

**Energy Research and Development Division
FINAL PROJECT REPORT**

**OFFSHORE MEMBRANE
ENCLOSURES FOR
GROWING ALGAE (OMEGA)**

**A Feasibility Study for Wastewater to
Biofuels**

Appendices

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PREFACE

The California Energy Commission Energy Research and Development Division supports public interest energy research and development that will help improve the quality of life in California by bringing environmentally safe, affordable, and reliable energy services and products to the marketplace.

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OMEGA (Offshore Membrane Enclosures for Growing Algae) - A Feasibility Study for Wastewater to Biofuels is the final report for the Algae OMEGA: Offshore Membrane Enclosures for Growing Algae project (contract number PIR-08-047) conducted by NASA Ames Research Center. The information from this project contributes to Energy Research and Development Division's Transportation Program.

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ABSTRACT

The biofuels community has shown considerable interest in the possibility that microalgae could contribute significantly to providing a sustainable alternative to fossil fuels. Microalgae species with high growth rates and high yields of oil that can be grown on domestic wastewater using nonarable land could produce biofuel without competing with agriculture. It is difficult to envision where the cultivation facilities would be located to produce the quantity of algae needed for fuels, given that these facilities must be close to wastewater treatment plants to save energy.

Researchers investigated a possible solution called Offshore Membrane Enclosures for Growing Algae for coastal cities. This system involved growing fast-growing, oil-producing freshwater algae in flexible, inexpensive clear plastic photobioreactors attached to floating docks anchored offshore in naturally or artificially protected bays. Wastewater and carbon dioxide from coastal facilities provided water, nutrients, and carbon. The surrounding seawater controlled the temperature inside the photobioreactors and killed any algae that might escape. The salt gradient between seawater and wastewater created forward osmosis to concentrate nutrients and to facilitate algae harvesting. Both the algae and forward osmosis cleaned the wastewater, removing nutrients as well as pharmaceuticals and personal care products, so-called compounds of emerging concern.

This report provided the results of two years of research into the feasibility of the Offshore Membrane Enclosures for Growing Algae system in which prototype systems were studied, built, and tested in seawater tanks. A 110-liter floating system was developed and scaled up to 1,600 liters. Algae's ability to grow on and treat wastewater was described. The impact of biofouling on photobioreactors and forward osmosis membranes floating in the marine environment was considered. Life-cycle and techno-economic analyses provided a perspective on what must be done to make this system commercially viable. Outreach efforts have carried the concept worldwide.

Keywords: biofuels, microalgae, algae, OMEGA, offshore systems, carbon sequestration, aquaculture, wastewater treatment, biofouling, life cycle analysis, techno-economic analysis

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**Appendix A:
Microalgae Cultivation Using Offshore Membrane
Enclosures for Growing Algae (OMEGA)**

Microalgae Cultivation Using Offshore Membrane Enclosures for Growing Algae (OMEGA)

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Abstract

OMEGA is a system for cultivating microalgae using wastewater contained in floating photobioreactors (PBRs) deployed in marine environments and thereby eliminating competition with agriculture for water, fertilizer, and land. The offshore placement in protected bays near coastal cities co-locates OMEGA with wastewater outfalls and sources of CO₂-rich flue gas on shore. To evaluate the feasibility of OMEGA, microalgae were grown on secondary-treated wastewater supplemented with simulated flue gas (8.5% CO₂ V/V) in a 110-liter prototype system tested using a seawater tank. The flow-through system consisted of tubular PBRs made of transparent linear low-density polyethylene, a gas exchange and harvesting column (GEHC), two pumps, and an instrumentation and control (I&C) system. The PBRs contained regularly spaced swirl vanes to create helical flow and mixing for the circulating culture. About 5% of the culture volume was continuously diverted through the GEHC to manage dissolved oxygen concentrations, provide supplemental CO₂, harvest microalgae from a settling chamber, and add fresh wastewater to replenish nutrients. The I&C system controlled CO₂ injection and recorded dissolved oxygen levels, totalized CO₂ flow, temperature, circulation rates, photosynthetic active radiation (PAR), and the photosynthetic efficiency as determined by fast repetition rate fluorometry. In two experimental trials, totaling 23 days in April and May 2012, microalgae productivity averaged 14.1 ± 1.3 grams of dry biomass per square meter of PBR surface area per day ($n = 16$), supplemental CO₂ was converted to biomass with >50% efficiency, and >90% of the ammonia-nitrogen was recovered from secondary effluent. If OMEGA can be optimized for energy efficiency and scaled up economically, it has the potential to contribute significantly to biofuels production and wastewater treatment.

Keywords: Biofuels, wastewater treatment, microalgae, photobioreactor, CO₂ mass transfer, fast repetition rate fluorometry, instrumentation and control

1. Introduction

Microalgae are currently under consideration as a significant source of sustainable biofuels because of their fast growth rate and ability to produce oil that can be readily transformed into fuel [1,2]. These microscopic, sin-

gle-cell organisms can be cultivated on non-arable land, lessening competition with agriculture and thus giving them an advantage over other biofuel crops [3-5]. On the other hand, microalgae require fertilizer and supplemental carbon dioxide (CO₂) for optimal growth, which can generate more environmental pollution and green-

house gas emissions than cultivation of more traditional biofuel feedstocks, such as switchgrass, canola, and corn [6-8]. Several authors have noted that these environmental drawbacks can be ameliorated by linking microalgae cultivation to wastewater treatment plants (to provide water and nutrients) and flue gas sources (to provide CO₂), which also improves the economics and energy return on investment (EROI) [6, 9, 10]. The feasibility of constructing microalgae cultivation facilities close to existing wastewater plants to avoid the prohibitive costs of pumping water long distances will depend on the location [11]. For most metropolitan areas, installing large microalgae ponds or fields of photobioreactors (PBRs) on land would significantly disrupt urban infrastructure. For coastal cities, however, which use offshore wastewater outfalls, a system of floating photobioreactors (PBRs) called Offshore Membrane Enclosures for Growing Algae (OMEGA) may resolve this difficulty [12].

The proposed OMEGA system is designed to grow freshwater microalgae in wastewater contained in flexible, clear, plastic PBRs attached to a floating infrastructure anchored offshore in protected bays [12-14]. The offshore placement allows the system to be in close proximity to wastewater treatment plants and sources of flue gas, eliminating the need to pump these wastes long distances to remote locations where land resources for algae cultivation may be available. By using wastewater for water and nutrients and by not using arable land the OMEGA system avoids competing with agriculture or disrupting urban infrastructure in the vicinity of wastewater treatment plants. On a scale relevant to biofuels, OMEGA will be intrusive in the marine environment, although it is possible that a large flotilla of PBRs may have beneficial effects in coastal areas. The OMEGA system would remove nutrients from the wastewater that is currently discharged into coastal waters and may thereby mitigate "dead-zone" formation. The infrastructure would provide substrate, refugia, and habitat for an extensive community of sessile and associated organisms [15]. It is known that introduced surfaces in the marine environment become colonized and can form "artificial reefs" or act as "fish aggregating devices," which increase local species diversity and expand the food web [16, 17]. A large-scale deployment of OMEGA systems may also act as floating "turf scrubbers" and function to absorb anthropogenic pollutants, improving coastal water quality [18].

The technical feasibility of the OMEGA concept however, has yet to be evaluated at any scale. Here a prototype, 110-liter OMEGA system was developed and tested in a seawater tank, using freshwater microalgae and secondary-treated wastewater. The details of the system design are described, including the gas exchange and harvesting system as well as the essential monitoring

and control instrumentation. This OMEGA prototype maintained viable microalgae cultures, recovered ammonia-nitrogen (NH₃-N) from wastewater, and sustained areal productivities at levels similar to those reported for other cultivation systems. Furthermore, the prototype utilized supplemental CO₂ with greater efficiency than other cultivation systems. These results support the proposal that offshore microalgae cultivation, co-located with waste resources, can contribute to the production of biofuels without competing with agriculture [12, 13].

2. Materials and Methods

2.1. Seawater Tank and Microalgal Cultures

Experiments were conducted in an 8,800-liter seawater tank at the California Department of Fish and Game, Marine Wildlife Veterinary Care and Research Center in Santa Cruz, CA (Lat: 36° 57' 13", Long: -122° 3' 56"). The tank was covered at night with a thermal pool blanket to minimize heat loss. A mixed culture of green microalgae used as the system inoculum was dominated by *Desmodesmus* sp. and grown in 19-liter glass carboys containing either BG11 medium (ATCC) or secondary wastewater effluent. The carboys were aerated continuously with a regenerative blower (Model VFC084P-5T, Fuji Blowers, Saddle Brook, NJ) and periodically injected with pure CO₂ to lower the culture pH and provide a source of carbon.

2.2. PBR System

Tubular PBRs contained swirl vanes to enhance mixing by creating a spiral flow and were connected by pipes and fittings to each other and to the rest of the circulation system (**Figure. 1**). The PBRs were constructed by welding sheets of 15-mil clear linear low-density polyethylene (LLDPE) into tubes (I.D. 11.4 cm ! 3 m long) using an AIE double impulse foot heat sealer (Industry, CA). The swirl vanes, improvised from polyethylene grain augers (Lundell Plastics Corporation, Odebolt, IA) were fixed inside a transparent schedule 40 polyvinyl chloride (PVC) collar (O.D. 11.4 cm ! 5.1 cm long) with a steel pin. The sharp edges of the PVC collar were removed with a bench grinder to prevent damaging the LLDPE. The swirl vanes were spaced 0.9 m apart and held in place using cable ties wrapped around the collar on the outside of the PBRs.

The ends of the PBR tubes were attached to cam-lock fittings (Model 400D, Banjo Corporation, Crawfordsville, IN) and connected in series by a U-shaped manifold constructed of two schedule 40 PVC 90° elbows (10.2 cm). The 10.2-cm cam-lock fittings on the PBR inlet and outlet were reduced to 5.1 cm to accommodate the transparent flexible PVC tubing that

was connected to the suction and discharge side of a centrifugal pump (Model 1MC1D5D0,

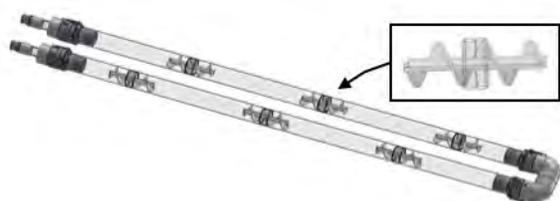


Figure 1. OMEGA photobioreactor (PBR) tubes with swirl vanes. PBRs were made of flexible, clear LLDPE connected with cam-lock fittings to a U-shaped PVC manifold. The six swirl vanes (see insert enlargement) directed the flow into a helical path to improve mixing and light exposure of the microalgae.

ITT-Goulds, Seneca Falls, NY). The speed of the centrifugal pump was adjusted using a 1-HP GS-2 variable frequency drive (Automation Direct, Cumming, GA). A sensor manifold located before the pump inlet housed a paddlewheel flow meter (Model 2537, Georg Fischer LLC, Tustin, CA), pH probe (Model 2750, Georg Fischer LLC, Tustin, CA), and dissolved oxygen (DO) sensor (Sensorex, Garden Grove, CA) and provided connection to the gas exchange and harvesting column (GEHC) (Figure 2).

2.3. Gas Exchange and Harvesting Column (GEHC)

The GEHC shown in Figure 3 was designed to: (1) manage concentrations of DO using an oxygen stripping device (OSD) based on a design by Barnhart [19], (2) supply CO₂ to the microalgae culture and control pH, and (3) provide a settling chamber to collect aggregating microalgae for harvesting. Approximately 5% of the total system volume was diverted to the GEHC per minute, using a 12 VDC SHUR-FLO diaphragm pump (Model 2088-343-135, SHUR-FLO, Costa Mesa, CA). The pumping rate into the GEHC was adjusted by changing the voltage setting on the variable DC power supply (Model HY3005D, Mastec Power Supply, San Jose, CA).

The culture from the PBR entered the GEHC through the OSD section and cascaded over five stacked PVC plates (20 cm² each) housed in a pipe (schedule 40 PVC: 15.2 cm diameter ! 0.3 m) attached to the top of the GEHC with a rubber coupling (model 1056-63, Fernco Inc., Davidson, MI). After the OSD, the culture entered the gas-injection pipe (schedule 40 clear PVC

7.6-cm diameter ! 2.13 m), containing a CO₂ diffuser made from soaker hose (22 cm²) located 1.8 m from the top of the column. The compressed CO₂ source was a mixture of 8.5% CO₂ in air (V/V) to simulate the concentration of CO₂ in typical flue gas [20]. The CO₂ input was regulated by a pH/temperature sensor (GF Signet 2750 pH sensor electronics, Georg Fischer LLC, Tustin, CA).

After the gas-injection section, the culture enters the settling chamber, which consisted of a section of clear pipe (schedule 40 PVC 15.2 cm diameter ! 0.91 m) with a ball valve (1.3 cm) drain at the bottom. The culture entered from the gas-injection pipe, which protruded 0.3 m into the settling chamber, and was capped to direct the outflow to the sides and prevent resuspending biomass collected at the bottom of the chamber. The culture returned to the PBRs from the settling chamber through a pipe (schedule 80 PVC 1.3 cm diameter) with a flow meter (model F-40377LN-8, Blue-White Industries LTD, Huntington Beach, CA) and a pneumatic pinch valve (1.3 cm VMP Series, AKO Armaturen & Separations GmbH, Germany). The pinch valve maintained a constant liquid height in the GEHC, using a feedback signal generated by a pressure transducer (model PTD25-10-0015H, Automation Direct, Cumming, GA) in the settling chamber.

2.4. Instrumentation and Control

A custom instrumentation and control (I&C) system was constructed for process automation and data logging (Figure 4). The pH and temperature sensors in the PBR and GEHC were connected to a GF Signet model 8900 multi-parameter transmitter (Georg Fischer LLC, Tustin, CA). Output signals from the transmitter, GEHC pressure transducer, flow meter, and photosynthetically active radiation (PAR) sensor were attached to inputs of a DL06 programmable logic controller (PLC) (Automation Direct, Cumming, GA). The PLC transferred data to a human-machine interface (HMI) created using LookoutDirect software (Automation Direct, Cumming, GA) that displayed real-time data and allowed operators to specify desired setpoints for the GEHC pH and liquid level. Feedback control loops generated PLC output signals based on the difference between the actual value and the desired setpoint entered into the HMI. When the pH in the GEHC exceeded the setpoint, the PLC output signal adjusted CO₂ injection rates through an Aalborg mass-flow controller (MFC) (Aalborg, Orangeburg, NY). Similarly, a current/pressure (I/P) transducer (Model IP610-060-D, OMEGA Engineering Inc. Stamford, CT), regulated by the PLC output signal, varied the pinch valve position as needed to maintain the desired liquid level in the GEHC. The objective of both control loops was to minimize the difference between the

column. The mass of CO₂ dissolved into solution was determined by measuring the pH change in the water

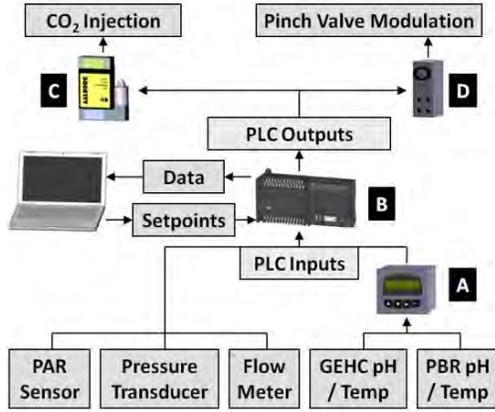


Figure 4. Components of the I&C system. Inputs from the sensors were routed through a multi-parameter transmitter (A) or directly into a PLC (B) were transferred to a computer database. Setpoint values established using an HMI modulated PLC outputs that controlled a mass flow controller for CO₂ injection (C) and an I/P transducer (D) to regulate pinch valve positioning.

column using the stoichiometry of the acid-base reaction relationship between the NaOH and H₂CO₃^{*} described in Equations (2) and (3).



The CO₂ uptake efficiency is the amount of CO₂ absorbed in the GEHC column divided by the amount supplied. The amount of CO₂ absorbed was determined indirectly by measuring pH changes in the water column. The total moles of CO₂ injected into the test column was determined using Equation (4), which allowed the calculation of the mass transfer efficiency with Equation (5). For this experiment, the mass transfer efficiency was calculated based on the amount of CO₂ required to change the pH of the solution from 10 to 9, 9 to 8, 8 to 7 and below 7.

$$M_{CO_2} = \frac{Q \cdot t \cdot p_{CO_2}}{RT} \quad (4)$$

$$CO_{2\text{Eff}} = \frac{M_{NaOH}}{2M_{CO_2}} \cdot 100 \quad (5)$$

A comparison of the CO₂ mass transfer rate in the GEHC and carbon consumption rate of microalgae in the PBR gave a “detention time ratio” that estimates the amount of time the culture can remain in the PBR before carbon replenishment is needed. The overall mass

transfer coefficient ($K_L a$) and subsequent CO₂ mass transfer rate in the GEHC were calculated from the titration data using Equations (6) and (7), whereas the carbon uptake rate in the PBR was approximated with Equation (8).

$$K_L a = \ln \left(\frac{C^* - C_2}{C^* - C_1} \right) / (t_2 - t_1) \quad (6)$$

$$\frac{dc}{dt} = K_L a (C^* - C) \quad (7)$$

$$C_{Uptake} = \frac{P_{Algae} \cdot f_{Carbon} \cdot A_{PBR}}{D_{Solar} \cdot 60 \cdot M_{Car} \cdot PBR_{Vol}} \quad (8)$$

Results from Equations (7) and (8) were used to calculate the detention time ratio between the GEHC and the PBR with Equation (9).

$$DTR = \frac{GEHC_{Xfer\ Rate}}{C_{Uptake}} \quad (9)$$

2.6. System Inoculation, Sampling Protocol, and Harvesting Procedures

Final plant effluent (FPE) was collected from the Santa Cruz wastewater treatment facility mixed with inoculum in a plastic barrel, and weighed with an Ohaus Defender scale. The contents of the barrel were transferred into the GEHC using a submersible pump. As the liquid level in the GEHC approached the setpoint, the I&C system opened the pinch valve and diverted liquid into the PBR. The volume required to fill the entire system (~110L) was determined by weight.

The optical density (OD₇₅₀), NH₃-N (Hach method 10031), NO₃-N (Hach method 8039), and total suspended solids (TSS) concentration (method 2540D) [25] were measured on samples collected daily from a port located on the discharge side of the PBR circulation pump. Differences in the OD₇₅₀ before and after physically shaking the PBR to resuspend settled biomass were used to determine the percent sedimentation within the PBR using Equation (10).

$$SED_{\%} = \frac{OD_{750\text{After}} - OD_{750\text{Before}}}{OD_{750\text{After}}} \quad (10)$$

The GEHC was drained into a barrel and refilled with fresh FPE when the ammonia concentration approached zero. The barrel was weighed to determine volume (assuming a density of 1 kg l⁻¹) removed from the GEHC and samples were collected for TSS analysis. The volume of water remaining in the PBR was determined by subtracting the harvest volume from the total system volume. This enabled calculation of the total

biomass produced between harvest periods, the biomass concentration factor in the GEHC, and areal productivity (Equations 11-13).

$$A_{Growth} = TSS_{GEHC} \cdot H_{Vol} + TSS_{PBR} \cdot PBR_{Vol} - I_{Mass} \quad (11)$$

$$HCF = \frac{TSS_{GEHC}}{TSS_{PBR}} \quad (12)$$

$$P_{Algae} = \frac{A_{Growth}}{APBR \cdot D_{Harvest}} \quad (13)$$

The result from Equation (11) and the totalized volume of gas injected into the GEHC recorded by the I&C system were used to calculate the CO₂-to-biomass conversion efficiency with Equation (14).

$$CO_{2Conv} = \frac{A_{Growth} \cdot f_{Car}}{\left(\frac{V_{Gas} \cdot p_{CO_2}}{RT} \cdot \frac{12g C}{mol CO_2} \right)} \quad (14)$$

3. Results and Discussion

3.1. System Design and Performance

A 110-liter prototype OMEGA system was constructed with two tubular PBRs floating in a seawater tank, connected to an external GEHC and an instrumentation and control system (**Figure 5**). The system components (PBRs, GEHC, and I&C) are described in the Materials and Methods. The PBRs, made of inexpensive plastic (LLDPE), were tested for their ability to support photosynthesis. The GEHC served to control DO, provide CO₂, and remove and harvest microalgal biomass. The I&C system monitored or controlled pH, temperature, flow rate, and DO concentrations, recording sensor outputs every three minutes.

Temperature and pH were measured both near the outlet of the PBR in the sensor manifold (**Figures 2 & 5**) and in the GEHC (**Figure 3**). The two monitoring sites provided comparative data, and the GEHC pH sensor served to control CO₂ injection rates, using a setpoint of pH 7.60. The I&C system also included measurements of photosynthetically active radiation (PAR) and the effect of light on cultures using FRRF, a rapid, nondestructive, technique that detects variable chlorophyll fluorescence in real time [26]. A decrease in the ratio of variable fluorescence to maximum fluorescence (F_V/F_M) indicates a decreased quantum yield resulting from damage to photosystem II and is used as an index for photoinhibition [27]. Reported F_V/F_M ratios in cultures exposed to high irradiance indicated up to 90% photoinhibition [27, 28].

To limit sedimentation of microalgae in the PBRs, cultures were circulated at velocities ranging from 14 to 21 cm sec⁻¹, flow rates that reportedly prevent sedimentation in open ponds [29].

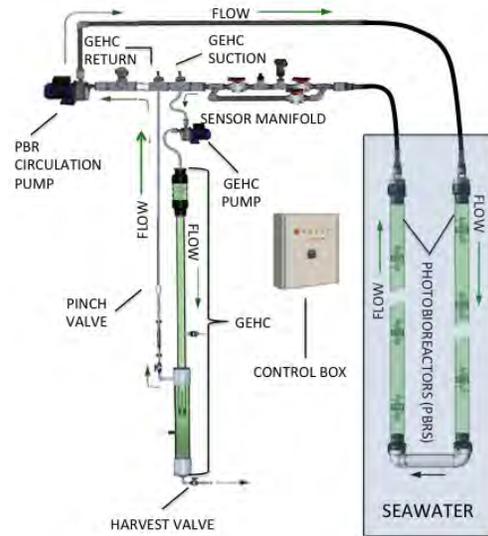


Figure 5. Component and flow diagram of the OMEGA system showing the circulation through the PBRs, sensor manifold, and side loop for the GEHC.

Microalgae suspension and mixing were enhanced by swirl vanes, which imparted a helical flow pattern. With the combination of flow rates and swirl vanes, microalgae settling in the PBRs never exceeded 14% of the total biomass. The swirl vanes also increased turbulence, which is known to improve nutrient exchange rates and light exposure in PBR cultures [30]. In cultures grown in laminar flow systems photoinhibition and light limitations are observed, both of which suppress productivity [28-30]. While swirl vanes may have improved suspension and light availability and hence productivity, two difficulties noted with the swirl vanes tested were 1) increase biofouling on the walls of the PBR in their vicinity and 2) increased drag, which increased pumping energy.

To assess the performance of the prototype OMEGA system, two consecutive experiments were conducted in April and May 2012. Experiment 1 lasted 13.5 days and experiment 2 lasted 8.6 days. In both experiments 1 and 2, the comparisons of hourly mean DO vs. PAR and DO vs. F_V/F_M are shown in **Figure 6**. The increase in photosynthetically generated DO correlates well with PAR from sunrise (06:00) to late afternoon (16:00), although the DO curve is artificially flattened at peak solar irradiance (~12:00) because the DO values exceeded the upper threshold for the oxygen sensors (212% saturation) (**Figure 6, DO saturation**). After 16:00, the decline in DO was due to a combination of decreased photosynthesis, respiration, and DO removal by the OSD in the

GEHC (see Materials and Methods: GEHC). The relative contribution of these different factors was not determined.

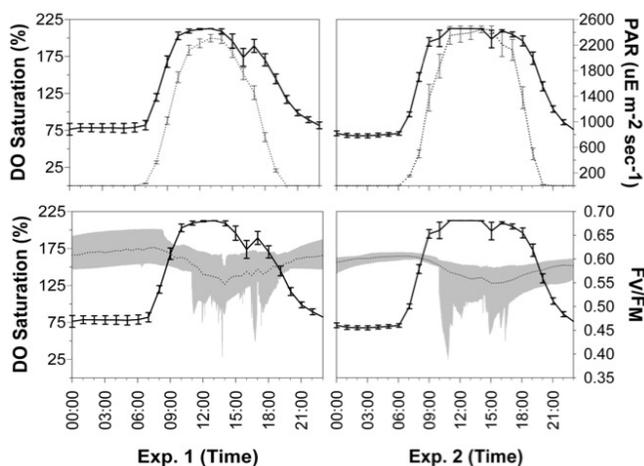


Figure 6. DO concentration, PAR and FV/FM values for experiment 1 (left) and experiment 2 (right). (Top) Mean hourly (\pm SE) concentration photosynthetically generated DO (solid line) increases and decreases as a function of PAR (dotted line). (Bottom) The mean hourly F_v/F_m ratio (dotted line) overlaying the range of data points (shaded area) measured by FRRF indicates that the culture has maintained high photoconversion efficiency. The slight suppression of the F_v/F_m ratio during mid-day is a result of photoinhibition caused by PAR intensity and elevated concentrations of DO (solid line).

At peak DO production and peak irradiance, there was a slight photoinhibition indicated by F_v/F_m measurements, which dipped to 0.49 in experiment 1 and 0.54 in experiment 2 (Figure 6, bottom). Rubio and co-workers [31] noted that in long tubular PBRs DO buildup at high irradiance caused photoinhibition and they identified this as one of the greatest constraints on the scale-up of PBRs. The solution for the OMEGA system is to adjust the ratios of residence time in the PBR to the transfer frequency to the GEHC, which depends on PBR length, the number of GEHCs, and the flow rate. In the OMEGA system the tested residence time of the culture in the PBRs was 20 min, based on a PBR length of 3.1 m, a 4.5% transfer to the GEHC, and a PBR flow rate of 86–130 lpm. In the future, DO as it relates to photoinhibition can be managed for PBRs of a given length using real-time FRRF and DO data in the control logic algorithm to modify GEHC input and flow rates. The size and configuration of the OSD can also be modified to increase the exchange of DO. In addition to DO management, the GEHC was where CO₂ was injected into the culture, both as a source of inorganic carbon for microalgae growth and to control the culture pH. Both carbon availability and pH control are dependent on

efficient CO₂ delivery, and both are critical to the productivity and economics of large-scale microalgae cultivation [23, 32–35]. Beal *et al.* [36, 37] have shown that commercial CO₂ supply is one of the biggest contributors to overall energy use and cost of microalgal biofuel production.

Traditionally CO₂ delivery systems, using sparging tubes bubbling into shallow cultures, resulted in 80–90% losses of CO₂ to the atmosphere [21, 38]. Diffusion methods, using silicon membranes or hollow fibers reduce CO₂ loss to the atmosphere but are cost prohibitive and prone to biofouling [21, 33, 39, 40]. Bubble columns, like the GEHC, are simple, low cost, and capable of reducing CO₂ losses to less than 20% [21, 38].

3.2. GEHC Mass Transfer Efficiency and Recycle Rate

The CO₂ mass transfer efficiency in a gas exchange column is influenced by the pH of the receiving liquid, by the height of the liquid column, which determines bubble contact time, by the size of the bubbles, which determines contact area, and by the CO₂ content of the gas bubbles. Experiments with the GEHC indicated that higher pH and a taller column increased CO₂ mass transfer efficiency (Figure 7). In the OMEGA system tested here, however, site restrictions limited the gassing portion of the GEHC to 1.8 meters, which gave a mass transfer efficiency of approximately 50% for the operating pH range in the GEHC of between pH 7.0 and 8.25. The overall volumetric mass transfer coefficient (K_{La}) was 0.21 min⁻¹ (SE 0.01, n=3), and the mass transfer rate of CO₂ was 1.69 ! 10⁻⁴ mol l-min⁻¹ (SE 1.03 ! 10⁻⁵, n=3).

Assuming an areal productivity of 20 g m⁻² day⁻¹, the carbon consumption rate in the PBR was calculated to be 8.72 ! 10⁻⁶ mol l-min⁻¹. Balancing the mass transfer rate in the GEHC with the carbon consumed by microalgae would require one minute in the GEHC for every 20 minutes in the PBR. Therefore, 5 lpm (4.5% total system volume per minute) were diverted from the PBR to the GEHC for gas exchange. This pumping rate provided the GEHC with an overdesign factor of 1.5 to ensure that carbon consumption in the PBR did not exceed the injection capacity and limit microalgae growth.

3.3. GEHC Operation

Diverting only a portion of the culture for CO₂ injection resulted in a pH differential between the PBR and GEHC (Figure 8, top). This differential was greatest at times of the highest photosynthetic activity, which correlated with the highest PAR and highest gas injection rate during the day when most inorganic carbon was consumed (Figure 8, bottom). The control system could maintain the pH near the setpoint (7.60), indicating that the mass transfer rate of CO₂ in the GEHC was not exceeded by

the rate of carbon removal in the PBR. Thus the control system could monitor and deliver the amounts of CO₂

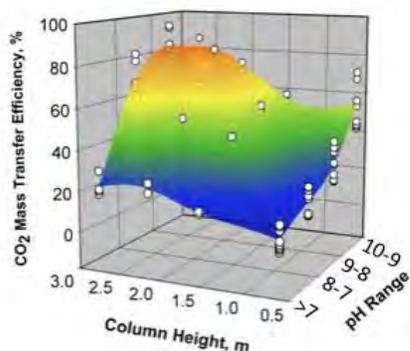


Figure 7. Efficiency of CO₂ mass transfer in the GEHC relative to the height of the column and the pH of the solution. Data were obtained (n=76) experimentally using tap water, pH adjusted (>11.0) with NaOH. For practical reasons a maximum column height of 1.8 meters was used.

demanded by the microalgae. Furthermore, this system reduced CO₂ losses as compared to “on-off” systems that produce hysteresis and potentially large variations from the desired pH setpoint [22, 32]. Further improvements in process control may be realized using predictive models to control pumping rates. Rubio et al. [31] developed a predictive model capable of estimating carbon depletion in tubular bioreactors based on pH differential, which could be adapted for the OMEGA system by comparing pH in the PBRs versus the GEHC. Further research is needed to determine how such pumping controls could improve energy efficiency and biomass productivity.

The details of harvesting intervals, biomass production, and carbon utilization for both experiments 1 and 2 are given in **Table 1**. Harvesting occurred every 0.83 to 2.79 days, triggered by the depletion of NH₃-N (see below). It was noted that microalgae accumulated in the settling chamber at the bottom of the GEHC hence the biomass in the GEHC was higher than in the PBRs by a factor of 2.0 ± 0.1 (n=7) in experiment 1 and 1.4 ± 0.1 (n=7) in experiment 2. These calculated concentration factors were based on the total volume of the GEHC however, and therefore do not represent the concentrations at the bottom of the settling chamber.

Harvesting efficiency in the GEHC could be improved by adding coagulants or by integrating an electrocoagulation (EC) system, which produces coagulants *in situ* [41, 42]. The EC system is well suited for OMEGA because it has no moving parts and is easily automated [42, 43]. Furthermore, by adding a small amount of seawater to the culture isolated in the GEHC, which would increase its ionic strength, would lower the

power required for EC and electrolysis would produce electrolytic chlorine, which could contribute to

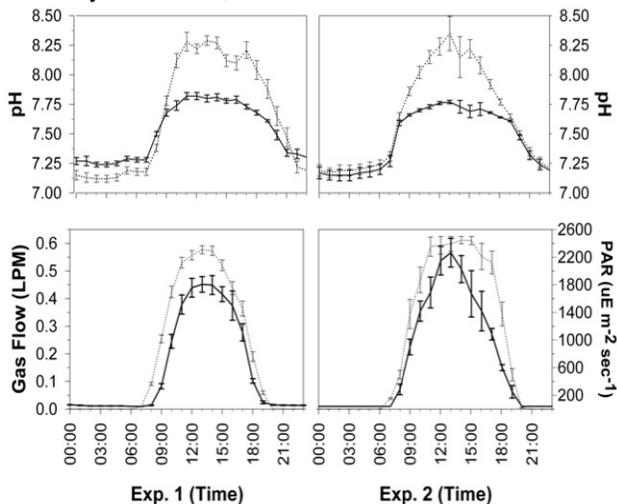


Figure 8. The mean hourly (\pm SE) pH, gas flow, and PAR recorded during experiment 1 (left) and experiment 2 (right). **Top:** pH values measured inside the GEHC (solid line) compared to pH in the PBR (dotted line). The differential between the GEHC and PBRs increases during the day due to carbon assimilation for photosynthesis. The rate of CO₂ injection was controlled to maintain the GEHC pH setpoint during the day. The slow decrease in pH at night is attributed to respiration. **Bottom:** Gas flow rates (solid lines) indicating CO₂ demand correlated with PAR (dotted lines), and inferred rates of photosynthesis. The pH of the GEHC and PBRs equalize at night due to respiration.

disinfecting the residual water before release into the environment [43, 44]. Additional research is needed to assess the EC harvesting process for the OMEGA system.

