GRI-79/0079

MARINE BIOMASS PROGRAM

ANNUAL REPORT FOR 1979

Gas Research Institute,
10 West 35th Street,
Chicago, Illinois 60616

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MARINE BIOMASS PROGRAM

ANNUAL REPORT FOR 1979

Prepared by

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For

GAS RESEARCH INSTITUTE
10 West 35th Street
Chicago, IL 60616

September 1980

RE-ENTRY SYSTEMS DIVISION
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GENERAL ELECTRIC

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16. Abstract (Limit: 200 words)

The Marine Biomass Program is a Research and Development Program which has as its overall objective the development of integrated processes for production and harvesting of seaweed in the ocean and conversion of that seaweed to methane at costs competitive, on a commercial scale, with other alternate energy production systems.

The General Electric Company has been the prime contractor in the conduct of this R&D Program for the Gas Research Institute since December 1976. The United States Department of Energy has also sponsored research on this program by funding to the California Institute of Technology, and has provided additional support to the program through a cooperative grant made to General Electric in 1978.

Experimental data 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. This report expands upon this data base with emphasis on the technical and economic requirements of the critical parameters associated with biomass yield and overall energy balance.

17. Document Analysis a. Descriptors
Biomass, Macroalgae, Kelp, biogasification Methane, methanogenesis, anaerobes, fermentors; Macrocystis.

b. Identifiers/Open-Ended Terms
inoculum-development; Biomass-conversion; anaerobic-digestion, marine-inocula; digestion-reactors; synthetic-fuels, off-shore-test-farms. Deep-water-upwelling.

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GRI COMMENTS

The work described in GE's Marine Biomass Program Annual Report for 1979 was accomplished while the program was at a relatively low funding level due to cofunding delays. During this 'maintenance phase', the program was kept intact though relatively little new research was initiated. Despite this problem, GE and its subcontractors performed excellent work and even achieved significant milestones by demonstrating that adult plants can reproduce in the open ocean and that growth can be stimulated by artifically upwelled deep ocean water in juvenile and adult kelp plants. This program will remain in a 'maintenance' mode until cofunding is obtained and the test farm is modified. It is anticipated that farm modification will be initiated during the summer of 1980.

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1. RESEARCH SUMMARY

Title

Marine Biomass Program
GRI Code: IU-115-2
GRI Contract Number: 5010-323-0014

Contractor

General Electric Company

Principal Investigator

Alan N. Tompkins, Program Manager, Biomass Programs

Time Span

January 1, 1979 to December 31, 1979

Major Achievements

- The Biological Test Farm, deployed approximately five miles off the coast of California at Laguna Beach in September 1978, was maintained at operational readiness during 1979. The reliability of the test farm's upwelling pumping system was raised to the required level of 85% through modifications incorporated into the fuel feed system, engine control system and electrical system. Changes were made in the test farm dispersion system to preclude damage to kelp plants caused by their ingestion into pump discharges during periods of pump shut down.

- A series of design studies were conducted which resulted in identifying several potential modifications which are expected to minimize the problem of kelp abrasion and entanglement encountered after the first test farm planting.

- In May 1979, an extensive population of juvenile plants was observed on all underwater surfaces of the test farm substrate. These juveniles were the result of the recruitment of spores released by the initial crop of adult plants, and their early growth was stimulated by strong natural upwelling thru June. A modification was incorporated into the test farm dispersion system to provide upwelled water directly to a portion of these juvenile plants located on one of the substrate spider pole arms. By August 1979, juvenile plants receiving nutrients from this upwelled water had grown to lengths of 25 feet before they became damaged by interaction with the test farm structure.

- In August 1979, three adult kelp plants were attached directly to one of the mooring lines connecting the machinery buoy to one of the mooring buoys. As of the end of December 1979, these three plants had survived the rigors of the ocean environment without any evidence of damage.

- Throughout 1979, gas conversion experiments continued at the 1 and 10 liter scale. In January 1979, 50 and 100 liter digesters were brought on line signalling the first step in the scale-up of the bioconversion process.

Recommendations

- As a result of experiences with the test farm through 1979, it is clear that modifications to the test farm must be implemented which will preclude entanglement and abrasion of kelp plants with the structure of the test farm. These modifications can take any one of several forms, and all options must be studied. The results of the experiments with the three adult plants attached to the mooring line indicate that if adult plants can be isolated from the edges and rough surfaces of the test farm, they will survive the violence of the ocean environment. Further work on kelp plant dynamics is recommended to provide data on the effects of water motion on kelp plants. This data is necessary for the design of more effective kelp plant tether systems.

- As a result of a peer group review of the status of the gas conversion experiments, it is recommended that the gas conversion experiments be expanded to include work on the effects of continuous feeding of digesters, innovative digester design concepts including plugflow system, packed bed system, and fluidized bed system. It is also recommended that additional effort be applied to identifying and characterizing the organism responsible for the acetate to methane conversions present in kelp fermentation as this chain provides 70% of the methane produced.

Description of Work Completed

- Although the Marine Biomass Program is a multi-year R&D Program with work on many program tasks spanning several years, several key elements of work were completed during 1979. These include:

  - Preliminary definition of concepts for minimizing kelp entanglement and abrasion on the test farm.

  - Development of experiments to be conducted on kelp plants dynamics at the test farm.

  - Upgrading of test farm pumping system reliability.

  - A preliminary evaluation of juvenile recruitment.

  - Steady-state operation of 50 and 100 liter kelp digesters.

2. OVERALL PROJECT OBJECTIVE

The overall objective of the GRI Marine Biomass Project is to investigate and develop those integrated processes including feedstock production, harvesting and conversion to produce methane from seaweed that is cost competitive on a commercial basis with other alternative sources of energy. To accomplish this objective, quantitative determinations are being made through direct experimentation and evaluation of concepts for the feedstock production, harvesting, conversion, by-product/co-product recovery, and the essential supporting technologies for complete systems. The technical, economic and energy requirements of the system are being determined so that the feasibility of producing cost-competitive commercial energy products from marine biomass farms can be fully established.

From the standpoint of availability of arable land, water, and fertilizer, biomass produced by a marine farm is a renewable biomass energy source that has the potential of contributing large quantities of Substitute Natural Gas (SNG) to the national gas supply. Due to the low solar efficiency of photosynthesis, any energy farm (terrestrial or marine), must cover very large areas in order to provide significant amounts of feedstock material. Table 1 illustrates the required farm areas to produce 100 percent of the current national gas supply as a function of yield per acre.

TABLE 1. PROJECTED MARINE FARM SIZE FOR NATIONAL SNG PRODUCTION

Biomass Yield (Dry-Ash Free Tons/ Acre-Year)	Required Farm Size to Produce 100% U.S. Gas Needs ($Miles^2$)	Miles Per Side
25	112,225	335
50	55,225	235
75	40,000	200

As can be seen from this table, the marine farm concept requires utilization of a relatively small portion of the open ocean. The concept of a marine farm (with a yield of 50 dry tons/acre-year) producing 10 percent of the national gas supply and occupying an ocean area of about 5500 square miles is a manageable one when the expanse of available open ocean area is considered. In addition, the nutrients required to sustain optimal growth rates are available at depths starting at 100 meters. Preliminary estimates indicate that the deeper nutrients can be delivered to an ocean farm at relatively low energy cost. In some areas, sufficient nutrients occur in the surface waters. These overall considerations have led the Gas Research Institute and DOE to sponsor investigation of a marine farm concept as a prime candidate for development as a major renewable energy source.

Over the next two to three years, the objectives which must be met are (1) to determine if macroalgal feedstock can be obtained in sufficient quantity and yield to demonstrate a strong economic future of ocean farm systems, (2) to confirm that ocean farms can provide net energy gains, and (3) to determine that macroalgae can be harvested and converted to methane or other fuels at costs competitive with other sources of energy. General concepts investigated during 1979 in the marine farming area include growing kelp on suspended artificial substrates positioned in deep ocean waters, utilizing nutrients obtained from artificially upwelled deep ocean water.

In the biomass conversion area, approaches being investigated include (1) development of inocula capable of increasing methane generation and yield, (2) development and operation of scaled-up digester systems producing optimum methane yields, (3) development of processes for pre- and pos-treatment of the biomass to improve process efficiency and to identify and recover useful by-products.

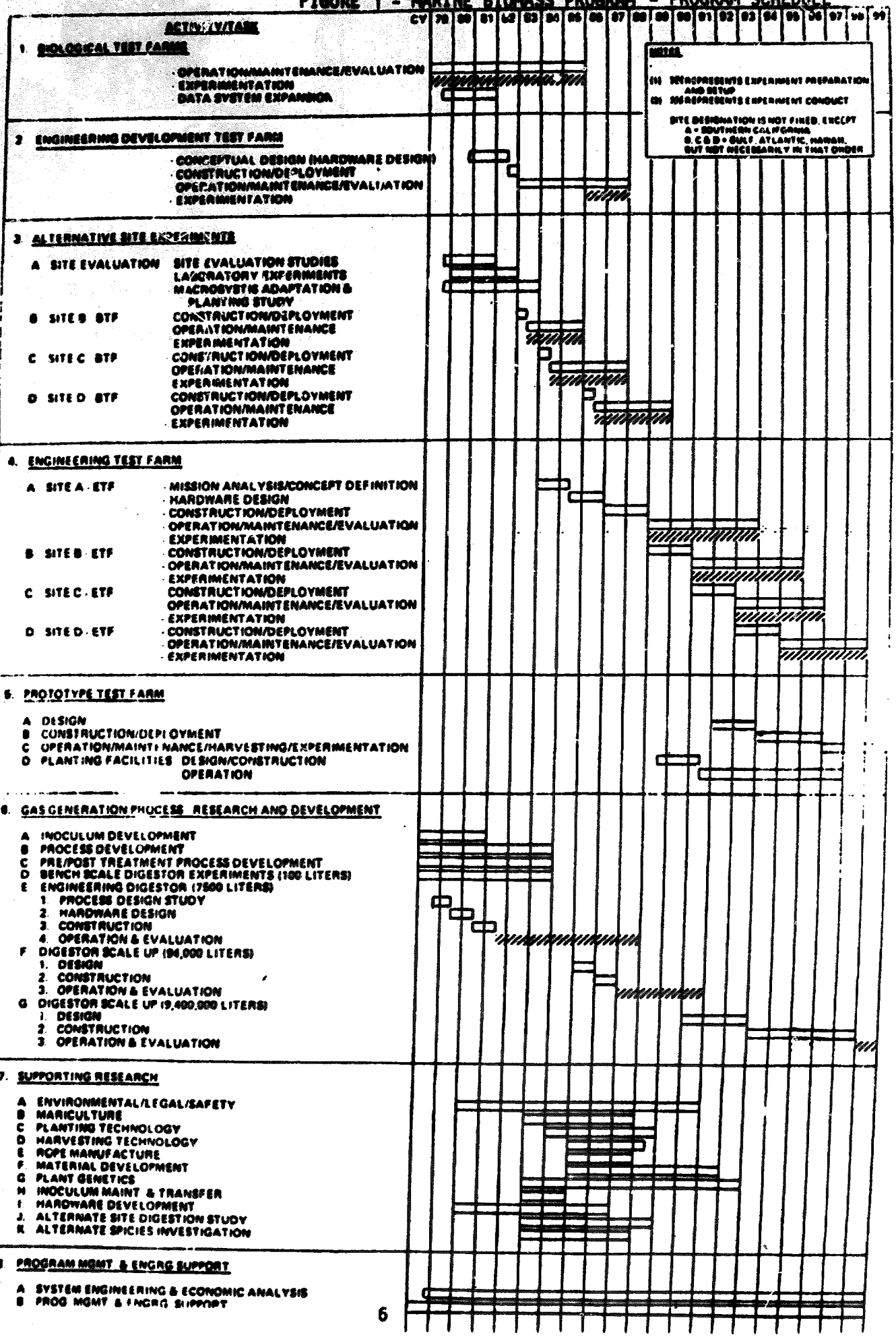
3. SUMMARY OF ALL PREVIOUS WORK PERFORMED

The Marine Biomass Program prior to 1979 emphasized acquisition of the biological and engineering data that are critical to making an accurate determination of the technical and economic feasibility of the marine biomass concept. The overall program is shown in Figure 1. All previous work has been focused in the concept validation phase which includes kelp growth and nutrition, ocean engineering, and methane generation studies.

KELP GROWTH AND NUTRITION

The investigation of kelp growth and nutritional requirements was funded by the Department of Energy and performed under the leadership of Dr. Wheeler North, Professor of Environmental Sciences, California Institute of Technology at Corona del Mar, California. The objective of this work

FIGURE 1 - MARINE BIOMASS PROGRAM - PROGRAM SCHEDULE



was to determine the nutritional requirements for Macrocystis pyrifera and to provide information on the physiological and biological requirements for optimizing kelp growth utilizing the open ocean test farm. Through extensive laboratory and field observations, Dr. North has specified a set of biological criteria which must be met in order to produce commercially acceptable yields on the ocean farm. Laboratory and field experiments investigating the growth response of juvenile Macrocystis sporophytes to nutrition by continuous immersion in deep water were performed. The significance of the specific micro-nutrients manganese and iron to increase kelp growth were investigated by Dr. North. Preliminary investigation indicates that trace quantities of these metals may play a major role in obtaining maximum kelp growth and yield. 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 will allow consideration of a range of design and operational strategies for supplying optimum levels of nutrition to the energy farm.

OCEAN ENGINEERING

Test Farm Design. The ocean engineering task of the concept validation program was performed by Global Marine Development Incorporated, Newport Beach, California. The objectives of this program were to provide an open ocean test bed for Macrocystis cultivation and to develop a series of designs for prototype or pilot commercial farms. The design for the test farm was completed in January, 1978. Construction began in April, 1978, followed by deployment during September, 1978.

The design of the test farm presented several engineering challenges, for example:

- The structure must be open and yielding, rather than rigid, so as not to offer substantial resistance to the ocean currents, winds, and waves.
- The structure must survive a 100 year storm with minimal damage.
- The structure must be completely compatible with the biological requirements for Macrocystis.
- Personnel safety and operational reliability are prime design constraints.
- 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 satisy the biological requirements of providing ambient levels of 3 microgram-atoms of nitrogen/liter throughout the farm volume.

GAS GENERATION RESEARCH

Unlike terrestrial plants, Macrocystis pyrifera has not evolved the lignin cellulose biopolymer for structural support. Instead, the plant is supported by specialized flotation members (pneumatocysts) attached to the base of the blades. The absence of lignin is a decided advantage for bacterial digestion. Figure 2 is a diagram of the average composition of Macrocystis pyrifera including percentage composition of water, inorganic salts, and volatile solids. The water content of the fresh plant, the absence of lignin and the presence of volatile solids that are biologically degradable, were the reasons for selecting the anaerobic digestion process.

The program tasks in anaerobic digestion are directed toward research and development in three major areas; Pre/Post Treatment, Inoculum Development, and Anaerobic Digestion Process Development.

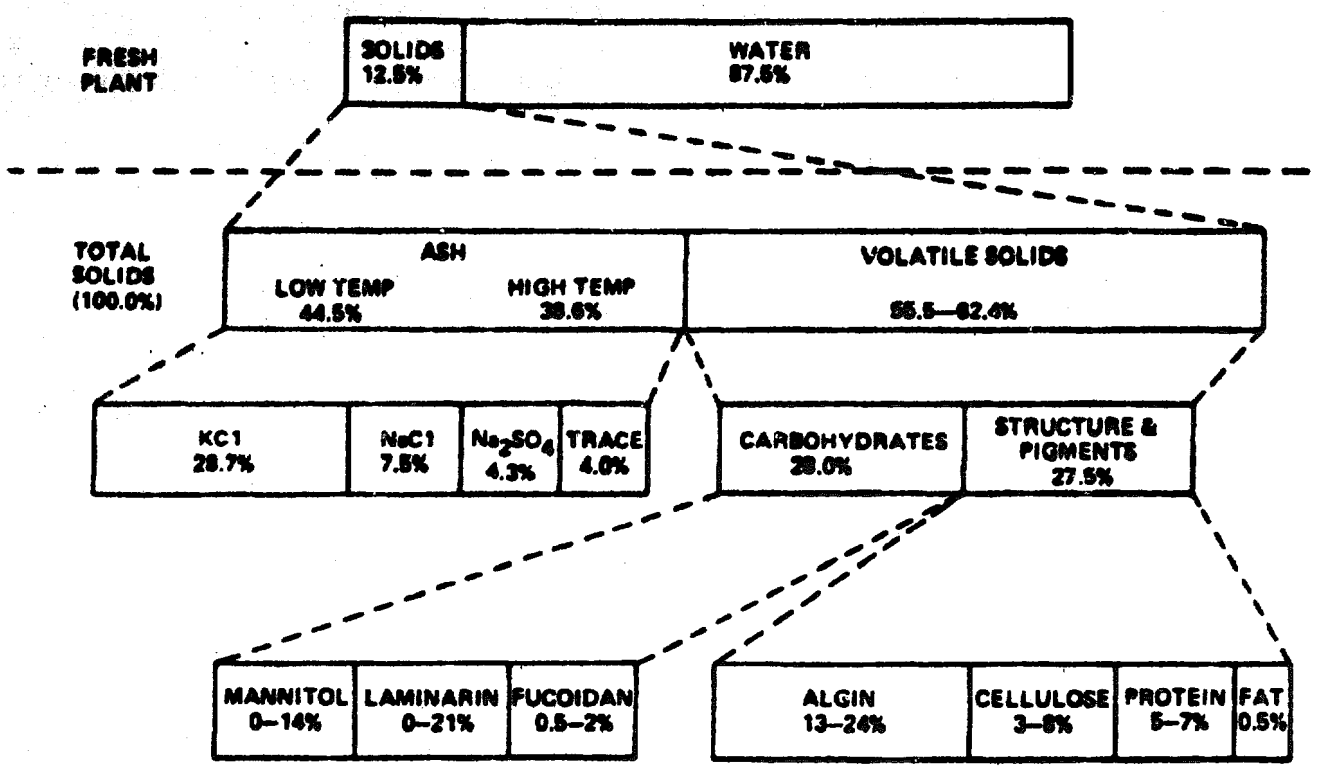


Figure 2. Macrocyctis Composition

Pre/Post Treatment Processing

This work was conducted at the Western Regional Research Center, United States Department of Agriculture, Albany, California, and was concerned with the definition and evaluation of mechanical and chemical pre/post treatment process steps which may increase the bacterial digestibility of kelp. Mechanical pretreatment studies have centered on the least capital and energy intensive methods to increase surface areas and cell rupture.

Additional studies related to this task were to investigate the potential of the digester solid effluent as an animal feed supplement.

Inoculum Development

The research activity, conducted at the General Electric Company, Re-entry Systems Division, centered on the development of an optimized anaerobic inoculum incorporating microorganisms which have been derived from the marine environment. The potential results of this research could have major impact on several significant cost centers in the gas generation process. Since the kelp substrate is of marine origin, it was felt that a collection of marine derived organisms, which decompose kelp in nature, will have the requisite enzyme systems for rapid depolymerization of kelp cellulose and algin and for utilization of the resulting degradation products.

Anaerobic Process Development

The objective of this work was to define and optimize the anaerobic digestion process for conversion of Macrocystis pyrifera to methane. The kelp digestion work was conducted at the Institute of Gas Technology (IGT), Chicago, Illinois.

3.1 MAJOR PROBLEMS AND SOLUTIONS

Problems and solutions relating to start-up and operation of the Test Farm are summarized below.

1. The Test Farm must be modified to increase its ability to provide "plant protection" to reduce plant abrasion as well as provide nutrient containment.
2. The operation of Engine #1 in the upwelling pumping system has been unsatisfactory. Action is underway to replace the engine under the manufacturer's warranty.
3. The modified Test Farm must be replanted with healthy plants obtained directly from natural beds at the same water depth as the Test Farm substrate.
4. An experimental start-up period of at least three months, after farm planting with healthy plants, is essential before data evaluation can begin to insure that the plants are acclimatized to the new environment.
5. Storm induced waves and currents cause relative motion and contact between the plants and the structure resulting in abrasion to the plants. Instrumentation must be provided to measure the motions of the Test Farm structural elements.
6. To preclude abrasion and tangling of the plants on the farm structure during heavy current conditions, a protective system will be designed and installed on the underwater portion of the farm.
7. Automatic fail/safe controls in the upwelling pumping system have activated properly, thereby turning off the engines as required, but resulting in periods of up to five days without upwelling. Continuous monitoring (recording and alarm) of the upwelling pumping system must be provided in order to minimize long periods of time without nutrient.

3.2 MAJOR ACCOMPLISHMENTS

Test Farm

1. The test farm upwelling system has performed as designed providing 9000 gpm of water containing from 25 to 32 microgram atoms per liter of nitrogen in the form of nitrates and nitrites.
2. The test farm's mechanical structure has performed as designed, and the integrity of the test farm's mechanical structure continues to be sound.
3. The curtain successfully worked as a device to reduce currents near the plants and thereby protect the plants and retain nutrients in .4-.5 knot current.

Gas Generation Research

1. Digestion has been conducted at ambient temperature with marine derived inocula, at the liter scale, with no apparent loss in gas production.
2. Digestion has been conducted at salt concentration of up to 7 percent (seawater is 3 percent), at the 10 liter scale using sewage-derived inocula, with equivalent methane yields. This data indicates that seawater dilution of kelp feedstock is possible, if necessary.
3. Based on the published literature, the methane yield from the anaerobic digestion of kelp is higher than for other types of biomass.
4. Ground, undiluted, raw kelp can be added directly to the digesters.
5. The digester solid effluent has considerable potential as a feed supplement and is chemically comparable in protein value to soy protein.

4. SPECIFIC OBJECTIVES FOR 1979

The major objectives for 1979 were to:

-- Assure continuous operation of the nutrient supply system, navigation marker equipment, and data collection system.

-- Perform preventative maintenance and corrective maintenance as required on the Biological Test Farm.

-- Continue biological experiments at Cal Tech related to plant selection, attachment, recruitment, mortality, nutrient analyses, and observation of associated flora and fauna.

-- Maintain stock and baseline cultures.

-- Continue Microbial Enrichment/Isolation studies.

-- Continue Biomass Conversion Process Development Experiments.

