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FINAL PROJECT REPORT

OFFSHORE MEMBRANE ENCLOSURES FOR GROWING ALGAE (OMEGA)

A Feasibility Study for Wasterwater to Biofuels

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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 technoeconomic 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, technoeconomic analysis

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EXECUTIVE SUMMARY

Introduction

Finding sustainable and environmentally compatible energy technologies that go beyond fossil carbon sources is a challenge for current and future generations. Many researchers believe that biofuels appear to be the alternative liquid fuel of choice, although in a world of seven billion people it is clear that biofuels production must not compete with food production. There areserious questions about how to achieve this, given the quantities of biofuel that will be required and that proposed biofuels crops are either food crops (e.g., corn, soy, and sunflower) or compete with food crops for water, fertilizer, and land (e.g., jatropha, canola, and palm). Microalgae include species that are good oil producers (as shown in Figure 1) and may be the best source of biofuels if they can be cultivated using wastewater as the source of water and fertilizer, but what about land?

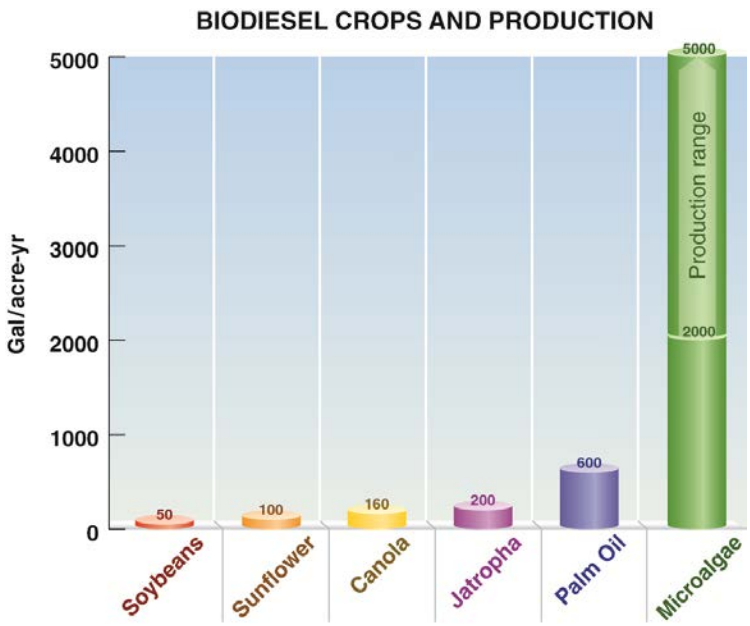

Figure ES-1: Production of Biodiesel by Different Feedstocks

Indicates that some species of microalgae may be the best source of biofuels, but only if they can be grown without competing with agriculture.

The solution for coastal cities may be to cultivate oil-producing microalgae in floating enclosures called photobioreactors (PBRs) on the domestic wastewater dumped off-shore through outfalls. The offshore system would have to be hundreds or thousands of acres in coastal areas to accommodate the amount of wastewater and to make enough fuel to be worthwhile. Such a system would require overcoming significant challenges inherent in large-scale algae cultivation as well as the challenges presented by the coastal marine environment

itself. The marine environment challenges engineers to design systems able to cope with strong and variable forces from waves, currents, and winds. It also requires ingenuity in developing materials that can withstand these strong forces along with corrosion, weathering, and biofouling. In some places there are also formidable social challenges in the rules and regulations imposed on developments in coastal regions such as permits and permission from the many coastal regulatory organizations that monitor and control use and environmental impact.

Many of these engineering, materials, and even social challenges have been addressed and mastered by the oil companies in their ongoing efforts to recover fossil fuels from the deep sea in some of the most difficult marine environments in the world (e.g., the Gulf of Mexico and the North Sea).

Project Purpose

The goal of this project was to develop a sustainable and environmentally compatible alternative that focuses on the offshore development of large-scale algae cultivation in a system called Offshore Membrane Enclosures for Growing Algae (OMEGA) by building on the knowledge gained from deep sea drilling. The OMEGA project had two related objectives: 1) to assess the feasibility of growing microalgae in floating photobioreactors (PBRs) using domestic wastewater and a plausible source of carbon dioxide on both theoretical and practical levels; and 2) to disseminate the OMEGA concept to motivate others to further develop and commercialize the technology. Researchers evaluated the feasibility of the floating PBR system in light of the urgency and importance of biofuel development and believed it was critical that the components (pipes, plastic, pumps, microbes, instrumentation, and feedback control systems) were adapted from existing and affordable materials and technology. Researchers wanted to demonstrate that the project was not dependent on developing new materials, genetically engineered super-organisms or special sensors and control systems.

Project Results

The OMEGA team consisted of scientists and engineers from a variety of public and private organizations. The team attempted to maintain an "open source" model in the dissemination of their results and welcomed contributions from colleagues and collaborators with interests in marine biology, ecology, engineering, environmental studies, economics, and public policy.

The project was divided into three phases. In the first phase, ideas about possible OMEGA materials and designs, deployment and operation, as well as environmental constraints and concerns were considered and discussed, which led to technical memoranda assembled into a report. In the second phase, a functional floating 110-liter prototype system was developed in a seawater tank at a research facility in Santa Cruz and then scaled up to 1,600 liters in seawater tanks at a wastewater treatment plant in San Francisco. In the third phase, the results were

evaluated and reported in a series of technical papers based on experiments and analyses in phases I & II. This report and a series of technical papers were the focus of Phase III.

Phase I focused on team building to establish two working groups. Collaboration between the two teams led to the compilation of a paper study, which included PBR designs and engineering considerations, possibilities for offshore sites, hypothetical considerations of the energy return on energy invested (EROI), and permitting issues, among other topics. During Phase I the project outreach was supported by the NASA Ames Public Affairs Office (PAO) and the project scientist gave many public presentations and interviews in a wide range of venues, including universities, conferences, television, radio, and newspapers.

In Phase II preliminary OMEGA research began in 2009-2010 in small (1-2 liter) PBRs built in the laboratory and tested in small tanks and laboratory sinks at NASA Ames Research Center in Moffett Field, CA. Through trial-and-error, a 110-liter system became the functional model system. The functional system was scaled up to a 1,600-liter system tested in seawater tanks. Field experiments were conducted to study biofouling at Moss Landing Harbor in collaboration with Moss Landing Marine Laboratories using their dock facilities. A 110-L prototype system was designed and constructed with floating PBRs made of inexpensive polyethylene, a circulation system made of polyvinyl chloride (PVC) pipes and pumps, and an instrumentat and control (I&C) system made with off-the-shelf sensors and controllers. PBRs with internal swirl-vanes or external furrows directed flow into a spiral pattern and provided mixing. The PBRs were floated in a 24,000-liter seawater tank for testing. A separate multifunction column (GEHC) was used for gas exchange to control critical soluble gases (CO_2 and oxygen) and for harvesting. During two consecutive experiments microalgae growing on wastewater were monitored for photosynthetic efficiency (FRRF), photosynthetic active radiation (PAR), dissolved oxygen (DO), temperature, water flow, gas flow, pH, and pressure. The flow, pressure, pH, and CO_2 input were automatically controlled within setpoints of the I&C system. The GEHC system removed DO during the day and provided DO at night. It provided CO_2 from a flue-gas simulant with less than 50 percent of the CO_2 injected converted into biomass compared to 10-20 percent conversions reported for conventional sparging systems. A role for the GEHC in harvesting was indicated by increased concentrations of microalgae in the settling chamber. Improved recoveries could be achieved using microalgae that naturally aggregate or by adding coagulants. Electrocoagulation (EC) could be incorporated into the GEHC to enhance harvesting efficiency and generate chlorinated compounds that would disinfect the wastewater prior to discharge. Secondary-treated wastewater provided nutrients for the microalgae and was effectively treated by the microalgae, which removed over 90 percent of ammonia nitrogen (NH_3-N). This suggested a role for the OMEGA system in wastewater treatment.

The prototype system developed at CDFG was scaled up from 110 liters with two PBRs to 1,600 liters with four PBRs floating in 300,000-liter seawater tanks at the Southeast Wastewater Treatment Plant in San Francisco, CA. The SEP system was run continuously for two months

using on-site and remote monitoring methods. Samples were taken over a 13-day period to monitor the complete microbial diversity and dynamics using high-throughput metagenomic techniques. Approximately five million sequences of 16S and 18S recombinant deoxyribonucleic acid (rDNA) were evaluated. Samples were evaluated for the impact of the OMEGA system on the removal of heavy metals, pharmaceuticals, personal care products, and other organic contaminants.

One of the most significant challenges for a successful OMEGA system deployed in the marine environment will be biofouling caused by marine organisms colonizing the outside of PBRs that will influence buoyancy, drag, and potentially light availability for microalgae cultured inside the PBRs. Two floating PBR designs (flat-panel and tubular) were deployed to study the rates of biofouling and to determine the impact of external biofouling on internal algae growth at a field site in Moss Landing Harbor, Moss Landing, CA.

Another environmental concern tested at Moss Landing Harbor was the interaction between PBRs and marine mammals and birds. The research team collected data over a four-month period using captive pinnipeds for behavioral studies with PBRs in tanks and using continuous infrared video to record day and night interactions of animals with the same floating PBRs used to study biofouling in Moss Landing Harbor.

This report and a series of technical papers were the focus of OMEGA Phase III. The goal of this phase was to communicate in the peer-reviewed scientific literature the details of a functional floating algae cultivation system and to present its pros and cons, advantages and disadvantages compared to alternative land-based systems.

The OMEGA project focused on the feasibility of offshore algae cultivation based on researchers' observations of the efficiency and parsimony of life-support systems. It was demonstrated that the microalgae cultivated on wastewater died in seawater, that the microalgae biomass could be used for biofuels, and that the system treated wastewater, even removing some of the pharmaceuticals and personal care products, the so-called compounds of emerging concern.

The OMEGA system consisted of flexible plastic enclosures with reinforced plastic on the underside and clear plastic on the upper (sunny) side. OMEGA modules were floating PBRs used in either batch or continuous-flow modes with inputs of non-saline wastewater and carbon dioxide (CO_2) along with an inoculum of microalgae. A gas-exchange column removed excess oxygen, provided a supply of CO_2, and constantly harvested algae into a settling chamber. A forward osmosis (FO) membrane system could be incorporated to concentrate nutrients and stimulate algae growth while dewatering the algae to facilitate harvesting. The mature, concentrated algae suspension was pumped back to shore, thickened, and dewatered, while the lipids were extracted and converted to fuels.

The system focused on wastewater treatment, which included removing nutrients that contributed to the creation and growth of dead zones as well as heavy metals and other potential toxins adsorbed or degraded by microbes included in the OMEGA biological consortium.

The OMEGA project was focused on the challenge of growing sufficient quantities of algae to substitute for fossil fuels, but it was also a demonstration of how technologies can be integrated to form an "ecology of technology" in which wastes from one part of the system were viewed as resources for another part.

OMEGA focused on using all aspects of its products and processes as a demonstration of technology ecology. As a biofuels system OMEGA focused on growing algae species with high lipid content for making jet fuels and rapid growth rates for producing biomass. The non-oil biomass was considered a source of fertilizer, animal feed, or specialty algal product. OMEGA provided services in addition to products, including wastewater treatment, carbon sequestration, and environmental remediation. The value of OMEGA products and services will depend on local conditions and needs and a host of downstream considerations, such as processing requirements, transportation, market considerations, and what can be considered secondary consequences; for example, the impact of wastewater treatment on fisheries or health or tourism.

OMEGA was an algae-cultivation system that relied on wastewater for nutrients to grow algae and seawater for temperature control, containment, and forward osmosis (FO). OMEGA could grow any desired strain, species, or community of freshwater algae that could utilize wastewater and cope with local ocean temperatures but that cannot thrive in saltwater. Within these constraints the OMEGA system could be used for any product or co-product associated with algae farming.

There were no general barriers to installing OMEGA systems provided there was access to salt water in a naturally or artificially protected bay, wastewater, sunshine, and the large quantities of relatively inexpensive materials needed for OMEGA construction such as plastic, pipes, valves, and moorings. The relevant engineering and fabrication skills were borrowed from the fields of marine engineering, plastics, aquaculture, wastewater treatment, and oil refining. The local requirements were a source of wastewater discharge into the ocean and a nearby source of CO_2, such as a near-shore power plant. The OMEGA system provided advanced wastewater treatment and quantifiable capture of CO_2, in addition to the algae products described above.

Specific barriers included restricted access to coastal zones due to lengthy and expensive permitting processes and expensive logistics for obtaining the wastewater effluent and CO_2 required for algae growth. Ultimately, rising sea level inundating coastal cities and low-lands may help motivate and facilitate the development of OMEGA systems.

Project Benefits

This project demonstrated that the OMEGA system has potential for growing algae species that could be used for biofuels and that the system could treat wastewater. Biofuels can play a role in lessening California's dependence on fossil fuels, which will help to reduce greenhouse gas emissions that contribute to climate change as well as other emissions that cause air pollution.

Chapter 1:
Is the Future of Biofuels in the Bag? Are Algae the Answer?

1.1 Summary

It appears that at least for coastal cities, the most plausible answer to the question of how to make the massive amounts of biofuels needed to displace significant quantities of fossil fuels without competing with agriculture will be to 1) use microalgae as the feedstock, 2) grow the microalgae on domestic wastewater, and 3) locate the cultivation system offshore in the vicinity of existing wastewater outfalls. The feasibility of an enormous offshore algae cultivation system will depend on overcoming major challenges inherent in algae cultivation, in finding appropriate sites and engineering offshore systems that can cope with extreme conditions at these sites, and in many countries, navigating the environmental and political bureaucracies, which may pose the greatest difficulty in testing the new technology. It is well established that the economic challenges for biofuels are daunting if not impossible to overcome. The OMEGA system addresses the economic challenge by integrating into the same offshore platform biofuels production, wastewater treatment, solar, wind, and wave-energy generation, as well as aquaculture.

Ultimately, rising sea level inundating coastal cities may help motivate the development of OMEGA systems as these cities are redesigned for sustainability.

1.2 Introduction

In the last few years there has been much excitement about microalgae for biofuels. Indeed, species of microalgae are among the fastest growing biomass and the best oil producers, but if something seems too good to be true, it usually is. There remain many fundamental questions and no clear consensus about how and where to cultivate microalgae on a scale that would be relevant to biofuels (Andres Clarens and Lisa Colosi, Putting algae's promise into perspective, Biofuels (2010) 1(6), 805–808 Pienkos, P.T. Darzins, A. The promise and challenges of microalgal derived biofuels. Biofuels, Bioprod. Biorefin. 2009, 3 (4), 431_440). There is a growing consensus however that the only sensible way that microalgae can be used for biofuels production will be to link their growth with wastewater treatment plants (see: Park et al. 2011[1] and Lundquist et al. 2010[2]).

[1] Park, J.B.K., R.J. Craggs, and A.N. Shilton, 2011. Wastewater treatment high rate algal ponds for biofuel production, Bioresource Technology 102 (2011) 35–42

[2] Lundquist, T.J., I.C. Woertz, N.W.T. Quinn, J.R. Benemann (2010). A realistic technology and engineering assessment of algae biofuels, Energy Biosciences Institute Report: http://www.renewableenergyworld.com/rea/news/article/2010/11/algae-for-biofuels-moving-from-promise-to-reality-but-how-fast

Wastewater provides both water and fertilizer for algae cultivation and algae provide desirable treatment for the wastewater. Using wastewater instead of synthetic fertilizer to grow algae saves energy and money and these savings are compounded by using algae instead of other methods for biological nutrient removal in wastewater. It has been recently calculated that linking algae cultivation and wastewater treatment moves the energy return on investment (EROI) above 1 (Beal et al. 2012), which means it is possible to get more energy out of the overall system than went into it—an essential feature for any would-be biofuel. This vital linkage between algae and wastewater, raises a very important practical question: How will large-scale algae farms be incorporated into wastewater treatment plants? We propose that the answer, at least for coastal cities, is OMEGA (Offshore Membrane Enclosures for Growing Algae). Here's why.

Wastewater treatment plants are an essential part of all modern cities and are usually embedded in urban or suburban infrastructure. It is safe to assume that these plants cannot be relocated or rebuilt to accommodate algae facilities. EROI considerations dictate that wastewater can only be pumped short distances to the algae facility. If the facility involves algae ponds, level land is required, and if it involves photobioreactors (PBRs), in many locations, a cooling system will be required; this usually involves evaporative cooling, which requires a large supply of clean water (refs). How big an area will be required for the algae facility?

The size of the algae facility will depend on the volume of wastewater, which in turn depends on the population of the city. In the USA, average per capita water use is about 100 gal (379 liters) per person per day. In San Francisco, for example, with an estimated population of 856,095,[3] the three wastewater treatment plants, fed by a network of over 900 miles (1450 km) of sewer pipes, have a daily (dry-weather) wastewater discharge into SF Bay and the Pacific Ocean of about 85 million gallons (321,760 cu meters). To locate an algae production facility in the vicinity of the SF Southeast Treatment Plant (SEP), which averages about 65 million gallons (236,025 cu meters) of wastewater per day, would require an area of approximately 1,500 acres (600 ha).[4] Figure 1 shows the "footprint" of a hypothetical 1,500-acre (600 ha) algae production facility in the vicinity of the SF SEP—on shore and offshore.[5]

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[3] Sheya, Daniel (May 2010). "E-1 Population Estimates for Cities, Counties, and the State with Annual Percent California Department of Finance. http://www.dof.ca.gov/research/demographic/reports/estimates/e-1/2009-10/.

[4] This calculated acreage is based on a 5-day algal growth cycle (holding capacity = 5 da x 65 Mgal/da = 325 million gallons or 1,230,125 cu meters). Assuming the open ponds or photobioreactors have channels 0.7 ft (0.2m) deep and 33 ft (10m) wide, their length is 382 (615 km). (0.7ft x 33ft x 20M ft = 43,450,000 cu ft x 7.5 gal/cu ft = ~325 M gal. The area = 33ft x 2M ft = 66M sq ft x 0.000023 acre/sq ft = 1,518 acres.

[5] To put this area in perspective, it is covering/displacing approx 5% of the city of San Francisco, which is (29,887 acres or 12,095 ha). In contrast, in 2007, about 8% of farms in the US were >1,000 acres and 3.6% >2,000 acres. http://www.ers.usda.gov/statefacts/us.htm

Figure 1: Co-Locating Wastewater Treatment and Large-Scale Algae Production Facilities.

In San Francisco, as in other coastal cities, the wastewater treatment plant (red rectangles) is in the city and near the coast. The entire Bay Area peninsula is developed and heavily populated (top center & right). The area (1,520 acres) required to contain 5-days of wastewater from the Southeast Treatment Plant is shown on land and in the water (green rectangles). The solid red arrow (pointing right) indicates where the wastewater effluent is currently discharged and the dashed red arrow (pointing left) indicates the pipeline needed to pump effluent inland. Inserts show enlargements of an open pond system on land (left) and an OMEGA system of floating photobioreactors (right).

It is obvious that to build a 1,500-acre algae production facility on the land adjacent to the SF SEP (Fig. 1, red rectangle) would require impossible infrastructure changes; roads, freeways, bridges, buildings, and many homes would be displaced, replaced, or otherwise impacted. An aerials view of the Bay-Area peninsula indicates there is no adequate undeveloped real estate for miles on land (cf. Fig. 1, top-center image). In the SF Bay-area, the only possibility for a large-scale algae facility is in the bay itself. In this offshore location the facility would not only be in the vicinity of the wastewater treatment plant, it could use the existing outfall infrastructure.

Assuming there are sufficient nutrients in the wastewater to yield 23 g-algae/meter^2/day, the 1,500 acres facility would annually yield an estimated 3 million gallons of biodiesel if the algae are 20 percent oil and nearly 8 million gallons if the algae are 50 percent oil.[6] There are also biogas and other algae byproducts. (In contrast, 1,500 acres of soybeans will yield 76,000 gal/yr, using a reported yield of 50 gal/ac/yr). With regard to concerns about impact on the bay, a 1,500-acre facility would occupy <1 percent of its surface area and the installation could be designed to minimize its impact on existing harbors, shipping lanes, and fishing or recreational sites.[7]

In the USA, 23 of the 25 most densely populated counties are located on the coasts, accommodating about 53 percent of the total population, and all these areas discharge large quantities of wastewater effluent into adjacent coastal waters.[8] The question then arises, *"Is it feasible to develop large-scale offshore algae production facilities in coastal cities?"*

The OMEGA project:

With support from NASA and the California Energy Commission, the OMEGA team has been investigating the feasibility of OMEGA. The team engineered and tested various floating PBR designs in seawater tanks. The PBRs included systems for monitoring and controlling pH, CO_2, dissolved oxygen, temperature, water pressure, and pump speeds. Algal physiology was monitored using fast repetition rate fluorometry (FRRF-ref). Growth rates, nutrient utilization, grazers, pathogens and total coliforms were monitored, as well as the biomass and composition of the biofouling community on both the inside and outside the PBRs. The impact of floating PBRs on local marine mammals and seabirds was studied using infra-red video observations of a PBR system deployed in a harbor. The estimated EROI of the system and economic analyses are underway.
It is envisioned that in the OMEGA system oil-producing freshwater algae are grown in flexible, clear plastic PBRs attached to a floating infrastructure anchored offshore in a protected bay. Wastewater and CO_2 from coastal facilities provide water and nutrients. The surrounding seawater controls the temperature inside the PBRs and kills algae that escape from the system. The salt gradient between seawater and wastewater drives forward osmosis, to concentrate nutrients and facilitate algae harvesting. The OMEGA infrastructure also supports aquaculture and provides surfaces for solar panels and access to offshore wave generators and wind turbines. Integrating algae cultivation with wastewater treatment, CO_2 sequestration, aquaculture, and other forms of alternative energy creates an *ecology of technologies* in which the wastes from one part of the system are resources for another. In addition, the parts

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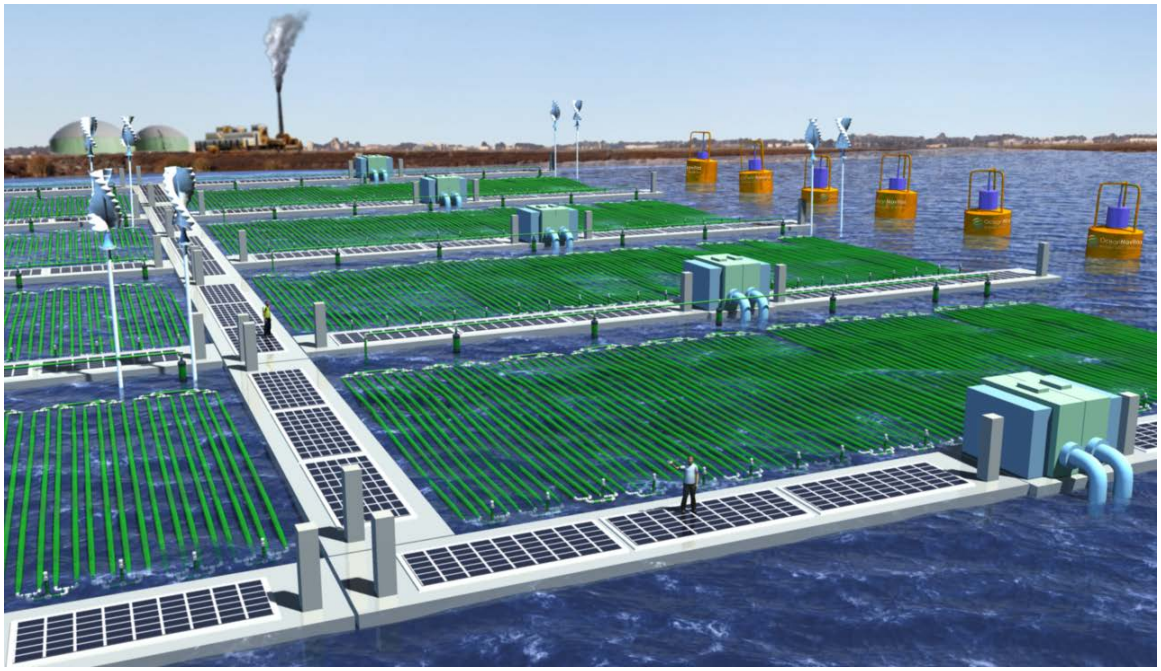
[6] Calculating: (23 g algae/m2/day/0.2m) = 0.115 kg/m3/da x 1,230,125 m3 OMEGA 5-da vol = 141,464 kg algae/da x 0.2kg oil/kg algae = 28,293kg oil/da x 365 da/yr = 10,326,899 kg oil/yr / 3.369 kg oil/gal = 3,065,271 gal oil/yr; i.e.,

3.065M gal oil/yr (assuming 20% algae oil content) or x 0.5/0.2 (kg/kg) = 7.7 M gal oil/yr (assuming 50% oil content)

[7] The SF Bay has an estimated area of between 250,000 and 1,000,000 acres, depending on how many small bays are included in the calculation, which means 1,520 acres would occupy between 0.6% and 0.15% of the area.

[8] It is estimated that 40% of the global population lives within 100km of the coast and that this percentage will increase in the future (*sedac.ciesin.columbia.edu/es/papers/Coastal_Zone_Pop_Method.pdf*).

economically support the integrated whole. By treating wastewater, sequestering CO_2, and providing a marine habitat, the system is environmentally friendly.



1.3 Conclusion

It appears that the only rational answer to the question of how to make the massive amounts of biofuels needed to displace significant amounts of fossil fuels without competing with agriculture will be to: 1) use microalgae as the feedstock, 2) grow the microalgae on wastewater, and 3) locate the cultivation systems offshore in the vicinity of existing wastewater outfalls. Even this will only begin to address global needs for liquid fuels. Predictions for algal biodiesel production are between 2,000 and 5,000 gal/acre/yr, while the average oil well in the USA yields 144,000 gal/well/yr. This means between 30 and 70 acres of algae will be needed to equal a single average oil well and the world's current usage of oil will require nearly 10 million of these oil wells; an area roughly the size of France and Spain, assuming an optimistic 5,000 gal/acre/yr.

The feasibility of such an offshore system globally distribute will depend on overcoming local challenges inherent in algae cultivation, engineering offshore systems, and mitigating environmental and political problems. Though these challenges are daunting, there are many incentives to proceed, not the least of which are the accelerating rates of social and environmental change, precipitated by population growth, resource limitations and allocations, and the escalating use of fossil fuels. Is this possible?

If people were sufficiently motivated progress could be made with economic drivers based primarily on wastewater treatment and aquaculture. In the long-term, there will be other drivers due to sea-level rise that will flood coastal lowlands, providing additional OMEGA sites that can be integrated into restructured coastal cities.

While OMEGA is in part meant for biofuels, the algae biomass can also be used for other products (such as fertilizer, animal food, cosmetics, or biochar), and the system provides services (such as wastewater treatment, carbon sequestration, alternative energy and aquaculture support). The point of OMEGA is that it is a system of systems, an "ecology of technology," in which the "waste" from one part of the system becomes a resource for another part. For example, domestic wastewater becomes a source of nutrients for algae and algae treat the wastewater. Algae produce oxygen as a waste product and the oxygen is made into ozone for the wastewater plant. The plant produces CO_2 by burning CH_4 and the CO_2 feeds the algae. The plant dumps phosphate and the algae capture phosphate to be used for fertilizer on land. The fertilizer is used to produce food that is eaten by people that produce waste that goes back to the wastewater treatment plant. Beyond this convoluted cycle, the OMEGA system itself requires a large floating infrastructure to support the algae cultivation system and the infrastructure itself can become a platform for offshore alternative energy (solar, wind, wave) and aquaculture. The aquaculture produces wastewater and requires oxygen.

With population growth, resource limitations, and the inevitable sea level rise, systems like OMEGA will be essential and widespread in the future. The current goal is to get started on its development.

"The greater danger for most of us lies not in setting our aim too high and falling short; but in setting our aim too low, and achieving our mark." Michelangelo

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

2.1 Summary

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 CO_2-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 percent CO_2 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 percent of the culture volume was continuously diverted through the GEHC to manage dissolved oxygen concentrations, provide supplemental CO_2, harvest microalgae from a settling chamber, and add fresh wastewater to replenish nutrients. The I&C system controlled CO_2 injection and recorded dissolved oxygen levels, totalized CO_2 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 CO_2 was converted to biomass with >50 percent efficiency, and >90 percent 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.

2.2 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, single-cell organisms can be cultivated on nonarable 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 (CO_2) for optimal growth, which can generate more environmental pollution and greenhouse 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 CO_2), 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 (NH_3-N) from wastewater, and sustained areal productivities at levels similar to those reported for other cultivation systems. Furthermore, the prototype utilized supplemental CO_2 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].

2.3 Materials and Methods

2.3.1 Seawater Tank and Microalgae Cultures

Experiments were conducted in an 8,800-liter seawater tank at the California Department of Fish and Wildlife, 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 CO_2 to lower the culture pH and provide a source of carbon.

2.3.2 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).

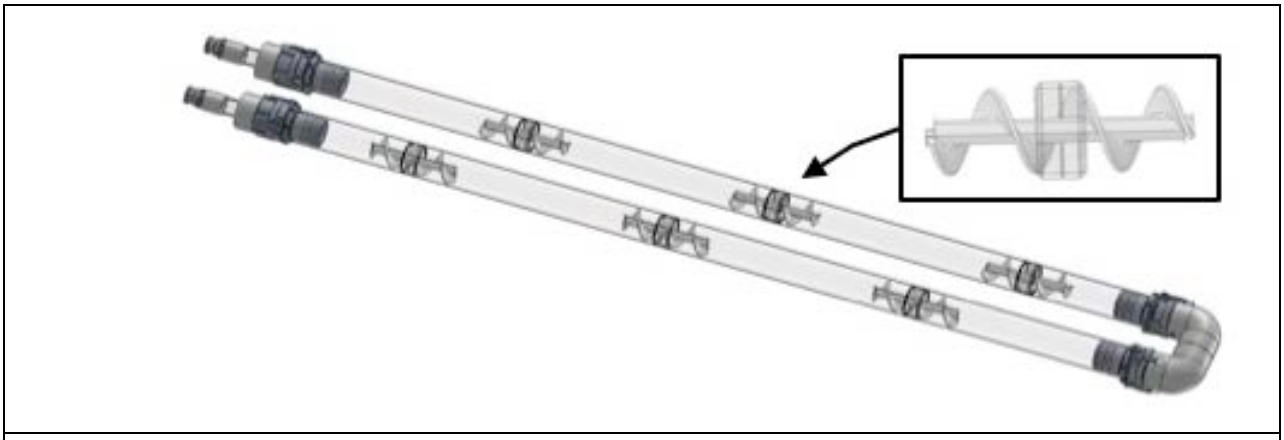


Figure 2: OMEGA Photobioreactor (PBR) Tubes with Swirl Vanes

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.

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, 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).

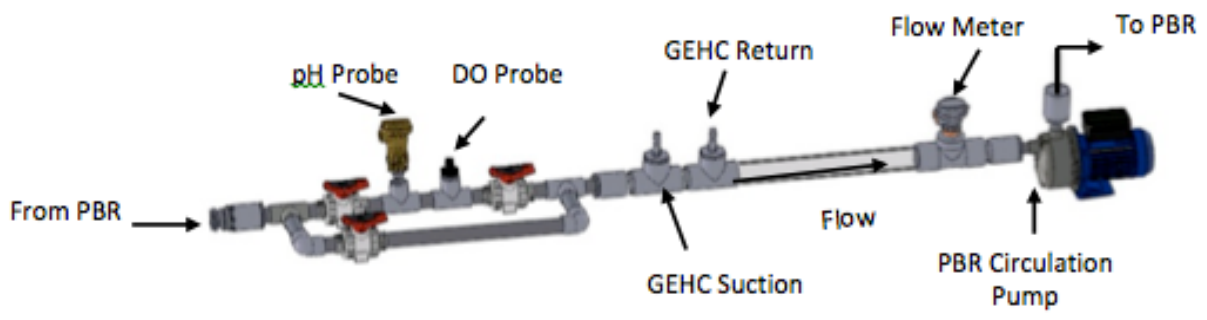


Figure 3: Inline Sensors for pH, Temperature, DO, and Flow Rate

The culture was pumped from the PBR past the sensors. Part of the circulating flow was diverted to the GEHC (see Figure 3) at the GEHC suction fitting by a positive displacement pump (not shown) and returned to the PBR flow at the GEHC return. The valved bypass was used to isolate the sensors for cleaning and maintenance without disrupting the overall circulation.

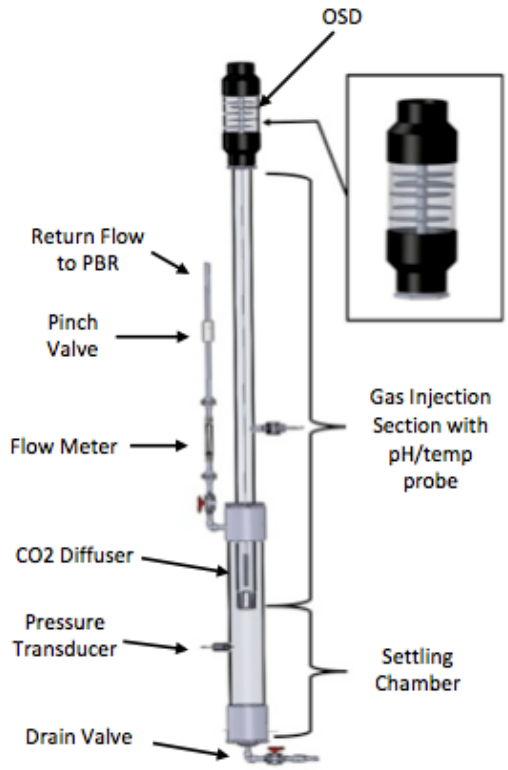
2.3.3 Gas Exchange and Harvesting Column (GEHC)

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 CO_2 to the microalgae culture and control pH, and (3) provide a settling chamber to collect aggregating microalgae for harvesting. Approximately 5 percent 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 cm^2 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 CO_2 diffuser made from soaker hose (22 cm^2) located 1.8 m from the top of the column. The compressed CO_2 source was a mixture of 8.5 percent CO_2 in air (V/V) to simulate the concentration of CO_2 in typical flue gas [20]. The CO_2 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.

Figure 4: Gas Exchange and Harvesting Column (GEHC) Controls pH, removes settled microalgae and provides a location for wastewater addition into the PBR system. An oxygen stripping device (OSD, top) designed to remove excessive concentrations of photosynthetically generated dissolved oxygen was built into the GEHC. CO_2 is added by gas bubbles injected with the diffuser at a rate controlled by pH. Biomass collected in the settling chamber is removed, whereas suspended microalgae are returned to the PBR (return flow pipe, left). The pressure transducer controls a pinch valve position to maintain a consistent liquid level in the GEHC. The volume of the GEHC was periodically harvested from the drain valve at the bottom and replaced with wastewater to replenish nutrients in the PBRs.



2.3.4 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 CO_2 injection rates through an Aalborg mass-flow controller (MFC) (Aalborg, Orangeberg, 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 actual value and the desired setpoint. DO was measured using a Sensorex DO probe (Sensorex, Garden Grove, CA) and data were recorded using a Craig Ocean Systems (Ben Lomond, CA) data logger. The physiological condition of the microalgae was monitored continuously using a fast repetition rate fluorometer (FRRF) set up for flow-through operation.

2.3.5 CO_2 mass transfer

The CO_2 mass transfer efficiency for the GEHC was calculated based on the column height and the gas flow rate required to sustain a target microalgae productivity of 20 g m^{-2} day^{-1}, in line with the average productivity cited by Putt *et al.* [21]. Several authors have noted that microalgae biomass is approximately 50 percent carbon [22-24], a value corroborated by elemental analysis of the algae grown in the OMEGA system (data not shown). These values, together with a 2× overdesign factor, were used in Equation (1) to estimate a peak gas injection rate of 0.5 lpm into the GEHC.

$$Q_{Gas} = \frac{P_{Algae} \cdot f_{Carbon} \cdot A_{PBR} \cdot RT}{D_{Solar} \cdot 60 \cdot M_{Car} \cdot pCO_2} \quad (1)$$

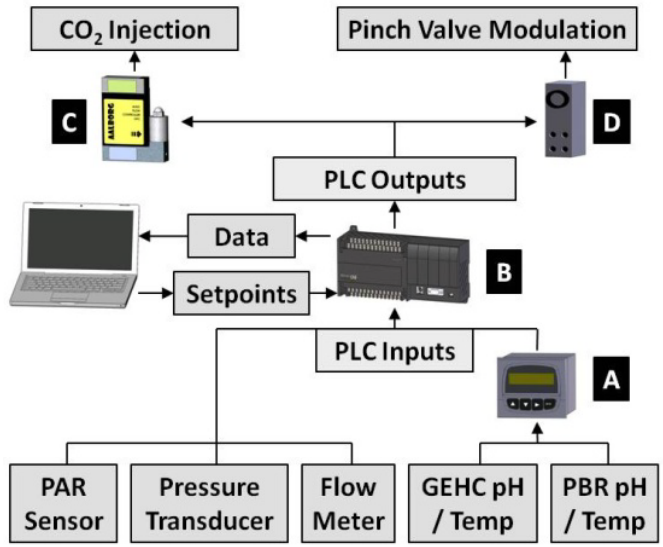


Figure 5: Components of the I&C System

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 CO_2 injection (C) and an I/P transducer (D) to regulate pinch valve positioning.

The CO_2 mass transfer efficiency was quantified for six different GEHC water column levels (0.3 m, 0.6 m, 0.9 m, 1.8 m, 2.1 m or 2.7 m) using a transparent PVC test column (3 m × 7.6 cm). A diffuser (described above), used to inject CO_2 (8.5 percent in air, V/V) into solution, was lowered to the bottom of the test column. The 0.5-lpm gas injection rate (from Equation 1) was controlled using a precision rotometer (Model WU-03218-52, Cole Palmer, Vernon Hills, IL) calibrated with an Agilent ADM1000 Flowmeter (Agilent Technologies Inc., Wilmington, DE). Tap water contained in a plastic barrel was weighed using an Ohaus Defender scale (Ohaus Corporation, Pine Brook, NJ) and the pH was adjusted to >11.00 with a known mass of NaOH. The mass of water corresponding to the desired liquid height was removed from the barrel and added to the test column. The mass of CO_2 dissolved into solution was determined by measuring the pH change in the water column using the stoichiometry of the acid-base reaction relationship between the NaOH and $H_2CO_3^*$ described in Equations (2) and (3).

$$H_2O + CO_2 \leftrightarrow H_2CO_3^* \quad (2)$$

$$H_2CO_3^* + 2NaOH \leftrightarrow Na_2CO_3 \quad (3)$$

The CO_2 uptake efficiency is the amount of CO_2 absorbed in the GEHC column divided by the amount supplied. The amount of CO_2 absorbed was determined indirectly by measuring pH changes in the water column. The total moles of CO_2 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 CO_2 required to change the pH of the solution from 10 to 9, 9 to 8, 8 to 7 and below 7.

$$M_{CO_2} = \frac{Q \cdot t \cdot pCO_2}{RT} \quad (4)$$

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

A comparison of the CO_2 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_La) and subsequent CO_2 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).

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

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

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

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

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

2.3.6 System Inoculation, Sampling Protocol, and Harvesting Procedures

Final plant effluent (FPE) was collected from the Santa Cruz wastewater treatment facility[2] 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 (OD_{750}), NH_3-N (Hach method 10031), NO_3-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 OD_{750} before and after physically shaking the PBR to resuspend settled biomass were used to determine the percent sedimentation within the PBR using Equation (10).

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

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).

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

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

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

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 CO_2-to-biomass conversion efficiency with Equation (14).

$$CO_{2_{Conv}} = \frac{A_{Growth} \cdot f_{Car}}{\left(\frac{V_{Gas} \cdot pCO_2}{RT} \cdot \frac{12gC}{mol\,CO_2}\right)} \quad (14)$$

2.4 Results and Discussion

2.4.1 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 CO_2, 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 CO_2 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, 29]. 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 [30]. Reported F_V/F_M ratios in cultures exposed to high irradiance indicated up to 90 percent photoinhibition [27, 28, 30].

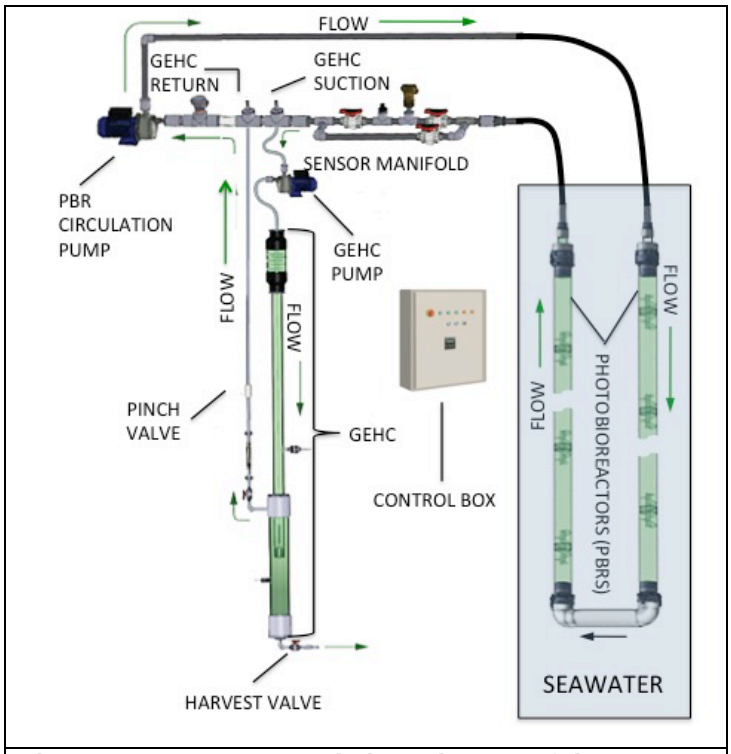


Figure 6: Component and Flow Diagram of the OMEGA System

Showing the circulation through the PBRs, sensor manifold, and side loop for the GEHC.

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 [26]. 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 percent of the total biomass. The swirl vanes also increased turbulence, which is known to improve nutrient exchange rates and light exposure in PBR cultures [27]. In cultures grown in laminar flow systems photoinhibition and light limitations are observed, both of which suppress productivity [26-28]. 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. Fv/Fm 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 percent 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.

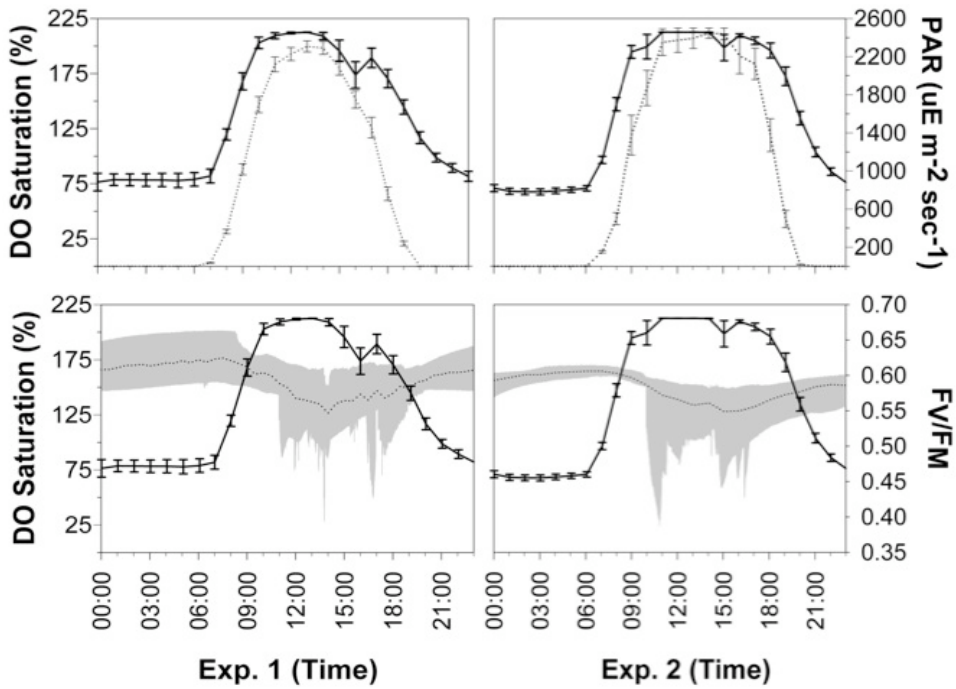


Figure 7: DO Concentration

PAR and FV/FM values for experiment 1 (left) and experiment 2 (right). (Top) Mean hourly (± 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 3.05 m, a 4.5 percent 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 CO_2 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 CO_2 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 CO_2 supply is one of the biggest contributors to overall energy use and cost of microalgal biofuel production.

