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A custom 3D printed paddlewheel improves growth in flat panel photobioreactor

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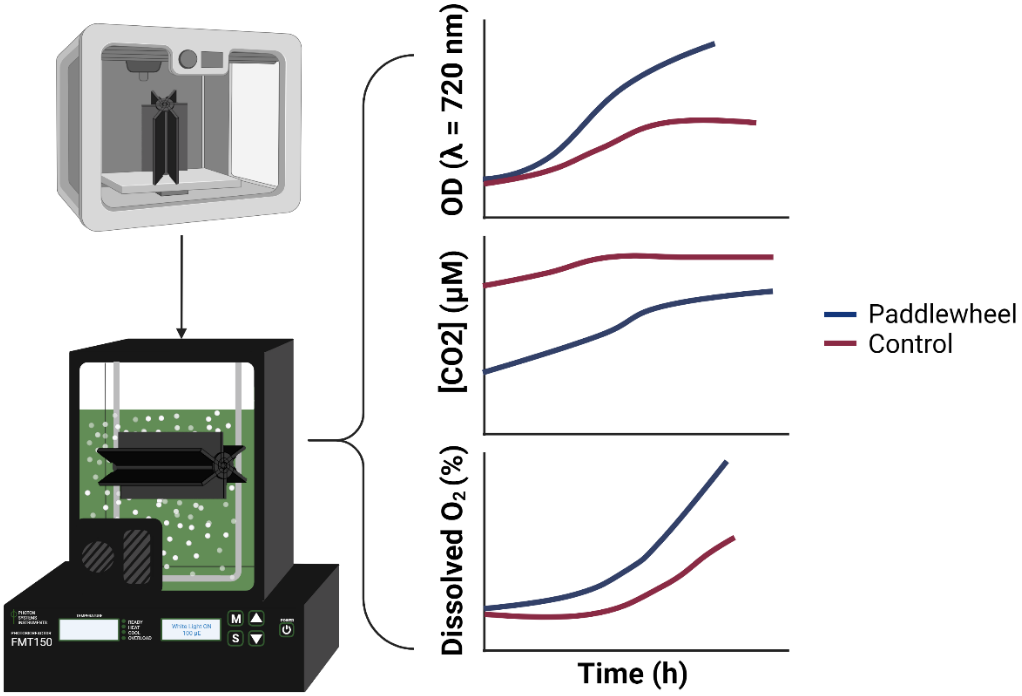
Graphical Abstract



Abstract

One of the main challenges with using flat panel photobioreactors for algal growth is uneven mixing and settling of cells in corners, especially when bubbling is the only method used for mixing. In order to improve mixing in our flat panel reactor, we designed a custom paddlewheel.

Paddlewheels are frequently used in outdoor algae raceway ponds to improve mixing and we are taking advantage of the same principle for mixing in the reactor. The paddlewheel is easily integrated into our PSI FMT150 1 L flat panel photobioreactor and is printed on a 3D printer using high temperature poly lactic acid (HT-PLA). With the inclusion of an annealing step, the paddlewheel is autoclavable. Addition of the paddlewheel in the reactor minimized cell settling and improved algal growth, as evidenced by a nearly 40 percent increase in oxygen production rates. Nutrient dispersion and utilization in the culture was also improved as evidenced by a corresponding 38 percent decrease in CO_2 concentration. The paddlewheel device presented here is a cost-effective method for improving algal growth in a flat panel photobioreactor.

Introduction

Flat panel photobioreactors are commonly used for the growth of single cell phototrophic organisms. One main advantage of these reactors is their short light path and large illumination surface that maximizes light usage and minimizes dark zones within the culture [9]. A major pitfall of these photobioreactors is the lack of adequate mixing. Due to the short light path and therefore very narrow reactor vessel, it is typical to rely on the movement of the sparged gas bubbles for mixing, which is insufficient to create homogenous conditions throughout the culture volume. A computational study performed by Loomba et.al. simulated fluid velocities in a 1 liter flat panel photobioreactor by Photon Systems Instruments (PSI) [1]. They showed very low fluid velocities through much of the bioreactor volume, particularly in the corners of the vessel and unfortunately, increasing gas flow rates was not sufficient to induce adequate mixing [1]. Some work has been done to improve mixing in flat panel photobioreactors. A common approach is the installation of baffles on the walls of the reactor vessel [10-14]. In this method, as bubbles rise around the baffles, they induce swirling flow patterns that increase mixing in the culture. However, this method requires a specially made photobioreactor vessel, and cannot be implemented as an addition to existing photobioreactors which leads to higher reactor costs. One other study has implemented a bubble-driven paddlewheel design in tube reactors, however these mixers were not autoclavable, and laboriously crafted by hand [15].

In work presented here, we have designed and implemented a simple 3D-printed paddlewheel that can be easily installed in the PSI FMT150 model bioreactor to increase mixing and improve algal growth. Using high temperature poly-lactic acid filament, we were able to produce a fully autoclavable paddlewheel. Our study here focuses on the design of building a bubble-driven paddlewheel and the impact of its implementation on gas availability and algal growth rates.

