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

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ABSTRACT

OMEGA is a system for cultivating microalgae using wastewater contained in floating photobioreactors (PBRs) deployed in marine environments and thereby eliminating competition with agriculture for water, fertilizer, and land. The offshore placement in protected bays near coastal cities co-locates OMEGA with wastewater outfalls and sources of 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% 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% 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% efficiency, and >90% of the ammonia-nitrogen was recovered from secondary effluent. If OMEGA can be optimized for energy efficiency and scaled up economically, it has the potential to contribute significantly to biofuels production and wastewater treatment.

Keywords: Biofuels; Wastewater Treatment; Microalgae; Photobioreactor; CO_2 Mass Transfer; Fast Repetition Rate Fluorometry; Instrumentation and Control

1. Introduction

Microalgae are currently under consideration as a significant source of sustainable biofuels because of their fast growth rate and ability to produce oil that can be readily transformed into fuel [1,2]. These microscopic, single-cell organisms can be cultivated on non-arable land, lessening competition with agriculture and thus giving them an advantage over other biofuel crops [3-5]. On the other hand, microalgae require fertilizer and supplemental carbon dioxide (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

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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. Materials and Methods

2.1. Seawater Tank and Microalgal Cultures

Experiments were conducted in an 8800-liter seawater tank at the California Department of Fish and Game, Marine Wildlife Veterinary Care and Research Center in Santa Cruz, CA (Lat: 36˚57'13", Long: −122˚3'56"). The tank was covered at night with a thermal pool blanket to minimize heat loss. A mixed culture of green microalgae used as the system inoculum was dominated by *Desmodesmus* sp. and grown in 19-liter glass carboys containing either BG11 medium (ATCC) or secondary wastewater effluent. The carboys were aerated continuously with a regenerative blower (Model VFC084P-5T, Fuji Blowers, Saddle Brook, NJ) and periodically injected with pure CO_2 to lower the culture pH and provide a source of carbon.

2.2. PBR System

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

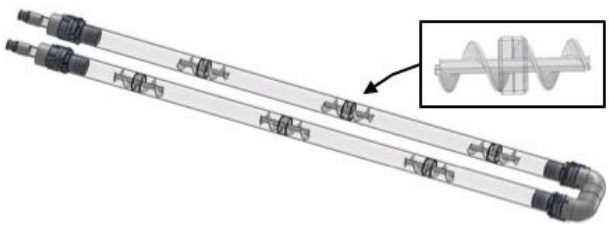

Figure 1. OMEGA photobioreactor (PBR) tubes with swirl vanes. PBRs were made of flexible, clear LLDPE connected with cam-lock fittings to a U-shaped PVC manifold. The six swirl vanes (see insert enlargement) directed the flow into a helical path to improve mixing and light exposure of the microalgae.

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**).

2.3. Gas Exchange and Harvesting Column (GEHC)

The GEHC shown in **Figure 3** was designed to: 1) manage concentrations of DO using an oxygen stripping device (OSD) based on a design by Barnhart [19]; 2) supply CO_2 to the microalgae culture and control pH; and 3) provide a settling chamber to collect aggregating microalgae for harvesting. Approximately 5% of the total system volume was diverted to the GEHC per minute, using a 12 VDC SHUR-FLO diaphragm pump (Model 2088-343-135, SHUR-FLO, Costa Mesa, CA). The pumping rate into the GEHC was adjusted by changing the voltage setting on the variable DC power supply (Model HY3005D, Mastec Power Supply, San Jose, CA).

The culture from the PBR entered the GEHC through the OSD section and cascaded over five stacked PVC plates (20 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% 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.