Traditionally CO_2 delivery systems, using sparging tubes bubbling into shallow cultures, resulted in 80-90 percent losses of CO_2 to the atmosphere [21, 38]. Diffusion methods, using silicon membranes or hollow fibers reduce CO_2 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 CO_2 losses to less than 20 percent [21, 38].

2.5 GEHC Mass Transfer Efficiency and Recycle Rate

The CO_2 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 CO_2 content of the gas bubbles. Experiments with the GEHC indicated that higher pH and a taller column increased CO_2 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 percent 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 CO_2 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 percent 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.

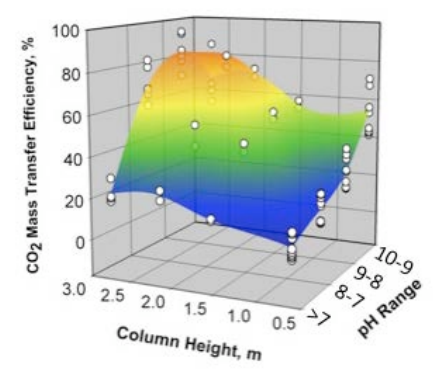


Figure 8: Efficiency of CO_2 mass transfer in the GEHC

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.

2.6 GEHC Operation

Diverting only a portion of the culture for CO_2 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 CO_2 in the GEHC was not exceeded by the rate of carbon removal in the PBR.

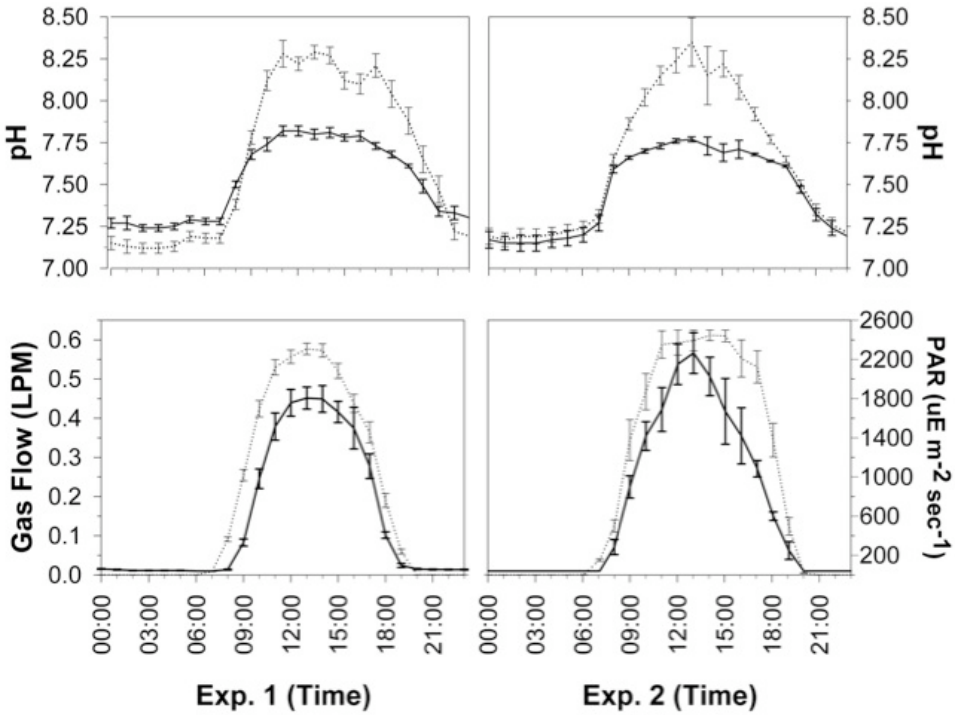


Figure 9: The Mean Hourly (± SE) pH, Gas Flow, and PAR Recorded During Experiment 1 (left) and Experiment 2 (right)

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.

Thus, the control system could monitor and deliver the amounts of CO_2 demanded by the microalgae. Furthermore, this system reduced CO_2 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 1and 2 are given in Table 1. Harvesting occurred every 0.83 to 2.79 days, triggered by the depletion of NH_3-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.

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.

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}
Experiment 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)**

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 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.

2.7 Carbon Utilization and Biomass Production

The totalized volume of simulated flue gas (8.5 percent CO_2/91.5 percent 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 CO_2. 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 CO_2 demand. The biomass produced relative to the amount of CO_2 injected was used to calculate the CO_2 utilization efficiency (Table 1): For experiment 1 the mean efficiency was 53.8 percent ± 4.0 percent (n=9) and for experiment 2 it was 60.2 percent ± 4.7 percent (n=7), with values from both experiments ranging from 31.6 percent to 80.9 percent. These measured CO_2 conversion efficiencies correspond well to the CO_2 solubility values obtained in the titration experiment (see section 3.3).

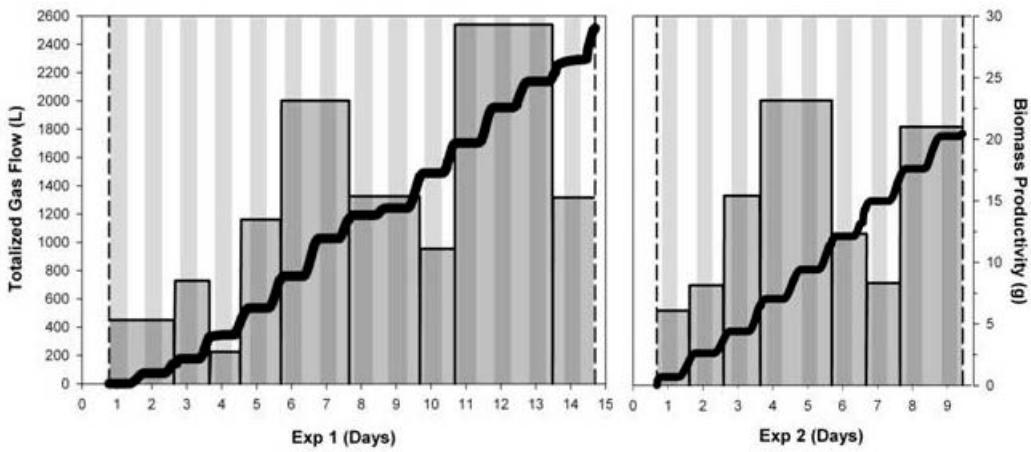


Figure10: Microalgal CO_2 utilization and productivity in experiment 1 (left) and experiment 2 (right) with the day/night cycle indicated by vertical stripes.

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).

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 CO_2 concentrations. The site restricted column height, available equipment determined the bubble size, and the CO_2 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.

2.8 OMEGA and Wastewater Treatment

The OMEGA system used secondary wastewater effluent as a source of nutrients for microalgae cultures and the concentrations of ammonia [NH_3] and nitrate [NO_3^-] were monitored (Figure 10). The rapid utilization of NH_3 required periodic replacement of spent culture medium with fresh wastewater. Between 16 percent and 34 percent of the total system volume was harvested from the GEHC and replenished to increase the concentration of [NH_3] (Figure 10; top). While

[NH_3] followed a consistent pattern of utilization and replenishment, the corresponding [NO_3^-] showed increases, decreases, or no change (Figure 10, middle). The increases in [NO_3^-] were attributed to nitrification by ammonia-oxidizing bacteria, which are known to be present in wastewater [48]. The decreases in [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].

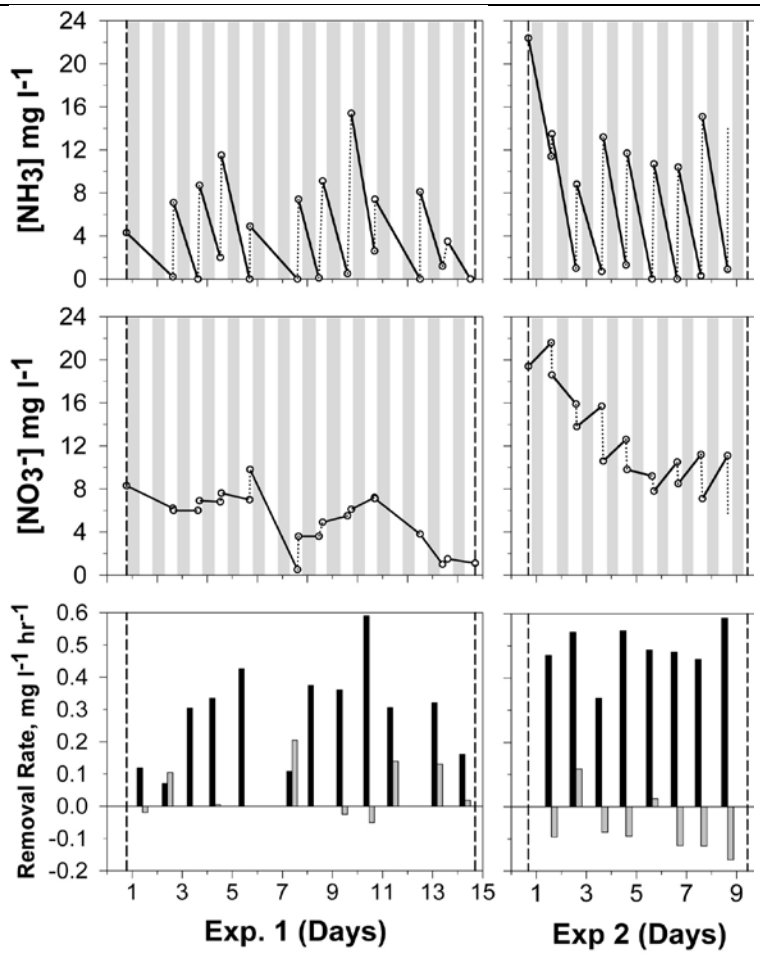


Figure 11: Time Course for the Addition and Utilization of [NH_3-N] (top), [NO_3^-N] (middle), and Removal Rates (bottom) for Experiment 1 (left) and Experiment 2 (right).

As in Fig. 10 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 NO_3^-N removal rates (grey bar) were occasionally negative due to nitrification. The microalgae preferred NH_3-N as their nitrogen source and consume NO_3^-N once the supply of NH_3-N was exhausted.

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) mg 1^{-1} 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].

2.9 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 NH_3-N in wastewater and had a CO_2 conversion efficiency of >50 percent; better than the 10-20 percent conversions in other systems [21, 38]. The areal productivity of the system averaged 14.1 g m^{-2} day^{-1} overall with peaks above 20 g m^{-2} day^{-1} values consistent with reported U.S. average microalgae productivity of 13.2 g m^{-2} day^{-1} [58]. The microalgae consistently removed >90 percent of the NH_3-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 the energy return on investment (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} =	Microalgae 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-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_{CO2} =	Moles of CO_2
t =	Time, minutes
$CO_{2\,Eff}$ =	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} =	g ,Total biomass produced
TSS_{GEHC} =	Total suspended solids content of culture harvested from GEHC, mg l^{-1}
H_{Mass} =	Mass of culture harvested from GEHC, kg
TSS_{PBR} =	Total suspended solids content of the culture in the PBR, mg l^{-1}
PBR_{Mass} =	Mass of culture in the PBR, kg
I_{Mass} =	Initial mass of solids in the system, g
HCF =	Harvesting concentration factor, unitless
$D_{Harvest}$ =	Harvesting frequency, days
$CO_{2\,Conv}$ =	CO_2 to biomass conversion efficiency, %
V_{Gas} =	Volume of gas injected into the GEHC between harvest periods

Chapter 3:
Techno-Economic Assessment of the Prototype OMEGA System for Large-Scale Algae Cultivation and Wastewater Treatment

3.1 Summary

In the last chapter, the components and operation of a functional experimental OMEGA system are described in which microalgae were grown in wastewater in a 110-liters two-photobioreactors (PBRs) system in Santa Cruz. This experimental system is used to model a much larger, full-scale OMEGA system that would substitute for biological nutrient removal (BNR) in a wastewater treatment plant. Here, we model wastewater treatment with BNR (WWT-BNR) and with OMEGA (WWT-OMEGA), assuming the systems would both process 37.9 million liters of wastewater per day. Both systems would also produce electricity and heat from biogas and fertilizer from biosolids, but in addition, the OMEGA system would produce aviation fuel from algae. The hypothetical OMEGA system would contain a volume of 190-million-liters of wastewater and consist of 204,320 floating PBRs that cover an area of 420 ha. The techno-economic analysis was used to estimate energy return on investment (EROI), revenue required (RR), and greenhouse gas (GHG) emissions. For the conventional WWTP-BNR the EROI was 0.32 with 6 percent of the input energy for primary treatment, 14 percent for sludge treatment, and 80 percent for secondary treatment mostly for aeration and BNR. For the WWT-OMEGA system the EROI was 0.28 with 60 percent of the input energy for pumping and 19 percent for harvesting. The revenue required (RR) for the WWTP-BNR was $9.7 million/yr primarily for membranes (34 percent), BNR (28 percent), and aeration/nitrification (20 percent). The RR for the OMEGA system was $100 million/year primarily for PVC components (38 percent), pumps (31 percent), and offshore infrastructure (21 percent). The GHG emissions for the WWTP-BNR system was 635.76 kg CO_2 equivalent/million-liters wastewater and for the OMEGA system it was 1,629 kg CO_2 equivalent/million-liters of wastewater. While this analysis clearly indicated it was impractical to directly scale-up the experimental OMEGA system described in Chapter 2, it also indicated that with simple design changes in the gas exchange and harvesting column (GEHC), harvesting system, and configuration of the PBRs, a more practical WWT-OMEGA system could be built. The design changes include: changing the flow configuration to eliminate PVC manifolds and swirl vanes, increasing the pipe diameter for flow from the GEHC to the harvester, , and recalculation of the CO_2 concentration in flue gas (using stoichiometry results in 14 percent (vol/vol) CO2 in flue gas from combusted biogas instead of 5 percent), the WWT-OMEGA EROI increases to 1.02, the RR decreases to $16M/y, and the GHGs decreases to -19.14 kg CO_2e/ML. Other plausible improvements in the OMEGA system could include algae strain selection and the expanded use of the offshore platform to include alternative energy production (solar, wind, or wave generation) and aquaculture. It is

suggested that this integrated system further supports the feasibility of developing sustainable and profitable OMEGA systems.

3.2 Introduction

With the current interest in using wastewater for growing microalgae to produce biofuels and other products, wastewater treatment plants are being transformed from waste generators to resource depots (Olguin 2012). It has been suggested that the production of microalgae using the nutrients in wastewater and CO_2 from boilers and digesters not only creates algae products without competing with agriculture for water or fertilizer, it also improves the treatment of wastewater, reduces greenhouse gas (GHG) emissions, and provides revenue for the wastewater treatment plants (Cristenson 2011) (Pittman 2011) (Craggs 2012). There are established methods for using microalgae in place of the more traditional biological nutrient removal (BNR) (Hu, 2012) and, in addition to providing advanced wastewater treatment, these algae-based systems can produce biofuels (Li 2011) (Woertz 2009). Thus combining algae cultivation with wastewater treatment seems to have the potential to address the ever more stringent regulatory requirements for wastewater plants, while reducing their overall costs by providing both environmental benefits and useful products.

While several studies have demonstrated the mutual benefits of coupling algal wastewater treatment with algal biofuel production, few have considered the limitations of actually integrating the two systems logistically and economically (Fortier 2012) (Lundquist 2010) (Beal 2012b). For many metropolitan wastewater treatment plants, it would be logistically impractical and prohibitively expensive to install land-based algae cultivation systems, such as high-rate ponds (raceways) or photobioreactors (PBRs), in close proximity to existing plants (Fortier 2012) (Richardson 2012). In addition to the capital and operating costs, such installations would significantly impact or displace urban infrastructure surrounding most wastewater plants (Trent 2012). Nor would it be economically feasible for cities to pump wastewater to undeveloped areas beyond city limits; in addition to the cost of pipelines, if pumping distances exceed a few tens of kilometers, the energy required for pumping exceeds the energy contained in the algae, which negates the energy benefit from growing algae (Wigmosta 2011) (Pate 2011) (Sturm 2011). There have been a number of life cycle analyses (LCAs) evaluating microalgae cultivation in open ponds and land-based PBRs, which confirm that indeed it will be difficult to justify using algae for producing biofuels (Clarens 2010) (Richardson 2012).

It was suggested in Chapter 1 that OMEGA may be an alternative algae production system for coastal cities with established offshore outfalls, which would neither disrupt urban infrastructure nor require long-distance wastewater pumping, and would provide both algal wastewater treatment and algae products (Trent 2012). For wastewater treatment, the proposed OMEGA system may have several potential benefits: 1) it could replace the costly biological nutrient removal (BNR) processes and utilize the nitrogen, phosphorus, and dissolved carbon in wastewater to make algae products; 2) it could improve the revenues for the treatment plant by producing biofuels and other valued algae products, and 3) the algae use CO_2 which could

reduce the emissions of this GHG by the plant from boilers or anaerobic digesters—the CO_2 is fed to the algae.

Here we created models of a conventional wastewater treatment plant with BNR or with a full-scale OMEGA system substituting for conventional activated sludge (CAS) and BNR. We used the models to calculate the energy return on investment (EROI), revenue required (RR) for projected capital and operating costs, and GHG emissions from these two hypothetical systems. The hypothetical OMEGA system modeled was based on a scaled up version of the 110-liter described in Chapter 2. The 110-liter system was explicitly designed to study floating PBRs and algae growth on wastewater, to develop and test control systems, and to investigate biofouling and cleaning protocols and it was not designed for efficient energy use, or for minimizing capital, operating costs, and GHG emissions. Despite these acknowledged shortcomings the models provided a perspective on major energy and economic sinks and GHG sources in wastewater treatment using conventional secondary treatment (WWT-BNR) and a hypothetical OMEGA system (WWT-OMEGA). The immediate goal was to identify sources and sinks for energy, revenue, and GHG and for WWT-OMEGA to explore the relative importance of design features, hydraulics, and biological considerations (e.g., algae productivity and lipid production). The ultimate goal was to further understanding of the feasibility of developing a functional, cost-effective, and environmentally acceptable OMEGA system for treating wastewater and producing biofuels.

3.3 Methods

3.3.1 Techno-Economic Analysis

The models, developed in Excel spreadsheets, compared conventional wastewater treatment starting with raw wastewater and ending with treated liquid effluent, bio-solids as fertilizers, and in addition, for the OMEGA system only, aviation biofuels. The model systems included the energy inputs and outputs, the capital and operating costs, and the GHG impacts. The credits for net energy and GHGs for the outputs (hydrotreated renewable jet fuel (HRJ), naphtha, propane, and nitrogen fertilizer) were calculated by the displacement method (Wang et al.,2011).

The boundaries for the models included both on-site and "upstream" considerations. Upstream refers to energy, costs, and impacts of obtaining raw materials (e.g., at a mine, well, or reservoir) and to the conversion of those materials to useful products (e.g., electricity generation, nitrogen fertilizer production, chemical processing, etc.). Data use in upstream energy input for calculating EROI and for GHG emissions were obtained from the GREET and Ecoinvent databases (Wang, 2011; Simapro, 2007).

The model does not account for the net energy or GHGs generated in the production of materials used in the construction of the wastewater treatment plant or the OMEGA system, such as plastics (PVC and LLDPE) infrastructure for docks or machinery used in harvesting, lysing, separations, and hydrotreatment.

The outputs of the model are energy return on investment (EROI), the revenue required (RR) to construct and operate the facility, and the net CO_2-equivalent GHG emissions impact (GHGs).

3.3.1.1 Energy Return on Investment

The EROI is the ratio of the energy outputs to the energy inputs for any energy production system. For the WWTP, the EROI was defined as,

1 $$EROI_{WWTP-BNR} = \frac{\acute{E}O + \acute{E}I_F}{\acute{\tilde{E}}_{PT} + \acute{\tilde{E}}_{ST} + \acute{\tilde{E}}_{SP}} \quad \left[\frac{kJ/L_{PW}}{kJ/L_{PW}}\right]$$

where

$\acute{E}O$ = energy offset credit that is applied to heat and electricity produced onsite

$\acute{E}I_F$ = indirect energy output from fertilizer

$\acute{\tilde{E}}_{PT}$ = energy input for primary treatment (pumping, the grit chamber, and primary clarification)

$\acute{\tilde{E}}_{ST}$ = energy for secondary treatment (aeration, nitrification, BNR, secondary clarification, mixing/filtration, and chlorination/dechlorination)

$\acute{\tilde{E}}_{SP}$ = energy for sludge processing (secondary sludge thickening, anaerobic digestion, solids handling, and cogeneration).

The energy offsets refers to the portion of resources generated onsite, such as heat and electricity, which avoids obtaining these resources offsite and the upstream impact. Excess heat is assigned an energy equivalent of zero.

For the OMEGA extrapolated scenario, the $EROI$ is defined as,

2 $$EROI_{OMEGA\ Extrapolated} = \frac{(\acute{E}I_F + \acute{E}O) + (\acute{E}D_{HRJ} + \acute{E}D_{NA} + \acute{E}D_{PR})}{\left(\acute{\tilde{E}}_{PT} + \acute{\tilde{E}}_{SP}\right) + \left(\acute{\tilde{E}}_G + \acute{\tilde{E}}_H + \acute{\tilde{E}}_P + \acute{\tilde{E}}_R\right)} \quad \left[\frac{kJ/L_{PW}}{kJ/L_{PW}}\right]$$

where

$\acute{E}I_F$ = indirect energy output from fertilizer

$\acute{E}O$ = energy offset credit that is applied to heat and electricity produced onsite

$\acute{E}D_{HRJ}$ = energy output HRJ

$\acute{E}D_{NA}$ = energy output naphtha

$\acute{E}D_{PR}$ = energy output propane

$\acute{\tilde{E}}_{PT}$ = energy for primary treatment (pumping, the grit chamber, and primary clarification)

$\acute{\tilde{E}}_{SP}$ = energy for sludge processing (secondary sludge thickening, anaerobic digestion, solids handling, and cogeneration)

$\acute{\tilde{E}}_G$ = energy for growth (including commercial CO_2, CO_2 distribution with compressors, and circulation of wastewater through PBRs and GEHCs with pumps)

$\acute{\tilde{E}}_H$ = energy for harvesting (including discharge water recycling/treatment)

$\acute{\tilde{E}}_P$ = energy for processing (including cell lysing, and lipid separation)

$\hat{E}_R$ = energy for refining (hydrotreatment of biocrude to produce HRJ)

3.3.2 Revenue Required

The capital costs estimates for the conventional WWT focused on the secondary treatment (conventional activated sludge, nitrification, and BNR), which is the part of the system for which the OMEGA system substitutes. The capital costs were obtained from the software CapdetWorks and include: plug flow activated sludge with nitrification, BNR removal (modified 5-stage Bardenpho reactor), clarifiers, membrane filtration and an alum feed system. The capital cost for the BNR membranes was based on an American Membrane Technology Association technical fact sheet at $1.13 per treated gallon of wastewater (American Membrane Technology Association). Not included in the capital cost estimates were the components for primary treatment (raw wastewater pump stations, aerated grit chamber, and primary sedimentation unit), for chlorination and dechlorination for solids handling (anaerobic digester and belt filter press), and for the combined heat and power system.
Capital costs for the OMEGA system (PBRs, GEHC, pumps, and floating docks) were estimated based on vendor quotes. The transport of flue gas and the costs of harvesting, lysing, separations, and hydrotreatment were based on previously published values (Campbell et al., 2011; Beal et al. 2012a).

The operating costs (materials and energy) for both the WWTP-BNR and OMEGA extrapolated were based on vendor quotes, published values, or standard engineering estimates (Davis et al., 2011).

The capital and operating costs as well as the revenues generated by the two model systems went into the calculation of the revenue required (RR). The RR represents the total revenue required to break-even, which means the revenue required in addition to the revenue generated. Some costs were not considered in these calculations, such as maintenance costs, taxes, labor, and regulatory fees, as well as additional revenue sources, such as subsidies and tax credits. The RR was calculated as,

3 $$\boldsymbol{RR} = \frac{\boldsymbol{TLCC}\ [\$/y]}{\mathrm{Q_{WW}}\ [L/y]}$$

where

$TLCC$ = annual total life cycle costs (including capital and operating costs)

$\mathrm{Q_{WW}}$ = annual output of the system, which, in this case, is liters of wastewater processed per year.

$TLCC$ was calculated as shown in the following equation:

4 $$\boldsymbol{TLCC} = \boldsymbol{C_{acap}} + \boldsymbol{C_{aop}} - \boldsymbol{R_a} \quad [\$/L]$$

where

C_{acap} = annualized capital cost

C_{aop} = annual operating cost

R_a = annual revenue

The total annual capital cost is amortized to obtain the annualized capital cost for any equipment according to,

5 $$C_{acap} = C_{cap} \cdot CRF(i, \mathrm{n}) \ \ [\$/y]$$

where

C_{cap} = total capital cost

CRF = capital recovery factor

The CRF is defined as,

6 $$CRF = \frac{i*(i+1)^n}{(i+1)^n - 1}$$

where

i = interest rate

n = equipment lifespan

Operating costs are calculated as,

7 $$\acute{C}_i = \acute{x}_i \cdot X_i \ \ \left[\frac{\$}{L}\right]$$

where

$\acute{x}_i$ = amount of each energy and material input

X = associated market price

The annual operating costs for the WWTP-BNR are calculated as,

8 $$C_{aop} = \left(\acute{\tilde{C}}_{PT} + \acute{\tilde{C}}_{ST} + \acute{\tilde{C}}_{SP}\right) \cdot \mathrm{Q_{WW}} \ \ [\$/y]$$

where

$\acute{\tilde{C}}_{PT}$ = cost of primary treatment per liter of WW

$\acute{\tilde{C}}_{ST}$ = cost of secondary treatment per liter of WW

$\acute{\tilde{C}}_{SP}$ = cost of sludge processing per liter of wastewater

$\mathrm{Q_{WW}}$ = annual wastewater flowrate

The annual operating costs are calculated as,

9 $$C_{aop} = \left(\left(\acute{\tilde{C}}_{PT} + \acute{\tilde{C}}_{SP}\right) + \left(\acute{\tilde{C}}_{G} + \acute{\tilde{C}}_{P} + \acute{\tilde{C}}_{R}\right)\right) \cdot Q_{WW} \ [\$/y]$$

where

$\acute{\tilde{C}}_{PT}$ = cost of primary treatment per liter of WW

$\acute{\tilde{C}}_{SP}$ = cost of sludge processing per liter of wastewater

$\acute{\tilde{C}}_{G}$ = cost of growth per liter of WW

$\acute{\tilde{C}}_{P}$ = cost of processing per liter of WW

$\acute{\tilde{C}}_{R}$ = cost of refining per liter of WW

Q_{WW} = annual wastewater flowrate

The revenue generated from the WWTP-BNR is for electricity generated via CHP and fertilizer solids. This electricity is consumed onsite and is assumed to have the same price as grid electricity, thereby canceling the cost of an equal quantity of electricity input. Thus, the annual revenue for the WWTP-BNR, R_a, is,

10 $$R_a = (\acute{P}_e \cdot X_e + \acute{P}_F \cdot X_F) \cdot Q_{WW} \ [\$/y]$$

where

$\acute{P}_e$ = amount of electricity produced per liter of WW

$\acute{P}_F$ = amount of fertilizer produced per liter of WW

X_e = market price of electricity

X_F = market price of fertilizer

The annualized revenue includes the production of HRJ, naphtha (NA), and propane (PR) from hydrotreating biocrude. Therefore, the annual revenue is calculated as,

11 $$R_a = \left(\left(\acute{P}_e \cdot X_e + \acute{P}_F \cdot X_F\right) + \left(\acute{P}_{HRJ} \cdot X_{HRJ} + \acute{P}_{NA} \cdot X_{NA} + \acute{P}_{PR} \cdot X_{PR}\right)\right) \cdot Q_{WW} \ [\$/y]$$

where

$\acute{P}_e$ = amount of electricity produced per liter of WW

$\acute{P}_F$ = amount of fertilizer produced per liter of WW

$\acute{P}_{HRJ}$ = amount of HRJ produced per liter of WW

$\acute{P}_{NA}$ = amount of naphtha produced per liter of WW

$\acute{P}_{PR}$ = amount of propane produced per liter of WW

X_e = market price of electricity

X_F = market price of fertilizer

X_{HRJ} = market price of HRJ

X_{NA} = market price of naphtha

X_{PR} = market price of propane

3.3.3 GHG Impacts

The GHG impact of the WWTP with BNR or with the OMEGA pathways was evaluated by calculating the GHG emissions (GHG_{emis}), GHG captures (GHG_{cap}), and GHG offsets (GHG_{off}) for each process. The primary metric used is the net GHG impact ($\acute{GHG}_{net}$) per liter of wastewater (Lww), which was calculated,

12 $$\acute{GHG}_{net} = \sum_{all\ proc}\left(\widehat{\acute{GHG}}_{emis} - \widehat{\acute{GHG}}_{cap} - \widehat{\acute{GHG}}_{off}\right) \left[\frac{g\ CO_2eq}{L_{WW}}\right]$$

where

$\widehat{\acute{GHG}}_{emis}$ = GHG emissions in units grams of CO_2 equivalent per liter of WW

$\widehat{\acute{GHG}}_{cap}$ = GHG captures in units grams of CO_2 equivalent per liter of WW

$\widehat{\acute{GHG}}_{off}$ = GHG offsets in units grams of CO_2 equivalent per liter of WW

There are two portions of the GHG emissions for each process: 1) on-site emissions of carbon dioxide, methane, and nitrous oxide, and 2) off-site ("upstream") emissions associated with the production of energy and the manufacturing of materials that are consumed on-site. Therefore, the $\widehat{\acute{GHG}}_{emis}$ was calculated as:

13 $$\widehat{\acute{GHG}}_{emis} = \widehat{\acute{GHG}}_{on-site} + \widehat{\acute{GHG}}_{off-site} \left[\frac{g\ CO2eq}{L_{WW}}\right]$$

$\widehat{\acute{GHG}}_{on-site}$ = GHG emissions generated on-site in grams of CO_2 equivalent per liter of WW

$\widehat{\acute{GHG}}_{off-site}$ = GHG emissions generated off-site in grams of CO_2 equivalent per liter of WW

The emissions contain contributions from carbon dioxide, methane, and nitrous oxide, and each of these emissions was normalized to grams of CO_2 equivalents using the global warming potentials (GWPs) for a 100 year time horizon relative to CO_2, which are assumed 25 for methane (CH_4) and 298 for nitrous oxide (N_2O),

14 $$\widehat{\acute{GHG}}_{emis} = \widehat{\acute{GHG}}_{emis_{CO_2}} + \widehat{\acute{GHG}}_{emis_{CH_4}} + \widehat{\acute{GHG}}_{emis_{N_2O}} \left[\frac{g\ CO_2eq}{L_{WW}}\right]$$

where

$\widehat{\acute{GHG}}_{emis_{CO_2}}$ = emissions contribution from CO_2 in units grams of CO_2 equivalent per liter of WW

$\widehat{\acute{GHG}}_{emis_{CH_4}}$ = emissions contribution from CH_4 in units grams of CO_2 equivalent per liter of WW

$\widehat{\acute{GHG}}_{emis_{N_2O}}$ = emissions contribution from N_2O in units grams of CO_2 equivalent per liter of WW

The on-site processes include biogenic greenhouse gas (GHG) emissions due to liquid and solid treatment, CO_2 losses from the GEHC and separations processes, and from fertilizer inputs from wastewater.

The off-site emissions include the production of grid electricity and chemicals that are used on-site and the associated emissions was calculated,

15 $$\widehat{GHG}_{off-site} = \sum_i \acute{x}_i \cdot EF_i \quad \left[\frac{g\ CO_2eq}{L_{WW}}\right]$$

where

$\acute{x}_i$ = amount of the chemical or energy consumed per liter of wastewater

EF_i = emission factor associated with the production of chemical or energy

Emission factors were obtained from the GREET database (Wang, 2011). For chemicals not available in the GREET database, the emissions factors was obtained from Simpapro software, Ecoivent v.2.0 database, GWP-100 year library (Simapro, 2007)

3.4 Results and Discussion

To compare the energy, cost, and GHG impacts of a conventional wastewater treatment plant with secondary treatment including BNR to a hypothetical treatment plant in which an OMEGA system is substituted for BNR, two model systems were developed.

Engineering Process Models

The WWT system was assumed to have an average flow of 37.9 million liters of raw influent per day and a typical wastewater composition: 140 mg of total organic carbon (TOC)/L, 75 mg HCO_3/L, 5 mg CO_3/L, 40 mg total N/L, 7 mg total P/L, and 430 mg chemical oxygen demand (COD)/L (Tchobanoglous 2003). The treatment process used conventional primary treatment with anaerobic digestion combined with either a secondary treatment pathway with BNR (WWT-BNR) or with a hypothetical OMEGA pathway (Figure 1). Both pathways lead to final plant effluent (FPE) that meets all state and local requirements for environmental release of treated wastewater and both pathways produce valued by-products. The two pathways start with primary treatment, which includes clarification to concentrate solids and a solids-handling step using anaerobic digestion to produces methane gas, which is used in "co-gen" to make electricity and heat. The electricity and heat are used to maintain temperature in digesters, among other things.

In the conventional WWT pathway, secondary treatment includes aeration, BNR, secondary clarification with the sludge produced contributing to anaerobic digestion (Fig. 1 top). The water in secondary treatment may be chemically flocculated, mixed, and filtered before being disinfected by chlorination/dechlorination before release into the environment as final plant effluent (FPE).

In the hypothetical OMEGA system, the primary effluent was disinfected

(chlorinate/dechlorinate) and circulated through PBRs for cultivating algae, which remove nutrients, toxins, and other contaminants (Fig. 1 bottom). The algae are systematically harvested to produce biomass and the nutrient-depleted water is discharged as FPE. The algae biomass is processed by cell lysis and separations. The cell debris is processed by anaerobic digestion and the cell extract is processed by hydrotreatment to produce jet fuel, naphtha, and propane (Fig. 1: OMEGA pathway). Other pathways from algae biomass to biofuels may be more cost effective are acknowledged (Frank 2012) but were not used in calculations reported here.

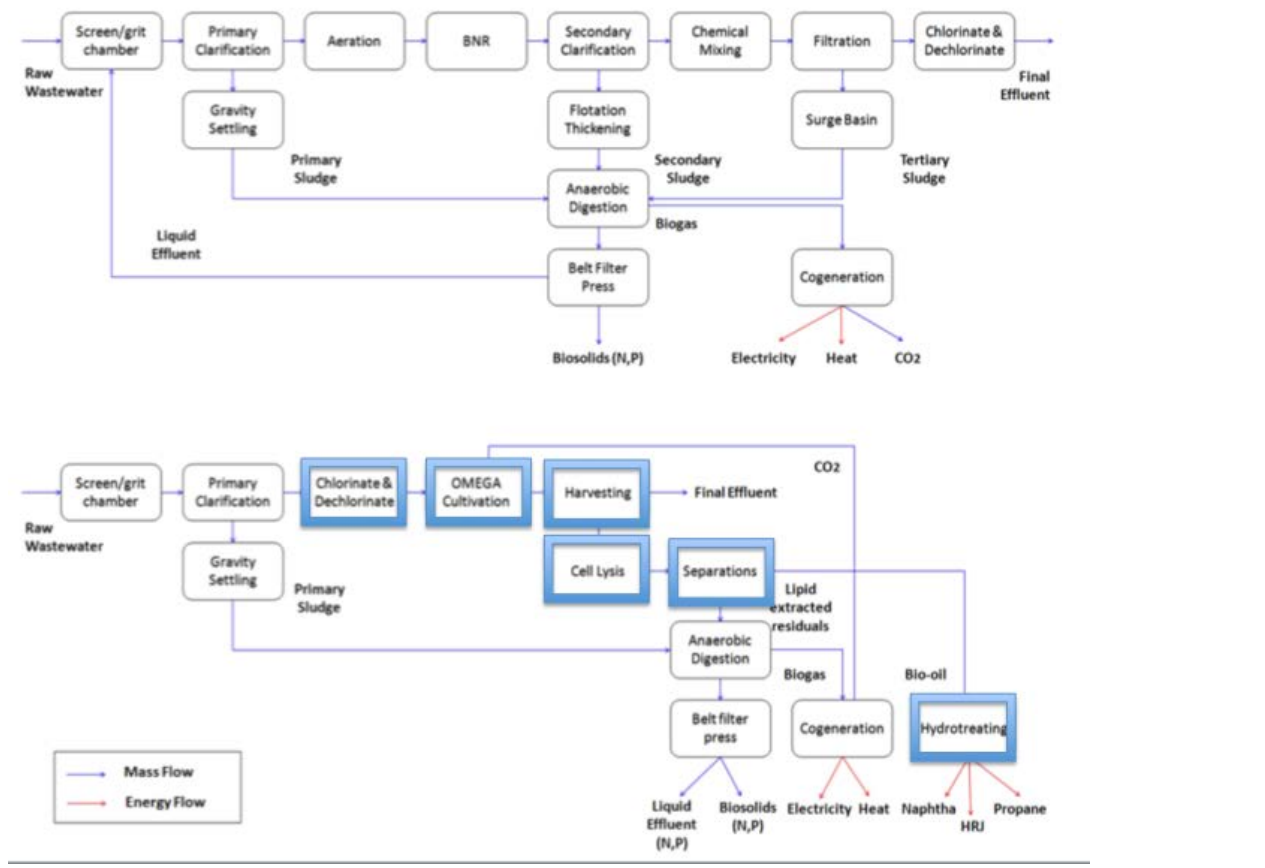


Figure 12: The Process Diagram for Wastewater Treatment

By primary treatment with anaerobic digestion and with secondary treatment by either a convention pathway with BNR to "final effluent" or with an OMEGA pathway that yields final effluent, jet fuel, naptha, and propane. Both pathways lead to fertilizer and biogas production from sludge, and the biogas is converted to electricity via co-generation. The blue lines indicate mass flow and the red lines energy flow. The bold boxes indicate the steps that are unique to the OMEGA pathway.

3.4.1 Wastewater Treatment with BNR Model

In the conventional wastewater processing pathway, raw influent is pumped through bar screens and a grit chamber into the primary clarifier to produce sludge and primary effluent. Sludge is processed by anaerobic digestion, which converts 50 percent of the carbon supplied to biogas (35 percent CO_2 and 65 percent methane by volume (Tchobanoglous 2003) and produces a residual liquid (centrate) that is recycled to the headworks of the plant. The anaerobic

digester generates solids that are used as nitrogen fertilizer. The liquid from the clarifier, the primary effluent, has a 40 percent reduction in chemical oxygen demand (COD), 40 percent reduction of the total organic carbon (TOC), 36 percent reduction of the carbon, 25 percent reduction of the phosphorus, and 34 percent reduction of the nitrogen, compared to the raw influent. The PE is further processed through secondary treatment along the BNR pathway to the final plant effluent. The sludge, recovered from secondary clarifiers, is also processed by anaerobic digestion.

The net energy required for wastewater treatment (input) and the energy returned from methane used to produce electricity or heat (cogen) or from fertilizer (output) is shown in Figure 2. The net energy includes the raw materials and energy for the production of electricity (U.S. mix) and chemical inputs (Wang, 2011).The input energy from primary treatment is primarily from pumping raw effluent (133.35 J/L), some energy is also used in primary settling (14.74 J/L), and for the grit chamber (12.75 J/L) and bar screens (0.19 J/L) (Goldstein and Smith, 2002). The energy input for sludge is primarily for thickening of secondary solids by floatation methods (192.32 J/L) and for heating the anaerobic digester (161.69 J/L) (Goldstein and Smith, 2002). There is also sludge energy input for pumping secondary solids to the digester (48.32 J/L), dewatering the sludge with a belt filter press (43.47 J/L) and gravity thickening (2.38 J/L) (Goldstein and Smith, 2002).

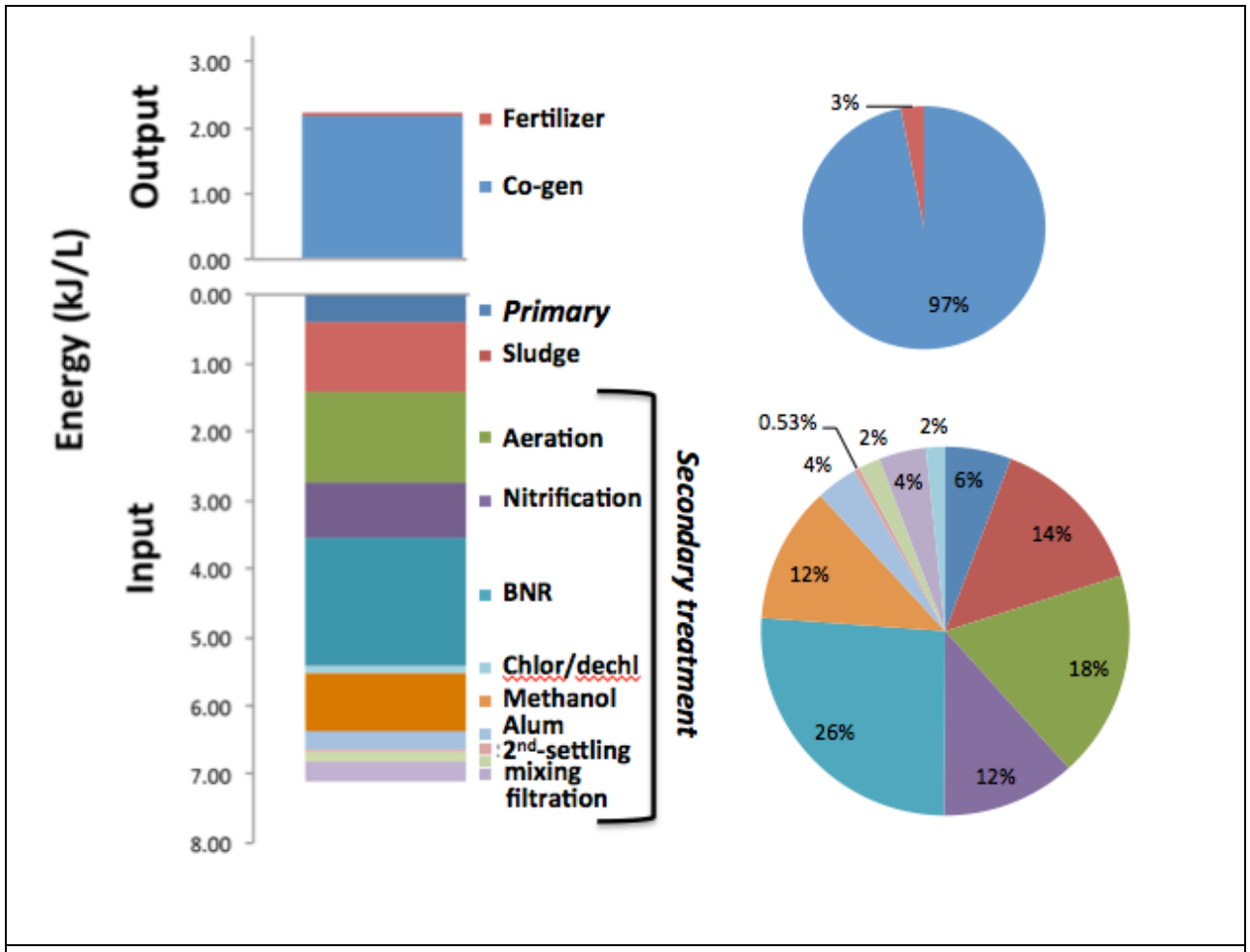


Figure 13: For Conventional Wastewater Treatment, the Energy Input (bottom-left) and Outputs (top left) in Kilojoules per Liter of Wastewater Treated and the Percentage Contribution of Inputs (bottom right) and Outputs (top right).

The energy input requirements for primary treatment, sludge processing, and the components of secondary treatment as well as the energy output from methane burned for electricity and heat generation (co-gen) and fertilizer production.

