes into a single strain of Botryococcus is indeed a formidable and long-term task requiring a coordinated effort of strain selection and improvement along side with development of mass culture techniques.

B. braunii hydrocarbon production process must, at this stage, be almost entirely conceptual, constrained by available data but assuming that current limitations could be overcome through directed basic and applied R&D. Of course, the major focus of such an effort would be to develop B. braunii strains that meet the requirements of very high photosynthetic efficiency, high content of hydrocarbons (at least 50%), adaptation to the pond environment and to local conditions (water quality and temperatures). In the development of this conceptual process it is assumed that such strains can be developed. Indeed, a major objective of the present exercise is to set the requirements for such a strain development program.

It is assumed that B. braunii would be, like most algae cultivated in such mass culture systems and selected for high biomass productivity (which does not equate with high competitiveness), be subject to invasions and contamination. This suggests that the culture would need to be replaced on a regular basis, which requires an inoculum production system. Inoculum production this is the major new component described in this Section, not considered in Section 8.

9.3.2. B. braunii Production Process

In the case of B. braunii the ability of the algae to float, due to the presence of hydrocarbons, has been suggested as a method for harvesting. However, this has not been demonstrated for laboratory cultures, only natural blooms have been shown to float. In any event, the same type of harvesting process would be used as discussed above, that is a deep pond into which the algal culture harvested from the growth ponds once a day would be placed, and the flotation (rather than settling) process allowed to take place as a batch process. Although the system would be of somewhat different design (e.g. with surface take-off for the algal product), and the amount of volume handled may be somewhat smaller (as the maximum harvest rate may only be about 33% per day), the overall cost of this system would not be significantly different.

In the lipid production process reviewed, the algal biomass recovered from the settling ponds would be concentrated further by centrifugation with simultaneous oil extraction. The cost of the centrifuges were allocated to the processing subsystem and the cost of algal oil extraction was based on the centrifugation costs. Although this is based on a similar system for recovery of beta-carotene from Dunaliella, actual costs, for a large-scale system, are somewhat speculative, as there is little or no data on which to develop cost data for an algal lipid extraction process (See Section 8).

It has been suggested that the hydrocarbon producing cultures could be recycled to the growth ponds after extraction of the hydrocarbons. Although his approach is appealing, it appears to be far ahead of the data and present knowledge and is not considered herein. Another suggestion is to utilize the putative ability of the alga to grow on sediments or in biofilms as a method for cultivation. Again, this is far ahead of current information, and we have no examples of non-planktonic algal mass culture. Thus, this is also eliminated from consideration.

The other support systems of this process, including methane fermentation of the residual biomass, water recycle, CO_2 supply, etc., are similar to those of lipid production systems (Section 8) with some modifications discussed below.

Herein a very favorable conceptual Botryococcus production process is proposed. The fundamental assumption is that the achievement of the assumed productivities is possible with the current state-or-the-art of biotechnology. However, achieving these objectives would be a long-term R&D project, requiring some "breakthroughs" which although logical and plausible, can not be clearly or confidently forecast. Here, the prior work is applied to the specific aspects of the production of Botryococcus, which, in essence, differs by the requirement of a large inoculum system.

9.3.3. Existing Inoculum Production Processes

The major objective of the present section is to establish the relative costs of an inoculum production subsystem, which was not previously included in the concepts for microalgae fuels production. Asides from inoculum production, the same overall process (ponds, harvesting, processing, water and CO_2 supply, etc.) is specified to be the same as in the triglycerides production in Section 8.

There is almost no prior analysis of inoculum production. For the commercial production of Spirulina, the companies claim (to the extent it is discussed) that they do not re-inoculate their ponds once the process is underway, but, rather, allow continuous algal production to go on indefinitely. If the culture in one pond is abandoned for any reason it is inoculated with the culture from another pond. It is difficult to ascertain the actual operations, however it is certainly the case that any inoculum production, if any, is a rather small part of the overall production effort for Spirulina.

For the one Dunaliella production company in the U.S., inoculation is practiced and that company claimed, build during 1995 a fairly extensive inoculum production system. This consists of, essentially, a series of open, paddle wheel mixed, raceway ponds, of increasing size. The medium, at over 150 g/l NaCl, is very selective, and, thus, there is little danger of contamination. (It is, however, not clear if there is contamination by "wild type" Dunaliella strains blowing in from the surrounding soil). In any event, in this system inoculation can be carried out with rather minimal effort, as there is no major effort required to produce unialgal cultures for inoculation purposes.

As already discussed in Section 2, the situation is quite different for Chlorella , where rather large volumes of inoculum are produced first under laboratory conditions in hundreds of flasks, which are then used to inoculate small indoor ponds under artificial illumination, and then larger ponds in greenhouses. This culture is then used to inoculate outdoor ponds, which yield only a few batches of algae before the process must be repeated. Inoculum production is clearly a major overall cost factor in such systems. However, the commercial nature of the process has resulted in essentially no technical details (such as the amount of inoculum production required) having been published is over three decades.

There are is one industrial application of microalgae that is currently producing relatively large amounts (several hundred kg/month) under relatively controlled conditions: the production of microalgae for aquaculture purposes (Benemann, 1992). The algae typically produced, such as Isochrysis galbana, Tahitian strain, can not be grown (for any length of time) in mass culture (probably due to poor temperature adaptation of this strain), and this forces its cultivation under controlled conditions. Several large operations are carried out in the U.S. (e.g. Donaldson, 1991), which involve the cultivation of such algae in circular ponds

under greenhouse covers and with additional artificial lights. Costs of production are high, about $400/kg (updated costs) for an optimized system able to produce a about 10 kg per day (Walsh, 1987). This could a model for an intermediate scale-up step of a Botryococcus inoculum culture, following the laboratory scale.