3.4. Carbon Utilization and Biomass Production

The totalized volume of simulated flue gas (8.5% CO₂/91.5% air V/V) injected into the GEHC and the biomass produced during experiments 1 and 2 are shown in **Figure 9**. The changes in gas utilization, which appear as a “staircase” in the plot, reflect the day/night cycles and the on-demand input of CO₂. The curve slopes upward during light periods due to increased gas flow required to satisfy the carbon demand for photosynthesis by the microalgae. The curve plateaus during dark periods when there is no CO₂ demand. The biomass produced relative to the amount of CO₂ injected was used to calculate the CO₂ utilization efficiency (**Table 1**): For experiment 1 the mean efficiency was $53.8\% \pm 4.0\%$ (n=9) and for experiment 2 it was $60.2\% \pm 4.7\%$ (n=7), with values from both experiments ranging from 31.6%

to 80.9%. These measured CO₂ conversion efficiencies correspond well to the CO₂ solubility values obtained in

Table 1. Harvesting frequency, biomass yields and mass of carbon injected into the GEHC used to calculate carbon conversion efficiency and areal biomass productivity during experiment 1 and 2.

Experiment 1						
Elapsed Time, Days	Days Between Harvest	Biomass Produced, g	Carbon Required, g	Carbon Injected, g	Carbon Conversion Efficiency, %	Biomass Productivity, g m ⁻² day ⁻¹
1.85	1.85	5.2	2.6	5.8	45.0	4.0
2.83	0.98	8.4	4.2	8.2	51.3	12.3
3.66	0.83	2.6	1.3	4.1	31.6	4.5
4.79	1.13	13.4	6.7	13.1	51.1	17.0
6.73	1.94	23.1	11.5	18.0	64.2	17.1
8.75	2.02	15.3	7.7	12.9	59.4	10.9
9.68	0.93	11	5.5	10.1	54.5	17.0
12.5	2.79	29.3	14.7	19.6	74.9	15.1
13.5	1.06	15.2	7.6	14.5	52.5	20.6
Mean (SE)		13.7 (4.6)	6.9 (1.4)	11.8 (1.8)	53.8 (4.0)	13.2 (1.9)
Experiment 2						
0.92	0.92	6.1	3.0	7.1	42.7	9.5
1.87	0.95	8.1	4.1	6.4	63.7	12.3
2.89	1.02	15.4	7.7	11.4	67.7	21.7
4.89	2.00	23.1	11.6	19.3	59.9	16.6
5.88	0.99	12.3	6.2	11.0	56.2	17.8
6.82	0.94	8.3	4.2	8.3	50.2	12.7
8.61	1.79	21.0	10.5	13.0	80.9	16.8
Mean (SE)		13.5 (2.5)	6.8 (1.3)	10.9 (1.7)	60.2 (4.7)	15.3 (1.6)

the titration experiment (see section 3.3). Gas transfer in the OMEGA GEHC could be improved by using a taller column (greater contact time for rising bubbles), smaller bubbles (greater surface-to-volume ratio), or higher CO₂ concentrations. The site restricted column height, available equipment determined the bubble size, and the CO₂ concentration was chosen to simulate flue gas to determine if it would be adequate to support microalgae cultures in the prototype system.

The observed productivity, normalized to PBR surface area per day, averaged 13.2 g ± 1.9 (n=9), in experiment 1 and 15.3 g ± 1.6 (n=7) in experiment 2 (**Table 1 & Figure 9 bars**). In experiment 1, sampling periods one and three had low biomass yields. The initially low yield, 4.0 g m⁻² day⁻¹, may have been due to a period of culture acclimation. The second low yield on the third harvest cycle (4.5 g m⁻² day⁻¹) was due to a short incubation period with minimal light exposure (**Figure 9**). Despite these limitations, the average observed areal productivities were within the range of values reported for open ponds [10, 45, 46], although somewhat less than

those reported for other PBR systems [5, 47]. This disparity with other PBRs may be due to lower nutrient concentrations in the unsupplemented wastewater, the presence of grazers and/or pathogens, or to other limiting culture conditions (e.g., time of year or culture temperature). Long-term experiments are required to determine the limiting factors in the OMEGA system and its potential yields.

3.5. OMEGA and Wastewater Treatment

The OMEGA system used secondary wastewater effluent as a source of nutrients for microalgae cultures and the concentrations of ammonia [NH₃] and nitrate [NO₃⁻] were monitored (**Figure 10**). The rapid utilization of NH₃ required periodic replacement of spent culture medium with fresh wastewater. Between 16% and 34% of the total system volume was harvested from the GEHC and replenished to increase the concentration of [NH₃] (**Figure 10; top**). While [NH₃] followed a consistent pattern of utilization and replenishment, the corresponding [NO₃⁻] showed increases, decreases, or no change

(Figure 10, middle). The increases in $[\text{NO}_3^-]$ were attributed to nitrification by ammonia-oxidizing bacteria,

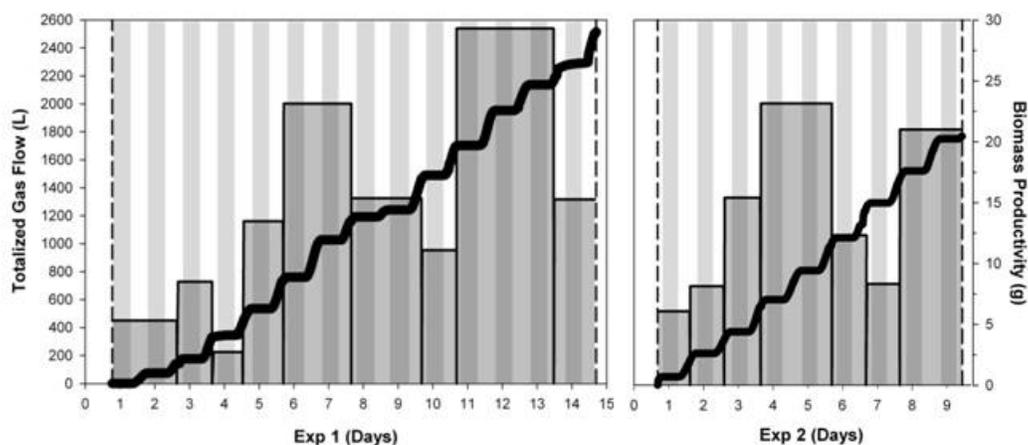


Figure 9. Microalgal CO_2 utilization and productivity in experiment 1 (left) and experiment 2 (right) with the day/night cycle indicated by vertical stripes. Totalized gas flow (8.5% CO_2 V/V) (bold black line) and biomass production (histogram). The totalized gas flow has a “staircase” shape because CO_2 was injected on demand; photosynthesis caused injection during the day (slope up), but not at night (plateaus). The histogram shows biomass production in the height of bars (right axis, g) and the time between harvesting in the width of the bars (bottom axis, days).

which are known to be present in wastewater [48]. The decreases in $[\text{NO}_3^-]$ observed in experiment 1 (days 5-8) and experiment 2 (days 1-3 and 4-6) were attributed to the depletion of NH_3 and the utilization of NO_3^- as the microalgae’s secondary nitrogen source (Figure 10, middle). Changes in preferred nitrogen sources have been observed for other microalgae [49].

The calculated rates of ammonia removal varied, but were positive, whereas the rates of nitrate removal were both positive and negative; a “negative removal” rate means nitrate production (Figure 10, bottom). The NH_3 removal rate averaged 0.29 ± 0.04 ($n=12$) and 0.49 ± 0.03 ($n=11$) $\text{mg l}^{-1} \text{hr}^{-1}$ for experiments 1 and 2, respectively. In contrast, NO_3^- removal rates were predominantly positive during experiment 1 but predominantly negative in experiment 2. In both experiments the actual nitrate concentrations represented the combination of production and utilization at each sampling point. A more effective utilization of total nitrogen may be achieved with longer retention times.

These results indicate that microalgae growing in a prototype OMEGA system can contribute to biological nutrient removal in wastewater treatment. It is well established that microalgae in ponds and other PBR designs can effectively remove nutrients from wastewater [50-53]. It has also been demonstrated that microalgae can remove heavy metals [53, 54] and organic contaminants, including surfactants, phenols, and hydrocarbons [53, 55-57]. Research reported elsewhere indicates that the OMEGA system can also contribute to the removal

of pharmaceuticals and personal care products as well as compounds of emerging concern [58].

Combining microalgae cultivation with wastewater treatment can improve water quality and provide biomass for biofuels or other products, but it remains to be demonstrated that the economics and EROI of the combined systems support its development [6, 9, 14].

4. Conclusion

OMEGA has the potential of co-locating microalgae cultivation with two major waste-streams from coastal cities: wastewater and CO_2 . By situating OMEGA systems in the vicinity of offshore wastewater outfalls and CO_2 sources, such as near-shore power plants, OMEGA can transform these waste streams into resources that produce biofuels and treat wastewater without competing with agriculture for water, fertilizer, or land [12]. The experiments presented here explored the technical feasibility of OMEGA, using a 110-liter prototype system that was built and tested over a 23-day period. Microalgae in secondary-treated wastewater circulated through PBRs floating in seawater tanks and through a gas exchange and harvesting column, while a custom I&C system monitored and controlled critical culture parameters. Analyses indicated that the system was supersaturated with dissolved oxygen during the day due to photosynthesis, but at the highest light levels there was only slight photoinhibition. The system rapidly used the $\text{NH}_3\text{-N}$ in wastewater and had a CO_2 conversion efficiency of

>50%; better than the 10-20% conversions in other systems [21, 38]. The areal productivity of the system aver-

aged $14.1 \text{ g m}^{-2} \text{ day}^{-1}$ overall with peaks above 20 g m^{-2}

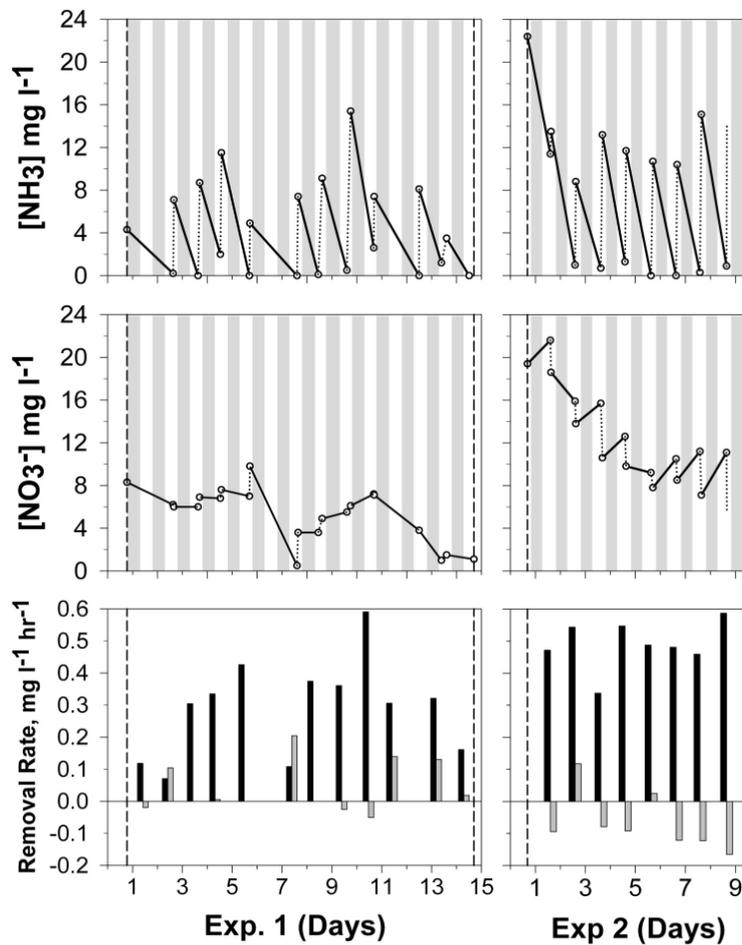


Figure 10. Time course for the addition and utilization of $[\text{NH}_3\text{-N}]$ (top), $[\text{NO}_3\text{-N}]$ (middle), and removal rates (bottom) for experiment 1 (left) and experiment 2 (right). As in Fig. 9 the day/night cycle is represented by white/gray shading and each line segment (top/middle) shows changes in nutrient concentration from the time of wastewater addition to harvesting, corresponding to “biomass production” bars in Fig. 9. Removal rates (bottom) are shown as positive when nutrients were depleted or negative when nutrient concentrations increased. The NH_3 removal rates (black bars) were always positive, but $\text{NO}_3\text{-N}$ removal rates (grey bar) were occasionally negative due to nitrification. The microalgae preferred $\text{NH}_3\text{-N}$ as their nitrogen source and consume $\text{NO}_3\text{-N}$ once the supply of $\text{NH}_3\text{-N}$ was exhausted.

day^{-1} , values consistent with reported U.S. average microalgae productivity of $13.2 \text{ g m}^{-2} \text{ day}^{-1}$ [58]. The microalgae consistently removed >90% of the $\text{NH}_3\text{-N}$ from the secondary-treated municipal wastewater tested. This result, combined with observations that the OMEGA system can remove other wastewater contaminants [59], suggests that a scaled-up system could provide effective wastewater treatment services.

Many open questions remain with regard to the feasibility of large-scale OMEGA systems. The small-scale prototype OMEGA system was intended for experimentation and was not designed for energy efficiency or economical scale up. For large-scale OMEGA deployment dense configurations of PBRs, improved hydrodynamics, optimized pumping and mixing, and more sophisticated process control algorithms will be needed to increase yields, improve EROI, and lower operating costs. In addition to the EROI and economics,

questions about the impact of biofouling, concerns about engineering systems that can cope with marine environments, and environmental issues around both environmental impact and environmental regulations will need to be answered. It remains to be seen if the need for sustainable biofuels will drive the innovation necessary to address these questions to develop large-scale OMEGA systems.

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Nomenclature

Q_{Gas}	= Gas flow rate, lpm
P_{Algae}	= Microalgal productivity, g m ⁻² day ⁻¹
f_{Carbon}	= Fraction carbon in biomass
A_{PBR}	= Area of the PBR tubes, m ²
R	= Ideal gas constant, 0.08206 L-atm mol ⁻¹ K ⁻¹
T	= Temperature, K
f_{Abs}	= Fraction CO ₂ absorbed
D_{Solar}	= Length of solar day, hours
M_{Car}	= Molar mass of carbon, g mol ⁻¹
pCO_2	= CO ₂ partial pressure, atm
M_{CO_2}	= Moles of CO ₂
t	= Time, minutes
CO_{2Eff}	= CO ₂ mass transfer efficiency, %
M_{NaOH}	= Moles of NaOH
K_{La}	= Overall volumetric mass transfer coefficient, min ⁻¹
C^*	= Equilibrium [CO ₂], mol l ⁻¹
C	= [CO ₂], mol l ⁻¹
PBR_{Vol}	= Volume of PBR tubes, l
DTR	= Detention time ratio, unitless
$GEHC_{Xfer Rate}$	= GEHC CO ₂ mass transfer rate, mol l ⁻¹ min ⁻¹
C_{Uptake}	= Carbon uptake in the PBR, mol l ⁻¹ min ⁻¹
A_{Growth}	= Total biomass produced, g
TSS_{GEHC}	= Total suspended solids content of culture harvested from GEHC, mg l ⁻¹
H_{Vol}	= Volume of culture harvested from GEHC, l
TSS_{PBR}	= Total suspended solids content of the culture in the PBR,

mg l⁻¹

I_{Mass} = Initial mass of solids in the system, g

HCF = Harvesting concentration factor, unitless

$D_{Harvest}$ = Harvesting frequency, days

CO_{2Conv} = CO₂ to biomass conversion efficiency, %

V_{Gas} = Volume of gas injected into the GEHC between harvest periods

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**Appendix B:
Potential Impact of Biofouling on the Photobioreactors
of the Offshore Membrane Enclosures for Growing
Algae (OMEGA) System**



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Potential impact of biofouling on the photobioreactors of the Offshore Membrane Enclosures for Growing Algae (OMEGA) system [☆]



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HIGHLIGHTS

- OMEGA deployed in the marine environment is subject to biofouling.
- Biofouling differed on the clear and opaque plastics of photobioreactors (PBRs).
- Rectangular and tubular PBRs had similar biofouling patterns—mostly on wetted sides.
- Biofouling attenuates light, decreases algae productivity, requires cleaning.
- The OMEGA system will be a floating reef in coastal waters.

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ABSTRACT

The influence of PBR composition [clear polyurethane (PolyU) vs. clear linear low-density polyethylene (LLDPE) (top) and black opaque high-density polyethylene (bottom)] and shape (rectangular vs. tubular) on biofouling and the influence of biofouling on algae productivity were investigated. In 9-week experiments, PBR biofouling was dominated by pennate diatoms and clear plastics developed macroalgae. LLDPE exhibited lower photosynthetic-active-radiation (PAR) light transmittance than PolyU before biofouling, but higher transmittance afterwards. Both rectangular and tubular LLDPE PBRs accumulated biofouling predominantly along their wetted edges. For a tubular LLDPE PBR after 12 weeks of biofouling, the correlation between biomass, percent surface coverage, and PAR transmittance was complex, but in general biomass inversely correlated with transmittance. Wrapping segments of this biofouled LLDPE around an algae culture reduced CO₂ and NH₃-N utilization, indicating that external biofouling must be controlled.

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1. Introduction

Strong environmental, social, ethical, and economic incentives support the development of biofuels as a sustainable alternative to petroleum-based liquid fuels (Caspeta et al., 2013; Running, 2012; Tillman et al., 2009). Microalgae appear to be the most promising of the many different feedstocks for making biofuels,

particularly if cultivation is coupled to wastewater treatment (Brennan and Owende, 2010; Pittman et al., 2011), which significantly improves the overall economics of the system (Beal et al., 2012; Lundquist et al., 2010). To avoid the excessive costs of pumping water long distances, however, algae cultivation facilities must be close to existing wastewater plants (Fortier and Sturm, 2012). Unfortunately, most cities cannot build traditional algae-cultivation ponds or “raceways” in proximity to existing treatment plants, because they are surrounded by urban infrastructure that would be prohibitively expensive to move or modify. Coastal cities, however, could use the proposed OMEGA system, in which floating photobioreactors (PBRs) are filled with municipal wastewater from offshore outfalls, meeting the requirement for proximity without disrupting urban infrastructure (Trent et al., 2012). Furthermore, 16

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OMEGA does not compete with agriculture for land, water, or fertilizer.

The OMEGA system has a number of additional advantages over land-based algae cultivation systems. For example, because the wastewater-filled PBRs float offshore, they are surrounded by seawater, which provides buoyancy for structural support, a heat-sink to prevent overheating [a major PBR problem on land (Carvalho et al., 2006)], and creates a containment system; i.e., the cultivated freshwater algae cannot thrive in seawater if they accidentally leak out. Furthermore, the salt gradient between seawater and wastewater can be used for forward osmosis (FO)—a process that removes clean water from the PBRs (Buckwalter et al., 2013). FO concentrates both nutrients in the wastewater, which stimulates algae growth, and algae, which facilitates harvesting. Moreover, FO cleans the wastewater, creating opportunities for capturing and reusing the otherwise wasted water (Claxton and Trent, personal communication).

To be implemented successfully, OMEGA must overcome a number of challenges, one of which is the biofouling that inevitably occurs on any exposed surface in the marine environment (Durr and Thomason, 2010). The rate and extent of biofouling depends on the nature of the surface, the location, and local conditions, such as depth, currents, water clarity, season, and extant biology. The process of biofouling is dynamic and frequently sequential (Briand et al., 2012; Zardus et al., 2008) with chronological steps that reportedly include: (1) the formation of an organic coating on the exposed surface, (2) microfouling with colonies of bacteria, cyanobacteria, protists, diatoms, and other unicellular algae, and (3) macrofouling with filamentous cyanobacteria, multicellular algae, and invertebrates (Bravo et al., 2011; Railkin, 2004).

The negative consequences of biofouling include increased drag, decreased buoyancy, accelerated degradation or corrosion, impaired function, and significant costs associated with equipment maintenance, repair, or replacement (Edyean, 2010; Schultz et al., 2010). Furthermore, PBRs require light to support algae growth and biofouling can influence both the quantity and quality of light that penetrates transparent PBR materials that provide natural light (Brush and Nixon, 2002; Wong et al., 2011) or critical regions of optical fibers that have been suggested for distributing light inside PBRs (Xue et al., 2013). There has been considerable research into the prevention and remediation of biofouling, using coatings, textures, and various cleaning methods (Inglis et al., 2012). Some of these antifouling methods have adverse environmental impact, although others, based on biomimicry non-toxic chemicals, or mechanical treatments are effective and relatively benign for the environment (Callow and Callow, 2011; Bixler and Bhushan, 2012).

It will be possible to use established antifouling methods on most OMEGA components, such as floating docks, moorings, pipes, and pumps (Dobretsov and Thomason, 2011). It is not clear, however, what biofouling will develop on OMEGA PBRs, its impact, and which, if any, antifouling methods will be applicable. Some methods for the control or removal of biofouling from marine equipment with transparent optical windows may be applicable to rigid PBRs, but not necessarily to the flexible plastic materials and specific designs proposed for OMEGA PBRs.

To address the question of biofouling on OMEGA PBRs we considered: (1) the identity of the biofouling organisms that attach to candidate PBR plastics; (2) the influence of PBR design and shape on the distribution and accumulation of biofouling; (3) the correlation between biofouling biomass, surface coverage, and light transmittance; and (4) the impact of biofouling on algae cultures inside the OMEGA system. Although biofouling will be site specific, these results provide a general understanding of the biofouling issues related to OMEGA and provide insights into methods to mitigate their effects.

2. Methods

Experiments were conducted to investigate: (1) the impact of selected PBR plastics on biofouling (experiment 1, referred to as “PBR plastic”), (2) the impact of PBR shape on biofouling (“PBR shape”), and (3) the impact of biofouling on algae productivity inside PBRs (“algae productivity”). The experiments were conducted at two different sites and used overlapping methods.

2.1. Experimental sites and algae cultures

Experiments were conducted in the Monterey Bay area in California between September 2010 and February 2012. Experiment 1 (PBR plastics) and experiment 3 (algae productivity) were conducted at the California Department of Fish and Wildlife (CDFW) Marine Wildlife Veterinary Care and Research Center (Lat: 36° 57' 13", Long: -122° 3' 56") using 950-liter and 8800-liter tanks filled with sand-filtered seawater refreshed at a rate of approximately 100 liters min⁻¹. Experiment 2 (PBR shape) was conducted at Moss Landing Harbor, Moss Landing (Lat: 36° 48' 6", Long: -121° 47' 13") using the dock and facilities provided by Moss Landing Marine Laboratories.

An inoculum culture used in experiment 3 (algae productivity), dominated by *Scenedesmeaceae*, was maintained in the laboratory in BG-11 medium (American Type Culture Collection) in shaker flasks at 22 °C in a lighted incubator with agitation and supplementary CO₂. The inoculum was added to PBRs containing treated wastewater [final plant effluent (FPE)] obtained from the Santa Cruz Wastewater Treatment Facility.

2.2. PBR plastics and PBR construction

The plastics for PBRs were either obtained as sheets and cut and welded in the laboratory using an AIE double impulse foot heat sealer (Industry, CA) or custom ordered from Raven Industries (Sioux Falls, SD). For experiment 1 (PBR plastics), two types of hexagonal PBRs (25 cm across) were made in the laboratory. The first was made of 0.2-mm non-permeable clear polyurethane (PolyU) obtained from American Polyfilm (Branford, CT). The second was made from 0.5-mm translucent linear low-density polyethylene (LLDPE) (top layer) and 1-mm black opaque high-density polyethylene (HDPE) (bottom layer) obtained from Gundle/SLT Environmental Inc (GSE Lining Technology, LLC, Houston, TX) or from Raven Industries. All hexagonal PBRs had a bulkhead fitting (3.75 cm diameter) in the bottom center for filling and to access the upper layer for periodic photosynthetically active radiation (PAR) light transmittance measurements (see below).

For experiment 2 (PBR shape), rectangular (flat panel) and tubular PBRs were purchased from Raven Industries. The flat-panel PBRs (9.5 m × 1.3 m) were made of 0.5-mm translucent LLDPE (top) and opaque black 1.0-mm LLDPE (bottom). The tubular PBRs (0.20 m diameter × 9.1-m length) were made of translucent (clear) 0.38-mm LLDPE tapered at the ends to 11.4 cm to allow attachment to polyvinyl chloride (PVC) pipes, which were used to secure the PBRs to the dock. Buoyancy for both flat-panel and tubular PBRs was provided by high-density foam floats and by filling the PBRs with freshwater. PBRs filled through bulkhead fittings was facilitated by air vents.

For experiment 3 (algae productivity) tubular LLDPE PBRs were recovered from Moss Landing Harbor after 12 weeks of exposure by cutting them into flat sheets and transporting them wet from Moss Landing to Santa Cruz. Two separate PolyU PBRs were constructed immediately before the experiments and installed in the OMEGA system using cultures grown on FPE as described above. Two segments of the LLDPE PBRs from Moss Landing, which were

long enough to cover the PolyU PBRs, were cut—one with biofouling intact (experimental) and the other cleaned with a brush and freshwater (control). The biofouled and cleaned LLDPE segments were wrapped around the PolyU PBRs and secured on the underside using plastic tie-wraps to monitor the impact of biofouling on algae growth. The sheets were switched after 3 days to confirm that observed differences in the cultures were due to the wrapping sheets and not to differences in the cultures themselves. Both sheets were removed briefly on day 5 to shake and homogenize the two cultures.

2.3. Identification of biofouling organisms

In experiment 1 (PBR plastic), all PBRs were observed weekly for 9 weeks. The major groups of organisms were identified using a Leica MZ125 dissecting microscope (Leica Microsystems GmbH, Wetzlar, Germany) or a Leica DMRX compound microscope. The relative abundances of organisms on PBRs were estimated, but not systematically quantified.

2.4. Photosynthetically active radiation (PAR) light transmittance

PAR was measured simultaneously in air and through PBR plastics with Li-COR Li-190 and Li-192 Quantum Sensors and the Li-1400 Datalogger (Li-COR Biosciences, Lincoln, NE). Transmittance (T) of the plastic was calculated using the equation $T = I/I_0$, where I_0 is the intensity of the incident radiation in air and I is the intensity of the radiation passing through the experimental plastic.

In experiment 1 (PBR plastic), transmittance of top layers was measured before the twelve hexagonal PBRs were exposed to seawater and weekly after deployment in the seawater tank for 9 weeks. For PolyU PBRs, transmittance was also measured through both top and bottom layers in regions with external fouling intact and through cleaned (2.5 cm × 2.5 cm) regions; the measurements were compared using paired t -tests (SigmaPlot, Systat v.12, San Jose, CA). For LLDPE/HDPE and PolyU PBRs transmittance was measured through the LLDPE top layer by inserting the PAR sensor through the bulkhead fitting.

Transmittance was also measured through the cut LLDPE PBR sheets, both biofouled and cleaned, used in experiment 3 (algae productivity). Such measurements were made in twelve locations along each of three transects across the width of the sheets. The three transects represented regions that were estimated to contain high, low, and intermediate levels of biofouling.

2.5. Biofouling surface coverage and biomass accumulation

Experiment 2 compared the biofouling distribution and accumulation on two widely used PBR shapes: (1) a rectangular “flat panel” shape and (2) a tubular shape (described in Section 2.2 above). On both shapes weekly samples were taken from within reference grids, during consecutive 9-week periods, August to November 2011 for the flat-panel PBR and November 2011 to January 2012 for the tubular PBRs. The grid for the flat-panel PBR was a 70 cm × 70 cm PVC frame, divided into 10 cm × 10 cm squares with monofilament. The grid for the tubular PBRs was a 61 cm × 61 cm section of flexible plastic fencing material divided into twelve rows of 5 cm × 4.75 cm rectangles and wrapped around a section of the PBR lifted out of the water with oars. The grid was removed from both flat-panel and tubular PBRs after each sampling and undisturbed areas were sampled at each time point.

The percent surface coverage was calculated from digital photographs (Olympus FE-310, 8-megapixel) of regions undisturbed by sampling. For the flat-panel PBR, the top (east and west sides) and the bottom were photographed. For the tubular PBR, photographs of two rows were taken around the circumference of the

PBR. All photographs were optimized for color, contrast, and saturation thresholds and analyzed with “ImageJ” software (National Institutes of Health, Bethesda, MD). Two to six photographs were analyzed for each sampling, depending on the quality of the photographs.

Biomass was sampled from both grids by scraping biofouling material within a grid square into a clean 50-ml Falcon tube for transport to the laboratory. The biomass was transferred to pre-weighed foil trays or glass microfiber filters (0.45 μm pore size; Whatman, Springfield Mill, UK), dried and stored in a desiccator, and weighed on an analytical balance (Ohaus, Parsippany, NJ).

2.6. Biofouling impact on PBR function

In experiment 3 (algae productivity) the impact of biofouling on algae was determined for algae growing on FPE wastewater in an OMEGA system developed at CDFW operating between January 27 and February 4, 2012 (for details of the system see: Wiley et al., 2013). Two 120-liter algae cultures were contained in newly constructed PolyU PBRs floating in a seawater tank. The cultures circulated at 75 liters min⁻¹ with 10% of the system volume passing through a gas exchange and harvesting column (GEHC) (Wiley et al., 2013). The PolyU PBRs were wrapped with segments of the LLDPE PBR recovered from Moss Landing Harbor with either 12 weeks of biofouling intact or cleaned by brushing. The two sheets wrapped around the PolyU PBRs were secured on the bottom with tie-wraps.

The system was monitored for pH, temperature, optical density (OD₇₅₀), NH₃-N, NO₃-N, and reactive PO₄³⁻ (Wiley et al., 2013). The CO₂ injection rates were monitored and the totalized or cumulative use of CO₂ was recorded every three minutes. A fast repetition rate fluorometer (FRRF) was used to determine the efficiency of light utilization (F_v/F_m), the photosynthetic quantum yields, and the rates of photosynthetic electron transport (Kolber et al., 1998), providing information about the photosynthetic performance of the culture in real time. F_v/F_m data range from 0.0 to 0.7, with >0.5 indicating lack of any significant photosynthesis stressors.

To confirm that the observed effects were the result of the LLDPE wraps and not variations in the algae cultures themselves, on day three the wraps were exchanged; the biofouled plastic was moved to the PBR previously wrapped with the cleaned plastic and vice versa. To suspend settled algae and homogenize the two cultures, on day 5 of the experiment, the wraps were temporarily removed, the PolyU PBRs were manually shaken, the two cultures were intermixed until equilibrated (OD₇₅₀), and the wraps were reattached.

3. Results and discussion

3.1. Identification of biofouling organisms

To determine the impact of possible OMEGA plastics on the diversity of biofouling, 12 hexagonal PBRs made of PolyU, LLDPE, and HDPE were sampled weekly on top and bottom. Brown biofilms developed in the first week and persisted on all PBRs throughout the experiment. These biofilms were the only noticeable biofouling on the opaque HDPE, but filamentous green macroalgae developed on the clear PolyU and LLDPE plastics and was abundant by week 9.