2.4. Instrumentation and Control

A custom instrumentation and control (I&C) system was constructed for process automation and data logging (**Figure 4**). The pH and temperature sensors in the PBR and GEHC were connected to a GF Signet model 8900 multi-parameter transmitter (Georg Fischer LLC, Tustin, CA). Output signals from the transmitter, GEHC pressure transducer, flow meter, and photosynthetically active radiation (PAR) sensor were attached to inputs of a DL06 programmable logic controller (PLC) (Automation Direct, Cumming, GA). The PLC transferred data to a human-machine interface (HMI) created using LookoutDirect software (Automation Direct, Cumming, GA) that displayed real-time data and allowed operators to specify

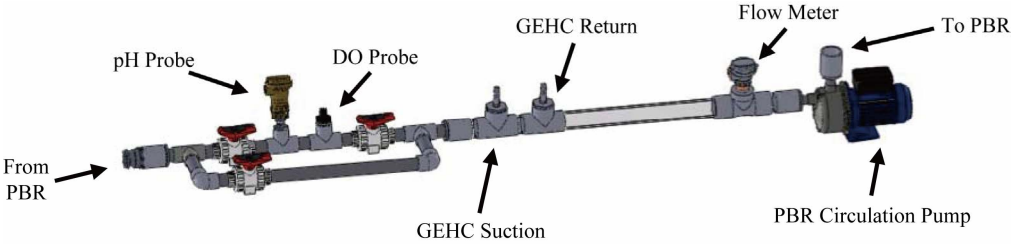

Figure 2. Inline sensors for pH, temperature, DO, and flow rate. The culture was pumped from the PBR past the sensors. Part of the circulating flow was diverted to the GEHC (see Figure 3) at the GEHC suction fitting by a positive displacement pump (not shown) and returned to the PBR flow at the GEHC return. The valved bypass was used to isolate the sensors for cleaning and maintenance without disrupting the overall circulation.

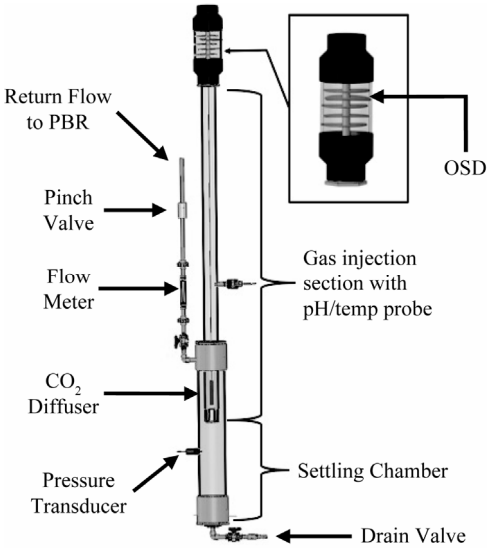

Figure 3. Gas exchange and harvesting column (GEHC) controls pH, removes settled microalgae and provides a location for wastewater addition into the PBR system. An oxygen stripping device (OSD, top) designed to remove excessive concentrations of photosynthetically generated dissolved oxygen was built 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.

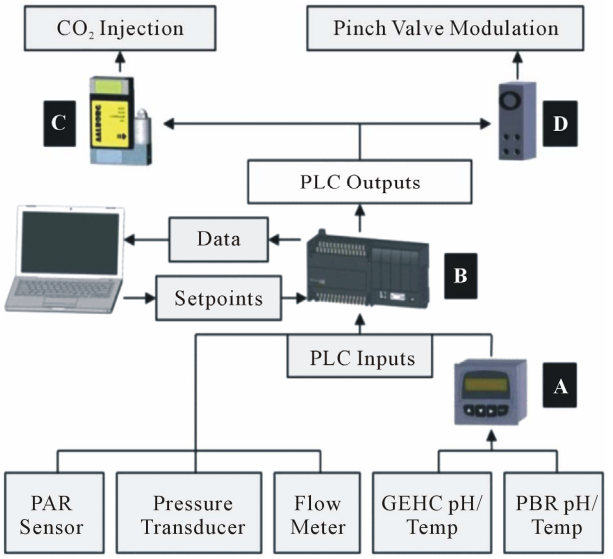

Figure 4. Components of the I&C system. Inputs from the sensors were routed through a multi-parameter transmitter (A) or directly into a PLC (B) were transferred to a computer database. Setpoint values established using an HMI modulated PLC outputs that controlled a mass flow controller for CO_2 injection (C) and an I/P transducer (D) to regulate pinch valve positioning.

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 set point. 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.

2.5. 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 $g \cdot m^{-2} \cdot day^{-1}$, in line with the average productivity cited by Putt *et al*. [21]. Several authors have noted that microalgae biomass is approximately 50% 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 × over design factor, were used in Equation (1) to estimate a peak gas injection rate of 0.5 lpm into the GEHC.