9.3.4. Level of Inoculum Required

In the conceptual system for microalgae production for fuels discussed in Section 8, the implicit assumption was made that inoculum production would be a very minor requirement: relatively small inoculations (about 1% of the standing culture) would be sufficient to start-up a culture and that this culture would be able to be continued for several months, or longer. Also, inoculum production would not require significant contamination control past the laboratory stage. Thus, inoculum production would amount to be less than 0.1% of the total biomass produced, and would not be a significant cost factor in such operations. Thus, it was neglected in the above analysis.

However, the validity of the assumption that such minimal cost inoculum production could be applied to the concept of large-scale, low-cost microalgae fuel production is questionable. If a very high productivity strain is to be developed, the actual inoculum level into the culture would likely be high, and must start with a more controlled culture than a small open pond. Every effort must be made to prevent opportunities (niches) for invading algal species. Also, the stability of the culture would not likely to be very high, and the culture would need to be restarted every few weeks Thus, it is likely that in such systems inoculum production would need to be significant, perhaps as much as 1% of total biomass production.

In the case of Botryococcus, inoculum production may need to be even higher. Indeed, it is assumed, as an initial working hypothesis, that the inoculum would need to be about 5% of the total biomass produced. The reason for this large inoculum production is that the metabolic drains imposed by the large amounts of hydrocarbon produced as well as the need for control of the photosynthetic pigments (see Section 5), could allow ample room for invasions by potential contaminants. (Of course, this argument could also apply to the production of high lipid algae). A 5% inoculum would suggest that the inoculum production system would need to be about 10% of the surface area of the biomass production ponds, as inoculum production is not likely to be as productive as the final cultures if only because of the initial inoculum used (10%), would result in some light sufficient growth period. Most important, of course, inoculum production should not increase overall costs unduly, as the overall economics are already close, or exceed, to the allowable limits. A total increase in capital/operating cost of only 10% is the cost goal for the inoculum process. would be a necessary goal for such a process. The objective of the remainder of this section is to explore if such a cost goal for a large inoculation system is, at least conceptually, plausible.

9.3.4. Inoculum Culture Systems: Scale-up and Costs

The major issue is the functional nature of the inoculum production system: how "clean" would it need to be. It is not possible to grow algae axenically (bacteria free, or, even, with defined bacterial consortia) beyond the very small laboratory stage. This stage would be limited to the production at most a hundred liters of algal inoculum daily grown under strictly controlled conditions, or perhaps 50 g of algal biomass per day. This corresponds to roughly the equivalent of a 1 m^2 of outdoor production system. For a 400 ha system, this corresponds to a millionth of the biomass required for a 5% inoculation rate. The actual productivity during the inoculum production stages would be lower, as discussed above. The laboratory-scale culture would be a very minor operation and need not be addressed. It is the scale-up from the laboratory to the final inoculum production that is the issue.

Basically the procedure for scale-up would be to transfer the laboratory culture through six stages, to build it up to the final inoculum level. The key issue is the design of these various stages of the process, and, most important, their cost. As stated, the goal would be to produce a 5% inoculum without increasing total production costs more than about 10%.

Inoculum production does not carry with it the costs of the harvesting, processing, media recycle, infrastructure and other costs, which represent about two-thirds of total fuel production costs. Only the actual inoculum production systems would need to be constructed, and for operations the marginal cost of labor and power added. (Fertilizers and CO_2 itself would be captured in the final product). The inoculum cultures, as soon as grown up (three to four doublings per stage), would be transferred in its entirety to the next stage. As capital costs dominate, they can be used as the primary guide in this analysis. Thus, based on the above, the question arises of whether an inoculum system could be built for about this cost.

Assuming that on average the productivity of the inoculum system is half that of the production system, then the inoculum area must be equal about 10% of the area devoted to the production ponds, for a 5% inoculation level. The capital cost per unit area for the growth ponds, as estimated in Section 8, is between \$1.8 and 2.8 /m^2 (depending on CO_2 and productivity assumption, with an average of \$2.5/m^2 used here). However, overall system costs are about \$7 to 10.4/m^2 (Table 8.2). This, actually an average of about \$9/m^2, is the cost target for the inoculum system, which, at 10% of the total growth pond area, or 40 hectares, would add 10% to total capital costs. (As the inoculum adds to production in the growth ponds, a small credit would accrue, but that is neglected here).

The concept proposed here is a system of six sequential stages, each ten times larger then the next, starting, after the laboratory stage, with 4 m^2, and ending with 40 hectares. Each stage would be of decreasing complexity, with decreasing unit cost and more limited control over the culture and potential contamination.

Assuming that costs decreased by a factor of three, on average, between stages, while their scale increases by ten fold, then, for such a model to fit the above cost constraint (of $\$10/m^2$, average cost), the unit cost of the final stage would need to be about $\$6.5/m^2$, that of the penultimate stage $\$20/m^2$, and so on. This is the cost constraint for the proposed inoculum production system. This suggests that at least the final stages would need to be relatively low cost. This cost constraint suggests that the final inoculum production stage would be a plastic lined pond system, with unit pond size similar, or only slightly smaller (e.g. 5 ha) than the growth ponds. Assuming a cost of $\$2.5/m^2$ for the basic pond, the liner would add about $\$4/m^2$, within the cost constraints calculated above. A plastic liner would allow cleaning of the pond between inoculum production runs, a critical aspect of any such system.

The next smaller stage in the inoculum production system, a total of 4 ha, would still be a paddle wheel mixed pond design, but of smaller unit size (maybe 1 ha), and both plastic lined and covered, with simple plastic sheeting, to prevent at least some of the more common forms of contamination (e.g. dust, etc.). Such a cover could be provided for about $\$10/m^2$, meeting (once smaller pond sizes are factored in) the constraint of about $\$20/m^2$.