Using microscopy, the dominant groups of organisms were identified (Table 1). The brown biofilms were predominantly pennate diatoms and cyanobacteria. The pennate diatoms were predominantly in the genera *Navicula* and *Achnanthes*, and the cyanobacteria were filamentous forms in the genera *Blennothrix* and *Schizothrix*. Pennate diatoms and cyanobacteria were present

Table 1

The dominant biofouling organisms on hexagonal PBRs made of clear polyurethane (PolyU) or clear linear low-density polyethylene (LLDPE) (top) and opaque (black) high-density polyethylene (HDPE) (bottom) in 9 a week seawater tank experiment. Dominant taxa are in bold and other taxa are listed in descending order of surface area coverage (weeks 1 and 2 were excluded due to low biofouling).

Plastic	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
PolyU	<ul style="list-style-type: none"> • Cyanobacteria • Pennate diatoms • <i>Ulva intestinalis</i> 	<ul style="list-style-type: none"> • Cyanobacteria • Pennate diatoms • <i>U. intestinalis</i> 	<ul style="list-style-type: none"> • Pennate diatoms • Cyanobacteria • <i>U. intestinalis</i> 	<ul style="list-style-type: none"> • Pennate diatoms • <i>U. intestinalis</i> • Cyanobacteria • <i>Prasinocladus marinus</i> 	<ul style="list-style-type: none"> • Pennate diatoms • <i>U. intestinalis</i> • Cyanobacteria • <i>P. marinus</i> • <i>Ulva lobata</i> 	<ul style="list-style-type: none"> • <i>U. intestinalis</i> • Pennate diatoms • Cyanobacteria • <i>U. lobata</i> 	<ul style="list-style-type: none"> • <i>U. intestinalis</i> • Pennate diatoms • Cyanobacteria • <i>U. lobata</i>
LLDPE	<ul style="list-style-type: none"> • Cyanobacteria • Pennate diatoms 	<ul style="list-style-type: none"> • Pennate diatoms • Cyanobacteria • <i>U. intestinalis</i> 	<ul style="list-style-type: none"> • Pennate diatoms • Cyanobacteria • <i>U. intestinalis</i> 	<ul style="list-style-type: none"> • Pennate diatoms • Cyanobacteria • <i>U. intestinalis</i> • <i>P. marinus</i> 	<ul style="list-style-type: none"> • Pennate diatoms • <i>U. intestinalis</i> • Cyanobacteria • <i>P. marinus</i> 	<ul style="list-style-type: none"> • Pennate diatoms • <i>U. intestinalis</i> • Cyanobacteria 	<ul style="list-style-type: none"> • Pennate diatoms • <i>U. intestinalis</i> • Cyanobacteria • <i>P. marinus</i>
HDPE	<ul style="list-style-type: none"> • Pennate diatoms 	<ul style="list-style-type: none"> • Pennate diatoms 	<ul style="list-style-type: none"> • Pennate diatoms 	<ul style="list-style-type: none"> • Pennate diatoms 	<ul style="list-style-type: none"> • Pennate diatoms 	<ul style="list-style-type: none"> • Pennate diatoms 	<ul style="list-style-type: none"> • Pennate diatoms

on all plastics throughout the 9-week experiment and in most samples pennate diatoms were dominant. The unique abundance of pennate diatoms on the bottom of the opaque-black HDPE may be attributed to the limited light available—a condition under which they are known to thrive [for review see (Molino and Wetherbee, 2008)].

The green macroalga *Ulva intestinalis* (Linnaeus) was conspicuous on the tops and bottoms of the clear PolyU PBRs starting in week 3, and on the clear tops of LLDPE PBRs starting in week 4 (Table 1). In week 6, the microalga *Prasinocladus marinus* (Cienkowski) appeared as a thin green layer on both PolyU and LLDPE PBRs, although it remained a minor component of the total biofouling assemblage. In week 7, both *Ulva intestinalis* and *U. lobata* (Kützinger) were present on PolyU PBRs, whereas *U. intestinalis* dominated LLDPE PBRs. By the end of week 9, the top layers of PolyU PBRs were noticeably more biofouled with macroalgae than the top surfaces of the LLDPE PBRs.

3.1.1. Change in PAR light transmittance

The hexagonal PBRs were also used to measure changes in PAR light transmittance (Fig. 1). Before biofouling, the transmittance in air of PolyU was 97% and of LLDPE was 92% (the photometer probes were calibrated in air to 100% transmittance) (Fig. 1, time 0; PolyU = grey bars; LLDPE = black bars). In the seawater tank, transmittance decreased with time for both plastics, but notably more for PolyU than for LLDPE. The transmittance reached its lowest value for PolyU in weeks 5–7 with an average overall decrease of 34%

(Fig. 1, grey bars), whereas the lowest value for LLDPE transmittance was in week 3 with an average decrease of <10% (Fig. 1, black bars). Although the patchy distribution of biofouling created a high standard deviation, the average transmittance of PolyU was significantly lower than that of LLDPE in weeks 7 and 8 (Student *t*-test, week 7: $p = 0.04$; $n = 6$, and week 8: $p = 0.02$; $n = 6$).

To determine the relative contributions of biofouling and UV-induced plastic “aging” to changes in PolyU PAR transmittance small regions of the PolyU PBRs were carefully cleaned with a washcloth and probed for transmittance throughout the 9-week experiment. The results indicated that no significant changes occurred in the PolyU plastic itself during that time period (Student *t*-test, $p = 0.58$; $n = 6$); the change in transmittance was entirely due to biofouling.

The experiment was limited to 9 weeks because this period was considered to be a reasonable PBR cleaning cycle. Published studies with polyolefin plastics, which included LLDPE, indicate that continuous biofouling for 1 year significantly influences material properties of the plastic (such as surface roughness, weight, surface charges, and tensile properties) (Sudhakar et al., 2007), suggesting that PAR transmittance may also be affected, although it was not measured. It remains to be determined if repeated fouling and cleaning cycles would similarly degrade LLDPE plastic in a way that would influence its function as a PBR. Also, previous studies showed that PAR was significantly reduced by biofouling by color morphs of sponges and tunicates growing as epiphytes on eelgrass (Wong and Vercaemer, 2012); earlier work showed that other epi-

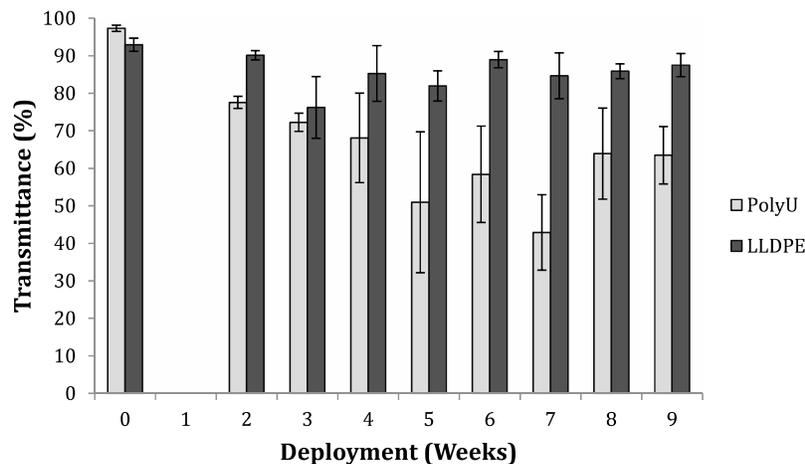


Fig. 1. Percent transmittance of photosynthetically active radiation (PAR) through hexagonal PBRs made of PolyU (grey column) and LLDPE (black column). Measurements were made through the top layers of PBRs with “week 0” the unbiofouled transmittance relative to air, which was set to 100%. The variance in weeks 2–9 reflects the patchy distribution of biofouling on the top of the PBRs (mean ± standard error; $n = 3$).

phytes also caused selective light attenuation [for review see: Brush and Nixon, 2002]).

Although PolyU initially had a slightly higher PAR transmittance as compared to LLDPE, PolyU had more fouling, was more difficult to clean, and cost significantly more than LLDPE. Hence, later experiments to determine the influence of PBR shape on biofouling used PBRs constructed from LLDPE.

3.2. The influence of PBR shape on biofouling

3.2.1. Biofouling surface coverage and biomass accumulation

To determine the impact of PBR shape on biofouling, two typical PBR designs, rectangular (flat-panel) and tubular, were made of LLDPE and deployed in Moss Landing Harbor (see Section 2.2 for design details). The goal was to determine the rates of accumulation, density, and distribution of biofouling on each shape, not to compare the shapes directly—they were not deployed concur-

rently. Biofouling was periodically photographed for image analysis to determine surface coverage or sampled by scraping defined regions within a reference grid to determine biomass.

On both PBR shapes, biofouling was predominantly along the wetted edges extending a short distance underwater and into a splash zone above the waterline. For the 1.3-m-wide flat-panel PBR most biofouling accumulated within 10 cm of the edge, notably less accumulated between 20 and 30 cm from the edge, and little to none accumulated more than 30 cm from the edge toward the center of the PBR. For the tubular PBR, the biofouling accumulated along the submerged sides and diminished toward the central top region. For both shapes, this distribution was attributed to patterns of wetting, periodic drying, and exposure to UV light, all factors known to impact biofouling (Bravo et al., 2011).

Image analysis of photographs indicated that, during the 9-week experiment, the percent surface coverage of biofouling was occasionally greater on the east edge of the flat panel PBRs, but

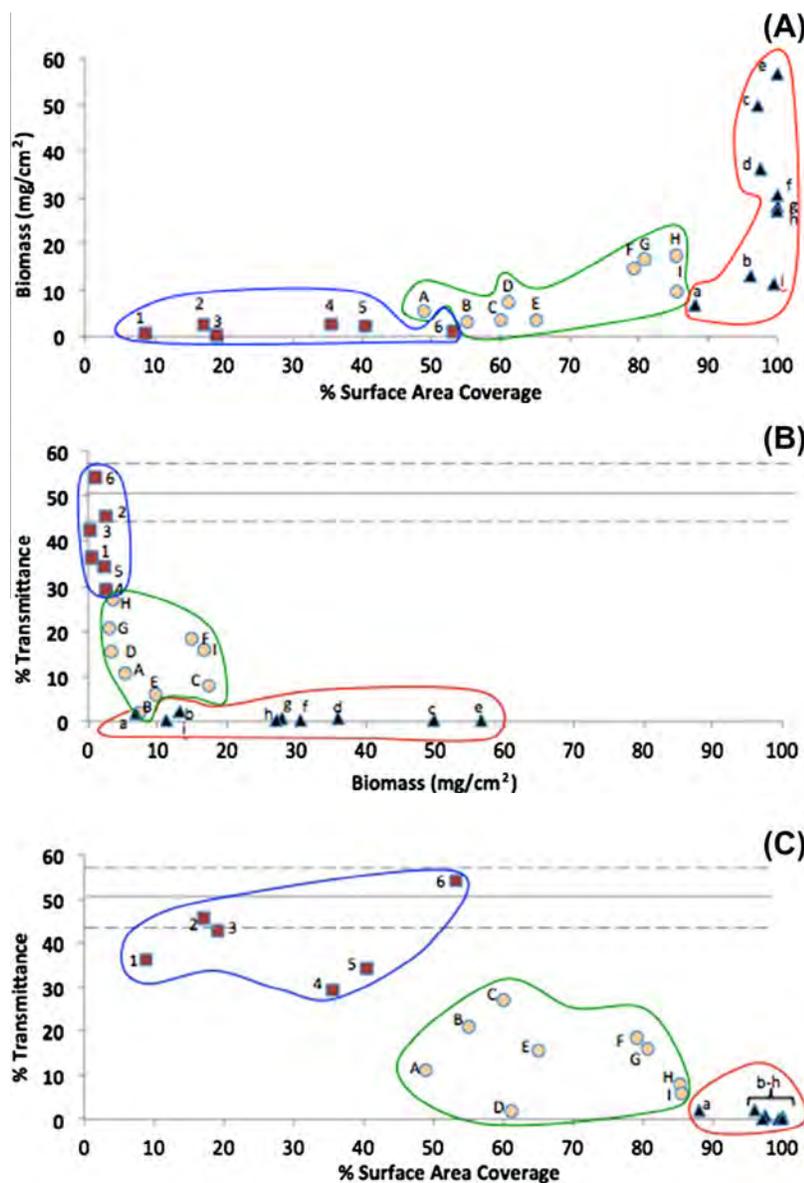


Fig. 2. Biomass vs. percent surface coverage (A), biomass vs. percent PAR transmittance (B) and percent surface coverage vs. transmittance (C) were measured at each of 2420 points on a PBR recovered from Moss Landing Harbor after 12 weeks of biofouling. For clarity, data points are grouped with different symbols and outlined. The numbered and lettered points indicate the results for each sampling site. The solid and dotted lines in (B) and (C) represent percent transmittance of the PBR cleaned of biofouling (mean \pm SD, $n = 36$).

more uniformly distributed around the tubular PBRs. On the east side of the flat-panel PBR after 1 week 30% of the surface was covered and by 9 weeks 68%. In contrast, on the west side it took 4 weeks to reach 39% coverage and by 9 weeks there was 66% coverage. On the bottom of the flat-panel PBR, during the first 2 weeks biofouling was undetectable; by week 3, however, it reached 10% coverage, and by week 9 it was 81% coverage.

For the tubular PBRs, over the 9-week period the surface coverage was calculated from photographs for six equal-size regions around the circumference of the PBRs (top, bottom, and two regions on each side). The coverage ranged from 0.2% to 96.2%. By week 3, there was detectable biofouling around the full circumference of the PBRs and in one upper-side region the coverage was >70%. In all seven weeks of photos analyzed (weeks 3–9), there were regions around the circumference of the PBRs that exceeded 70% coverage. In six out of the seven, the top region had the lowest surface coverage; only in week 9, the coverage was lowest on two of the side regions. In weeks 7 and 8, five out of six of the regions around the circumference exceeded 70% coverage.

The biomass accumulation on PBRs, based on scrapings and dry weights of recovered material, ranged from 0 to 37.8 mg cm⁻² on the flat-panel and from 0 to 18.5 mg cm⁻² on the tubular design. Although the greatest biomass accumulation was observed in week 9, the measurements were highly variable because of the patchy biomass distribution along the length of the PBR. As expected from the results with the small hexagonal PBRs, relatively little biomass accumulated on the bottom of the flat-panel PBR (made of opaque

black LLDPE), although a biofilm of pennate diatoms was ubiquitous and barnacles and bryozoans were abundant. Biomass was not quantified in this region, but surface-area coverage was and found to be high.

3.3. Biofouling impact on PBR function

The impact of biofouling on algae productivity inside tubular PBRs floating in a seawater tank with circulating wastewater and a gas-exchange column (Wiley et al., 2013) was determined by wrapping these PBRs with sheets of biofouled or cleaned LLDPE PBRs. The biofouled (experimental) and cleaned (control) sheets were made from a PBR recovered from Moss Landing Harbor after 12 weeks of exposure. Before the OMEGA PBRs were wrapped, the biofouled PBR segment was sampled to measure biomass density and photographed to calculate percent surface coverage; both the biofouled and cleaned segments were probed to determine PAR-light transmittance.

3.3.1. Correlations between biomass, percent surface coverage, and PAR light transmittance

To correlate biomass density, percent surface coverage, and PAR-light transmittance, all three parameters were measured on the 12-week biofouled PBR at 24 locations along two transects within 5 cm × 4.75 cm sample areas. The data are shown in Fig. 2A–C with the points numbered or lettered to indicate the correlated data for each specific location (i.e., triangle 'a' refers to the

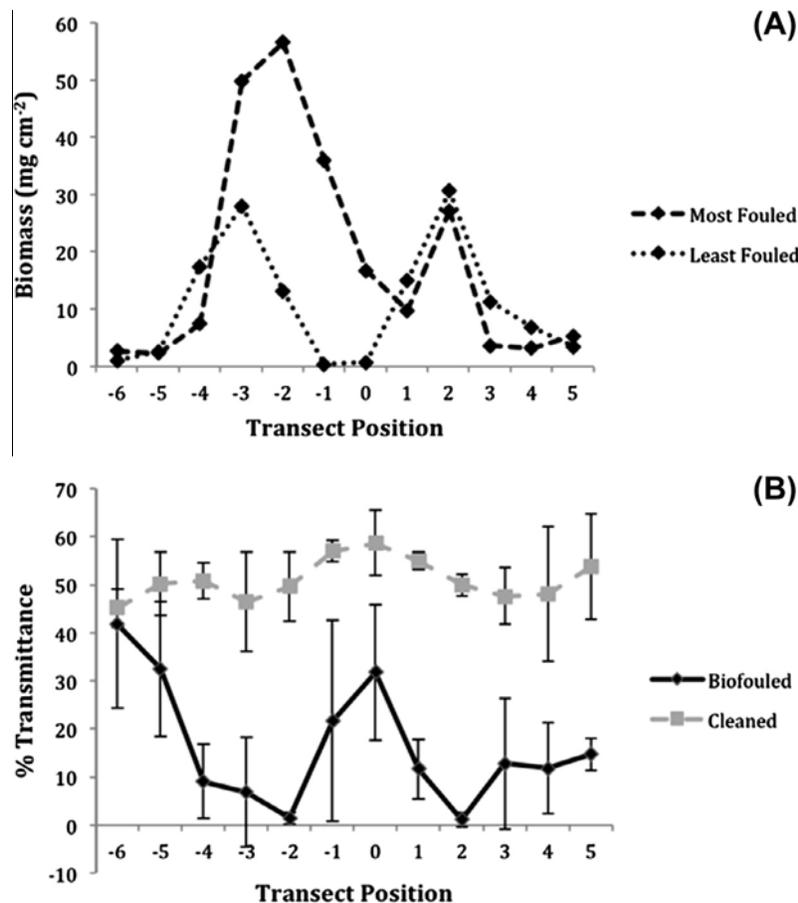


Fig. 3. Biomass distribution (A) and percent transmittance (B) along transects across cleaned and biofouled LLDPE sheets cut from PBRs recovered from Moss Landing Harbor (see Fig. 4). The recovered tubular PBR was cut along the bottom between positions –6 and 5, creating a sheet with position zero at the center, which was the top of the PBR. Biomass was sampled from along three transects in regions identified as the most fouled, least fouled, and average. Percent PAR transmittance was determined in biofouled and cleaned regions. Data represent mean ± 1 standard deviation, n = 3.

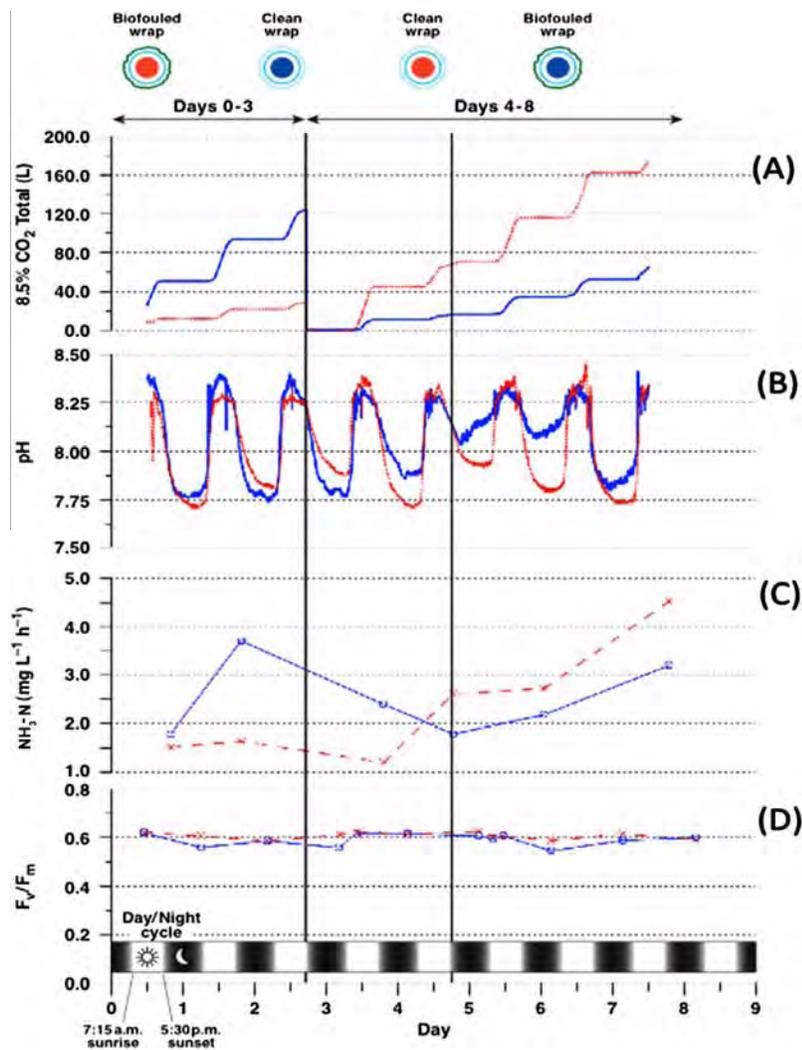


Fig. 4. Effects of biofouled and cleaned sheets wrapped around algae cultures in PBRs from an OMEGA system. Measurements of total CO₂ utilization (A), changes in pH (B), rate of ammonia consumption (C), and photosynthetic efficiency (F_v/F_m) (D) were taken during an 8-day experiment with the day/night cycle indicated at the bottom. The configuration for days 1–3 for the biofouled-wrapped culture (red curve) and cleaned-wrapped culture (blue curve) was switched for days 4–8, indicated as biofouled-wrapped culture (blue curve) and the cleaned-wrapped culture (red curve). On day 5, the contents of the two PBR systems were shaken and intermixed to homogenize the culture.

same region in Fig. 2A–C). To identify trends, the data are grouped into three categories designated by different symbols and outlined. In one category (squares), consistently low biomass densities (<5 mg cm⁻²) correlated with surface coverage ranging from 9% to 53% (Fig. 2A) and transmittance of 30–55% (Fig. 2B and C: squares). In a second category (circles), biomass density between 3 and 18 mg cm⁻² correlated with surface coverage of 47–85% (Fig. 2A) and with transmittance of 3–30% (Fig. 2B and C: circles). In the third category (triangles), a wide range of biomass densities (7 to 57 mg cm⁻²) correlated with >85% surface coverage (Fig. 2A) and in all cases low (<5%) transmittance (Fig. 2B and C: triangles).

The results indicate that for biofouling with high biomass densities (>25 mg cm⁻²) and high surface coverage (>90%), light transmittance was low (<5%) (Fig. 2: triangle points c–h). Conversely, the lowest biomass and the lowest percent surface coverage corresponded with the highest transmittance (Fig. 2: squares). However, at low biomass densities (<15 mg cm⁻²) and high surface coverage (>89%), the light transmittance can also be low (Fig. 2: triangles a, b, i) because of thin films that absorbed light well. At biomass densities < 20 mg cm⁻² and percent coverage ranging from 47% to 87%, transmittance was occasionally below 30% (Fig. 2: circles). In gen-

eral, both biomass and percent surface coverage were inversely correlated with PAR transmittance, but there were many exceptions as a result of thin, light-absorbing films or thick, dispersed clumps.

The cleaned LLDPE PBR had PAR transmittance of 51% ± SD 7% (n = 36) (Fig. 2B and C), which was significantly lower than the initial transmittance of 83% ± 0.04% of the LLDPE. It was not determined if this 32% decrease was the result of changes in the surface caused by biofouling, the harsh brushing used for cleaning, and/or weather-induced changes in the plastic during the 12-week exposure.

To quantify the biofouling distribution around the circumference of the PBR at 12 weeks, biomass and PAR transmittance were measured at twelve locations along three transects across the flattened PBR segment (Fig. 3). The biomass samples were taken from regions visually determined to contain the highest and lowest biomass densities on the PBR segment. Relatively low biomass was present in the region corresponding to the top (Fig. 3A: position '0 ± 1') and the bottom of the PBR (Fig. 3A: positions -5 ± 1 and 4 ± 1). The biomass was highest in the regions corresponding to the sides (Fig. 3A: positions -3 to -1 and 1 to 3). The biomass

was symmetrically distributed in the region with the least biofouling and skewed to one side in the region with the most biofouling.

In general, average transmittance was inversely related to the biomass. Hence, transmittance was highest in areas corresponding to the top and bottom of the PBR and lowest in the areas corresponding to the sides (Fig. 3B). The transmittance for the cleaned plastic averaged $51\% \pm \text{SD } 7\%$; $n = 36$ (Fig. 3B; grey dashed line) as described above.

3.3.2. Impact of biofouling on algae productivity

The OMEGA-system PBRs wrapped with either the biofouled (experimental) or cleaned (control) LLDPE PBR sheets (analyzed above) were used to determine the impact of biofouling on algae productivity. For eight day–night cycles the wrapped cultures were monitored for CO_2 utilization, changes in pH, ammonia uptake, and photosynthetic efficiency as measured by Fast Repetition Rate Fluorometry (FRRF) (Fig. 4A–D). The cumulative flow of CO_2 entering both cultures [which increased during the day, but not during the night (Fig. 4A)] and the utilization rates of ammonia (Fig. 4C) indicate the relative levels of photosynthesis. The CO_2 used by each culture was controlled by a pH feedback system, such that CO_2 was injected to maintain the pH below 8.25. As expected, the pH followed a diurnal cycle (Fig. 4B). During the day, pH rose as CO_2 was consumed by photosynthesis, but at night pH fell as CO_2 was released by respiration. The control system maintained the culture pH between a nighttime low of 7.75 and daytime high of 8.5 (Fig. 4B). Ammonia consumption followed the changes observed in photosynthesis (Fig. 4C). The photosynthetic efficiency (F_v/F_m) in both cultures remained between 0.54 and 0.63, indicating that the light levels and pH did not affect the photo-physiology of the algae under the experimental conditions (Fig. 4D).

During the first three light periods, the algae in the PBR wrapped with the biofouled sheet used an average of 77% less CO_2 and 60% less $\text{NH}_3\text{-N}$ than the algae in the PBR wrapped with the cleaned LLDPE sheet (Fig. 4A&C, red vs. blue curves). To insure that the observed differences were a result of the wraps and not differences between the cultures themselves, at the end of third light period, the biofouled and cleaned wraps were swapped. This manipulation led to a significant decrease in CO_2 use and rate of $\text{NH}_3\text{-N}$ uptake in the newly biofouled PBR (Fig. 4A and C; blue curve) and corresponding increase in these parameters in the clean PBR (Fig. 4A and C; red curve). The biofouled-wrapped PBR used an average of about 59% less CO_2 and about 30% less NH_4 than the cleaned-wrapped PBR. From days 4 to 8, the optical density (OD_{750}) of the culture in the biofouled PBR increased about 2-fold compared to a 2.4-fold increase for the culture in the cleaned PBR (data not shown).

3.4. Biofouling and the OMEGA system

These data clearly indicate that biofouling significantly reduced algae productivity because of light attenuation and confirm that OMEGA PBRs will require a cleaning system. Known antifouling coatings or cleaning methods might be adapted for OMEGA, provided the coatings have good light transmittance and the cleaning methods do not damage the PBRs (Callow and Callow, 2011). Some naturally occurring antifouling compounds have relatively low environmental toxicity and may be useful for OMEGA if large enough quantities can be obtained. Silicone materials with microtopographies that reduce biofouling (Petronis et al., 2000) may also be applicable, but the cost and light transmittance of these materials require evaluation. Mechanical cleaning methods are likely to be the best solution for the OMEGA system, if they are efficient, energy and cost effective, and non-damaging. Whether these requirements are met will depend on the frequency of cleaning, which will

be site-specific and seasonal. On the basis of the observations reported here, a monthly cleaning cycle may be expected.

Although biofouling on the upper surface of the OMEGA PBRs is problematic, biofouling on the bottom of the PBRs and the OMEGA support structures may have environmental and economic benefits. Submerged OMEGA surfaces provide substrate, refugia, and habitat for sessile and associated organisms and a large-scale OMEGA deployment may help control eutrophication by acting as a floating “turf scrubber” (Mulbry et al., 2010). Algae can effectively remove nutrients (Christenson and Sims, 2012), heavy metals and other pollutants (deBashan and Bashan, 2010). By removing nutrients from coastal waters, OMEGA may help prevent unwanted algae blooms; by removing other pollutants, the system may improve coastal water quality.

In addition to improving water quality, the OMEGA flotilla will act as a “fish aggregating device” or an “artificial reef,” both of which increase local species diversity and expand the marine food web (Kerckhoff et al., 2010). Observations at Moss Landing Harbor indicated that even the small OMEGA PBRs deployed there provided sites for marine birds and sea otters to forage, rest, and play.

4. Conclusion

Biofouling on candidate OMEGA PBR plastics indicated that clear LLDPE had less biofouling, was easier to clean than PolyU, and that opaque LLDPE and HDPE developed only thin biofilms in 9-week experiments. Two LLDPE PBR designs (rectangular and tubular) both accumulated biofouling primarily on their wetted sides. Correlations between biomass, surface coverage, and light transmittance revealed that both thick and thin biofouling layers impact light transmittance, as does a harsh cleaning method. Twelve weeks of biofouling on LLDPE decreased algae productivity, suggesting the need for a cleaning cycle. OMEGA biofouling may improve coastal water quality and increase local biodiversity.

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Appendix C: Dewatering Microalgae by Forward Osmosis

Dewatering microalgae by forward osmosis

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Highlights

- ✍ Microalgae cultures were dewatered using forward osmosis membranes and seawater
- ✍ Average dewatering rates of 2 l/m²·hr
- ✍ Marine biofouling did not impact dewatering rates unless it damaged membranes

Abstract

Microalgae are known to be an excellent source of biofuels, but there are many issues with the scale and economics of their cultivation. In particular, dewatering methods, such as centrifugation and tangential flow filtration, are prohibitively energy intensive. In this study forward osmosis (FO) is considered as a partial dewatering method for microalgae growing on wastewater in a marine environment. Using artificial seawater as the draw solution average dewatering rates of 2 l/m² membrane/hr (range 1.8-2.4 l/m²!hr) were observed and volumes decreased by 65-85%. For a single membrane, daily-dewatering rates did not significantly change in 14 consecutive experiments. Hourly dewatering rates did not gradually decrease, as might be expected, instead the dewatering rate oscillated throughout each experiment. Exposing an FO membrane in the ocean for 45 days, caused significant biofouling on its surface, but its dewatering did not change. Exposing three FO membranes in the ocean for 52 days also caused significant biofouling, but in this experiment all membranes developed leaks that allowed saltwater to pass. These experiments suggest that FO may be an energy-saving step in dewatering freshwater microalgae if an appropriate draw solution is available and if conditions are controlled to prevent leakage.

Keywords: osmosis, microalgae harvesting, dewatering, biofouling, biofuels, OMEGA

INTRODUCTION

The growing interest in microalgae as a feedstock for biofuels has focused attention on engineering large-scale cultivation systems that do not compete with agriculture for water, fertilizer, or land^[1]. One approach that has been proposed for coastal cities is to grow freshwater microalgae in photobioreactors (PBRs), floating in seawater, using municipal wastewater from offshore outfalls^[2]. The system called “OMEGA” (Offshore Membrane Enclosures for Growing Algae) is designed to use wastewater for nutrients, a nearby source of CO₂ for carbon, and it uses the heat-capacity of the surrounding water to cool the PBRs, wave energy for supplementary mixing, and the salt content of the surrounding seawater to prevent the cultivated freshwater algae from becoming invasive species in the marine environment^[3]. It has also been suggested that the salt gradient between wastewater and seawater can be used for forward osmosis (FO) to assist in the algae dewatering process^[3].

FO has been applied to a variety of dewatering processes, including biosolids separation in wastewater treatment^[4]^[5], concentration of industrial wastewater, and dewatering of landfill leachate^[5]^[6]. FO is also used in food processing, for example, to thicken tomato juices for the production of ketchup and

Abbreviations: FO is forward osmosis; BG-11 a common microalgal growth medium; l/m²!hr liter per square meter of membrane per hour

to make fruit juice concentrates^{[7] [8] [9]}. The FO process uses an osmotic gradient across a semipermeable membrane to draw liquid (permeate) through the membrane, while concentrating a broad range of solutes that do not pass through the membrane (retentate). The draw solution produces a trans-membrane osmotic pressure that pulls water through the membrane, while the membrane itself acts as a barrier to most salts, organics, and particles. The principle of FO and its applications have been recently reviewed^[5].