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

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% 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 ADM-1000 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.

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

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

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 titraion data using Equations (6) and (7), whereas the carbon uptake rate in the PBR was approximated with Equation (8).

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

$$\frac{dc}{dt} = K_La\left(C^* - C\right) \quad (7)$$

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

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

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

2.6. System Inoculation, Sampling Protocol, and Harvesting Procedures

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

The optical density (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).

$$SED_{\%} = \frac{OD_{750_{After}} - OD_{750_{Before}}}{OD_{750_{After}}} \cdot 100 \quad (10)$$

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 $kg \cdot l^{-1}$) removed from the GEHC and samples were collected for TSS analysis. The volume of water remaining in the PBR was determined by subtracting the harvest volume from the total system volume. This enabled calculation of the total biomass produced between harvest periods, the biomass concentration factor in the GEHC, and areal productivity (Equations (11)-(13)).

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

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

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

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).

$$CO_{2_{Conv}} = \frac{A_{Growth} \cdot f_{Car}}{\left(\frac{V_{Gas} \cdot pCO_2}{RT} \cdot \frac{12g\ C}{molCO_2}\right)} \cdot 100 \quad (14)$$

3. Results and Discussion

3.1. System Design and Performance

A 110-liter prototype OMEGA system was constructed with two tubular PBRs floating in a seawater tank, connected to an external GEHC and an instrumentation and control system (**Figure 5**). The system components

(PBRs, GEHC, and I&C) are described in the Materials and Methods. The PBRs, made of inexpensive plastic (LLDPE), were tested for their ability to support photosynthesis. The GEHC served to control DO, provide 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]. A decrease in the ratio of variable fluorescence to maximum fluorescence (F_V/F_M) indicates a decreased quantum yield resulting from damage to photosystem II and is used as an index for photoinhibition [27]. Reported F_V/F_M ratios in cultures exposed to high irradiance indicated up to 90% photoinhibition [27,28].

To limit sedimentation of microalgae in the PBRs, cultures were circulated at velocities ranging from 14 to 21 $cm \cdot sec^{-1}$, flow rates that reportedly prevent sedimentation in open ponds [29]. Microalgae suspension and mixing were enhanced by swirl vanes, which imparted a helical flow pattern. With the combination of flow rates and swirl vanes, microalgae settling in the PBRs never exceeded 14% of the total biomass. The swirl vanes also increased turbulence, which is known to improve nutrient exchange rates and light exposure in PBR cultures [30]. In cultures grown in laminar flow systems photoinhibition and light limitations are observed, both of which suppress productivity [28-30]. While swirl vanes may have improved suspension and light availability and hence productivity, two difficulties noted with the swirl vanes tested were 1) increase biofouling on the walls of the PBR in their vicinity and 2) increased drag, which increased pumping energy.

To assess the performance of the prototype OMEGA system, two consecutive experiments were conducted in April and May 2012. Experiment 1 lasted 13.5 days and experiment 2 lasted 8.6 days. In both experiments 1 and 2, the comparisons of hourly mean DO vs. PAR and DO vs. 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