Methods

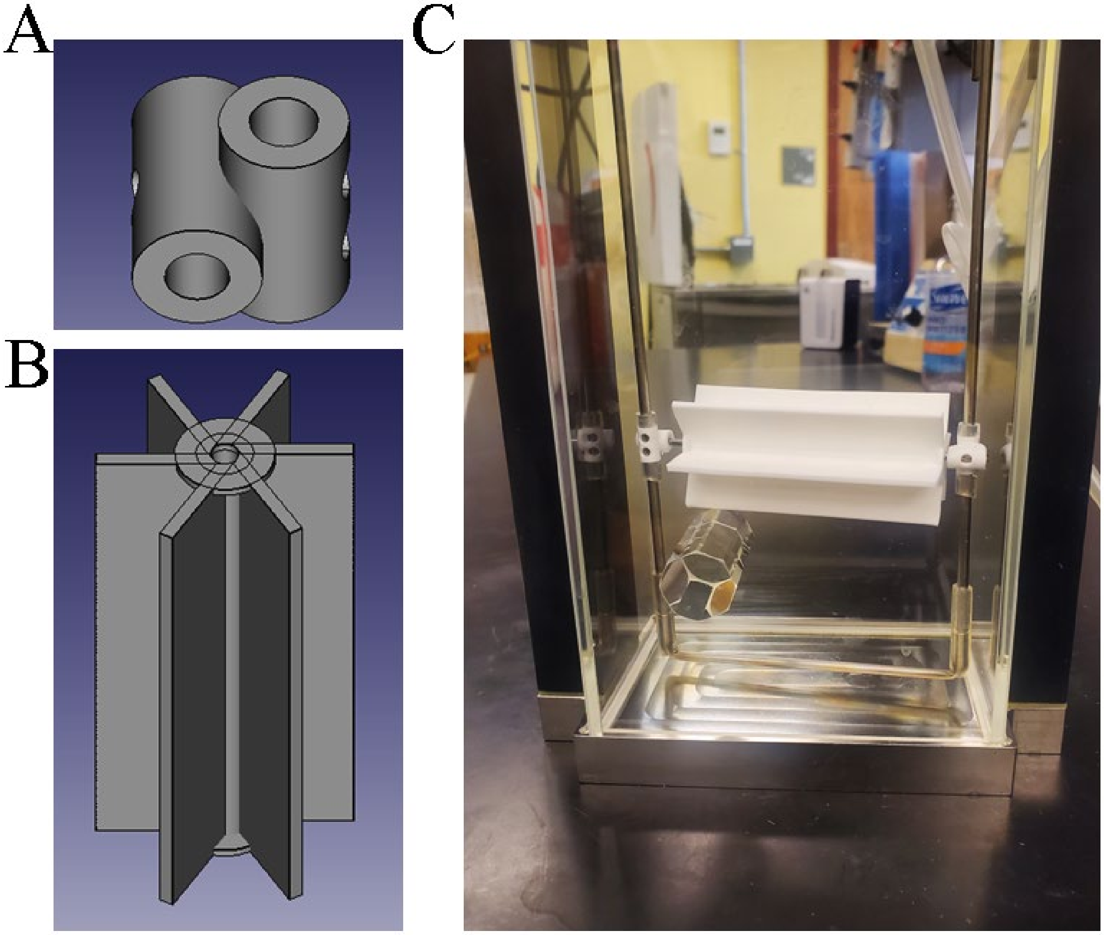


Figure 1. Paddlewheel design for the PSI 1 Liter photobioreactor. A.) axel clips. B.) paddle wheel. C.) assembled and installed apparatus

CAD design

FreeCAD was used to create models for both the wheel and the axle fixtures. The complete design files are provided in Supplemental Files 1 and 2. The paddlewheel was designed to fit within the growth vessel of the PSI (Photon Systems Instruments) model FMT150 1 Liter photobioreactor with a stainless-steel rod to act as an axle for the paddlewheel with connectors designed to attached the axle to the gas sparge line. The paddlewheel design was based on paddlewheels used in largescale algal raceway ponds, with large flat fins designed to catch rising bubbles from the sparge line and turn the wheel. The final CAD designs and paddlewheel apparatus are shown in Figure 1.

Choice of Material

The most commonly used polymers for 3D printing are acrylonitrile butadiene styrene (ABS), polylactic acid (PLA), acrylonitrile styrene acrylate (ASA), and polyethylene terephthalate (PET) [2]. ABS and ASA have similar properties, both relatively flexible and tough and resistant to water degradation and temperatures ranging from -20°C to 80°C, however, neither of these plastics are biodegradable and can release harmful vapors during the printing process [2]. PET, also sold in variants such as PETG, PETE, and PETT, is also resistant to water damage and is nontoxic [2]. However, neither ABS nor PET variants are able to withstand the temperature extremes that are used during autoclaving. PLA, however, can be heat treated after printing to anneal the polymer chains into a crystalline structure. High temperature PLA (HTPLA) is made to be annealed and is a composite material with nanoparticles dispersed within the PLA to act as nucleation sites during the annealing process. This increases the heat resistance of the plastic such that it can be autoclaved. This property, in addition to the fact that it is nontoxic and biodegradable makes HTPLA the recommended plastic for this design.

3D Printing

CAD designs were processed using Ultimaker Cura printing software to create a gcode for printing. During this processing, 3D models are sliced into layers for printing, and print settings are specified. The paddlewheel and axle clips were printed using a 0.2 mm layer height and 100% infill. Wall line width was adjusted to half the width of each fin on the paddlewheel. This ensures that each wheel fin is comprised of straight wall only, with no infill pattern present which increases the structural durability of the fins. The printing temperature was specified at 215°C. All other printing parameters were left on default options. After slicing, designs were printed on an Ender 3 v2 3D printer outfitted with a BL touch leveling sensor. High-temperature poly-lactic acid (HTPLA) filament from Protopasta was used to print the paddlewheel and axle fixtures, which were thermally annealed after printing before use in growth experiments.