The secondary treatment includes aeration, nitrification, BNR, and material inputs of methanol, alum, chlorine The primary energy input is for pumping (gas, liquids, and solids), with some energy for mixing and filtration. Secondary treatment uses 80 percent of the total energy input in conventional WWT and the BNR-process by itself, accounting for 42 percent of the total energy input. The aeration process requires energy for air compression and pumping water (506 J/L) (Goldstein and Smith, 2002). Similarly, the nitrification process uses energy for compressing air and pumping water (327.76 J/L) (Goldstein and Smith). The BNR energy input includes power for pumping water and returning activated sludge and compressing air (723.80 J/L) (Sturm and Lamer, 2011). Methanol (65.16 mg/L) is added as a supplemental source of carbon for nitrogen removal (Fleischer et al., 2005). The upstream energy for methanol (854.26 J/L) is included for steam reformation of methane into synthesis gas and subsequent catalytic synthesis of methanol from synthesis gas (Wang, 2011). Alum (85.21 mg/L) was used as a chemical precipitant for phosphorous(Fleischer et al., 2005). The upstream energy for the alum is included for extraction and chemical production (258.32 J/L) ((Simapro, 2007). Similarly, the

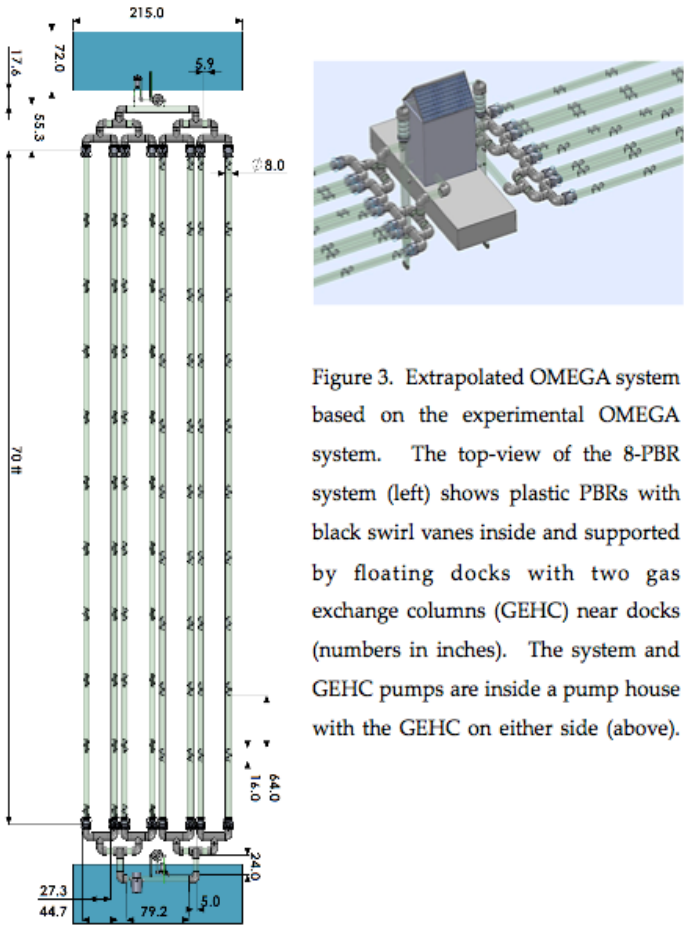


Figure 3. Extrapolated OMEGA system based on the experimental OMEGA system. The top-view of the 8-PBR system (left) shows plastic PBRs with black swirl vanes inside and supported by floating docks with two gas exchange columns (GEHC) near docks (numbers in inches). The system and GEHC pumps are inside a pump house with the GEHC on either side (above).

use of chlorine (102.95 J/L) for disinfection and sulfur dioxide (11.31 J/L) for dechlorinating are upstream energy inputs for extraction of raw materials and transformation as well as some local input for pumping water to chlorination unit (2.57 J/L). The energy input for filtration is from pumping with a feed pump (36.6 J/L), a filter media pump and standard pumps for transferring material to the surge basin and transferring solids from surge basin to disposal (78.2 J/L) (Goldstein and Smith, 2002). The energy for secondary settling is also primarily pumping settled effluent (14.74 J/L). Finally, the energy input for both mechanical and chemical mixing (52.50 J/L) is included in the calculation (Goldstein and Smith, 2002).

The energy output of the conventional system is primarily from methane produced by the anaerobic digester used to make electricity and from nitrogen fertilizer (Fig. 2, top). Clearly, the treatment process requires more energy than it produces and the percentages of the total input energy indicates the large energy requirements for secondary treatment (Fig. 2 right). The calculated EROI for WWT-BNR was 0.32.

3.4.2 Wastewater treatment with the OMEGA system

The features of the experimental OMEGA system are described in Chapter 2. These features include circulating algae in wastewater through PBRs and controlling dissolved gases and periodically harvesting algae in a "gas exchange & harvesting column" (GEHC) (Figure 3). Instead of treating secondary wastewater as described in Chapter 2 (Wiley et al., 2013), the extrapolated case considered here treats primary wastewater. The full-scale system, is based on a WWTP influx of 37.9 million liters of raw influent per day, the modeled OMEGA system needed an estimated holding capacity of 189.5 million liters. This accommodates 136.4 million liters for an assumed 3.6-day retention time with 53.1 million-liters (28 percent of total volume) added as reserve capacity.

The model assumes the PBRs are made of the same flexible linear low-density polyethylene as the experimental PBRs and they have the same diameter (0.2 m), but their length increases from

10 m to 22 m. The total number of tubes in the system increased from 4 experimental tubes tested to 204,320 in the model system. The PBRs were arranged in units of eight-PBR with a volume of approximately 6,500-L per unit. Instead of the series configuration as described in Chapter 2 (Wiley et al., 2013), the extrapolated system consists of 4-parallel flow PBRs that flow through a manifold (constructed of large diameter els and tees) for the return circuit. The volume was circulated by appropriately sized pumps with separate pumps to divert approx. 0.5 percent of the volume into GEHCs. This flow rate to the GEHC was reduced by a factor of 10 from 5 percent reported in (Wiley 2013) based on theoretical demand for CO_2 in the PBR. The flow through the PBRs was directed into a helical motion by swirl vanes spaced at 2.4 m intervals as described in Chapter 2 (Wiley, 2013). The swirl vanes are shown equally spaced in Fig. 3.

The marine infrastructure was assumed to use conventional floating docks (Fig. 14). It was calculated that an OMEGA system of this volume combined with the support infrastructure would cover 420 ha. In the extrapolated OMEGA case the areal efficacy was 22 percent giving a photosynthetic area for the PBRs of 90 ha.

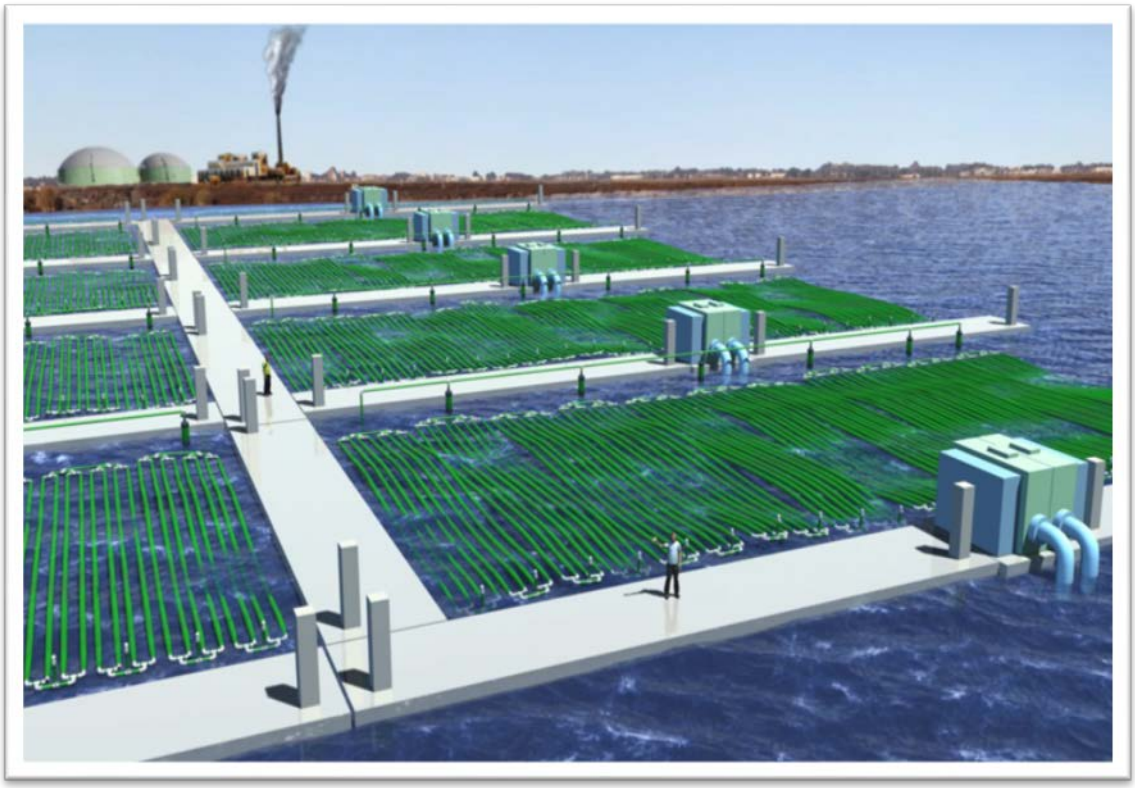


Figure 14: An Artist's Conception of the Large-Scale OMEGA Wastewater Treatment Facility Showing the Wastewater Treatment Plant and Flue Gas on-Shore

For the biology, the Redfield ratio ($C_{106}H_{181}O_{45}N_{15}P$) was used to represent the chemical stoichiometry of algae and for calculating resources required to support productivity (Clarens 2010). To replenish nutrients (N & P), 28 percent of the culture volume (roughly 1,800 L) was harvested daily and replaced with an equal volume of primary effluent. It was estimated based on the Redfield ratio that at each harvest the culture would contain an excess of 1.2 mg of phosphorus per liter, which was precipitated with the harvested algal biomass. With no supplementary nitrogen and 26.4 mg N/L_{PE} the expected algae yield was 304 mg algae/L_{PE}. The algae productivity assumed a growth rate of 15 g/m^2-d and a neutral lipid production based on 10 percent of the dry weight. It was also assumed the algae were able to access 100 percent of the soluble carbon in the wastewater (100 mg/L_{WW}) and 50 percent of the carbon dioxide provided by the GEHCs. Carbon was identified as the limiting nutrient and the system was therefore supplemented with commercial CO_2 to insure removal of nitrogen and phosphorus from the wastewater to meet discharge requirements. Thus, carbon is supplied to the culture in three forms: 1) soluble carbon in the primary effluent, 2) flue gas from electricity generation, and 3) commercially procured CO_2 from monoethanolamine (MEA) scrubbing.

Energy for Wastewater treatment with the OMEGA system

The net energy input and the energy output of the WWT system with OMEGA is shown in Figure 5 along with the much lower energy values for the conventional WWT described above (Fig. 5 gray bars). The net energy includes the raw materials and energy for the production of electricity (U.S. mix) and chemical inputs (Wang, 2011). The major energy input for the system is pumping, which had three components: 1) circulation of the culture through the PBRs, 2) pumping a portion of the culture through a separate GEHC, and 3) CO_2 transport and input. The energy required for pumping was calculated by estimating the head loss through every component in the system.

By far the greatest energy input for the OMEGA system itself is the "main system pumping" (circulation of the culture through the PBRs) representing 5,389.29 J/L and 60 percent of the total energy input (Fig. 5 blue bar left and wedge right). Other significant pumping energy is required for transferring culture to the GEHC (489.77 J/L) and from GEHCs to harvest algae (955.10 J/L).

There are also energy inputs for compression of CO_2 captured from flue gas (5 percent vol/vol) (243.75 J/L) and for upstream energy for commercial CO_2 captured during the MEA scrubbing process (780.84 J/L).

3.4.3 Separations and Biofuel Processing Model

The harvesting and processing methods modeled have been described in detail elsewhere (Beal et al. 2012b) (Beal et al. 2012a). Briefly, harvesting used chemical flocculation (requiring lime and CO_2), with 95 percent of the algae recovered at a 65X concentration factor. The energy

inputs harvesting include extraction and transformation of chemicals to produce lime used to flocculate algae (111.27 J/L) and electricity for harvesting (500 J/L).

The "processing" energy includes water recycling, lysing, and separations. Water recycling includes chlorine (102.95 J/L) and sulfur dioxide (11.31 J/L) for disinfection and chlorine removal as well as pumping water (2.54 J/L). The algal cells are lysed using electromechanical pulsing with an assumed efficiency of 95 percent. Lysing requires electricity (30.38 J/L) for the lysing unit and for pumping culture to lysing unit (3.54 J/L). Neutral lipid are separated using a membrane contactor with 90 percent. Separations use distillation energy (188.24 J/L), chilling water (41.91 J/L), water supply (14.99 J/L), and the organic solvent (heptane) used in extraction of the bio-oil (4.81 J/L); and separation electricity (0.01 J/L).

Finally, "refining" refers to the process in which lipids are upgraded to hydrotreated renewable jet fuel (HRJ) using the hydrotreatment process from the GREET model (Wang, 2011). The hydrotreatment unit requires heat (139.54 J/L), electricity (3.88 J/L), hydrogen for transformation of bio-oil to hydrotreated renewable jet fuel (HRJ) (0.04 J/L), and pumping of the lipid extracted algae remains to the anaerobic digester (0.01 J/L).

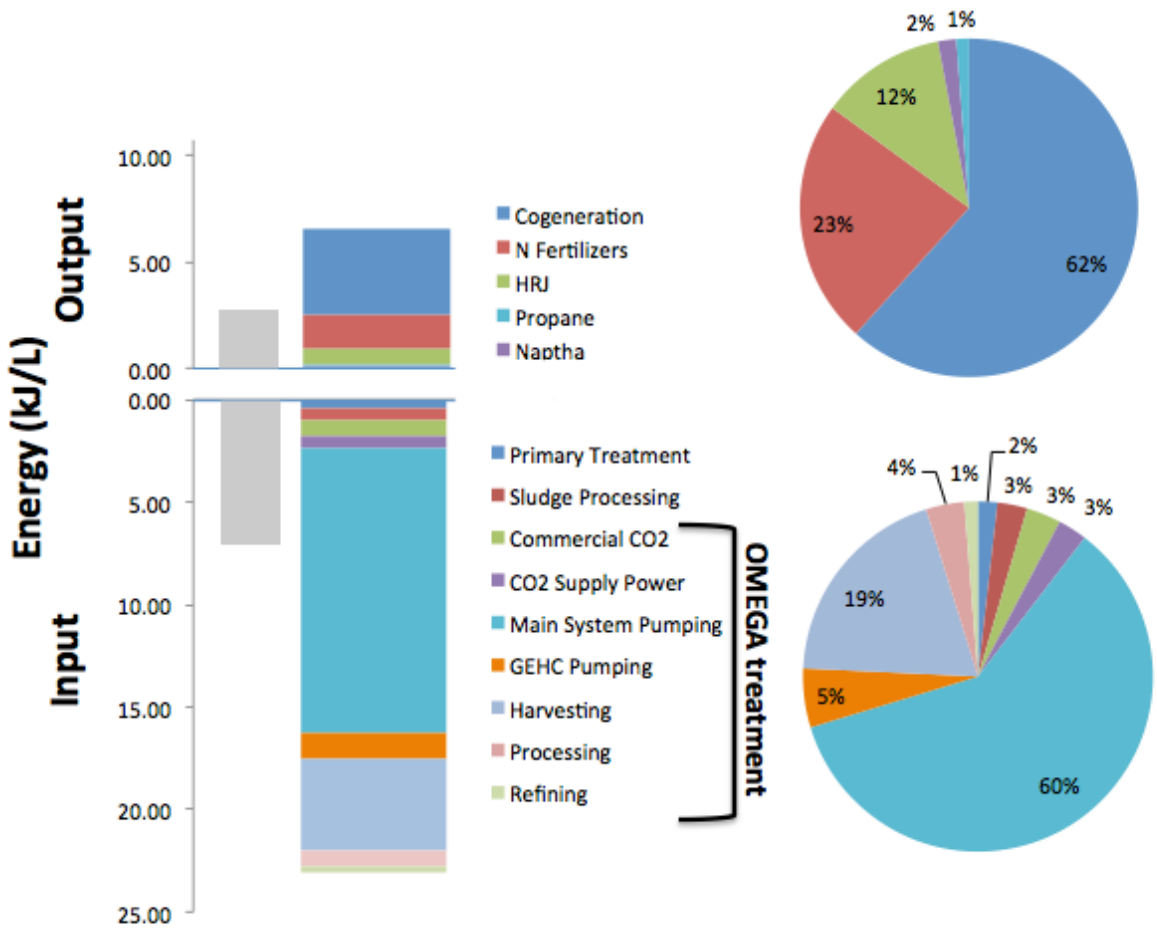


Figure 15: WWT with OMEGA

The energy input (bottom-left) and outputs (top left) in kilojoules per liter of wastewater treated and the percentage contribution of inputs (bottom right) and outputs (top right). The energy input requirements for primary treatment, sludge processing, and the components of the OMEGA system, as well as the energy output from methane burned for electricity and heat generation (co-gen), fertilizer and biofuels production. The grey bars (left) represent the values for the WWT-BNR shown in detail in Fig. 4.

The energy output from the WWT with OMEGA includes the energy from cogeneration of electricity from biogas produced in the anaerobic digester multiplied by the CHP turbine electricity generation efficiency (30 percent) (1,361.64 J/L) and from heat, also derived from the biogas produced in the anaerobic digester and multiplied by the turbine heat generation efficiency (70 percent) (425.28 J/L on-site heat). The excess heat (2,876.27 J/L) was not credited to the system.

Another energy output is calculated from the nitrogen fertilizer produced from 80 percent of the liquid effluent from the anaerobic digester (29.94 mg/L). The energy in this recycled N fertilizer is calculated as displaced commercial fertilizer produced using the Haber-Bosch process (steam reformation of methane) with a total energy content of 1,382.10 J/L. There is also the remaining

20 percent of the nitrogen in the bio-solids in which 40 percent is bio-available (2.99 mg/L). Here too, the energy derived from this recycled N fertilizer is based on displaced commercial fertilizer produced using the Haber-Bosch process (138.23 J/L). The biofuel outputs include 0.72 kg of HRJ, 0.10 kg of naptha, and 0.07 kg of propane produced from 1 kg of neutral lipids. A detailed summary of the material and energy flows of WWT-OMEGA is provided in Supplementary Table 1 at end of this chapter.

Taken together, the energy input clearly exceeds the energy output for a wastewater treatment plant with the OMEGA system. The calculated EROI was 0.28.

3.4.4 Costs, revenue, and revenue required for WWT with BNR or OMEGA

The costs for the two modeled treatment systems were based on vendor quotes, published values, or standard engineering estimates. The project lifetime was assumed to be 20 years, the internal rate of return 10 percent, and values for materials used in operating both systems are listed in Table 1 (Davis et al., 2011).

Table 2: Unit Prices of Components Used in System Operations.

Item	Price/Unit
Alum	$400/Ton
Chlorine	$250/Ton
Commercial CO2 (MEA scrubbed)	$40/Ton
Commercial Nitrogen	$1.29/Kg
Grid Electricity (U.S. Mix)	$0.1/kWh
Heptane	$3/L
HRJ	$6.32Kg
Hydrogen	$1.5/Kg
Lime	$100/Ton
Methanol	$0.4/Kg
Naphtha	$0.093/Kg
Propane	$0.7Kg
Sulfur Dioxide	$400/Ton

The estimated annual capital costs (Cap) plus the operating (Op) costs minus the revenue (Rev) allowed the revenue required (RR) to be calculated and compared (Figure 6). The direct costs include overhead 15 percent, mobilization 2 percent, site preparation 3 percent, site electrical 5 percent, instrumentation and control 3 percent, and lab and administration buildings 4 percent of capital costs (Capdet Works, 2007).

The indirect costs include 15 percent engineering design fee, 5 percent miscellaneous costs, 2 percent administration/legal, 2 percent inspection, 10 percent contingency, and 2 percent for technical (Capdet Works, 2007). Construction was assumed to take place over 3 years.

While the OMEGA system revenue was significantly higher than the WWT-BNR system (Fig. 6-left "Rev"), both the capital and operating costs were also much higher and therefore the revenue required was higher. The

direct capital costs for construction, engineering fees, and other costs gave a direct total for the WWT-BNR ($45.4M) and for WWT-OMEGA ($547M), which were divided into different cost sectors (Fig. 6: pie charts-right). The three largest direct cost sectors for the WWT-BNR were membranes (34 percent), BNR (28 percent), and aeration/nitrification (20 percent), which account for 82 percent of the total costs (Fig. 6, bottom-right). The three largest direct costs for the OMEGA capital costs were PBR-PVC components (38 percent), the pumps (31 percent), and the offshore platform (21 percent), which account for 90 percent of the total (Fig. 6, top-right).

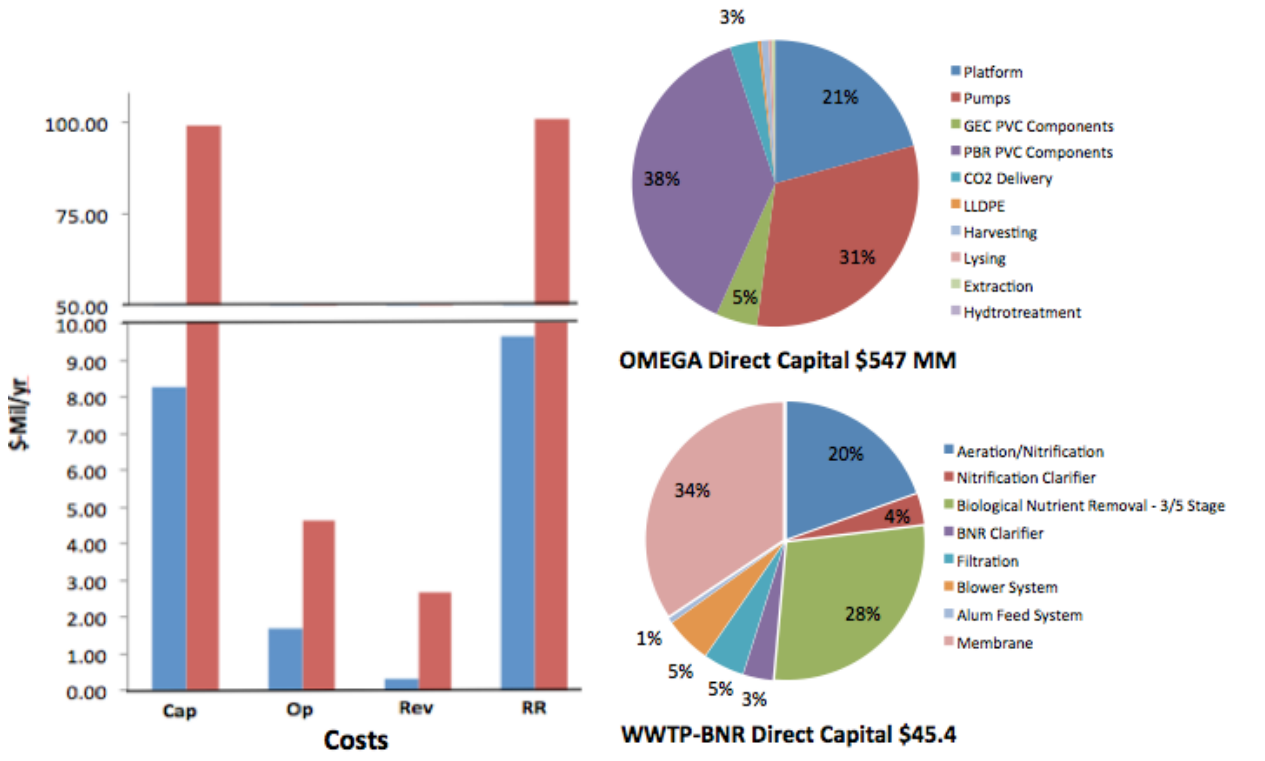


Figure 16: Annual Capital Costs (cap), Operating Costs (Op), Revenue (Rev), and Net Revenue Required (RR) to Achieve a 10% Rate of Return for WWTP-BNR (blue bar) and OMEGA (red bar).

The direct capital cost allocation for wastewater treatment with OMEGA (top) and with conventional BNR (bottom).

Obviously, the full-scale WWT-OMEGA system, based on the extrapolated experimental system, was prohibitively expensive, but the purpose of this study was to adhere to a direct scale-up of the experimental OMEGA design. This cost analysis raises questions about the importance of PVC components, the number and types of pumps employed, and the costs associated with the platform.

3.5 GHG estimates for WWT-BNR and WWT-OMEGA

The system boundaries for the GHG emissions follow the process diagram of Figure 1 and include on-site and upstream sources. Disposal of final solids and the use of co-products are not included in the system boundaries. The emissions associated with facility construction (pumps, buildings, PVC etc.) are also not included in the calculations for greenhouse gas accounting. A full life cycle accounting, from cradle-to-grave, is outside the scope of this work.

On-site emissions were estimated from published values and calculated mass balances for each unit process (Chandron et al., 2010) (RTI, 2010). For WWT-BNR, the on-site emissions included methane released from oxidation of organic carbon compounds (during aeration/nitrification and BNR), fugitive methane emissions from anaerobic digestion, and nitrous oxides from treatment processes during nitrification and denitrification (aeration and BNR). For the OMEGA system, the on-site emissions include nitrous oxide produced by autotrophic bacteria in wastewater during cultivation, carbon dioxide that escapes from the gas exchange and harvest system, fugitive methane and carbon dioxide emissions from the anaerobic digester, carbon dioxide emitted during processing of algae biomass (deflocculation), and combustion of biogas in the CHP.

Upstream impacts include the emissions for extraction of raw materials and conversion of those materials to useful products. The upstream GHG emissions, include electricity, heat, and materials (methanol, alum, chlorine, sulfur dioxide, lime, and commercial CO_2 derived from the monoethanolamine (MEA) scrubbing process) and were obtained from the GREET and Ecoinvent databases (Wang, 2011) (Simapro, 2007). An example of an upstream GHG emission is nitrogen fertilizer. The emissions result from the production of the fertilizer in the Haber-Bosch process rather emissions (N_2O) released from the use of the fertilizer. The nitrogen fertilizer production process includes extraction of crude oil, distillation of crude oil to make methane, steam reformation of methane to produce hydrogen gas, high-pressure reaction of hydrogen and nitrogen gas to produce ammonia, and processing of ammonia to make fertilizers(urea and ammonium nitrate).

GHG credits were given by the displacement method in which emissions credits are subtracted from the total emissions burden for the product substitution (e.g. bio-electricity produced from anaerobic digestion instead of electricity produced from a coal-burning power plant), co-products such as hydrotreated renewable jet fuel (HRJ), naphtha, propane, and nitrogen fertilizer (Wang et al., 2011).

The model estimates GHG emissions from the WWT-BNR and WWT-OMEGA systems for onsite, offsite, for offsets and the net values are shown in Figure 7. The offsite emissions of OMEGA are nearly four times higher than the WWT-BNR system due, primarily, to the large energy requirement for the OMEGA system. The offsets refer to the displaced GHG from co-gen or fertilizer. The WWT-OMEGA system (Fig. 7: red bar) is larger than the WWT-BNR (Fig.

7: blue bar) because there was greater biogas production from the anaerobic digestion of residual algae biomass and because of the biofuels production.

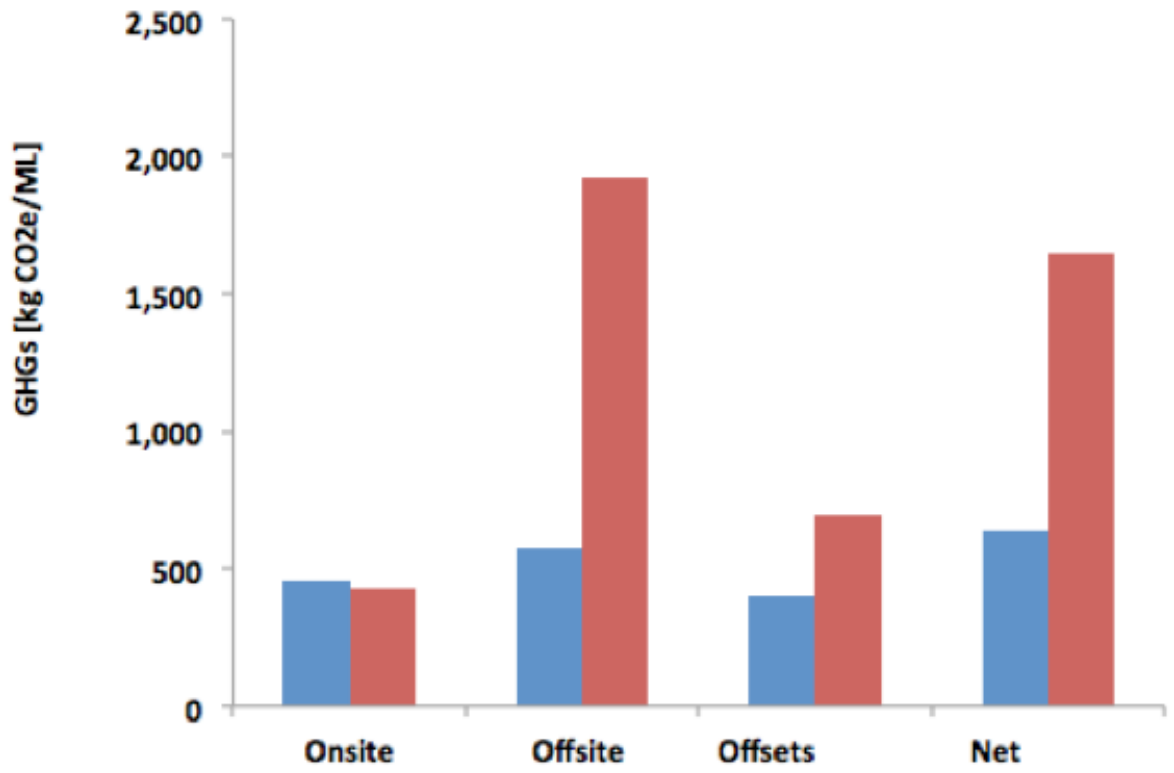


Figure 17: Comparison of GHGs Produced Onsite, Offsite, Offsets, and Net Emissions in Units Kilograms CO_2e Per Million Liters Treated Primary Wastewater by WWTP-BNR (blue bars) and by WWT-OMEGA (red bars).

3.5.1 Wastewater Treatment with BNR versus OMEGA

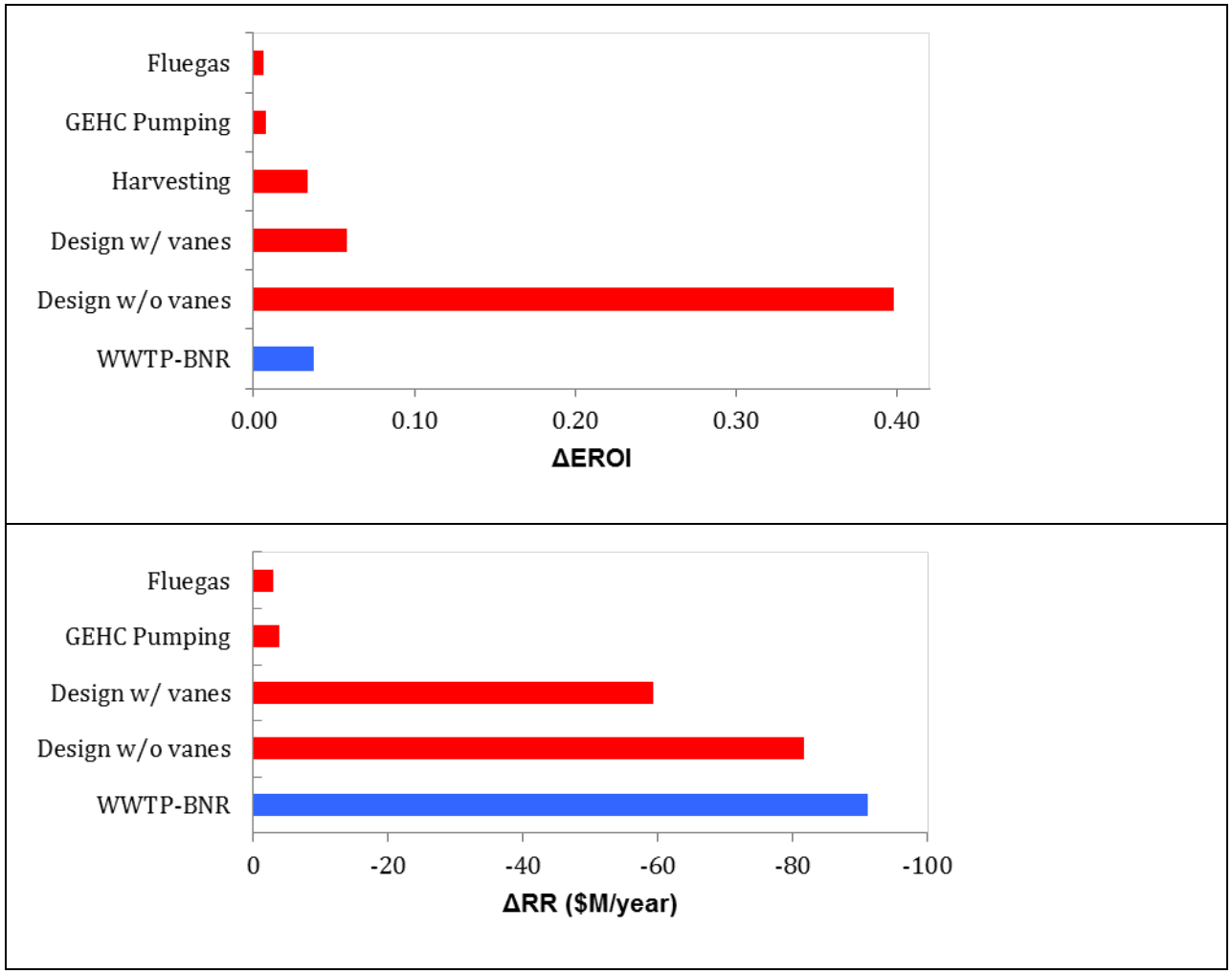
The WWT-BNR system had an EROI of 0.32, a RR of $700.6/ML of wastewater, and a net GHG emissions of 636 kg CO_2eq/ML of wastewater. Secondary treatment (including BNR) contributes 80 percent of the total energy input, 57 percent of the total capital cost, 90 percent of the operating cost, and 73 percent of the total GHG emissions. The BNR-process by itself, accounts for 42 percent of the total energy input, 39 percent of the total capital cost, 65 percent of the operating cost, and 26 percent of the total GHG emissions.

In comparison, the WWT-OMEGA system had an EROI of 0.28, a RR of $7,313/ML of wastewater, and a net GHG impact of 1,629 kg CO_2eq/ML of wastewater. The model therefore clearly indicates that substituting OMEGA for the BNR secondary treatment based on the previously published experimental OMEGA design extrapolated to full size made the WWT system less energetically efficient, more expensive, and produce higher GHG emissions. In other words, the model clearly indicates that it is obviously impractical to extrapolate the

experimental OMEGA system described in Chapter 2 to large scale unaltered. The model does however, provide insights into the alterations in the OMEGA system that would have the greatest impact on its successful deployment.

3.6 *Examples of parameters that impact the WWT-OMEGA system*

A few of the possible changes in the WWT-OMEGA system that significantly impact the outcome of the techno-economic model are shown in Figure 8. In each of the examples, the model was changed for single variable listed and the impact plotted as the change in energy return on investment (ΔEROI), the change in the revenue required (ΔRR), and the change in greenhouse gas emissions (ΔGHG) (Figure 8: top, middle, bottom, respectively).



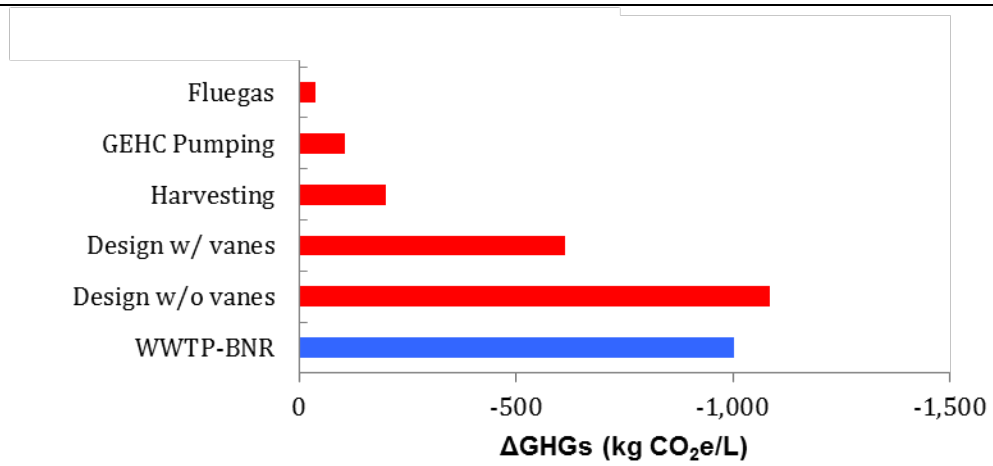


Figure 18: The Impact of Changes in Plausible Parameters in the WWT-OMEGA System on the EROI (top), the RR (middle) and the GHG (bottom).

The details of the parameters are given in the text. For comparison, the difference between the WWT-BNR and the WWT-OMEGA system modeled here is included (blue bars).

Revised flue gas calculations (fluegas) were based on a recalculation of the CO_2 concentration in the flue gas (from 5 percent to 14 percent (vol/vol)) based on the stoichiometry of combusted biogas rather than pure methane, decreased depth of the gas diffuser in the GEHC (from 2.74 m to 1.83 m) which reduces energy needed for the compressor from 0.674 J/mg CO_2 to 0.284 J/mg CO_2, and estimating flue gas capital costs with vendor quotes instead of using published values. These changes increase the EROI by 0.01 to 0.29, decrease the RR by $2.3 M/yr (from $100.8 M/y to $98.5M), and reduce the GHGs emissions by 29 kg CO_2e/ML wastewater (from 1,629 to 1,600 kg CO_2e/ML wastewater). The direct capital costs were reduced by $13M or 2.4 percent (from $547M to $534M).

Revised GEHC calculations (GEHC pumping) were based on changing the GEHC construction material from clear schedule 40 PVC to white schedule 40 PVC, changing the experimental ball valve to an economy ball valve ($20 instead of $100), decreasing the height of GEHC (from 3 m to 1.5 m) results in less specific energy for pumping (from 0.489 kj/l to 0.251 kj/l). These changes increase the EROI to 0.29, decrease the RR by $3.3M or 3 percent (from $100.79M/y to $97.5M), and decrease the GHG emissions by 49 kg CO_2e/ML wastewater or 3.6 percent (from 1,629 to 1,580 kg CO_2e/ML wastewater). The direct capital costs were reduced by $17M (from $547M to $530M).

Revised harvesting calculations (harvesting) were based on increasing the diameter of the pipe pumping culture to the harvester (from 0.0103 m to 0.0508 m), which reduces the specific energy for pumping from 0.955 kj/l to 0.050 kj/l. This change increases the EROI to 0.31, has no

appreciable impact on the RR, but decreases the GHG emissions by 189 kg CO_2e/ML wastewater or 12 percent (from 1,629 to 1,440 kg CO_2e/ML wastewater).

Revised PBR design with swirl vanes calculations (design w/ vanes) were based on creating the flow pattern by welding plastic sheets into channels and eliminating the PVC manifolds increasing the packing density of PBRs and the surface area efficiency from 22 percent to 70 percent. This change increased the EROI to 0.34; decreased the RR by $59.3M (from $100.8M/y to $41.5M/y); and decreased the GHG emissions by 322 kg CO_2e/ML wastewater or 20 percent (from 1,629 kg to 1,307 CO_2e/ML wastewater).

Revised PBR design and removed swirl vanes calculations (design w/out vanes) were based on changing the configuration as described above and eliminating the inserted plastic swirl vanes. These two design changes increase the EROI to 0.68, decrease the RR by $81.6M or 81 percent (from $100.8M/y to $19.2M), and decrease GHG emissions by 1,094 kg CO_2e/ML wastewater or 59 percent (from 1,629 to 535 kg CO_2e/ML wastewater). The direct capital costs were reduced by $440M or 80 percent (from $547M to $107M).

More specifically, the combination of changing the PBR design and eliminating swirl vanes had such significant impact on the model because it affected:

- Increased the surface area efficiency from 22 percent to 70 percent.
- Reduced the number of floating docks and therefore their cost from $94M to $32.9M.
- Eliminated most of the PVC.
- Eliminated swirl vanes at $15 each.
- Design change reduced the pressure drop for each PBR by decreasing minor loss from 846 Pa to 0 Pa, which increases the allowable number of PBRs from 8 to 256 per pump.
- Reduced the number of pumps from 25,540 to 814.
- Reducing the flow rate from 1,211 to 302.8 l/min, which reduced the cost per pump from $2,904 to $700.
- Reduced the number of circuits made by the culture from 269 to 2.4 per day, which reduces the specific energy for pumping from 5.4 kj/l to 0.17 kj/l
- Eliminated the need for pump houses at $750 each.

Examples of other simple design changes that would significantly change the results of the model include pump duty cycle (reducing the operating time to 8 hours would result in a 66 percent energy reduction), and improvements in the systems controls that could improve the CO2 uptake efficiency by decreasing the size of the gas bubbles (Zimmerman, 2012)
By combining changing in the flow configuration, eliminating the PVC manifold and the swirl vanes, by revising the operations of harvesting, the GEHC, and the distribution of flue gas, the EROI increases to 1.02, the RR decreases to $16M/y, and the GHGs decreases to minus 19.14 kg CO_2e/ML (McKuin and Trent, in preparation).

Other revisions focus on the biological assumptions of the model presented here. For example, the algae productivity was assumed to be 15 g/m^2-da and lipid production 10 percent of dry wt., both of which could be significantly increased through strain selection or genetic engineering (Beal 2011). For example, increases in algal biomass of 50 to 80 percent have been demonstrated by artificially increasing the expression of genes that encode components of the carbon-concentrating mechanism, normally induced only under low CO2 conditions (Scott 2010) (Spalding 2011). The assumed lipid content of 10 percent could conceivably be increased to 50 percent by enhancing appropriate enzymatic activities (Courschesne 2009) (Turchetto-Zolet 2011). In addition to improvements in the biology, there are developments with chemical transformation systems for making biofuels that use the whole biomass rather than lipids, which may provide near-term improvements in energy recovery (Gouveia, 2011) (Wiley 2011).

3.7 Concluding remarks

Although several studies of algal-based wastewater treatment and biofuels production have considered land, water, nutrients, and energy balance, few studies have considered all four requirements simultaneously (Lundquist 2010) (Fortier 2012) (Pienkos 2009) (Pate 2011) (Wigmosta 2011) and none have considered the resources required for an offshore cultivation system. In the model tested here the possibility of substituting an OMEGA system for biological nutrient removal (BNR) in a conventional wastewater treatment pathway was considered and the OMEGA system produced biofuels (jet fuel, naphtha, and propane) as a byproduct. The design of the OMEGA system modeled was based on the system described in Chapter 2, directly scaled up to meet the needs of the wastewater plant. The calculated EROI, RR, and GHG emissions indicated that in fact it is not practical to directly scaled-up the experimental system and that there are plausible modifications that greatly improve the performance of a WWT-OMEGA system.

It has been suggested that biofuels with an EROI <3 are impractical to deploy (Beal 2012c), noting that fossil-oil based gasoline has an overall EROI of 5 to 10. It therefore remains to be seen if algae biofuels can reach such EROI values and meet the demands of profitability and environmental compatibility. For the OMEGA system, leveraging the platform with other renewable energy sources (including wind, solar and wave energy) and aquaculture may be the most promising approach for the future (McKuin 2013).

