MARINE BIOMASS PROGRAM

ANNUAL REPORT FOR 1980

Gas Research Institute
8600 West Bryn Mawr Avenue
Chicago, Illinois 60616

Solar Energy Research Institute
1617 Cole Boulevard
Golden, Colorado 80401

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ANNUAL REPORT FOR 1980

PREPARED BY

A.N. TOMPKINS

GENERAL ELECTRIC COMPANY
RE-ENTRY SYSTEM DIVISION
3198 CHESTNUT STREET
PHILADELPHIA, PA. 19101

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DR. LAWRENCE RAYMOND,
BRANCH CHIEF
BIOMASS PROGRAM

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TABLE OF CONTENTS

LIST OF FIGURES

REPORT DOCUMENTATION PAGE	1. REPORT NO. GRI-80/0026	2.	3. Recipient's Accession No.
4. Title and Subtitle MARINE BIOMASS PROGRAM ANNUAL REPORT FOR 1980			5. Report Date 1/27/81 6.
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16. Abstract (Limit: 200 words)

The Marine Biomass Program is an integrated research and development program that is directly involved in the development of integrated processes for the growing of a natural resource - in this instance, kelp - specifically for the production of methane as a substitute for natural gas.

Previous experimental data has shown that the concept of growing kelp in the open ocean is technically feasible and that methane can be derived by the anaerobic decomposition of this biomass. This report broadens upon this data base, emphasizing the economic as well as the biological and technical requirements that, when solved, will lead to processes for the conversion of kelp to methane that are competitive with other sources of energy.

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LIST OF FIGURES (Cont)

LIST OF TABLES

LIST OF TABLES (Cont)

LIST OF TABLES (Cont)

1. RESEARCH SUMMARY

TITLE	Marine Biomass Program GRI Contract Number 5010-323-0014; SERI Contract Number XK-0-9350-1
CONTRACTOR	General Electric Company Re-entry Systems Division
PRINCIPAL INVESTIGATOR	Alan N. Tompkins, Program Manager, Biomass Programs
TIME SPAN	January 1, 1980 - December 31, 1980

MAJOR ACHIEVEMENTS

This report describes research which has been conducted during 1980 to help assess the feasibility of growing marine biomass in the open ocean and converting it to methane. Previous experimental data has shown that kelp can be grown from spores to adult plants using nutrient-rich deep ocean waters and then converted to methane. During 1980, the data base was broadened by adding to the knowledge of kelp survival requirements in the open ocean and improving the understanding of the relationship between kelp composition and methane production. Major achievements this year included:

- Demonstration that adult kelp plants, when prevented from abrading against the test farm structure, could survive severe weather conditions.
- Production and verification of a model which predicts methane production based on elemental composition and mannitol content.
- Production of hybrid kelp species which were outplanted and grown at a nearshore farm site.

RECOMMENDATIONS

General Electric recommends that:

- Modifications to Test Farm must be implemented and upgraded to preclude entanglement and abrasion of kelp plants with Test Farm structure. Further ocean experiments are recommended on plant dynamics to provide data on effects of water motion on Macrocystis.
- Growth and production rates of selected hybrid species of Macrocystis should be further evaluated and documented under various environmental conditions.
- Continuing studies are required to recover maximum methane yields from digester solids, and to improve feed/chemical value of digester effluent.
- Innovative digester design concepts including plug flow systems, fluidized bed systems and packed-bed systems, should be further expanded to upgrade and optimize gas conversion.
- Another large scale kelp harvest should be undertaken in late 1981 to supply researchers with a homogenous biomass supply for continued research.
- Continuing efforts should be applied to further characterize and optimize the microorganism(s) responsible for optimum conversion of acetate to methane.

DESCRIPTION OF WORK COMPLETED

- Although relegated to a "maintenance mode" operation due to relatively low funding levels and co-funding delays through August 1980, significant milestones were nevertheless achieved by the various Marine Biomass subcontractors during the course of this 1980 contract. These achievement include:

- Preliminary concepts definition to preclude entanglement and abrasion of kelp plants on Test Farm Structure.
- Maintenance and refurbishment of test farm hardware to operational readiness during 1980.
- Preliminary evaluations of Macrocystis hybrids for optimizatior of plant characteristics.
- Preliminary definition of nitrogen and micronutrient requirements of Macrocystis plants.
- Greenhouse facilitization and candidate species selection of seaweeds indigenous to New York State waters.
- Preliminary evaluation of innovative digester designs and autofeeder.
- Completion of mannitol studies at IGT.

GRI COMMENT

The thrust of the work GE has performed under the GRI/SERI contract since September 1980 has been to obtain kelp yield data through modifications to the existing offshore test platform or through the acquisition of nearshore facilities. Regardless of how yield data is obtained, the program will emphasize near-shore studies on fundamental questions such as nutrient uptake and storage, as well as plant improvement. In addition, the relationship between kelp growth and digestibility will continue to be an important focus. Another thrust of this program has been, and will continue to be, work on innovative digester systems, as well as pre- or post-treatment and inoculum development in order to increase methane production rates.

2. OVERALL PROJECT OBJECTIVE

The Marine Biomass Program has, as its primary objective, the development of optimized and integrated processes for the production of methane from seaweed cultivated in the ocean that are cost competitive with alternative sources of energy. This objective will be accomplished through direct experimentation and evaluation of concepts for the feedstock production, harvesting and conversion process systems. The technical, economic and energy requirements of the processes will be determined so that the feasibility of producing methane on a competitive commercial scale from marine biomass can be fully established.

Over the next 2-3 years the specific objectives that must be met include: (1) determining that production of macroalgal biomass can be sustained such that the harvest yields demonstrate a strong economic future for ocean farm systems, (2) confirming that ocean farming of biomass can provide net energy gains, and (3) determining that macroalgae can be economically harvested and converted on a basis that is competitive with alternate sources of methane and other synthetic fuels.

The biomass of central interest in the current program is the giant kelp, Macrocystis pyrifera. This species has been studied extensively and its high growth rate, physical size and structure, long life and ease of harvesting have made it a leading candidate for ocean energy farms. Other candidate macroalgal species will also be investigated during the next 2-3 years and their suitability for marine biomass farming will be ascertained. The initial emphasis towards meeting the specific program objectives over the next 2-3 years will be on marine farming concepts using the giant kelp as the "benchmark" species. The concept to be investigated is the growing of kelp on suspended artificial substrates positioned in the open ocean with nutrients provided by upwelling deep seawater.

Previous work has shown that controlled cultivation of macroalgae is feasible and that fuels can be derived from marine biomass feedstocks. Extensive work with

Macrocystis has indicated that it can be grown in the open ocean when fertilized by artifically upwelled deep ocean waters. Kelp thus derived has been shown to be favorably suited to methane production by the process of anaerobic conversion. Gas yields attained have been generally higher than those obtained from other biomass forms. Thus some key aspects of this project have been shown to be technically feasible. However, sufficient data have not been generated to show that these concepts can be effected in such a way as to generate cost-competitive energy products.

The work will expand upon the previous data base with emphasis on the technical and economic requirements of the critical parameters associated with biomass yield and overall energy balance. While the development of significant quantities of energy products derived from marine biomass is expected to require a long term continuous effort, on the order of twenty years, the specific work to be done over the next two or three years will justify or refute the value of continuing this work towards commercialization.

3. SUMMARY OF PREVIOUS WORK PERFORMED

Prior to 1980 the program emphasized acquisition of biological and engineering data that was critical to making an accurate determination of the technical and economic feasibility of the concept. Previous work has been directed towards validation of the basic concepts involved in the engineering of marine farm systems (Ocean Engineering); the growth and nutrition of Macrocystis (Biological Studies); and the conversion of Macrocystis to methane (Bioconversion). The following sections summarize the status of the research in each of the major research areas.

OCEAN ENGINEERING

The ocean engineering activities were conducted by Global Marine Development Incorporated, commencing in 1978, under subcontract to the General Electric Company. The objectives of this initial work were to provide an open ocean test structure for controlled cultivation of Macrocystis. The test structure was not intended as a miniature version of a commercial farm, but rather was designed to enable the biological experiment team to gather the requisite data for determining the growth, yield and nutritional requirements of kelp. The constraints imposed on this initial test structure were:

- The structure must be open and yielding, rather than rigid, so as not to offer substantial resistance to ocean currents, winds and waves.
- The structure must survive a 50 year storm (40 foot waves, 2 knot current) with a minimal amount of damage.
- The structure must be completely compatible with the biological requirements of Macrocystis and in itself must be non-polluting.
- Personnel safety and operational reliability would be prime considerations.
- The structure must be moored, for test purposes, in approximately 2000 feet of water.

- Water must be reliably pumped up, or upwelled, from a depth of 1500 feet in order to satisfy the biological requirement of providing ambient levels of 3 microgram atoms of nitrogen/liter throughout the farm volume.

The following are details of the design philosophy and descriptions of the major components of the Test Farm that was deployed off the coast of Southern California. The initial experiences with this farm are also discussed.

Test Farm Description

The equipment configuration shown in Figure 1 was selected after an extensive series of computer analyses and model testing. The test farm was designed to initially support approximately 100 adult Macrocystis plants. The base of the plants are attached to the horizontal ropes strung between the arms of the substrate. This structure maintains the base of the plants at a depth of approximately 50 feet. The plants then grow up to the surface and subsequently form a canopy which floats on the surface. Upright growth and flotation result from the presence of specialized buoyant parts of the plant, called pneumatocysts, which act as flotation bladders. The Test Farm has the capability for providing upwelled water from a depth of 1500 feet through a two foot diameter polyethylene pipe. (Details of the unique upwelling pipe will be discussed later.) Conventional 15 horsepower diesel engines are used for power. Instrumentation, power and pumping systems are contained in the central spar buoy. This machinery buoy also houses the fuel supply for the upwelling pumps, auxiliary batteries for navigation aids and a system for supplying trace quantities of micronutrients to the farm if required. The lower portions of the system are isolated from motions caused by wave action by gimbals between the machinery buoy and the substrates as well as between the substrate and the upwelling pipe.

Mooring System

The Test Farm is positioned in approximately 2000 feet of water by a three point catenary mooring system approximately 4.5 miles offshore from Laguna Beach in Southern California. The mooring consists of three (3) identical 16 foot diameter spring buoys each of which is connected via wire rope to 1350 feet of 2 inch chain and a 15000 pound anchor on the ocean floor. The spring buoys are positioned at vertices of an equilateral triangle 520 feet on a side. The Test Farm is moored at the center of this triangle by wire ropes connected from the mooring swivel on the structure to the spring buoys at a point 60 feet below the water line. The general arrangement of the Test Farm is depicted in Figure 2.

Upwelling Pipe - Design and Fabrication

A major ocean engineering challenge encountered in the initial design task developed from the structural and deployment requirements for the upwelling system. In order to maintain an ambient nitrogen concentration within the farm volume of 3μ g-atoms/liter (in up to 0.1 knot currents), the upwelling rate of deep water with a nitrogen content of 30μ g-atoms/liter was set at approximately 9000 gallons/minute. In order to attain the flow at reasonable horsepower (30 HP), a two foot diameter pipe was selected. The pipe length was set at 1500 feet in order to reach a stable level of nitrogen concentration. To minimize structural rigidity in the ocean environment, the pipe was designed to be attached at the upper end only. In this way, the total farm structure is able to yield, within specified limits, both laterally and axially in response to waves and currents. Computer simulation of the upwelling pipe indicated that wave motion would be transmitted, with minimal damping, from the top of the pipe structure to the bottom, and large bending moments would be set up along the entire length of the pipe. After detailed evaluation of several conventional marine structural materials (steel, aluminum, concrete) and several plastic materials (fiberglass

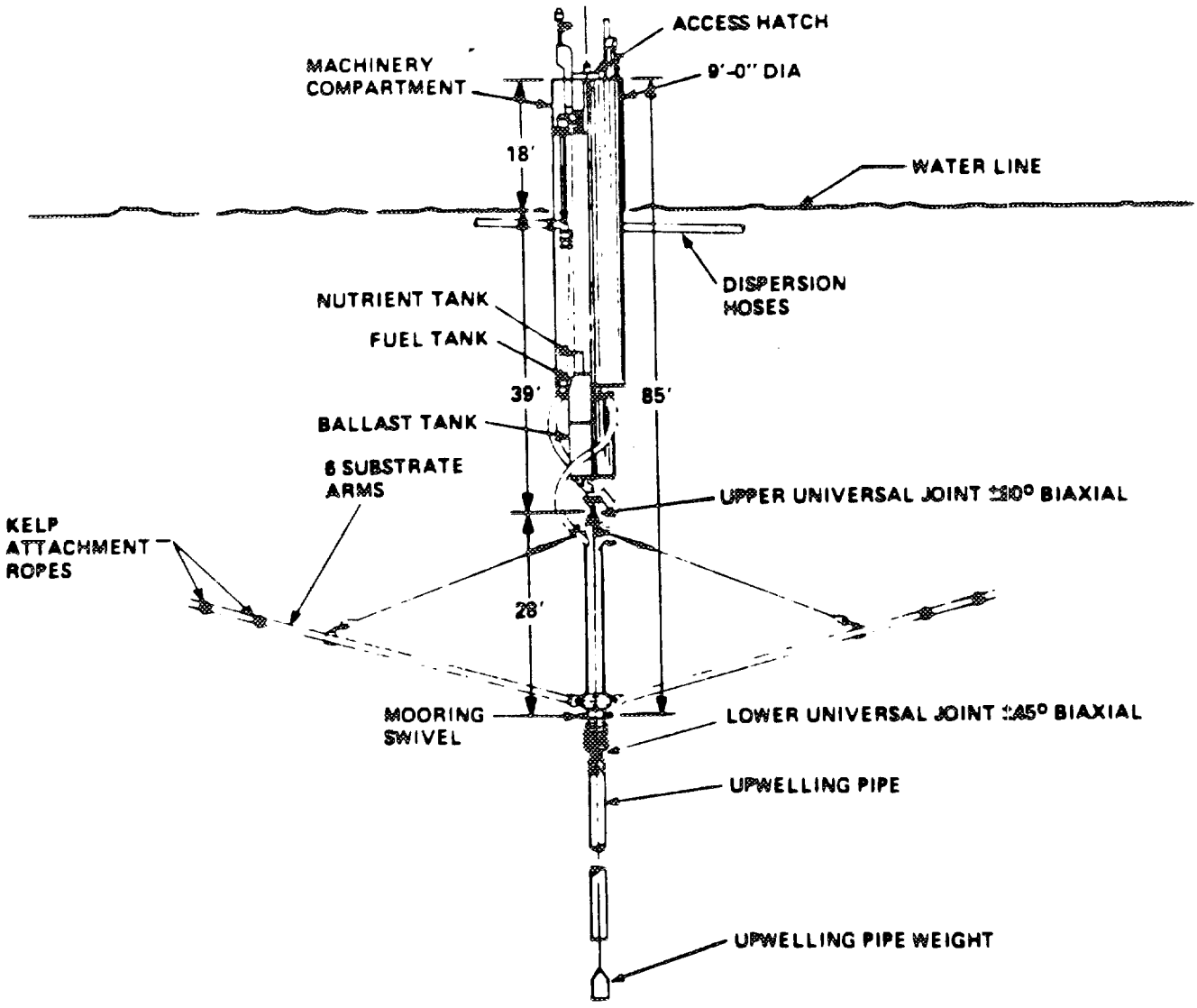

Figure 1. Test Farm Detail Profile

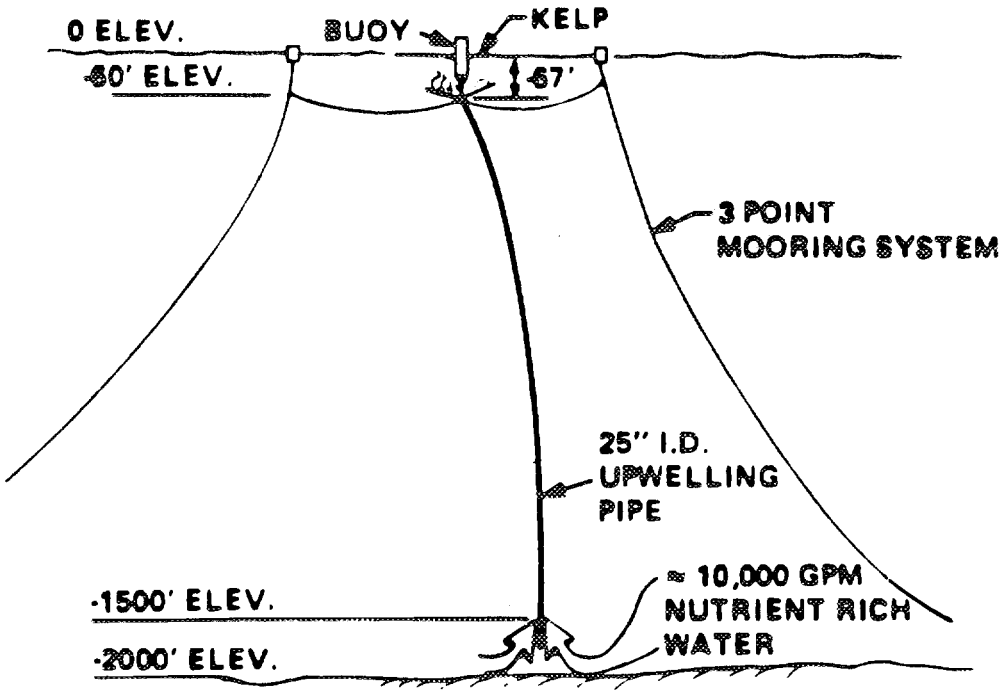

Figure 2. Test Farm General Arrangement

reinforced plastic and high density polyethylene), it was determined that the most viable candidate material in terms of flexibility (reduced bending moments), weight, cost, and availability, was polyethylene. Figure 3 provides details of the upwelling pipe design.

The pipe was fabricated from 60 foot sections by fusing them into one continuous element. The individual polyethylene sections were manufactured by DuPont of Canada marketed under the trade name of Sclairpipe. Sclairpipe has previously been used in marine applications for sewer outfalls and water lines by laying the pipe on the floor of the ocean or lake. The application of this material in the Test Farm was unique in that the fabricated pipe string is held vertically by the farm structure and weighted at its lower end to maintain the vertical attitude. Thus, the pipe was to be fabricated into a continuous 1500 foot horizontal string; towed to the Test Farm site horizontally; upended to a

vertical attitude; then attached to the lower position of the Test Farm. Individual pipe sections were joined (fused) on land, then deployed into a protected harbor at Dana Point, California. The pipe string was staged in the harbor until the complete assembly was towed to the Test Farm.

Test Farm Operation

The accumulation of mature plants for use on the Test Farm began while the Test Farm was being deployed and continued through the mechanical shakedown and debugging period of the farm equipments. All plants were deemed satisfactory at the time of collection but few, if any, were in excellent condition simply because Southern California kelp beds typically experience seasonal nutrient lows during early to mid-fall each year. When satisfied that the support equipment was operational, the Cal-Tech experiment team transferred 100 adult plants from shallow waters near shore, where they had been stored, to the Test Farm. A curtain designed to retain upwelled nutrients was then installed around the farm. The curtain was designed as a barrier to reduce ambient external currents to 0.1 knot or less inside the farm planting area. In currents exceeding approximately 0.5 knot, the curtain was designed to lift so as not to impose unacceptable loads on the mooring system. It is important to note that on large scale commercial farms, sized in the thousands of acres, the need for a protective curtain to reduce currents and contain nutrients, would not be necessary because of its larger size. Currents in a large farm would be significantly reduced simply because of the presence of the dense biomass - the phenomena being somewhat analogous to the "edge effect" of a large wheat field exposed to high winds.

After the curtain was installed, a period of observation and data collection was begun including weekly measurments of the growth of juvenile fronds, analysis of dissolved nutrients in water samples and blade-tissue analysis. Initial measurements of nitrates/nitrites in the upwelling system discharge varied from 25

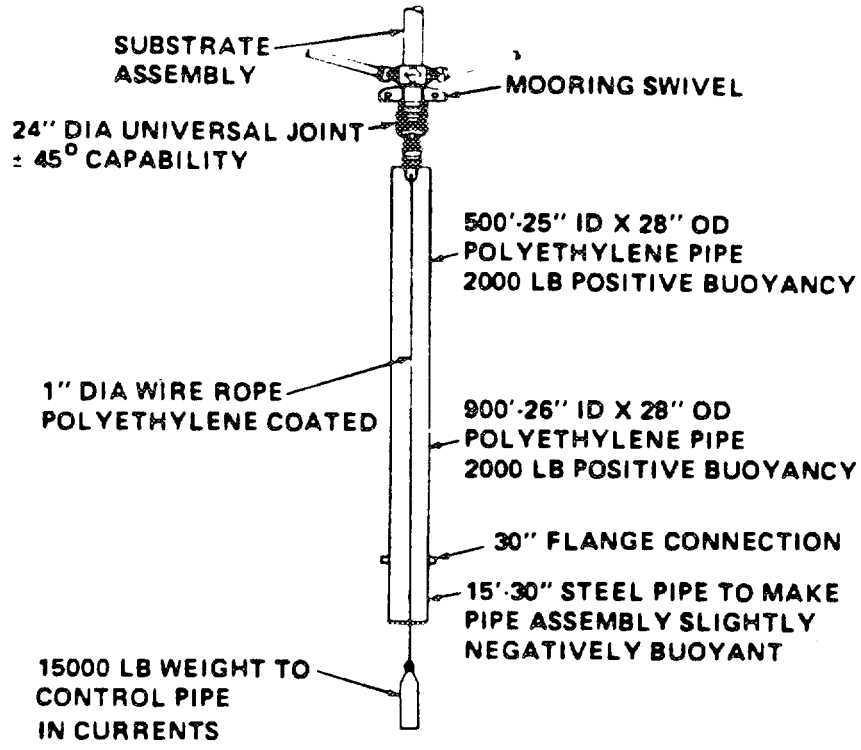

Figure 3. Upwelling Pipe

to 32 microgram atoms of nitrogen per liter as compared to background levels during the same period of 0.05 to 4.46 microgram atoms per liter, measured at a control station approximately two miles from the Test Farm at depths ranging from surface to 60 feet. Water temperature from the upwelling system was on the average 6.5°C colder than background levels at the control station which ranged from 7 to 10°C as opposed to 13.5 to 16.7° at the Test Farm in the surrounding surface water for the same time period. These data proved the feasibility of the upwelling system concept and verified the operability of the equipments used. Initial observation of curtain performance based on underwater photographs and plant tissue analysis provided evidence of the effectiveness of the curtain in reducing currents and containing nutrients. Figure 4 shows a positive effect of the upwelling and nutrient containment system on plant composition. The upwelled flow rate shown in the figure is the accumulated operating time of the pumps

recorded between maintenance visits to the farm and then averaged on a daily basis over the maintenance period. The tissue nitrogen data shown is the average of data points collected on a particular day. From these limited data we conclude that nutrient-rich water was being effectively upwelled and contained within the Test Farm during this period as evidenced by initial plant tissue nitrogen values which peaked at about 3 percent by dry weight. Nitrogen content of kelp tissue from natural beds used as a control were reported to be fairly constant and at the lower end of a range of 1 to 2 percent. As shown in the figure, the apparent reduction in tissue nitrogen content as the average upwelling rate was reduced is evidence that the upwelling system had provided some nutrients to the plants. The lag in plant response to the reduction in upwelling is not surprising since the ability of Macrocystis to store nutrients for future use is a known, albeit not fully understood, phenomemon. The effects of nitrogen starvation would not be manifested immediately if plants had an opportunity to build up reserves of N.

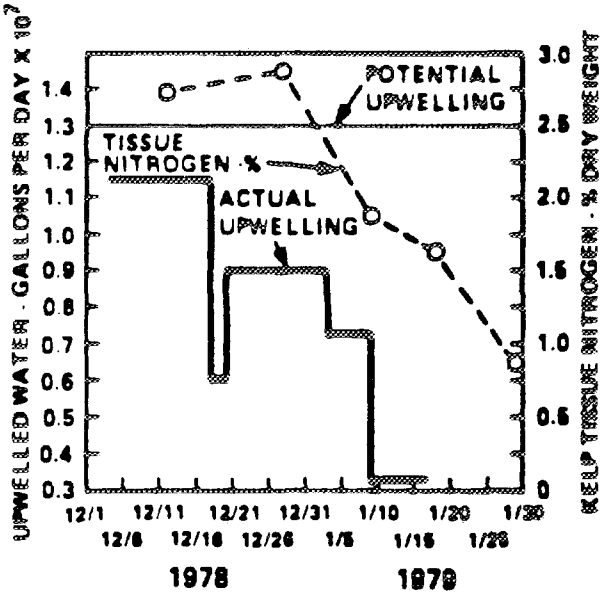

Figure 4. Kelp Tissue Nitrogen Content and Pump Flow Rate

Heavy storms during the first month of operation after the current retardant barrier was installed began tearing the curtain at its points of attachment, and within a month most of the curtain was lost. The effect of the loss of the curtain, combined with the storm-induced currents and waves and resulting motions

of the farm, was the abrasion of many of the plants against Test Farm structure. The intensity of the storms increased during the next month with winds of 70 to 100 mph reported off the Dana Point area. This caused further attrition of the plants until there were no viable transplants left on the farm. However, the initial planting exercise was instructive in a number of key areas and the data collected and operational experience acquired will be used in development of an effective plant protection system for the test farm experiment.

A significant development following this initial planting was the determination that Macrocystis juveniles were growing in profusion on almost all surfaces of the marine Test Farm structure. The juveniles developed from spores that were released by the initial adult plants which had been attached to the farm. The Cal-Tech experimentors conducted an evaluation of the population density and estimated the initial count to be in the neighborhood of 36,000 new juvenile plants. At the time of this initial evaluation the plants ranged in size up to two feet in length. The majority of the juvenile plants on the Test Farm most probably developed from spores released during the time the protective curtain was intact around the Test Farm. The protective curtain was clearly effective in retaining the upwelled water within the farm area during that time as evidenced by the decrease in water temperature. North, Sanbansuga and Neushul have reported the effect of chilled seawater to stimulate spore release in Macrocystis, and a similar phenomena probably occurred with the initial transplants on the Test Farm.

In order to capitalize on the presence of the juvenile plants on the test structure, action was taken to direct the upwelled water to the new growth in order to allow the collection of data on recruitment, population density, juvenile growth rate in the sea and survivability. The upwelling system was originally designed to deliver nutrients to canopies of adult plants at the surface rather

than to juveniles at 30 to 60 foot depths. The likelihood of sufficient nutrients "sinking" to the vicinity of the juveniles in order to promote vigorous growth was questionable. To verify this hypothesis and to map the distribution of upwelled water from the surface, a fluorescein dye dispersion test was run. Figure 5 depicts the approximate dye distribution pattern in a current between 0.3 to 0.5 knots. A side note of interest is the marked difference in temperature between the plume of upwelled deep water and the surrounding environs. This dispersion pattern data confirmed that it was necessary to modify the upwelling system and extend one of the surface dispersion hoses down into a selected area of juvenile plants on one of the structure's radial arms in order to assure sufficient ambient nutrient levels. A flow-splitting device was added to the exit of the deep dispersion hose to direct water in both directions along the radial arm. A second dye dispersion test was run, and the approximate dispersion patterns are depicted in Figure 6. Currents measured from the surface down to 60 feet varied from 0.1 knot to 0.35 knot, with the peak current at about 30 feet.

Measurements of growth and nutrient uptake by the new juvenile plants, which were being supplied with artificially upwelled water, indicated that they survived and grew well in the Test Farm environment. Later data surveys indicated that some juveniles had grown to lengths of 25 to 30 feet. This occurrence was important in demonstrating that recruitment and development of juvenile plants can take place on a structure in the open ocean.

In summary then, the use of the Test Farm hardware as a device to provide upwelled water for supporting the study Macrocystis in the "desert" of the open ocean has been verified by over two years of in-situ operation. The ability to continuously upwell nutrient rich deep water and the capability to present an adequate nutrient environment for plant growth has been established. The engineering challenge of design, fabrication, vertical deployment and continuous

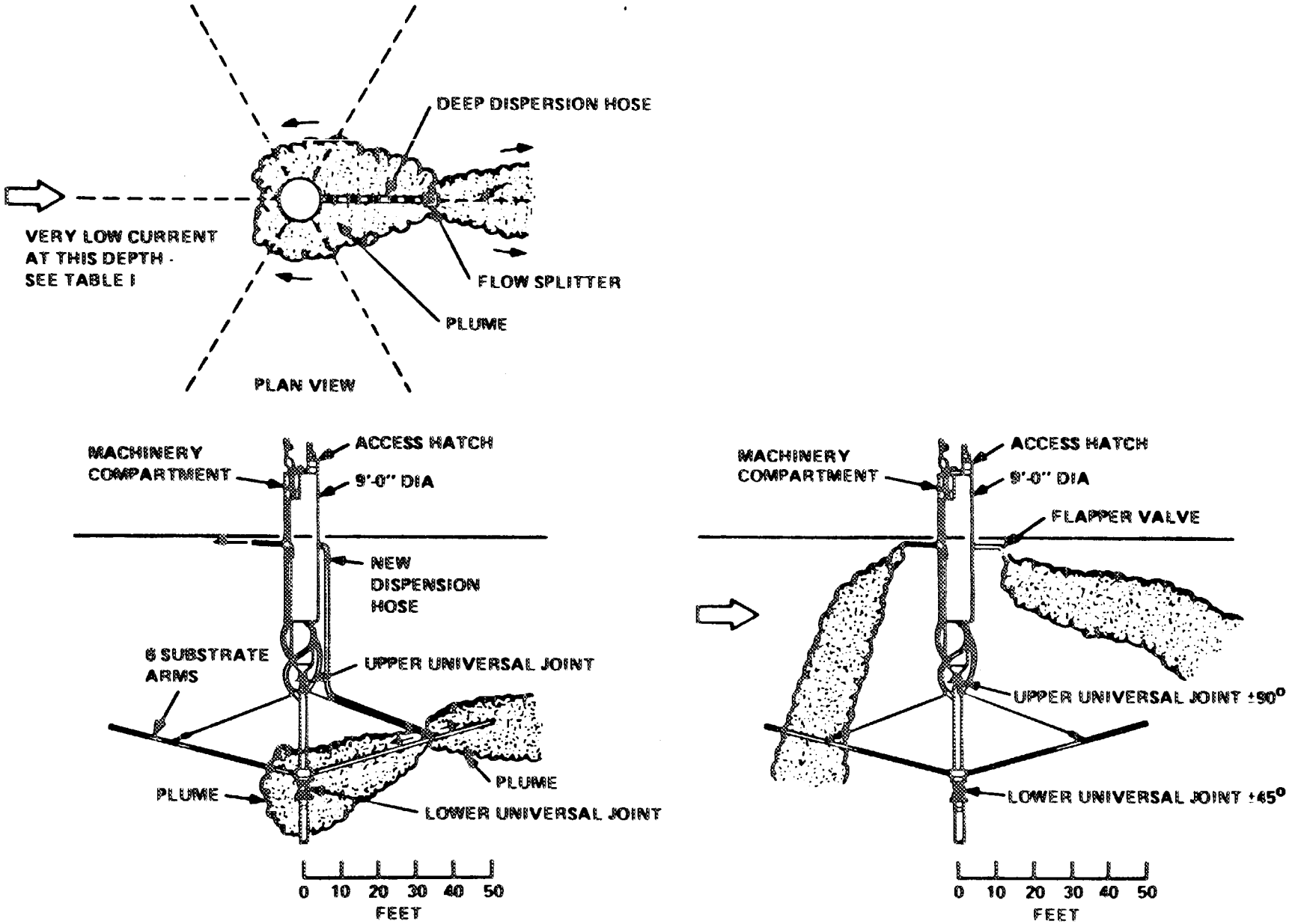

Figure 6. Fluorescein Dye Dispersion Test - Surface Plus Deep Water Release

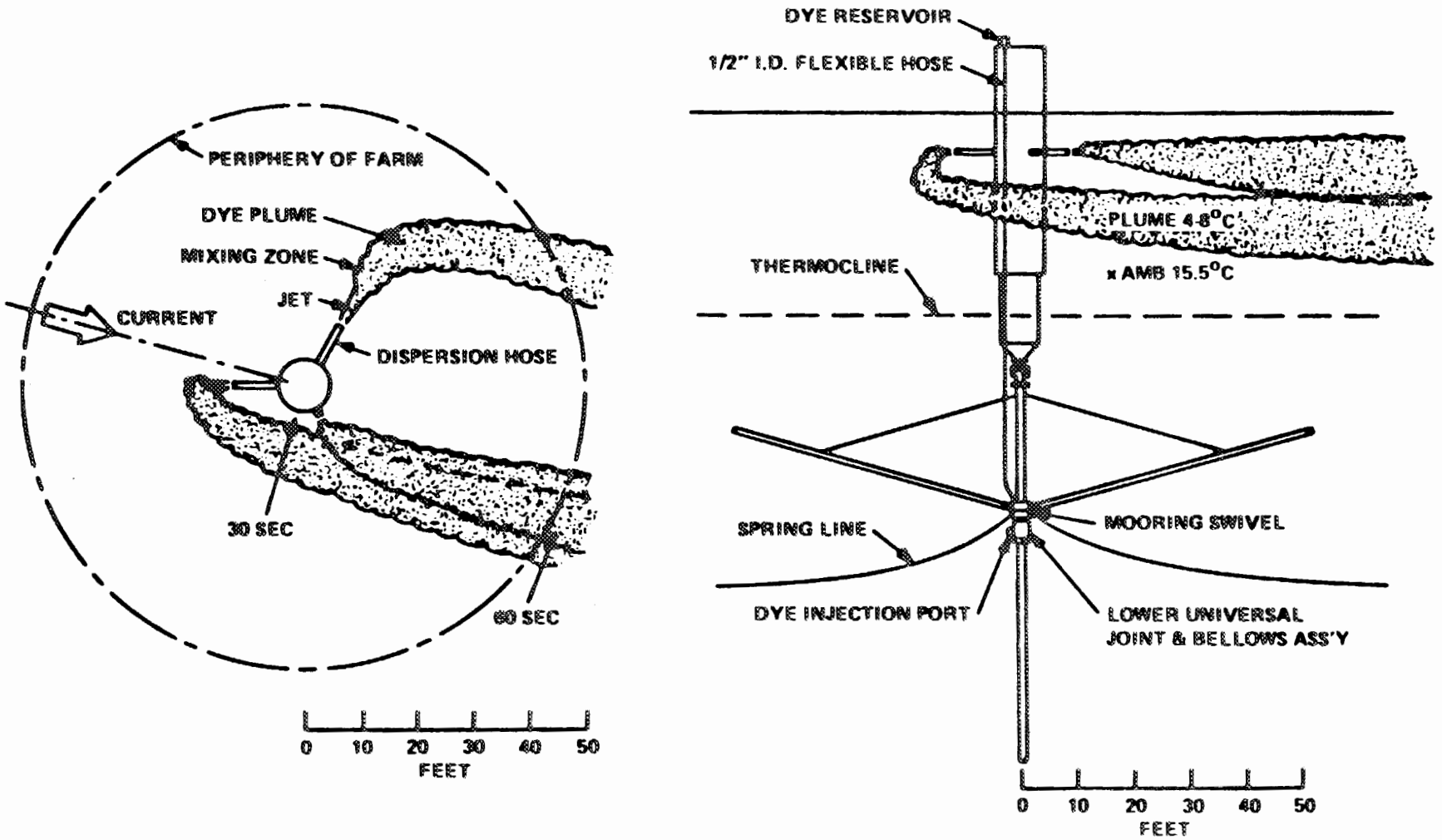

Figure 5. Fluorescein Dye Dispersion Test Surface Release

operation of a flexible, deep water pipe has been successfully met and, has had important synergistic effects with other energy programs. The upwelling system has survived the extreme environment of the ocean including storms with winds up to 100 mph and sustained waves of 10-12 feet. With the exception of the initial flexible current barrier, the mechanical structures of the Test Farm have been unscathed by these severe winter storms. The critical need now is to modify the structure so that plants will not be damaged after they are attached to it.

BIOLOGICAL STUDIES

The marine biomass selected for initial evaluation of marine farming concepts is the giant brown kelp, Macrocystis, which is the largest of the marine algae, attaining lengths of 200 feet in the adult plant. It occurs along the entire Pacific coastline from Baja, California to Alaska and on coasts of China, Japan and Korea. This kelp is one of the fastest growing plants known to man with growths of up to 2 feet per day common in adult plants. The plant generally occurs on rocky bottoms and attaches to solid substrate by means of its holdfast. Fifty to sixty percent of the mass of an adult plant is found in the top ten feet of the water's surface and new growth is continuously generated from the holdfast to replenish the harvested canopy. Figure 7 is a schematic of an adult Macrocystis plant.

The investigation of kelp growth and nutritional requirements has been performed under the leadership of Dr. Wheeler North, Professor of Environmental Sciences at the California Institute of Technology. The objective of this work has been to determine the nutritional requirements of Macrocystis and to provide information on the physiological requirements for optimizing kelp growth in an open ocean cultivation system. Through extensive laboratory and field observations, Dr. North has developed a set of biological criteria which must be met in order to achieve commercially acceptable yields. It has been shown that the

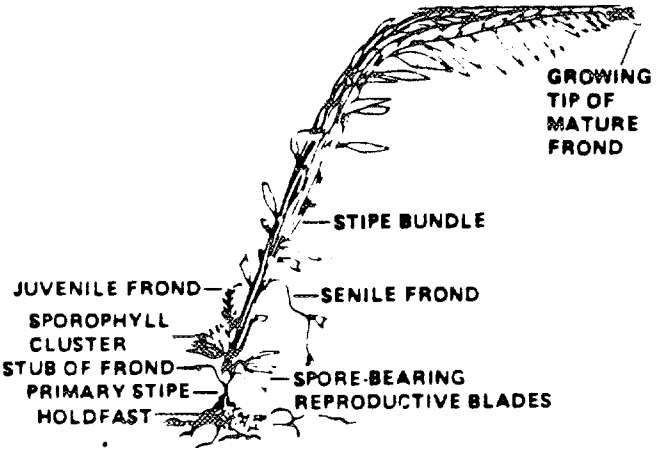

Figure 7. Diagram of an Adult Macrocystis Plant

growth rate of juvenile plants fertilized by water taken from 1000 feet is at least twice that of juvenile plants taken from natural kelp beds which were fed by natural upwelling. The difference in growth rate was shown to be due to continuous exposure of the test plants to this deep water. Plants in natural beds are fed only intermittently by upwelled deep water and the resulting limitation in nitrogen availability is responsible for the slower growth. Preliminary investigations by Dr. North have also indicated that trace quantities of the specific micronutrients manganese and iron play a major role in obtaining maximum kelp growth and yield. Dr. North's studies have shown that iron and manganese concentrations generally appeared adequate in surface waters but inadequate in these trace metals in deep water. Laboratory work to date has indicated that a mixture of deep and surface waters should provide the near-optimal medium for fertilizing plants in oceanic farms. Dr. North and others have also obtained data that indicates that Macrocystis has the capability to store nutrients for future use. This information has major program impact in that it may allow consideration of a range of design and operational strategies for supplying optimum levels of nutrition to the biomass energy farm. For the offshore test farm experiment (OSTF) Dr. North has specified the maintenance of a nutrient level of

3μ g-atoms/liter of nitrogen throughout the farm as an operational requirement. This level affords an adequate background of dissolved nutrients in the manganese and iron-rich surface waters.

The work at Cal Tech has also led to development of techniques for transplanting healthy adult Macrocystis plants directly from natural beds onto the OSTF and experimental techniques for the controlled field evaluation of kelp growth, yield, recruitment and state of health for the open ocean experiment. Recent work has also led to further important understanding of plant dynamics.

BIOCONVERSION

Unlike terrestrial plants, Macrocystis does not contain the lignin cellulose biopolymer for structural support. The plant instead is supported by flotation members (pneumatocysts) attached to the base of the blades. From the standpoint of bacterial digestion, the absence of lignin is a decided advantage. Figure 8 is a diagram showing the average composition of Macrocystis including percentage composition of water, inorganic salts and volatile solids. The content of water in the fresh plant, coupled with the absence of lignin and the presence of volatile solids constituents that are biologically degradable, were the key factors that have focused the conversion research on the anaerobic digestion process.

The research in bioconversion has been concentrated in three major areas: Pre-Post Treatment, Inoculum Development and Anaerobic Process Development.

Pre-Post Treatment

This work has been conducted at the Western Regional Research Center, United States Department of Agriculture, Albany, California. These efforts have been directed toward the definition and evaluation of mechanical and chemical pre-post treatment process steps which may increase the bacterial digestibility of kelp.

Mechanical pretreatment studies centered on the development of least capital and least energy intensive methods to increase surface areas and cell rupture. Baseline processes and process equipment have been evaluated and a material balance for the selected process has been developed. Particle size reduction was examined using pilot scale and commercial scale equipment and correlations of particle size with energy consumption have been developed. Various grinding mechanisms have been studied and demonstrations of grinding using pilot scale equipment have indicated that hammer-mill grinding is the least energy-intensive method for the range of particle sizes of interest. Anaerobic digestion studies showed an insensitivity to reductions in particle size beyond a d_{70} value of 4 mm (d_{70} is the diameter at which 70 percent of the weight passes through a 4 mm screen). In these pilot scale studies using a hammer mill, the energy required to produce this size particle from wet kelp was measured at 1.1 KWH/Ton of raw kelp. This represents about 1 percent of the energy available in the feedstock. These studies have also shown that there is no energy advantage to multi-stage grinding approaches.

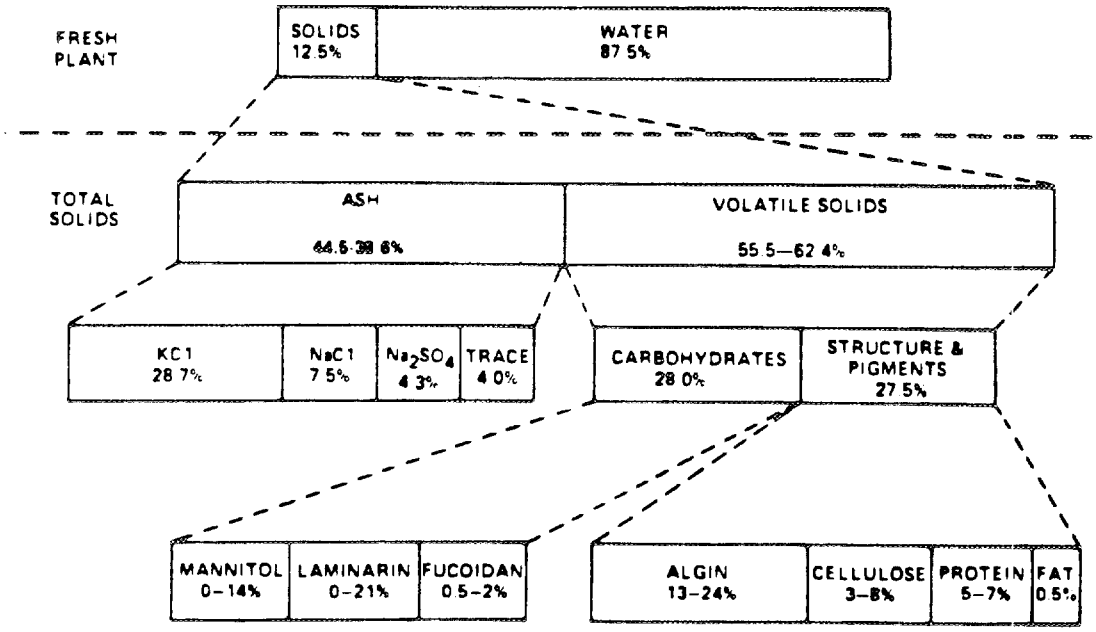

Figure 8. Macrocystis Composition

Additional studies have resulted in an assessment of the potential of the use of digestor solid effluent as an animal feed supplement. Data from these studies indicate that the effluent has a crude protein content of about 37 percent and has good potential as an animal feed supplement. The feed is chemically comparable in protein value to soy protein and may, in fact, exceed the nutrient value of any plant protein in terms of its amino acid composition. However, in-vitro examination of digestibility indicated that additional pre-treatment will be needed prior to use of the residue in animal feeds.

Inoculum Development

The research activity in specialized inoculum development has been conducted by the General Electric Company, Re-entry Systems Division. The objective of the work is the development of an optimized anaerobic inoculum incorporating micro-organisms derived from the marine environment. The potential results of this research could have major impact on several significant cost centers in the bioconversion process. Since the kelp substrate is of marine origin, a collection of marine derived organisms, which decompose kelp in nature, is expected to have the requisite enzyme systems for rapid depolymerization of kelp cellulose and algin and for utilization of the resulting degradation products.

Enrichment cultures derived from marine sediment, rotting kelp and marine organisms that are known to digest kelp, have been incubated on raw kelp substrates. Isolations have been subsequently made of the micro-organisms in the enrichment culture that degrade the various components of the substrate. Results to date have led to the identification of the specific enzyme requirements for the degradation of major constituents of kelp as well as the optimum pH values for maximum enzymatic degradation of certain major constituents. This research has also resulted in successful development of micro-organism enrichments which can produce methane from the kelp constituents: algin, cellulose, mannitol and

fucoidan. Preliminary tests on enriched anaerobic cultures composed of marine micro-organisms have indicated that such inocula can be incubated at ambient temperatures rather than at the usual mesophilic range of 35°C without sacrificing reaction rates as might normally be the case with conventional sludge-derived inocula currently in use. The significance of this data, once satisfactorily verified by additional experimental work, is a potential reduction in processing system costs and energy requirements.

Anaerobic Digestion Systems Development

Work on the development of an optimized digestion process has been conducted by the Institute of Gas Technology. The objective of this work is the definition and optimization of the anaerobic digestion process for conversion of Macrocystis to methane. The significant results of this task include the demonstration at the bench side of stable, reproducible methane production from raw kelp by an inoculum adapted for kelp digestion. This has been accomplished by the use of baseline inocula and detailed experimental investigations of physiochemical and biochemical parameters key to maximizing methane yield and process efficiency. Basic information on kelp digestion kinetics has been developed and will continue to be developed as part of this research activity.

Ongoing experimentation at IGT has resulted in methane yields of 4.9 to 5.0 SCF/lb of VS. This yield represents approximately 75 percent of that which is theoretically attainable and, on the average, exceeds those of any other known biomass as documented in literature. Table 1 compares methane yields of raw kelp relative to other various types of biomass.

In addition to the goal maximizing methane yields, work has also been conducted at IGT to further effect system economies by increasing the loading rate and reducing detention times. Digesters at IGT have been stabilized at a loading rate of 0.15 lb VS/Ft^3-day and 15 days retention time improved over earlier

TABLE 1. PERFORMANCE DATA FOR ANAEROBIC DIGESTION OF VARIOUS TYPES OF BIOMASS

Reference	Biomass Type	Methane Yield SCF/lb VS Added	Special Conditions
IGT, Oct. 1978	Raw Kelp	4.9	T[b]-34, L[c]-0.15, DT[d]-15
Pfeffer	MSW-Sludge[a]	2.42	T-55, DT-20
Pfeffer	MSW-Sludge	1.58	T-35, DT-20
McCarty et al.	MSW-Sludge	3.26	T-35, L-0.23, DT-15
Ghosh and Klass	MSW-Sludge	3.80	T-35, L-0.14, DT-12
Ghosh et al.	Grass Mixture	3.10	T-35, L-0.12, DT-16
Bryant et al.	Feedlot Cattle Waste	4.10	T-60, L-0.54, DT-9
Bryant et al.	Feedlot Cattle Waste	2.74	T-60, L-1.62, DT-3
Converse et al.	Dairy Manure	3.33	T-35, L-0.27, DT-15

[a]Municipal Solid Waste-Sewage Sludge [b]Temperature, °C

[c]Loading, lb VS/cu ft-day [d]Detention time, days

values of 0.1 lb VS/Ft^3/day and 18 days retention time. With pretreatment process steps added and with the process design and operation optimized, it is expected that a loading rate of 0.3 lb VS/Ft^3/day or higher, a retention time of six days or lower and a methane yield of 5.5 SCF/lb of VS, or higher, will be achieved.

Additional results and significant accomplishments from this bioconversion research that directly affect reduction in process economics are:

- Macrocystis is degradable in saline culture and can be fed directly to digestors in its raw, undiluted state and effectively converted to methane. Fresh water is not needed and salt does not inhibit the digestion process.

- The addition of supplementary nutrients to the reactor are not required for active digestion.
- Mannitol is a major and most rapidly biodegradable component of kelp and a relationship between methane yields and mannitol has been experimentally developed. The potential for "tailoring" cultivated Macrocystis that is high in mannitol could further increase methane yields at reduced retention times.

SUMMARY

As can be seen from the previous discussions, the concept of marine farming is fairly complex in its research and development needs. It is the feeling of the researchers involved, however, that the potential for large scale production of renewable energy produced from biomass grown in the ocean, warrants the extensive work required to determine the technical and economic feasibility of commercial cultivation of seaweed as a renewable energy source.

Because of necessity for precise identification and understanding of the parameters and the interaction among parameters involved, the energy production system demands an integrated approach for the management of the proposed R&D program. The organizations contributing to this research program form a highly capable and effective team for this purpose. With this team, the R&D capability has been developed, within one program, to address the many questions that must be satisfactorily answered.

Questions that have been answered to date include the very critical ones of: the capability for growth and reproduction of kelp in the deep ocean away from its natural near-shore environment; the ability to sustain kelp growth with nutrients derived from the deep ocean; and the capability of the anaerobic digestion process for the high yield conversion of kelp to methane. Previous R&D efforts, conducted in concert with the development and application of the systems model, has focused

the project on those tasks having the highest cost, energy, and biological impacts within the concept framework.