-- Evaluate pre- and post-treatment techniques

-- Continue kelp supply for on-going experiments

-- Continue program management

5. 1979 WORK PLAN BY TASK AREA

This section describes the actual effort expended in the "performance" mode of this contract. To provide clarity for the reader, the areas reported will be presented as individual sections as follows:

5.1 Biological Test Farm Maintenance and Operations (GMDI)

5.2 Kelp Biological Studies (Cal Tech)

5.3 Inoculum Development (GE/RSD)

5.4 Conversion Process Development (IGT)

5.5 Pretreatment and Posttreatment Studies (USDA-WRRC)

5.1 BIOLOGICAL TEST FARM MAINTENANCE AND OPERATIONS

GLOBAL MARINE DEVELOPMENT INC.

GENERAL REPORT

The 1979 GMDI project objective was to provide a minimal maintenance program for the Biological Test Farm (BTF). The overall project objective was to provide support, maintain, and repair the test farm in the open ocean upon which Macrocystis pyrifera was to be grown.

Prior to 1979, the BTF was designed, fabricated, and deployed off Laguna Beach, California. Kelp transplanting was completed in December 1978.

The specific objective of 1979 was to maintain the BTF. In actuality, minimal maintenance plus raising the system reliability was accomplished. The reliability of the machinery buoy system was raised from below 50 percent to above the required 85 percent during the year. Minor additions were made to the farm including the addition of check valves to the dispersion system and extending one dispersion hose down to the substrate to feed juvenile plants.

Forty-four maintenance visits were made to the BTF in 1979. The engines on the BTF consumed 150 drums of diesel fuel and 155 gallons of lube oil. One diesel engine was replaced after suffering major internal damage from an ingested foreign object. A second diesel engine was rebuilt on board after a similar problem. The problem causing element, the air filter system, was replaced with a sea cap. The vibration mounting hardware on the engines was modified to solve a mounting bolt failure problem. The main system fuel pump was replaced solving fuel starvation and fuel flooding problems. The original pump case exhibited structural weaknesses. Redundant engine starting and shutoff systems were eliminated to prevent accidental system shut off and electrical short circuits from wiring failures.

A formal maintenance schedule and check list system was instituted providing better controls, documentation and maintenance planning. A qualified diesel mechanic was added to the work party. He has rebuilt an engine onboard and has increased the engine reliability through his constant checking for parts deterioration and performance. A lube oil analysis program was initiated to monitor the diesel engines. The lab reports provide quantitative data on impurities found in the lube oil alerting us to internal fuel leaks and abnormal engine wear.

The year 1979 was a successful year for the BTF in regard to operational readiness. The system is now well understood and the maintenance tasks have become more predictable than not. The next year, 1980, will bring some different problems as the system has been deployed for a year and a half. Methods and procedures will have to be developed to predict and handle problems that arise attributable to time. One problem is that of replacing sacrifical anodes in situ. Another potential problem is the accumulation of water in the fuel tank on the machinery buoy due to condensation over time. There are also potential wear problems in mooring lines and structural attachments. Planning is in process to recognize and solve these potential problems under general maintenance instead of emergency repair.

The specific objectives for 1980 are to keep ahead of any maintenance problems and keep the BTF under positive control. The aim is to continue working toward having a facility that can be efficient and safe in terms of functional performance and reliability. Modifications must be made to the BTF in order to meet the primary objective of growing kelp in the open ocean. GMDI will continue to support the 1980 Marine Biomass Program in any capacity requested by General Electric Company.

5.2 KELP BIOLOGICAL STUDIES

CALIFORNIA INSTITUTE OF TECHNOLOGY

GENERAL REPORT

Kelp biological studies continued during 1979 in a variety of areas including operations at the test farm, in the general area of the ocean surrounding the test farm and in the laboratory. The results of these studies were reported during January 1979 through June 1979 in a series of monthly reports to the various agencies supporting this effort. For the period July 1, 1979 thru December 31, 1979, a similar series of monthly progress letters were generated by which results were documented. These reports may be obtained from the California Institute of Technology. By permission, a verbatim extract from a compilation of July through December 1979 Monthly Progress Reports is included herein as pages 39 through 50.

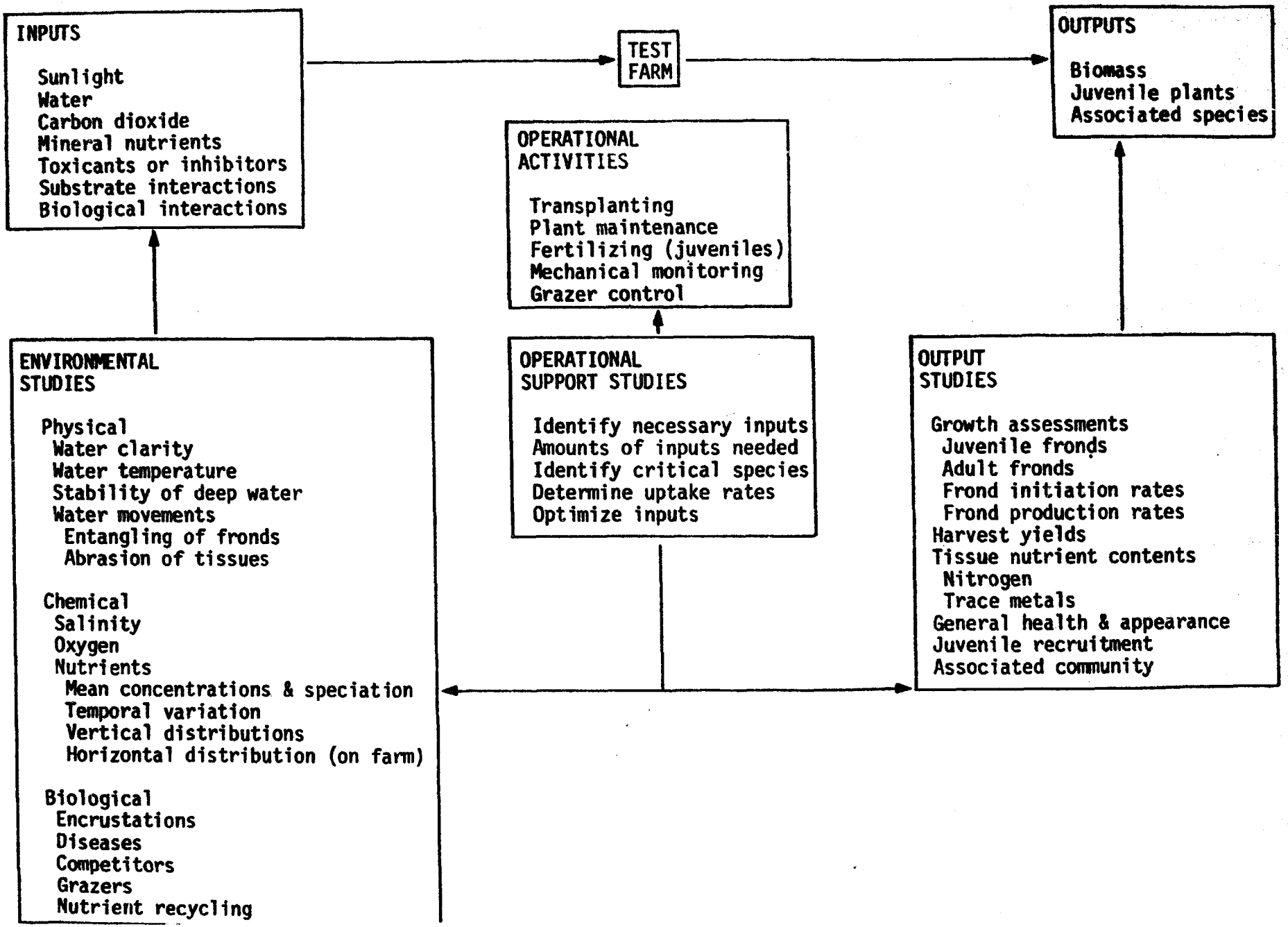
1978-79 IN RETROSPECT

The principal event of the past year was the initiation of scaled-up field operations in a two-year experiment that involves assessing results from fertilizing about 100 adult Macrocystis plants with water artificially upwelled from 450 m depths. The study, designated the Test Farm Experiment, had been in the planning and implementation stages for about three years. Much of our previous work, as well as ongoing activities, were related directly or indirectly to the Test Farm Experiment (Table 2). In this summary review of our 1978-79 activities, we will be presenting results in an organizational framework that emphasizes relationships either directly to the Test Farm or to similar prototype farms of the future. The primary categories of activities shown in Figure 1 will be used as the main subdivisions of our discussion.

Operational Activities

Our principal accomplishments in this category involved transplanting adult Macrocystis to the Test Farm in November-December 1978, disentangling fronds of these plants from the structures around them in the subsequent two months, and providing information regarding mechanical operations to our collaborators at General Electric Company (GE) and at Global Marine Development Inc. (GMDI). We also attempted to fertilize a crop of juvenile plants on the Farm in late Spring, but this activity is more appropriately discussed below under Output Studies. We will discuss here only the transplantation operation.

TABLE 2. RELATIONSHIPS BETWEEN FLUXES OF ENERGY AND MATERIALS AT THE TEST FARM AND THE PRINCIPAL GROUPINGS THAT CONSTITUTE OPERATION AND MONITORING OF THE FARM BY STAFF OF THE CALIFORNIA INSTITUTE OF TECHNOLOGY



We began stockpiling adult kelp transplants in early August 1978, in anticipation of deployment of the Test Farm structure during Fall. We had already laid out about 1000 ft of scrap chain, secured at intervals to engine blocks, on the bottom at depths of 40 to 50 ft off Reef Point (about two miles northwest from Laguna Beach, California, Figure 3). The chain was designed to receive and hold about 150 kelp transplants. The location was selected because it lay in that portion of the coast closest to the Test Farm site and also supported a natural stand of kelp. We installed a second similar but shorter stockpiling chain off Laguna Beach in October. Most of the transplants were by then being gathered off Laguna Beach, and the second chain eliminated the necessity for towing these plants all the way to Reef Point. About 117 plants had been moved to the two stockpiling chains by November 18, 1978. These were the "best available" in the local beds at that time. All were deemed satisfactory at the time of collection but few if any were in excellent condition, simply because sourthern California kelp beds typically reach their seaonsal lows during early to mid-Fall each year.

The Test Farm structure was successfully installed at the 1800 ft depth contour by GE and GMDI personnel during September 1978 (Figure 3). Operational testing and modification occupied the next two months. The structure was in readiness to receive kelp transplants by latter November except that a protective curtain had not been deployed to reduce effects of current and increase retention of the artificially upwelled water on the farm.

During October we attached small, numbered "planting" buoys to the concentric cables at the outer portion of the substrate arms, to serve as tethering points for our transplants (Figure 4). We began moving our stockpiled transplants to the Test Farm on November 30. We installed a total of 103 plant

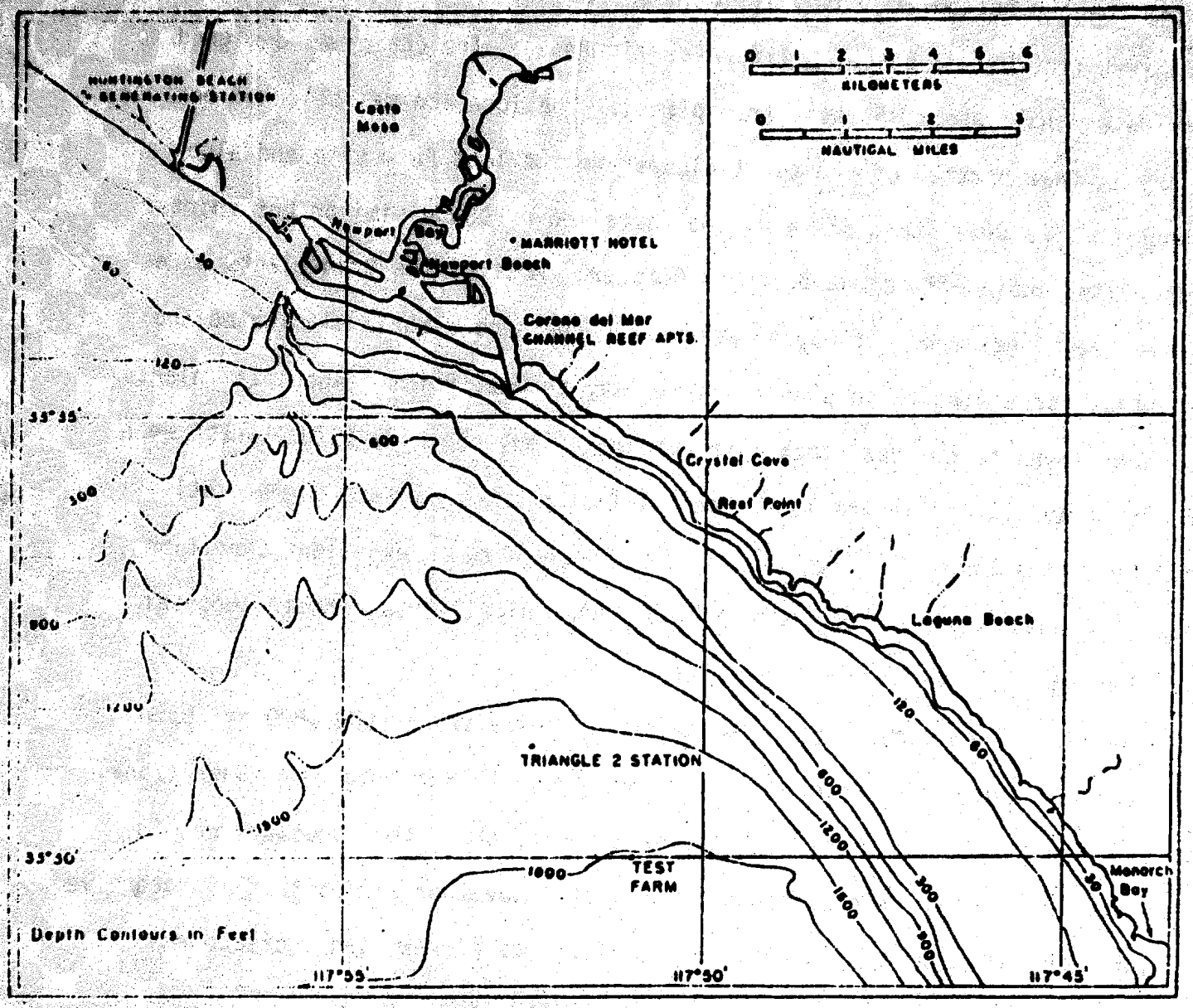


Figure 3. Chart of the southern California coastline from Huntington Beach to Monarch Bay showing locations of the Test Farm and other geographical features described in the text.

on the Test Farm by December 6, including 18 removed that day directly from the Laguna Beach kelp bed (i.e. not stockpiled). We had collected these additional 18 specimens because condition of 32 of the stockpiled transplants was considered unacceptable after they had been moved to the Farm. Given a suitable environment, we had no doubts that all of the 103 plants finally installed would flourish.

GMDI installed a protective curtain around the western border of the Farm on December 12, to reduce effects of currents on the transplants and the dispersed upwelled deep water. The curtain was lost to storms within the following week. Strong currents (> 0.5 knot) were frequently observed at the Test Farm. Entanglement of the plants with cables and other features of the Farm structure occurred whenever current bent the kelp fronds over at large angles to the vertical. Entangled fronds soon perished from abrasion against the solid structures as the substrate heaved in passing swells. Upper portions of all plants were gone by the end of January, leaving only holdfasts and a few tattered frond remains.

Output Studies

Investigations concerned with biological outputs from the Test Farm encompassed two defined time periods: A. December 1978 to January 1979 when our adult transplants existed at the Farm; B. May and June 1979 when dense populations of juvenile plants appeared, presumably offspring arising from spores liberated by the adult transplants five months previously. The most important conclusions and results from our December-January monitoring studies were:

1. Growth rates

   Juvenile fronds: A series of seven weekly determinations between December 12 and January 29 yielded mean standard growth rates ranging from 5.4 to 7.4 percent elongation per day. These are within the

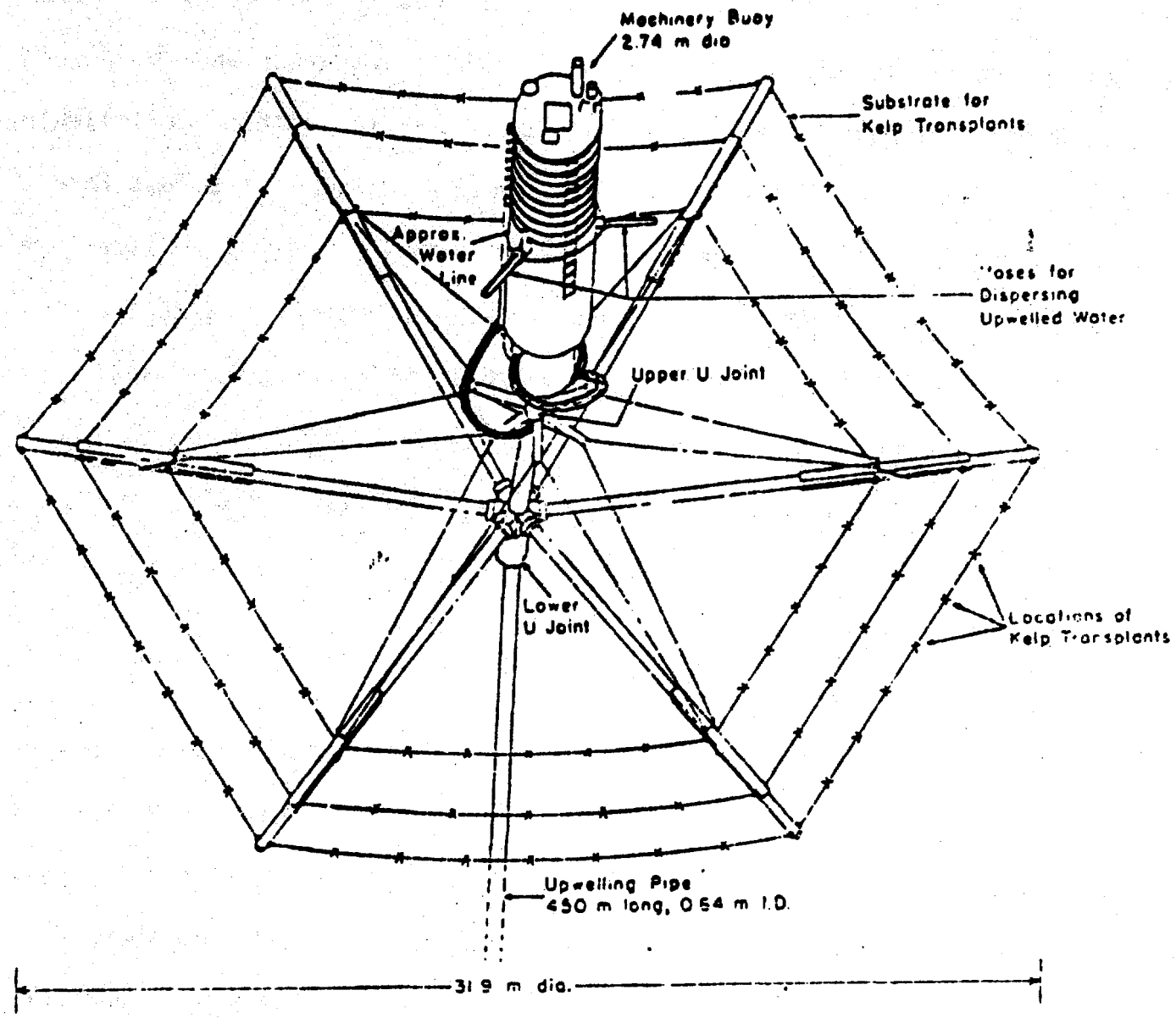


Figure 4. Diagram of the Test Farm structure showing principal features and dimensions.

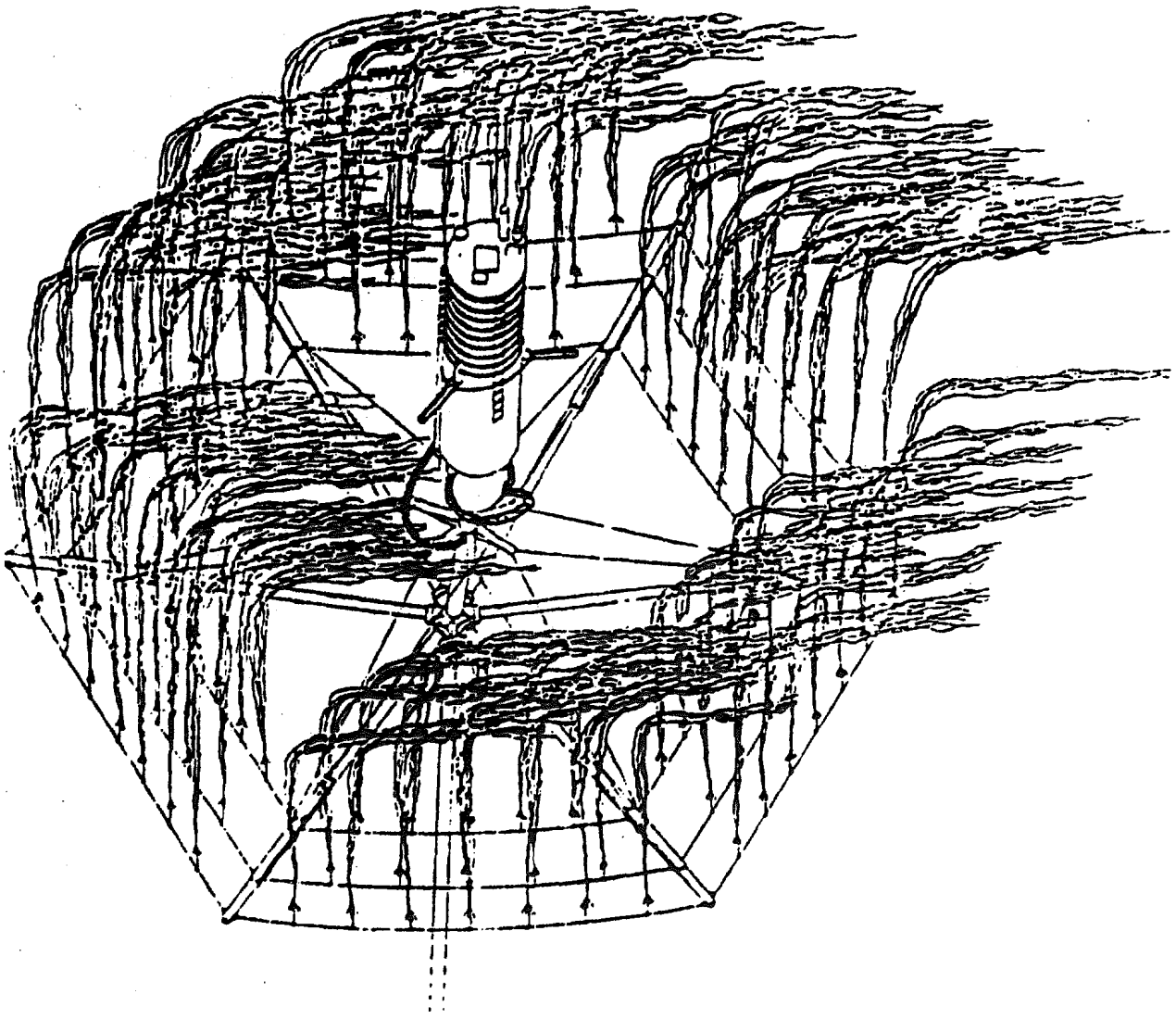


Figure 5. The Test Farm structure as shown in Figure 4 but with diagrammatic representations of kelp transplants added. The holdfasts were tethered by lengths of nylon rope about 6 mm diameter and 60 to 100 cm long, leading to styrofoam buoys positioned just beneath each holdfast to reduce tendencies for interactions with the peripheral concentric cables.

range of normality for natural kelp beds at this time of year but tend to lie in the lower portion of the range. Percent of fronds showing abnormally slow growth rates among tagged juveniles ranged from 6 to 44% of the tagged recoveries, a relatively high proportion of abnormal juveniles. Abnormally slow growth in juvenile fronds often results from severe damage to or loss of the parent adult frond that nourishes growth of the juvenile through translocation of photosynthate.