The 0.4 ha stage could be more complex (and expensive) system, such as a totally enclosed plastic "sleeve" type tubular (20 to 50 cm diameter) reactor system, in which the algae would be maintained under highly controlled and clean conditions. Such units are currently being tested for astaxanthin production by Haematococcus a notoriously easy to contaminate organism. Systems of several hectares are being considered, and, thus, this scale is not unrealistic. A total cost of $\$60/m^2$ for such units is estimated (Benemann, 1996, in preparation).

Finally, the last two stages, of 400 and 40 m^2, could allow for much higher unit (per m^2) costs, while contributing relatively little to total system costs. Designs could be based on either plastic or glass tubes of about 5 cm in diameter, with many different designs to choose from (Benemann, 1996, in preparation).

As described above, each stage of the inoculum production system would involve a change in design, in costs and in ability to prevent contamination. Of course, not only design but also operations would differ between stages. Thus, early stages may well be operated with carefully filtered, even U.V. disinfected water and media. Even the CO_2 used could be filtered. The systems (e.g. tubular reactors) would be cleaned and disinfected between uses. At the larger scales these requirements could be relaxed, with less frequent and rigorous cleaning, and not as great attention to prevention of contamination. Although few economic studies have been carried out for such production systems, overall costs appear reasonable based on existing commercial experience, literature data, and independent analysis. Indeed, costs can be adjusted by reducing the amount of inoculum production specified. The point is that it is plausible to produce a substantial amount of inoculum, without increasing costs unduly. Table 9.1 summarizes this system.

TABLE 9.1. PROPOSED BOTRYOCOCCUS INOCULATION PROCESS

Stage:	Area	Unit Cost $/m^2	Total Cost
Starter Culture	--	>5,000	5,000
Laboratory Cultivation	< 1 m^2	2,000	2,000
Enclosed sterilizable reactors	4 m^2	2,000	8,000
Tubular Cultures	40 m^2	300	12,000
Tubular Reactors	0.04 ha	200	80,000
Plastic Sleeve Reactors	0.4 ha	50	200,000
Covered Ponds	4 ha	20	800,000
Open Lined Ponds	40 ha	6.5	2,600,000
Open Unlined Growth Ponds	400 ha	2.5	10,000,000
TOTAL SYSTEM	445 ha	3.4*	13,700,000

* Cost per m^2 based on actual growth pond area of 400 ha.

9.4. BOTRYOCOCCUS PRODUCTION: HARVESTING AND PROCESSING

Compared to the inoculum production systems, biomass production would be similar to that described for algal lipid production, Section 8, and will not repeated here. The harvesting system is also similar to that used in the algal lipid production process, in that it involves a deep pond into which the culture to be harvested is placed on a daily basis. However, instead of the algae settling, they would be allowed to float, based on their hydrocarbon content. This would require some adjustments in the design, but relatively little in terms of overall costs.

The cost estimates for the hydrocarbon extraction and processing are taken directly from the above estimates for lipid production by microalgae. However, these were very imprecise as there is essentially no prior information on which to base such a process. In a rare experiment on B. braunii hydrocarbon extraction with hexane, Frenz et al. (1989), found that it required about 0.5 to 1 hour to reduce the hydrocarbon content from about 18 to 15%, and that extraction efficiency falls of when larger quantities of cell paste are contacted with the hexane. This obviously indicated a contacting problem, and the authors concluded that "in a commercial application ... achieving good mixing of the solvent phase with free cells may pose a serious design problem". Obviously the extraction process requires much more work, and present cost estimates are highly uncertain.

Another aspect which is not addressed in this feasibility analysis is the upgrading of the hydrocarbons extracted to a useable liquid transportation fuel. Again, some experimental data is available: Hillen et al., (1982) reported that the hydrocracking of botryococcene hydrocarbons produced 67% gasoline fractions, 15% aviation fuel, 15% diesel oil, and only 3% residual oil. This preliminary study suggested that the oils of this alga are suitable as a feedstock material for cracking to transportation fuels.

The amount of biomass residue remaining after hydrocarbon extraction is about half of the total dry weight (but only about 40% of total C and one third of the heat of combustion). The residue would be treated by anaerobic digestion. As above, a major uncertainty is the digestibility of the Botryococcus biomass. This alga is reported to be relatively resistant to chemical, and perhaps also to biological, degradation (Derenne et al., 1989). However, this requires further experimental study. For the present no change in the methane yield, compared to a algal lipid production system is proposed. The residues of the methane digestion process would be fed back into the ponds (along with any CO_2 produced during biogas production and use, if feasible), as in prior designs.

Overall, a more detailed analysis is clearly required, but not necessary at this stage in the process.

9.5. CONCLUSIONS AND R & D NEEDS

The cost analysis of the conceptual process as outlined above is only an initial and preliminary assessment of the relative economics of such a system, and many details still remaining to be explored and addressed.

The major differences for B. braunii production are the inoculation system, which provides a large amount of biomass produced under controlled conditions to minimize the threat of culture contamination. Although it increases costs significantly, it does not appear to be a major limiting factor, if the inoculum system is kept at about 10%, the covered ponds about 1%, and the closed photobioreactors less than 0.1% of the total area.

There are many uncertainties in the above feasibility and cost analysis. For example the cost of hydrocarbon extraction and processing is very uncertain. The cost estimates for the tubular reactors and covered ponds are sketchy. The harvesting system should be redesigned, as it uses a settling pond design for what would be actually a flotation process. Thus, there is considerable work that would need to be done on the process design and cost estimates to better define the projected costs of hydrocarbon production with Botryococcus. Although such an exercise would certainly advance this objective, such feasibility analysis are based on many assumptions which greatly influence the performance of the system. Thus, the most critical need is for a better definition of the achievable range for some of the parameters that most influence the overall economics of such a process. This will require, for the foreseeable future, mainly laboratory and small-scale (1 to 3 m^2) cultivation work on Botryococcus braunii. The major need is in experimental work on specific physiological, biochemical, and genetic aspects of this alga.