Oleaginous microalgae, which range in size from 2 to 50 μm may have cell densities of <1.0 g/L, and must be concentrated or even brought to near dryness for making biofuels^[10]. Current dewatering methods involve multiple steps that usually include chemical or biological flocculation^[11], centrifugation^{[12] [13]}, and/or filtration^[14] as well as some form of spray or thermal drying^[15]. It is estimated that these dewatering and drying steps account for 20-30% of the total energy costs in microalgae biofuel production. In the ongoing effort to reduce production costs, large scale, low energy, and low cost dewatering methods are of interest. FO membranes can be used for large-scale dewatering in a wide range of contexts provided 1) there is a readily available or easily produced draw solution, 2) the substance of interest does not pass through the FO membrane, and 3) the contents do not inactivate or damage the membrane. For industrial purposes, draw solutions are typically salts that naturally occur in brackish water, seawater, or hypersaline water, but they can include any osmolyte, such as glucose and fructose^[16].

To use FO for dewatering microalgae growing in wastewater in the proposed OMEGA system would require that the FO membrane is not influenced by the microalgae or their growth medium and that the FO system functions in the marine environment. Here, we investigated flat-sheet cellulose triacetate FO membranes for dewater freshwater microalgae, *Chlorella vulgaris*, grown in either an artificial medium or municipal wastewater, using artificial or natural seawater as draw solutions. We also investigated the impact of biofouling on FO membranes by comparing dewatering rates through membranes exposed in the ocean environment or stored in the laboratory for 45 and 52 days. We discuss the potential use of FO as an initial dewatering step in large-scale microalgae harvesting and for advanced wastewater treatment.

MATERIALS AND METHODS

Cultures, media, and membranes

A culture of *Chlorella vulgaris*, obtained from Arizona State University, was grown in either BG-11 medium prepared as described by the American Type Culture Collection (ATCC) or in filtered wastewater effluent obtained from the Sunnyvale Municipal Wastewater Treatment Plant (Sunnyvale, CA). It has been proposed that large-scale algae cultivation will use wastewater effluent, which is why it was used as a growth medium. Algae concentrations on both BG11 and wastewater ranged from 0.5-2.0 gram per liter (after 10-day incubations).

Experiments were done using either intact X-Pack Hydration bags produced by Hydration Technology Innovations (HTI) or using FO test chambers made by modifying X-Pack Hydration bags. Intact X-Pack bags have FO membranes, consisting of an active layer of cellulose triacetate on a robust non-woven polyester polyethylene backing that is enclosed in a plastic envelope. FO test chambers are modified X-Pack bags in which the plastic exterior envelope is removed, exposing the inner FO chamber. The membranes have a nominal pore size of 3-5 \AA and an area of approx. 0.09 m². The algal culture was put inside the modified "FO chamber" against the active layer of the membrane and the backing layer was exposed to the saltwater outside. Prior to use, each membrane was rinsed inside and out with deionized (18 m Ω) water.

FO dewatering tests

Preliminary dewatering tests were done with the intact X-Pack hydration bag. Feed solutions (250 ml) consisted of either BG-11 media or a culture of *C. vulgaris* in BG-11. The draw solution (750 ml) was 35 g/l NaCl in water. Dewatering rates were obtained by measuring the increase in volume of the draw solution. For each volumetric measurement, the draw solution was emptied into a graduated cylinder and then poured back into the bag. The X-Pack bag experiments were not agitated and were considered complete when the volume inside the chambers decreased by at least 80% from the starting volume.

FO performance tests

Fouling tests were done using FO test chambers filled with 1 liter of culture and floated in a bath containing

190 liters of saltwater (35.5 g/l NaCl). The first four tests were conducted with *C. vulgaris* grown in BG-11 and the subsequent 10 tests were conducted with *C. vulgaris* grown in wastewater effluent. The salt bath was agitated with a wave paddle to prevent stratification and the FO test chamber floated on the surface during testing. Dewatering rates were calculated by weighing the FO chambers approximately every hour until the volume inside the chamber had decreased by at least 80%, approximately six hours. Weights were determined by removing the FO chamber from the saltwater bath, drying it with paper towels, and then weighing the FO chamber using a top-loading microbalance (Ohaus I-10, Florham Park, NJ). The error due to drying and weighing was calculated by repeatedly wetting, drying, and weighing the same chamber. Between experiments, FO chambers were rinsed three times with deionized (18 m^Ω) water and stored wet at below 10°C.

Dewatering rates for the laboratory and ocean experiments are reported as the average dewatering rate over the first four hours of dewatering. While the data may not have been taken precisely on the hour for each experiment, the values reported were calculated to correspond to hourly measurements using a weighted average. The weighted average was calculated with the two measurements taken before and after the four-hour mark. The difference in time between each measurement and the hour was the basis for weighting a value.

Ocean experiments

For the first ocean experiment, three FO chambers were filled with 1 liter of water collected from Soquel Creek. The FO chambers were attached to a weighted rope, and lowered to approximately 1m below the surface in the Monterey Bay off the lower dock of Capitola pier (Capitola, CA). The FO chambers were oriented vertically throughout the experiment. Salinity of the Soquel Creek water and seawater in Capitola were measured using conductivity and calculated in accordance with the AWWA Standard Method 2520B Electrical Conductivity^[17].

Dewatering rates were monitored approximately every hour for at least four hours by retrieving the FO chambers and measuring the residual volumes of creek water. The volume was measured by transferring the contents of the chambers to a 1000 ML graduated cylinder. After each measurement, the creek water was returned to the same FO chamber and re-submerged. This process was repeated until 85-95% of the creek water was removed by osmosis.

For ocean fouling experiments, FO chambers were soaked for 17 hrs in deionized water at ambient temperatures before one FO chamber was attached approximately 0.5 meters below the surface on an offshore buoy. The other two FO chambers were stored in the laboratory at ambient temperature in a closed bucket filled with seawater from Capitola. After 45 days, the FO chamber on the buoy was retrieved and the three FO chambers were tested for dewatering rates.

In a follow-up experiment, three FO chambers were attached to the offshore buoy. Approximately every two weeks one FO chamber was removed and photographed. After 52 days of ocean exposure all three FO chambers were removed and tested for dewatering rates.

Dewatering rates are reported as the average rate over the first four hours of each experiment. If the volume was not measured at exactly four hours, a weighted average (described above) was calculated to approximate the volume at exactly four hours.

RESULTS AND DISCUSSION

Dewatering of *Chlorella vulgaris* with FO membranes

The dewatering rates of *C. vulgaris* were measured using commercially available FO systems called X-Pack bags and modified versions of these FO bags referred to as "FO chambers" (see Materials and Methods). The FO membranes in both the commercially available bags and the FO chambers have a smooth cellulose triacetate side and a plastic-backing side. Preliminary experiments indicated the microalgae were entrapped in the backing, making it difficult to clean, but were not entrapped on the smooth side of the membrane. Therefore experiments were conducted with the microalgae on the smooth

side and the draw solution on the rough side of the FO membranes.

Two initial dewatering experiments were conducted to determine if the algae impacted the dewatering rates of FO membranes using artificial seawater as the draw solution. In one experiment the dewatering rates of *C. vulgaris* in BG-11 growth medium was measured and in the second experiment BG-11 alone was measured. The conductivity of the BG11 was measured to be 2.6 ms/cm and the addition of *C. vulgaris* did not significantly change the conductivity. For comparison, the conductivity of seawater is 5.0×10^5 ms/cm. For *C. vulgaris* in BG-11 the rates ranged from 1.4 to 2.4 l/m²!hr (avg = 1.9 Std. Dev. 0.5; n= 3) and for BG-11 alone the rates ranged from 0.9 to 2.2 l/m²!hr (avg = 1.6 Std. Dev. 0.9; n= 2). The variation in rates may have been due to mixing, which was difficult in the whole X-pak. There was no significant difference between the dewatering rates, which indicated the algae did not interfere with forward osmosis.

Dewatering performance of a single FO membrane with repeated use:

To determine the reproducibility of dewatering rates and the performance of an FO membrane, a single FO chamber was tested sequentially 14 times (Fig. 1). The average dewatering rate over the 14-day period was 2.1 l/m²!hr with a standard deviation of 0.17 l/m²!hr. In the first four tests, the dewatering rates of 1 liter of *C. vulgaris* (stationary phase culture) in BG11 decreased from 2.4 to 1.9 l/m²!hr (avg. = 2.2; Std. Dev. 0.47; n=4)(Fig. 1 light gray bars). The gradual decrease in rates was not significant in light of the observed variability. The next ten dewatering tests were done with *C. vulgaris* in to municipal wastewater (effluent) from Sunnyvale, CA. In these tests, dewatering rates ranged from 2.2 to 1.8 l/m²!hr (avg = 1.9; Std. Dev. 0.17; n= 10). Tests 5 and 11 had rates that were nearly as high as test 1 and only tests 8 and 12 had rates below 2 l/m²!hr. Over the 4-hour period in these experiments, the algal cultures were dewatered by between 65% and 85%. This is about a 21% increase in algae concentration per hour and a doubling in concentration in 2.4 hours. In these experiments the difference between the salt content of both BG11 and wastewater compared to artificial seawater allowed FO dewatering to continue beyond 85% without a noticeable change in flux rates. We did not determine the point at which the salts in BG11 or wastewater stopped the FO process. (The conductivity of a wastewater sample was measured to be 1.5 ms/cm).

Previously reported FO dewatering rates for wastewater are significantly higher than the rates reported here. For example, Holloway and coworkers^[18] observed FO dewatering rates of approximately 5 to 9 l/m²!hr, using filtered and unfiltered water from anaerobic digesters and 35-70 g/L NaCl as the draw solution. Rates of 18 l/m²!hr and 24 l/m²!hr were reported for 3x and 2x wastewater concentrates respectively, using 100 g/l NaCl as the draw solutions^[5]. These differences may be attributed to the higher salt concentrations in the draw solutions (we used 35 g/l to approximate seawater) and to differences between the systems. The systems mentioned above both used FO membranes in a modified SEPA cell in which tangential flow induces shear, which clears particles and ions causing clogging or concentration polarization. For inexpensive drying, transportation, and processing algal biomass must be dewatered to in some cases to approximately 20% solids and in other cases to dryness depending on the specific downstream process^[19].

For each of the 14 dewatering tests, there were four dewatering rate measurements made at hours 1, 2, 3, and 4. The avg rate for the first hour was 2.6 l/m²!hr with a std. dev. = 0.43 (n=14), for the second hour the avg was 2.3 l/m²!hr, std. dev. = 0.45, for the third hour the avg was 1.9 l/m²!hr, std. dev. = 0.57, and for the fourth hour the avg was 1.1 l/m²!hr, std. dev.= 0.61. The average hourly dewatering rates showed a significant decreasing trend from the first hour (2.6 l/m²!hr) to the fourth hour (1.1 l/m²!hr) (Student t-test, t<0.05). There was however, an observed variability between samples that could not be explained, although it was determined not to be due to methodical errors. It was determined that change in the salinity of the water bath was <0.35 g NaCl!!⁻¹ and the maximum error due to drying and weighing the FO chamber was <1% of the weight of a dry FO chamber,. While it is difficult to account for the observed variability, it may have been due to microalgae, bacteria, and inorganic precipitates which reversibly interact with the surface of the FO membrane and influence its effective surface area and charge characteristics. Variations in FO dewatering rates are typically attributed to membrane fouling and clogging, which creates internal and external concentration polarization, effectively reducing the active surface area of the membrane and therefore the membrane flux rates^[5].

The dewatering potential FO is compared to other methods used for harvesting microalgae in Table 1. While FO has relatively low energy requirements, the harvesting rate and maximum solids concentration were lower than established harvesting methods. Suspended air flotation has the lowest energy requirements, while centrifugation has the highest concentration potential. The optimal harvesting method or combination of methods will depend on the strain of microalgae and the requirements of the downstream processes (% solids or dryness). Other considerations, would include the location of the algae cultivation facility and if wastewater treatment is part of the process. In the case of OMEGA, both the offshore location for algae cultivation and wastewater treatment are part of the proposed process, suggesting FO could be a useful part of the process, if the FO membranes can function in the marine environment.

Table 1: Comparison of forward osmosis with established microalgae dewatering methods.

Microalgal dewater methods	Energy input (kWh/m ³)	Solids concentration	Relative harvesting Rate	Reference
Forward Osmosis	0.3	up to 2%	Slow	Semiat, 2010
Sedimentation	0.1	up to 3%	Very slow	Uduman et al., 2010
Dissolved Air Flotation	1.5 to 20	up to 5%	Medium	Wiley et al., 2009
Suspended Air Flotation	3 x 10 ⁻³	up to 5%	Medium	Wiley et al., 2009
Tangential flow filtration	2.06	up to 4%	Medium-Fast	Uduman et al., 2010
Centrifugation	8	up to 12%	Fast	Schenk et al., 2008

Dewatering performance after ocean exposure

Three FO chambers filled with freshwater from Sequel Creek were suspended in the Monterey Bay for six weeks. The dewatering rates were observed to be between 1.3 l/m²!hr and 2.4 l/m²!hr for the first two tests (Fig. 2, Day 1 and 2). After 5.4 hours the FO chambers were on average 87% dewatered (range 82%-92%). For each experiment, dewatering rates increased for the first two hours and then steadily decreased with time, indicating that maximum flux is reached after the membrane pores have been thoroughly wetted. The salinities for the Sequel Creek water and seawater were 0.5 g/l and 33.6 g/l, respectively. After the preliminary dewatering tests, one of the FO chambers was attached to a buoy in the ocean and the other two were stored in the laboratory. After six weeks in the laboratory, the FO chambers were relatively clean (Fig. 3a), while the bay-exposed FO chamber accumulated biofouling, including a layer of macroalgae on the outer surface, which was the plastic backing (Fig. 3b). This biofouling was not removed from the membrane prior to the dewatering test. In these experiments with six weeks of either marine exposure or laboratory storage, the observed dewatering rates did not significantly decrease compared to the first two runs (Fig. 2, Day 45). After 4.7 hours, the bay-exposed FO chamber was 80% dewatered and the laboratory-stored FO chambers were 80% and 84% dewatered.

This initial experiment indicated that biofouling on the outside of the FO membrane did not decrease flux rates, but a second experiment in which three FO chambers were attached to a buoy for 52 days, resulted in heavier biofouling, which included a conspicuous layer of seaweed and invertebrates, including crustaceans. In this experiment all three of the membranes developed leaks (Fig. 3c). It was not determined what caused these leaks, although observations under the microscope suggested the holdfasts

from seaweed or crustacean claws could puncture the cellulose triacetate layer directly or put added mechanical strain on the membrane and induce leaks through repeated flexing due to water motion. This result suggests that to be used for dewatering in an exposed marine setting, sheets of FO membranes must be engineered to withstand intrusive biofouling and mechanical damage. Future experiments with protective netting around the FO membranes could help resolve this issue and minimize the effects of biofouling.

CONCLUSION

Here, it was demonstrated that currently available FO membranes could provide an initial dewatering step for harvesting microalgae provided 1) slow rates of dewatering are acceptable and 2) problems with biofouling and mechanical damage of the membranes can be overcome. Previous forward osmosis studies using seawater as a draw solution to concentrate and recover heavy metals from dilute industrial wastewater, revealed the limitation of the system due to internal concentration polarization and reverse solute flux^[5]. In future studies on large-scale algae cultivation in conjunction with wastewater facilities, the combined effects of algae and FO on wastewater quality and the impact of reverse solute flux on algae products should be investigated. For both wastewater treatment and algal biofuels, low-energy FO methods may improve the quality of wastewater released into the environment and may be combined with other harvesting methods to increase the techno-economic potential of biofuels.

Acknowledgements

We thank Michael Flynn and Jack Herron for consultation and the staff of the Capitola Boat and Bait Store for assistance. We thank Brandi McQuin and Shirley Fauth for editorial assistance. The research was funded through a collaborative project with Google called "Global Research into Energy and the Environment at NASA (GREEN)."

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Figures

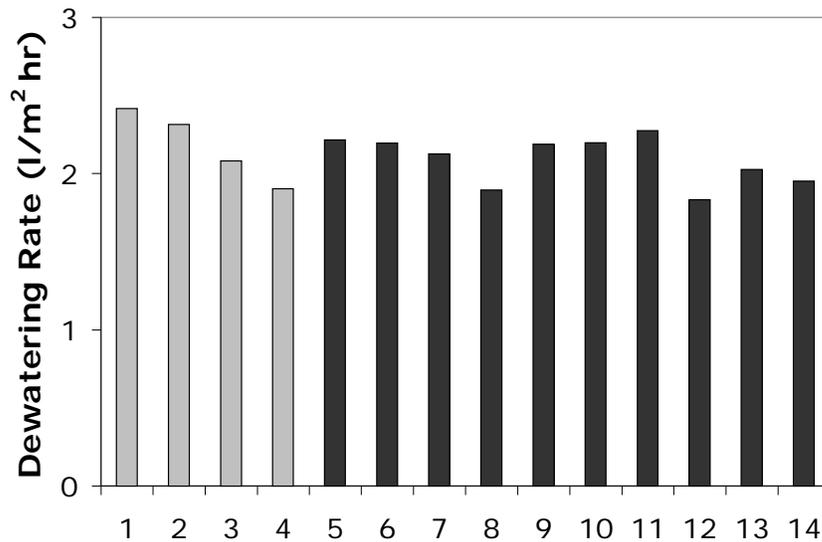


Figure 1: Dewatering performance of a single FO membrane with 14 consecutive dewatering tests. *Chlorella vulgaris* in BG11 medium (gray bars) or added to secondary wastewater effluent from Santa Cruz Wastewater Treatment Facility in Santa Cruz, CA (black bars). Dewatering rate is calculated as the average dewatering rate over the first 4 hours of dewatering. The average dewatering rate was 2.1l/m²!hr, std. dev. 0.17 l/m²!hr.

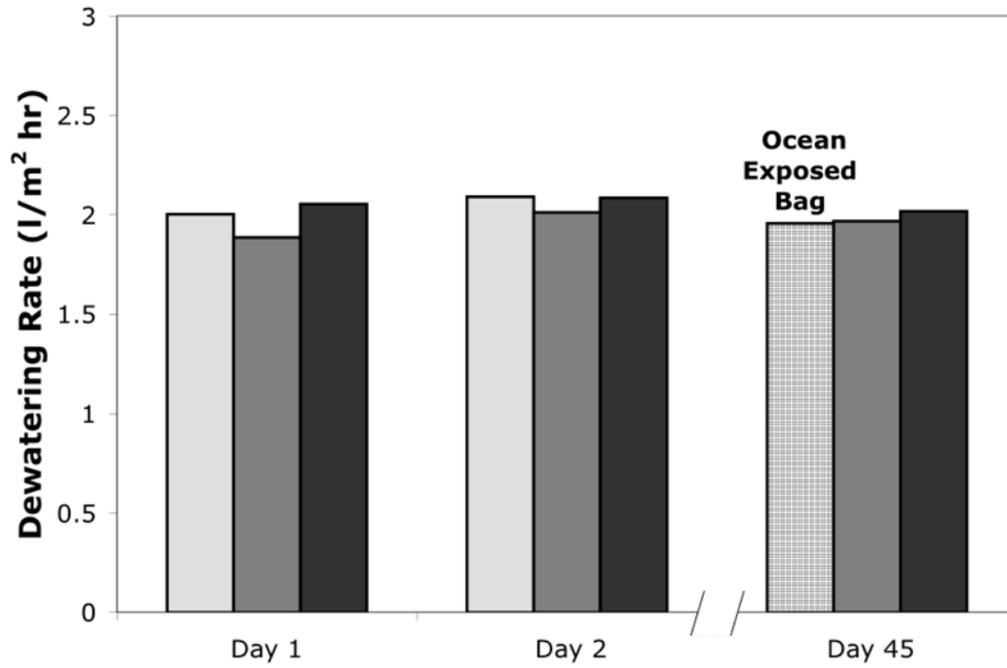


Figure 2: FO membrane performance. Average dewatering rates after four hours for Soquel Creek water using seawater as the draw solution. day 45 shows the ocean-exposed chamber and the two laboratory-stored chambers. The grey and black bars represent the laboratory-stored chamber and the light grey bar represents the ocean-exposed chamber.



Figure 3: Appearance of FO bags after exposure to the marine environment. (a) FO chamber stored in artificial seawater in the laboratory for 45 days, (b) FO chamber in recovered from Monterey Bay after 45 days (3/20/09-5/4/09) and (c) FO chamber recovered from Monterey Bay after 52 days (10/11/09-12/2/09).

**Appendix D:
Wireless ISFET pH Sensor Network for Offshore
Microalgae Cultivation Proceedings of the ASME 2012
International Mechanical Engineering Congress &
Exposition IMECE 2012 November 9-15, 2012,
Houston, Texas, USA**

APPENDIX D:

Wireless ISFET pH sensor network for offshore microalgae cultivation

*Proceedings of the ASME 2012 International Mechanical Engineering Congress & Exposition
IMECE2012 November 9-15, 2012, Houston, Texas, USA*

Summary

Microalgae technology continues to show tremendous promise for becoming a major source of renewable transportation fuel in the coming decades. However, for microalgae to provide a significant fraction of the current US demand for fuel, their cultivation will be required on an enormous scale. One of the many formidable challenges that must be met to achieve this scale is the development of appropriate sensor networks to provide information about the growth conditions and the algae themselves. These sensors would monitor the heterogeneity of a) environmental parameters, such as pH, oxygen, and nutrient levels and b) algal characteristics such as size, oil content, and viability. Here we present a wireless sensor network to measure the local pH in NASA OMEGA project (Offshore Membrane Enclosures for Growing Algae). The pH is measured using Ion Sensitive Field Effect Transistor (ISFET) technology, which is more robust and has a faster response than traditional glass pH electrodes. A custom circuit drives the ISFET sensor and interfaces with an ANT wireless network system. The wireless network consists of a network hub which can service up to 8 sensor nodes and a series of relays to transmit the data to a PC. The data is logged with a custom LabVIEW program. In this work, we demonstrate operation of this network using a single ISFET pH sensor, one hub, and two relay units. The performance of the pH sensor network is evaluated and compared in parallel with an existing wired glass electrode based pH monitoring system at the NASA OMEGA project.

INTRODUCTION and motivation

One of the preeminent challenges facing scientists and engineers in the 21st century has been and will likely continue to be the development of economically and technically feasible renewable energy technologies. While many of these efforts, such as wind, solar, and geothermal, address electricity generation, there are relatively few options to consider for transportation fuels which account for over 1/3 of the US energy needs [1]. Biofuels, from corn or other plant products, have tremendous promise as they can serve as a drop-in replacement for use in our existing infrastructure. However, there is real concern over whether “conventional” bio-feedstock can be viable replacements for fossil fuels due to their need for arable land, high water usage, and relatively long growth cycle. Microalgae, on the other hand, does not suffer from these same limitations and many researchers

For the experiment presented in this work, one wireless pH sensor was constructed to be compatible with standard 3/4" PVC fittings, located on a sensor manifold in the OMEGA system that sampled the flow at the exit of the photobioreactors. To facilitate more in-depth troubleshooting of the circuit board, the circuitry was not enclosed in a PVC pipe section for this experiment. Instead, the board was left open in a small plastic box with a plastic sheet to prevent rain entering the compartment. The PVC barrier was left in place. No leaks through this barrier were observed through the course of this experiment.

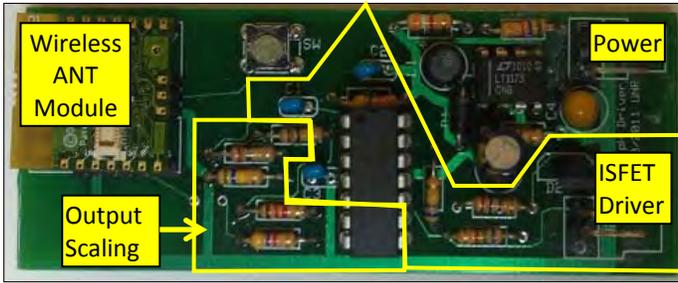


Figure 3: Simplified block diagram of primary ISFET sensor electronic components

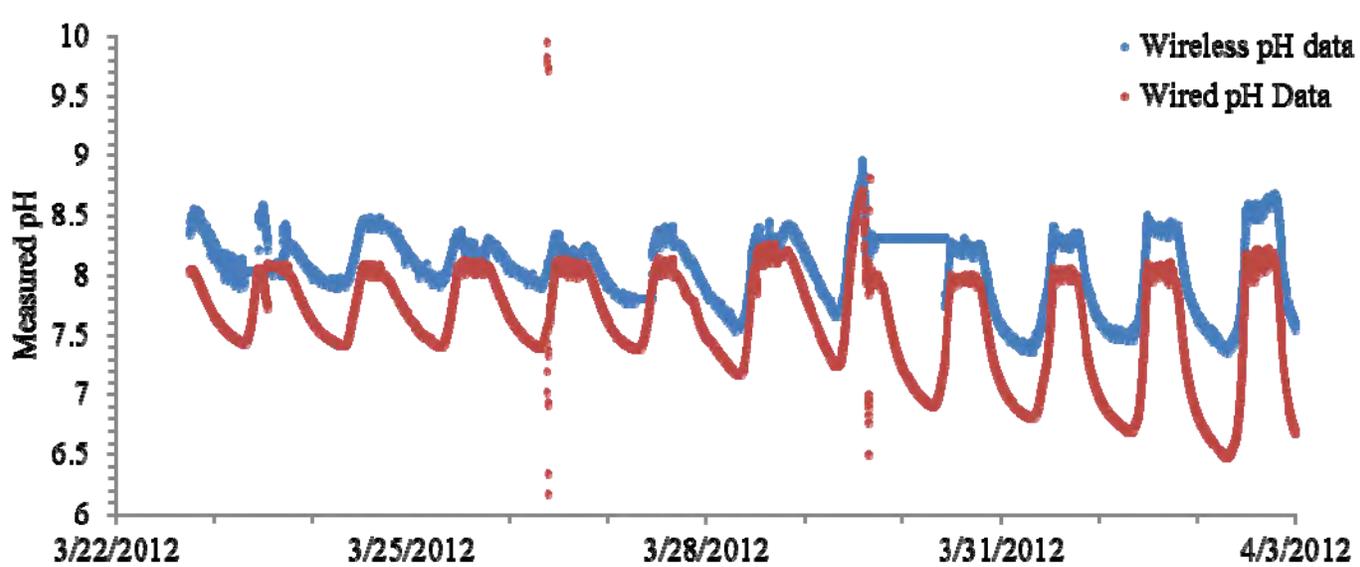
This pH sensor was installed on the sensor manifold in parallel with the existing OMEGA system, which used a pH electrode supplied by Cole-Parmer (Vernon Hills, IL). The wireless system logged data from 22 March 2012 to 2 April 2012, stopping only when the battery in the sensor was depleted. In addition, the sensor circuit's calibration was checked on 20 March 2012 and 6 April 2012.

DISCUSSION OF RESULTS

The evaluation of calibration stability was accomplished by measuring the voltage output of the sensor circuit in standard pH buffer solutions of pH 7 and 10, at a time before and after the main data collection run of this experiment. These data are given below in Table 1. This demonstrates a very stable calibration for the ISFET sensor, moving less than 2 percent in 17 days.

Table 1: ISFET sensor output voltage in pH calibration standards before and after the present experiment

<i>Date</i>	<i>pH</i> <i>7</i>	<i>pH</i> <i>10</i>		
20 March			1.594	1.052
			V	V
6 April			1.599	1.038
			V	V



In the time between these checks of the calibration

n, the network was configured to save the data beginning on 22 March 2012. This data logging continued with l

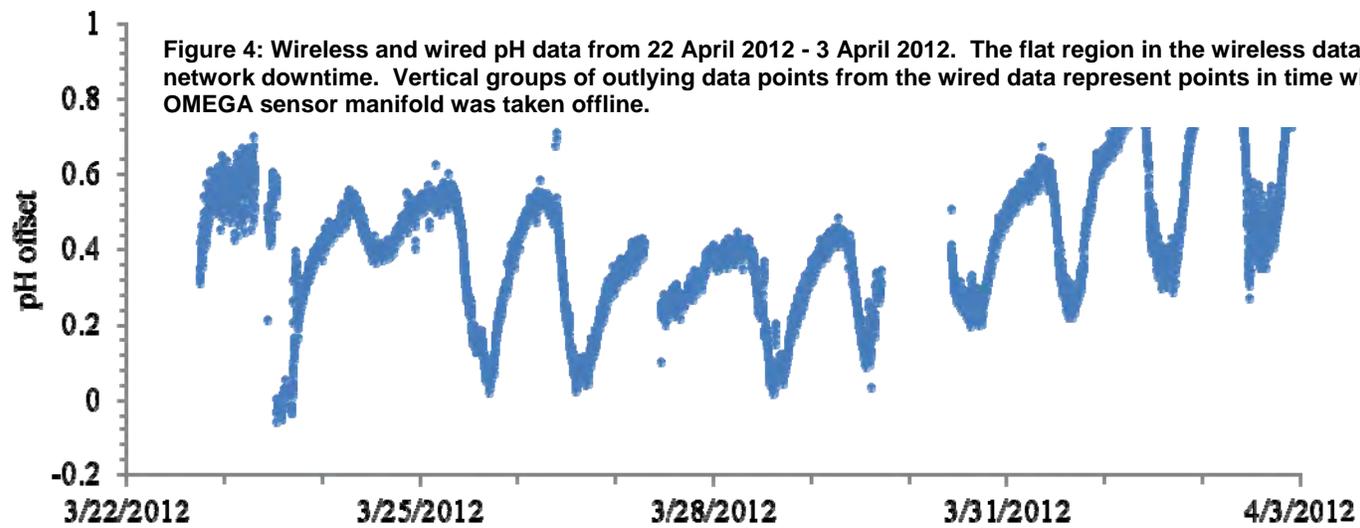


Figure 4: Wireless and wired pH data from 22 April 2012 - 3 April 2012. The flat region in the wireless data represents network downtime. Vertical groups of outlying data points from the wired data represent points in time where the OMEGA sensor manifold was taken offline.

limited interruptions until 2 April

2012, when the battery

Figure 5: Offset pH vs. time. This gives the difference between the wireless and wired data as a function of time.

in the sensor node was depleted. The 3-volt CR123A battery cells were also used in the hub and relay units, and were not depleted at the end of this experiment. The observed interruptions in the data were due to a program failure in one of the relay units. Programming-based solutions for this problem

are currently being explored. Interruptions in the data can be seen as uncharacteristically flat portions of the wireless data signal in the plot given in Figure 4.

Figure 4 is the plot over time of the wireless pH data alongside the existing wired system at OMEGA. From this figure it can be seen that the general trend of the wireless pH data matches the daily cycling of the wired pH data, but has a significant positive offset from it. This offset, the difference between the wireless and wired pH data, is plotted in Figure 5. This also exhibits a daily cyclical variation, and a close inspection of the data shows that the pH offset is greater at lower measured values of pH, giving a suggestion of a nonlinear response in one or both of the sensor systems. Subsequent work is planned to characterize this condition in more detail.

The physical integrity of the system remained excellent throughout the duration of the experiment. The enclosures for each component remained intact and sealed during exposure to wind and rain. There were no long wires that risked damage in this configuration, while the sensor cables for the wired system needed to be retrofitted to prevent water entry at its connection points. A larger scale experiment with more components, with a duration of at least one complete maintenance cycle for a wired system would verify the superiority of a wireless system from a reliability and maintenance perspective.

CONCLUSIONS AND FUTURE WORK

In this work, a wireless ISFET pH sensor has been demonstrated in operation in the context of the OMEGA microalgae cultivation system designed for offshore use and prototyped in conditions replicating a near-shore protected waterway. The calibration stability has been characterized, and the difference in measurement between the wireless device and a parallel wired system has been examined. The performance characteristics of the sensor and the reliability of the data communication show promise for this technology to be featured in monitoring systems for large scale algae cultivation operations, including those located in marine environments.

