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FINAL REPORT

GTI PROJECT NUMBER 20960
DOE PROJECT NUMBER DE-FE0002640

Macroalgae for CO_2 Capture and Renewable Energy – A Pilot Project

Reporting Period:
January 15, 2010 – October 31, 2010

Report Issued:
January 31, 2011

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Abstract

The objective of this project was to demonstrate, at a pilot scale, the beneficial use of carbon dioxide (CO_2) through a technology designed to capture CO_2 from fossil-fuel fired power plant stack gas, generating macroalgae and converting the macroalgae at high efficiency to renewable methane that can be utilized in the power plant or introduced into a natural gas pipeline.

The proposed pilot plant would demonstrate the cost-effectiveness and CO_2/ NO_x flue-gas removal efficiency of an innovative "algal scrubber" technology where seaweeds are grown out of water on specially-designed supporting structures contained within greenhouses where the plants are constantly bathed by recycled nutrient sprays enriched by flue gas constituents.

The work described in this document addresses Phase 1 of the project only. The scope of work for Phase 1 includes the completion of a preliminary design package; the collection of additional experimental data to support the preliminary and detailed design for a pilot scale utilization of CO_2 to cultivate macroalage and to process that algae to produce methane; and a technological and economic analysis to evaluate the potential of the system.

Selection criteria for macroalgae that could survive the elevated temperatures and potential periodic desiccation of near desert project sites were identified. Samples of the selected macroalgae species were obtained and then subjected to anaerobic digestion to determine conversions and potential methane yields. A Process Design Package (PDP) was assembled that included process design, process flow diagram, material balance, instrumentation, and equipment list, sizes, and cost for the Phase 2 pilot plant. Preliminary economic assessments were performed under the various assumptions made, which are purposely conservative. Based on the results, additional development work should be conducted to delineate the areas for improving efficiency, reducing contingencies, and reducing overall costs.

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Executive Summary

The overall objectives of the project are to demonstrate the beneficial use of carbon dioxide (CO_2) through a technology designed to capture CO_2 from fossil-fuel fired power plant stack gas, generating photosynthetically fixed biomass (macroalgae), and converting the macroalgae at high efficiency to renewable methane that can be utilized in the power plant or introduced into a natural gas pipeline.

A closely allied objective is to demonstrate the cost-effectiveness and CO_2 / NO_x flue-gas removal efficiency of an innovative "algal scrubber" technology where seaweeds are not grown submerged in seawater, but out of water on specially designed supporting structures contained within a greenhouse in which the seaweed are constantly sprayed by recycled nutrient sprays enriched by flue gas constituents.

The overall project is divided into two phases: Phase 1 – Process Optimization and Conceptual Design; and, Phase 2 – Pilot System Engineering Design, Construction, and Operation. The scope of work for Phase 1 focuses on the preliminary design activities for the proposed macroalgae for CO_2 capture and renewable energy system. At this time, Phase 2 has not been initiated because of siting and co-funding issues. The scope of work associated with Phase 2 of this project would have been on demonstrating the technology at a pilot plant located at a site adjacent to a natural-gas-fired power plant. Phase 2 would have focused on two key elements: 1) Using CO_2 from the power plant flue gas to produce macroalgae, and 2) converting the macroalgae into renewable fuel, specifically, pipeline quality natural gas.

Potential candidates for macroalgal species selection were evaluated for adaptation to spray cultivation systems and seasonally elevated ambient temperatures anticipated within greenhouse enclosures set at the primary project site in Escondido, CA. The criteria for selecting, screening, and culturing a seaweed species (or consortia of species) to optimize biomass yields under the expected conditions have been delineated. Recommended high priority taxa includes various species of *Gracilaria*: *G. pacifica, G. vermiculophylla, G. tikvahiae,* and *G. cervicornis* (formerly known as *G. ferox*). Other eurythermal red algal taxa that meet most of the selection criteria and are targeted for collection and testing include the amphi-atlantic tropical species *Solieria filiformis (= tenera),* the Northeast American warm-temperate species, and *Agardhiella subulata.* Backup eurythermal and euryhaline species for consideration include *Ulva expansa, U. rigida, U. clathrata, Porphyra perforata, P. umbilicalis*, and *P. dioica*.

Material for cultivation of several of these species including *G. vermiculophylla* (3 strains), *G. tikvahaie* (2 strains), *G. cervicornis,* and *G. pacifica* have been secured. Unialgal cultures of each are established and are in the process of vegetative propagation for mass cultivation in the Seaweed Marine Biotechnology Labs of UCONN. *G. pacifica*, which UCSD-SIO obtained from a commercial aquaculture operation in San Diego County, CA has been isolated and cultured as well.

Other high priority eurythermal red algal taxa targeted for isolation include the Amphi-Atlantic tropical species *Solieria filiformis (=tenera)* and the Northeast American warm-temperate species, *Agardhiella subulata.* Other eurythermal and euryhaline species of Ulvalean and

Rhodophycean algae are being considered. Ulvalean species should include *U. expansa*, *U. rigida* and *U. clathrata*. Other Rhodophycean macroalgae should include *Porphyra perorata*, *P. umbilicalis*, and *P. dioica*.

An assessment of the potential convertibility via anaerobic digestion of several macroalgae feedstocks was performed using an assay procedure developed by GTI. Methane production data from the small batch test reactors were collected for *Ulva*, *Sargassum*, and *Gracilaria* feedstocks over a 60-day test period. Methane production was also measured using the same assay on control batch reactors fed with AVICEL cellulose material. Compositional analyses were conducted on the AVICEL cellulose and the three macroalgae feedstocks. Methane yield results from control batch reactors indicated that approximately 6.66 SCF of methane per lb of volatile solids (VS) added was achieved which is reasonably close to a theoretical methane yield of 7.0 SCF/lb VS added from pure cellulose. Results from test reactors indicated that *Gracilaria* has the potential for the highest methane yield amounting to over 5.2 SCF/lb VS added; this represents a methane yield that is among the highest measured for marine biomass feedstocks.

A detailed conceptual process design for site-specific installation of the Macroalgae-Derived Renewable Energy (MADRE) pilot plant for Phase 2 of the overall project has been completed. The conceptual process design includes an overall material balance for generating renewable natural gas (biomethane) from macroalgae grown in a greenhouse supplied with CO_2 from the flue gas from a natural-gas-fired power plant, process flow diagram, equipment list with sizes, piping and instrumentation diagrams, electric power requirements, and layouts for two potential project sites (Palomar Power Plant, Escondido, and Moreno Compressor Station, Moreno Valley, both in California). Cost estimates for the equipment, installation, and operation were also completed for the potential Phase 2 work.

In addition, a process design package (PDP) or Design Information Document was compiled as a deliverable for the project. The PDP consists of fifteen sections: Project Report, Cost Estimate, Procurement and Construction Schedule, Process Flow Diagrams, Piping and Instrumentation Diagrams, Utility Diagrams, Site Preparation, Foundations, Structural, Layout, Equipment, Instrumentation, Piping, Electrical, and Planning Documents. The PDP includes the information required for proceeding with the Phase 2 MADRE pilot plant procurement, installation, and operation including details of the permitting requirements for the Escondido site and the Moreno Valley site.

A process design and material balance for an Aspen Plus simulation was prepared so that a techno-economic evaluation and life-cycle assessment could be conducted. The techno-economic evaluation and life-cycle assessments were originally based on three scenarios described below.

In the first scenario, the substitute natural gas to be generated in the Phase 2 MADRE pilot plant would be used to off-set a portion of the natural gas required at the nominal 550-MW natural-gas-fired power plant. A slip stream of the flue gas from the plant would be directed to the greenhouse. At the scale of operation of the MADRE pilot plant, this represented 0.006 percent of the total natural gas requirement for full-load operation. The costs for the pilot-scale equipment were then scaled up for the second scenario.

In the second scenario, the scaling calculations matched the proposed power plant capacity of 550 MWe. All process flow streams were linearly scaled so that the total higher heating value of natural gas / methane supplied to the power plant was equal to the proposed capacity (550 MWe) divided by the expected conversion efficiency of the combined cycle power plant (50.5% HHV).

The second scenario further considers commercial deployment in which all (100 percent) of the CO_2 produced from power plant operations is utilized in a series of MADRE greenhouses. This scenario assumes a fairly low-density growth of macroalgae in the greenhouses. The third scenario assumes a higher-density growth of macroalgae.

The calculated cost to generate electricity under the assumptions and the three scenario listed above are $0.056, $1.111, and $1.043 per kW-hr, respectively. Based on the assumptions, the land area devoted to the greenhouses and anaerobic digesters for the three scenarios is 2 acres (Phase 2 pilot), 25,450 acres (low density), and 20,410 acres (high density), respectively. The above costs do not include any credit for CO_2 abatement.

In reviewing the results above, the assumptions underlying the two techno-economic assessments were revisted. With most parameters held constant, the forth scenario assumes 25 percent of the CO_2 in the flue gas would be directed to the MADRE greenhouses. Also, the density of the macroalgae produced in the greenhouses was assumed to be significantly higher than the second or third scenarios above. This would be accomplished by stacking macroalgae supports up to 6 meters high on both sides of the support. The objective was to maximize macroalgae growth thereby reducing the amount of land required to generate the macroalgae. Harvesting the macroalgae would be mechanized in the N^{th} commercial plant. In this scenario, the overall plant required 2,350 acres for the power plant as well as the macroalgae greenhouses and anaerobic digestions systems.

The resulting cost of electric power from the high-high density scenario was calculated to be $0.318 per kW-hr, which is still considerable higher than electricity produced from conventional power plants.

At the suggestion of DOE NETL during a project review webinar, GTI included the potential credit for CO_2 abatement. The CO_2 credits considered were $10, $25, and $50 per ton of CO_2 removed/abated. Spreading the credit over the electricity supplied by the plant resulted in a fractional (less than 1 cent/kW-hr) reduction in electricity cost.

In summary, selection criteria for macroalgae that could survive the elevated temperatures and potential periodic desiccation of near desert project sites were identified. Samples of the selected macroalgae species were obtained and then subjected to anaerobic digestion to determine conversions and potential methane yields. A Process Design Package (PDP) was assembled that included needed process design, instrumentation, and equipment list, sizes, and cost for the Phase 2 pilot plant. Preliminary economic assessments were performed under the various assumptions made, which are purposely conservative. Based on the results, additional development work should be conducted to delineate the areas for improving efficiency, reducing contingencies, and reducing overall costs.

Approach

Two areas of research are discussed in this section: (1) Macroalgae species selection and optimization experiments and (2) macoralgae anaerobic digestion studies. The UCSD-SIO, UCONN, Heifetz team led the macroalgae species selection activities and optimization experiments. Based on an initial screening of candidates, *Gracilaria* as well as two other strains were sent to GTI for anaerobic digestion studies. The purpose of these studies was to evaluate macroalgae biodegradability and potential methane yield. The results from these activities will also serve as basis for the design, construction, and operation of a pilot unit.

Macroalgae Species Selection

Genus / Species Selection

A comprehensive set of species selection criteria was established based on input from team members (UCSD-SIO, UCONN, and Heifetz BioConsulting, M. D. Hanisak) and other experts in the field. Rationale included both direct experience of the team with different macroalgae species as well as information available in the literature. Meteorological data for the proposed Escondido site, Escondido municipal wastewater composition and published information regarding enclosed greenhouse systems developed for microalgae utilizing flue gas fertilization were considered in the development of baseline environmental parameters. Taxa with upper survival temperatures greater than 25°C (Yarish *et al.*, 1987; Lüning and Freshwater, 1988; Garza-Sánchez *et al.*, 2000) were favored.

Seaweed Collection and Permitting

Once suitable species were selected, the UCSD-SIO team determined where they could be collected and which permits were required for collecting and growing these species in the SIO laboratories and at the Escondido site. Both state and federal agencies were contacted.

Seaweed Culturing

In situ and pond-based marine macroalgae cultivation techniques are standardized and routine, except for spray culture techniques, which have been sparsely practiced by only a few research groups, with no known pilot or commercial-scale systems deployed anywhere. Different taxa require different farming methods. Although some seaweed species need one-step farming through vegetative propagation, others require a two-step cultivation process, where propagules must be started from spores, given that the adult form cannot survive if propagated vegetatively. *Gracilaria* species are propagated vegetatively (one step), whereas *Porphyra* and *Ulva* species are started from spores (two-step), (Hanisak, 1987; Hanisak, 1990; Sahoo and Yarish, 2005; Yarish and Pereira, 2008; Pereira and Yarish, 2010). Although large-scale open water cultivation of some species has been carried out in many Asian countries, other species are cultivated in tanks and ponds. For example, *Gracilaria* is being cultivated in tanks and raceways in the U.S., Israel, and Portugal. Epiphytes, fouling, and critical nutrient requirements are serious issues to consider in any cultivation system, especially for submerged culture in tanks.

The major scientific challenges for attaining successful spray cultivation at larger scales are:

(1) Site selection and availability of high quality seawater – or artificial seawater;
(2) Spray culture system design, construction and operations, including application of recirculation systems for minimizing water loss (especially if cultivation is away from the sea);
(3) Knowledge of the reproductive biology of the culture species;
(4) Selection of the best strains;
(5) Control of the environmental variables including temperature, pH and CO_2 availability, light, salinity and desiccation;
(6) Nutrient uptake and seawater exchange/nutritional requirements; and
(7) Optimizing stocking density in relation to available light within the culture module.

Critical reviews of these factors can be found in the works of Hanisak (1987), Craigie and Shacklock (1995), Yarish and Pereira (2008), Kim *et al.* (2009), and Kim and Yarish (2010).

Testing of Vertical Substrates with *Gracilaria*

UCSD-SIO performed initial experiments to test different horizontal and vertical substrates for use in the proposed pilot scale propagation facility and greenhouse. The purpose of these experiments was to test different growth orientations to determine what would be used in the proposed pilot plant unit. *G. pacifica* branches obtained commercially in San Diego County were fragmented into 1-2 inch pieces and settled onto frames constructed from netting material and one of four sizes of plastic mesh screening lying horizontally in a tub and covered with 2 inches of enriched seawater. After 5 days of settling time, the substrate materials were lifted to the vertical position in air, and the number of seaweed fragments remaining attached to each substrate type were counted and tabulated.

Optimization Experiments with *Gracilaria*

Several optimization experiments were planned to test the performance of selected species under various conditions of spray, nutrients, temperature, irradiance, salinity and CO_2 availability using an array of 12 each laboratory-scale (3.8-L capacity) "mini" spray-culture modules designed and built by UCSD-SIO.

Anaerobic Biogasification Potential and Methane Yield Measurements of Candidate Macroalgae Species

The objective of this work was to evaluate biodegradability and methane yield of up to three macroalgae species as identified by the UCSD-SIO/UCONN/Heifetz team using Anaerobic Biogasification Potential (ABP) assays. Since the composition of the test feed samples significantly affect biodegradability, the results from ABP assays will help the project's species selection and optimization program for selecting appropriate species for high conversion efficiency in the anaerobic digestion process. The results will also serve as a basis for design, construction, and operation of the MADRE pilot unit in Phase 2.

Both semicontinuous and batch-fed techniques were used to evaluate the anaerobic digestion performance for the conversion of *Gracilaria pacifica*. A batch bioassay conducted using a flask or serum bottle is simple and equipment needs are minimal. The test has been widely used as an effective, economical laboratory technique for the assessment of biomass conversion efficiency and methane yield. Upon completion, these tests were followed by the operation of a semi-continuous anaerobic digester that was fed with *Gracilaria.*

Anaerobic Biogasification Potential (ABP) Assay

The Anaerobic Biogasification Potential (ABP) assay assesses the biodegradation potential (expressed as methane yield) of biomass candidates. If yields from the ABP assay are acceptable (greater than approximately 60% of the corrected theoretical yield), the candidate is further evaluated under conditions of baseline anaerobic digestion using a stirred tank reactor (STR) to better define performance characteristics (e.g., loading, retention time, and solids recycle). Other parameters with the potential to influence methane production rates and yields should also be evaluated, including nutrient requirements, mixing, feeding frequency, temperature, inoculum, toxicity problems, and pretreatment.

GTI used a 100-mL ABP batch digestion assay to determine the ultimate biodegradability of feedstocks as measured by gas production and volatile solids (VS) reduction following a 60-day incubation period with a sludge inoculum at 35°C. The assay was performed in a defined nutrient growth medium to ensure an adequate supply of macro- and micronutrients and growth factors for proliferation of the organisms catabolizing the feedstock under study, as well as minimization of nutritional variability when screening biodegradability of candidate biomass species. The slopes of the methane production versus time curves provide a relative measure of the reactivity rate for the various macroalgae samples investigated. Bulk samples of macroalgae species were obtained from UCSD-SIO and shipped to GTI for processing and anaerobic digestion studies.

Results and Discussion

Task 2.0 - Optimization of Process Conditions for Macroalgal Production and Conversion to Biomethane

Genus / Species Selection

The candidate species may be collected from either the natural marine or estuarine waters of Southern California, and/or natural marine or estuarine waters of other regions of the world ocean, and/or obtained from established culture collections.