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Supplementary TABLE 1. Material and Energy Inputs for WWT-OMEGA system

Inputs	Value	Units
Screens/Grit Chamber		

Pumping	133.35	J·L-1
Bar Screens	0.19	J·L-1
Grit Chamber	12.75	J·L-1
Primary Treatment		
Primary Clarification	14.74	J·L-1
Chlorination/Dechlorination		
Water Recycling/Treatment Energy	2.54	J·L-1
Water Treatment Chlorine	7.89	mg·L-1
Dechlorination (Sulfur Dioxide)	1.97	mg·L-1
OMEGA Algae Cultivation		
Commercial CO2	86.95	mg·L-1
CO2 Supply Power	243.75	J·L-1
Main System Pumping	5389.29	J·L-1
GEHC Pumping	489.77	J·L-1
Harvesting		
Pumping	955.1	J·L-1
Flocculants (lime)	30.42	mg·L-1
Deflocculants (CO2)	76.06	mg·L-1
Additional Harvesting Energy	500	J·L-1
Lysing		
Pump	3.54	J·L-1
Power Supply	30.38	J·L-1
Separations		
Separations Energy	0.01	J·L-1
Distillation Energy	188.24	J·L-1
Chill Water	4.23	mL·L-1

Chilling Energy	41.91	J·L-1
Heptane Loss	0.00012	mL·L-1
Hydrotreatment		
Hydrotreatment Electricity	3.88	J·L-1
Hydrotreatment Heat	139.54	J·L-1
Hydrotreatment Hydrogen	0.04	mg·L-1
Solids Handling		
Lipid Extracted Biomass Pumping	0.01	J·L-1
Primary Solids Pumping	32.21	J·L-1
Anaerobic Digester Heat	285.73	J·L-1
Dewatering of digested solids	76.81	J·L-1

Chapter 4:
The Role of OMEGA PBRs in Advanced Municipal Wastewater Treatment

4.1 Summary

Offshore Membrane Enclosures for Growing Algae (OMEGA) is a system of floating photobioreactors (PBRs) that grow microalgae on municipal wastewater. Using municipal wastewater allows biofuels to be made from algae without competing with agriculture for water and nutrients and the algae may also enhance municipal wastewater treatment. To determine the treatment capabilities of the OMEGA system, samples were collected from 110- and 1600- L PBRs and analyzed for pharmaceuticals, personal care products, steroid hormones, metals, nutrients, pathogens, and other water quality parameters. Concentrations of about half of the wastewater-derived pharmaceuticals, personal care products, and steroid hormones decreased by >80 percent, presumably by sorption and biotransformation mechanisms. Concentrations of some recalcitrant contaminants (e.g. carbamazepine, gemfibrozil) did not decrease, although partial (40 percent) attenuation of the recalcitrant compound primidone suggests a unique removal mechanism in the OMEGA system. Concentrations of lead, chromium, and mercury decreased by >60 percent and copper and zinc by >45 percent, whereas nickel concentrations remained unchanged. Although organic carbon was depleted (50-60 percent), biochemical oxygen demand increased by 155 percent during production, indicating an alteration in dissolved organic matter. The OMEGA PBR system is an effective option for removal of numerous wastewater-derived contaminants

4.2 Introduction

Growing algae offshore, using municipal wastewater as a carbon and nutrient source, provides a potential means of producing biofuels that does not compete with agriculture for water, fertilizer, or land [1-3]. In fact, the U.S. Department of Energy National Algal Biofuels Roadmap (2010) specifically points to the importance of combining wastewater treatment and algal biofuels production [4]. In addition to their role as a biofuel feedstock, algae also provide ancillary municipal wastewater treatment that significantly improves water quality [5]. Algae, grown in circulating channels called "high-rate ponds" (HRPs) or in enclosures called photobioreactors (PBRs), improve water quality by reducing biochemical oxygen demand (BOD), total suspended solids (TSS); nutrients that cause eutrophication [6-9]; metals, including toxic heavy metals [10, 11]; and organic contaminants, surfactants, phenols, and hydrocarbons [12-14].

Algal treatment systems augment conventional wastewater treatment because they add a diversity of physical, chemical, and biological treatment mechanisms. For example, PBRs are designed to maximize solar irradiation for algae growth, which also enhances photochemical

transformations (UV exposure and photochemical oxidant production) not included in conventional wastewater treatment systems [15-17]. Algae cause diurnal changes in pH that may range from pH 6 to 11, which can enhance chemical transformation mechanisms such as hydrolysis that may even be capable of disinfecting wastewater by creating alkaline conditions [6, 12]. High concentrations of algae biomass, which can exceed 1 g/L in PBRs, and related metabolic activity can provide additional biological treatment mechanisms [18]. Algal biomass may be lipid-rich, thus partitioning and sequestrating hydrophobic contaminants [2, 19]. Algal metabolism may degrade contaminants by enzymatic pathways distinct from those of other microbial systems [13, 20]. The combination of mechanisms, some of which are unique to algal systems, may be a useful addition to wastewater treatment, particularly for the diverse classes of wastewater-derived constituents that are otherwise difficult to remove by traditional wastewater treatment processes.

Although the benefits of coupling algal-biofuel production with wastewater treatment plants have been elucidated [21], it is unclear how large-scale algae cultivation facilities can be installed in the vicinity of existing treatment plants, most of which are surrounded by extensive urban infrastructure [22]. Co-location of algae production and wastewater treatment facilities is essential because pumping wastewater more than a few tens of kilometers requires more energy than can be extracted from algae [21]. A solution for coastal cities is the Offshore Membrane Enclosures for Growing Algae (OMEGA) system, which cultivates lipid-rich algae in floating PBRs deployed in protected bays in proximity to existing wastewater outfalls [22]. The proposed OMEGA algal cultivation system uses wastewater for water and nutrient supply, a local source of waste carbon dioxide as a carbon source, buoyancy to support the PBRs, the heat capacity of the surrounding water for cooling, wave energy for mixing, and the wastewater-seawater salinity gradient to drive forward osmosis [22].

In this study, two prototype OMEGA PBR systems (which did not include forward osmosis) were evaluated for their impact on municipal wastewater treatment. Both prototype systems cultivated algae on wastewater effluent—that is, the final plant effluent (FPE)—that was ready for discharge into the marine environment. The concentrations of pathogens, nutrients, metals, organic carbon, and wastewater-derived organic contaminants including pharmaceuticals and personal care products (PPCPs) were analyzed by comparing FPE from the treatment plant with effluent from the OMEGA PBRs.

4.3 Materials and Methods

Algae were grown in two different PBR systems for 11 or 13 days. During the course of these experiments, CO2 injections maintained the pH of the cultures near a set point of 7.7 (see Ch. 2 for a description of the pH control system). Temperature was maintained at 19.2 to 22.8 °C. PBRs were inoculated with a culture at OD_{750} of 0.2 to 0.5. At the end of experiments, the PBR contents were discharged to the sewer for treatment.

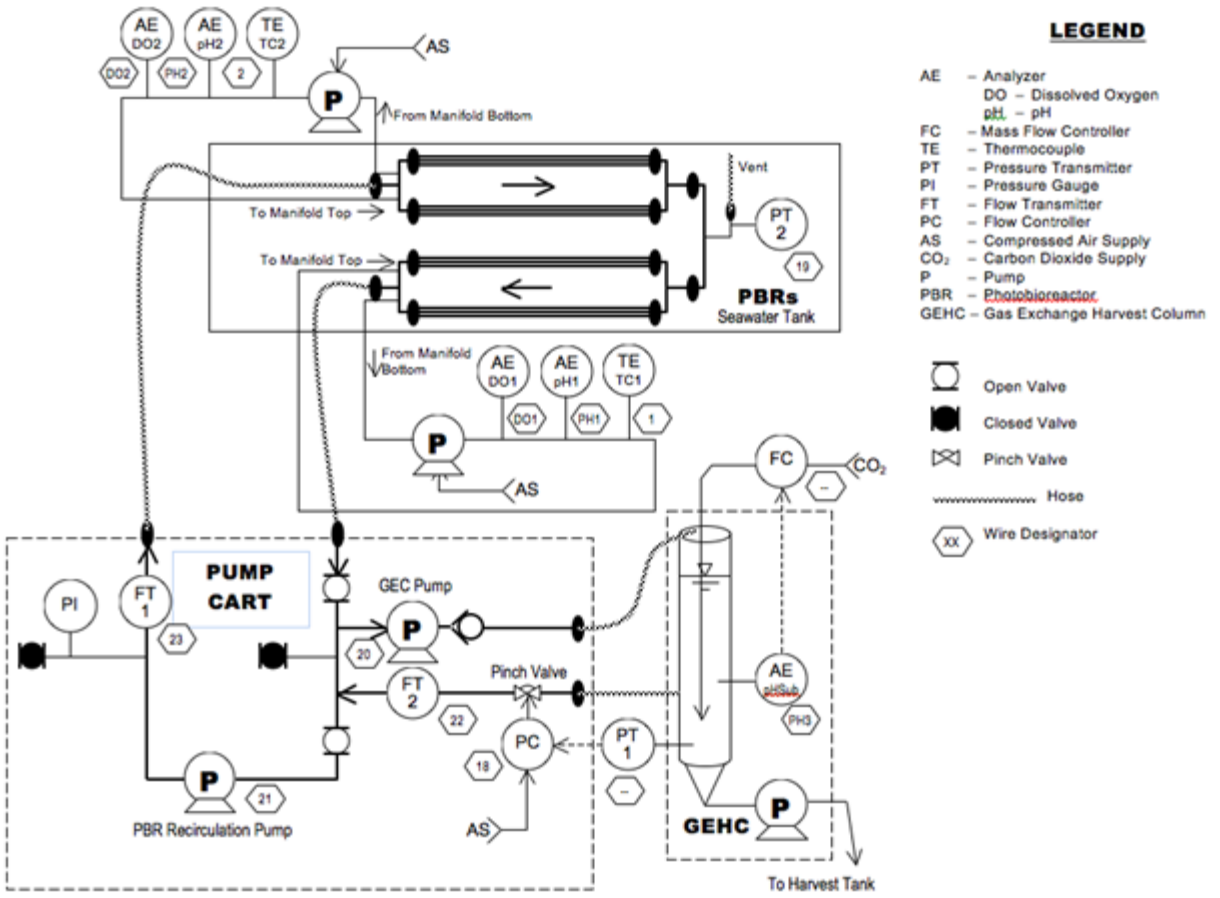

Figure 19. The Generic OMEGA System Showing the Circulation Through the PBRs, Sensor Manifold, and Side-Loop for the Gas Exchange and Harvesting Column.

In Santa Cruz (SC OMEGA system) there was one PBR in each direction and in San Francisco (SF OMEGA system) there were two as shown. Sensor data storage and controls for regulating flow rates, head pressure (pinch valve/pump), and CO_2 input are mediated by an electronic control system.

4.3.1 Prototype OMEGA Systems

Two OMEGA systems of similar design, but of different sizes and at different locations, were used both of which had an overall design shown in Figure 1. The smaller system (110-L), which included two PBRs, each 3 m long x 11.4 cm in diameter, was at the California Fish and Wildlife Laboratory in Santa Cruz, CA ("SC OMEGA system"). The PBRs were filled with FPE from the Santa Cruz Wastewater Treatment Facility (38,000 m^3 day^{-1} trickling filter process) [23]. The larger 1600 L system ("SSF OMEGA system") had four PBRs, each 9.1 m long x 20.3 cm in diameter, at the Southeast Water Pollution Control Plant (250,000 m^3 day^{-1} pure oxygen activated sludge process) in San Francisco, CA, [24] (see Chapter 5). Wiley et al. [23] give a detailed description of the functional OMEGA system. Briefly, algae circulate continuously through PBRs, which are made of flexible, clear polyethylene and fitted with swirl vanes for mixing. Approximately 5 percent of the culture volume is diverted through a gas exchange/harvesting column (GEHC: see Chapter 2) that removes dissolved oxygen (DO), adds CO_2, and collects algae that aggregate or otherwise settle and are harvested. A control system monitors pumping

rates, temperature, photosynthetically active radiation, DO, and pH. A feedback system adds CO_2 to maintain the pH between 7.1 and 8.5. Nutrient concentrations were measured daily and maintained by wastewater additions in the SC system and by centrate addition in the SSF system.

Details of the experiments described herein are given by Wiley et al. [23] for the SC system and by Carney et al. [24] for the SSF OMEGA system. For both systems, algae cultures (dominated by *Scenedesmus* sp. and *Desmodesmus* sp.) were added to the wastewater. The enrichment cultures were grown in flasks containing BG-11 medium (prepared using the American Type Culture Collection protocol) mixed with FPE; the cultures were kept in lighted incubators (4,500 lux) with shaking and CO_2-bubbling to provide carbon and maintain pH between 7.6 and 8.2. These enrichment cultures were scaled up to 150 L in carboys, also with CO2 bubbling. The cultures were added to the OMEGA systems in log-phase growth at an optical density (OD_{750}) of between 0.1 to 0.4. Carney and co-workers provide a detailed description of the SF culture by metagenomic analysis (24). (Carney et al. in preparation).

4.3.2 Sample Collection

Data were obtained from SC OMEGA system from May 21 to 31, 2012 and the SSF OMEGA system from May 22 to June 4, 2012. For routine water quality analyses, samples were collected and preserved using standard protocols [25]. Between 50 and 1000 mL samples were collected and stored in prewashed amber glass bottles without preservation. Samples for metals analysis were collected in acid-washed fluoropolymer plastic containers. Samples (1 L) for PPCP and steroid analysis were supplemented with ascorbic acid (50 mg/L) and sodium azide (1 g/L) for preservation [26].

Samples were collected every 1 to 4 days. For the 1,600-L SSF OMEGA system the volume of the samples (2-6 L daily) minimally impacted the total volume of the system; this volume loss was somewhat offset by the periodic addition of centrate. For the 110-L SC OMEGA system, however, a large percentage volume, 20 to 80 L, was periodically removed and replenished with wastewater to re-supply nutrients. This exchange significantly impacted the overall volume of the system. To account for these additions, paired samples were collected from the 20 – 80 L of water used to refill the SC system (feed) and the volume removed (harvest). Sample processing varied with the analysis. SSF samples analyzed at the San Francisco Public Utilities Commission (PUC) were immediate chilled and delivered to the laboratory for analysis. PPCP samples were stored at 4 °C for up to 10 days prior to shipping or shipped overnight on ice for next day analyses.

4.3.3 Sample Analyses

Subsets of samples were analyzed to determine concentrations of PPCPs; steroid hormones (17β-estradiol, estrone, 17α-ethinyl estradiol, progesterone, and testosterone); metals (antimony, beryllium, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, thallium, and zinc); pathogens (fecal coliforms, E. coli, and enterococci); nutrients (ammonia, nitrate, and orthophosphate); and bulk parameters such as dissolved carbon (total, organic, and inorganic), pH, UV_{254}, and BOD.

The PPCP and steroid hormone analysis was performed by solid-phase extraction and isotope dilution liquid chromatography-tandem mass spectrometry as described elsewhere [26]. Reporting limits for PPCPs ranged from 0.25 to 25 ng/L. QA/QC protocols included matrix

spikes, duplicate samples to assess and monitor analytical precision, and blanks that indicated whether there were analytical or contamination issues (none were found). EPA Method 200.8 was used for analyses of all metals except mercury, for which EPA Method 1631 was used [27, 28]. The San Francisco PUC Water Quality Laboratory analyzed trace metals and did microbiology (MPN/100 mL for fecal coliforms and enterococci) using Standard Methods 9221E and 9223, respectively [25]. Concentrations of the nutrients ammonium (NH4+-N), nitrate (NO3—N), and reactive phosphate (PO4) were determined using a Hach DR 2700 spectrophotometer (methods #10031, 8039, and 8048, respectively).

Total carbon (TC), dissolved organic carbon (DOC), and inorganic carbon (IC) were analyzed with Standard Method 5310B, using a Shimadzu TOC analyzer [25]. 5-day BOD was quantified in the SSF OMEGA system samples with Standard Method 5210B [25]. Before additional analysis, samples were filtered through 0.7 μm glass fiber filters. To characterize organic carbon further, absorbance at 254 nm (UV_{254}, Cary 300 Bio, Varian, Palo Alto, CA) was measured. Using similar methods to those of Chen et al., excitation-emission (EEM) fluorescence spectra were also measured with a CARY Eclipse Fluorescence Spectrofluorometer (Varian) equipped with a xenon lamp; 1 cm Quartz 6Q cuvettes were used [29].

4.4 Results and Discussion

The OMEGA systems at the two sampling sites had different volumes and different functional deployments. The SSF OMEGA system contained 1600 L of cultures in wastewater. It was filled on day 0 and harvested regularly for 13 days with small additions of centrate (<20 L total, ~1 percent of system volume) on days 5, 8, and 10 to replenish nutrients. Without correcting for the added centrate, the hydraulic retention time (HRT) nearly matched the sample collection days. During the 13-day experiment at SF, the culture reached a maximum cell density of 4.8 x 10^7 cells/mL on day 10 and remained in stationary phase thereafter.

The SC OMEGA system was operated as a semi-batch system with 18 percent to 84 percent of the total system volume withdrawn at one to three day intervals and replace with fresh wastewater to maintain nutrient concentrations above 20 mg/L ammonia. Because the withdrawal volume varied, the effective HRT of the system also varied, ranging from 2.1 to 4.3 days with an average of 3.0 days. To assess water quality, withdrawal volumes were tracked and the SC OMEGA system harvest data were compared to a volume-weighted average of the feed composition, effectively creating a "composite feed" concentration for comparison.

4.4.1 PPCPs

A variety of PPCPs occur in nearly all wastewater effluents and are known to persist to varying degrees after conventional wastewater treatment. The analytes studied here were carefully selected to represent a range of chemical properties and degradation rates, which had been previously observed in wastewater activated-sludge and in the environment [30-34]. These PPCPs provide information about the performance of the OMEGA system and insights into the possible physical and chemical mechanisms responsible for their attenuation. For example, the PPCPs chosen vary by several orders of magnitude in their octanol-water partition coefficients (*i.e.* log K_{ow}) and in their biotransformation rate constants. Based on these properties, the analytes chosen would be expected to include labile compounds that would be effectively sequestered or transformed during treatment, moderate removal compounds with intermediate

sequestration or transformation, and recalcitrant compounds with poor removal or little sequestration and transformation. Most engineered treatment systems will demonstrate a complete range in performance outcomes from poor removal to near complete removal within a set of analytes, with the moderate compounds being particularly effective metrics for performance assessment.

4.4.2 PPCPs—Steroid hormones

The concentrations of three common wastewater hormones were measured at both sampling sites. In the feed sample of the SSF OMEGA system, estrone was present at 10 ng/L, 17β-estradiol at 3.7 ng/L, and progesterone at 2.6 ng/L, whereas 17α-ethinyl estradiol and testosterone were not detected. Over the first three days of the trial, all steroids became undetectable (Fig. 2), due to a combination of partitioning to algal biomass, phototransformation, and biochemical transformations, all mechanisms capable of attenuating steroids [40-42]. In the SC OMEGA system, only estrone was detected in feed samples, at concentrations of 11 to 73 ng/L (n = 8). Over the 2 to 4 day HRT characteristic of the SC OMEGA system, estrone concentrations declined by one to two orders of magnitude usually reaching undetectable concentrations (not shown). The maximum estrone concentration observed in SC OMEGA system samples—which occurred when algal population densities were lowest—was 1.9 ng/L, suggesting a strong influence of algal population density or activity on steroid removal.

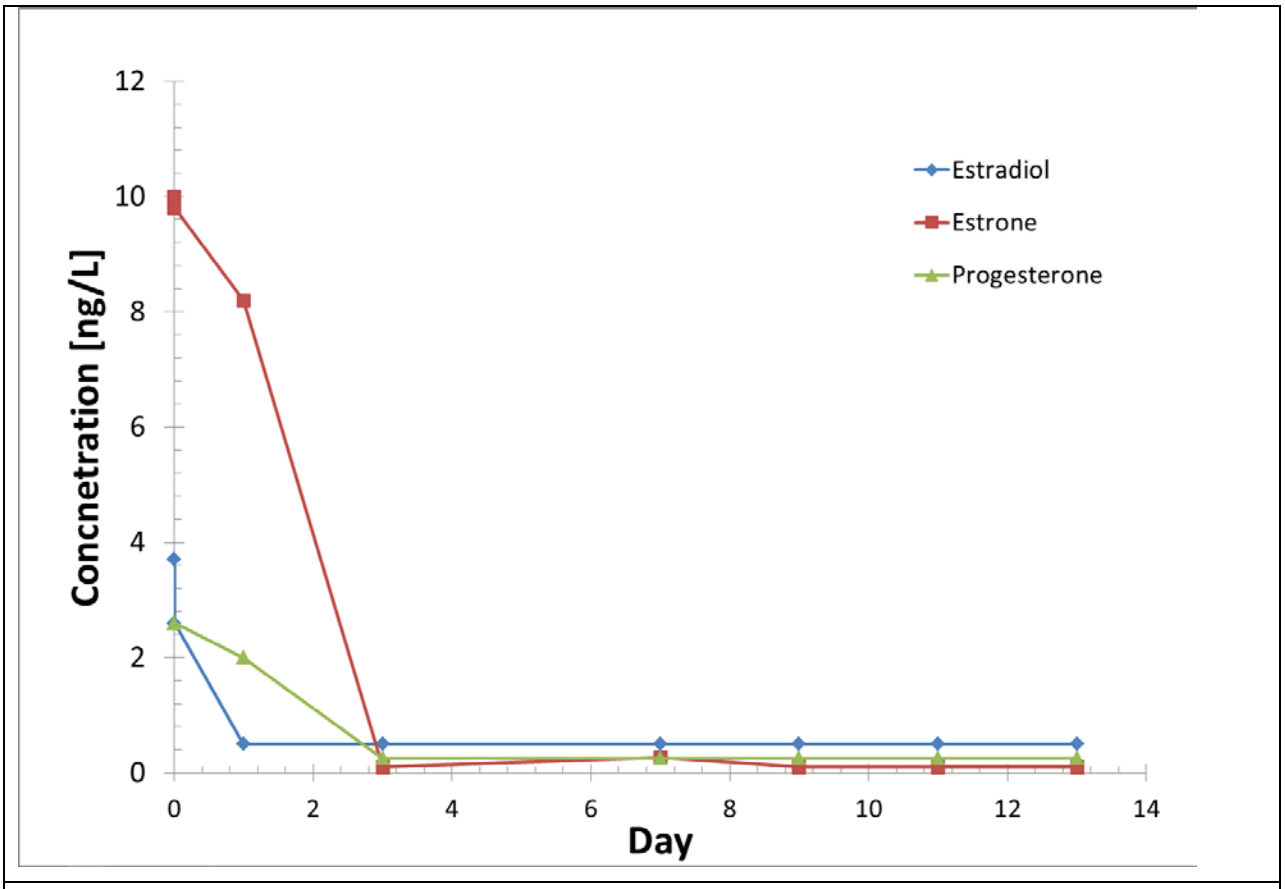

Figure 20: Changes in the Ratio of Final Concentration (C) to Starting Concentration (Co) of Steroid Hormones in FPE During the 13-Day Experiment at SSF System.

4.4.3 PPCP clearance in the SSF OMEGA System

Twenty wastewater-derived PPCPs were detected in the FPE (normally discharged into San Francisco Bay), which was used as the feed in the SSF system (Fig. 3). Concentrations of triclocarban were low (approximately 9 ng/L), whereas concentrations of caffeine and sucralose were high (21,000 ng/L and 25,000 ng/L, respectively). The concentrations of most of the other analytes studied ranged from 170 to 1800 ng/L.

Eight of the PPCPs studied (triclosan, triclocarban, DEET, acetaminophen, fluoxetine, naproxen, ibuprofen, and caffeine) were effectively removed over the 13-day period with concentration decreases of >80 percent; most reductions exceeded 93 percent (Figure 3). Moderate removal was observed for atenolol (53 percent) and primidone (43 percent). Primidone is known to be recalcitrant to aerobic activated-sludge, soil-aquifer treatment, ozonation, and activated carbon adsorption systems, although advanced treatment led to partial removal [30, 35, 36]. Removal was low for sucralose (13 percent), trimethoprim (19 percent), and TCEP (24 percent). No significant removal occurred for the remaining four compounds (meprobamate, gemfibrozil, carbamazepine, and sulfamethoxozole) (t-test, $p>0.05$).

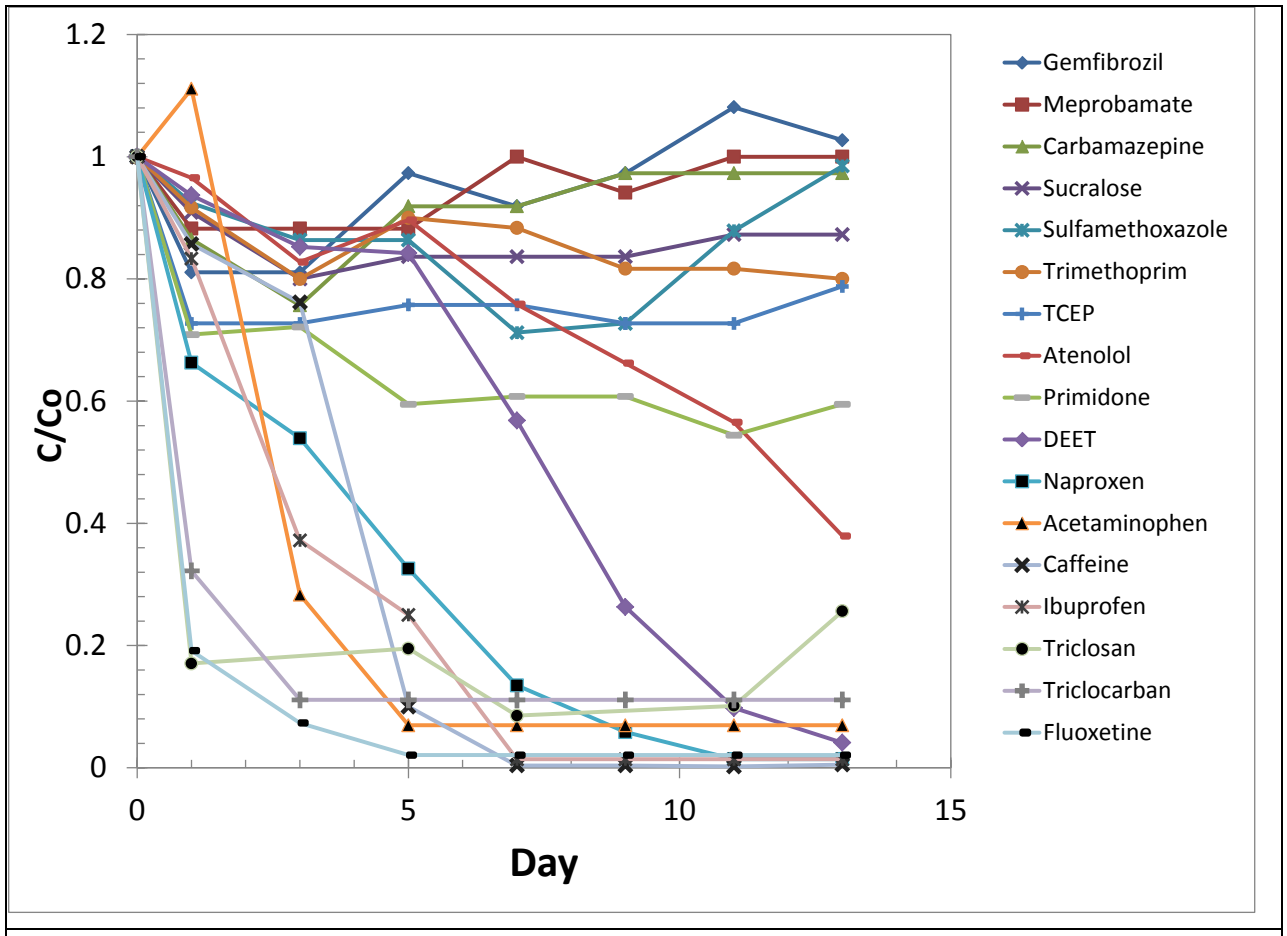

Figure 21: Changes in the ratio of final concentration (C) to starting concentration (Co) of PPCPs in FPE during the 13-day experiment at SSF OMEGA system.

The order of the analytes in the legend is generally consistent with their stability; Gemfibrozil was most stable, not removed, and Fluoxetine was removed most quickly.

4.4.4 PPCP clearance in the SC OMEGA system

In the SC FPE (feed samples), 17 wastewater-derived PPCPs and one steroid hormone were detected (n =8). Concentrations ranged from 25 ng/L for triclocarban to 39,000 ng/L for sucralose. Most of the other analytes were present at concentrations that ranged from 130 to 2000 ng/L with concentrations generally slightly lower than values observed at the SSF site (Fig. 4).

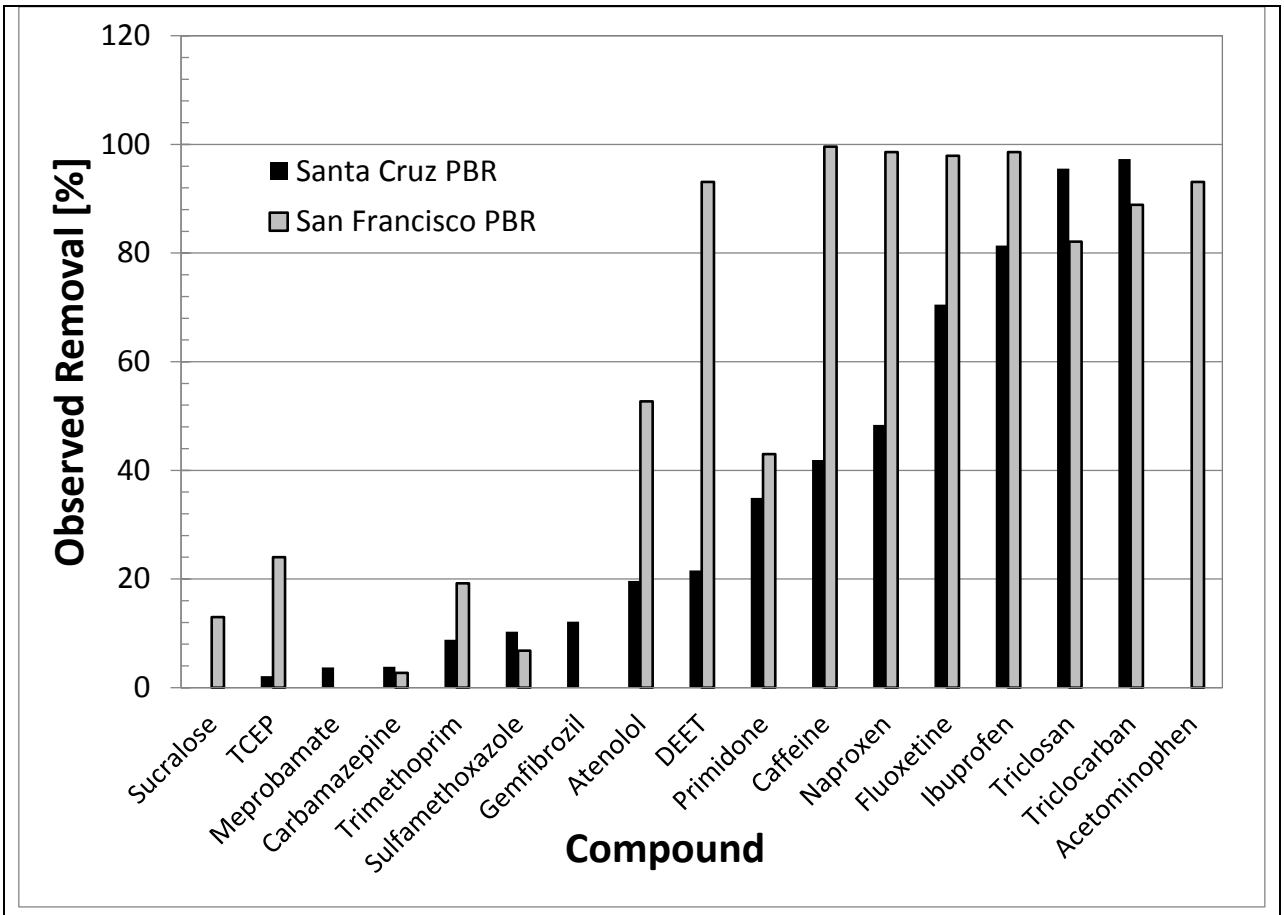

Figure 22: Percent Removal of Pharmaceuticals and Personal Care Products (PPCP) in Final Plant Effluent from San Francisco and Santa Cruz after 13 or 10 days in the SSF OMEGA System and the SC OMEGA System, Respectively.

4.4.5 Comparison of OMEGA systems

Comparison of harvest sample concentrations with a volume-weighted composite feed concentration was used to estimate removal performance. Nearly all PPCPs were less effectively removal by the SC OMEGA system than the SSF system (Figure 2). For example, of the eight PPCPs undergoing effective removal (>80 percent) in the SSF OMEGA system (triclosan, triclocarban, DEET, acetaminophen, fluoxetine, naproxen, ibuprofen, caffeine), only ibuprofen, triclosan, and triclocarban underwent concentration decreases of >80 percent in the SC OMEGA system, suggesting that these three PPCPs are likely the most easily attenuated compounds during algal treatment. Similarly, removal dropped for DEET (from 93 percent to 22 percent), fluoxetine (98 percent to 71 percent), naproxen (98 percent to 48 percent), and caffeine (>99 percent to 42 percent). In addition, lower removal was observed for both primidone and atenolol in the SC OMEGA system. Similar to results from the SSF system, no significant removal ($p>0.05$) was observed for meprobamate, carbamazepine, or sulfamethoxozole, with sucralose, TCEP, trimethoprim, and gemfibrozil also undergoing no or poor (<25 percent) removal in the SC system.

The SC OMEGA system probably removed PPCPs less effectively than the SSF system because of its shorter HRTs were insufficient for this process. Figure 3 demonstrates the effect of HRT

on PPCP removal in the SSF OMEGA system. The dissipation kinetics of compounds such as ibuprofen, caffeine, fluoxetine, DEET, atenolol, primidone and naproxen indicate that there was likely insufficient time to attain higher removals in the relatively short 2 to 4 day HRT of the SC OMEGA system. Certain PPCPs that are amenable to transformation, such as caffeine, DEET and atenolol, were effectively removed after a lag phase. In the case of caffeine, substantial removal was only observed after 4 days, whereas 7 days were required for reductions in DEET and atenolol concentrations. These lag periods are longer than the longest HRT experienced in the SC OMEGA system trial and the exchanged volume of this system during the experiments was more variable for algal harvest and nutrient replenishment. The volume exchange ranged from ~18 percent to ~84 percent.

PPCP removal varies widely in conventional advanced wastewater treatment [36]. Treatment processes, such as ozonation or reverse osmosis, rapidly remove nearly all wastewater-derived PPCPs, but are energy intensive [36, 40-43]. Other, less energy intensive processes that rely on sequestration using activated carbon, soil aquifer systems, or constructed wetlands can remove >95 percent of PPCPs [35, 41, 44]. For many PPCPs, the removal in the OMEGA system is similar to biological treatment systems such as wetlands and soil aquifer treatment systems [42] and mechanistically it is likely that similar sequestration and biotransformation mechanisms are involved [36, 42].

4.4.6 Metals

Metals were measured in the SSF OMEGA system over the 13-day experiment (Fig. 5). Starting values for chromium were 0.6 μg/L, copper 9.9 μg/L, lead 0.8 μg/L, mercury 0.009 μg/L, nickel 3.5 μg/L, and zinc 110 μg/L. These values decreased in concentration for all metals except nickel, which did not significantly decline ($p > 0.05$). Zinc and copper concentrations declined by 48 percent and 55 percent respectively, whereas mercury, chromium, and lead concentrations decreased by 77 to 88 percent. Fitting the concentration data to a first order kinetic model yielded linear fits and allowed metal half-lives to be estimated. Lead had the shortest (2.8 day) half-life in the SSF OMEGA system; the half-lives of chromium (3.9 d), mercury (4.3 d), zinc (8.5 d), and copper (12 d) were longer. The most toxic heavy metals (mercury, chromium, and lead), were among the most effectively removed by the OMEGA system.

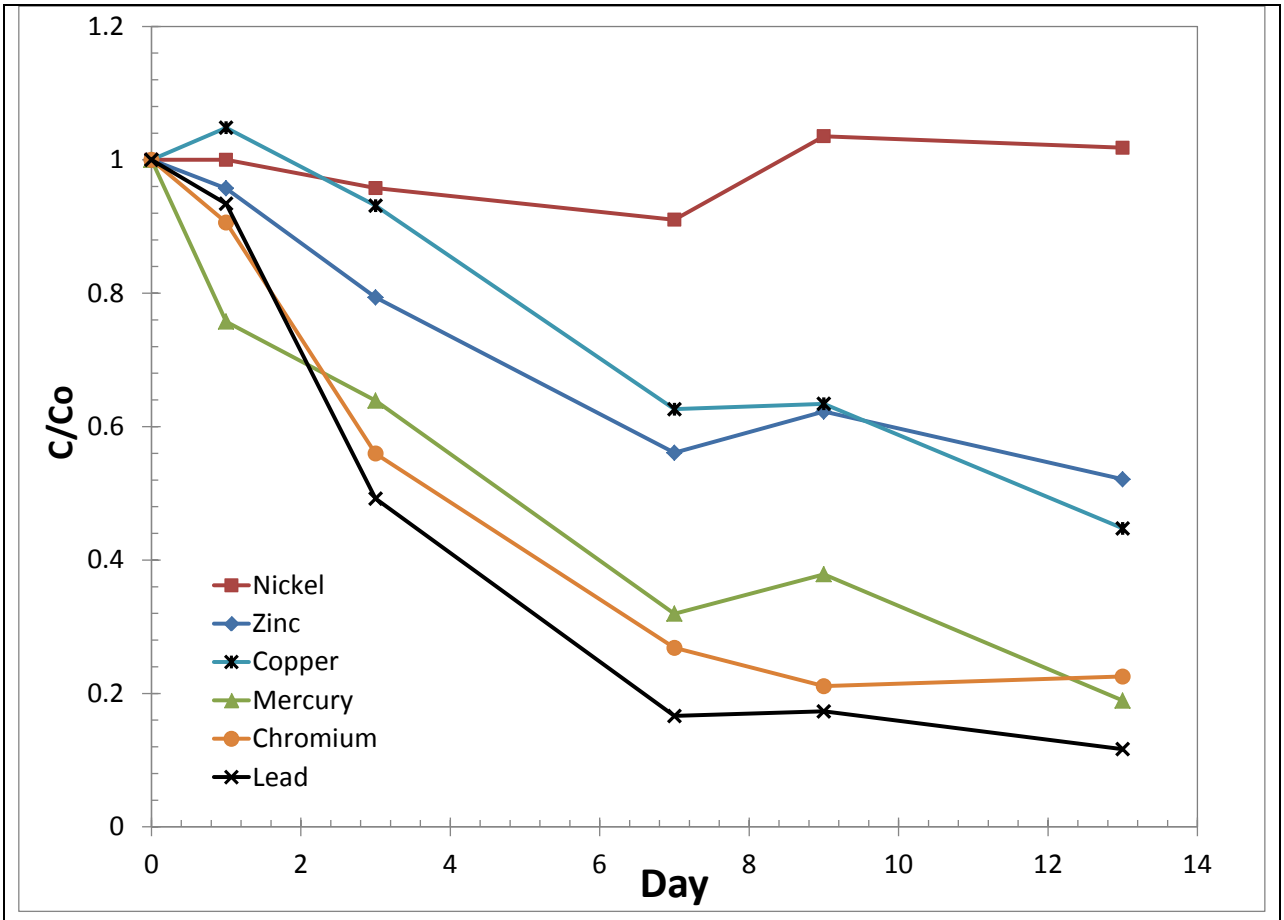

Figure 23: Changes in the Ratio of Final Concentration (C) to Starting Concentration (Co) of Metals in FPE During the 13-Days in the SSF OMEGA Dystem.

The lead, mercury, and zinc values reported after Day 8 are below the method detection limits and the ratios are based on estimated concentrations .

Removal of dissolved metals by algae is well-documented [10, 44] and reported mechanisms include biosorption to cell surfaces, chelation to extra-cellular polymers, ion exchange, redox transformations, and precipitation [11, 12, 46]. It is reported that algal sorption capacities for lead and mercury can account for >80 percent of sequestration [11, 12]. Slight reductions in removal rates with time (*e.g.*, chromium) may represent saturation of chelating and sorption sites for passive biosorption, with remaining removal depending on cellular uptake mechanisms [11]. Considering the low initial metal concentrations relative to algal biomass concentrations, it is unlikely that removal was limited by saturation of biosorption and chelation sites. The dominant removal mechanism depends on the chemical properties of the specific metal [11, 12, 46] and it is difficult to attribute mechanisms to the OMEGA system, which includes algae, bacteria, plastic surfaces, bubbling, and a dynamic flow rate.

The results suggest the OMEGA system is capable of "polishing" wastewater effluents by reducing metal concentrations, although additional research should validate whether these results extend to higher metal concentrations. Observed removal performance in the PBRs is similar to results reported for conventional advanced wastewater treatment processes, although performance again depends on the specific technology. Although their long term performance

is uncertain, similar removals are observed for many metals in biological treatment systems like subsurface flow constructed wetlands [44, 47].

4.4.7 Dissolved Organic Carbon (DOC), Inorganic Carbon (IC), and Pathogens

In the SSF OMEGA system, total carbon (TC), composed of DOC and IC decreased over the 13-day experiment, whereas BOD increased (Figure 6). The decrease in DOC and IC and the increase in BOD suggest that the composition of the organic matter was changing, presumably due to production of algal biomass.

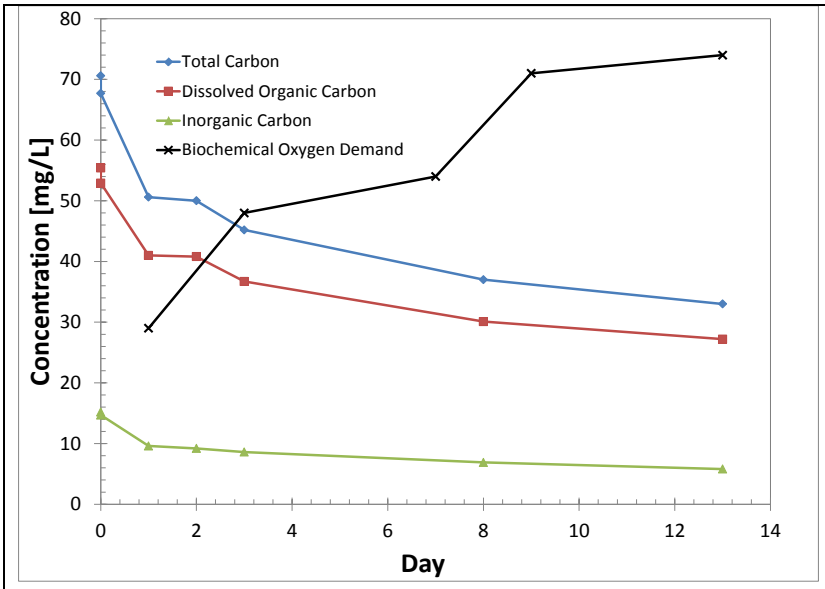

Figure 24: Concentrations of Total Carbon, Dissolved Organic Carbon, Inorganic Carbon and Biochemical Oxygen Demand (BOD) in the SSF OMEGA Dystem.

Additional research into the organic matter dynamics in the OMEGA systems is needed to clarify the relationship between organic matter composition and BOD.

4.5 Conclusions

Recent recognition of the need for alternative energy sources, especially those sources that meet "carbon neutral", "sustainable, or "renewable" criteria, has led to extensive research on algae cultivation for biofuels. Energy and economic considerations have established the need to integrate and co-locate algae cultivation with wastewater treatment facilities, which for coastal cities can be done with OMEGA. In two experimental OMEGA systems (~110 and ~1600 L) 22 pharmaceuticals and personal care products (PPCPs), including three steroids, and six metals were investigated. Some PPCPs were reduced by >95 percent, while others by <10 percent . Concentrations of zinc, copper, chromium, mercury and lead were all significantly reduced, but not nickel. DOC concentrations decreased, although increases in BOD and alteration in organic carbon composition were observed, suggesting that post-treatment to meet discharge requirements may be necessary. OMEGA, as well as other algae cultivation systems, warrant further investigation for their contribution to reducing wastewater-derived contaminants and advanced wastewater treatment.