In the development of a hydrocarbon production process a major, immediate, issue is the choice of algal strain, or strains, on which to base future development efforts. There are probably about a dozen strains that have been studied to at least some extent in the laboratory. Most of these have been kept in the laboratory for a relatively long period, which could call into question whether genetic selection and even drift under such conditions may have affected actual physiological capabilities and genetic diversity and adaptation potential. Although such strains are useful in terms of hydrocarbon biosynthesis studies, and for laboratory studies, they may be deficient in terms of characteristics useful in outdoor production, including adaptation to fluctuating light and temperature regimes.

However, it must be recognized that such adaptations would not likely be present to a large extend even in natural populations, which would have been selected for over the past few (even many) hundreds of million years that Botryococcus is recognized in the fossil record in the natural environment. Of course, we do not know what these

selective pressures were, as we do not know either the function of the hydrocarbon produced, nor the major habitat for this alga (e.g. planktonic or benthic, for example), nor of the actual physico-chemical conditions that favor the development of blooms of this alga.

Although this severe lack of knowledge may argue for the need for much more basic research on the ecophysiology of this alga, which is certainly of interest, it can also be argued that whatever the actual adaptations of the natural strains, the environment in a mass culture system is well enough defined, and sufficiently different from natural environments, that wild type algal populations would need to adapt, and be selected for, such conditions in any event.

Thus, one approach to the selection of a suitable strain of Botryococcus braunii would be to use mass cultures to select for a well adapted strain. Alternatively, laboratory cultures could be set up that simulated to the extent realistically feasible, the outdoor pond environment in the laboratory. The major parameters that should be simulated are light, pH, pO_2, pCO_2 and temperature, over the diurnal cycle. There are significant advantages to any laboratory selection process, as it is more controllable and easier to carry out. However, outdoor pond "self-selection" may prove to be more realistic. The best alternative is not yet clear.

The major objective would be to select for cultures that maximize productivity (cell, or more correctly, pigment density). Note that the objective is not to select for the fastest growing alga, and thus dilution rate should be kept within bounds. A 33% per day dilution rate, corresponding to a doubling of biomass every three days, would appear to be the maximum dilution to be aimed at, with initial work starting out at lower dilutions (to avoid washing out the cultures). Too fast of a growth rate may result in rapid loss of hydrocarbon production potential. Thus, these type of experiments must be carefully monitored for both total biomass and hydrocarbon productivity.

This system could be used for more than just selection for high productivity under simulated mass culture conditions. For example a recycle mode could select for flotation capabilities, a central issue of the overall process. In such a concept, the entire culture would be subjected, on a daily basis, to flotation, with only the algal cells that floated being recycled back to the culture vessel. This would very quickly result in selection for algae that exhibit flotation. Indeed, if, as expected, flotation is related to hydrocarbon content it could be used to also select for this parameter. (However, due to Stokes law, floatation it is also a function of colony size, which is not necessarily a desirable parameter to maximize).

The above outlined selection process, would have for its objective to develop strains of algae that would be well adapted to the requirements of mass culture.

Two major objectives are to maximize productivity and hydrocarbon content to near the biological limits, and the problem of culture contamination.

How to actually achieve near theoretical (e.g. 10%) productivities at high light intensities is a subject for long term R&D. It will require a fairly intimate knowledge of the physiology and biochemistry of the alga being studied. This is a general problem in algal mass cultures and not further addressed here (see Benemann, 1992). However, the unique attribute of Botryococcus, the ability to produce large amounts of hydrocarbons, is of more specific interest to the present feasibility analysis.

The basic issue is whether to produce the hydrocarbons constitutively or induce them through media (nutrient) manipulations, as is proposed for triglyceride production by other green algae and diatoms. In the above feasibility study a hybrid process is proposed: a 20% constitutive hydrocarbon content which is increased to 50% by nutrient limitation. Obviously such a process would require a more detailed understanding of the algal physiology, in particular hydrocarbon content in response to nutrient limitations. Selection of strains with high hydrocarbon content may take advantage of density differences.

Many other aspects of the proposed process require investigation. For example hydrocarbon extraction and processing must be investigated. It can be argued that alternative processes, such as those using algal cultures in biofilms or artificially immobilized, could be more advantageous. For another example, it has been suggested that the algae can be re-used after hydrocarbon extraction. Although these may have potential, they are far from current practice, data is too limited, and such proposals do not allow any realistic assessment of their feasibility.

The major conclusion of this Section is that Botryoccoccus braunii should be considered as a viable subject for future development in any algal-energy and CO_2 mitigation program. This alga has the potential for most directly producing a (relatively) high value fuel. From the above analysis and literature review it would appear that the inherent problems in the mass culture of this alga (as well as many others) can be overcome.

these power plants, or remote locations can be considered. Indeed, FPL owns at least two large tracts that could be considered for locating algal facilities (L.G. Coakley, FPL, personal communication). For another example, Tampa Electric owns over 3000 acres of land adjoining one of its power plants on Tampa Bay, currently rented for agricultural production, but suitable for establishment of a CO_2 capture/utilization project, particularly as there is available seawater at that site.

Still, obtaining the data required to allow a detailed national resource assessment would, indeed, be a major effort, which is not required at present or warranted by the current state of the art. Certainly, the direct coupling of microalgae systems with power plants, required if flue gas is to be used directly, would severely limit the resource base. And even remote siting is constrained by climate which would restrict this technology to about one fourth to one third of the power plant CO_2 emissions. Considering other likely constraints, the resource potential is likely in the order of 10% of power plant CO_2 emissions. This is perhaps the best overall assessment of the long-term potential of this technology currently possible.

With an output per 400 ha pond system (total area about 25 to 50% larger), of, roughly, 1 to 2 x 10^{12} BTU/yr (depending on productivity), it would take five hundred to one thousand such systems, or half a million to a million acres of ponds to produce one quad of energy. Although a large land area, this is a small fraction of the land (and water) resources being considered for other biomass production systems, whose productivities are projected one order of magnitude lower than those expected for microalgae systems.