Additional experiments are planned along several lines of inquiry. Additional data on the ISFET performance can be collected by running additional data collections in parallel with other monitoring systems and

by comparison with a frequently calibrated pH measurement standard. The analog/digital conversion capability of the ANT modules suggests that the wireless communication method can be extended to other types of sensors of interest to algae cultivation operations, including temperature and dissolved oxygen probes. Further programming refinements with the ANT modules can allow more complex and larger networks of sensors, and additional safeguards to programmatically ensure robust autonomous operation.

ACKNOWLEDGMENTS

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**Appendix E:
OMEGA Outside Endorsements**

Appendix E: OMEGA Outside Endorsements

- 1. Phase I Review letter to NASA Ames Center Director (Pete Worden) from Moss Landing Marine Laboratories**
- 2. Navy Memorandum of Understanding (January 2011)**
- 3. Environmental Protection Agency Interest Letter (June 2012)**
- 4. Chesapeake Bay**



Moss Landing Marine Laboratories

8272 Moss Landing Road, Moss Landing, CA 95039-9647 USA Tel: (831) 771-4400 Fax: 632-4403

(<http://www.mlml.calstate.edu>)

June 2, 2010

Dr. S. "Pete" Worden, Center Director
NASA Ames Research Center
Mail Stop I:200-3
Moffett Field, CA 94035-1000

Re: Letter of Support for OMEGA program

Dear Dr. Worden,

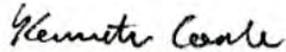
We are writing as members of the OMEGA Executive Level Review Board, convened at the request of Associate Center Director, Dr. Steve Zornetzer to provide an independent assessment of the OMEGA program and guidance to the project team. We are familiar with the program, having received written materials, electronic resources and two briefings to date. Although familiar with many aspects of marine science, none of us have any direct fiscal or current scientific interest in this program. Although we are not conflicted in this regard, we remain keenly interested in the potential that this project seeks to deliver.

Because the oceans can provide unique aspects for growing biofuels in terms of cooling, osmotic dewatering, mixing, conservation of arable land, etc., this project is of particular interest and concern for the marine scientist and policy experts. In our opinion the technology development that the OMEGA project seeks to achieve is highly worthy of pursuit. Unique opportunities exist to combine science with engineering, engage a multidisciplinary team that will address a socially relevant issue that represents one of the greatest challenges of our time. The Review Board feels strongly that a pilot scale program should go forward recognizing that the next phase will answer the question of how well microalgal biofuels can meet the our aviation needs of the future. The project has been well developed conceptually and demonstrated with some limited laboratory experiments and preliminary engineering studies, yet remains at a crossroads and cannot advance to the level of demonstration without resolving some of the larger design issues. It is important to do this study so that known issues can be resolved and unknown issues, identified. We note also that, within the marine science and policy arenas, the issue of marine spatial planning is moving ahead quickly. It will be important for the OMEGA program to articulate its requirements within that context.

It should be mentioned that the goal of this project is more important than some of the problems and resistance that will be encountered. In our opinion, it is appropriate that NASA takes a leadership role, including the commitment of resources and personnel (as well as reputation) to solve the sustainable energy challenges that face the nation. This is a tremendous opportunity and one that will advance the US on a path of leadership in engineering, innovation, sustainability. We look forward to strong commitment from NASA to help insure the success of this project.

In this regard, we find the OMEGA project to resonate strongly with the NASA National Plan for Aeronautics Research and Development as articulated in the recent Aeronautics Science and Technology Subcommittee report to the NST Council in 2007. Here, the need for a sustainable, economical, secure and reliable source of aviation fuel was articulated throughout this document as an environmental and national security imperative. It is completely appropriate that NASA take a leadership role towards the realization of these important objectives, and we strongly feel that the OMEGA program represents a bold step in this direction. Please feel free to contact any of us if you have any questions or concerns regarding this assessment.

Best regards,



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Mr. Luke Nachbar
Congressional Affairs Specialist
National Oceanic and Atmospheric Administration
Luke.nachbar@noaa.gov

Cc: Dr. S. Zornetzer

OMEGA

NASA-Navy: a strategic planning discussion

Norfolk, VA

Thursday, March 25, 2010

!

!



Project Scope Overview

The primary goals of this project plan are to demonstrate the feasibility of the OMEGA system for: 1) production of biofuels and fertilizer, 2) processing of wastewater, and 3) sequestration of CO₂. To reach these goals, a diverse OMEGA implementation team will address technical, environmental, and economic challenges.

Technical approach

An experimental test-bed system will be developed in which prototype OMEGA configurations can be evaluated for material and functional integrity as well as filling and harvesting logistics. Various OMEGA designs will be tested for algae growth in wastewater, fouling on internal and external surfaces, and structural integrity against leakage, breakage, and component failures. The necessary facilities to design, build, and test OMEGA systems will be constructed at the California Fish and Game (CFG) facility in Santa Cruz, CA.

Environmental approach

In the CFG outdoor tanks and field sites, the impact of OMEGA on the environment will be evaluated both by long-term exposures and by simulating OMEGA failures that release treated wastewater and freshwater algal biomass. In addition to experimentation, OMEGA will be presented to environmentalists and to state and local government agencies to determine what social, policy, and permitting issues will need to be addressed to deploy OMEGA on various scales in different offshore locations. This process will involve presentations and discussions as well as applications for actual permits.

Economic approach

The life-cycle costs and effects of OMEGA will be analyzed and compared to other technologies to show that the system is robust and economically viable. Life cycle analysis will be used to complete a lifecycle impact assessment for OMEGA system parameters (e.g., energy, CO₂, solid waste and materials) from cradle to grave. The pros and cons of OMEGA relative to other alternative energies will be quantified and evaluated alongside capital and operating cost for design and deployment, products (e.g., aviation fuel and fertilizer) and services (wastewater treatment and CO₂ sequestration). The ideal footprint for the OMEGA system, which includes not having to compete for agricultural land or freshwater resources, uses untapped wave energy for mixing, and utilizes waste products, will be essential in showing economic viability and positive environmental impacts.

MILESTONE – OMEGA Preliminary Engineering Analysis

During this task a set of design criteria and guidelines will be established. Based on these criteria a preliminary engineering analysis of the bio-reactors will be performed, followed by a scale model used to complement the findings obtained analytically. The preliminary engineering analysis will focus on the enclosure for the OMEGA bio-reactors. Analysis of mooring structures, anchors and connection to outfall pipe is not covered in this task. This task will be carried out in a series of subtasks as follows:

Preliminary engineering analysis of major structural elements of the closed photo-bio-reactors

URS will perform a preliminary engineering analysis of major structural elements following the criteria set forth in the previous sub-task. Engineering analysis performed during this sub-task will be limited to: (1) bio-reactor's enclosure, (2) plastic welds, (3) cable and hoses, and (4) enclosure/hoses connections.

Preliminary engineering analysis will be performed for the three enclosure configurations defined in the previous sub-task. This analysis will be limited to the structural integrity of the bio-reactor's enclosure. Analysis of the following items is not included in this sub-task: (1) hydraulic modeling of the bio-reactor filling, harvesting, and dewatering, (2) anchors, (3) wincher, (4) outfall pipe and manifold, (5) bio-reactor/outfall connection, and (6) mooring structure.



Preliminary engineering analysis of major structural elements of the bio-reactor enclosure will be performed in two stages: (1) analytical procedures, and (2) computer modeling.

In the first stage, reasonable assumptions will be used to simplify the problem, such that analytical methods can be used (e.g. using basic formulations that can be solved by hand, spreadsheet or MathCAD routines). These simplified analytical procedures will be used as a first approach to guide the second stage towards the analysis of those items that result more critical from a design standpoint. In the second stage, advanced computer modeling (e.g. Finite Element Models) will be used to model particular conditions that are likely to control the design of the bio-reactor's enclosure.

Two deliverables will be prepared within this subtask: (1) Draft preliminary engineering analysis – technical memorandum and (2) Preliminary engineering analysis – technical memorandum. Following submittal of the draft technical memorandum, a review meeting will be held to discuss comments to the document. The preliminary engineering analysis will be issued once comments received during the review meeting are incorporated into the draft preliminary engineering analysis. Two copies of each deliverable will be provided.

Test Procedure and Data evaluation of scale model

NASA will build and operate a scale model of the bioreactor. URS will prepare a test procedure for the scale models based on the findings of the preliminary engineering analysis. The test procedure will define the scenarios to be tested, as well as those variables to be measured.

Test results for each of the scenarios tested will be provided to URS for their analysis. Data will be analyzed to establish the validity of the results and evaluate how the results of the model compare to those obtained analytically.

Four deliverables will be prepared within this subtask: (1) Draft scale model procedure – technical memorandum, (2) Scale model procedure – technical memorandum, (3) Draft scale model data evaluation – technical memorandum, and (4) Scale model data evaluation – technical memorandum. Following submittal of draft technical memorandums, a review meeting will be held to discuss comments to the document. The final versions will be issued subsequently. Two copies of each deliverable will be provided.

The preparation and execution of the actual scale model is not included within URS' scope of work. Execution of activities within this sub-task is dependent on collection of data from the scale model. Schedule changes might arise as a result of delays on the construction and operation of the scale model.

Environmental and Permitting Reconnaissance

NASA will identify three potential sites for an Algae OMEGA pilot plant installation. URS will conduct permitting reconnaissance of these three sites to determine what permits might be required for such an installation at each of the sites as well as the cost and time required to secure all of the required permits. The first step in this process will be to research each to determine whether there are any obvious fatal flaws that would preclude issuance of appropriate permits. Upon completion of the fatal flaw evaluation, URS will identify the lead agency for each site. The lead agency has the responsibility for undertaking the NEPA/CEQA review. We will consult with the probable lead agency and determine what permits will be required at the candidate sites. Once the lead agency has been identified and a list of permits drawn up, URS will develop an estimated cost to complete the NEPA/CEQA review process and acquire the required permits.

Life Cycle Analysis (LCA)

URS will undertake a scalable lifecycle analysis for OMEGA and two baseline alternatives. The lifecycle analysis will compare the full range of environmental and social effects assignable to the multiple



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Life Cycle Analysis (LCA)

URS will undertake a scalable lifecycle analysis for OMEGA and two baseline alternatives. The lifecycle analysis will compare the full range of environmental and social effects assignable to the multiple



The purpose of this task is to provide an estimate of the mixing of the enclosure contents with the ambient waters and the corresponding dilution. This task consists of the following five subtasks.

Mixing of a discharge into an ambient water body is usually divided into two different regimes, the near-field and the far-field. In the near-field the mixing is due to the characteristics of the discharge. For example the discharge flow rate, effluent density relative to ambient conditions, size of the discharge and effluent velocity. In the far-field the dilution is due to the characteristics of the ambient environment, e.g., temperature and salinity, and current speed and direction. In developing discharge permits, such as NPDES permits, the regulatory agencies are most often concerned about near-field dilution. For spills and unintended discharges agencies may be also concerned about the far-field dilution since that may be necessary to accurately estimate the size of the impact.

Risk Management

The proposed pilot project has a number of risk factors in the financial, technical and environmental fields. They will be managed by utilizing a project specific risk register which quantifies the cost and scheduling issues associated to each potential hitch. The risk register will be developed and updated regularly throughout design and construction.



products produced by OMEGA and compare these to alternative technologies or production systems (e.g., ethanol). It involves an assessment of raw material production, manufacture, distribution, use and disposal including all intervening transportation steps necessary or caused by the product's existence.

The first phase of the LCA involves formulating the goal and scope of the study. The goal and scope should address the overall approach used to establish which unit processes are included in the LCA (e.g., energy, CO₂, and solid waste). The lifecycle inventory is the next phase. It involves data collection and modeling of the product system, as well as description and verification of data. This encompasses all data related to environmental (e.g., CO₂) and technical (e.g., intermediate chemicals) quantities for all relevant unit processes within the study boundaries. The final important phases are the lifecycle impact assessment and interpretation of the LCA results, some of which may be valued in the benefit cost analysis below.

Benefit Cost Analysis

URS will use benefit cost analysis (BCA) to compare the lifecycle benefits and costs of OMEGA for four (4) different scenarios. This task will combine information generated in other tasks to determine the economic attractiveness of the OMEGA system relative to the baseline alternatives identified in the LCA. For this task, URS will use discounted cash flow analysis to develop a tool to compare both the financial and net social benefits of OMEGA. The financial analysis will model project cashflows including revenues (including carbon credits), operating and capital and expenses, taxes and costs of finance. All assumptions used in developing the financial model will be tested using sensitivity analysis. This analysis will answer the question "how profitable and what is the return on investment for the OMEGA system". Alternatively, the net social benefit cost analysis will identify and quantify wherever possible, the public and private economic, environmental and social (i.e., green job creation) benefits and costs associated with the OMEGA system. This analysis will answer the question "when all economic, environmental and social tradeoffs are considered, is investment in OMEGA economically justified?" Once again sensitivity analysis will be used to test key assumptions.

The analyses will identify the mix of public and private beneficiaries and the magnitude of these benefits. Such information is critical for developing financing/funding and cost sharing arrangements. URS will write a technical memorandum documenting this task and the assumptions used.

Develop Business Case

URS will work with NASA Ames to prepare a business case that "tells the story" for the development of the OMEGA system from the initial industry and political drivers, to the alternatives analyzed, and recommendations for further development and implementation. The OMEGA business case is a concise document that will advocate a particular course of action to decision makers, drawing together all the analyses and arguments available in support of the advocated decision. It will summarize all of the information obtained from the engineering, environmental and economic approaches, and additional information including recommended procurement, financing, implementation and timing.

Energy Return on Investment Study

URS will estimate the energy return on investment for the OMEGA system by combining information on energy use from the LCA (Task 3.8), and annualized lifecycle costs identified in the Benefit Cost Analysis (Task 3.9). The return on investment provided by OMEGA will be benchmarked with the unit cost of energy production for other alternative energy technologies.

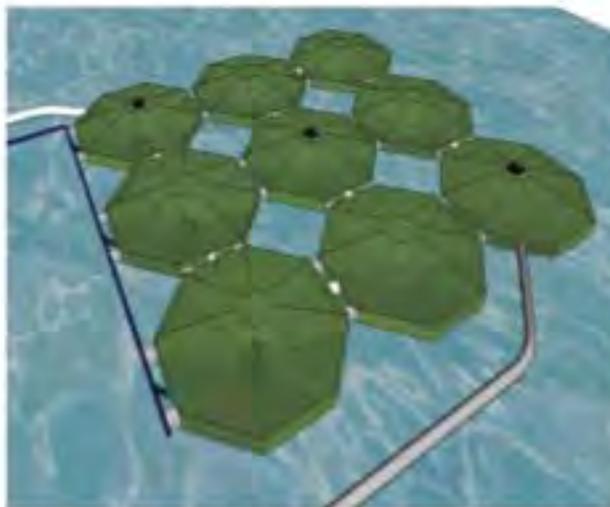
Dilution Modeling

Under normal operating conditions the OMEGA enclosures will exchange oxygen and carbon dioxide between the algae enclosure and the ambient water through gas permeable membranes. There will be no discharge from the enclosure into the ambient waters. However, if there were a failure of the membranes or damage to the enclosure there could be a discharge of the enclosure contents into the ambient waters.

Photobioreactor designs known as batch and continuous flow are under engineering review for materials, hydraulics, and function. The continuous flow systems are currently favored, focusing on designs configured as plug-flow (a) and completely mixed systems (b). In the coming weeks, prototypes of both of these systems will be constructed, using high-density polyethylene (HDPE) for tank testing to monitor strength, durability, mixing, and functionality.



(a) Plug-flow system



(b) Completely mixed system



NASA and the U.S. Navy collaboration on OMEGA for the fuel of the future

The National Aeronautics and Space Administration and the U.S. Navy share a practical and profound interest in "green" fuels based on their commitments to air transportation and national security on the one hand and global warming and the need to find alternatives to fossil fuels on the other. It is now widely acknowledged that the best source of alternative fuels or biofuels is algae or more specifically microalgae. Algae, like other plants, use CO₂ and sunlight to grow and therefore in the process of growing remove this green-house gas from the atmosphere. The productivity of algae greatly exceeds all other biofuel crops by a wide margin. It is estimated, for example, that algae could provide the entire annual supply of aviation fuels for the U.S. (~21 billion gallons) in a cultivation area half the size of Connecticut, which is <2% of the U.S. agricultural land currently under cultivation. Furthermore, it has been suggested that large quantities of algae can be produced without competing with agriculture for land, water, or fertilizer. Algae do not require agricultural land because they are traditionally cultivated in open ponds or closed photobioreactors, which can be located away from agricultural land—in deserts, for example. Algae require water and fertilizer, but they can grow on the water and residual nutrients in treated domestic wastewater—water not currently used for agriculture. If all this is true, why aren't algae a major contributor to biofuel production?

The major reasons algae are not currently contributing to biofuels have to do with logistics and economics. The logistics problems stem from the scale of algae farms required for producing the quantities of biofuel needed to meet the economics. Such farms will use millions of acres of non-agricultural land and require huge quantities of wastewater. The logistics problems are apparent, if we equate non-agricultural land with deserts and consider that big cities are the best sources of wastewater. Most big cities are not near deserts, which means wastewater will need to be transported great distances—transporting water is not only difficult, it is prohibitively expensive.¹ On the other hand, perhaps we do not need to equate non-agricultural land with deserts.

Most major cities in the U.S. are located on the coasts and these cities are indeed producing copious amounts of wastewater (>11 billion gallons per day). Currently, most of this wastewater is dumped into the oceans through offshore outfalls and both the water and associated nutrient are not only lost at sea, in some cases the nutrients in the wastewater cause harmful algae blooms in coastal zones—wastewater, algae blooms, and there's no agriculture going on offshore.

NASA scientists and engineers are proposing OMEGA (Offshore Membrane Enclosures for Growing Algae) to meet the needs of large-scale algae cultivation for biofuels in a way that does not compete with agriculture for land, water, or fertilizer, and will not only help sequester CO₂ from the atmosphere, but will directly improve environmental conditions in marine ecosystems by preventing harmful algae blooms. The OMEGA concept involves floating photobioreactors made of flexible plastic modules filled with treated domestic wastewater from existing offshore outfalls. Located offshore, OMEGA obviously does not compete for cultivatable land and using wastewater, it does not compete for water or fertilizer, but OMEGA has many other advantages over land-based algae cultivation systems. For example, land-based systems require energy for temperature control and mixing the algae culture, but OMEGA uses the heat capacity and thermal inertia of the surrounding seawater for temperature control and it uses wave power to induce mixing. In addition, OMEGA takes advantage of the salt gradient between wastewater and seawater to protect the environment from the cultivated algae and for a process called forward osmosis, which stimulates algae growth, saves energy during harvesting, and cleans the water released into the marine environment. The salt gradient protects the environment from the cultivated algae,

¹ Arguably, if wastewater is transported to deserts, it should be used for growing food rather than algae to meet the world's food needs rather than our need for biofuels.



because OMEGA will cultivate freshwater algae in wastewater and if these algae escape into the surrounding seawater they will die. In other words, the algae in OMEGA cannot become invasive species in the marine ecosystem.

Forward osmosis uses the osmotic pressure between the low salt concentration of wastewater and the high salt concentration of seawater to move water across a membrane that excludes solutes and particles. The OMEGA system uses forward osmosis to slowly move water out of the OMEGA system, concentrating the nutrients and the algae in the process. The concentrated nutrients stimulate algae growth and the concentrated algae save energy needed for harvesting. In addition, both the forward osmosis and the algae inside OMEGA remove nutrients and other components of the wastewater, which cleans the water that is released into the surrounding environment. This process can help prevent harmful algae blooms and improve conditions in coastal ecosystems.

OMEGA has the potential to grow the vast quantities of algae that will be needed for the production of significant amounts of biofuels needed by our society. It can do this without competing with agriculture and while having a positive impact on the environment by providing advanced wastewater treatment and CO₂ sequestration. What then is needed to implement OMEGA on a scale and in time to make a difference in the world?

OMEGA faces the intrinsic challenges of all algae cultivation systems and some of its own. These challenges include biological, engineering, economic, and environmental challenges. Biological challenges include algal growth rates, invasive (weed) species, pest control, and the effects of pathogens. Engineering challenges include the and above all economics. OMEGA also has its own unique challenges associated with its offshore deployment. These include materials and designs that can withstand the rigors of the marine environment, logistics for filling and harvesting OMEGA modules under varying conditions, and finding locations for large OMEGA farms required for biofuels that will not impact the aesthetics of our coastal areas, fishing, and ship traffic of all kinds.

While these challenges are formidable and the rates of social and environmental change suggest we do not have a lot of time to meet them—perhaps less than ten years. On the other hand, the potential impact of OMEGA on the strategic energy future of the United States and on marine ecosystems, directly by wastewater treatment and indirectly through its effects on climate change, are all strong incentives to push forward with OMEGA at all costs. The success of OMEGA will depend on unprecedented innovation and highly integrated systems engineering combined with a profound understanding of the marine environment. With this in mind, what two organizations are better suited to take on the challenges of OMEGA and to put the US in a leadership role in biofuels, than NASA and the US Navy?

an acceptable system for producing biomass for use as a biofuel feedstock to meet the Navy's needs for future operations. Areas of potential additional cooperation include:

- Navy consultation and assistance in furtherance of NASA's OMEGA prototype designs.
- Navy consultation and assistance in furtherance of NASA's evaluation and testing of OMEGA materials and subsystems.
- Navy support for performing tests or analyses using Navy facilities and equipment. Any use of Navy facilities or equipment by NASA shall be pursuant to separate agreements specifically outlining the terms thereof.
- Identification and assessment of potential OMEGA deployment locations that facilitate the Navy's entry into further biofuel projects designed to meet long-term Navy objectives.

III. AUTHORITY

NASA enters into this MOA in accordance with the National Aeronautics and Space Act of 1958, as amended (42 U.S.C. § 2473(c)). The Navy enters into this MOA in accordance with 10 U.S.C. Section 5013(b)(4). NASA and the Department of the Navy may be individually referred to as a "Party" and together as the "Parties."

IV. POINTS OF CONTACT

The Parties designate the individuals identified below as their respective single points of contact (POC), who have the responsibility and authority to coordinate and execute the provisions of this MOA. POCs will serve as liaisons and have full authority to coordinate with their counterparts to ensure successful execution of this MOA.

Department of the Navy

Office of the Deputy Assistant Secretary of the Navy for Energy
Mr. Chris Tindal, Director of Operational Policy

Navy Energy Coordination Office (OPNAV N43E)
CAPT James Brown Jr., Director

NASA

NASA Ames Research Center
Thomas Edwards, Ph.D., Director of Aeronautics

V. FUNDING & LIABILITY

- A. This MOA does not constitute an obligation or commitment of funds or a basis for the transfer of funds. Each Party shall fund its own participation in this MOA, and each

Party's participation shall be subject to the availability of appropriated funds. No provision of this MOA shall be construed to require provision of resources in violation of the Anti-Deficiency Act, 31 U.S.C. 1341, *et seq.*, or any other applicable statute or regulation.

- B. Each Party agrees to assume liability for its own risks associated with activities undertaken in this MOA. Nothing in this MOA constitutes a guarantee by either Party of any obligation assumed by the other Party or of an agreement by either Party to indemnify the other Party for any liability arising out of activities undertaken in this MOA.

VI. USE AND RELEASE OF TECHNICAL DATA, PROTECTION AND SECURITY OF INFORMATION

- A. The Parties intend that the information and data exchanged in furtherance of the activities under this MOA will be exchanged without Federal-use restrictions, unless required by national security regulations or otherwise agreed to by the Parties for specifically identified information or data.
- B. The Parties agree that they will take appropriate measures to protect proprietary, privileged, classified, or otherwise confidential information that may come into their possession as a result of this MOA.
- C. Release of information associated with joint activities carried out under this MOA will appropriately recognize each Party and will be coordinated between the Parties.

VII. INTELLECTUAL PROPERTY

Unless otherwise agreed by the Parties, custody and administration of inventions made as a consequence of, or in direct relation to, the performance of activities under this MOA will remain with the respective inventing Party. In the event an invention is made jointly by employees of the Parties or an employee of a Party's contractor, the Parties will consult and agree as to future actions toward establishment of patent protection for the invention.

VIII. DISPUTE RESOLUTION

The Parties may consult on any matter arising out of this MOA. An issue concerning the interpretation or implementation of the terms of this MOA shall first be referred to the POCs for the Parties. If they are unable to come to agreement on any issue, the dispute will be referred to the MOA signatories or their designated representatives for resolution.

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IX. MODIFICATIONS

This MOA may be modified upon the mutual written consent of both Parties. Modifications must be signed by the original signatories to the MOA, or their designees or successors. No oral statement by any person shall be interpreted as modifying or otherwise affecting the terms of this MOA.

X. TERM

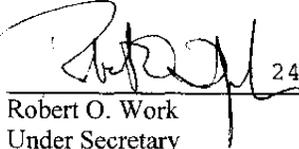
This MOA shall be effective when signed by the authorized representatives of both Parties. Unless terminated by either Party as provided in section XI, it shall remain in effect until the completion of all obligations of both Parties hereto, or at the end of the first quarter of the Federal Government's fiscal year 2012, whichever comes first.

XI. TERMINATION

Either Party may terminate this MOA at any time, with or without cause and without incurring any liability or obligation to the terminated Party, by giving the other Party at least thirty (30) days prior written notice of termination.

XII. APPROVALS

In consideration of the foregoing, the undersigned hereby execute this MOA:


24 JAN 2011
Robert O. Work
Under Secretary
Department of the Navy


Lori B. Garver
Deputy Administrator
National Aeronautics and Space Administration



Department of Energy

Washington, DC 20585

November 10, 2010

The Honorable Thomas McLain Middleton
Chairman, Chesapeake Bay Commission
60 West Street, Suite 406
Annapolis, Maryland 21401

Dear Senator Middleton:

Thank you for your October 1, 2010 letter to Energy Secretary Chu regarding the Chesapeake Bay Commission's interest in the deployment of the NASA-funded OMEGA project. We share the Commission's recognition of the critical need to find technologies that can address the combined issues of water quality, environmental sustainability, and energy in watershed regions throughout our country.

The OMEGA off-shore system concept for growing microalgae for biofuel feedstock using treated wastewater effluent is an interesting complement to the research, development, and deployment investments currently being made in the Department of Energy's (DOE) project portfolio, which have on-shore focus. Staff members from the Department's Biomass Program currently serve on the design review panel for the NASA-OMEGA project. The current assessment of our experts is that the technologies and logistics for OMEGA remain in the research and development stages of development and considerable supporting research, incremental development, and scale-up testing and evaluation are still needed on a range of topics that include algae growth and nutrient utilization in a highly variable wastewater environment, cultivation system design and materials compatibility, and overall technical and economic performance on a life cycle basis.

DOE will continue to maintain cooperative engagement with NASA as the OMEGA project moves through its planned phases of design, development, testing, and evaluation while looking for potential opportunities for future collaboration that could possibly include incremental scale-up, testing, and demonstration as part of a broadly supported effort of the relevant governmental agencies representing constituencies around the Bay.

We would also be pleased to engage in broader discussions to seek solutions to Bay contamination problems. Algae has the potential to be utilized to reduce nutrient loading of wastewater and natural surface waters, such as the Bay, while potentially producing biomass that can be used as a feedstock for biofuels production. There are numerous technologies under development to treat wastewater and recycle nutrients from agricultural wastewater streams, including turf scrubbing technologies that may be of interest. If desired, we could arrange to meet with you to further discuss technology options and possible collaboration in deployment of commercial technology or development and trial testing of new concepts.





UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

REGION IX

**75 Hawthorne Street
San Francisco, CA 94105-3901**

JUN 29 2012

Ms. Lori B. Garver
Deputy Administrator
NASA Headquarters
Washington D.C. 20546-000

Dear Ms. Garver:

This letter is to inform you of the U.S Environmental Protection Agency (EPA) Region 9's interest in the progress of the NASA OMEGA Project. Jonathan Trent, the Project's principal investigator, gave a seminar under the auspices of the Region 9 Regional Science Council in our San Francisco office about a year ago.

Several EPA staff environmental scientists and specialists have been tracking the progress of the OMEGA project over the past few years, and recently visited the pilot study site at the Southeast Water Pollution Control Plant in San Francisco. Because OMEGA is designed as an integrated system with several environmental components, this project has attracted interest from EPA staff with expertise in renewable energy, wastewater treatment and toxicology, algal bioengineering, and environmental indicators. Staff across these disciplines has been impressed, not only by the vision to create multiple environmental benefits, but also by expertise of the team leading the research effort and by the progress made over the past few years and recent months.

The research goals of the OMEGA project are well-aligned with several EPA priorities, including response to climate change and protection of America's waters. EPA has an interest in seeing development of viable low-carbon fuels, assessed on a lifecycle basis, that meet the mandate for increased renewable fuels under the Renewable Fuel Standard 2. Low-carbon fuels produced in a renewable manner can be a part of the response to global climate change. Another major EPA goal is to reduce nutrient and toxic compound loading in wastewater discharge. If successful, the OMEGA system could provide a low-cost treatment option to produce high quality recyclable water and a source of biofuels. Finally, the potential of the system design, to create multiple environmental co-benefits ranging from marine ecosystem restoration to use and production of multiple renewable energy sources is most promising.

EPA Region 9 staff was very much impressed with the high quality of work and dedication of the OMEGA research team. We look forward to reading publications that they are preparing discussing the results of the OMEGA experiments at Santa Cruz and San Francisco, CA. We believe that the information will be of great interest to a large community of scientists, engineers and public utility professionals working to improve wastewater quality, to reduce greenhouse gas emissions and to meet energy needs in an integrated, sustainable life support system. Realizing the potential benefits, we at EPA Region 9 wish to express our deepest appreciation for NASA's continued support of such far-reaching research efforts.

Sincerely,

A handwritten signature in black ink, appearing to read "Thomas J. McCullough".

Thomas J. McCullough
Assistant Regional Administrator

Printed on Recycled Paper

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Appendix E: OMEGA Articles

Marine Scientist - February 2010

The Oceans: The world's energy frontier?

Currents: The Navy's Energy and Environmental Magazine - Spring 2011

NASA and the Navy Developing the Fuel for the Future

Algae Industry Magazine – August 2011

NASA's OMEGA Scientist, Dr. Jonathan Trent

New Scientist – September 2012

Grow your own Energy

The oceans: The world's energy frontier?

Jonathan D. Trent, PhD
NASA Ames Research Center
Jonathan.D.Trent@nasa.gov

Plants produce copious amounts of oil, and refining methods for transforming vegetable oils into fuels have been known since well before fossil forms of oil took over. Soybeans, for example, produce oil with a yield of approximately 50 gal/acre/year, while some species of palm produce 600 gal/acre/yr. The best oil producers are among the microalgae, which produce between 2,000 and 5,000 gal/acre/year.

Unfortunately, oil-producing plants in general, and algae in particular, cannot be cultivated on scales that make their oil economically competitive with fossil oil without significantly competing against agriculture for land, water, and fertilizer.¹ It has been suggested that this can be solved by growing algae in deserts or other remote sites using seawater and municipal wastewater and for these and other reasons, it is argued, that of all the oil-producing plants, only algae are practical for biofuels.²

Typically, algae are grown in shallow ponds called "raceways" or in enclosures known as photobioreactors (PBRs). Ponds can be excavated in deserts and PBRs can be set up almost anywhere. So what is the hold up? Why aren't algae the primary source of biofuels?

As is often the case, the devil is in the details. For example, if raceways and PBRs are set up in remote locations that do not compete with agriculture, it's not trivial to transport the required

water, wastewater, and CO₂, and where will the harvested algae be processed? It is possible to pump, truck, or ship water, compressed gas, or the dried algae from one place to another, but all this requires energy—a lot of energy.³ In addition, raceways are open to the air, which means there is an issue with evaporation. It is estimated that to replace the evaporated water and maintain appropriate salinities for growing marine algae on a scale relevant to biofuels would take trillions of gallons of freshwater—this competes with agriculture.

Evaporation may not be a problem for PBRs, which can be closed and sealed chambers. But algae require light and if the light comes from the sun, there is also heat. Sealed PBRs are functionally solar-thermal collectors and to maintain temperatures suitable for growing algae, they need to be cooled. It is possible to use evaporative cooling, but using freshwater for evaporative cooling again competes with agriculture, and pumping cooling water requires energy. PBRs already use a lot of energy for mixing and circulating algae cultures. Indeed, the energy involved in operating costs and the materials used for PBRs, make them prohibitively expensive for biofuels' production.