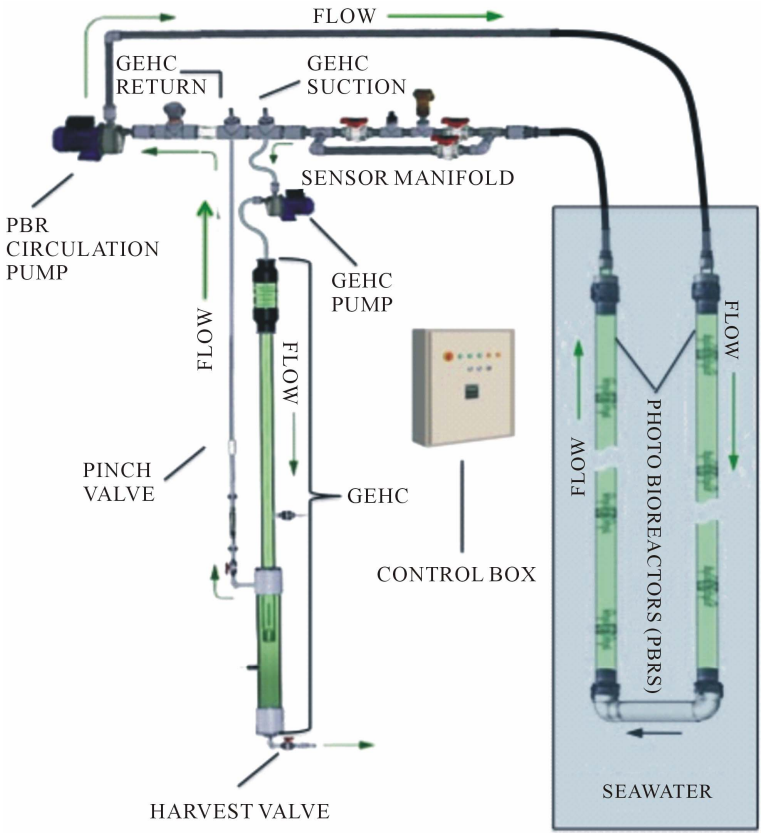

Figure 5. Component and flow diagram of the OMEGA system showing the circulation through the PBRs, sensor manifold, and side loop for the GEHC.

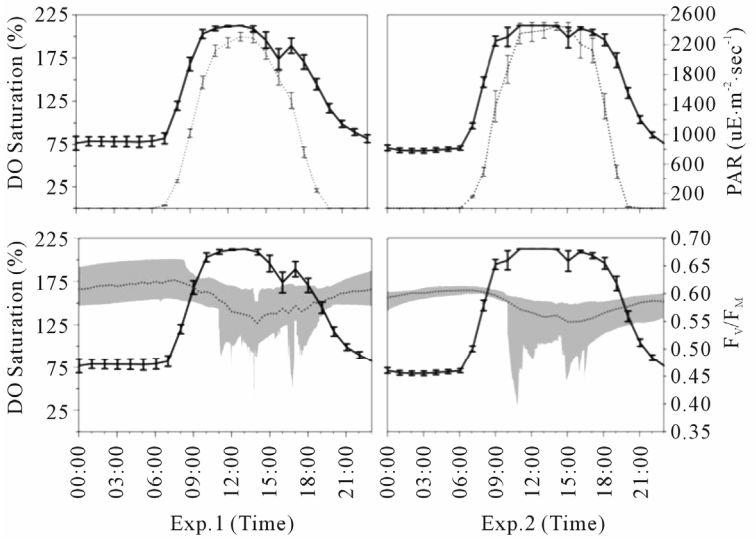

Figure 6. DO concentration, PAR and F_V/F_M values for Experiment 1 (left) and Experiment 2 (right). (Top) Mean hourly (± SE) concentration photosynthetically generated DO (solid line) increases and decreases as a function of PAR (dotted line). (Bottom) The mean hourly F_V/F_M ratio (dotted line) overlaying 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).

the DO curve is artificially flattened at peak solar irradiance (~12:00) because the DO values exceeded the upper threshold for the oxygen sensors (212% saturation) (**Figure 6**, DO saturation). After 16:00, the decline in DO was due to a combination of decreased photosynthesis, respiration, and DO removal by the OSD in the GEHC (see Materials and Methods: GEHC). The relative contribution of these different factors was not determined.

At peak DO production and peak irradiance, there was a slight photoinhibition indicated by F_V/F_M measurements, which dipped to 0.49 in experiment 1 and 0.54 in Experiment 2 (**Figure 6**, bottom). Rubio and co-workers [31] noted that in long tubular PBRs DO buildup at high irradiance caused photoinhibition and they identified this as one of the greatest constraints on the scale-up of PBRs. The solution for the OMEGA system is to adjust the ratios of residence time in the PBR to the transfer frequency to the GEHC, which depends on PBR length, the number of GEHCs, and the flow rate. In the OMEGA system the tested residence time of the culture in the PBRs was 20 min, based on a PBR length of 3.1 m, a 4.5% transfer to the GEHC, and a PBR flow rate of 86 - 130 lpm. In the future, DO as it relates to photoinhibition can be managed for PBRs of a given length using real-time FRRF and DO data in the control logic algorithm to modify GEHC input and flow rates. The size and configuration of the OSD can also be modified to increase the exchange of DO. In addition to DO management, the GEHC was where 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% 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% [21,38].