Annealing and Deformation Measurements

The protocol used for thermal annealing of printed pieces was taken from Wach et.al., who found that heating for 15 minutes at 95°C followed by slow cooling gave optimal results for increasing the degree of crystallinity in PLA [3]. To test this annealing process, twelve identical test pieces with a width of 2 mm were printed with HTPLA. Six of these pieces were thermally annealed by heating as described, after which the oven was turned off without opening it to allow slow cooling of the samples overnight. The annealed and non-annealed control samples were then tested by heating at various temperatures for 30 minutes. Upon removal from the oven, each piece was bent by hand as far as possible without breaking to test the structural integrity of the PLA, and the extent of deformation was measured using a digital caliper.

Algal growth trials

Chromochloris zofingiensis cultures were generously provided by the Niyogi lab at UC Berkeley, and were grown in an optimized media designed by researchers in the Roth lab at UC Berkeley. This media consists of $NaNO_3$ (22.5 mM), $MgSO_4$ (2.5 mM), $CaCl_2$ (0.08 mM), K_2SO_4 (1.5 mM), $FeCl_3$ (0.2 mM), $MnCl_2$ (0.015 mM), $CuCl_2$ (0.012 mM), $ZnSO_4$ (0.0175 mM), Na_2CO_3 (0.22 mM), Na_2EDTA (0.291 mM), Na_2SeO_3 (0.03 µM), $(NH_4)_6Mo_7O_{24}$ (0.057 µM), K_2HPO_4 (5.7 mM), and KH_2PO_4 (0.3 mM). Cultures were grown in the FMT 150 1 liter photobioreactor from Photon Systems Instruments at 25°C with 100 μE continuous light for 5 days. During growth experiments, 1% CO_2 in nitrogen was bubbled into the vessel at a rate of 0.5 vvm. Optical density measurements were taken at 720 nm and 680 nm every 5 minutes using the built-in optical density monitoring function of this photobioreactor. Temperature, pH, carbon dioxide, and dissolved oxygen measurements were also recorded every 5 minutes. Data collection and storage was automated using the provided data collection software for the photobioreactor. This experiment was repeated with and without the paddlewheel to assess its impact on algal growth and nutrient distribution in the algal culture. Probes for monitoring pH, carbon dioxide concentration, and dissolved oxygen concentration were freshly calibrated for each experiment to maintain consistency.

Results and Discussion

Annealing improves heat tolerance

To determine the impact of the annealing process on deformation of the HTPLA, we measured the deformation of a 2 mm test strip before and after heating at 6 different temperatures (see Figure 2). Prior to annealing, each test piece was 2 mm wide but after the annealing process, the width of our test pieces increased to 2.2 mm indicating that some deformation naturally occurs during the annealing process. Interestingly, during heat testing, annealed pieces showed no further deformation up to 130°C (also see supplemental Figure S1), while the non-annealed (control) pieces showed increased deformation at all temperatures except 110°C. The bioreactor growth vessel is autoclaved at 121°C for 30 minutes prior to running growth experiments, so minimizing deformation of the HTPLA is important to maintain performance of the paddlewheel. By thermally annealing the printed pieces, the molecular structure of the polymer is reorganized into a semi-crystalline structure that greatly improves heat resistance properties of the material. By including this step, the paddlewheel apparatus becomes fully autoclavable, and can even be put through multiple cycles. Our results here indicate that thermal annealing is required prior to the first use/ sterilization of the paddlewheel to ensure reliable performance.

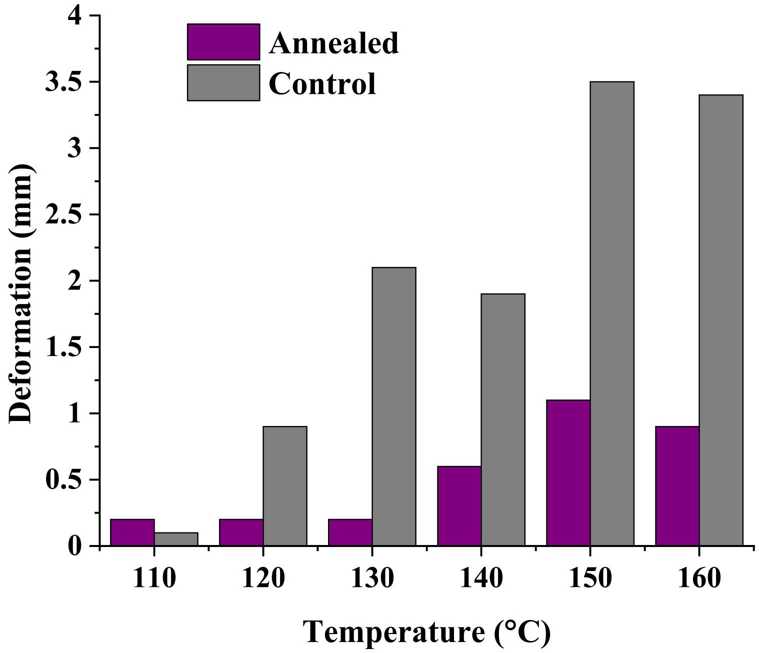


Figure 2. **Annealing HTPLA leads to smaller deformation.** Annealing experiment results comparing deformation of annealed and non-annealed control pieces of HTPLA after heating at temperatures ranging from 110°C to 160°C. Initial printing width for test pieces was 2mm. Some deformation was experienced during the annealing process, causing these pieces to start at a width of 2.2 mm. Each bar in this figure represents a single measurement.

Impact on growth and photosynthetic activity

The need for additional mixing in the PSI FMT 150 1-liter photobioreactor was first evident when we switched from growing *Chlamydomonas reinhardtti*, a green alga with flagella capable of both photo- and chemo-taxis, to *Chromochloris zofingiensis*. Our initial growth studies were limited to extremely dilute cultures because there was significant accumulation of cells in the corners of the reactor vessel (see Figure 3A). As a terrestrial alga, *C. zofingiensis* is prone to sedimentation when grown in liquid culture. This settling leads to consistently low optical density measurements, even when the culture is growing, as shown in Figure 4A. Due to the short light path of the reactor, the number of possible solutions to increase mixing in the reactor and keep cells in solution was limited.