Adult fronds: Damage and mortality among adult fronds interfered with assessments so that a statistically adequate evaluation of growth rate was not achieved. It was established, however, that some of the tagged specimens generated reasonable rates of production of new blades.

2. Plant mortality

About 2/3 of the initial complement of transplants were lost from December 5 to January 5. Mortality during the next 20 days declined, as only about a third of the remaining plants disappeared. A short but violent squall on January 30 destroyed the last of our transplants. Tangling with and abrasion on various parts of the Test Farm structure were the sole causes of plant mortality.

3. Nitrogen contents of blade tissues

Except for the final week of January (when all of the remaining plants had suffered significant damage), N-contents remained above one percent of the dry weight. In our experience, this represents a healthy nutritional condition. Of the 82 blade samples taken, 71% were above 1.5% in N-content. The highest N-contents were around 2.5% and came from canopy blades during the period when the curtain was most effective in retaining the upwelled water within the Farm. We concluded that, unlike our previous experimental oceanic farms, the transplants on this Test Farm did not suffer from inadequate nutrition.

During January 1979, we observed small juvenile Macrocystis plants attached to several of the planting buoys. One month had elapsed since the transplants had been introduced. We therefore presumed that these juveniles reached the Test Farm as established microscopic-sized plants and did not arise from spores liberated at the Test Farm. Usually at least three months are needed for development of barely visible juveniles from settled kelp spores, and the time may be longer if light or nutrients are not optimal.

In late April 1979, we observed small plants developing near the ends of the Test Farm dispersion hoses. By May, large numbers of juveniles were appearing on most of the solid surfaces of the Test Farm structure down to depths as great as 30 m. Concentrations were sparse, however, below the level of the transplanting substrate (20 m). Development by most of these plants was probably stimulated not by the artificially upwelled deep water but by natural upwelling which usually is maximal during late Spring. The juvenile recruits were studied intensively to gather ecological information that might be useful for encouraging and assisting kelp reproduction on this and on other oceanic farms. Several noteworthy results emerged.

1. Total plant population on the substrate arms, cables, and planting buoys was estimated to be 36,000 individuals.
2. Temporal changes in nitrogen contents of kelp blades paralleled changes in ambient nitrate concentrations (nitrate is a good measure of natural upwelling in this instance) and correlated with changes in rates of plant elongation.
3. Greatest plant mortality occurred on the smooth plastic-coated cables. Plants were probably easily dislodged by water movements from this type of substrate. High mortality rates also occurred among plants on upwelling hoses where barnacle encrustations proliferated and created

extremely abrasive surfaces. Intermediate degrees of mortality occurred on the planting buoys and substrate arms. Lowest mortality appeared among plants attached to the moderately rough surfaces provided by polyester ropes.

4. Tissue nitrogen concentration and growth were enhanced slightly by "spraying" a group of juveniles on a substrate arm, twice weekly with 1 M ammonium sulfate. Even greater enhancement occurred among plants close to bags of Osmocote pellets affixed to the side of a substrate arm. The pellets slowly released nitrogen and phosphorus into the surrounding water.

In summary, perhaps the most revealing result thus far from the Test Farm experiment was our failure to observe increased growth rates among the juvenile fronds during the period when the curtain was retaining the nutrient-rich deep water and hindering effects by currents. This very preliminary finding suggests that growth of juvenile fronds may be limited by the rate at which photosynthate could be translocated downward from the canopy and not from limited availability of nutrients (in this particular case). While we need more experimentation to establish this hypothesis, the possibility has important implications for optimizing biomass productions. If translocation rate is important as a limiting factor in juvenile frond growth, the best strategy would involve trying to achieve a condition where availability of light becomes the principal limiting factor. Presumably this could be done by increasing frond density on the Farm (i.e. placing the plants more closely together).

For the future, our collaborators at General Electric will be installing a more durable protective curtain at the periphery of the Test Farm in late 1979. We will then be able to resume our studies monitoring health and measuring productivity of adult kelp plants being held in the artificially upwelled deep water.

5.3 INOCULUM DEVELOPMENT

GENERAL ELECTRIC COMPANY/RSD

OBJECTIVE

The primary objective of this program is to develop from marine sources an alternative, optimized microbial culture for the conversion of kelp (Macrocystis pyriferia) to methane gas. To this end, it becomes necessary to develop a sufficient undertanding of the microbiological reactions and interactions such that effective control of the conversion process is possible.

KELP DIGESTER STUDIES (BASELINE)

During the course of this development study, kelp to methane digester systems established solely with marine derived inocula, have been maintained as a study base and as a source of microbial species of interest. These systems were charged, as described previously, on an alternate day basis. Beginning about February 1, 1979, settled effluents solids, obtained by gravity-settling digester effluent under nitrogen for one half hour, were recycled at the 20 percent (by volume) level with the incoming feed. In early May, the feedstock was switched from Lot #3 to Lot #47, which, because of its low mannitol content (5.18 percent) was supplemented with exogenous mannitol to approximate that of Lot #3 (18.6 percent). Lot #48 (9.06 percent mannitol), used since September 3, was likewise supplemented. In order to approximate a marine-like environment in the digesters and to be more consistent with probable large scale operating situations, sea water (Instant Ocean) was used as a feedstock diluent beginning July 23.

Performance of these digesters (MF-6 and MF-21) is presented in Tables 3 and 4 and graphically depicted in Figures 6 and 7. Corresponding volatile fatty acids (VFA) and pH values are given in Tables 5 and 6.

Toward the end of 1978 and during the first few weeks of 1979, the performance of these baseline digesters deteriorated significantly - methane yields dropped, volatile fatty acid levels rose, and pH levels could not be maintained. The cause of this decreased performance was not immediately apparent, but was not associated with the problems experienced earlier by IGT and WRRC since only one lot of kelp (Lot 37) has been used at GE through that period. Institution of corrective action resulted in a resumption of gas production.

Digester MF-6 initially gave higher gas yields than digester MF-21, producing on the average > 4 SCF/lb VS added (~ 3.5 SCF/lb VS for MF-21) and for a two week period during late March produced > 5 SCF/lb VS added. Between August 6 and 17, gas production dropped to < 3 SCF/lb VS and remained so for essentially the remainder of the period. This drop was coincident with a shift in feed from 0.10 lbs VS ft^3 to 0.123 lbs VS ft^3 but also occurred within a short period after initiating dilution with sea water. Digester MF-21, although operating under slightly different conditions of loading and hydraulic retention time (HRT) (0.1 lbs VF/ft^3, 30 day HRT), performed similiarly, but recovered to produce > 3 SCF/lb VS. An examination of the pH and VFA levels in these digesters (Tables 5 and 6) shows that in MF-6 a decrease in average pH and an increase in propionate levels was coincident with the decrease in gas output. In contrast, the pH values in MF-21 were generally lower and propionate levels higher (or comparable) throughout the same period. Acetate levels were also higher in MF-21. Other volatile fatty acids, though present in both systems, were in relatively minor concentrations.

TABLE 3 GAS PRODUCTION - KELP DIGESTER MF6 (Continued)

FISCAL WEEK	SCF/LV VS ADDED(1) CH_4	SCF/LV VS ADDED(1) TOTAL GAS	REMARKS
1	1.64	3.92	Kelp - Lot 3
2	1.90	4.10	
3	1.27	2.90	Stopped feeding for 4 days
4	2.78	4.55	
5	3.33	6.03	
6	3.88	6.80	0.07 lbs VS/ft^3 20% Solids Recycle 40 Day HRT
7	3.53	6.40	
8	4.03	7.33	
9	4.23	7.63	
10	4.70	8.08	
11	4.10	7.23	
12	4.03	7.35	
13	5.63	9.17	
14	5.78	9.20	
15	4.27	7.73	
16	4.18	7.33	0.1 lbs VS/ft^3
17	3.50	6.67	
18	4.13	7.48	
19	4.27	7.77	
20	4.10	7.25	Kelp - Lot 47
21	3.23	5.93	
22	4.55	8.48	
23	3.83	7.33	30 Day HRT
24	4.30	7.65	
25	4.97	8.70	
26	4.50	7.87	

(1) Weekly Averages

TABLE 3. GAS PRODUCTION - KELP DIGESTER MF6 (Concluded)

FISCAL WEEK	SCF/LB VS ADDED CH_4	SCF/LB VS ADDED TOTAL	REMARKS
27	4.65	8.40	
28	4.55	8.20	
29	3.70	6.63	Leak in Gas Hose
30	4.45	8.33	Sea Water Diluent(2)
31	3.73	7.43	
32	4.10	8.70	0.125 lbs/VS/ft^3
33	2.50	5.15	
34	2.23	4.95	
35	2.20	5.07	
36	2.28	5.13	Kelp Lot 48
37	2.70	5.10	
38	2.63	5.27	
39	2.86	5.90	
40	2.85	5.90	
41	2.67	5.56	
42	2.58	5.70	
43	3.03	6.40	
44	2.93	6.15	
45	2.83	6.13	
46	2.70	5.73	
47	2.50	5.33	
48	2.58	5.62	
49	2.50	5.60	
50	2.60	5.60	

(2) Instant Ocean

TABLE 4 GAS PRODUCTION - KELP DIGESTER MF21

FISCAL WEEK	SCF GAS/LB VS (1) CH_4	SCF GAS/LB VS (1) TOTAL	REMARKS
12	3.1	6.2	0.07 lbs VS/ft^3 20% Solids Recycle 40 Day HRT
13	4.3	7.5	Kelp Lot 3
14	3.7	6.8	
15	3.5	6.8	
16	4.0	7.2	
17	3.4	6.5	
18	3.6	6.7	
19	3.9	7.1	
20	3.6	6.5	Kelp Lot 47
21	3.0	5.7	
22	3.4	6.6	
23	3.7	6.9	
24	3.6	6.8	
25	3.9	7.3	
26	3.7	6.9	
27	3.8	7.1	
28	3.7	7.2	
29	2.9	5.5	
30	3.4	6.9	
31	2.4	5.5	
32	2.5	6.1	
33	1.8	4.3	
34	2.4	5.2	
35	2.3	4.8	
36	2.4	5.4	Kelp Lot 48
37	2.7	5.3	
38	2.7	5.4	
39	3.2	6.4	
40	3.3	6.4	
41	3.3	6.4	
42	3.1	6.5	
43	3.4	6.7	
44	3.5	7.1	
45	3.2	6.7	
46	3.2	6.5	
47			
48	3.6	7.2	
49	2.8	6.2	
50	3.2	6.6	

(1) Weekly Averages

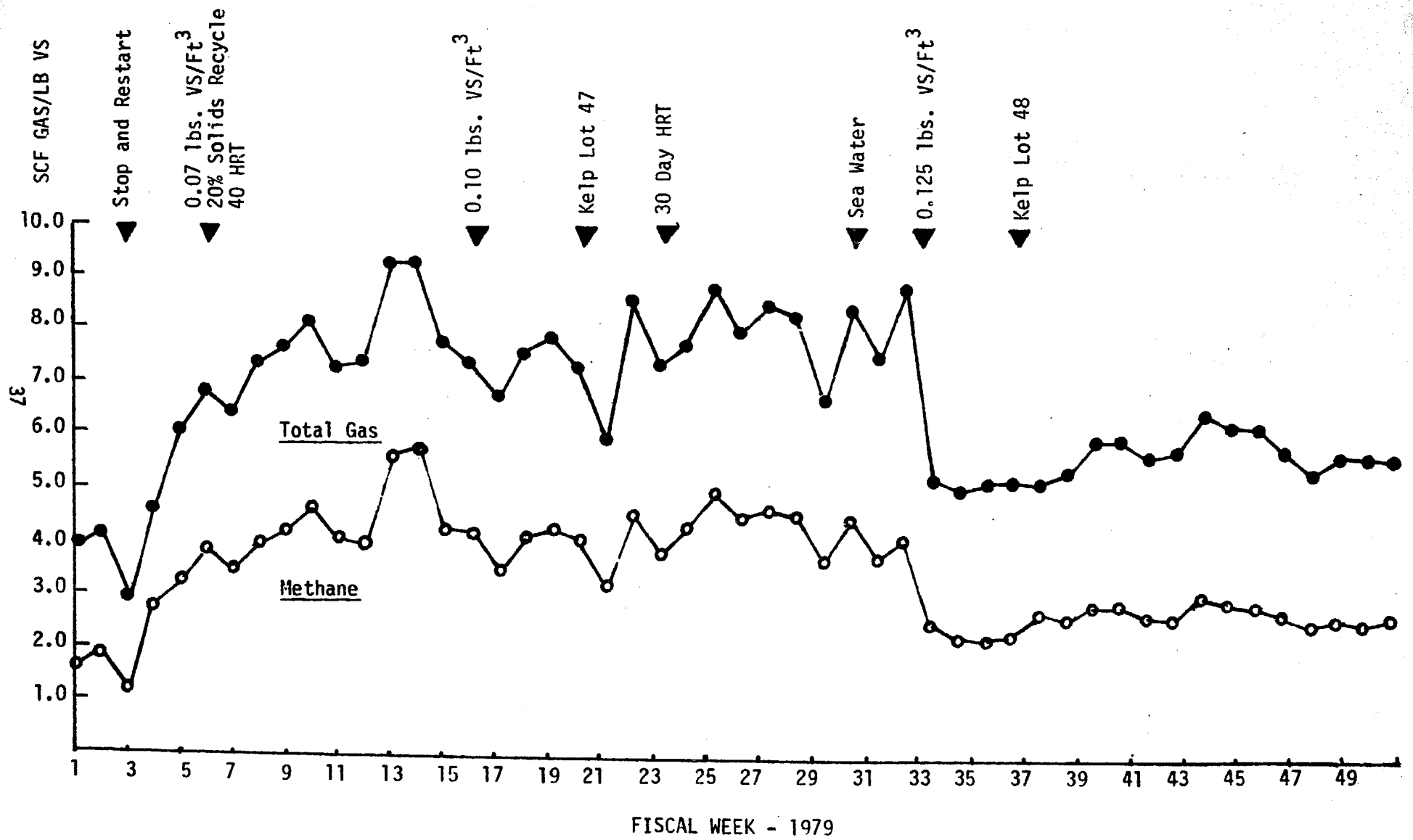


Figure 6. Gas Production - Kelp Digester MF-6
(SCF Gas/lb Volatile Solids Versus Time)

GAS PRODUCTION - KELP DIGESTER MF-21

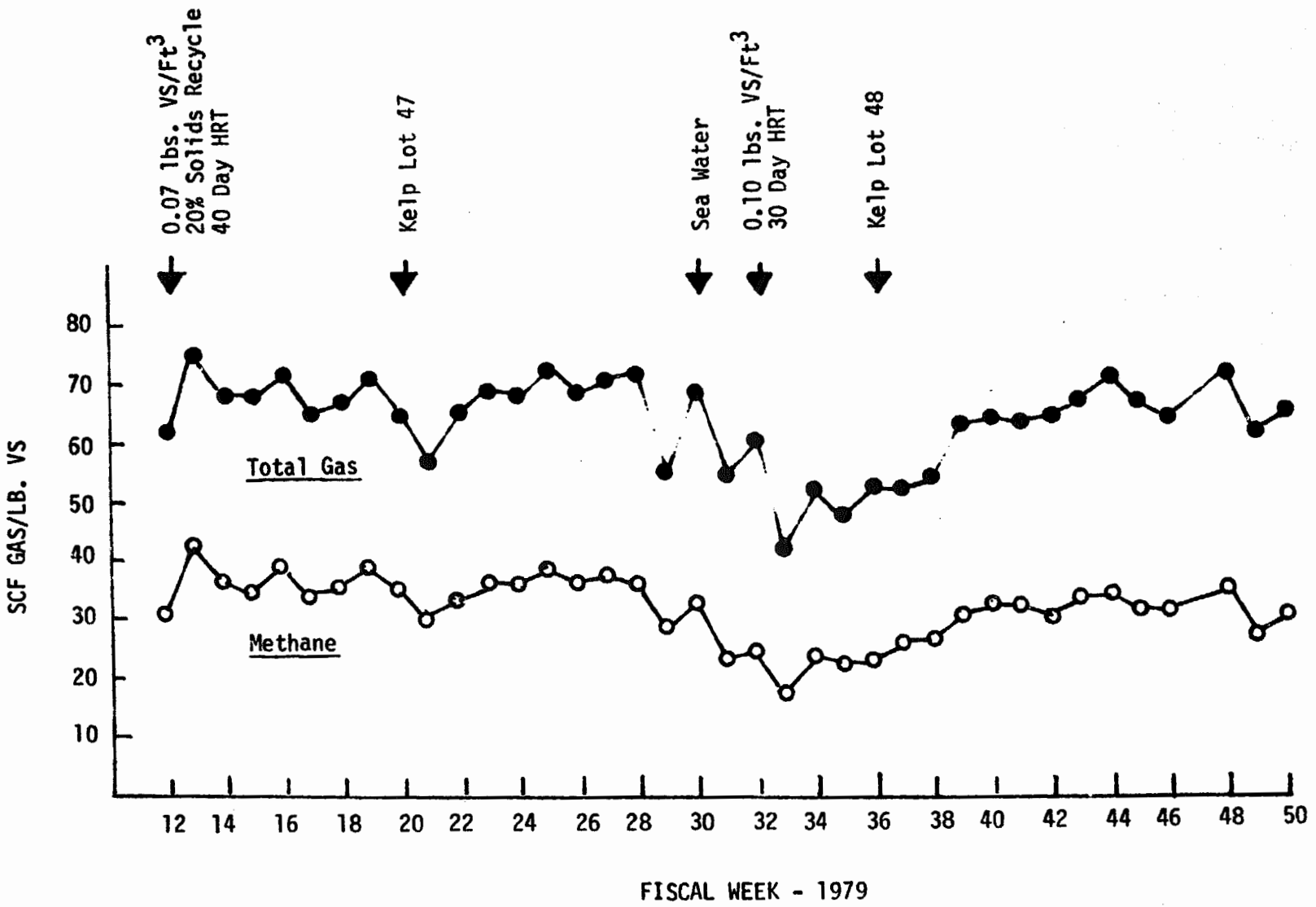


Figure 7. Gas Production - Kelp Digester MF-2
(SCF Gas/lb Volatile Solids Versus Time)

TABLE 5 VOLATILE FATTY ACID AND pH LEVELS IN KELP DIGESTER MF-6

FISCAL WEEK	ACID CONCENTRATION (milligrams/liter) ACETIC	PROPIONIC	ISO-BUTYRIC	BUTYRIC	ISO-VALERIC	VALERIC	CAPRUIC	pH
3	3073	1691	60	504	128	376	204	6.5
4	2210	1241	44	315	75	258	20	6.9
5	2622	1599	74	375	136	332	61	6.9
6	1796	1243	57	184	102	226	47	6.9
13	141	1562	65	-	57	29	-	7.2
14	184	154	-	-	52	-	-	7.5
15	199	157	24	-	59	37	-	7.3
16	684	609	87	201	287	107	107	7.5
17	291	179	31	27	106	47	31	7.0
18	155	341	22	-	39	-	-	7.2
19	251	417	39	-	65	30	-	7.0
20	192	926	15	-	43	4	-	7.3
21	238	231	19	-	35	-	-	-
22	54	255	8	-	83	75	-	7.4
23	133	704	2	-	23	16	2	7.2
24	263	757	30	-	59	27	-	7.2
25	128	576	-	-	25	-	-	7.2
29	94	37	13	-	6	-	22	7.2
30	48	19	-	-	7	-	-	7.2
31	108	133	-	-	13	14	17	7.2
33	102	980	28	8	53	16	25	7.1
34	121	1577	29	-	49	5	-	7.0
37	137	3216	60	23	205	4	-	7.0
38	163	3132	68	47	225	8	10	6.8
49	146	2948	76	110	233	154	11	-

TABLE 6 VOLATILE FATTY ACID AND pH LEVELS IN KELP DIGESTER MF-21

FISCAL WEEK	ACID CONCENTRATION (milligrams/liter) ACETIC	PROPIONIC	ISO-BUTYRIC	BUTYRIC	ISO-VALERIC	VALERIC	CAPRUIC	pH
12	1066	1652	65	61	136	143	160	6.8
13	886	1597	65	61	132	146	150	-
14	522	1625	83	45	180	204	-	7.0
15	590	1700	77	27	133	74	-	6.9
16	1178	2595	142	289	745	155	203	6.9
17	1029	2555	111	148	697	118	112	6.9
18	625	1697	64	14	132	55	-	7.1
19	713	1672	76	30	129	73	-	6.9
20	597	1666	74	11	125	49	-	7.0
21	521	1477	79	15	127	42	-	-
22	576	1567	65	10	112	52	48	6.9
25	456	1436	110	19	150	96	-	7.1
29	561	2144	92	1	200	106	18	7.1
30	521	2037	87	1	159	111	-	6.9
31	531	1714	84	18	154	115	93	7.1
33	428	945	53	25	86	-	36	7.0
37	313	2761	70	58	121	150	39	6.6
49	319	2710	87	84	249	205	9	-

Attachment of particular significance to these observations must be tempered with the fact that no attempt has been made to optimize the performance of these digesters and the operating conditions (alternate day feeding) imposed unusual stresses on these systems (i.e., shock loading).