Based on the criteria established by the project team (see below) the genus *Gracilaria* was selected for further evaluation and optimization of specific species/strains. Species to be evaluated include *G. pacifica* (native to San Diego area, but less warm-adapted – see Figure 1); *G. vermiculophylla* (widespread in Pacific and Atlantic, including tropics); *G. cervicornis* (formerly known as *G. ferox*) demonstrated to be capable of high productivity in culture in tropical environments.

Figure 1. *Gracilaria pacifica*, S. California Native Species (Obtained by UCSD-SIO from Carlsbad Aquafarms, Inc., San Diego County, CA)

Criteria for Macroalgae Species Selection

Survival Under Expected Escondido Greenhouse Conditions

- Temperature tolerate up to 35°C; broad optimal temperature range species preferred
- pH in the range of 6 to 10
- Desiccation tolerance (anticipated suitability for spray culturing)
- Salinity fluctuations (½ to 2 times seawater salinity; range of 17-70‰ or ppt)

- Trace metal tolerance (Escondido reclaimed municipal wastewater assay range)

Productivity

- Low stocking density (goal is to regenerate holdfast structures from small fragments)
- Rapid growth and nutrient uptake in culture (*Gracilaria* in tumble-tank culture capable of sustained growth exceeding 40 g dry weight per m^2 per day)
- Robustness of growth versus changing conditions or on edge of environmental envelope

Creation of Natural Genetic Diversity

- Small gametophyte or isogamous to facilitate high-throughput screening approaches
- Sexual cycle with availability of interfertile but geographically distinct strains/species
- Reasonable expectation of experimental tractability for selection

Morphology

- Robustness with respect to design of system and harvesting method. Vertical orientation of system to enhance areal yield will limit types of seaweed. Blade-type structures without rigidity unlikely to be suitable, but branched morphologies like *Gracilaria* are ideal.

Harvestability

- Proliferate vegetatively from holdfast via basal meristem to facilitate harvest and regrowth cycles

Digestibility

- Compatible with GTI anaerobic digestion process (Phase 1)

Seaweed Collection and Permitting

Collection permits were obtained from the California Department of Fish & Game (CF&G) allowing UCSD / Mitchell Laboratory personnel to collect kilogram quantities of native seaweed along the coastline of San Diego County. A permit was also obtained from San Diego Bay National Wildlife Refuge to collect unlimited amounts of *Ulva* spp. and smaller amounts of other species adapted to the warm water hypersaline conditions of the Tijuana Estuary. Per email correspondence with CF&G it was determined that a separate import permit will be required for each species/origin shipped to the Escondido plant for use in commercial-scale aquaculture. No aquaculture permit will be required for the non-commercial scale demonstration project planned for Phase 2. This will facilitate transfer of *G. cervicornis* (formerly known as *G. ferox*) and *G. vermiculophylla* from UCONN to UCSD.

Practical Methods Applicable to the Selection and Adaptation of Algae Strains

Methods were developed for the selection and adaptation of algal strains for optimized survival and proliferation under defined environmental conditions applicable to the selected candidate seaweed species when cultured under the expected culture conditions of the proposed Phase 2 spray-culture greenhouse-enclosed culture module. Such adaptation is a core component of strain selection and is required in order to facilitate propagation in the proposed spray-culture greenhouse-enclosed culture module.

Culturing of *Gracilaria*

UCONN carried out isolation of different taxa of *Gracilaria*. One taxon is confirmed as *Gracilaria vermiculophylla* (Figures 4, 5, and 6), one is tentatively identified as *Gracilaria cervicornis* (Figures 2 and 3), one is identified as *Gracilaria pacifica*, one identified as *Gracilaria tikvahiae* (RI) (Figure 7) and another presumably as *Gracilaria tikvahiae* (CT). Besides this taxon, UCONN also established cultures of two other strains of *G. vermiculophylla* (from CT) and one tentatively identified as *Gracilariopsis longissima* (all these have pending molecular confirmations of their identification). The isolation of these cultures was performed according to the method described by Sahoo and Yarish (2005) and Kawai *et al.* (2005).

For the species *G. vermiculophylla*, UCONN has in culture isolates of female gametophytes, male gametophytes and tetrasporophytes. Two strains of male and female gametophytes were selected as being the ones that presented faster growth rates during preliminary germination experiments (Abreu *et al.*, unpublished). These cultures were propagated vegetatively, in preparation for work planned under Subtask 2.1.3. More effort was put in the culture conditions of female gametophytes. Unlike other stages of the life cycle, cultures of female gametophytes ensure no loss of material due to formation and release of reproductive material. For this species, UCONN also has in culture at least two other strains of *Gracilaria vermiculophylla* isolated from non-reproductive material from Long Island Sound.

For the species *G. cervicornis*, UCONN has in culture a vegetative strain originally isolated in Florida (Capo *et al.* 1999). This culture was treated to eliminate algal epiphytes and was subjected to positive taxonomic confirmation using DNA technologies.

For the species *Gracilaria pacifica*, UCONN has in culture material collected by the partners at UCSD-SIO. For the species presumably identified as *G. tikvahiae*, UCONN has in culture material collected in Potters Pond, South Kingston, RI, by the members of the UCONN team and one other isolate from Holly Pond, Stamford, CT. Finally, UCONN intends to establish a unialgal culture of a strain tentatively identified as *Gracilariopsis longissima* (Seaside Beach, Bridgeport, CT)

All *Gracilaria* cultures are being maintained in von Stosch's Enriched Seawater medium (VSE), in environmental chambers with temperature, photoperiod and light control (Ott, 1965; Carmona *et al.* 2006). In all environmental chambers, illumination is provided by cool white, high-output, linear fluorescent bulbs (F48T12CW-HO). Cultures of *G. vermiculophylla* are presently

maintained at 10°, 15°, and 20°C, under photon flux densities between 30-40 µmol photons m^{-2} s^{-1}. Cultures of G. cervicornis are presently maintained at 10°, 15°, 20° and 25°C, under photon flux densities between 30-40 µmol photons m^{-2} s^{-1}. Cultures of *G. tikvahiae* are maintained at 10° and 15°C under 30 µmol photons m^{-2} s^{-1}. Cultures of *Gracilariopsis* are presently maintained at 15°C and 30 µmol photons m^{-2} s^{-1}.

Cultures selected for propagation and biomass production are gently aerated under 15°, 20°, and 25°C, day neutral photoperiod (12:12, L:D) and approximately 60-80 µmol photons m^{-2} s^{-1} Figure 8. Detail of Culture Units Used for Biomass Production of *Gracilaria*(Figure 8). For the cultures of *G. pacifica*, *G. cervicornis*, *G. vermiculophylla* and *G. tikvahiae* there are also some culture units under long-day conditions (16:8, L:D) and similar photon flux densities.

Using UCONN methods, UCSD-SIO will clean up and batch culture several species of *Gracilaria* with the intention of using it for future optimization experiments.

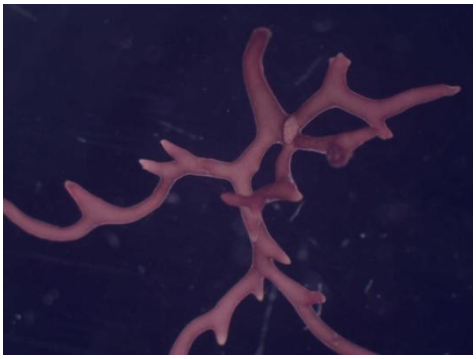

Figure 2. *Gracilaria cervicornis* in Culture

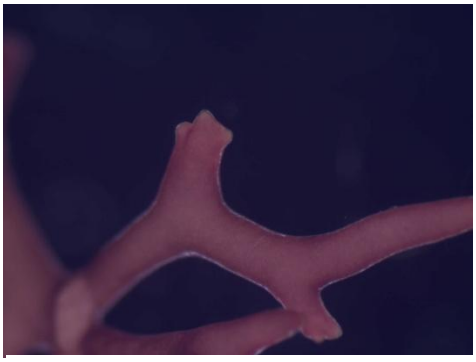

Figure 3. *Gracilaria cervicornis*, Branch Formation

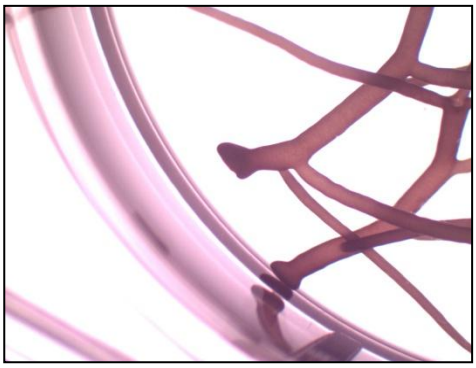

Figure 4. *Gracilaria vermiculophylla*, Branch Formation

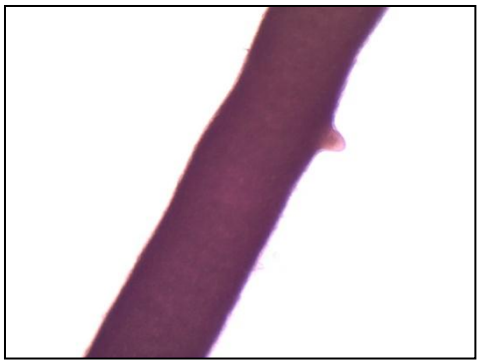

Figure 5. *Gracilaria vermiculophylla* in Culture

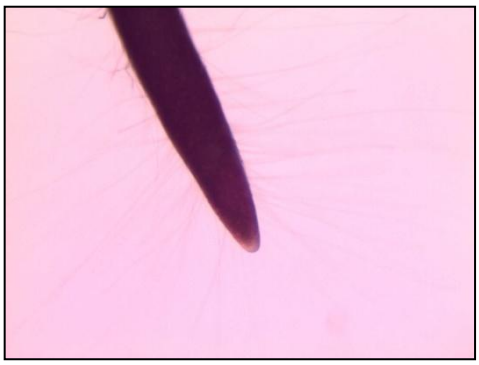

Figure 6. *Gracilaria vermiculophylla* in Culture, Apical Area with "Hair-Like" Cell Formation

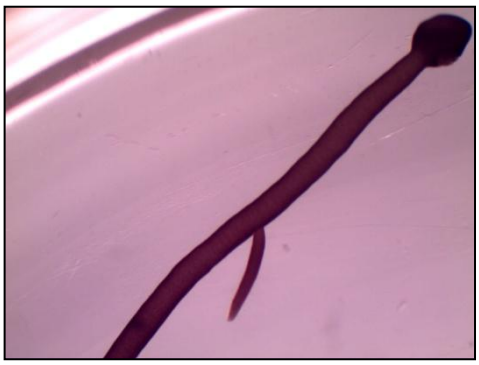

Figure 7. *Gracilaria tikvahiae* in Culture, Detail of Branch Formation and Healing Area

Figure 8. Detail of Culture Units Used for Biomass Production of *Gracilaria* in the Laboratory (From left to right, strains of *G. vermiculophylla, G. pacifica* and *G. cervicorni)*

Several of the other taxa recommended for testing were also isolated. UCONN has isolates of *Porphyra umbilicalis* and *P. dioica*, as well as capabilities to isolate and select new strains of *P. perforata* from the west coast of North America. While *P. perforata* is known to have high temperature tolerance, *P. dioica* has the advantage of possible vegetative propagation, as described by Pereira *et al.* (2006).

As for the remaining taxa recommended, UCONN, with the assistance of UCSD-SIO have a network of experts, that have the capabilities to isolate and select strains of other species including *Gracilaria pacifica*, *Solieria filiformis (=tenera)*; *Agardhiella subulata, Ulva expansa, U. rigida, U. clathrata*. These taxa have upper survival temperatures greater than 25°C (Yarish *et al.*, 1987; Lüning and Freshwater, 1988; Garza-Sánchez *et al.*, 2000).

UCONN has worked on a review of the environmental parameters that will be critical for future follow-up studies. The main environmental conditions that need to be tested in order to select strains for any future work are temperature, pH or CO_2 availability, nutrients, light, salinity, desiccation, and epiphytism.

Experimental protocols have been designed to compare temperature tolerances and temperature optimum for the *Gracilaria* species. These experiments will be followed by others that will investigate the combined effects of light (photosynthetic photon flux density) and nutrients under selected temperatures. For the light factor, particular attention will be given to the minimum light requirements to maximize growth and nutrient uptake. For the nutrient factor, particular attention will be given to the uptake capacity of nitrate, ammonium, phosphate as well as to the effects of CO_2 enrichment. For desiccation experiments in future studies, we will follow the protocols outlined by Kim *et al.* (2009) and Kim and Yarish (2010).

Testing of Vertical Substrates with Gracilaria on Test Mesh Panels

UCSD-SIO conducted tests with different substrate materials to determine how well the fragments of seaweed (*Gracilaria*) could be retained. Given favorable results, the substrate materials could be candidates for the vertical support structures. In their experiments, at least 50% of seaweed fragments were retained on all substrates once they were set to a vertical position. Polyester netting stretched on PVC frames each about 13 cm by 15 cm (5 by 6 inches) square retained 81 to 85% (Figure 9) of the fragments.

UCSD will test other substrate materials and configurations in Phase 2. They will also plan to test down-scaled spray-culture module utilizing 12-each miniature spray tanks (Figure 10). In these spray-culture modules, the saltwater spray flow rate will be about 0.1 gallon per minute.

Figure 9. Test Panels of Polyester Netting and Plastic Aquaculture Mesh with Pieces of *Gracilaria pacifica* Entangled into Mesh

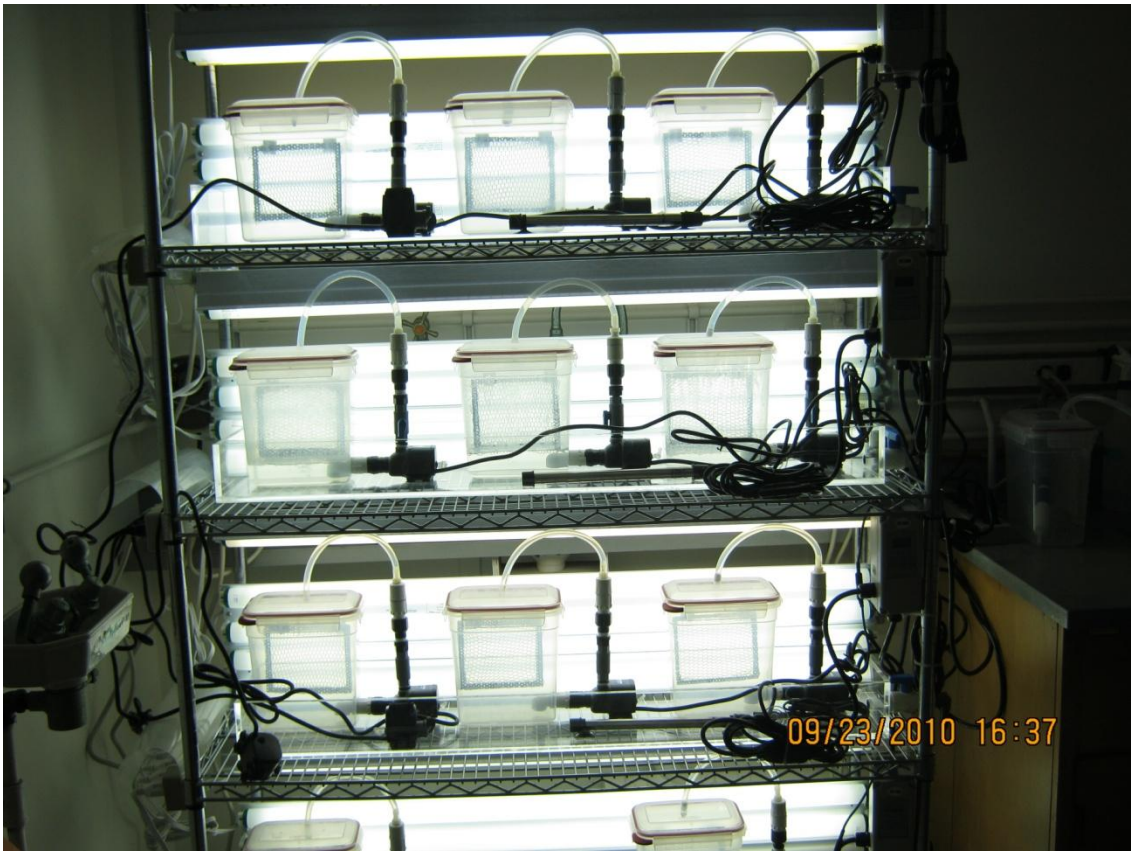

Figure 10. Twelve-Place, 4-Tier Mini-Spray Culture System (UCSD-SIO)

Gracilaria Species Comparison and Optimization using Mini Spray Culture System

Experimental Objectives

The objective was to perform a comparative analysis of performance of different *Gracilaria* species/strains under greenhouse-analog conditions in the laboratory (UCSD-SIO and UCONN). Results will establish the baseline for Phase 2 strain optimization and selection experiments.

Experimental Approach

Thalli from the chosen experimental species / batches are fragmented and then attached to net frame replicates within 3.8-L plastic containers with attached spray nozzles and re-circulating media. Each fragment was mapped to species and tracked.