Additional work has led to the determination that commercially available equipment and systems can be used to process kelp feedstock on a large scale. Harvesting, size reduction, and transport equipment already exist and could be scaled up for use in a commercial kelp cultivation and processing system.

In the preliminary investigation of by-products, two potentially valuable products resulting from the anaerobic digestion process have been identified and are under evaluation: an animal feed ingredient having high protein content, with an excellent amino acid profile; and potash, which has potential application for agricultural uses.

Information developed as a result of the anaerobic digestion process development task has given us confidence that the task of scaling from bench scale reactors to engineering sized digesters can be accomplished. The potential is high for attaining significant increases in conversion process yield and rate.

The isolation and characterization of microorganisms active in the digestion process, and the determination of their reaction kinetics on the individual constituents of kelp, represent a unique approach to the development of optimized inocula. Results to date from this research give confidence that still lower digester retention times and increased yields can be expected as a result of this microbial work.

While the past work has answered several critical questions, it has also raised additional considerations. The interaction of the kelp plants with the growing structure has emerged as a key area to be addressed. These interactions of the kelp plants with the growing structure must be minimized so that the test facility can be used to acquire critical yield data.

Previous work has shown the Macrocystis is sufficiently tough and flexible to survive the violent water motions during typical Southern California storms. We believe that if the plant is isolated from rigid structures and allowed to react only to the ocean - regardless of the ocean's violence - the plant will survive. The variation of harvest yield as a function of crop density, harvest frequency and upwelled water application is a critical parameter that must be understood.

Future research and development will focus on these and other considerations. The primary task will be the determination of the productive capacity and the sustained yields that may be attained during controlled cultivation of kelp.

3.1 MAJOR PROBLEMS AND SOLUTIONS

Problems related to overall operation of Marine Biomass Program, and corrective actions which either have been or will be taken, are summarized within.

1. Plant protection is needed at the Test Farm to reduce abrasion and entanglement as well as to provide nutrient containment. Action taken has been a Concept Design Study and a Preliminary Design Review, resulting in the tentative acceptance of a Hemi-dome configuration if model wave tank tests support the use of this structure at the offshore test site.
2. Water and microbial growth continually clogged the fuel lines aboard the machinery buoy during early months of 1980. Action taken was to install a combination water separator and fuel filter together with addition of an anti-microbial agent.
3. Encrustation of underwater farm components by marine growth allowed only a gross observation of machinery buoy/substrate structure. Action taken was initiation of cleaning procedures to remove growth.

4. Differing motions of substrate and plants caused abrasion and entanglement of the kelp. Action taken was the initiation of experimentation using an elastic cord and damping plate to isolate plant motion from substrate motion.
5. High summer temperatures and reduced upwelling caused expansive kelp canopy losses causing cessation of routine analysis. This problem will be solved when growing conditions return to the coastal beds.
6. Grazing predators caused extensive growth losses among Test Farm experimental plants. Start-up of a larger and healthier plant population after Test Farm modification should reduce grazing problems.
7. IGT experienced three mechanical problems during the early part of the year - a defective speed controller on a gyratory skaker; an autofeeder not delivering a specified volume; and high temperatures in the feed slurry tank. These problems were quickly corrected shortly after they occurred, and presented no slippage in milestone schedules or cost overruns.
8. Heavy metal toxicity due to leaching in the kelp storage reservoir at WRRC resulted in poor digester performance. The run was subsequently terminated and a new storage tank designed.

3.2 MAJOR ACCOMPLISHMENTS

1. The Biological Test Farm, deployed off the coast of Southern California in September of 1978 continued to maintain its structural integrity after nearly 2 1/2 years in the open ocean, thus giving credence to the farm design and fabrication.
2. Design studies further narrowed potential farm modifications expected to minimize kelp abrasion and entanglement on the farm structure.

3. Adult kelp plants attached to a mooring line in August 1979 survived the rigors of the ocean environment during the winter and spring of 1980, validating the hardiness of the Macrocystis plant.
4. Steady-state mannitol experiments verified the model proposed to predict methane yield on basis of elemental composition and mannitol content.
5. Several innovative digesters were designed, fabricated and tested, signalling the start-up of advanced digester studies.
6. An autofeeder was designed, constructed and successfully tested for feeding undiluted raw kelp to digesters.
7. Facilitization of pier laboratory and establishment of coastal test farm marked initiation of interspecific hybrid production and outplanting between species of Macrocystis.
8. Candidate species selection, test site evaluation and greenhouse facilitization signalled start-up of Biomass activities in East Coast waters contiguous to New York State.
9. Growth enhancement of quiescent juvenile fronds was demonstrated by lowering into nutrient-rich ocean waters.

4. SPECIFIC OBJECTIVES FOR 1980

Specific objectives of the work performed during 1980 on the Marine Biomass Program are highlighted below under the five major areas of endeavor.

4.1 BIOLOGICAL TEST FARM MAINTENANCE/OPERATION

- To assure continuous operation of nutrient dispersion supply system, navigational aid marker equipment, and data collection system.
- To perform preventive maintenance and corrective maintenance on the Biological Test Farm as required
- To maintain the Test Farm in an operational "ready mode"

4.2 KELP BIOLOGICAL STUDIES

- To continue environmental monitoring at the offshore control site and at the natural kelp bed control station
- To continue to monitor transplants at the Test Farm
- To monitor the biological community associated with the Test Farm
- To perform physiological studies to determine relationship between environmental factors such as nutrient concentration and light intensity upon mannitol content
- To perform experiments determining the micronutrient uptake rates in *Macrocystis*
- To initiate characterization and seedstock production of *Macrocystis* species
- To integrate foreign technology research

4.3 INOCULUM DEVELOPMENT

- To maintain stock and baseline cultures
- To continue Microbial Enrichment/Isolation studies

- To initiate particulate studies to correlate cell growth with measurable parameters including ATP, F_{420}, methane production, and Most Probable Numbers techniques
- To initiate studies on digestibility of kelp and marine inoculum development

4.4 CONVERSION PROCESS DEVELOPMENT

- To elucidate factors that limit fermentation rates and methare yields
- To determine operational parameters that produce the highest methane yields and production rates
- To develop a kinetic model that will predict fermentation rates and yields under different operating conditions, including loading, detention time, and effluent solids recycle

4.5 PRE-TREATMENT AND POST-TREATMENT STUDIES

- To continue evaluations of pre- and post-treatment techniques
- To maintain baseline and stock cultures
- To collect, process and distribute kelp to subcontractors as needed
- To collect effluent for experimental tests

5. 1980 WORK PLAN BY TASK AREA

The following sections describe the experimental work expended during the course of this contract - the 8 month period covering January 1, 1980 through August 31, 1980. In addition, a brief synopsis of the first four months' work under the SERI contract - covering the period September 1 through December 31 - is included where applicable. To provide clarity to the reader, each major section listed below is presented as an individual section of work:

5.1 Biological Test Farm Maintenance and Operations

5.2 Kelp Biological Studies

5.3 Inoculum Department

5.4 Conversion Process Development

5.6 New York State Site and Species Selection

5.7 Kelp Cultivation, Characterization and Genetics

5.1 BIOLOGICAL TEST FARM MAINTENANCE AND OPERATIONS

GLOBAL MARINE DEVELOPMENT INC.

5.1 BIOLOGICAL TEST FARM MAINTENANCE AND OPERATIONS

OSTF MAINTENANCE AND OPERATION

Primary objective of GMDI's Maintenance and Operation Task was to continue the maintenance and operation of the OSTF according to operations plans and procedures prepared by GMDI and approved by GE-RSD. These plans and procedures were to include, but not necessarily be limited to, the maintenance and/or operation of the diesel engines and upwelling pumps, engine fueling, farm hardware inspection, normal repairs, damage repairs, navigation aid maintenance, spares inventory maintenance, logistics support, insurance and applicable drawings.

The specific tasks to be performed during this period are:

1. Three maintenance trips per month to the machinery buoy to refuel, inspect and maintain machinery and NAV/Aid System. The work party will consist of a construction engineer, a mechanical and electrical engineer, Submarine Engineering Associates crew, and Mike Newell, diesel mechanic. One of the trips each month will include an underwater inspection performed by an S.E.A. dive team.
2. Planning the three trips as to defining specific tasks to be performed by crew and providing necessary spare/replacement parts. Determining fuel requirements, procuring services and hardware necessary for these tasks.
3. Performing necessary engineering to properly maintain the Biological Test Farm. Analyze problems, define solutions, supervise work parties.
4. Performing maintenance tasks on machinery buoy to clean up the buoy electrical power system. Bring electrical drawings up to date to establish present Test Farm configuration.
5. Build a spare parts inventory, based on 1979 history, to minimize 1980 down time when repairs are needed. Replace auxiliary non-critical worn components on the OSTF that have been deferred due to 1979 lack of funding.

6. Replacing the retro reflective tapes on all four buoys. Coast Guard Safety Requirements dictate that the buoys be so banded. Engineering will develop a more permanent method of banding the buoys. The new process will then be implemented.

Toward this goal, twenty-nine maintenance visits including eight dive inspections by Submarine Engineering Associates crew were made to the OSTF during the eight month period. The main objective of these visits was to keep the machinery operational and satisfy Coast Guard Nav Aid requirements. The system has begun to show signs of aging with water showing up in the fuel system, sacrifical anodes reducing to 30 percent of their original size and the underwater structure becoming encrusted with marine growth.

The major problem during the year was one of fuel contamination. Normal condensation and perhaps water in the fueling drums was the major cause of the problem. The water supported bacteriological growth in the fuel tank which in turn clogged the fuel filters. After diagnosis of the problem the main buoy fuel tank was pumped dry and cleaned thoroughly. A new fuel filtration/water separator unit was procured and is used in all fuel transfer operations. The unit can be put on board the buoy to allow removal of condensed water from the fuel tank by pumping fuel from the tank bottom through the device and back into the fuel tank. A chemical additive is now added during fueling operations to prevent the possibility of bacterial growth in the system. The problem was not anticipated, but now that it is understood, the new hardware and revised procedures will prevent recurrence.

Another minor modification was made to the fuel system to aid system diagnosis. A pressure gauge was added to allow direct readout of the pressure drop through the fuel filter. This gauge indicates filter condition in a normal mode. A valve was added upstream of the gauge to allow checking pressure

capability of the main fuel pump. This simple change allows quantitative evaluation of the fuel system hydraulics.

The on-board spares inventory proved to be adequate during the year. There were no major problems requiring engine removal or prolonged shutdown due to lack of spares.

Eight dive inspections were performed during the eight month period. The encrustation of the underwater hardware by marine growth allowed only a gross inspection of the machinery buoy/substrate structure. The dive inspections usually include some useful underwater maintenance or special test support in order to maximize program returns.

MARINE BIOMASS, SERI CONTRACT

The work during the period from September 1 through December 31, 1980 was covered by the SERI contract. Normal maintenance continued during this period along with several additional tasks. The additional tasks under the new contract are Refurbishment and Repair, Concept Trade-Off Analysis, Model Testing, Hardware Design, Fabrication and Installation, Instrumentation, Dispersion Modeling and Test and Geometry and Material Tests.

The Refurbishment and Repair task covers the deferred maintenance jobs on the OSTF. Buoy painting, underwater cleaning of critical areas, and replacing the upwelling hoses were the major tasks. The cleaning has been completed, the painting started and the upwelling hoses have been ordered. The cleaning of the upper universal joint revealed some wear in the pin joints not previously detected through the marine growth. This was discovered in December and a study has been initiated to determine the best fix. Engineering dive inspections determined that the joint is not causing an immediate emergency but repairs should be made in the near future.

The Concept Trade-Off Analysis was a task designed to study proposed modifications to the test farm. Several concepts were initially evaluated and the number reduced to six for final consideration on November 17 and 18 when a Preliminary Design Review was held at GMDI to present the findings. A decision was made at that time for GMDI to concentrate on the Hemi-dome Design. The Hemi-dome is a hemispherical membrane filled with sea-water. This concept provides maximum protection to the kelp plants and better control over nutrient concentration than the other concepts.

Chicago Bridge and Iron Company was given a letter of intent in late December for testing the Hemi-dome concept. This testing is scheduled to start in late January.

The Hardware Design task was started in December. The next major milestone in this task is the Critical Design Review (CDR) in late March, 1981. The Fabrication and Installation task will commence after CDR.

The Dispersion Modeling and Test task supports General Electric in designing, installing and testing different dispersion schemes on the Test Farm. The hardware will be completed and installed, ready for testing by mid January.

The Geometry and Material tests also support General Electric in designing, installing and testing two small scale kelp attachment structures. One device, a motion limiting spring-coupled sphere was installed in mid December. The other device, a buoyant fifteen foot square grid attached by bungees, (stretchable cables), will be ready for installation in mid January, 1981.

The Geometry and Material Test hardware will be instrumented to determine dynamic motions and attitudes. This hardware was designed and procured. The instrumentation sensors are installed on the machinery buoy. Data recording was started in later December on the buoy motions.

5.1.1 "MYRTLE" EXPERIMENTS WITH ELASTIC TETHERS AND DAMPING PLATE - GENERAL ELECTRIC COMPANY

INTRODUCTION

Three adult Macrocystis plants attached to substrate floats approximately 40 feet below the surface and tethered near the mid-points of one of the 300-foot catenary cables had been the subject of biological studies for several months. The first of the group to be installed by the Cal Tech team was given the name "Myrtle", hence the designation. They were attached to the catenary to avoid any potential abrasion or tangling contact with the OSTF structure or mooring buoys. Although exposed to severe storms in January and February (wave heights up to 20 feet and winds exceeding 40 knots were reported) the plants sustained very little damage. This, in effect, answered one of the primary questions concerning the ability of Macrocystis to survive in the open ocean. It should be noted that the floats used in the Myrtle group had a much higher buoyancy (by a factor of ten) than those used originally in the OSTF. This higher buoyancy prevented snapping action in the tether and contributed to the survival of the Myrtle plants. However, the problem of juvenile frond abrasion remained and appeared to be aggravated by the use of high buoyancy floats. The Myrtle experiments described herein were undertaken to study techniques to alleviate this potential abrasion problem. Such techniques would be useful for future kelp transplanting and attachment operations.

Divers fed the Myrtle plants periodically with a concentrated nutrient solution by enclosing each plant temporarily in a plastic sleeve. Certain fronds were tagged and growth data obtained by measuring the elongation of the blades. This was translated into increase in biomass by means of an empirical length-mass correlation factor.

Divers noted that new growth which originated in the vicinity of the holdfast was being damaged, apparently by abrasive contact with the float and/or tether to which the holdfast was attached. It appeared that this was caused by the cyclical downward flow of water around the float arising from the combined action of the waves and the heave of the catenary cable to which the tethers were attached. An early Wheeler North estimate was that perhaps 40 percent or more of the juvenile fronds were being lost by this means. Since the emphasis of the program is in biomass yield, losses of this magnitude are intolerable. The problem, therefore, was how to eliminate this effect, or at least reduce it to an acceptable level.

ENVIRONMENTAL APPROACH

The objectives of these experiments were: 1) to substantiate the visual observations made by the divers concerning the abrasive action between the juvenile plants and the subsurface floats, and 2) to determine if this behavior could be alleviated by means of an elastic tether and/or damping arrangement. Concerning the latter, the float-substrate attachment was changed on two of the plants. The inelastic tether on one plant was replaced by an elastic cord and damping plate while an identical elastic cord without a damping plate was substituted for the other. The diameter of the plate and spring-constant of the tether were chosen to produce an over-damped system in order to minimize the relative motion between the float and its surroundings. The values selected for these parameters were based partially on the results of a simple 1/6 scale model test conducted at GE, Valley Forge, using the apparatus previously constructed under the 1979 IR&D wave pump program. The third plant "Myrtle" retained the original inelastic or "hard" tether. The experimental plan was to observe and record on video tape the relative behavior of the three attachment schemes for a given sea state. To expedite setting up this experiment it was decided to use the existing floats and plants.

EXPERIMENTAL DETAILS AND CALCULATIONS

The choice of elastic tether material was guided by the following considerations: 1) When in static equilibrium with the float the extension of the material should be around 50 percent of the maximum range, 2) the maximum elastic range must exceed the anticipated 4.0 foot dynamic range and 3) the spring constant, based on the model tests and rough calculations should be of the order of 5 to 10 lbs/ft.

The most promising and readily available material for this application was rubber shock parachute cord, sometimes referred to as "bungee cord". Elongation measurements were made on various samples using a 5-inch gauge length for convenience. No elongation occurred until the load exceeded 19 pounds, whereupon the length increased in a nearly linear manner. Measurements with smaller diameter cords indicated that the maximum elastic range is about equal to the initial length. If the initial length is L the force required to stretch it an additional amount ΔL is:

$$F = \left(\frac{1}{L}\frac{dF}{dT}\right)\Delta L + Fo \qquad (1)$$

where 1 is the gauge length. The quantity within brackets is equal to the spring constant, k, which appears in the conventional Hook's Law relationship $F = kx$, where x is the displacement. A duplicate empty barrel (actually a 1/2 keg) with the chain hitch and associated fittings was found to be 40 pounds. A full 1/2 keg weighs almost 163 pounds, thus the buoyant force would be 123 pounds. Allowing an additional 9 pounds for the plywood disk and kelp plant, the net buoyancy comes to approximately 132 pounds. For the extension to be near mid range at 132 pounds two 1/2 inch cords were used in parallel, thus the right hand side of Equation No. 1 must be multipled by 2.0, i.e.,

$$F = 2\left(\frac{1}{dl}\frac{dF}{}\right)\frac{\Delta L}{L} + 2\,Fo \qquad (2)$$

Substituting l = 5 inch, $\frac{dF}{dl}$ = 15.5 lbs/in., F = 132 pounds and Fo = 19 pounds, we obtain $\Delta L/L$ = 0.6 which was close enough to the mid point for experimental purposes. The longest practical length for the extended cord, namely 24 feet, was determined by the working distance from the floats to the catenary cable. Thus we have $L \pm \Delta L$ = 24 feet. Substituting $\Delta L/L$ = 0.6 from foregoing calculation gives L = 15 feet as the initial length. The spring constant for 15 feet of double cord turns out to be 10.33 lbs/ft, which is an acceptable value, although in the high end of the range. The force is plotted as a function of total length where the "operating point" is the equilibrium displacement.

The damping plate, 4 foot diameter x 1/2 inch thick plywood, was given several coats of a resin and acetate mixture to delay delamination. The bungee cords were cut to length and all terminations and interconnections were made in the marine laboratory in preparation for deployment.

DEPLOYMENT

The plan decided upon was to use a block and tackle to haul each float down far enough to tie the double bungee to the float shackle and the catenary. The original tether would then be cut free. The block and tackle would then be slacked off and removed after the strain was taken up by the bungees. A life line would be added between the floats and the catenary to prevent loss of the plants should the bongees part.

DISCUSSION

The motion of the water relative to the float is the resultant of the substrate motion and the motion induced by surface wave actions. The extension of the elastic tether is the sum of the two, assuming the float follows the local water movement. The contribution made by wave action can be estimated from theory. For simple harmonic waves the total vertical displacement of the water

(i.e., a water particle) at a depth d is H exp ($-2\pi d/\lambda$) where H = 4 feet, λ = 100 feet and d = 38 feet the total displacement will be 4 exp (-2π 38/100) = 0.37 feet. This is negligible compared with the 6 foot displacements which were observed. It must be concluded that the displacement is due mainly to the heave of the catenary cable. From the few observations of the OSTF substrate motion by the divers it appears that the catenary motion is more severe. Observations so far suggest that the kelp plants have very little effect on the dynamic behavior of the beer-keg floats. This is not surprising since the buoyancy of the float is at least twenty times that of the attached plant. The only noticeable effect is the deflection of the 30 foot long attachment system due to drag.

CONCLUSIONS

1) The experiments conducted on the Myrtle group met all objectives.
2) Video tape records show that conditions for the abrasion and destruction of juvenile fronds are generated by the use of a hard tethered attachment system.
3) It was demonstrated that the use of an elastic tether and damping can solve the problem of juvenile frond loss by drastically reducing the relative motion between the float (hold-fast) and the surrounding water.
4) The relative velocity is due primarily to the motion of the substrate.
5) The plants themselves had very little effect on the dynamic behavior of the high buoyancy floats used in these experiments.
6) The use of TV for real-time monitoring and recording has proved to be an extremely efficient, effective and accurate diagnostic tool, both from an operational as well as an analytical point of view.

5.1.2 DETERMINATION OF DRAG ON KELP PLANTS FROM DEFLECTION MEASUREMENTS - GENERAL ELECTRIC COMPANY

INTRODUCTION

One of the primary concerns with respect to the practicability of an ocean farm for growing Macrocystis is the survivability, physically, of these plants in the open sea. Indeed, there is a question whether or not the plants could survive even if there were no mechanical structure on which they could abrade or become entangled. To answer this question, Dr. Wheeler North deployed several mature plants far enough from the existing BTF structure to avoid mechanical interaction. The holdfast of each plant was attached to a subsurface float and tethered to one of the catenary cables. The first of these plants to be deployed (in October 1979) is referred to affectionately as "Myrtle". Divers fed the plants periodically with a concentrated nutrient solution while each plant was enclosed temporarily in a plastic sleeve. In effect, this provided an elementary nutrient containment scheme. Even though the plants have been exposed to high seas and currents (wave heights up to 20 feet), there has been no evidence of any significant damage. This is a particularly important observation since the subsurface floats move vertically with large amplitudes sending S-shaped kinks up the length of the plant, and yet no deleterious effects have been noted. Plants in a natural bed never experience this mode of mechanical excitation, except possibly during an earthquake.

The effect of current on the plants is interesting. Dr. North and colleagues noted that the deflection of the plants is very large compared to the angle subtended by the float tether. Underwater photographs taken under high current conditions show very little deflection of the float although the plants are almost horizontal. No quantitative data can be obtained from the photographs taken to date due to the absence of reference coordinates. However, Dr. North estimates that for currents in the range of 1 to 1 1/2 knots, the angular displacement of

the float was perhaps 10-15^{0} and the plants were "nearly" horizontal. Using this semi-quantitative data, it is possible to make some assessment of the magnitude of the drag and buoyancy of a mature plant. In addition, it is reasonable to suggest future experimental procedures for obtaining more quantitative information.

Theory:

If the system is in equilibrium, we have

$$\epsilon Fx = D_f + F_p \cos\phi - T \sin\theta = 0 \quad (1)$$

$$\epsilon Fy = F_p \sin\phi - T \cos\theta + F_B - W = 0 \quad (2)$$

The quantity of primary interest is $F_p \cos\phi = Dp$ which is the horizontal component of the force which the plant exerts over the supporting structure. Dividing Eq. 1 by Eq. 2 and solving for D_p leads to the following expression

$$D_p = \frac{(F_B - W) \tan\theta - D_F}{1 - \tan\theta \times \tan\phi} \quad (3)$$

Calculation of D_p:

It so happens that the float to which Myrtle is firmly attached is an empty 1/2 beer barrel, fortuitously available. A full 1/2 keg. contains 15.75 gallons and weighs 160 to 165 pounds depending on the material of construction (aluminum or stainless steel). For subsequent calculations we assume a weight of 160 pounds. The specific gravity of beer (Budweiser) at 15^{0} C is 1.01, therefore

$$W = 160 - 1.01 \times 62.4 \times 15.75/7.48 = 27.3 \text{ pounds.}$$

When empty the buoyance of the barrel in sea water will be at least

$$F_B = 15.75 \times 64/7.48 = 134.8 \text{ pounds}$$

neglecting the volume displaced by the metal. The fluid dynamic drag on the barrel is $D_F = 1/2\ C_D \rho A V^2 = \frac{\pi}{8} C_D \rho d^2 V^2$ (4)

where d is the "equivalent diameter" of the barrel, i.e., the sphere having the same drag. Now a sphere containing 15.75 gallons has a diameter of 1.59 feet.

Adding to this, 0.15 foot to account for the combined thickness of the holdfast and metal shell gives 1.74 feet or d. Substituting this and $C_D = 0.5$ into Eq. 4 gives $D_F = 1.19V^2$, thus for Myrtle, Eq. 3 becomes

$$Dp = \frac{134.8 \tan\theta - 1.19V^2}{1 - \tan\theta \times \tan\phi} \qquad (5)$$

Using $\theta = 12.5^o$ and V = 1.25 knots, the median of Dr. North's estimates, and setting $\phi = 10^o$ (the author's guess) we have

$$Dp = \frac{134.8 \tan 12.5^o - 1.19 (1.69 \times 1.25)^2}{1 - \tan 12.5^o \times \tan 10^o} = 25.6 \text{ pounds}$$

The drag on the barrel itself is 5.3 pounds. The buoyancy of the plant, B_p, is just 25.6 $\tan\phi$ or 4.5 pounds, or 0.09 lbs./ft. if the plant is 50 feet long. If the volume of a typical pneumatocyst is 0.4 in^3, the buoyancy is 0.0143 pounds. If this is the only source of buoyancy for the plant, Myrtle must have approximately 0.09/0.0148 or 6.1 blades per foot, assuming the tissue density is the same as sea water.

FUTURE MEASUREMENTS

Program plans for 1981 include tasks for the direct measurement of drag on plants both in natural beds and in the open sea. Current meters and three-axis force measuring devices have been proposed as the basic instrumentation for otaining continuous detailed information on the forces acting on the holdfast over a wide range of current and sea state. One objective of measurements taken in natural beds is to ascertain the effect of number density on the drag of individual plants, i.e., the interference effects. The actual magnitude of the drag will depend on the velocity profile and this will vary considerably with depth due to boundary effects caused by the ocean floor. This will complicate the calculation of the drag coefficient and most certainly will introduce a considerable uncertainity in the derived values. Other tasks which involve the

dynamic behavior in response to sea state would have greater validity. It appears that measuring float and plant deflection angles provides a rapid, inexpensive technique for determining drag with a reasonable degree of accuracy for single plants for selected oceanographic conditions. It is envisioned that measurements would be made by a diver in the course of other scheduled work for the sake of economy.

COMMENTS ON SUBSURFACE FLOAT BUOYANCY

The use of a 1/2 keg was a fortunate choice because of the large reserve buoyancy. Indeed, it appears that buoyant force was large enough to prevent the tether line from going slack even in high seas or because of changing tension in the catenary cable. As a consequence, Myrtle and her companions were not subjected to the large impulsive forces which could arise if the slack is taken up while the float and tether attachment point were moving in opposite directions.

CONCLUSIONS

1) Deflection measurements provide a practical, inexpensive means for determining kelp drag with a sufficient degree of accuracy for engineering design purposes.
2) The drag of an adult plant in a 1 knot current is approximately 25 pounds.
3) The buoyancy of an adult plant is of the order of 0.1 lbs/ft. based on semi-quantitative data.

5.2 KELP BIOLOGICAL STUDIES

CALIFORNIA INSTITUTE OF TECHNOLOGY

5.2 KELP BIOLOGICAL STUDIES

The specific objectives for CIT during 1980 were to collect pertinent oceanographic data and monitor the oceanic environment near the Test Farm; continue appropriate experimentation at the Test Farm that assesses feasibility of growing Macrocystis at the site; conduct supporting laboratory studies needed to comprehend, guide, and improve observations and experiments at the Test Farm.

ENVIRONMENTAL MONITORING

In order to provide a continuing assessment of the nutritional status and physical condition of near-surface layers in the vicinity of the Test Farm and at control stations, samples and measurements were conducted weekly for determination of NO_3 and PO_4, temperature profiles, and Secchi disk depth to depths of about 60 meters. During the first six months of the current reporting period, monitoring activities also included a monthly oceanographic phase that extended the sampling and data collections to depths of 500 meters. These activities were terminated at the close of the upwelling season in June. The temperature and salinity profiles from these six months continued to indicate relative instability in the top 300 meters of the water column, adding further justification to the decision to draw the upwelled water for the Test Farm from the more stable depth of 450 meters.

The shallower oceanic layers that constitute the environment of the Test Farm displayed the usual seasonal conditions associated with winter, spring, and summer for this region. Although the general conditions were as expected, certain features deserve special comment because of their intensity or other noteworthy characteristics. Considerable rainfall occurred during the winter of 1980. Dilution effects from the resulting runoff caused a decline in surface salinity. Runoff can be high in nutrients and the surface nitrate and phosphate levels peaked during this period at both the coastal and offshore sampling sites. Runoff

also can affect water clarity, as determined by Secchi disk depth. The upwelling season began in early March as indicated by rises in nitrate and phosphate contents of water from 50 meter depth. Shortly thereafter, nutrient contents of the shallower levels also began rising as upwelling intensified. Upwelling peaked in late May, subsided into July, and then occurred as sporadic events during summer and fall. The intensity of upwelling at any given time can be assessed roughly by examining the depth of the 12.5°C isotherm. This isotherm reliably marks the boundary between overlying nutrient-poor water and the underlying nutrient-rich deep water. Upwelling moves the cold deep water toward the surface so that the depth of the 12.5°C isotherm shoals. Comparisons of the 12.5°C isotherm depths between 1979 and 1980 suggest that upwelling intensity was reduced during 1980 except for July and September (Figure 9).

Upwelling and runoff both furnish nitrogen to kelp plants. N contents of kelp tissues thus serve as indicators of the combined effects of these two physical processes. N contents were monitored within a control kelp bed at Cameo Shores during 1979 and 1980 to assess the total nitrogen supplies available locally as well as to estimate healthiness of this bed, since it might serve as a source of transplants for installation at the Test Farm. During 1980, N contents of canopy blades at Cameo Shores peaked during the winter, the time of maximal runoff, while the peak among basal blades occurred in early July, near the close of the upwelling season (Figure 10). The beds were not sampled for canopy fronds after August because warm surface temperatures during the summer destroyed almost all of the upper portions of the kelp plants.

TEST FARM OPERATIONS

To provide the information required to assess the relative and potential value of open-ocean marine farms as a source of biomass energy, a major activity at the Test Farm in 1980 was the continuation of observing three adult kelp plants

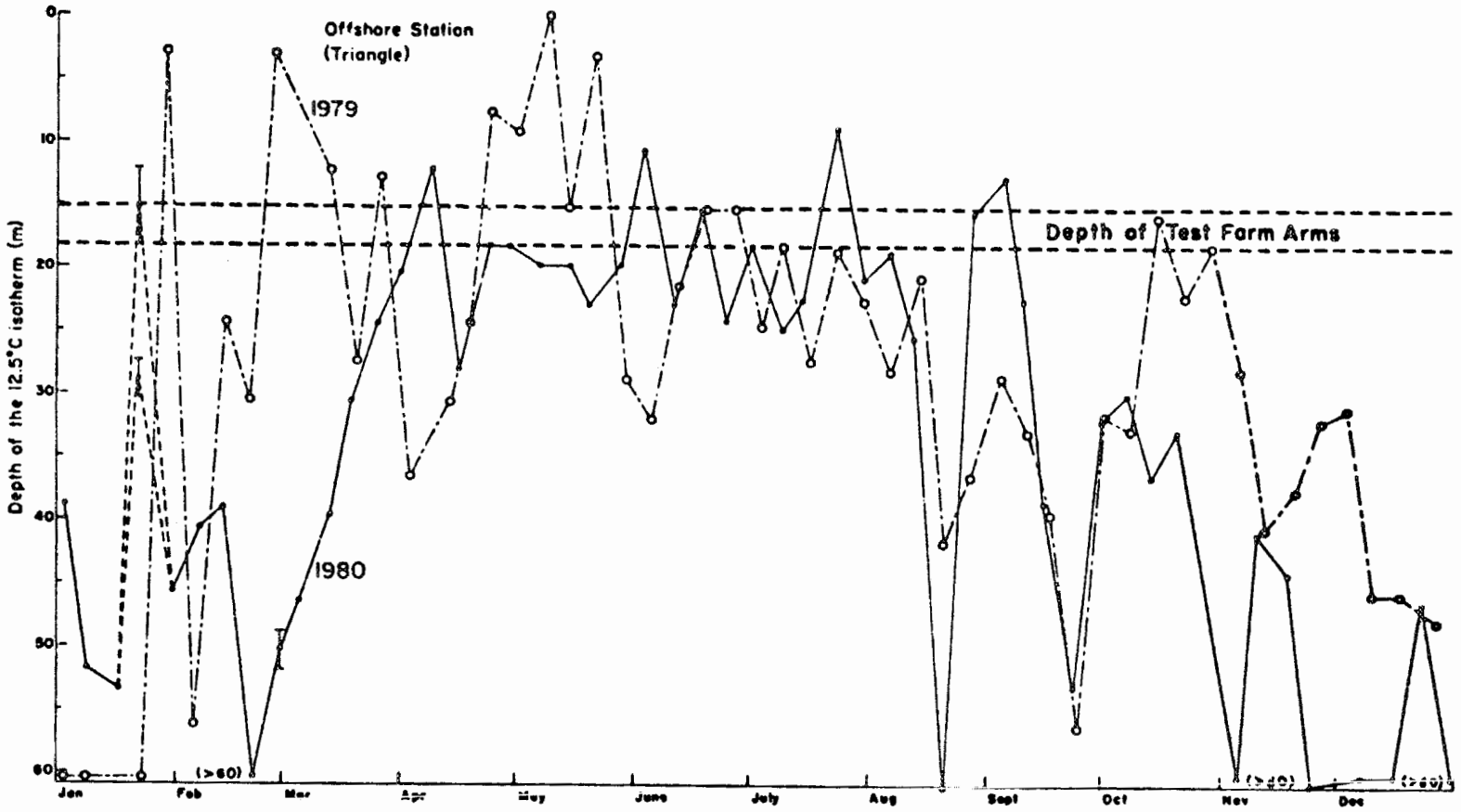

Figure 9. 1979 and 1980 12.5°C Isotherm Fluctuations

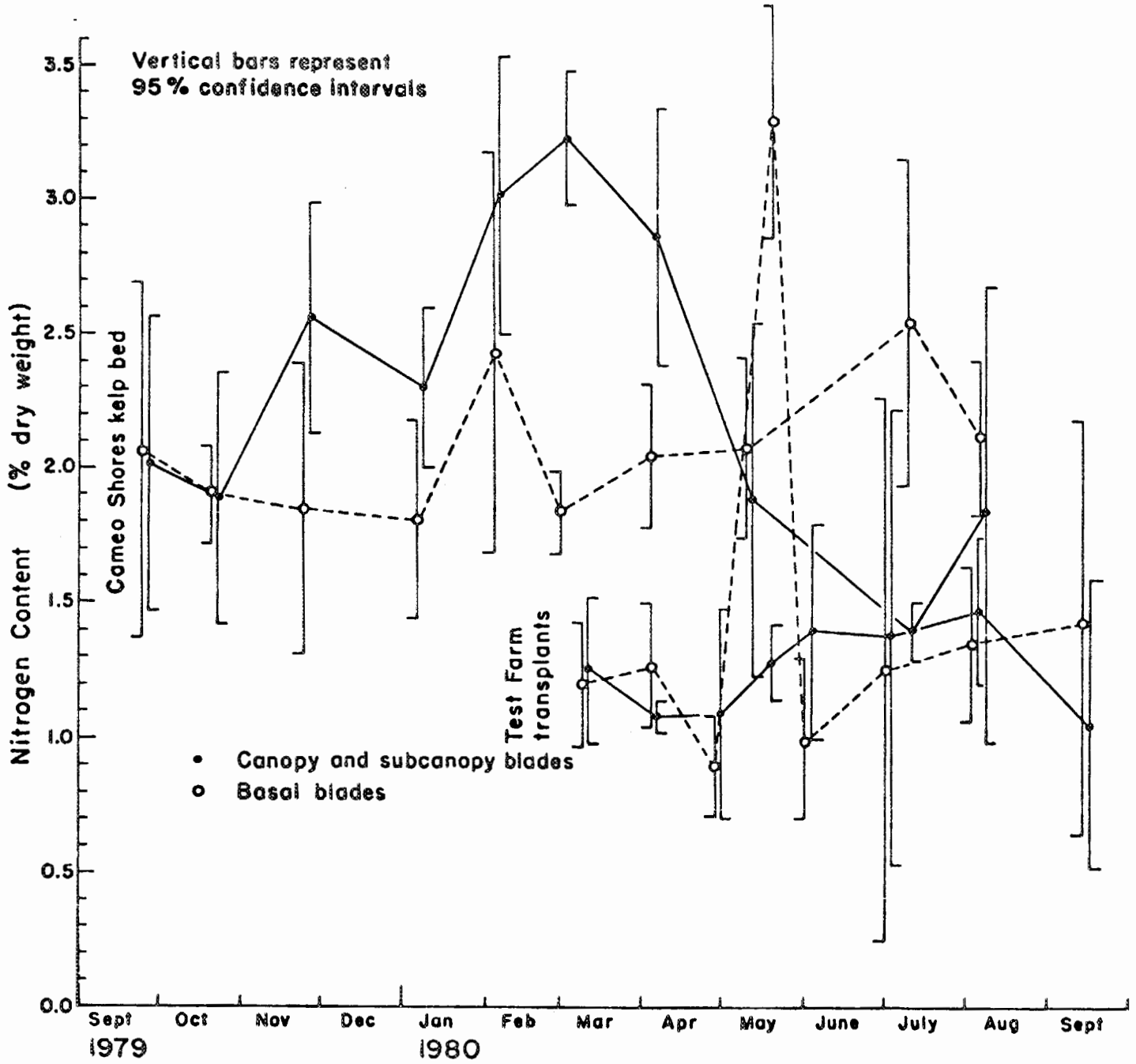

Figure 10. Comparisons of N contents

that had been installed in the fall of 1979. The transplants were fastened to stainless steel barrel buoys which in turn were attached to the south mooring cable of the Farm, approximately midway between the spring buoy and the tips of the arms projecting from the machinery buoy. The 100+ lbs buoyancy provided by each barrel prevented the tether rope from tangling with its mooring, and the distance from any obstructions in midwater prevented tangling by the kelp fronds. Periodically, numbers of juvenile and adult fronds for each transplant, N contents of selected blades, and growth rates of juvenile fronds were recorded. Two of the transplants were fertilized from November into January, March, and again in June. Fertilizing was accomplished by enclosing the fronds in a long tubular plastic bag and pumping ammonium sulfate into the bag, allowing an exposure of 20-30 minutes to the fertilizer. The ammonium damaged some of the apical and immature blades at the frond tips, so fertilizing occurred only when the blades appeared pale. The holdfasts were initially moored at depths of 15 meters. It was realized in mid-spring that upwelling intensity was quite moderate. Consequently, the holdfasts were lowered to 17 meter depths on May 13 and finally to 21 meters deep on July 11th in the hope that the deeper portions of the transplants would obtain nutrients from the cold deep water.

Several significant findings emerged from this study:

1. The transplants survived the winter-spring period of stormy weather, indicating the tissue strength sufficed to withstand the violence of the oceanic water movements.
2. A sharp increase in tissue N of the basal blades was observed immediately after lowering the holdfasts on May 13th (see Figure 11). This increase was accompanied by a spurt in growth rate and was the first indication of growth stimulation in an adult plant as a result of enhanced nutrition.

3. N content of basal blades returned to the more usual value for these plants of 1-1.5% dry weight during June and July as upwelling intensity slackened. Nonetheless, good growth rates were maintained. Probably the accumulated N was rapidly diluted by new tissue formation so that the overall N content remained fairly low. This finding strengthens our previous position that a N content in the range of 1 to 1.5 percent represents a lean but healthy condition for the adult plant.

Warm surface temperatures during late July and early August caused canopy deterioration which was exacerbated by fish grazing not only on encrustations but also on bits of kelp tissue, leading to destruction of the entire blade. By the end of August, most of the blades had been stripped from the mature fronds. Frond numbers and growth rates declined drastically, although N contents remained in the 1-1.5 percent range. All living tissue was destroyed on one plant by October, but the two other plants persisted and displayed a few tattered juvenile fronds through November.

Individual growth rates were examined for each transplant to determine whether any effects were apparent from the differing fertilizing regimes. Variability was so high that no patterns or differences were discernible.

The solid structures of the Test Farm were examined at least monthly for appearances of any juvenile kelp plants. No 1980 crop was seen except for a few individuals on the barrels used to moor the transplants. About 200 small plants persisted through spring from the 1979 juvenile recruitment. All responded vigorously to the spring upwelling. Most, however, succumbed to fish grazing in August and September. A few individuals from 1979 were attached to the mooring cables away from the machinery buoy, and still remain.

Totals of 17 plant species and 50 animal species associated with the Test Farm were tallied in 1980 (Table 2).

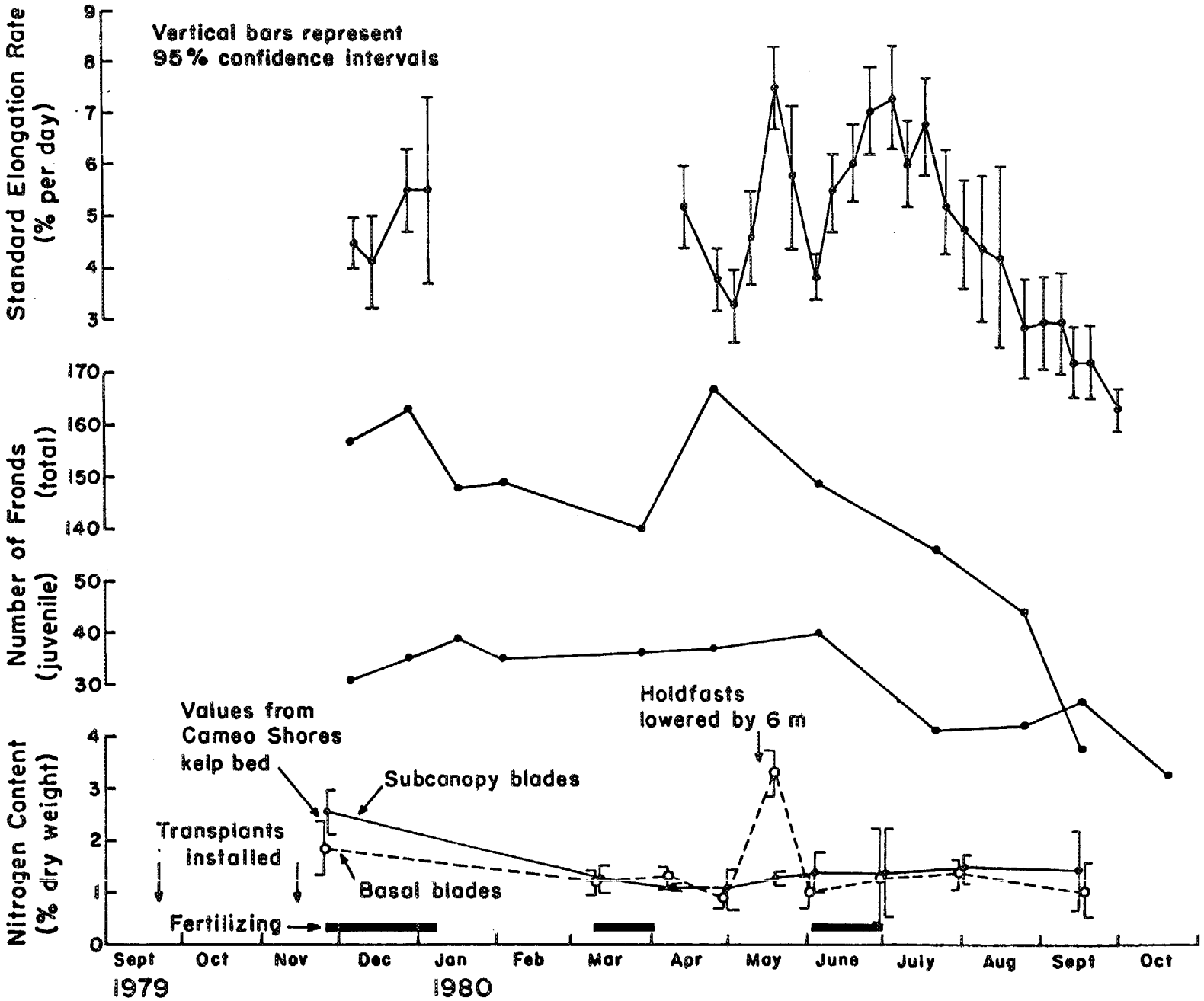

Figure 11. Parametric Fluctuations of Test Farm Transplants

TABLE 2. TEST FARM SPECIES

Species observed at the Test Farm during the first nine months of 1980 by W.J. North and Karen Rossberg.

PLANTS

Chlorophyta
- Bryopsis - like
- Cladophora sp
- Codium fragile

Phaeophyta
- Colpomenia sinuosa
- Dictyota sp
- Egregia laevigata
- Eisenia arborea
- Hydroclathrus clathratus
- Macrocystis pyrifera

Rhodophyta
- Acrochaetium thureti
- Ceramium gracilimum
- Corallina officinalis
- Gigartina canaliculata
- Jania crassa
- Lithothrix aspergillum
- Polysiphonia pacifica
- Pterosiphonia dendroidea

ANIMALS

Porifera
- Leucetta losangelensis
- Leucosolenia nautica
- Rhabdodermella nuttingii

Coelenterata
- Anthopleura elegantissima
- Corynactis californica
- Metridium senile
- Unident. Hydroids

Bryozoa
- Bugula sp
- Diaporoecia sp
- Disporella sp
- Hippodiplosia insculpta
- Lagenipora sp
- Membranipora sp
- Rhyncozoon rostratum
- Thalamoporella californica

Annelida
- Chaetopterus variopedatus
- Dexiospira spirillum
- Salmacina tribrachiata
- Unident. Serpulid
- Unident. Sabellid

ANIMALS (cont'd)

Mollusca
- Dendronotus sp.
- Hermissenda crassicornis
- Hinnites multirugosus
- Leptopecten latiauritus
- Mitrella carinata
- Mytilus edulis
- Triopha maculata

Arthropoda
- Balanus tintinnabulum
- Jassa falcata
- Lepas sp
- Policipes polymerus

Echinodermata
- Ophiothrix spiculata
- Pisaster giganteus
- Pisaster ochraceus
- Strongylocentrotus franciscanus
- Strongylocentrotus purpuratus

Tunicata
- Didemnum carnulentum
- Pyura haustor
- Styela montereyensis
- Unident. colonial sp. A
- Unident. colonial sp. B

Pisces
- Chromis punctipinnis
- Hypsoblennius sp
- Medialuna californiensis
- Mola mola
- Paralabrax elathratus
- Sebastes sp
- Trachurus symmetricus
- Unident. Cottid
- Unident. Perciform

PHYSIOLOGICAL STUDIES

Purposes of the laboratory studies were to obtain the information necessary to assure the complete nutrition of kelp and to learn how best to produce kelp tissue with the chemical composition most likely to maximize the value of kelp feedstock for anaerobic digestion and by-product processing operations.

Micronutrient Uptake

The delineation of the chemical parameters needed for the maximization of growth of Macrocystis is not complete unless it includes the study of micronutrients. The following results are from a study of the uptake parameters of iodide, iron, zinc, cobalt, and manganese in an artificially defined seawater (Aquil) by blade tissue of juvenile sporophytes obtained from rope culture. A flow diagram of the culturing methodology is seen in Figure 12. A brief outline of the methodology is included herein:

Free space is that portion of the tissue which is extracellular. It is a functional term which is derived from observations from uptake and efflux experiments. Free space includes water free space (WFS) and Donnan free space (DFS), which are also function terms, both of which have a structural basis of existence. WFS is an extension or film of moisture of the external solution lining the extracellular space. Material freely diffuses in the WFS. DFS is a functional term describing the restriction of movement of material through the extracellular components (i.e., cell wall material).