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Chapter 5:
Microbiome Analysis of a Microalgal Mass Culture Growing in Municipal Wastewater in a Prototype OMEGA Photobioreactor

5.1 Summary

Large-scale cultivation of microalgae for biofuels may avoid competing for agriculture, water, and fertilizer by using wastewater and avoid competing for land by using the Offshore Membrane Enclosures for Growing Algae (OMEGA) system. Some microalgae thrive in wastewater, however wastewater also contains a complex mixture of organisms. An algae culture in wastewater from a San Francisco Treatment Facility in a 1600-L OMEGA culture system was monitored by standard methods. *Prima facie* evidence of a chytrid infection prompted a detailed investigation of the microbiome over a 13-day period using second generation sequencing of hypervariable regions of the small subunit rDNA genes. The observed bacteria, initially dominated by γ-proteobacteria, shifted to Cytophagia, Flavobacteria, and Sphingobacteria after addition of exogenous nutrients. The dominant algae genera introduced with the inoculum, *Desmodesmus* and *Scenedesmus* remained over 70 percent of the sequence reads on day 13, although the optical density and fluorescence of the culture declined. Nonalgal eukarya, initially dominated by unclassified alveolates, chrysophytes, and heliozoan grazers, shifted to chytrid fungi on day 5 and continued to day 13. The results of this microbiome analysis can facilitate the development of probe based surveillance systems for routine monitoring of large-scale microalgae cultures.

5.2 Introduction

The growing interest in microalgae-based biofuels has focused attention on the potential benefits of large-scale cultivation systems that use wastewater for nutrients and water [1,2]. Economic and energetic considerations show clear benefits from integrating algae cultivation with wastewater treatment, and under some circumstances net greenhouse gas emissions may be reduced [3,4,5,6]. An algal cultivation system proposed for coastal cities is Offshore Membrane Enclosures for Growing Algae (OMEGA) [7].

Many challenges to maintaining stable and productive large-scale microalgal cultures in wastewater exist. In developing pond management systems, detailed information about the complex and variable microbial consortium, particularly algae "weed species", grazers, and pathogens, would potentially be useful. Ample evidence indicates that some organisms may stimulate algal productivity [8,9], while others may be inhibitory or even catastrophic, such as viruses and bacteria [8,10,11], fungi [12,13,14], and grazers (e.g., Park et al. [5]). Although it is calculated that annualized algal production could reach 33-42 g m^{-2} day^{-1} [15], the actual U.S.

average annualized production is 13.2 g m^{-2} day^{-1} [16]. The discrepancy is, in part, due to pathogens and grazers (Ben-Amotz, personal communication).

Biomass losses in algae cultures can be rapid. For example, within three days, chytrid parasites can infect 100 percent of a *Haematococcus* culture [14], and rotifer grazers can ingest 200 algal cells min^{-1} $rotifer^{-1}$ [17] while doubling their numbers in 24 hours [18]. Therefore, efforts to maintain large-scale algae cultures will benefit from routine, detailed, fast and cost-effective monitoring methods for the community as a whole or for a subset of species that are indicative of the state of the culture. Potential monitoring methods include microscopy (e.g., Rasconi et al. [19]), PCR assays (e.g., Lakaniemi et al. [20] and Lefèvre et al. [21]), and flow cytometry coupled with digital imaging [22,23]. Each of these methods has limitations: optical methods cannot identify bacterial or viral agents, and may miss unusual morphotypes of eukaryotic agents. Probe based methods such as PCR require *a priori* knowledge of the biocontaminant and therefore cannot detect unusual or unanticipated species. When these methods fail, a more complete analysis of the microbial community may be required to identify the deleterious species the results of which can then be used to eliminate the blind spots in the routine monitoring process.

Microbiome analysis using second-generation sequencing (SGS) methods has been used to characterize naturally occurring algae blooms and to detect novel and unexpected species [24,25,26]. Current SGS methods that use the MiSeq platform (Illumina, San Diego, CA, USA) can generate >5 million 150-base DNA sequences in ~24 hours [27]. Sequences of hypervariable regions in rDNA genes provide measures of community diversity and relative abundance of archaea, bacteria [28,29] and eukarya [30,31]. Employing methods for the analysis of multiple samples in a single lane in a rapid sequencer (i.e., multiplexing) can provide the low-cost, rapid analysis applicable to the forensic analysis of algal cultivation operations. Here we demonstrate this principle by analyzing archived samples from a mixed algal culture grown in secondary-treated wastewater in a prototype OMEGA system over a 13-day period coincident with increasing and decreasing productivity and biomass indicative of a contaminated culture.

5.3 Materials and Methods

5.3.1 PBR Culture Conditions and Monitoring

Algae growth experiments were conducted in a prototype OMEGA system at the Southeast Wastewater Treatment Plant in San Francisco, CA (SEP) from May 22 to June 4, 2012. The system at SEP was based on a smaller prototype described in detail elsewhere [32]. The system at SEP was 1600 L circulating at 10 cm s^{-1} through four floating PBR tubes (0.2 m × 9.1 m) made of translucent 0.38-mm linear low-density polyethylene with a nylon core (Raven Industries, Sioux Falls, SD). Approximately 5 percent of the culture was continuously diverted through a gas exchange/harvesting column that removed dissolved oxygen (DO), added CO_2, and collected algae that settled in the column. A custom instrumentation system (CSS-Dynamac, Fairfax, VA) controlled CO_2 injection and monitored pumps, temperature, irradiance, DO, pH,

and CO_2 consumption (see Wiley et al. [32]). The system at SEP was repeatedly flushed with "#2 water," which is sand-filtered final plant effluent (FPE) treated with sodium hypochlorite (4-8 mg L^{-1}), maintaining a chlorine residual of 4 mg L^{-1} for 12 hours to eliminate contaminating microorganisms. The system was then refilled with #2 water and the hypochlorite neutralized with equimolar sodium metabisulfite (verified using a Hach spectrophotometer DR 2700 and Hach method #8167).

Stock cultures of mixed green algae were maintained in BG-11 medium (prepared using American Type Culture Collection protocol) in a room temperature, lighted incubator (4500 lux) with shaking. The stock culture consisted primarily of *Scenedesmus* sp. and *Desmodesmus* sp. (Family: Scenedesmaceae, 99.6 ±0.06 percent of reads from second-generation sequencing of the hypervariable small subunit rDNA V4 region – see description of methods in section *2.2. Sampling for Microbiome Analyses*). Stock cultures were scaled up to 150 L in carboys maintained in a greenhouse with aeration. CO_2 levels in the incubator and greenhouse inoculum cultures were manually maintained at pH 7.6-8.2. The OMEGA system was inoculated to a cell density of ~2.3×10^7 cells mL^{-1} [optical density (OD_{750nm}) = 0.34, total suspended solids = 0.124 g L^{-1}]. Cell density was inferred for inoculation from a linear fit for OD_{750nm} and cell densities generated for previous OMEGA experiments. On days 5, 8, and 10, nutrient-rich wastewater (18 L) from an anaerobic digester (centrate) was added to supplement nutrients. Cultures were analyzed daily for changes in optical density (OD_{750nm}), photosynthetic efficiency (i.e., fluorescence - Fm) using a fast repetition rate fluorometer (FRRF) [33] and nutrient concentrations using a Hach DR 2700 spectrophotometer (methods #10031, 8039, and 8048, respectively): ammonium (NH_4^+-N), nitrate (NO_3^-N), and reactive phosphate (PO_4^{3-}). During the experiment, CO_2 injections maintained the pH near a set point of 7.7 (see Wiley et al. [32] for description of pH control system).

Samples of the culture were evaluated daily by microscopy using bright field or phase contrast optics, with an Olympus IMT-2 inverted microscope (Olympus, Center Valley, PA) and a Leica MZ125 dissecting microscope (Leica Microsystems GmbH, Wetzlar, Germany). Formaldehyde fixed samples or unfixed samples were used for fluorescence imaging. For fixed samples, 15 mL of culture was centrifuged (1600 × g, 10 min) and the pellet resuspended in 1 mL Dulbecco's phosphate-buffered saline (Gibco). 340 µL 16 percent formaldehyde (methanol free, Thermo Pierce) was added and the samples stored at 4°C until imaging. For fluorescence imaging, 190 µL sample was mixed with 5 µL calcofluor white stain (Sigma) and 5 µL 10 N NaOH before viewing using the DAPI filter on an Olympus Leica DMRX compound microscope. Micrographs were recorded with a SPOT Idea 3 camera (Diagnostic Instruments, Inc., Sterling Heights, MI).

5.3.2 Sampling for Microbiome Analyses

Samples (50 mL) were collected from the PBR 11 times over the 13-day culture period. Samples were also taken from the inoculum, the neutralized #2 water used as the algal media, from the

FPE, and from a biofilm that formed on the swirl vanes inside the PBR. Biomass from the 50 mL samples was harvested by centrifugation (1600 × g, 10 min) and preserved with RNAlater® (Qiagen, Valencia, CA, USA). Preserved samples were shipped overnight on ice to Sandia National Laboratories for microbiome analyses.

5.3.3 Sequencing Library Preparation

Genomic DNA was purified from each sample using the ZR Fungal/Bacterial DNA MiniPrep™ Kit (Zymo Research, Irvine, CA, USA) from a pellet obtained from 20 mL PBR culture (< 200 mg pellet weight). Sequencing libraries were prepared by amplifying the hypervariable region V4 of the eukaryotic small subunit (SSU) rDNA and the hypervariable region V6 of the bacterial SSU rDNA. The V4 libraries were prepared directly from purified genomic DNA using primers TAReuk454FWD1 and TAReukREV3 and conditions described by Stoek et al. [34], except that the primers were modified to include a hexanucleotide barcode and Illumina adapters. The V6 libraries were prepared using a nested PCR process using the chloroplast-excluding primer set 799F and 1492R [35,36] in the first round. Amplification reactions contained 17.2 μL Platinum® PCR SuperMix (Life Technologies, Grand Island, NY, USA), 0.2 μM final concentration of each F/R primer, and 20 ng genomic DNA in a volume of 20 μL. Cycling conditions were an initial denaturation at 94°C for 3 min; 24 cycles of 94°C for 30 s, 53°C for 30 s decreasing 1°C each cycle, and 72°C for 1 min; 12 cycles of 94°C for 30 s, 45°C for 30 s, and 72°C for 2 min; and a final 10-min extension at 72°C. PCR products were visualized by agarose gel electrophoresis, cleaned and concentrated to 20 μL using a DNA Clean & Concentrator™ kit (Zymo Research). The second round of PCR was carried out on the purified product using V6 specific primers [37] to which a barcode and Illumina adapters were added as above.

The amplification conditions for V6 followed Gloor et al. [37] except with a starting annealing temperature of 51°C. Barcoded PCR products were cleaned and concentrated as above. Each sequencing library was generated in triplicate as technical replicates of the amplification process. Multiplexed V4 and V6 libraries were prepared from one replicate (rep 1) prepared by pooling 20 ng of each of 18 barcoded products (4 of which were not included in this study) and concentrating again into 20 μl of nuclease-free water, resulting in a final library concentration of 3-4 ng μL^{-1}. Combined multiplexed libraries (30 barcoded products each) were created from the remaining two technical replicates (rep 2 and 3).

5.3.4 Second-Generation Sequencing (SGS) methods and Data Analysis

Each multiplexed library was sequenced (150 bps, paired-end) in a single lane of a MiSeq flow cell (Illumina, San Diego, CA, USA). For V4 (575 bp in length), the library was diluted to 8 pM in HT1 buffer (Illumina) and mixed 3:1 with PhiX 174 (8 pM) paired-end library DNA (Illumina). For V6 (262 bp in length), the library was diluted to 5.5 pM in HT1 buffer and mixed 7:3 with PhiX 174 (5.5 pM) library. Demultiplexed FASTQ files containing sequence and qualities were produced by the MiSeq RTA software and saved for downstream analysis.

Raw reads were processed by Qfilter, a custom preprocessing Perl script. Low-quality tails were trimmed from reads in the manner of BWA [38], library primer sequences (entire or partial) were then trimmed from reads, and low-complexity sequence was masked with DUST [39]. Reads were rejected if length was < 30 nt, if ≥ 3 ambiguous bases were present, or if average positional quality score was < 30. Low-complexity sequence was then unmasked for the retained reads. Read pairs were rejected if either of the mates were rejected. We downloaded Silva release 111 SSU Ref rRNA database [40] and edited it by removing sequences with poorly resolved and uninformative taxonomic assignments. Accepted reads were aligned to our edited Silva index (modified from Pruesse et al. [40]) using Bowtie2 [41] in local mode (stringency set to "L,0,1"). Resulting alignments ("hit candidates") were scored by the BLAST raw bitscore convention, and those scoring less than the maximum candidate for each read were rejected. When the retained accepted hits spanned multiple taxa, final taxonomic assignment was resolved by Lowest Common Ancestor (LCA) analysis. Because assignments based on the 18S rDNA gene are not robust to species [42], we reported assignments to the genus level when possible. Read pairs were assigned to a single genus, or multiple genera when there was a tie (max. 5 genera); otherwise, read pairs were assigned a rank higher than genus (reported as "unplaced to genus"). The read pairs that did not have a match to our edited version of Silva 111 were then aligned to the full version of Silva. Hits to the full database are described further when a large portion of the reads were assigned to a single sequence. Correlation analysis was performed to determine the reproducibility of microbiome analysis. For each sample/timepoint, the observed raw taxonomic count was used to calculate the Pearson correlation coefficient between technical replicates. The calculations were performed at the family and the genus taxonomic levels.

5.4 Results and Discussion

5.4.1 OMEGA PBR Culture Conditions

Wiley et al. [32] described the details of the prototype OMEGA system, including the style of PBR, circulation system, sensors, and the control system, that was scaled up to 1,600 L and adapted for use at the San Francisco Southeast Treatment plant (SEP). During the 13-day experiment described here, final plant effluent (FPE) that was chlorinated and filtered (known as #2 water) was dechlorinated for use as growth medium in the OMEGA system. During the experiment, the temperature was maintained within 4°C (19.2-22.8°C), pH between 7.1 and 8.5, DO between 76 percent and 300 percent saturation, and the control system provided CO_2 on demand (Fig. 1). Temperature in the OMEGA system (Fig. 1a) was buffered from fluctuations in air temperature (12.6°C to 21.0°C) by the heat capacity and hysteresis of the 330,000-L seawater tank in which the PBR floated. The fluctuations in DO, pH and CO_2 (Fig. 1b-d) followed the diurnal (light-dark) cycle, reflecting the alternation between photosynthesis and respiration (Fig. 1: light-dark cycle indicated by vertical shaded and non-shaded bars).

During the day, DO production (Fig. 1b) and rates of utilization of CO_2 (Fig. 1d) indicated that photosynthesis was occurring throughout the 13-day experiment. FRRF showed no signs of light-limitation, photo-inhibition, or nutrient deprivation (data not shown). During the first four days, DO production was high and correlated with gradual increases in optical density (OD_{750nm} 0.4 to 0.6), maximum fluorescence (Fm 2000 to 8000), and the utilization of phosphate and ammonia (Fig. 2a&b). On days 5, 8, and 10, centrate was added to replenish nutrients. Although these added nutrients were utilized, neither OD_{750nm} nor Fm indicated significant increases in productivity during the last 7 days of the experiment (Fig. 2 days 5-13).

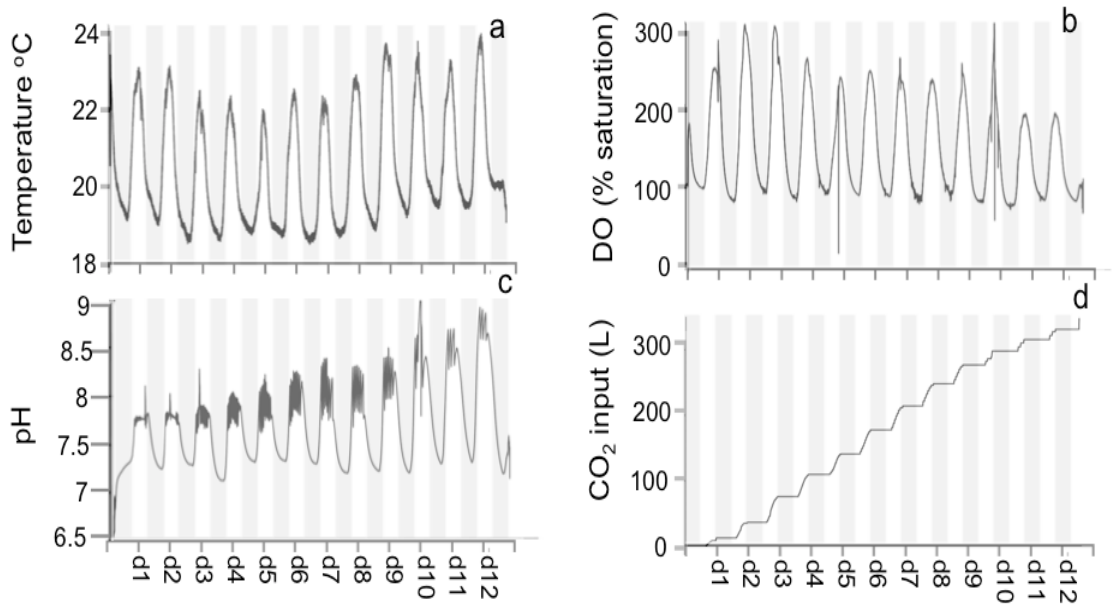

Figure 25: Variation in Photosynthetic Parameters Through Day-Night Cycles Over the Course of the Experiment

Data from the OMEGA control system were logged from the PBR at one-minute intervals. Parameters included a) temperature, b) percent DO, c) pH, and d) totalized CO_2 input to the PBR. Shaded areas correspond to night.

Throughout most of the experiment OD_{750nm} increased gradually, reaching the maximum (OD_{750nm} 0.65) on day 10 (Fig. 2a) and declining thereafter along with Fm (Fig. 2a), nutrient utilization (Fig. 2b), DO production (Fig. 1b), and CO_2 utilization (Fig. 1d).

The analysis of the OMEGA culture at SEP using standard physical and chemical PBR monitoring methods (temperature, pH, DO, CO_2, nutrients, OD_{750nm}, FRRF, and microscopy) provided information about changes that were relevant to the algae culturing conditions and background information for the detailed analysis of microbial diversity by metagenomic methods.

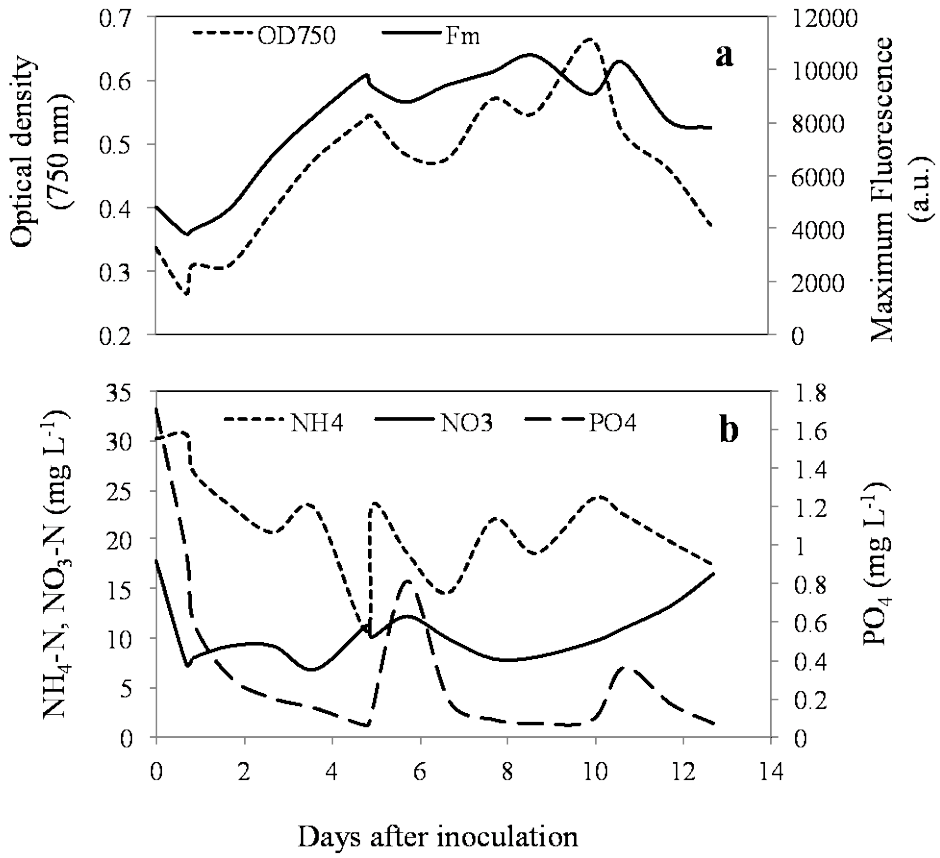

Figure 26: Algae Growth and Nutrient Utilization Over the 13-Day Experiment in the Prototype OMEGA PBR. (a) Optical Density (OD 750 nm) and Maxiumum Fluorescence (Fm). (b) Concentrations of Ammonium (NH_4-N), Nitrate (NO_3-N), and Phosphate (PO_4). Nutrient Rich Wastewater (Centrate) Were Added on Day 5, 8 and 10.

5.5 Microbial diversity determined by microbiome analysis

In the microbiome analyses, millions of samples of taxonomically diagnostic fragments of the hypervariable region of rDNA genes were generated by PCR, sequenced using the Illumina MiSeq flow cell, and characterized using the Silva 111 rDNA database (Table 1). Over 4.3 million read pairs were generated each for the bacterial (V6) region and the eukaryal (V4) region for samples presented in this study. Of the generated read pairs, 92 percent of V6 and 93 percent of V4 were analyzed, of those analyzed 80 percent of V6 and 90 percent of V4 were assigned to sequences in the Silva database, and of the pairs in the database, about 90 percent of both V4 and V6 could be assigned genus-level identities. Approximately 9 percent of the total read pairs analyzed were reported as "unplaced to genus," either because the associated sequences in the Silva database were without known taxonomy (i.e., classified as "uncultured organisms") or they were assigned to >5 genera and therefore identified with a taxonomic level above genus. The 19 percent of read pairs analyzed for V6 and 9 percent for V4 that could not be assigned to sequences in Silva using our criteria were reported as "unhit in Silva". Replicate databases 2 and 3 generated for each variable region were identical (r > 99.9 percent), thus only

correlation analyses between rep 1 and 2 are reported. Replicates for both variable regions were highly correlated at the family level (mean correlation [± 1SD] for V6: r = 87.4 ±9.8 percent; for V4: r = 99.9 ±0.06 percent). However, only the V6 datasets were highly correlated at the genus level (mean correlation [± 1SD] for V6: r = 88.7 ±8.7 percent; for V4: r = 41.7 ±29 percent). The poor correlation for V4 was a technicality of the fact that the rRNA sequences in that region for the two dominant algae genera were indistinguishable and therefore assigned arbitrarily in each dataset. Lumping *Desmodesmus* and *Scenodesmus* in these datasets for the correlation analysis resulted in much higher reproducibility for V4 (r = 92.2 ±8.2 percent).

The relative proportions of different taxa in the FPE; the FPE after chlorination, filtration, and dechlorination (chl #2); in samples from the PBR during the 13-day time series; and in the biofilm on the walls of the PBR (biofilm) are shown for bacteria (Fig. 3) and eukarya (Fig. 4). Many of the organisms identified are commonly found in wastewater [43,44].

Table 3: Counts of Paired-End rRNA Gene Sequences Obtained From the Illumina MiSeq Flow Cell for 18 Samples at Each Metagenomic Analysis Step.

	V4	V6
Read pairs generated[9]	5,825,393	4,908,888
Read pairs analyzed with pipeline	5,435,182	4,523,540
Read pairs with hit to full Silva	4,848,865	3,732,340
Read pairs mapped to a genus	4,381,500	3,355,505
Median sample coverage	339,929	269,447

[1] Read pairs retained after initial quality filters and processed using the metagenomic data analysis pipeline and ultimately assigned to genus level taxonomies. Undetermined reads, including those contributed by PhiX, were excluded from further data analysis and from above counts. Once read pairs were passed through initial quality filtering and the analysis pipeline, 75% for V4 and 68% for V6 were successfully assigned to the genus-level. Approx. 8% of the total read pairs generated were either assigned to sequences in the Silva 111 rRNA database that did not have a taxonomy (i.e., uncultured organisms) or were assigned to more than 5 other genera and, thus ultimately assigned to the next highest common taxonomic level. In both cases, reads were reported as 'unplaced to genus'. The remaining 10% of read pairs for V4 and 16% for V6 could not be assigned to any sequences in Silva (reported as 'unhit in Silva') and may have been sequence artifacts due to PCR errors (Acinas et al., 2005).

5.6 Bacterial diversity and changes with time

In the bacterial analysis, a large proportion of the sequences obtained throughout the study were not found in Silva (unhit to Silva), not identified at the genus level (unplaced to genus), or classified as "minor genera" (Fig. 3: gray bars). Of the read pairs assigned at the genus level, *Clostridium* and γ-proteobacteria dominated the FPE, #2 water, and PBR on the day of inoculation (Fig. 3). During the first three days, *Clostridium* abundance was greatly reduced and γ-proteobacteria increased in the proportion of reads, with *Shewanella*, a common wastewater microbe [44], in greatest abundance (Fig. 3). As the γ-proteobacteria steadily declined, the flavobacteria and the α-proteobacterium *Rhizobia* became abundant. *Rhizobia*, which are usually soil bacteria associated with nitrogen fixation, was by far the most abundant genus recovered from the inside surface of the PBR (Fig. 3: biofilm 13 days after inoculation). Notably, none of the groups that developed in the PBR were human pathogens.

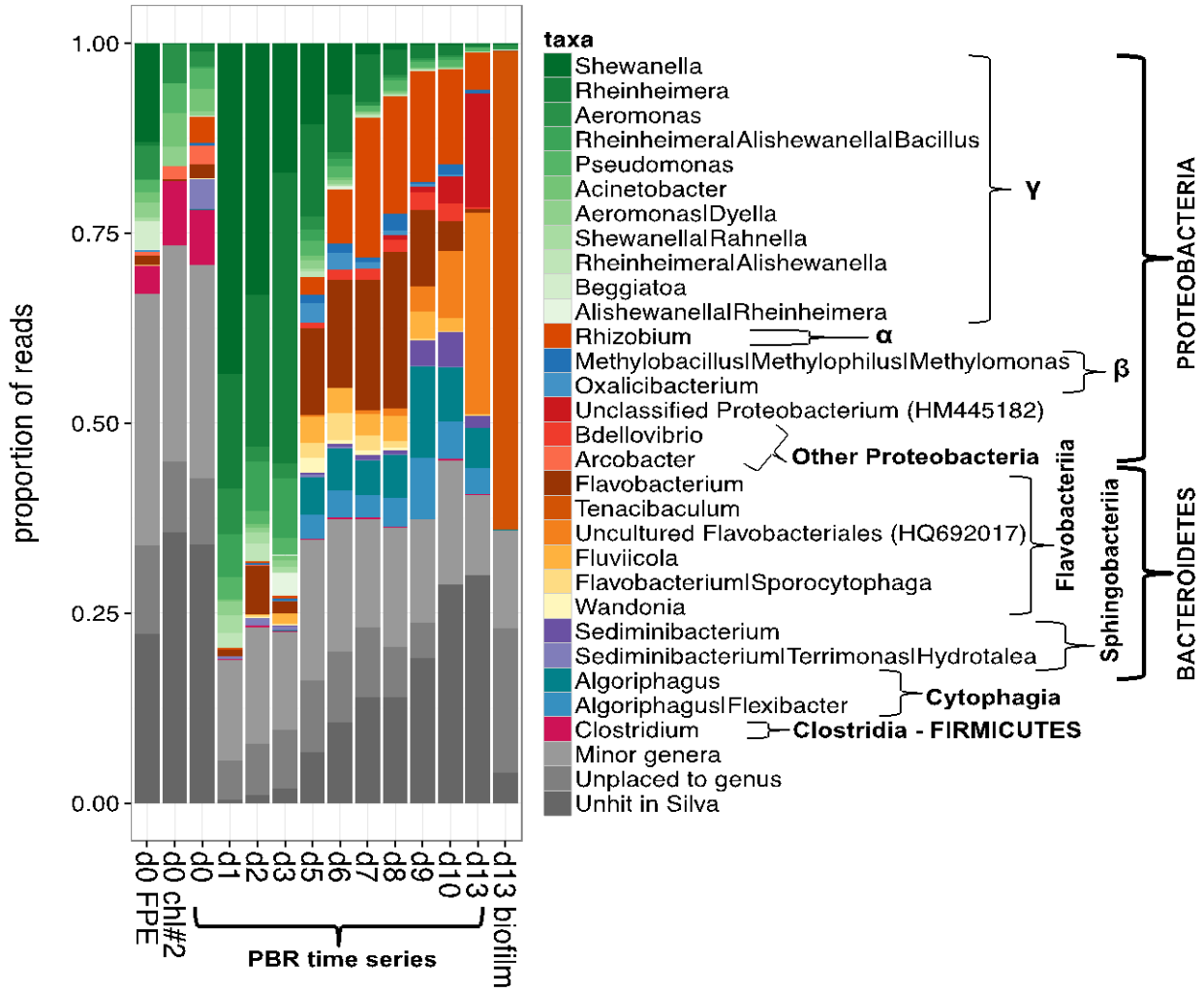

Figure 27: Metagenomic Genus-Level Identifications of Bacterial Sequences.

Results are based on the bacterial hyper-variable region V6 and are shown in order of decreasing abundance (Class and Phylum in bold) for samples described in Figure 3. When sequence assignments

were tied between fewer than six genera, genera are listed connected by " | "; genera comprising < 1.8% of at least one sample were grouped into 'minor genera'; read pairs that passed quality and were run through the data analysis pipeline but were assigned a taxa not including genus are indicated by 'unplaced to genus'; and, read pairs not assigned a taxa (i.e., no hit) are indicated by 'unhit in Silva'. General groups are identified by brackets (right).

The bacteria introduced with the wastewater decreased in abundance within the first few days of the 13-day experiment and bacteria associated with algae became the most abundant (Fig. 3). Specifically, the γ-proteobacteria were initially most abundant, with a peak in the widely distributed *Rheinheimera* three days after inoculation [45]. By day 5 the community began to diversify to other types of proteobacteria, Flavobacteriia and Cytophagia (Fig. 3). Species within the *Flavobacterium* and *Rhizobium* genera are known to stimulate the growth of some algae [9,46], while members belonging to Cytophagia and the genus *Bdellovibrio* are potentially pathogenic. *Bdellovibrio* has been reported to lyse green algae (reviewed by Cole [8]), but it could not be determined if this strain or other lytic strains (genus *Algoriphagus*) contributed to decline of algal biomass after day 10 (see Figs. 1&2). By the end of the experiment, bacteria with unknown taxonomies were most abundant (Fig. 3; an unclassified *Proteobacterium* and an uncultured member of the Flavobacteriales). *Pseudomonas*, originating from the wastewater, was consistently present at low levels in all samples and is known to stimulate productivity through glycoprotein production [47], but also compete with algae for phosphate [8] and have a direct algicidal effect [48].

5.7 Eukaryotic diversity and change with time

In the eukarya analysis (Fig. 4), a much smaller proportion of unhit, unplaced, and minor genera (Fig. 4: gray bars) was found. Prior to treatment and inoculation with microalgae, the FPE was dominated by "uncultured eukaryotes," with 50.3 percent of the read pairs matched to an Alveolate Accession # AY919735 [49] (Fig. 4: FPE). The alveolate superphylum includes ciliates, dinoflagellates, and other groups of photosynthetic and heterotrophic organisms that are expected to thrive in wastewater. AY919735 may be closely related to the parasitic protest perkinsea [50]. After the FPE was treated to become chl #2 wastewater, a different aveolate (DQ244038) was present and the dominant organisms were grazers, primarily *Carchesium* and *Epistylis*, which are peritrich ciliates known to be important bacteriovores and planktivores [43, 51,52,53]. As expected, the dominant eukarya in the freshly inoculated PBR were microalgae identified as *Desmodesmus* or *Scenedesmus* based on sequence information and most likely in the genus *Desmodesmus* based on microscopy (Fig. 4: day of inoculation).

During the 13-day experiment, the green algae in the genera *Desmodesmus* and *Scenedesmus* (Family: Scenedesmaceae) continued to dominate the diversity of eukaryotes in the PBR. Another green spherical alga detected was *Mychonastes* (Family: Sphaeropleales), but it was present at relatively low abundances, as were algae in the genus *Tetraselmis* (Family: Chlorodendraceae) and diatoms (Family: Bacillariaceae). As expected, pennate diatoms, in the

genera *Cymbella*, *Achnanthes*, or *Amphora*, which are known to associate with surfaces, were represented in the biofilm sample (Fig. 4: biofilm 13 days after inoculation).

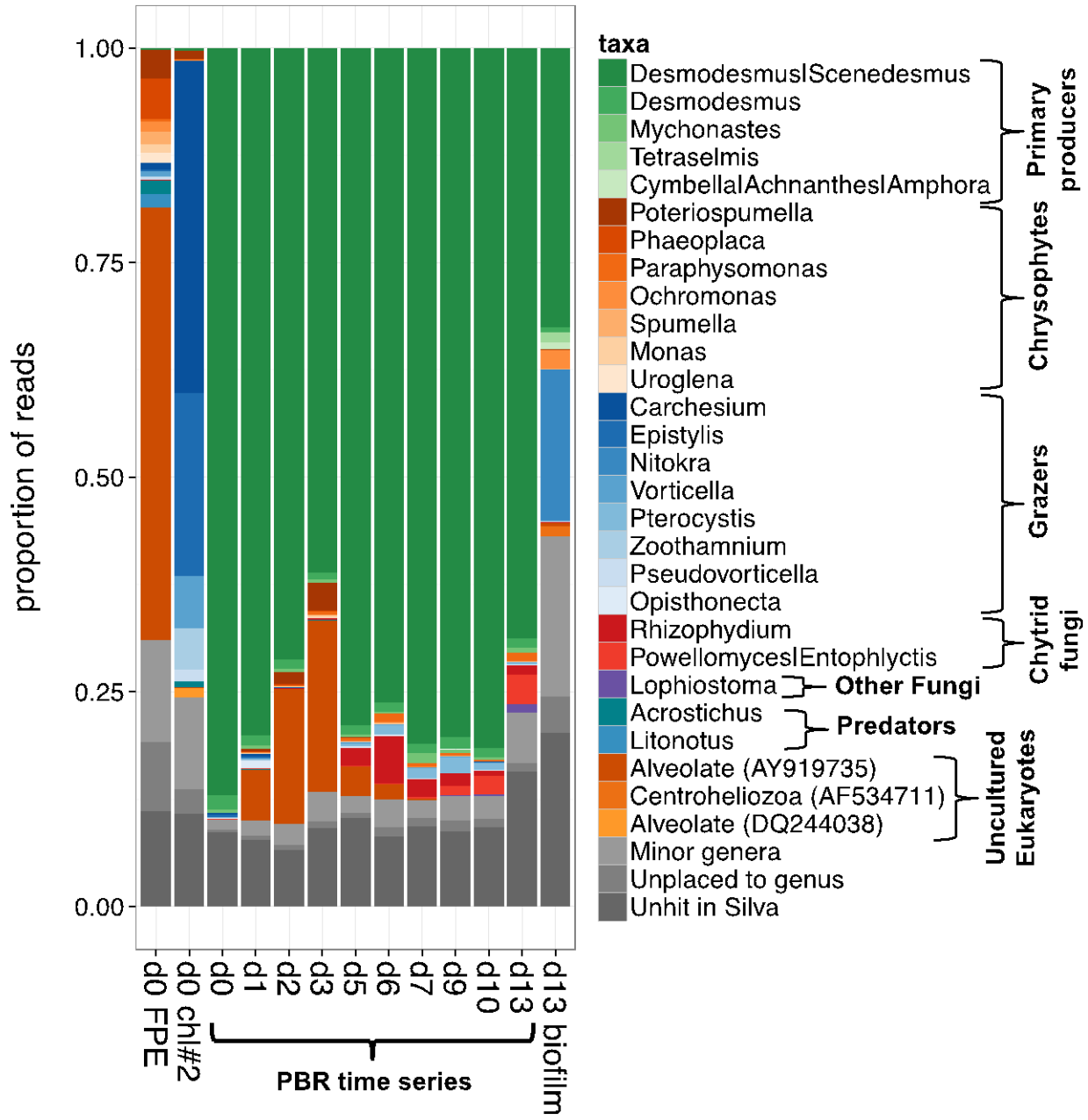

Figure 28: Metagenomic Genus-Level Identifications of Eukaryotic Sequences.

Results are based on the eukaryotic rRNA hyper-variable region V4 and are shown in order of decreasing abundance for the FPE, chlorinated #2 wastewater (chl#2), the 13-day time series (d0-d13), and the biofilm collected from the inside surface of the PBR after harvesting. Genera comprising < 0.8% of at least one sample were grouped into 'minor genera'.

5.8 Parasites and grazers

Microscopy on samples from the PBR confirmed the abundance of green spherical algae as well as potential parasites (Fig. 5). Cells with morphologies indicative of *Desmodesmus* were abundant (Fig. 5a), but as the culture progressed, empty cells became apparent (Fig. 5b) and calcofluor-white-stained samples from day 5 viewed by fluorescence (Fig. 5c) revealed the presence of chitin within the remnants of lysed algae cells and structures resembling both the encysting zoospores of chytrids [54] (Fig. 5d-f) or aphelids, an understudied basal group of fungi that parasitize green algae [55]. However, aphelid sequences were not detected by comparison to the Silva 111 database or to a known aphelid sequence (Accession # JX507298).

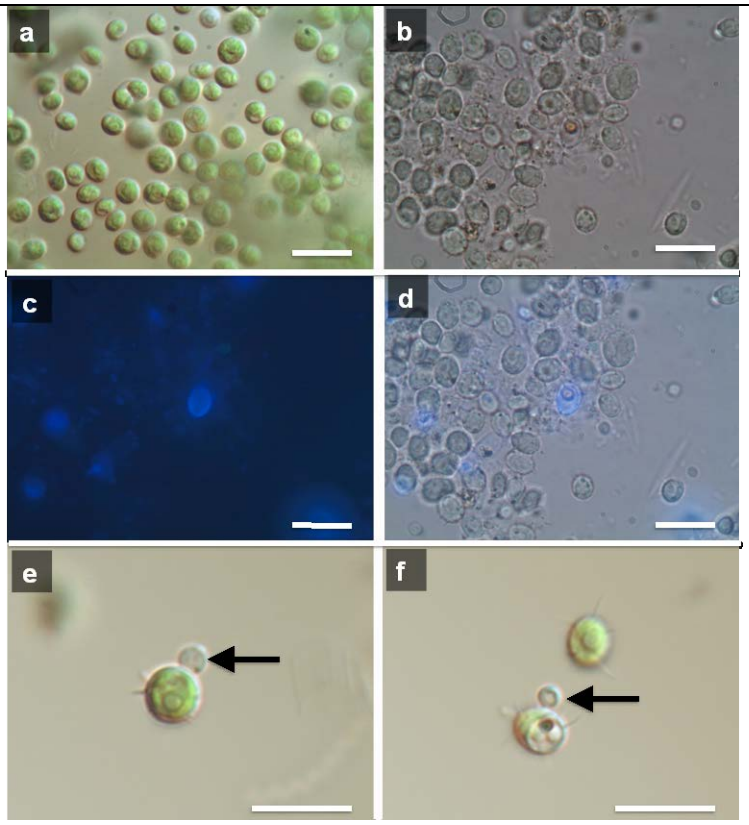

Figure 29: Microscopy of OMEGA Culture Samples.

a) Nomarski image of stock inoculum on the day of inoculation; b) bright field image of cells from the PBR fixed with formaldehyde and stained with calcofluor white; c) same field, fluorescence image showing staining indicative of chitin; d) same field, overlay of brightfield and fluorescence; e) Nomarski image of an algae cell with an encysted zoospore attached to its surface; f) later stage of fungal infection; this image shows an algae cell with a chytrid-like structure attached to its surface, which might be an emerging zoospore. Scale bars in all images, 10 µm. b-f, 5 days after inoculation.

One day after inoculation the most common taxa of eukaryotic heterotrophs present in the PBR was the alveolate also identified in the FPE (Figs. 4 and 6). The relative abundance of these organisms increased from days one to three and then the population declined rapidly from day five, onward.

The most prevalent eukaryotic heterotrophs identified to genus level in the PBR were chytrid fungi (Figs. 4 and 6). Notably, a member of the genus *Rhizophydium*, which includes obligate parasites of green algae [56,57], was identified and is known to occur in both open and closed cultivation systems [12,14]. A BLAST search was performed on a haphazardly selected group of

read pairs that were assigned to genus *Rhizophydium*, resulting in 100 percent identity with sequences from two species known to attach to green algae: *R. chlorogonii* [58] and *Rhizophydium* sp. JEL 317 (isolated from a green alga growing in a commercial PBR; J. Longcore, personal communication). *Rhizophydium* was detected 5 days after inoculation, peaked in relative abundance on day 6, and decreased in abundance thereafter (Fig. 6). Increasing *Rhizophydium* abundance appeared to coincide with a decrease in productivity (Fig. 2a). Another chytrid, either *Powellomyces* or *Entophlyctis*, appeared and increased in abundance from day 6 through the end of the experiment (Fig. 6). These two genera are in the orders Spizellomycetales and Chytridiales, respectively [54,59], which includes both algal parasites and saprobes living on organic matter [54].

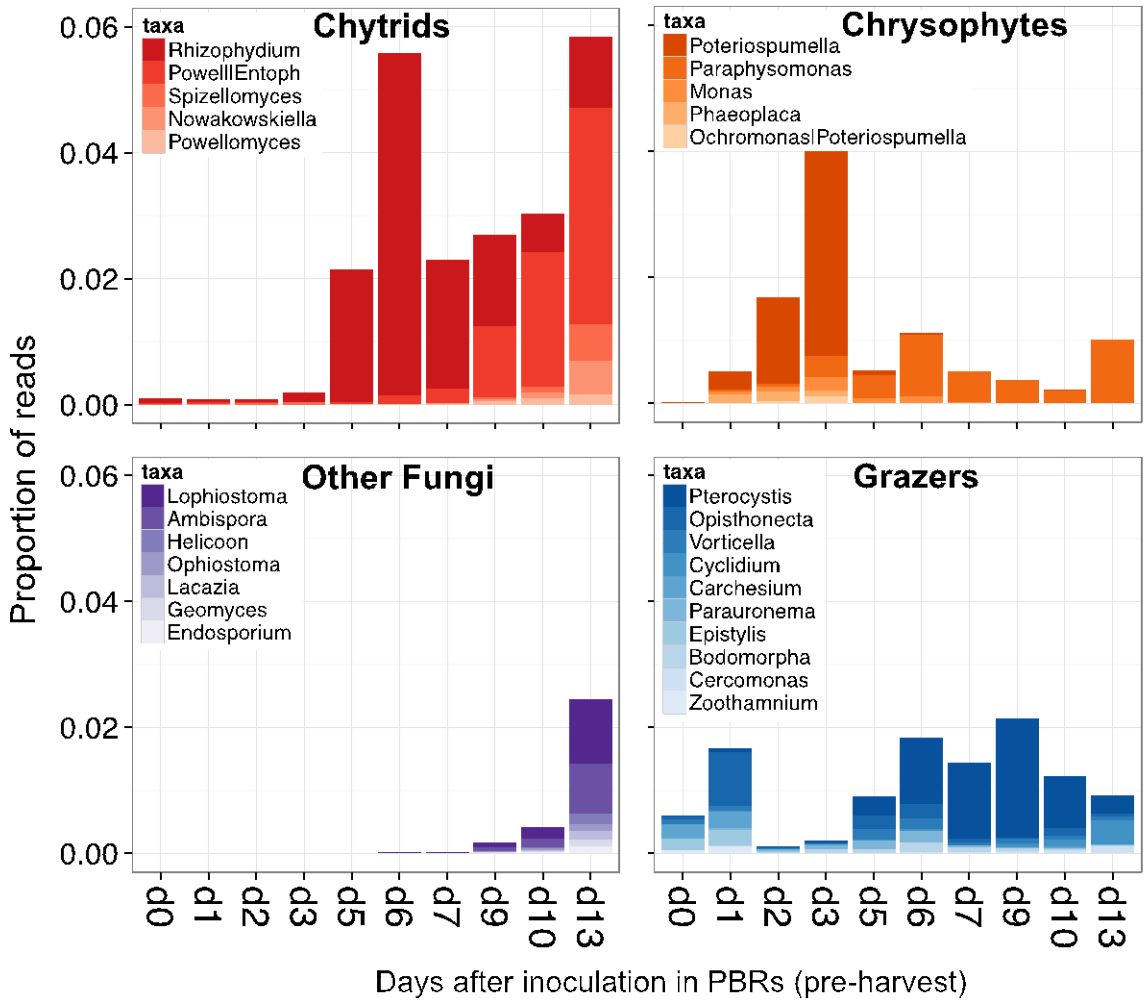

Figure 30: The Most Abundant Eukaryote Groups (Excluding Obligate Autotrophs) in the PBRs Shown in Figure 3.