3.2. 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% 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% total system volume perminute) were diverted from the PBR to the GEHC for gas exchange. This pumping rate provided the GEHC with an overdesign factor of 1.5 to ensure that carbon consumption in the PBR did not exceed the injection capacity and limit microalgae growth.

3.3. GEHC Operation

Diverting only a portion of the culture for 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. 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

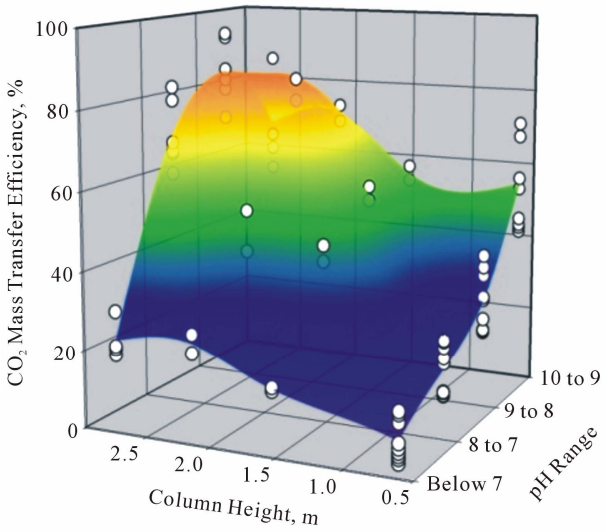

Figure 7. Efficiency of CO_2 mass transfer in the GEHC relative to the height of the column and the pH of the solution. Data were obtained (n = 76) experimentally using tap water, pH adjusted (>11.0) with NaOH. For practical reasons a maximum column height of 1.8 meters was used.

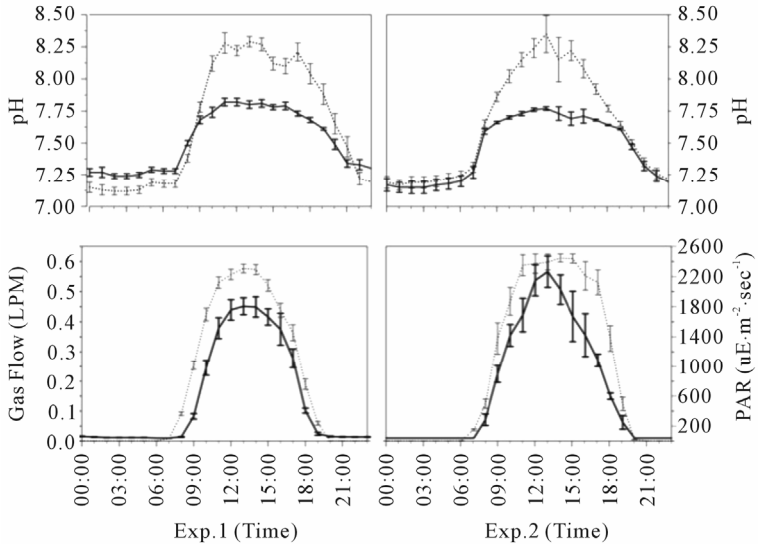

Figure 8. The mean hourly (± SE) pH, gas flow, and PAR recorded during Experiment 1 (left) and Experiment 2 (right). Top: pH values measured inside the GEHC (solid line) compared to pH in the PBR (dotted line). The differential between the GEHC and PBRs increases during the day due to carbon assimilation for photosynthesis. The rate of CO_2 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 CO_2 demand correlated with PAR (dotted lines), and inferred rates of photosynthesis. The pH of the GEHC and PBRs equalize at night due to respiration.

energy efficiency and biomass productivity.

The details of harvesting intervals, biomass production, and carbon utilization for both Experiments 1 and 2 are given in **Table 1**. Harvesting occurred every 0.83 to 2.79 days, triggered by the depletion of 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.

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.