Free space uptake (FSU), primarily due to the cation exchange properties of the cell wall (largely alginic acid and sulfated polysaccharides), represents a significant proportion of the cationic gross uptake after 10 minutes (Table 3). The chemical speciation, relative solution concentrations, the relative binding affinities with extracellular material, and the cellular uptake rates all influence the percent FSU in these experiements (uptake periods, tissue quality

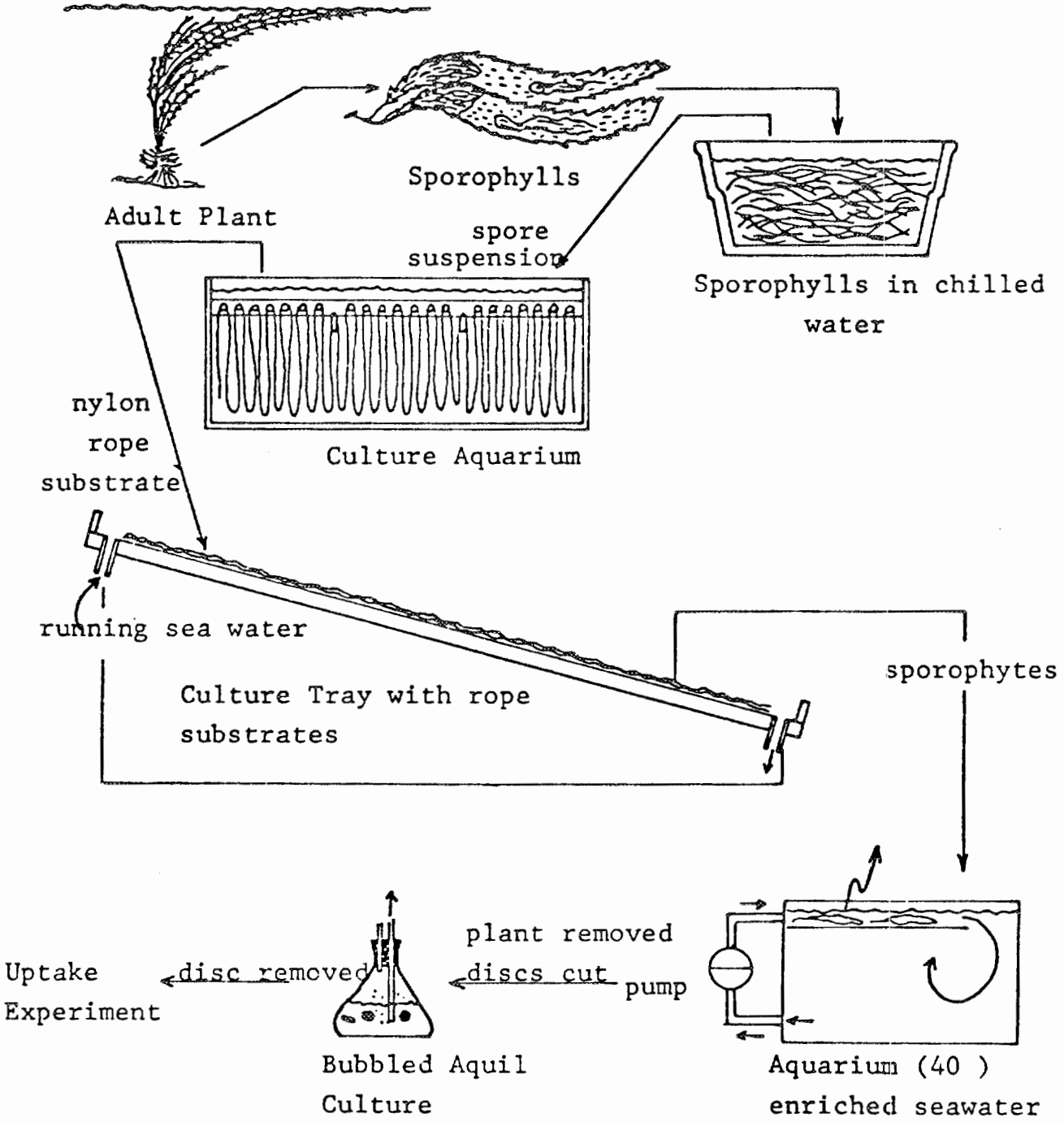

Figure 12. Flow Diagram for Culturing Macrocystis

and quantity were constant). It is, therefore, difficult to determine exact reasons for percent FSU differences. The percent FSU for Co and Mn were very similar. The percent FSU for Zn was very small as compared to Co and Mn even though the solution concentration was the same (10 nM). The reported binding constants for these three cations to alginic acid are very similar. Their speciation is also similar. The relative solution concentration was not a factor since it should tend to lower the % FSU for Co and Mn as compared to the Zn value. The difference may, therefore, be due to cellular uptake rates or the accessibility of the ions to the carrier.

Uptake of the nonmicronutrient Ni was overwhelmingly from cell wall binding; 95% FSU at 100 nM (net uptake=50 f-moles . $cm^{-2}.min^{-1}$). Iodide exhibited significant FSU which was exchangeable and not considered water-free space. FSU for iodide was due to anion exchange sites, either released from cells ruptured during cutting or from glycoproteins associated with the cell wall. Iron had a relatively low % FSU as compared to the other cations even though it was present in larger amounts. Also, iron was less readily exchangeable. The slow exchange rate may reflect a greater affinity for Fe^{+3} by the ion exchange sites or may be related to cellular uptake mechanism.

The uptake velocity of Co, Zn, Mn, and I was a function of distance from the base of the blade which either may reflect a physiological difference due to cell age (rapidly dividing near base) or perhaps a slight increase in blade thickness (Table 3). Any increase in I^- uptake rate at the blade tip was not explained. Iodide uptake by M. pyrifera was a light-independent, active process, not involving iodate and was similar to other algae in this regard. Analysis of v vs $[I^-]$ (Figure 13) revealed a clear deviation from Michaelis-Menten kinetics, displaying biphasic uptake that may, but not necessarily, reflect the presence of two separate carriers. 2,4-Dinitrophenol (DNP) is an uncoupler of photo- and

oxidative phosphorylation and also causes cell depolarization, and 3-(3,4-diclorophenyl)-1,1-dimethyl urea (DCMU), is an electron transport inhibitor in the region of photosystem II.

Iron uptake is light independent and was not inhibited significantly by DCMU. Iron uptake was inhibited by DNP which suggests active uptake. However, since it was seen only after preconditioning with DNP for 1 hour, it is difficult to conclude whether this decrease is indicative of active transport of iron, cell depolarization, or general tissue weakening. It is also possible that the tissue is impermeable to these substances. the immediate decrease in iron uptake in the presence of BPDS (bathophenanthroline disulfonate) without preconditioning supports the mechanism of a ferric to ferrous reduction prior to uptake, since BPDS readily complexes with ferrous ion.

Mn and Zn uptake was not decreased significantly in the dark or in the presence of DCMU. Inhibition by DNP, after 1 hour preconditioning, was very slight for Zn and insignificant for Mn. This suggests that their uptake is not active (not against a free energy gradient). Their uptake probably requires an electrical potential across the membrane and thus is energy dependent. Co uptake was significantly decreased in the dark and by DNP and DCMC. This suggests that uptake may be active. However, since the effects of the inhibitors required preconditioning, it is difficult to draw any conclusions. Further experiments are needed to characterize the uptake of these metals in Macrocystis. The primary purpose of these experiments was to determine if cellular uptake could be measured and to determine some parameters of their uptake. The methods utilized do measure cellular uptake; however, significant variability is evident. Refinements of procedure are in progress and further and more exacting uptake experiments, utilizing mature blade tissues, are planned so that the question of micronutrient-limited growth can be resolved.

TABLE 3. UPTAKE PARAMETERS FOR MICRONUTRIENTS

a) Values in parenthesis are concentrations of that micronutrient during its FSU determination, all other micronutrients at standard value

b) after 10 min uptake

c) L = saturating light, D = dark, numbers indicate distance (cm) from base of blade

d) Preconditioned for 1 hr in 50 μM DNP or DCMU, except for iodide which was preconditioned for 20 min

e) BP = biphasic uptake I or II to distinguish which phase value applies, for iodide see text

Micronutrient		I	Fe	Zn	Co	Mn
[a]Conc. in Aquil (nM)		100	350	170(10)	70(10)	30(10)
[b]FSU, % gross		8	19	20	79	76
Net uptake velocity ($f\text{-moles}\cdot cm^{-2}\cdot min^{-1}$)	[c]3L	1100±69	1001±106	66.8±6.2	21.5±8.7	1.72±0.32
	6L	722±24 (100%)	1120±62 (100%)	50.9±4.2 (100%)	2.8±0.2 (100%)	1.29±0.22 (100%)
	12L	1009±59	1090±95	51.1±4.5	4.7±0.6	1.37±0.39
	6D	722±13 (100%)	1170±7 (104%)	41.9±3.9 (82%)	1.8±0.2 (64%)	1.06±0.07 (82%)
	[d]DNP	378±9 (52%)	280±34 (24.9%)	40.2±2.1 (79%)	1.8±0.3 (64%)	1.08±0.02 (84%)
	[d]DCMU	-	701±13 (62.6%)	47.7±6.2 (94%)	1.9±0.2 (68%)	1.32±0.25 (102%)
	BPDS	-	170±61 (15.2%)	-	-	-
[e]K_m (μM)		11.3-I 0.155-II	2.5	6.5	1.05-B.P.I	39.6-B.P.II
[e]V_{max} $\left(\frac{p\text{-moles}}{cm^2\cdot sec}\right)$		33.3-I 5.65-II	98.8	4.44	0.127	4.95

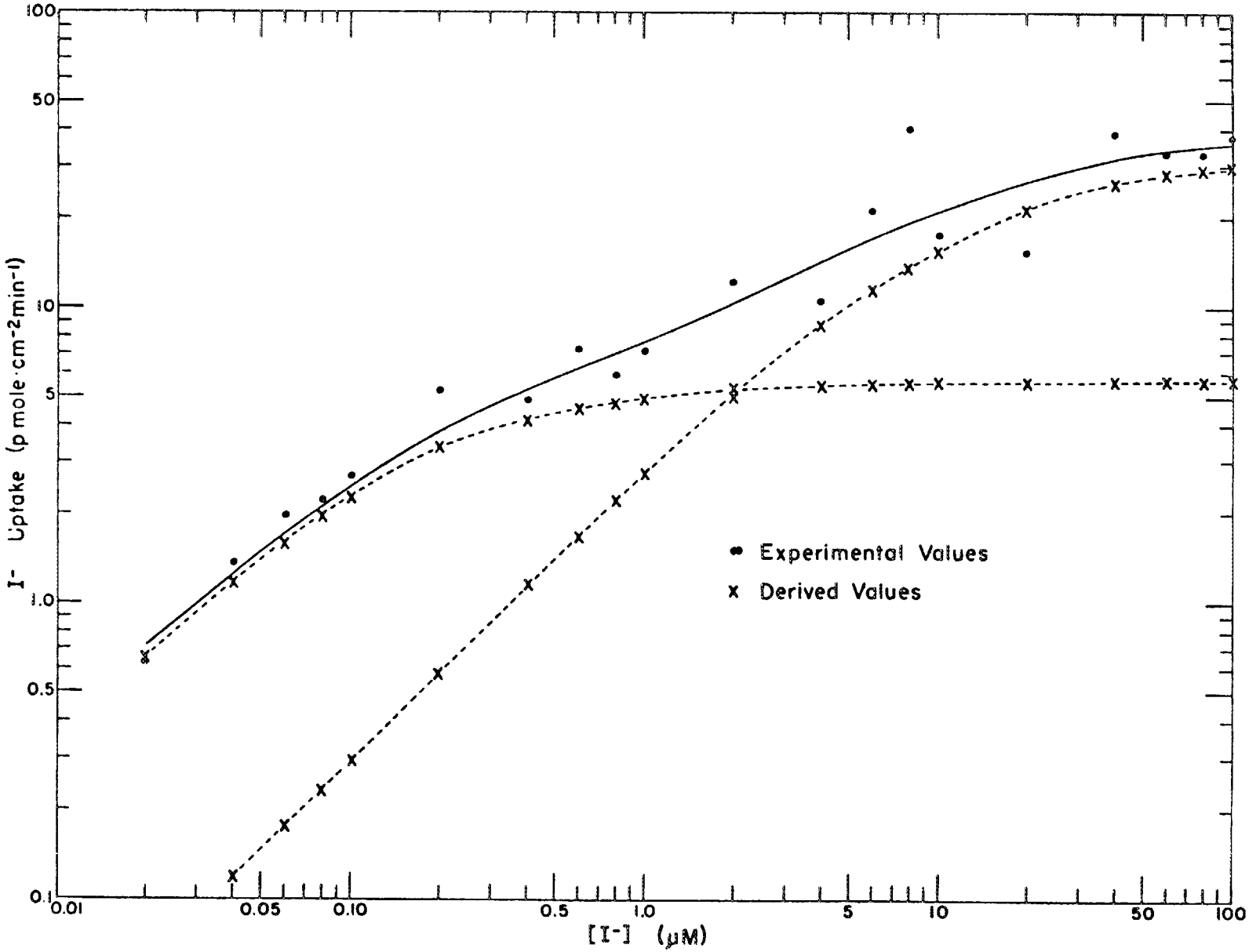

Figure 13. Relationship Between Iodide Uptake Rate and Concentration for Juvenile Macrocystis Sporophytes. Hypothetical curves depicted by X values are also shown as a possible explanation of the biphasic character of the empirically derived curve.

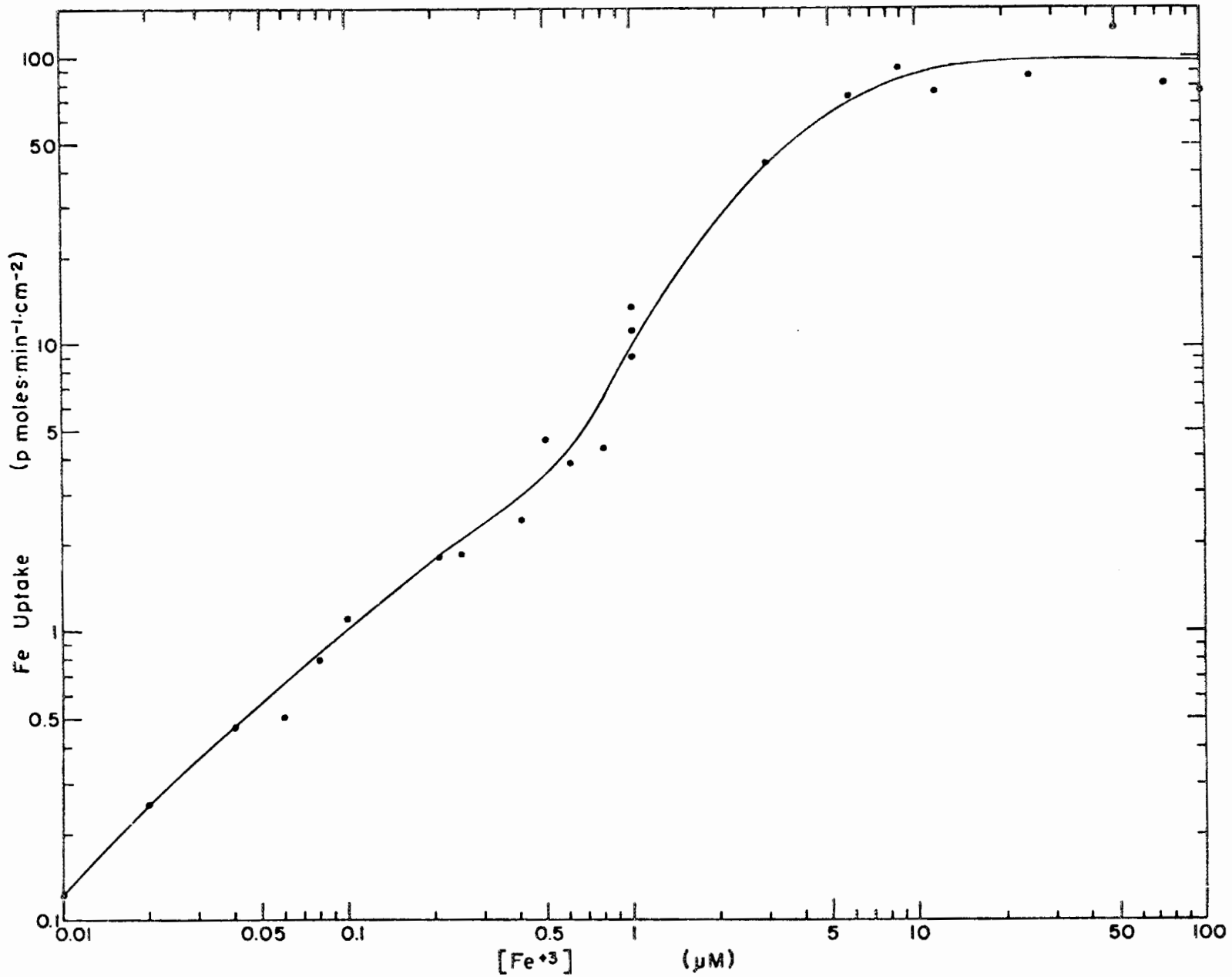

Figure 14. Curve Showing Variation in Uptake Rate of Ferric Iron as the Iron Concentration Changes. Experiements were conducted in Aquil media, using EDTA to ensure complete solubilization of the iron.

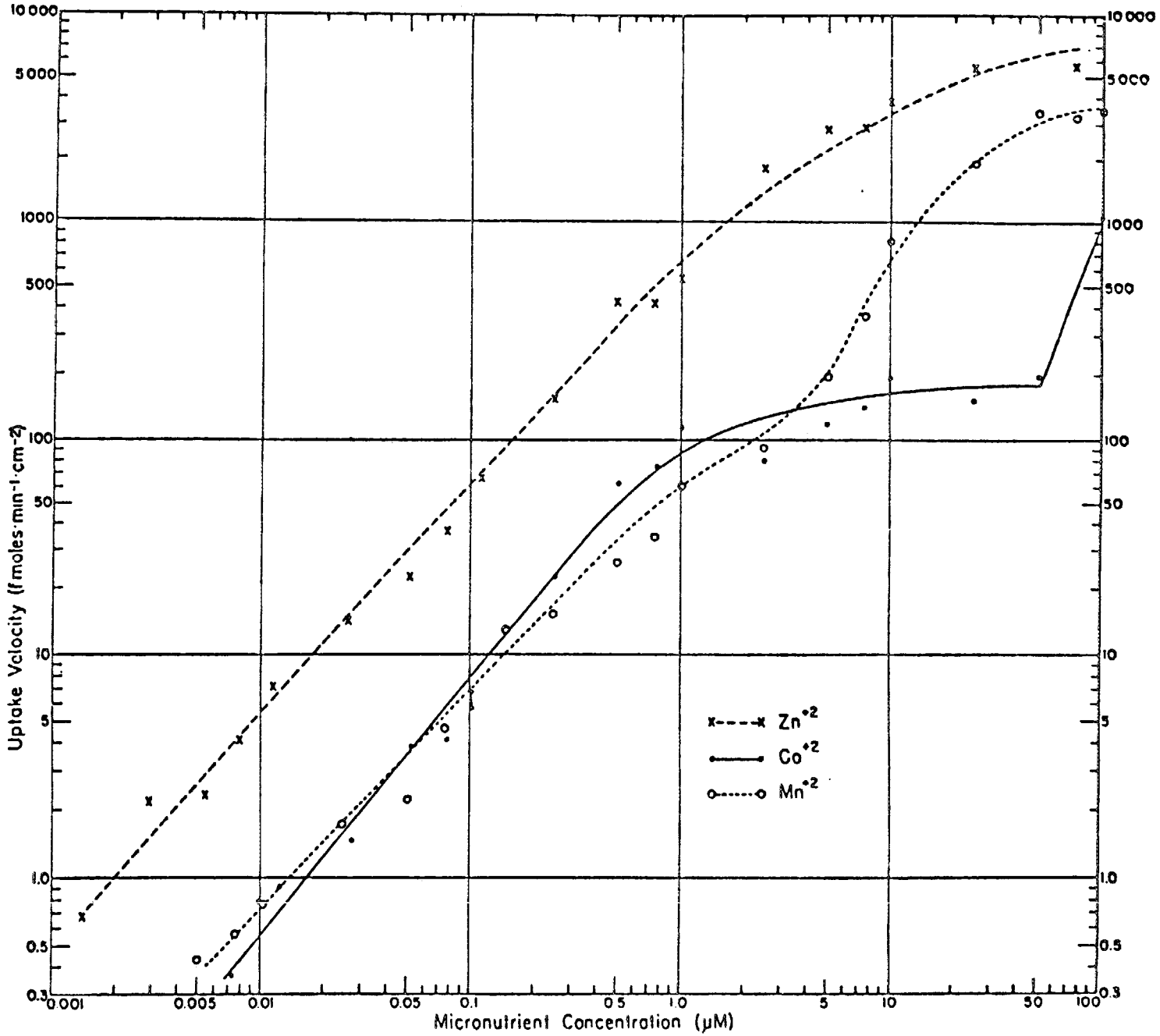

Figure 15. Uptake Velocity vs Micronutrient Concentration for Zinc, Cobalt, and Manganese

N-Budget Experiment, (Preliminary Results)

This experiment involved monitoring tissue N content and growth of an adult Macrocystis plant held in a low nitrogen environment. The objectives were to determine (1) initial distribution of nitrogen within various tissues of a plant from a relatively high N environment, (2) whether tissue N content is diluted by growth under low ambient N conditions, (3) whether N is translocated within the plant to support growth of certain tissues under low ambient N conditions, and the (4) critical tissue N content below which frond growth rates decline under low ambient N conditions.

During the first week of July, an adult Macrocystis plant was selected from the inshore forest at Cameo Shores (Corona del Mar, California). Every frond 50 cm or more in length was tagged and measured giving a total of 16 fronds. Two laminae were collected from each of three juvenile fronds (50-400 cm long) as well as two basal laminae, two subcanopy laminae, and two sporophylls from each of three mature fronds (400-1000 cm long). Sections of stipe (30-50 cm long, pneumatocysts included) were collected from three juvenile fronds and from basal and subcanopy sections of three mature fronds on an adjacent kelp plant. Core samples 2-3 cm deep were collected from the holdfast of the experimental plant. All of these tissue samples were analyzed for total N content.

The experimental plant was then moved to the offshore Test Farm, and the holdfast attached to a buoy at 11 meter depth. Lengths of all fronds greater than 50 cm were measured twice weekly. Lamina samples were collected weekly from juvenile and mature fronds for N analysis. Frond condition was noted during every visit.

Temperature profiles down to 18 meter depth were recorded at the Test Farm site 3-4 times per week. From 9-16 July, the 12.5^{o}C isotherm remained below 15 meter depth, and the experimental plant was exposed only to warm, nitrate-poor

water. On 17 July, the 12.5°C isotherm rose from below 18 meter to 14 meter depth, indicating upwelling of nitrate-rich water. The holdfast of the experimental plant was then brought up to 8 meter depth in order to maintain a low N environment. The isotherm remained between 7 and 8 meters for the duration of the experiment.

During the third week of July, 41 fronds (40-1200 cm in length) were collected from the inshore forest at Cameo Shores near the original location of the experimental plant. All of these fronds had intact apical blades, minimal lamina sloughing, and minimal epiphyte encrustation. Length, lamina wet weight, and stipe wet weight (including pneumatocysts) were recorded for each frond. Fronds more than 400 cm long were divided in half by length; lamina and stipe weights were recorded separately for apical and basal halves. These measurements were used to estimate frond weights from length data and in calculations of frond N content.

On July 28th, the experiment was terminated. The experimental plant was brought to the laboratory, and length-weight measurements were made on each tagged frond. The holdfast was cleaned of dead hapteral tissue and infauna, and weighed. Lamina and stipe samples were collected from juvenile and mature fronds, and holdfast cores were collected for N analysis.

Only growth and tissue N data from July 7-14th are presented herein. After July 14th, natural upwelling of nitrate-rich water and damage to the experimental plant by fish grazing (Medialuna californiensis) made interpretation of results difficult. For example, fronds more than 300 cm long showed decreased elongation rates after July 21st. This decrease was probably due to grazing damage and loss of photosynthetic area, rather than to low N availability. Tissue N samples collected after 14 July were not analyzed.

The relationship between frond length (x) and wet weight (y) is described by the power curve, $y = ax^b$, where $a = 0.32$ and $b = 1.26$ ($r^2 = 0.93$). Other pertinent morphometric data are shown in Table 4.

When initially sampled, N content ranged from 1.4 to 3.1 percent dry weight in various tissue types of the experimental plant (see Table 5). Stipe tissues were all relatively low in N. Of three lamina types sampled, laminae from juvenile fronds had the highest N content. Mean N content of sporophylls was not significantly different than that of basal laminae on mature fronds, so no distinction was made between these tissue types. Holdfast tissue was relatively high in N. Only lamina tissues were collected for N analysis at the end of the first week of the experiment. N content of all three lamina types decreased during that week. Laminae on juvenile fronds showed the greatest N decrease. For N distribution calculations, all tissue types were assumed to have a dry content 10 percent of wet weight.

Distribution of total N within the experimental plant on 7 July is shown in Table 6. Total N of each juvenile frond was estimated from a length measurement as total wet weight x % lamina (and stipe) weight (Table 4) x 10% dry weight x % N (Table 5). Similar calculations were made for apical and basal halves of mature fronds. Total wet weight for the plant was estimated to be 16.2 kg. Frond biomass composed approximately 60 percent of total weight, and holdfast tissue ca. 40 percent. However, almost 50 percent of the plant's 38 gm total N was contained in holdfast tissue. Eighty percent of the total frond N was in lamina tissue.

Frond wet weights and lamina N were calculated using length and N content data collected July 14th for comparison with initial status calculations (see Table 7). Frond wet weight increased from 8.7 to 10.4 kg (not including frond #4 which was lost during that week), giving a daily growth estimate of 3 percent.

TABLE 4. MORPHOMETRIC RESULTS OF FROND WET WEIGHT MEASUREMENTS (N = 41, $\bar{X}$ SHOWN)

Frond weight analysis

juvenile fronds = 67% lamina weight
33% stipe weight

mature fronds (>400 cm)

apical half = 72% of total frond weight
78% lamina weight
22% stipe weight

basal half = 28% of total frond weight
57% lamina weight
43% stipe weight

TABLE 5. N CONTENTS OF TISSUE SAMPLES FROM THE EXPERIMENTAL PLANT (N = 3, $\bar{X}$ SHOWN)

Tissue type	N content (% dry wt) 7/7/80	7/14/80
juvenile fronds:		
laminae	3.1	2.1
stipe	1.5	---
mature fronds:		
apical laminae	2.3	2.0
apical stipe	1.6	---
basal laminae	2.0	1.7
basal stipe	1.4	---
holdfast	2.6	---

TABLE 6. DISTRIBUTION OF WET WEIGHT AND TISSUE N CALCULATED FOR EXPERIMENTAL PLANT ON 7 JULY 1980

Frond #	Length (cm)	Estim. wet wt. (gm)	N content (gm) laminae	stipe	total
juvenile					
1	95	98	0.2	0.05	0.3
2	218	280	0.6	0.1	0.7
3	123	136	0.3	0.1	0.3
4	353	513	1.1	0.3	1.3
5	102	107	0.2	0.1	0.3
7	265	357	0.7	0.2	0.9
8	224	289	0.6	0.1	0.7
10	107	114	0.2	0.1	0.3
11	174	211	0.4	0.1	0.5
14	387	576	1.2	0.3	1.5
mature					
9	457	710	1.1	0.3	1.4
15	406	611	1.0	0.3	1.2
26	802	1440	2.3	0.6	2.9
28	773	1375	2.2	0.6	2.8
29	772	1373	2.2	0.6	2.8
30	631	1065	1.7	0.4	2.2
Frond total		9.3 kg	16.0 gm	4.3 gm	20.3 gm
Holdfast		6.9 kg			17.9 gm
TOTAL		16.2 kg			38.2 gm

TABLE 7. FROND LENGTHS, WEIGHTS, AND LAMINA N COMPARED FOR 7 AND 14 JULY

Frond #	7/7/80 Length (cm)	7/7/80 Est. wet weight (gm)	7/7/80 Lamina N content (gm)		7/14/80 Length (cm)	7/14/80 Est. wet weight (gm)	7/14/80 Lamina N content (gm)	
juvenile								
1	95	98	0.2		118	129	0.2	
2	218	280	0.6		251	334	0.5	
3	123	136	0.3		152	178	0.3	
4	lost	--	--		--	--	--	
5	102	107	0.2	4.4 gm	118	129	0.2	3.9 gm
7	265	357	0.7		310	435	0.6	
8	224	289	0.6		248	329	0.5	
10	107	114	0.2		127	142	0.2	
11	174	211	0.4		203	256	0.4	
14	387	576	1.2		*447	690	1.0	
mature								
9	457	710	1.1		517	829	1.2	
15	406	611	1.0		454	704	1.0	
26	802	1440	2.3	10.5 gm	906	1679	2.3	10.8 gm
28	773	1375	2.2		868	1591	2.2	
29	772	1373	2.2		907	1681	2.3	
30	631	1065	1.7		726	1270	1.8	
Total		8.7 kg	14.9 gm			10.4 kg	14.7 gm	

frond wt. increase = 1.7 kg = 20%/wk = 3 %/day

Δ lamina N content = -0.2 gm = 1%

*Frond #14 was treated as a juvenile frond, although its length on 14 July was greater than 400 cm.

Total lamina N did not change significantly; juvenile fronds showed a small decrease in lamina N, and mature fronds a small increase.

When initially sampled, the experimental plant showed tissue N contents characteristic of plants from a moderate N environment. N content of laminae from inshore plants typically ranges between 1 and 4 percent of dry weight. In juvenile plants, N contents below 1 percent indicate N starvation, and values greater than 1.5 percent indicate accumulation of stored N reserves. Laminae on juvenile fronds of inshore adult plants often have higher N contents than basal laminae on mature fronds, despite similar positions and ambient nutrient conditions. This was found to be true initially for the experimental plant. The relatively low N contents of stipe tissues indicate that stipes may function in N transport, but not storage. The high N content of holdfast tissue and the large proportion of total plant N contained within the holdfast suggest the possibility of N storage. However, mobility of holdfast N has not yet been demonstrated.

During one week in a low N environment, lamina tissues of the experimental plant showed dilution of tissue N in proportion to growth. Stipe samples at the end of one week would probably have also shown a decrease in N content. There was no indication of significant N translocation in or out or between fronds. However, on July 14th, all laminae still had N contents greater than 1.5 percent of dry weight. When tissue N content drops below 1 or 1.5 percent, either translocation of N reserves or decreased frond growth rates are predicted in a low N environment. Given an experimental plant with nutritional status similar to the one described here, at least 2-3 weeks in a low N environment will be necessary to obtain further information about N storage, translocation, and growth in adult Macrocystis.

Aquil Studies

Activities were continued during 1980, directed toward developing the optimal artificial seawater for culturing Macrocystis gametophytes and sporophytes. The formulation known as Aquil for the basal medium that contains the major ions of seawater (Cl, Na, SO_4, Ca, P, etc.) was chosen because a computer program is available for calculating concentrations of all the chemical species present in any given mixture of major ions plus micronutrients. A formulation of micronutrients in Aquil was devised in 1979 by James Kuwabara of Cal-Tech, which was the first such medium that successfully sustained growth by Macrocystis gametophytes through their normal life cycle to its completion by gamete production and appearance of embryonic sporophytes. Mr. Kuwabara then experimented with variations in concentrations of the micronutrients during latter 1979 and early 1980, until he achieved what appeared to be an optimal formulation as follows: NO_3 - 20, PO_4 - 2, Fe - 0.35, Mn - 0.03, Cu - 0.01, Zn - 0.17, Co - 0.07, I - 0.10, Mo - 0.10 (concentrations in uM).

Additional studies using Mr. Kuwabara's medium began in the spring 1980. These experiments were directed toward culturing small Macrocystis sporophytes to see if requirements were similar to those of gametophytes. Initial studies sought to determine optimal concentrations of Fe and Mn. Replicate experiments tested four combinations of the two elements, each at two different concentrations (Mn 5 - Fe 100 nM, Mn 5 - Fe 500 nM, Mn 25 - Fe 100 nM, and Mn 25 - Fe 500 nM). Plant wet weights were measured every three days. Three plants were used per Erlenmeyer flask container. Experiments were terminated after 17 days because biomasses of the sporophytes were straining the capacity of the support system. Considerable variability in growth rates prevented reaching any general conclusions regarding optimal concentrations of Fe and Mn. In spite of the variability problem, the first experiments did provide some useful information. Except for varying Fe and

Mn, the formulation of the Aquil medium used duplicated the composition of the medium successfully devised by Mr. Kuwabara for culturing Macrocystis gametophytes. The present experimental series clearly showed that Mr. Kuwabara's medium also works well with sporophytes. Likewise, a number of extraordinarily high growth rates were recorded. Out of a total of 144 growth rate measurements (three-day periods) nine fell in the range 40 to 50 percent/day, exhibited by a plant held in 5 nM Mn and 100 nM Fe. High growth rates do not necessarily translate into high yields because the latter also requires consideration of biomass density. We can tentatively conclude that Mr. Kuwabara's Aquil is a better growth medium than surface seawater enriched with NO_3 and PO_4. This gives confidence in the Aquil formulation and provides hope that the growth-stimulating properties of seawater may be enhanced by further nutrient additions.

Growth Inhibition Studies

During May and June 1980, six materials transmitted by GMDI were tested for possible growth inhibiting effects among juvenile Macrocystis sporophytes in DT aquaria. Two of the materials were panels of PVC-coated Dacron cloth (Shelter Rite 7028 and 8130 XR-5), one was Glarescreen - a smooth-surface plastic netting (Julius Koch #6517), and three were samples of industrial nylon laundry bag netting (Apex Mills). The three nylon netting samples were identified by the letters A, B, and C. All samples were acid rinsed, then soaked in Q water prior to testing. A group of 30 juvenile sporophytes was preconditioned for ten days commencing May 28, 1980, in a medium of offshore surface seawater enriched daily with 15μ M NO_3 and 1μ M PO_4 and held beneath an illumination of 24000 ergs/ cm^2 sec. The plants were divided into groups of six, cultured in five different DT aquaria. On June 7, four of the GMDI materials were introduced separately to each aquarium for a two-week exposure period. The materials were removed June 21,

and the two remaining samples were separately introduced to two of the cultures that had been least affected by the initial exposure. None of the materials caused a significant growth reduction during the first week of testing, although one of the two PVC samples (Shelter Rite 7028) came close to being associated with a decline in growth rate that was significant at the 95% Confidence Interval.

By the end of the second week, highly significant declines in growth had occurred in both cultures exposed to Shelter Rite PVC. A nonsignificant decline was apparent among plants exposed to the Julius Koch Glarescreen. All these plants displayed abnormal appearances to varying degrees (pale blade ends, deformed blade surfaces, poor tissue strength). No ill effects were apparent from the culture exposed to nylon laundry bag netting Sample A. We removed the first four materials from the aquaria at this point and introduced Samples B and C of laundry bag netting into the aquaria that formerly contained the Julius Koch Glarescreen and Sample A. We reasoned that Samples B and C would probably not be inhibitory. Any increases in growth rates among the cultures formerly exposed to PVC and to Glarescreen would serve to strengthen the hypothesis that these substances were inhibitory, by eliminating the possibility that some unknown factor was causing the observed steady decline in growth rates. Both cultures exposed to Shelter Rite PVC showed substantial increases in mean growth rates during the following week. Some of the abnormal features persisted in outer blade regions. This represents tissue already laid down the previous week. The inner blade regions containing new tissues appeared normal. The remaining two cultures exposed to nylon netting, as expected, did not differ significantly from the controls.

The two samples of Shelter Rite PVC were then soaked in running seawater for one month to determine whether the growth-inhibiting property can be removed by leaching. A follow-on series of experiments was completed during the next quarter

which examined effects from these same two materials after the leaching. Means of the test plants never differed significantly from those of the controls during the three weeks of testing. In contrast, exposure to Shelter Rite 7028 in the earlier experiments had caused significant growth inhibition after one week, and both types of PVC/Dacron were associated with highly significant growth reductions after two weeks of exposure. Latest results thus indicate that one month of leaching sufficed to eliminate the growth-inhibitory property previously recorded for these materials.

In was anticipated that a modest degree of growth inhibition might be encountered in these experiments which would relate the phenomenon to possible effects on tissue N. It was observed that blade color deepened during the earlier experiments as growth inhibition became more apparent. Consequently, it was surmised that the growth inhibitory effect was not affecting N-uptake rates. If so, the deepening color was a manifestation of rising N content that occurred because of reduced effects of N dilution by growth. A similar effect was previously observed when testing the growth inhibiting effects of Cu. While color has been a reliable qualitative indicator of N content, quantitative documentation of these changes was necessary. Accordingly, a few plant tissues were analyzed for N contents at the end of each experimental week to document trends that might appear. The first set of samples was collected July 26th at the end of the first experimental week. By the close of the second week, however, it became clear that no growth inhibition was occurring. The results are presented herewith because of their general interest, with no relation to the previously intended objective of documenting changes in N content. Also included are analyses for two other groups of plants in this experimental series that were being held for comparisons in surface seawater enriched with 30μ M NO_3 and 2μ M PO_4, twice the concentrations being used in the aquaria containing plants exposed to PVC/Dacron.

There was no significant difference in the mean N contents of the three groups of plants held in 15μ M NO_3 nor between the two groups held in 30μ M NO_3. As expected, significant differences occurred between all groups held in 15μ M NO_3 and either of the two groups held in 30μ M NO_3. There was, however, one instance of a significant difference within the 15μ M NO_3 groups as well as within the 30μ M NO_3 groups. Possibly, plant ages and growth rates or biomass densities need to be taken into account, but such factors remain for future study. It appears that N-content analysis is able to distinguish effects from twofold differences of NO_3 concentrations in media. A rather substantial variability can occur, possibly due to unknown influences on some aspect of N metabolism, so that caution should be used in drawing conclusions from culturing where differences in NO_3 concentrations are small.

Growth Studies

A culturing experiment, the DT-61 series testing effects on growth from altering the nitrate level at constant phosphate concentration was underway at the start of the reporting period. The DT-61 series of experiments was completed on March 15, 1980. The first portion of the DT-61 series began in November 1979. The series has investigated effects on growth rates of juvenile sporophytes from combinations of four nitrate concentrations (3, 6, 15, and 30μ M) and three illuminations (5300, 24000, and 53000 ergs/cm^2 sec). Phosphate concentration was kept constant at 2μ M. Somewhat similar experiments were conducted previously except that the N/P ratio had been held constant at 15 (i.e., P was allowed to vary with N). It was impossible in these early experiments to separate possible effects on growth of N limitation from P limitation. The DT-61 series did not have the possible complication of effects from P limitation.

Growth rates tend to decline moderately as juvenile plants age. Hence, it was useful to compare experimental results on a week-by-week basis. During the

first week following preconditioning, no significant differences were found in growth rates between cultures held at the same light intensities but at differing NO_3 concentrations. There were, however, significant differences between cultures illuminated at 5300 ergs/cm^2 sec and those at 53000 ergs/cm^2 sec for all nitrate concentrations tested except 30μ M. Differences between the 5300 ergs/cm^2 sec and the 24000 ergs/cm^2 sec cultures were significant only at 15 μM NO_3. There were no significant differences between the 24000 and the 53000 ergs/cm^2 sec groups at any nitrate concentration. This was not surprising because both these illuminations were well above the saturating value of about 13000 ergs/cm^2 sec. One might expect significant increases in growth rates as nitrate concentration increased, particularly at high illuminations. Probably all plants still retained sufficiently high levels of N reserves as a consequence of three weeks of preconditioning in 30μ M NO_3. Effects on growth were evident in data from subsequent weeks as the N reserves began dwindling in the media supplied with low NO_3 concentrations.

Results from the second week of the experimental period showed a clearly defined rising trend as nitrate increased to 15μ M among the groups receiving 53000 ergs/cm^2 sec of illumination (Figure 16). Mean growth at 30μ M was not significantly different than at 15μ M NO_3, suggesting a saturation type of phenomenon. It is possible, however, that growth is slightly inhibited at 30μ M, particularly at low light intensities. The significantly low value obtained at 30 μM NO_3 and 5300 ergs/cm^2 sec in Figure 17 are illustrative. Mean growths among cultures held at 5300 and 24000 ergs/cm^2 sec did not differ significantly from each other except at 30μ M NO_3. Failure of the curves to show rising trends as NO_3 concentration increased may signify that N reserves were still not depleted during the second week.

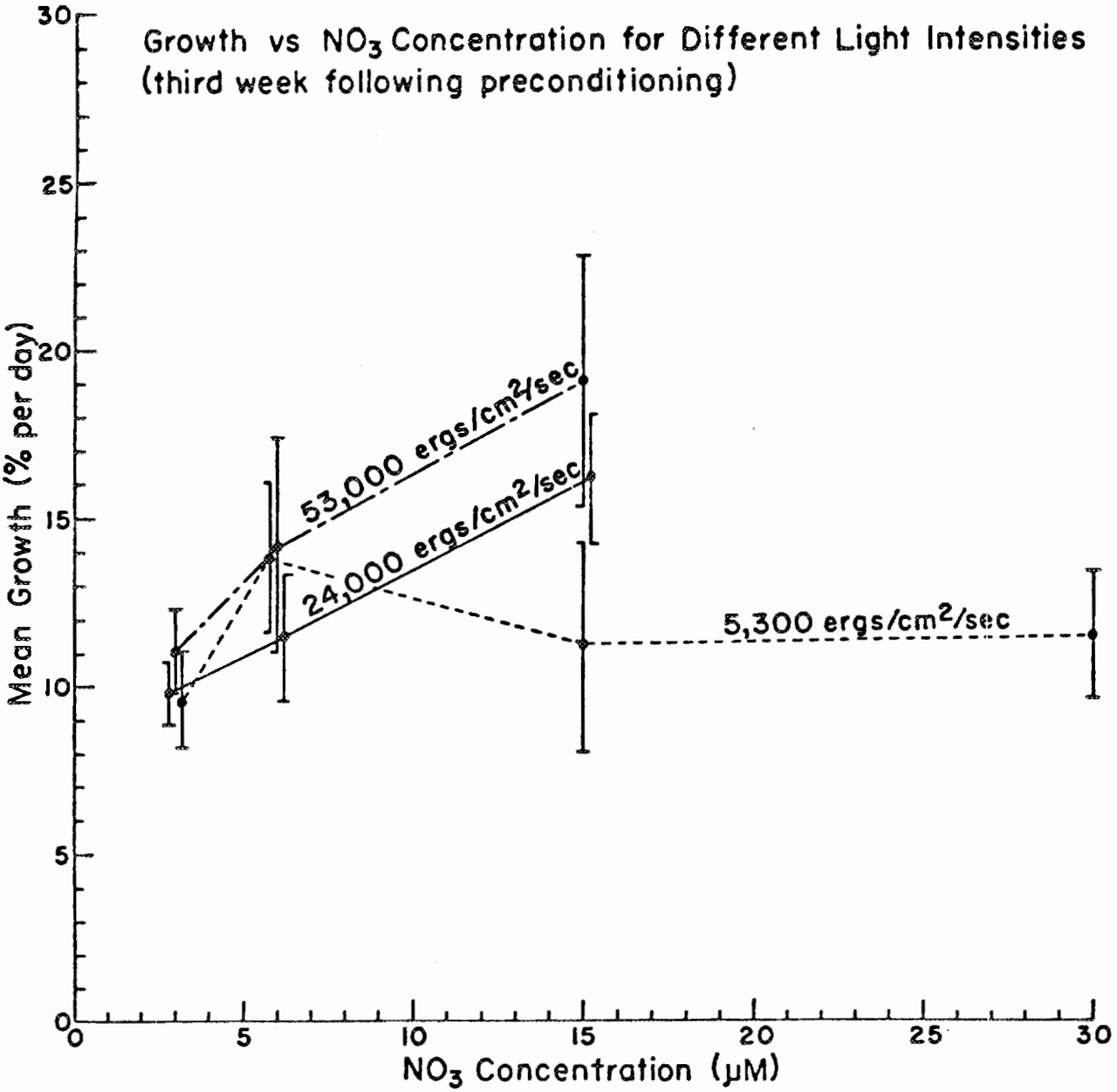

Figure 16. Mean Specific Growth Rate as a Function of Concentration of Nitrate in the Medium for Three Levels of Illumination. Media Were Offshore Surface Water Enriched with 2 μM Phosphate. Vertical Bars Represent 95% Confidence Intervals.

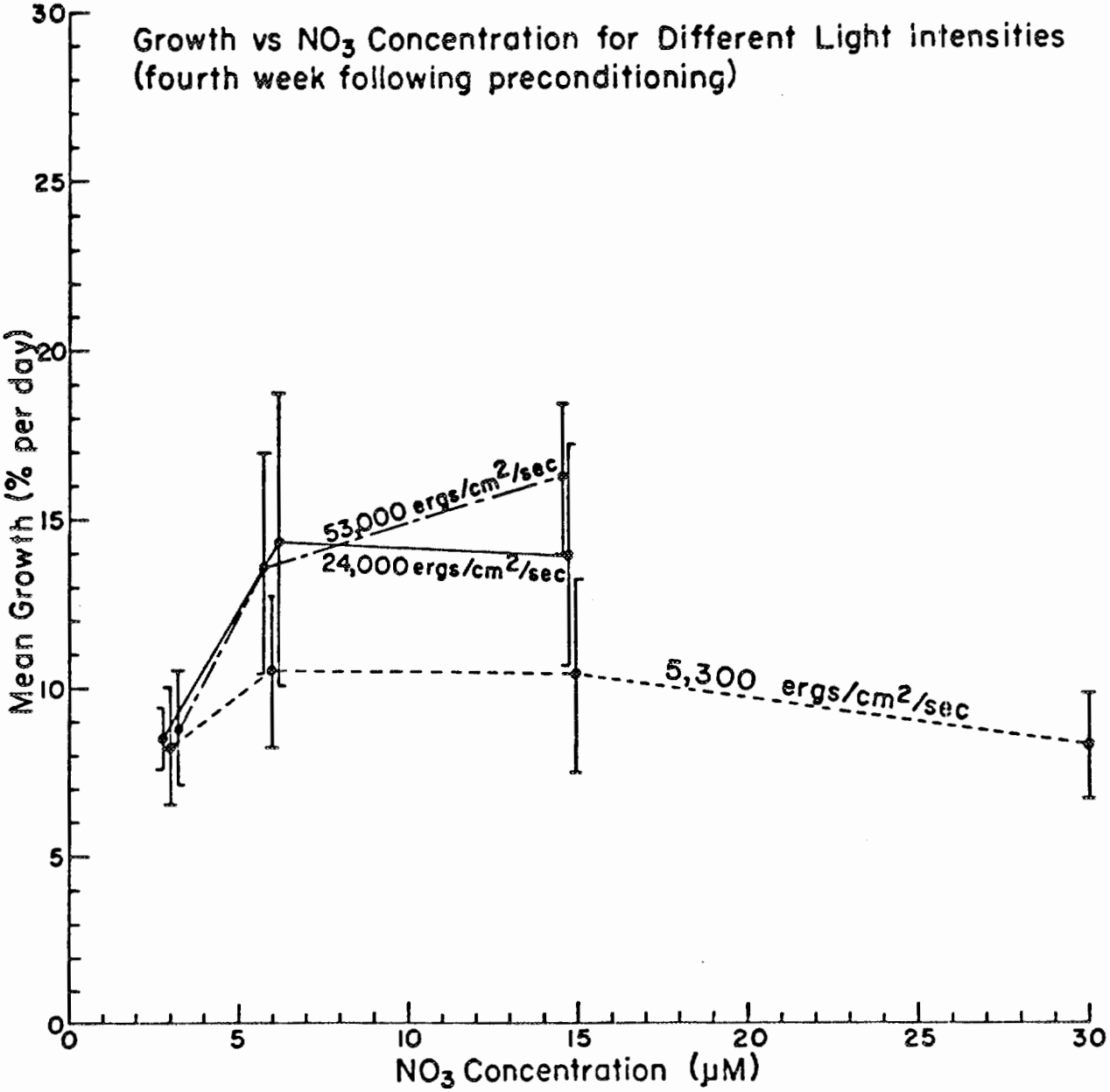

Figure 17. Effects on Mean Growth Rates of Juvenile Macrocystis Sporophytes From Various Combinations of Nitrate Concentrations and Illuminations. Basal Media Were Offshore Surface Seawater Enriched with 2 μM Phosphate. Vertical Bars Represent 95% Confidence Intervals.

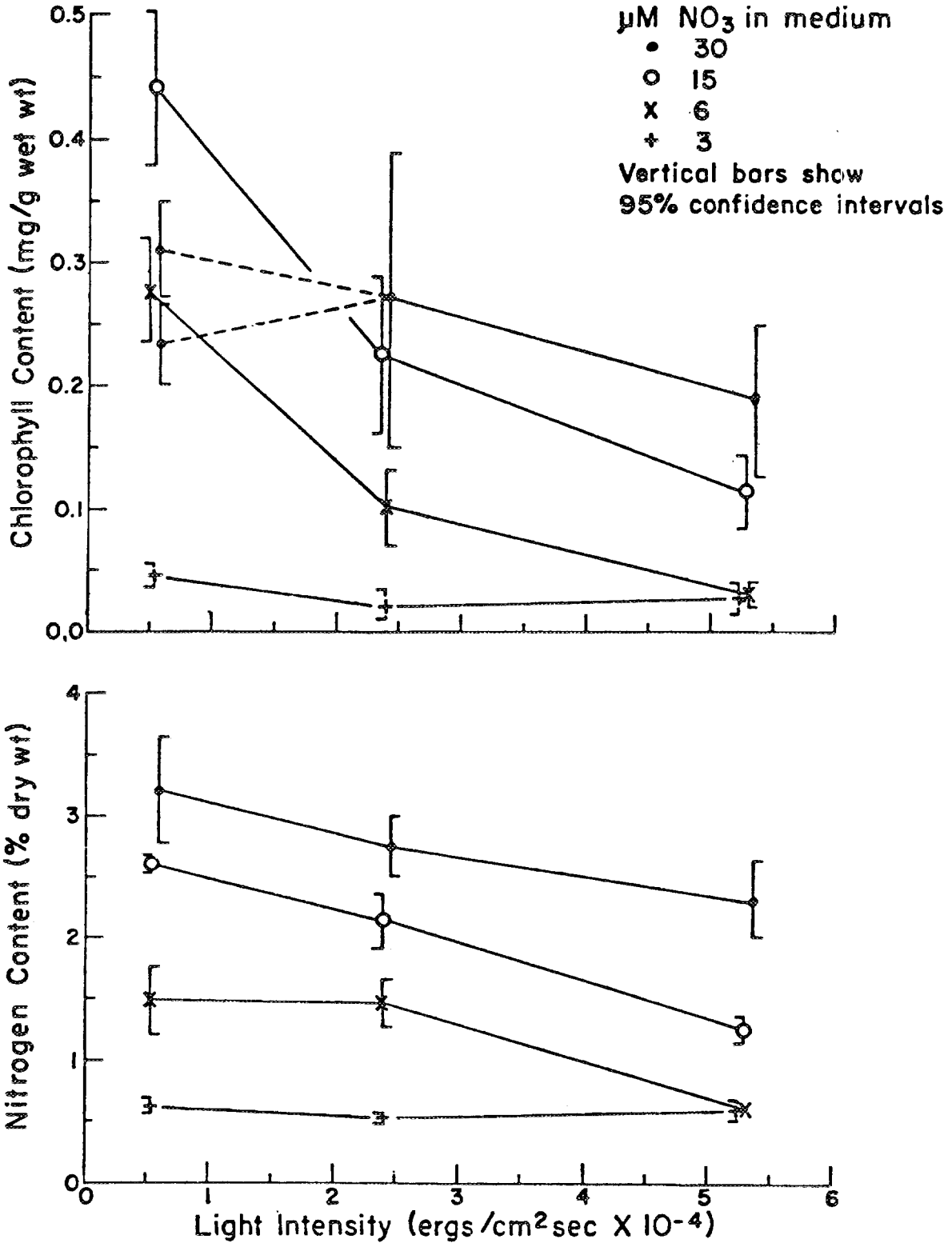

Figure 18. Relationship Between Chlorophyll, Nitrogen, Illumination, and Nitrate

Results from the third and fourth weeks of the experimental period showed rising trends in growth rates as NO_3 concentrations increased from 3 to 6 μM for both the 24000 and the 53000 ergs/cm^{2}sec groups of cultures and from 6 to 15 μM for the 53000 ergs/cm^{2}sec group (see Figures 16 and 17). Third and fourth week data for cultures held in 30 μM at the two highest illuminations was accumulated during the next report period. Mean growths among the cultures held at 5300 egs/cm^{2}sec did not vary significantly with changes in NO_3 concentration. This suggests that these groups were strongly limited by available light. Hence reducing NO_3 concentration to as low as 3 μM did not alter growth significantly. This is somewhat surprising because earlier experiments indicated that growth at 5300 ergs/cm^{2}sec was well up on the shoulder of the graph showing variation in growth rate with light intensity. Likewise, N contents of plants held under 5300 ergs/cm^{2}sec at 3 μM and 6 μM NO_3 were both substantially reduced below cultures held in 15 μM and 30 μM at this illumination (Figure 18). The explanation may lie in the apparent ability of Macrocystis to increase its chlorophyll content at low illuminations and perhaps thereby increase its photosynthetic capacity. Evidence of this capability will be presented below. Both 3 and 6 μM NO_3 were apparently growth-limiting concentrations at 24000 and 53000 ergs/cm^{2}sec. At 15μ M NO_3, growth rates were always greater for the 53000 ergs/cm^{2}sec culture vs the 24000 ergs/cm^{2}sec group. Differences between the two, however, were significant only during the second week. Results from the 24000 ergs/cm^{2}sec culture seemed anomalous during the second week and this portion of the study was eventually repeated.