Genera comprising < 0.106% of at least one sample are not shown; read pairs assigned to both *Powellomyces* and *Entophlyctis* are shown as 'Powell|Entoph'.

Because parasitic chytrid genera were the most abundant eukaryotic heterotrophs by the end of the experiment and appeared to increase in relative abundance as algal productivity decreased

~6 and 10 days post-inoculation (Fig. 2a), chytrids were implicated in the reduction of algal biomass. Non-chytrid saprobic fungi were also detected during the final days of the experiment (Fig. 6), suggesting that these fungi were exploiting the accumulation of decaying algae.

Several non-algal eukaryotes (ciliates, small flagellates, nematodes, and rotifers) were detected throughout the 13-day experiment. The SGS-based microbiome analysis detected, identified, and to some extent quantified a diversity of eukaryotes, including protozoan grazers and fungi (Fig. 4). The unclassified alveolate (Accession # AY919735) detected in the FPE was abundant during the first 5 days of culture (Fig. 4). The planktonic heliozoan grazer *Pterocystis* [60] peaked in relative abundance on day 9. Heterotrophic nanoflagellates were the most abundant grazers. *Poteriospumella* [61] increased through day 3, and *Paraphysomonas* [62] increased slowly throughout the experiment (Fig. 6).

5.9 Conclusions

Conditions in a prototype OMEGA system, including temperature, pH, DO, optical density, fluorescence, CO_2, and nutrient concentrations, were monitored over a 13-day period and compared to a detailed analysis of microbial diversity and dynamics using second generation sequencing (SGS). By analyzing sequences of millions of amplified gene fragments, microbiome analysis provided a comprehensive view of microbial diversity, including low-abundance species and bacteria, which cannot easily be characterized in detail by other sequence-based methods or microscopy. The SGS method used here had the advantage over probe-based methods, such as PCR, of not requiring advanced knowledge of the organisms present. Further, by multiplexing samples a single SGS MiSeq system can be highly efficient at generating sequence information to identify organisms.

Combining the conventional algae culture measurements of OD, fluorescence, and nutrient utilization with the detailed information from SGS provides insights into the development of the culture in the context of the microbial community. In the experiment described here with the OMEGA system, the conventional methods revealed three distinct culture conditions or periods. During the first period (days 1-5), the microalgae proliferated; during the second period (days 5-10), the culture was in a relatively stable state with only transient decreases in biomass; and the third period (days 10-13) was a time of microalgae decline (Figure 2).

Superimposed on these apparent periods of rise, stability, and decline of the algae culture, SGS analyses revealed dynamic microbial community structure. For example, a few bacterial genera dominated during the first three days of the culture and diversified after day 3 with organisms capable of stimulating algal productivity (e.g., *Rhizobium*, *Flavobacterium*) as well as competitors, parasites, and pathogens (e.g., *Bdellovibrio* and *Algoriphagus*). Alveolates and grazers of algae, such as the heliozoan, *Pterocystis*, and the chrysophytes, *Poteriospumella* and *Paraphysomonas*, abundant from day 1 to day 3, were supplanted by chytrid fungi after nutrient (centrate) addition on day 5. Although the relative abundance of alveolates was high during the first

phase of the culture it does not appear that they had a discernable effect on algal productivity. Chytrids, which were low in abundance initially (Fig. 5), spiked in abundance on days 6 and 7 and after day 10, coinciding with reductions in optical density and fluorescence of the culture (Fig. 2). This increase in parasite load may have been responsible for ultimately reducing algae productivity.

In the process of characterizing the dynamics of a microbiome within a single cycle of a closed algae cultivation system, we have demonstrated that microbiome analysis can be instrumental in determining the identity and relative abundance of critical organisms in the microbial community. We have shown that, in a case where a PBR performance is sub-par or atypical, a retrospective microbiome analysis of archived samples can provide explanations that could help guide management strategies in the future. In general, timely information about the diversity, relative abundance, and dynamic changes of key organisms will be helpful in managing the stability, productivity, and thereby the overall performance of any algae cultivation system in PBRs or open ponds.

As the speed and accuracy of HTS continue to increase and the costs decrease, its use for routinely monitoring of complex communities, such as microalgae growing in wastewater, will become feasible for different cultivation systems and a valuable database on microbial communities will emerge for each system. These databases will inform the development of specific probes or biomarkers for both advantageous and deleterious species, which will allow rapid near-real-time monitoring of production systems, tracking organisms of interest, and providing a rational basis for effective management practices.

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Chapter 6:
Marine Mammal and Seabird Interactions With Model OMEGA Photobioreactors

6.1 Summary

OMEGA (Offshore Membrane Enclosure for Growing Algae) is a system to produce biofuels and treat wastewater in offshore photobioreactors (PBRs) that will require an enormous floating infrastructure in marine bays. The potential impact on marine mammals and birds was investigated using 1.5 x 1.5 m flat-panel PBR's in tanks with captive pinnipeds and using 9 x 1.3 m flat panel and 9.5 x 0.2 m tubular PBRs at a boat slip in Moss Landing Harbor (36.804°N, -121.785°W) to observed wild animals. A trained harbor seal (*Phoca vitulina richardii*) and two trained California sea lions (*Zalophus californianus*) followed most commands to bite, drag, and haul-out onto PBRs, but there were no uncommanded interactions observed. Wild animal interactions with PBRs were recorded day and night for 2,424hrs (October 10, 2011 to Jan 22, 2012) using digital infrared video, and documented one interaction with *Z. californianus*, 94 interactions with the sea otters (*Enhydra lutris*) and 1350 interactions with birds, mostly coots (*Fulica americana)* and gulls (*Larus occidentalis* and *L. californicus*). Otter and bird interactions, characterized as passive (feeding, walking, resting, grooming, and social activity), or proactive (biting, pecking, investigating, and unspecified manipulating) were mostly during the day. Otter interactions were 5x more likely to be observed as proactive than bird interactions, which were predominantly passive. The duration of interactions ranged from 1 to 190 min were not significantly different between mammals and birds, but varied between bird species. Most (96 percent) otter interactions occurred in the winter (4 percent in fall), whereas most (73 percent) bird interactions in fall; the times when otters and birds are most abundant in the harbor.

All otter and bird interactions with PBRs were benign for both animals and PBRs, although Instron experiments with a sea otter canine tooth and gull beaks indicated that with sufficient force these animals are capable of penetrating the 15-mil linear low density polyethylene (LLDPE) PBR plastic and that weathered plastic (12-weeks of exposure in harbor) was weaker than new plastic. Longer studies with larger OMEGA systems are needed to determine if a full-scale OMEGA systems will have net positive or negative environmental impact.

6.2 Introduction

The biofuels community has shown considerable interest in the possibility that microalgae could contribute significantly to providing a sustainable alternative to fossil fuels that would not compete with agriculture (Chisti 2008). Indeed, microalgae species can be grown on domestic wastewater to avoid competing for freshwater and fertilizer (Woertz et al. 2009) and the OMEGA system has been proposed for large-scale algae cultivation located offshore that avoids competing for land (Trent *et el.* 2012). In the OMEGA systems, fast-growing, oil-producing freshwater algae are grown in flexible plastic photobioreactors (PBRs) attached to floating docks, anchored offshore in naturally or artificially protected bays. The PBRs use wastewater and CO_2 from coastal facilities to provide water, nutrients, and carbon for the algae and the algae contribute to wastewater treatment by removing nutrients as well as toxins and contaminants (Mulbry et al. 2010). The surrounding seawater controls the temperature inside the PBRs and kills any algae that might escape (i.e., if a PBR module accidentally leaks, the freshwater algae that can grow in wastewater cannot grow in the surrounding salt water). It is suggested that the network of OMEGA photobioreactors and floating docks will support biofuels production, advanced wastewater treatment, solar photovoltaic installations, offshore wind turbines and wave generators, as well as aquaculture (McKuin and Trent, in preparation).

The OMEGA system of PBRs and docks in coastal waters represents a huge surface area that will inevitably be colonized by a diversity of sessile and associated organisms known to opportunistically attach to floating objects (Cole, Glasby, & Holloway 2005; *Connell 2000, Inger et al 2009, Dempster 2005, Reubens et al 2013b*). The biofouling organisms on model OMEGA PBRs and their impact on PBR function has been recently reported (Harris *et al*. 2013), but the interaction of coastal marine mammals and birds with OMEGA PBRs has yet to be described. Human activities in coastal areas, such as the construction of coastal cities with harbors, marina, breakwaters, dredging, ship traffic, and offshore platforms change the environment in ways that significantly impact the local ecology (Rosenberg et al. 2000; Bochetti et al. 2008; Kim et al. 2008; Saensgupavanich 2011). These changes can cause radical shifts in circulation and wave patterns, sediments, substrate availability, and many other features that impact the abundances of organisms, biodiversity, and community structure of coastal habitats (Piper *et al* 2002; Love & Schroeder 2004; Inger et al 2009; Lowe *et al* 2009, Reubens *et al* 2013). It is well documented that marine mammals and birds are influenced by both offshore and near-shore structures (e.g., piers, breakwaters, docks, wind turbines, oil & gas platforms, and wave generators). In some cases these structures are utilized as alternative resting, feeding, and/or breeding sites (Connel 2000), whereas in other cases they pose risks of collisions, entanglement, oiling, acoustic and electromagnetic "noise," habitat fragmentation, and foraging potential (Carstensen 2006; Burke *et al.* 2012; Martin *et al.* 2011; Murphy *et al.* 2012; Thompson et al 2013).
To treat wastewater from large coastal cities and produce significant quantities of biofuels, the OMEGA system will cover hundreds or thousands of hectares on the surface of protected bays

(Trent et al. 2012). Such an imposing floating infrastructure will, among other things, limit light penetration into the water column and to the benthos (Glasby 1999; Perkol-Finkel 2008), alter sea surface currents, and surface temperatures, create an artificial floating reef (Jaquemet et al 2004) and mitigate excess nutrients and toxins from sewage outfalls. For marine animals, it will limit access to the water column and change foraging opportunities. In addition, animals may damage the OMEGA system by biting or pecking the PBR plastic, hauling out on PBRs or docks and scratching, covering, or fouling surfaces.

Here we describe interactions of marine mammals and birds with small PBRs, using captive pinnipeds and wild marine mammals and birds. Captive, trained pinnipeds were observed and commanded to interact with floating and tethered PBRs in seawater tanks. Wild marine mammals and birds were observed in 96 days of day and night recordings of PBRs tied in a boat slip in a harbor using infrared video. The animals in the recordings were identified, and the nature, duration, and timing of their interactions were characterized. Laboratory experiments determined the forces required for the dominant animals to bite or peck through new and weathered PBR plastic.

These small-scale experiments suggest the interactions between coastal animals and OMEGA PBRs are benign, but larger-scale studies will be required to effectively manage the ecological impacts of full-scale OMEGA systems.

Materials & Methods

Observations of captive pinnipeds with PBRs

The responses of three pinnipeds to model PBRs was investigated in a seawater tank (7.6m diameter and 1.7m meters deep), using a 23 year-old male harbor seal (*Phoca vitulina richardii)*, and two 2 and 25 year-old, female California sea lions (*Zalophus californianus)* from the Cognition Laboratory at Long Marine Laboratory of the University of California Santa Cruz. All three pinnipeds were trained, but naïve to PBR's prior to testing.

Two PBRs (1.52m x 0.91m) made by Raven Industries (Souix Falls, SD) had translucent linear low-density polyethylene (LLDPE) on top and black LLDPE on bottom. They were filled with 114 liters of freshwater with some residual air to approximate the buoyancy of OMEGA PBRs. For the seal and younger sea lion, one tethered and one free-floating PBR was used. For the older sea lion both PBRs were tethered. Each animal was given previously learned and commonly performed commands including fetching the PBR by dragging it with its mouth, fore-flipper slapping the PBR, resting its head on the PBR, and jumping over, partly onto, or hauling-out on the PBR. The responses of each animal were recorded as a success or failure to perform the command with the PBR and the PBR was inspected for damage.

Video recordings of wild animals with PBRs

Nearly continuous video was recorded of PBRs from October 10, 2011 to January 22, 2012 using an infrared camera (Foscam FI8905W Outdoor Wireless IP Camera, Shenzhen, China). The wireless infrared camera was mounted adjacent to the PBRs and filmed 24 hours per day to opportunistically record animal interactions within its field of view. Two different PBR designs were observed: flat panel and tubular both made as prototypes by Raven Industries (Souix Falls, SD). The flat-panel PBR (9.5m x 1.3m) was made of 0.5mm translucent LLDPE (top) and opaque black 1.0mm LLDPE (bottom), whereas the tubular PBR (0.20m diameter x 9.1m) was made of 0.38mm translucent LLDPE (top and bottom). Both PBR designs were filled with freshwater, tethered to high-density floats to insure positive buoyancy, and secured alongside the dock at the Marine Operations Facilities of Moss Landing Marine Laboratories (36.804°N, -121.785°W).

The raw video was stored on a hard drive (Blue Iris Professional, Shenzhen, China) and transferred to a video-editing program (Adobe Premiere Pro-CS5; Ottawa, ON, CAN) to view at 7x normal speed and mark segments with animals. Animal segments were transferred to a separate digital file and viewed in real-time for (1) animal identity (genus and species when possible), (3) the nature of the interaction (behavior), (3) the duration of the interaction, (4) the time of day (morning, afternoon, evening, and night) and (5) the date. For animal identities, marine mammal and bird experts were consulted in case of ambiguity or the animals were characterized as "unidentified."

The nature of the interactions between animals and PBRs was described as passive, which includes feeding, walking, resting, grooming, or socializing) or proactive, which includes directly pecking, biting, rolling on, investigating, or potentially damaging PBRs. These behavior codes are shown in Table 1.

Table 4: Descriptions of Mammal and Bird Behavior Codes

Code	Category	Description
	Passive	
1	Feeding	masticating food items on or near PBR
2	Walking	ambulating, crawling, hopping, hauling out (mammals) or flying across PBR (birds)
3	Resting	perching (birds), sleeping, sitting, or floating motionless on top of PBR
4	Grooming	preening feathers (birds), or rubbing fur (mammals)
5	Social behavior	preening, beak fighting, and food parasitism (birds), or vocalization with another animal or group of animals interacting on the PBR
	Proactive	
6	Investigative, or, potentially harmful	Directly pecking, biting, rolling, or investigating PBR or associated tethers and buoys
7	Unspecified contact, or manipulation	Swimming underneath, or unspecified behavior associated with PBR

The time of day for each interaction was recorded to the nearest minute and compiled as morning (0600-1159), afternoon (1200-1759), evening (1800-2359), and night (0000-0559). Duration also was recorded for each interaction, and the proportion of time mammals and birds interacted with PBRs daily also was described.

Forces required to puncture PBR plastic

The force required for a sea otter tooth and seabird beaks to puncture new and weathered PBR plastic (LLDPE) was measured using an Instron 3342 compression testing system (Instron, Norwood, MA). A canine tooth from a dead beach-cast sea otter (*Enhydra lutris*) and beaks from a glaucous-winged gull (*Larus glaucescens*) and California gull (*L. califonicus*) were used to puncture test samples of new and weathered LLDPE—the weathered plastic was recovered from an OMEGA PBR that was in Moss Landing Harbor for 12 weeks. Samples of LLDPE (17.8 cm x 17.8 cm) were secured to a moving force transducer and lowered onto the beak or tooth mounted in the base clamp of the Instron and peak forces were recorded following the manufacturer's instructions.

Data analyses

Statistical analyses (PSAW Statistics: 19.0, IMB, USA) used criteria for parametric tests unless stated otherwise. Statistical significance was assumed for an alpha level <0.05, and results are expressed as mean ± standard error (SE).

Differences in the time and numbers of observed interactions between animals and PBRs were tested using individual Peasron's chi-squared tests (or Cochran's chi-squared if *df*=1). Student's t-tests were used to compare means of the interaction durations, but interactions <30 seconds were excluded from analyses. The means of the interaction durations for the different groups of birds and for the mammals were compared to birds using a fixed factor ANOVA. Logistic regression was used to test if animal type (mammal or bird) accurately predicted passive or proactive interactions with the PBRs. Model fit for logistic regression was assessed using log-likelihood ratio, the Homer and Lemeshow test statistic, and odds ratios to determine the magnitude of effects (Peng et al 2002).

For experiments with the Instron, the mean forces required for breaking new and weathered plastic with an otter tooth or seabird beaks were compared using Student's t-tests. Additionally, a fixed factor ANOVA was done to compare the mean forces required for breaking new and weathered plastic by different species.

RESULTS

Captive pinniped interactions with PBRs

One harbor seal and two sea lions were confined in a seawater tank with either tethered or floating PBRs to determine the interactions these animals could have with PBRs spontaneously or by command and to gauge the impact of these behaviors on the PBRs (**Figure 1**). During each approx. 45-min experiment, there were no indications the animals were self-motivated to interact with the PBRs, although the uncommanded time for the animals was short—maximum 8 minutes. All three animals responded to commands with a success rate of 91 percent (n=23).

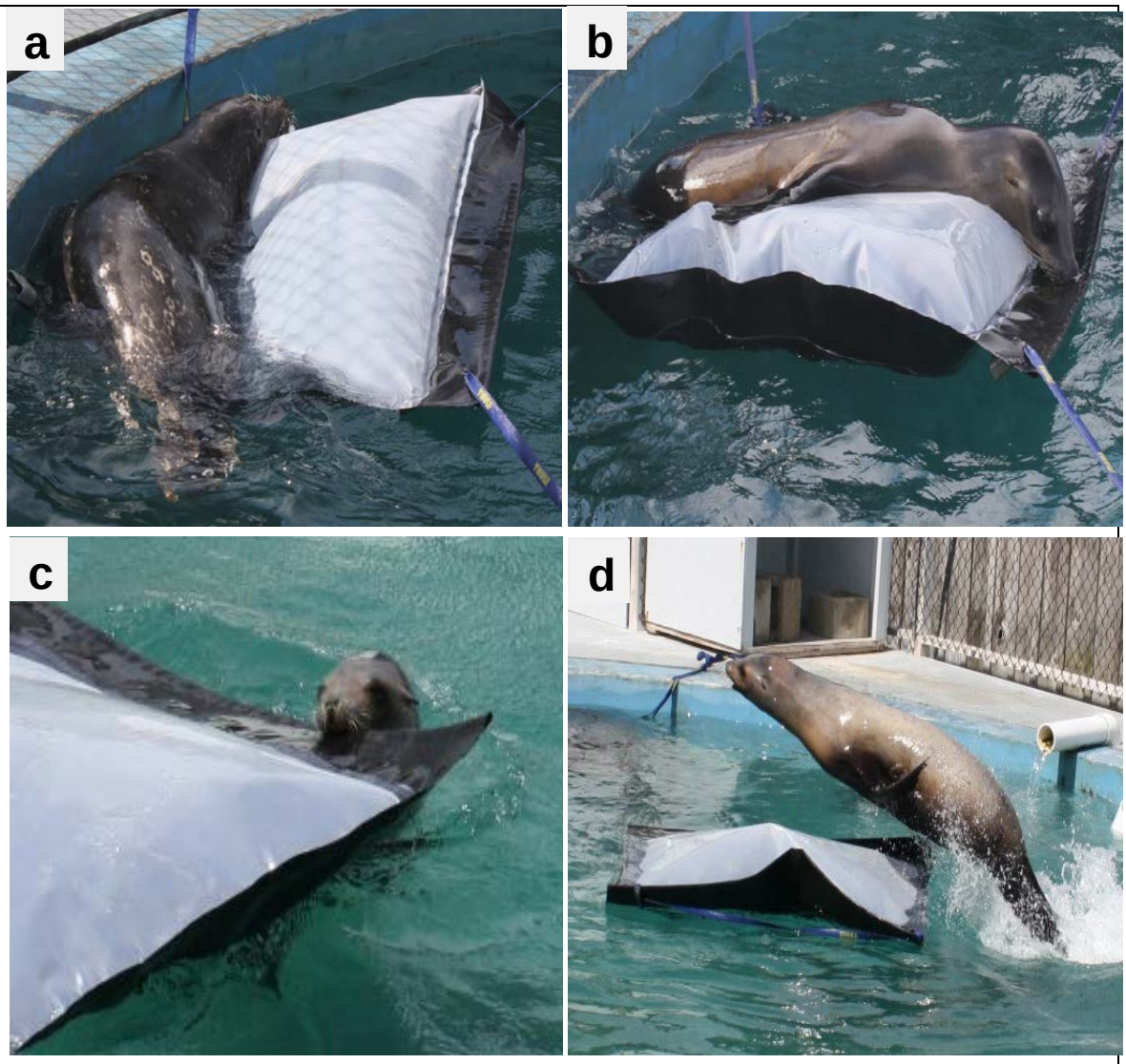

Figure 31: Captive Pinnipeds Executing Commands to Interact with Photobioreactors (PBRs).

Hauling out onto the PBR by the harbor seal (*Phoca vitulina richardii*) (a) and the young sea lion (*Zalophus californianus*) (b), fetching the PBR by a second older sea lion (c) and jumping onto or over a floating PBR (d).

There were individual differences in responses due to differences in training and abilities. For example, the harbor seal always fulfilled the commands (n=3) to swim under the PBR, rest his head on the PBR, or to haul out on the PBR (Fig. 1a), although he has limited vision from advanced cataracts. The younger sea lion responded 89 percent successfully to these same commands as well as to the command to haul out (Fig. 1b), fetch the PBR by biting and pulling (Fig. 1c), jumping over the PBR, which usually involved landing on and submerging about half the PBR (Fig. 1d). The young alnimal also did flipper slaps on the surface (not shown) (n = 9). The older sea lion also responded 91 percent successfully to the same commands as the young sea lion (n=11). It should be noted that all these animals were more interest in the tethers securing PBRs than in the PBRs themselves. This was indicated by their swimming patterns

when entering the tank and between commands. Only the young sea lion was given uncommanded time to interact with the PBR (approx. 8 min.) and during this time the animal showed no overt interest in the PBR. From these limited observations there is no reason to believe pinnipeds would attempt to use small OMEGA PBRs for haul-out or resting sites, although larger PBRs and floating docks, like those proposed for large-scale OMEGA, may be used.

Moss Landing harbor observations of marine mammal and bird interactions with PBRs: The species, the number, the nature, and duration of interactions:

During the nearly 4-month observational experiment, (10 Oct 2011 to 22 Jan 2012) almost continuous IR video was taken of PBRs floating in a boat slip belonging to Moss Landing Marine Laboratories in Moss Landing Harbor. In the 2,424 hrs of video recordings there were <140 total hrs of animal interactions observed; i.e., <1 percent of the total video time showed marine mammals and about 4.7 percent showed birds.

A total of 1,441 separate interactions were analyzed to identify the animals interacting with the PBRs, to characterize the nature of the interactions as passive or proactive (see Table 1 Materials and Methods for description), and to measure the duration of the interactions (Table 2). The predominant mammals interacting with the PBRs were sea otters (*E. lutris*), which were classified as invertebrate divers and observed in a total of 94 interactions. These interactions were mostly (67 percent) characterized as "proactive," indicating a purposeful interaction with the PBRs, although they were mostly brief, averaging about 3 minutes. There was one animal observed to rest on the PBR for 44 minutes.

	Interactions			Duration	
Identity/feeding group	*Passive*	*Proactive*	Total	mean±SE	Max.
Mammals					
Invertebrate divers					
Sea otter (*Enhydra lutris*)	32	66	98	3.1±0.6	44
Fish					
California sea lion (*Zalophus californianus*)	0	1	1	-	1
Birds					
Omnivorous surface feeders					
American coot (*Fulica americana*)	426	175	601	3.9±0.3	127
Unidentified gull	303	126	429	7.1±0.9	137
Western gull (*Larus occidentalis*)	145	47	192	5.6±1.2	170
Unidentified duck	13	8	21	1.7±0.1	4
Mallard duck (*Anas platyrhynchos*)	0	3	3	-	-
Carnivorous diving/wading feeders					
Black-crowned night heron (*Nycticorax nycticorax*)	3	1	4	1.5±0.5	2
Double-crested cormorant (*Phalacrocorax auritus*)	2	0	2	-	11
Western grebe (*Aechmophorus occidentalis*)	0	1	1	-	1
Great blue heron (*Ardea herodias*)	2	0	2	6.0±4.0	10
Unidentified cormorant	0	2	2	1	1
Unidentified birds	59	34	93	13.0±3.1	190

Table 5: The Animals Interacting with PBRs in Moss Landing Harbor. The Nature of the Inaction (Passive or Proactive) and the Duration.

There was also a California sea lion (*Z. californianus*), classified as a fish and invertebrate diver, observed interacting, but only once and proactively focused on a PBR float. The interaction lasted one minute.

The bird interactions with PBRs totaled 1350. The birds were divided into the feeding groups: omnivorous surface-feeders, carnivorous-diving/wading-feeders, and a remaining group of "unidentified birds" (Table 2; bottom). The most frequently observed interacting birds were American coots, *Fulica americana*, (n=601) and unidentified gulls (n=429), both of which were classified as omnivorous surface feeders. The coots were mostly (71 percent) passively interacting with the PBRs for average durations of approx. 4 min and a longest duration of about 2 hours. Unidentified gulls were also mostly (71 percent) passively interacting with PBRs, with average durations of about 7 min and a maximum interaction of a little over two hours. Also in this category, Western Gulls, *L. occidentalis,* were observed 192 times with mostly passive behaviors (76 percent), with average interaction durations of about 6 min and with a maximum duration of nearly three hours. The other omnivorous surface feeding birds were ducks, which were observed a total of 24 times, about equally characterized as passively or proactively interacting with the PBR, for average durations of about 2 min and max duration of 4 min. Most of these duck interactions were with a single white bird that is resident at the harbor and easily identified.

Among the carnivorous-diving/wading-birds the Black-Crowned Heron (*Nycticorax nycticorax)* was seen on four occasions, whereas the other four species were seen only once or twice (Table 2). The Great Blue Heron (*Ardea herodias*) had the longest duration of interactions both on average (6 min) and maximum (10 min). In the "unidentified bird" category there were 93 observations of interactions between birds and PBRs with 63 percent of these interactions classified as passive, average durations of 13 min and a maximum duration of over three hours (Table 2).

In general, the number of interactions with PBRs observed for birds was significantly greater than that of mammals ($X^2_{0.05,\,1}$=1086.1, P< 0.001) The duration of interactions of sea otters with PBRs (X=3.1, SE=0.5, n=95) and birds with PBRs (X= 5.7, SE= 0.4, n=1186) was not significantly different after the data were corrected for outliers to meet the assumption of equal variances (t (1259)=2.056, P=0.22). There were however, differences in the nature of the interactions between animals and PBRs.

The nature of PBR interactions and animal behaviors:

The passive and proactive interactions between animals and PBRs were further evaluated in the videos and characterized with behavior codes as "feeding" (code 1), "walking" (code 2), "resting" (code 3), "grooming" (code 4), "social activity" (code 5), "investigating PBR" (code 6), and "manipulating PBR" (code 7). In this system, codes 1-5 are considered passive and codes 6-7 proactive—the proactive behaviors could pose a risk of damage to the PBRs. The numbers of

discreet behaviors (single digit codes) and combinations of behaviors (multi-digit codes) are presented in Figure 2.

Although sea otters were 5 times more likely to interact proactively with the PBR than birds (Omnibus $X^2_{0.05,\,1}$= 53.861, P<0.001), the observed behavior patterns of bird were more elaborate than sea otters. For sea otters there were 19 behaviors coded (Fig. 2 top) and for birds there were 50 (Fig. 2 bottom). For example, the most common sea otter behavior observed was "investigating PBRs" (code 6) and this behavior was occasionally combined with "walking" (code 2,6), "resting" (code 3,6), "grooming" (code 4,6), and "social activity" (code 5,6), as well as "feeding + resting" (codes 1,3,6), and "walking + resting" (codes 2,3,6) (Fig. 2 top).

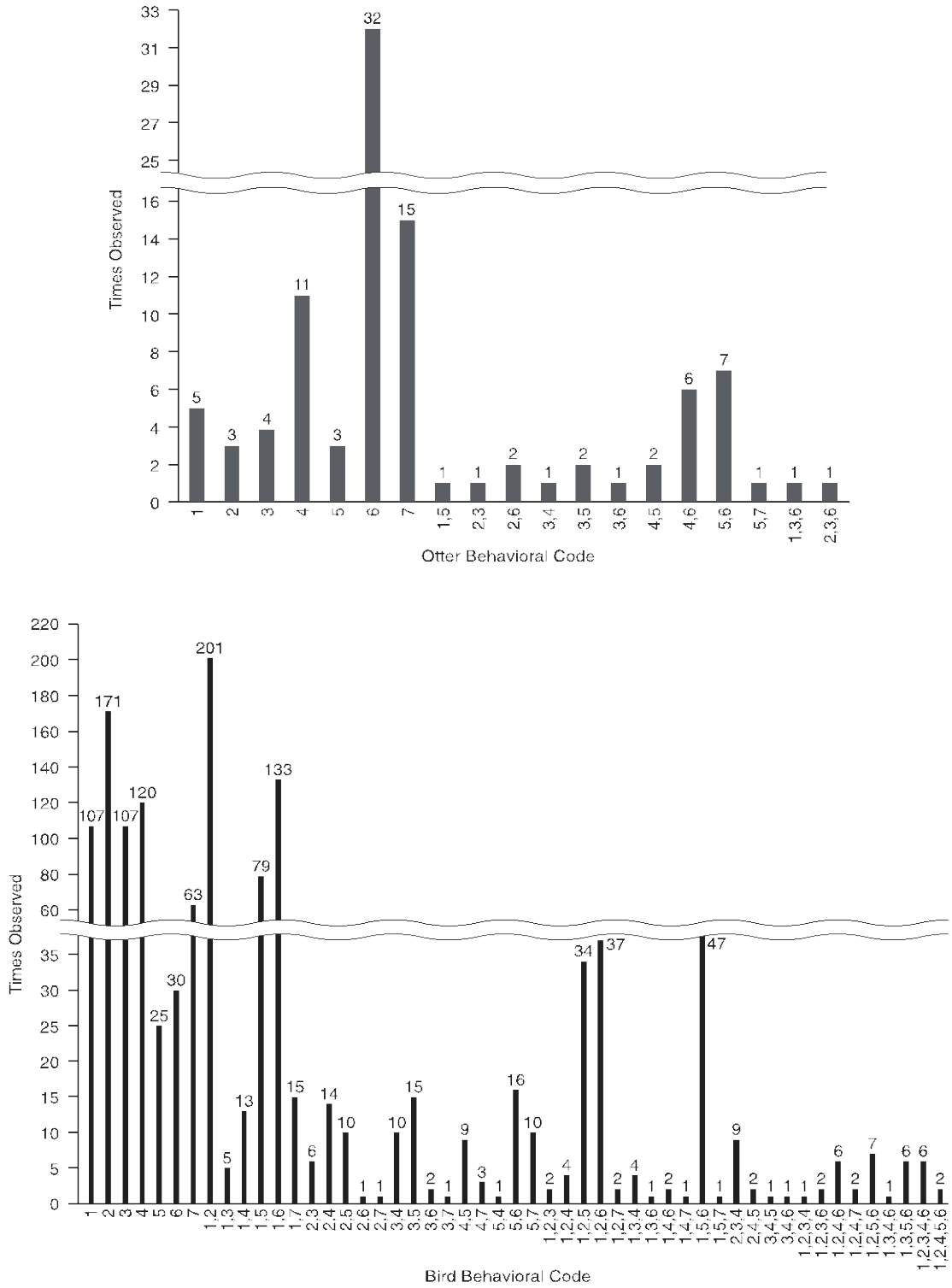

Figure 32: The Number of Times Different Behaviors Were Observed in Sea Otters (above) and All Birds (below).

In the numerical code 1=feeding, 2=walking, 3=resting, 4=grooming, 5=social activity, 6=investigating, and 7= manipulating.

For birds, the most common behaviors observed were "walking" (code 2) and "feeding + walking" (codes 1,2), but there were also >100 observations for resting (code 3), grooming (code

4), and feeding + investigating (codes 1,6) (Fig. 2 bottom). Sea otter behavior was described with three codes, whereas bird behaviors combined up to five codes (1,2,3,4,6).

Forces required for a sea otter tooth and seabird beaks to puncture PBR plastic

Most animal behaviors were passive and unlikely to damage the PBRs or the OMEGA system. It is possible however, that proactive behaviors in which the animals bite or peck at the PBR plastic, could result in scratches or punctures in the upper LLDPE plastic. An Instron instrument was used to measure the force require for an otter canine tooth or gull beaks to puncture LLDPE plastic (Fig. 3). Both "new" (Fig. 3: black columns labeled "a") and weathered plastic (Fig. 3: gray columns labeled "b") were tested. The new plastic was not exposed to outside conditions, whereas the weathered plastic was recovered from PBRs that floated in Moss Landing Harbor for 12 weeks. The mean force required for an otter tooth to puncture the new plastic was 92.8 Newtons (±1.7, n=6) and to puncture weathered plastic was 50.5 Newtons (±1.8, n=7). The mean force required for the California Gull beak to puncture new plastic was 71.8 (±2.8, n=7) and weathered plastic 61.5 (±1.2, n=7). The mean force require for the Glaucus-winged gull beak to puncture new plastic was the highest at 129.3 N (5.0, n=4) and weathered plastic was 87.2 (±2.8, n=9).

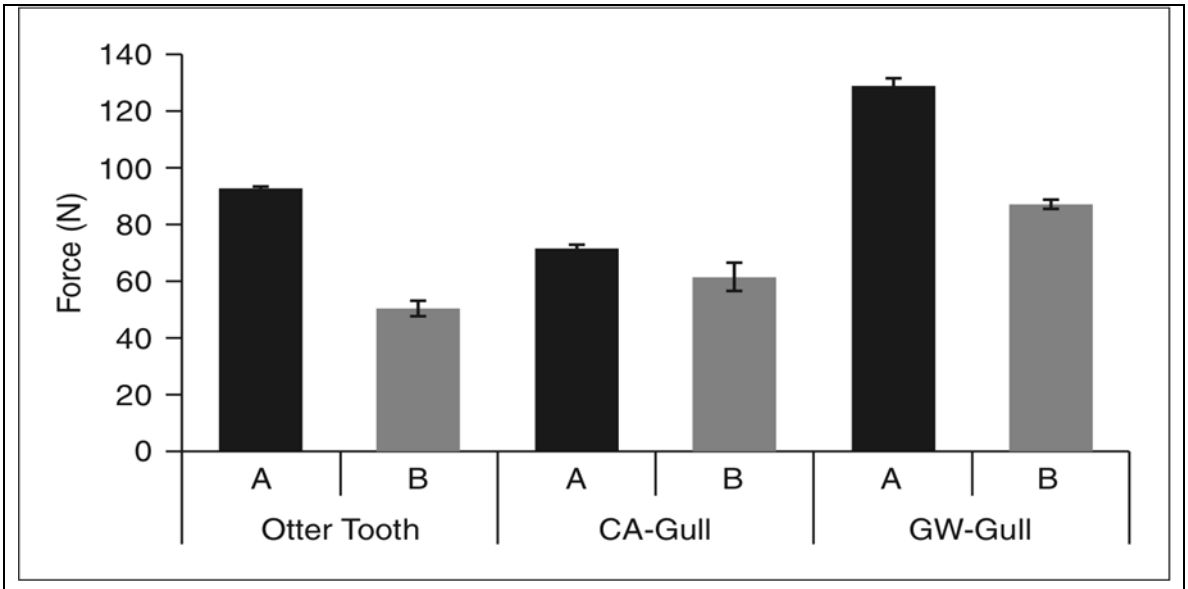

Figure 33: Maximum Force in Newtons (N) Required for a Sea Otter Canine Tooth (Otter Tooth), California Gull (CA-Gull), Glaucous-Winged Gull (GW-Gull) Beaks, to Penetrate New (A) and Weathered (B) PBR Plastic--Clear Linear Low Density Polyethylene LLDPE.

The weathered LLDPE was recovered from PBRs that had been used in experiments in Moss Landing Harbor for 12 weeks. The overall mean force was 78.6 N (±3.7, n = 40).

The mean forces required for the otter tooth and gull beaks to puncture new versus weathered plastic were significantly different ($t(38)=3.55$, $P=0.001$). Furthermore, the mean forces required for the different species to puncture new ($F_{2, 14} = 81.7$, $P \leq 0.001$) and weathered ($F_{2, 20} = 76.88$, $P \leq 0.001$) plastics were significantly different.

Time-dependent interactions of marine animals with PBRs

Both the sea otters and the birds interacted with PBRs more during the day than in the evening and at night (Figure 4). For the sea otters 76 percent of the 94 total observed interactions were in the morning and afternoon with only 24 percent in the evening and night. Similarly, for birds 71 percent of the 1346 total observed interactions were in the morning and afternoon with only 29 percent in the evening and night.

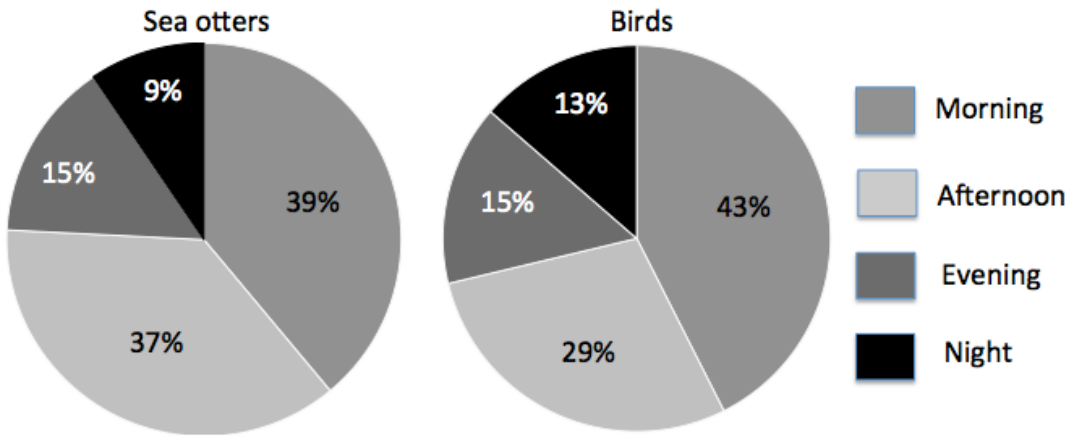

Figure 34: Percentage of Sea Otter (left) and Bird (right) Observations with PBRs Distributed Over the Day from Morning (6am to 12pm), Afternoon (12pm to 4pm), Evening (4pm to 10pm) and Night (10pm to 6am).

For both sea otters and birds this day versus night difference in interactions with PBRs was significant ($X^2_{0.05,\,1}$=26.8, P< 0.001).

Unlike the similarity of day/night interactions of sea otters and birds with PBRs, on the time-scale of months the interactions were significantly different, comparing October to November through January ($X^2_{0.05,\,1}$=191.5, P< 0.001) (Figure 5). Approximately 4 percent (n=4) of otter interactions occurred in fall, and 96 percent (n=91) in winter, whereas 73 percent, (n=978) of birds interactions were observed in the October and 27 percent, (n=368) in the winter months.

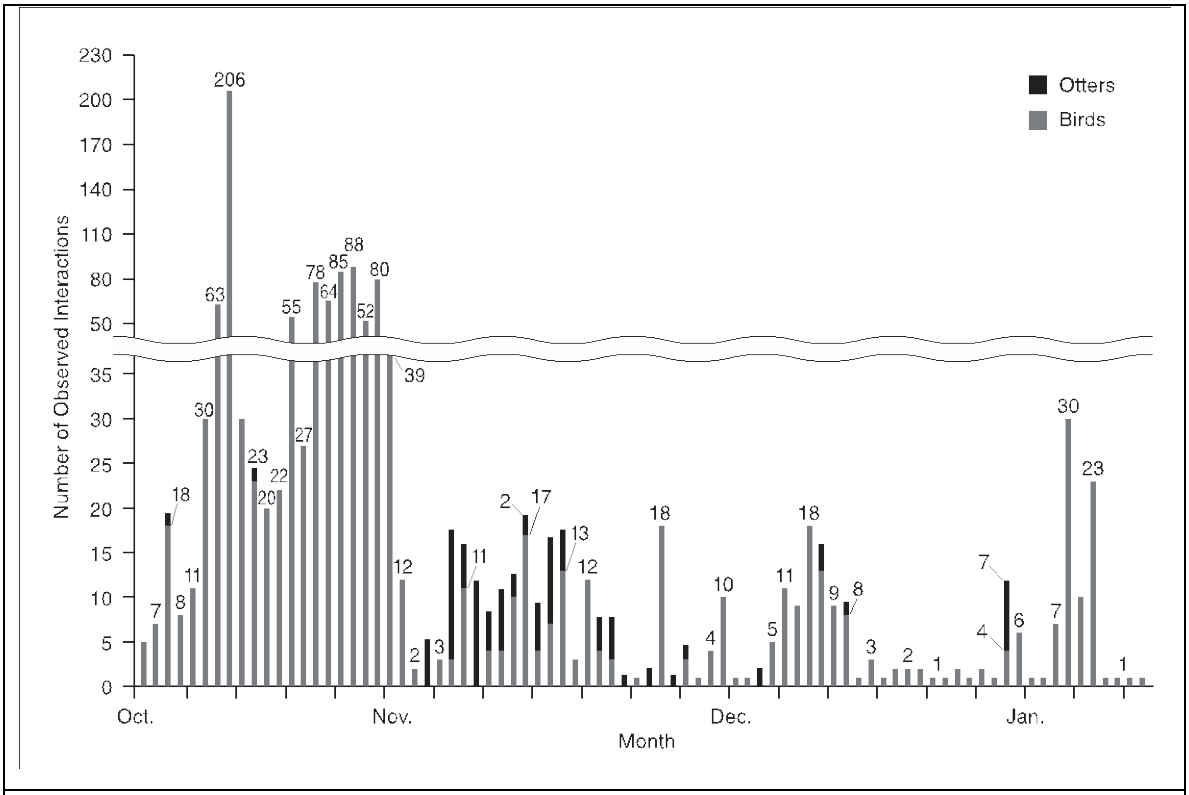

Figure 35: The Number of Daily Sea Otter and Bird Interactions with PBRs Observed Between 11 Oct 2011 and 22 Jan 2012.

Sea otter interactions shown in black and bird interactions in grey. The total number of interactions is 1444.

In November, there were 17 days in which multiple otter interactions with the PBRs were observed (Fig. 5, middle: black bars). Ten of these days were consecutive and nine of the ten involved multiple observations on the same days. Throughout the study period there were bird interactions with the PBRs with distinctly more interactions in October. During October, there were five or more interactions each day with a maximum of 206 interactions observed on October 19.

With the exception of the resident duck at the Moss Landing Harbor, it was not possible for most animal interactions with the PBRs to distinguish between repeated interactions by a single individual and novel interactions by many different animals.

Discussion

In coastal environments marine mammals, seabirds, and some aquatic birds opportunistically use floating objects (Connell 2000; Piper et al 2002; London et al. 2012) and the proposed OMEGA system of floating PBRs and docks will undoubtedly be a focus of marine mammal and bird activity. The small PBRs tested with captive, trained animals in tank experiments indicated that trained seals and sea lions interacted with PBRs only on command and these interactions were not harmful to the animals nor did they damage the PBRs. These captive pinnipeds showed no undirected interest in the small PBRs and in the nearly four months of video recordings of PBRs floating in Moss Landing Harbor there was only a single brief interaction recorded between a wild sea lion and the PBRs.