Conditions are maintained at optimal temperature (20°-24°C), initially at low irradiance levels, e.g., 50 µmol photon m^{-2} s^{-1}, which is set using multiple layers of plastic window screen between the bank of fluorescent lights and the mini-spray tanks. The nutrient seawater-based media is full strength von Stosch's Enriched Seawater medium (VSE).

Replicates are weighed every 2 to 3 days to assess biomass yield, including any pieces that may become caught-up in the in-line filter screens.

Individual fragments were scored visually for health and relative proliferation over 2 weeks in order to compare the suitability of species for spray culture.

Species Optimization for Survival and Proliferation

Experimental Objectives

For each candidate strain the range of temperature, salinity, pH, nutrients, irradiance, CO_2 availability, desiccation and epiphytism that allow (a) survival and (b) optimal proliferation was dtermined.

Initial experiments determined the upper limit temperature tolerance for the different species/strains isolated at UCONN during Phase 1 (Table 1) using a temperature controlled gradient table already fabricated at UCONN (Figure 11). Each plate of the gradient table can hold up to 64 Petri dishes (60 x 10 mm). The different replicates are randomly distributed as shown.

Experimental approach

- Determine the upper lethal temperature for the following *Gracilaria* species / strains:
    - *Gracilaria vermiculophylla* [female strain, Portugal (PT)]
    - *Gracilaria vermiculophylla* (CT)
    - *Gracilaria tikvahiae* (RI)
    - *Gracilaria cervicornis* (FL)
    - *Gracilaria pacifica* (CA)
- Temperatures tested: 26° to 33°C in 1°C increments
- Photon flux density: 100 µmol photons m^{-2} s^{-1}
- Culture medium used: VSE renewed every 4 days, using the same batch of seawater with the VSE throughout the whole experiment
- Culture units: Small Petri dishes (60 x 10 mm)
- Biomass: 3 apices per Petri dish (0.3±0.1 mm length)
- Replicates: 4 Petri dishes per temperature
- Observations and digital imaging at days 0, 1, 3, 6, 9, 12 and 15.

For each species/strain, the biological material to be used was acclimated beforehand at the lowest temperature of interest (26°C). At least 15 apices (cut tips of thalli) from each species/strain will be selected, cleaned with cotton swabs and dragged through seawater agar. The apices selected and excised were 0.3±0.1 cm in length and no signs of ramifications. These apices were left to heal for at least 3 days prior to the beginning of the experiment. At day 0, groups of 3 apices were selected randomly from the 15 prepared for each species/strain and distributed into each Petri dish. A digital image was captured for every sampling day and the

area of *Gracilaria* determined with the appropriate software. Growth was determined by photographing the apical fragments with a "pixeLINK" digital camera, under the dissection microscope and use the software to determine area and, consequently, growth. Photos were taken at day 0, day 1, 3, 6, 9, 12, 15.

Table 1. Experimental Design for Temperature Tolerance Experiments at UCONN

Species	Samples per Temperature [C]								Total Samples
	26	27	28	29	30	31	32	33	
G. vermiculphylla - strain PT	4	4	4	4	4	4	4	4	32
G. vermiculophylla - strain CT	4	4	4	4	4	4	4	4	32
G. tikvahiae - strain RI	4	4	4	4	4	4	4	4	32
G. cervicornis - strain FL	4	4	4	4	4	4	4	4	32
G. pacifica - strain CA	4	4	4	4	4	4	4	4	32
TOTAL									**160**

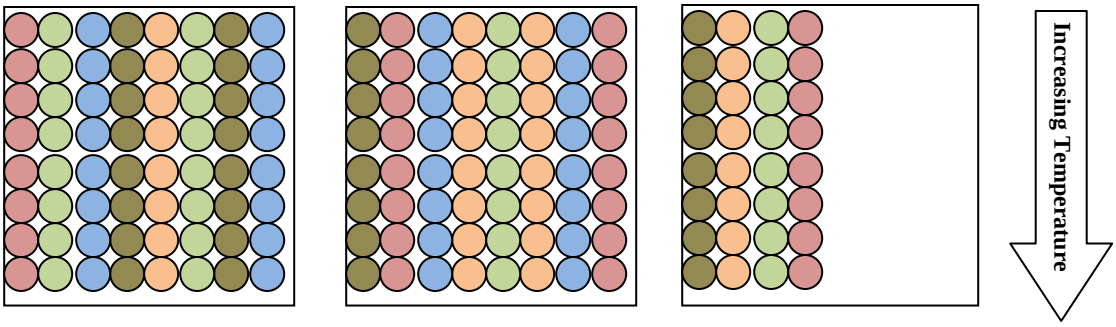

Figure 11. UCONN Temperature Gradient Table with 64 Petri Dishes per Plate (One species per gradient line)

Results

Figure 12 presents the results from the temperature tolerance experiments. In general, the three species of *Gracilaria* tested presented a considerable thermal tolerance up to 34°C. All the species presented survival rates between 0 and 10% after 1 day of exposure to 39°C. A similar result was observed at 36°C, except that in this case *Gracilaria vermiculophylla* showed resistance during some time.

High thermal tolerance for the 3 species tested was observed. *Gracilaria vermiculophylla* is clearly the best prepared for survival and growth at these range of temperatures. It had higher survival rates at 34°C and capacity to resist a 2 day exposure to 36°C. It also had higher growth rates of all the species tested between 22 and 32ºC.

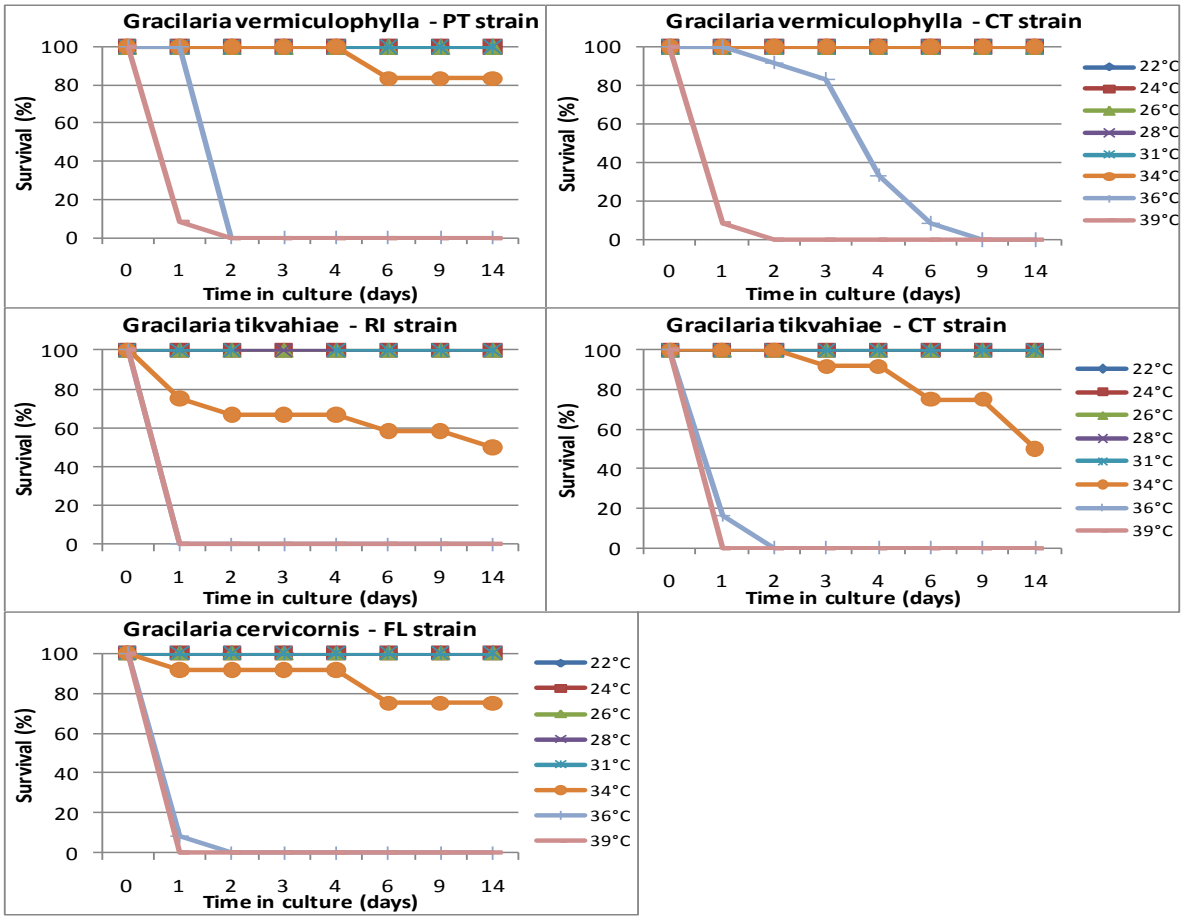

Figure 12. Results from Temperature Tolerance Experiment

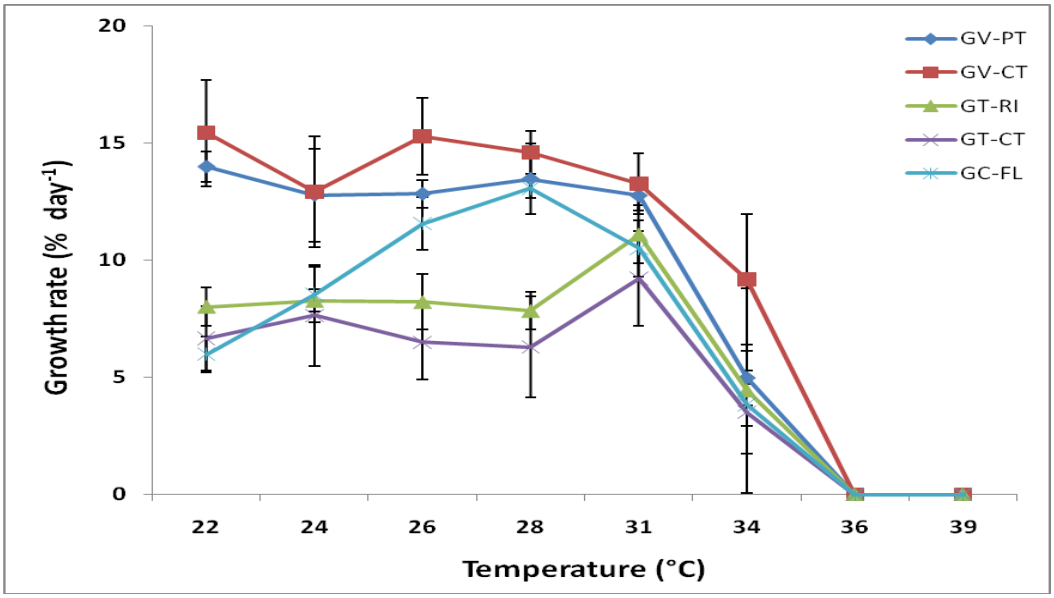

Figure 13. Comparison of Growth Rate of Gracilaria Species

Optimization of Propagation Process for Gracilaria

Experimental Objectives
The objective of this task was to design biological processes for the propagation laboratory. This includes determining the minimum size of fragments that are required for optimal regeneration, optimal conditions for maximizing proliferation, etc.

Experimental Approach
The ability of *Gracilaria* spp. to regenerate on vertical matrices by testing a range of fragment sizes on net frames was evaluated. The optimal size and time for scale-up from milligram to gram to kilogram quantities by quantifying productivity was determined and biomass yield on vertical frames was compared between species. This work was to be continued in Phase 2.

Evaluation of Selected Gracilaria Species Under Spray-Culture Conditions

Experimental Objectives
The objective was to test survival of *Gracilaria* spp. under spray conditions while testing other environmental parameters relevant to the Phase 2 scale-up system.

Experimental Approach
Subject thalli from two candidate *Gracilaria* species was transferred to spray conditions in laboratory-scale "mini-spray" apparatus.
Under culture spray conditions, UCSD-SIO performed a series of experiments each testing one pair of the following environmental parameters simultaneously: Temperature from 21° to 35°C, irradiance of 50 µmol photon m^{-2} s^{-1} after initial acclimation (3-5 days) to spray modules, and spray flow rate of low/high, and intermittent/continuous. Optimal conditions of nutrients (full strength VSE seawater medium), salinity of 30 ppt, and pH of 8 will be maintained.

Every 2 to 3 days netting replicates with seaweed thalli was weighed to assess biomass yield and individual fragments were scored visually for health.

Methods and Apparatus
For the laboratory-scale spray culture experiments, the USCD-SIO team constructed a 12-place "mini"-spray culture system utilizing 3.8-L cap Sterilite translucent containers (see Figure 10). The system comprises 4 temperature-controlled clear acrylic water baths placed on one of four metal-wire shelves. Each shelf is lit by three horizontally arranged, dual 4-foot long fluorescent bulb light fixtures (6 bulbs total per shelf; Philips F40T12/DX, 6500 K cool white 84 CRI). Measured full light levels inside each of the containers range from 200-205 µmol-photons m^{-2} s^{-1}.

Each water bath accommodates three 3.8-L mini-spray modules placed side-by-side. Each container accommodates one vertically oriented, 13 cm x 15 cm (5 x 6 inch) net substrate (1 cm lightweight polyester seine netting) for supporting an experimental quantity of seaweed thalli. Each of the culture modules is fitted with a single, centrally mounted interior mini-spray nozzle attached to flexible silicone tubing in line with a small, external aquarium water pump. Also in-line is a flow-control valve and a mini-strainer basket to capture any small pieces of seaweed thalli that break loose from the net substrate from clogging the spray nozzle. In operation, the

module is filled with approximately 600 ml of VSE seawater medium. The pump continuously circulates the media producing a gentle spray over the seaweed thalli.

UCSD-SIO designed, fabricated and assembled and wet-tested the mini-spray module system in their laboratories. The 12-module system accommodates the testing of two parameters simultaneously under spray culture conditions (e.g., temperature and irradiance).

UCSD-SIO received various batches of *Gracilaria* spp. from UCONN (*G. vermiculophylla*, *G. cervicornis*, and *G. tikvahiae)*, and evaluated them for suitability in the mini-spray culture apparatus. Based on its morphological attributes and range of temperature tolerance (as determined in the UCONN tests), it was decided to test the very hardy and high-temperature tolerant *G. vermiculophylla* strain from the CT collection site. The southern California-collected strain *G. pacifica* was also selected for testing, given that it is endemic within the same bio-geographical zone as is the site chosen for the proposed Phase 2 pilot-scale deployment of the MADRE technology.

Anaerobic Biogasification Potential Assay Results of selected Macroalgae Species

The objective of this task was to evaluate biodegradability and methane yield of three macroalgae species using ABP (anaerobic biogasification potential) assays. Since compositional characteristics of the feedstock significantly affect the biodegradability, the results from the ABP assays will help with strain selection and optimization program (being conducted by UCSD-SIO and UCONN) for selecting appropriate macroalgae traits for high conversion efficiency in the anaerobic digestion process. The results served as a basis for design, construction, and operation of a pilot unit.

Seed Bioreactor Operation

Reactor Inoculum

The inoculum seed for the laboratory-scale reactor was digested effluent sludge from the Methanogenic Phase digester from the DuPage County Woodridge-Greene Valley Wastewater Treatment Plant, which is operated at a 15-day hydraulic retention time (HRT) with a loading rate of 0.1 lb VS/ft^3-day (1.6 g VS/L-day).

Reactor Feedstock

Waste Activated Sludge (WAS) was obtained from the DuPage County Woodridge-Greene Valley Wastewater Treatment Plant. According to the treatment plant, the feed WAS contains 6 to 7% total solids (TS) or 60-70 g/L of which 80% is VS. Using g/L, the VS content in the WAS is 48-56 g/L.

The reactor was fed at a loading rate of 0.025 lb VS/ft^3-day (or 0.4 g VS/L-day) beginning on Friday, January 29, 2010. At a culture volume of 2.5 L, this loading rate is equivalent to 1.0 g VS/day for the seed reactor. The reactor was fed three times a week after removing an equivalent volume of culture from the reactor effluent port.

Seed Bioreactor Start-up and Loading Rate

The 5-L stirred tank reactor (STR, 2.5-L culture volume) is equipped with feeding and wasting ports, temperature control (mesophilic, 35°C), continuous mixing, and an American® Wet Test Meter (Model AL-17) for measuring gas production, gas temperature, and pressure.

Before inoculation, the STR was pressure-tested for leaks at 3 psig overnight. When no leaks were detected, the head-space in the digester was filled with helium gas, and, under continuous helium outgassing, 2.5 L of fresh inoculum (methanogenic phase effluent sludge) was pumped into the reactor. The reactor head space was thoroughly outgassed with helium to ensure the displacement of air from the system. The temperature of the reactor was set at 35°C and was controlled using a BioFlo110 Fermentor/Bioreactor (New Brunswick Scientific). The reactor was started on Wednesday, January 27, 2010 in GTI's Microbiology Lab. The reactor was fed with WAS and wasted semicontinuously three times a week with a loading rate of 0.025 lb VS/ft^3-day or 0.4 g VS/L-day.

The effluent of the seed reactor was used to set up ABP assays for various seaweed species obtained from UCSD-SIO.

