Pilot Reactor Operation Of The Oxygenic Photogranule (OPG) Wastewater Treatment Process

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Abstract -Treating wastewater is imperative for maintaining public health and sanitation as well as the natural environment. In the United States and developed countries around the globe, the activated sludge (AS) process has been paramount in effectively treating wastewater for over a century. However, high energy consumption due to required mechanical aeration of AS limits its potential as a sustainable process. The oxygenic photogranule (OPG) wastewater treatment process utilizes the photosynthetic ability of OPGs made possible by the symbiosis between heterotrophic AS and filamentous cyanobacteria and algae in dense, spherical biogranules. This research presents pilot operation of the OPG wastewater treatment process, supporting its scalability and effective nutrients removal. Four pilot OPG reactors were operated in sequencing batch reactor (SBR) mode with alternating light/dark cycles, achieving a maximum volume of 30 L (20-times scale-up factor) and maximum operation time of 152 days. Pilots A & B (Phase I) and Pilot D (Phase III) achieved soluble chemical oxygen demand (sCOD) removal efficiencies of 65%, 73%, and 81%, respectively, without any mechanical aeration. Pilot C (Phase II) employed a hybrid light/aeration system, attaining 83% sCOD removal with 12 hours of light (day) and aeration only (night).

1.1 Introduction
The activated sludge (AS) process has been the gold standard for operating large-scale wastewater treatment plants (WWTPs), providing continuous, high-rate degradation of nutrients (commonly referred to as chemical oxygen demand, or COD). This ubiquitous biotechnology involves a diverse community of bacteria and protozoa (sludge) that feeds on COD in wastewater. The OPG biomass in SBR operation settled very quickly, effectively separated from the treated effluent water, and was experimentally determined to be very energy-rich compared to AS; OPGs produced more COD (in the form of biomass) than the amount of COD they consumed (1.4 g COD produced/g COD consumed), suggesting that atmospheric CO₂ may have been sequestered by the photosynthetic microorganisms.

1.2 Research Objectives
The main objectives of this research are as follows:
(1) Demonstrate the ability of the OPG process to treat real wastewater without aeration at the pilot scale (> 10 L reactor volume);
(2) Track and analyze pilot reactor performance under varying environmental conditions (such as temperature and wastewater strength), and;
(3) Explore and evaluate multiple OPG reactor operation parameters, designs, and scaleup strategies to optimize biomass growth and nutrients removal.

1.3 Literature Review

1.3.1 Current Wastewater Treatment Methods
Recently, many researchers have focused on developing novel wastewater treatment bioprocesses without AS altogether; alternatives to AS include algae, aerobic heterotrophic granules, and novel OPGs (Park et al., 2011; Manheim and Nelson, 2013).

1.3.2 Aerobic Sludge Granules and Algae Ponds
The main challenges associated with AS are its poor settleability, separability, and dewaterability, which limit the minimum clarifier (settler) size, cause high sludge waste volume, and in an extreme case can cause discharge of sludge in the effluent (Houghton et al., 2000; Rittmann and McCarty, 2008; Grady et al., 2011).

1.3.3 The OPG Wastewater Treatment Process
The discovery and characterization of the oxygenic photogranule (OPG) began with a static cultivation of AS biomass under natural sunlight that produced discrete biogranules with an outer layer of photosynthetic algae and cyanobacteria encapsulating a core of AS (Park and Butler, 2013; Park and Dolan, 2015; Milferstedt et al., in review). The static cultivation of OPGs was repeatable using AS from multiple locations around the world.

1.3.3.1 OPG Biomass Characteristics
Artificial (fluorescent) light providing photosynthetically active radiation (PAR) values ranging from 90–200 µmol/m²-s supported the static formation of OPGs, slightly higher than 40–130 µmol/m²-s used for biodiesel production.

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(p)-2276-285
cyanobacteria and Oscillatoria sp. cultivations (Post et al., 1985; Anderson and McIntosh, 1991)

1.3.3.2 Nutrients Removal
Evidence of a limited aerobic zone through the depth of cyanobacterial-based microbial mats is supported by the literature, allowing for simultaneous aerobic and anoxic degradation of nutrients within the biofilms (Stal, 1995; Stauch-White, 2016).