3.4. Carbon Utilization and Biomass Production

The totalized volume of simulated flue gas (8.5% CO_2/ 91.5% air V/V) injected into the GEHC and the biomass produced during Experiments 1 and 2 are shown in **Figure 9**. The changes in gas utilization, which appear as a "staircase" in the plot, reflect the day/night cycles and the on-demand input of 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

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, $g \cdot m^{-2} \cdot day^{-1}$
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)

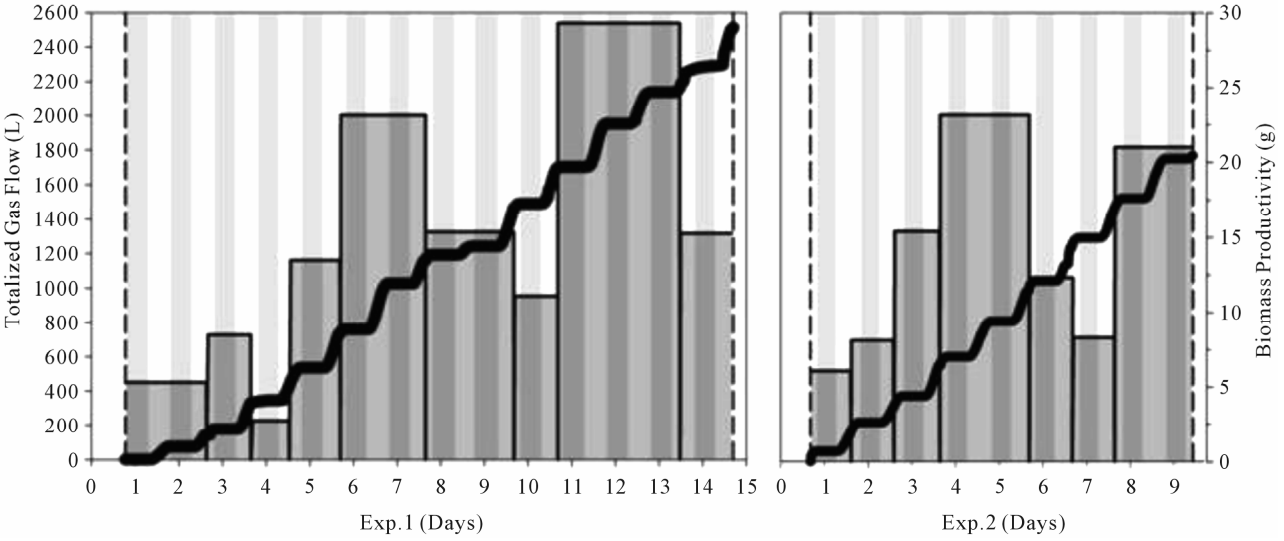

Figure 9. Microalgal CO_2 utilization and productivity in Experiment 1 (left) and Experiment 2 (right) with the day/night cycle indicated by vertical stripes. Totalized gas flow (8.5% CO_2 V/V) (bold black line) and biomass production (histogram). The totalized gas flow has a "staircase" shape because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

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% ± 4.0% (n = 9) and for Experiment 2 it was 60.2% ± 4.7% (n = 7), with values from both experiments ranging from 31.6% to 80.9%. These measured CO_2 conversion efficiencies correspond well to the CO_2 solubility values obtained in the titration experiment (see Section 3.3). Gas transfer in the OMEGA GEHC could be improved by using a taller column (greater contact time for rising bubbles), smaller bubbles (greater surface-to-volume ratio), or higher 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 $g \cdot m^{-2} \cdot day^{-1}$, may have been due to a period of culture acclimation. The second low yield on the third harvest cycle (4.5 $g \cdot m^{-2} \cdot day^{-1}$) was due to a short incubation period with minimal light exposure (**Figure 9**). Despite these limitations, the average observed areal productivities were within the range of values reported for open ponds [10,45,46], although somewhat less than those reported for other PBR systems [5,47]. This disparity with other PBRs may be due to lower nutrient concentrations in the unsupplemented wastewater, the presence of grazers and/or pathogens, or to other limiting culture conditions (e.g., time of year or culture temperature). Long-term experiments are required to determine the limiting factors in the OMEGA system and its potential yields.

3.5. OMEGA and Wastewater Treatment

The OMEGA system used secondary wastewater effluent as a source of nutrients for microalgae cultures and the concentrations of ammonia [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% and 34% 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].