In a later experimental series (DT-62), it was impossible to compare growth rates among the plants in the 30 μM NO_3 media with a control culture growing in 10 μM NO_3. The objective was to confirm the finding described above; that increasing the nitrate concentration above 15 μM did not result in higher growth

rates when light intensity is well above the saturation level of about 13000 ergs/cm^{2}sec. The recent study yielded results similar to our previous findings during the first quarter of 1980. It should be noted that the mean growth rates achieved from the more recent study (ranging from 23.1 to 31.0 percent/day) were well above values recorded during the earlier studies (14.0 to 22.1 percent/day). It is conjectured that the increased rates resulted from improvements in the culturing technique.

Several record growth rates from individual plants were obtained during the DT-62 experimental series. The highest value was 43.5 percent/day obtained from plant DT-62-60 during preconditioning (July 12-19).

Chlorophyll analyses and determinations of N contents among the plants in the DT-61 series were also conducted. Tissue N values were significantly different from each other, comparing cultures held at differing nitrate concentrations except for the two highest intensity/lowest nitrate cultures (3 and 6 μM NO_3 at 53000 ergs/cm^{2}sec, Figure 18). Comparing cultures at differing light intensities but similar nitrate concentations, the 15 μM NO_3 series was the only one consistently showing significant differences between all intensities. The chlorophyll content values showed overlapping confidence intervals for all the 30 μM NO_3 cultures for all intensities. Similar overlapping occurred in the 3 μM NO_3 cultures while the 6 μM and 15 μM NO_3 series were distinct at the various intensities.

Figure 18 also illustrates the anomalous chlorophyll levels occurring in plants raised in 30 μM NO_3 and beneath 5300 ergs/cm^{2}sec. The first such cultures was analyzed December 10, 1979. The findings seemed so unusual that the experiment was repeated. A second set of analyses on February 21, 1980 confirmed the anomalous characteristic previously noted. None of the mean growth rates observed at 30 μM NO_3 under any illumination were significantly better than

observed at 15 μM and usually they were slighly lower (Figure 16 to 17). Thus, it appears that concentrations as high as 30 μM NO_3 are no better than at 15 μM NO_3. Earlier experiments had shown a general rise in growth rate as NO_3 concentration was increased in the medium to 30 μM. A major difference between that and the present series was the frequency of nutrient renewal (once per 2 days for the 1979 series vs daily for the 1980 study). Thus, the 30 μM NO_3 medium in the 1979 series was equivalent to the 15 μM NO_3 cultures of the present study. Hence, both studies were in agreement in finding that kelp growth rate increased up to 15 μM NO_3 added daily or its equivalent. At low illuminations, 30 μM NO_3 produces an irregularity in chlorophyll content whose significance is still unknown. Possibly, NO_3 at 30 μM added daily stresses the enzyme systems reducing the N and transferring it to amino acids, particularly at low illuminations where growth does not act as effectively to "dilute" the incoming N with new tissue.

Thus, the optimal nitrate concentration for batch culturing of Macrocystis sporophytes appears to be around 15 μM NO_3 for illuminations of 53000 ergs/cm sec or less. The Nitrate Uptake Model (NUM) has predicted that a flowing medium containing 3 to 6 μM NO_3 (the exact value depending on biomass concentration) was the equivalent of a batch culturing medium renewed with 15 μM NO_3 every other day with respect to total amounts of nitrogen provided to the plants. Evidence thus suggests that the NO_3 concentration used as a design criterion for the Test Farm would be suitable provided biomass concentrations of around one wet gram of kelp per liter of seawater were achieved. Typical biomass concentrations in Macrocystis canopies may range from 5 to 30 grams per liter.

Mannitol Studies

During May and June, mannitol contents were analyzed in 55 samples of dried Macrocystis blades, representing tissues from two DT aquarium studies conducted

during early summer 1979. The samples had thus spent almost one year in storage between times of collection and analysis. The samples were material remaining from blade tissues preserved for N content analyses. They all had been cultured in media strongly enriched with nitrogen, but the cultures represented a wide range of illuminations. Difficulties were experienced with 21 of the analyses because of insufficient sample size. Thus, suitable data were recovered for 34 samples. No relation was found between mannitol content and the illuminations used during culturing (Table 8). There was roughly a two-fold difference between average mannitol contents computed for groups of plants coming from the same aquarium. Several plants from the same aquarium tended to show a characteristic mannitol content that might differ substantially from another group. Thus, plants 7 and 8 from the June 3 and June 10 sampling were quite distinct from plants 13 and 14 from the same dates, and they maintained this distinction over a lapse of one week. It was conjectured that some other variable might be influencing mannitol content. Specific growth rates and N contents were examined, but no obvious correlations were found as to the nature of the supposed variable. The N content data indicated all plants contained large reserves of nitrogen. Obviously, the next step is to analyze specimens raised at lower concentrations of nitrate in the medium. Plant 20 was cultured in an ammonium-enriched medium (all the rest were in nitrate-enriched media), but it did not yield an unusual mannitol content.

Nitrate Uptake

Knowledge of the nutrient dynamics of Macrocystis is essential to our ability to predict its survival and productivity under different nutrient conditions and to formulate strategies for efficient fertilizing of both inshore and offshore populations. Our current knowledge of NO_3^- uptake by Macrocystis comes from measurements made with tissue pieces or detached blades from adult plants

TABLE 8. SUMMARY OF MANNITOL CONTENTS

Summary of mannitol contents determined for juvenile Macrocystis sporophytes raised under various culturing conditions as indicated. Nutrients were renewed daily in the culturing media. Plants from the same aquarium shown with no spacing between rows.

Plant No.	Date	$[NO_3]/[PO_4]$ uM	Light Intensity Ergs/cm^2 sec	Specific Growth Rate %/day	% Dry Wt as N	% Mannitol	Ave % Mannitol
DT-59-7	6/3/79	30/2	33000	11.5	2.90	7.88	8.47
59-8				11.6	3.00	9.06	
DT-59-9	6/3/79	30/2	52000	22.1	2.50	8.25	8.03
59-10				16.0	2.56	7.13	
59-11				19.3	2.50	8.71	
DT-59-13	6/3/79	30/2	53000	22.8	2.48	3.60	3.64
59-14				19.5	2.32	3.60	
59-15				16.0	2.15	3.59	
59-16				-	2.21	3.77	
DT-59-20	6/3/79	20/2*	2700	5.86	2.05	4.44	4.44
DT-59-37	6/9/79	60/4	13000	16.5	3.34	4.73	4.73
DT-58-51	6/9/79	60/4	53000	20.7	3.17	4.55	4.55
DT-59-1	6/10/79	60/4	13000	13.3	2.97	3.61	4.86
59-2				11.0	2.91	5.56	
59-4				16.0	3.22	5.42	
DT-59-6	6/10/79	60/4	33000	15.9	3.11	8.78	8.31
59-7				13.3	3.11	8.40	
59-8				12.6	2.91	7.76	

TABLE 8. SUMMARY OF MANNITOL CONENTS (Cont)

Plant No.	Date	$[NO_3]/[PO_4]$ uM	Light Intensity Ergs/cm^2 sec	Specific Growth Rate %/day	% Dry Wt as N	% Mannitol	Ave % Mannitol
DT-59-13	6/10/79	60/4	53000	23.0	3.22	3.85	3.92
59-14				-	3.18	3.98	
DT-59-21	7/22/79	30/2	5300	14.7	2.64	4.38	4.69
59-24				12.3	2.87	5.51	
59-25				12.6	2.76	5.07	
59-29				9.7	2.47	3.79	
DT-59-31	7/22/79	30/2	15000	16.9	2.83	3.45	4.24
59-34				17.2	3.14	3.70	
59-35				13.5	2.84	5.18	
59-39				19.5	2.92	5.44	
59-40				17.5	3.02	3.43	
DT-59-46	7/22/79	30/2	22800	11.1	3.57	4.00	3.76
59-48				9.0	3.20	3.62	
59-49				8.9	3.51	3.66	
DT-59-51	7/22/79	30/2	24000	23.1	2.86	4.18	4.50
59-56				17.9	2.46	4.82	

*The medium used for plant DT-59-20 was enriched with NH_4 instead of NO_3 which was used for all the other cultures shown.

(Wheeler, 1978) and with whole juvenile plants (Haines and Wheeler, 1978) under laboratory conditions. Such measurement could differ significantly from actual *in situ* uptake by intact adult plants. A method of accurately measuring NO_3^- uptake *in situ* has now been developed and used to compare rates of uptake by different types of intact adult plant tissues. The method has also been used to examine the influence of various environmental factors on NO_3^- uptake rates.

Several experiments were designed to test the accuracy of this *in situ* method. During April and May when phytoplankton standing stocks were relatively high, control incubations were carried out with no kelp tissue. Eight controls showed an average apparent change of only 0.1 μM from initial NO_3^- concentrations between 22 and 25 μM during a 1 hour incubation period. In the second experiment NO_3^- uptake by mature blades on canopy fronds was measured for incubation periods ranging from 0.3 to 2.2 hours. Results indicated a linear relationship between uptake and incubation period for the range tested (Figure 19). To determine whether water motion within the bag was limiting to NO_3^- uptake, the third experiment compared uptake by mature blades on canopy fronds in bags that were continuously shaken by divers to unshaken controls. Uptake rates at two ranges of initial concentration were significantly different for the two treatments (Table 9). Although ambient sea state was calm during this experiment, water motion within the unshaken bags was probably similar to ambient water motion (Gust, 1977). Furthermore, movement of the kelp blades within the unshaken bags was perceptible during the slightest swells. The final test of the method involved rinsing incubated tissue in low-NO_3^- seawater to determine what proportion of measured uptake was due to diffusion into tissue free space. Four mature blades on canopy fronds were incubated for 1 hour at high NO_3^- concentrations (22-30 μM), then rinsed for 20 minutes. During the first 5 minutes, 2-6 percent of the NO_3^- taken up was rinsed from the blades. No NO_3^- was removed during the next 15 minutes of rinsing.

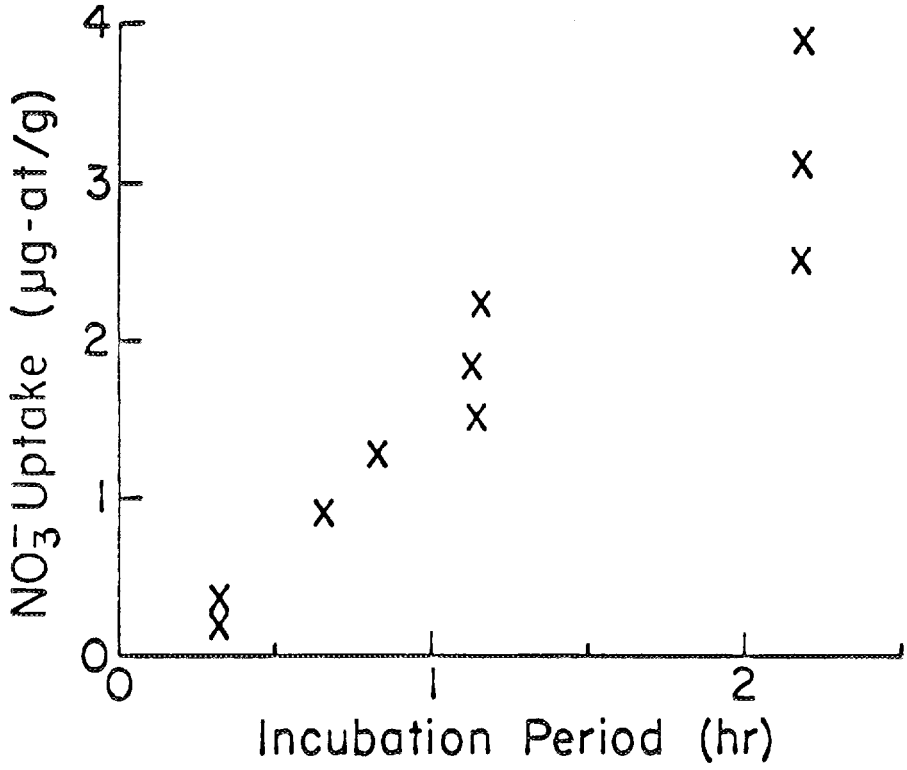

Figure 19. NO_3^- Uptake by Mature Blades on Canopy Fronds of Macrocystis During Incubation Periods of Varying Length. Initial NO_3^- = 21-26 µM

The relationship between NO_3^- concentration and uptake rate was determined experimentally for mature blades on canopy fronds. NO_3^- uptake followed saturation kinetics. Kinetic parameters calculated from the Hanes-Woolf plot were V_{max} = 2.9 µg-at/gm/hr or 70 µg-at/cm^2/hr and K_s = 17.2 µM. These values were comparable to those determined under laboratory conditions by Haines and Wheeler (1978) for juvenile Macrocystis sporophytes and by Wheeler (1978) for lamina discs and detached blades from adult plants.

NO_3^- uptake rates measured during the spring upwelling period were similar to rates measured during fall when coastal upwelling was less prevalent (Figure 20). These results contrasted with the findings of Haines and Wheeler (1978) that juvenile Macrocystis plants grown in nutrient-rich seawater had a higher average V_{max} than plants from a nutrient-poor background. Temperature, nutrient concentration, and N content of kelp tissues are all influenced by upwelling

TABLE 9. Mean Rates of NO_3^- Uptake by Mature Blades on Canopy Fronds (A,B) and Apices on Juvenile Fronds (C) Compared for Two Experimental Treatments. $\bar{x} \pm 1$ SD Shown

A	No Shaking		Continuous Shaking	
n	Initial $[NO_3^-]$ (µM)	Uptake rate (µg-at/gm/hr)	Initial $[NO_3^-]$ (µM)	Uptake rate (µg-at/gm/hr)
3	3.5	0.7 ±.1	3.4-3.7	0.7 ±.1
6	10.1-12.0	1.1 ±.2	10.1-11.8	1.2 ±.8
B	**Intact**		**Detached**	
3	2.8-3.1	0.4 ±.2	3.0	0.6 ±.3
4	21.3-23.1	2.1 ±.4	21.6-24.9	2.1 ±.2
C	**Intact**		**Detached**	
3	21.8-24.3	0.8 ±.3	21.9-25.4	0.8 ±.2

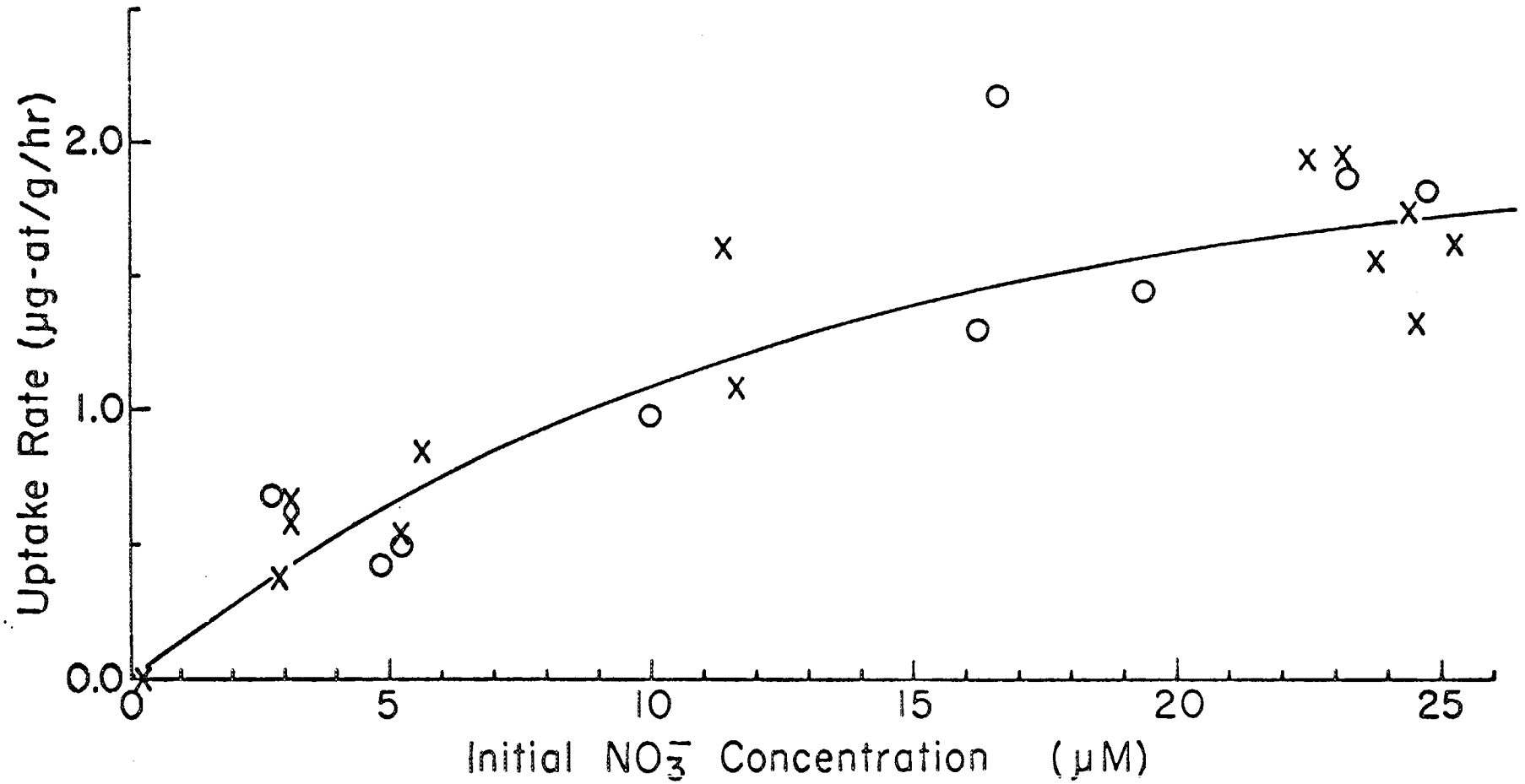

Figure 20. Relationship between NO_3^- Concentration and Uptake Rate for Mature Blades on Canopy Fronds of Macrocystis. Incubations Were Carried Out During April-June (X) and October (O).

(North et al., in press; Gerard, unpublished data). On different experimental days, ambient temperatures ranged from 10 to 18°C, and ambient NO_3^- ranged from 0.05 to 8.9 μM. N content of individual experimental tissues varied between 1.5 and 4.4 percent of dry weight. No obvious changes in NO_3^- uptake rates occurred in conjunction with fluctuations in any of these parameters. Similarly, differences in epiphyte cover, which varied between 0 and 30 percent of total surface area, had no obvious relation to NO_3^- uptake. However, more data are necessary to adequately test the influence of these factors.

Rates of NO_3^- uptake by seven tissue types were compared at near-saturation concentrations (initial NO_3^- = 20-34 μM). Mean uptake rates ranged from 0.1 μg-at/gm/hr for holdfast tissue to 2.1 g-at/gm/hr for apices of canopy fronds (Table 10). Uptake rates did not vary with tissue age; e.g., young basal blades on juvenile fronds and old basal blades on canopy fronds had comparable uptake rates. Tissues located deeper in the water column had lower uptake rates than shallow tissues, even for similar tissue types, e.g., apices of juvenile and canopy fronds.

Several experiments were designed to determine whether differences in rates of NO_3^- upake by different types of tissue were physiological or environmentally influenced. All tissues used in these experiments were detached from the plant prior to incubation. Detachment had no significant effect on NO_3^- uptake by mature blades or frond apices (Table 9). During incubations carried out both at the surface and at the bottom (10-12 meter depth), canopy frond apices took up NO_3 2-3X faster than juvenile frond apices (Table 11). These results indicate physiological differences between the two types of tissue. However, both tissue types showed significantly higher uptake rates at the surface than at the bottom, indicating an environmental influence as well.

TABLE 10. COMPARISON OF *IN SITU* NO_3^- UPTAKE RATES BY VARIOUS TISSUES OF ADULT *MACROCYSTIS PYRIFERA*. RATES WERE MEASURED AT NEAR-SATURATION CONCENTRATIONS (INITIAL $[NO_3^-]$ = 20-34 μM)

Tissue	n	NO_3^- uptake rate (µg-at/gm/hr) range	NO_3^- uptake rate (µg-at/gm/hr) $\bar{x}$ ±SD	NO_3^- uptake rate (ng-at/cm^2/hr) range	NO_3^- uptake rate (ng-at/cm^2/hr) $\bar{x}$ ±SD
canopy frond					
apex	7	1.6-3.0*	2.1* ±.6	40-156*	68* ±40
mature blade	9	1.3-1.9	1.7 ±.2	31-48	43 ±5
basal blade	6	0.0-0.8	0.4 ±.3	0-28	13 ±10
sporophyll	3	0.2-0.2	0.2 ±0	6-10	7 ±2
juvenile frond					
apex	17	0.3-1.4	0.8 ±.3	10-64	36 ±15
basal blade	9	0.0-1.0	0.4 ±.4	0-30	11 ±11
holdfast	3	0.1-0.1	0.1 ±0	--	--

*NO_3^- concentrations in bags at end of incubation periods were relatively low (10-19 µM); therefore, values reported may be significantly lower than actual saturation uptake rates.

TABLE 11. RATES OF NO_3^- UPTAKE BY MACROCYSTIS FROND APICES COMPARED FOR FOUR EXPERIMENTAL TREATMENTS. INITIAL NO_3^- CONCENTRATIOLNS RANGED FROM 22-25 μM. SIGNIFICANCE LEVELS INDICATED ARE RESULTS OF T-TESTS FOR DIFFERENCES BETWEEN MEANS.

Tissue type	Treatment	n	NO_3^- uptake rate (μg-at/gm/hr) range	$\bar{x} \pm$ SD
apex of juvenile frond	surface incubation	3	1.0-1.8	1.3 ± .5
"	bottom incubation	6	0.6-0.9	0.8 ± .1
apex of canopy frond	surface incubation	5	3.0-4.2	3.4 ± .5
"	surface incubation, shaded	3	2.1-2.8	2.5 ± .4
"	bottom incubation	5	1.3-1.7	1.5 ± .2
"	bottom incubation, shaded	3	0.8-1.1	1.0 ± .2

p<0.05 (1.3 ± .5 vs 0.8 ± .1)
p<0.01 (1.3 ± .5 vs 3.4 ± .5)
p<0.05 (3.4 ± .5 vs 2.5 ± .4)
p<0.01 (3.4 ± .5 vs 1.5 ± .2)
p<0.01 (2.5 ± .4 vs 1.0 ± .2)
p<0.01 (0.8 ± .1 vs 1.5 ± .2)
p<0.05 (1.5 ± .2 vs 1.0 ± .2)

The rate differences found at different depths were not related to temperature or ambient NO_3^- concentration, which varied from surface to bottom by less than 1^oC and 1 μM, respectively, on the experimental days. Therefore, light was examined as a potentially influential factor. Uptake rates were measured for apices of canopy fronds incubated in dark bags at both the surface and bottom. Light within the bags was reduced to 5 percent of ambient intensity. Uptake by shaded apices was 26-33 percent lower than uptake by unshaded apices at both depths (Table 11). This rate reduction was comparable to that found by Wheeler (1978) for dark vs. light incubations in the laboratory. However, it is critical to note that shaded apices incubated at the surface had significantly higher uptake rates than unshaded apices incubated at the bottom, even though the latter were exposed to higher light intensities (estimated at 100 $\mu E/m^2/sec$) than the shaded tissues (estimated at 50 $\mu E/m^2/sec$). It must be concluded that NO_3^- uptake was influenced by some depth-related environmental factor other than temperature, ambient NO_3^-, or light intensity. That factor may have been pressure.

The in situ method described herein provided accurate measurements of NO_3^- uptake by adult Macrocystis plants. Because the experimental tissues underwent minimal handling - then were not cut into pieces, exposed to air, scraped clean of epiphytes, etc. - the resulting data involved little artifactual error. The results were comparable to results of previous laboratory studies (Haines and Wheeler, 1978; Wheeler, 1978). This was surprising in view of evidence that surface-related functions such as respiration (Hatcher, 1977) and exudation of dissolved organic carbon (Fankboner and de Burgh, 1977; Moebus and Johnson, 1974) were affected by damage and handling of brown algae.

The importance of depth to NO_3^- uptake could only have been determined by in situ experimentation. Whatever the environmental factor that influences uptake

kinetics in synergism with light intensity, knowledge of the overall effect of depth is critical to our understanding of Macrocystis nutrient dynamics. For example, the efficiency of a fertilizing project could be reduced by 50 percent or more if currents held the plants in a horizontal position at depths of 10-12 meters.

Accurate measurements of NO_3^- uptake by different tissue types can be used to estimate uptake by whole adult Macrocystis plants under a variety of conditions. Overall capacity must depend to some extent on population structure. For example, a population with a high proportion of juvenile fronds which have relatively low uptake rates should have a lower uptake capacity than a population with a high proportion of canopy fronds. Such differences occur seasonally in inshore kelp populations (Gerard, 1976). Removal of canopy tissues during harvesting should significantly reduce uptake capacity of a kelp population, because those tissues have the highest uptake rates.

The effect of vertical stratification of nutrients in the water column on nutrient uptake by adult Macrocystis plants can also be predicted using the rate measurements reported herein for different tissue types. High NO_3^- concentrations throughout the water column would certainly provide maximum uptake, but not necessarily maximum fertilizing efficiency. Uptake should be greatly reduced by a stable midwater thermocline, a common occurrence in summer when nutrients are depleted and remain low in surface waters. Although Jackson (1977) hypothesized that growth of canopy fronds was supported by upward translocation of nitrogen taken up by deeper portions of kelp plants, low rates of NO_3^- uptake by deeper tissues make this less than probable. Conversely, high rates of uptake by shallow tissues enhance the efficiency of fertilizing methods which expose only the kelp canopy to high NO_3^- concentrations. A mathematical model is now being developed to predict actual uptake rates by adult Macrocystis plants under different population and nutrient conditions.

CONCLUSIONS

Upwelling intensity in 1980 was substantially reduced compared to 1979. Technical feasibility now seems well established that adult Macrocystis plants can be maintained throughout an average year in an oceanic environment even as isolated individuals. A population of thousands of plants would undoubtedly constitute a less stressful situation compared to isolated individuals. Adult Macrocystis plants displaying average values of tissue N in the range of 1 to 1.5 percent dry weight can sustain normal growth rates. This range apparently represents a condition where nitrogen reserves are completely lacking. All nitrogen accumulated goes into newly created tissue, so the average N content of the entire plant remains constant. The average N content apparently remains in this range during moderate nitrogen limitation, but growth rates of juvenile fronds decline. N contents below 1 percent, which we have observed among some kelp tissues in previous years (i.e. San Onofre kelp bed in 1976-77), probably represent a condition of severe nitrogen starvation. Macrocystis gametophytes responded optimally to an Aquil medium enriched with the following micromolar concentrations of nutrients: 20 NO_3, 2 PO_4, 0.35 Fe, 0.03 Mn, 0.07 Co, 0.01 Cu, 0.17 Zn, 0.1 I, 0.1 Mo. Iron was actively taken up by a frond from an adult Macrocystis. The optimal Aquil medium for Macrocystis gametophytes also supports superlative growth rates among juvenile sporophytes. An adult Macrocystis plant in a nitrogen-deficient medium was able to grow at about a 3 percent daily weight increase. Nitrogen reserves were apparently drawn from mature tissues and translocated to support production of new tissue in the meristematic areas. N content of the entire plant remained constant. Fresh PVC-containing material inhibits Macrocystis growth under the restricted conditions of aquarium culturing. The growth-inhibiting properties were eliminated by leaching the PVC panels for one month in running seawater. Enrichments of 15μ M NO_3^- and 1μ M

PO_4 support kelp growth rates equivalent to 30μ M NO_3^- and 2μ M PO_4 under batch culturing conditions. Chlorophyll content of Macrocystis (and possibly photosynthetic capacity) is influenced by nitrate concentration and light intensity used in culturing.

Work Performed from September to December 1980

Upwelling intensity was greatly reduced during the Fall. Depth of the 12.5°C isotherm frequently occurred deeper than 150 feet and was deeper than the arms of the Test Farm most of the time. Fish grazing completely destroyed one of the three transplants and left only a few small fronds on the other two. Three new transplants were introduced to decoy the fish, but blades on these plants were completely stripped off in about a month. Attempts were unsuccessful to eliminate the fish by gill netting and by introducing a raft to attract sea lions. Iron was found to be concentrated severalfold within the exudate from a stipe of an adult Macrocystis frond compared to the external concentration bathing the blades. This strongly suggested that iron is actively accumulated by Macrocystis. Variability was controlled to reasonable proportions in our Aquil culturing involving juvenile sporophytes. Two culturing series were completed examining effects of nitrate and phosphate concentration and of light intensity on mannitol content of juvenile sporophytes. High mannitol in the juvenile may be a transient response to a reduction in nutrient availability. No effects were observed on mannitol content from various light intensities. High mannitol contents occurred in the mature blades of an adult frond. Three dye dispersal studies were conducted and showed a substantial reduction of ocean current by kelp beds but enhanced vertical mixing in the vicinity of adult plants. Studies of buoyancy and drag were conducted using an adult plant.

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5.3 INOCULUM DEVELOPMENT

GENERAL ELECTRIC COMPANY/RE-ENTRY SYSTEMS DIVISIONS

5.3 INOCULUM DEVELOPMENT

The primary objective of the Inoculum Development Phase of the Marine Biomass Program continues to be the development of optimized anaerobic microbial cultures for the bioconversion of kelp (Macrocystis pyrifera) to methane gas. In order to accomplish this objective, it is necessary to explore and define the fundamental microbiological reactions and interactions in the process to the degree that it becomes possible to effectively maximize methane yield and productior rate.

Consistent with this objective, various tasks were outlined and conducted during this contract period. These studies centered mainly upon substrate competition, development of techniques to monitor microbial food chain populations, and factors affecting kelp digestibility.

BASELINE DIGESTERS

Baseline digester systems established with materials obtained from various marine environments were maintained as experimental test systems, and as sources of selected microbial strains involved in the conversion of kelp to methane. These small (1.5 liter liquid volume) CSTRs* were charged with kelp diluted with artificial sea water (Instant Ocean) on a 3-times-per week schedule, with performance being monitored by gas production, gas composition and pH measurements. Digested solids (gravity settled for 20 minutes under an atmosphere of flowing nitrogen gas) were recycled with the feed on a 20 percent (V/V) basis. The kelp lots utilized during this reporting period were Lot #48 (supplemented with mannitol to give a final mannitol concentration of 18.58 percent) and Lot #49 (supplemented with ammonium chloride to give a C/N ratio of 13). No attempt was made to optimize performance during this period. All digesters had periodic withdrawals of samples for various experimental purposes. Therefore, actual gas production data was not significant and is not presented herein.

*Continuously stirred Reactors

A general observation is worth noting, however. When kelp Lot #49 was employed as a feedstock, the pH of the digesters had to be controlled in the operating pH range of 6.7 to 7.0 by the addition of sodium hydroxide. This is in contrast to the performance with Lot #48 where the pH was routinely in the 7.0 - 7.1 range without base addition. The reason for this difference may lie in the higher mannitol content in Lot #49 (24 percent vs. 18 percent in Lot #48) which, in turn, may result in enhancement of acidogenic reactions with a concommitant drop in pH. This remains to be verified.

DIGESTION TO COMPLETION

The objective of these studies was to determine the limitations on the biological degradation of kelp with the purpose of maximizing the extent and rate of substrate conversion to methane.

Previous studies performed on steady-state digester effluents (15-18 day hydraulic retention times) had shown incomplete utilization of several organic components, particularly the structural polymers. Development of strategies for further conversion of these materials requires that a determination be made of the composition of the residual fraction after cessation of methanogenesis. Studies of this kind provide insight as to whether incomplete degradation is a function of the activity and rate of the microorganisms involved.

Initial studies were aimed at examining the extent of kelp degradation under long-term digestion conditions. Both CSTR and serum bottle techniques were employed as experimental vehicles. CSTR units were being used to provide sufficient material to develop correlation between gas yield data and compositional changes during digestion of kelp and at the cessation of methanogenesis. Serum bottle studies explored the effect of various additives on increasing ultimate gas yields and evaluated possible procedures to reinitiate methane production after it has ceased.

In order to address the residual composition problem, feed to an active 1.5 liter kelp-to-methane digester was discontinued. This digester had been charged with artificial seawater (Instant Ocean) diluted kelp (Lot #48, supplemented to 18.5 dry weight percent mannitol) at a loading of 0.1 pound VS/ft^3 on an alternate-day basis and a retention time of 30 days. A 20 percent settled solids recycle was also incorporated with the feed.

Gas measurements (gas volume and composition) and digester content samples were obtained at zero time and at regular intervals. Analyses were perfcrmed to determine the pH, solids content (total solids and volatile solids), volatile fatty acids, mannitol, algin and fiber content of these samples. After a period of several weeks, an apparent slow leak developed in the gas collection system. Attempts to locate and repair the leak were unsuccessful; and although no oxygen could be detected in the system, the methane concentration continued to drop, and the experiment had to be terminated.

This experiment has been redesigned to include an assessment of inoculum source and temperature on digestibility (biomethanation) and is scheduled to be performed at a later date.

The other aspect of this study is to ascertain whether the residue remaining after cessation of methanogenesis is biologically inert, or, given the proper organisms or nutritional factors, is further degradable. A screening test was developed to evaluate certain nutritional aspects on long-term digestion. These studies included both pre- and post-methanation cessation effects. Although previous short term studies (15-18 days) have shown no improvements in biomethanation by nutrient addition (unless nutrient limited, i.e., C/N >15), long term culturing could result in certain factors either being destroyed or made unavailable for further use.

A series of serum bottle cultures were established from an active kelp digester being charged with N - supplemented kelp Lot #49 diluted with artificial seawater. One set received no further additions, while the second set received one of the nutritional supplements listed in Table 12. Each set was monitored for methane content until gas production ceased. All cultures were then purged with O_2 - free nitrogen to remove all traces of methane from the headspace. Those that had received additions in the first phase were given an identical supplement. Those which had initially received no additions were also challenged with supplements (except controls). The results of the first phase are presented in Table 12.

These data indicate that initial nutrient supplementation does not significantly increase long-term gas yields from kelp. The slightly higher levels obtained with certain additions are a result of biomethanation of the addition. For example, additions of mannitol at a final concentration of 0.1 gm/l should theoretically increase gas yield by 3.0 percent. The actual value found was 3.1 percent, well within experimental error. The increase observed with the addition of an acetoclastic enrichment can be attributed to the consumption of the residual acetate and is not due to any added microbial activity, as the non-viable culture gave essentially the same gas yields as the viable culture. The slight stimulation by vitamins and high concentrations of yeast extract may indicate some minor nutritional requirements, but this will have to be explored later.

Phase 2 reinforced the results obtained in the first part of these experiments. While slightly higher levels of methane production were observed with certain additions, these increases could be attributed to degradation of the nutrient supplements.

TABLE 12. EFFECT OF SELECTED SUPPLEMENTS ON EXTENDED BIOMETHANATION OF KELP

Supplement	% Change From Control*
NH_4Cl (1 gm/l) + K_2HPO_4 (0.4 gm/l)	- 3.4
Yeast Extract (0.1 gm/l)	0
Yeast Extract (0.5 gm/l)	+ 8.7
**Vitamin Solution (5 ml/l)	+ 5.2
Mannitol (0.1 gm/l)	+ 3.1
Mannitol (0.5 gm/l)	+13.3
Methanogenic Acetate Enrichment - 5% V/V	+19.2
Methanogenic Acetate Enrichment (killed) - 5% V/V	+18.2

* Average of Replicate Samples

** Wolin et al. (1963)

It thus appears that nutrients per se are probably not a limiting factor in the extent of kelp digestion (biomethanation) and that other factors are restricting the extent of conversion. An analysis of the residual fraction as planned may provide information regarding these factors.

MIXED CULTURE STUDIES

Studies of the interactions between microorganisms are an important factor in developing and controlling bioconversions requiring more than one microbial species (i.e., a food chain). Reactions of a specific strain are often very different when grown in co-culture, and it is these differences which can control the rate and extent of degradation.

Initial studies were designed to investigate simple binary mixtures consisting of acidogenic microorganisms (i.e., alginolytic, cellulolytic) and aceticlastic methanogens, thus establishing food chains in which a highly polymeric carbohydrate and other kelp constituents are converted to methane.

Two strains of acetate utilizing methanogens (aceticlastic methanogens) were obtained from Dr. Robert Mah, UCLA. These strains are members of the genus Methanosarcina (Strains 227 and W) and were isolated from enrichments developed from sewage sludge digesters (Hyperion waste treatment facility, El Segundo, California). These cultures were received in liquid basal media containing methanol as the methanogenic substrate.

Due to the fact that the non-methanogenic strains to be used in these experiments were of marine origin and growing in media containing 3 percent NaCl, it was necessary to modify the methanogenic growth medium to also contain 3 percent NaCl. After an initial lag period, Strain 227 grew well in the presence of salt with methanol as the growth substrate and was likewise adapted to grow readily in calcium acetate plus NaCl. Although we also have been able to achieve good growth of this strain in sodium acetate (Figure 21), we have not been successful in getting it to grow in sodium acetate fortified with NaCl.

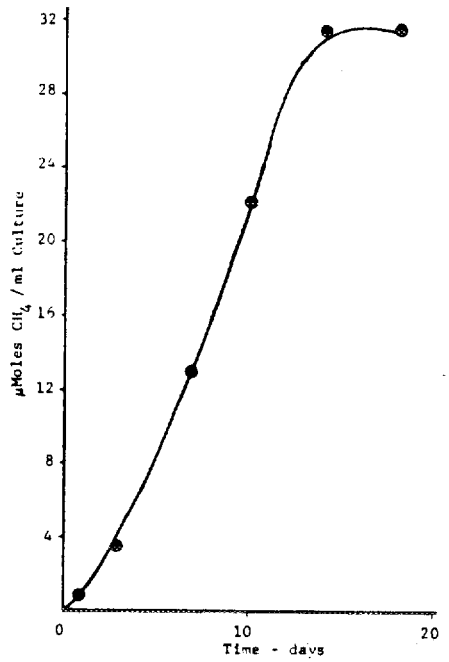

Figure 21. Methanogenesis by Methanosarcina Strain 227 Growing in Sodium Acetate

Strain W, which grows on methanol and slowly on calcium acetate, will not grow in 3 percent NaCl and adaptation experiments in which transfer was made into media with increasing salt concentration were unsuccessful. A summary of the growth characteristics of these two strains is shown in Table 13.

These data indicate that additional work is required to adapt these methanogenic strains to a completely soluble medium containing salt prior to mixed culture studies (see Note 1).

Other aceticlastic methanogens are actively being sought, particularly those from marine environments. Acetate utilizing methanogenic enrichments developed from kelp digesters (marine inoculum), along with digester effluent samples, were forwarded to Dr. Mah. From those sources, Dr. Mah has indicated that he has isolated, and is now characterizing, several new salt-tolerant methanogenic strains which will be utilized in this culture development study.

FOOD CHAIN STUDIES

Previous efforts on the isolation of mannitol degrading microorganisms in marine derived anaerobic digestion systems have been reported. These organisms

TABLE 13. COMPARISON OF GROWTH OF TWO METHANOSARCINA STRAINS

Strain	3% NaCl			No NaCl		
	Methanol	Calcium Acetate	Sodium Acetate	Methanol	Calcium Acetate	Sodium Acetate
227	+	+	-	+	+	+
W	-	-	-	+	+ (slow)	-

*Growth as measured by methane production in medium of Mah et al.

+ = Growth; - = No growth

Note 1: Mixture of calcium acetate medium with alginate medium will result in the formation of insoluble calcium alginate, that will precipitate from the medium and limit its availability.

were found to be mainly clostridial-like in morphology with some producing spores. These reports also described a Cytophaga sp. which appears to be the major alginate-utilizing organism in these systems. We have further examined this species and have found that it will also utilize mannitol as a growth substrate. Experiments were established to determine how the presence of a readily soluble methanogenic substrate such as mannitol, affects the utilization (degradation) of algin by this species. Cells were grown in a batch mode in a defined medium with either algin (0.25%, W/V) or algin (0.25%) + mannitol (0.25%) as growth substrates. Samples were taken at various time intervals for measurement of growth (optical density at 650 nm) and analysis of substrates (algin and mannitol). Figure 22 shows the results of this growth study. Algin degradation was nearly identical in the presence or absence of mannitol - both in terms of the time profile and extent of utilization (~95%). Decrease in optical density after 80 hours is due to cell lysis. The corresponding mannitol consumption curve is shown in Figure 23. In addition, the growth curves were very similar, the only difference being, as anticipated, the higher cell yield obtained because more substrate is available in the algin + mannitol medium. The initial disappearance of algin before noticeable growth was probably due to very active alginase activity introduced with the inoculum.

The results of these batch fermentation studies indicate that algin degradation is apparently unaffected by the presence, and simultaneous consumption of mannitol. Cell growth rates, algin breakdown rates and total amount of algin utilized were the same in both cases. It should be noted that the mannitol concentration employed (0.25%, W/V) is roughly equivalent to the concentration of mannitol expected in a digester being fed at a maximum loading. It might be expected, therefore, that alginolytic activity in kelp digester systems is also unaffected by the mannitol content of the kelp, particularly since there are many

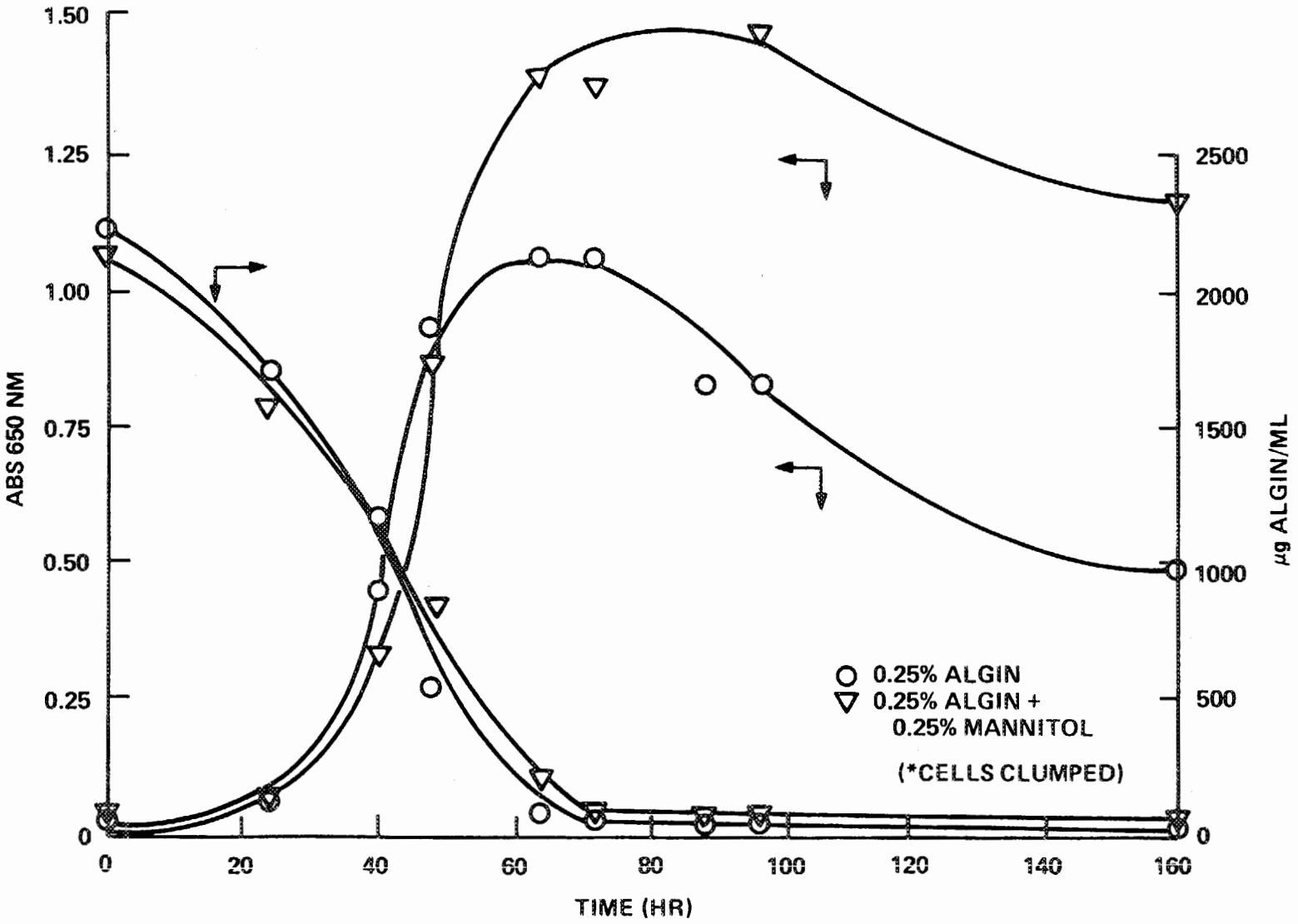

Figure 22. Effect of Mannitol on Cell Growth and Algin Utilization by a Cytophaga Sp.

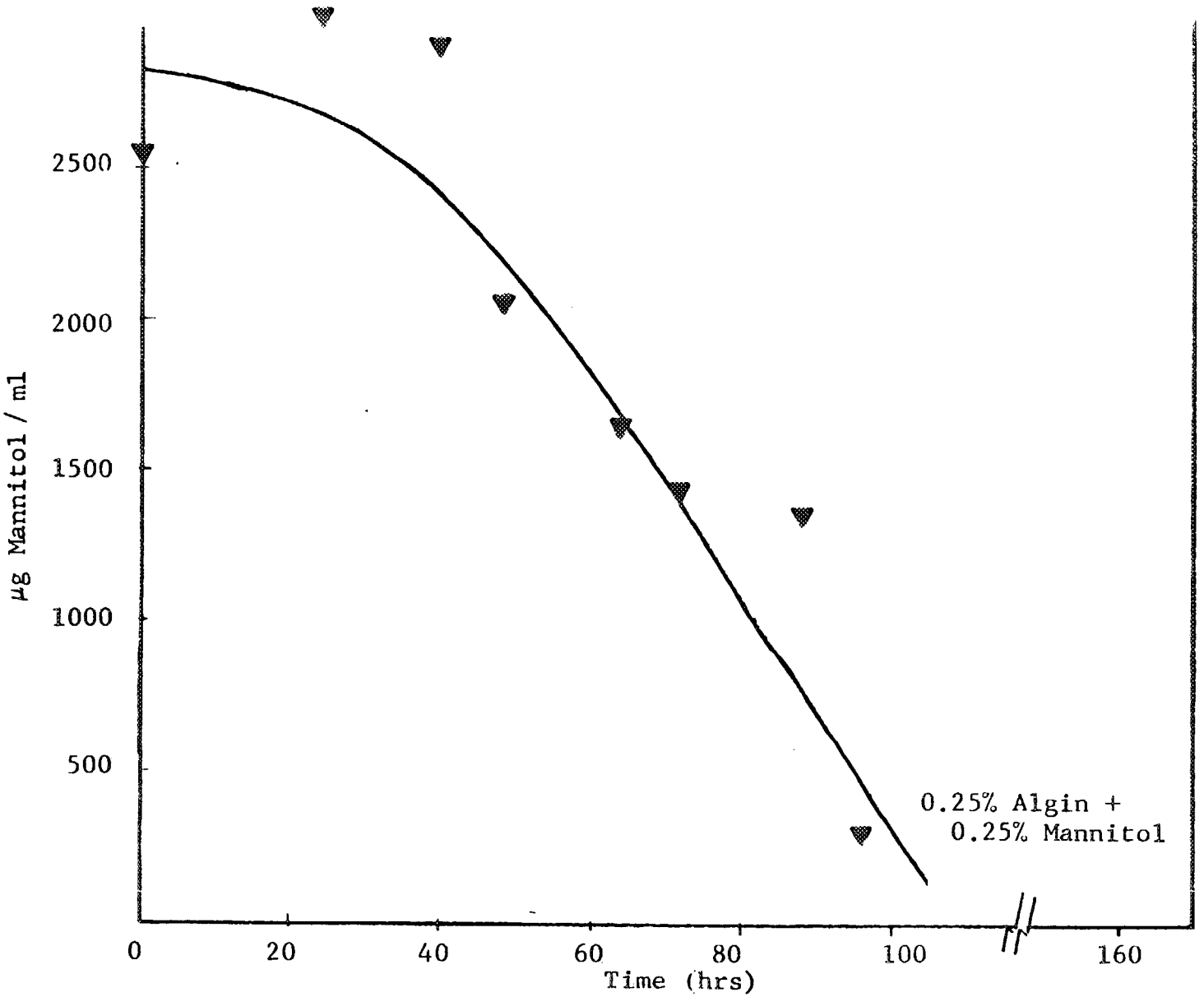

Figure 23. Mannitol Utilization During Growth on Alginate

other microorganisms competing for mannitol and the actual steady-state level of this compound is probably much lower. This, of course, must be verified.