1.3.3.3 Bench-Scale Reactor Operation
Operating the OPG wastewater treatment process in SBR mode with alternating light/dark sequences treated real wastewater with no external aeration, regardless of the lighting sequence (Abouhend et al., in preparation). Successful SBR operation was achieved with four 6-hr cycles, 3.5 hours of light and 2.5 hours of dark within each

PILOT REACTOR OPERATION:

2.1 Pilot A
To explore the outcomes of scaling up and operating the oxygenic photogranule (OPG) process for wastewater treatment, we collaborated with the Amherst Wastewater Treatment Plant (WWTP) to install the first pilot OPG reactor within the municipal facility. We used the WWTP primary clarifier effluent as influent chemical oxygen demand for 53 days at a maximum volume of 30 L, demonstrating that the OPG process can be scaled up by at least 20 times.

2.1.1 Materials and Methods

2.1.1.1 Pilot Housing: The Shed
The pilot OPG reactors were housed and operated within the Amherst WWTP (Amherst, MA). We assembled the shed adjacent to the north side of a concrete junction station with access to wastewater flowing from the primary clarifier to the aeration basin (Figure 1). Using Superstrut steel framing channels, we constructed a shed providing a space of approximately 3 ft x 4 ft x 5 ft. Two ventilation ducts were installed to reduce the greenhouse effect within the covered pilot housing. Heavy duty tarps were secured to the frame using bungee cords and metal clamps to protect the pilot equipment from rain and snow, and were also necessary for providing dark conditions for light/dark cycling during the daytime.

2.1.1.2 Electrical Power and Pilot Equipment
Electrical power was provided by the Amherst WWTP for the pilot studies. The electrician at the treatment facility (Fred Hartwell) courteously constructed a 3-phase, 4-wire branch circuit with eight covered 20A, 125V duplex receptacles to power the pilot OPG system. The containers used were a 10-L rectangular glass tank (30 cm x 18 cm x 22 cm), followed by a 50-L rectangular clear plastic container (58.4 cm x 41.3 cm x 31.1 cm). Mixing was provided by an overhead digital mixer (IKA RW20, USA) and a single flat paddle (6 cm x 2 cm) throughout the operation. 900-lumen bright white compact fluorescent lamp (CFL) bulbs were used to provide light to the pilot reactor.

2.1.1.3 Reactor OPGs
The seed OPGs used were formed using 10 mL of municipal activated sludge inoculum in 20 mL vials under static, batch conditions with continuous light provided from above the vials (150 µmol/m²·s PAR value) at a constant temperature of 20 °C for 25-35 days (Milferstedt et al., in review).

2.1.1.4 Pilot Operation
The first pilot OPG reactor, Pilot A, was initiated in the laboratory on July 21, 2017. We used 1 L of OPG biomass from reactor 1, and 1.5 L of biomass from reactor 2; both reactors had been operating in SBR mode treating real wastewater for 150 days. We filled the 10 L rectangular glass reactor with the 2.5 L of mixed biomass, 3.5 L of primary effluent, and 4 L of effluent from the lab scale reactors. Light was provided to the sides of the reactor by 2 CFL bulbs. Using the lux (lx) meter, we adjusted the distance between the bulbs and the reactor to provide light intensity of 10 klx on the reactor surface.

2.1.1.5 Analytical Methods
Two 10 mL mixed liquor (ML) samples of the pilot OPG biomass were taken three times per week to measure total suspended solids (TSS) and volatile suspended solids (VSS) concentrations using Standard Methods for Water and Wastewater Analysis (APHA, 2005). In addition, 50 mL unfiltered samples of the pilot influent wastewater and effluent were taken at the end of the noon cycle to measure soluble chemical oxygen demand (COD) three times per week.