Even water resources are not necessarily a limiting factor. The water use efficiency of microalgae crops is much higher than those of irrigated land plants, indeed relatively as high as biomass productivities. More importantly, algal systems can use saline, brackish, and wastewaters, not useable by conventional agriculture. Thus, there should be relatively little competition for water, or for that matter land, between microalgae and crop production.

Thus, in conclusion, the issue raised in the past (e.g. MIT, 1993), that microalgae systems have too limited a resource potential for consideration as a CO_2 mitigation technology, can now be laid to rest. Although direct flue gas utilization would , indeed, have significant limitations, likely being limited to about a percent or two of total power plant CO_2 emissions, by remotely siting algal ponds, an order of magnitude increase in resource potential is plausible. Thus microalgae CO_2 utilization could be a major technology for future CO_2 capture and recycling, should such technologies ever be required.

The issue thus, is not potential resource base, but the technology itself, and the required R&D effort to develop it. That is the subject of the remainder of this section.

10.3. CO_2 UTILIZATION IN MICROALGAE WASTEWATER TREATMENT

The advantage of CO_2 utilization in algal wastewater treatment is that waste treatment credits would significantly improve the economics of such a CO_2 utilization process. Or, conversely, CO_2 abatement credits would accrue to help support the economics of wastewater treatment. More importantly, CO_2 utilization would significantly improve overall process performance, by allowing more stable and productive algal cultivation, reducing land areas required and nutrients in the effluents.

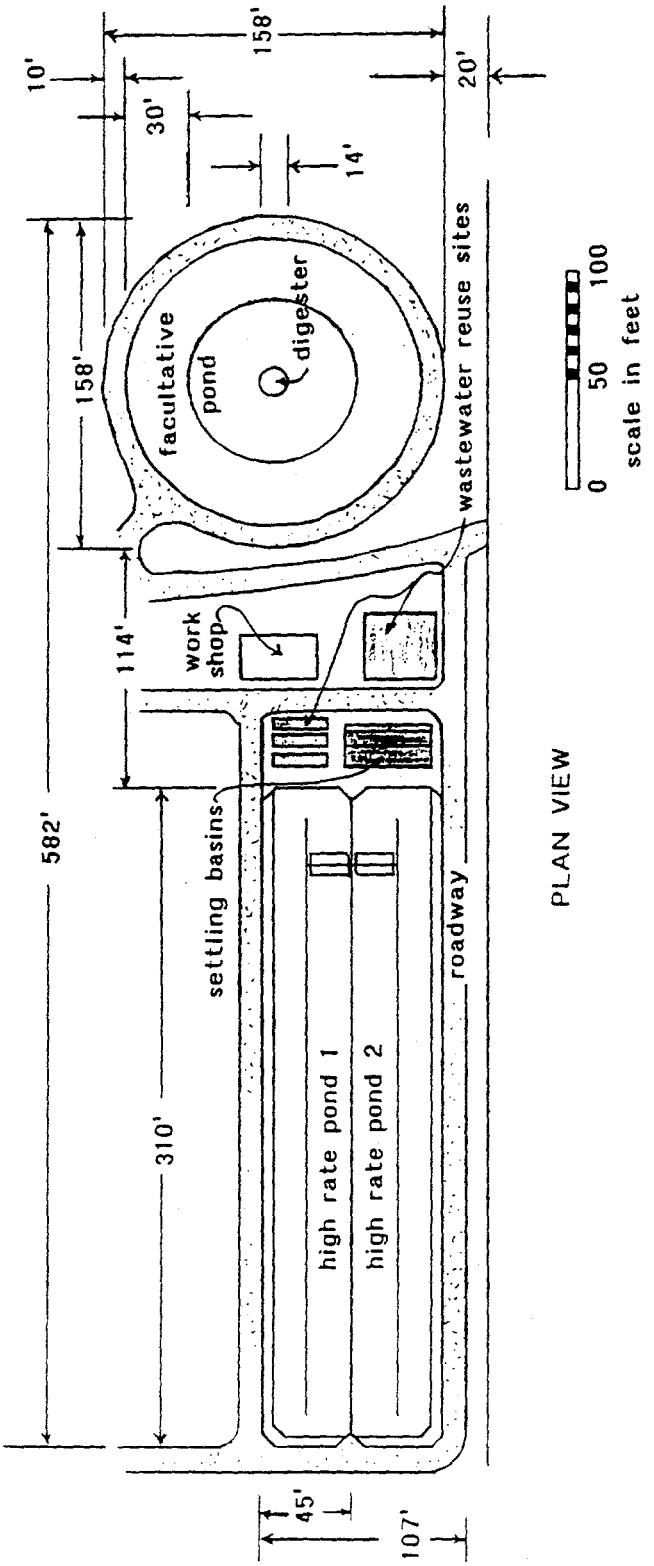
Of course, overall potential in terms of CO_2 abatement, would be modest. Based on incomplete data, Augenstein et al. (1994) recently estimated that the biogas from digestion of sludges produced by U.S. municipal wastewater treatment plants, could support the power generation of about the equivalent of only a handful of large coal-fired power plants. And due to climatic and land availability limitations, microalgae processes would only capture a fraction of this potential. The major advantage of a combined microalgae wastewater treatment/CO_2 abatement process is the potential for early commercialization of such technology, due to favorable economics at smaller scales. This is the major justification for emphasizing this aspect of the technology as the highest priority for near term R&D.

As reviewed in Section 3, microalgae are widely used in waste water treatment, generally using unmixed deep ponds ("oxidation ponds") in which the algal species are not controlled and the biomass produced is not harvested. Raceway ponds have been applied in wastewater systems, where they allow more efficient treatment (Oswald, 1978, 1988). However, raceway ponds for wastewater treatment are not a widely practiced technology, in part because of the relative expense of harvesting of the algal biomass from wastewater ponds.