Feedstock and Effluent Analysis

Feedstock (WAS) and reactor effluent were analyzed for the purposes of experimental set-up and material balance calculations. The feedstock and effluent samples were analyzed for total moisture, ash, C, H, N, S, P, O (by difference), gross calorific value, pH, alkalinity, total solids (TS), volatile solids (VS), ammonia, chemical oxygen demand (COD), and volatile fatty acids (VFA's).

The compositional results of the WAS feedstock are summarized in Table **2**. The initial WAS analysis showed a VS content of 5.15% (or 51.5 g/L), which was close to the wastewater treatment plant value. However, periodic analysis of the WAS stored in a 20-L plastic container at 4°C indicated that the VS content declined to 4.32% after 14 days in storage probably due to slow settling. The whole content of WAS in a 20-L jar was completely mixed and sampled at Day 43, and stored at -20°C until use. The VS content of the sample was 2.91%. The feeding volume of WAS to the seed bioreactor was calculated and adjusted accordingly over time based on the VS content to maintain a loading rate of 0.025 lb VS/ft^3-day.

Table 2. Results of Analyses of WAS Feedstock for Seed Digester

Date	2/22/2010	3/8/2010 (4C)	3/8/2010 (-20 C)	4/6/2010
Sample Log#	101089-001	101165-002	101165-003	101248-001
Total Moisture, wt %, as received	94.18	95.00	94.90	-
Ash (550°C), wt %, dry basis	18.99	19.45	18.76	-
Ash (550°C), wt %, dry basis, SO_3 corr.	18.54	18.90	18.20	-
Carbon, wt %, dry basis	46.46	47.33	45.76	-
Hydrogen, wt %, dry basis	6.17	6.14	6.19	-
Nitrogen, wt %, dry basis	6.49	6.65	6.24	-
Sulfur, wt %, dry basis	0.62	0.53	0.60	-
Heating Value, BTU/lb, dry basis	8850	9170	8600	-
pH, as received	7.00	8.00	6.00	-
Total Solids, wt %, as received	6.62	5.37	5.68	3.6
Volatile Solids, wt %, dry basis	77.82	80.58	82.33	80.94
Volatile Solid (VS), %	5.15	4.32	4.68	2.91
Ammonia, wt %, dry basis	1.80	2.40	1.60	-
Chemical Oxygen Demand, mg/g, dry basis	1600	150	150	-
Phosphorus, wt %, dry basis	1.55	1.41	1.34	-
Alkalinity, wt %, dry basis	4.90	5.60	4.30	-
Oxygen, wt %, dry basis	21.27	19.90	22.45	-
Oxygen, wt %, dry basis, SO_3 corr.	21.72	20.45	23.01	
VFA, mM, as received	74.46	65.00	51.00	-
Acetic	41.41	35.44	30.18	-
Propionic	14.64	13.62	11.12	-
Isobutyric	2.63	2.45	1.40	-
Butyric	9.39	8.71	5.30	-
Isovaleric	3.84	3.29	2.09	-
Valeric	0.94	0.75	0.51	-
Iso-caproic	0.82	0.47	0.29	-
Caproic	BDL	BDL	BDL	-
Heptanoic	0.78	BDL	BDL	-
BDL: Below Detection Limit				
-: not analyzed				

The compositional results of the seed reactor effluent samples are summarized in Table **3**. The effluent composition indicated the increase of water content and a decrease in VS content over time. The moisture increased from 97.2% on February 8, 2010 to 98.5% on March 3, 2010, and VS content dropped from 1.83% to 0.61% during the same period. The period corresponded to the slow deterioration of reactor performance because less WAS VS was actually added to the reactor every week due to the slow precipitation of WAS solids and/or degradation during storage (4°C). Therefore, the feeding volume of WAS was adjusted several times over the experimental period according to the VS content data (Table **2**) to maintain a loading rate of 0.025 lb VS/ft^3-day.

Table 3. Results of Analyses of Seed Reactor Effluent

Date	2/8/2010	2/15/2010	3/8/2010	3/12/2010	4/7/2010
Sample Log#	101089-002	101104-001	101165-001	101175-002	101248-002
Days of operation	Day 13	Day 20#	Day 41	Day 45#*	Day 71
Total Moisture, wt %, as received	97.23	97.90	98.90	98.50	-
Ash (550°C), wt %, dry basis	33.49	33.70	32.62	32.77	-
Ash (550°C), wt %, dry basis, SO_3 corr.	32.09	32.60	31.80	31.70	-
Carbon, wt %, dry basis	36.98	36.50	36.84	36.82	-
Hydrogen, wt %, dry basis	4.92	5.10	5.00	4.98	-
Nitrogen, wt %, dry basis	6.20	6.00	5.80	5.71	-
Sulfur, wt %, dry basis	1.17	1.30	1.05	1.15	-
Heating Value, BTU/lb, dry basis	7070	7020	6970	7020	-
pH, as received	8.00	9.00	8.00	8.00	-
Total Solids, wt %, as received	2.71	2.70	1.30	1.22	1.2
Volatile Solids, wt %, dry basis	67.59	68.90	68.17	49.58	87.025
Volatile Solid (VS), %	1.83	1.86	0.89	0.61	1.044
Ammonia, wt %, dry basis	8.90	12.00	18.00	13.00	-
Chemical Oxygen Demand, mg/g, dry basis	1100	1100	1180	930	-
Phosphorus, wt %, dry basis	3.55	4.19	3.58	2.95	-
Alkalinity, wt %, dry basis	30.00	35.00	65.00	46.00	-
Oxygen, wt %, dry basis	17.24	17.40	18.69	18.57	-
Oxygen, wt %, dry basis, SO_3 corr.	18.64	18.50	19.51	19.64	
VFA, mM, as received	17.17	20.12	0.93	0.81	-
Acetic	13.90	16.56	0.90	0.73	-
Propionic	2.99	3.16	0.01	0.03	-
Isobutyric	0.08	0.10	0.01	BDL	-
Butyric	0.03	0.04	BDL	BDL	-
Isovaleric	0.10	0.17	BDL	0.03	-
Valeric	0.03	0.04	0.01	0.01	-
Iso-caproic	0.03	0.05	BDL	BDL	-
Caproic	BDL	BDL	BDL	BDL	-
Heptanoic	BDL	BDL	0.00	BDL	-
BDL: Below Detection Limit					
#: Used to prepare ABP tests for *Ulva* and *Sargassum* (#1), 200 mg of VS were added to each ABP bottle.					
#*: Used to prepare ABP tests for *Gracilaria* (#2), 200 mg of VS were added to each ABP bottle.					
-: not analyzed					

Gas Monitoring and Analysis

Gas production, temperature, and atmospheric pressure were monitored with a wet test meter and recorded daily (Monday through Friday). The reactor temperature was controlled and monitored using a BioFlo110 Fermentor/Bioreactor temperature probe and heating tape. The pH of the effluent was measured on feeding days (3 times a week). During the experiment, the effluent pH was maintained in the range of 7.5 to 8.0. Primary product gas constituents (CO_2, N_2, and CH_4) were analyzed periodically by gas chromatography (GC).

The major components of raw gas from the seed reactor are summarized in Table 4, and the daily methane production from the seed reactor is in Figure 14.

Table 4. Major Components of Raw Gas from Seed Reactor

Date	Day	CO_2, vol %	CH_4, vol %
2/15/2010	20	37.1	62.9
3/8/2010	41	35.8	64.2

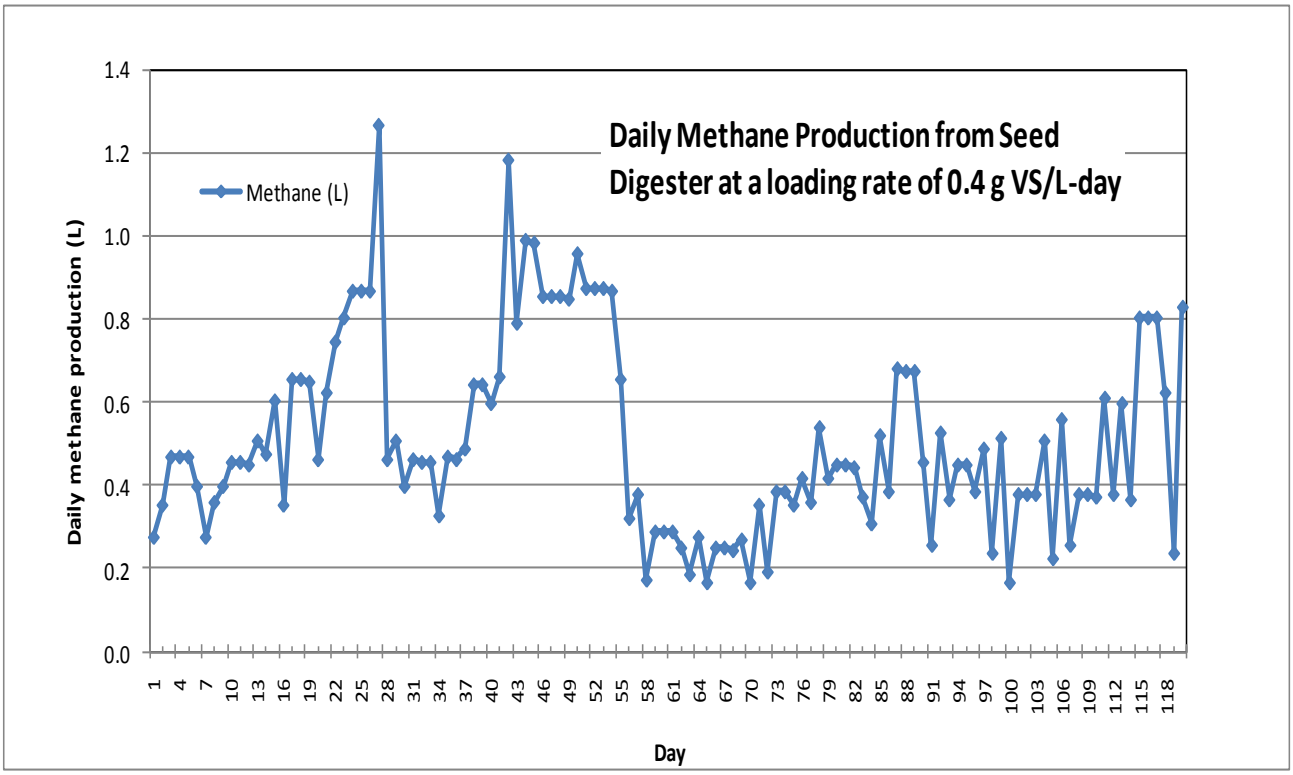

Figure 14. Daily Methane Production from Seed Digester
(Loading Rate of 0.4 g VS/L-day)

Growth Medium for ABP Assay

The ABP assay was performed in a defined nutrient growth medium (Table 5) to ensure an adequate supply of macro- and micronutrients and growth factors for proliferation of the organisms catabolizing the feedstock under study, and to minimize nutritional variability when screening the biodegradability of candidate biomass species.

Table 5. ABP Assay Growth Medium

Macronutrients	Gram / L
$(NH_4)_2HPO_4$	0.072
NH_4Cl	0.36
KCl	1.17
$MgCl_2 \cdot 6H_2O$	1.62
$CaCl_2 \cdot 2H_2O$	0.225
$FeCl_2 \cdot 4H_2O$	0.333

100X Trace Elements stock (add 10 ml to 1 L)	Milligram / 100 mL
$MnCl_2 \cdot 4H_2O$	180
$CoCl_2 \cdot 6H_2O$	270
H_3BO_3	50
$CuCl_2 \cdot 2H_2O$	24
$NaMoO_4 \cdot 2H_2O$	23
$ZnCl_2$	19

100X Vitamins stock (add 10 ml to 1 L)	Milligram / 100 mL
Biotin	0.2
Folic Acid	0.2
Pyridoxine Hydrochloride	0.9
Riboflavin	0.45
Thiamin	0.45
Nicotinic Acid	0.45
Pantothenic Acid	0.45
Vitamin B_{12}	0.009
p-Aminobenzoic Acid	0.45
Thioctic Acid	0.45

10X Reducing Solution stock (add 1 ml to 1 L)	Gram / 10 mL
Sodium Thioglycolate	1
Sodium Ascorbate	1
Distilled water	10 mL
Filter sterilize	
Distribute aliquots into sealed, N_2 purged, and autoclaved bottles	
Store at 4°C	

Substrates for ABP Assays

The purpose of the ABP assays is to determine the anaerobic biogasification potential of seaweed candidates. The first two species of marine seaweeds (*Ulva* and *Sargassum muticum*) were received from UCSD-SIO on February 10, 2010 that had been collected on February 9, 2010. *Ulva* was cut and homogenized with a blender to a paste-like material; *S. muticum* was cut to one inch long pieces and homogenized after adding 20% de-ionized water. However, the homogenization process failed to product the desired paste-like consistency of *Sargassum*. The blended *Sargassum* was further cut and mixed manually to generate relatively uniform feed material (up to 5 mm long). The third marine seaweed (*Gracilaria*) was received from UCSD-SIO on March 12, 2010. The *Gracilaria* was cut to 0.2-0.5 inch long pieces and crushed. However, the end product of this action with *Gracilaria* was not the desired paste-like material. Relatively uniform *Gracilaria* material was used in the ABP assay. Aliquots of blended seaweed materials were frozen and stored at -20°C until used. One fresh aliquot was analyzed for the

purpose of determining digester loadings, predicting potential nutrient requirements, and calculating theoretical yields.

In addition, a sample of AVICEL cellulose was also subjected to the ABP. Previous studies at GTI with microcrystalline cellulose (AVICEL) as a feedstock showed very consistent methane yield. Therefore AVICEL is used as a positive control to assess the variability of the ABP assay due to potential system perturbations from inoculum handling and incubation conditions. An average methane yield of 6.66 SCF/lb (0.42 L/g) VS added was achieved for cellulose in previous GTI studies compared to a theoretical yield of 7.0 SCF /lb (0.44 L/g) VS added.

Set-up of ABP Assays

The effluent from the seed reactor was used as inoculum for ABP assays of various seaweed species. The ABP assay protocol and preparation procedures are outlined in Figure **15** and Figure 16. The ABP assays for *Ulva* and *Sargassum* were set up on February 16, 2010 and for *Gracilaria* on March 18, 2010.

After the growth medium was purged with helium gas that had been passed through a heated, reduced copper column to remove oxygen, the inoculum (i.e., effluent) from the seed bioreactor was anaerobically transferred to purged growth medium resulting in a diluted inoculum to contain approximately 200 mg of inoculum VS in 100 ml. For the *Ulva* and *Sargassum* ABP assays, Day 20 effluent from the seed reactor (Table **3**) was used for preparation of diluted inoculum – the effluent was diluted 1:9.3 with growth medium to make diluted "medium+inoculum" containing 2.0 g of VS/L. For the *Gracilaria* ABP assay, Day 45 effluent from the seed reactor (Table **3**) was used for the preparation of diluted inoculums (1:3.05 dilution with growth medium). A sample of the diluted inoculum was retained for solids analyses.

The blended seaweed substrates were weighed and transferred to 250-mL Wheaton serum bottles (in triplicate) to provide 200 mg of seaweed VS in each bottle. The bottles were then purged with helium gas and sealed.

An aliquot (100-mL) of diluted inoculum was transferred to the serum bottle containing the seaweed substrates in an anaerobic glove box. This resulted in a 1:1 ratio of feed to inoculum VS content. Finally the sealed serum bottles were purged with helium gas through the sampling port, and the head space was filled with helium gas at atmospheric pressure. The bottles were incubated at 35°C in an inverted position to minimize gas leaks.

The ABP assays were conducted for 60 days to ensure complete biodegradation of the organic substrate, thereby providing an accurate assessment of ultimate biodegradability. The ultimate biodegradability of the substrate is determined by two methods, methane yield and VS reduction. Methane yields are calculated from the mineralization of the substrate to methane and carbon dioxide measured during the incubation period. VS reductions are calculated based on the non-biodegradable substrate remaining at the end of the incubation period. Methane yields are expressed as SCF/lb (or L/g) of VS added.

In addition, an inoculum negative control and an AVICEL cellulose positive control were prepared and incubated concurrently with the macroalgae feedstocks.