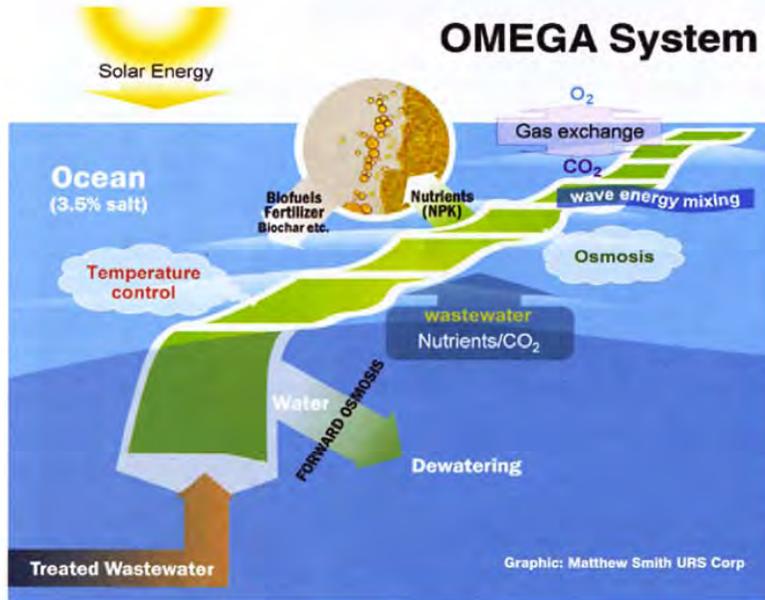
Algae seem to have great potential, but growing algae in raceways or in standard PBRs on a large enough scale to produce biofuels at a cost that can compete with fossil fuels remains an elusive challenge.

Would it be possible to grow oil-producing microalgae without competing with agriculture for land, freshwater, or fertilizer, without pumping water great distances, and in a way that benefits the environment?

What about OMEGA (Offshore Membrane Enclosures for Growing Algae)?

The OMEGA system consists of individual modules that are closed photo-bioreactors made of inexpensive clear, flexible plastic with inserts of semi-permeable forward osmosis (FO) membranes. The modules are filled at coastal outfalls of domestic wastewater and towed off to incubation areas "OMEGA farms." Each OMEGA module floats just beneath the sea surface. The algae absorb sunlight, but unlike PBRs on land, which have problems with heat and energy use, OMEGA modules transfer heat to the surrounding seawater, while mixing and circulating the algae is done using wave energy. The OMEGA system also uses buoyancy, gravity, and osmosis. Gas-filled bladders in the OMEGA structure maintain buoyancy and diffuse CO₂ into the culture through gas-permeable membranes. Water-filled bladders adjust the buoyancy and provide structural stability. Patches of FO membranes on the bottom of the plastic enclosures allow water to diffuse out into the surrounding saltwater. This process is driven by the salt gradient and during an

Against a backdrop of peak oil, climate change and ocean acidification Jonathan Trent, lead researcher and scientist on NASA's OMEGA Project, takes an optimistic view of the future, focusing on what can be done using an innovative approach to replacing fossil fuels with carbon neutral, and sustainable biofuels.



Location, location, location

OMEGA farms will be located in marine (salty) environment in the vicinity of a source of nutrient-rich freshwater and a source of CO₂, such as a coastal power plant. Other conditions, including temperatures, light, water clarity, frequency and severity of storms, geography, boat traffic, wildlife conservation, will all influence how the OMEGA farm will be configured and what algae will be cultivated. The plan is to cultivate only freshwater algae and to use wastewater that meets regulations for release into the marine environment. Therefore, if an OMEGA system accidentally leaks, the algae will be unable to survive and wastewater will have acceptable impact on marine ecosystem, at least by today's standards.



Each OMEGA module floats just beneath the sea's surface. The white squares in this polyethylene prototype, small scale OMEGA are forward osmosis membranes (Photo: Jonathan Trent/NASA Ames Research)

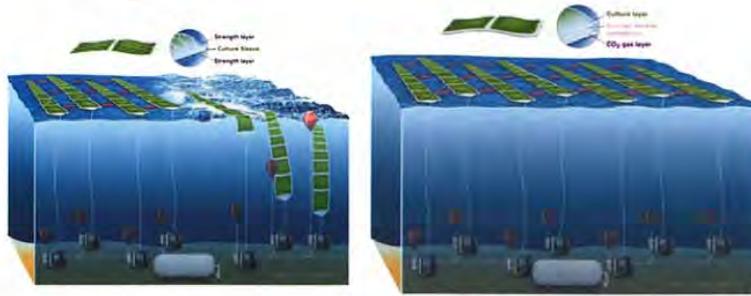
incubation period of 10-20 days, >85% of the bulk water can be removed. The FO process stimulates algal growth by concentrating nutrients in

the wastewater and assists in harvesting by initiating dewatering of the algae. Furthermore, the water passing through the FO membrane is clean (bacteria, viruses, and pollutants do not pass FO). This will help remediate dead zones in polluted coastal areas.

In addition to FO for partial dewatering, harvesting the algae from an OMEGA module can take advantage of buoyancy, wave power, and gravity. One scenario uses a float rolling under a module, pushing the algae slurry into a collection barge, where the dewatering process continues using gravity, wave, and possibly wind energy, while the algae are transported to onshore refineries.

To accommodate sea birds and marine mammals, the OMEGA modules in a farm will be separated. This also allows light to penetrate into the water column between the modules. The number of OMEGA modules in a farm will depend on the location, shipping lanes and the amount of wastewater to be processed in a given location.

Obviously, OMEGA farms will be easier to build in protected bays, in areas surrounded by floating or otherwise constructed breakwaters, or in places with existing marine infrastructure such as offshore oil platforms or wind farms. In the case of wind farms, OMEGA farms would use the infrastructure for anchorage and organization and could



OMEGA is a strong, flexible system and individual module strings can be retracted in bad weather. A tube provides CO₂ from a submerged tank

benefit from the source of local energy for pumping water, air, and CO₂, as well as producing artificial light. In turn, the wind-farm benefits by using the algal biomass as a way to solve the problem of long-term storage of wind energy.

As designs and methods for OMEGA farming develop, exposed open ocean locations may also be used. These will require robotic systems for transporting wastewater from the major sewage outfalls and for return algae biomass to shore. On the other hand, with global warming and rising sea levels, OMEGA farms may take advantage of flooded coastal zones, forming a new coastal infrastructure for algae production, wastewater treatment, and carbon sequestration.

Is OMEGA feasible and scalable?

A team of scientists, engineers, ecologists and economists, supported by NASA ARMED and the California Energy Commission, is carrying out a demonstration project to determine if OMEGA is feasible and scalable. This demonstration is meant to determine if OMEGA, as an example of "technology ecology" (an integrated system where wastes become resources), is feasible: technically, biologically, environmentally, and economically, and if it can be done on a scale that



OMEGA farms can take advantage of the infrastructure of offshore wind-farms

impacts the current use of fossil fuels. OMEGA is a complicated system of systems and the feasibility studies are non-trivial. The economics, for example, are based on a life-cycle analysis of products and services. Algae products include biomass, oil, fertilizer, animal fodder and high-value products such as dyes, nutraceuticals, cosmetics, and drugs. Services include wastewater treatment, dead-zone remediation, and CO₂ sequestration. OMEGA uses the "wastes" from one process as resources for another, and integrates solar, wave, and wind energy with gravity, buoyancy, heat-capacity, and osmosis. Analyses include considerations of the production of all materials and uses of these materials after OMEGA (cradle to cradle, rather than cradle to grave). The economic consequences of not doing OMEGA and the continued

use of fossil fuels, are also considerations.

The goal of the OMEGA project is to demonstrate feasibility and scalability, and that OMEGA products and services do not compete with agriculture or damage marine ecosystems. Indeed, OMEGA is meant to improve the marine environment by removing nutrients that are currently contributing to the formation of dead zones and by sequestering CO₂ that is contributing to ocean acidification. The hope is that based on the results of the demonstration, people around the world will be motivated to develop OMEGA systems for their locations. Projects like OMEGA will require local experts supported by government agencies and private investors who all recognize the magnitude of global problems and the urgency of finding solutions. If such experts can be mobilized and openly share information, it is estimated that OMEGA technology can be developed to significant scales within 10 years. It is impossible to predict if OMEGA will contribute significantly to the needs of the rapidly growing population for declining resources in a changing environment, but, something needs to be done and failure is not an option. ☉

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- (Ronald Pate. 2007. Techno-economics, siting and resource use challenges for onshore algal biofuels production. In *Wind, Sea, Algae Proceeding*, Ed. J. Trent p. 103; http://wind-sea-algae.org/?page_id=305)

NASA & the Navy Developing the Fuel of the Future

Joint Effort Investigating Algae Farms in the Ocean

IN FEBRUARY 2011, the National Aeronautics and Space Administration (NASA) signed a Memorandum of Agreement (MOA) with the Navy to test a system for producing what many believe to be the fuel of the future, using algae grown in the ocean.

“Changing the way energy is used and produced in our country is the right thing to do,” said Navy Secretary Ray Mabus, upon signing the agreement. “It’s the right thing to do for

in sediments. Under some conditions, with appropriate temperatures, pressures, and rock formations, they form oil that accumulates in reservoirs. Once discovered, these reservoirs can be tapped to meet our fossil fuel needs.

Fortunately, plants living today can also produce oil. For example, 50 gallons of fuel oil can be produced per year from an acre of soybeans, and 600 gallons can be produced from an acre of palm trees. The plants that produce the most

oil. On 22 April, Earth Day, 2010, the Navy flew an F/A-18 Super Hornet fighter jet at Mach 1.2, powered by a blend of conventional jet fuel and alternative aviation biofuel made from camelina oil. More recently, the Navy tested its RCB-X combat boat on a blend of conventional diesel and algae biodiesel, and flew an SH-60 helicopter on a similar blend. The important conclusion is that biofuels work—they function without re-designing engines and equipment.

Changing the way energy is used and produced in our country is the right thing to do.

—Navy Secretary Ray Mabus

our security, it’s the right thing to do for our economy, and it’s the right thing to do for our environment.”

The Basics About Oil

The oil we use today comes from plants that lived in ancient times—mostly microscopic, single-celled, plants called microalgae, which lived in seas and lakes. When they died, they settled to the bottom and were buried

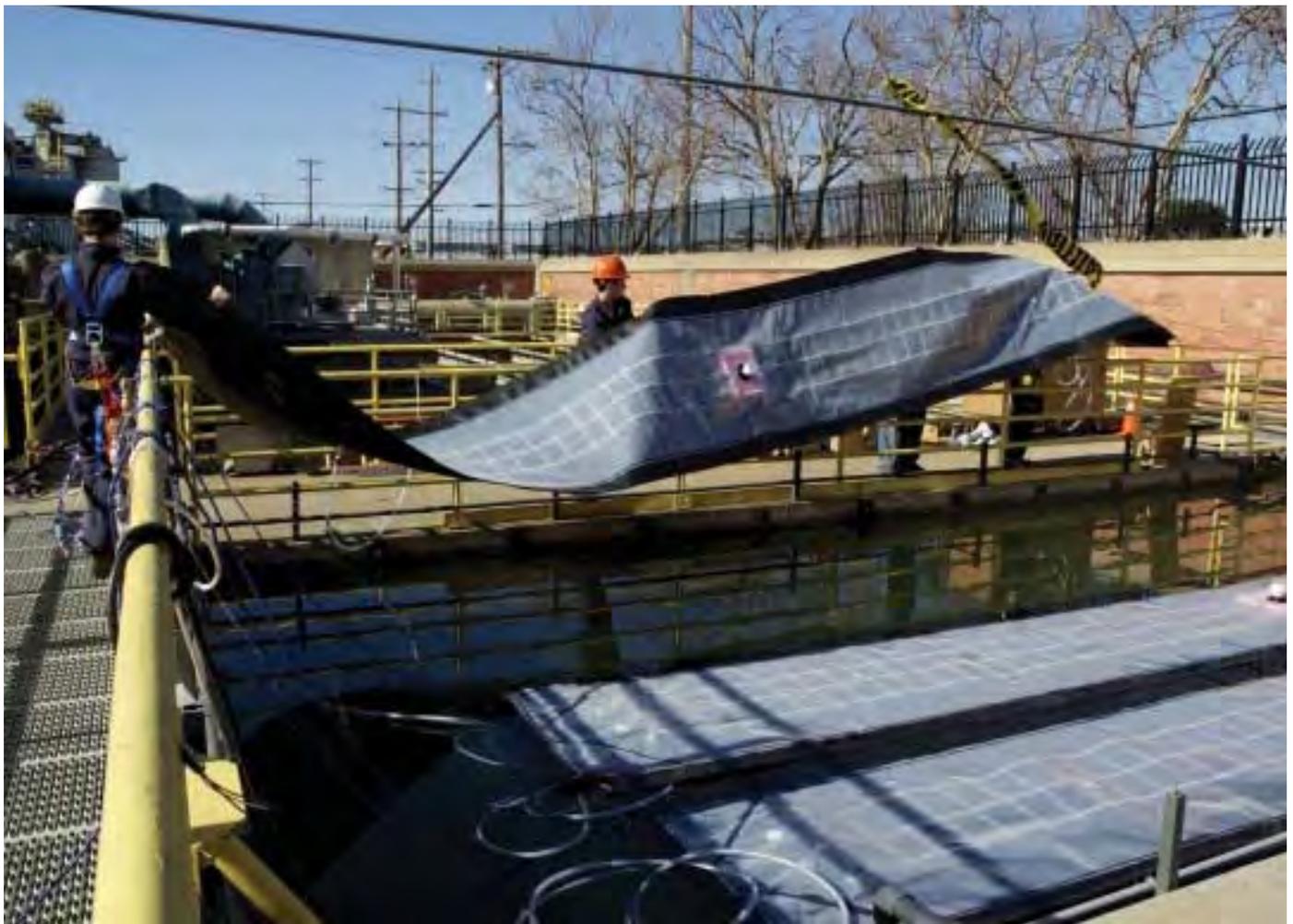
oil, however, are the modern versions of the microalgae that made most of our fossil oil. From among the thousands of known types of microalgae, researchers have discovered some species that can support production of between 2,000 and 5,000 gallons of oil per acre per year.

The Navy has taken the lead in demonstrating that it is possible to make functional fuel out of vegetable

The next questions are, how do we produce enough of this fuel to meet our needs? How do we produce biofuels economically? How do we produce them without competing with agriculture for land, water, and fertilizer?

Transform Wastelands into Fuel Farms?

The Navy is testing fuels like camelina and algae because they are not food

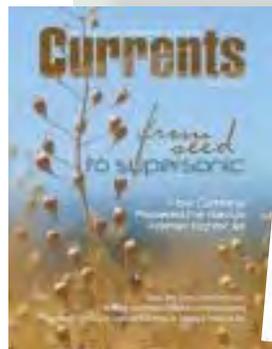


Prototype OMEGA photobioreactors being deployed in seawater tanks to grow microalgae on wastewater from the nearby sewage treatment plant.
 URS Corporation

crops and their production will not compete directly with agriculture. In this regard, microalgae is a superb potential source of biofuels, because it produces the most oil, it grows in water, and marine algae can even grow in seawater. At present, microalgae are commonly grown in shallow circulating channels called “raceways” or in transparent enclosures known as photobioreactors (PBR). To produce biofuels, thousands of acres of raceways and tens of thousands of PBRs will be required. In principle, to avoid competing with agriculture for land, raceways and PBRs can be located in deserts or on unusable fallow land. For water, they can use seawater and cultivate oil-producing marine algae.

For More Information

FOR MORE INFORMATION about the RCB-X demonstration, see our article entitled “Navy Fuels Great Green Fleet Vision: Latest Milestone on the Road to Energy Security” in the winter 2011 issue of *Currents*. For more insights into the Navy’s success in flying an SH-60 helicopter on a blend of conventional and algae biodiesel blend, see our article entitled “Navy Tests New Fuel in Seahawk Helicopter: Demo Provides “Off Ramp” from Petroleum-Based Fuels” on page 16 of this issue of *Currents*.



quantities of biofuels using traditional raceways or PBRs.¹ The question then arises—is there another process that can be developed more quickly to grow the large quantities of oil-producing microalgae that the United States needs?

The OMEGA System

The Navy is teaming up with NASA to investigate a radical new approach to large-scale algae cultivation using a system called Offshore Membrane Enclosures for Growing Algae (OMEGA). The OMEGA system consists of floating PBRs filled with wastewater from existing offshore sewage outfalls and deployed in protected marine environments. The

individual OMEGA modules are constructed of flexible plastic, clear on top, to allow light penetration for photosynthesis, and reinforced white plastic on the bottom, for strength. The modules are filled with secondary-treated wastewater and inoculated with freshwater algae. If the system leaks, it minimally impacts the environment because:

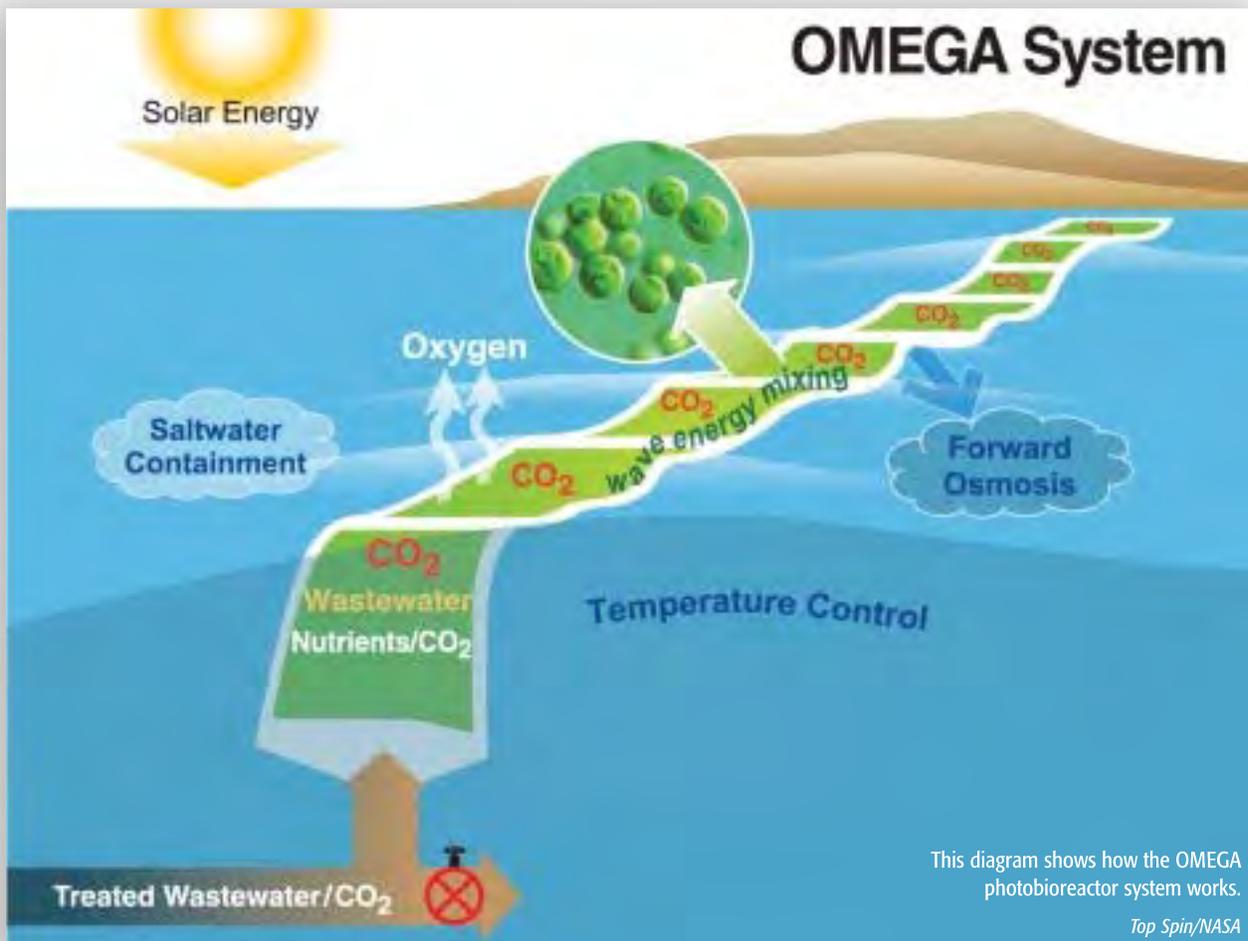
1. The wastewater is already approved for release into the ocean
2. The freshwater algae cannot survive in seawater

OMEGA utilizes virtually nothing except natural energy—solar energy to initiate photosynthesis and wave energy to maximize algae exposure

to sunlight and to mix nutrients. Unlike land-based PBRs that can overheat, OMEGA uses the surrounding water for temperature control. It uses the salinity difference between wastewater and seawater both to prevent algae that escape from becoming invasive species and to drive forward osmosis (FO).

Forward osmosis is the process by which water moves across a selective semi-permeable membrane in the direction of concentrated salts. The OMEGA system uses FO to:

1. Concentrate nutrients in the wastewater, stimulating algae growth
2. Dewater the algae, facilitating harvesting



focus on efficiency and parsimony that OMEGA began.

The feasibility and scalability of the OMEGA system will be determined by combining NASA expertise with the knowledge and expertise of naval engineers, university professors and industry.

The State of OMEGA

Supported by the California Energy Commission and NASA's Aeronautics Research Mission Directorate, the team of NASA scientists, Navy engineers, civil engineers from URS Corporation,



Samples from two different OMEGA PBRs. After three days floating in seawater, microalgae grew in both photobioreactors, but the one with forward osmosis membranes significantly changed volume due to the loss of water into the seawater. This concentrates the microalgae.

Jonathan Trent



The inside of a PBR. PBRs are one of two ways that algae is farmed on land.

John Benemann

I don't want us to be remembered as the generation of locusts, who consumed everything and left nothing for future generations. I'd much rather have us be known as the "regeneration generation."

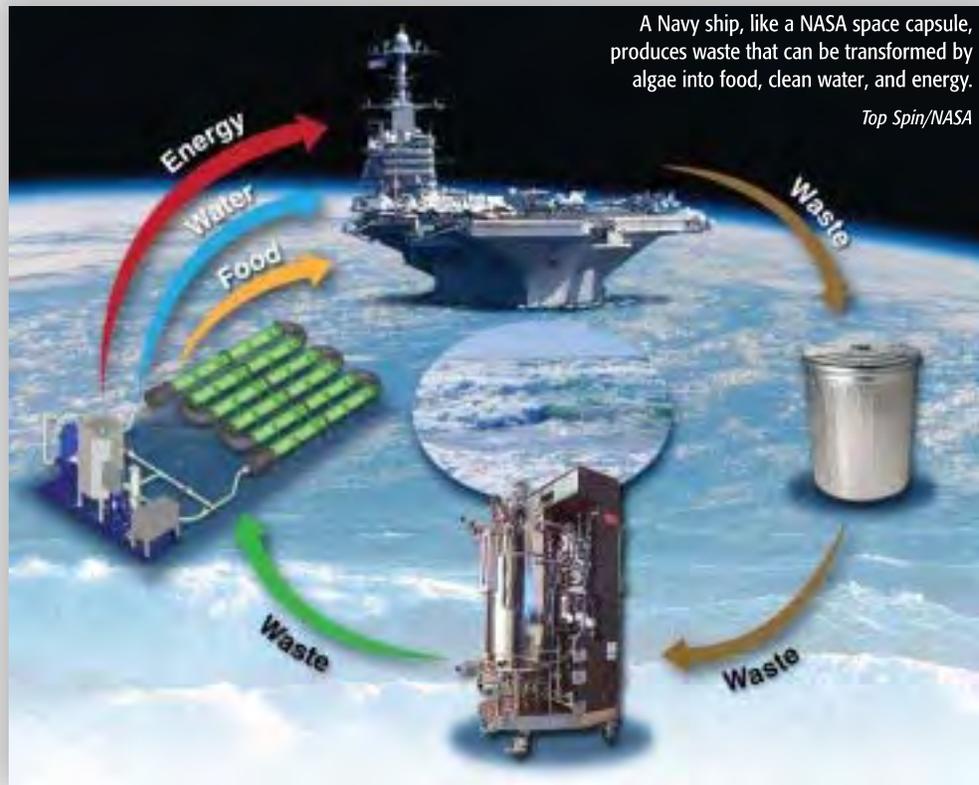
—Rear Admiral Phillip Cullom

and algae experts from the University of California at Santa Cruz, are building small-scale OMEGA PBRs in California. Floating these PBRs in seawater tanks, they are determining operating conditions for growing algae and treating wastewater. Naval engineers, using tow tanks and wave tanks, will determine how an OMEGA system will ultimately fare in the marine environment. These studies will guide the further development of durable PBR designs that can withstand the rigors of the marine environment, while providing information about the energy return on investment and the feasibility of commercializing OMEGA systems.

If successful, the project has the potential to help the Navy reach its goal of finding an alternative to fossil fuels, energy independence and a more sustainable future. Rear Admiral Phillip Cullom said in a recent lecture: "I don't want us to be remembered as the generation of locusts, who consumed everything and left nothing for future generations. I'd much rather have us be known as the 'regeneration generation.'"² [↴](#)

¹ Ronald Pate. 2007. *Techno-economics, Siting and Resource Use Challenges for Onshore Algal Biofuels Production*. *Wind, Sea Algae Proceedings*. <http://wind-sea-algae.org?page.id=305>.

² RADM Phillip Cullom, October 2010. *Keynote lecture at a meeting of the Algae Biomass Organization, Phoenix, AZ.*



A Navy ship, like a NASA space capsule, produces waste that can be transformed by algae into food, clean water, and energy.

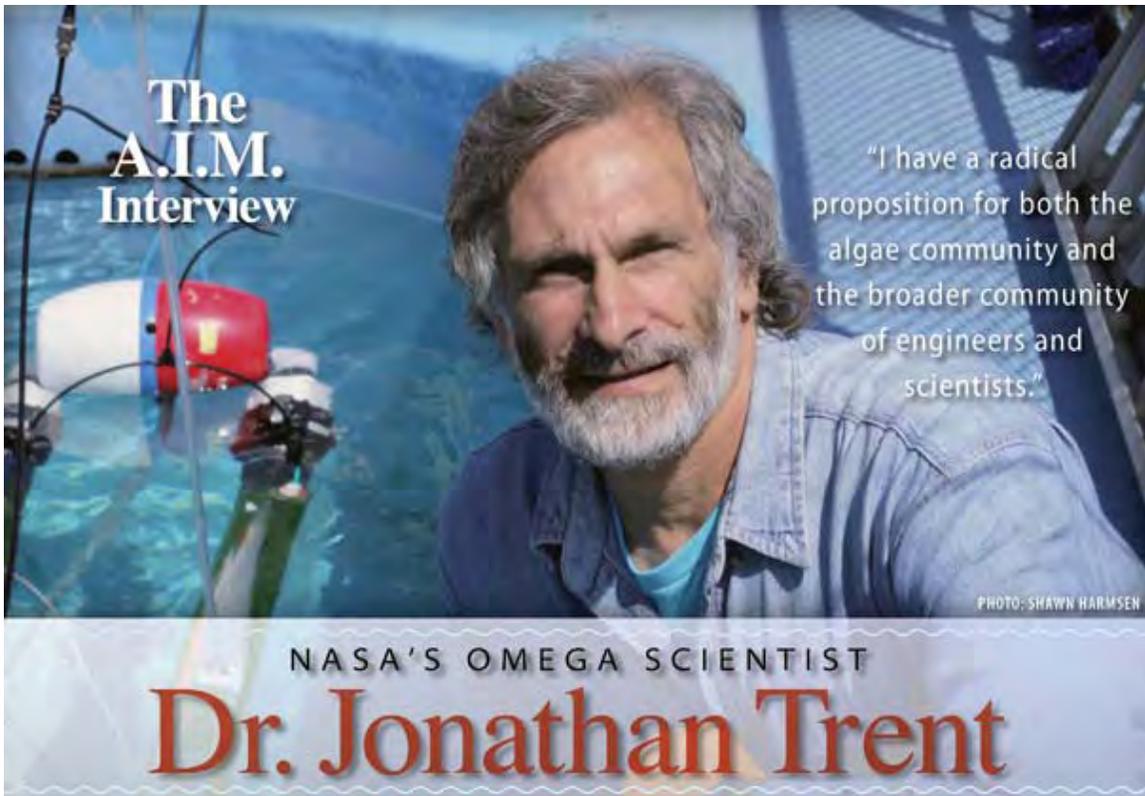
Top Spin/NASA

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August 21, 2011, by David Schwartz AlgaeIndustryMagazine.com

NASA scientist – the inventor, heart, and soul of the OMEGA system (Offshore Membrane Enclosures for Growing Algae) – Dr. Jonathan Trent received his PhD in biological oceanography at Scripps Institution of Oceanography. He went on to post graduate work in Europe studying the biochemistry and molecular biology of microorganisms living in geothermal hot springs, the so-called “extremophiles.” He continued his work on extremophiles at Yale Medical School and discovered a class of proteins in these unusual organisms that is closely related to a class of proteins in humans.

Dr. Trent moved on from the medical school to Argonne National Lab where he studied environmental usages for extremophiles, mostly for cleaning up toxic wastes. He got involved with NASA shortly after they started a program in astrobiology in the late 90s. “It was a perfect job for me,” he says, “NASA was looking for people studying the most extreme organisms on this planet to understand if there could be life on other planets.”

Taking on the NASA job in 1998, he soon got involved in nanotechnology. “I basically was taking the robust molecules from extremophilic organisms and using their innate molecular recognition that allows them to self-assemble and using a bit of genetic engineering, we created some interesting structures and extremely tiny, devices.”

We spoke with Dr. Trent recently to get an update on where things are currently with the OMEGA project and his view of its, and our, future.

How did the OMEGA program get started at NASA?

One of the interesting projects of my nanotechnology group at NASA was self-assembling multi-enzyme arrays on a stable molecular scaffold we borrowed from an extremophile. One of the arrays we were working on was to improve the degradation of cellulose, using a variety of enzymes in that pathway. It was an interesting project and brought my attention to biofuels. You know there are two “holy grails” for biofuels, one is cellulose degradation and utilization and the other is microalgae. With my background in marine science, microalgae was a natural for me and I quickly dug into that literature. I realized almost immediately that one of the biggest hurdles for making algae into biofuels is the problem of scale and that’s what I wanted to address.

If you consider the scale of algae cultivation required to meet our current appetite for fuels and you put that in the context of the growing world population with food and water requirements, it is clear that whatever we do to make algae biofuels cannot compete with agriculture. For me this meant that we can’t use freshwater and we can’t use fertilizer, and in my view we can’t even use land. I don’t buy the argument about using the so-called non-arable land for algae cultivation, because if we made all the effort of transporting water and fertilizer to non-arable land to grow algae, why wouldn’t we make it arable land and start growing food on it?

I suppose if we were pumping seawater to the non-arable land it would be another story, but in general pumping water is energy intensive and not cost effective. In any case, back in 2008, thinking about all the problems associated with super-large-scale algae cultivation, I had the inspiration for Offshore Membrane Enclosures for Growing Algae (OMEGA). We’ve been working ever since then to prove or disprove the feasibility of this offshore approach.

Give us your elevator pitch on the OMEGA System.

Well, given that some species of microalgae are the fastest growing biomass on the planet and the best oil producers, we can probably agree that algae are the organism of choice for biofuels. If we further agree that biofuels production cannot compete with agriculture for freshwater or fertilizer, which means to me we have to use domestic wastewater to grow them, then let’s consider our options.

I think the fact that in all our coastal cities we already have the infrastructure for “disposing” of our wastewater offshore, we need to consider the possibility of using this wasted water and the existing infrastructure for growing microalgae offshore. In addition to using wastewater from existing offshore outfalls for developing algae systems, there are other good reasons for OMEGA, I mean float photobioreactors (PBRs) in seawater. For example, there’s the heat-capacity of the seawater that can be used to control the temperature of the PBRs

–temperature control of PBRs on land is a huge and expensive problem. The sea provides other energy savings also. Wave action can be used for mixing and the salt gradient can be used for forward osmosis, which not only cleans the wastewater released into the sea, it also concentrates the algae for harvesting. If the freshwater algae cultivated in wastewater escape into the surrounding seawater they die (freshwater algae can't survive in salt water), which means they will not become invasive species in our coastal waters. The OMEGA structure itself can be used as an enormous substrate for developing aquaculture to grow edible seaweeds, mussels, oysters, or some other marine “crop” appropriate for the local conditions.