PARTICULATE STUDIES

Previous data generated in this laboratory, as well as in those of others, indicate that significant improvements in methanogenic activity could be realized by culturing methanogens on particulate materials. In this manner, the methane generation rate, which is dependent upon the number of methanogenic bacteria in the system, is maximized as these bacteria are concentrated and not washed out in flowing systems.

Many factors are involved in this attachment process. It is the objective of this research to define these growth and adherence phenomena, both in terms of the individual bacterial species, and the particle type.

An important parameter in studies of this nature (and also in mixed culture studies) is to ascertain the concentration of viable cells present as a function of process status (or as a function of total populations, etc.) Many techniques are available to determine cell numbers (microscopic counts, plating, etc.), but these methods are not suitable for use with particulate matter.

An approach to this problem, which is being investigated in this program, is to attempt to utilize the fact that methanogens have a unique co-factor, Factor 420 (F_{420}), which is a component of their electron transport system. Not only is the compound unique to methanogens [1], but it has been found in all methanogens described to date. Correlation of this component with cell numbers would provide a direct measurement of the methanogenic population attached to particulates or in mixed cultures. Total population counts can readily be determined using an ATP analysis (Luciferin-Luciferase reaction).

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(1) F_{420} has recently been found in the non-methanogen, Streptomyces griseus, but this organism is an aerobe and does not function under conditions conducive to methanogenesis (Eker et al., FEMS Letters 8:161-165, 1980).

A sample of purified F_{420} obtained from Dr. J. G. Ferry, Anaerobe Laboratory VPI, was used to verify a F_{420} extraction technique described by Delafontaine et al[1], and to establish standard values. Emission and excitation spectra of pure F_{420} in 1N NaOH, as obtained using an Hitachi-Perkin Elmer Fluorescence Spectrophotometer, are shown in Figure 24. The excitation maximum is at 420 nm, and the emission maximum is at 464 nm. Less than 1×10^{-9} grams/ml is readily detectable using this technique, making it as sensitive as, if not more sensitive than, the ATP analysis technique.

Studies to determine the concentration of F_{420} per cell, and the effect of growth conditions on this concentration, are being conducted. Initial efforts were conducted with Methanosarcina Strain 227 growing on methanol. A comparison of F_{420} with optical density (650 nm), as shown in Figure 25, indicated that F_{420} is growth related, but does not correlate with optical density per se. This is not unexpected, however, as optical density is a measure of both viable and non-viable cells. Total cell count becomes higher than viable count as the culture ages.

At the present time the Most Probable Number (MPN)* technique is being evaluated as a means of estimating viable cell counts. Actual counts determined by this procedure may be low due to the fact that Methanosarcina strains do not grow as single, isolated cells, but rather as variously sized clusters of attached cells which are not readily dissociated.

ADDITIONAL STUDIES

Autoclaved (Sterilized) Kelp as a Substrate

Although many studies have been conducted using pure microbial isolates growing on "pure" substrates, no effect to date has been made to examine the

[1] Delafontaine et al. (Biotechnol Letters 1:71-73, 1979)

*Standard Methods for the Examination of Water and Waste-water APHA 13th Ed. 1971.

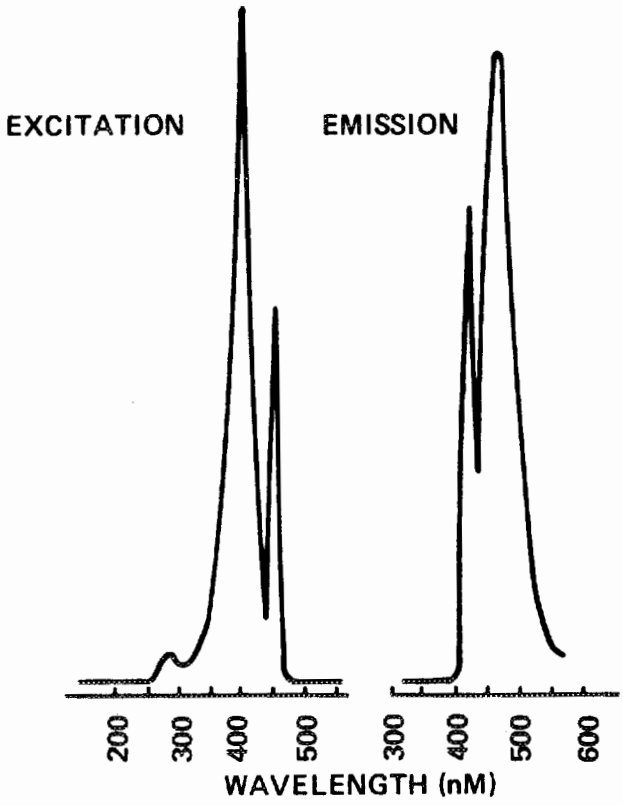

Figure 24. Emission and Excitation Spectra of F_{420}

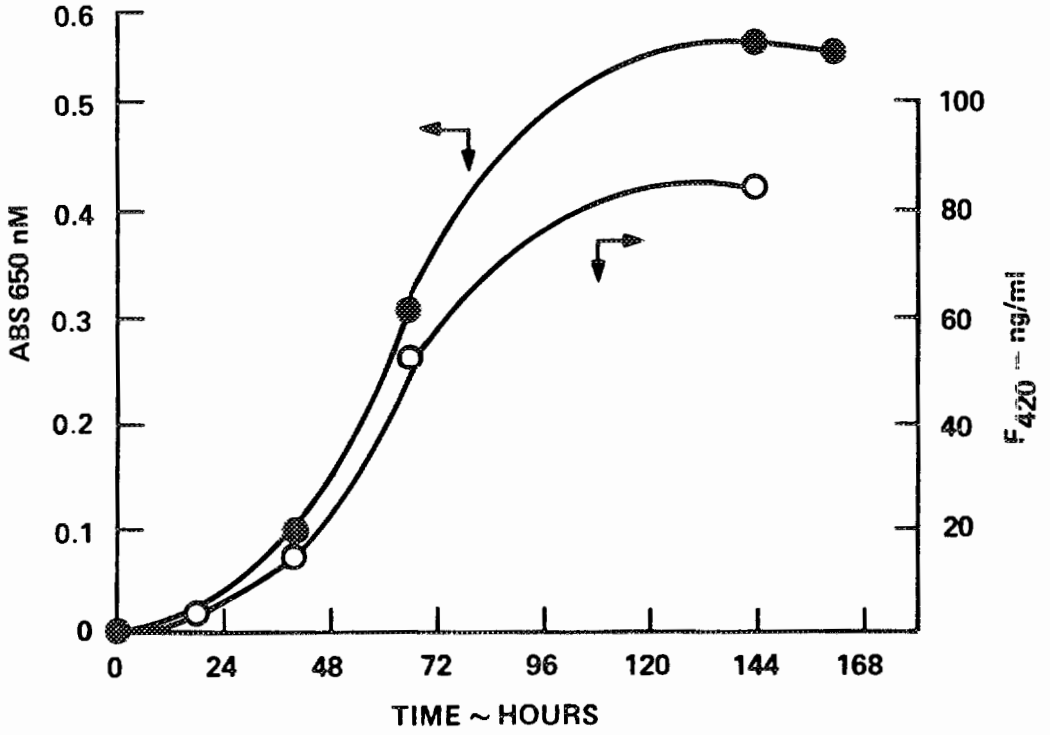

Figure 25. Relationship Between F_{420} and Growth in Methanosarcina 227

growth and substrate utilization of pure strains growing on kelp. In order to accomplish this, it is necessary to destroy the microbial population naturally associated with the biomass. Sterilization of kelp by autoclaving is a convenient procedure, but some alteration of the biomass can be expected; the question being to what extent? One measure of "fitness" of autoclaved kelp as a model "natural" substrate would be its convertibility to methane. A comparative bioassay of raw and sterilized kelp (15 PSI, 121^{o}C, 15 minutes) was performed using kelp digester effluent as an inoculum. Methane yields for raw and autoclaved kelp were 3.09 SCF/lb. VS and 3.18 SCF/lb. VS, respectively, indicating little if any change in degradability by this treatment. Autoclaved kelp thus may be used as a test substrate for pure culture studies.

Preliminary experiments indicate that the alginolytic Cytophaga sp. will grow in autoclaved kelp + artificial seawater, but data on algin and mannitol consumption are not available.

SUMMARY OF ACTIVITIES

SEPTEMBER 1, 1980 TO DECEMBER 31, 1980

BASELINE DIGESTERS

All baseline digesters were converted to an undiluted feed regime at a continued loading of ~ 0.1 lb. VS/ft^3. This effectively increased the detention time to ~ 100 days. Difficulties in controlling pH developed and gas production performance deteriorated.

In preparation for Temperature/Inoculum evaluation studies, two additional digesters were established with effluent from IGT's baseline digester (sewage inoculum). These were charged as above and appeared to suffer from the same pH control and gasification problems.

The feedstock for all digestors was switched from kelp Lot #49 to Lot #53-1 in mid December. The digesters began gradually returning to a satisfactory performance.

Effluent from these digesters will be used as inocula for digestibility studies, and for the temperature/inoculum experiments.

MIXED CULTURE STUDIES (Methanogenic Strains)

A new salt tolerant (3% NaCl) Methanosarcina strain isolated by Dr. Robert Mah from materials provided by this laboratory has been received. Preliminary evaluations of this strain indicate that it grows readily in methanol, and in calcium acetate, but only very slowly in sodium acetate. As a result, it may have a limited value in mixed culture studies. Additional marine derived strains are expected shortly.

PARTICULATE STUDIES (F_{420}/ATP Studies)

Initial attempts to correlate F_{420} and ATP with viable counts of Methanosarcina have been completed. These data indicate an average of 2 x 10^{-16} gm ATP per methane forming unit (cell?), which include recovery losses (to be

determined), and is consistent with levels found in other bacterial cells. F_{420} levels average 1×10^{-15} gm per methane forming unit. Due to the fact that Methanosarcina grows in clusters rather than as isolated single cells, actual values per cell cannot be determined. It therefore becomes more appropriate to correlate the F_{420} and ATP levels of these organisms with methanogenic colony-forming units. Variations in the levels of these two components as they relate to the physiological state of the microorganism will be investigated.

TEMPERATURE/INOCULUM STUDIES

In preparation for a comparative evaluation of marine derived and sewage derived inocula for the biomethanation of kelp, and for an evaluation of methane yields achievable at ambient temperature with those at 37°C, four 10-liter CSTR reactors are being constructed. The design of these reactors is the same as that used by IGT for their studies. Digester cultures are being readied for this experimental evaluation. Both types of cultures will also be utilized to assess long term digestibility and recalcitrant fractions.

5.3.1 ISOLATION AND PURE CULTURE WORK ON ACETATE-USING METHANOGENS FROM KELP

ENVIRONMENTAL RESOURCES GROUP

5.3.1 ISOLATION AND PURE CULTURE WORK ON ACETATE-USING METHANOGENS FROM KELP

INTRODUCTION

One of the key reactions in the bioconversion area which has not as yet been fully defined is the anaerobic conversion of acetate to methane and carbon dioxide. The isolation and characterization of the nutritional and physiological requirements for growth and acetate methanogenesis by these organisms is crucial to the improved applications for methane formation by production scale fermentors.

The primary objective of this isolation and pure culture work task by ERG is to isolate, characterize, adapt and preserve acetate-using methanogens for further studies which will lead to an understanding of the fermentation processes involved in the anaerobic conversion of acetate to methane and carbon dioxide.

To this end, ERG received two kelp inocula samples from Dr. John Forro of the General Electric Company which were to serve as a source for the isolation of aceticlastic methanogens. One was an acetate enrichment inoculum obtained from a kelp digester and subsequently carried through several enrichment transfers. The other was from a kelp digester maintained by Dr. Forro.

The anaerobic media used for growing these methanogens were prepared according to the usual Hungate method [1], with the two media composition being modified to include a higher concentration of NaCl and KCl in order to simulate the salt content of the inocula. The composition of the two media are as follows:

1. Roll Tube Methods for Strict Anaerobes. Methods in Microbiology Vol. 3B. J.R. Norris and D.H. Ribbons (eds) Academic Press N. York 1969.

Medium I		Medium II	
NH_4Cl	1.0 g	NH_4Cl	1.0 g
$K_2HPO_4 \cdot 3H_2O$	0.4 g	$K_2HPO_4 \cdot 3H_2O$	0.4 g
$MgCl_2 \cdot 6H_2O$	0.1 g	$MgCl_2 \cdot 6H_2O$	0.1 g
Yeast Extract	2.0 g	Yeast Extract	2.0 g
Trypticase	2.0 g	Trypticase	2.0 g
KCl	15.0 g	NaCl	30.0 g
Na Acetate	6.8 g	Ca Acetate	4.0 g
Cysteine	0.5 g	Cysteine	0.5 g
0.1% resazurin	1.0 ml	0.1% resazurin	1.0 ml
Tap Water	1000 ml	Tap water	1000 ml

Both media were adjusted to pH 6.7 and dispensed in 50 ml aliquots before autoclaving. The following were added just prior to inoculation:

0.5 ml 1% Na_2S

0.5 ml 1% $CaCl_2 \cdot 2H_2O$

0.25 ml 10% MeOH

The inocula were diluted serially into roll-tube media, incubated at 37°C, and aceticlastic colonies presumptively identified by their characteristic appearance of color, size, and shape (and later, in older colonies, of calcium carbonate precipitation). Several likely colonies were picked and transferred after three weeks of incubation. From these, pure cultures were isolated after approximately ten successive transfers, each requiring "picking" of an isolated colony and inoculation into fresh roll tube media followed by incubation at 37°C for 10-20 days.

Three pure cultures of aceticlastic methanogens were obtained by these methods. All three organisms were of the Methanosarcina type. The first isolate was a slow growing organism which required NaCl, grew poorly on Sodium acetate,

but grew without difficulty on calcium acetate. The second isolate was a gas vacuolated pseudosarcina originally isolated in medium containing the composition of Medium II supplemented with 33 percent sludge supernatant. On subsequent culture transfer, the sludge supernatant was eliminated from the medium. Although the isolate grew adequately on this medium, it lost the characteristic for gas vacuole formation. It seems likely that the genetic trait for gas vacuole formation may depend, for phenotypic expression, on some unknown factor(s) present in the sludge supernatant. The third isolate is a Methanococcus mazei type of pseudosarcina, exhibiting part of the life cycle of our previous isolates, but differing in that it requires a high salt concentration for optimal growth.

The slow-growing, NaCl-requiring organism and the KCl-requiring organism have both been sent to John Forro for further experimental work.

5.4 CONVERSION PROCESS DEVELOPMENT

INSTITUTE OF GAS TECHNOLOGY

5.4 CONVERSION PROCESS DEVELOPMENT

The initial objective of IGT's effort in Conversion Process Development is to optimize the anaerobic digestion process for producing methane from kelp. Toward this objective, near-term goals have been developed and the performance of an on-going comprehensive research program on the anaerobic digestion of kelp is in progress. These goals, directed toward achievement of the project objective are as follows:

- Evaluation of the performance of kelp under conventional baseline conditions of anaerobic digestion
- Optimization of methane yields and production rates by:
  - identification of factors that affect methane yields and production rates
  - investigation of unconventional digester systems
  - evaluation of operating conditions
- Development of process design

Although the overall project objective and near-term goals remain unchanged, the specific objectives of individual contract periods have been modified as required by budgetary constraints. The results of this study will be implemented in the design, construction and operation of large-scale biomethanation systems under the management of IGT.

Digester Design and Operation

Sixteen digesters were continued in operation or initiated during this report period. An outline of each digester and its mode of operation or status during the culture maintenance or process development protocols of this report period is presented in Table 14. These digesters received undiluted or freshwater-diluted kelp, depending upon the specific objective of each experiment.

TABLE 14. DIGESTER OPERATION DURING CULTURE MAINTENANCE AND PROCESS DEVELOPMENT PROTOCOLS

Run No.	Digester Type	Operation During Culture Maintenance Protocols	Operation During Process Development Protocols
41	10-ℓ CSTR	Maintenance	Dialysis evaluation
42	10-ℓ CSTR	Maintenance	Acid-phase operation
43	10-ℓ CSTR	Maintenance	Baseline for BFR
44	10-ℓ CSTR	Maintenance/kelp lot evaluation	Baseline system
39	10-ℓ CSTR	Maintenance/Autofeeder	Maintenance/Autofeeder
8	1.5-ℓ CSTR	Maintenance/mannitol studies	Mannitol studies
138	1.5-ℓ CSTR	Maintenance/mannitol studies	Mannitol studies
136	1.5-ℓ TR*	Inoculum G	Inoculum G
139	1.5-ℓ TR	Inoculum H	Inoculum H
140	1.5-ℓ TR	Inoculum A	Inoculum A
50	50-ℓ CSTR	Construction/maintenance	Baseline for UFSB
51	10-ℓ BF	Construction/maintenance	Shakedown/Operation
52	5-ℓ UFSB	Design	Construction/Operation
53	5-ℓ EBR	Design	Construction/Shakedown
54	5-ℓ PBR	Design	Construction/Shakedown
00	50-ℓ drum	Stock Culture	Stock Culture

*TR-non-stirred tank reactor.

Unless otherwise specified, all of the several types of digester units used were operated at 35°C, with gases collected and measured by displacement of an acid-salt solution from calibrated gas burettes, and were fed daily. One digester type (Runs 8 and 138) consisted of Plexiglass CSTR units with a culture volume of 1.5 liters that were shaken continuously at approximately 130 rpm by using a New Brunswick gyratory shaker, and incubated in a thermostatically-controlled environmental chamber. Another CSTR digester type (Runs 41, 42, 43, 44 and 39) had a culture volume of 10 liters, was maintained at a constant temperature with heating tape connected to a temperature controller, and was mechanically mixed. Other digesters, together with gas collection devices, autofeeders, and supportive equipment, are discussed below.

Table 15 shows the analytical schedule employed for maintenance-level digester operation. The frequency of operational analyses was reduced during the culture maintenance phase, with feed slurry and effluent samples stored for future analysis, and the normal schedule was resumed during the process development protocol.

TABLE 15. ANALYTICAL SCHEDULE FOR DIGESTER OPERTION*

Analysis	Frequency
Gas Production Measurement	Daily
Digester Temperature	Daily
Digester Effluent pH	Daily
Volatile Acids	Weekly
Gas Composition	Weekly
Conductivity	Weekly
Alkalinity	Bi-Weekly

*All methods used as described in the Annual Report, Project 6118, February 1978.

Three cultures were evaluated as sources of inoculum for the anaerobic digestion of kelp. These cultures, obtained from different sources, are referred

to as inocula A, G, and H. Inoculum A, originally derived from a mixture of effluents from an IGT pilot-scale digester that was receiving municipal solid waste/sewage sludge and from a municipal high-rate digester, has been gradually converted to as-received raw kelp under a variety of operating conditions. This inoculum is currently used for most of the experimental work reported on this project. Inoculum G was developed at room temperature from anaerobic marine sediment received from Kerckhoff Marine Laboratory. During the initial period of development, the digester received sodium acetate and undiluted kelp. The feed was gradually converted to undiluted raw kelp alone after the successful development of inoculum. Inoculum H was developed at room temperature from the anaerobic marine sediment collected in Newport Harbor, Calif. In the beginning, the digester received a mixture of sodium acetate, sodium formate, and yeast extract. Later, it received increasing pulses of undiluted raw kelp and mixtures of sodium acetate and sodium formate. Finally, the feed was converted to undiluted raw kelp alone.

Raw kelp was used in all digester runs during this time period. Kelp was either commercially harvested in southern California or harvested by the Western Regional Research Center (WRRC), then drained of physical water, chopped, ground, and frozen prior to use.

Digester Design and Techniques Study

In order to improve the loading rates and methane yields by utilizing innovative digester designs as combined or as two-phase systems, four unconventional digesters, baffle-flow, upflow solids blanket, expanded bed, and packed-bed - were selected for preliminary concept and process evaluation. Emphasis was placed on retaining the organisms in the reactor and maintaining low hydraulic retention times. Table 16 outlines some of the schemes under consideration in this program and particular units under evaluation.

TABLE 16. OUTLINE OF DIGESTER DESIGN AND TECHNIQUES STUDY SCHEMES

Scheme No.	Feed	Combined Phases	Acid Phase	Separation	Methane Phase
1	Undiluted kelp	CSTR*	→CSTR*	Settling, vacuum filtration	CSTR
2	Undiluted kelp	CSTR*	→CSTR	Dialysis*	Packed bed*
3	Undiluted kelp	Upflow solids blanket*	→Upflow	Settling, vacuum filtration	Packed Bed
4	Undiluted kelp	Baffle flow	→Baffle flow		
5	Seawater-diluted kelp	Expanded bed*			
6	Seawater-diluted kelp				Expanded bed
7	Undiluted kelp		→CSTR		Upflow solid blanket

*Under evaluation during 1980.

Baffle-Flow Digester

A 10 liter baffle-flow digester was designed, fabricated, and initiated with the inoculum from the baffle-flow digester which was in operation last year. The design is based on unmixed plug-flow with baffles to promote solids settling and prevent short-circuiting. The objective of the design was to minimize cost and to promote phase separation and solids retention time (see Figure 26). The serpentine configuration of the digester develops a velocity gradient across the channel at any length of the flow, which causes longitudinal shear. However, the average velocity of all flow streams parallel to the channel is the same. The laminar flow pattern develops longitudinal shear, which generated localized mixing.

Upflow Solids Blanket Digester

A prototype reactor (Figure 27) for upflow solids blanket, packed-bed, and expanded-bed bench-scale digestion studies was designed, and three units were

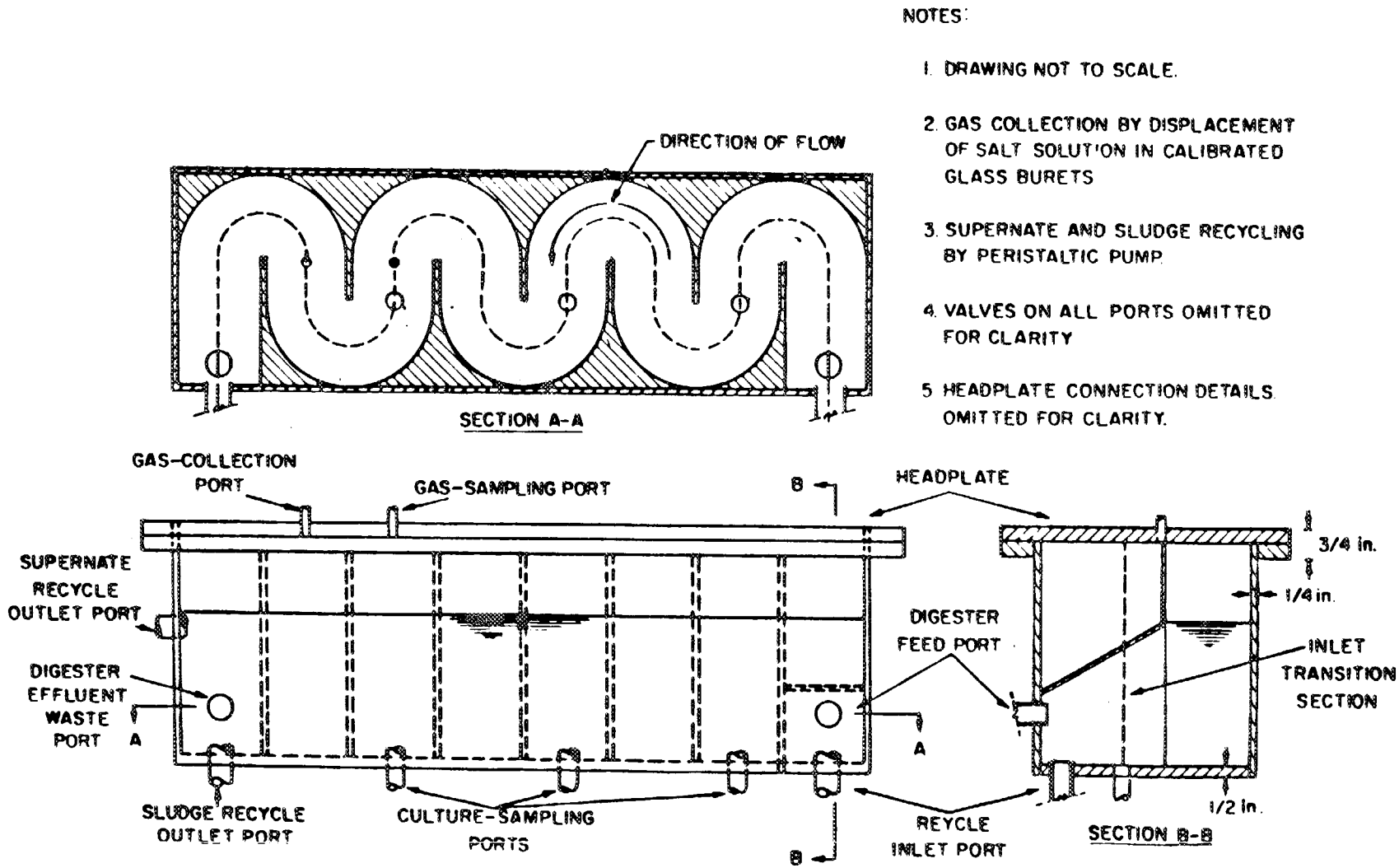

Figure 26. 10-ℓ Baffle-Flow Anaerobic Digester

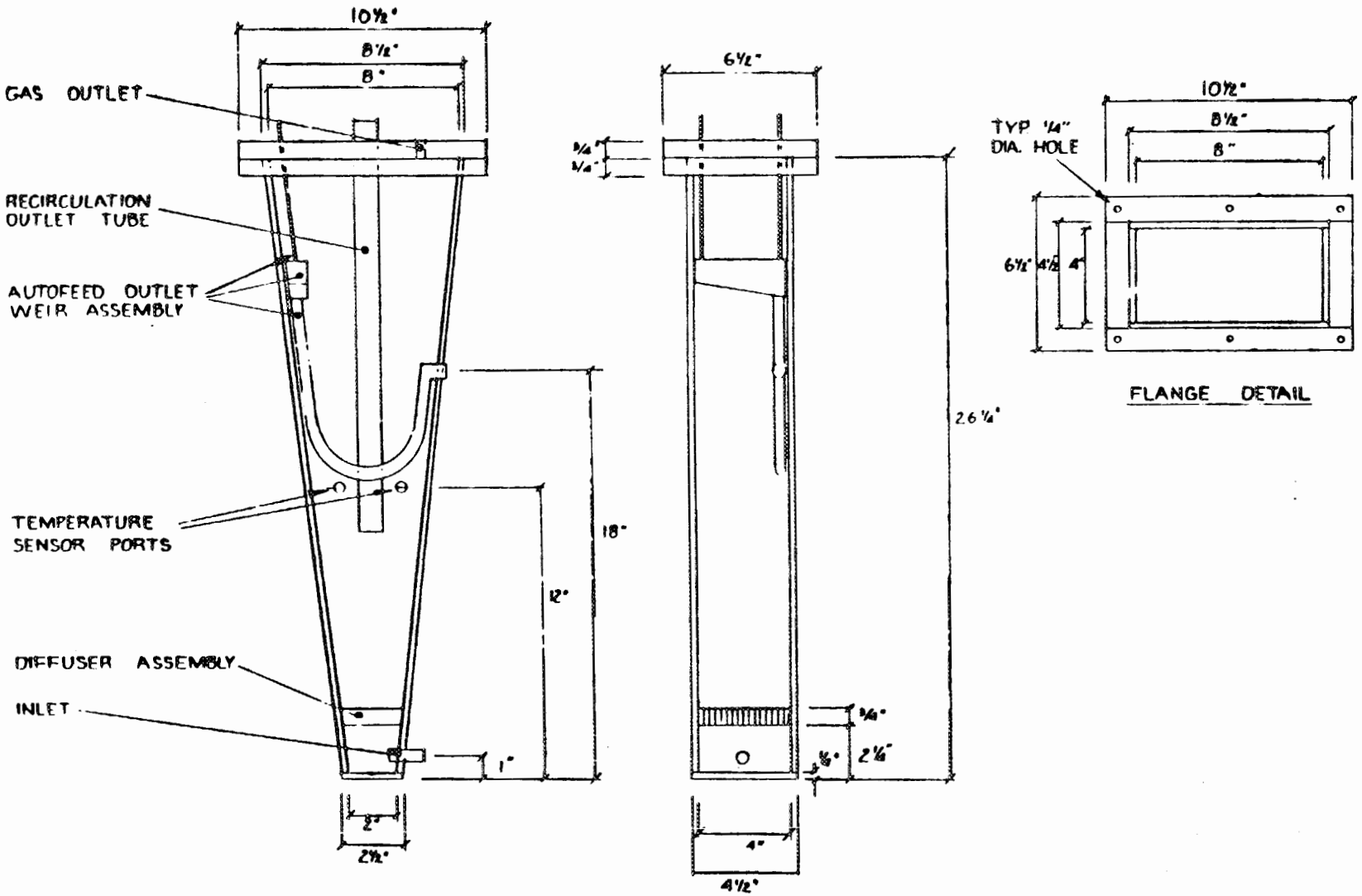

Figure 27. Basic Design of Upflow Digester

constructed. The basic unit consists of 1/4 inch Plexiglass, except for the 3/4 inch head plate. The unit, which is funnel-shaped, contains a lower diffuser ring and several peripheral rings to prevent short-circuiting and an adjustable weir assembly to allow for variations in the culture volume. These units were wall-mounted and equipped with temperature controllers and gas burettes similar to those used on other digesters.

Expanded-Bed Digester

A prototype expanded-bed digester, employing the upflow design described above, was constructed and is shown in Figure 28. This digester contained sand as a microorganism support medium and utilized a newly designed automatic gas collecting-and-wasting-system. A fully self-priming, flexible propeller pump of 1 inch NPT port size (Gelber No. 14540-0001) was selected and connected to a 1/4-hp variable-speed motor (Bodine Nos. 594 and 598) for fluid recirculation.

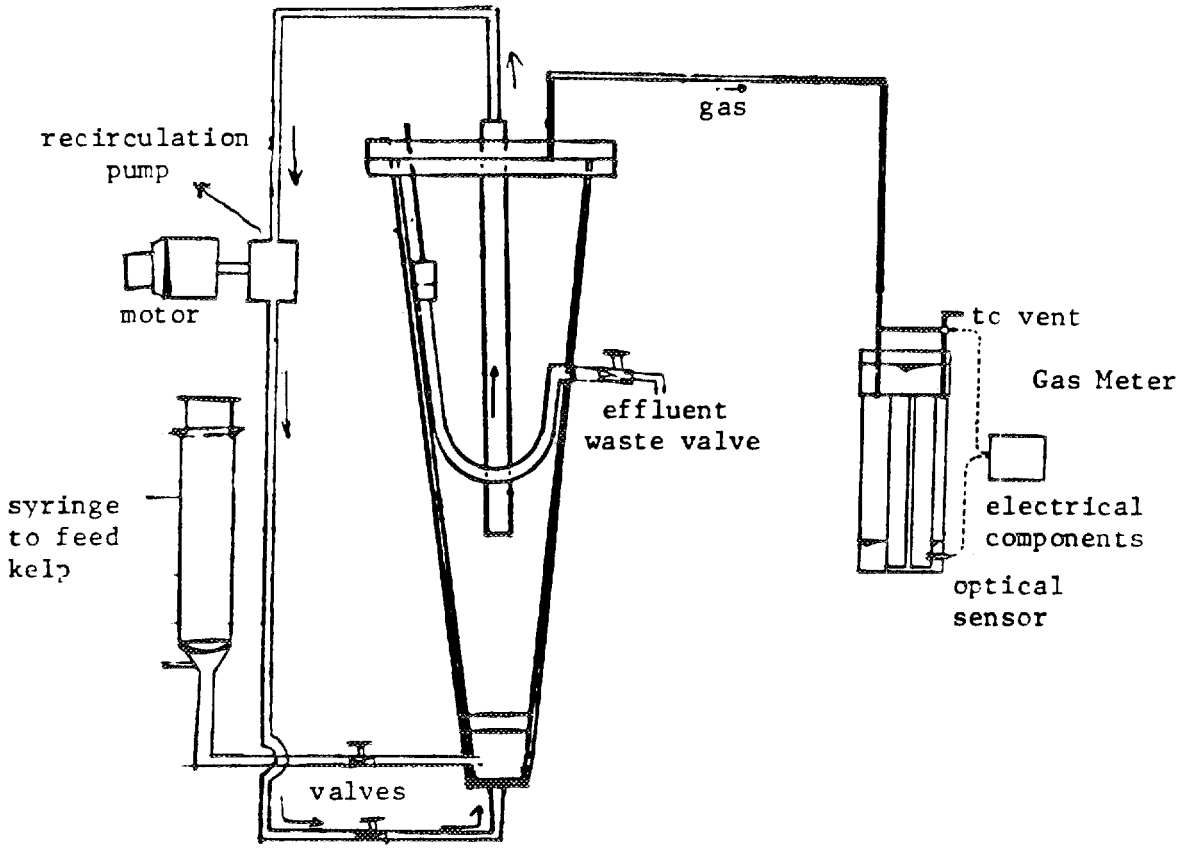

Figure 28. Schematic Diagram of Expanded-Bed Digester

Packed-Bed Digester

Using the upflow solids blanket reactor design described above, a packed-bed digester, utilizing porcelain raschig rings as a support medium, was constructed. Figure 29 shows the schematic diagram of the packed-bed digester with a connected valveless metering pump (Cole Palmer No. C-7115-70).

Dialysis Unit

A portable unit for dialysis separation for anaerobic microbial phases, similar to that reported by Hammer and Borchardt,[4] was designed and fabricated. This unit, shown in Figure 30, consists of two circular chambers (4.5 in. diameter) separated by one 0.20- μm pore size polytetrafluoroethylene (PTFE) membrane filter (Schleicher & Schuell, Keene, NH, TE 35 Type). This membrane filter was designed to perform at high or low temperatures with corrosive chemicals. The PTFE is supported with non-woven polypropylene and a stainless

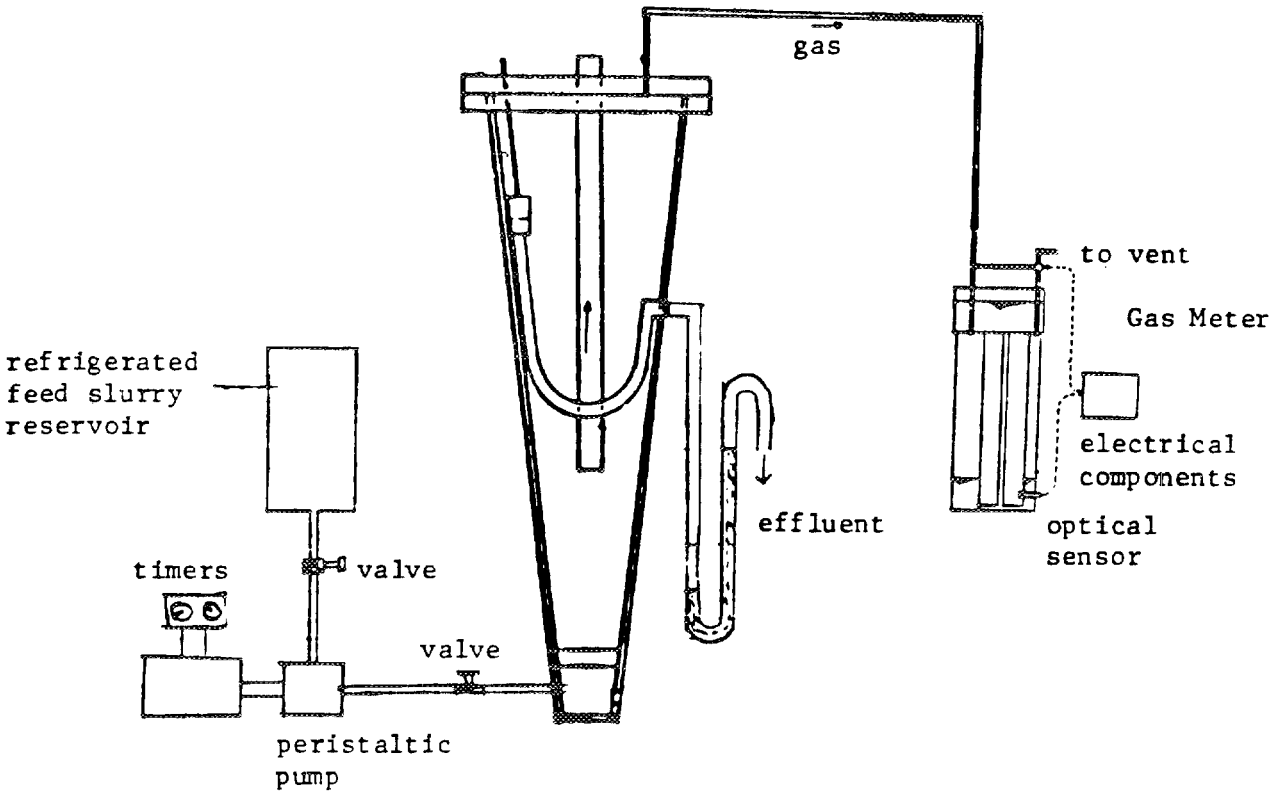

Figure 29. Schematic Diagram of Autofeed Packed-Bed Digester

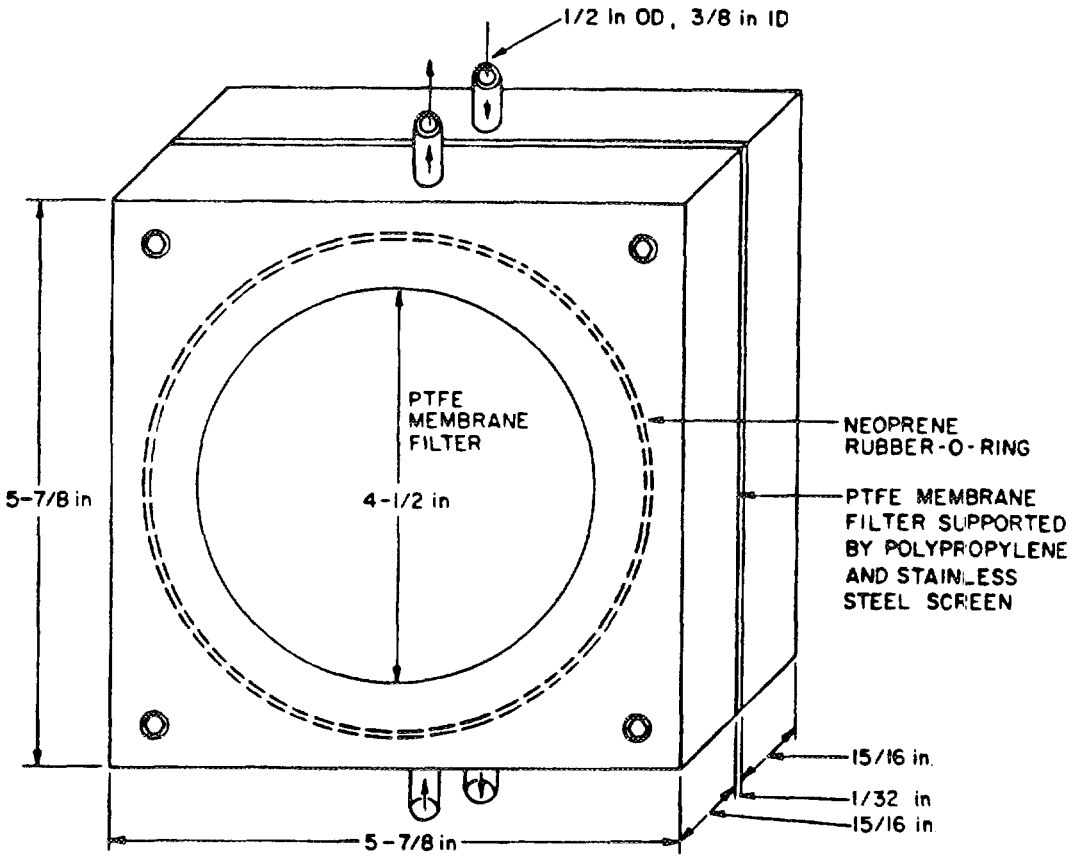

Figure 30. Basic Design of Dialysis Unit

wire screen on both faces for better stability. Both circular chambers have an inlet and outlet and are fastened together with O-ring seals and four bolts.

Autofeeder

A 10 liter baseline digester was connected to an autofeeder (Figure 31).

The digester also was equipped with an automatic gas-collecting and wasting system, and the effluent was withdrawn automatically through an overflow tube connected to the digester at the 10 liter mark. A serpentine configuration of the overflow tube prevents any gas loss through the tube. The autofeeder was modified and installed to feed fresh-water-diluted kelp with a built-in option to add nutrient or caustic. It fed kelp Lot 49 at a loading of 0.10 lb VS/ft^3-day and a retention time of 15 days (Table 17). The feeding frequency of the autofeeder at this operating condition is 21 times per day. Note that this autofeeder design

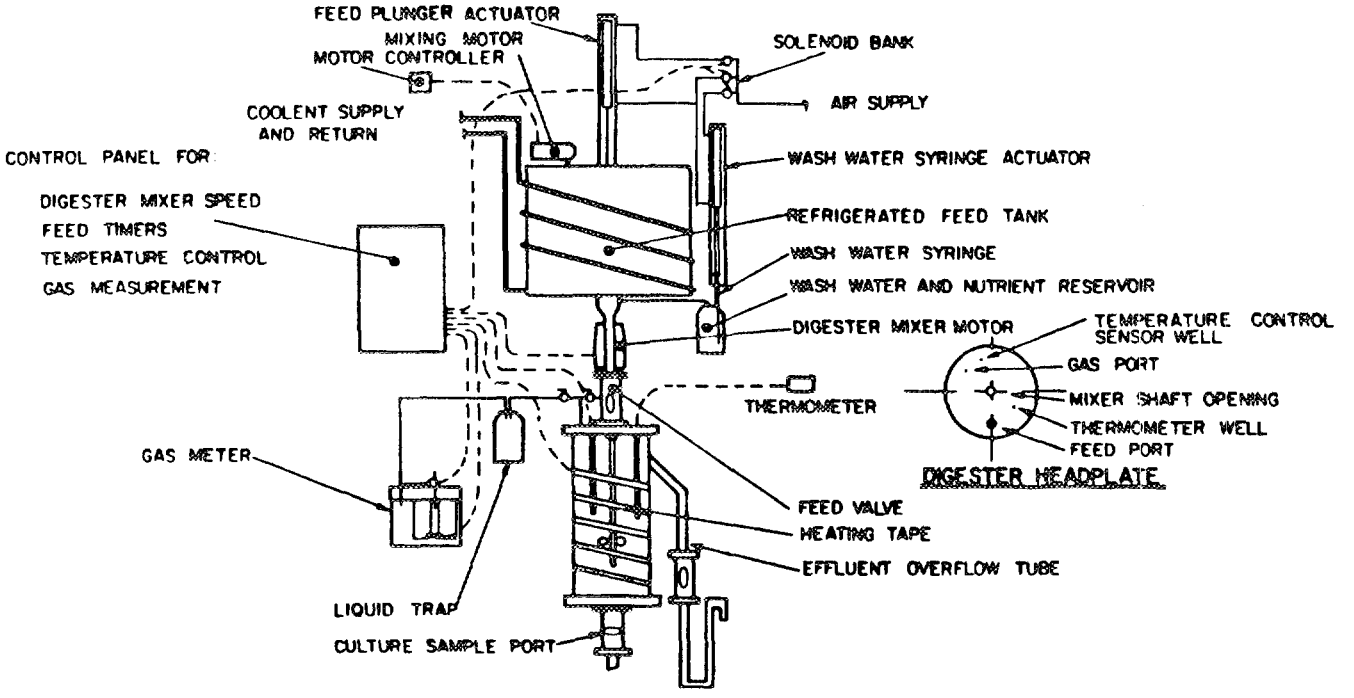

Figure 31. 10-ℓ Anaerobic Digester with Autofeeder

cannot be used for undiluted kelp because of the rinse cycle, which necessarily adds water to the feed.

The feed tank is a 12-inch-diameter stainless steel tank with a pneumatically activated plunger. It is equipped with a mixing paddle and small variable-speed motor to ensure proper concentration of feed going into the digester. The size of the paddles was increased from that of the original design to facilitate better mixing. The feed tank is wrapped with a coil connected to a refrigeration unit. The whole tank is insulated to maintain a low feed temperature.

Just below the feed plunger is a funnel connected to the digester feed valve. The inside diameter of the funnel outlet was increased from the original design to allow thick feed slurry to be used. A wash-water syringe is connected to the top of the funnel to rinse the funnel and to add nutrient or caustic to the digester. The two actuators, one for the feed plunger and the other for

wash-water syringe, are pneumatic cylinders connected to three solenoids. The two cylinders are operated by one single solenoid during the upward stroke and by two solenoids during the downward strokes (one solenoid for each cylinder). This allows a time delay in wash-water plunger. Timings for the feed cycle are controlled by electronic timers. All electronic components are assembled inside the control box.

Automatic Gas-Collecting and Wasting System

The solenoid valve (#100) acts as a set of two synchronized valves. In the "normally open" position, the digester is connected to the gas collector (while gas is collected) and the vent is isolated. Gas evolving from the digester displaces the water (or red solution) in the gas collector until the level in the side arm passes below the sensor, at which time the solenoid and counter are actuated for a length of time (about 12 seconds, determined by control). During this time, the digester is isolated from the gas collector and the vent, the fluid returns from the reservoir to the collector, and the collected gas is expelled out the vent. By the end of the interval (about 60 seconds), the liquid must have returned to the datum level. With deactivation of the solenoid, the cycle begins again as digester gas is admitted to the collector.

For automatic operation, the collector volume, V (from the datum level to the sensor level), is determined by calibration and gas production at the ambient temperature, and the hydrostatic head, h, is calculated by:

$$\text{gas collected } (T, h) = n \times V$$

where n is the number of cycles counted. Subsequent adjustment can be made for the presence of water vapor and/or the equivalent gas produced at a set of standard conditions.

To bring the positive pressure digester to ambient pressure, the "auto-off-man" switch is activated to the "man" position as required; gas expelled

during this operation must be manually accounted for. (It would be convenient to feed just after a recycle. Perhaps an alarm could be added for this purpose). Figure 32, shows the design details of the system. An optical sensor, activated by gas displacement fluid level, activates solenoid valve, which regenerates volume and frequency of gas collection. The gas is expelled from the system at the termination of the cycle.

A pressure relief valve was provided to allow the release of pressure from the digester in the event of prolonged actuation of the solenoid valve. This valve also can be utilized at feed and waste times to ascertain the status of the pressure in the digester.

Feed Characteristics

The physical and chemical characteristics of all lots of kelp used during this report period are listed in Table 17. All kelp lots had a similar moisture content; however, Lots 48 and 50 were significantly lower in volatile solids,

TABLE 17. COMPOSITION OF KELP FEEDS USED DURING REPORT PERIOD

Analyses	Lot 48*	Lot 49*	Lot 50*
Harvest Date	4/29/79	10/16/79	5/6/80
Moisture, %	88.9	87.9	88.3
Total Solids (TS), %	11.1	12.1	11.7
Volatile Solids (VS), % TS	53.8	62.7	56.3
Ash, % TS	46.2	37.3	43.7
Elements, % TS			
C	25.5	31.4	26.5
H	3.39	4.01	3.53
N	1.89	0.96	1.78
P	--	0.20	0.28
S	1.01	1.10	1.12
Mannitol, % TS**	9.06	23.9	8.27
Heating Value, Btu/lb dry	4072	5032	4275

* Raw kelp, commercially harvested by Stauffer Chemicals, and processed by U.S. Department of Agriculture, Western Regional Research Center (WRRC), Albany, Cal., and shipped to IGT in Trans-temp. containers at -5° to -15°C.
** Analysis of WRRC.