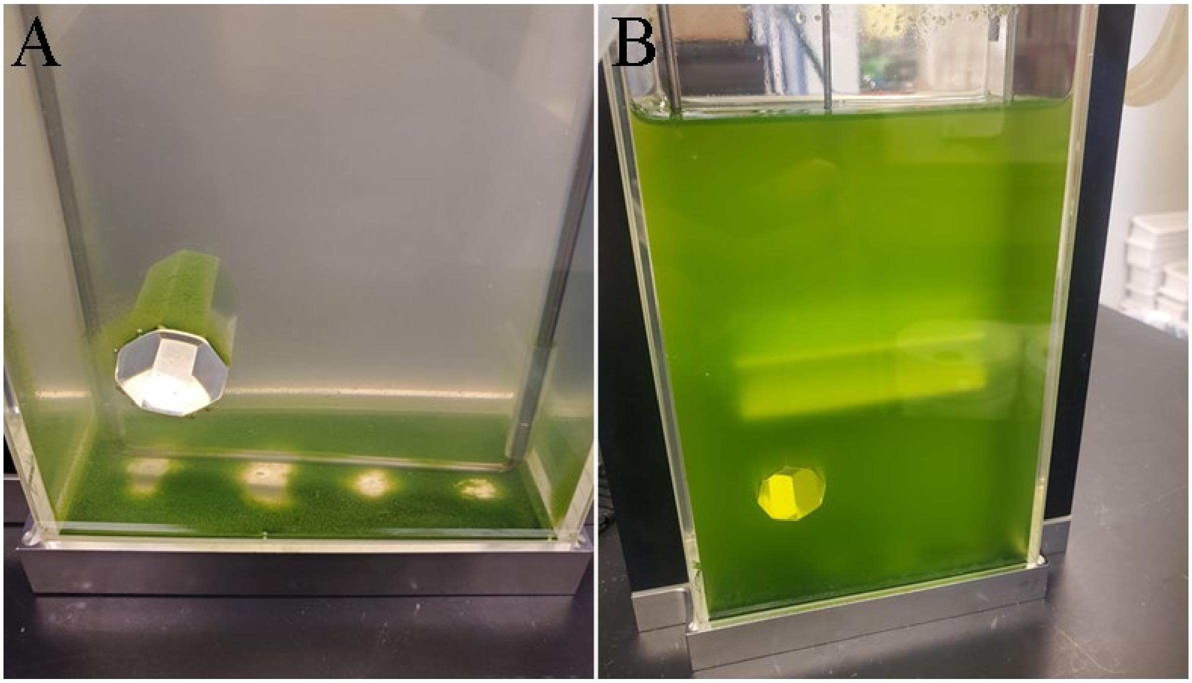


Figure 3. Growth of terrestrial algae *Chromochloris zofingiensis* with (B) and without (A) the inclusion of the paddlewheel. A) Cells settle to the bottom of the reactor vessel when no paddlewheel is included due to inadequate mixing. B) Cells remain dispersed in liquid media when sufficient mixing is provided.

Taking advantage of the gas sparging line in the FMT photobioreactor, we designed a paddlewheel to increase mixing and keep cells in solution. We tested the impact on the growth of *C. zofingiensis* by monitoring the optical density at two different wavelengths (λ = 680 nnm and λ = 750 nm), CO_2 concentration, O_2 concentration and pH. We ran several different growth trials, one representative set of data is shown in Figure 4, we have added others in supplemental Figure S2. With the paddlewheel, growth as measured by optical density increases over 100% as compared to growth without the paddlewheel. This can partially be attributed to the cells being kept in solution rather than settling on the bottom of the vessel, but we also see a 39.5% increase in the oxygen evolution rate and a 38.6% decrease in carbon dioxide concentration in the culture vessel. This increase in oxygen and decrease in carbon dioxide are both indicative of higher photosynthetic activity in cultures grown with the paddlewheel. In both cultures, the pH remains stable throughout the growth period.

The inclusion of this paddlewheel allows for a more homogenous culture volume (see Figure 3B), and provides adequate mixing for the cells to remain suspended within the liquid culture. This inclusion greatly improves experimental design and makes it possible to grow non-planktonic algae more efficiently in liquid culture. This increased homogeneity of the culture volume is important in experiments where a tight control of conditions throughout the culture volume is needed such as synchronization studies while growing under diurnal conditions. An increased homogeneity of the culture would also be necessary in isotopically in-stationary ^{13}C metabolic flux analysis experiments, where once an isotopic label is introduced, it must be rapidly distributed throughout the culture volume.