Perhaps one of the longest and best documented pilot-scale pond operations of interest to this project were carried out during the late 1970's at the P.I.'s laboratory, where two 1,000 m^2 ponds were operated, with hydraulic retention time the major variable. This facility (Figure 10.1) was recently renovated and is now again fully operational. Table 4.1 summarized the productivity and harvestability data obtained at that time. Productivity measured over the year is equivalent to a productivity of 70 t/ha/y. (All data on productivity is organic dry weight). After compensating for heats of combustion, and non-algal solids, this is only about half of the "achievable" productivity of over 100 mt/ha/yr of high lipid algal biomass projected in this report. The reason for this is both the less favorable climate at Richmond, but, perhaps most importantly, the limitation to biomass productivity due to insufficient CO_2 supply in these algal ponds.

In wastewater treatment, large diurnal pH swings demonstrate a rapid utilization of CO_2 and subsequent CO_2 limitation. This alone may not account for the entire difference, and other factors must be considered (e.g. the effect of turbidity, which

FIGURE 10.1.
Pilot -Scale Microalgae Production System at the Richmond Field Stations of the University of California Berkeley



would decrease productivity; other nutrient limitations; grazing and other biotic effects, etc.). However, CO_2 supply is likely the single most important limiting factor and CO_2 supply should significantly increase overall productivity in waste treatment ponds. Indeed, for microalgae cultures grown on brackish or seawater, with CO_2 supplementation, productivities are generally about 50% higher than with algal wastewater treatment algal systems. (Such a comparison is very difficult to make, as there are no side-by-side comparisons). Again, a large part of this difference can be attributed to CO_2 supplementation.

The amount of CO_2 utilization achievable during wastewater treatment would be one of the major objective of any future R&D. By adding CO_2 to municipal wastewaters the nature of the limiting nutrient (after light) is changed: from C to N. This could allow about a doubling to tripling of total production of algal biomass possible on a given volume of wastewater (e.g. g algae/liter of wastewater), and more importantly, a large increase also in the areal productivity. Actual CO_2 utilization will depend on whether the algal culture is grown to the N growth potential of the wastewater. By capturing most of the nitrogen in the wastewater in the algal biomass, this would not only maximize the amount of CO_2 utilized, but would also result in a wastewater treatment plant effluent much lower in N, and, reduced in P, the goals of advanced, or "tertiary" wastewater treatment. Such advanced treatment processes are required by increasing numbers of cities. Conventional advanced treatment processes are more expensive than secondary treatment and could support any increased costs due to CO_2 utilization. Growing the algae to the N growth potential would also aid in harvesting the algae through settling, and in the production of a high lipid (or high carbohydrate) algal biomass. The increased productivity expected from CO_2 supplementations should allow accomplishing such objectives without an increase in the size of the algal ponds.

Demonstration such a process, using the existing facility at the University of California Berkeley, Richmond field station, is the central, and highest recommendation of this report. Several experiments would be planned to explore and demonstrate this process, with both two-stage processes (in which algae are grown in a first stage, harvested, and then a second batch of algae are grown in the effluents), and single-stage (in which a fraction of the clarified effluent is returned to the ponds after the algae are harvested), along with, of course, controls (without CO_2 supplementation). During this work a major objective would be to demonstrate the harvesting processes, including settling and DAF (dissolved air flotation) systems. Finally, this project would also demonstrated at least the feasibility of converting the algal biomass to methane with a simple covered pond.

Such a R&D project would greatly aid both the development of a practical CO_2 mitigation process as well as a potentially near-term application of this technology, which could be cost effective even in the absence of any CO_2 mitigation credits.

10.4. R&D NEEDS TO MAXIMIZE MICROALGAL PRODUCTIVITIES

Each of the assumptions on which the proposed microalgae CO_2 utilization processes are based - bioflocculation, species control, large-scale earthen ponds, etc. - has a foundation in existing experience, laboratory, pilot-scale and full-scale commercial applications. However, the main factor in these analysis is the ability to achieve very high productivities. This must be the a central goal of future R&D.

The first priority would be to demonstrate the feasibility of achieving the very high productivities, equivalent to 10% conversion efficiency at full sunlight intensities, projected in this report. The basic rationale for this is described fully in chapter 5, and need not be repeated here. Suffice it to state that the work already carried out by Prof. A. Melis, at the University of California Berkeley, provides the basis for this recommended research effort. In the longer-term this research would aim to develop algal strains that are highly productive in open ponds. More immediatedly, the basic feasibility of this approach must be demonstrated.

As discussed previously, a major advance in the technology of algal mass culture has been the ability to mass culture diatoms on seawater in open ponds (Weissman, unpublished). Diatoms are the most likely organisms for future production of lipids and large-scale CO_2 mitigation. Seawater is a potentially major resource for microalgae cultures. In addition, cultivation of diatoms on seawater has potential applications in aquaculture (Benemann et al., 1992) and in the production of high value lipids from microalgae, currently being produced fermentatively (Barclay et al., 1994; Radmer et al., 1994). The immediate need is to demonstrate the currently achievable productivities in this system, as a basis for future development.

Finally, for the long-term, the development of microalgae strains that producte lipids in high quantities and rates is of central importance. The work at NREL on the genetics and genetic engineering of lipid biosynthesis and high lipid algal strains provides a foundation for future R&D, required if the U.S. is to maintain its technological lead in this area of great importance commercially in the near-term and for the development of CO_2 mitigation processes in the longer-term.