If you see where this is going, OMEGA is a system of systems or an “ecology of technologies” – in which the concept of waste disappears: a waste product from one part of the system becomes a resource for another part. As far as possible the whole system, which includes the environment, is in balance.

In other words, we use algae to treat wastewater and wastewater to grow algae. We use carbon dioxide to grow algae and algae to sequester carbon dioxide. We use the inside of the OMEGA PBR to contain algae and the outside to produce aquaculture crops. We use the salinity gradient to prevent algae from becoming invasive species and to drive forward osmosis and to further clean the wastewater. We use solar energy, wave energy, and the heat capacity of the water. It's all rather exciting and it's very much like what NASA is developing for closed life-support systems for long-duration space exploration.

Well, I realize that was a long elevator pitch, but this is an important topic to consider on many levels of detail! I guess we'll need a very tall building to do an elevator pitch for OMEGA!

So how far along is the project at this point?

The project was initially generously funded by the California Energy Commission, which was enough to get us started. And then by luck and serendipity I had a chance to present the OMEGA concept to Lori Garver, the Deputy Administrator of NASA. Lori immediately understood that not only was this technology an important spin-off from the kinds of closed life support systems that NASA has been developing for decades, but it is precisely the kind of technology that NASA gives back to society and to the world. Lori's insight and understanding of the potential of OMEGA led to additional funding through the “Green Aviation” initiative at NASA.

Within a few months we completed Phase One, a 400-page paper study that considered possible materials and designs, hypothetical deployment locations and logistics, and estimates of energy return on investment, life-cycle analysis, etc. Based on Phase One results and an external review, we were encouraged to proceed with Phase Two, which is in progress and focuses on building and testing prototype PBRs as well as the OMEGA system components in the lab and in seawater tanks.

Phase Two is underway at two locations: a California Fish and Game lab in Santa Cruz and a wastewater treatment plant in San Francisco. The Santa Cruz lab is our “skunkworks,” where we are experimenting and testing floating PBR and system designs. We have two large seawater tanks and thirty-four 250-gallon tanks in which we are studying biofouling on different types of plastic.

Prototype floating PBRs in seawater tank at Cal. Fish and Game OMEGA laboratory in Santa Cruz, CA. Various flow-through PBR designs were tested either with internal gas sparging or with external gas exchange columns. Starting cultures were grown in an aquarium on wastewater stored in the beige tank. The orange ball is a wave generator. (Photo: Susanne Trent)

We grow algae on wastewater from the Santa Cruz wastewater treatment plant, which we collect in 50-gal drums and pump into our floating PBRs. We bubble the algae with an 8-10% CO₂/air mixture to mimic flue gas. In one of the large tanks we have a wave generator and analytical equipment for the PBRs to continuously monitor pH, dissolved oxygen, temperature, photosynthetically active radiation (PAR) and photosynthetic efficiency using Fast Repetition Rate Fluorometry (FRRF). The inventors and developers of FRRF, Zbigniew Kolber and Sasha Tozzi, are on our team and their instrument has been a boon to our research. It continuously takes the photosynthetic “pulse” of the algae cultures, indicating biomass accumulation and the effects of light, nutrient, and oxygen. It’s an important tool in our studies.

Laboratory monitoring system for algae growing on wastewater.

Continuous measurements of temperature, pH, dissolved oxygen, and conductivity (shown) will soon be supplemented with a system to monitor photosynthetic efficiency using Fast Repetition Rate Fluorometry (FRRF). (Photo: Sigrid Reinsch)

We also have an instrument called a zetameter, which tells us about the surface charge of the algae and indicates when to harvest them.

The other location we have for experiments is in San Francisco at one of the wastewater treatment plants. We have an agreement with the city of San Francisco’s Public Utilities Commission to use four big tanks there. They were dissolved air flotation tanks that haven’t been used in years. With help from the plant workers and our contractors, these four tanks were cleaned out and filled with SF bay water. With a bit of additional plumbing for wastewater and flue gas we are preparing to do experiments with floating PBRs in these tanks.

The goal is to test our designs and ideas developed on a small scale in Santa Cruz on a larger scale in San Francisco.

Our current funding gets us through Phase Two, which should culminate in some reasonable designs for scalable floating PBRs, some algae growth data in small-scale PBRs, an energy return on investment supported with actual data, and some estimates for what it will take to obtain permits and do a commercial-scale system. Our broad objective is to complete this pragmatic analysis of OMEGA

feasibility based, not just on biofuels, but on other products and services as well, by the end of 2011.

What strains are you working with, and is the system optimized for any particular strains?

We're working primarily with *Chlorella vulgaris*, because it's one tough bug and grows really well in wastewater, but dies quickly in seawater. We wanted to test an organism that is well known and is a natural strain – not a genetically modified organism.

I should add, however, that the OMEGA system is agnostic with regard to what algae go into the system provided: 1. The strain grows well on wastewater and 2. It dies in saltwater; as I said, the key is that if the OMEGA system leaks, it is not introducing invasive species into the marine environment. In fact, the freshwater algae will not only die in seawater, they are also bio-degradable.

How is the algae harvested?

There are lots of people working on improving harvesting methods and this is outside the scope of the OMEGA project. We are testing some different harvesting methods however, because ultimately we'd like to find or adapt a method that we can incorporate into the continuous, flow-through system we are developing. There are a lot of clever possibilities emerging.

Describe a little more about the physical properties of the system.

The OMEGA system we are now testing on a small scale consists of manifolds connected to floating clear flexible plastic tubes, pH/dissolved oxygen/temperature sensors control systems for pH, gas exchange columns, and harvesting systems. Wastewater is the source of nutrients and photosynthesis occurs primarily in the plastic tubes. Dissolved oxygen is removed as the culture falls through an airspace in the gas exchange column, while the pH is controlled and CO₂ is added by bubbling flue gas through the water in the column. The OMEGA system: Treated wastewater from an offshore outfall and CO₂ pumped into a floating photobioreactor (PBR) to grow microalgae, which use the nutrients in wastewater and solar energy to fix CO₂, producing biomass, oil, and oxygen. Temperature in the PBR is controlled by the heat capacity of the surrounding seawater and the salinity gradient between wastewater and seawater is used for forward osmosis to dewater the algae and to clean the wastewater. The salt water also provides containment in case of an algae spill—the freshwater algae growing in wastewater cannot survive in saltwater. (Illustration: Tom Esposito, TopSpin Design Works, NASA)

When the algae reaches a density that limits photosynthesis, it is shunted to an experimental forward osmosis chamber to pre-concentrate, and then to a harvesting chamber. Wastewater is added back to the system to maintain a supply of nutrients and a concentration of algae optimum for photosynthesis. In other words, we want to make sure that the algae never gets so dense that we're

just harvesting photons in the upper few millimeters of our bioreactor, but we are harvesting enough algae biomass to cover the energetic costs of harvesting. To optimize mixing and light exposure, the culture is pumped passed swirl veins, which move the algae along a helical path down the tube. At commercial scale each module would be between 50 and 100 feet long. Obviously, pumping water through the system is going to have the biggest energy requirement. We're looking at wind, wave, and solar energy to supply most of this energy.

Is OMEGA wastewater dependent at this point?

Many of us in the algae community agree that we have to use wastewater for large-scale algae cultivation so as not to compete with agriculture, but also to meet economic requirements. If you look at our major cities, the wastewater systems tend to be embedded in the city. Take San Francisco, for example. It's about 45 square miles and there are three wastewater treatment plants. The plant at Hunters Point, handles 65 million gallons a day. If you tried to build ponds around the wastewater plant, you'd have to displace freeways and all kinds of infrastructure. Just to deal with five day retention time you need about 1200 to 1500 acres of ponds, and it has to be on level land, which is really hard to find near San Francisco.

On the other hand, if we were to somehow float algae photobioreactors in San Francisco Bay and use the wastewater currently pumped offshore, we would use less than one percent of the huge area of the Bay and in the worst case we would displace a few fishermen – actually we'd probably improve the fishing in the Bay.

Using wastewater for algae growth in San Francisco: As in other coastal cities, the SF treatment plant (red rectangle) is embedded in the city and existing outfalls are offshore (solid red arrow). To accommodate the 65 million gallons per day (MGD), assuming a 5-day retention time for algae growth requires 325 million gallon ponds or photobioreactors. This would require approx 2.3 sq miles of area (green rectangle) on land or offshore (Illustration: Tom Esposito, TopSpin Design Works, NASA)

Well, then the issue is, can we do this? Can we figure out how to cultivate algae in offshore environments? There will undoubtedly be somewhat different solutions for each location and some places will be impossible, but what do the easiest solutions look like?

I'm hoping to be able to get support for the next Phase of OMEGA, which will be the first marine deployment in a bay somewhere. I'm hoping to do this with the US Navy, but time will tell where it will happen.

What are the biggest obstacles you've been dealing with in getting the OMEGA system into full deployment?

I think that there are four major areas with formidable hurdles some of which

apply to all algae systems and some of which are particularly true for OMEGA because it's not an established technology.

Those four "obstacle" areas (in no specific order of importance) are:

1. Biology, which includes finding the right strains of algae that grow well in wastewater and form a stable community. For OMEGA, they also have to die in seawater.
2. Engineering, which is a problem in the OMEGA system because the marine environment is daunting both in terms of materials and corrosion as well as strength and longevity with 5, 10, and 100 year storms. This depends on where you are, but even in places like the North Sea there is some pretty amazing engineering going on to pursue oil in deep water. In addition to deepwater oil drilling platforms, there are plans for large floating airports and even floating cities, being developed in Holland to anticipate sea level rise. I somehow think our engineering ingenuity is up to the challenge of developing OMEGA systems at least in protected bays for now, in the new bays that will form in the future with sea-level rise, and maybe someday in the open ocean.
3. Economics, the OMEGA project itself is facing an economic crisis of sorts because we are going to run out of money at the end of this calendar year and we are looking for funding for our next Phase, but that's not relevant to the overarching economic challenge. In general, the economics of large-scale algae cultivation for a commodity like biofuel, is considered a major issue. I would argue that the economics of an OMEGA system will be based on the integrated system of both products and services. The products include algae biofuels, biogas, fertilizer, and aquaculture harvests. The services include wastewater treatment and carbon sequestration and to some degree environmental remediation, if OMEGA can be used like the "turf scrubber" system.
4. Environmental obstacles, which have environmental impact and social components. The marine component is how OMEGA impacts the local marine environment. The fact that it's going to clean up wastewater outfalls is a positive impact, but there are open questions about marine mammals and sea birds, and shading the local eco systems. I think the overall impact will be positive, but that remains to be determined.

The "social environment" component involves obtaining permits, and jurisdiction, and competition for space with stakeholders like shipping companies, fishermen, and recreational boaters. All these issues depend on where we are and how sensitive we are to the conditions in the marine environment.

What would you say are the significant breakthroughs or major refinements needed to make this system a more elegant solution?

It would be great if one of our industry colleagues came up with a really good oil producing strain of algae that grows well on wastewater and outcompetes

everything else. But those kinds of breakthroughs I leave to others. From our perspective, we have been working on how to get the hydraulics of the flow-through system to work and how to control gas exchange so we don't poison our algae with oxygen, and we provide them with adequate supplies of CO₂. We've got a system working now that we've developed at our "skunkworks" where we are measuring and monitoring how quickly the algae are removing the nutrients from the wastewater, and how we can balance our wastewater input to keep the algae growing, and balance the harvesting and the gas exchange. We think we've cleared – just in the last month or so – some major hurdles with regard to the hydraulics and the whole biological balancing act that we need to do to keep the algae growing. Time will tell if this system is stable over the course of months.

The good news is the system we have now seems to be quite scalable at least in principle. In a natural environment, there are going to be issues with materials and design to cope with stresses from currents, waves, and wind as well as biofouling. But I'm more optimistic than ever about the feasibility of OMEGA.

If you mean the algae industry as a way to make biofuels, my personal opinion is that the US should be investing the kind of money and brainpower that we invested in the Manhattan project and Apollo. The Manhattan project was an investment of something like \$22 billion (in 2008 dollars) over a five year period. And the whole Apollo program was about \$98 billion over 14 years. They were amazing government-funded programs that mobilized the best and the brightest, actually from all over the world to reach socially and scientifically important goals. Given the importance of liquid fuels, not only to the transportation industry, but to so many aspects of our society, and considering both the limited availability (peak oil and the location of reserves) and desirability (environmental impacts and national security) of fossil fuels, it's time we make the transition away from fossil fuel dependence. The fossil fuel industry is nearly 150 years old and it represents some \$5 trillion a year in revenue.

I think if we want to maintain a semblance of our lifestyle in the future, we need to seriously ask ourselves what it will take to replace the bulk of the fossil fuels we are currently using with sustainable, carbon neutral biofuels and can we do this in the next five to ten years? Then, we as a nation, should take on that enormous challenge with the determination of the Manhattan Project and the enthusiasm of the Apollo mission. With our current focus on the "economic crisis" I don't know if the U.S. is up to this challenge. On the other hand, if we can invest over \$1.2 trillion in the last ten years for wars in the middle east, perhaps we can find the resources to secure our own energy sources, energize a green economy, and make those wars obsolete.

Are you passionate about algae?

Ha ha! Well I guess if you haven't noticed by now I'm passionate about algae, I'm

passionate about the oceans, I'm passionate about the environment and I'm passionate about finding a way forward for the growing population of human beings that is sensitive to the environment and responsible on the global scale. Above all, I'm passionate about finding a sustainable, carbon-neutral energy supply and I think algae can be part of that supply.

Why? Because, while I know it's incredibly difficult to make meaningful predictions, the Intergovernmental Panel on Climate Change (IPCC) has made some daunting predictions about global changes that seem plausible to me as a scientist. Among other things, the IPCC is predicting that we are changing the climate, acidifying the oceans, and that our activities threaten 40% of known species with extinction by the end of the century! But even if we ignore all these incredibly important issues, we're also talking about literally burning through the global reserves of fossil carbon in a little over a century and having no viable alternative plan for the future. In other words, this isn't about "tree hugging" *per se*, it's about seeing folks getting ready to cut the last tree on Easter Island and thinking: what next?

Well, I'm thinking OMEGA. It's a fundamentally different way to think about resources and technology embedded in the local environmental context. It's about not just mining resources for technology, it's about thinking in terms of waste products as resources and the environment as part of the system.

I am passionate about this system-level thinking because in the case of OMEGA it is focused on self-sustaining cycles. By the way folks, there's a lot at stake and so little time left for procrastination.

Anything else you'd like to put out there?

I have a radical proposition for both the algae community and the broader community of engineers and scientists. I'd like to propose that we come together and openly collaborate to meet the challenge of replacing fossil fuels in the next decade.

I think we need to critically evaluate the idea of developing algae as an alternative fuel and we need to start thinking out of the octagon – or at least out of the pond and conventional PBR. There is no doubt that we can grow algae in ponds and bioreactors and it's a viable industry for small quantities of high-value products, but we need to face the problem of scale needed for algae-energy facilities and accept the fact that pumping seawater or wastewater to remote sites is not energetically feasible.

I'd like to see the algae community, wastewater engineers, marine engineers, oceanographers, aquaculturists, city planners, and knowledgeable scientists take on the question of whether or not we can use existing offshore outfalls and floating PBRs to grow algae offshore in at least some locations?

The OMEGA project is supported by state and federal grants. I'm a civil servant, which means I don't have investors to please, shareholders, or production quotas to meet. In other words, I'm in a good position to critically evaluate this

technology. Honestly, the more I look into it, the more difficulties and challenges I discover, but I see the broader vision of a truly integrated system, combining solar, wind, and wave energy, with algae cultivation, wastewater treatment, carbon sequestration, and aquaculture. I hope others will share and help to realize this vision.

From the broadest perspective, it seems to me we're standing on a threshold now that is arguably one of the most important in the history of civilization, comparable to the transition from hunting and gathering our food to cultivating it. We now need to make that same transition for energy. We can no longer hunt and gather it, we need to cultivate it and we need to cultivate it in sustainable and environmentally conscious ways. If we can find the pathway to this transition – and we don't have much time to do it – it will be our legacy for future generations. If we do not at least try, then what?

For additional information:

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Article from

NewScientist

Grow Your Own Energy

A NASA-backed experiment harvests algae for oil, releases fresh water.

By **Jonathan Trent** | Posted Monday, Sept. 3, 2012, at 7:15 AM ET



All algae produce natural oils. We may just need to harness the right ones for an energy source.
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Before we run out of fossil oil, we will thoroughly tap the sea floor, find and frack wells wherever they may be, and excavate and extract the most recalcitrant of oil shales. In so doing, we will fuel our lifestyle for a few more decades at the cost of releasing vast amounts of carbon dioxide, adding to global warming, melting ice caps, raising sea levels, acidifying oceans—and setting course for a future for which there are few optimistic scenarios.

In the face of all this, scientists are racing to find alternatives. Biofuels are my passion, but they have had rather a bad press, from complaints about displacing food production to the inefficiency of soybeans and the carbon footprint of ethanol. Microalgae have a low profile but they deserve a much higher one, since the fossil oil we mine mostly comes from microalgae that lived in shallow seas millions of years ago—and they may be key to developing sustainable alternative fuels.

Algae are single-celled organisms that thrive globally in aqueous environments and convert CO₂ into carbohydrates, protein, and natural oils. For some species, as much as 70 percent of their dry weight is made up of natural oils. Through transesterification (the process of adding three molecules of alcohol to one molecule of natural oil), the algae oils can be transformed into renewable fuels.

Advertisement



Microalgae hold great promise because some species are among the fastest growing plants alive and are therefore



growing process and are therefore one of the best sources of biomass, while other species have been estimated to produce between 18,700 and 46,750 liters of oil per hectare per year, nearly a hundred times more than soybeans' 468 liters per hectare per year.

But there are big unsolved problems at which governments should be throwing funds and brainpower as if we were involved in a Manhattan project. For example, since few species of microalgae have been domesticated, we don't know

how to grow them reproducibly or economically. At what scale will algae farming be efficient? To put this in perspective, U.S. planes use 80 billion liters of fuel per year. To supply this fuel from microalgae at the lower end of the estimated production rate would take 4.2 million hectares—twice the area of Wales.

Luckily, there may be a good way to cultivate this much algae while solving the ethical problem of producing biofuel without competing with agriculture. Freshwater algae can be grown in wastewater (effectively, water with fertilizer), or marine algae can be grown in a blend of seawater and wastewater. In both cases, wastewater provides a growth medium and the algae clean the wastewater by removing nutrients and pollutants from it. So there's no competition for fresh water needed elsewhere, no reliance on synthetic fertilizer, and the environment benefits.

The United Nations estimates that the world produces around 1,500 cubic kilometers of wastewater annually, of which more than 80 percent is untreated. This means there is an ample supply of nutrient-rich water for the algae, while algae treatment is available to offset the environmental impact of wastewater.

There remains the question of how and where to grow the algae. A few species are cultivated commercially on a small scale, in shallow channels called raceways or in enclosures called photobioreactors (PBRs). Raceways are relatively inexpensive, but need flat land, have lower yields than PBRs and problems with contamination and water loss from evaporation. PBRs have no problems with contamination or evaporation, but algae need light, and where there is light, there is heat: A sealed PBR will cook, rather than grow, algae. And mixing, circulating, and cleaning problems send costs sky high.

Assuming we can fix this, the question of siting remains. In order not to compete with agriculture, PBRs must use nonarable land reasonably close to a wastewater treatment plant. But in most cities, wastewater plants are surrounded by infrastructure, so installing PBRs on thousands of hectares around the plants would affect roads, buildings, and bridges—again driving up costs prohibitively.

A solution occurred to me: For coastal cities, we should try a system I call OMEGA: Offshore Membrane Enclosures for Growing Algae. Some 40 to 60 percent of Earth's population lives near a coast, most of the biggest cities are near a coast, and nearly all coastal cities discharge wastewater offshore.

How does OMEGA work? It uses PBRs made from cheap, flexible plastic tubes floating offshore, and filled with wastewater, to grow freshwater, oil-producing algae. It would be easier to build the systems in protected bays, but breakwaters could also be constructed to control waves and strong currents. The water need not be deep or navigable, but a few things are crucial, including temperature, light, water clarity, frequency and severity of storms, boat traffic,

nature and wildlife conservation.

Beyond solving the problem of proximity to wastewater plants, there are other advantages to being offshore. OMEGA uses buoyancy, which can be easily manipulated, to move the system up and down, influencing exposure to surface waves and adjusting light levels. And the overheating problem is eliminated by the heat capacity of the surrounding seawater.

The salt gradient between seawater and wastewater can also be exploited to drive forward osmosis. Using a semipermeable membrane, which allows water, but not salt, pollutants, or algae to pass through, wastewater is drawn into the saltwater with no added energy. In the process, algae are concentrated in preparation for harvesting and the wastewater is cleaned, first by the algae, and then by forward osmosis. This produces water clean enough to release into the marine environment or recover for reuse.

If OMEGA's freshwater algae are accidentally released, they die in seawater, so no invasive species can escape into the ecosystem. In fact, OMEGA can improve conditions by providing a large surface for seaweed and invertebrates to colonize: part floating reef, part floating wetland. Then there are the extra possibilities of developing wind or wave power and aquaculture, growing food such as mussels.

OK, if it's so good, where is it? For the past two years, backed by NASA and the California Energy Commission, and about \$11 million, we have crawled over every aspect of OMEGA. In Santa Cruz, Calif., we built and tested small-scale PBRs in seawater tanks. We studied OMEGA processing wastewater in San Francisco, and we investigated biofouling and the impact on marine life at the Moss Landing Marine Laboratories in Monterey Bay.

I'm now pretty confident we can deal with the biological, engineering, and environmental issues. So will it fly economically? Of the options we tested, the OMEGA system combined with renewable energy sources—wind, solar, and wave technologies—and aquaculture looks most promising. Now with funds running out and NASA keen to spin off OMEGA, we need the right half-hectare site for a scaled-up demonstration. While there is enthusiasm and great potential sites in places ranging from Saudi Arabia to New Zealand, Australia to Norway, Guantanamo Bay to South Korea, as yet no one has committed to the first ocean deployment.

We could be on the threshold of a crucial transition in human history—from hunting and gathering our energy to growing it sustainably. But that means getting serious about every option, from alpha to OMEGA.

This article originally appeared in *New Scientist*.

**Appendix F:
OMEGA Data Synopsis Project Posters**

Appendix F: OMEGA Data Synopsis Project Posters



OMEGA: Offshore Membrane Enclosures for Growing Algae



Preparation of Infrastructure at South East Plant

SEP Mechanical and Electrical Support, URS, NASA

Preparation of Dissolved Air Flotation (DAF) Tanks	Installation of CO ₂ Flue Gas Source	Instrumentation & Electrical Installation
 <p>Four Tanks to be Used for NASA Experiment</p>	 <p>Flue Gas Tap From Boiler Building Runs Underground to DAF Tanks</p>	 <p>FBR Algae Sample System</p>
 <p>Removal of Chain and Drive System</p>	 <p>Chillers and Heat Exchangers Installed</p>	 <p>DAF Tank Pump Controls</p>
 <p>Alternative CO₂ Source Installed (Gas Burner)</p>	 <p>Main Electrical Power</p>	 <p>Lab Trailer Power</p>

Summary of SEP Site Infrastructure Modifications

- 1) Installed Bay water pump to bring salt water to DAF tanks
- 2) Installed Plant Effluent piping from North side of SEP to DAF tanks
- 3) Installed flue gas heat exchanger, chiller and piping
- 4) Installed Gravity Bell Thickener filtrate pump
- 5) Installed transformer, lighting panel, conduit and wire for power to DAF recirculation pumps, Bay water pump, and wavemaker system
- 6) Installed wave maker system
- 7) Installed electrical, sewer, and water service to trailer



OMEGA: Offshore Membrane Enclosures for Growing Algae

OMEGA Project Scientist: Jonathan Trent, Ph.D.
email: jonathan.d.trent@nasa.gov



Sparging v. Gas Exchange Column: Comparing the Performance of OMEGA Module Configurations

Project Team: Patrick Wiley and OMEGA Project Team

Motivation

Algae require CO₂ to grow and for delivering CO₂ to algal cultures with minimal atmospheric losses are needed to improve the economics of industrial-scale production. This work evaluated the CO₂ mass transfer efficiency and physiological condition of algae grown in OMEGA modules using either an internal bubbling network (sparging) or an external gas exchange column (GEC).

Materials & Methods

Determination of CO₂ mass transfer efficiency

- 1) CO₂ was bubbled at a controlled rate through a column of water (heights ranging between 1 and 9 feet) containing a known mass of NaOH
- 2) The stoichiometry of the acid-base neutralization reaction between NaOH and CO₂ was used to determine the mass of dissolved CO₂, which combined with the volume of gas injected allows calculation of the mass transfer efficiency.

Module Construction and Evaluation



- 1) 100-liter OMEGA modules were deployed and inoculated with wastewater containing *Chlorella* sp.
- 2) Cultures were grown in either sparging modules or GEC modules with swirl vanes (right). (The GEC is shown in the results section).
- 3) Algae responses were evaluated using Fast Repetition Rate Fluorometry (FRRF).
- 4) Sedimentation was quantified by measuring the OD₇₅₀ before and after physically shaking the modules.

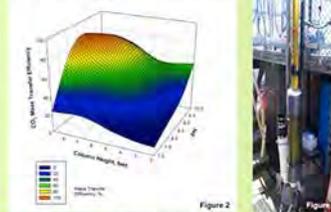
Goals

Determine which OMEGA module performs most favorably with respect to:

- 1) CO₂ mass transfer efficiency;
- 2) Algal culture viability as indicated by FRRF measurements;
- 3) The ability to maintain a well-mixed algal suspension within the module.

Results

CO₂ Mass Transfer Efficiency as a Function of pH and Column Height



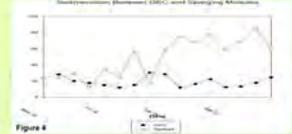
- 1) The gas transfer efficiency improves with increasing contact time between gas and liquid phases (Figure 2).

Column Height (feet)	GEC CO ₂ Mass Transfer Efficiency at Variable pH Ranges			
	11 to 10	10 to 9	9 to 8	8 to 7
9	85.6 ± 3.8	83.2 ± 4.3	86.4 ± 2.1	13.9 ± 2.5
7	75.7 ± 2.3	80.6 ± 2.9	47.9 ± 5.3	25.2 ± 7.4
5	54.9 ± 2	54.0 ± 1.8	43.8 ± 2.1	28.8 ± 0.5
2	42.9 ± 4.2	25.3 ± 2.6	17.8 ± 2.0	8.8 ± 1.7
1	41.2 ± 10.5	23.1 ± 2.8	15.7 ± 2.5	7.0 ± 0.8

pH Range	Sparging CO ₂ Mass Transfer Efficiency at Variable pH Ranges			
	11 to 10	10 to 9	9 to 8	8 to 7
	49.9 ± 3.1	34.4 ± 4.3	18.3 ± 8.1	6.8 ± 0.11

Results cont.

- 2) The sparging system provided less effective mixing (Fig. 4) and generated a less viable algal culture (Fig. 5) compared to the GEC system.



Impact & Implications

- 1) The GEC system provides greater CO₂ mass transfer efficiency, better mixing, and more viable algal cultures than sparging
- 2) This evaluation provided valuable performance data needed to determine which OMEGA module design to develop.



Real-Time Fluorescence-based Methodology to Optimize Algae Production Rates

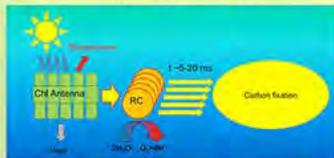
Project Team: Zbigniew Kolber and Sasha Tozzi

Motivation

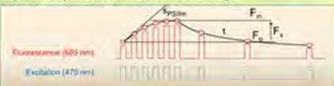
A large number of algal species display fast growth rates and high oil content. Despite these properties, the average rates of algal biomass production rarely exceed $20 \text{ g m}^{-2} \text{ d}^{-1}$, yielding about $2\text{--}3 \text{ L m}^{-2} \text{ year}^{-1}$ of oil. Such low yields, coupled with relatively high cost of infrastructure, maintenance, and processing currently renders the algae-based biofuels commercially nonviable. The biggest obstacle toward commercial viability is the relatively low efficiency of solar light utilization, averaging about 2.5%. Higher efficiencies, although theoretically possible, are difficult to achieve and maintain due to the fact that algae down-regulate their light utilization in response to a wide range of environmental stresses, including pH shifts, nutrient and micronutrient limitation, excess of solar radiation, and excess of dissolved oxygen. Here we present a fluorescence-based methodology and instrumentation to measure, in real-time, a range of photophysiological parameters in algae such as efficiency of solar light utilization, the yield of photosynthetic charge separation, and the rates of photosynthetic electron transport. These parameters define the level of physiological performance under current growth conditions, and are used to optimize delivery of nutrients and CO_2 to define the required level of mixing rates to minimize photoinhibitory damage, and to assess the adequacy of gas exchange in maintaining the dissolved oxygen level within the physiologically-safe range.

Methodology

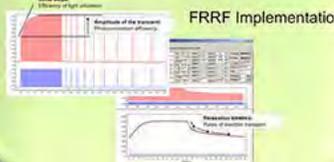
Fast Repetition Rate Fluorescence (FRRF) Technique



Most of the light absorbed by the photosynthetic pigments (Chl Antenna) is transferred to the PSII reaction centers (RC), stimulating charge separation and ensuing photosynthetic electron transport. A portion of the absorbed energy, however, is re-emitted in the form of fluorescence. The level of photosynthetic activity dictates the amplitude of this fluorescence signal.

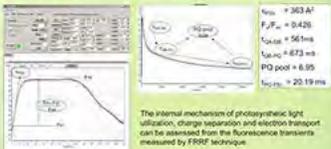
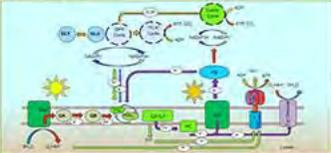


In the FRRF technique this activity is modulated by a series of excitation flashes (blue trace) produced at a varying duty cycle. As a high duty cycle the excitation energy exceeds the capacity of the photosynthetic electron transport, causing a transient increase in the fluorescence signal (red trace). The initial slope of this transient defines the efficiency of photosynthetic light utilization (Φ_{PSII}). The amplitude of the transients normalized to the maximum fluorescence signal, F_v/F_m , is a measure of the photosynthetic yield. Following the saturation transient, the time interval between flashes is gradually increased, resulting in relaxation of the fluorescence signal with a kinetics defined by the time constant of photosynthetic electron transport (τ).



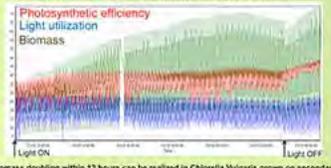
Goals

1. Understand the mechanisms by which growth conditions affect the of photosynthetic light utilization, charge separation, and electron transport in photosynthesis.
2. Develop instrumentation that allows assessing these effects in real time.
3. Apply this instrumentation in a process control loop to optimize the light utilization and algal biomass growth.

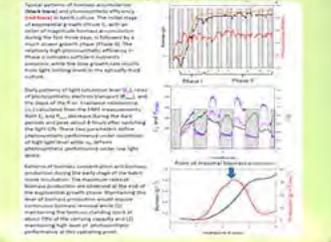


Results

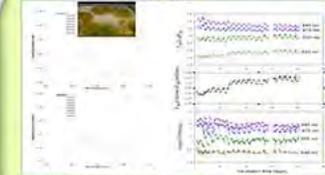
14 hours snapshot of PBR operation



Biomass doubling within 12 hours can be realized in *Chlorella vulgaris* grown on secondary wastewater under optimal conditions of light, CO_2 supply and oxygen removal.



Goal 3



Changes in the spectral properties of *Chlorella* related to oil accumulation can be detected in the fluorescence excitation spectra, as well as in the specially-resolved FRRF signal. These changes are due to accumulation of non-photosynthetic carotenoid pigments.