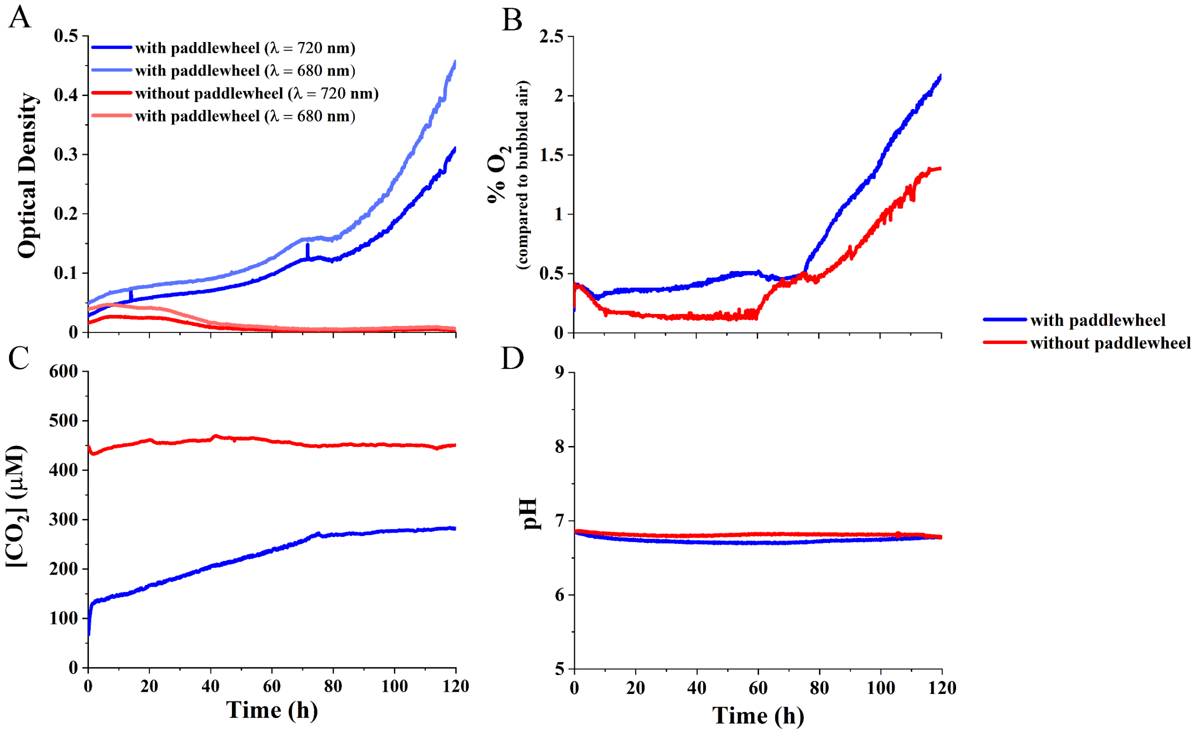


Figure 4. Growth data from *C. zofingiensis* with and without the inclusion of the paddlewheel. A) Optical density readings taken at 720 nm and 680 nm. B) Dissolved oxygen data. C) Dissolved carbon dioxide data. D) pH probe data. Measurements were taken every 5 minutes for 5 days for both experiments.

Conclusions

One of the major advantages of growth in traditional bioreactors is the assumption of the reactor being a well-mixed vessel. Unfortunately, this assumption cannot be assumed in the operation of a flat panel photobioreactor without mixing due to the accumulation of cells in the corners of the reactor. This is especially problematic if the cells do not have flagella to 'swim' and keep themselves a float. In an effort to improve the homogeneity of our reactor, we designed and a bubble-driven paddlewheel that can easily be manufactured via 3D printing. Inclusion of the paddlewheel improved the growth and photosynthetic activity of the algae, shown as increased oxygen and lower carbon dioxide concentrations. The paddlewheel also enables us to grow cells at higher density, which helps minimize sample volumes needed for any type of systems biology characterization. This study also highlights the utility of 3D printing in the lab: the ability to quickly and easily design and test new devices in the lab is invaluable for improving experimental approaches efficiently.

Author Contributions

Michelle Meagher: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – Original Draft, Writing – Review & Editing

Jacob Tamburro: Investigation, Methodology, Validation, Writing – Original Draft

Nanette Boyle: Funding Acquisition, Project Administration, Resources, Visualization, Writing – Original Draft, Writing – Review & Editing

Acknowledgements

MM was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0019171 and JT was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0023027.

Conflict of Interest Statement

The authors declare no conflict of interest for this article.

Data Availability

All relevant data is available in the supplemental files provided with this manuscript.

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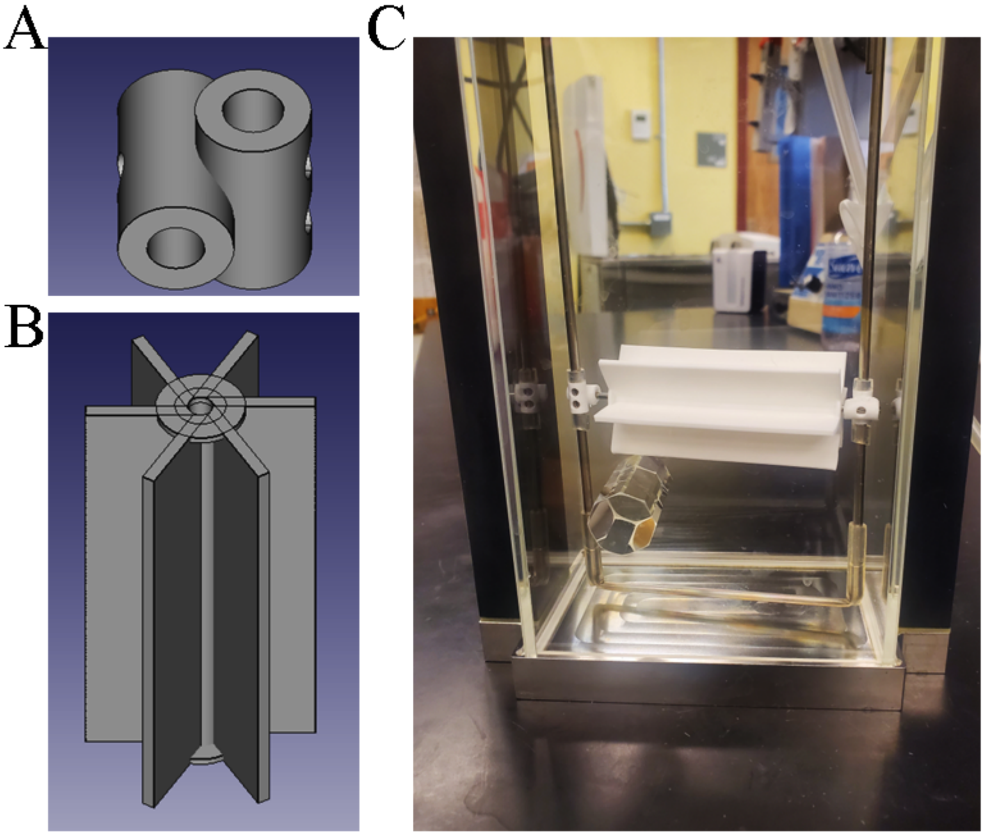