These data do show, however, that sea water can be used effectively as a feedstock diluent and that these digesters quickly adjust to supplementation of low mannitol-containing kelp with exogenous mannitol.

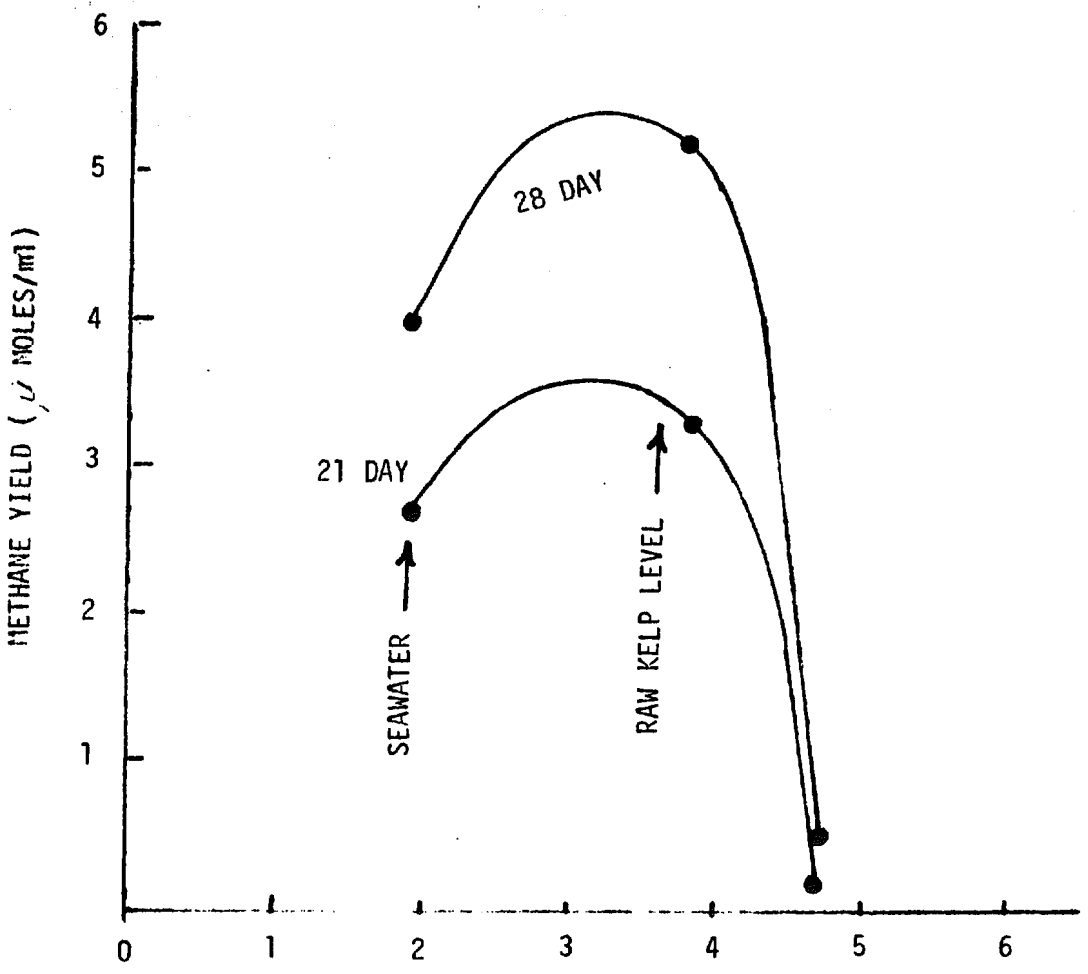
SALT TOLERANCE OF METHANOGENIC ENRICHMENTS

Experiments were performed to ascertain the tolerance of marine derived methanogenic enrichment cultures to the levels of salts that may be present if undiluted kelp were used as a feedstock, for as the feedstock concentration is increased, the diluent level is decreased and the salt levels will approach that of the feedstock. Table 7 compares the values of NaCl, KCl, and Na_2SO_4 found in sea water and undiluted kelp. It is to be noted that kelp has a particularly high KCl level (3.6 percent) as compared to sea water (1.9 percent), but has reduced levels of NaCl and Na_2SO_4. The reduction in $SO_4^=$ may actually improve CH_4 yields as $SO_4^=$ can be an electron acceptor for non-methanogens capable of utilizing acetate.

Experiments were conducted using marine derived methanogenic enrichments utilizing acetate as sole carbon source. The medium of Baresi et. al. (1978), except modified to contain sea water, was used as a baseline and supplement with KCl as required. Figure 8 shows the methane production obtained in relation to the KCl content of the growth medium. Clearly, methane yields from acetate are uneffected by the KCl levels found in raw kelp and are noted to be slightly

TABLE 7 SALT CONCENTRATIONS IN SEA WATER AND RAW KELP

	NaCl		KCl		Na_2SO_4	
	Molarity	%	Molarity	%	Molarity	%
Sea Water	0.51	3.0	0.26	1.9	0.09	1.3
Kelp	0.15	0.94	0.48	3.6	0.04	0.55



METHANE GENERATION IN ACETATE ENRICHMENTS
EFFECT OF KCl CONCENTRATION
(CALCIUM ACETATE MEDIUM OF BARESI ET AL., 1978)
MADE WITH SEAWATER

Figure 8. Methane Generation In Acetate Enrichments (etc.)

above those cultures grown in normal sea water. As KCl concentrations exceed 4 percent, methanogenesis rapidly approaches 0.

It can be concluded then that the terminal reaction in the kelp to methane food chain will not be adversely affected by salt levels possible in digesters feed undiluted kelp at maximum loading. It does remain, however, to verify these data with the H_2/CO_2 utilizing methanogens.

ALGINATE UTILIZATION

Extrapolations from pure culture data to the alginate degradation mechanisms in a mixed population fermenter require, at a minimum, that the organisms in the pure culture represent the major classes of such organisms in the fermenter. To this end, roll tube dilutions were made from kelp digester effluents, and the colony types were evaluated. The colony counts (Table 8) indicated approximately 10^8 alginate-utilizers per ml of effluent. All isolated colonies on 10^7 and 10^8 dilutions were indistinguishable from the previously isolated and characterized Cytophaga-like culture designated 39-1. These data indicate that this isolate represents the major class of organisms responsible for the primary degradation of alginate, the major kelp biopolymer.

CELLULOSE DEGRADATION STUDIES

As described previously, cellulose is present in kelp and apparently represents one of the major recalcitrant fractions under the present digestion conditions. Effort, therefore, is being devoted to the study of marine derived anaerobic cultures involved in cellulose hydrolysis. Enrichment cultures were established using Walseth cellulose as substrate in a basal

TABLE 8 ROLL TUBE COUNTS OF ALGINATE UTILIZING ORGANISMS IN KELP DIGESTERS

DILUTION	COUNT	EVALUATION
10^{-6}	TNC	--
10^{-7}	12	All morphologically identical to *Cytophaga* strain 39-1
10^{-8}	1	Morphologically identical to 39-1

medium (Weimer and Zeikus, 1977) and digester effluent as an inoculum. Both methanogenic and non-methanogenic enrichments were obtained. Roll tube dilutions have yielded five cellulolytic isolates (CW 13 - CW 17), which exhibited zone clearing of embedded cellulose. Two of these (CW 14 and CW 15) appear to be pure cultures, while the remaining three exhibit sufficient pleomorphism[1] to warrant further purity evaluations. All cultures exhibit active motility[2] and all degrade both Walseth cellulose (acid swollen amorphous cellulose) and Avicel (microcrystalline cellulose). A description of these cultures is provided in Table 9.

Large amounts of acid are produced as metabolic end-products by these cultures during growth on cellulose and the final pH is generally less than pH 5.0 in a phosphate buffered mineral salts medium. Table 10 shows a profile of the volatile and non-volatile acids produced in batch culture by these isolates and four additional mixed culture cellulolytic enrichments (MPYR, SM+K, MF6, and MF19*), one of which is methanogenic. The predominant volatile acid is acetic in all cultures with significant levels of propionate also appearing in the enrichments. The production of other volatile and non-volatile acids is variable and generally are less than 100 ppm in concentration.

An initial analysis of gas production by these cultures is presented in Table 11. Cultures CW 13, CW 16, and CW 17 and enrichment MF 6 produce H_2 while enrichments MF 19 and MF 23 produce methane - No H_2 or methane has been detected in CW 14, CW 15, MPYR, or SM+K.

Kinetics of cell growth and cellulose utilization have yet to be determined and it will be necessary to utilize a more effective buffering system or pH controlled fermentors to moderate or eliminate the severe depression of pH in batch cultures.

(1) Pleomorphism - The occurrence of several independent stages in the life cycle of an organism.

(2) Motility - The power of motion.

TABLE 9 ANAEROBIC CELLULOLYTIC ISOLATES FROM KELP DIGESTER INOCULUM

Culture	Description
CW 13	Chain forming rod, some long filaments with no obvious cross walls. Motile Some cells exhibit phase dark terminal ends.
CW 14	Rods in short chains and singles. Motile Grows as compact mat at bottom of culture
CW 15	Rods which become sphaeroplast-like in older culture. Motile
CW 16	Non-chain forming rods. Motile
CW 17	Chain forming rods. Motile

TABLE 10 VOLATILE AND NON-VOLATILE ACID PRODUCTION BY CELLULOLYTIC ISOLATORS AND ENRICHMENTS (1)

ACID	CW 13	CW 14	CW 15	CW 16	CW 17	MPYR	SM+K	MF6	MF19*
VOLATILE									
FORMIC	-	-	183	-	-	-	-	-	-
ACETIC	946	431	892	972	971	1304	1419	1124	1168
PROPIONIC	58	69	58	66	65	485	436	809	310
ISO-BUTYRIC	-	19	17	-	18	20	17	16	15
BUTYRIC	-	-	14	-	-	34	86	54	46
ISO-VALERIC	-	-	-	-	-	-	-	72	19
TOTAL VOLATILE	1004	519	1164	1038	1054	1843	1958	2075	1558
NON-VOLATILE									
PYRUVIC	89	-	-	32	33	-	63	93	-
LACTIC	65	37	46	48	63	-	49	-	-
OXALACETIC	36	-	22	-	-	20	-	-	-
OXALIC	42	-	-	-	-	-	32	25	21
MALONIC	-	-	-	-	-	-	-	40	18
FUMARIC	-	21	37	-	-	-	14	19	-
SUCCINIC	16	26	12	23	13	379	-	211	86
TOTAL NON-VOLATILE	248	84	117	103	109	399	158	388	125
TOTAL ACIDS	1252	603	1281	1141	1163	2242	2116	2463	1683

(1) CW cultures are from isolated colonies exhibiting zones of cellulose hydrolysis. All others are enrichment cultures.

TABLE 11 GAS PRODUCTION BY CELLULOLYTIC ISOLATES AND ENRICHMENTS

Culture	Gas Produced	
	H_2	CH_4
CW 13	+	-
CW 14	-	-
CW 15	-	-
CW 16	+	-
CW 17	+	-
MPYR	-	-
SM+K	-	-
MF6	+	-
MF 19	-	+
MF 23	-	+

METHANOGENIC ENRICHMENTS

In continuation of studies begun last year, methanogenic enrichment cultures were examined further. Acetate utilizing cultures were carried in the calcium acetate medium of Baresi et. al. (1978), with gas production and acetate consumption being monitored as shown in Figure 10. After an initial transfer lag, methane was generated at a rate of about 4.2 micromoles/milliliter-day and acetate was consumed at a rate of about 4.3 micromoles/milliliter-day. The cultures thus show the expected one-to-one relationship of acetate to methane. The observation notes methane production rates were also substantially higher than reported last year (1.9 micromoles/milliliter-day) and reflects the continued enrichment of methanogen in these cultures.

Microscopic examinations revealed the predominant microbial form to be a large sarcina-like organism similar to that described by Mah et. al. (1978). Roll tube dilutions were made into the low yeast extract, calcium acetate medium of Mah et. al. (1978) with a N_2 atmosphere. In this medium, methanogens form rock-hard colonies composed of cells and deposited calcium carbonate. Subsurface colonies are also recognizable by the production of large gas bubbles which form between the agar and the glass of the tube.

All of the methanogenic isolates examined to date appear to be morphologically identical to Methanosarcina sp. as described by Mah et. al. (1978). Pure cultures of these organisms, however, have not been obtained. Contaminants appear to be limited, but are either a motile, curved rod-shaped organism or a large, rod-shaped bacterium. The difficulty in purification results from the association of "contaminant" with the methanogenic conglomerate in the original medium which is not being effectively dissociated during dilution plating.

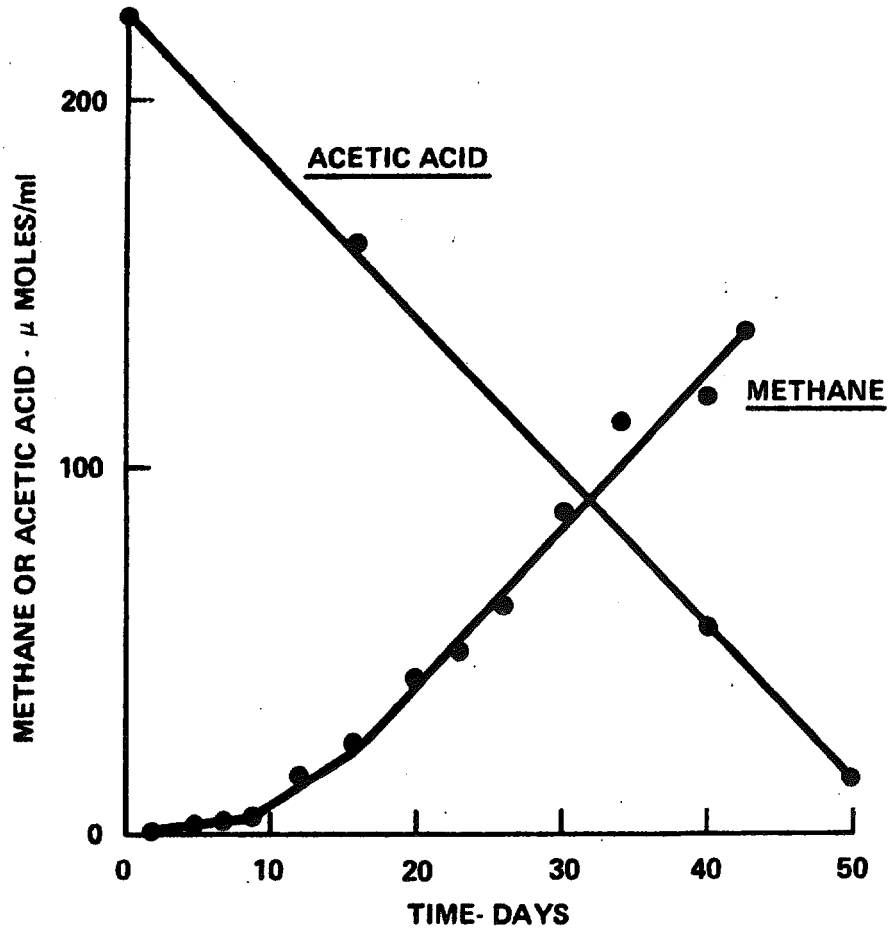


Figure 9. Methand Generation and Acetate Consumption in Acetate Enrichment Culture

Several of these enrichment cultures and kelp digestion effluent were forwarded to Dr. Robert Mah, UCLA, for his evaluation and it has been learned that he has succeeded in purifying the Methanosarcina. Further, he has found this to be a highly gas vacuolated strain, in contrast to his previously reported isolates which are non-vacuolated. He will be sharing his findings with us and will provide cultures for future microbial interaction studies.

As described above, acetate enrichment culturing had been carried out in the calcium acetate medium of Baresi et. al. (1978), a medium highly buffered with insoluble calcium carbonate. Attempts to establish active cultures in the low yeast extract, sodium acetate medium without calcium carbonate (Mah et. al., 1978) were generally unsuccessful. If, however, inert particulates were added to the medium, methanogenesis (growth) was similar to that in calcium acetate. Prompted by these results and by others (Jewel et. al., 1978), which suggest that attachment to particles allows methanogens to be retained in high rate digesters without washing out, a preliminary study was made of the effect of various particulate types of methanogenesis by enrichment cultures growing in sodium acetate medium. Particulates employed were ordinary sand, marble chips, activated carbon, and diatomaceous earth (Chromosorb P). Results of these studies are shown in Figure 10 Control media (no particles) and media containing carbon supported only low levels of growth and methanogenesis. Marble chips were not much better. Sand proved to be an excellent material with high rates of methane production. No activity was obtained when Chromosorb P was used as a support and these data are omitted from this Figure. The mechanism for observed increased activity with certain particulates will be explored in the future. The increased activity is probably related to two major factors: 1) increased surface

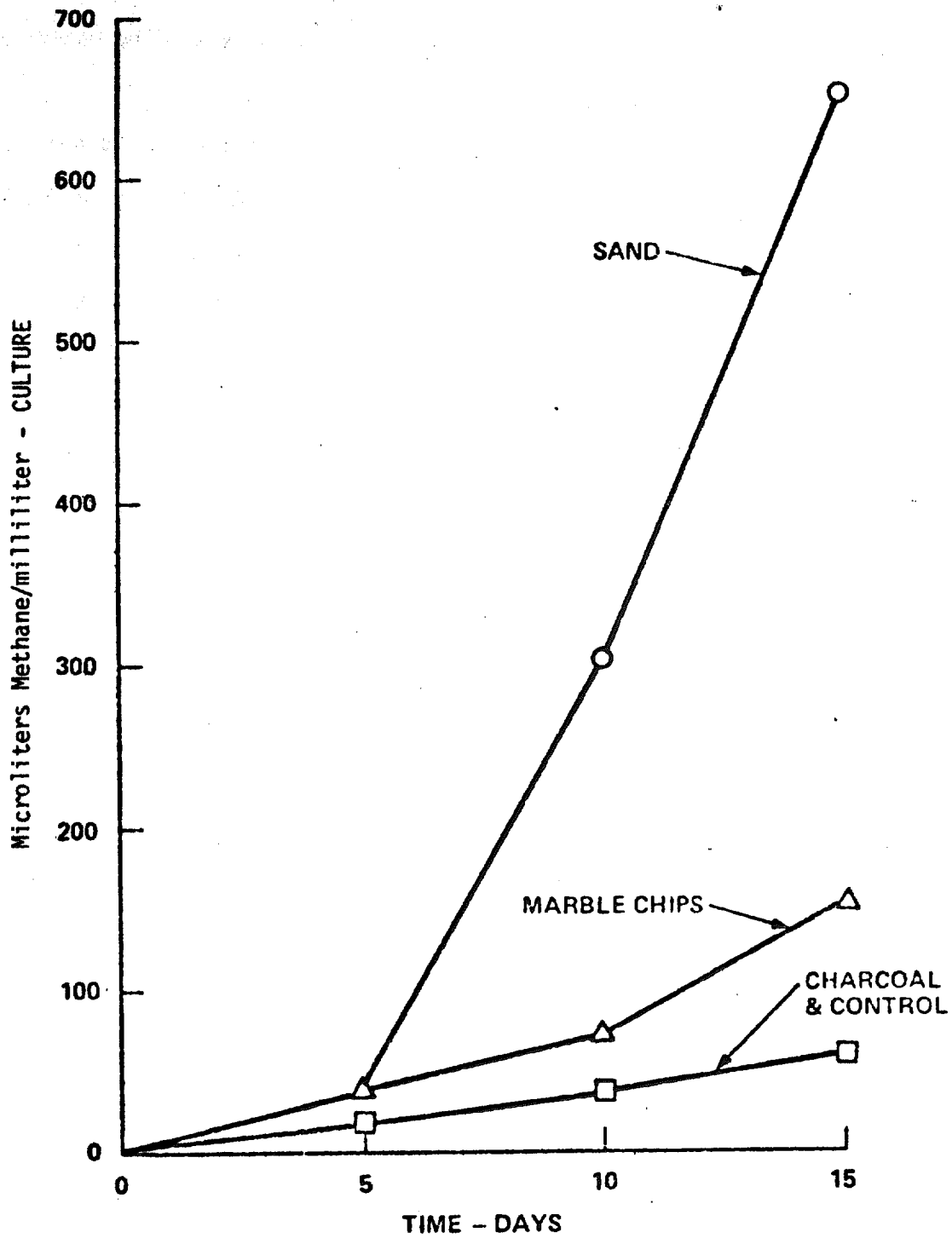


Figure 10. Methane Generation by Acetate Enrichments Effect of Added Particulates

area and 2) fixed communities of synergistic microorganisms. Why certain materials do or do not support these associations is unknown at this time, but may be due to absorption, toxicity, surface roughness and porosity, etc. Efforts will be undertaken to examine this area more thoroughly.

Several small (less than 1 liter volume) expanded bed column systems as depicted in Figure 11 were constructed to study particulate attachment and methanogenesis under flow conditions. The expanded bed design was chosen as it is, by definition, essentially free from plugging, a problem with packed bed reactors. Successful operation has, however, not been achieved to date due to mechanical problems associated with liquid recycle.

In addition to the studies on the conversion of acetate to methane described above, research efforts are being focused on the reactions involved in the interconversion of low molecular weight intermediates, such as butyrate, lactate and propionate to acetate. Initial approaches have been similar to those being used in the study of acetate and have involved the establishment of enrichment cultures from kelp digesters and other sources, which use these compounds as a sole carbon source. Multiple transfers result in a selection for the organisms involved in these particular food chains, thereby facilitating subsequent studies and isolations.

Butyrate enrichments were established in the medium of Baresi et. al. (1978) by substituting calcium butyrate for calcium acetate. Batch kinetic data with these enrichments show as expected that butyrate is converted stoichiometrically to methane via acetate by these food chains (Figure 12). Methanogenic lactate consuming enrichments were also established, but these require yeast extract, suggesting a co-factor requirement by the food chain. Likewise, propionate cultures will grow with yeast extract, but it has not yet been established whether these are utilizing the propionate.