Of course, many other potential subjects for R&D in this field could be suggested. However, compared to the R&D needs outlined above, these would be of lower priority. For example, the development of photobioreactors, for inoculum production (Chapter 9) is not a high priority at present, and such reactors are, anyway, being developed in many laboratories around the world (Benemann, 1996). Determining the effect of flue gases on algal cultures has been studied sufficiently in the laboratory (Chelf and Brown, 1994, Zeigler et al., 1995) and in outdoor ponds (Ikuta et al., 1994), to not require further study at this time. Similarly, the genetics of CO_2 fixation by algae is not a high priority. Thus, the above outlined R&D projects and subjects would be sufficient to meet both the near- and long-term objectives of a microalgal CO_2 mitigation R&D program.

10. CONCLUSIONS AND R&D NEEDS

10.1. INTRODUCTION

This, as well as the prior feasibility analysis were based on a number of technical assumptions:

1. The engineering feasibility of using very large (>5 hectares), simple earthwork construction (e.g. no plastic liners) ponds;
2. The ability to control the algal pond culture such that desirable algal species and strains are maintained for long periods in the ponds;
3. The harvesting (concentration) of the microalgae with a simple (low cost) process involving, essentially, the sedimentation of the algal cells;
4. The conversion of the biomass to a fuel which would substitute for fossil fuels and thus mitigate CO_2 emissions;
5. The recycle of the nutrients tied up in the biomass after the fuel conversion step, and also the recycle of most of the water used in such a process; and
6. The achievement of productivities as close as possible to the maximal theoretical for photosynthesis (about 200 t/ha/y dry organic matter basis for a high lipid biomass).

Each of the above assumption is based on experience and/or theory, as reviewed in Section 2 to 7 above:

1. Large unlined ponds are already being used in practical applications.
2. Some algal species can be cultivated readily in open ponds.
3. Harvesting by settling has been demonstrated in pilot scale operations.
4. Algal lipids and methane fermentations have been extensively studied.
5. Water recycle is done in commercial systems and nutrient recycle has been has been demonstrated in laboratory and small outdoor experiments.
6. There is strong theoretical basis for achieving very high productivities.

The engineering and cost analysis presented in Section 8, updates, supports, and extends prior studies, and arrived at preliminary costs estimates that suggest that this technology is at worst competitive with alternative CO_2 capture/disposal options, and has the potential of being a low-cost technology if, indeed, high productivities are achieved and energy prices escalate modestly.

Of course the practical development of this technology still requires long-term R&D, both of a fundamental and applied nature. The problem of CO_2 mitigation provides an opportunity for such R&D. The potential practical applications of this technology in the production of higher value products and wastewater treatement provides an opportunity to achieve some intermediate goals in the near-term in the development of this technology. This is the recommended strategy for future R&D in this field. First, however, the potential resource based is considered briefly.

10.2. RESOURCE POTENTIAL

The resource potential of microalgae fuel production has been the subject of considerable discussion and some controversy for over twenty years. Compared to ocean farming of seaweeds, where systems of "500 miles square" (25,000 mi^2) were proposed (Flowers, 1977), land-based microalgae systems were perceived as being of limited potential. Indeed, the initial cost analysis (Benemann et al., 1978) was directed to be for 100 mi^2 (25,600 ha) systems. That report did identify several sites in the U.S. where such large-scale systems could be established: the Salton Sea area in S. California, where algal systems could be used to help manage salinity of this inland water body, and the San Joaquin Valley, also in California, with its extensive agricultural drainage water, laden with Se and nitrate contamination, which could also benefit from algal processes. (Indeed, a project is currently underway at this laboratory for Se removal from this drainage water, see Lindquist et al., 1994). However, there is not need for gargantuan systems: economics of scale are achieved at a few hundred hectares, similar to many agricultural operations. And at such scale the resource potential would greatly increase.

Of course, the question still is, what is the potential. Early pronouncements that the potential for U.S. microalgae energy production could be 100 quads (Raymond, 1981) can be rejected as fanciful. Considerable work has been carried out by NREL on resource assessments, in particular of saline water, CO_2, and land, resources (Hill et al., 1984; Vigon et al., 198?, Feinberg and Karpuk, 1988; Maxwell et al., 1985, Lansford et al., 1990). Few of these studies ventured any specific predictions regarding resource base, although clearly these are relatively large, with multi-quad potential for algal fuel production. However, any detailed generic resource assessment is unlikely to be cost-effective at present. As shown by the recent work of Kadam (1994b) for a specific power plant in New Mexico, it requires considerable effort to obtain site-specific data on land availability, water resources, and other inputs for the siting such a system.

For another example, as part of this project, a review was undertaken of the resource potential associated with one utility, located in a climatically favorable region of the U.S., Florida Power and Light Co. (FPL). The FPL generation stations, along with some operating data, and land resources owned by the utility, are listed in Table 10.1. The oil and gas (and some coal) used by these power plants provide a basis for an estimation of the total CO_2 outputs. From Table 10.1 it is clear that only a minority of the power plant sites are potential candidates for such systems, due to site size limitation (< 100 acres). Of the remaining, several (e.g. St. Lucie Power Plant in Ft. Pierce) are located in environmentally sensitive areas, where any land management (e.g. replacing existing vegetation with algal ponds) would not be possible. Even sites that have large tracts of land, may only allow a portion of the land to become become available for algae culture: at the Martin Power Plant 6,700 acres are taken up by the cooling reservoir, and some other areas are maintained as natural wetlands. Of course, other land surrounding

TABLE 10.1. FLORIDA POWER AND LIGHT POWER PLANTS

PLANT NAME	ACRES	SYSTEM	MW	FUEL	USE
Cape Canaveral	42	Conventional	760	Fuel oil	1915
				Natural Gas	15073
Cutler	86	Conventional	197	Gas	4930
Fort Myers	460	Conventional	504	Res. oil	3395
		Gas Turbines	756	Diesel/oil	
Lauderdale	764	Conventional	1126	Low S oil	227
				Natural gas	1162
Manatee	8,000	Conventional	1566	Res oil	7396
Martin	11,360	Conventional	1566	Fuel oil	2411
				Natural gas	24031
Port Everglades	93	Conventional	1142	Low S oil	2041
				Natural gas	1423
		Peaking	439	Distillate	2059
				Natural gas	12394
Putnam	87	Combined Cycle	520	Low S Dist.	No data
				Natural Gas	No data
Riviera	21	Conventional	544	Oil	2060
				Natural gas	10846
St. Lucie	1,132	Nuclear	1678	Nuclear	not appl.
St. Johns River	No data	Unknown	250	Coal	No data
Stanford	1718	Conventional	504	Natural gas	1902
				Low S Res	1696
Turkey Point	22,295	Conventional	734	Low S oil	2241
				Natural gas	23735
		Nuclear	1333	Uranium	not appl.