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

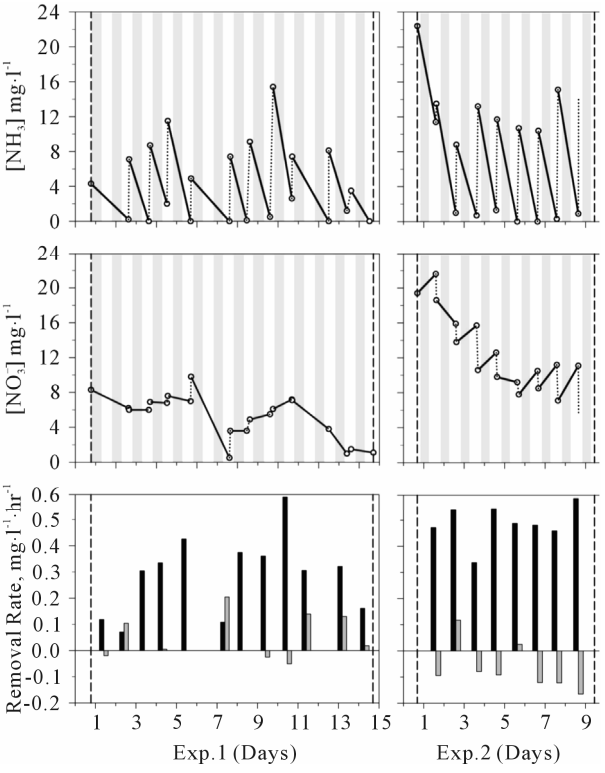

Figure 10. Time course for the addition and utilization of [NH_3^-N] (top), [NO_3^-N] (middle), and removal rates (bottom) for Experiment 1 (left) and Experiment 2 (right). As in Figure 9 the day/night cycle is represented by white/gray shading and each line segment (top/middle) shows changes in nutrient concentration from the time of wastewater addition to harvesting, corresponding to "biomass production" bars in Figure 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.

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

These results indicate that microalgae growing in a prototype OMEGA system can contribute to biological nutrient removal in wastewater treatment. It is well established that microalgae in ponds and other PBR designs can effectively remove nutrients from wastewater [50-53]. It has also been demonstrated that microalgae can remove heavy metals [53,54] and organic contaminants, including surfactants, phenols, and hydrocarbons [53,55-57]. Research reported elsewhere indicates that the OMEGA system can also contribute to the removal of pharmaceuticals and personal care products as well as compounds of emerging concern [58].

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

4. Conclusions

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%; better than the 10% - 20% conversions in other systems [21,38]. The areal productivity of the system averaged 14.1 $g \cdot m^{-2} \cdot day^{-1}$ overall with peaks above 20 $g \cdot m^{-2} \cdot day^{-1}$, values consistent with reported US average microalgae productivity of 13.2 $g \cdot m^{-2} \cdot day^{-1}$ [59]. The microalgae consistently removed >90% 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 [58], suggests that a scaled-up system could provide effective wastewater treatment services.

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

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

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Nomenclature

Q_{Gas} = Gas flow rate, lpm
P_{Algae} = Microalgal productivity, $g \cdot m^{-2} \cdot day^{-1}$
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, $g \cdot mol^{-1}$
pCO_2 = CO_2 partial pressure, atm
M_{CO_2} = 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 $[CO_2]$, $mol \cdot l^{-1}$
C = $[CO_2]$, $mol \cdot l^{-1}$
PBR_{Vol} = Volume of PBR tubes, l
DTR = Detention time ratio, unitless
$GEHC_{Xfer\ Rate}$ = GEHC CO_2 mass transfer rate, $mol \cdot l^{-1} \cdot min^{-1}$
C_{Uptake} = Carbon uptake in the PBR, $mol \cdot l^{-1} \cdot min^{-1}$
A_{Growth} = g, Total biomass produced
TSS_{GEHC} = Total suspended solids content of culture harvested from GEHC, $mg \cdot l^{-1}$
H_{Vol} = Volume of culture harvested from GEHC, l
TSS_{PBR} = Total suspended solids content of the culture in the PBR, $mg \cdot l^{-1}$
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