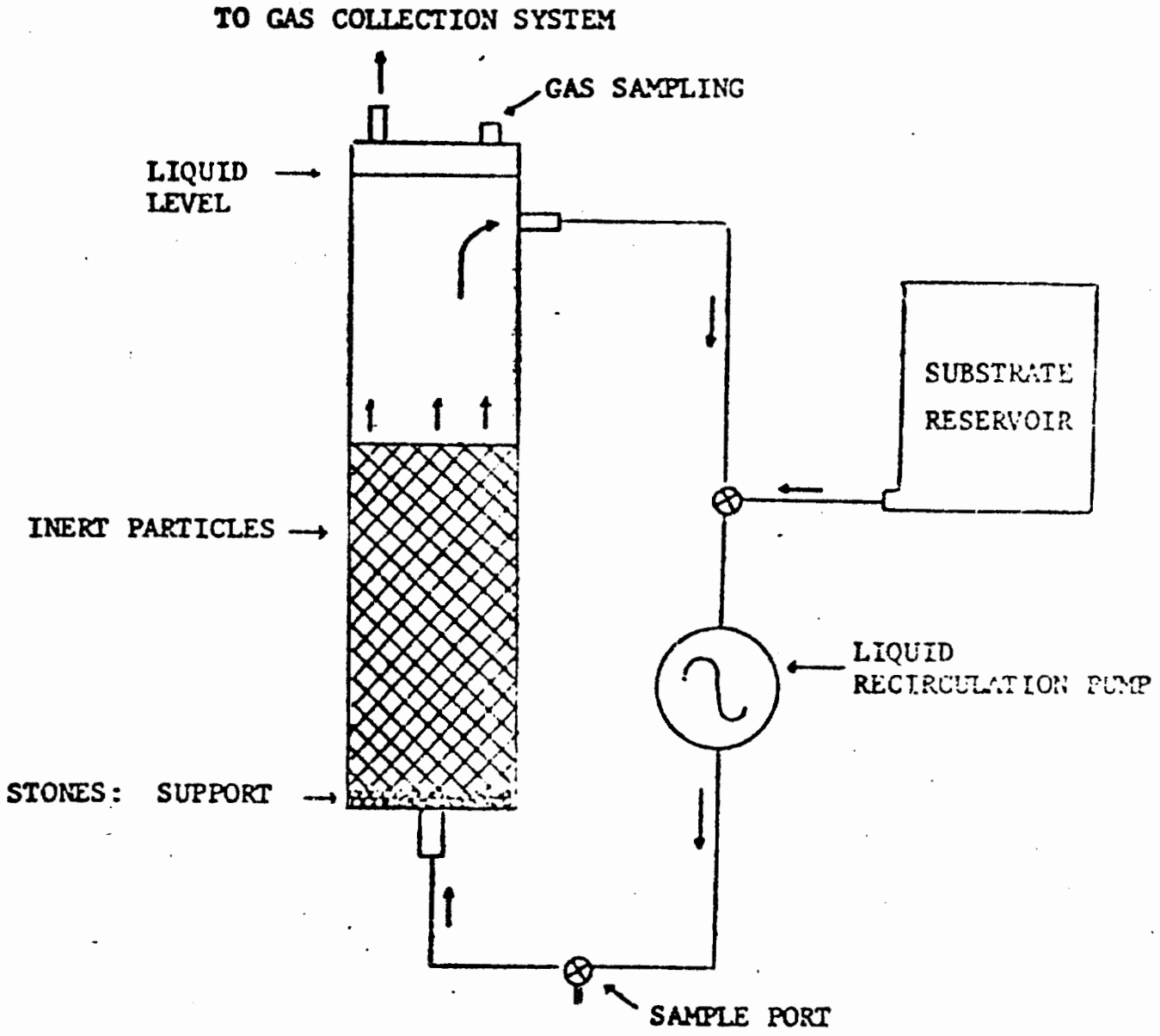


Figure 11. Schematic of Column Test System

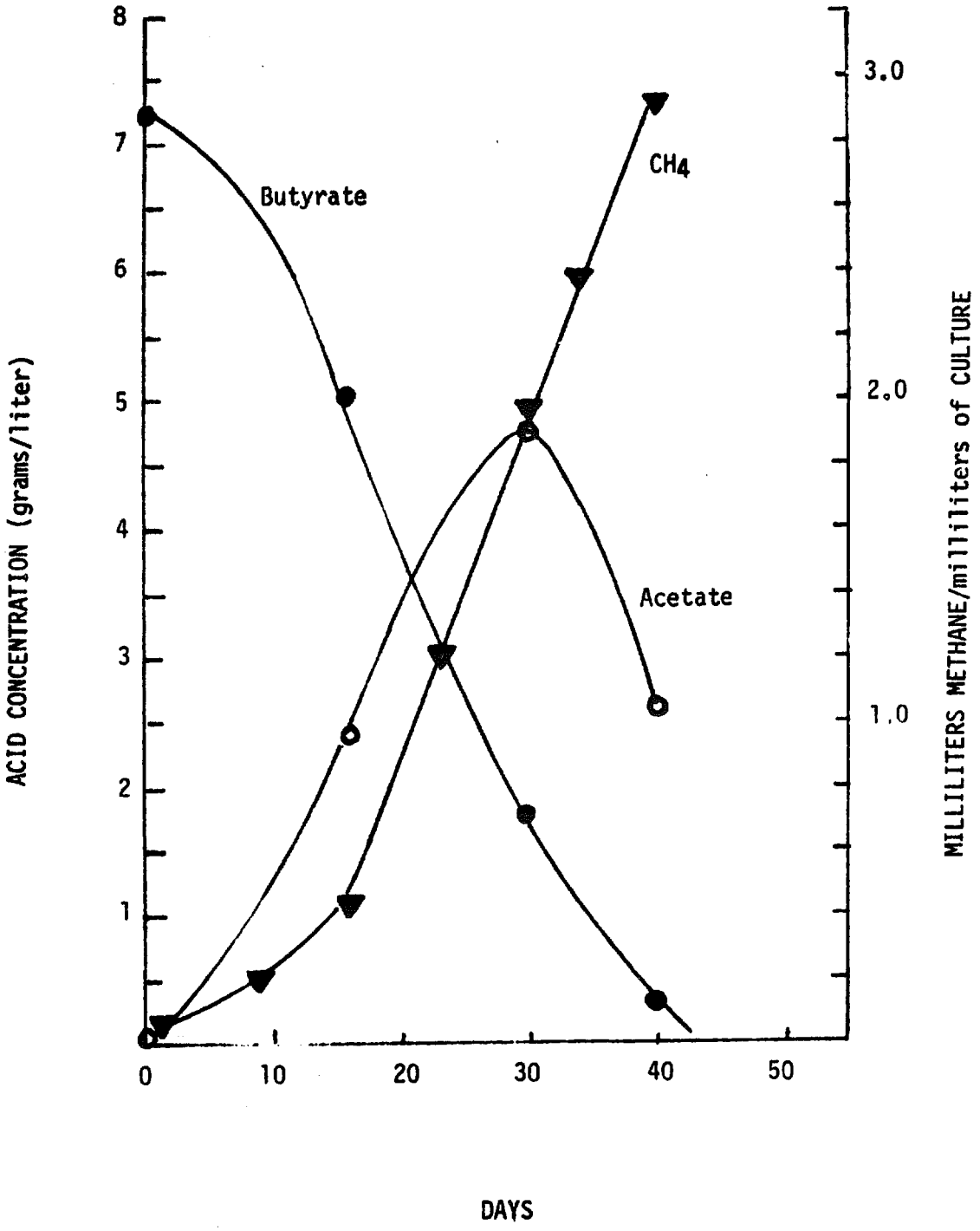


Figure 12. Production of Methane and Turnover of Butyrate and Acetate in Butyrate Enrichments

Further studies are obviously necessary in this area and will involve attempts at isolation and characterization of the microbial forms involved in these important reactions.

ANALYTICAL TECHNIQUES

The following analytical techniques were employed during the course of this study.

1. Volatile Fatty Acids

Volatile fatty acids were determined by acidifying, extracting the sample with diethyl ether in the manner described in Holdeman and Moore (1975) except the ratio of ether sample was doubled, and Na_2SO_4 was used to dehydrate the ether extracts. Chromatography was performed on a Varian 3700 gas chromatograph using the following conditions:

Detector: Thermal Conductivity, current 195 mA

Carrier Gas: 60 ml/min Helium

Column Temperature: 100°C, Hold 0, ramp 8°C/min to 182°C, Hold 0

Column: 1/4" OD X 4 mm ID X 6' long glass

Packing: 15% SP 1220/1% H_3PO_4 on 100/120 Chromosorb W AW

2. Non-Volatile Acids

The methyl ester derivatives were prepared in the manner described in Holdeman and Moore (1975) and chromatographed on the same column as were the volatile fatty acids. The column temperature was isothermal at 115°C. A Flame Ionization detector was used in place of a Thermal Conductivity detector because it was found to yield both better sensitivity and better resolution of the early peaks from the solvent front.

3. Permanent Gases

All gas samples are taken with gas tight, valved syringes. The volume reading on the syringe is the volume of gas injected at the pressure in the sampled container. These values are corrected to a standardized temperature and pressure (60°F, 760 mm mercury) as are all calibration data. The pressure within the sampled containers is determined either manometrically, by the method of Balch and Wolfe (1976) or by calculation from the syringe piston displacement method of Miller and Wolin (1974).

A. When not monitoring hydrogen: 200 µℓ is injected into a Fisher Hamilton Model 29 Gas Partitioner equipped with two columns in series. Gas detection is with two matched pairs of thermal conductivity cells, with one pair determining the gases exiting the first column (6' X 1/4" aluminum tubing, packed with 30 percent DEHS on 60/80 mesh Chromosorb P), and the second pair determining those gases exiting the second column (6.5' X 3/16" aluminum tubing packed with 40/60 mesh Molecular Seive 13X).

B. When monitoring hydrogen:

Instrument: Varian 3700

Detector: TCD at 195 mA

Carrier: 30 ml/min Helium

Column: 6' X 1/8" OD stainless packed with 80/100 mesh sperocarb

Temperature: 140°C isothermal

Note: The calibration curve is linear up to 40µℓ hydrogen per injection (20%). Above this level, the hydrogen peak area declines, and eventually goes negative. Methane and CO_2 can be quantitated simultaneously with hydrogen.

70 LITER ANAEROBIC DIGESTER

The construction and testing of a second 70 liter digester system, identical to that described in the 1978 Final Report, was completed and the unit was shipped to the Western Regional Research Center, Albany, California. It will be used for their substrate pretreatment and digester effluent evaluation studies.

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5.4 CONVERSION PROCESS DEVELOPMENT

INSTITUTE OF GAS TECHNOLOGY

INTRODUCTION

This is an annual report for 1979 of Institute of Gas Technology (IGT) Project 20502, "Reserach Study to Determine the Feasibility of Producing Methane Gas From Sea Kelp." This research is an integral part of the Energy From Marine Biomass Program sponsored by the Gas Research Institute (GRI) and managed by the General Electric Company (GE). The objective of the overall program is to determine the technical and economic feasibility of large-scale commercial production and harvesting of kelp in the open ocean and its conversion to methane and valuable by-products such as food and fertilizer. The initial objective of IGT's part of this program is to perform the laboratory-scale fermentation studies necessary for the design and operation of a large-scale biomethanation process for production of methane from kelp.

The goals of the laboratory-scale kelp anaerobic digestion studies at IGT are the following:

-- To elucidate factors that limit fermentation rates and methane yields

-- To determine operational parameters that produce the highest methane yields and methane 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.

The results of this study will be implemented in the design, construction, and operation of large-scale biomethanation systems under the management of IGT.

The work reported here is a continuation of research initiated at IGT in 1974 through an in-house project. This ultimately became part of the Energy From Biomass Program sponsored first by the A.G.A. and now GRI through its

prime contractor, GE. The detailed results of this work appear in four annual reports and are summarized in several publications. Previous studies evaluated digester performance on various forms of kelp, including raw kelp, pressed kelp (juice removed), and kelp juice under conventional operating conditions and both mesophilic and thermophilic temperatures. Based on the results of these studies, the research reported here focused on mesophilic digestion of raw kelp.

The kelp digestion studies at IGT were reduced to a culture maintenance level in 1979 because of budgetary constraints. However, the need for inoculum maintenance and for several active digesters ready for resumption of the normal experimental program was recognized. Therefore, instead of just maintaining several digesters under standby operating conditions, IGT decided to conduct experimental digester runs with minimal sample and data analysis. Feed and effluent samples were frozen and data stored for future analysis when funding becomes available. The 1979 work plan reflecting the conduct of several experimental digester runs is outlined in Table 12 and discussed below.

Baseline and Stock Cultures

Three baseline cultures were maintained during the report period, two on diluted and one on undiluted kelp. These runs provided baseline performance data on different kelp lots and served as controls for several experimental runs.

Determination of Nitrogen Requirements

During 1978, reduced digester yields and stability were observed with certain kelp lots; these problems could be a result of feed-nitrogen deficiencies. Because

TABLE 12. OUTLINE OF IGT DIGESTER RUNS

Run	
Nutrient Experiments	
Feed Slurry of Lot 42	
39	Feb. 25 to March 31
41	March 11 to April 8
42	Feb. 18 to March 25
43	March 11 to April 8
44	Feb. 18 to March 18
132	Feb. 25 to April 8
133	Feb. 18 to March 18
134	April 1 to April 29
Mannitol Experiment	
138	Nov. 26 to Dec. 30
Plug Flow	
PF-1	Aug. 20 to Sept. 16 Oct. 21 to Nov. 18
Hydrogen Repression Experiment and Ambient Temperature Marine Inocula Development	
8	Sept. 1 to Nov. 13 + Feed Slurry of Lot 48
136	Sept. 1 to Sept. 30 Oct. 7 to Dec. 15
139	Aug. 26 to Sept. 30 Oct. 7 to Dec. 15
Baseline Culture and Evaluation of Different Lots of Kelp	
8	Feb. 11 to March 8 Feed Slurry of Lot 46 Dec. 15 to Dec. 30 (diluted Lot 0)
121	Feb. 18 to March 25 (L = 0.15) Feed Slurry of Lot 0 Sept. 9 to Oct. 7 (L- 0.10)
42	Sept. 23 to Oct. 28 (Undiluted Lot 48)
44	Sept. 16 to Oct. 8 (Diluted Lot 48)

the effect on digester performance is dramatic and has significant total systems implications, several digester runs were conducted to quantitatively evaluate the relationship between kelp nitrogen content and digester performance.

One 50-ℓ stock culture was developed and maintained on kelp during the report period. This culture was fed once per week and maintained at a low loading and high retention time.

Mannitol Experiments

In 1979, a kelp lot was received that resulted in stable digester performance but reduced methane yields. The low mannitol content of this lot was thought to be the cause of the low yields because mannitol is the major biodegradable kelp component. The relationship between mannitol content and theoretical and experimental methane yields for previous IGT kelp feeds and digester runs was evaluated. A new experimental run was begun to evaluate the effect of added mannitol content on digester performance.

Baffle Flow Digester

In response to a review meeting on anaerobic digestion held at GE on June 12 and 13, 1979, and based on recent reports by DOE contractors, a new digester was designed, constructed, and subjected to preliminary evaluation. The design is based on unmixed plug flow with baffles to promote solids settling and to prevent short circuiting. The purpose of the design was to minimize cost and promote phase separation and solids retention.

Inoculum Evaluation

Marine inocula derived from anaerobic sediments near kelp beds may be capable of improved kelp conversion. Performance of runs conducted in 1978

was similar for marine and sludge inocula on freshwater diluted kelp. The objective of this task was to compare the performance of the two types of inocula on undiluted kelp, which has a higher salt content than freshwater - diluted kelp. It is possible that fresh water diluted kelp resulted in selection away from halophilic[1] bacteria in the 1978 experiments.

Loading-Retention Time-Solids Recycle

Although high methane yields and stable digester performance have been achieved with both undiluted and freshwater diluted kelp, selection of one or the other feed types will depend upon associated digestion kinetics. The kinetic parameters for both feed types will be determined by observing digestion performed at different loadings and retention times. Because solids recycle improves digestion kinetics, parallel runs with solids recycle will be included as part of this task.

During 1979, digesters were developed to and maintained at the first set of operating conditions for these experiments. These experiments were not conducted because of spoilage (at WRRC due to refrigeration breakdown) of the kelp lot used to develop these runs and because of uncertainties about the validity of using discontinuous daily feeding for kinetic studies.

Digesters

Two types of digester units were used for most of the runs reported here. One type consisted of glass units with a culture volume of 1.5 liters, shaken continuously at approximately 130 rpm using a New Brunswick gyratory shaker and incubated at 35° C in a thermostat-controlled environmental chamber. The other type had a culture volume of 10 liters, was maintained at a constant temperature using heating tape connected to a temperature controller, and was mechanically mixed continuously at 150 rpm. All digesters were operated at

[1] halophilic - halogen or salt-loving

35°C unless otherwise specified. Gas collection and measurement were accomplished by displacement of acid-salt solution from calibrated gas burets. All digesters were fed daily except where otherwise specified. The details of digester design and operational procedures were presented in the February 1978 annual report for this project.

A baffle flow digester was made from 1/4 inch plexiglass, as shown in Figure 13. The digester had a culture volume of 9.8 l, was fed with a 50 ml syringe, and maintained at 35°C in a thermostat-controlled environmental chamber. This unit was not mixed mechanically and gas collection and measurement were accomplished by displacement of acid-salt solution from calibrated gas burets as described in previous report of this project.

A 50 l stock culture was maintained at room temperature in a 15 gallon polyethylene drum. This digester received undiluted kelp at a loading of 0.025 lb VS/ft^3 day. Feeding frequency was once per week. On a daily schedule, gas was released and the digester shaken manually. Periodically (about once per month) samples were taken for measurement of pH and volatile fatty acids.

Feeds

Raw kelp (RK) was used in all runs during this report period. This feed was harvested by WRRC, drip drained of physically-attached sea water, chopped, ground, and frozen prior to use. Table 13 lists shipments of kelp received from WRRC and used for this study. The identity of different kelp feed lots used for specific runs is shown in Table 14. All kelp feeds were analyzed for moisture, total solids, volatile solids, ash, heating value, and the elements C, H, and N.

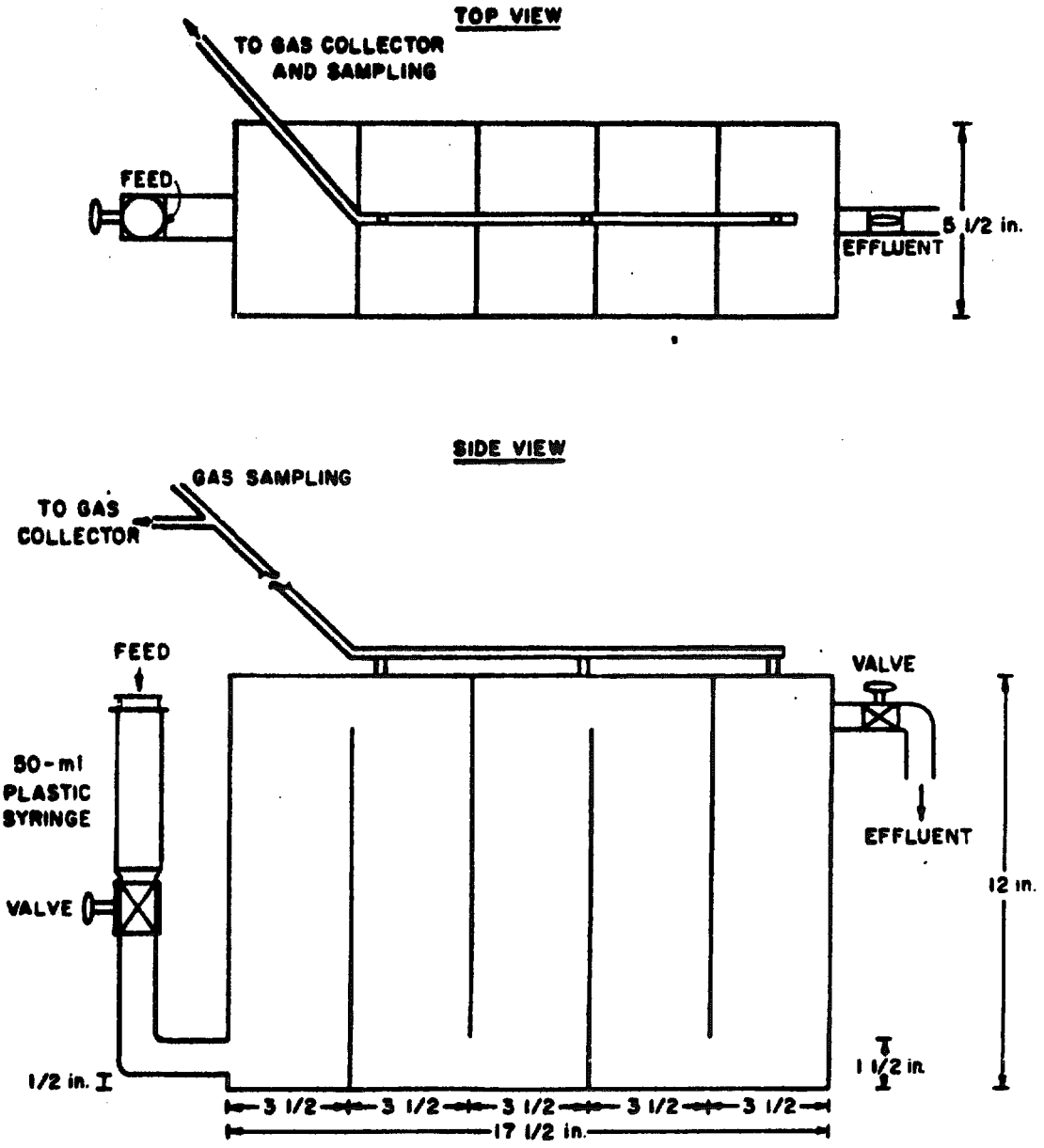

Figure 13. BAFFLE-FLOW DIGESTER

Table 13. SHIPMENTS OF RAW KELP RECEIVED FROM WRRC* AND USED DURING 1979

Date Harvested	Date Received	WRRC Kelp Lot	Number of Cartons
9/13/78	1/15/79	42	36
1/24/79	1/30/79	46	36
1/24/79	4/25/79	46	18
3/21/79	4/25/79	47	18
4/29/79	5/4/79	48	18
4/29/79	5/16/79	48	36
4/29/79	7/12/79	48	46
3/21/79	7/12/79	47	8
4/29/79	8/24/79	48	46
3/21/79	8/24/79	47	8
4/29/79	10/2/79	48	36
4/29/79	10/25/79	48	17
10/16/79	10/25/79	49	19
10/16/79	11/2/79	49	36
4/29/79	11/20/79	48	65

* U.S. Department of Agriculture Western Regional Research Center, Albany, Calif.