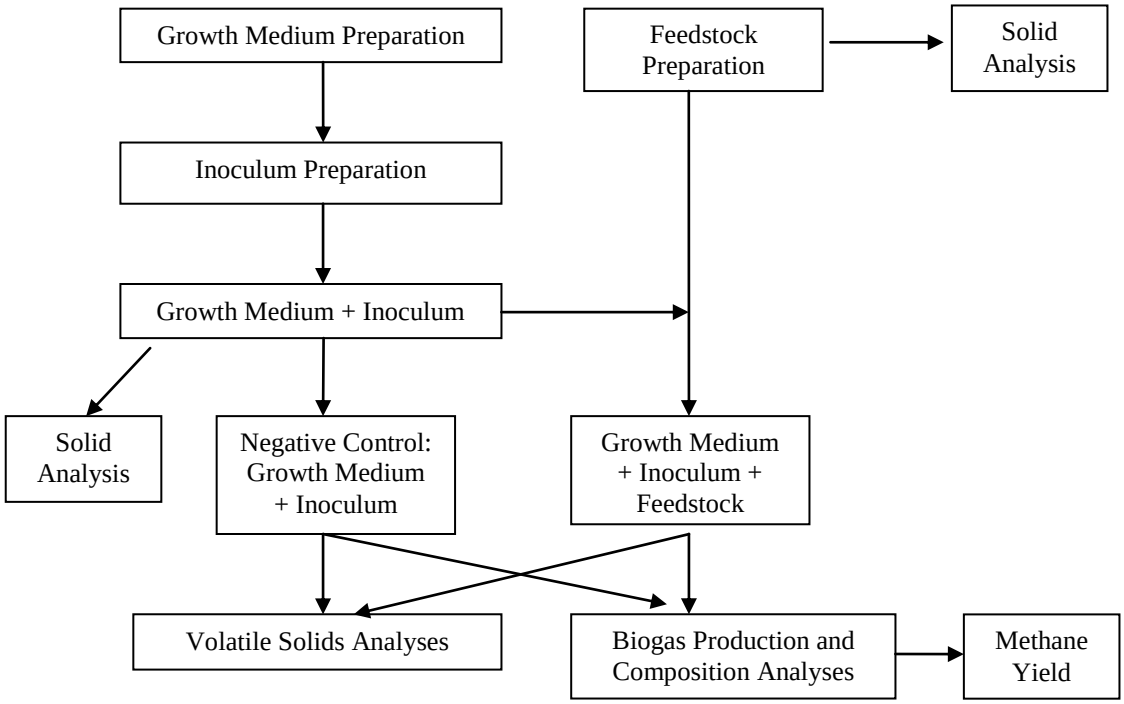

Figure 15. ABP Assay Protocol

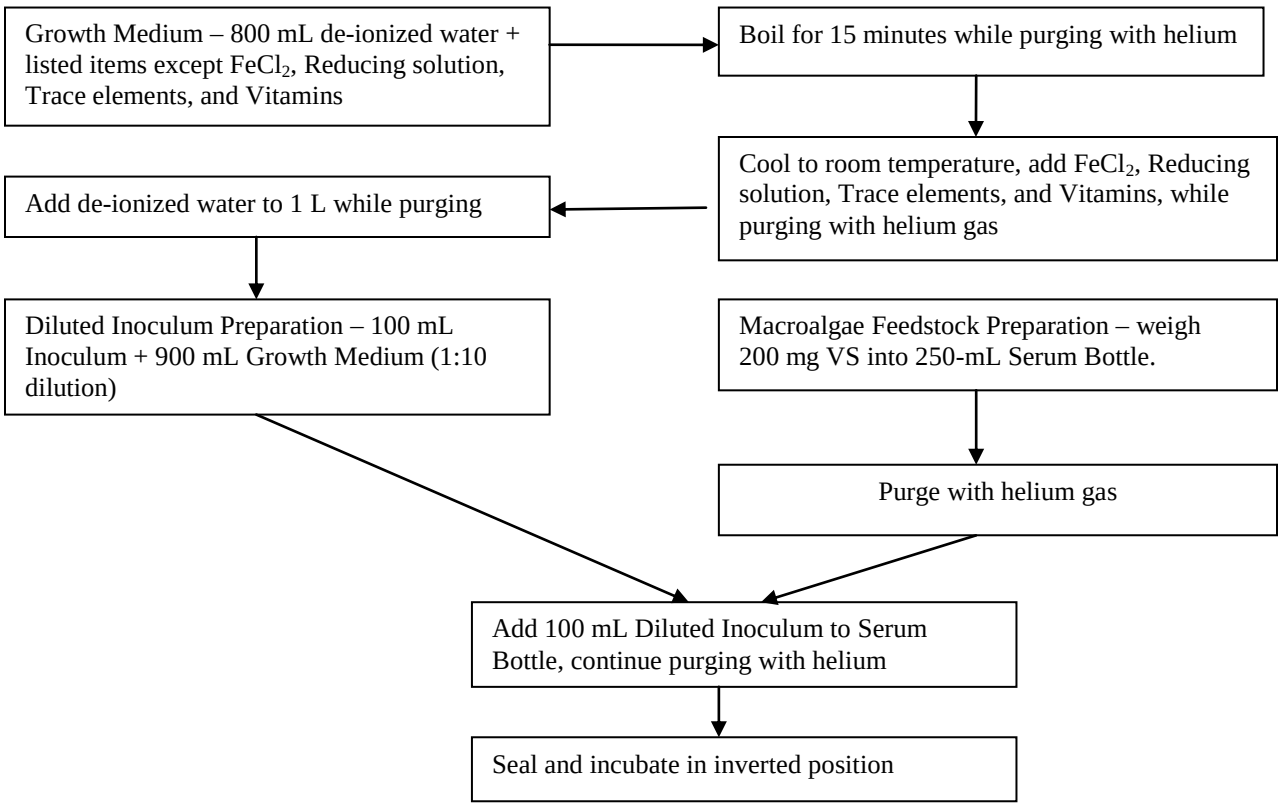

Figure 16. ABP Assay Procedure

Feedstock and Digestate Analysis

Feedstocks (seaweeds and cellulose) were analyzed for the purpose of experimental set-up, material balances, calculation of theoretical yields, estimation of nutrient availability for bioconversion, and determination of feed variability (if multiple feed lots are involved). Seaweed samples were analyzed for total moisture, ash, C, H, N, S, P, O (by difference), gross calorific value, pH, alkalinity, total solids (TS), volatile solids (VS), ammonia, chemical oxygen demand (COD), and volatile fatty acids (VFA's). These analyses enable the determination of methane yield, methane production rate, VS reductions, solids material balance, empirical carbohydrate formula, and stoichiometric methane yield. The digestate samples from each ABP assay bottle at the end of the 60-day incubation period were analyzed for TS and VS.

The analyses of seaweed species and cellulose are summarized in Table 6. The VS contents for *Ulva*, *Sargassum*, *Gracilaria*, and cellulose were 7.7%, 9.9% (after 20% dilution), 6.81%, and 99.91%, respectively. The data were used to calculate the quantity of each substrate needed to provide 200 mg of VS for each ABP assay bottle. The results of digestate samples from the ABP assays after 60 days of incubation are summarized in Table 7.

To evaluate the performance of each seaweed species and positive cellulose control during anaerobic digestion and to provide a basis for establishing target methane yields, we calculated the maximum theoretical methane yields (stoichiometric methane yield) for these seaweed species and cellulose. These calculations were based on the empirical formula of each species

determined from chemical analyses. Ignoring nitrogen and sulfur and assuming that the carbon, hydrogen, and oxygen in the feed reacted with water to form CO_2 and CH_4, the following equation was used:

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O = \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4$$

Using the above stoichiometric equation, C, H, and O content (Table 6) were used to determine the empirical formula of seaweed species and cellulose. Oxygen content was calculated by difference from the balance remaining from the sum of ash, C, H, N, and S. Due to the high sulfur content, the SO_3-corrected ash was used to derive the oxygen content in the seaweeds, and SO_3-corrected oxygen then was used for the determination of the empirical formula of the seaweed species.

Ulva	$C_6H_{10.02}O_{5.46} + 0.7686\ H_2O \rightarrow 3.1118\ CO_2 + 2.8882\ CH_4$
Sargassum	$C_6H_{8.95}O_{4.44} + 1.5416\ H_2O \rightarrow 2.9914\ CO_2 + 3.0086\ CH_4$
Gracilaria	$C_6H_{9.86}O_{3.39} + 1.8417\ H_2O \rightarrow 2.6145\ CO_2 + 3.3855\ CH_4$
Cellulose	$C_6H_{9.98}O_{5.00} + 1.0047\ H_2O \rightarrow 3.0033\ CO_2 + 2.9967\ CH_4$

Using the above empirical formulae, stoichiometric methane yields were calculated and are presented in Table 8. These yields represent the upper possible methane yield from these feeds without correction for bacterial synthesis or refractory components.

Table 6. Results of Analyses of Seaweed Species and Cellulose

Date	2/10/2010	2/10/2010	3/12/2010	3/12/2010
Sample Log#	101094-002	101094-001	101175-001	101175-003
Seaweed Species	***Ulva***	***Sargassum****	***Gracilaria***	***Cellulose***
Total Moisture, wt %, as received	88.79	85.60	90.30	3.94
Ash (550°C), wt %, dry basis	40.63	33.81	46.52	0.06
Ash (550°C), wt %, dry basis, SO_3 corr.	32.39	30.49	41.30	NA
Carbon, wt %, dry basis	26.67	31.37	27.46	44.38
Hydrogen, wt %, dry basis	3.71	3.90	3.76	6.15
Nitrogen, wt %, dry basis	2.21	2.26	3.62	0.07
Sulfur, wt %, dry basis	2.69	1.02	3.19	0.01
Heating Value, BTU/lb, dry basis	4,530	5,090	4930	7470
pH, as received	7.00	7.00	7.00	5
Total Solids, wt %, as received	12.20	15.06	12.34	NA
Volatile Solids, wt %, dry basis	63.14	66.20	55.18	99.91
Volatile Solid (VS), %	7.70	9.90	6.81	99.91
Ammonia, wt %, dry basis	0.24	0.27	0.80	NA
Chemical Oxygen Demand, mg/g, dry basis	800	1000	94	NA
Phosphorus, wt %, dry basis	0.21	0.36	0.55	< 0.001
Alkalinity, wt %, dry basis	0.60	0.75	< 1	NA
Oxygen, wt %, dry basis	24.09	27.64	15.40	49.33
Oxygen, wt %, dry basis, SO_3 corr.	32.33	30.96	20.67	NA

*Sargassum was diluted by 20% of distilled water during blending.

NA: not analyzed

Table 7. Results of TS and VS Analysis from ABP Assays After 60 Days of Incubation

	1st ABP setup			2nd ABP setup		
Sample ID	*Ulva*	*Sargassum*	Negative	*Gracilaria*	Cellulose	Negative
Sample Log#	101094-002	101094-001	101175-001	101326-1~3	101326-4~6	101326-7~9
TS, %	0.605	0.35	0.467	0.737	0.638	0.616
VS of TS, %	44.7	93.65	53.85	34.92	58.01	55.2
VS, %	0.254	0.341	0.233	0.256	0.366	0.393

Table 8. Stoichiometric Methane Yields Calculated from Empirical Formulae

	Ulva	***Sargassum***	***Gracilaria***	**Cellulose**
L/g VS	0.38	0.44	0.56	0.41
SCF/lb VS	6.12	7.10	8.93	6.64

Gas Monitoring and Analysis

Gas production and composition were monitored periodically throughout the incubation period of the ABP assay tests to provide data on the rate of substrate biodegradability. Gas production measurements for ABP bottles were performed using a glass syringe equipped with a 20-gauge needle. To conduct the gas production measurements, the ABP assay bottles were first equilibrated to room temperature. The glass sample syringe was lubricated with de-ionized water and flushed with helium gas prior to taking the gas reading. Gas volume determinations were made by allowing the pressure in the syringe plunger to equilibrate between the bottle and atmospheric pressure. The composite gas samples from triplicate bottles were analyzed for major gas components (CO_2, N_2, and CH_4) using a GC.

Cumulative methane production from each substrate was calculated after adjustment for raw gas volume with the negative control (inoculum and growth medium only) and methane content in the raw gas. The average methane contents of raw gas from ABP assays of *Ulva, Sargassum, Gracilaria*, and cellulose were 68.7, 70.1, 69.5, and 69.5 mol %, respectively. Finally, methane yields were calculated in terms of SCF/lb (or L/g) of VS added.

The stoichiometric methane yield for cellulose was calculated from the empirical formula to be 6.64 SCF/lb VS (Table 8), and the cumulative methane yield from the cellulose positive control was 6.76 SCF/lb VS after the 60-day incubation period at 30°C (Figure 17).

Stoichiometric methane yields calculated using the empirical formulae for *Ulva, Sargassum,* and *Gracilaria* were 6.12, 7.10, and 8.93 SCF/lb VS, respectively (Table 8). The cumulative methane yields from *Ulva, Sargassum*, and *Gracilaria* were 3.40, 3.55, and 5.26 SCF/lb VS after 60 days of anaerobic digestion, respectively (Figure 18). The VS conversions for *Ulva, Sargassum*, and *Gracilaria* were 55.6%, 49.9%, and 58.9%, respectively.

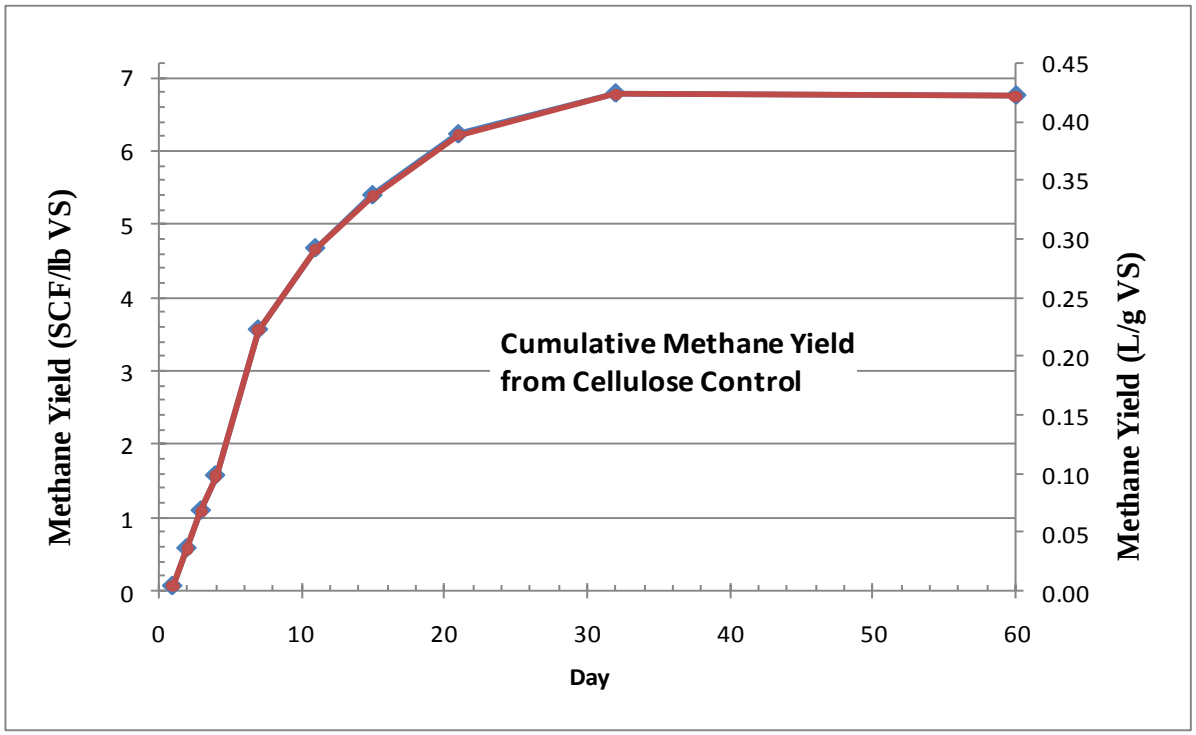

Figure 17. Cumulative Methane Yield from Anaerobic Digestion of AVICEL Cellulose

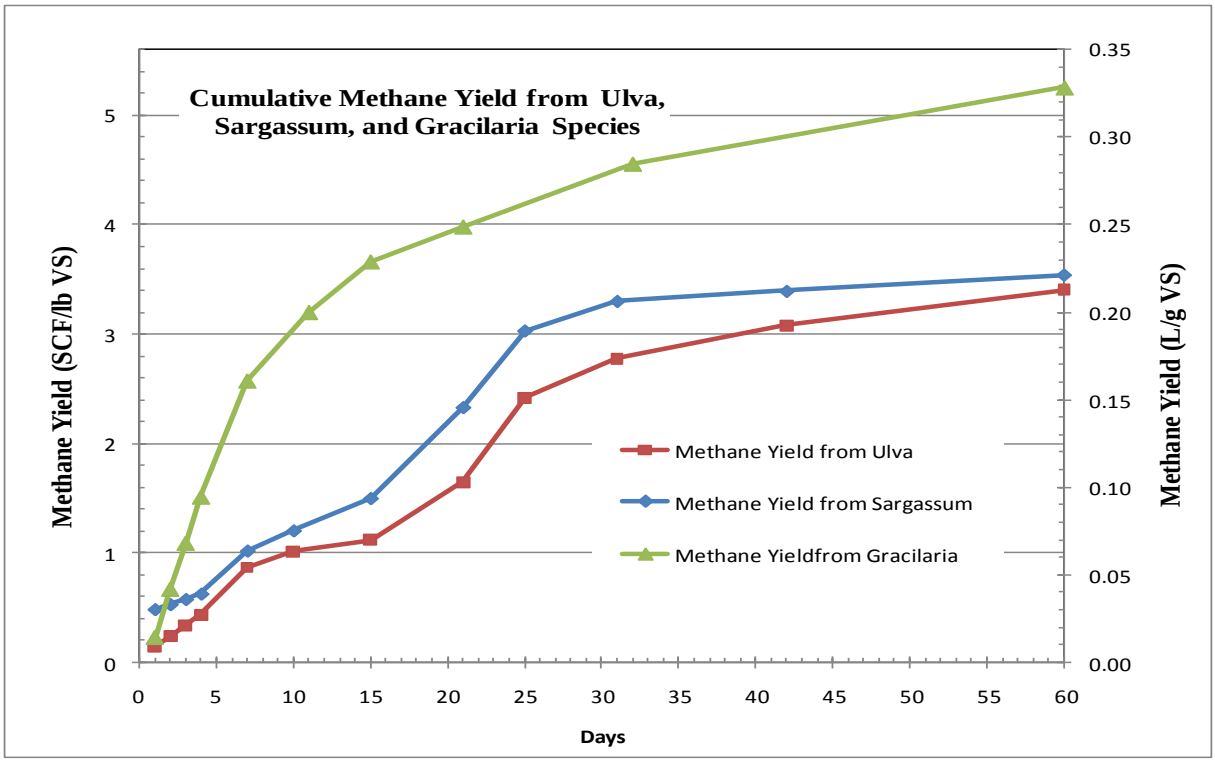

Figure 18. Cumulative Methane Yield from Anaerobic Digestion of *Ulva*, *Sargassum*, and *Gracilaria*

Semi-Continuous Digester Performance

Results from the ABP assay show that *Gracilaria* has the potential for the highest methane yield amounting to over 5.2 SCF/lb VS added; this represents a conversion efficiency of nearly 75%, which is among the highest conversion efficiencies measured for marine biomass feedstocks.