Figure 1.tif

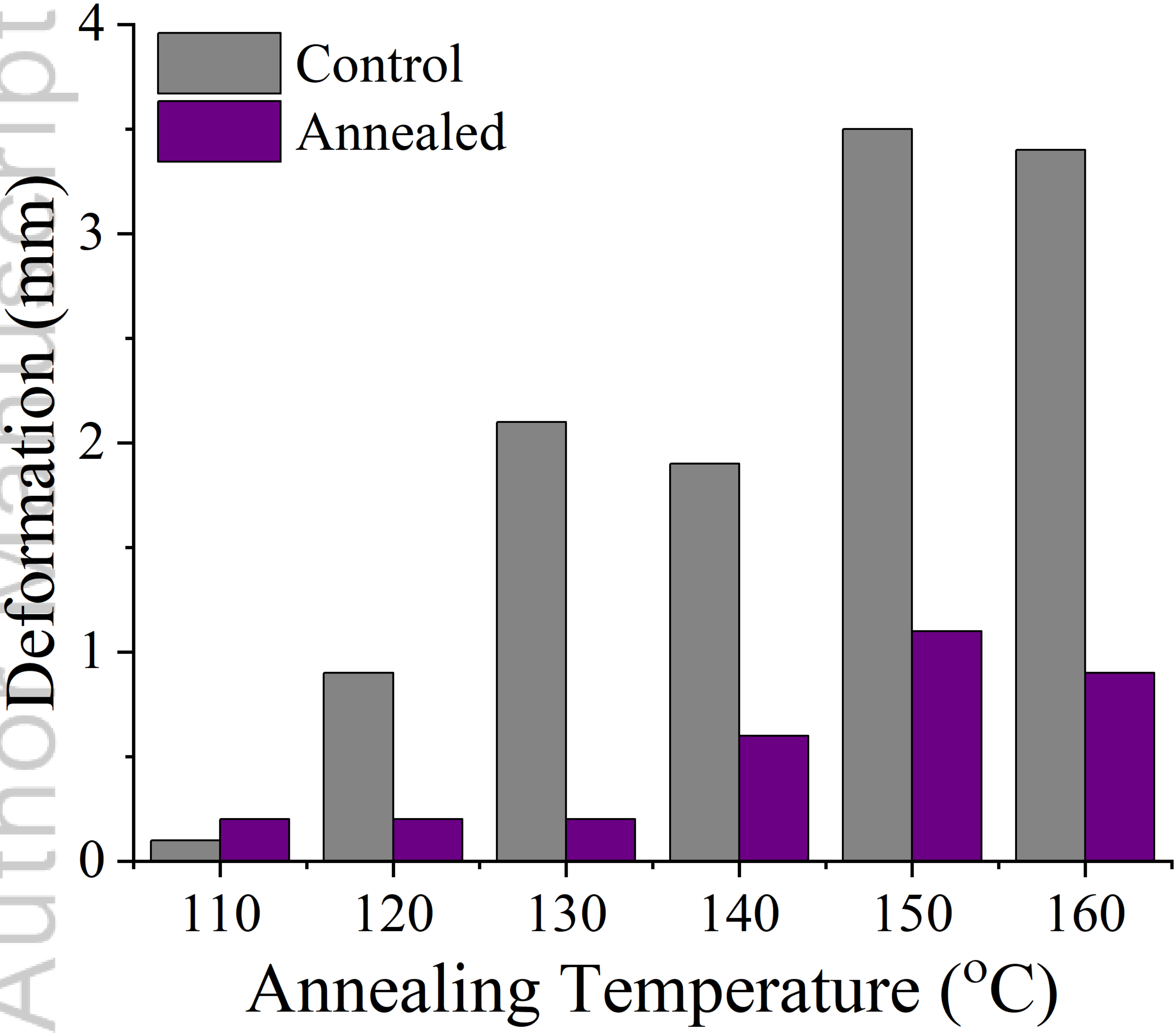

Control
Annealed
Deformation (mm)
0
1
2
3
4
110
120
130
140
150
160
Annealing Temperature (°C)