2.1.2 Results

2.1.2.1 OPG Biomass Growth
Changes in OPG biomass concentration (measured in mg/L TSS) and reactor volume versus operation time for Pilot A are shown in Figure 4a. Although biomass growth patterns changed throughout pilot operation, the ratio of g VSS/g TSS was consistent at 0.83 ± 0.01. Pilot A was split into four unique growth periods over the course of operation, and linear regression curves were used to determine linear concentration-based (LCB) OPG biomass growth rates during these periods (Figure 4b).
2.1.3 Discussion

2.1.3.1 Reactor Operation and OPG Growth

The 53-day operation of Pilot A provided us with useful data as well as optimism for future pilot operation and scale ups. The first scale up from 1.5 L to 10 L SBR reactor, a 6.7-times dilution, dramatically reduced the OPG biomass concentration (TSS) to 715 mg/L. The initial 24-hour light batch mode with limited feeding (2.5 d HRT for the first 7 days, and 4 d HRT on the 8th day) provided good conditions for the OPG biomass to grow linearly at 152 mg/L-d (Figure 4b). The trend continued until after day 10 when the biomass leveled off at around 2,000 mg/L over the next 16 days.

2.1.3.2 Organics Removal

Increasing the number of light bulbs between days 28–36 may have slightly improved COD removal at that time, but did not have a noticeable effect on the removal efficiency throughout the rest of pilot operation.

2.1.3.3 Robustness to Varying Conditions

Since F/M ratio remained consistent during this period, the increase in OPG biomass growth rate may have been due to: (1) available space and increased light availability inside the reactor after the 6.7-times dilution, (2) higher operating temperature, or a combination of (1) and (2). While faster OPG growth is a clear advantage for scaling up the system, operating above 25 °C has its own set of challenges.

2.1.4 Conclusion

Pilot A was a successful plunge into the unknown of scaling up the novel OPG biotechnology. It demonstrated that the OPG wastewater treatment process is robust and can substantially remove COD from real wastewater of varying strengths without any external aeration. However, the sCOD removal efficiency (65%) was well below EPA regulations (> 85%) and the bench scale OPG reactors (85–95%), potentially due to low TSS concentration (700–2,300 mg/L, compared to 2,000–3,500 mg/L during the benchscale study). Periods of increasing F/M ratios between 0.05–0.1 (mg sCOD/mg VSS-d) corresponded with improved removal efficiency, but F/M ratios above 0.15 hindered COD degradation. Two successful scale ups (1.5 to 10 L, 10 L to 30 L) led to the quick formation of new OPG biomass, although their progression was hindered at several points during operation.

2.2 Pilot B

Although Pilot A demonstrated the ability to treat real wastewater of varying strength at above-average temperatures (26° C), the system removal efficiency and OPG biomass concentration never exceeded 76% and 2,300 mg/L, respectively. In contrast to Pilot A, Abouhend et al. operated OPG reactors at higher biomass concentrations, maintaining average TSS concentrations of approximately 3,000 mg/L for both reactors. To evaluate the effect of higher biomass concentration on the pilot OPG system using the bench-scale light configuration, we initiated Pilot B on October 5, 2017.

2.2.1 Materials and Methods

2.2.1.1 Pilot Housing and Equipment

The same reactor housing and equipment used to operate Pilot A were used to operate Pilot B. The reactor was changed from the 10-L rectangular glass reactor to an 8L cylindrical glass reactor (22.5-cm diameter x 18-cm height). We eventually switched from the cylindrical glass...
reactor to a white plastic rectangular reactor (44.5 cm x 36.2 cm x 17.8 cm).

2.2.1.2 Reactor OPGs and Operation
Amherst primary effluent was added to the OPG biomass up to a volume of 3.5 L in the glass reactor, yielding a TSS concentration of 4,300 mg/L. Mixing was provided at 90 rpm to completely suspend the OPGs. We operated Pilot B in batch mode for one day, and on day 2 we moved it to the shed and increased the volume to 5 L. To investigate the inverse light scheme (dark first, then light), we started operating in SBR mode with 1-d HRT and a light sequence of 2-hr dark followed by 4-hr light using 2 LED bulbs on opposite sides of the reactor. To provide more light availability due to the appearance of sludge flocs in the reactor, we added more light bulbs between days 4–6, including overhead fluorescent lamps (Figure 7). Light configuration used for Pilot B from days 6–33.

2.2.1.3 Analytical Methods
Please refer to section 2.1.1.5 as the methods used to analyze Pilot B were identical to those used for the analysis of Pilot A.