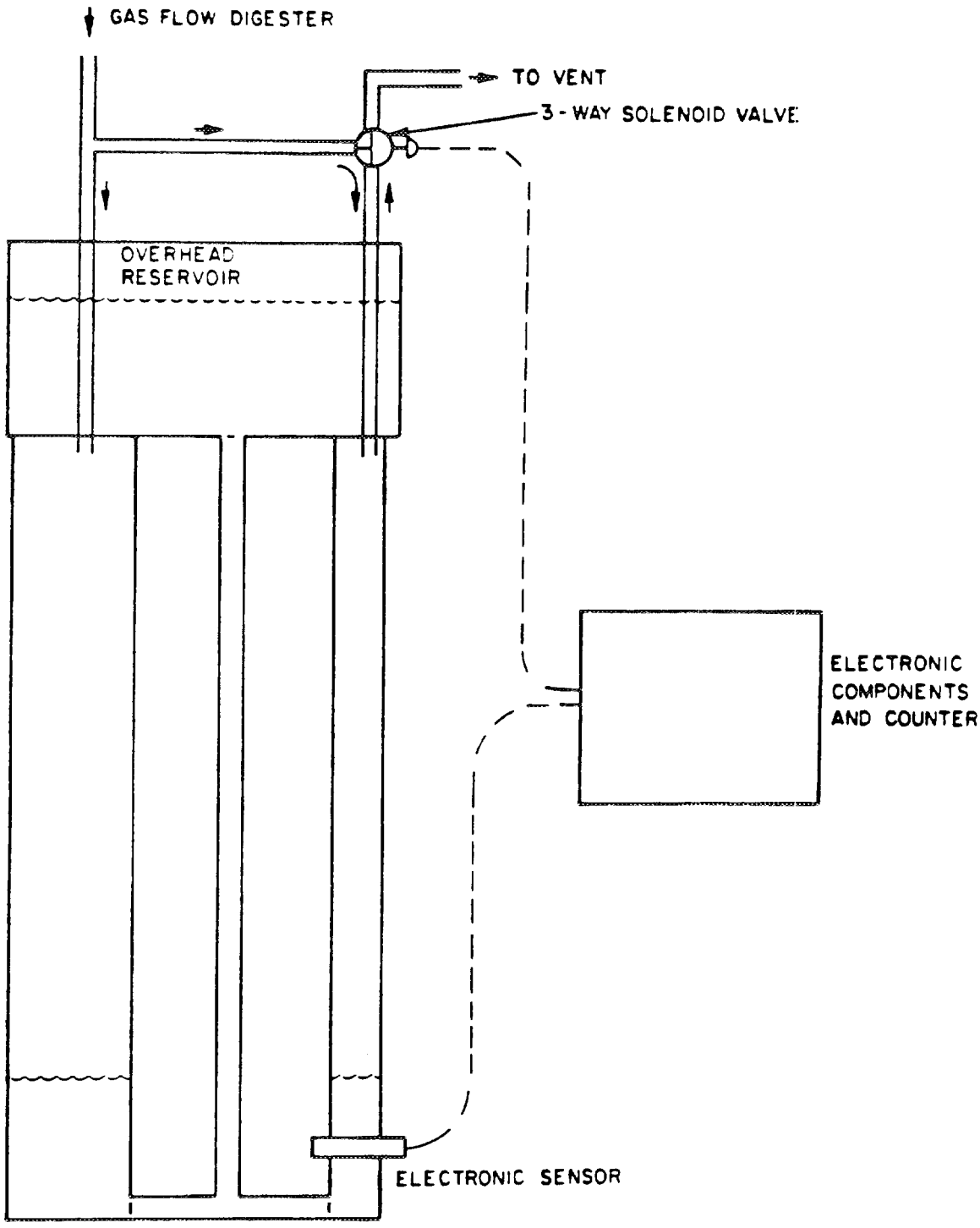

Figure 32. Automatic Gas-Collecting and Wasting System

carbon content, and heating value. Lot 49 had high volatile solids, carbon content, and heating value, but it was deficient in nitrogen and required nitrogen supplements for stable digestion. The mannitol content of the different lots showed considerable variability.

To evaluate the performance of kelp digestion runs and to provide a basis for establishing target yields, maximum theoretical yields of methane from the biomethanation of different lots of raw kelp (RK) were calculated from the data in Table 17. These yields were determined as follows: Compositional data were used to calculate the empirical formulas of each RK lot. Stoichiometric equations for the conversion of each feed to methane and carbon dioxide were determined. The resulting molar yields of methane were expressed as SCF/lb VS added. Because a fraction of organic matter in all bacterial fermentations is converted to bacteria, these yields were corrected for cell synthesis by using the data reported by McCarty and Speece[12], and McCarty[13] for the anaerobic digestion of pure carbohydrate and protein. The final theoretical methane yields are reported below in Table 18.

TABLE 18. EMPIRICAL FORMULA AND THEORETICAL METHANE YIELD FOR KELP USED DURING REPORT PERIOD

Kelp Lot	Empirical Formula[a]	Stoichiometric Yield,[b] SCF/lb VS added	Corrected Yield,[c] SCF/lb VS added
48	$C_{2.13}H_{3.39}O_{1.38}$	8.12	6.72
49	$C_{2.61}H_{4.01}O_{1.58}$	8.58	6.97
50	$C_{2.21}H_{3.53}O_{1.44}$	8.02	6.62

[a] Excluding N, P, and S.

[b] Assumes that the reactants are kelp and H_2O and the products are CH_4 and CO_2.

[c] Corrected for bacterial production, assuming 20% of soluble carbohydrate and 7% of soluble protein for cell maintenance.

The theoretical yields for the kelp lots used during this report period ranged frm 6.62 to 6.97 SCF/lb VS added. It is not surprising that these yields are directly related to volatile solids, carbon content, and heating value. The relationship between mannitol content and methane yields is discussed later.

Inocula Evaluation

Three 1.5 liter digesters (Runs 136, 139 and 140) were used to develop and evaluate the performance of the three described inocula under the same operating conditions. Runs 136 and 139, operated on anaerobic marine sediment inocula G and H, respectively, were started during 1979. Run 140, started with IGT's Inoculum A, was initiated at the end of February 1980. These runs, maintained at ambient room temperature, received undiluted raw kelp at a loading of 0.10 lb VS/ft^3-day.

Summaries of the performance of the three inocula, A, G and H, on kelp Lots 48 and 50, are presented in Tables 19 and 20, respectively. Using kelp Lot 48 feed, the methane yields were higher in the inocula G and A cultures than in inocula H. Although no substantial difference in the methane yields were observed in the three digesters after changing to kelp Lot 50 (Table 20), the highest volatile acids concentrations were found in the inoculum G digester. Observation of the performance of these three digesters, as shown in Figure 33, suggests, however, that the performance of inoculum A was more stable than that of the other two inocula when operated under similar conditions.

Mannitol Experience

During 1979, kelp Lot 46 gave a low methane yield even though digester performance was stable. The low mannitol content (5.19 percent dry weight) of this lot was hypothesized to be a major factor contributing to these low yields because mannitol is the major biodegradable kelp component. To evaluate this hypothesis, the relationship between mannitol and the theoretical and experimental methane yield for previous IGT kelp feeds and digester runs was evaluated. Based

TABLE 19. COMPARISON OF DIGESTER PERFORMANCE OF IGT INOCULUM A AND MARINE INOCULA G AND H MAINTAINED AT ROOM TEMPERATURE RECEIVING KELP LOT 48* (Culture Volume 1.5ℓ; Loading 0.10 lb VS/ft^3-day; Retention Time 40 days)

Analyses	Inoculum G	Inoculum H	Inoculum A
Run No.	136	139	140
Date Initiated	5/3/79	7/26/79	2/22/80
Data Period	5/26-6/1/80		
No. of Retention Times in Progress	4.9	4.9	2.5
Gas Yield, SCF/lb VS added	6.40	5.80	6.70
Methane Content, mol %	46.5	39.0	46.3
Methane Yield, SCF/lb VS added	2.98	2.26	3.10
Methane Production Rate, SCF/ft^3 culture-day	0.30	0.23	0.31
Total Volatile Acids, mg/ℓ as acetic	4880	5920	2930
Alkalinity, mg/ℓ as $CaCO_3$	11,040	12,800	10,660
Mean Caustic Added, meq/ℓ feed	0	0	0
Conductivity, μmhos/cm	14,000	11,700	12,500

*All runs were converted to Lot 50 in the first week of June because of the shortage of Lot 48.

TABLE 20. COMPARISON OF DIGESTER PERFORMANCE OF IGT INOCULUM A AND MARINE INOCULA G AND H MAINTAINED AT ROOM TEMPERATURE RECEIVING KELP LOT 50 (Culture Volume 1.5ℓ; Loading 0.10 lb VS/ft^3-day; Retention Time 40 days)

Analyses	Inoculum G	Inoculum H	Inoculum A
Run No.	136	139	140
Data Period	8/18-8/31/80		
No. of Retention Times in Progress	1.2	1.2	1.2
Gas Yield, SCF/lb VS added	6.32	6.03	6.76
Methane Content, mol %	49.2	49.5	47.2
Methane Yield, SCF/lb VS added	3.06	2.99	3.20
Methane Production Rate, SCF/ft^3 culture-day	0.31	0.29	0.32
Total Volatile Acids, mg/ℓ as acetic	9610	4160	4540
Mean Caustic Added, meq/ℓ feed	0	0	0
Alkalinity, mg/ℓ as $CaCO_3$	9600	8570	10,100
Conductivity, μmhos/cm	20,400	19,400	18,700

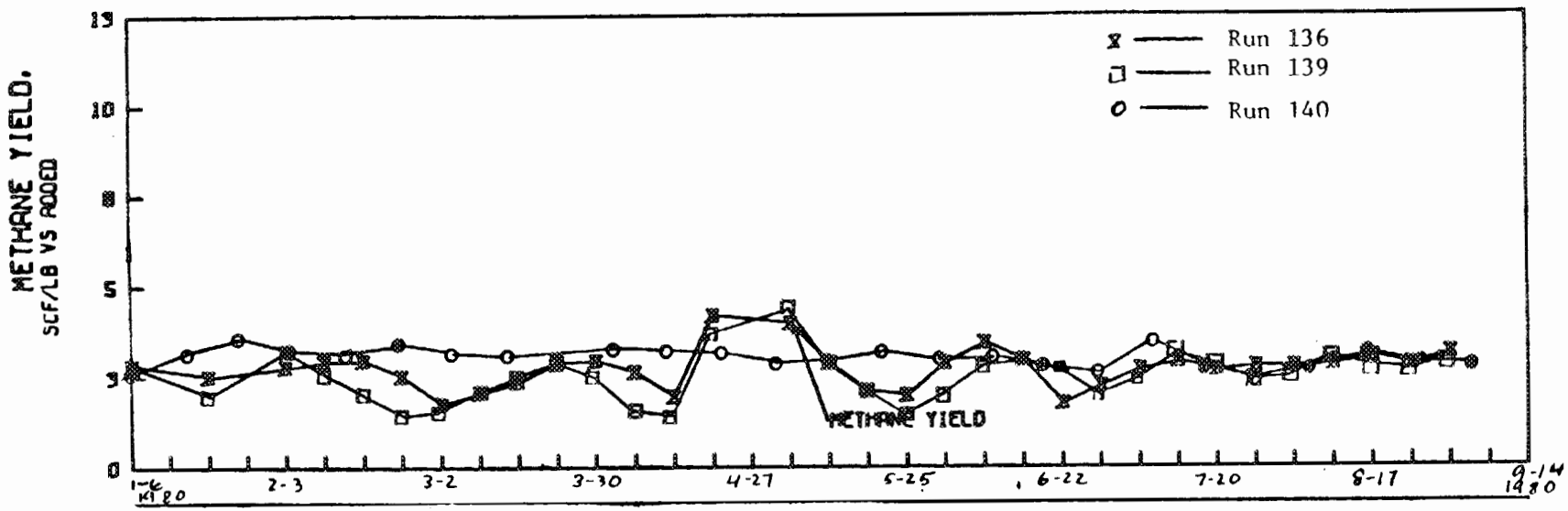

Figure 33. Methane Yield of IGT Inoculum A and Marine Inocula G and H at Room Temperature Receiving Kelp Lot 50

on the data from previous studies, a linear relationship was developed for predicting methane yield, given the kelp empirical formula (estimated from C, H, and O analysis), and the mannitol content:

$$Y_E = Y_T \quad 0.02(X_m) + 0.375 \qquad (1)$$

where

Y_E = experimental methane yield, SCF/lb VS added

Y_T = theoretical methane yield, SCF/lb VS added

X_m = mannitol concentration, % dry wt.

To experimentally confirm this model, two 1.5 liter digesters, Runs 8 and 138, were used to evaluate the effect of mannitol content on digester performance. Run 8 was initially a control run, receiving freshwater-diluted kelp Lot 48, which 9 percent dry weight of mannitol. Run 138 received the same kelp supplemented with mannitol to a total concentration of 12 percent dry weight. After collecting the steady-state data and samples in April, Runs 138 and 8 received kelp Lot 48 supplemented with mannitol to a total concentration of 15 and 20 percent dry weight, respectively. These runs reached steady state at the end of August and, for verification of this model at very high mannitol concentrations, a final set of mannitol studies were initiated by supplementing Runs 8 and 138 with 25 and 30 percent dry weight mannitol, respectively.

The performance of two digesters (Runs 8 and 138) receiving kelp feed Lot 48, containing mannitol concentrations of 9 and 12 percent dry weight, respectively, is presented in Table 21. Run 8 was fed as-received kelp, having a mannitol concentration of 9 percent dry weight, while Run 138, was fed kelp with the mannitol concentration adjusted to 12 percent. Methane yields of 3.88 and 4.23 SCF/lb VS added were observed in Runs 8 and 138, respectively. These findings are in close agreement with the calculated values, based on the model presented above, of 3.76 and 4.14 SCF/lb VS added for digesters receiving kelp containing 9 percent

TABLE 21. STEADY-STATE PERFORMANCE AND OPERATING CONDITIONS FOR DIGESTER RECEIVING KELP LOT 48 WITH 9 AND 12% MANNITOL
(Culture Volume 1.5ℓ; Loading 0.10 lb VS/ft^3-days; Retention time 15 days)

Run No.	8	138*
Data Period	4/28-5/25/80	
No. of Retention Times in Progress	4.7	2.1
Total Mannitol Concentration, % dry wt	9	12
Gas Yield, SCF/lb VS added	6.95	8.20
Methane Content, mol %	56.3	55.2
Methane Yield, SCF/lb VS added	3.88	4.23
Methane Production Rate, SCF/ft^3 culture-day	0.39	0.45
Total Volatile Acids, mg/ℓ as acetic	250	140
Mean Caustic Added, meq/ℓ feed	0	0
Alkalinity, mg/ℓ as $CaCO_3$	5300	5220
Conductivity, μmhos/cm	8500	8500

*Received feed supplemented with mannitol to a total mannitol concentration of 12% dry wt.

and 12 percent mannitol, respectively. The methane yields for the same digesters with mannitol concentrations adjusted to 15 percent and 20 percent are shown in Table 22 and were found to be 4.57 and 4.99 SCF/lb VS added. These yields are very similar to the calculated values of 4.52 and 5.21 SCF/lb VS added for kelp digesters containing 15 percent and 20 percent mannitol, respectively. When plotted on a curve (Figure 34) obtained from the previous work and used to develop the predictive model, these experimental data verify the model at the mannitol concentrations studied.

Baseline Digestion System

The objective of this on-going task, which was initiated at the end of July, is to operate a digester at optimal demonstrated performance levels. The baseline digestion system (Run 44), with 20 percent mannitol, a C/N ratio of 15, and a loading of 0.175 lb VS/ft^3-day, were initiated on kelp Lot 50 in August and preliminary data are presented in Table 23. The methane yield after 0.5 retention

TABLE 22. STEADY-STATE PERFORMANCE AND EFFLUENT QUALITY DATA FOR DIGESTER RECEIVING KELP LOT 48 WITH 15 AND 20% MANNITOL
(Culture Volume 1.5ℓ; Loading 0.10 lb VS/ft^3-day; Retention Time 15 days)

Run No.	138	8
Data Period	8/25-9/21	
No. of Retention Times in Progress	5.0	5.0
Total Mannitol Concentration, % dry wt	15	20
Gas Yield, SCF/lb VS added	8.05	8.59
Methane Content, mol %	56.7	58.0
Methane Yield, SCF/lb VS added	4.57	4.99
Methane Production Rate, SCF/ft^3 culture-day	0.46	0.50
Total Volatile Acids, mg/ℓ as acetic	160	320
Mean Caustic Added, meq/ℓ feed	0	0
Alkalinity, mg/ℓ as $CaCO_3$	4750	4780
Conductivity, μmhos/cm	17,000	19,000

TABLE 23. PRELIMINARY PERFORMANCE DATA AND OPERATING CONDITIONS OF BASELINE DIGESTION SYSTEM DURING AUGUST 1980

Run No.	44
Culture Volume, ℓ	10
Data Period	8/11-8/17
Kelp Lot	50
Loading, lb VS/ft^3-day	0.175
Residence Time, days	15
No. of Retention Times in Progress	0.5
Gas Yield, SCF/lb VS added	6.65
Methane Content, mol %	54.6
Methane Yield, SCF/lb VS added	3.63
Methane Production Rate, SCF/ft^3 culture-day	0.81
Total Volatile Acids, mg/ℓ as acetic	2680
Mean Caustic Added, meq/ℓ feed	<10
Alkalinity, mg/ℓ as $CaCO_3$	5640
Conductivity, μmhos/cm	15,600

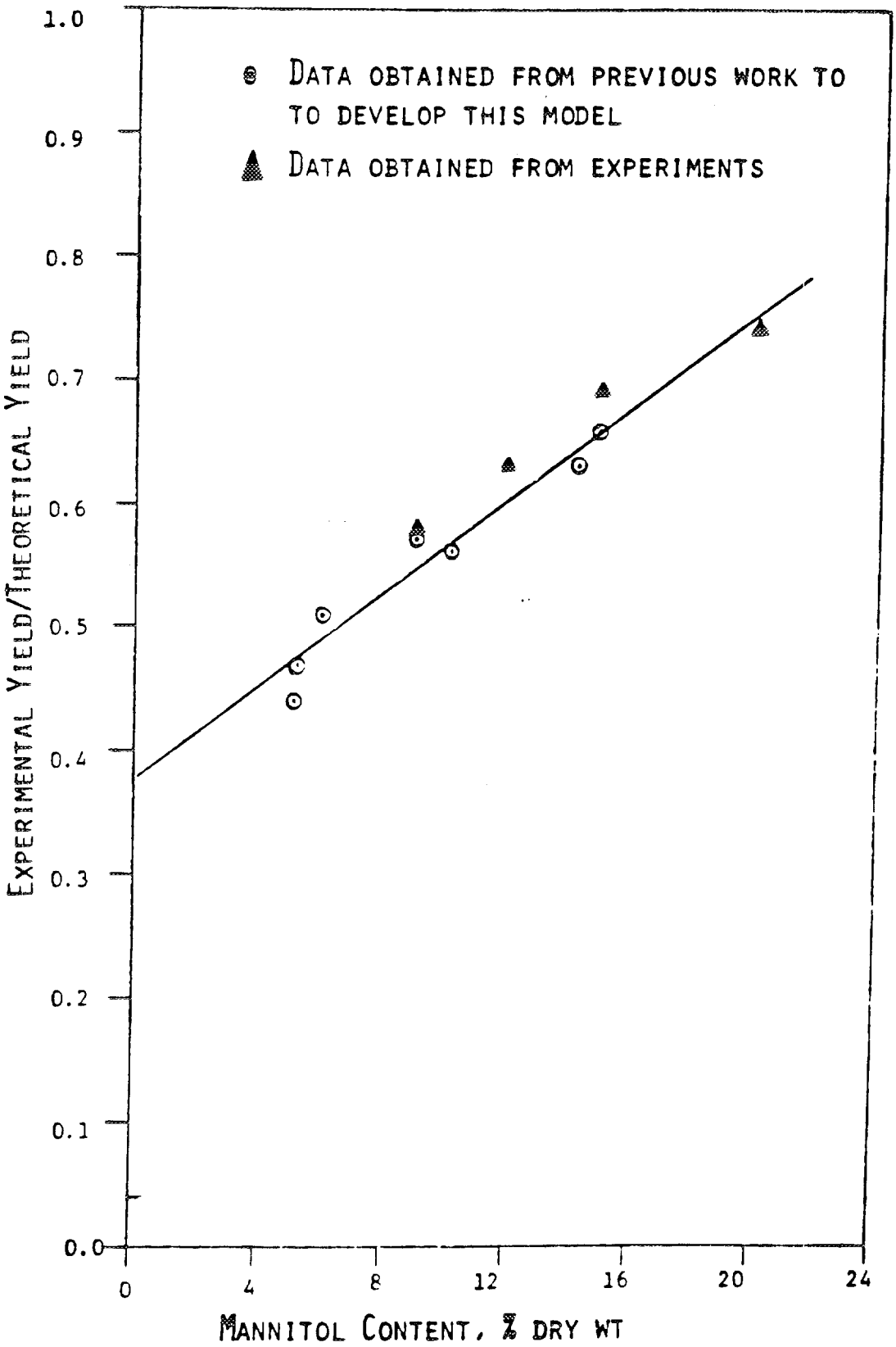

Figure 34. Effect of Mannitol Content on Methane Yield

times was 3.63 SCF/lb VS added. At the end of August, a program decision was made to redefine the baseline conditions to those which have given the highest stable methane yields from kelp on a sustained basis. These conditions, based on Run 8 with Lot 37, which showed a 4.5 SCF/lb VS methane yield and was reported in the 1978 Annual Report for this project,[3] are a mannitol concentration of 15 percent, a C/N ratio of 15, a 0.1 lb VS/ft^3-day loading, and an 18-day residence time. The baseline digestion system was adjusted to all these operating conditions, except that the residence time was maintained at 15 days. Data are not available for this report period on the performance of this digester under the redefined operating conditions.

Baseline Stock Culture Maintenance and Operation

Five 10 liter digesters, Runs 39, 41, 42, 43 and 44, were maintained for kinetic studies during the first and second quarters of 1980. However, as the program status changed from culture maintenance to normal operation, the objectives and utilization of these runs were reassessed and changed accordingly. During the first quarter, Runs 39 and 41 through 44 were maintained on kelp Lot 49, supplemented with nitrogen and phosphorus, at a loading of 0.10 lb VS/ft^3-day. Runs 41 and 42 received undiluted kelp, while Runs 39, 43 and 44 received freshwater-diluted kelp. During the second quarter, Run 41 was maintained on a twice-per-week feeding schedule at a loading of 0.05 lb VS/ft^3-day, while Runs 39, 43 and 44 were continued at a 0.10 lb VS/ft^3-day loading.

To evaluate a new kelp lot prior to its use in new digestion studies, Run 44 was converted to Lot 50 and its performance, during July, is presented in Table 24. A methane yield of 3.70 SCF/lb VS with stable digester performance confirmed the suitability of kelp Lot 50 for inclusion in other digestion studies.

TABLE 24. DIGESTER PERFORMANCE DATA AND OPERATING CONDITIONS
RUNS 44 AND 50 RECEIVING KELP LOT 50
(Loading 0.10 lb VS/ft^3-day; 15 Day Retention Time)

Run No.	44	50
Culture, Volume, ℓ	10	50
Data Period	7/7-7/27	6/9-6/22
No. of Retention Times in Progress	3.0	2.0
Gas Yield, SCF/lb VS added	6.6	6.9
Methane Content, mol %	55.6	53.0
Methane Yield, SCF/lb VS added	3.70	3.66
Methane Production Rate, SCF/ft^3 culture-day	0.37	0.37
Total Volatile Acids, mg/ℓ as acetic	1580	3450
Mean Caustic Added, meq/ℓ feed	40	0
Alkalinity, mg/ℓ as $CaCO_3$	5970	10,740
Conductivity, μmhos/cm	14,500	20,000

Another 50 liter digester was modified and installed in March 1980. It was equipped with a variable-speed motor to provide mixing in the digester, a proportional controller to maintain constant digester temperature, and an automatic gas-collecting and wasting system, as shown in Figure 32 above. This digester, operated as Run 50, was started in March from the inoculum stored in the 15-gallon polyethylene drum and received undiluted kelp Lot 49 at a loading of 0.10 lb VS/ft^3-day. Due to unstable digester performance on kelp Lot 49, this digester was converted to Lot 50 at the end of July 1980. As shown in Table 12, methane yields similar to those found in Run 44 were observed with kelp Lot 50 in Run 50. The methane yield in Run 50 when operated at a 0.10 lb VS/ft^3-day loading was 3.66 SCF/lb VS added. After the process development protocol was initiated, the other baseline digesters were operated as controls for

unconventional digesters, a baseline digestion system, or as a digester for continuous feeding studies.

One 50 liter stock culture, maintained in a 15-gallon polyethylene black drum at room temperature, received kelp Lot 49 once per week at a loading of 0.025 lb VS/ft^3-day. The digester was shaken manually once a day and the gas was permitted to escape through a water column; except once per month when the gas composition, volatile acids, and pH were measured.

Digester Design and Techniques Study

Baffle-Flow Digester

The 10 liter baffle-flow digester, Run 51, was initiated at the end of March 1980 utilizing the 50 liter room temperature digester culture as an inoculum. After initial inoculation, the baffle-flow digester received undiluted raw kelp Lot 49, supplemented with nutrients (nitrogen and phosphorus) to C/N and C/P ratios of 15 and 75, respectively, at a loading of 0.10 lb VS/ft^3-day. During the second quarter of 1980, the digester effluent was collected after settling, and the supernatant (about 50 percent of total effluent by volume) was recycled with the raw kelp as feed. After digester imbalance was observed, the supernatant recycling was terminated. During July the run was converted from the nutrient-limited kelp Lot 49 to Lot 50 and was maintained at a loading of 0.10 lb VS/ft^3-day without nutrient addition.

The baffle-flow digester did not achieve steady-state operation during this report period. During June of the initial operation of the digester, with effluent supernatant recycling, increases in volatile acids suggested digester imbalance. In an effort to reduce this imbalance, the effluent supernatant recycling was discontinued. The methane yield from this digester, with and without effluent supernatant recycling, was 2.97 and 3.32 SCF/ft^3-day, respectively, and is shown in Table 25. Although steady-state was not attained,

TABLE 25. PRE-STEADY STATE PERFORMANCE DATA AND OPERATING CONDITIONS FOR BAFFLE FLOW DIGESTER (RUN 51) AND A CONTROL CSTR DIGESTER (RUN 43) (Culture Volume 10-ℓ; Loading 0.10 lb VS/ft^3-day, Kelp Lot 50; 40-day Retention Time)

Run No.	51	43
Data Period	7/14-7/27	8/4-8/31
Gas Yield, SCF/lb VS added	7.09	6.73
Methane Content, % mol	46.9	47.5
Methane Yield, SCF/lb VS added	3.32	3.2
Methane Production Rate, SCF/ft^3-culture-day	0.33	0.32
Total Volatile Acids, mg/ℓ as acetic	7,329	4,720
Mean Caustic Added, mg/ℓ feed	22	10
Alkalinity, mg/ℓ as $CaCO_3$	8,670	8,150
Conductivity, μmhos/cm	11,500	18,100

the performance of the baffle-flow digester is compared to that of the CSTR control digester (Run 43), operated under similar conditions, in Table 25. Run 43 had a methane yield of 3.2 SCF/lb VS added, which was not substantially different from that observed in the baffle-flow digester. During July, a program decision was made to increase the loading from 0.10 to 0.175 lb VS/ft^3-day. However, this change caused substantial digester instability and a reduction in overall performance. Consequently, the loading was readjusted to 0.10 lb VS/ft^3-day during the last week of the report period.

Upflow Solids Blanket Digester

A 5 liter upflow solids blanket digester (Run 42) was initiated during June with the room temperature inoculum. The digester was fed undiluted raw kelp Lot 50 at a loading of 0.15 lb VS/ft^3-day. The methane yield of this digester and its control CSTR digester (Run 50) was relatively stable after one retention

time. As shown in Table 26, however, this methane yield was about one-third higher, 3.22 versus 2.19 SCF/lb VS added, and the volatile acids were almost 50 percent lower, 2940 versus 5570 mg/ℓ as acetic, in Run 52 than in Run 50 at 0.15 lb VS/ft^3-day loading. These comparative data shown relatively low yields with kelp Lot 50 at 0.15 lb VS/ft^3-day loading, but suggest that an upflow solids blanket digester may perform better at higher loadings than a CSTR. Studies with other kelp lots at high loadings may be necessary to attain higher methane production rates in kelp digestion.

Expanded-Bed Digester

After preliminary testing of support media, recirculation techniques, and the automatic gas collecting and wasting system, the expanded-bed digester, Run 53, utilizing sand as a support medium for microorganisms, was placed into operation during August. This digester was inoculated with room temperature culture and received kelp Lot 50 diluted in seawater to a feed slurry of 2 percent total

TABLE 26. PRE-STEADY STATE PERFORMANCE AND OPERATING DATA OF UPFLOW SOLIDS BLANKET DIGESTER (RUN 52) AND CONTROL CSTR DIGESTER (RUN 50) (Loading 0.15 lb VS/ft^3-day; Kelp Lot 50, 27 day Retention Time, 1 Retention Time in Progress, Data Period 8/18-8/31)

Run No.	52	50
Culture Volume, ℓ	5	50
Gas Yield, SCF/lb VS added	6.58	4.41
Methane Content, mol %	49.0	49.6
Methane Yield, SCF/lb VS added	3.22	2.19
Methane Production Rate, SCF/ft^3 culture-day	0.48	0.33
Total Volatile Acids, mg/ℓ as acetic	2940	5570
Mean Caustic Added, meq/ℓ feed	0	0
Alkalinity, mg/ℓ as $CaCO_3$	10,250	12,640
Conductivity, μmhos/cm	40,000	48,000

solids concentration, and was operated as a combined phase digester at a temperature of 27°C, slightly above room temperature. Due to a design defect that caused uneven solids accumulation within the reactor, it was redesigned and a cylindrical digester, without baffles, was constructed and placed into operation under the conditions described. Since this digester was started very late in this report period and was subjected to shakedown operation, data are not reported.

Packed-Bed Digester

The packed-bed digester (Run 54) was initiated late in the project period as a methane-phase digester after inoculation with a room-temperature inoculum. The digester was operated at 35°C on the effluent supernatant from an ongoing baseline digester (Run 50). During the initial shakedown stages, the heating tape used to control digester temperature caused a weakening of a glued joint of the reactor. Consequently, the digester was shut down, the joints were reinforced, and the heating mechanism was redesigned. Although the digester was restarted, reportable data are not available for this report period.

Acid-Phase Digester

To achieve feed hydrolysis and acids production for subsequent conversion in a methane-phase digester, an acid-phase digester (Run 42) was started by increasing the loading rate. As shown in Figure 35, the volatile acids concentrations in the digester increased to approximately 13,530 mg/liter as acetic and then dropped sharply. As the pH dropped below 5.0, the conventional indicators of digester performance, including acids and gas production, suggested microbial inhibition in the digester. After critical evaluation of the digester performance, it was left, without pH adjustment, until evidence of anaerobic digestion was absent. An additional acid-phase digester, with pH controlled at approxmately 6.0, is planned during the next contract period to maintain high

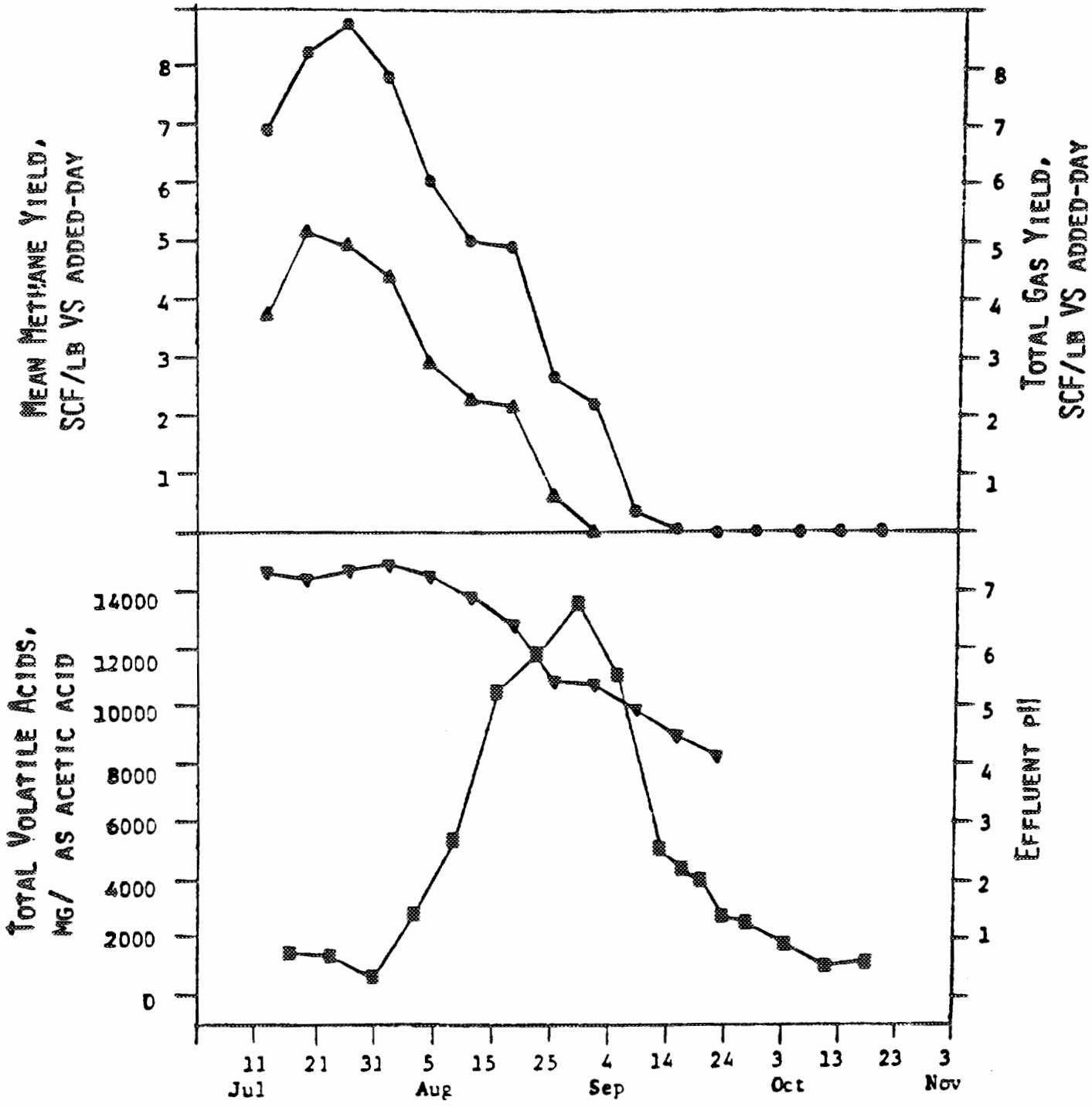

Figure 35. Acid Phase CSTR Digester Performance Curves
(Undiluted Kelp, L = 0.3)

acids concentrations in the system. More analytical work is needed to characterize the operation of acid-phase digesters.

Dialysis Unit

The objective of this task was to select the optimum membrane for acid dialysis and microbial phase separation. The dialysis of an acid-phase digester effluent with a selectively permeable membrane will allow passage of the soluble acids through the membrane concurrently with solids retention. This procedure can be used to remove acids from imbalanced digesters or to optimize digestion kinetics by promoting effective phase separation of the two-phase digestion process.

The first preliminary testing and shakedown of this unit was performed with the configuration illustrated in Figure 36. The capacity of the system to dialyze volatile acids from an undiluted kelp-fed digester was evaluated. The acids were dialyzed into degassed distilled water to which sodium sulfite had been added (0.2 g/liter) to remove dissolved oxygen that would inhibit activity in the digester. During July and August Run 41 received undiluted kelp Lot 20 on a daily feeding schedule at a loading of 0.10 lb VS/ft^3-day and was used for dialysis studies. Several types of membranes were examined in the process of selecting the optimum membrane. Out of approximately 15 selected types tested, the best performance was obtained by a regenerated cellulose membrane (Sartorius membrane filter, Catalog No. 11533), pore size less than 0.005 rpm. Due to change in the program of study at IGT, this experiment was interrupted during September 1980.

Autofeeder

After a short period of continuous operation, the autofeeder developed several problems that affected both its operation and digester performance. The autofeeder did not deliver the design volume of 26 ml feed slurry into the digester with each feed cycle. This problem was corrected by modifying the rinse

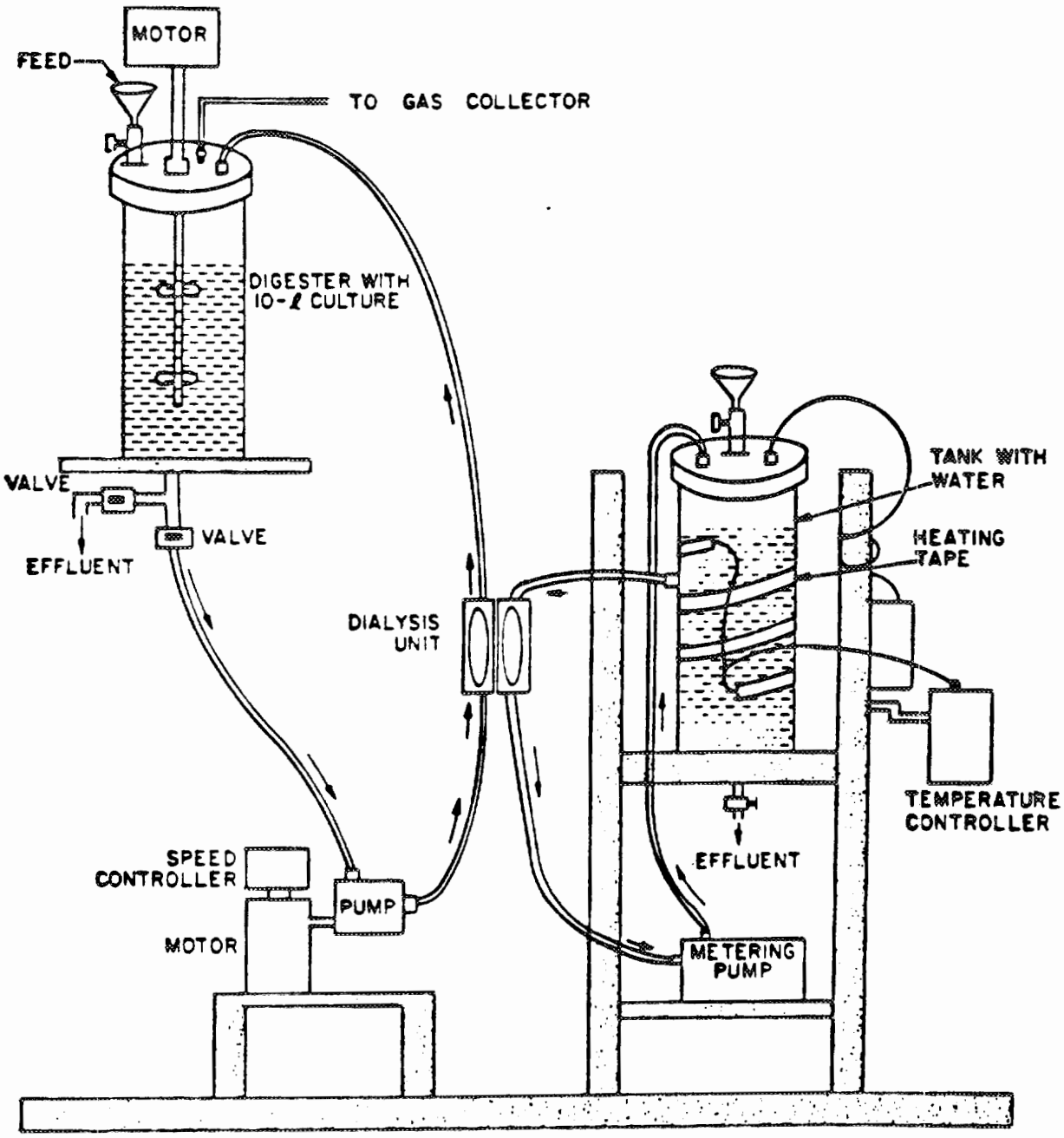

Figure 36. Schematic Diagram of Dialysis Unit for Microbial-Phase Separation or Acids Removal

line and replacing the conical funnel with a cylindrical connector. The minimum achievable temperature in the feed tank was about 12°C, which was not low enough to prevent degradation of the kelp prior to feeding. The refrigeration capacity of the system was improved by additional copper tubing around the feed tank and by a mixture of ethanol, methanol, and water as the coolant. The feed slurry can now be maintained in the temperature range of 2° to 3°C.

Run 39, a 10 liter baseline digester, was connected to the autofeeder during the first quarter. This digester received kelp Lot 49 supplemented with nitrogen and phosphorus nutrients, to a target loading of 0.10 lb VS/ft^3-day and a retention time of 15 days. The digester gas composition, weekly effluent quality data, daily pH, and temperature were collected regularly.

Because of several defects in the autofeeder, the loading and retention times were not stabilized. The digester performance (Run 39) was, however, generally poor and suggested the presence of either gas leaks or microbial toxicity. After assuring the absence of leaks in the system, a bioassay was performed to determine potential toxicity of the storage tank feed slurry to a viable microbial inoculum. The results of this bioassay showed a 50% reduction in total gas production with the storage tank feed compared with a freshly prepared feed slurry. This reduction in total gas production was attributed to toxicity resulting from heavy metals, particularly Fe, Cr, and Ni, were found in the feed after storage in the autofeeder reservoir. Because of these problems, Run 39 was disconnected from the autofeeder on July 28 and was operated on a new lot of kelp, Lot 50, on a daily-fed basis. Additional designs for autofeeder construction, with consideration of feed milling to reduce materials handling problems, were initiated.

TABLE 27. HEAVY METALS IN KELP, STORED AND UNSTORED IN AUTOFEEDER RESERVOIR

	Fe	Cr	Ni
	μ g/g of feed		
Unstored	320	87	4.8
Stored	2800	303	12

WORK PERFORMED UNDER SERI CONTRACT

(SEPTEMBER 1980 TO DECEMBER 1980)

The anaerobic digestion systems design and process development protocol continued to address the technologies for the improvement of methane production rates by increasing organic loading rates without sacrificing methane yields. Toward this goal, the following activities were initiated or continued during the period of September 1980 through December 1980.

1. Stock Culture Maintenance and Baseline Culture Operation

This task is intended to ensure the availability of stock and baseline cultures to participating investigators in all related areas of research and development. This maintenance of a bank of cultures must continue at several other locations also, to ensure against catastrophic events which may impose serious threats to program progress. This task involves maintenance of stock cultures on raw kelp feedstocks and continuous operation of conventional digesters as baseline cultures to serve as controls for the various unconventional digestion systems under investigation.

2. Baseline Digestion System

The objective of this task is to operate a digester at optimal demonstrated performance levels.

3. Digester Design and Technique Study

The objective of this task is to improve loading rates and methane yields by utilizing innovative digester designs operated as combined or as two-phase systems. Digester designs to be studied include CSTR, plug-flow, upflow solids blanket, expanded-bed and packed-bed as combined or single-phase systems. This task involves the construction of several digesters and associated equipment and operation of these unconventional digesters in the configuration shown in Table 28.

TABLE 28. OUTLINE OF PROPOSED DIGESTER DESIGN AND TECHNIQUES STUDY SCHEMES

Combined	Acid Phase	Methanation Phase	Comments
1. --	CSTR	CSTR	Solids separation by settling.
2. --	CSTR	Packed bed reactor	Solids separation by settling and vacuum filtration, or by centrifugation.
3. Upflow solid blanket reactor	Upflow solid blanket digester		
4. Plug flow			--
5. Expanded bed reactor			Seawater-diluted kelp feed.
6. --	CSTR	Expanded bed reactor	Solids separation by settling.
7. --	Upflow solid blanket reactor	Packed bed reactor	Solids separation by settling and vacuum filtration, or by centrifugation.

4. Effect of Mannitol Concentration

Ongoing experiments on studying the effect of mannitol concentration on methane yield under baseline conditions were completed during this time frame. This task involved continuous operation of two digesters up to December 1980.

5. Autofeeder Design

An autofeeder was designed, constructed, and tested for feeding undiluted kelp to bench-scale reactors. It involved developing a few concepts and selecting one for complete evaluation.

6. Continuous Feeding Studies

This task will be initiated after the completion of Task 5. The objective of this task is to determine the effect of feeding frequency and provide a correlation for data between semicontinuous or batch feeding and continuously fed digesters.

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5.5 PRE-TREATMENT AND POST-TREATMENT DEVELOPMENT

WESTERN REGIONAL RESEARCH CENTER

5.5 PRE-TREATMENT AND POST-TREATMENT DEVELOPMENT

The overall objectives for WRRC were to develop the necessary processes and supportive engineering and cost data for the storage, transportation, and preparation of kelp for fermentation and for the utilization of fermenter effluent.

Due to low level funding, WRRC was asked to operate in a maintenance mode in 1980. Consequently, 1980 objectives were to obtain and supply suitable prepared kelp, maintain existing digesters, and collect and accumulate liquid effluent for future studies. These have been formalized as Kelp Supply; Bench Reactor Maintenance; and Digester Effluent Supply and are outlined below.

Kelp Supply

The various agencies (GE, IGT, and WRRC) investigating kelp fermentation have operated their digesters on kelp grown in natural beds off the California coast. Because of its proximity to the kelp beds and its processing facilities, WRRC was the logical agency to supply kelp for the Marine Biomass Program needs. To meet these needs, WRRC has made a considerable number of relatively small harvests between 1975 and the present. In effect, this has resulted in a somewhat random sampling of the kelp beds WRRC has access to.

A substantial data base has been developed over this period regarding the natural variation in kelp composition and its affect on fermentation. When a digester is fed a new kelp lot, which differs substantially in composition from its previous feed, an extended period of adaptation occurs before steady conditions return. Kelp lots with a nitrogen content such that their C to N ratio is less than 15 have caused severe digester upset. Kelp lots with a high mannitol content (e.g. 24%) have also caused digester upset during the adaptation period. However, mannitol is almost completely digested (95%+) under current operating conditions, and both a high mannitol and high volatile solids content (VS) are desirable from the standpoint of potential methane yield per pound of kelp. Now

that this essential data base has been developed, it is apparent that further work can more efficiently be done by utilizing much larger kelp lots. A single kelp lot, chosen for a desired composition, might supply all agencies for one or more years of digester operations. All agencies would then be using the same kelp lot, and direct comparison of results could be made. Time lost for research while a digester adapts to new feed would be minimized. Finally, digester upset caused by kelp composition could be avoided. Because of these considerations, WRRC performed a large scale-production harvesting operation in the latter part of 1980 in addition to its regular smaller scale research harvests.

Kelp Compositional Requirements

Kelp, like all other agricultural crops, exhibits considerable seasonal variation as shown in Tables 29-32. Nitrogen contents have varied from 0.88 to 2.62 percent, mannitol from 5.18 to 23.97 percent, and volatile solids from 52.65 to 64.84 percent. Some generalizations can be made from the data, although considerable individual variations are present. Monthly mean values from all harvests were computed and plotted. More sampling would probably be necessary to support a statistical evaluation of the data. However, the trends seem consistant. Nitrogen content was highest in the winter months of December through February and lowest in the summer months of June through August. Mannitol and volatile solids were similar to each other, being lowest in late winter and highest in late summer. It should be emphasized that these conclusions are tentative. However, it does appear that late summer or early fall would be the most likely time to obtain a composition relatively high in mannitol and volatile solids yet with enough nitrogen to achieve a C/N ratio of 15.

Bench Reactor Maintenance

In 1980, WRRC operated a 100 liter and a 50 liter digester for the entire year and operated a 10 liter digester for part of the year. This task consisted of

maintaining these cultures under baseline conditions of a loading of 0.15 lbs VS/ft^3-day (using diluted feed), a 15 day hydraulic retention time, and 35^{o}C. These conditions will be changed in 1981, upon the recommendations of IGT. Undiluted feed will be used and loading will be reduced to 0.10 lbs VS/ft^3-day. It is felt that the reduced loading will alleviate, to some extent, the increased stress resulting from using undiluted feed.

Several kelp lots were utilized as feed over the course of the year, with all digesters being fed from the same kelp lot in use at a particular time. Diluted Lot 48 kelp was fed at the beginning of the year with Lots 50 and 49 fed at successively later dates during the year.

The 100 liter digester was operated with continuous stirring throughout the year. It was very stable up to the end of the third quarter even though several kelp lots were used as feed. However, these kelp lots, 48 and 50, were very similar in composition. When Lot 49, containing much higher mannitol content and requiring nitrogen supplementation, was introduced, severe digester upset occurred. Volatile fatty acid (VFA) content rose to about 5000 mg/l as HOAc. Feeding was stopped at that time, and the culture was starved until VFA content dropped below 2000 mg/l. At that time, loading, using a 50/50 mixture of Lots 49 and 50, was resumed at a low level and slowly brought up to 0.10 lb VS/ft^3-day. The kelp mixture was used to minimize the adverse effect of Lot 49 with the goal of gradually converting to 100 percent Lot 49 as the culture became adapted to the new feed. However, a later decision has been made that all agencies will use Lot 53 if an IGT test fermentation of Lot 53 proves successful.

During stable operation (first quarter throgh the 9th week of the third quarter), mean gas yield rose from 6.3 SCF/lb VS to 6.6 SCF/lb VS while mean-methane yield dropped from 2.9 SCF/lb VS to 2.7 SCF/lb VS. Methane content

of the gas dropped from about 47 percent to about 42 percent. VFA content varied between 1600-2000 mg/l.

A 50 liter digester was just being started at the beginning of the year and full loading was not reached until late in the first quarter. Shortly after this, the digester operation was changed from continuous stirring to intermittent stirring (5 min/hr) in order to observe the effect of intermittent stirring. Stable operation ensued for the second and most of the third quarter until Lot 49 was fed. Severe digester upset then developed with the VFA content eventually rising to over 7000 mg/l. Feeding was stopped, and the digester has been held this way to force down the VFA content. Although it has dropped substantially to about 3500 mg/l, it is still too high to resume feeding. Efforts to restore this digester to satisfactory operation are continuing.