Table 14. **KELP LOTS USED FOR 1979 DIGESTION RUNS** (Continued)

Run	WRRC* Kelp Lot	Date	Comment
8	44 (687-158-0)	11/21/78-2/5/79	
	46	2/6/79-5/1/79	
	47	5/2/79-10/9/79	
	44 (687-158-0)	10/10/79 to present	
128	42	10/18/78-1/22/79	Run terminated
129	44	11/21/78-1/22/79	Run terminated
130	42	1/4/79-1/22/79	Run terminated
132	42	1/23/79-5/2/79	Run terminated
133	42	1/23/79-3/28/79	
	46	3/29/79-5/2/79	Run terminated
134	42	1/23/79-6/14/79	Run terminated
135	46	3/25/79-5/17/79	
	48	7/12/79-7/25/79	Run terminated
136	46	5/3/79-5/17/79	
	48	9/13/79 to present	
137	48	6/15/79-10/10/79	Run terminated
138	46	7/16/79 to present	
139	48	9/12/79 to present	
119	42	10/2/78-3/24/79	Run terminated
121	44 (687-158-0)	12/11/78-19/9/79	Run terminated
PF-1	48	7/13/79 to present	
39	42	9/29/78-4/25/79	
	47	4/26/79-5/1/79	
	46	5/2/79-5/30/79	
	47	5/31/79-7/5/79	
	48	7/6/79 to present	
41	42	9/22/78-3/28/79	
	46	3/29/79-5/5/79	
	48	5/6/79-6/13/79	
	49	10/30/79 to present	
42	42	9/22/78-3/13/79	
	46	3/14/79-5/5/79	
	48	5/6/79-10/29/79	
	49	10/30/79 to present	

* U.S. Department of Agriculture Western Regional Research Center, Albany, Calif.

Table 14.Cont. **KELP LOTS USED FOR 1979 DIGESTION RUNS** (Concluded)

Run	WRRC* Kelp Lot	Date	Comment
43	42	9/22/78-3/28/79	
	46	3/28/79-5.5.79	
	48	5/6/79-10/29/79	
	49	10/30/79 to present	
44	42	9/22/78-3/13/79	
	46	3/14/79-5/5/79	
	48	5/6/79-6/13/79	
	46	6/14/79-7/11/79	
	48	7/12/79-10/29/79	
	49	7/30/79 to present	

*U.S. Department of Agriculture Western Regional Research Center, Albany, Calif.

Inocula

Inoculum A

Inoculum A was 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. This inoculum was gradually converted from a feed of municipal solid waste/sewage sludge to raw kelp, has received raw kelp under a variety of operating conditions since July 1975, and is currently the inoculum used for most of the experimental work reported here.

Inoculum G

This inoculum 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 yeast extract. Later on, it received increasing pulses of sodium acetate and undiluted kelp. The feed was gradually converted to undiluted raw kelp alone after the successful development of inoculum.

Inoculum H

Inoculum H was developed at room temperature from the anaerobic marine sediment collected in Newport Harbor, California. In the beginning, the digester received mixtures of sodium acetate and sodium formate and yeast extract. Later on, it received increasing pulses of undiluted raw kelp and mixtures of sodium acetate and sodium formate and finally the feed was converted to undiluted raw kelp alone.

Analytical Methods

The analytical schedule employed for maintenance level digester operation is shown in Table 15. The frequency of operational analysis was reduced and feed slurry and effluent samples were stored for future analysis.

Table 15. ANALYTICAL SCHEDULE FOR MAINTENANCE-LEVEL DIGESTER OPERATION*

Analysis	Frequency
Gas Production Measurement	Daily**
pH	Daily
Temperature	Daily
Volatile Acids	Bimonthly
Gas Composition	Bimonthly
Conductivity	Monthly
Alkalinity	Monthly
Feed Slurries and Effluent Samples of Runs at Steady State	Frozen for future analysis

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

**Not corrected for changes in temperature and atmospheric pressure.

Calculations

Equations for calculating performance parameters were presented in last year's report. Daily gas production data were not corrected for fluctuations in temperature and atmospheric pressure. Instead, they were all normalized to the average temperature and pressure for this area, i.e., 25°C and 29.8 in. Hg. Although this method reduced data processing time, it could result in a ±10% error factor in data presented.

RESULTS AND DISCUSSION

Introduction

This section is based primarily on mean performance data of digester runs during select periods of stable or steady-state performance. Detailed weekly and steady-state performance data will be made available when funds are available for more thorough analysis including analysis of frozen effluent samples and correction of gas production data for fluctuation in ambient temperature and barometric pressure.

Characteristics of Feeds

The physical and chemical characteristics of all lots of kelp used during the report period are listed in Table 16. All kelp lots had a similar moisture content, however, Lots 46, 47, and 48 were significantly lower in volatile solids, carbon, and heating value.

Lots 42 and 49 were both deficient in nitrogen and required nitrogen supplement for stable digestion. Considerable variability was observed in the mannitol content of different lots. The effects of the differences in properties of kelp lots on theoretical and experimental methane yields and digester performance are discussed below:

Table 16. COMPOSITION OF KELP FEEDS USED DURING 1979

Analysis	Lot 42[a,b]	Lot 44 (687-158-0)[a,b]	Lot 46[a,b]	Lot 47[a,b]	Lot 48[b,c]	Lot 49[b,c]
Harvest Date	9/13/78	11/1/78	1/24/79	3/21/79	4/29/79	10/16/79
Moisture, %	88.1	89.7	89.9	89.5	88.9	87.9
Total Solids (TS), %	11.9	10.3	11.1	10.5	11.1	12.1
Volatile Solids (VS), % TS	62.9	60.4	55.3	54.6	53.8	62.7
Ash, % TS	37.1	39.6	44.7	45.4	46.2	37.3
Elements, % TS						
C	28.30	27.9	25.5	32.87	25.5	31.4
H	3.61	3.51	3.24	3.50	3.39	4.01
N	1.18	1.89	1.95	2.22	1.89	0.96
P	0.22	0.29	--	--	--	0.20
S	1.35	1.62	1.37	1.33	1.01	1.10
K	3.4	4.0	--	--	--	--
Na	12.0	13.0	--	--	--	--
Ca	1.4	1.7	--	--	--	--
Heating Value, Btu/lb dry	4544	4515	4112	4330	4072	5032
Mannitol, % TS[d]	13.0	10.2	5.19	5.18	9.06	23.9

[a]Harvested and processed by U.S. Department of Agriculture, Western Regional Research Center, Albany, Calif., and shipped to IGT in Trans-Temp containers at −5°C to −15°C.

[b]Raw kelp-freshly harvested kelp drained of free water, chopped, ground in hammer mill equipped with a 0.188-in. screen, and frozen.

[c]Same as footnote b, but commercially harvested.

[d]Analysis of WRRC.

Maximum Theoretical Yields and Heat of Reaction

To evaluate performance of kelp digestion runs and provide a basis for establishing target yields, calculations of maximum theoretical yields of methane from the biomethanation of different lots of raw kelp (RK) were made using the data in Table 17. These yields were calculated as follows: Compositional data were used to calculate the empirical formulas of each raw kelp (RK) lot. Stoichiometric equations for the conversion of each feed to 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 using the data reported by McCarty and Speece and McCarty for the anaerobic digestion of pure carbohydrate and protein. The final theoretical methane yields are reported in Table 17.

Theoretical yields for kelp lots used during the report period ranged from 5.84 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.

Stock/Baseline Cultures

Three runs were maintained on freshwater diluted kelp as stock or baseline cultures during the report period. Run 8, operated at a loading of 0.1 lb. VS/Ft^3 day and retention time of 15 days, exhibited mean methane yields of 3.14 and 3.20 SCF/lb VS added on kelp lots 44 and 46 respectively. (See Table 18).

Table 17. THEORETICAL METHANE YIELDS FOR KELP USED DURING 1979

Kelp Lot	Empirical Formula[a]	Stoichiometric Yield, SCF/lb VS added[b]	Corrected Yield, SCF/lb VS added[c]
42	$C_{2.36}H_{3.57}O_{1.77}$	7.11	5.84
44 (687-158-0)	$C_{2.33}H_{3.48}O_{1.57}$	7.59	6.24
46	$C_{2.13}H_{3.24}O_{2.14}$	6.45	5.34
47	$C_{2.25}H_{3.50}O_{2.07}$	7.29	6.07
48	$C_{2.13}H_{3.39}O_{1.91}$	7.11	5.89
49	$C_{2.61}H_{4.01}O_{1.58}$	8.58	6.97

[a] Excluding N, P, and S.

[b] Assumes that the reactants are kelp + 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.

Table 18. STEADY-STATE PERFORMANCE AND EFFLUENT QUALITY DATA FOR STOCK CULTURE RUN 8 WITH KELP LOTS 46 AND 44
(Culture Volume 1.5 ℓ; Temperature 35°C; Loading 0.10 lb VS/ft^3-day)

Analysis	Lot 46	Lot 44 (687-158-0)
Data Period	2/12-3/11/79	11/26-12/30/79
Retention Time, days	18	15
Retention Times in Progress	0.5	3.1
Gas Yield, SCF/lb VS added	5.98	5.81
Methane Content, mol %	53.5	54.0
Methane Yield, SCF/lb VS added	3.20	3.14
Methane Production Rate, SCF/ft^3 culture-day	0.32	0.31
Total Volatile Acids, mg/ℓ as acetic	1020	850
Alkalinity, mg/ℓ as $CaCO_3$	—	5080
Mean Caustic Added, mg/ℓ feed	0	0

One stock culture was maintained on undiluted kelp (Lot 44) at two different loadings. The methane yields were 3.71 and 2.34 SCF/lb VS added for loadings of 0.1 and 0.15 lb VS/ft^3 day respectively. As was observed in 1978, undiluted kelp digesters were stable but had high concentrations of volatile acids. Note that the methane yields of undiluted kelp Run 121 were higher than that of Run 8, which received diluted kelp at the same loading. (See Table 19). This result may be attributed to the longer retention time associated with the undiluted kelp feed mode.

Gas production measurements were not made on the 50 liter stock culture maintained at room temperature on undiluted kelp in a polyethylene drum. The pH was stable above 7.0 and volatile acids concentration below 200 mg/l (as acetic) in this culture.

Nutrient Experiments

In 1978, it was shown that certain lots of kelp were nitrogen deficient resulting in digester instability and reduced methane yields. Addition of supplementary nitrogen to a C/N ratio of 15 restored performance to normal. Phosphorus was apparently not limiting because supplemental phosphorus has no observed effect on performance in the presence of excess nitrogen. Because kelp nutrient content is so significant to conversion yield, and performance is undoubtedly influenced by nutrient concentrations in the growth environment, experiments were conducted to define the critical C/N ratio for kelp fermentation.

Table 19. STEADY-STATE PERFORMANCE AND EFFLUENT QUALITY DATA FOR RUN 121 WITH UNDILUTED KELP LOT 44 (687-158-0) AT TWO DIFFERENT LOADINGS
(Culture Volume 1.5 ℓ; Temperature 35°C)

Analysis	Loading, 0.15 lb VS/ft^3-day	Loading, 0.10 lb VS/ft^3-day
Data Period	2/12-3/11/79	9/3-10/7/79
Retention Time, days	25	38
Retention Times in Progress	3.7	1.8
Gas Yield, SCF/lb VS added	4.93	6.87
Methane Content, mol %	47.5	54.1
Methane Yield, SCF/lb VS added	2.34	3.71
Methane Production Rate, SCF/ft^3 culture-day	0.35	0.37
Total Volatile Acids, mg/ℓ as acetic	7800	3500
Alkalinity, mg/ℓ as $CaCO_3$	--	9800
Mean Caustic Added, mg/ℓ feed	0	0

Several digester runs were initiated to determine the minimum C/N ratio needed to give non-nitrogen-limited performance. Six runs operated at a loading of 0.1 lb VS/ft^3 day and a retention time of 15 days received nitrogen supplements sufficient to adjust the C/N ratio to a range of 14 to 24. Two additional runs received phosphorus to further substantiate 1978 data that had shown that phosphorus was not limiting. The kelp lot used for these experiments, Lot 42, had a C/N ratio of 24 and C/P ratio of 129. The results of these runs are presented in Table 20 and Figure 14.

All runs receiving feed C/N ratios greater than 14 exhibited reduced methane yields, instability, and accumulation of high concentrations of volatile acids.

Although data and effluent samples from these runs will require further analysis, preliminary results suggest that the critical C/N ratio for kelp digestion is 14. As was observed before, phosphorus supplement did not result in improved digester performance.

Data reported by Lindner et. al. indicates that seasonal fluctuations in the nitrogen content of kelp occurs and is related to nutrient concentration in surrounding waters. With respect to the kelp farming concept under evaluation in the Marine Biomass Program, some control over kelp nitrogen content might be exercised by variation in upwelling waters.

Effect of Mannitol on Theoretical and Experimental Yields

During 1979, a kelp lot (Lot 46) was received and tested which gave a low methane yield even though digester performance was quite stable. Further evaluation of

Table 20. STEADY-STATE PERFORMANCE OF DIGESTER RUNS FOR NUTRIENT EXPERIMENTS
(Kelp Lot 42; Loading 0.1 lb VS/ft^3-day; Retention Time 15 days; Temperature 35°C)

Run No.	41	134	39	132	44	133	42	43
Date Initiated	1/3/79	1/23/79	1/23/79	1/25/79	1/3/79	1/23/79	1/3/79	1/3/79
Data Period	3/19-3/25/79	4/2-4/8/79	3/19-4/8/79	3/19-4/8/79	2/19-3/11/79	2/19-3/11/79	3/19-3/25/79	3/19-4/8/79
Number of Retention Times in Progress	5.0	4.6	3.7	3.8	3.1	1.8	5.0	5.0
Nutrients Added	None	0.25X-N (67.5 mg N/ℓ)	0.5X-N (135 mg N/ℓ)	0.74X-N (202 mg N/ℓ)	1.0X-N* (270 mg N/ℓ)	1.25X-N (337 mg N/ℓ)	1X-(N + P) (270 mg N/ℓ) (39 mg P/ℓ)	1X-P+ (39 mg P/ℓ)
Total N Present in Feed, mg/ℓ feed	450	518	586	653	720	788	720	450
Total C Present in Feed, mg/ℓ feed	10,814	10,814	10,814	10,814	10,814	10,814	10,814	10,814
Adjusted C/N	24	20.9	18.5	16.5	15	13.7	15	24.0
Adjusted C/P	129	129	129	129	129	129	87.8	87.8
Methane Yield, SCF/lb VS added	0.30	1.93	1.39	2.85	3.08	3.21	3.33	0.5
Methane Production Rate, SCF/ft^3-culture-day	0.03	0.19	0.14	0.29	0.31	0.32	0.33	0.05
Methane Content, mol %	40.0	47.1	43.9	50.0	52.8	52.4	53.0	35.3
Total Volatile Acids, mg/ℓ as acetic	3510	2380	3250	1320	1350	760	480	2980
Mean Caustic Addition, meq/ℓ feed	150	43	105	30	16	0	0	125

*1X-N = 270 mg N/ℓ feed volume as NH_4C.

+1X-P = 39 mg P/ℓ feed volume as KH_2PO_4.

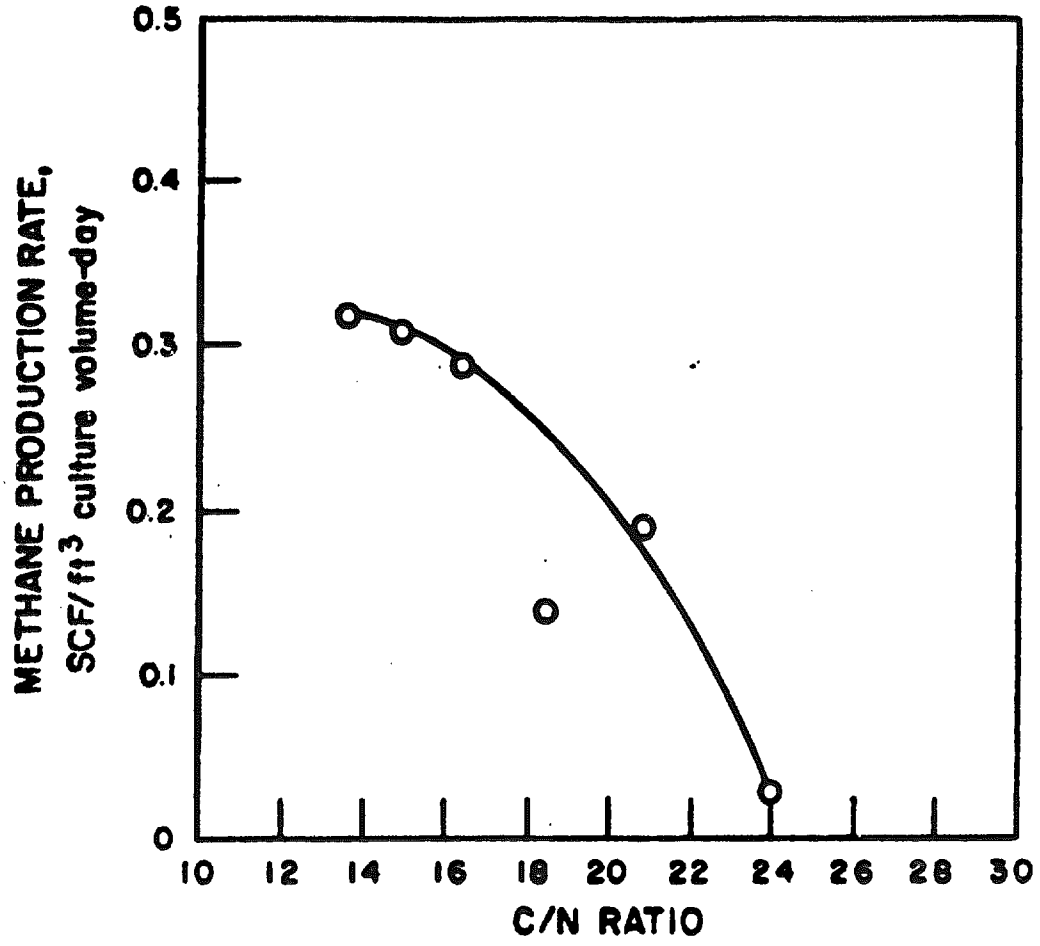

Figure 14. THE EFFECT OF C/N RATIO ON BIOMETHANATION OF KELP

the composition of that lot disclosed that it was low in mannitol content (5.19% dry weight) compared to that of the other lots used previously in this program which was typically greater than 14% dry weight. Because mannitol is the major biodegradable component of kelp, the lower yields were attributed to the low mannitol content. To further evaluate this effect, previous data obtained on kelp digestion at IGT were evaluated for a relationship between feed mannitol content and theoretical and real methane yields. (See Table 21 and Figures 15 and 16.) Figure 15 shows that, in general, both theoretical and experimental yields increase with mannitol content; however, the data points are scattered and thus not suitable for predicting this effect. Figure 17 shows a plot of the ratio of experimental to theoretical yields versus mannitol concentration. This linear relationship, with a coefficient determination of 0.93, can be used to predict methane yield given the kelp empirical formula (estimated from CHO analysis) and the mannitol content, as shown in Equation 1.

$$Y_E = Y_T\ (0.02\ [Xm] + 0.375) \qquad \text{Equation 1}$$

Y_E = Experimental Methane Yield, SCF/lb VS added

Y_T = Theoretical Methane Yield, SCF/lb VS added

[Xm]= Mannitol Concentration, % dry weight

In order to further evaluate the above equation for predicting the effect of mannitol on methane yield, Run 138 was established on Lot 46 which has a low mannitol content of 5.19 weight percent. This run is operated at a loading of 0.1 lb VS/ft^3-day and retention time of 15 day has stabilized at a methane yield of 3.0 SCF/lb VS added (see Table 22). This run will receive mannitol supplements in a stepwise manner to determine the methane yields at 10, 15, and 20 percent mannitol at the same loading and retention time of the initial control run.

Mannitol concentration also appears to be inversely related to digester stability. Although this effect has not been documented quantitatively,

Table 21. THE EFFECT OF MANNITOL CONTENT ON THEORETICAL AND EXPERIMENTAL METHANE YIELDS

Lot	Mannitol Content,* % dry wt	Experimental Methane Yield, SCF/lb VS added	Theoretical Methane Yield, SCF/lb VS added
47	5.18	2.70	6.07
46	5.19	3.00	5.34
1	6.30	3.57	7.01
48	9.06	3.30	5.89
44	10.20	3.50	6.24
42	13.04	3.10	5.84
26	14.30	4.20	6.72
37	14.89	4.50	6.77
RK	18.7	4.50	6.77
49	23.9	--	6.97

* Analysis conducted at WRRC.