Objective

The objective of this task is to confirm the performance of the ABP assay in an engineered continuous stirred tank reactor (CSTR). The CSTR was to be fed with *Gracilaria* at a 0.05 lb VS/ft^3-day (0.8 g VS/L-day) loading rate and operated at a 15-day hydraulic retention time (HRT) to estimate the methane yield achievable in a twice-a-week feed digester.

Digester Inoculum

Seed material was obtained on August 31, 2010 from a thermophilic [130°F (54°C)], continuous-feed digester operated by the DuPage County Woodridge-Greene Valley Wastewater Treatment Plant (WWTP) at a 15-day HRT and a loading rate of 0.1 lb VS/ft^3-day (1.6 g VS/L-day). The digested sludge material had a pH of 7.9.

Digester Feedstocks

Fresh *Gracilaria pacifica* (food grade) was purchased from Carlsbad Aquafarms, Inc., San Diego, CA and received on August 31, 2010 via overnight shipping. The *Gracilaria* was cut to fine branches and a well-mixed sample was then submitted to GTI's Analytical Laboratory for chemical analysis. The analyses include total moisture, ash, C, H, N, S, P, O (by difference), gross calorific value, pH, alkalinity, total solids (TS), volatile solids (VS), ammonia, and chemical oxygen demand (COD).

Waste Activated Sludge (WAS) was also obtained from DuPage County Woodridge-Greene Valley Wastewater Treatment Plant. A sample of the WAS was mixed with *Gracilaria pacifica* at the startup of CSTR so that the microbial population in the inoculum seed could adapt to seaweed biomass gradually.

The compositions of *Gracilaria pacifica* and WAS feedstocks are shown in Table **9**. The VS content of the *Gracilaria pacifica* sample was 3.96%.

To evaluate the performance of the anaerobic digestion of the *Gracilaria pacifica* feedstock, and to provide a basis for establishing target methane yields, the maximum theoretical yield (stoichiometric methane yield) for *Gracilaria pacifica* was calculated. These calculations are based on the empirical formula of each species determined from compositional analyses. Ignoring N and S, and assuming that the C, H, and O in the feed reacted with water to form CO_2 and CH_4, the following equation is used:

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O = \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4$$

Using the above stoichiometric equation, C, H, and O content (Table **9**) were used to determine the empirical formula of *Gracilaria pacifica*. Oxygen content was calculated by difference from

the sum of ash, C, H, N, and S. Due to high sulfur content, the SO_3-corrected ash was used to calculate the oxygen content in *Gracilaria pacifica*, and SO_3-corrected oxygen then was used for the determination of the empirical formula of *Gracilaria pacifica*.

The empirical formula of *Gracilaria pacifica* conversion to methane is:

$$C_6H_{10.3}O_{2.05} + 2.91\ H_2O \rightarrow 2.22\ CO_2 + 3.77\ CH_4$$

Using the above empirical formula, the stoichiometric methane yield was calculated to be 11.76 SCF/lb (0.73 L/g) VS added. The yield represents the upper ultimate yield from this batch of *Gracilaria pacifica* without correction for bacterial synthesis or refractory components. The nature of the empirical formula for *Gracilaria* biomass is highly reduced and saturated compared to most other types of biomass feedstocks that have empirical formula similar to that of cellulose ($C_6H_{12}O_6$). Therefore, *Gracilaria* biomass has a higher methane yield upon anaerobic digestion than other biomass types that are less saturated.

Table 9. Analyses of *Gracilaria pacifica* and Waste Activated Sludge (WAS)

Date	August 31, 2010	4/6/2010
Sample Log #	101573-001	101248-001
Seaweed Species	*Gracilaria pacifica*	WAS
Air-Dry Moisture, wt %, as received	91.73	
Total Moisture, wt %, as received	92.14	-
Ash (550ºC), wt %, dry basis	58.07	-
Ash (550ºC), wt %, dry basis, SO_3 corr.	51.84	-
Carbon, wt %, dry basis	24.53	-
Hydrogen, wt %, dry basis	3.51	-
Nitrogen, wt %, dry basis	2.23	-
Sulfur, wt %, dry basis	6.69	-
Heating Value, Btu/lb, dry basis	3,760	-
pH, as received	6.0	-
Total Solids, wt %, as received	8.66	3.6
Volatile Solids, wt %, dry basis	45.68	80.94
Volatile Solid (VS), %	3.96	2.91
Ammonia, wt %, dry basis	0.38	
Chemical Oxygen Demand, mg/g, dry basis	776	
Phosphorus, wt %, dry basis	0.20	
Alkalinity, wt %, dry basis	< 1.1	
Alkalinity, mg/L	<865	
Oxygen, wt %, dry basis	4.97	
Oxygen, wt %, dry basis, SO_3 corr.	11.2	

Digester Start-up and Operation

The 6-L CSTR (3-L culture volume) was equipped with feeding and wasting ports, temperature control, continuous mixing, and an American® Wet Test Meter (Model AL-17) for measurement of gas production, gas temperature, and pressure.

Before inoculation, the CSTR was pressure-tested for leaks at 3 psig overnight. When no leaks were detected, the head-space in the digester was filled with helium gas, and, under continuous helium purging, 3 liters of fresh inoculum (methanogenic phase effluent sludge) were pumped into the digester using a Moyno progressive cavity pump. The digester head space was thoroughly purged with helium to ensure the displacement of air from the system. The temperature of the digester was set at 45°C at startup, and was controlled using a BioFlo110 Fermentor/Biodigester (New Brunswick Scientific). The digester was started on September 3, 2010 in GTI's Microbiology Lab.

The next day (September 4), a 200 ml mixture of *Gracilaria pacifica* and WAS (25:75 VS ratio) was fed into the digester. The initial loading rate was 0.025 lb VS/ft^3-day (0.4 g VS/L-day) at an HRT of 15 days. The same volume of effluent was wasted from the digester each time prior to feeding. Initially the digester was fed daily, but it was found that daily feeding interrupted digester performance. Subsequently, the digester was fed three times a week (Monday, Wednesday, and Friday).

The initial feedstocks consisted of 25% *Gracilaria pacifica* and 75% WAS to provide 0.025 lb VS/ft^3-day loading rate. The feedstock ratio was sequentially changed to 50:50, 75:25, and finally only *Gracilaria pacifica* was fed to the digester at 0.025 lb VS/ft^3-day loading rate. The acclimation process took 26 days to reach stable digester performance. Then the loading rate was increased to 0.05 lb VS/ft^3-day, and after the digester performance was stabilized, the digester temperature was gradually reduced from 45° to 35°C in 12 days. A mesophilic temperature of 35°C was chosen for CSTR operation because the ABP assays for *Gracilaria pacifica* were performed at 35°C.

Effluent Analysis

Samples of digester effluent were analyzed for total moisture, ash, C, H, N, S, P, O, heating value, pH, alkalinity, total solids (TS), volatile solids (VS), ammonia, chemical oxygen demand (COD), volatile fatty acids (VFA's). The pH of the effluent samples was measured on feeding days. The analytical results of digester effluent samples are summarized in Table 10 and Table **11** below for *Gracilaria pacifica*.

The effluent composition and VFA data indicated a classic case of digester failure – note the high concentrations of ammonia and total VFA's, low pH, and low alkalinity.

Table 10. Chemical Analyses of Digester Effluent

Date	10/13/2010	10/15/2010	10/18/2010
Sample Log #	101655-001	101662-001	101665-001
Seaweed Species	Effluent#1	Effluent#2	Effluent#3
Air-Dry Moisture, wt %, as received	98.56	98.74	98.23
Total Moisture, wt %, as received	98.62	98.81	98.36
Ash (550ºC), wt %, dry basis	42.12	44.06	37.92
Ash (550ºC), wt %, dry basis, SO_3 corr.	40.87	43.09	36.05
Carbon, wt %, dry basis	32.74	32.71	34.84
Hydrogen, wt %, dry basis	4.57	4.5	4.74
Nitrogen, wt %, dry basis	4.13	4.12	4.55
Sulfur, wt %, dry basis	1.66	1.08	1.21
Gross Calorific Value, Btu/lb, dry basis	6,220	6020	6180
pH, as received	6.9	6.6	6.1
Total Solids, wt %, as received	1.57	1.59	1.32
Volatile Solids, wt %, dry basis	68.78	65.05	61.26
Volatile Solid (VS), %	1.08	1.034	0.809
Ammonia, wt %, dry basis	4.64	4.45	2.5
Chemical Oxygen Demand, mg/g, dry basis	1880	2270	670
Phosphorus, wt %, dry basis	2.05	2.42	1.81
Alkalinity, wt %, dry basis	11.6	11.8	12.8
Alkalinity, mg/L	1600	1400	2100
Oxygen, wt %, dry basis	14.78	13.53	16.74
Oxygen, wt %, dry basis, SO_3 corr.	16.03	14.5	19.61
Total VFAs, mg/L	1527.8	1578.7	2580.4

Table 11. Variation of VFA's, pH, and Methane Production from 3-L Digester Effluent

ID	Effluent #1		Effluent #2		Effluent #3*		Effluent #4*		Effluent #5*		Effluent #6**	
Sampling date	10/13/2010		10/15/2010		10/18/2010		10/21/2010		10/22/2010		10/25/2010	
pH	6.92		6.64		6.14		6.0		6.01		7.76	
Ave. Daily raw gas before pH measurement (L)	0.49		0.55		0.33		0.20		0.13		0.08	
Component	mM	mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	mg/L
Acetic	7.39	443.8	8.43	506.2	14.3	858.7	23.5	1411.2	21.0	1261.1	25.2	1513.3
Propionic	13.1	970.4	12.7	940.8	21.1	1563.1	25.6	1896.4	21.9	1622.4	16.3	1207.5
Isobutyric	0.39	34.4	0.44	38.8	0.46	40.5	0.40	35.2	0.36	31.7	0.47	41.4
Butyric	0.2	17.6	0.22	19.4	0.27	23.8	0.40	35.2	0.41	36.1	0.61	53.7
Isovaleric	0.55	56.2	0.69	70.5	0.86	87.8	0.76	77.6	0.69	70.5	0.82	83.7
Valeric	0.03	3.1	0.03	3.1	0.04	4.1	0.08	8.2	0.08	8.2	0.11	11.2
Iso-caproic	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Caproic	0.02	2.3	BDL	BDL	0.02	2.3	0.03	3.5	0.03	3.5	BDL	BDL
Heptanoic	BDL	BDL	BDL	BDL	BDL	BDL	0.04	5.2	BDL	BDL	BDL	BDL
Total	21.7	1527.8	22.5	1578.7	37	2580.4	50.8	3472.6	44.5	3033.4	43.5	2910.9
BDL: Below Detection Limit												
*: Samples taken after using calcium carbonate to adjust pH of digester culture												
**: Sample taken after using both calcium carbonate and magnesium hydroxide to neutralize digester culture pH												

Gas Monitoring and Analysis

Gas production, temperature, and atmospheric pressure were monitored with a wet test meter and recorded daily except for weekends. The reactor temperature was controlled and monitored using a BioFlo110 Fermentor/Bioreactor temperature probe and heating tape. The headspace gas sample was taken with a Tedlar bag for analysis of major gas components (CO_2, N_2, and CH_4) using a GC. The major components of raw gas from the digester are presented in Table 12, and the daily methane production from the digester is presented in Figure 19.

Table 12. Major Components of Raw Gas from Digester

Date	Days at 0.05 lb VS/ft^3-day	Days at 35°C	CO_2, %	CH_4, %	H_2S, %
10/15/2010	12	3	68.3	28.7	3.0
10/18/2010	15	6	80.9	17.8	1.3
10/20/2010	17	8	81.0	15.5	3.5
10/21/2010	18	9	84.0	12.5	3.5
10/22/2010	19	10	85.5	11.0	3.5
10/25/2010	22	13	36.5	59.4	4.1

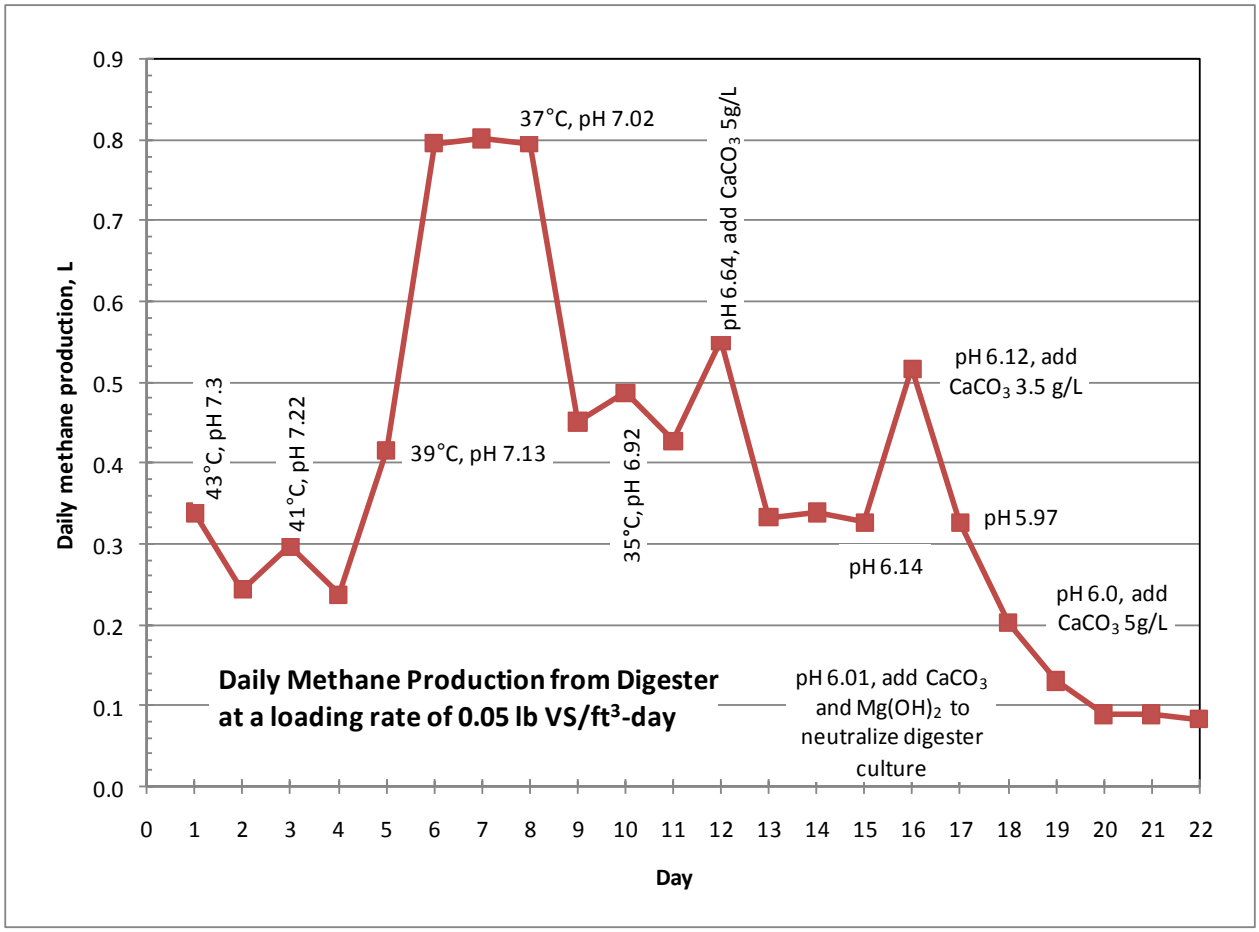

Figure 19. Daily Methane Production from Digester
(Loading Rate: 0.05 lb VS/ft^3-day)

Discussion of Anaerobic Digestion Studies

The digester was operated for 22 days at a 0.05 lb VS/ft^3-day loading rate (0.8 grams VS/L-day), and fed three times a week. The culture temperature was initially maintained at 43°C (109°F) and was gradually reduced to 35°C (95°F) by Day 10. This temperature (35°C) was maintained for the rest of the operation. Beyond the first four days of startup to Day 12, the CSTR digester averaged approximately 0.6 liters of methane production per day. Normalized methane production rate averaged about 0.2 liters of methane per liter of digester volume per day. If this level of methane production could be sustained in a continuous digester, the apparent methane yield would equate to about 4 ft^3/lb (0.25 L/g) VS added. This methane yield is consistent with that achieved (5.2 ft^3/lb VS added) with the small batch ABP assays.