2.2.2 Results
2.2.2.1 OPG Biomass Growth
The OPG biomass concentration (TSS) and reactor volume versus operation time of Pilot B are shown in Figure 8a. The VSS/TSS ratio of Pilot B was 0.85 ± 0.02, slightly higher than that of Pilot A. The unique growth periods were selected and linear regression curves were used to determine the LCB growth rates of the granules during these periods (Figure 8b).

Figure 8. (a) Pilot B TSS concentration and reactor volume over the operation period. (b)

OPG biomass grew very quickly when the volume was 3.5 L, but leveled off between 4,000–4,600 mg/L after scaling up to 6.5 L (omitting the 3,200 mg/L outlier data point measured on day 19). Even after scaling up to 10 L, the biomass concentration exceeded 4,000 mg/L.

2.2.2.2 Organics Removal
Pilot B operated with an average influent sCOD concentration of 157 mg/L, ranging from 133 to 182 mg/L. The average effluent sCOD during the pilot operation was 41 mg/L, and ranged from 35 to 49 mg/L. Figure 9 below displays influent and effluent sCOD concentrations over the operation period of Pilot B.

2.2.3 Discussion
2.2.3.1 Reactor Operation and OPG Growth
OPG biomass grew most rapidly between days 3–10, when TSS increased from 1,800 mg/L to 4,000 mg/L with an LCB growth rate of 288 mg/L-d (Figure 8). The high growth rate was most likely due to the scale up from 3.5 L to 5 L which provided space for new OPGs to form.

2.2.3.2 Organics Removal
The F/M ratio initially fell from 0.11 to 0.05, and remained around 0.05 until day 26 (Figure 10b). The increase to 0.08 that followed correlated with an increase in removal efficiency, although by this time sludge flocs were present and the reactor which inhibited the formation of OPG biomass.

2.2.4 Conclusion
Pilot B achieved greater sCOD removal than Pilot A – most likely due to higher TSS concentration – suggesting that OPG biomass concentration plays a significant role in reactor performance. Yet it fell short of the bench scale studies, indicating that conditions in the pilot were not optimal. The initial scale up from 3.5 L to 5 L led to the very rapid LCB growth rate (nearly twice the highest rate achieved in Pilot A), possibly due to high (> 1,800 mg/L) biomass concentration after the volume increase.

2.3 Phase I General Findings
Pilots A and B were exciting and powerful indicators that the OPG process for wastewater treatment is scalable, reaching a maximum volume of 30 L (20-times increase from bench scale studies). Uniquely, a pattern of strong linear growth during the first 1–2 weeks was observed three times immediately after three different scale ups ranging from 1.4–6.7-times volume increase. The LCB growth rate was a surprisingly consistent measurement of growth in the pilot reactors, and may hold significance in determining optimal OPG scale-up conditions. Although the data collected was useful for growth analysis, recording additional parameters – such as chlorophyll concentration in the OPGs, and dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) removal efficiency – is recommended for improved overall analysis of the OPG biomass and pilot conditions.
PILOT REACTOR OPERATION: PHASE II

3.1 Pilot C

Phase I pilot scale operation of the OPG system exhibited significant removal of organics (65–73% sCOD removal), periods of high biomass growth (71–288 mg/L-d), and the ability to operate the OPG process at high ambient temperatures (over 25 °C). With the ultimate goal of using natural sunlight as the main energy source for the photosynthetic OPGs, we brainstormed many possible designs that utilize natural diurnal light to provide DO to the system during the day. Realizing the cost benefits of designing an OPG system that minimizes infrastructure changes to typical continuously-stirred tank reactor (CSTR) municipal WWTP configurations, we elected to investigate an OPG system design that utilizes bottom diffusion aeration during the nighttime only. On November 13, 2017, we commenced operation of the first hybrid light/aeration OPG wastewater treatment process: Pilot C.

3.1.1 Materials and Methods

3.1.1.1 Pilot Housing: The New Shed

The shed used for Pilots A and B was uncomfortably small, difficult to work in, and its durability in the snow was not to be tested. A heavy-duty portable shed with dimensions 12 ft x 16 ft x 8 ft (width x length x height) was purchased (ShelterLogic, USA) and constructed during late September of 2017 (Figure 11).