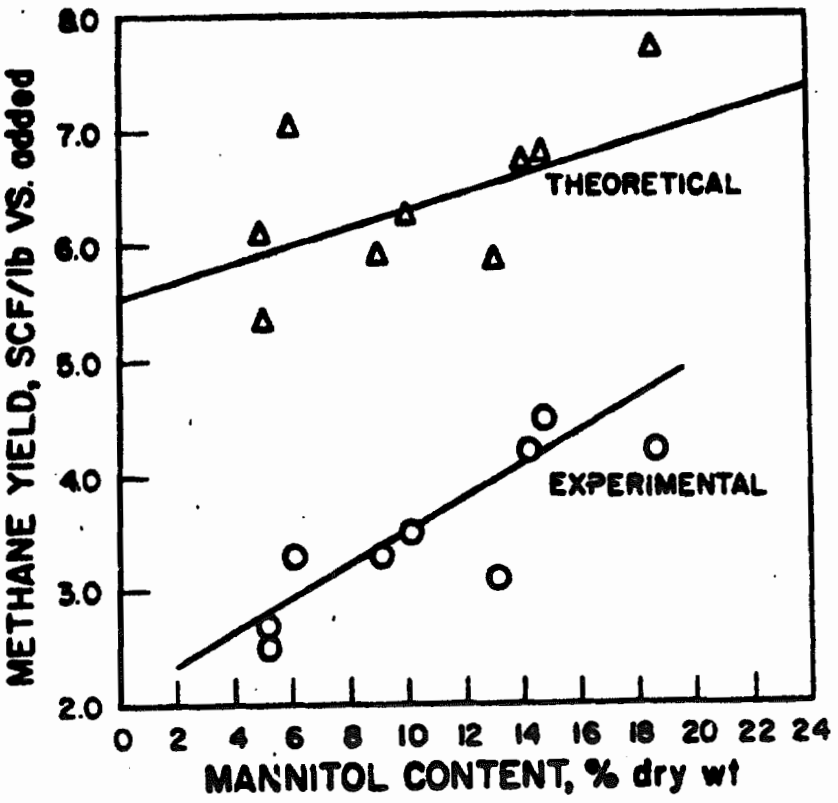

Figure 15. THE EFFECT OF MANNITOL CONTENT ON BIOMETHANATION OF KELP

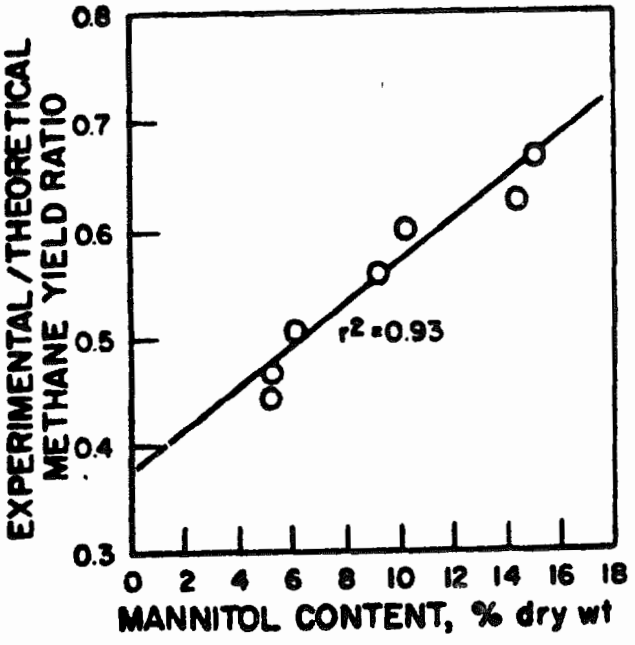

Figure 16. THE EFFECT OF MANNITOL CONTENT ON THE EXPERIMENTAL/THEORETICAL METHANE YIELD RATIO

Table 22. STEADY-STATE PERFORMANCE AND EFFLUENT QUALITY DATA FOR CONTROL RUN 138 OF MANNITOL EXPERIMENT WITH KELP LOT 46
(Loading 0.10 lb VS/ft^3-day; Retention Time 15 days; Culture Volume 1.5ℓ ; Temperature 35°C)

Analysis	Run 138
Date Initiated	7/16/79
Data Period	12/3-12/30/79
Number of Retention Times in Progress	9.3
Gas Yield, SCF/lb VS added	5.43
Methane Content, mol %	55.3
Methane Yield, SCF/lb VS added	3.0
Methane Production Rate, SCF/ft^3 culture-day	0.30
Total Volatile Acids, mg/ℓ as acetic	407
Alkalinity, mg/ℓ as $CaCO_3$	4770
Mean Caustic Addition, mg/ℓ feed	0

digesters receiving kelp lots with a high mannitol content appear to be less stable when either changing operating conditions or when operating at high loadings. We hypothesize that this instability is related to the fact that mannitol is rapidly decomposed, and with a daily feeding schedule, the products of its rapid decomposition result in a daily shock to the fermentation. This shock might explain instability observed in high loading runs, including several thermophilic experiments. This effect should be carefully documented by conducting daily-feed and continuous-feed experiments under the same operating conditions. Continuous feeding should eliminate the shock effect of rapid mannitol metabolism, permit operation at higher loadings, and result in stable performance. These experiments might also point out the invalidity of using daily-feed digester runs for kinetic studies.

Baffle Flow Digester

A 10 l baffle flow digester was constructed from an old settling tank and operated on freshwater-diluted kelp at a loading of 0.1 lb VS/Ft^3-day and hydraulic retention time of 15 days with 20 percent volumetric effluent recycle. The objective of this run was to evaluate performance of a digester design that is simple, does not require mechanical mixing, results in microbial phase separation (non-methanogenic and methanogenic phases), and maximizes retention of feed solids and microorganisms.

The performance of this digester is shown in Table 23 and Figure 17 The methane yield reached 4.5 SCF/lb VS added, compared with 3.0 SCF/lb VS for CSTR digesters. Run 44, which was a CSTR digester, operated under the same conditions. Performance in the baffle flow run was also very stable. (See Table 24.)

Operation of the baffle flow digester was terminated in mid November due to structural failures which were not repairable. IGT has proposed

Table 23. STEADY-STATE PERFORMANCE AND EFFLUENT QUALITY DATA FOR RUN PF-1 WITH FRESHWATER-DILUTED KELP LOT 48 AT TWO DIFFERENT LOADINGS
(Culture Volume 9.8 ℓ; Temperature 35°C; Retention Time 15 days)

Analysis	Loading, 0.05 lb VS/ft^3-day	Loading, 0.10 lb VS/ft^3-day*
Date Initiated	7/13/79	7/13/79
Data Period	8/20-9/2/79	10/15-11/18/79
Number of Retention Times in Progress	2.5	1.0
Gas Yield, SCF/lb VS added	6.20	7.98
Methane Content, mol %	55.3	56.2
Methane Yield, SCF/lb VS added	3.43	4.48
Methane Production Rate, SCF/ft^3 culture-day	0.26	0.45
Total Volatile Acids, mg/ℓ as acetic	570	570
Alkalinity, mg/ℓ as $CaCO_3$	2960	3420
Mean Caustic Added, mg/ℓ feed	0	0

* 20% of the effluent by volume was recycled.

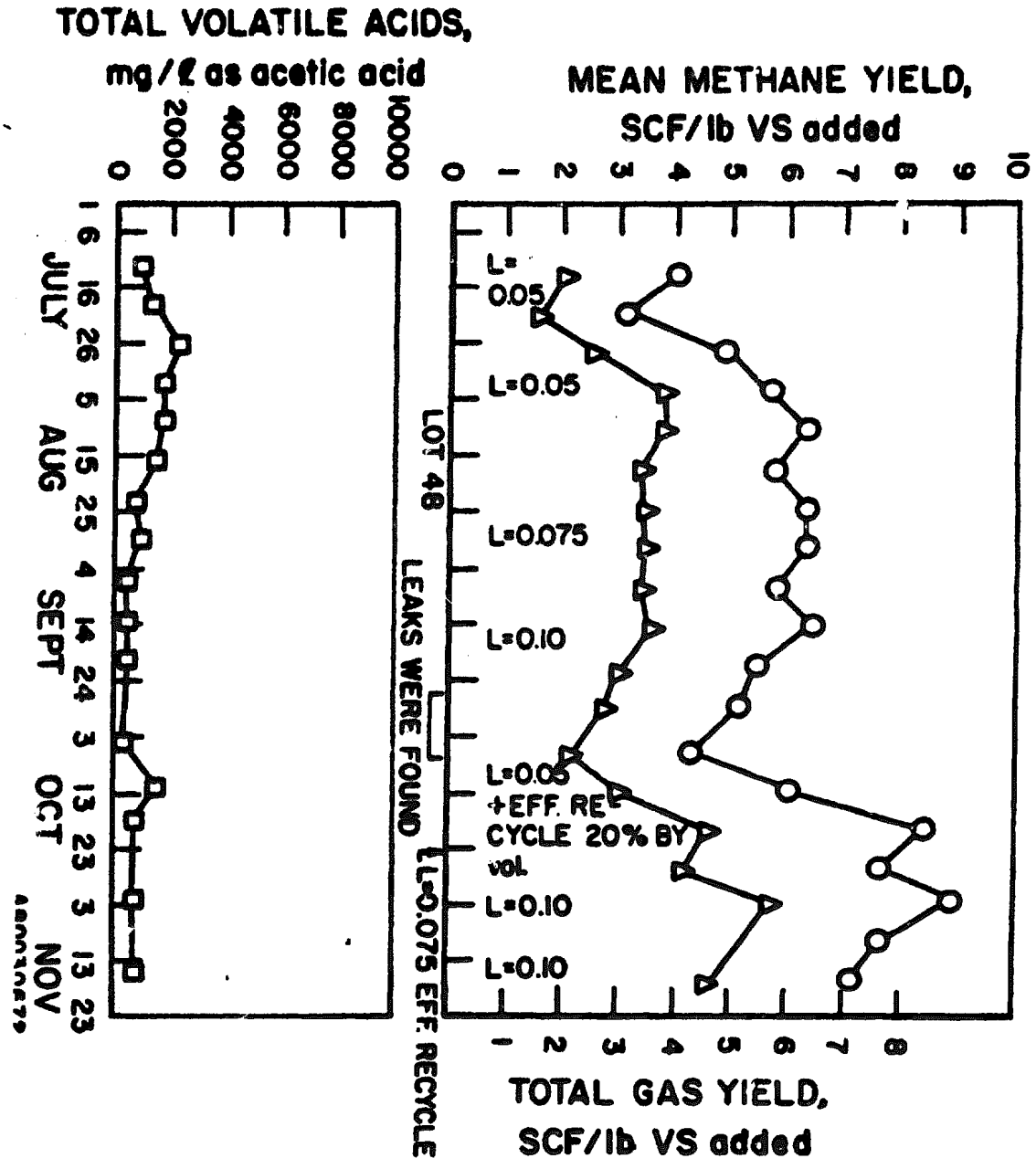

Figure 17. Performance of Baffle-Flow Digester (Run PF-1) With Kelp Lot 48 (Loading 0.10 lb VS/ft^3-day; Retention Time 15 days; Plug Flow 35°C)

Table 24. STEADY-STATE PERFORMANCE DATA FOR RUNS 43 AND 44 WITH FRESHWATER-DILUTED KELP LOT 48 (Culture Volume 10ℓ; Loading 0.15 lb VS/ft^3-day; Temperature 35°C; Retention Time 15 days)

Run No.	43	44
Date Initiated	5/6/79	5/6/79
Data Period	9/10-10/14/79	9/10-10/14/79
Number of Retention Times in Progress	1.0	1.0
Loading, lb VS/ft^3-day	0.15	0.15
Gas Yield, SCF/lb VS added-day	5.83	5.68
Methane Content, mol %	52.9	52.8
Methane Yield, SCF/lb VS added-day	3.08	3.00
Methane Production Rate, SCF/ft^3-culture-day	0.46	0.45
Total Volatile Acids, mg/ℓ as acetic	2890	2570
Alkalinity, mg/ℓ ac $CaCO_3$	8970	9680
Mean Caustic Added, meq/ℓ feed	50	48

construction and evaluation of this and other simple digester systems for 1980, including an expanded bed digester design.

Marine Inocula

Runs 136 and 139 represent experiments with two marine inocula derived from anaerobic marine mud at room temperature (ca. 26°C) on undiluted kelp. Previous marine inocula evaluated at IGT were developed on freshwater-diluted kelp, which may have resulted in selection away from halophilic bacteria. Stepwise increases in kelp loading for the current runs were preceded by pulses of acetate (in Run 136) or a mixture of acetate and formate (in Run 139). The objective of these pulses was to precede buildup of acid-formers by an artificial build-up of methane-formers, thus minimizing the shock normally observed with increasing kelp loading. Data in Figures 18 and 19 indicate that this technique proved successful for buildup of the culture. No significant differences were observed with the use of acetate alone or in combination with formates. Both runs achieved steady state after about 3.5 months at a loading of 0.1 lb VS/Ft^3-day. The methane yields as shown in Table 25 were 2.62 and 2.44 SCF/lb VS added for Runs 136 and 139 respectively. These are lower than the yields of 3.21 and 3.43 observed for Runs 41 and 42 which were operated under similar conditions but at a higher temperature of 35°C. Note that all of these runs receiving undiluted kelp had high concentrations of volatile acids. These results emphasize the need for further investigation of factors that limit conversion of volatile acids to methane.

Maintenance of Digesters for Kinetic Studies

Runs 41 and 42, which received undiluted kelp, discussed above, and Runs 43 and 44, which received freshwater-diluted kelp, were maintained during the report period under the first set of conditions for kinetic studies.

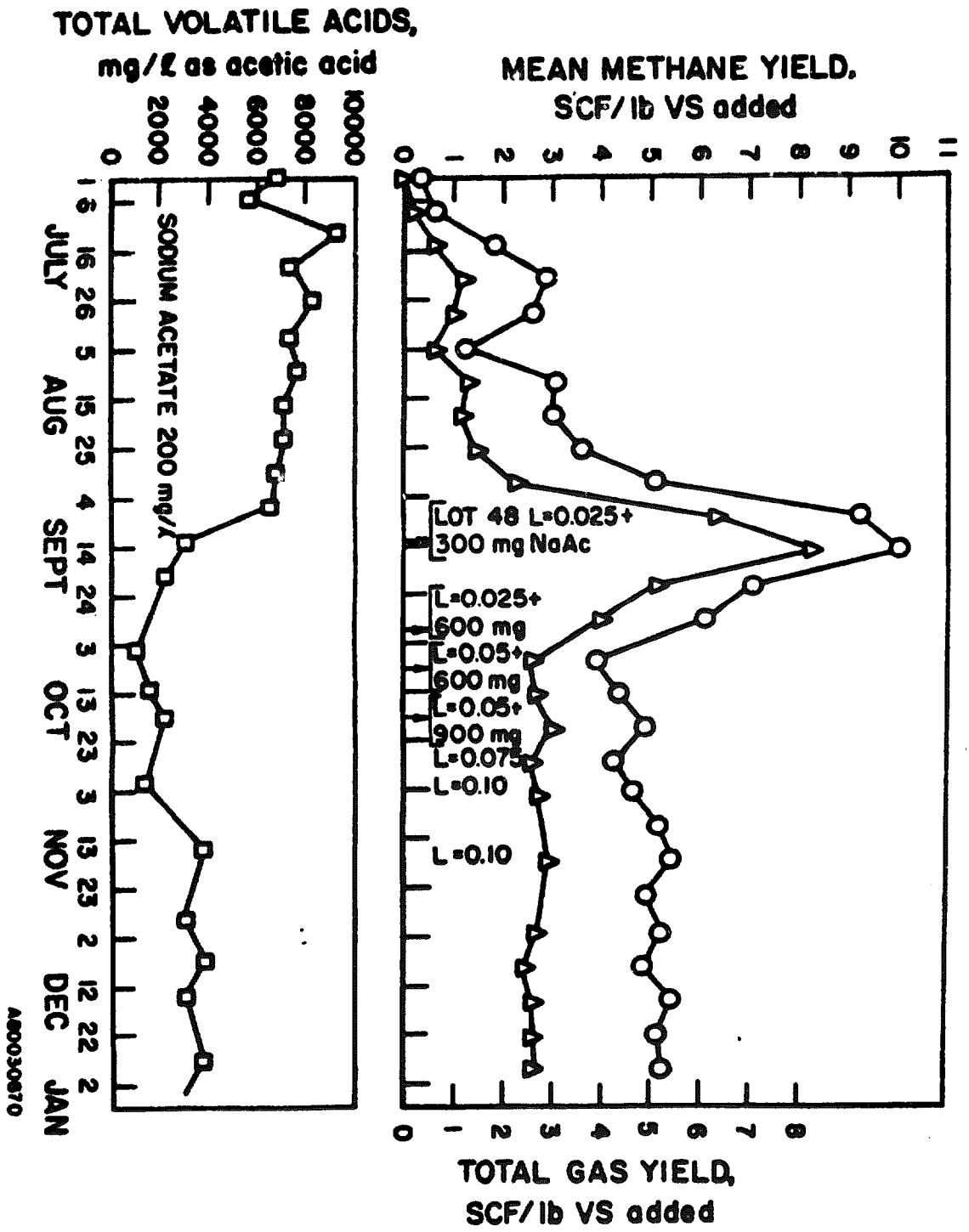

Figure 18. Performance of Run 136 at Room Temperature With Undiluted Kelp Lot 48

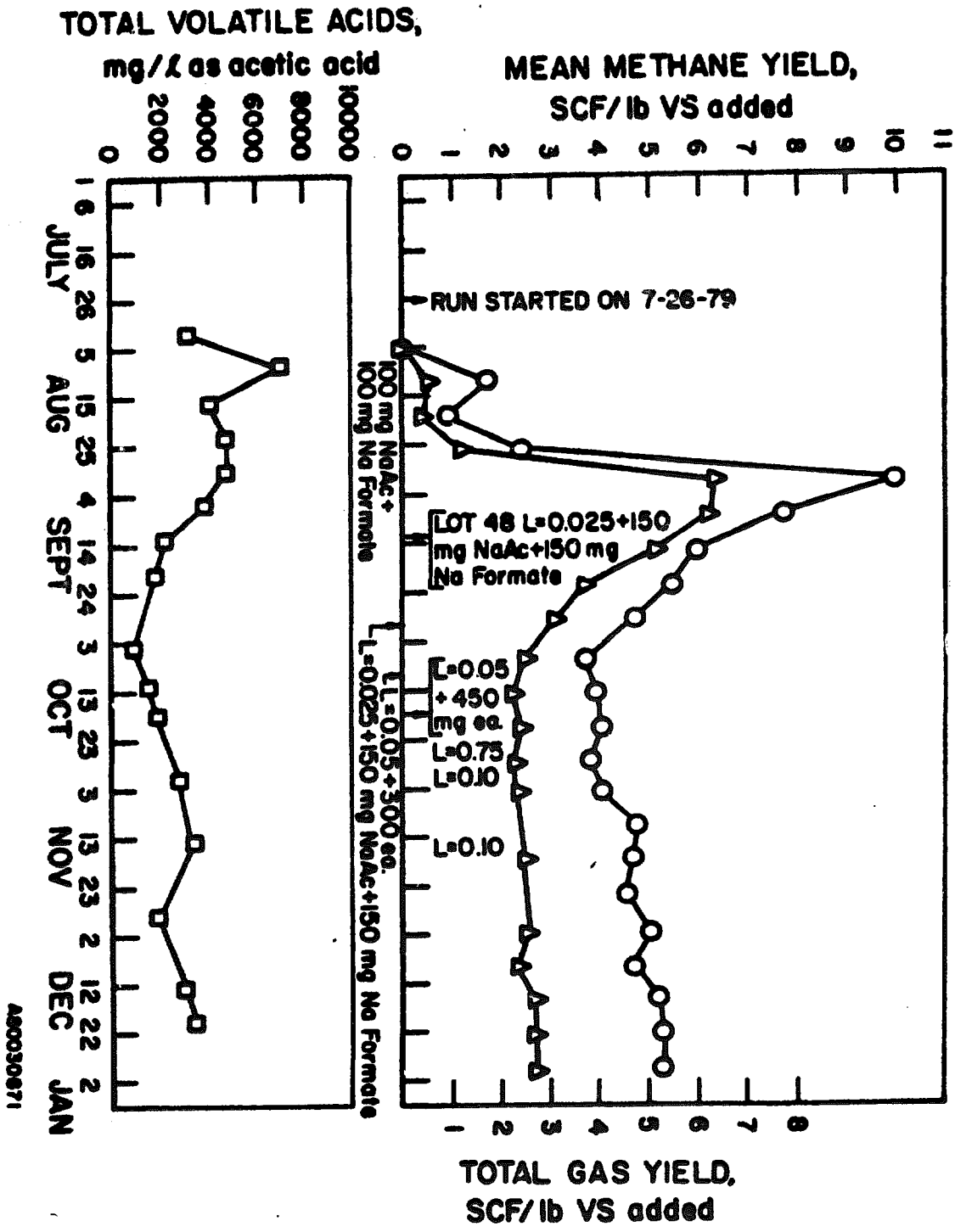

Figure 19. Performance of Run 139 at Room Temperature With Undiluted Kelp Lot 48

Table 25. COMPARISON OF DIGESTER PERFORMANCE OF RUNS 136 AND 139 STARTED FROM MARINE SEDIMENT AND RUNS 41 AND 42 STARTED FROM IGT'S INOCULUM A (Undiluted Kelp; Loading 0.10 lb VS/ft^3-day; Retention Time 38 days)

Run No.	136	139	41	42
Date Initiated	5/3/79	7/26/79	5/6/79	5/6/79
Data Period	11/12-12/16/79	11/5-12/9/79	9/24-10/28/79	9/24-10/28/79
Kelp Lot	48	48	48	48
Gas Yield, SCF/lb VS added	5.24	4.84	6.20	6.62
Methane Content, mol %	50.0	50.4	51.8	51.8
Methane Yield, SCF/lb VS added	2.62	2.44	3.21	3.43
Methane Production Rate, SCF/ft^3 culture-day	0.26	0.24	0.32	0.34
Total Volatile Acids, mg/ℓ as acetic	3360	2170	3400	3390
Alkalinity, mg/ℓ as $CaCO_3$	11,360	13,370	13,550	12,730
Mean Caustic Addition, meq/ℓ feed	0	0	18	13

Accidental spoilage of kelp Lot 47 intended for these studies prevented conversion to the next higher loading of 0.15 lb VS/ft^3-day for the undiluted kelp runs and 0.175 for the diluted kelp runs. We now believe that kinetic studies should be conducted using autofed digesters capable of continuous feed. IGT plans to renovate and place into operation the 10-l autofeeder system supplied by GE in 1977. In addition, we recommend that IGT's 400-l autofed digester be placed into operation early in 1980. These digesters could be used to document the effect of continuous feed on kinetics and digestor stability as well as to develop the kinetic data needed for process scaleup.