Recommendations

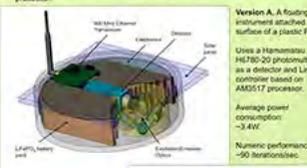
1. Optimize algae growth conditions toward maximal slope of the growth curve by controlling nutrients supply, CO_2 delivery and oxygen removal.
2. Using continuously-acquired FRRF data operate PBR at the "sweet spot" of optimal light utilization and (30-90% of the maximum biomass stock).
3. Harvest the biomass at rates equal to production rates, stabilizing the "sweet spot" conditions.



How?

Field FRRF instrument

Solar powered FRRF instrument with wireless communication and rechargeable LiFePO₄ batteries. Internal processor/controller with hardware floating point math processor.



Version A. A floating instrument attached to the surface of a classic PBR. Uses a Hamamatsu H6780-20 photomultiplier as a detector and Linux computer based on AMD517 processor.

Average power consumption: ~3.4W. Numeric performance: ~90 iterations/sec.

Version B. An instrument interfaced to a 4" plastic pipe. Uses Semi Micro SL60000 as a detector and STM32174 controller. Average power consumption: 0.32 W. Numeric performance: ~34 iterations/sec.

Performance/power consumption tests indicate that solution B will be more appropriate for OMEGA applications. We are currently working on this version of field FRRF instrument.

High growth and biomass accumulation rates in algae are possible, but maintaining such growth rates requires continuous monitoring of algal photosynthetic performance. New FRRF to provide such monitoring are currently under development.



OMEGA with Forward Osmosis

Tra-My Justine Richardson and The OMEGA Team

Motivation

Forward osmosis (FO) membrane processes are considered for OMEGA to 1) concentrate wastewater nutrients before and during the algal growth cycle, and 2) concentrate algae as an initial dewatering step for harvesting. Here, we show the FO system used and report on experiments to examine FO performance (i.e. flux rates) and algae growth.

Materials & Methods

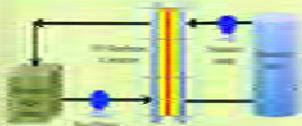


Figure 1: FO Experimental Setup

Feed: *Chlorella vulgaris* cultures in 40 gallons of secondary treated wastewater.

Draw solvent: Seawater

FO module: Hydration Tech Innovation (HTI) spiral wound with wide spacers.

Mixing: Sparging at the bottom of the fish tank.

pH control: carbon dioxide in sparging tube diffuser.



Figure 2: Spiral wound module in clear PVC case

Recirculate algae culture on the feed side and seawater on the brine side. Measure the weight of seawater to determine water flux. Monitor optical density, Fw/Fm, pH, conductivity, cations, and anions.

Goals

- Determine if FO impacts algal growth (presumably by increasing nutrients, not toxins)
- Determine if algae impact FO flux rates.

Results

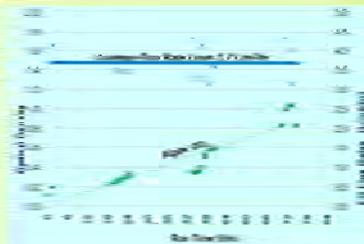


Figure 3: Optical density of algae culture and FO flux over 78 hours period

Figure 3 shows increases in algal biomass (OD_{680}) and that FO membrane performance (flux rates) does not decrease significantly over 78 hours of operations.

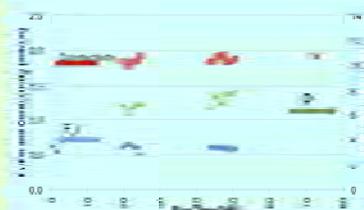


Figure 4: Fw/Fm, pH, and conductivity during 78-hour growth period

Figure 4 shows that Fw/Fm value remain stable and the salt concentration in the feed remain constant over 78 hours of operation.

Results cont.



Figure 5 shows algal fouling on FO membrane before and after operation. Fouling lessened by periodic washing with wastewater.

FO membrane testing



Figure 6: A flat sheet FO setup used for membrane testing

Comparison of six different FO membranes indicate a range in membrane performance and contaminant rejection.

Impact & Implications



FO processes can be used in the OMEGA system to concentrate wastewater nutrients and dewater algae cultures. Further studies needed to establish efficacy.



Growth of *Chlorella* in effluent concentrated by Forward Osmosis (FO)

Sigrid Reinsch, Tsage Embayo, Hironori Kagawa, Justine Richardson, and Jonathan Trent, Ph.D.

Goals

- Determine whether productivity is enhanced by concentrating nutrients in wastewater using FO
- Assess if FO concentrates compounds with toxic effects

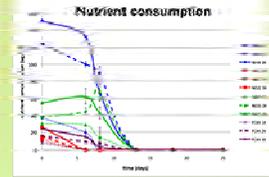
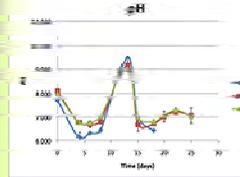
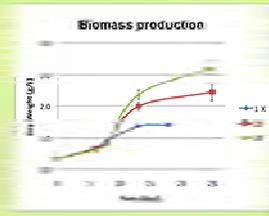
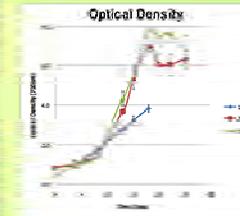


Materials and Methods

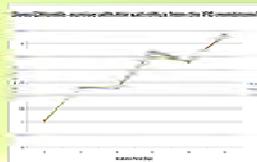
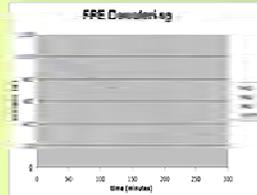
- Concentrate wastewater by FO (1x, 2x, 3x) – FRI X-pack with FPE and place in salt water tank for dewatering
- Determine nutrient concentration and salt backflux of initial sample and at discrete times during growth
- Grow in triplicate in 250 ml volumes under light/dark 14/10 hours cycles with CO₂
- Measure biomass by OD and dry weight – monitor pH



Results



Results



- Estimated salt reflux from FO membrane is 0.8 g/L
- *Chlorella* grew well at twice as high salt concentration of the estimated reflux.



Conclusion and impact

- FO concentration enhanced growth of *Chlorella*.
- At the concentrations used there is no negative impact of concentrating final effluent using FO
- FO could be a valuable process at the front of our system



OMEGA: Offshore Membrane Enclosures for Growing Algae



Biofouling and Marine Animal Interactions

Project Team: Linden Harris, Sasha Tozzi, Colleen Young, Marilyn Cruickshank, Will Fennie, Julie Kuo, Shawn Harmsen, Jonathan Trent, Sigrid Reinsch, and Patrick Wiley

Motivation

OMEGA photobioreactors (PBRs) will inevitably accumulate biofouling on exposed surfaces. Biofouling may limit the light available to cultures and therefore limit algae productivity. To determine rates of biofouling and its impact on productivity, and to assess the impact of OMEGA on seabirds and marine mammals, we deployed PBRs in the harbor at Moss Landing, CA for three months.

Materials & Methods

- 1) Biofouling was monitored weekly on two PBR designs (D1&D3) floating at a dock for 9 weeks each (Sept 2011-Jan 2012) in Moss Landing Harbor.
- 2) Grids used to quantify attached algae were photographed and analyzed by Image-J or used as scraping templates for measuring biomass by dry weight.



- 3) The impact of 9-weeks of biofouling on productivity was measured in a tank by wrapping a fouled (experimental) or a cleaned (control) PBR around PBR containing growing cultures.



- 4) Animal interactions were based on 3 months of day/night video at MLH. Captive animals were also observed interacting with small OMEGA PBRs.

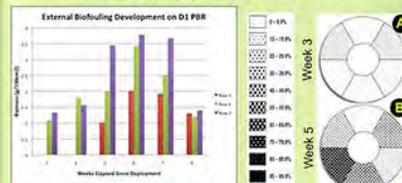
Goals

- 1) Quantify biofouling on PBRs.
- 2) Measure the effects of external biofouling OMEGA productivity.
- 3) Determine how animal interactions with OMEGA will impact animals and the OMEGA system.

Goal 1

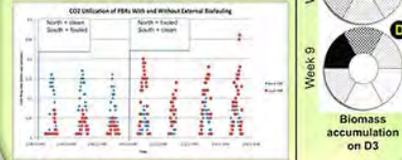


Above: Progression of biofouling on D1 (left) and D3 (right) in Moss Landing Harbor



Biofouling changed with time and varied in different areas of the PBRs. The center of the PBRs (rows S&E) were lower than the edges (row N). All rows peaked in weeks 6 & 7 and decreased by week 9.

Goal 2



Biofouling decreased algae carbon consumption.



Goal 3



Captive sea lion interacted with PBRs on command. Animals were not independently interactive.



Seabirds and otters fed, groomed and rested on PBRs. No pinniped interactions were observed.

Impact & Implications

- Biofouling was unevenly distributed on PBRs
- The shape of the PBR impacted biofouling
- 2 months of external fouling decreased algal growth rates inside PBRs
- PBRs must be cleaned periodically to maximize growth of contained algae
- Animals rest, groom, and feed on PBRs were not adversely impacted

POC: jonathan.d.trent@nasa.gov

- OMEGA PBRs will need to be cleaned periodically to maximize biofuels production
- Marine animals may benefit from OMEGA.



Biofuels from Fatty Acid Methyl Esters (FAME): Algae production, methods for extraction and purification

Project Team Tsogeread Embaye, Sigrid Reinsch, Jonathan Trent and OMEGA Project Team

Motivation

It is known that algae produce oil and that growth conditions can influence the amount of oil they produce. We investigated the conditions that induce the production of oil in *Chlorella vulgaris* grown in wastewater. We also investigated oil extraction methods and analyzed the types of oils extracted.

Materials & Methods

Algae cultivation and oil production, extraction, purification

- 1) *Chlorella vulgaris* was grown in wastewater for approx. 10 days or until nitrogen was depleted.
- 2) Cells were centrifuged and freeze dried.
- 3) Dry cells mass was determined.

Extraction & purification

- 1) Two extraction methods were compared: Bligh and Dyer (BD) vs. direct transesterification (DT)
- 2) B&D standard methods: briefly added methanol (10pts), chloroform (5pts), DI water (4pts); shaken 1hr; centrifuged; supernatant phase separated with chloroform:water (1pt:1pt); chloroform dried and lipids resuspended in methylene chloride.
- 3) DT: Dried cells resuspended in methanol:toluene (1:1); added 0.2N KOH-Methanol; sealed heated 50°C 2 hrs; neutralized with 1M acetic acid pH 6.2; added 2pts water; extracted with hexane; azeo and resuspended in methylene chloride

Analyses

- 1) TLC plates
- 2) Bands recovered eluted, dried, and analysed by GCMS

Goals

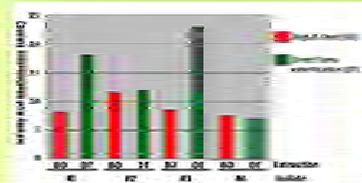
Determine

- 1) Conditions that optimize algae oil production
- 2) Best extraction methods for FAME from isolates
- 3) Analyze quality and quantity of FAME
- 4) Study the impact of forward osmosis on algae growth; on TAG synthesis and accumulation levels (not shown).
- 5) Impact of iron, heterotrophic growth, salt, pH, and light on lipid production (not shown)

Results



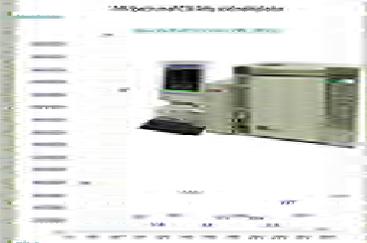
1) Algae cultivated with limited nitrogen increase synthesis of TAG from 8% to 30%.



2) Direct transesterification without extraction by BD of total lipid provided higher yields of FAME for three algae isolates (#1-#3) and one mixed community (#4).

Results-cont.

3) Analyses of FAME by gas chromatography mass spectroscopy (GCMS) indicates C18:1 and C16 predominate.



MS provides "fingerprint" for each fatty acid methyl ester—C16 shown here.

Conclusions

- 1) *C. vulgaris* produces up to 30% biomass; grows in wastewater.
- 2) Good C-chain length for biodiesel.
- 3) Direct transesterification is superior to B&D—faster, less solvent, less energy, direct to end product.



OMEGA: Offshore Membrane Enclosures for Growing Algae



Management Strategies for Algae Cultivation: Grazers, Competitors, Parasites, and Probiotics

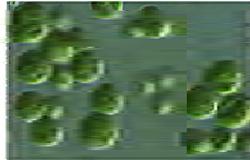
Kit Clark, Tege Embaye, Shawn Harmsen, Sigrid Reinsch, Jonathan Trent and the OMEGA team

Chlorella vulgaris:

a robust, well-studied green microalga (chlorophyte) that grows well in wastewater and dies in seawater.



Chlorella cell (5-7 μm) with a human hair. SEM image.



Chlorella culture phase contrast image.

Strategies used to control pests in algae cultivation systems

Ozone gas? Requires high doses for viruses, spores, and cysts. Highly reactive, needs corrosion resistant.

UV irradiation? Variable efficacy due to water color, turbidity, and chemistry.

Microfiltration? Expensive infrastructure, difficult to scale, may not be suitable for fungi, bacteria, & viruses.

Antibiotics? Expensive, difficult to scale, selective and creates antibiotic resistant microbes.

High or Low pH? Effective values for pests may damage algae.

Bleach? Highly caustic, effective, potential safety and environmental hazards.

Smells Shock? "Freshwater pests found in wastewater die in seawater, chlorella, yes, but is it effective?"

GRAZERS

Algae grazers rarely establish themselves in OMEGA systems. Ciliates are most abundantly observed.

Ciliates:



Limnolobus sp.

Hymenostoma

Rotifers:



Paramecium

amoeba

Ectocela

Branchionid

Copepods:



Cyclopoid female with eggs

larval nauplius

larval copepodite

COMPETITORS

A diversity of microalgae found in wastewater are welcome in OMEGA systems. *Scenedesmus* is usually the most abundant competitor.

Scenedesmus sp. Cyanobacteria:



S. quadricornis

S. dimorphus

filamentous

coccoid (spheres)

Diatoms:



pennate

chain

centric

Date	Waste water organism	10% (1 foot)	20% (2 feet)	30% (3 feet)	40% (4 feet)	50% (5 feet)
03-2011	μm. Flagellates (Kinetoplastid)	+	+	+	+	-
	μm. ciliate (swimming Rhizomonads)	+	+	+	+	-
	μm. Flagellates (Cryptophytes)	+	+	+	+	-
	Navicula sp. (penate diatom)	+	+	+	+	-
	S. quadricornis sp. (swimming ciliate w/ trunk)	+	+	+	+	-
	Scenedesmus sp. (Cyclopoid)	+	+	+	+	-
03-01-11	μm. Flagellates (Cryptophytes)	+	+	+	+	-
	med. Ciliates (Hydrocotyle)	+	+	+	+	-
	μm. ciliate (Rhizomonads)	+	+	+	+	-
	Phlocladia sp. (crawling Rotifer w/ rostrum)	+	+	+	+	-
	Navicula sp. (penate diatom)	+	+	+	+	-
03-01-11	μm. Flagellates (Kinetoplastid)	+	+	+	+	-
	Scenedesmus sp.	+	+	+	+	-
	Navicula sp. (penate diatom)	+	+	+	+	-
	Scenedesmus sp. (Cyclopoid)	+	+	+	+	-
	amoebae	+	+	+	+	-
	Limnolobus sp. (swimming ciliate w/ trunk)	+	+	+	+	-
	+	+	+	+	+	-
	+	+	+	+	+	-

Table of observations using chemical shock as a means of pest control. Samples of community were antibiotic and treated to seawater. Organisms were observed under compound and associated microscopes for at least 1 minute, 1 hour and 1 day after community treatment.

PROTOZOAN BACTERIOVORES

Flagellates graze bacteria and fungi and are welcome in the OMEGA system.



Stalked ciliates

Flagellates

Vorticella

Ciliated organism

Stalked ciliated organisms are indicated by arrows. They have the ability to change their morphology.



Coliform Bacteria and Antibiotic-resistance

Nitomi Kagawa, Sigrd Reinsch, & Jonathan Trent

Coliform bacteria are used as an indicator of wastewater quality. We determined if the OMEGA system impacts the number of coliform bacteria and the profile of antibiotic-resistant coliforms using standard plating methods.

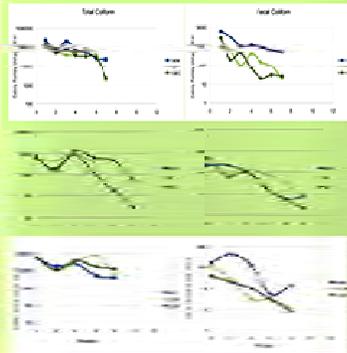
Coliform Bacteria Plating



Left photo: Glittering "total coliform" colonies on mEnzo agar includes *Citrobacter*, *Enterobacter*, *Haemophilus*, *Klebsiella*, *Serratia* and fecal coliforms.

Right photo: Blue "fecal coliform" colonies on mFC agar is almost exclusively *Escherichia coli*.

Three experiments showing coliform counts in wastewater with or without algae in two types of PBRs.

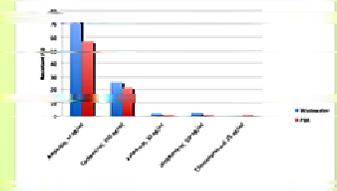


Left graph: total coliform counts
Right graph: fecal coliform counts.

Coliform counts in different PBRs (light & dark green curves) compared to wastewater without algae (blue curve).

In ten days, both total and fecal coliform counts decreased 10x to 100x, although there was no significant difference between PBRs with algae and wastewater without algae.

Antibiotic-resistance total coliform



Antibiotics: ampicillin & carbenicillin (cell wall synthesis inhibitors) kanamycin, streptomycin, and chloramphenicol (protein synthesis inhibitors).

Blue bars: resistant coliform in wastewater. Red bars: resistant coliforms in OMEGA PBRs.

Ampicillin, 70% resistant in wastewater compared to 50% in PBR with algae. Carbenicillin, 20% in wastewater and in PBR with algae. Kanamycin, streptomycin, and chloramphenicol, <2% resistant in wastewater and in PBRs with algae.

Antibiotics resistance correlates with frequency of use (ampicillin and carbenicillin common)

The antibiotic-resistance was not significantly different between wastewater and PBRs suggesting PBRs do not modify antibiotic resistance.



OMEGA: Offshore Membrane Enclosures for Growing Algae



Techno-economic Analysis

Brandi McKuin, Colin Beal, Patrick Wiley, Russel Adams, and Jonathan Trent

Table 1
OMEGA growth assumptions

Algae Productivity (g/m ² /day)	25
Algal cell density (g/L)	0.3
Liquid Yield (dry weight)	25%
DWG Production (g/m ² /day)	3,275
Dewatering (g/m ² /day)	365

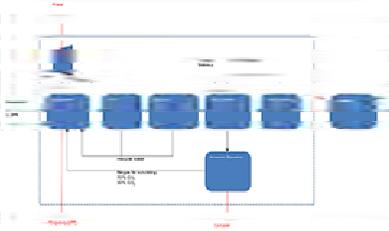


Figure 1 Schematic of algae biofuel process model. Dashed lines represent system boundaries. Bars in red represent products and services revenues.

Table 2 Inventory of capital costs

OMEGA	US\$ per hectare of PBRs
Capital Cost Items Direct Values:	
Land	Not included
Membrane	3,553
Extraction	8,100
Dewatering	11,788
Insulation	10,000
Electrical system & management	2,000
TRP System	40,000
Total Direct Capital Invested Capital	76,441
30% contingency	22,932
Working Capital (25%)	19,408
Total Capital Investment	118,781

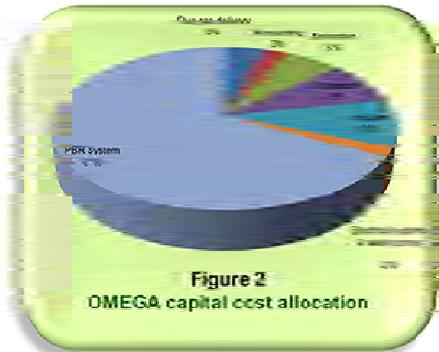


Figure 2 OMEGA capital cost allocation

Summary of Economic Assumptions

1. Conceptual estimate (Lundquist et al., 2010)
2. Based on Independent Power Projects (IPPs), rather than regulated utility projects.
3. The cost of production is a simple levelized cost of energy which includes taxes on profits but includes Corporate Tax Credit and MACRS + bonus at (35% tax rate).
4. Assume no construction ramp up, no cost-of-fuel escalation, and nominal interest rate
5. 100% investor financing (i.e. 100% debt, 0 equity) of capital plus 25% of operating costs;
6. 20 year plant life

Table 4 Non-liquid value of services

Value of Services	\$/year
Wastewater Treatment (Wang et al., 2011)	49,500
Nitrogen (Liquid & Solid Digestates) (\$407/ton)	1,912
Phosphate (Liquid & Solid Digestates) (\$422/ton)	519
Compost (\$/dry ton)	0
Recycle LLDPE (\$0.20/lb)	8,958
Totals	56,816

Table 5 Preliminary Results



Equations used in the analysis

If a photo-bioreactor were built with capital cost, C , to be recovered in t years, with an annual rate of return i , the required annual payment, Q , would be:

$$Q = \frac{C(i+1)^t}{(1+i)^t - 1}$$

Q must be less than or equal to the annual revenue from the system minus the annual expenses:

$$Q \leq R_1 + R_2 - Q_1 Q_2 + Q_3 Q_4 - Q$$

where
 R_1 : Revenue from photo-bioreactor (\$/y)
 R_2 : Value of lipids produced (\$/y)
 R_{wtr} : Value of non-liquid biomass (biogas electricity sold back to the grid) (\$/y)
 V_{wtr} : Value of services (wastewater treatment (WWT), fertilizer or compost, recycles LLDPE) (\$/y)
 P_{wtr} : Production rate (gal/y)
 A : Annual operation costs (labor, electricity, heating, maintenance) (\$/y)

Conclusion

To achieve a 10% rate of return, the required product selling prices were found to be \$20.30 and \$4.82/gal of TAG for case 1 (not including wastewater treatment revenues), and case 2 (including wastewater treatment revenues) respectively. Given the current petroleum diesel production cost of \$2.73/gal (March 2012, EIA), the results suggest the economics of microalgae biofuel production are currently not competitive with traditional fossil fuels unless wastewater is an added value of the fuel production system.

Table 3 OMEGA operating cost allocation

OMEGA	US\$ per hectare of PBRs
Operating Costs	\$/yr
LLDPE (30.00 \$/lb)	41,537
Electricity (20¢/kWh)	4,000
Compost (2¢/dry ton)	7,400
Maintenance	2,000
Insurance	2,000
WWT (30¢/gal)	2,937
Lab-leasing	600
Vehicle maintenance	100
Lab/office supplies	125
Employee training	100
Total Operating Costs	65,802



Figure 3 OMEGA operating cost allocation



Coliform Bacteria and Antibiotic-resistance

Nitomi Kagawa, Sigrd Reinsch, & Jonathan Trent

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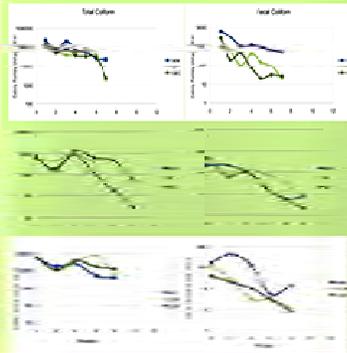
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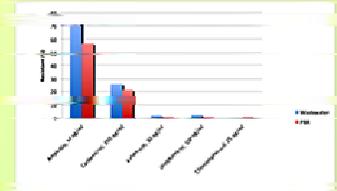


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Development of a wireless ISFET-based pH sensor network: Proof-of-Concept Testing

Project Team: John Malinowski and OMEGA Project Team

Motivation

A microalgae cultivation facility requires accurate and precise sensor information; most notably information on pH, dissolved oxygen content, and temperature. The marine environment envisioned for project OMEGA creates a need for durable, autonomous sensor equipment. It is proposed in this work that a wireless system can provide a reliable, autonomous pH measurement. Additionally, this project proposes the use of ISFET-based pH measurement for enhanced durability and accuracy of sensor performance.

Key Components

ISFET pH Sensor



The ISFET (Ion-Sensitive Field Effect Transistor) pH sensor is more durable and rugged than the commonly used glass pH electrodes. The electrode does not require wet storage, and can be cleaned easily without risking damage to a glass bulb.

Further development of this sensor network will analyze the long-term performance of the ISFET as a pH sensor in terms of drift, temperature dependence, and effects of biofouling.

Key Components, cont.

ANT Wireless networking system

The ANT system is a low-cost, low-power wireless sensor communications platform. The extremely low power consumption enhances the autonomy of the system, while providing sufficient data handling capabilities. A simple scripting language is included for interfacing any type of sensor to an ANT module with A/D conversion capability.



The ANT system transmits a signal in the 2.4 GHz range (ISM band, known for Bluetooth devices, among others) across a range of up to 15 meters in clear air.

Proof-of-Concept Experiment

One ISFET pH sensor module was installed to operate in parallel with an existing wired pH sensor in the sensor manifold for OMEGA's D3 algae cultivation system. The sensor data was relayed to a PC in the laboratory trailer through a hub unit (designed to communicate with several sensors) and two relays to send the information back to the PC. The data were recorded over a time period from 22 March to 2 April 2012. Calibration of the ISFET sensor occurred on 20 March, and was rechecked on 6 April. The data from the wired and wireless pH units is given below.

Summary of Results

The wireless pH system captured data from the OMEGA system for a period of approximately 11 days, when a battery failure in the sensor ended this run. Some interruptions were observed; shown in the graph as an abnormally flat line. These events were due to a faulty power connection on one of the relay units. A gradually varying offset of ~0.5 pH from the wired system is clearly visible. Subsequent experiments are planned to analyze accuracy and drift characteristics in more detail.

In the 17-day period between calibrations of the ISFET module, the calibration in pH standards moved by less than 2%, as shown in the following table.

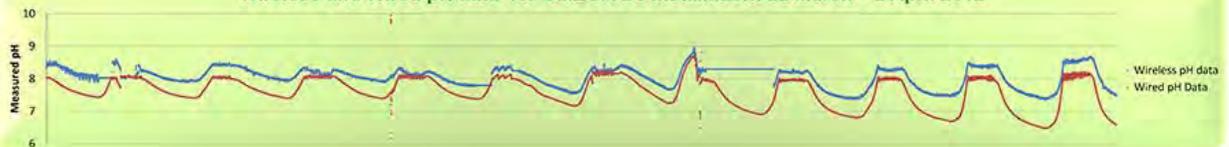
Calibration: Sensor output in pH standards

Date	pH 7	pH 10
20 March	1.594 V	1.052 V
6 April	1.599 V	1.038 V

Acknowledgements

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Dr. Jonathan Trent and the OMEGA team

Wireless and Wired pH data for OMEGA D3 installation: 22 March – 2 April 2012





OMEGA: Offshore Membrane Enclosures for Growing Algae



Integration with Wastewater Treatment: Energy, Cost, and Greenhouse Gas Assessments

Collin Beal, Brandt McHuff, Patrick Wiley, Russel Adams, and Jonathan Trent

Motivation

To evaluate the commercial feasibility of the OMEGA system, several questions must be answered:

- 1) What is the Energy Return on (Energy) Investment?
- 2) What is the Financial Return on Investment?
- 3) What are the GHG impacts of the system?

Key Points

Wastewater Provides Critical Nutrients

In addition to supplying water for cultivating algae, the wastewater is a rich source of carbon, nitrogen, and phosphorus, all of which are required nutrients for algal cultivation.

Avoidance of Costly BNR Processes

Implementing the OMEGA system would allow WWT facilities to avoid costly BNR processes because the algal biomass effectively removes nutrients from the wastewater.

Jet Fuel Production

Neutral lipids can be extracted from algae using wet extraction techniques and upgraded into fuel substitutes.

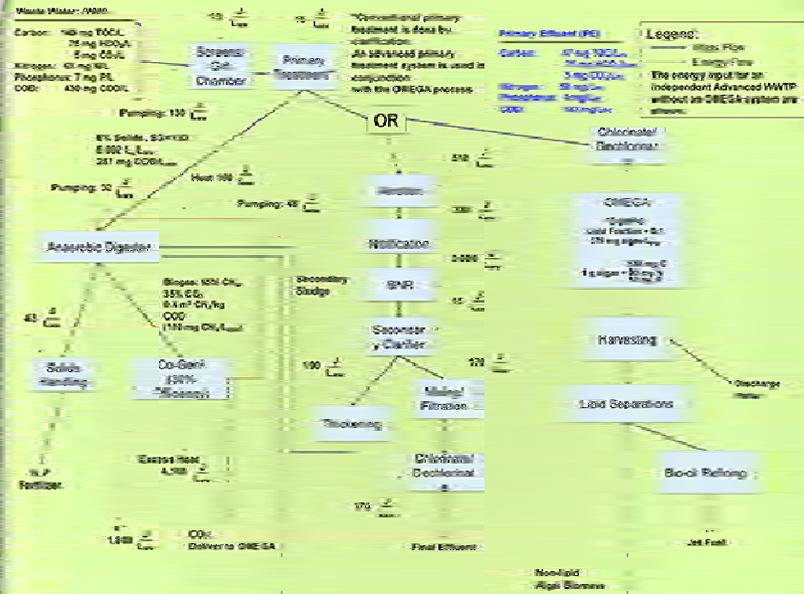
Direct, Indirect, and Capital Inputs

The energy, cost, and GHG analyses consider not only direct inputs, but also indirect (upstream) inputs and capital expenses. These analyses are currently being conducted.

Mutual Benefits

When operated independently, wastewater treatment is typically an energy negative process. Similarly, algal biofuel production has been shown to be energy negative. However, by coupling the two systems, it is possible to transform two energy sinks into a collective energy source!

The OMEGA system avoids costly BNR processes and yields valuable fuels



References

This work builds on research conducted at the University of Texas at Austin that will be published in Water Environment Research: 1. Beal C.M., Stillwell A.S., Cohen S., Berberoglu H., Connolly R., King C.W., Bhattarai R., Heber R.E., Webber M.E., "Energy Return on Investment for Algal Biofuel Production Coupled with Wastewater Treatment," Water Environment Research, Accepted.

Much of the data shown above was presented by Goldstein and Smith (2002): Goldstein, R., Smith, W. (2002) Water & Sustainability (Vol. 4): U.S. Electricity Consumption for Water Supply & Treatment. EPRJ #1009737.

**Appendix G:
Contributors**

Appendix G: Contributors

CHAPTER 1: Biofuels

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Acknowledgements: The authors thank all OMEGA team members, in particular S Ord, E Austin, A Wong, Z Kolber, T Embaye, L Harris, H Kagawa, K Clark, R Adams, K Long, A Nazzal, M Primack, S Fauth, J Malinowski, J Richardson, J Rask, S Harmsen, E Geiger, M Claxton, S Eckhart, S Martin, P Buckwalter, J VanGelder, R Takrit, K Acierto, Z Hall, B Smith, S Toy-Choutka, S Zimmerman, S Marwood and C Beal, as well as the California Department of Fish and Game, Moss Landing Marine Laboratory (CA, USA) and Southeast Wastewater Treatment Plant (CA, USA) staff. Research support was provided by the California Energy Commission and by NASA.

CHAPTER 2: Microalgae Cultivation Using Offshore Membrane Enclosures for Growing Algae (OMEGA)

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CHAPTER 3: Techno-economic assessment of the prototype OMEGA system for large-scale algae cultivation and wastewater treatment

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CHAPTER 4: The role of OMEGA PBRs in advanced Municipal Wastewater Treatment

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CHAPTER 5: Microbiome analysis of a microalgal mass culture growing in municipal wastewater in a prototype OMEGA photobioreactor¹

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CHAPTER 6: Marine mammal and seabird interactions with model OMEGA photobioreactors

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CHAPTER 7: Why OMEGA is needed and how to get there from here...

An Environmental Business Case

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