During its 22 days of operation, the *Gracilaria pacifica* digester exhibited characteristics of a low buffered system. The pH of the digester effluent steadily declined from 7.3 to less than 7.0 at Day 10 (October 13, 2010) and continued to fall to 6.6 at Day 12 (October 15, 2010). The addition of 5 grams of calcium carbonate per liter of culture volume on Day 12 did not reverse the decrease in pH. On October 18, 2010 (Day 15), the digester pH further declined to 6.1. At the same time, the gas production from the digester decreased significantly from an average of 0.82 L/day between Days 9 and 12 to an average of 0.56 L per day between Days 12 and 15.

The decline in pH in the digester represented a critical challenge to this short digester experiment. The decrease in pH resulted from an accumulation of VFA's (Table **11**). The concentration of total VFA's increased from about 1500 mg/L at Days 10 and 12 to 2580 mg/L on Day 15 and 3472 mg/L on Day 18 even after the addition of 5 gram and 3.5 g/L calcium carbonate per liter of culture volume on Days 12 and 18, respectively. Among VFA's, the accumulations of acetic and propionic acids were significant, indicating the unstable digester performance and a kinetic uncoupling between acid producers (acetogenic bacteria) and consumers (acetoclastic methanogens). It has been reported that VFA's and H_2S are toxic to methanogens when pH is below 7. Propionic acid is more inhibitory to methanogens than any other VFA, especially when its concentration is greater than 900 mg/L. Ammonia is also toxic to methanogens at greater than 150 mg/L concentration. The accumulation of VFA's and the drop in digester pH corresponded to a dramatic decrease of methane production. The digester clearly "soured."

Under optimal digester operation, the microbial populations need time to adapt to new feedstocks, feeding schedules, temperature, organic loading rate, and addition of buffering chemicals. Unfortunately, the results clearly indicated that the *Gracilaria* digester was not able to reach stable performance within the 22 days of operation. Digester instability was supported by the following analytical results – reduction in gas production rate, reduction in methane gas content, increase in VFA concentration, drastic decline in pH, reduction in alkalinity, and increase in ammonia concentration. The periodic calcium carbonate addition in an attempt to gently correct digester pH failed to stabilize the digester performance in the 22 days of operation. The methanogenic population clearly required more time to recover after a pH decline of this magnitude to reduce VFA concentration in the digester and achieve stable digester performance.

Task 3.0 – Conceptual Design

Conceptual Design for Integrated Macroalgae to Methane System

As part of the overall project work, GTI completed a comprehensive literature review of biomass and macroalgae-related research. Much relevant literature was gleaned from proceedings of previous symposia sponsored in part by GTI, which were convened yearly from 1976 to 1999.

Since the early 1970's, GTI has conducted significant basic and applied research in biological conversion of different biomass types to biogas (a mixture of methane and carbon dioxide). The types of biomass include water hyacinth, hybrid poplar, sorghum grasses, municipal solid wastes, and macroalgae (*Macrocystis pyrifera* – giant sea kelp), among others. Based on this literature review, GTI was able to assemble/collect the general anaerobic digestion characteristics of *M.*

pyrifera including typical loading rates, hydraulic retention times, effects of temperature, particle size, among other characteristics.

GTI prepared a general process flow diagram for the Macroalgae Derived Renewable Energy (MADRE) pilot plant including material balance for a base case scenario. In this scenario, the main greenhouse is sized to produce 50 pounds (dry basis) per day of macroalgae. The anaerobic digestion system is sized to process this quantity of macroalgae per day and generate an estimated 315 ft^3/day of biogas with 60 percent methane content. The methane yield used for the MADRE pilot plant design was 5.44 SCF CH_4/lb VS added.

From the standpoint of carbon capture, each pound of biomass generated in the greenhouse would require 2 net pounds of CO_2. So the MADRE pilot plant to be built in Phase 2 would convert approximately 100 pounds of CO_2 per day into biomass.

Because of the strict requirements for the natural gas being combusted in the power plant, the biogas would not be upgraded to pipeline quality for this pilot plant, but will be flared or otherwise beneficially used.

A complete description of the overall process was developed and included in the Process Design Package. In the overall process, a slipstream of flue gas from the natural-gas-fired power plant is brought to the macroalgae greenhouse, where it is incorporated into the biomass. Biomass (macroalgae) is harvested daily, ground, and fed to a solids concentrating anaerobic digester (the solids retention time is much longer than the hydraulic retention time). The biomass is converted to biogas – a mixture of methane and carbon dioxide with trace amounts of hydrogen sulfide and water vapor. The H_2S is removed with a sulfur sorbent, such as iron sponge, and the water is removed by cooling/condensing or dehydration.

Phase 2 Seaweed Culture System Concept

The conceptual design for the Phase 2 macroalgae spray-culture greenhouse culture module envisions specially selected seaweed biomass growing on net frames attached to vertically oriented, rectangular-shaped (nominally 3-ft wide x 3-ft high) plastic or coated wire mesh culture panels arranged into parallel rows and placed across the width of a greenhouse (about 150 ft long x 26 ft wide x 12 ft high/tall). Overhead and above the seaweed culture panels an array of water sprayer nozzles would distribute nutrient-rich artificial seawater to irrigate and fertilize the seaweed plants growing on the panels. Any culture water not taken up by the growing seaweeds falls onto a sloping cement or rubber-covered floor and hence into a central drain and then on to a harvest pit located at one end of the greenhouse.

During harvesting, which is envisioned to occur on a daily basis during the operations phase, the growth increment of seaweed would be mechanically cut from the growing mat on the vertical culture panels and would fall to the floor. Filtered high velocity sprays of culture water pumped from the harvest sump in a continuous re-cycled loop would direct the harvested bits of thalli towards the central drain where the flow of water would carry them into the harvest sump. The cut bits would be conveyed by a mesh-belt conveyor up to a screen basket located on a transport cart which would drive the weekly harvest increment to the input hopper of the anaerobic

digester unit. All culture water that drips from the harvest units would be collected and recycled to the harvest sump, where an internal continuously operating low flow-rate filtration loop would remove the small bits and condition the water using a series of in-line bio-filters.

The digested seaweed would produce a portion of methane gas that could be cleaned and recycled as burnable fuel for the power plant to offset a portion of natural gas. CO_2 (also produced in the digester) could be recycled to the seaweed culture greenhouse(s) to augment increased biomass production in the process.

A portion of the flue gases (in the primary case obtained from a San Diego Gas & Electric natural gas-fired nominal 550 MW dual-cycle electric power generating plant, Escondido, CA) would be piped into the head-space of the greenhouse(s) to provide gaseous CO_2 to the growing seaweed to support high-rate photosynthesis and plant biomass growth. In this manner, a portion of the CO_2 produced by the power plant would be captured and recycled into seaweed biomass. The alternative location for the Phase 2 MADRE pilot plant would the Moreno Compressor Station, Moreno Valley, CA.

The Phase 2 pilot demonstration module will allow for accurate process documentation for GTI to refine its estimates for capital and operating costs for a scaled-up commercial-sized seaweed-based CO_2 capture and renewable fuels facility located in the Western region of the United States.

Seaweed Propagation Laboratory

The seaween propogation laboratory includes exterior and interior / laboratory design elements required and useful for supporting seaweed propagule production and scale-up for the proposed Seaweed Propagation Laboratory, including (a) tumble tank array and greenhouse cover, (b) propagation house exterior / interior layout, and (c) water table / spray-culture starter system, all components of the proposed Phase 2 macrophyte culture module.

Propagation House – Concepts:

The propagation facility must be isolated from the outside environment to protect the seaweed starter stock from possible contamination. To achieve this degree of isolation a greenhouse cover for the facility is envisioned. To be able to stock the main greenhouse culture panels in a period of 3 to 4 months from the projected date of commencement of Phase 2 culture activities, a layout of 24 water table tanks will be required (see Item 3 below). These tanks could be housed in two, 30-ft x 60-ft greenhouses constructed as PVC bow-frame, flexible polyethylene-covered greenhouses with gravel floors.

Tumble Tanks: An array of 4 each about 1,800 gallon fiberglass tumble tanks have been conceived to serve the biomass production needs for stocking the culture panels and screens in the propagation house. These 4 tanks would be covered with their own small (15-ft x 35-ft) PVC bow-framed greenhouse similar to those built for the propagation houses.

Water Table Tanks / Spray Culture Starter System: An array of 12 each 12-ft x 3.5-ft x 3-ft deep fiberglass tanks per propagation house has been conceived and designed. The re-circulating water table tanks would be served by under-tank bio-filters that will allow for closed-system operation and conservation of water resources. In operation, the now horizontally-placed mesh seaweed culture panels would be seeded with small plantlets (cut from tumble-tank-produced biomass). Over a period of from 1 to 2 weeks the plantlets would grow and inter-twine through the mesh of the culture substrates. At that time, the substrates would be lifted from the water table tank and hung vertically overhead on hooks to be sprayed by nutrient mix media in preparation for being stocked into the main culture greenhouse.

Estimation of Performance and Cost at Full Scale/Techno-Economic Analysis

GTI updated and revised the process flow diagram generated by the Aspen Plus® process simulation software package for commercial-scale application of the MADRE process. The material balance generated by Aspen Plus as well as the process flow diagram were transmitted to UCSD-SIO, which conducted the preliminary techno-economic analysis of the MADRE process. The following discusses the techno-economic analysis of the MADRE process prepared by UCSD-SIO. The Aspen Plus flow diagram for the MADRE process is included in Figure 20.

Engineering-economic and lifecycle analytic models of the proposed macroalgae production system have been developed to characterize system wide technical and economic performance and expected greenhouse gas emission impacts. This work integrates expectations for technical and economic performance parameters developed by the project team with key results from detailed systems analysis developed using the software package Aspen Plus and lifecycle emissions factors from the California Modified GREET model (per California Air Resources Board). Models were developed for three system configurations to enable comparative analysis of: 1) a baseline natural gas-fired combined-cycle power plant, 2) a power plant integrated with the proposed macroalgae production system, and 3) a power plant integrated with a high density macroalgae cultivation system. Another configuration was developed in which the quantity of flue gas from the power plant was reduced to 25 percent and a high-high density macroalgae cultivation system (N^{th} plant) was considered. The modeling approach is characterized below while the underlying assumptions and detailed model results are provided in the model.

Further, the effect of CO_2 abatement credits in the range of $10, $25, and $50 /ton of CO_2 removed/abated on the electric power cost was considered (these values for CO_2 credits were suggested by NETL during the project final conference webinar (December 13, 2010).

The engineering-economic model consists of the following components: i) a Master Assumptions table that drives most of the analysis; ii) key results from systems analysis using Aspen Plus, which provides the basis for many of the technical assumptions in the Master Assumptions table as well as capital cost scaling; iii) a capital cost schedule defining total capital requirements; iv) a schedule of process component cost scaling for the proposed aquaculture system; v) an operating cost schedule; schedules detailing labor and yield expectations for the two proposed aquaculture configurations; and cash flow schedules for each configuration.

The Master Assumptions is divided into two sections: Technical analysis assumptions and financial analysis assumptions. A few assumptions were based on the judgment of various team members, but most are derived from specific analysis or literature sources. The basis for each assumption is provided in its description.

Selected results from systems analysis using Aspen Plus are reported as originally output from Aspen and scaled in two distinct ways to support utilization in the current analysis. The first of

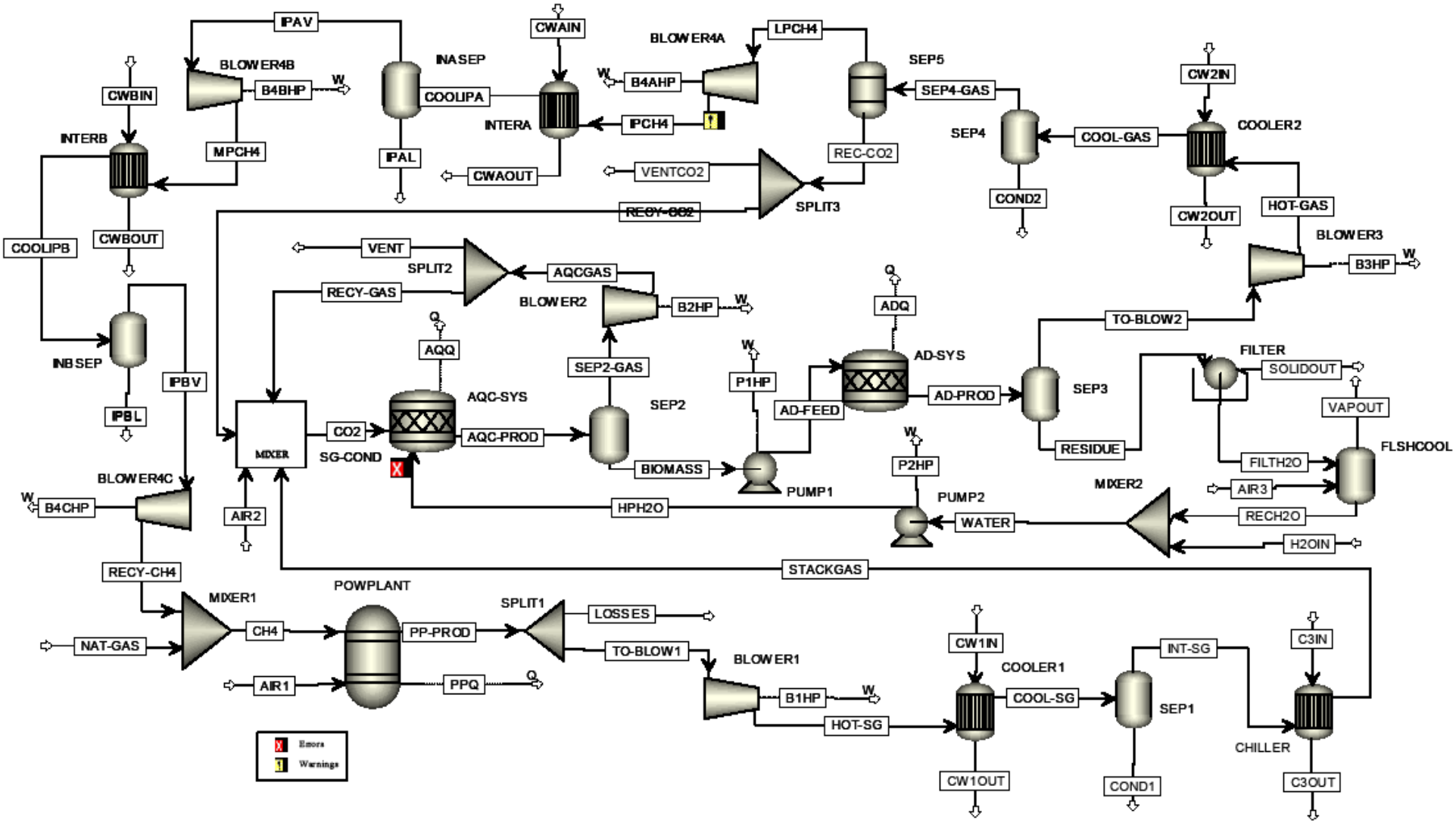

Figure 20. Aspen Plus® Process Flow Diagram for Commercial Application of MADRE Process

these is to match the proposed system utilization of power plant stack gas. The original Aspen simulation assumed only a very low level of stack gas utilization (0.006%), consistent with the potential Phase 2 pilot plant to grow about 50 pounds (dry basis) per day of macroalgae.

For the first pass at the commercial feasibility, it was assumed that a commercial deployment would utilize all of the CO_2 produced from power plant operations. As such, scaling to reflect proposed CO_2 utilization is achieved in two steps. The first is to scale all downstream process flows linearly from the ratio of CO_2 utilization (100% / 0.006%). The second step is to scale down the natural gas supply linearly to maintain equal carbon inputs to the power plant and aquaculture system.

The second set of scaling calculations is to match the proposed power plant capacity of 550 MWe. All process flow streams were linearly scaled so that the total higher heating value of methane supplied to the power plant was equal to the proposed capacity (550 MWe) divided by the expected conversion efficiency of the combined cycle power plant (50.5% HHV). The process flow data is provided for four resulting process flow scales: 1) original scale, 2) scaled to increased CO_2 utilization, 3) scaled to proposed capacity, and 4) scaled to proposed capacity and increased CO_2 utilization. In fact, only the last of these cases is directly integrated into the model. Results for the others are provided for completeness. Detailed accounting of calculations for total capital requirements (TCR) for all three system configurations were determined. The baseline, low density, and high density configurations have expected TCR's of approximately $561 million, $14.8 billion, and $13.7 billion, respectively. The high estimated TCR's for the aquaculture configurations reflect, among other things, the high expected component costs and relatively conservative contingencies appropriate for such a novel system. Additional research and development should substantially reduce these contingencies.