Conclusions

Approximately 35 digester runs were conducted in 1979, 20 of these achieved steady state. The crude gas production data on these runs provided the following information:

- -- Methane yields from several kelp lots with different compositions.
- -- Additional data on diluted and undiluted kelp digestion.
- -- Preliminary definition of the critical maximum C/N ratio for kelp digestion.
- -- Development of a model that predicts the relationship between methane yield and mannitol content.
- -- Evidence that an unmixed baffle-flow digester design gives higher yields and more stable performance than a CSTR digester.
- -- Performance of marine inocula developed at room temperature on undiluted kelp.

Although a crude evaluation and interpretation of data obtained can be made, a valid assessment will depend upon future analysis of frozen feed slurry and effluent samples and of corrected gas production data. These runs and associate sample were identified in a letter to GE and proposed for 1980 effort.

5.5 PRETREATMENT AND POSTTREATMENT STUDIES

U.S. DEPARTMENT OF AGRICULTURE

WESTERN REGIONAL RESEARCH CENTER

Introduction

The overall objective of the Energy from Marine Biomass Program is the development of a system for large-scale commercial production and harvesting of kelp (Macrocystis pyrifera) in the ocean and its conversion to methane and valuable by-products such as food, feed, and fertilizer. WRRC's current objective in the program is to develop treatments to increase methane production by increasing substrate digestibility and to determine by-product value of fermentor effluent as animal feed or fertilizer.

Previous contributions by WRRC to the program have included the development of a process to reduce the water and ash content of kelp. Grinding, thermochemical treatment, and pressing are used to reduce the ash and water content by about 70% while retaining about 70% of the volatile solids. WRRC has investigated and specified standard analytical procedures used on kelp. Existing analyses were adapted for use and new procedures were developed where necessary. WRRC has used these analyses to choose candidate treatments to increase substrate digestibility and to estimate solid effluent feed value and finally WRRC has correlated grinding energy with substrate particle size for both impact and altrition mills.

Due to limited funding, WRRC operated in a maintenance mode during calendar year 1979. No research was undertaken. Tasks for 1979 were a) obtain and maintain existing cultures; b) obtain and process kelp into suitable substrate and supply, as needed, to other contractors; and c) collect and accumulate effluent for possible future small animal feeding studies.

Work performed during 1979

A. Bench reactor operation and maintenance

During 1979, WRRC operated three fermentors, a 100 liter, a 50 liter, and a 10 liter size. Both 100 and 10 liter fermentors were established during 1978 and their operation was continued under steady-state conditions during 1979. The 50 liter fermentor was supplied to WRRC by GE and it was subsequently assembled and installed with some modification, charged with active culture from the 100 liter fermentor, and brought to steady-state conditions.

100 liter fermentor operation. Operating parameters at the beginning of the year were a loading rate of 0.1 lb. vs/day/cubic ft. and a retention time of 20 days. Gradually the loading rate was increased and the retention time decreased until by mid-April, the base-line steady-state conditions of 0.15 lb. vs/day/cubic ft. loading rate and 15 days retention time were reached. Typical steady-state gas yield was about 6.0 SCF/lb. vs, with a methane content of about 45% Operating conditions are summarized in Figures 20 and 21

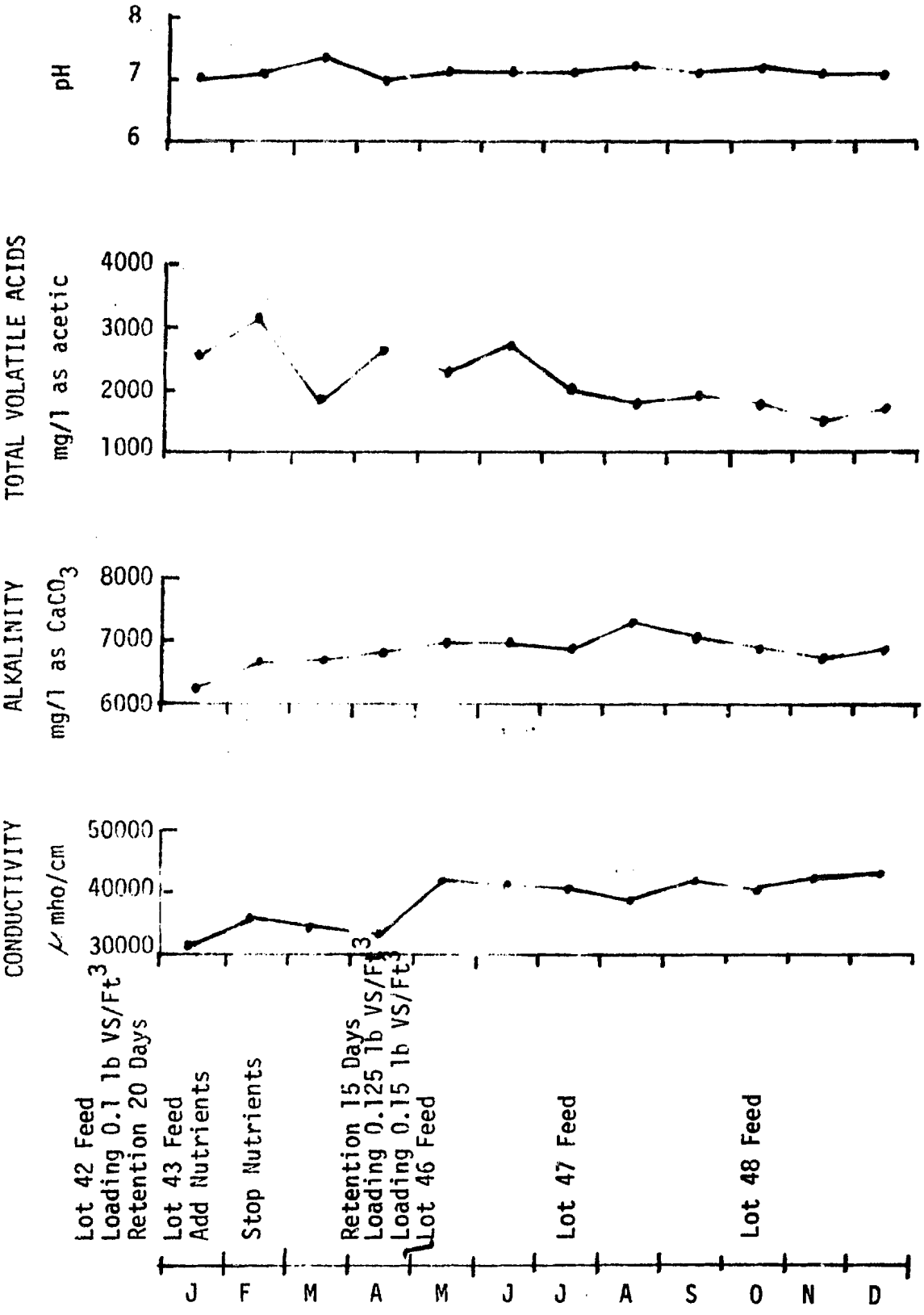

Figure 20. Typical Operating Conditions for 100 Liter Fermentor (Mid-Month Values Plotted)

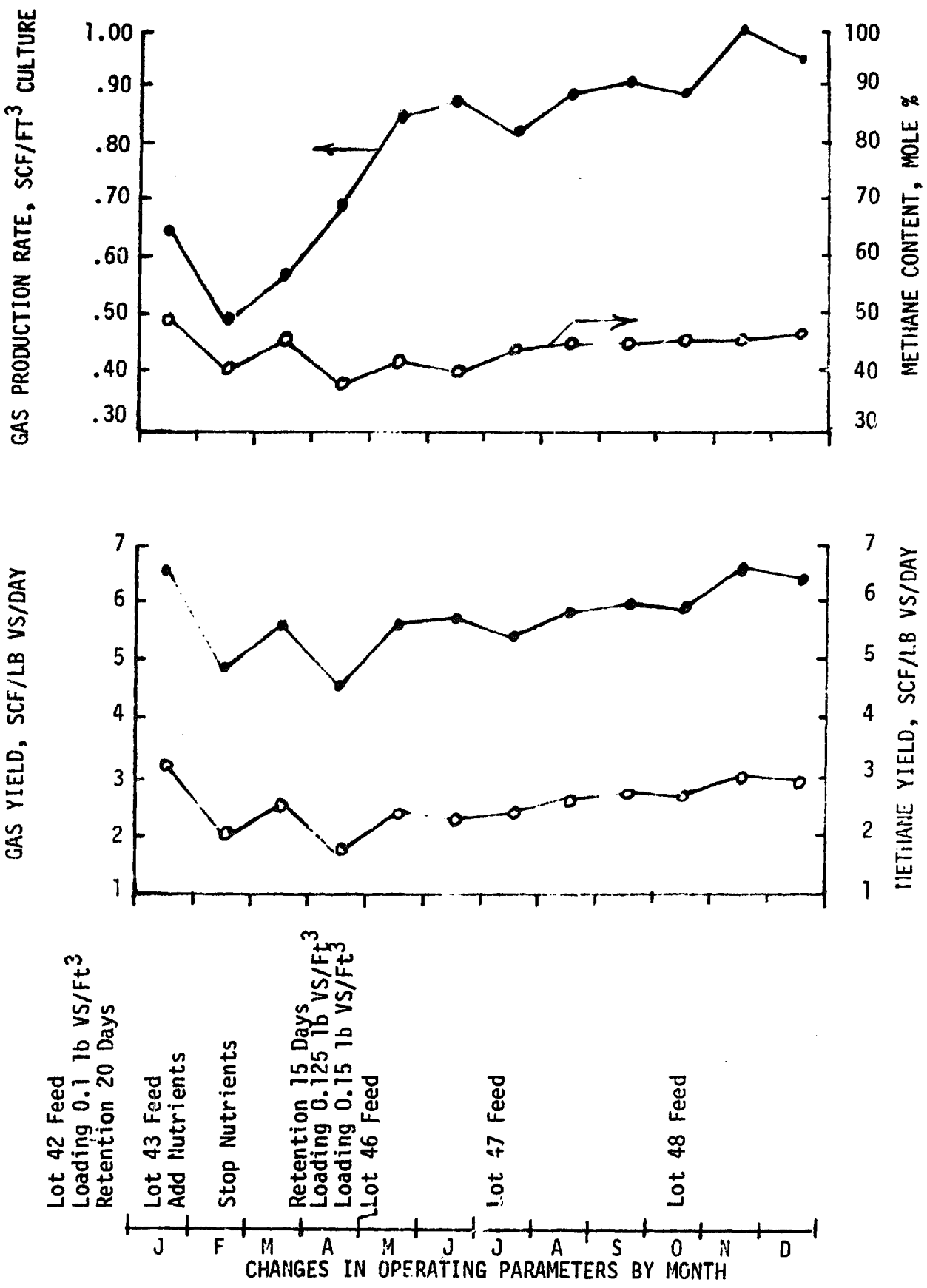

Figure 21. Typical Methane Yields for 100 Liter Fermentor (Mid-Month Values Plotted)

10 liter fermentor operation. Difficulty was experienced in the early part of the year due to equipment malfunction resulting in two shutdowns. After some equipment renovation and careful culture establishment, problems were remedied by mid-April at which time base-line steady-state conditions were reached. Typical steady-state gas yield was 5.5 SCF/lb. vs with a methane content of about 45%. Operating conditions are summarized in Figures 22 and 23.

50 liter fermentor operation. This fermentor was received from GE in early 1979. The fermentor was designed with large feed and exit port openings to accomodate large kelp particle sizes. This fermentor will be used to correlate particle size with fermentor performance. The fermentor was installed and slightly modified to improve operation. Installation of associated plumbing and gas collection and monitoring equipment was completed by December. The culture was introduced in late December and had not had enough time to reach base-line steady-state conditions by the end of the calendar year.

Bioassay. Major modifications were made in the system to facilitate culture transfer and feeding and eliminate leaks. Initial testing was undertaken.

B. Kelp supply

Four harvests were completed and processed during the year, two from local kelp beds in Monterey Bay, and two from Southern California beds harvested by Stauffer Chemical Company. Table 26 lists pertinent data on these harvests. Lot 49 kelp was set aside for exclusive use by IGT because its mannitol content was judged more suitable for experimental runs. Lots 46-48 contained unusually low mannitol contents and were used by WRRC for maintenance of our cultures.

C. Effluent accumulation

Samples of effluent were frozen and stored for possible later use in animal feeding studies.

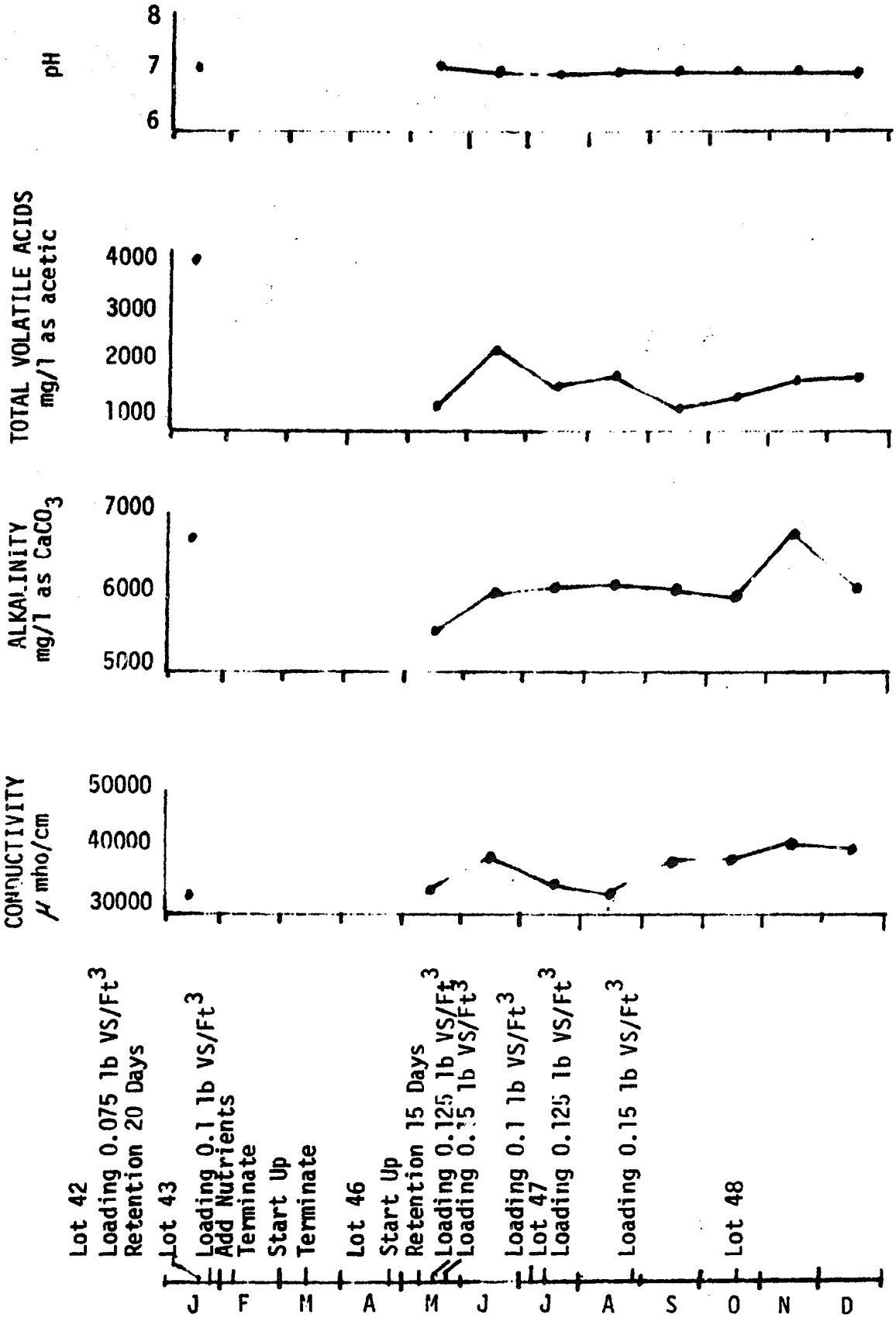

Figure 22. Typical Operating Conditions for 10 Liter Fermentor (Mid-Month Values Plotted)

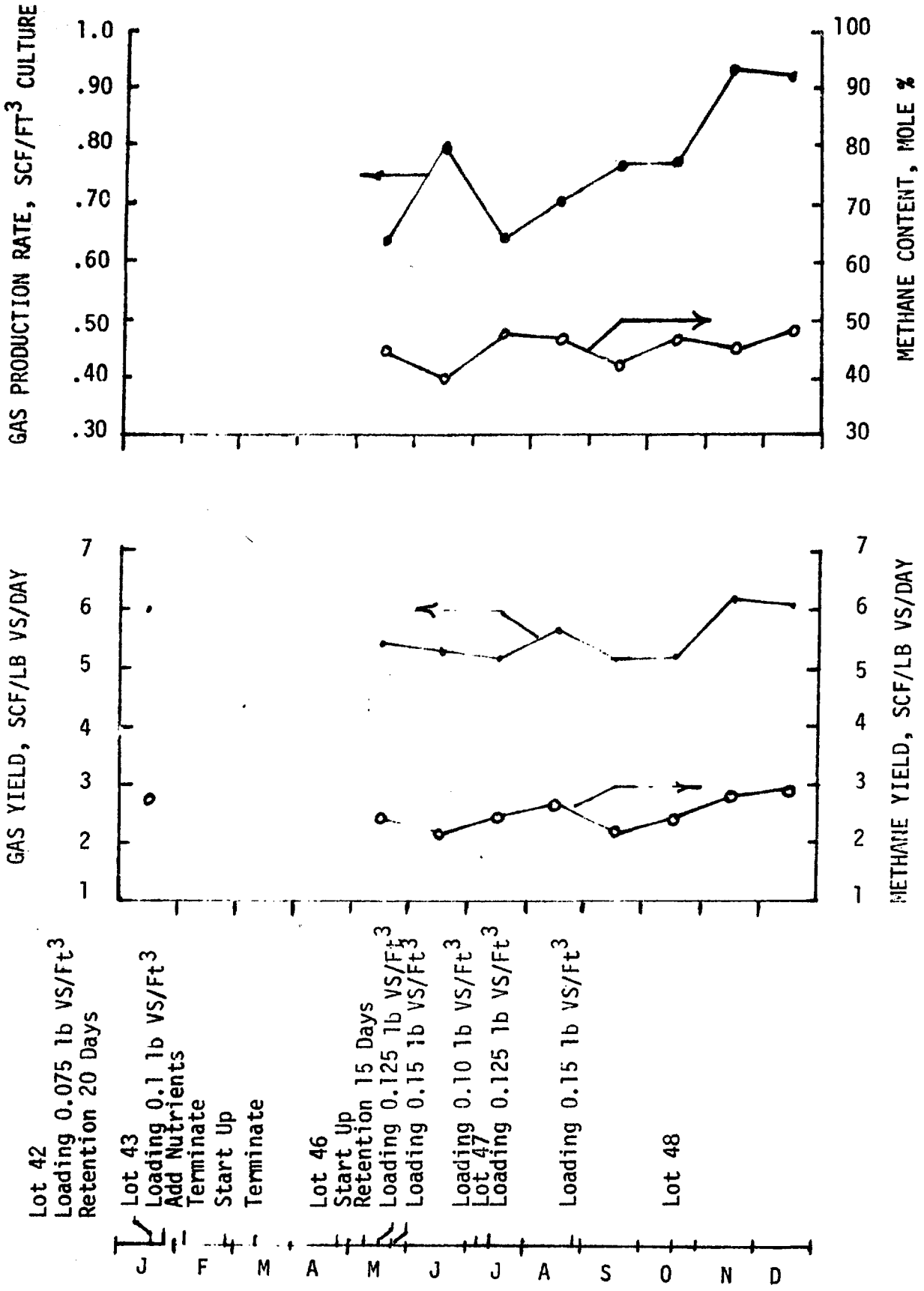

Figure 23. Typical Methane Yields for 10 Liter Fermentor (Mid-Month Values Plotted)

TABLE 26

SUMMARY OF HARVEST DATA AND COMPOSITION

Harvest Date	1/24/79	3/21/79	4/29/79	10/16/79
Harvester	WRRC	WRRC	Stauffer	Stauffer
Location	Santa Cruz	Santa Cruz	So. Calif.	So. Calif.
Lot Number	46	47	48	49
Sample No.	687-162	687-163	687-164	687-166
Total Solids, %	10.31	10.45	11.12	12.00
Dry Matter Composition				
Ash, %	47.01	45.44	46.17	37.10
Volatile Solids, %	52.99	54.56	53.83	62.90
Nitrogen, %	2.03	2.15	1.73	1.06
Fat, %	1.55	0.89	1.51	0.20
Fiber, %	6.57	5.32	6.20	5.30
Algin, %	18.97	18.87	14.18	14.45
Mannitol, %	5.19	5.18	9.06	23.97

6. SPECIFIC OBJECTIVES AND WORK PLAN FOR 1980

The following section describes in detail those objectives and activities planned for 1980 and for the next several years as many of the tasks are longer term continuing efforts. If the required funding becomes available, several new tasks will be initiated which are also included in the following description. These tasks include (1) additional marine test farms in near-coastal waters, both natural and man-made, (2) scale-up digester design, (3) system analysis and engineering, etc. As of this writing, however, the Marine Biomass Program is still funded at a level which does not provide for initiation of these additional tasks.

A detailed plan for the conduct of farm modification(s) conceptual trade-offs has been developed. Further, a preliminary specification identifying the requirements to which subsequent designs will be produced has been prepared and reviewed with GRI and SERI technical personnel. In addition, a concept scoring system has been developed. Both of these documents will be forwarded to GRI for review and approval by May 1, 1980.

WORK PLAN - 1980

A. MARINE FARM SYSTEMS

1. Maintenance
2. OSTF Refurbishment/Repair
3. OSTF Design Concept Analysis and Trade-off
4. OSTF Mods Model Testing
5. OSTF Mod Design
6. OSTF Mods Construction
7. Instrumentation System
8. Dispersion System
9. Material/Hardware Tests
10. Drag Model Development

B. KELP YIELD/BIOLOGICAL STUDIES

1. OSTF Yield Measurements
2. Laboratory Studies
3. Planting Technology Development
4. Methods Development

C. KELP BIOMASS CONVERSION

1. Inocula Research/Development
2. Digestion Systems R/D
3. Pretreatment and Posttreatment R/D

D. SYSTEMS ANALYSIS

E. ENGINEERING SUPPORT AND CONSULTATION

F. NYS-ERDA

1. Species Selection/Collection
2. Species Evaluation
3. Site Selection
4. Systems Analysis