In the second quarter, mean gas and methane yields and methane content were virtually identical to those of the 100 liter digester and ranged from 2500-3000 mg/l. In the third quarter, until Lot 49 was fed, mean gas and methane yields were 7.0 and 3.0 SCF/lb VS, respectively, slightly higher than the corresponding 100 liter digester values of 6.6 and 2.7 SCF/lb VS. Methane content was virtually identical to the 100 liter digester while VFA content dropped to less than 1000 mg/l, substantially better than the VFA content of the 100 liter digester. Based on these parameters, an intermittently stirred fermenter performed at least as well as a continuously stirred fermenter.

The 10 liter digester was not as stable as the 100 liter digester. A mild upset occurred at the beginning of the first quarter. Loading was reduced to 0.125 lb VS/ft^3-day for a short time, coupled with pH control, to regain stable operation. A substantial gas leak developed in the second quarter at which time the culture was discarded and the digester shut down for reconditioning. Reconditioning has been completed, and this digester can be reactivated when needed by charging with several days' effluent from the 100 liter digester.

Methane content was very similar to that of the 100 liter digester during the period of operation. However, mean gas and methane yields were 5.8 and 2.8 SCF/lb VS, respectively, substantially lower than those of the 100 liter digester. These lower values were probably a result of a gas leak which became apparent in the second quarter. Methane content was very similar to the 100 liter digester. VFA content was somewhat higher, varing from 2500-3000 mg/l.

Digester Effluent Supply

Liquid effluent was to be separated from solid effluent and accumulated for eventual use in studies utilizing it as a nutrient source for kelp. This task was initiated shortly before the digesters experienced their severe upset at the end of the third quarter. Collection of liquid effluent was suspended until the digesters return to steady-state at which time accumulation will be resumed.

WORK PERFORMED UNDER SERI CONTRACT

(SEPTEMBER 1 - DECEMBER 31, 1980)

Research Harvests

During 1980, WRRC performed four small scale harvest/processing runs. Local kelp (Santa Cruz area) was hand-harvested by WRRC personnel. California issues harvesting permits for scientific purposes but restricts harvesting to hand methods. A 32 foot boat, the "Scammon", was graciously provided, at cost, by the Marine Studies Department of the University of California at Santa Cruz, for harvesting. The kelp was cut and approximately 50 pounds placed in plastic bags. Harvesting was done in the morning; afternoon winds and swells made harvesting too difficult. Upon returning to dock, the kelp was iced and trucked to WRRC, arriving by mid-afternoon. Kelp was stored overnight under ice for next day processing.

Southern California kelp was obtained from a commercial harvester, Stauffer Chemical Company. Stauffer Co. was most helpful and cooperative, allowing us to obtain their last harvested kelp (first unloaded). Kelp was placed in wooden bins, covered with a tarpaulin, and trucked overnight under ambient temperature (50-60^{o}F) to WRRC.

Processing was typically done the day after harvesting. Kelp was ground, using a Master Crusher impact mill, and collected in 50-200 gallon tanks. The slurry was then packaged and frozen in WRRC's blast freezer. Frozen kelp was stored at WRRC and distributed as needed.

Production Harvests

Three year requirements of the various agencies indicated that a total of 50,000 pounds would suffice. Examination of previous data indicated September throgh October would give the best chance of obtaining the most desirable

composition. Further considerations were the weather and the condition of the kelp beds. Winter storms and rough seas generally begin during or shortly after October in this area, and they greatly reduce the kelp canopy. Not only would this make harvesting very difficult, there might not be enough kelp to supply the desired amount after October. Consequently, the decision was made to proceed with harvesting in October, even though this presented considerable difficulties in terms of scheduling conflicts with other research at WRRC and very little time to procure necessary harvesting equipment.

Before the production harvesting began, it was necessary to sample the local kelp beds in order to determine kelp composition and identify kelp species. Both local kelp beds (Soquel Point and Santa Cruz Point) were sampled and composition determined as suitable. Marine biologists from Neushul Mariculture identified Santa Cruz Point as containing Macrocystis pyrifera and Soquel Point as containing M. integrifolia. The Santa Barbara area, where Stauffer would harvest, was principally M. augustifolia. Consequently, harvesting was limited to the Santa Cruz Point bed, the only available source of M. pyrifera.

Since 50,000 pounds was much too large to harvest and process in one day, it was decided to extend operations over several days. Composition of an individual bed was unlikely to change much over a short period of time. Goal of the operation was to complete the harvesting in 10 days or less.

The basic harvesting and processing procedure, described for the research harvests, had to be extensively modified in order to achieve the greatly increased amounts envisioned for the production harvest. The Scammon's first trip to the kelp bed began each morning between 7:30 - 8:00 am; three to four trips were made each day to the kelp bed. Three people were employed by the University and, with a WRRC employee, comprised a harvesting crew of four members. Two crewmen cut the kelp and pulled it onboard. The remaining crewmen piled the kelp on a 12 foot

square cargo net spread out on the deck. A maximum of two nets could be filled at a time, after which the boat returned to the dock. A crane was used to hoist each net, holding about 950 pounds of kelp, and carry it to a truck where it was loaded. Kelp in the truck was covered with a tarpaulin. Harvesting ceased upon the onset of afternoon winds and heavy seas. Each day's harvest was trucked to WRRC, arriving between 3:00 - 5:00 pm, and was transferred to wooden bins and covered for overnight storage at ambient temperature (50-60^{o}F).

Each day's harvest was processed the following day by grinding it through a Master Crusher impact mill. Ground slurry was collected in a 200 gallon tank. Emough kelp was ground at a time to fill the tank. Slurry was packaged by pumping out of the tank into waxed paper tubs with pull tab lids. Pulp and juice fractions of the slurry separate with time so occasional mixing was required. When mixing was needed, the slurry was continuously recirculated by pumping back into the tank. A composite sample of each day's harvest was obtained by taking abount 50 cc of slurry from approximately every 20th tub. Filled tubs were boxed and palletized. Processing was finished by approximately 2:00 pm each day at which time the boxed kelp was trucked to a commercial facility for freezing and storage. Packaged kelp was in 0^{o}F storage by 4:30 pm each day.

Harvesting was done on October 21, 22, and 28, and processing done on October 22, 23, and 29. One harvest, scheduled for October 27, was cancelled due to rental equipment failure. No other harvests could be scheduled between October 22 and 28 due to the required use of WRRC pilot plant facilities for other research programs, and no harvests were made after the 28th because of a drop in mannitol content of the kelp.

Composition of harvests on October 21 and 22 was very similar (Lots 53-1 and 53-2 in Table 30). Both nitrogen and mannitol were relatively high, 1.8 percent

and 19-21 percent, respectively, which was desirable. Nitrogen content of the October 28 harvest (Lot 54) was even higher at 1.9 percent, but mannitol content was substantially lower at 15 percent than Lots 53-1 and 53-2. Lots 53-1 and 53-2 are similar enough to be combined as one lot. This will be distributed to the agencies. Lot 54 was kept separate and stored for possible future use.

A total of approximately 10,800 pounds was the combined net weight of Lots 53-1 and 53-2. IGT will receive approximately 6,700 pounds by refrigerated truck, approximately 900 pounds to GE will be shipped by air freight, and the remainder will be kept by WRRC. Lot 54 weighed approximately 3,100 pounds.

Although the combined harvest fell short of its goal, enough kelp was prepared for about one year of operation. The experience gained should result in a shorter harvest time per boat load in the future. More trips could be made in a day-daily harvests of about 5 tons appear more feasible. With enough lead time, a number of consecutive harvest days could be scheduled so that a substantial amount of kelp could be obtained before kelp composition shows much change.

In addition to the above work, all 50 and 100 liter digesters were maintained during the September through December time period.

TABLE 29. COMPOSITION OF KELP LOTS HARVESTED OFF SOQUEL PT., CALIFORNIA
RESULTS REPORTED ON A DRY BASIS

Lot No.	Harvest Date	% Ash	% Volatile Solids	% Nitrogen	% Fat	% Fiber	% Mannitol	% Algin
12	11-17-75	--	--	2.59	1.45	5.12	11.50	9.50
13	11-19-75	36.37	63.63	2.36	0.75	4.42	14.10	9.40
14	12-09-75	39.91	60.09	2.34	1.50	4.71	13.60	9.30
15	12-12-75	44.40	55.60	2.62	2.00	4.97	9.70	8.40
16	02-09-76	47.35	52.65	2.37	1.47	4.87	6.30	8.60
17	02-10-76	45.56	54.44	2.46	0.82	4.60	8.60	9.40
18	02-23-76	47.44	52.56	2.40	1.19	5.27	7.00	9.10
19	03-26-76	40.08	59.92	1.35	1.40	5.18	18.10	10.40
20	05-06-76	46.14	53.86	1.94	0.88	5.64	--	--
21	05-20-76	43.14	56.86	1.23	1.45	5.80	15.40	12.50
22	06-09-76	38.81	61.19	1.02	0.86	5.63	19.90	11.80
24	06-30-76	40.63	59.37	1.79	0.92	6.15	14.90	10.60
26	09-30-76	41.59	58.41	1.59	1.22	6.08	14.30	17.04
37	10-25-77	38.88	61.12	1.90	1.06	6.18	14.89	17.69
42	09-12-78	40.00	60.00	1.24	0.57	6.76	13.05	17.49
43	10-11-78	43.75	56.25	2.47	1.30	6.53	8.64	12.63
44	11-01-78	43.61	56.39	1.87	1.03	5.44	10.20	16.90
46	01-24-79	47.01	52.99	2.03	1.55	6.57	5.19	18.97
47	03-21-79	45.44	54.56	2.15	0.89	5.32	5.18	18.87
51	08-18-80	37.04	62.96	1.00	1.22	5.21	24.86	18.84
52-2	10-01-80	36.36	63.64	*	*	*	22.37	14.14

* Analyses not completed.

TABLE 30. COMPOSITION OF KELP LOTS HARVESTED OFF SANTA CRUZ PT., CALIFORNIA
RESULTS REPORTED ON A DRY BASIS

Lot No.	Harvest Date	% Ash	% Volatile Solids	% Nitrogen	% Fat	% Fiber	% Mannitol	% Algin
4	08-20-75	39.27	60.73	1.87	0.74	6.37	--	--
5	09-03-75	35.86	64.14	2.17	0.75	5.70	--	--
6	09-17-75	39.44	60.56	1.93	1.11	5.75	--	--
7	09-30-75	36.55	63.45	1.84	1.07	5.69	19.50	--
10	11-05-75	39.04	60.96	2.10	1.59	5.84	13.80	12.00
52-1	10-01-80	39.53	60.47	1.44	*	*	20.01	16.23
53-1	10-20-80	39.35	60.65	1.80	0	5.67	21.45	12.38
53-2	10-21-80	38.98	61.02	1.83	0	6.07	19.66	12.54
54	10-28-80	41.78	58.22	1.91	*	*	15.35	13.08

* Analyses not completed

TABLE 31. COMPOSITION OF KELP LOTS HARVESTED OFF SOUTHERN CALIFORNIA
RESULTS REPORTED ON A DRY BASIS

Lot No.	Harvest Date	% Ash	% Volatile Solids	% Nitrogen	% Fat	% Fiber	% Mannitol	% Algin
41	06-06-78	38.53	61.47	1.52	0.93	5.89	19.67	14.26
45	11-28-78	37.37	62.63	0.88	0.99	5.65	21.64	19.48
48	04-28-79	46.85	53.15	1.95	1.08	6.24	8.83	13.21
49	10-16-79	37.10	62.90	1.06	0.20	5.30	23.97	14.45
50	05-06-80	43.74	56.26	1.92	0.68	5.96	8.27	19.06

TABLE 32. COMPOSITION OF KELP LOTS HARVESTED OFF MONTEREY, CALIFORNIA
RESULTS REPORTED ON A DRY BASIS

Lot No.	Harvest Date	% Ash	% Volatile Solids	% Nitrogen	% Fat	% Fiber	% Mannitol	% Algin
1	07-16-75	35.16	64.84	1.16	0.57	5.75	--	--
2	07-23-75	33.93	66.07	0.87	1.04	5.81	--	--
3	08-06-75	36.70	63.30	1.11	1.01	5.64	21.80	--
8	10-23-75	46.30	53.70	2.20	2.03	7.67	--	--
23	06-24-76	39.17	60.83	1.90	1.20	6.31	11.70	12.85
25	07-13-76	39.00	61.00	1.34	0.84	5.53	18.89	11.05
27	01-17-77	44.50	55.50	2.23	1.11	6.05	--	--

5.6 NEW YORK STATE SITE AND SPECIES SELECTION

NEW YORK SEA GRANT INSTITUTE
AND
GENERAL ELECTRIC COMPANY

5.6 NEW YORK STATE SITE AND SPECIES SELECTION

The overall objective of the Marine Biomass Program in New York is to demonstrate the economically competitive commercialization of the methane-from-marine-biomass concept in waters contiguous to New York State. Natural gas is widely used in New York for industrial and domestic purposes thereby creating a ready market for methane. The capability and expertise exists to grow seaweeds for conversion to methane and New York's coastal waters are rich enough in nutrients to support highly productive growth. The work tasks encompassed the following areas:

Species Evaluation

Field Collection

Species Compositional Analysis

Gasification

Plant Growth

Test Site Evaluation

Engineering Systems Analysis

Species Evaluation

This task was concerned with the evaluation of seaweeds as potential candidate feedstocks for the bioconversion process. Selection criteria were established to determine which marine macroalgal species would be collected from New York waters. Data on Macrocystis pyrifera served as the baseline for evaluation and ranking of candidate biomass feedstock.

A comprehensive selection criteria list was developed jointly by GE-RSD and NYSGI personnel. This list includes:

1) Productivity

2) Composition

3) Digestibility

4) Plant Characteristics

5) Environmental Impact

6) Harvesting/Planting Technology

7) By-Products

Although this list was deemed comprehensive, it has not yet been prioritized. At this time, the most important criterion from an economic viewpoint, may be the technology of the culture system itself (#6 above). A preliminary list of potential candidate macroalgae species for the New York Biomass Program was compiled. This list includes:

Fucus vesiculosus
Ascophylum nodosum
Ulva lactica and *U. rigida*
Agardhiella tenera
Gracilaria verrucosa
Chondrus crispus
Palamaria palmata
Codium fragile
Laminaria agardhii

In order to develop baseline information against which the various biologic and culturability characteristics of East Coast species could be compared, *Macrocystis pyrifera* was added to the list.

The above list was further expanded to include macroalgal species which are found in waters nearby to the state of New York and could be potentially cultivated - These are:

Agarum cribosum
Alaria esculenta
Laminaria saccharina
Sargassum sp.

Field Collection

The initial field collections were for compositional analysis. Two, 2 pound (wet weight) samples of the following species had been forwarded to GE by 31 March 1980.

Fucus vesiculosus

Ascophyllum nodosum

Ulva lactuca

Chondrus crispus

Laminaria agardii

Palmaria palmata

During the second quarter, two 2 pound samples each of Fucus, Alaria, Agarum, Ascophyllum, Laminaria, Ulva, Chondrus, and Palmaria were collected from a number of sites around Long Island and from the waters near Nahant, Massachusetts and shipped.

During the third quarter, two 2 pound samples each of Chondrus, Ulva, Codium, and Laminaria were collected from the eastern Sound (Orient Point) and shipped to GE for analysis. Additionally, two 2 pound samples each of Ulva, Codium, Fucus, Ascophyllum, and Laminaria were collected and frozen. Specimens of Sargassum sp and Agardhiella were not found during any of the collection periods. A history of the harvesting conditions for each of these samples is provided in Table 33.

Composition Analysis

This task dealt with the determination of the chemical composition of candidate marine macroalgal species to assess the amount of biodegradable organic matter that would contribute to methanogenesis. The selection of the basic processes by which marine biomass is converted to methane is dependent on the biomass composition. However, the chemical composition of plant material varies considerably among species and often within species as a function of its

TABLE 33. HARVESTING HISTORY OF MACROALGAL SPECIMENS RECEIVED FOR ANALYSIS BY GE-RSD

Species	Type[1]	Lot	Harvest Conditions: Water Temperature °C	Harvest Conditions: Date 1980	Harvest Conditions: Location[2]	Comments
Alaria esculenta	B	1	10	5/3	Nahant, Mass.	Drift sample from rocky beach; not dessicated
Aqarum cribosum	B	1	10	5/3	Nahant, Mass.	Drift sample from rocky beach; not dessicated
Ascophyllum nodosum	B	1	4	3/9	Flax Pond, L.I.	At low tide, in mud and Spartina grass
		2	4	3/23	Orient Point, L.I.	Drift sample; not dessicated
		3	-	5/3	Nahant, Mass.	From rocks at low tide
Chondrus crispus	R	1	5	3/-	Conn.	Extensive subtidal bed
		2	3	2/24	Orient Point, L.I.	Mixed species bed; plants healthy, small to medium size
		3	24	7/22	Orient Point, L.I.	Large plants with some pigment loss; rocky bottom
Codium fragile	G	1	22	6/4	Long Beach Bay, L.I.	Intertidal; scattered mature 2nd young plants; pebble bottom
Fucus distichous	B	1	24	7/22	Orient Point, L.I.	Rock bottom; 2-3 m deep; healthy, variable pigment

TABLE 33. HARVESTING HISTORY OF MACROALGAL SPECIMENS RECEIVED FOR ANALYSIS BY GE-RSD (Continued)

Species	Type[1]	Lot	Harvest Conditions: Water Temperature °C	Harvest Conditions: Date 1980	Location[2]	Comments
Fucus vesiculosus	B	1	-	1/11	Flax Pond, L.I.	Above low tide mark; rock; extensive healthy bed
		2	25	7/24	Flax Pond, L.I.	Healthy; mixed with Spartina grass
Laminaria agardhii	B	1	-	2/6	Orient Point, L.I.	1.5 m, in surf zone; medium sized, healthy
Laminaria saccharina	B	1	-	3/23	Orient Point, L.I.	Drift sample from beach; large, complete plants
		2	3	4/9	Orient Point, L.I.	2-3 m deep; sand/rock bottom; large, healthy
		3	-	7/22	Orient Point, L.I.	2-4 m deep; rocky; plants large; some epiphytism
Macrocystis pyrifera	B	48	-	4/29/79	Southern California	Stauffer Lot 687-164
		49	-	10/16/79	Southern California	Stauffer Lot 687-166
Palmeria palmata	R	1	5	3/-	Conn.	Subtidal rock; patchy bed; healthy
		2	24	7/22	Orient Point, L.I.	Rocky; healthy, but some pigment loss
Ulva Lactuca	G	1[3]	-	2/6	Flax Pond, L.I.	Pebble bottom; 1 m deep; extensive bed; mature, healthy

TABLE 33. HARVESTING HISTORY OF MACROALGAL SPECIMENS RECEIVED FOR ANALYSIS BY GE-RSD (Continued)

Species	Type[1]	Lot	Harvest Conditions: Water Temperature °C	Harvest Conditions: Date 1980	Harvest Conditions: Location[2]	Comments
Ulva Lactuca	G	2	-	1/11	Flax Pond, L.I.	Mud flats at low tide; long, mature, healthy
		3[3]	24	7/22	Orient Point, L.I.	Rocky bottom; 2-3 m deep; healthy

Footnotes:

(2) L.I. = Long Island

(3) May be Ulva rigida instead of U. lactuca

(1) Type B = Brown Algae

R = Red Algae

G = Green Algae

physiological state. Therefore, laboratory analyses were performed to determine the constituents, qualitatively and quantitatively of the samples collected.

Prior to the initiation of quantitative analyses, it was necessary to develop and verify an analytical protocol specific to the wide variety of constituents expected in the candidate macroalgae. Verification where necessary was accomplished by "spiking" Macrocystis pyrifera samples with known amounts of expected constituents and assessing recovery. The flow chart developed during this study for materials handling and analyses is presented in Figure 37.

A compilation of the compositional data is presented in Table 34.

Discussion of Analytical Results

1. Dry Weight

From the data it is evident that Ascophyllum nodosum has the highest percent dry weight (ca. 24%), but Aqarum cribosum, Alaria esculenta, and Chondrus crispus also average over 20 percent. Macrocystis pyrifera at 11-12 percent, and Codium fragile at 7.8 percent, show the lowest dry weight values.

2. Volatile Solids

On a percent of dry weight basis, Fucus distichous has the highest volatile solids (organic content), but Aqarum cribosum, Alaria esculenta, Ascophyllum nodosum, and Palmaria palmata all have values in the 70 percent plus range. Codium fragile, Laminaria agardhii and M. pyrifera contain volatile solids below the 60 percent level. A high percent volatile solids (low percent ash) is an obviously desirable feature for biomass conversion. With the exception of Ulva lactuca, and Palmaria palmata, the differences within a species seem less than those between species. The Ulva lactuca, Lot 2 sample appears to be much different than Lots 1 and 3 in this set of determinations. Dr. Brinkhuis feels that Lot 2 is actually Ulva lactuca, while Lots 1 and 3 are probably representatives of the related, but much hardier species, Ulva rigida. The weight

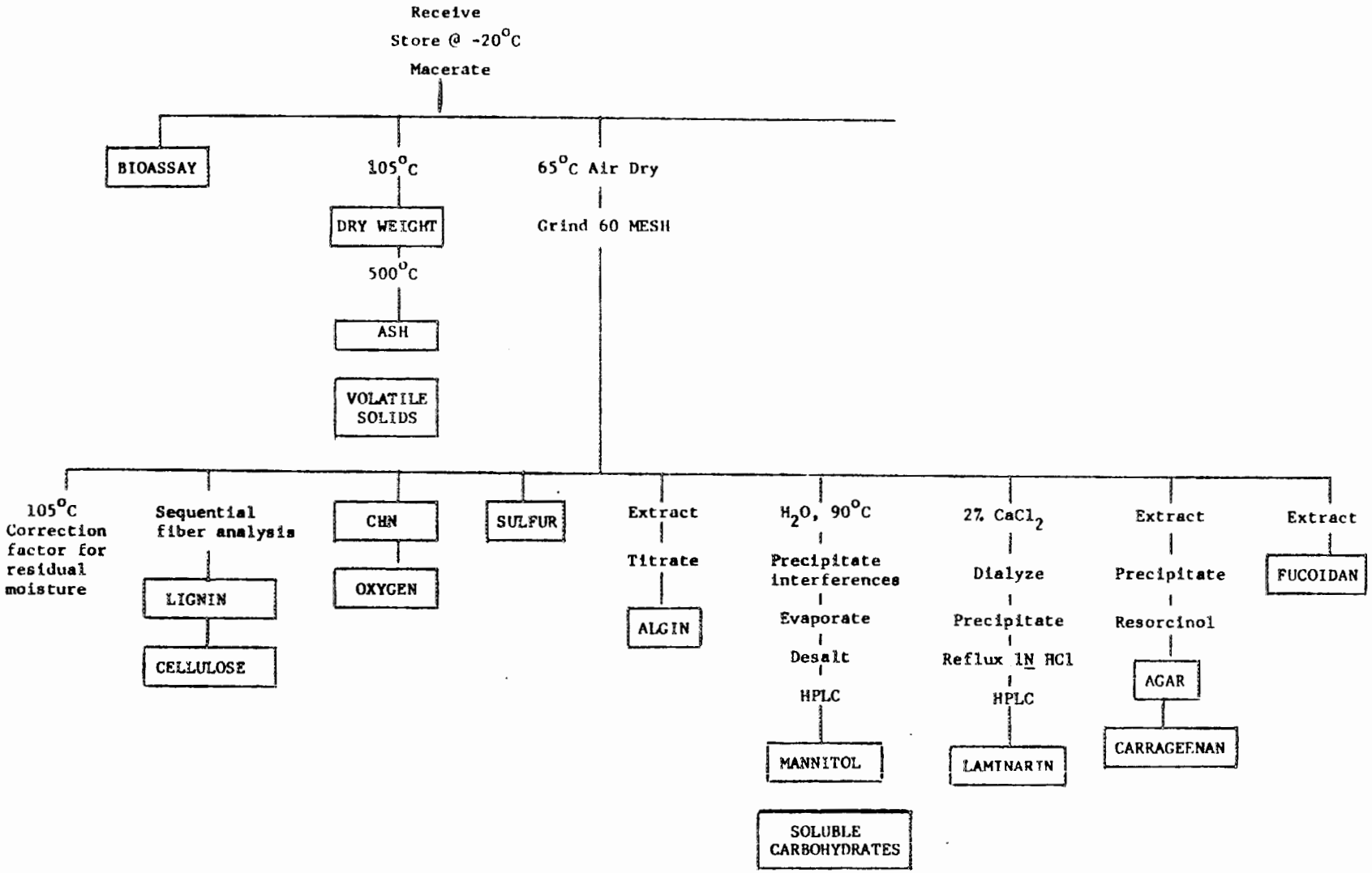


Figure 37. Protocol for Seaweed Analysis

TABLE 34. MACROALGAL ANALYTICAL DATA

Percent of Dry Weight

	Agarum cribosum	Alaria esculenta	Ascophyllum nodosum			Chondrus crispus			Codium fragile	Fucus distichous	Fucus vesiculosus		Laminaria agardhii	Laminaria saccharina			Palmaria palmata		Ulva lactuca			Macrocystis pyrifera	
Type (B, G, R)	B	B	B			R			G	B	B		B	B			R		G			B	
Lot Number	1	1	1	2	3	1	2	3	1	1	1	2	1	1	2	3	1	2	1[1]	2	3[1]	48	49
% Dry Weight	22.2	21.8	24.0	22.3	24.5	19.8	25.9	19.1	7.8	23.8	18.1	19.4	13.0	13.0	14.4	17.5	10.0	17.0	20.1	11.6	17.6	11.4	11.9
Volatile Solids	69.9	74.0	76.4	78.3	76.9	72.7	65.6	64.3	59.8	82.5	73.0	71.1	52.7	64.2	70.1	82.1	71.2	79.8	79.1	50.7	74.3	55.7	63.2
Ash	30.1	26.0	23.6	21.7	23.1	27.3	34.4	35.7	40.2	17.5	27.0	28.9	47.3	35.8	29.9	17.9	28.8	20.2	20.9	49.3	25.6	44.3	36.8
Carbon	30.5	31.9	35.7	33.7	33.1	30.7	24.7	24.6	19.2	38.1	34.5	31.2	23.8	27.5	29.8	33.9	30	34.9	35.4	18.4	29.2	23.7	27
Hydrogen	4.0	4.7	5.0	4.5	4.9	4.5	3.6	4.0	3.4	5.1	4.3	4.3	2.8	3.8	4.1	5.4	4.5	5.6	5.0	2.4	4.9	2.8	4.1
Oxygen (2)	32.2	33.7	32.9	37.9	35.6	32.8	33.2	32.5	35.6	35.4	30.8	32.8	23.5	30.2	31.8	39.7	32.9	35.1	33.8	27.4	37.4	27.5	31
Nitrogen	3.2	3.7	2.8	2.4	3.3	4.7	4.2	3.1	1.6	3.9	3.4	2.9	2.6	2.7	4.3	3.2	3.8	4.1	4.9	2.5	2.8	1.7	1.1
Sulfur	1.5	0.7	2.4	1.5	2.9	4.0	3.8	5.0	4.1	1.4	1.5	2	0.4	0.3	0.4	0.4	0.5	0.5	2.3	1.6	4.1	0.7	0.7
Algin	13.3	16.8	3.7	14.7	5.0	0.6	0.7	<2	<2	5.8	10.8	6.1	17.8	17.3	19.2	5.9	0.6	3.1	0.5	0.7	<2	11.5	12.4
Carrageenan (3)	-	-	-	-	-	22.6	11.0	13.1	-	-	-	-	-	-	-	-	30	15.6	-	-	-	-	-
Cellulose	5.5	3.2	2.9	4.0	1.6	2.0	3.3	4.8	4.1	7.3	5.7	5	8.0	6.8	2.1	4.0	4.4	4.0	2.6	1.3	11.9	6.4	4.7
Fucoidan	0.7	0.5	3.8	3.9	3.8	0.4	0.7	0.7	0.5	3.1	2.5	4.3	0.1	0.1	0.1	0.9	0.1	9.7	8.0	4.9	5.8	2.1	1.2
Laminarin	-	-	-	+	-	-	-	-	-	-	-	-		tr	-	9.1	-	-	-	-	-	-	-
Lignin	17.2	9.4	13.6	19.1	14.0	2.4	2.2	3.0	7.0	12.9	5.8	6.0	6.0	6.6	4.0	9.0	2.1	2.8	5.7	12.4	1.6	4.0	7.8
Mannitol	1.7	4.7	4.3	1.7	2.8	-	-	-	1.3	6.3	5.8	11.4	-	2.5	4.3	18.3	-	-	-	-	-	7.1	18.1
Total Anthrone (Carbohydrate)	14.2	28.4	23.8	25.3	22.2	43.5	40.5	54.9	38.2	28.6	14.4	21.1	13.6	16.3	14.1	39.6	23.1	60.9	42.8	24.3	58.5	18.5	18.8
Sugars/Alcohols (as mannitol)	6.4	3.8	2.2	-	1.2	6.0	-	1.0	1.2	11.7	-	1.3	-	2.3	19.4	5.0	19.4	27.1	3.4	-	1.8	12.3	-
C/N Ratio	9.6	8.6	12.9	13.9	10.0	6.5	5.9	7.9	10.0	9.7	10.0	10.9	9.3	10.3	6.9	10.8	7.9	8.5	7.2	7.4	10.6	13.6	23.7

- = Analysis not performed

(1) May actually be Ulva rigida instead of U. lactuca

(2) Oxygen by difference = VS - C-H-N

(3) Carrageenan/agar

Type: B = Brown
G = Green
R = Red

of volatile solids is calculated as lbs/wet ton. Values are shown in Table 35 and a number of the species show values above 300 lbs VS/wet ton. Macrocystis pyrifera averages about 140 lbs VS/wet ton.

3. Elemental Analyses

Elemental analyses were performed on each sample and the data obtained used to estimate the theoretical gas yields (to be discussed later) and to assess the ratio of carbon to nitrogen (C/N ratio). Generally a C/N ratio of <15 indicates that an active digestion of the biomass can be attained without nitrogen supplementation provided the nitrogen is biologically available. With the exception of Macrocystis pyrifera Lot 49, all specimens showed a C/N <15.

4. Algin

Algin, a major cell wall structural component in Brown seaweeds, is a random copolymer of D-mannuronic and D-guluronic acids. As expected, the Red and Green algae contain almost no algin (less than 1%). Among the Brown algae, the highest algin content ($>17\%$) occurred in Laminaria agardhii, and in two of the Laminaria saccharina specimens, and the lowest levels ($<5\%$) occurred in two Ascophyllum nodosum samples and in Fucus distichous. Most of the brown seaweed algin values, except for Laminaria saccharina and Macrocystis pyrifera, are below the ranges found in the literature.

5. Agar and Carrageenan

Agar and Carrageenan are sulfated polygalactans found in red macroalgae, and these compounds are quantitated by the improved resorcinol method of Yaphe and Arsenault (1965), which detects the 3,6 - anhydrogalactose moiety in these compounds.

Of the 12 species assayed, only Chondrus crispus and Palmaria palmata are red algae and as expected were found to contain varying quantities of agar/carrageenan. Interpretation of the Palmaria data presents a problem however

TABLE 35. COMPARISON OF ORGANIC CONTENT IN WET MACROALGAL SPECIMEMS

MACROALGAL SPECIMENS	#VS/WET TON
Agarum cribosum	310
Alaria esculenta	323
Ascophyllum nodosum	367
	349
	377
Chondrus crispus	288
	340
	246
Codium fragile	93
Fucus distichous	393
Fucus vesiculosus	264
	276
Laminaria agardhii	137
Laminaria saccharina	167
	202
	287
Palmaria palmata	142
	271
Ulva lactuca	318
	118
	262
Macrocystis pyrifera	127
	150

in that the sulfur content is unusually low for specimens containing sulfated polymers. It suggests that either the 3,6 anhydrogalactan polymer is non-sulfated or that other polymers i.e. xylans which are reportedly found in substantial amounts in Palmaria (Percival and McDowell, 1967) are assaying as 3,6 anhydrogalactose (sic agar/carrageenan). Further studies are required to resolve this anomoly, if found necessary.

6. Fucoidan

Fucoidan is a mucilagenous, viscous, sulfated polyfucose compound reportedly found in some brown seaweeds. It should be noted that all species examined contained fucoidan. The major amounts were found, as expected, in the brown macroalgae, but both Palmaria palmata (Lot 2) and Ulva lactuca seemed to contain significant quantities of material analyzing as fucoidan. According to the literature, these species do not contain this polymer.

7. Laminarin

Laminarin is a polyglucose reserve compound found in some brown seaweeds. In the specimens analyzed, a significant amount of laminarin was found in Lot 3 of Laminaria saccharina. The literature values for this species range from 6 to 21 percent. The low laminarin content found in Lots 1 and 2 of this same species is attributed to the fact that these lots were collected during the winter season and Lot 3 was obtained in the summer. Other brown seaweeds show low laminarin content, probably for the same reason. Macrocystis pyrifera does not contain any laminarin.

8. Mannitol

Mannitol, a 6 carbon sugar alcohol, is used for the storage and translocation of photosynthetic energy in many brown seaweeds. This compound is rapidly

digestible in kelp fermentations. With the exception of Codium fragile, mannitol was found only in the brown seaweeds.

9. Lignin and Cellulose

Lignin and cellulose was determined by the Forage Fiber Analysis acid detergent method of Goering and van Soest (1970) as modified by Hart et al. (1978) to correct for interference by algin. The lignin-like fraction in Macrocystis may not be a true lignin, but has been found to be refractory to anaerobic degradation. Cellulose is of interest because it is potentially convertible to methane, although this conversion appears to occur only slowly in Macrocystis fermentations.

Agarum cribosum, Ascophyllum nodosum, and Fucus distichous were found to exhibit the highest lignin levels (13-19%) while Chondrus crispus and Palmaria, both red algae, exhibited the lowest (ca. 2%). Macrocystis exhibited an intermediate level.

The highest cellulose value (8%) was found in Laminaria agardhii, while the lowest values (1-4%) were found in Alaria, Ascophyllum, Chondrus, Codium and Ulva.

10. Unidentified Sugars/Sugar Alcohols

With some macroalgal samples, unidentified peaks were observed during the mannitol determinations. These peaks eluted between glucose and mannitol, and concentrating value was determined by using the mannitol standard curve. As can be seen from the data in Table 34, some of the samples contained up to 27 percent of the dry weight as mannitol equivalents. Additionally, analytical determinations would need to be conducted to more positively identify these compounds.

11. Calorific Value

Calorific values by adiabatic bomb calorimetry were determined in order to estimate the total energy ultimately available in a given macroalgal specimen. This technique was being evaluated as another method for species selection.

The calorific data developed are presented in Table 36 as BTU/lb dry weight and in the normalized form (BTU/lb volatile solids) as recommended by Paine (1971). This latter method corrects for the widely varying (supposedly neutral) ash component. However, this ash component apparently interferes drastically with the determination of the energy content of the organic material. (Paine, 1971).

A regression fit of the bomb calorimeter results (normalized to volatile solids) against the percent ash content produced the following equation:

$$Y = -43.7\ x + 9124$$

where Y = bomb calorimeter results (BTU/lb volatile solids)

x = percent ash in sample

The correlation coefficient, R, of 0.658 is significant at the 2 percent level (2 tail probability = 98.6%). This relationship indicates a statistically significant negative correlation between the ash content and the calorific value determined by bomb calorimetry. The intercept value of 9124 BTU/lb VS is fairly close to the 9308 BTY/lb VS predicted from the mean calorific value calculated from heats of formation.

Because of these questionable variations in calorific content, it has been concluded that bomb calorimetry is of little value in screening studies of seaweed species as candidates for marine biomass feedstock.

Gas Yield Calculations

This subtask dealt with the determination of the theoretical gas (methane) yield from candidate marine macroalgal species to assess the efficiency with which methanogenic (methane-producing) bacteria convert the substrate to methane. This efficiency is a major factor in determining the economics of methane production from biomass.

TABLE 36. CALORIFIC CONTENT OF MACROALGAL SPECIMENS

SPECIES	TYPE	LOT	CALORIFIC CONTENT BTU/LB Total Solids	BTU/LB Volatile Solids
Ascophyllum nodosum	B	1	6740	8820
		2	6080	7770
Chondrus crispus	R	1	5780	7960
		2	4350	6620
Fucus vesiculosus	B	1	6160	8440
Laminaria agardhii	B	1	4120	7820
Laminaria saccharina	B	1	4880	7600
		2	5300	7550
Macrocystis pyrifera	B	48	4150	7160
		49	4690	7420
Palmaria palmata	R	1	5670	7960
Ulva lactuca	G	1	6440	8150
		2	3880	6680

In the Marine Biomass Program, theoretical gas yields are determined utilizing the equation of Bushwell and Meuller (1954) $C_mH_aO_bN_c$+

$$\left[M - \frac{a}{4} - \frac{b}{2} + \frac{7c}{4}\right] H_2O \longrightarrow \left[\frac{m}{2} - \frac{a}{8} + \frac{b}{4} - \frac{5c}{8}\right] CO_2 + \left[\frac{m}{2} \quad \frac{a}{8} \quad \frac{b}{4} \quad \frac{3c}{8}\right] CH_4 + [C] \quad NH_4HCO_3 \qquad (1)$$

where m, a, b, and c represent the molar quantities of carbon, hydrogen, oxygen and nitrogen, respectively, present in the sample. Carbon, hydrogen and nitrogen are determined experimentally and oxygen is determined by difference, i.e.,

$$\text{Oxygen} = \text{Dry Weight} - \text{Ash} - C - H - N - S \qquad (2)$$

Several problems are inherent in this approach. First, in many of the species examined in this study, sulfur is no longer a minor component existing in sulfur amino acids, etc. but is found in relatively high amounts as sulfate in the structural polysaccharides, i.e., agar, carrageenan, fucoidan and other sulfated galactans. Second, in utilizing equation 2, the sulfur component is actually being counted twice: once as the free sulfur and again as sulfate in the ash fraction. Third, sulfur (as sulfate) can be utilized as an electron acceptor by certain species of anaerobic bacteria with acetate (Desulfotomaculum) or H_2 (Desulfovibrio sp) as electron donors thereby reducing potential methane yields.

Hydrogen sulfide (H_2S) is a product of these reactions which in itself is a problem in that it dilutes product quality, is toxic, odorous and must be removed from the gas stream.

Taking these factors into account, the Bushwell Mueller equation can be modified to incorporate the sulfate term as follows:

$$C_mH_aO_bN_c + \left[SO_4\right] d^+ \left[M - \frac{a}{4} - \frac{b}{2} + \frac{7c}{4} - d\right] H_2O \longrightarrow \left[\frac{m}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8} - d\right] CH_4$$

$$+ \quad \left[\frac{m}{2} - \frac{a}{8} + \frac{b}{4} - \frac{5c}{8} + d\right] \quad CO_2$$

$$+ \quad [d] \quad H_2S$$

and to eliminate the double counting of sulfur, oxygen is more properly determined by:

$O = VS - C - H - N$ where VS = Dry Weight - ASH

A comparison of the theoretical gas yields as determined by the two methods described is given in Table 37. Methane yields, uncorrected for sulfate, range from a high of 7.35 SCF/lb VS for Fucus distichous to a low of 4.80 SCF/lb VS for Codium. and (when corrected for sulfate) from 6.61 to 2.72 SCF/lb VS. respectively. Clearly, the high levels of sulfur (sulfate) present in some of the specimens examined and the potential for sulfate reducing bacteria to metabolize at the expense of methanogenic reactions has a siginificant impact on the theoretical gas yields. These reductions range from around 3 percent in Laminaria saccharina (a low sulfur species).

A relative ranking of these macroalgae based upon the theoretical yields is given in Table 38. No definite conclusions can be drawn at this time because of the limited species samples and the wide variation in specimen growth history.

Plant Growth

Studies on the GRI Marine Biomass Program have shown that biomass yield, expressed in terms of dry-ash free tons per acre of farm per year, is that parameter to which overall system economics and energetics is most sensitive. Yield, or productivity, is also expected to be the driving force underlying the economic feasibility of systems established on the East Coast. Other factors associated with yield also significantly influence the suitability of a macroalgae for use in a marine farming system. These include size, growth patterns and growth as a function of season and growth as a function of the stage of a plant's reproductive cycle.

TABLE 37. THEORETICAL GAS YIELDS OF CANDIDATE MACROALGAL SPECIES
SCF CH_4/LB VS

SPECIES	LOT	UNCORRECTED FOR SULFATE	CORRECTED FOR SULFATE	% REDUCTIONS
Agarum cribosum	1	6.60	5.91	10.45
Alaria esculenta	1	7.04	6.58	6.53
Ascophyllum nodosum	1	7.93	6.51	17.91
	2	6.55	5.75	12.21
	3	7.10	5.54	21.97
Chondrus crispus	1	6.96	4.81	30.89
	2	5.51	3.57	35.21
	3	6.44	3.69	42.70
Codium fragile	1	4.80	2.72	43.33
Fucus distichous	1	7.35	6.61	10.07
Fucus vesiculosus	1	7.49	6.59	12.02
	2	6.98	5.79	17.05
Laminaria agardhii	1	6.64	6.31	4.97
Laminaria saccharina	1	6.40	6.19	3.28
	2	6.20	5.96	3.87
	3	6.37	6.18	2.99
Palmaria palmata	1	6.41	6.12	4.52
	2	7.18	6.86	4.46
Ulva lactuca	1	7.25	5.99	17.38
	2	4.61	3.58	22.34
	3	6.63	4.52	31.83
Macrocystis pyrifera	48	5.96	5.48	8.05
	49	6.84	6.31	7.75
	3	8.23	7.26	11.79

TABLE 38. RELATIVE RANKING OF MACROALGAL SPECIES BASED ON THEORETICAL GAS YIELDS*

UNCORRECTED FOR SULFATE			CORRECTED FOR SULFATE		
SPECIES	NUMBER OF SAMPLES	SCF CH_4/lb VS	SPECIES	NUMBER OF SAMPLES	SCF CH_4/lb VS
Fucus distichous	(1)	7.35	Fucus distichous	(1)	6.61
Fucus vesiculosus	(2)	7.24	Alaria esculenta	(1)	6.58
Ascophyllum nodosum	(3)	7.19	Palmaria palmata	(2)	6.49
Alaria esculenta	(1)	7.04	Macrocystis pyrifera	(3)	6.35
Macrocystis pyrifera	(3)	7.01	Laminaria agardhii	(1)	6.31
Palmaria palmata	(2)	6.80	Fucus vesiculosus	(2)	6.19
Laminaria agardhii	(1)	6.64	Laminaria saccharina	(3)	6.11
Aqarum cribosum	(1)	6.60	Ascophyllum nodosum	(3)	5.93
Laminaria saccharina	(3)	6.32	Aqarum cribosum	(1)	5.91
Chondrus crispus	(3)	6.30	Ulva lactuca	(3)	4.70
Ulva lactuca (rigida)	(3)	6.16	Chondrus crispus	(3)	4.02
Codium fragile	(1)	4.80	Codium fragile	(1)	2.72

* SCF/lb VS is average for number of samples cited.

The purpose of this task was to assess the upper limit of growth potential on the native species that offer promise after the initial screening.

The activities include evaluation of existing data, and where feasible, laboratory experiments on growth rates under controlled conditions.

Data Survey and Evaluation

A literature survey of candidate species was completed. Although it is preliminary, the survey provides insight for the prioritization of the candidate species list. A complicating factor is that growth, productivity, composition, nutrient response, and life histories may be area-or environment-specific and therefore of limited use.

A summation of these data coupled with some subjective information is presented in Tables 39 and 40.

A test plan detailing the experimental protocol for the seaweed biomass growth and productivity experiments was developed. This experimental procedure will:

1) determine growth rates of nine candidate species at one time under semi-natural conditions;
2) provide data on growth characteristics and response to culture conditions;
3) optimize culture methods for each species;
4) provide GE/RSD with samples for compositional analysis and gas yield experiments.

Construction bids for the greenhouse were opened April 17, 1980. Construction permits were cleared with the Department of Environmental Conservation and the local Crane Neck Property Owners Association. Construction commenced April, 1980 and was completed July, 1980.

The greenhouse is attached to the south wall on the western half of the Flax Pond laboratory. It has a floor area of approximately 20 feet x 40 feet.

TABLE 39. SELECTED LITERATURE VALUES FOR CANDIDATE MACROALGAE

	Ascophyllum	*Fucus*	*Laminaria*	*Macrocystis*	*Ulva*	*Codium*	*Chondrus*	*Gracilaria*	*Agardhiella*	*Palmaria*
* dry weight	11.1-30	18-31.9	23.5	13-14	18.7-22 22 22	7	20-24.6	8.3		13.8-21.8
* ash	17.8-24	13-22	21.1	36-38.5	15.6 15.6-21 13.5 (33)	42	25-28	3.55 5.52		26.9
* total carbohyd	56-62.8	62		40.6	50.6 72					
* algin		16-28	13-35	13-24						
* laminarin	1-7	1- 7	1-24	5.8-7.4						
* mannitol	3.8-12	8.3-16.1	6-23	5.5-25						
* total fiber	5.8-6.51	5.5	6.7 3.28	5.8	0.2			4.3		
* carbon	38	35		26-31	34.45			24.3-26.9		
* nitrogen	1-3	1-3	1.57-2.34	1.0-3.8	2.5 4.68		0.2-3.0	2.4-3.2		1.8-3.7
C/N ratio	13-38	13-38		8-26	7.36 7-40	12-35		8.4-10.1		

* % of ash free dry weight

TABLE 40. BIOASSAY EVALUATION OF CANDIDATE MACROALGAL METHANE YIELD (SCF/LB VS)

SPECIES	LOT	BIOASSAY*	THEORETICAL[2]	% of THEORETICAL
Agarum nodosum	1	0.55 (0.55)	5.91	9.3
Alaria esculenta	1	0.72 (0.75)	6.58	10.9
Ascophyllum nodosum	1	1.05	6.51	16.1
	2	1.28	5.75	22.3
	3	0.35 (0.35)	5.54	6.3
Chondrus crispus	1	1.73	4.81	35.9
	2	1.20	3.57	33.6
	3	0.51 (0.54)	3.69	13.8
Codium fragile	1	0.07 (0.07)	2.72	2.6
Fucus distichous	1	0.04 (0.04)	6.61	0.6
Fucus vesiculosus	1	1.94, 1.68	6.59	29.4, 25.4
	2	1.35 (1.36)	5.79	23.3
Laminaria agardhii	1	0.02, 0.16	6.31	0.3, 2.5
Laminaria saccharina	1	0.17	6.19	2.7
	2	0.06	5.96	1.0
	3	0.55 (0.64)	6.18	8.9
Palmaria palmata	1	0.96	6.12	15.7
	2	0.35 (3.37)	6.86	5.1 (66.1)
Ulva lactuca	1	0.66, 0.70	5.99	11.0, 11.7
	2	1.96, 2.01	3.58	5.47, 5.61
	3	1.51 (1.51)	4.52	33.4
Macrocystis pyrifera	48	1.97, 2.01	5.48	35.9, 38.3
	49	2.24	6.31	35.5

* 1. Two values for one lot were obtained in separate bioassay experiments. Values in ( ) are corrected for vented hydrogen as explained in text.

2. Theoretical values are corrected for sulfate.

Ten 3 foot x 6 foot and five 3 foot x 4 foot seawater tanks were installed. Each tank will hold four smaller culture tanks of seaweed. Seawater is pumped from the laboratory storage tank which is continuously filled by the main lab pumps. Separate pumps draw out water and pump it into the greenhouse. Two pumps and two seawater lines were installed and cross-connected to permit intermittent cleaning and maintenance of each system. Seawater flow in the greenhouse is regulated by manual valves supplying each of the small culture tanks. Flow depends on the water volume turnover rate desired. The turnover rate is dictated by ambient seawater nitrogen content, which is monitored twice weekly, and physical water flow needed for some species. Overflow of this seawater goes into the larger holding tanks.

In-tank culture of seaweeds began in July 1980. During that month 2 tanks of Fucus, 2 of Ascophyllum, 1 of Ulva, 1 of Codium and 1 of Chondrus were begun. More cultures have been started since then so that at present there are 3 tanks each of Laminaria, Ulva, Codium, Fucus, and Ascophyllum, 4 small tanks of Macrocystis, and 1 tank each of Chondrus and Agardhiella.

Bio-Conversion Process

This task is concerned with the early laboratory determination of the applicability of native marine species (macroalgae) as feedstock for the bio-conversion process. Specific steps in this validation demonstration will be:

- Process Definition
- Digestion Initiation
- Gas Yield Measurements.

Process Definition

This subtask deals with the process definition for anaerobic digestion of the candidate feed stocks.

The results of previous studies, plus the compositional analyses were to be used to define various aspects of the process flow, including but not limited to:

- Material storage
- Pre-treatment
- Inocula source
- Loading rate
- Detention time
- Temperature

It soon became evident, as the program evolved, that sufficient information on the macroalgal species was not available to develop logical processing scenarios (other than those being pursued in the biomethanation of Macrocystis pyrifera). Therefore, Process Definition and Digestion Initiation sub-tasks were deferred until the plant growth task was far enough along to provide sufficient feedstock with known growth history and composition.

Gas Yield Measurement

This subtask was concerned with the measurement of gas generated by anaerobic digestion of candidate feedstocks of selected species.

A bioassay technique was employed as a rapid screening procedure for a comparative assessment of the biomethanation potential of the candidate macroalgae. Actual digestion using bench-scale digestors was deferred to a later date when sufficient quantities of one or two of the most promising candidates is available from the plant growth studies.

The objective of the bioassay technique was to compare the methane yields of different candidate seaweeds under standardized, small batch conditions. The technique was that of Owen, et al (1979) with some minor modifications.