Reducing all process and project contingencies to 8% would reduce TCR's for the low and high density configurations to approximately $10.0 billion and $9.3 billion, respectively. **Error! Reference source not found.Error! Reference source not found.**Detailed accounting of aquaculture component cost scaling was also determined. This component cost scaling was achieved with a fairly simplistic approach, which likely represents a significant source of uncertainty. Additional analysis to refine component costs could substantially change resulting capital cost estimates. Detailed component cost estimates for the proposed pilot facility form the basis for component cost scaling. The original cost estimates for all components other than the greenhouse (which were assumed to have a fixed cost per square foot defined in the Master Assumptions table) are scaled based on the total stack gas stream diverted to the aquaculture system using a scaling factor of 0.65 (i.e., capital costs are assumed proportional to the ratio of stack gas stream throughputs to the 0.65 power).

Economies of scale are limited however by assumptions associated with assumed 10-acre farm modules for low, high, and high-high density configurations. This is implemented in the component cost scaling by defining scaled component costs as the product of the number of system trains and component cost per train. Component costs per train, are scaled as described above, except that the stack gas throughput is defined for the commercial scale as the total stack gas flow divided by the number of trains. The number of trains is constrained by an assumed three digesters per 10-acre farm module and is therefore defined as the total production area

divided by three digesters per 10-acres. This represents an important factor underlying the high expected capital costs of aquaculture systems. If equipment could be scaled up substantially and support 10 hectare (ha) modules, for example, then total component capital costs for the aquaculture systems could be cut roughly in half. Also, increasing the macroalgae growth density further would reduce the estimated capital costs. Detailed accounting of annual operating costs for each system configuration was determined. These results are calculated directly from parameter values in the Master Assumptions table. Labor and other operating costs for the gas turbine system are assumed to be included in the fixed and variable operating and maintenance (O&M) values provided under Maintenance costs. Additional detail regarding proposed 10-acre farm scale production modules for the low, high, and high-high density aquaculture production systems, respectively were determined. Key results are areal yields (mass algae per unit area), aquaculture labor costs, and validation of digester scale parameters.

Financial parameters were used to provide estimated cash flow projections for the baseline, low density, high density, and high-high density aquaculture systems. In addition to providing a cash flow schedule, these projections are used to compute estimates of levelized costs of electricity for each configuration. This is accomplished in Microsoft Excel with the solver add-in by setting the NPV of equity investments and net profits after taxes (NPAT) equal to zero by changing the cost of electricity. As such, the electricity sales price and associated total revenues are very different across these three schedules. Specifically, levelized costs of electricity for the baseline, low density, and high density aquaculture systems are estimated to be $0.056, $1.111, and $1.043 per kW-h, respectively. The electric power cost for the high-high density case in which 25 percent of the flue gas stream is utilized for macroalgae production was calculated to be $0.318 per kW-h.

All costs and electricity prices increase over time based on the escalation factors defined in the Master Assumptions table. Depreciation for the aquaculture system results in zero tax payments during the first five years of system operations, due to the assumption of accelerated depreciation for these assets. Losses are not, however, carried forward to offset future taxes.

A lifecycle analysis (LCA) of greenhouse gas emissions has been developed for the three system configurations. Detailed assumptions and calculations comprising this LCAwere determined. Emissions are estimated from natural gas supply, natural gas combustion, and fertilizer supply. Emissions from aquaculture production and digestion of macroalgae are not estimated directly as it is assumed that: 1) that all greenhouse gas emissions from aquaculture production are in the form of CO_2 not converted to methane for recycle back to the power plant, and 2) all carbon in the biosolids is effectively converted to CO_2. The effect of these assumptions is that the only direct effects on greenhouse gas emission from aquaculture production are reduced natural gas consumption and emissions from fertilizer supply. Emissions from biosolids transport and NOx emissions from biosolid degradation were not estimated.

In all cases, emissions factors are taken directly from the modified GREET model (from the California Air Resources Board). Greenhouse gas emissions are reported in mass units of CO_2 equivalent (CO_2 eq), where emissions of CH_4 and NOx are converted to CO_2 equivalent based on their 100-year climate forcing estimates, which are included in the Schedule. Emissions of volatile organics and carbon monoxide are assumed to be converted to CO_2 relatively rapidly.

The resulting CO_2 emissions are estimated based on the relative carbon ratios of each set of compounds (also reported in the Schedule).

Summary results from the techno-economic analysis and LCA are provided. In reporting the basic to key techno-economic and LCA results, this schedule reports renewable energy production, specific land requirements, and CO_2 mitigation costs. These are only reported for the aquaculture systems as the baseline system has zero renewable energy production and mitigation costs are computed as the difference in electricity costs divided by the difference in emissions between each of the aquaculture configurations and the baseline system. As presented, the results suggest that the proposed system is a relatively expensive means of producing renewable electricity or mitigating greenhouse gas emissions. These costs could be reduced by a number of strategies, including for example, more detailed analysis of component cost scaling; developing larger production trains; integrating with coal fired power plants (which have greater baseline CO_2 emissions); or integrating production of high value co-products.

Given the very high capital and operating expenses calculated for the low-density and high-density cases, it was decided to revisit the overall assumptions.

Instead of assuming that the entire (100%) flue gas stream would be directed to the macroalgae cultivation area, we assumed that 25 percent of the flue gas was utilized. The objective of this assumption was to significantly reduce the estimated capex as well as the required land area for the macroalgae cultivation, digestion operations. At the same time, we assumed that macroalgae cultivation and harvesting could be improved in the N^{th} plant design. This case is designated as the high-high density configuration. With these assumptions, the levelized cost of electricity becomes $0.318 per kW-hr and the areal extent of the project is 2,350 acres. Both of the these values are significantly lower than those of the other scenarios considered.

Finally, the cost of electricity was calculated assuming that various levels of credit could be obtained for reducing the net emission of CO_2 into the atmosphere. This CO_2 abatement credit was suggested by DOE NETL during the project review webinar held on December 13, 2010. DOE suggested that a range of CO_2 credits be considered, specifically, $10, $25, and $50/ton of CO_2 abated. Spreading the credit over the electricity supplied by the plant resulted in a fractional (less than 1 cent per kW-hr) reduction in electricity cost.

Task 4.0 – Permit Planning

Develop Plans for Securing Permits

The objective of this task is to identify the permits needed for the MADRE pilot plant and establish a schedule and protocol to securing the needed permits. Because of their familiarity with the geographical region and permitting experience in particular, Power Engineers, Inc. (POWER), was subcontracted by GTI to perform this task. POWER prepared the following

permitting plan for the MADRE site location in Escondido, California. The following summarizes the potential permitting requirements for the proposed Escondido site. It is expected that the use of the alternate plant site at the Moreno Compressor station (Moreno Valley) would result in similar permitting requirements.

1. Complete parcel lease and access agreement

2. Land use, zoning and geotechnical considerations, including soil composition, seismic zone, flooding, ground contamination considerations

3. Grading plan approvals/grading permit

4. State Water Board Stormwater Permit, as parcels larger than one acre require a SWPPP plan and filing of a notice of intent (NOI) for storm runoff discharges

5. General Construction Permit from the Regional Water Quality Control Board (RWQCB)

6. RWQCB Dewatering Permit (if needed)

7. San Diego Air Pollution Control District (APCD) permits for discharges from the seaweed greenhouse and the anaerobic digester

8. Sanitary Sewer connection permits for discharge of process wastewater if needed

9. Building permits for new construction of pilot facilities and concrete pads

10. Sign Permit/Sign Relocation and Encroachment Permit, if needed

11. Construction of street improvements (i.e., new access from cul-de-sac), infrastructure associated with utility connections, including sanitary sewer, storm water supply lines, electrical, and natural gas connections

12. California Energy Commission (CEC) and the Public Utilities Commission (PUC) will be consulted regarding any changes to the existing power plant.

13. Compliance with the California Environmental Quality Act (CEQA)

14. National Environmental Policy Act (NEPA)

15. UCSD-SIO investigated State of California Department of Fish & Game permitting requirements for collecting and culturing native and collecting, importing and culturing non-native seaweeds for the planned Phase 2 effort.

During their work, POWER approached appropriate State and Federal regulators to inform them of the potential Phase 2 project and to seek guidance on approach for permitting the pilot plant. Based on their work, POWER developed a permitting plan and approach for the potential Phase 2 project. The permitting plan prepared by POWER is discussed below.

The permitting effort includes conducting pre-application meetings with the host city (Escondido or Moreno Valley) to inform their respective planning departments of GTI's plans and expectations for the project. The initial permit would be a Conditional Use Permit (CUP) for

temporary operation of the Phase 2 MADRE pilot plant over the 3-year Phase 2 project period. After this pre-application meeting, the CUP application can be completed and submitted to the permitting agency.

GTI and POWER will prepare a detailed project description so that all project components are clearly defined and all potential impacts of the project are properly evaluated. The purpose and need will be defined for inclusion in the Initial Study/Negative Declaration (IS/ND) document.

GTI and POWER will prepare an environmental checklist as part of the California Environmental Quality Act (CEQA), which addresses: Aesthetics, agricultural resources, air quality, biological resources, cultural resources, hazards and hazardous materials, hydrology and water quality, geology and soils, land use, mineral resources, noise, population and housing and public services, traffic and transportation, utilities and service systems, among others.

Based on feedback from the permitting agencies, the IS/ND document will likely require modification and updating. There is also opportunity for the local community to review the IS/ND to assess the impacts of the project. Comments by the community will be addressed for follow up.

The overall schedule for permit planning and execution was prepared. From the initial steps, the overall schedule includes 9 months of activities to have the required permits issued for the project.

Task 5.0 – Preliminary Design

Prepare Design Information Document

GTI and its subcontractor, POWER, completed preparing the necessary documents and drawings to be included in the Design Information Document (Process Design Package or PDP), which is a deliverable for Phase 1 and necessary for the Phase 2 detailed design work. The complete Design Information Document was prepared. This document includes drawings, basis of design, process description, process flow diagrams, material balances, cooling tower calculations, piping and instrumentation diagrams, preliminary layout and general arrangement diagrams, single-line electrical diagram, and electric power load summary.

The Design Information Document or PDP consists of fifteen sections: Project Report, Cost Estimate, Procurement and Construction Schedule, Process Flow Diagrams, Piping and Instrumentation Diagrams, Utility Diagrams, Site Preparation, Foundations, Structural, Layout, Equipment, Instrumentation, Piping, Electrical, and Planning Documents. The PDP includes the information required for proceeding with the Phase 2 MADRE pilot plant procurement, installation, and operation including details of the permitting requirements for the Escondido site.

The PDP includes the primary conditions for the MADRE pilot plant including site location, range of ambient weather conditions, seismic code, specifications for the main greenhouse and anaerobic digester, among other details. The site characteristics include the primary project site at the SDG&E natural-gas-fired power plant in Escondido, CA and the secondary project site at the SDG&E Moreno Compressor Station in Moreno Valley, CA.

Among the diagrams prepared were three process flow diagrams (PFD's): Overall MADRE Pilot Plant (MA-F-400-E, rev C), Macroalgae Production (MA-F-401-D, rev C), and Biomass Gasification (MA-F-402-D, rev C). These PFD's include equipment names, equipment numbers, and simple description of each equipment item.

The PFD's also include material balances for the major components in the process streams with flow rates, temperature, and pressure.

Develop project Plan and Cost Estimates for Phase 2

The objective of this task was to construct the essential elements that can be utilized for the Phase 2 renewal application. The project team developed a project plan that would be assembled for the pilot demonstration effort of Phase 2. The plan included a discussion of the recommended configuration, options for alternative decisions, and the costs associated with the proposed system.

GTI and its subcontractor team completed preparing the necessary items for the Phase 2 application. However, as the May 17, 2010 proposal deadline neared, it became evident that the required co-funding as well as the site could not be definitively secured in time. Therefore, the application for Phase 2 renewal was not submitted.

GTI and POWER completed a detailed cost estimate for procuring and installing the MADRE pilot plant equipment items for the anticipated Phase 2 project, which is included in the Design Information Document. This cost is directed to the primary project site location at the SDG&E natural-gas-fired power plant at Escondido, CA. Please note that the cost to procure the equipment and install the MADRE pilot plant at the Moreno Compressor Station, Moreno Valley, CA was not estimated. However, the cost to install the MADRE pilot plant at the Moreno Compressor Station is expected to be comparable to that of the Escondido site.

There would be a notable difference in the Phase 2 operating costs at the Moreno Compressor Station compared with the Escondido site. The Moreno Compressor Station does not operate continuously and therefore, the source of CO_2-containing flue gas is NOT continuous at the Moreno site. A secondary source of CO_2 would need to be provided for continuous operation of the macroalgae greenhouse.

A simple line-item summary of MADRE pilot plant procurement costs developed for Phase 2 is presented in Table 13 below. As mentioned above, detailed costs are included in the PDP. In total the cost to procure and install the MADRE pilot plant for the Phase 2 project is estimated to be $4,109,900.

It should be noted that the plant operating labor, supervision, utilities, chemical analysis, project management as well as other operating costs expected to be incurred during the 3-year Phase 2 project are not included in this estimate.

Table 13. Cost Estimate for Phase 2 MADRE Pilot Plant

DIRECT COSTS	
Civil & Site Work	$580,730
Concrete	$365,490
Structural Steel	$28,840
Architectural & Buildings	$152,470
Engineered Equipment	$786,050
Engineered Equipment Installation	$117,240
Above Ground Piping	$361,210
Electrical	$166,500
Instrumentation	$354,370
Other	$20,000
Total Direct Costs	$2,932,900
INDIRECT COSTS	
Construction Indirects	$109,740
EPCM Services with Markup & Expenses	$965,160
Total Indirect Costs	$1,074,900
Other Costs	$16,700
TOTAL PROJECT COSTS (TPC)	$4,024,500
Environmental Services (Permitting)	$85,400
TPC & Environmental Services	$4,109,900

Summary and Conclusions

In summary, selection criteria for macroalgae that could survive the elevated temperatures and potential periodic desiccation of near desert project sites were identified. These criteria were used to identify several candidate species. A subset of these cultured and subjected to various comparative and optimization testing. Several *Gracilaria* species (*Gracilaria vermiculophylla PT, Gracilaria vermiculophylla CT, Gracilaria tikvahiae, and Gracilaria cervicornis*) were tested to determine temperature tolerance and the results demonstrated that *Gracilaria vermiculophylla* is clearly the best prepared for survival and growth at temperatures as high as 34°C. It also showed the capacity to resist a 2 day exposure to 36°C. *Gracilaria vermiculophylla* also had the highest growth rates at temperatures ranging from 22°C to 36°C.

Samples of the selected macroalgae species were obtained and then subjected to anaerobic digestion to determine conversions and potential methane yields. Macroalgae species *Gracilaria* was selected for anaerobic digestion studies based on tufted morphology, tolerance to desiccation, growth rate, and tolerance to temperature. The *Gracilaria* demonstrated a cumulative methane yield amounting to over 5.2 SCF/lb VS added in 60-days anaerobic digestion with a conversion efficiency of greater than 73%.

Two potential sites were characterized for the proposed pilot plant, a power plant in Escondido, CA (primary site) and a compressor station in Moreno Valley, CA (alternative site). A detailed conceptual process design for installation of the proposed pilot plant was completed for these two specific sites. The conceptual process design includes an overall material balance for generating renewable natural gas (biomethane) from macroalgae grown in a greenhouse supplied with CO_2 from the flue gas from a natural-gas-fired power plant, process flow diagram, equipment list with sizes, piping and instrumentation diagrams, electric power requirements, and layouts for two potential project sites (Palomar Power Plant, Escondido, and Moreno Compressor Station, Moreno Valley, both in California).

In addition, a process design package (PDP) or Design Information Document was compiled as a deliverable for the project. The PDP consists of fifteen sections: Project Report, Cost Estimate, Procurement and Construction Schedule, Process Flow Diagrams, Piping and Instrumentation Diagrams, Utility Diagrams, Site Preparation, Foundations, Structural, Layout, Equipment, Instrumentation, Piping, Electrical, and Planning Documents. The PDP includes the information required for proceeding with the Phase pilot plant procurement, installation, and operation including details of the permitting requirements for the Escondido site and the Moreno Valley site.

A process design and material balance for an Aspen Plus simulation was prepared so that a techno-economic evaluation and life-cycle assessment could be conducted. Preliminary economic assessments were performed under the various assumptions made, which are purposely conservative. Based on the results, additional development work should be conducted to delineate the areas for improving efficiency, reducing contingencies, and reducing overall costs. Specifically, the estimated capital and operating costs demonstrate the need to build and conduct Phase 2 MADRE pilot testing to confirm estimated greenhouse growth yields for macroalgae,

harvesting techniques to reduce operating costs, biogas yields in SOLCON (solids concentrating) digester and to reduce contingencies for cost estimates.

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