3.1.2 Results

3.1.2.1 OPG Biomass Growth

The OPG biomass concentration (TSS) and reactor volume over Pilot C operation are shown in Figure 12a. The granules grew slowly throughout Pilot C operation, and TSS never exceeded 4,500 mg/L. The highest LCB growth rates achieved were 80 mg/L·d ($R^2 = 0.51$), which occurred between days 1–11, and 72 mg/L·d ($R^2 = 0.49$), which occurred between days 89–105 (Figure 12b).

3.1.2.2 Nutrients Removal

The soluble COD accounted for 53–100% of the total effluent COD throughout operation, with an average of 73%. Influent sCOD strength varied widely, ranging from 56–330 mg/L with an average of 155 mg/L (Figure 13).

3.1.2.3 Chlorophyll

Chlorophyll-a and chlorophyll-b concentrations normalized by TSS of the OPG biomass and versus pilot operation time are shown in Figure 16a.. Chlorophyll-a decreased on day 103, but increased again until day 117. Average chlorophyll-a/TSS and chlorophyll-b/TSS mass percentages were 0.51% and

3.1.1.2 Electrical Power and Pilot Equipment

The white plastic rectangular reactor used previously in Pilot B was used during days 1–4 (10 L), followed by a white cylindrical plastic reactor from days 5–27 (10–12 L), a green cylindrical plastic reactor from days 28–105 (15.5–18 L), and the same rectangular clear plastic reactor used for Pilot A operation (30 L) between days 106–147.

3.1.1.3 Reactor OPGs and Operation

Pilot C was operated in SBR mode with four 6-hr cycles of feeding, mixing, settling, and decanting. Each cycle started with feeding (2.5 L of Amherst primary effluent per cycle, 70 min feeding period, 1-d HRT) and continuous mixing at 100 rpm. The mixer was turned off 40 min prior to the end of each 6-hr cycle to allow for 30 in of settling and 10 min of supernatant decanting, the periods of biomass wastage specified below, biomass removal was kept to a minimum and collected only when samples were needed for analysis (60–160 mL per week).

3.1.1.4 Analytical Methods

To determine TSS and VSS concentrations over the operation period, duplicate 10 mL (days 1–46) and 25 mL (days 49–147) ML samples of the pilot OPG biomass were collected immediately before the settling period prior to the 2 pm cycle two times per week and analyzed in the lab using the Standard Method (APHA, 2005).

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0.08%, respectively. The chlorophyll-a/chlorophyll-b ratio was typically between 6–7, but was found to be as low as 4 and as high as 10 (Figure 16b).

3.1.2.4 Dissolved Oxygen
Because the dark cycle was entirely outside of the Amherst WWTP facility’s normal hours, only daytime DO levels were measured (Figure 17). In general, dissolved oxygen was very high during the first half of Pilot C operation, except for some periods when DO was less than 2 mg/L. Between days 74–85, DO remained low (around 1 mg/L), but then increased to high levels (>2 mg/L) until day 110 when DO approached 1 mg/L for the remainder of operation.

3.1.2.5 Image Analysis
Petri dish and microscope images during Pilot C operation were cropped and arranged sequentially based on the unique growth periods, excluding days 1–11.

3.1.2.6 Combined Analysis
Figure 20 shows the removal efficiencies of sCOD, DOC, and TDN along with F/M ratio (Figure 20a) and chlorophyll-a/chlorophyll-b ratio (Figure 20b) versus the operation time of Pilot C.

3.1.3 Discussion
3.1.3.1 OPG Biomass Growth
The OPG biomass in Pilot C grew the fastest during 10 L reactor operation when the TSS concentration was high (3,300–4,300 mg/L) and the F/M ratio was consistently 0.04–0.05 g sCOD/g VSS-d (Figures 12 and 20). The increase in growth rate and biomass yield between days 133–144 can be attributed to the overgrowth of sludge flocs caused by reducing HRT (1-d to 0.75-d) after previously reducing nighttime aeration and beginning to waste on days 106 and 111, respectively.

3.1.3.2 Nutrients Removal
Nitrogen removal was active for TSS between 2,500–3,500 mg/L, and achieved the greatest efficiencies when F/M ratio was 0.04 and 0.10 (Figure 22b). When divided into the six unique growth periods, we see that nitrogen removal was the highest during days 1–11 (high TSS, high yield, low F/M ratio) and days 109–130 (moderate TSS, high F/M ratio, low yield, relatively high proportion of filamentous green algae).