Sources: FPL brochures and information and Utility Data Institute, 1989 data base. Fuel use is in 1,000 bbls for oil and 1000 MCF for natural gas. Notes: Data base incomplete. Data has not been checked for accuracy.

10.5. CONCLUSIONS

Mitigation of CO_2 through the utilization of this waste gas by microalgae cultures to produce fuels, is likely to be an expensive technology, compared to many alternatives, such as other biological systems involving forest preservation and biomass production (Benemann, 1996). However, perhaps not as expensive or difficult than some others, specifically alternative direct mitigation options that involve capture and disposal of CO_2. Indeed, the uncertain nature of the global warming problem, and the ultimately limited potential of any one technology, or even suite of technologies (e.g. terrestrial biomass systems), justifies the development of many alternative technolgies, which in aggregate could solve, or at least ameliorate the long-term consequences of global warming.

The co-combustion of wood and coal is a low cost option for direct CO_2 mitigation, as the credit for the reduction in fossil fuel use accrues directly to the power plant where the fuel is burned. This is likely the lowest cost of all the direct options. However, co-firing will, by definition, and resource limitations, always be only a partial solution to this problem, justifying, indeed requiring, the development of additional mitigation technologies.

R&D in microalgae CO_2 mitigation has several advantages compared to both conventional biomass processes (e.g. indirect methods involving growing trees and crops, for conversion to fuels) or other direct CO_2 mitigation options:

1. **Microalgae R&D is Fast.** The generation times of microalgae are hours to days, not years, as for trees. This allows a much faster development.
2. **Microalgae R&D is Cheap.** The factors affecting algal growth are much more controllable, compared to higher plants. Thus results at one site can be readily applied to other sites, requiring a much smaller R&D effort.
3. **Microalgae R&D is Easy to Scale-up.** Results from small ponds scale easily to larger ponds, thus not requiring large facilities.
4. **Microalgae R&D can Lead to Breakthroughs.** Examples are the recent work at ORNL on a new type of photosynthesis, and the concept outlined in Section 5, to maximize productivities. This could have wider implications.
5. **Microalgae R&D can be Applied in the Near Term.** Applications could come in wastewater treatment and even high value foods/chemicals productions.
6. **Microalgae R&D will Result in Technology Transfer.** Indeed, prior R&D funded by DOE has already spawned several companies and applications.
7. **Microalgae R&D will Preserve U.S. Competitiveness.** The U.S. has a strong technology lead in the industrial cultivation and production of microalgae, with this industry perhaps currently valued at a billion dollars.
8. **Microalgae R&D is Already Supported by DOE-PETC.** The U.S. DOE has invested over $25 million in this technology, providing a basis for future R&D.
9. **Microalgae R&D can be Integrated with other DOE Programs.** The DOE Hydrogen Program has overlapping interests with algal CO_2 mitigation R&D.
10. **Microalgae R&D can result in CO_2 mitigation.**

Of course, the limitations of microalgae in CO_2 mitigation are also clear, from a limited potential to high costs, to an undeveloped technology. However, none of these appears to be overwhelming.

To reiterate, the following are the highest priority R&D needs in microalgae CO_2 mitigation as developed in this report and proposed above:

1. Demonstrate and develop utilization of CO_2 in wastewater treatment.
2. Demonstrate and develop low cost harvesting (bioflocculation, DAF systems).
3. Demonstrate and develop mechanisms to overcome light saturation effect
4. Demonstrate and develop culture stability and productivity in seawater cultures.
5. Develop genetic engineering technology for high microalgae lipid productivity.

A relatively low budget R&D program in this area would preserve the U.S. lead in this technology as well as foster the long-term development of microalgae CO_2 mitigation technologies.

The present effort clearly indicated the potential of microalgae technology, and the limitations of any present engineering analysis, in the absence of more information. Thus, at present, further efforts along these lines, or more detailed resource assessments, would not appear required or warranted. Of course, in connection with the above recommended R&D activities, such as the development of CO_2 utilization during wastewater, specific technology assessments and economic analysis will be required. However those should wait until sufficient data has been obtained on which to base such engineering studies.

Microalgae technology has the potential to contribute to the long -term reduction in the risks of greenhouse warming. No clear warming trend is yet apparent, but the evidence for such a trend is building. Even though the risks of future global warming are unknown, the precautionary principle dictates that we reduce CO_2 and other GhG (greenhouse gas) emissions as much as possible at present at small cost, and develop technologies for possible future applications. Although nature currently mitigates about half of current anthropogenic net CO_2 emissions, through absorption into oceans and storage in forests, the future of these natural processes is uncertain, and they may fail us when we most need them during the next century, when the expected, unavoidable, increased fossil fuel utilization will greatly increase the risks of major adverse impacts. And, it should be further recognized that even a modest reduction in CO_2 greatly reduces the risks of adverse climatic impacts (see discussion in Benemann, 1991). All these arguments point to the need for initiating risk reduction now, using the least expensive options (energy conservation, preservation of C in the biota) while developing other mitigation technologies for the long-term. Microalgae fuel production should be one of those long-term options.

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