Moss Landing harbor is connected to the Elkhorn Slough, the third largest estuary in California, adjacent to Monterey Bay. During the year, the slough hosts many California sea lions, Pacific harbor seals, sea otters and over 250 species of birds (Roberson 1985; Silberstein & Campbell 1989; Ramer 1991; Caffery et al 2002; Harvey & Connors 2002). Although not all species in the slough move into the harbor, California sea lions, harbors seals, sea otters, and all the bird species observed interacting with PBRs are frequently observed in Moss Landing harbor (Greig 2005; Harvey, Helm & Morejohn 1995; Oxman 1995; Nicholson et al 2007). While the wild pinnipeds showed almost no interest in the PBRs in the harbor, the video revealed 94 interactions with sea otters, 805 interactions with seven identified species of birds and 545 interactions with unidentified birds. There are many shorebirds in the slough (e.g., sandpipers, dunlins, dowitchers, godwits, and willets), which are common on the mudflats adjacent to the harbor (Ramer 1985), but were never observed interacting with PBRs, presumably because the PBRs were out of their preferred habitat. For the animals that did interact, it was not possible to determine if the interactions were with unique individuals or repeated interactions with the same individuals. It was clear however, that at least these animals were quick to use the floating PBRs for a variety of purposes.

Sea otters are year round residents in Elkhorn Slough and the harbor with their greatest abundance between November and December (Feinholz 1998; Henkel & Harvey 2008), which coincides with their peak interactions with the PBRs. A group of tagged otters, routinely monitored in Moss Landing harbor, occasionally haul out onto kayaks, boats, and docks (SORAC, Carl Mayer, personal communication), setting a precedent for these animals interacting with man-made objects. It has been proposed that otters haul-out to conserve energy (Maldini et al 2012), and the PBRs may provide haul-out sites. The sea otters seen resting, grooming, and feeding on the PBRs were displaying typical behaviors (Packard & Ribic 1982; Kvitek et al 1988; Feinholz 1998). The proactive otter behavior of biting the nylon purge-valves on top of PBRs, rolling on the PBR surface, and investigating or manipulating the high-density foam floats with their paws and mouths were also typical of otter behavior. None of the observed behaviors caused harm to the animals or damage to the PBR components.

All the birds seen interacting with the PBRs are year-round residents in the slough, are commonly seen in the harbor, and in general are most abundant in Monterey Bay in the fall (Harvey & Connors 2002; Ford et al 2004; Henkel 2004). Resident gulls and coots nest locally, but over-wintering visitors arrive in early October and stay until mid-November (Roberson 1985), which may explain the October peak in bird interactions with PBRs. Gulls and coots are omnivores and well adapted to urbanized environments (Brinkley 2003; Lafferty et al 2013). They appeared to forage on the biofouling community that developed on the PBRs, which included diatom films, seaweeds, crustaceans, and bryozoans (Harris et al 2013), all of which are known to be food resources for gulls and coots (Roberson 1985; Brinkley 2003). Coots commonly aggregate while foraging (Trenchard 2013), which may explain the spike in coot interactions on the PBR on October 19th. Compared to coots and gulls, other birds (grebes, cormorants, ducks, and herons) were rarely seen on the PBRs, probably because the PBRs were outside their normal ambit and there was no foraging attraction.

For the PBRs, there was no indication that the animals, even those that were proactive in their interactions, damaged the PBRs. Instron results indicate that it is possible for otter teeth and bird beaks to puncture PBR plastic and the risk is greater when the plastic is weathered for 3

months. It is possible for sea otters to bite through the plastic and unlikely the birds tested would develop the pecking forces required to puncture even the weathered plastic.
For all animals, the interactions with the floating PBRs in the harbor was <4 percent of the total video recording time and the typical durations of interactions were brief (usually <4 minutes), indicating that at least these small experimental PBRs accounted for a small portion of the daily activity of the animals in this habitat. In an actual OMEGA deployment, the PBRs and associated support infrastructure will cover many hectares of coastal waters in protected bays (Trent et al 2012). Depending on the location, depth and water clarity, this large expanse can indirectly impact marine mammals and birds by shading the water column and benthos thereby impacting productivity and community structure. OMEGA anchoring or tethering systems may present entanglement problems for diving animals and cause drownings. It also possible that extreme weather and ocean conditions (e.g., hurricanes or tsunamis) could damage and release OMEGA structures into the environment and the plans must be developed to mitigate the impact of both plastic and associated debris both in bays and if it is swept out to sea (Derraik 2002; Moore 2008; Gregory 2009;).

Conclusion

Conversely, the large OMEGA infrastructure may have environmental and economic benefits. Submerged OMEGA surfaces provide substrate, refugia, and habitat for sessile and associated organisms. This may help control eutrophication by acting as a floating "turf scrubber" (Mulbry et al., 2010), removing nutrients (Christenson and Sims 2012), heavy metals and other pollutants (deBashan and Bashan 2010; Ghasemi et al., 2011) from the water column and improving coastal water quality. In addition, the OMEGA infrastructure may act as a "fish aggregating device" or an "artificial reef," both of which increase local species diversity and expand the local food web (Kerckhoff et al., 2010). Observations at Moss Landing Harbor indicated that even the small OMEGA PBRs provided sites for sea otters and birds to forage. While interactions of pinnipeds with small PBRs were negligible, large-scale PBRs deployments may become foraging areas for pinniped and other marine mammals and diving birds.
There are strong environmental, social, ethical, and economic incentives for developing sustainable alternatives to petroleum-based liquid fuels (Caspeta et al.,, 2013; Running 2012; Tillman et al., 2009) and microalgae appear to be the most promising feedstock for sustainable biofuels, particularly if their cultivation is coupled to wastewater treatment (Brennan and Owende 2010; Pittman et al., 2011). To avoid the excessive costs of pumping wastewater long distances, algae cultivation facilities must be close to existing wastewater plants (Fortier and Sturm 2012) and for coastal cities, which dispose of their wastewater offshore, the OMEGA system of floating PBRs is perhaps the only serious option (Trent et al., 2012). While the proposed OMEGA system could provide coastal communities with biofuels advanced wastewater treatment, fertilize and carbon sequestration from microalgae cultivation, as well as alternative energy from solar, wave, wind and expanded aquaculture products from the associated offshore platform, the functional OMEGA scale of hundreds of hectares will have major ecological impact in coastal waters (*Inger et al 2009, Lovich et al 2013)*. An effective coastal management scheme to accommodate this expanded use of this environment will require additional studies and careful planning.

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Chapter 7:
Why OMEGA is Needed and How to Get There From Here…An Environmental Business Case

7.1 Summary

In the recently released Draft of the "National Algal Biofuels Technology Roadmap," compiled and edited by experts from around the world for the U.S. Department of Energy, it is stated that algal biofuels could provide sufficient fuel feedstock to meet the fuels needs of the entire United States, while being completely compatible with the existing fuel infrastructure (refining, distribution, and utilization).

Although it is widely recognized that algae or microalgae offer "great promise" to contribute significantly to the production of renewable fuels, including green aviation fuels, current algae cultivation methods on land (in ponds or in bioreactors) compete with agriculture for freshwater and fertilizer; if they alternatively use wastewater as a source of water and nutrients, constructing algae cultivation facilities in proximity to existing wastewater treatment plants would not be economically or energetically feasible at the scale needed for commercial production of biofuels.

For coastal cities, however, the Offshore Membrane Enclosures for Growing Algae (OMEGA) system solves many of the problems inherent in land-based systems because (a) it is deployed offshore and does not compete for agricultural land; (b) it uses treated municipal wastewater and does not require increasingly scarce freshwater or the additional energy represented by fertilizer; and (c) it uses the ocean for structural support, temperature control, and to some extent mixing. In addition, OMEGA addresses the economic limitations of land-based algae systems by providing a diversity of products and services that optimize the use of local resources and available energy.

For example, after the algae oil is recovered for biofuels, the algal remains can be processed into fertilizer, animal food, or higher-value products such as cosmetics or nutraceuticals or the remains can be anaerobically digested to produce methane ("natural") gas to contribute to the energy output of the system. While producing these products, the OMEGA system is designed to provide services such as advanced wastewater treatment and carbon-capture for nutrient recovery and some amount of carbon sequestration simultaneously. The wastewater treatment is provided by the algae growing inside the OMEGA system, which removes nutrients, trace metals, and contaminants from the wastewater, and by a forward osmosis system, which use the salt gradient between wastewater and seawater to polish the wastewater that is released into the surrounding seawater; this advanced wastewater treatment captures and recycles nutrients that are currently polluting the oceans and requires no input of additional energy. Furthermore, OMEGA uses carbon dioxide (CO_2) to control the pH of the algae cultures and provide carbon for their growth. The algae carbon can be quantified and sequestered for centuries if the algal biomass is transformed into "biochar," which is a valued soil amendment. In addition to using the algae produced by the OMEGA system, the OMEGA infrastructure can

be used for generating electricity from solar panels, wind turbines, or wave generators. The infrastructure also provides access to aquaculture sites for producing food crops. Thus, OMEGA has a broad range of economic and environmental benefits that go beyond the production of fuels and it is therefore not dependent on this single product for economic viability.

The OMEGA system itself consists of an array of closed photobioreactors (PBRs) constructed primarily of inexpensive, clear, flexible plastic designed to be filled with treated wastewater from coastal cities' municipal wastewater plants and inoculated with freshwater oleaginous algae species. The OMEGA modules float just below the surface and use sunlight for the growth of algae from added CO_2 and wastewater nutrients. The OMEGA PBRs are constructed from flexible inexpensive plastic to control internal temperature by heat exchange with the surrounding water and promote mixing of the algae culture using surface waves.

Economic and financial evaluations, based on the limited data available, show that OMEGA compares favorably with other algae production systems. The advantage of OMEGA is that it eliminates land use, provides convenient access to wastewater and advanced wastewater treatment, contributes to carbon capture and sequestration (CCS), and creates a multifunctional offshore platform. An economic analysis shows that OMEGA may be able to produce lipids for less than land-based PBR technologies, and at a price competitive with open ponds, which are currently considered to be the lowest-cost method of algae production. At the current price of oil [$1.20/L ($4.54/gallon)], the integrated OMEGA system is even competitive with petroleum.

Assuming a 10 percent return on investment, the OMEGA system would produce only biofuel at a cost of $6.67/L. If the OMEGA system combines biofuel production with wastewater treatment, the cost of the biofuels is reduced by 13 percent to $5.80/L. If the system combines biofuel, wastewater treatment, and renewable electricity (from solar, wind, and wave energy), the cost of biofuel is reduced 24 percent to $4.20/L. If the system does all of the above and is combined with aquaculture (e.g., mussels), the cost of biofuels is reduced 41 percent to $1.43/L. In other words, as the OMEGA system becomes integrated and scaled up, fuel production costs reach parity with petroleum—a critical goal in the search for an end to the fossil-oil age.
The overarching goal of the California Energy Commission OMEGA project was to demonstrate the feasibility of the OMEGA concept and to motivate others to further develop the technology, in part by insinuating that this technology has commercial potential. This chapter is meant to demonstrate the environmental importance of the OMEGA technology and an economics context that is indeed justified. A more detailed integrated techno-economic analysis is in preparation for publication (McKuin and Trent) but this chapter of the report will suffice to raise public awareness.

The Global Predicament

Increasingly the world must look toward new energy technologies that are carbon neutral, that reuse waste products, and that have net positive impacts on the environment. In this section, background on the factors contributing to the energy crisis, policies developed to address

energy and climate issues, project goals, the project team, and the structure of a business case for the project are discussed.

7.2 Background

The world is projected to experience a devastating global energy crisis due to the continued dependency on non-renewable fossil fuels (IEA, 2009). The conditions that need to be considered in understanding the energy crisis include dwindling oil reserves, increased populations and energy use, and global climate change.

7.2.1 Peak Oil and the Need for Renewable Energy

In 1956 the theory of peak oil was introduced to the American Petroleum Institute by the geologist M. King Hubert (Hubert, 1956). His theory predicted that a geographic oil-producing region's production would begin slowly, build to a peak, and then decline at approximately the same rate it climbed. He theorized that discovery and production would follow similar curves, with approximately 20-40 years between peaks.

Hubert predicted that U.S. production would peak between 1965 and 1970 (it peaked in 1970). His forecast for the United States was based on the lower 48 states; he did not consider the Alaskan oil fields. Even with those oil reserves, his predictions for the United States have been called perhaps the most amazingly accurate long-range forecast ever. Today, U.S. production is down 40 percent from its peak, and oil shortfalls are being met with foreign oil (U.S. EIA, 2010). Foreign oil reserves will not last forever. According to current estimates, at current production rates, global sources of oil and gas will be exhausted within 40 to 60 years. And although production is down, the rise in global population is driving total energy consumption up.

7.2.2 Population Growth and the Demand for Energy

The future population growth of the world is difficult to predict. Birth rates and death rates can change drastically over the course of time. Recent forecasts have the world's population increasing to beyond 9.2 billion in 2050 from the current 7.0 billion (U.S. Census Bureau, 2010). Other projections are that the world's population will eventually peak, though it is uncertain when or how. In some scenarios, it will peak as early as around 2050 at fewer than nine billion due to gradually decreasing birth rates (Figure 1a).

The continued upward trend in the world's population can only mean a greater need for energy sources, which lately have been fossil fuels. World energy consumption is projected to increase from 508 quadrillion BTU (quad) in 2010 to 683 quad in 2030 (IEA, 2009). Most of that increase will occur in countries not affiliated with the Organisation for Economic Co-operation and Development (OECD), as shown on Figure 1b. The projected energy mix is also shown on Figure 1b. According to the U.S. Energy Information Administration, in 2006, fossil fuels supplied 85 percent of the U.S. energy consumption, with renewable energy supplying 7 percent (Figure 1c).

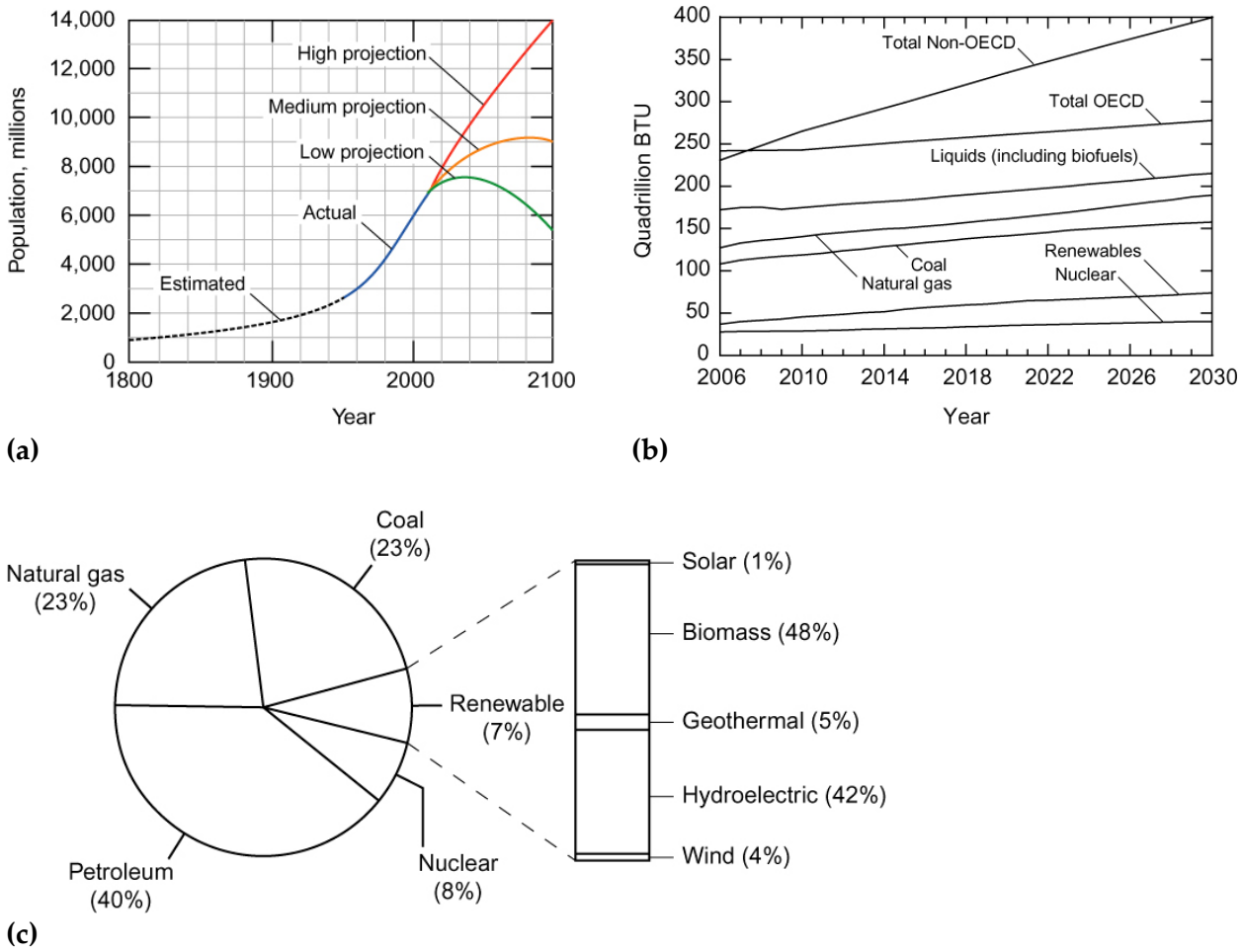


Figure 36: Summary of Trends

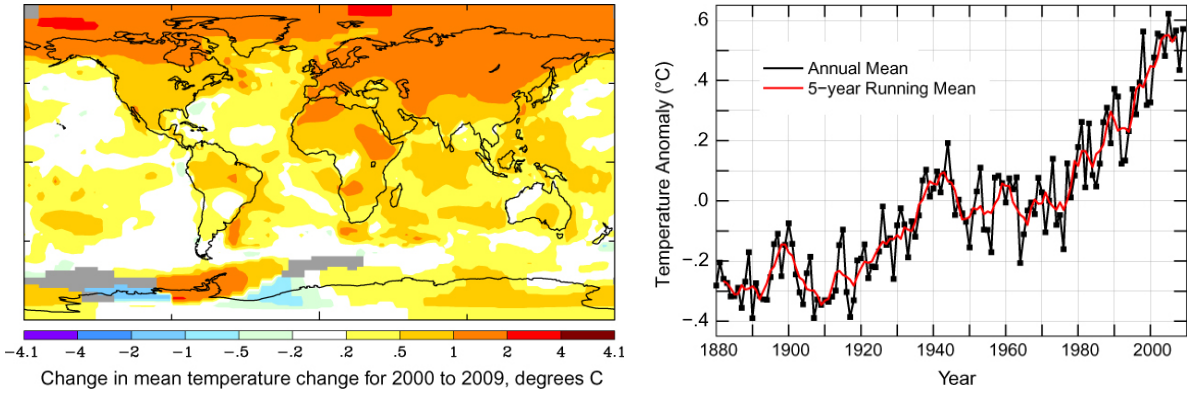
(a) World Population, (b) projected world marketed energy consumption for various fuels, including total consumption by countries affiliated with the Organization for Economic Co-operation and Development (OECD) and non-OECD countries, and (c) energy consumption in the United States for the year 2006 (U.S. Energy Information Administration, 2010).

7.2.3 Global Warming

The intergovernmental Panel on Climate Change (IPCC), the leading authority on global climate change issues, has reached consensus concerning the outcome of global climate change if mitigating measures are not taken to reduce the current and forecasted anthropogenic contribution of greenhouse gas (GHG) emissions to the Earth's atmosphere (IPCC, 2007). Although natural processes are the cause of the majority (97 percent) of GHG emissions, there is compelling evidence that the increase of the Earth's surface temperature is being influenced significantly by human activities; the IPCC concludes that the Earth is definitely warming and recent temperature increases are due in part to anthropogenic GHG emissions (see Figure 2). Natural systems are already being affected by climate change and the IPCC predicts increasing droughts, floods, and wildfires, with potentially devastating impacts on global ecosystems with serious implications for societies worldwide.

The impacts of climate change include melting glaciers, extinction of up to 40 percent of known species, and extreme weather events, with predictions of drought, floods, increased hurricane

intensity, and other changes from what have been decades of relatively benign weather. All of these changes are expected to impact agriculture and the availability of food, which in combination with continued population growth and immigration, has global implications for economic and national security.



(a) (b)

Figure 37: Summary of global temperature changes

(a) annual temperature abnormalities, in °C, between 1951 to 1980 and 2000 to 2009 and (b) global land ocean temperature index 1880 to 2010. (GISS, 2009)

The recent Stern Review (2006) provides a comprehensive analysis of the economics of climate change, taking into consideration the costs and benefits of actions to reduce the emissions of GHGs that cause climate change. The authors conclude:

> "The evidence gathered by the review leads to a simple conclusion: the benefits of strong early action considerably outweigh the costs of such action. Tackling the effects of climate change can be done in ways that do not cap the aspirations for growth of rich or poor countries. The earlier effective action is taken, the less costly it will be."

The conclusions of the Stern Review and many other scholarly evaluations (e.g., IPCC, 2007) of the current global predicament establish unequivocally that there are economic drivers that support mitigation of GHG emissions and strategic regulatory legislation should be expected to be forthcoming. Indeed, U.S. federal, state, and local governments are reacting accordingly; many climate change-related bills have already passed into law, with many others pending in Congress.

The potentially catastrophic impact of global climate, the associated legislative national and international mandates, and public awareness and concern, combine to create a special aspect to a business plan for technologies that address climate change.

7.3 Policy Context

A number of policies have been developed to promote the use of renewable fuels, reduce GHG emissions, and reduce the discharge of nutrients to surface waters. These topics are discussed below.

7.3.1 Renewable Fuels

Recognizing the need to reduce GHG emissions and develop renewable fuels, the Federal Government established the Energy Independence and Security Act of 2007 (EISA) fuel program, hereafter referred to as Renewable Fuel Standard (RFS2). The RFS2 mandates the use of 36 billion gallons of renewable fuel by 2022 – nearly a five-fold increase over the highest volume specified by the previous version of the Renewable Fuel Standard. The RFS2 also established four separate categories of renewable fuels (cellulosic biofuel, biomass-based diesel, advanced biofuel, and total renewable fuel that must be used in transportation fuel), each with a separate volume mandate and each with a specific lifecycle GHG emission threshold. The final 2010 standards are shown in Table 2.

Table 6: EISA Renewable Fuel Standards for the Year 2010

Fuel category	Amount of fuel required to be renewable (percent)	Volume of renewable fuel (billion gallons)
Cellulosic biofuel	0.004	0.0065
Biomass-based biodiesel	1.1*	1.15*
Total advanced biofuel	0.61	0.95
Renewable fuel	8.25	12.95

* Combined 2009/2010 biomass based diesel volumes applies in 2010

State Governments have also to varying extents implemented Renewable Fuel Standards. In California, Executive Order S-1-07, the Low Carbon Fuel Standard (LCFS) calls for a reduction of at least 10 percent in the "carbon intensity" of California's transportation fuels by 2020. Here, "carbon intensity" refers to the amount of lifecycle GHG emissions per unit of energy of fuel delivered.

7.3.2 Reducing GHG emissions

The regulatory environment governing the emission of GHG has changed substantially throughout the last decade. The Chicago Climate Exchange (CCX), which was developed in 2003, was one of the first markets set up to trade GHG emission allowances and thus reduce GHG emissions. However, because the CCX was completely voluntary, its success in reducing emissions was limited. More recently in the U.S., mandatory reporting and cap and trade bills are increasingly being passed by state legislatures that will substantially reduce future GHG emissions.

In 2007, the California Legislature passed the California Global Warming Solutions Act, AB-32, which was signed into law by then-Governor Arnold Schwarzenegger. The Act requires that Californians reduce their GHG emission to 1990 levels by 2020. Since then, seven U.S. states and four Canadian provinces jointly created the Western Climate Initiative (WCI), a regional GHG emissions trading system.

The American Clean Energy and Security Act (H.R. 2454), a cap-and-trade bill, was passed on June 26, 2009 in the U.S. House of Representatives by a vote of 219-212. The bill, which originated in the House Energy and Commerce Committee, was introduced by Rep. Henry A. Waxman (D-CA) and Rep. Edward J. Markey (D-MA) and is still in consideration in the Senate. The bill proposes a cap and trade system, under which the government sets a limit (cap) on the

total amount of GHG that can be emitted nationally. Companies then buy or sell permits to emit these gases, primarily CO_2. The cap is reduced over time to reduce total carbon emissions.
The U.S. EPA Mandatory Reporting Rule (MRR) was published in October 2009. The rule requires reporting of GHG emissions from large sources and suppliers in the United States, and is intended to collect accurate and timely emissions data to inform future policy decisions. Under the rule, suppliers of fossil fuels or industrial GHGs, manufacturers of vehicles and engines, and facilities that emit 25,000 metric tons or more per year of GHG emissions are required to submit annual reports to the EPA.

7.3.3 Wastewater Treatment: Nutrient Control

According to the U.S. EPA (2009): "In terms of growing drinking water impacts, expanding impairment of inland waters, and compromised coastal estuaries, nitrogen and phosphorus pollution has the potential to become one of the costliest, most difficult environmental problems we face in the 21st century."
Increasingly regulators are placing more stringent rules on nutrient pollution from urban stormwater runoff, municipal wastewater discharges, and agricultural livestock and row-crop runoff. U.S. municipal wastewater treatment facilities currently treat about 34 billion gallons of wastewater per day (U.S. EPA 2008). The estimated cost to upgrade all the publicly owned treatment works in the United States to achieve the more stringent technology-based nutrient limits—3 mg/L for nitrate and nitrite and 0.1 mg/L for phosphate—would be about $44 billion to remove nitrogen, about $44.5 billion to remove phosphorus, and approximately $54 billion to include capabilities to remove both nitrogen and phosphorus simultaneously (based on U.S. EPA 2008). Future regulatory changes, such as the complete or partial prevention of wastewater discharge to waterways, will involve further costs that must be met by stakeholders.

7.4 Renewable Transportation Fuel

As shown previously on Figure 1b, the consumption of liquid fuels is the single largest contributor to worldwide energy usage and is projected to rise sharply in the coming decades. According to the Energy Information Agency: "Liquid fuels are expected to remain the world's dominant energy source, given their importance in the transportation and industrial end-use sectors" (U.S. EIA, 2010).
World use of liquids and other petroleum is forecast to grow from 85 million barrels per day in 2006 to 91 million barrels per day in 2015 and 107 million barrels per day in 2030. In the absence of significant technological advances, liquids will continue to be the primary energy source in the world's transportation sector.
Biofuels, including ethanol and biodiesel, will be an increasingly important source of the unconventional liquids supply, reaching 5.9 million barrels per day in 2030. Strong growth in biofuels consumption is forecast for the United States, where production of biofuels is projected to increase from 0.3 million barrels per day in 2006 to 1.9 million barrels per day in 2030, largely due to legislation (including RFS2) that mandates increased use of biofuels.

7.5 Carbon-neutral Energies Based on Biomass

A variety of carbon-neutral energy processes are currently deployed or in development, including photovoltaic (PV) solar, concentrating *solar* power (CSP), wind, wave, hydropower, geothermal, and nuclear fission. All of these technologies are geared toward producing electrical power, and can do little else.

Only biomass can be used to produce liquid transportation fuels. Fuels that can be obtained from biomass include methane, ethanol and other alcohols, biodiesel, and other hydrocarbon fuels obtained via processes that include pyrolysis, Fisher-Tropsch synthesis processes, and gas-to-liquids (GTL) technology.

The advantages of biomass energy include:

- The production of liquid transportation fuels that can be readily introduced into the existing fuel infrastructure
- Fuels can be stored easily, contrary to electricity, heat, hydrogen, and most other forms of carbon-neutral energy. Fuels can also be efficiently transported over a continent-wide pipeline network.
- The mitigation of several other pressing environmental problems
- Aquatic as well as terrestrial biomass can be used to remove nutrients from wastewater.
- Land-based energy crops can provide wildlife habitat (for example, tree farms and switchgrass fields)
- Perennial energy crops (like switchgrass) can provide soil stabilization and net sequestration of carbon in their extensive below-ground biomass

The disadvantages of biomass energy include:

- Compared to PV and CSP, biomass has relatively low solar energy conversion efficiency.
- Biomass production can have substantial direct and indirect water and energy demands.
- If biomass production competes for acreage and resources with food and feed production, it can distort food markets and lead to substantial price swings due to the low elasticity of food prices.

Therefore, in developing carbon neutral fuels using biomass, technologies should:

- Not compete for land with food and feed crops;
- Be implemented so as to minimize water, energy, and chemical fertilizer inputs;
- Produce much more energy than it consumes; and
- Lead to significant reductions in net carbon emissions.

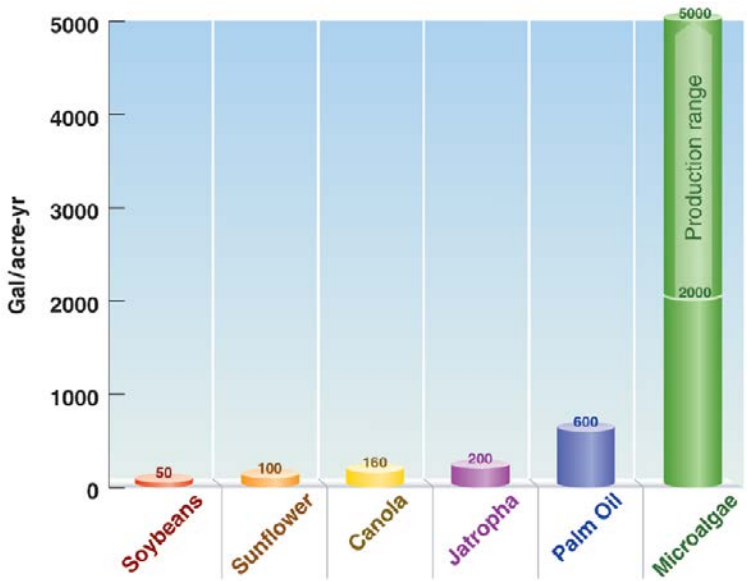


Figure 38: Relative Oil Production From Various Crops

[The graph is based on data from National Renewable Energy Laboratory (NREL), 2009.]

By many criteria, microalgae—single celled, aquatic algae species—represent the best choice of biomass for biofuels production. As shown on Figure 4, when used as a feedstock for biodiesel production, microalgae produce far more oil per acre than do terrestrial plants. Microalgae are also the fastest growing plants; this higher productivity (i.e., dry tons/acre/year; or dry grams/m^2/day) also contributes to biomass. Furthermore, algae can have a very substantial lipid content, and the chemical composition of these lipids is particularly appropriate for jet fuel production, contrary to the lipids produced by terrestrial crops.
Because individual algae cells have a very large surface area per unit of mass for gas and nutrient exchange and are supported and surrounded by their aqueous environment, these species have no need to devote energy to building structural fibers, they are never water-limited, and they can always be in intimate contact with dissolved CO_2 and nutrients, which helps maximize their metabolic efficiency.

Basic Requirements for Algae Growth Systems

Culture depth and circulation: The depth of algae cultures must be limited and the cultures must be agitated or circulated to provide adequate light for photosynthesis and allow for gas exchange.

- **Adequate light exposure:** The ultimate culture density (in grams dry algae biomass per liter) is ultimately limited by light availability. Algae cells must not be stuck in either darkness or direct sunshine. Thus, cultures are generally circulated or mixed. There are algae growth systems without circulation, such as Dunaliella ponds in Australia, which involve motile (swimming) algae species. Some species that float can be stirred only occasionally, as is done in some Spriulina farms.

- **Oxygen elimination:** When algae photosynthesize, they produce oxygen in proportion to their growth, but high oxygen combined with high light is harmful to the algae. Dissolved oxygen must be monitored and excess oxygen must be removed.

- CO_2 **delivery:** A fast-growing algae culture requires carbon supplementation to increase productivity CO_2 and to help control the pH. Bubbles of CO_2 supply additional carbon and lower the pH.

- **Cleaning or biofouling reduction:** If algae cells attach to the walls of PBRs, they can attenuate the light reaching the culture inside. Circulation, mixing, and bubbling can reduce the amount of fouling on the walls of the PBRs or ponds. Build-up of dead algae and other detritus on the bottom of the growth system can create problems that depend on culture nutrient levels and other culture parameters. The ability to clean an algae growth container easily is crucial to its long-term performance.

- **Temperature Regulation:** All algae species have a preferred temperature range for optimum growth. Although good illumination is generally required for high productivity, high light may also produce high temperatures. In open ponds, temperature is controlled by evaporative cooling that occurs inevitably through the exposed water surface. In closed PBRs, evaporative cooling is created by spraying the outside of the PBRs with water.

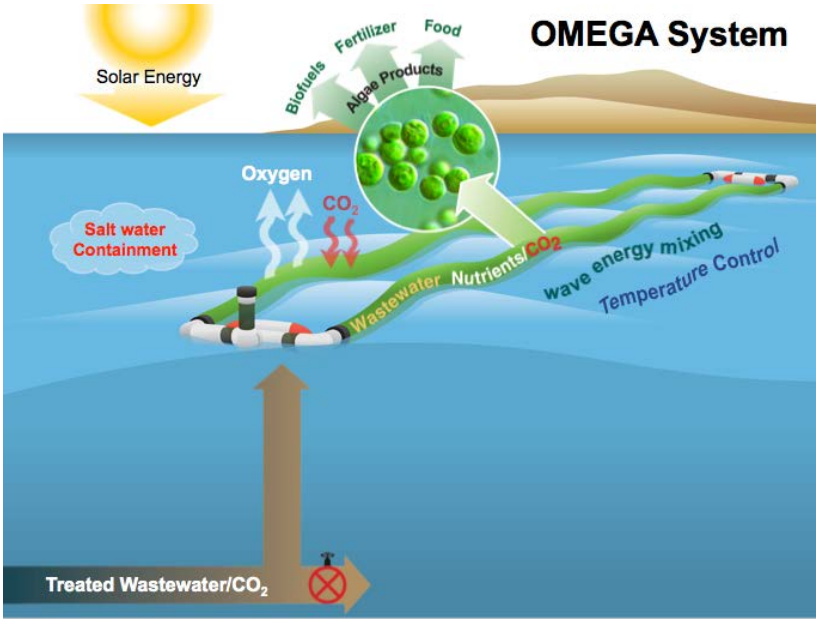
- **Harvesting:** Algae cultures are mostly water and for many processes the dry or nearly dry biomass must be recovered. Methods for sedimenting, floating, or centrifuging algae are standard for harvesting, but energy requirements and costs are of concern.

The Case for OMEGA

OMEGA (Offshore Membrane Enclosures for Growing Algae) is a system that uses PBRs made of flexible clear plastic tubes floating in protected offshore environments. The PBRs are filled with nutrient-rich water and CO_2 to cultivate microalgae. There is already a thriving industry using microalgae to produce high-value products (e.g., pharmaceuticals, nutraceuticals, and cosmetics) and a growing interest in using microalgae for producing biofuels, if they can be cultivated at large scale without competing with agriculture for water, fertilizer, or land. The OMEGA system is the answer.

OMEGA can use wastewater from the offshore outfalls of coastal cities to provide water and fertilizer for algae growth. Because OMEGA is located offshore, it is scalable without competing for land. In addition, OMEGA uses seawater to maintain the system's temperature and the salt gradient between wastewater and seawater to kill algae that escape from the system. It also uses the salt gradient to assist in harvesting the algae by a process called forward osmosis (FO). In addition to producing microalgae, OMEGA provides advanced wastewater treatment and carbon sequestration. Furthermore, depending on the location, the OMEGA infrastructure can provide surfaces for solar arrays and access to offshore sites that can be used for wind and wave energy generation, as well as offshore aquaculture. The economics of the OMEGA system will depend on the location, the scale, and local conditions, but the potential products and services are diverse and the impact on local job creation would be significant.

The OMEGA system is an array of closed PBRs constructed primarily of inexpensive, clear, flexible plastic designed to be filled with treated wastewater from coastal cities' municipal wastewater treatment plants.



OMEGA has passed the planning and testing stage. With support from the California Energy Commission (US $0.8M) and NASA (US $10M) for the last three years (2010-2012), the OMEGA team of scientists and engineers developed and extensively tested floating microalgae cultivation systems in seawater tanks. The technical results are available on request. These results confirm that OMEGA is feasible for growing microalgae on wastewater and suggest that in the right location the system could be scaled and highly productive.

Abundant supply of wastewater inputs
Approximately 34 billion gal/d of wastewater is presently treated in the US. Roughly half of this wastewater is produced in coastal counties and is therefore potentially available as a nutrient input to OMEGA.

Does not require irrigation water **or fertilizer**
Conventional terrestrial energy crops like corn ethanol or soy for biodiesel have very high direct and indirect water demands, ranging from 2,100 L water/L ethanol fuel to 14,000 L water/L soybean biodiesel. By contrast, the OMEGA project does not consume any irrigation water; instead it satisfies all its water needs using wastewater effluent that is currently discharged to the sea. Additionally, the product algae suspension delivered to shore is generally more than 99 percent water, which can be reclaimed. OMEGA uses no irrigation water, it needs no cooling water (contrary to land-based enclosed algae growing facilities), and the water used in the process can be reclaimed through a patent pending system called "Desalgae."

Synergies with carbon capture and storage (CCS) technologies
It is increasingly likely that CCS will generate massive amounts of high purity fossil CO_2 circulating in an extensive pipeline network, and that OMEGA will be able to connect to it for its CO_2 supply.
Offshore platform does not compete for land and can be used for alternative energy and aquaculture

Because OMEGA includes large arrays of floating docks in shallow coastal waters, these surfaces could be used for PV panels above water and water-cooled storage batteries below water to capture solar power. Sensitive motion generators could take advantage of small waves and vertical axis wind turbines can utilize low speed winds to generate power from wind energy. These local sources of energy can power the pumps of the OMEGA system, which are only needed during the day. The offshore structure also provides access for aquaculture development compatible with local conditions and markets. OMEGA is a technology that produces high-energy clean-burning renewable fuel, while removing nutrients from wastewater, thereby addressing a significant wastewater treatment issue. Contrary to any land-based terrestrial energy crop, it does not use any land, and does not compete for food and feed acreage. It uses no chemical fertilizers that are produced from energy- and water-intensive processes. An artist's conception of the integrated system is shown in Figure 5.

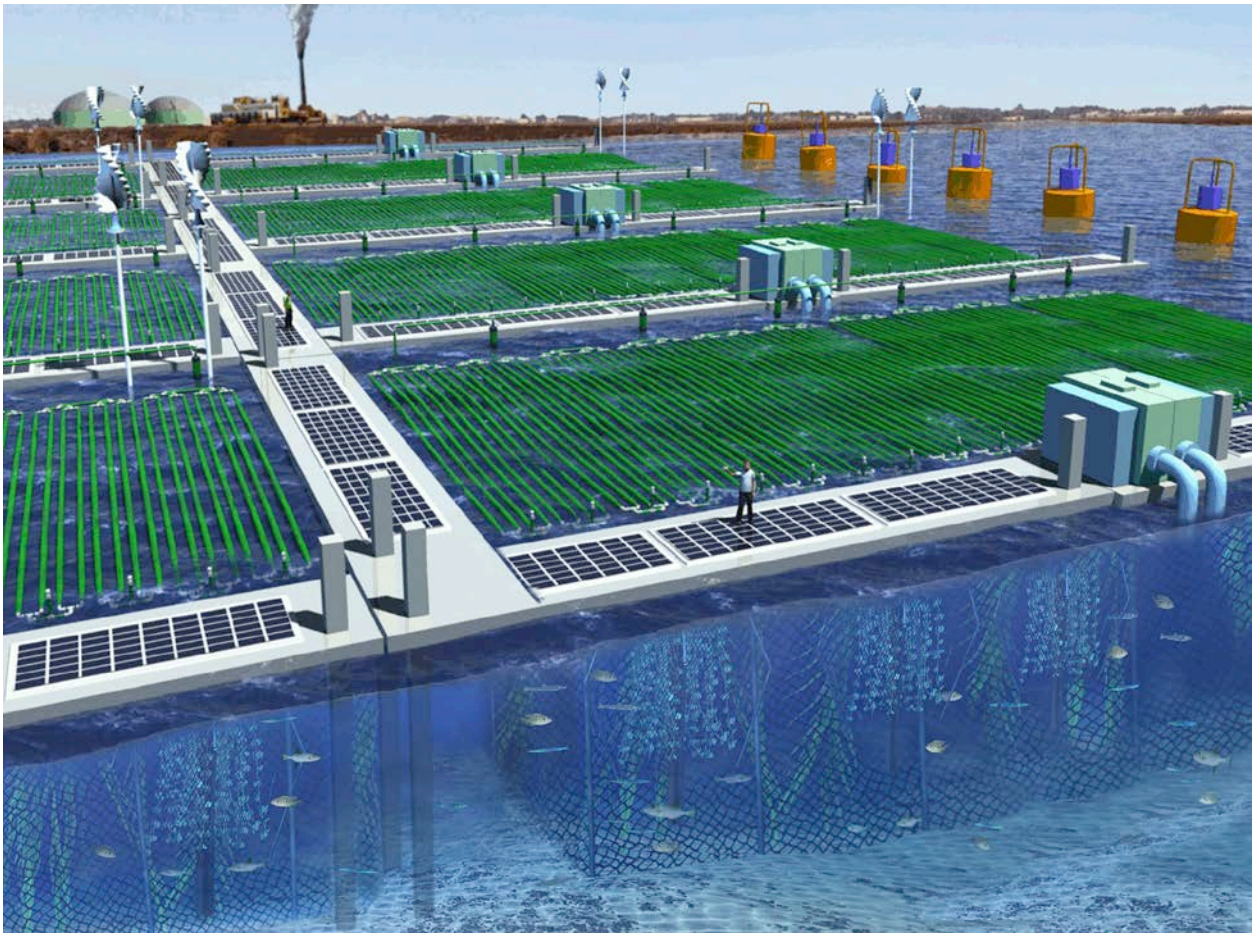


Figure 39: Conceptual View of Integrated OMEGA System

7.6 From here to there

Based on preliminary results, the OMEGA technology appears to be technically feasible, but whether it can be cost-effective, energy efficient, and have a net positive impact on the environment remains to be demonstrated.

This report outlines the business case OMEGA. The goal of the OMEGA project is to demonstrate that an ocean deployed, floating PBR inoculated with freshwater algae can produce sufficient lipids for conversion to fuel to be economically feasible and appropriately scalable so the technology may be transferred to commercial or other government sectors.

To achieve this overarching goal, five top-level objectives have been identified as:

- Design, develop, and build a modular algal cultivation system that utilizes and treats wastewater and is sufficiently scalable to be relevant for biofuels.
- In the next two years, deploy and operate an integrated OMEGA pilot system that combines algae cultivation, wastewater treatment, carbon sequestration, alternative energy (solar, wind, wave), and aquaculture at the scale of 1 hectare.
- Develop protocols to expand OMEGA systems into diverse marine environments.
- On the basis of actual field data, evaluate the OMEGA return on investment for both energy and economics, and conduct a complete life cycle analysis to determine its impact on the environment.
- Develop global partnerships with governments and industry to transition and implement large-scale OMEGA projects.

7.7 OMEGA Project Team

The successful development of OMEGA will involve a diverse group of engineers, scientists, aquaculturists, business and marketing people, as well as government officials, supporters, and consumers. Required personnel will include civil, mechanical, electrical, process, and marine engineers; university scholars, experimentalists, and students; industrial entrepreneurs, developers, marine architects, materials suppliers, builders, instrumentation and control systems providers, and a host of down-stream processing experts ranging from algae oil producers to wastewater analysts.
Developing the OMEGA Commercial System Addressing the Need and creating the market

7.7.1 Introduction

The concept of OMEGA emerged in the summer of 2008 as a way to manage nutrient rich agricultural runoff and wastewater spill that was causing large algae blooms in inland and coastal waters. NASA scientist Dr. Jonathan Trent proposed a system for removing fertilizer from the rivers using freshwater microalgae in large enclosures that effectively "filtered" out the

nutrients in the river water as it passed by the algae enclosures. When the algae culture was fully grown, the enclosure would be floated down river and into the ocean where FO would be used to dewater the algae in preparation for harvesting. Based on this original idea, he developed the OMEGA system to process domestic wastewater discharged offshore form coastal cities. Focusing initially on protected bays, the OMEGA system can reach scales that would be consistent with the production of biofuels.