PILOT REACTOR OPERATION: PHASE III
4.1 Pilot D
Interestingly, observed OPG biomass yields during Pilot C operation were very low, holding significance in its low sludge production. However, the growth rate of OPG biomass during Phase II operation was lower than for previous SBR operation, delaying scale ups. Since scaling up quickly is a main objective of this research, the hybrid light/aeration system was not ideal for pilot operation. On April 14, 2018, we initiated Pilot D, which successfully treated real wastewater with no external aeration for 152 days and reached a volume of 12 L.

4.1.1 Materials and Methods
4.1.1.1 Pilot Housing, Electrical Power, and Pilot Equipment
We used the new shed (section 3.1.1.1) to house Pilot D, as well as the circuit used in Phases I and II to provide electrical power (section 2.1.1.2). The white plastic rectangular reactor was used during days 1–27 (10–12 L volumes), followed by the green cylindrical plastic reactor from days 28–105 (15.5–18 L volumes), and the same rectangular clear plastic reactor used for Pilot A operation (30 L) between days 106–147. The mixing paddle used for Pilot D was identical to that used for Pilot C.

4.1.1.2 Reactor OPGs and Operation
Concentrated OPG biomass from Pilot C was brought to the lab and cleaned of sludge flocs using the washing method described in section 2.1.1.5. About 5 g of OPG biomass was salvaged, and we commenced Pilot D on April 14, 2018.
4.1.1.3 Analytical Methods
For TSS and VSS concentration analysis over the operation period, duplicate 10 mL (days 1–19) and 25 mL (days 22–152) mixed liquor samples of the pilot OPG biomass were collected immediately before the settling period prior to the 2 pm cycle two times per week and analyzed in the lab using the Standard Method (APHA, 2005). 25 mL samples of pilot effluent and influent water were collected beginning on days 22 and 70, respectively, and analyzed for TSS and VSS concentrations.

4.1.2 Results
4.1.2.1 OPG Biomass Growth
Changes in the OPG biomass concentration (TSS) and reactor volume versus Pilot D operation time are shown in Figure 23. Since scaling up was based on small incremental volume increases, TSS was maintained at 3,220 ± 410 mg/L (4,130 mg/L maximum) between days 1–78. After the large loss of biomass on day 83, TSS dropped below 1,700 mg/L and never exceeded 2,500 mg/L until after the volume reduction on day 106.

LCB growth rates for 11 different growth periods during Pilot D operation were calculated using linear regression models and then tabulated (Table 24). The OPG biomass grew the fastest between days 22–29 (after scaling up from 3 L to 4 L), 2–6 (beginning of operation), and 86–92 (after large accidental wastage).

Table 24. LCB growth rates for 11 unique periods during Pilot D operation.

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<th>Days</th>
<th>6</th>
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<th>7</th>
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<td>LCB G</td>
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<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
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4.1.2.2 Nutrients Removal
the average sCOD removal efficiency was 81% (excluding the last week of operation, 80% if included) and only achieved 85% removal for one quarter of Pilot D operation.

DOC concentrations in the influent and effluent ranged from 19–58 mg/L and 6–30 mg/L, respectively, with an average removal efficiency of 67% (Figure 26). Influent and effluent DOC levels were almost always proportional to influent and effluent sCOD.
Similar to Phase II operation, significant yet fluctuating nitrogen removal occurred throughout Pilot D operation. Influent and effluent TDN concentrations ranged from 15–57 mg/L and 9–52 mg/L, with averages of 33 and 27 mg/L, respectively (Figure 27). Removal efficiency reached a single-day maximum of 70%, but averaged just 21% over the operation period.

4.1.2.3 Chlorophyll
Chlorophyll-a and chlorophyll-b concentrations normalized by TSS of the OPG biomass versus operation time are shown in Figure 28a. Chlorophyll-a/TSS steadily increased from less than 0.35% to greater than 0.61% between days 12–48, but then decreased to about 0.4% after the large loss of OPG biomass operation. (b) Chlorophyll-a/chlorophyll-b ratio over the operation period.