The bioassay data for the macroalgal specimens examined are presented in Table 40. Sets 1, 2 and 3 represent three separate bioassay experiments. Hydrogen

was assayed for (only in set 3) and the data is shown corrected for hydrogen conversion to methane (i.e. $CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$). The extreme case was Palmaria, where the poor methane yield of 0.35 SCF/lb VS determined directly as methane becomes 3.37 SCF/lb VS when the hydrogen correction is applied.

Although overall gas yields were lower than anticipated, it should be noted that essentially no methane gas was produced from some species (i.e. Laminaria, Fucus, etc.). In fact, some of the interim data implied that actual inhibition of methanogenesis was occurring and therefore a study was initiated to determine if this was the case. This study examined the digestion of Laminaria agardhii alone and in combination with Macrocystis pyrifera by an active Macrocystis digesting culture. Macrocystis alone acted as a positive control. Figure 38 depicts the time course of this assay. Data plotted is net gas yield obtained after subtracting gas produced by inocula controls. Clearly, an inhibition of the activity of the inoculum occurs in the presence of Laminaria samples. This inhibition is unknown and needs to be explored in future studies.

There are several steps which are being explored to improve the bioassay screening procedure. It is possible that the Macrocystis - grown inoculum does not contain the requisite microorganisms for the degradation of the various constituents in these species. Additional inocula sources are being sought for integration into the mixed feedstock digesters. In addition, venting and non-venting techniques will be compared to examine the effects of hydrogen loss on methanogenesis.

In conclusions, the bioassay data obtained thus far indicates that several of the samples of species examined for methane yield are comparable to Macrocystis pyrifera using this same technique. These species were Chondrus crispus and Ulva lactuca. Refinements of the bioassay technique are required to improve this method of species evaluation.

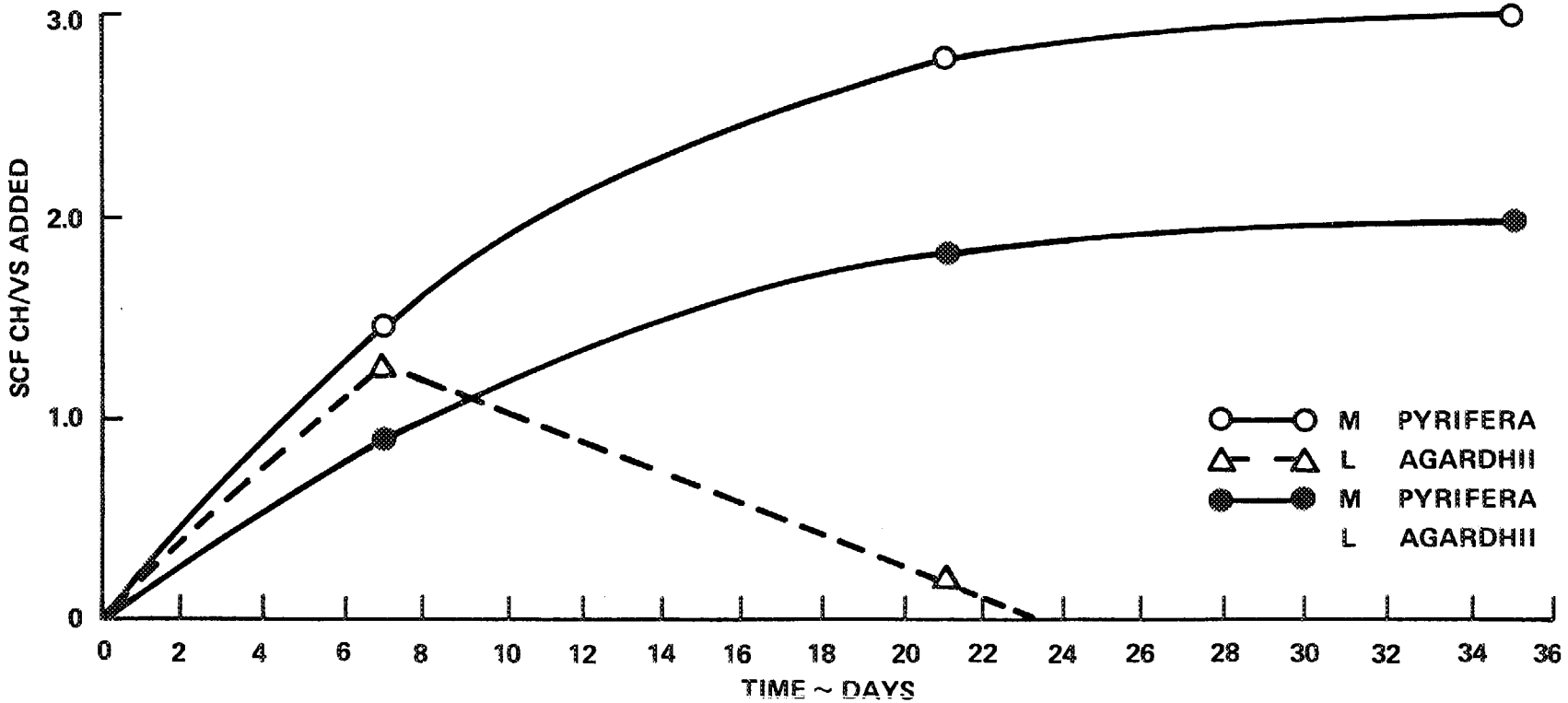


Figure 38. Bioassat Methane Production from Macrocystis Pyrifera and Laminaria Agardhii

Test Site Evaluation

The purpose of this task was to evaluate available data to determine candidate potential sites for future experimental ocean test farms. Based on analysis, additional field data will be acquired as required.

During the first quarter, the three basic water bodies being considered as a potential site for a test farm were Long Island Sound, Gardiners/Peconic Bays complex and the New York Bight. Much of the large-scale environmental and hydrographic data required to grossly characterize these areas are on file at the Marine Sciences Research Center. Lack of sufficiently protected sites effectively precluded the New York Bight as a site for the first biological test farm. It was anticipated that water quality and general hydrographic perameters of Long Island Sound and the Gardiners/Peconic Bay complex were sufficiently similar to permit consideration of a number of alternative sites. Current regime may be the most important environmental factor. Lack of a clearly superior site based on environmental considerations resulted in preliminary site selection being dependent upon the jurisdictional and legal regimes governing the use of these water bodies. While selection of a test farm site in close proximity to the Marine Sciences Research Center is desirable, it was not deemed a critical factor in site selection.

In order to proceed with the exercise of evaluating alternative area (as opposed to the actual selection of a test farm site), it was necessary to make several assumptions which would define area requirements of the test farm and duration of deployment and also limit the geographic scope of potential test farm locations. After determining that Long Island Sound and Gardiners/Peconic Bays would be the best sites, based on the criteria derived from the above assumptions, an additional screening of the two target areas was required to make application of site selection criteria more manageable. Three specific bays were selected for

additional evaluation: Smithtown Bay in central Long Island Sound, and Hog Neck Bay and Orient Harbor in Gardiners/Peconic Bays. Smithtown Bay was selected in part because it encompasses several jurisdictional scenarios. This bay also has relatively high water quality, but it is also subject to degradation on an episodic basis. The two sites in Gardiners/Peconic Bays have excellent water quality; conditions at these locations approximate those found in adjacent ocean waters.

Systems Analysis

Concurrent with the analytical and experimental activities previously discussed, a System Analysis Task was to be undertaken. The purpose of this task was to perform an early examination and evaluation of all program elements to insure system compatibility of individual technical areas and to identify down-range engineering or socio-economic road blocks.

Professor Reis and Coastal Law Fellows (supported by the Sea Grant Institute) prepared a series of Interim Reports as follows:

First Interim Report - Explored the broad areas of legal issues to be covered. This report was superseded by the Third and Fourth.

Second Interim Report - Analyzed specific issues to be dealt with in discussions with the Assistant Commissioner for Natural Resources, New York State Department of Environmental Conservation including introductions of exotic permits and related matters. Its content is superseded, in part, in later drafts.

Third Interim Report - Deals with the questions of bottom land ownership within territorial waters of New York State; federal, state and local environmental regulations to be observed; relationships to navigable water regulations.

Fourth Interim Report - Site specific information on the ownership of lands in the three primary sites identified is present.

These reports are available to interested parties.

Engineering Systems Analysis

An initial engineering systems analysis has been completed. This report examines substitute natural gas requirements, farm site locations, data requirements, experiment planning and species selection methodology.

5.6.1 ENGINEERING SYSTEMS ANALYSIS

1. Introduction and Task Purpose

Engineering Systems Analysis seeks the alternative solutions to problems associated with developing a Marine Biomass farm to provide Substitute Natural Gas for New York State. The selections of the "solution" depends upon the results of a number of scientific experiments. A system study seeks to:

a. Provide an early analog to direct research efforts.

b. Evaluate research efforts to establish the optimum solution.

The problem complexity requires an iterative solution, and one of the major aspects of the complexity is the interaction of the component parts of the solution. For example, the initial needs of the region must be evaluated to define the amount of gas to be produced. From the amount to be produced and the production rate we determine the biomass production and establish the farm size. We must then define the species to be selected, the species growth characteristics and nutrient requirements to achieve this growth. Species selection, which can be determined by a number of criteria, will dictate farm design and harvesting techniques. Methane production by anaerobic digestion requires a delivery (transport) of biomass to a source, probable pre-treatment requirements prior to digestion and disposal and/or use of residue from the production/gasification process.

Eventually the process which delivers the requisite amount of pipeline quality methane at or near competitive price levels can be selected.

The initial phases of the NYS/ERDA System Analysis has explored the requirements for natural gas, the selection of suitable species to provide these requirements and the data required to improve the confidence level of the eventual solution. The following pages define:

1. Establishing Gas Quantities
2. Exploration of Farm Sites
3. Data Required and Experiment Planning
4. Species Selection and Methodology

2. Substitute Natural Gas Requirments

Substitute natural gas requirements can be determined a number of ways, several of which depend upon forward projection of required energy levels, which will result in differing production quantities. It is appropriate to determine requirements based upon current utilization of Natural Gas within the locale. It is also appropriate to extend these uses to the future. In evaluating literature surveys of competing energy use one has to bear in mind a socio-political requirement to reduce dependency on sources not under direct control of the locale. This can be stated as a goal of energy self sufficiency.

It then appears natural to evaluate the past energy uses of the New York State region. From figures available for New York State through 1975, (Fig. 39), data shows that New York State in 1975 used approximately 0.6 quads of natural gas, distributed among residential, commercial and industrial customers and utility customers. Figure 39 also shows the trend in gas consumption, which is declining toward the end of the period. Principal reason for the decline is utility use which decreased rapidly from 0.1 quads to 0.02 quads in the 5 year

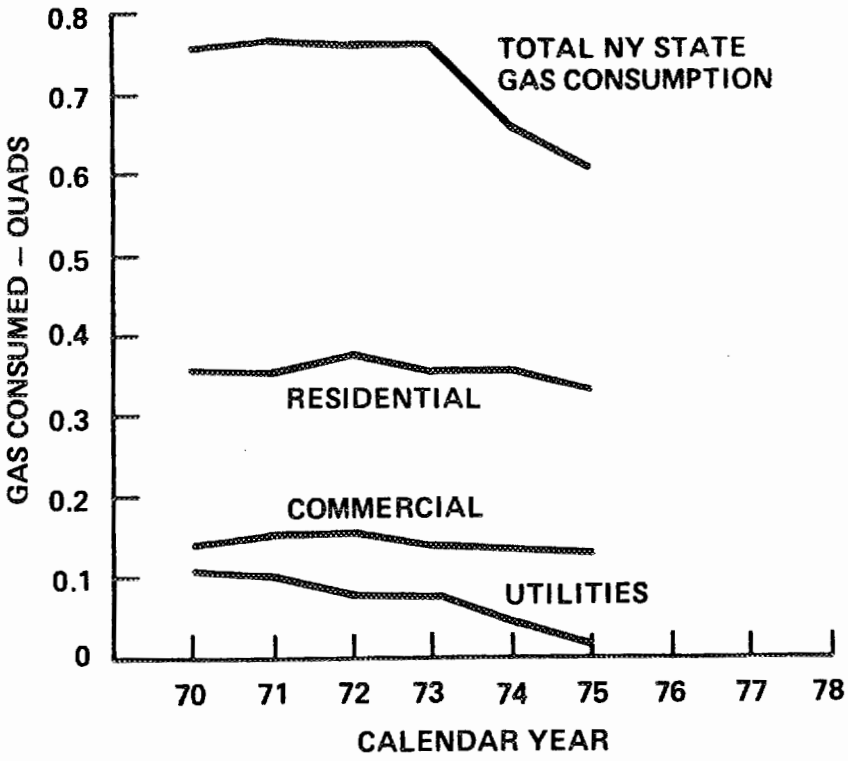


Figure 39. Gas Consumed in New York State for Calendar Years 1970-1975

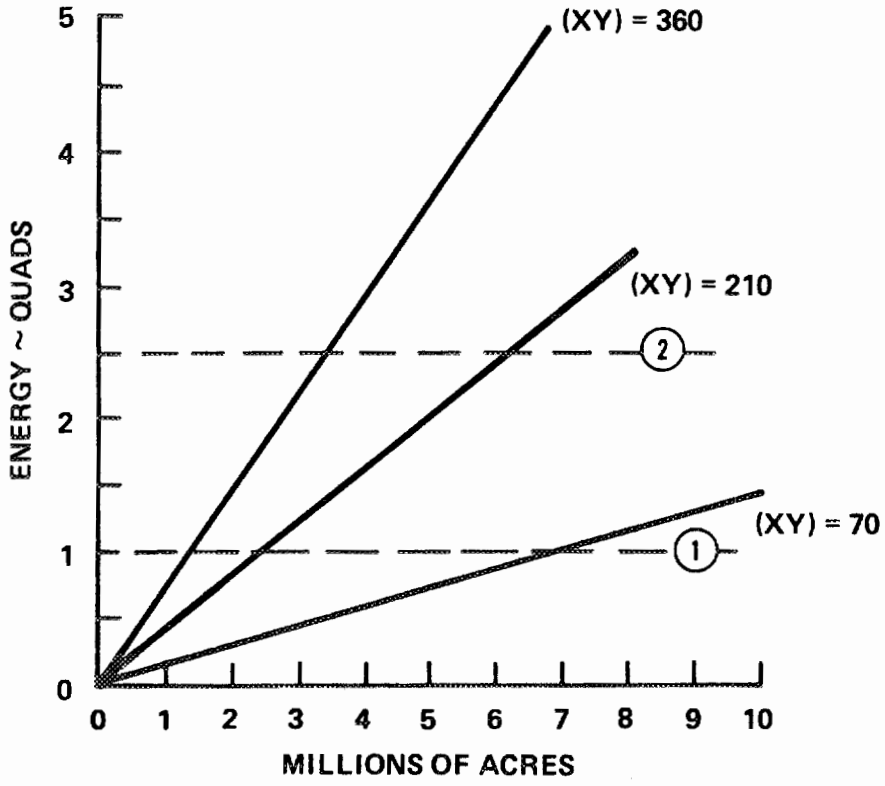


Figure 40. Farm Output vs Acres for 3 (XY) Combination. ② Represents Energy to Replace All Except Transportation and ① Reflects 25 Percent Growth in Present (1975) Use of N.G.

period. A corresponding increase in petro fuel and nuclear fuel is shown to offset gas use.

A further assessment shows energy use to reach 3.5 quads, with about 1 quad assigned to transportation. If we assume Natural Gas can account for energy requirements exclusive of transporation we set a goal of 2.5 quads.

A quick calculation to achieve these goals can be made. If gas production of x SCF/lb VS is assumed and y DAFT/ACRE YEAR predicated we can estimate farm size requirements.

Figure 40 shows farm size requirements for 2 energy levels (1.25 x present gas consumption and a second assumption that we use SNG to replace all energy except transportation). The xy values shown are achievable in marine farming.

Figure 40 can provide further insights into systems problems. Biomass yields in dry ash free tons/acre year result in requirements to harvest and transport wet weights which may vary between 2 and 5 times the dry weight.

At present it is too early to finalize energy requirements except broadly, since the species are not selected nor are the available farm sites studied.

3. Farm Site Locations

Marine farms are attractive since the farm will be placed in water locations ordinarily unused by comparison with land agriculture. Obviously ocean use is not completely absent, since sea lanes are required for marine traffic and shoreline use for recreation and commercial reasons is extensive. The initial plan called for establishing a marine farm in the New York Bight area.

The preliminary area estimate is made by excluding a region of water about 15 miles from any shoreline which provides a buffer zone to accommodate local use for pleasure boating, and excludes Long Island Sound from consideration. The remaining bight region contains roughly 20,100 square miles, and if a shipping

lane area of 3000 square miles is excluded the net usable area is 10.9×10^6 acres.

Sufficient area is available to provide a reasonable SNG production. To further evaluate the available area a preliminary estimate of environmental suitability should be initiated, including current speed level, wave height and bottom contours. Figure 41 shows the New York Bight region. Nutrients from the shoreline discharge into the Bight region with heaviest concentration from the Harbor entrance [Hudson River] and less concentrated discharge from local regions along Long Island and New Jersey.

Nutrients are in general heavier than water, and as expected, surface concentrations at discharge points are heavy, with a general setting of nutrients in deeper water. The bottom contour lines shown in Figure 41 are relatively shallow however. The 50 meter line is approximately 30 miles off shore, with the 200 meter line about 70 miles off shore.

A general flow from clean open ocean water enters the Bight region from a southeasterly direction and is turned south by the Jersey shoreline and northeasterly around Montauk Point. As a result of dispersion and flushing with clean water, the heavy concentration of Nitrates at the harbor mouth decreases with distance. A high surface value occurs at the New York Harbor mouth region with lower concentration levels for the positions at sea.

Two general conclusions can be drawn from this. The initial conclusion is that nutrient levels in the Bight region are relatively high inshore, and if 3.0 ug/liter provides heavy growth, the nutrients on the surface appear marginally adequate. A second conclusion is reflected at Diamond Point which is on the Peconic Bay system. Nutrient levels are reasonably consistent with the Bight area and under these conditions test farm growth data would be representative of open ocean area data.

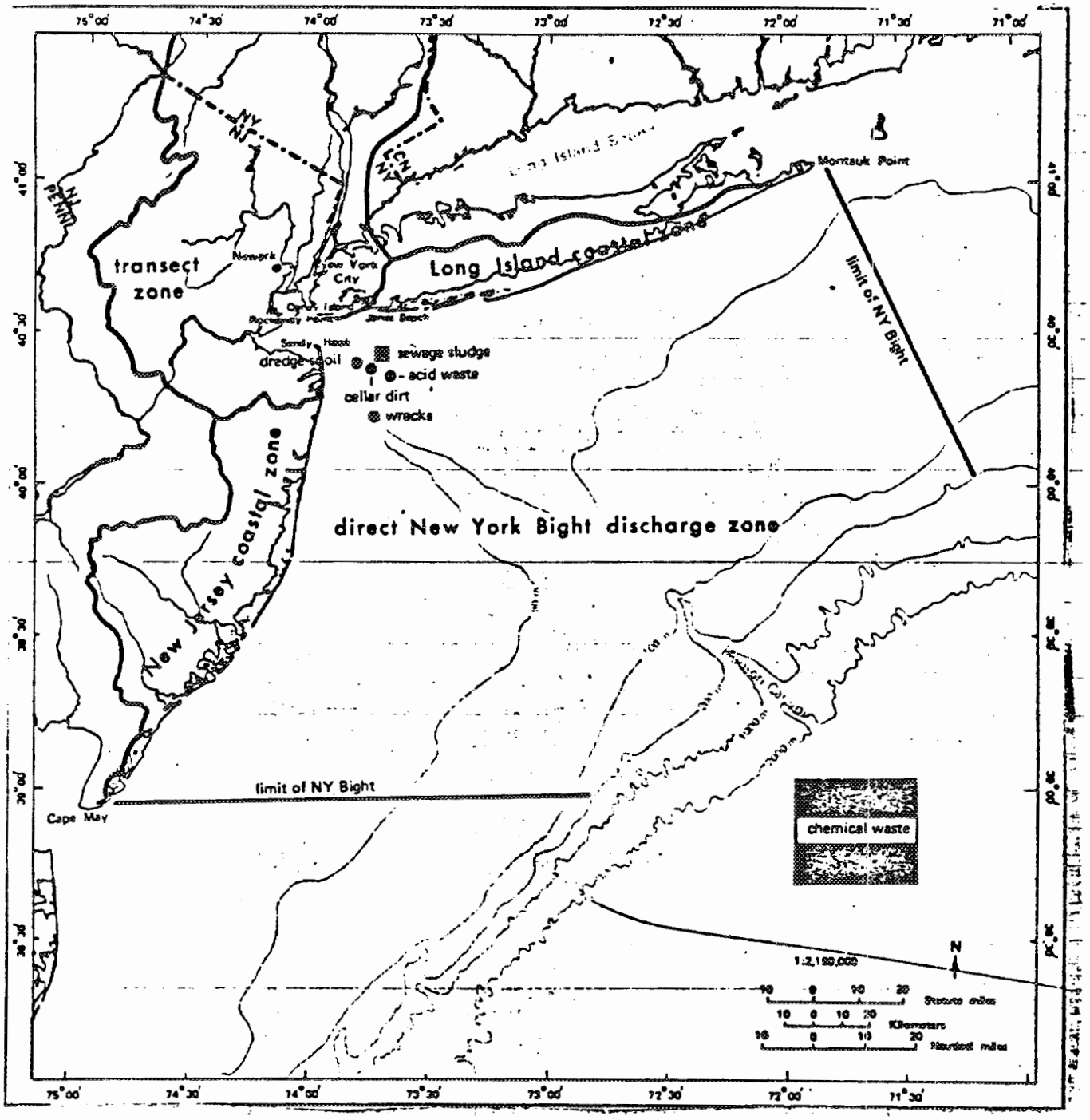


Figure 41. New York Bight Map

4. Data Requirements and Experiment Planning

Requirements for experimental data can be formulated and growth experiments planned without regard to the specific species. Our initial assumption on yield requirements in Section 2 provided limited information on [x y] as a combination, where x represented the gas quantity per pound of volatile solids and y represented yield in dry ash free tons/acre year. Preliminary information of species size and growth under normal [i.e., natural] conditions of temperature, sunlight, nutrient and water quality have been obtained and will be reported later. In essence, however, the yields per acre will be determined by several factors.

Selection of the optimum species of seaweed can depend upon the final site location which in turn will be determined by "human factors" such as compatability with local ecology, size and growth rate and proliferation. These factors have been included in a general sense in the early phases of the species selection, and with reasonable assurance of success the growth experiments can be initiated.

These experiments will have both long-term and near-term goals. Literature review shows that nutrient level, sunlight and temperature will in a large sense determine growth rates, with trace metal elements providing a possible catalytic effect. Yield rates* will depend upon nutrient uptake, photosynthetic activity, pH, and planting density. Plant size, age, water temperature, and traces of organics or non-organics will also influence the yield data.

In turn, the yield data will influence the cost or selling price of natural gas. In fact, based upon experience with the Biomass studies conducted in other programs, the farm costs are the major factor in establishing the selling price of substitute natural gas.

*John Ryther, "Cultivation of Macroscopic Marine Algae and Freshwater Aquatic Weeds" Woods Hole Oceanographic Institution, Progress Report for the Period May - Dec. 1979.

The preliminary site survey shows that seasonal variations in available sunlight, nutrient concentration, water temperature, salinity and turbidity will occur. Growth experiments which will be conducted to provide a reasonable analog for the definition of commercial farm yield must account for these effects. In a fashion similar to the expressions developed for the BioEc Computer Code we can model growth in an exponential format, i.e.,

$$W_n = (1 + \gamma)^n W_o \qquad (1)$$

where W_n is biomass at n days, W_o is the standing crop and γ is the compound growth rate of the species.

Assuming that all new growth is harvested:

$$W_n = (W_n - W_o) = W_o \quad [(1 + \gamma)^n - 1] \qquad (2)$$

where W_n is harvest weight, and other items are as in Equation (1). The compound growth rate is, of course, unrealistic and limit conditions on both γ and n must be established for the commercial farm region.

Growth experiment objectives should be structured to define the following points.

(1) Determine the influence of the following on the growth rates of the various species:
   (a) Solar energy level
   (b) Nutrient concentration
   (c) Planting density
   (d) Harvest period

(2) Determine the form of growth rate curve as a function of species size. (Determine a limit condition on growth.)

(3) Determine the limiting conditions on growth, and the degree of the interaction of growth variables.

Answers to the third question are required to establish the non-linearity factors which should be associated with normal growth. The non-linearities are the result of growth-limiting conditions and can influence the processing plant design. For instance, if the growth is influenced by nutrient level and sunlight conditions at the site, reduced growth and reduced yield levels would occur during the period of reduced sunlight. Reduced yield results in reduced harvest delivery and reduced output of SNG. The converse occurs during periods of greater solar energy. An overall system solution to non-uniform biomass delivery can be established when definition of growth rate effects are established.

Growth data obtained on the various species will strongly influence final species selection, and data to obtain a realistic growth value will establish the biomass yield. Based upon prior experience the yield of biomass will weigh heavily in establishing the economics of biomass for SNG. Other factors influencing these economics are volatile solids ratio, nitrogen content, theoretical gas yield and kinetics of biodegradation. These items will be addressed in future systems studies.

5. Species Selection Methodology

Evaluation of the list of species indigenous to New York waters which may provide adequate biomass yield must be made to establish an optimal material for the New York State application. This requires that a suitable method for effectively screening a large number of candidates be developed.

The species selection methodology must establish those qualities which influence the design of the farm substrate, the design of the harvesting subsystem and the design of the processing subsystem. In addition, the species selection methodology must concern itself with biomass yield and eventual Substitute Natural Gas output.

Description of Methodology and Preliminary Description of Candidate Species

Table 41 lists the physical, biological and chemical characteristics which are considered of importance in rating species as candidate material. The present effort concentrates on the physical aspects of the species, shown in Table 41. The differentation of candidates begins at the species level and, as shown, growth mode can initiate a farm conceptual design effort. Four configurations are possible, depending on whether the plant grows attached or unattached and whether it is a buoyant or non (neutral) buoyant.

A further breakdown expresses whether that species primarily flourishes in tropic, temperature or frigid environments, and further, what regions support growth.

Species Selection

Species of seaweed must be evaluated and a selection made to initiate the commercial farm. The method used in the selection provides an assurance that a non-viable selection will not be made, and that a cost-effective solution is a high probability. The species selected must provide a quantity of biomass capable of producing a given quantity of Substitute Natural Gas. Physical, biological and morphological characteristics, Table 41, can be cited as influencing the potential of any species. Further breakdown of the physical characteristics, (Table 42) are used to evaluate potential candidates and provide the framework for the screening of candidate species.

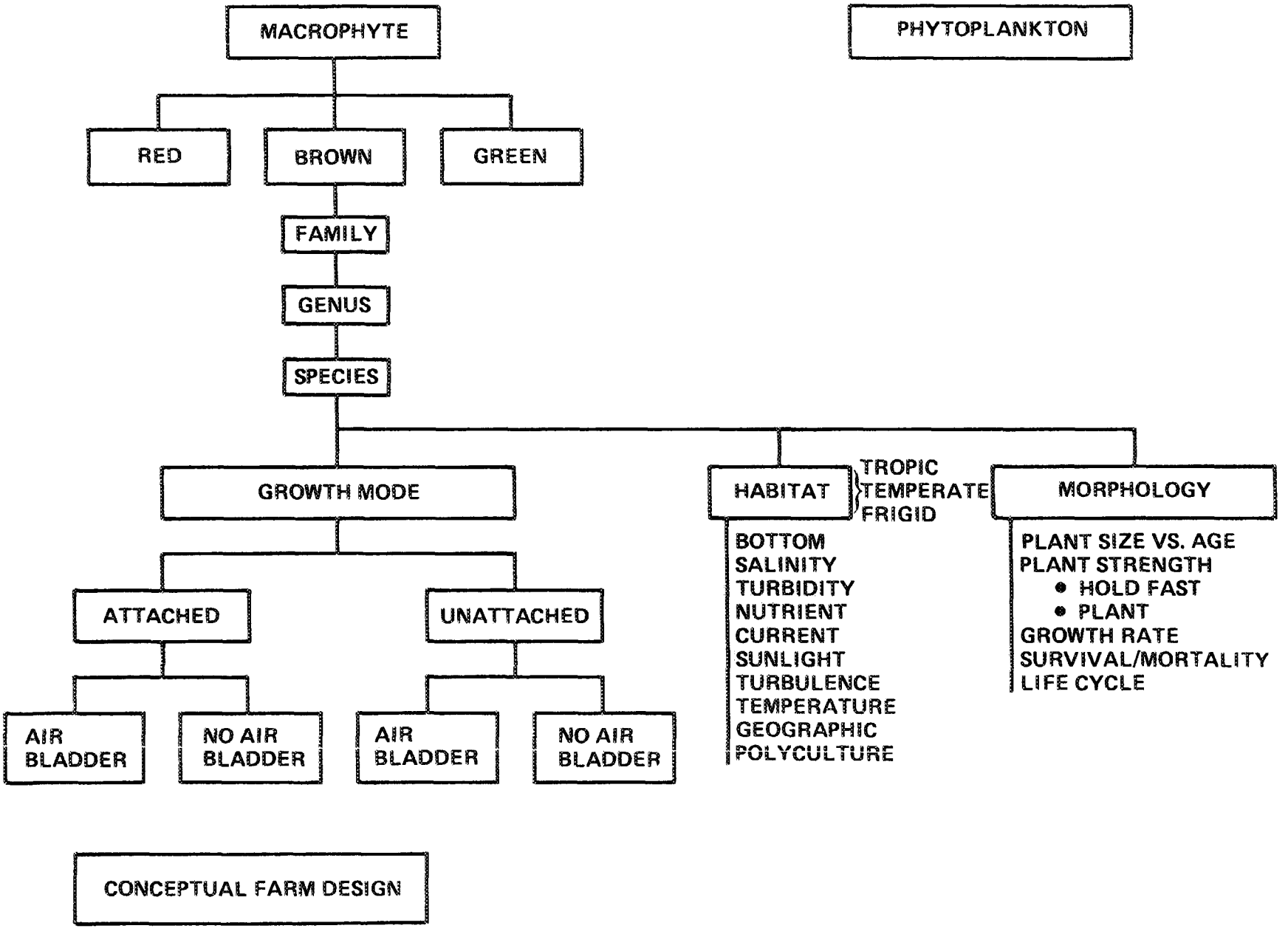
Physical Characteristics of NYS-ERDA Species

The initial concerns of plant physical characteristics will be the subject of the present writing. Morphology, growth mode and habitat are expended in Table 43. As indicated we are principally interested in Macrophytes of the Chlorophycophyta, Phaeophycophyta and Rhodopycophyta divisions. The important of color at present (from an engineering sense) is not known. All three contain

TABLE 41. PLANT CHARACTERISTICS CONSIDERED SIGNIFICANT FOR SPECIES SELECTION

PHYSICAL	BIOLOGICAL	CHEMICAL
1. MORPHOLOGY	1. POTENTIAL BIOMASS YIELD	1. METHANE YIELD
2. GROWTH MODE	2. LIFE CYCLE & REPRODUCTION	2. BIO DEGRADABILITY
3. HABITAT	3. ECOSYSTEM FUNCTION	3. RESIDUE FRACTION
	4. MORTALITY	
	5. GROWTH SYSTEM	
	6. POLYCULTURE COMPATIBILITY	
	7. CULTIVATED GROWTH	
	8. GENETIC PLIABILITY	

TABLE 42. SPECIES SELECTION METHODOLOGY PHYSICAL CHARACTERISTICS



the green chlorophylls (photosynthetic), and usually zanthophylls or carotenes. Phaeophyta and Rhodophyta contain brown and red pigments as well, and these may vary according to habitat and plant health. They do however indicate energy absorption differences and it may be of some significance when establishing the ocean farm requirements.

The plant genus and species within the genus are of importance. Identification of the species permits definition of many characteristics which establish the farm design, the harvesting technique and the processing requirements. Initially growth mode will be either attached or unattached with further distinctions of floating or neutral buoyancy species. This distinction requires conceptual farm designs of differing characteristics to supply an ocean substrate

To initiate conceptual design studies we need data on morphology. The physical outline of the plant along with size and strength are important in conceiving farm substrates. Growth characteristics of NYSERDA species native to New York waters are shown in Table 44. We can now add morphology characteristics, however, we do this for each individual plant in the NYSERDA list.

Species Description

The seaweed species shown in Table 44 are all native to New York State waters and provide minimum ecology perturbations. A more detailed description of each species in this list is given to initiate conceptual design studies.

Species Fucus vesiculosis

Fucus vesiculosis is of the brown division, Fucus genus. Common names such as Rockweed, Bladder Wrack Sea Ware, etc. abound. Fucus vesiculosis is found intertidally from North Carolina to Hudson Bay. The term vesiculosis results from the growth of vesicles (bladders) on the plant. This vesicle growth occurs in plants in quiet waters while plants subject to strong currents will not have these

TABLE 43. SUMMARIZATION OF CRITICAL DATA FOR CANDIDATE SPECIES ASSEMBLED FROM THE LITERATURE

	Ascophyllum	Fucus	Laminaria	Macrocystis	Ulva	Codium
Habitat	mainly intertidal but also to 8 m	mainly intertidal but also to 8 m	low tide to 18 m	intertidal to 40 m	low water to 25 m	low water to 25 m
Seasonal growth pattern (of macro stage(s) (in this area)	perennial	perennial	perennial	perennial	seasonal-annual alternation of generations	perennial
Size (length)	to 3 m	to 1 m	to 3 m	to 40 m+	to 3 m+	$<$1 m
Natural productivity $Kg/m^2/yr$ (d.w.)	to 5.2	to 15.3	-	17.2	-	0.14 (surely too low)
Culture productivity $Kg/m^2/yr$ (d.w.)	-	-	-	-	-	-
Strength of plant (estimated)	high	high	high	high	low	moderate
Reproduction (for culture purposes)	gametes fragments	gametes fragments	gametophytes (fragments?)	gametophytes	spores, gametes fragments	spores fragments
Probable culture Modes	attached, floating?	attached floating?	attached	attached	attached suspended	attached suspended, (floating?)
Estimated nutrient response	low	moderate	low	high	moderate	high
Other uses (if known)	algin seaweed meal	algin seaweed meal	algin seaweed meal (closely related species for food)	algin seaweed meal	food?	

TABLE 44. FIRST LEVEL EVALUATION OF NYSERDA SPECIES PHYSCIAL CHARACTERISTICS

SPECIES	GROWTH			
	A[1]	U[2]	B[3]	S[4]
FUCUS VESICULOSIS [B][5]	X	X	X	X
ASCOPHYLLUM NODOSUM [B]	X	X		X
ULVA LACTUCA [G][6]	X	X		X
CHONDRUS CRISPUS [R][7]	X	X		X
LAMINARIA AGARDHII [B]	X			X
GRACILARIA FOLIFERA [R]	X	X		X
CODIUM FRAGILE [G]	X	X	X	
NEOAGARDHIELLA BAILEYI [R]	X	X		X
RHODYMENIA PALMATA [R]	X			X

1 Attached

2 Unattached

3 Buoyant

4 Non-buoyant

5 Brown

6 Green

7 Red

growths. Therefore Fucus vesiculosis is classified as AUBS on Table 44, implying growth unattached or attached and buoyant or non-buoyant.

This plant presents several challenges in farm design. If we assume that we would like the plant attached and buoyant, a substrate should be provided to attach the holdfast. This requires protection from current forces and wave action if we are to establish vesicles.

Fucus vesiculosis is generally found from North Carolina to Arctic and in the mid to lower littoral. Size commonly ranges from 12-24 inches to 3 feet(2).

Species Ascophyllum nodosum

Ascophyllum nodosum, Rockweek, Knotted Wrach, etc. is one of the most common of intertidal algae, and is found on rocks exposed at low tide. Plant growth may reach 8 to 10 feet, and grow erect from a cylindrical holdfast. Growth was either attached or unattached as expressed by Brinkhuis, and the plant was described as neutrally buoyant or as a sinker. The description in Reference 2 refers to air bladders and states that Ascophyllum nodosum can be found as drift in southern waters (below New Jersey).

Ascophyllum nodosum will grow in conjunction with Fucus vesiculosis in the intertidal zone.

Species Ulva lactuca

Ulva lactuca can attach using a rhizoidal holdfast to submerged rocks, shell, pilings or other seaweed. Resembling a loose lettuce head, Ulva is found free-floating on the bottom of shallow pools or brackish ponds. Ulva may grow blades of up to 20 inches length.

(2) Kenneth L. Gosner, "A Field Guide to the Atlantic Seashore", Houghton Mitlin, 1979.

Species Chondrus crispus

Chondrus crispus is a member of the red (Rhodophyta) family, and grows attached to rocks from low tide level to depths of 3 or more fathoms. Heights of 6 inches and breadths of 4 inches are attained. Dense growth colonies occur naturally. The algae is distributed from New Jersey north, and is especially abundant north of Cape Cod.

Species Laminaria agardhii

Large, up to 10 feet in length, Laminaria agardhii is a dominant species south of Cape Cod. The plant is a perennial with a blade nearly one foot in width, and is thick and flat during the winter months. Laminaria agardhii has a many branched and tough holdfast with a stripe length dependent upon depth. The plant is especially resistant to strong surf common to rocky shores.

Species Gracilaria foliifera

Labeled Graceful Red Weed, Gracilaria foliifera grows to appproximately 1 foot with a central axis of 1/2 inch in diameter. It grows in shallow bays and sounds south of Cape Cod.

Species Codium fragile

The dark green tubular plants Codium fragile was first reported in 1957, from East Marion, Long Island, and is now common along the shores of a number of states. Shellfish beds have been threatened since Codium fragile sometimes covers the complete bed. Inter-tissue accumulation of oxygen in larger plants results in buoyancy of the plant, (hence the name "Oyster Thief") and results in floating aft of plant and attachment device. Growth up to 3 feet is reported. The plant is described as perennial, biennial or psuedo perennial, living for several years.

Neoagardhiella baileyi

No data is available at this time.

Palmaria palmata: (Rhodomenia palmata)

The Palmaria palmata generally attaches to rocks and shells by a disc-like holdfast and may reach 15-20 inches with broad (6 inch) blades. The thallus will be found from Northern New Jersey to Newfoundland and grows in cold waters. The depth quoted is from intertidal to deep waters.

Summary of Species Selection

Physical characteristics of the initial species list are described in the previous section. The data obtained can be used to indicated farm concepts designs (a task not yet addressed), however, conclusions and ranking of candidates should be attempted to direct initial research on plant growth. Table 45 lists the indigenous and nearby species selected for preliminary evaluation and includes Macrocystis sp as baseline. with summary of characteristics to date.

The growth mode, upper size limit, holdfast and nominal habitat column data are essentially literature results. All species are perennial and essentially all have some commercial history (except Sargassum sp).

A conceptual farm design with a seaweed attachment point, vertical growth at some prescribed depth (such as the test farm) appears difficult, since species size limit is relatively low. The largest specie (Alaria esculenta) is only 12 feet long and substrate depths would be relatively shallow in NYS/ERDA region waters to permit sufficient sunlight to penetrate and provide adequate photosynthetic activity. Chondrus crispus (0.5 feet maximum length) normally is found from low tide to 3 feet in depth, implying a substrate depth no greater that 3 feet for growth.

TABLE 45. INITIAL CANDIDATE SPECIES

	SPECIES		GROWTH MODE AF	GROWTH MODE UF	GROWTH MODE AN	GROWTH MODE UN	UPPER LIMIT SIZE (FT)	HOLDFAST	NOMINAL HABITAT GEOGRAPHICAL	NOMINAL HABITAT LOCAL
	MACROCYSTIS PYRIFERA	B	*				200	BRANCHING	CALIFORNIA COAST	COOL WATER, DEEP
INDIGENOUS	ASCOPHYLLUM NODOSUM	B	*	*		*	10	DISC	NORTH OF N.J.	INTERTIDAL
INDIGENOUS	FUSCUS VESICULOSIS	B	*	*	*	*	5	DISC	N.C. TO MAINE	INTERTIDAL
INDIGENOUS	LAMINARIA AGHARDII	B			*		10	BRANCHING	N.J. - MAINE	ROCKS, WHARFS, STRONG SURF
INDIGENOUS	CHONDRUS CRISPUS (L)	R			*		.5	DISC	NORTH OF N.J.	LOW TIDE LINE-TO 3' DEPTH
INDIGENOUS	GRACILARIA FOLIIFERI	R		*	*	*	1			
INDIGENOUS	NEOGHARDIELLA BAILEYI	R			*		1	DISC	TROPICS - MASS	QUIET WATERS
INDIGENOUS	PALMARIA PALMATA	R			*		1	DISC	N.J. - NEWFOUNDLAND	COOL WATERS - INTERTIDAL-
INDIGENOUS	CODIUM FRAGILE	G	*	*			3	DISC	{ NEW YORK }	COLD WATER
INDIGENOUS	ULVA LACTUCA	G			*	*	3	RHIZOIDAL	FLORIDA - NEWFOUNDLAND	
NEARBY	AGARUM CRISBOSUM	B			*		4	BRANCHING	MASS. - NORTH	DEEP WATER
NEARBY	ALARIA ESCULENTA	B			*		12	BRANCHING	MASS. - LABRADOR	ROCKY SHORES - STRONG SURF
NEARBY	LAMINARIA SACCHARINA	B			*		6	BANCHING	CAPE COD - NEWFOUNDLAND	
NEARBY	SARGASSUM SP	B	*	*			3	DISC	TROPICS - MASS.	LOW TIDE - 90'

NOTE:

ALL SPECIES PERENNIAL

ALL SPECIES (EXCEPT SARGASSUM) HAVE COMMERCIAL HISTORY.

AF - ATTACHED, FLOATER

UF - UNATTACHED, FLOATER

AN - ATTACHED, NON-FLOATER

UN - UNATTACHED, NON-FLOATER

5.7 KELP CULTIVATION, CHARACTERIZATION AND GENETICS STUDY

NEUSHUL MARICULTURE INCORPORATED

5.7 Kelp Cultivation, Characterization and Genetics Study

This section focuses on progress made in this task by Neushul Mariculture Incorporated (NMI) from May through August 1980. The work centered on the initiation of cultures, species characterization, laboratory cultures, field cultures, and a survey of foreign literature and industry. Progress also includes additional staff and equipment procurement.

In general, NMI has begun to genetically select, characterize, culture, cultivate, and grow giant kelp plants, as well as assess the potential for the cultivation of hybrid kelps. For example, the production of plants with consistently high mannitol levels should reduce digester retention times and increase methane yield. The cultivation of plants of uniform genotype should increase the predictability of plant responses to the induced environment. The hybridization work could pave the way for "engineering" plants for favorable growth, morphological and biochemical characteristics; and sterile plants could possibly be produced.

Licenses for growing kelp have been obtained and an expanded sea-floor site for growing large kelp plants has been leased at Ellwood Pier from the California Department of Fish and Game.

In addition to the laboratory and field studies, NMI has surveyed the literature generated by Japanese and Chinese studies. This literature is largely untranslated and could be a significant aid in the Marine Biomass Program. Kelp production has been increased dramatically in both countries as a result of genetic selection. In addition to this genetic work, a high level of activity exists in other areas of research and development, such as open ocean farming and productivity enhancement by conventional cultivation methods.

Initiation of Cultures

The objective of this task was to domesticate sporophytes of the three Macrocystic species native to California; M. angustifolia, M. integrifolia, and M. pyrifera to provide samples of the three species having a known genetic history. These samples can then be used in subsequent selection, hybridization, biochemical composition and growth studies. Using standard and new hybridization methods, isolations of each species have been maintained. By the end of August, fifty-five gametophytes derived from single spores were isolated from cultures taken from the sporophytes of three different stock plants of M. pyrifera collected from Point Loma. Thirty gametophytes were isolated from cultures initiated from two different stock plants of Macrocystis collected from Anacapa Island.

Species Characterization

The objective of this task is to provide a data base of morphological, physiological, growth pattern and biochemical characteristics of the three Macrocystis species. This base will allow trade-off studies of species characteristics and trait selection as well as aid in development of experimental plants for selection and hybridization.

Species characterization rationales for both morphology and chemistry were developed. Morphometic analysis of both pure-bred lines and hybrids was carried out along with the characterization of plants collected from San Diego. The three species were produced from gametophytic strains and hybrids were made between all three species (M. pyrifera X M. angustifolia, M. pyrifera X M. integrifolia and M. angustifolia X M. integrifolia).

A detailed scheme for characterization of juvenile sprophytes was developed and seventy-seven hybrids were characterized using this scheme prior to transplantation to the ocean. Twenty hybrid plants were subjected to a

"development with time" experiment which involves close examination of specific growth characteristics on a weekly basis.

Chemical characterization techniques are being investigated and developed in concert with a review of methodology in the literature.

Laboratory Culture

The objective of this task is to establish a bank of cultures of selected species and hybrids beyond the gametophytic stage. This bank is to provide specimens for evaluation of traits such as mannitol content, growth rate and morphological and growth patterns.

Laboratory cultures of sporophytes were established from gametophytic strains of all three species of Macrocystis. Bubble cultures were employed to hold these young sporophytes in flasks. Tank culture with running seawater was also carried out temporarily at the University of California at Santa Barbara greenhouse.

Work accomplished in July 1980 included the construction of a sporophyte holding facility at Ellwood Pier and the outplanting of pure lines and hybrids, as well as the rearing of many sporophytes in large bubble-culture containers. Approximately 200 hybrids were developed from bubble culture in the laboratory.

A specially designed sporophyte tank and culture system were under construction at the NMI laboratory during August 1980.

Field Culture

This task will allow the determination of growth characteristics and evaluation of selected stocks in the sea for potential application in marine farm systems.

Additional substrate systems were constructed and put into place at the Ellwood Pier Facility. Substrates were built to hold both young and mature sporophytes of Macrocystis at Campus Point. Macrocystis plants with a known culture history, that have been under observation for the past nine months, were

put in place on a new marine farm structure at Campus Point. Work completed included the establishment of reliable holding facilities for mature plants transplanted from Monterey and San Diego to Santa Barbara. Farm support spars were installed both in Goleta Bay and adjacent to Ellwood Pier on the new lease site. An electronic buoy with automatically adjusting depth control was successfully tested at the Pier.

The Goleta Point Farm was also increased by the addition of four new substrates. As of August, there were 157 juvenile sporophytes developed from cultures growing on six substrates and 19 mature transplanted plants (6 from Point Loma, 4 from Anacapa Island and 9 from the local waters of Santa Barbara) located on the farm. Seventy-six juveniles were characterized, photographed, and outplanted on substrates at the Point.

Survey of Foreign Literature

The objective of this task is to extract and compile significant data on kelp genetics and farm operations from the foreign literature. This data will provide a base which can be used to aid the development of genetic selection and kelp productivity improvement techniques for the Marine Biomass Program.

Lines of communication were established with Japanese investigators who are interested in introducing Macrocystis into Japanese waters. Mr. Masahide Inui, Manager of the Technical Division of Tokyo Kyuei Co., Ltd. visited NMI and was interested in possible collaborative work on the environmental impact of Macrocystis farming in Japan. A review of pertinent Japanese and Chinese literature was initiated. A bibliography was prepared which covers most of the foreign literature dealing with macroalgal culture in the Orient. At the Xth International Seaweed Symposium, it was possible to review some of the culture

work being done in Sweden by Dr. T. Von Wachenfeld and his co-workers. Special emphasis was placed on literature describing substrate construction, crop plant selection and cultivation, nutrient enrichment and genetics.

6. SPECIFIC OBJECTIVES AND WORK PLAN FOR 1981

As shown in the previous sections, significant progress has been made during 1980 toward the objective of determining the technical and economical feasibility of producing methane from seaweed grown in the open ocean. It is evident, however, that additional work is necessary in order to narrow the uncertainties and drive toward a positive determination of the feasibility of the overall concept.

A major goal during 1981 will be the hemi-dome modification at the Test Farm. This modification must be accomplished before kelp outplanting and subsequent biological experiments and data collection can be accomplished at the Test Farm. The near-shore and laboratory biological studies, kelp conversion studies, inoculum development and pre-treatment and post-treatment studies will be continuations of the research already underway. System analysis will integrate the data developed from this ongoing research into the updated model for evaluation of the overall concept.

WORK PLAN - 1981

A. MARINE FARM SYSTEMS

1. Test Farms Maintenance/Operations
2. OSTF Refurbishment/Repair
3. OSTF Modification Construction
4. OSTF Modification Deployment
5. Material/Hardware Tests
6. Instrumentation Tests
7. Nutrient Dispersion Studies
8. Drag Model Experiments
9. OSTF Modification Model Tests/Analysis
10. Biological Engineering Tasks (Kelp Help)
11. AS-CTF* Design/Fabrication/Installation

B. KELP YIELD/BIOLOGICAL STUDIES

Field Studies (OSTF and AS-CTF)

1. Environmental Monitoring
2. Mariculture Methods Development/Test
3. Yield Measurements
4. Foreign Technology Survey
5. Culturing Experiments

Laboratory Studies

Physiological Studies

1. Macro-Nutritional Requirements
2. Micro-Nutritional Requirements

*Artificial Substrate - Coastal Test Farm

Biological Studies

1. Hybridization Studies
2. Genetics Studies
3. Culturing Studies
4. Farming Methods Development

C. KELP BIOMASS CONVERSION

Inoculum Research/Development

1. Ultimate Digestibility Studies
2. Food Chain Synthesis Studies
3. Particulate Attachment Studies
4. Temperature Effects Studies
5. Aceticlastic Methanogen Studies

Anaerobic Digestion Systems Research/Development

1. Multi-Stage Digestion and Reactor-Design Evaluation
2. Feeding Rate Effects Studies

Pre- and Post-Treatment Studies

1. Kelp Supply and Distribution