With preliminary laboratory results from NASA Ames, a proposal funded by the California Energy Commission (CEC) in the spring of 2009 supported lab experiments through the summer of 2009. A briefing to the Deputy NASA Administrator, Lori Garver, presented at the end of August, 2009 led to support from NASA Headquarters and the NASA OMEGA project began in Jan 2010 with collaborations at University of California at Santa Cruz, California Fish, Wildlife, and Moss Landing Marine Laboratory, University of Nevada at Reno, and the San Francisco Wastewater Southeast Treatement Plant (SEP).

Using the lab and seawater tanks at the California Fish and Wildlife facility as a "skunk works," a functional system evolved at a scale of 100 liters, which was replicated at a scale of 1,600 liters at SEP (Wiley et al., 2013). These experiments confirmed that indeed algae can grow well on wastewater and that the OMEGA system is an effective way to remove nutrients and contaminants, such as pharmaceuticals and personal care products (Kolodziej et al. 2013). Experiments with forward osmosis (FO) indicated that the FO process can be used to concentrate microalgae from wastewater using seawater as a draw solvent (Buckwalter et al., 2013).
In this section, the OMEGA system is evaluated for the critical factors needed for commercial success and the plans for future pilot testing and development are presented.

7.7.2 Project Objectives and Scope

The OMEGA project proposes to use solar energy, nutrient-rich secondary wastewater effluent, and CO_2 to grow lipid-producing algae in ocean-deployed PBRs. The microalgal biomass will then be converted in to a hydrocarbon fuel spread focused on jet fuel. The modules will be located offshore to eliminate the use of valuable land, while utilizing the ocean's thermal stability, wave energy, and salinity to maximize lipid production and greatly reduce the energy required for algae production. The technology will be cost-effective and energy-efficient.

A basic process flow diagram for the assumed overall system is shown on Figure 6. Note that CO_2 is provided by a combustion source nearby or from anaerobic digesters. When needed, this would be supplemented from a CCS pipeline.

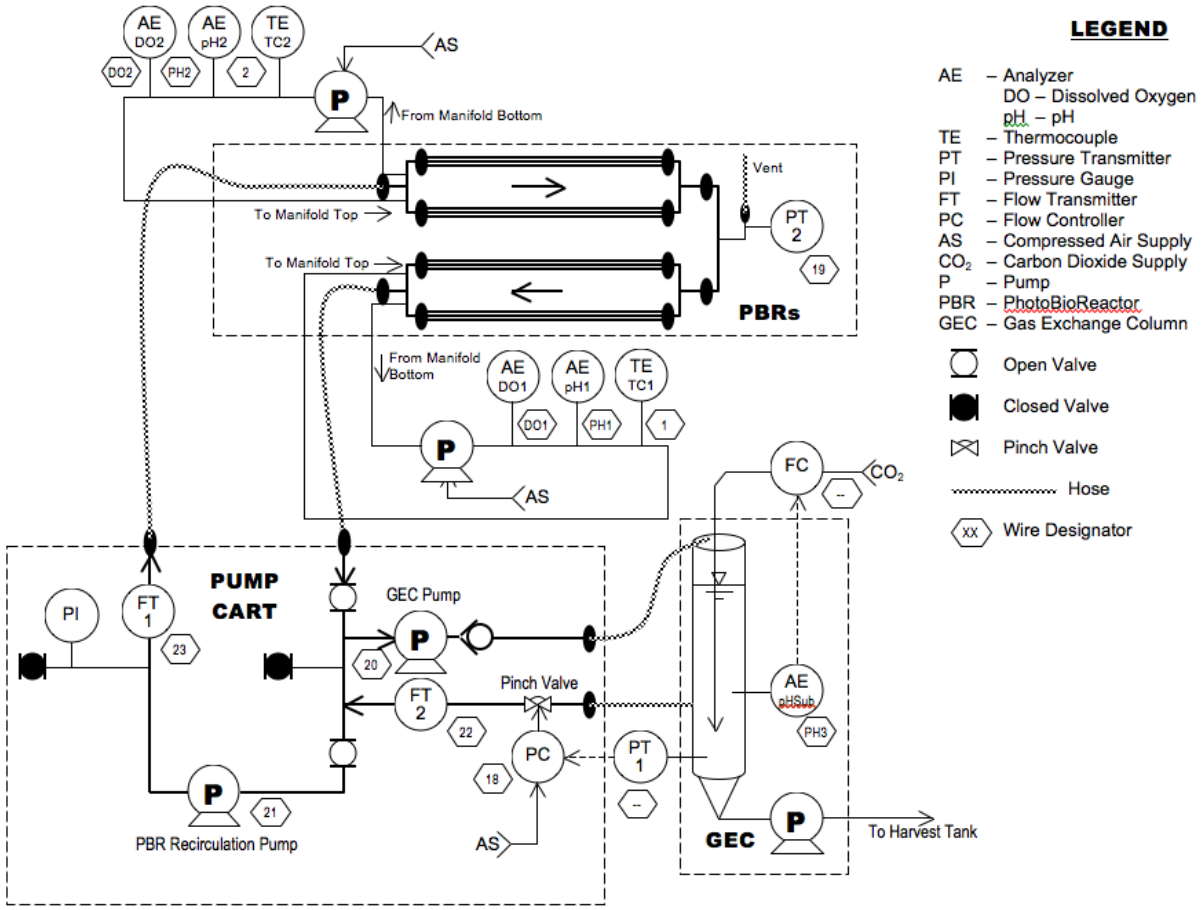


Figure 40: Schematic Representation of the OMEGA System

The full-scale OMEGA system will consist of an array of PBR modules floating on the ocean surface in naturally or artificially protected bays. A network consisting of valves, pipes, and pumps will supply the PBR modules with wastewater for nutrients and CO_2 for conversion to algae biomass (Wiley et al., 2013). Gas exchange and harvesting columns (GEHCs) will control dissolved oxygen and pH using controlled CO_2 addition. The algae slurry that accumulates at the bottom of the GEHC will be harvested and returned to shore for further dewatering, lipid extraction, and recovery of other biomass constituents. The major system-level operations required for the functioning of an OMEGA array can be summarized as filling of the PBRs, algae growth, and the harvesting of mature biomass from the PBRs.

Filling: includes the transfer of secondary-treated wastewater effluent and CO_2 gas to start the algae growth cycle in the circulating PBR modules. Operating costs for filling consist mainly of the cost of energy required for pumping. The energy required to fill the system is related to the infrastructure, pumping head required, and the volume to be moved. The wastewater would be provided directly from a landside treatment plant via new pressurized marine pipeline(s), or by existing wastewater outfall pipes.

Growth: the algae growth cycle is continuous with instrumentation that monitors pH controlling CO_2 input and dissolved oxygen determining the rate of transfer to the

GEHC. Wireless monitoring indicates various operating parameters to a landside computer for processing. This setup will aid in process control to optimize growth for prevailing conditions and in determining when to harvest. During this part of the production cycle, the initial effluent may be partially dewatered using some form of FO to concentrate nutrients.
Harvesting: the algae slurry is removed from the OMEGA GEHC regularly, further concentrated, and pumped as a slurry to shore for processing. The volume of the harvested culture will be reduced by FO dewatering and some form of flotation or sedimentation to reduce the energy required for pumping.

7.8 Critical Success Factors

For the OMEGA concept to be successful, certain critical success factors must be met. These factors include requirements for:

Sustainability: able to be sustained for an indefinite period without damaging the environment, without depleting local resources; all components are renewable or recyclable.
Economically feasibility: the production of biofuel must be economically feasible when considering all economic, social and environmental benefits and costs. Furthermore, the system must be cost-effective when compared with alternative technologies.
Scalable and able to produce high value fuels: scalable and able to produce an algal feedstock capable of making biofuel, while providing other services and products.
Able to use nutrient-rich wastewater effluent: thereby changing its perception from one of waste product to that of important process input. In so doing, saving energy intensive nutrient removal treatment costs, and preventing potential environmental impacts from discharge.
Minimal land and water footprint: continued population growth and urban sprawl are placing increasing pressure on valuable land and water resources. Land prices are increasing, and water availability is declining. A system that has a low land and water footprint will achieve longer-term sustainability.
Energy efficiency: the system must be capable of producing more energy than is used as an input during its production. An energy efficient system will have a ratio between energy outputs and energy inputs of >1.
Integration into a system of system: using local energy sources and resources to produce electricity and independent products. The system is capable of producing electricity using solar PV, wind turbine, and wave-energy generators. It uses seawater to cool batteries for electricity storage. It uses the offshore infrastructure to support aquaculture.

7.9 Functional Assessment

7.9.1 OMEGA Engineering Considerations

The purpose of the preliminary engineering analysis was to identify any fatal flaws and investigate possible OMEGA system components. Ultimately, OMEGA PBR module must be able to withstand the ocean environment, contain the freshwater algae, and transmit sunlight

for algae growth. The PBR modules must not only provide a suitable product from a biological standpoint but also be structurally sound and cost effective, requirements that are often in conflict with one another.

PBR Module Design

PBR designs are based on simplicity of fabrication, materials cost, suitability for cleaning, ability to maintain uniform culture depth, scalability, ease of modeling, compatibility with support structures, and adaptability of the overall process. Many designs have strengths and weaknesses that could not be appreciated fully without prototype testing. Some reactor designs were more compatible with specific locations and modes of operation. The key is to utilize the system that is best for each location.

Structural Analysis

The structural engineering analysis focuses on the external and internal stability of individual OMEGA modules. External stability includes those forces acting on the PBR, such as wave action, weight, and buoyancy. Internal stability refers to the analysis of forces within the module, such as hydrodynamic pressure on the inside of the plastic module enclosure. From calculations of wave and current loading, the possibility of single modules with few attachment points was ruled out as the stress due to drag forces alone would be very large. Assessment of the combined forces showed that many anchor points will be required per PBR to keep the plastic thickness in a reasonable range. A system design incorporating a supporting grid with many attachment points was conceived to distribute stress on the PBR modules. An analysis of buoyancy led to designs for flotation to supplement the natural buoyancy of the PBR network, if necessary. Several anchoring and mooring options were investigated; those used will depend on local conditions. Internal stability analysis revealed that internal forces due to movement of the liquid inside the PBRs will be significantly less than that due to external forces, and so will not be a major consideration. The PBR designs were evaluated for their compatibility with the supporting grid.

Infrastructure Analysis

The main infrastructure components that were identified include (a) pressurized undersea main from the wastewater treatment plant, (b) network of pressurized distribution piping and manifolds for the bioreactor array, (c) platform for mounting of manifold and valve equipment and for staging service operations, and (d) instrumentation and control systems. A full hydraulic design will be needed for sizing of pipelines, pumping systems, and control systems; however, the process design and PBR module configuration will have a significant impact on the overall infrastructure and power requirements. Therefore, it is recommended that further process and module selection be conducted iteratively and in parallel with the hydraulic design. Piping and distributions system components should be made of polyethylene, which is the best option when workability and strength are considered.

The strategic assessment selected to an all-fixed infrastructure; however, within that alternative many choices remain. It would be premature to down-select further at this point, because the technical feasibility of the various process designs is yet to be determined. Future proposed

field testing will provide the data necessary to determine technical feasibility, after which financial feasibility can be used to optimize alternatives.
The strategic assessment provided sufficient information from where the project team developed an OMEGA concept baseline that will be evaluated further using benefit cost analysis (BCA) to provide a preliminary estimate of financial feasibility. The BCA also identified those variables that have the greatest impact on financial feasibility, which will become the focus of future investigations.

7.10 OMEGA Evaluation

The OMEGA features identified for further evaluation is described in Table 4.

Table 7: Attributes of the OMEGA Baseline

Attribute	Description
System Architecture	Floating PBRs with integrated alternative energy and aquaculture facilities
Location	Offshore in protected bay in the vicinity of wastewater outfall and source of CO2 from flue gas
Production Capacity	100 million liters per day treated wastewater feed to the OMEGA system
Ocean Footprint	The pilot OMEGA field will be approx. 1 hectare; each OMEGA PBR will be approx. 20m x 20 cm in diameter.
System Mooring	There will be a dock and mooring support system for the OMEGA field and associated aquaculture.

In advancing OMEGA commercialization, a strategic assessment of the return on investment (ROI) must be based on reasonable assumptions or preferably actual measurements. In the research presented in this CEC Report, we have described the first steps in defining a functional OMEGA system and its implication for cultivating algae and wastewater treatment. An OMEGA pilot test in a commercial situation is required to produce the data necessary to resolve unanswered questions.

Such a strategic assessment can be conducted in three steps. First, identify the deployment site and evaluate accessibility of wastewater, CO2, algae processing facilities, social and political issues, and other features of the site. Secondly, assemble the local science and engineering team to create preliminary designs with an energy return on investment (EROI) to identify the preferred system architecture. Thirdly, the build test modules to evaluate the designs, engineering and logistics. The system will be implemented systematically to identify fatal flaws and investigate preferable OMEGA system components and conditions.

Techno-economics (TE) and Life cycle Assessment (LCA)

The preliminary TE and LCA should compare OMEGA's life cycle impact with that of other biofuel technologies (e.g. corn ethanol and biodiesel). Further, the LCA will identify the steps,

inputs, and outputs associated with OMEGA with the greatest environmental effects, highlighting opportunities to reduce those.

No results are presently available from the LCA; however, relative to other biomass systems, OMEGA will show considerable advantages with respect to the use of water and land resources.

7.10.1 EROI

A preliminary EROI will focus on the energy expended in the three major operations for the PBRs: filling, growth, and harvesting, as well as for all peripheral activities associated with alternative energy production and aquaculture. All of these operations are based on Energy Investment versus Energy Return, as follows:

ENERGY INVESTMENT

- Embodied energy (energy to manufacture, transport and assembly infrastructure for OMEGA system)
- Operations energy for each system of the system
- Processing and distribution

ENERGY RETURN

- Liquid biofuel production, electricity, and calories
- Biogas from non-oil biomass: energy recovered from anaerobic digestion of non-oil fraction of the algal biomass.
- Other sources of energy based on savings from alternative production methods.

7.11 Analysis of Risk

The OMEGA project has a number of technical, economic, and environmental risks associated with each phase of the project. Without additional data, it is not possible to give a meaningful assessment of risk, but it is useful to identify some of the key categories of perceived risk for different phases of OMEGA (Table 5 below). Risk is qualitatively classified according to:

- Prototype, pilot, commercial Scales
  - Site selection and evaluation
  - Logistics for supply and processing pathways
  - Permitting, purchasing, and environmental impact surveys
  - System construction, installation, deployment
  - System operation

- Project Elements/Sub-Element
  - Site characterization
    - Physical environment: weather, waves, currents, history, future
    - Distance to resources: sewage, CO_2, plastics, suppliers manufacturing, installation
    - Distance to markets: processing plants, customers
  - OMEGA system installation
    - Infrastructure: docks, piers, plumbing, pumps, solar, wave-energy, wind turbines

        - PBRs: Manufacture
- OMEGA system performance
        - Algae productivity
        - PBR cleaning protocols: inside (PIGs), outside (biofouling busters)
        - Harvesting: system comparisons
        - Carbon sequestration: quantification [life-cycle assessment (LCA)]
        - EROI, ROI
- Alternative Energy
        - Solar
        - Wind
        - Wave
- Aquaculture
        - Species selection: suitability, markets, logistics
        - Environmental impact
        - Requirements

Table 8: Risk Analysis of Factors Impacting OMEGA at Protoype, Pilot, and Commercial Scales.

Risk Scenario Index	Project Stage	Project Element	Project Sub-Element	Risk Scenario
1	Prototype-Scale Test Program (Phase I)	PBR Fabrication	Fabrication of Prototype-Scale PBRs	Delay in PBR fabrication due to low priority assigned by manufacturer
2	Prototype-Scale Test Program	Permitting	Environmental Permitting	Permitting issues delay testing
3	Prototype-Scale Test Program	Prototype-Scale Testing of Engineering Behavior	PBR Prototype	Test results show that substantial modifications to design/engineering analysis of PBR modules are required
4	Prototype-Scale Test Program	Prototype-Scale Testing of Bio-Chemical Behavior	Test Plan	Some key research questions may not be investigated
5	Prototype-Scale Test Program	Prototype-Scale Testing of Bio-Chemical Behavior	Prototype-Scale Process Testing	Test results show inconclusive or conflicting patterns regarding the effect of key process parameters on biomass growth and lipid production
6	Pilot-Scale Test Program (Phase II)	Site Selection	Pilot-Scale Test Site	Delay in getting agreement with site operator for use of site for pilot testing
7	Pilot-Scale Test Program	Site Selection	Pilot-Scale Test Site	Site doesn't have all required OMEGA inputs
8	Pilot-Scale Test Program	Site Selection	Wave-Tank Test Site	Delay in getting agreement with site operator for use of site for wave-tank testing
9	Pilot-Scale Test Program	Environmental Permitting	Pilot-Scale Test Site	Delay in getting necessary permits from regulatory agencies delays test program
10	Pilot-Scale Test Program	Site Modifications	Pilot-Scale Test Site	Delay or inability to perform site modifications delays test program
11	Pilot-Scale Test Program	PBR Design	PBR Design	Incomplete/ inadequate PBR design at time of pilot test
12	Pilot-Scale Test Program	PBR Design	PBR	Design is unable to be fabricated in timely manner
13	Pilot-Scale Test Program	PBR Fabrication	Fabrication of Pilot-Scale Prototype PBRs	Delay in fabrication due to low priority assigned by manufacturer
14	Pilot-Scale Test Program	PBR Logistics and Deployment	Pilot-Scale Test Site	Fabricated PBR is unable to be deployed and recovered; delay is due to special requirements
15	Pilot-Scale Test Program	PBR Infrastructure Design	Pilot-Scale Test Site	Infrastructure is incorrectly designed, causing delays in pilot testing

Risk Scenario Index	Project Stage	Project Element	Project Sub-Element	Risk Scenario
16	Pilot-Scale Test Program	PBR Infrastructure Design	Wave-Tank Test Site	Infrastructure is incorrectly designed, causing delays in pilot testing
17	Pilot-Scale Test Program	PBR Installation	PBR Installation at Pilot Test Site	Installation problems lead to design modifications and reinstallation
18	Pilot-Scale Test Program	I&C System	Performance	I&C system does not perform as designed, leading to design modifications and reinstallation
19	Pilot-Scale Test Program	Pilot-Scale Testing of Biochemical Behavior	Test Plan	Budget/Schedule constraints limit the number of experiments that could be carried out; Some key research questions may not be investigated
20	Pilot-Scale Test Program	Pilot-Scale Testing of Biochemical Behavior	Algae Biomass Growth	Test results show inconclusive or conflicting patterns regarding the effect of key process parameters on biomass growth; large variability/uncertainty in biomass growth projections
21	Pilot-Scale Test Program	Pilot-Scale Testing of Biochemical Behavior	Lipid Production	Test results show inconclusive or conflicting patterns regarding the effect of key process parameters on lipid production; large variability/uncertainty in lipid production projections
22	Pilot-Scale Test Program	Pilot-Scale Testing of Engineering Behavior	Piping Infrastructure	Piping infrastructure fails to perform as expected; requires design modifications
23	Pilot-Scale Test Program	Communications Protocol	Communications Between Science and Engineering Teams	Poor communication leads to improper testing and data use
24	Pilot-Scale Test Program	Pilot Testing	Test Plan	Test plan not completed on time leads to schedule slip
25	Pilot-Scale Test Program	Field Laboratory	Equipment	Delay in setting up field laboratory negatively impacts ability to implement test program
26	Pilot-Scale Test Program	Field Laboratory	Staffing	Lack of sufficient number of qualified personnel leads to lack of proper test procedures and lack of sufficient data collection
27	Pilot-Scale Test Program	Decommissioning	Pilot-Scale Test Site	Decommissioning requires more extensive provisions than anticipated
28	Develop 1 hectare (Phase III)	Commercial OMEGA System	PBRs	Test results indicate need for revised analytical methods
29	Develop 1 hectare	Commercial OMEGA System	PBRs	Test results indicate uncertainty regarding scalability to a fully commercialized system
30	Develop 1 hectare	PBR Models	FEA	FEA model is inconclusive due to lack of sufficient quality data
31	Develop 1 hectare	PBR Models	Hydrodynamic	Hydrodynamic model is inconclusive due to lack of sufficient quality data

Risk Scenario Index	Project Stage	Project Element	Project Sub-Element	Risk Scenario
32	Develop 1 hectare	Design	OMEGA Engineering Design Standards	OMEGA engineering design standards are inadequate which may lead to inadequate design (structural/ materials failure, process failure) or overly conservative design
33	Develop 1 hectare	O&M	O&M Protocols	OMEGA O&M protocols are unreliable or ineffective due to the use of insufficient or improper data
34	Feasibility Evaluation	Business Case	BCA	BCA model is inconclusive due to the lack of sufficient quality data
35	Feasibility Evaluation	Business Case	LCA	LCA model is inconclusive due to the lack of sufficient quality data
36	Feasibility Evaluation	Business Case	EROI	EROI model is inconclusive due to the lack of sufficient quality data
37	Feasibility Evaluation	Business Case	GHG	GHG model is inconclusive due to the lack of sufficient quality data
38	Feasibility Evaluation	Environmental Compliance & Permitting	Full Scale Deployment	Unclear permitting requirements leads to delay in full scale deployment

Currently the largest risks are those influenced by the uncertainty associated with the marine environment and the impact it has on the integrity and operation of the OMEGA system. Environmental issues will affect all phases of the project from system construction, installation, and deployment to system operation and performance. Other risks include those associated with interference to ship navigation channels, construction impacts on the marine environment, and costs and scheduling impacts due to permitting.

Risk from competition from existing algae companies that are making biofuel on land is not considered significant. Companies, such as Solazyme, Sapphire Energy, Aurora Algae, Origin Oil, Algae.Tech, Heliae, and many others claim to be working in the biofuels market, but simple calculations indicate their systems are not scalable or cost effective. Indeed, most of these companies are actually focused on algae products for high-value markets involving pharmaceuticals, nutriceuticals, cosmetics, or fine chemicals. OMEGA is focused on biofuels and therefore on scale, low costs, and energy efficiency. As indicated above, the scale-up strategy uses services (wastewater treatment and environmental remediation) and products unassociated with algae; i.e., alternative-energy electricity and aquaculture.

CHAPTER 8:
Evaluation of OMEGA Commercialization

8.1 Background

OMEGA offers many advantages that other biomass generating systems do not. These advantages include minimal use of land and water resources, synergies with CCS technologies, and the production of offshore alternative energy and food from aquaculture. In this section, the OMEGA technology is evaluated to see whether it meets the stated critical success factor of being economically feasible. OMEGA infrastructure is used for mariculture fish farming and crustacean, mollusk and aquatic plant cultivation in marine environments. PBR systems can become an integral product line for the vast global commercial aquaculture market. The system benefits could include supporting mussel, scallop, oyster, and abalone production, as well as fin-fish, crab, and shrimp production. The global multi-trophic aquaculture market sector is a multi-billion dollar industry.

8.2 Life cycle Assessment

A LCA is presently underway to determine how OMEGA's life cycle impact compares with that of other biofuel technologies (e.g. corn ethanol and biodiesel). Further, the LCA will identify the steps, inputs, and outputs associated with OMEGA with the greatest environmental effects, highlighting opportunities to reduce those.
No results are presently available from the LCA; however, relative to other biomass systems, OMEGA will show considerable advantages with respect to the use of water and land resources.

8.3 OMEGA cost analysis

A preliminary analysis is based on construction capital costs, operating costs and revenues over the investment lifecycle and was used to show the production costs after tax, depreciation, and amortization (Fig. 7). This analysis does not include indirect costs or contingencies. All costs, revenues, and yields were computed using a standard discounted cash flow (DCF) analysis. A summary of the discounted initial investment, depreciation, operating costs, revenues and oil yield is presented in Table 6. The financial period assumed a 20-year lifetime and used a 10 percent discount rate. A business tax credit of 30 percent and a tax rate of 35 percent were assumed. The operating costs include the material and energy required for the facility, but do not include labor.

The total lifecycle costs (TLCC) were calculated using the equation:

$$TLCC = I - (T \times PV_{DEP}) + PV_{OM}\,(1-T) - PV_{NLPS}$$

- where I is the initial investment
- T is the income tax rate

- PV_{DEP} is the present value depreciation cost
- PV_{OM} is the present value operation and maintenance
- PV_{NLPS} is the present value of non-lipid services
- The revenue required (RR) equation for a break-even scenario gives the dollar value per liter of triacylglyceride (TAG),
- RR ($/liter TAG)=TLCC ($M/project lifetime) / PVoil yield (liters/project lifetime)

Table 9: Summary of Discounted Cash Flows and Assumptions Used to Calculate the Total Life Cycle Costs and Revenue Required in Units Dollars Per Liter of Triacylglycerides (TAG)

Present Value	
Initial Investment ($M/project lifetime)	65.24
Depreciation ($M/project lifetime)	45.88
Operating Costs	
Electricity ($M/project lifetime)	1.65
Hexane ($M/project lifetime)	0.07
Non-lipid services	
Fertilizer ($M/project lifetime)	1.00
Oil Yield (Mliters/project lifetime)	4.54
Tax Rate (%)	35
Discount Rate (%)	10
Project Lifetime (years)	20

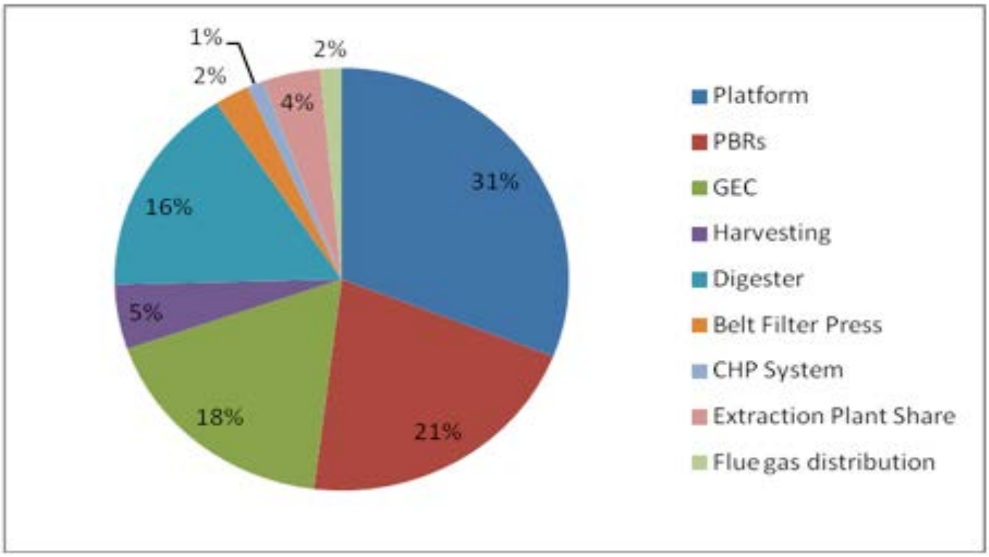


Figure 41: Allocation of Construction Capital Costs of OMEGA

The result for the production cost of TAG using the OMEGA base-case model is $6.67/liter TAG ($25.26/gallon). A study conducted by the National Renewable Energy Laboratory (NREL, 2009) evaluated the economics of producing microalgal derived oil using open ponds and closed PBR systems for three growth scenarios – base case, aggressive case and max growth case. The findings of the NREL study are summarized in Table 7.

Table 10: Economic Comparison of Base-Case OMEGA to Values Published by NREL (2009)

	OMEGA Base Case	**NREL Base Case**		**NREL Aggressive Case**		**NREL Max Growth Case**	
	PBR	**Open Pond**	**PBR**	**Open Pond**	**PBR**	**Open Pond**	**PBR**
Algal Productivity	25 $g/m^2/day$	25 $g/m^2/day$	1.25 $kg/m^3/day$	40 $g/m^2/day$	2.00 $kg/m^3/day$	60 $g/m^2/day$	3.00 $kg/m^3/day$
Cell Density [kg/m^3]	**0.3**	0.5	4	0.5	4	0.5	4
Lipid Yield [dry wt%]	25%	25%	25%	40%	40%	60%	60%
Overall Lipid Production Cost [$/gal]	**$25.26**	**$11.25**	**$19.49**	**$5.11**	**$10.07**	**$3.99**	**$6.62**

Land Cost [$/gal]		$0.38	$0.21	$0.12	$0.06	$0.07	$0.04
Capital Cost [$/gal]	**$21.94**	$5.10	$13.21	$2.16	$6.75	$1.63	$4.07
Operating Cost [$/gal]	**$3.30**	$5.86	$6.07	$2.83	$3.26	$2.29	$2.51

There are many potential benefits of OMEGA that were not quantified in this simple economic assessment that would significantly influence the outcome. For example, by reducing the nutrient, contaminants, and other pollutants in wastewater entering coastal waters, OMEGA will reduce the potential for natural algae blooms, which impact recreation and, in some cases, produce toxins or reduce dissolved oxygen in water and disrupt sensitive coastal ecosystems.

Techno-economic modeling indicates that converting algae to biofuels only will be difficult if not impossible to support economically. By leveraging the potential of the OMEGA platform for other services and activities, however, may change the economic picture. For example, combining algae cultivation for biofuels with wastewater treatment, renewable electricity, and aquaculture significantly changes the economics (Fig. 8).

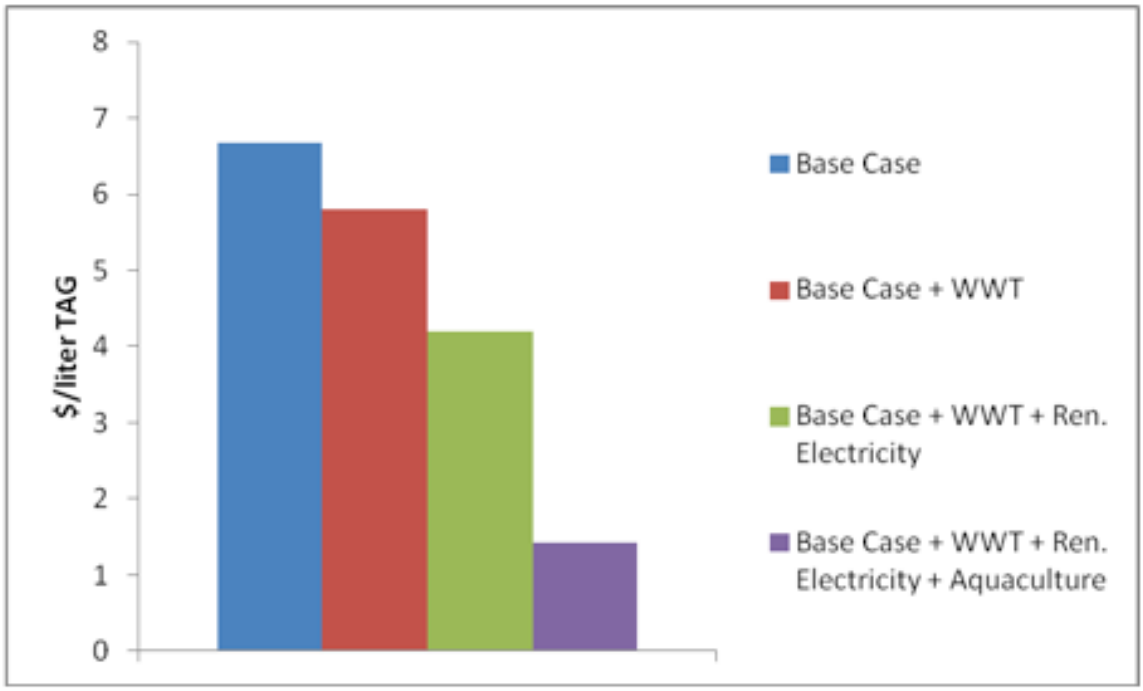


Figure 42: Economic Evaluation of OMEGA Base-Case Integrated With Wastewater Treatment (Nutrient Removal of Secondary Wastewater), Renewable Electricity (Wave-Energy, Wind, and Solar), and Mussel Aquaculture.

Collocating OMEGA with a municipal wastewater treatment plant provides nitrogen and phosphorus from wastewater as well as carbon from combustion of biogas. In turn, the algae provide biological nutrient removal and contaminant remediation for the wastewater. The floating docks supported aquaculture of mussel production and also provided power for the OMEGA system by providing surfaces for solar panels and access to vertical-axis wind turbines as well as power buoys. The locally generated power supported cultivation, harvesting, and bio-oil production with surplus electricity exported to the grid. Bio-oil production used traditional solvent extraction methods, but hydrothermal liquefaction could reduce the uncertainty of cost estimates. Using this "industrial symbiosis" system, and assuming a 10 percent return on investment, the cost of renewable diesel fell from $6.67/L (without symbiosis) to $5.80/L (13 percent reduction) with wastewater treatment, to $4.20/L (24 percent reduction) with the addition of renewable electricity sources, and to $1.43/L (41 percent reduction) with revenue from aquaculture. The economic impact of the integrated system represents a 78 percent reduction in costs (Fig. 8).

Sensitivity analysis: The financial results indicate key variables for both capital and operating costs are influenced by assumptions about currents and wave forces, lipid content of cells, the unit cost of the OMEGA PBR modules, the lifetime of the modules, and the cost of obtaining CO_2. The sensitivity analysis.

- An increase in PBR support and mooring capital costs by 50 percent, which could be caused by a 50 percent increase in the current and wave forces experienced by the system, decreases the NPV by 27 percent. This result implies that siting away from strong currents and waves will be a major concern for OMEGA.
- Doubling the unit costs of OMEGA PBR modules decreases the NPV by 26 percent, whereas halving the unit cost of plastic decreases the NPV by 13 percent.
- A PBR module lifetime of 1.5 years instead of the assumed 3-years decreases the NPV by 26 percent.
- If CO_2 can be obtained from an existing carbon capture system (i.e., zero cost), NPV increases by 18 percent
- Increasing the lipid concentration from 25 percent to 50 percent increases the NPV by 42 percent.

8.4 Conclusions and Next Steps

Results from the economic and financial analyses suggest that biofuel produced by OMEGA may be a reasonable proposition if OMEGA is combined with complementary activities, but the integrated system warrants further research. OMEGA has a negligible land footprint and it may provide improved treatment of wastewater, significant CCS, and remediation of algal blooms caused by wastewater runoff. Future research at the pilot-scale of a hectare, will help

address technical issues, external drivers, and biological, engineering, environmental, and economic challenges. Specific studies include analyses of GHG emissions, LCA, EROI, and risk. These analyses will broaden the foundation that supports further OMEGA development, while mitigating risks for any specific location. The identification and resolution of regulatory or environmental issues and the completion of an Environmental/Permitting Fatal Flaw Analysis will be important for developing OMEGA in California.

OMEGA converts wastewater into fertilizer and GHG into biomass. In addition to solar, wind, and wave energy, OMEGA uses the chemical energy between wastewater and seawater for forward osmosis. Environmentally, OMEGA remediates wastewater pollutants in coastal ecosystems and its floating infrastructure enhances local biodiversity. After a three-year feasibility study, OMEGA is ready for deployment in protected bays near cities with offshore sewage outfalls and a ready source of CO2 that is currently polluting the atmosphere. An integrated OMEGA system that produces biofuels, treats wastewater, produces renewable electricity, and supports mussel aquaculture is predicted to be financially viable and a step forward on the path beyond fossil-carbon energy.

Triple Bottom Line

- ***Environmental:*** OMEGA produces biofuels to replace fossil fuels, cleans and reuses wastewater, captures carbon to reduce global warming, and increases coastal biodiversity.
- ***Social:*** OMEGA provides a means for biofuel production that does not compete with agriculture and a platform for aquaculture for expanded food production and alternative energy generation (solar, wind, and wave) to augment non-fossil-fuel energy. The OMEGA system with all the associated industries will support thousands of jobs.
- ***Economic:*** On the basis of a techno-feasibility analysis, the OMEGA integrated system creates an "ecology of technologies" that supports both energy production and economic returns on investments.

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GLOSSARY

ARTICLES/Web articles		
Web Article	**Date**	**Title of Article**
Energy Next	5/1/13	India has great potential for developing OMEGA systems.
San Jose Mercury News	5/18/12	A green future: NASA's $10 million project explores algae of as fuel source
Green Chip Stocks	4/26/12	NASA Algae Biofuel System Ready for Commercial Use
AlgaeBiodiesel	4/26/12	Algae Biofuel Updates
Pune Mirror	4/18/12	NASA plans algae farms for jet fuel
Mountain View Patch	4/18/12	NASA Produces Biofuels with Wastewater
Algae Magazine - The Buzz	4/13/12	NASA Invites Media to Tour OMEGA System
Technology Review MIT	4/11/12	NASA Wants to Launch Floating Algae Farms
Algae Magazine	8/21/11	AIM Interview: NASA's OMEGA Scientist, Dr. Jonathan Trent
Post Carbon Institute-Energy Bulletin	6/21/11	Clean and Green Investment Forum:Biomass and Algae
Inhabitat	6/15/11	NASA's OMEGA Project Creates Carbon Neutral Food and Fuel
AJU Business Daily - Korea	6/10/11	(Needs translation)
A Matter of Degree	2010	Dr. Jonagthan Trent Nasa Senior Scientist The Omega Project (slide #7)
UCSC	10/11/10	Baskin School of Engineering highlights Research Advances on October 21, 2010
NASA Lunar Science Institute	4/1/10	OMEGA

Web Article	Date	Title of Article
The Green Economy Post	12/1/09	NASA Wants to Bag Biofuel from Sewage
AlgaeHub - Post Tagged	11/17/09	Trent (OMEGA) Algae
UCSC	8/21/09	Jonathan Trent launches innovative biofuel pilot in Santa Cruz
Sustainable Brands-The Bridge to Better Brands	5/20/09	Algae Tr(eats) Sewage, Produces Biofuel
Scientific American Magazine	5/12/09	NASA Aims for Future Fuel from Algae-Filled Bags of Sewage
New York Times	5/12/09	NASA bags algae, wastewater in bid for aviation fuel
Algae Bags	4/30/09	Proposing Algae Bags
Tech Briefs - Features	10/1/08	Dr. Jonathan Trent, Bioengineering Research Scientist, NASA Ames Research Center

Domestic Presentations/Lectures			
Date	**Organization**	**Event**	**Title of Talk/Presentation**
7/15/13	AIAA at San Jose Convention Center	Panel discussion "Emerging Pathways to Jet fuel"	TBD
6/20/13	NASA Ames	Student Intern Program	How did we get here and what's next?"
8/3/11	Stanford University	Leonardo ISAST - LASER	Space-time traveling in the here and now: How did we get here and what's next?"
8/1/11	UCSC	Center for Sustainable Energy and Poswer Systems - Solutions/Environmental Issues	Tools and Fuels for a Sustainable Future
6/6-7/2011	OPAL Financial Group	Clean & Green Investment Forum	Panelist: Algae

5/17/11	The Maritime Alliance	Greening of the Navy & Ports	The OMEGA Project
12/11-14/10	Honolulu, HI	Pacific Rim Summit Building Innovative Collaboration across the Pacific	Industrial Biotechnology & Bioenergy
10/21/10	Baskin Engineering UCSC	Research Review Day/Plenary Talks	Beyond Petroleum: The OMEGA Revolution
9/20/10	Algaetech	Visit by Algaetech at NASA Ames	OMEGA Presentation
6/8/10	National Marine Sanctuary Foundation	National Marine Sanctuary Foundation Annual Leadership Awards Dinner - 10th Anniversary	Ocean Energy Sources on the Horizon

International Presentations/Lectures

Date	Location	Organization/Conference	Title of Talk
6/10--11/2013	Lolland Denmark	International Algae Conference	Microalgae as a Scalable, Sustainable Source of Biomass for Biofuels
6/3-7/2013	Cph, Denark	21st Europeano Union Biomass Conf. and Expo	OMEGA Challenges
5/3-12/2013	So. Africa	MANGOSUTHU UNIVERISTY OF TECHNOLOGY	OMEGA presentation
4/23-25/2013	Mexico City	Congreso International Sustainability Ibero 2013	OMEGA for Biofuels, Wastewater Treatment, Carbon Sequestration, and Aquaculture
3/5-/2013	Japan	OIST (Biological Systems Unit (2 mo visit)	OMEGA: For cultivating a sustainable future
6/23-27/12	Scotland	TED Global2012	Radical Openness
10/14/11	Malaysia	MSAB Public Lecture/MSAB CRIM University Kebangsaan Malaysia	Algae for Sustainable Energy and Cean Environment Public Lecture
10/10-13/11	Korea	The Millennium Project Korea	The Universe and Me
10/2-16/11	Korea	KRIHS Transport and Maritime Affairs	Global Green Growth is Sustainable Economic Growth?

9/13-16/11	Singapore	International Green Building Conference 2011 - Advancements in Sustainability and Civil Infrastructure	OMEGA for Biofules, Wastewater Treatment, Carbon Sequestration, and Aquaculture
5/10/11	Korea	2011 3rd Global Green Growth Forum	Sustainable Economy comes from Sustainable Energy
10/28-29/10	Korea	International Forum on Developing the Green Waterfront Belt of Nakdong River	OMEGA: For cultivating a sustainable future
10/27-28/10	Korea	Global HR Forum Workshop "Open and Ready for Tomorrow" Future Foresight Workshop I	Replacing Fossil Oil with Carbon-neutral & Sustainable Oil: Algae Emerging Technology Climate Change & Renewable Energy
6/27-7/2/2010	Japan	Renewable Energy 2010	OMEGA Presentation
Dec. 2009	Denmark	The Bellona Foundation	101 Solutions "Algae - a new tool in the fight against global warming
12/1-2/2009	Hamburg, Germany	International Algae Congress	Growing algae in the ocean for biofules, watewater remediation and carbon sequestration.
4/20/09	Lolland Denmark	1st International Conference on Off-shore Algae Biofuels	Wind, Sea and Algae?

WEB Talks/Videos

Website	Date	URL
TEDGlobal Radical Openness	6/27/12	http://conferences.ted.com/TEDGlobal2012/program/speakers.php
KUSP Radio	6/19/12	N/A
Channel 7 KGO	4/17/12	http://abclocal.go.com/kgo/story?section=news/environment&id=8625389
NBC - Bay City News	4/17/12	http://www.nbcbayarea.com/news/local/NASA-Shows-Off-OMEGA-Project-147867395.html
NBC - Bob Reddell	4/17/12	http://www.nbcbayarea.com/news/local/NASA-is-147798805.html
Geek.com	4/13/12	http://www.geek.com/articles/geek-cetera/nasa-10-million-into-floating-algae-bags-20120413/
NASA-OMEGA Project You Tube	2/24/12	http://www.algaeindustrymagazine.com/nasa-omega-project/
Algaetech Insternational	10/17/11	http://algaetech.com.my/v1/2011/10/17/algae-talk-session-crim-

		conference-hall-ukm/
Press Release of Dr Jonathan Trent of NASA to Algaetech International Sdn Bhd	9/26/11	http://algaetech.com.my/v1/2011/09/26/press-release-of-dr-jonathan-trent-of-nasa-to-algaetech-international-sdn-bhd/
TedX Santa Cruz	7/27/11	http://www.youtube.com/watch?v=A6oekxl0JAs
TedX San Jose	4/18/11	http://www.youtube.com/watch?v=1_z-LnKNlco
Plenary Talk Energy	10/21/10	http://rr.soe.ucsc.edu/2010/program
SmartPlanet	7/6/2010	http://wn.com/Turning_algae_into_oil_the_NASA_way
World Trade Group	7/1/10	http://www.youtube.com/watch?v=oSM0OD7iYCc
NASA Lunar Science Institute	4/1/10	http://lunarscience.nasa.gov/?s=trent
YOU TUBE	3/18/10	http://www.youtube.com/watch?v=htggO_QOIEc
NAVY (Washington D.C)	11/16/09	http://www.energyconversation.org/conversation/can-algae-replace-petroleum-clean-low-carbon-homegrown-fuel-our-military-and-more
International Algae Congress	12/1/09	http://www.algaecongress.com/Congress-programme/page/1498/
NREL Workshop Presentation	2/19/08	http://www.nrel.gov/biomass/pdfs/trent.pdf