4.1.2.4 Dissolved Oxygen and Temperature
Dissolved oxygen levels were recorded consistently at the end of the light and dark cycles during pilot operation except between days 30–47 when the instrument was not working (Figure 29 during Pilot D operation. The instrument was not working between days 30–47.

4.1.2.5 Image Analysis
Petri dish and microscope images during Pilot D operation were cropped and arranged sequentially based on the unique growth periods, excluding days 1–11 (Figures 30 and 31).

4.1.2.6 Combined Analysis
Figure 32 shows the removal efficiencies of sCOD, DOC, and TDN along with F/M ratio (Figure 32a) and chlorophyll-a/chlorophyll-b ratio (Figure 32b) versus Pilot D operation time.

OPG biomass yield was also calculated over the operation period (excluding days 97–104 and 142–152 when the observed yields were negative) and for the unique growth periods (Figure 33).

GENERAL FINDINGS
5.1 Discussion
A maximum volume of 30 L was achieved with an overall scale factor of 20 times. Average COD removal was above 65% for Phase I, and exceeded 80% for Phases II & III. Phases I & III employed successful treatment of real wastewater without any aeration, and Phase II operation demonstrated a hybrid light/aeration system. Two extreme cases of observed biomass yield were found: extremely high (> 0.7 g VSS produced/g sCOD consumed), and extremely low (< 0.3 g VSS produced/g sCOD consumed). OPGs with higher yields typically contained large amounts of filamentous cyanobacteria, whereas the low yield biomass consisted of much more green algae. Low observed yield was recorded during hybrid light/aeration operation; therefore, further
investigation on a hybrid OPG process design is recommended.
TSS concentrations above 4,000 mg/L were not effective for treating the wastewater; TSS between 2,000–3,500 mg/L achieved the highest sCOD, DOC, and TDN removal efficiencies. LCB growth rates were identified as a means to quantify OPG biomass growth. Observed yield and LCB growth rate were not strongly correlated, although higher LCB rates were typically seen when the yield was high. LCB growth rates ranged from 15–300 mg/L-d, and no clear relationships with temperature, dilution factors, or TSS concentration were found. Low observed yield biomass holds potential in sludge reduction biotechnologies; however, because minimizing time required for scaling up the OPG process is imperative for its commercialization potential, studies focused on maximizing biomass growth rate are suggested at this time.

Compared to bench-scale studies, pilot OPGs were not as smooth and spherical. Hairy, loose OPGs reduced biomass settleability and light penetration, inhibiting new biomass growth and reactor performance. Large wastages, although sometimes reducing removal efficiency, helped improve light penetration and overall reactor conditions. Pilots C and D overcame large incidental wastages, proving that the OPG system can perform over a wide range of concentrations. Chlorophyll-a/TSS content was typically between 0.4–0.6%, with the best nutrients removal occurring at 0.5%, regardless of chlorophyll-a/chlorophyll-b ratios.

5.2 Conclusion
The OPG wastewater treatment process has the potential to revolutionize modern wastewater treatment by eliminating the need for costly mechanical aeration. The high density and superior settleability of OPG biomass can dramatically reduce the required footprint for wastewater treatment. Pilot operation of the OPG process supported previous bench-scale OPG studies and demonstrated the scalability of the novel biotechnology. Good effluent quality was achieved; high removal of sCOD and DOC was observed, while nitrogen removal occurred sporadically. Successful operation in aboveaverage ambient temperatures holds significance in developing the OPG process for lowcost/low-energy wastewater treatment in developing countries. Light availability, biomass concentration, and wastage were identified as important factors that most influenced reactor conditions and performance. F/M ratios may also impact OPG growth and biomass performance. TSS concentrations between 2,000–3,500 mg/L exhibited the best nutrients removal, while concentrations greater than 4,000 mg/L showed no advantage. Further research on both maximizing OPG growth rate for fast scaling up and minimizing observed yield for sludge reduction are recommended. The development and application of the OPG process will play a significant role in aeration-free wastewater treatment, a solar biotechnology that brings us one step closer towards sustainable treatment practices.

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optimize photosynthesis and enhance carbon burial in Lake