Figure 2.tif

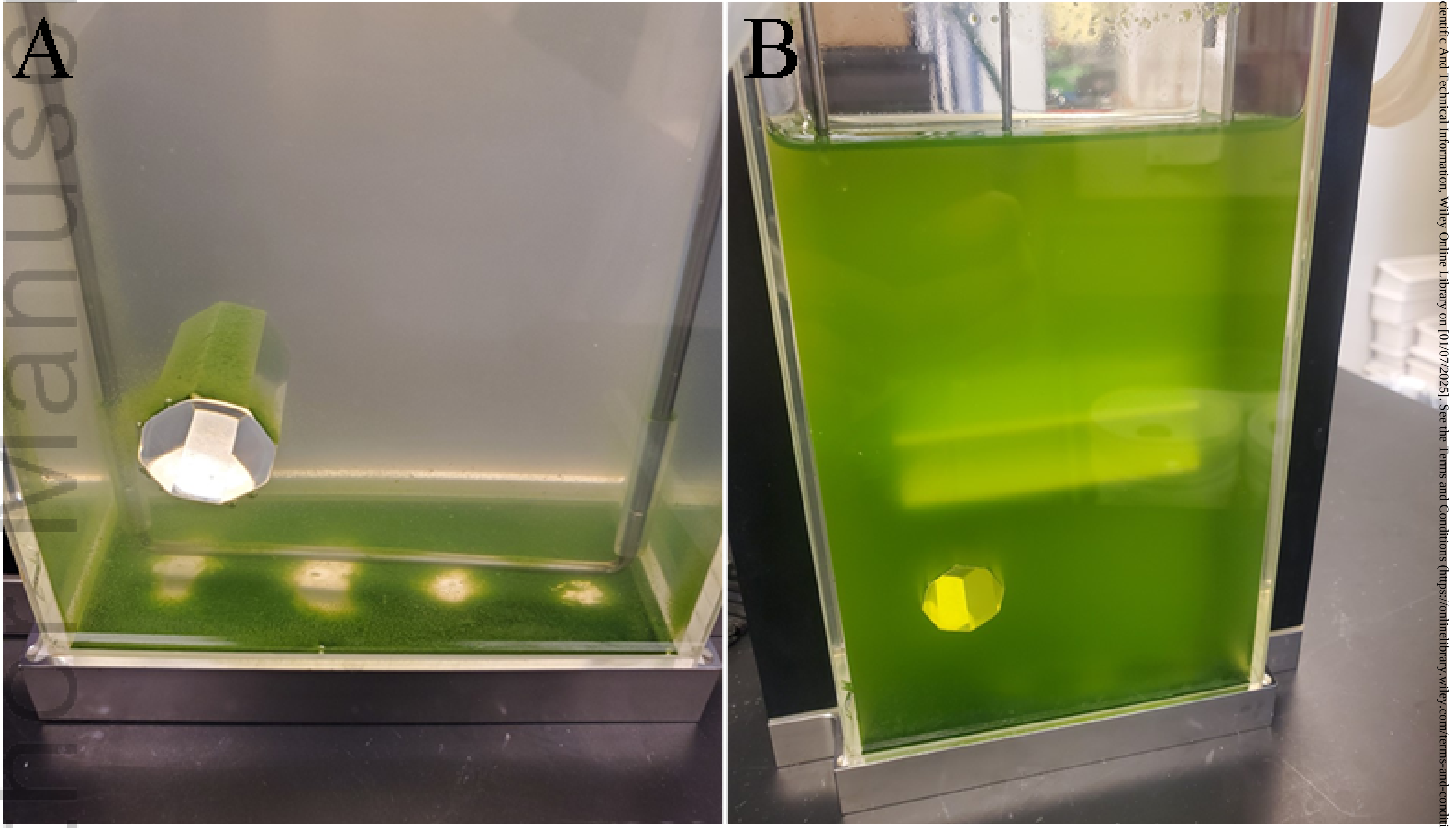


Figure 4.tif

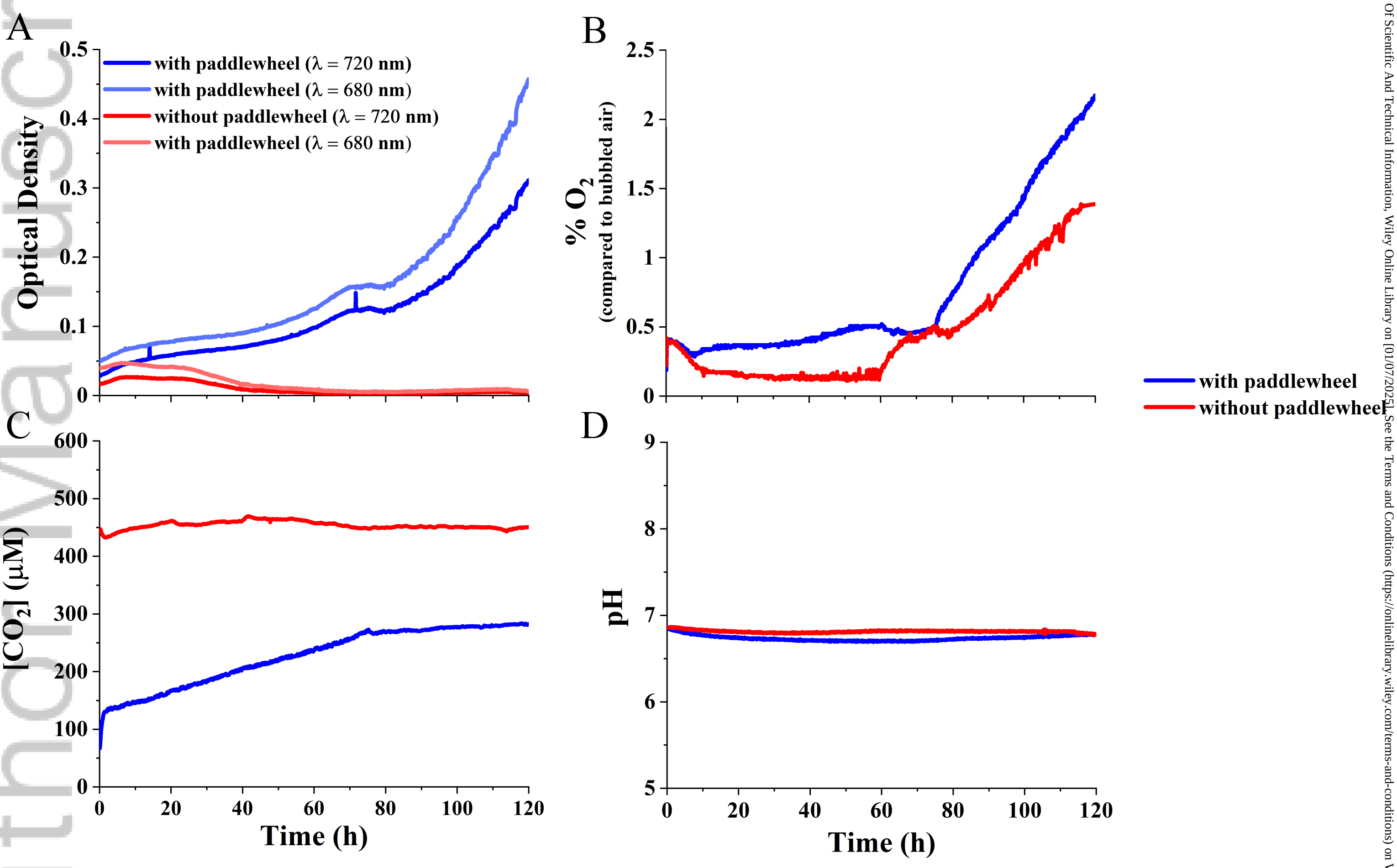
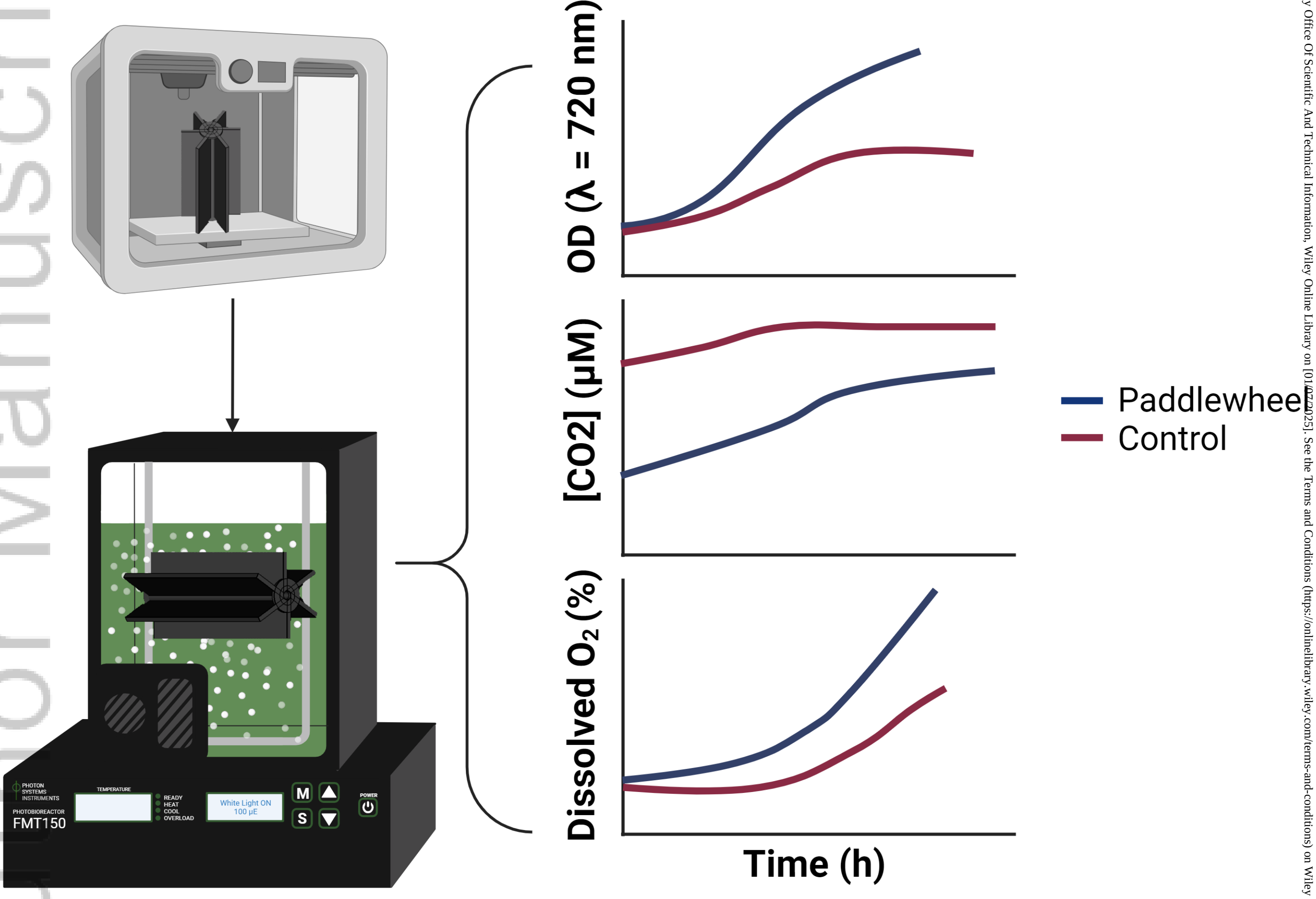

A
Optical Density
0.5
0.4
0.3
0.2
0.1
0
with paddlewheel (λ = 720 nm)
with paddlewheel (λ = 680 nm)
without paddlewheel (λ = 720 nm)
with paddlewheel (λ = 680 nm)
B
% O_2
(compared to bubbled air)
2.5
2
1.5
1
0.5
0
with paddlewheel
without paddlewheel
C
[CO_2] (μM)
600
500
400
300
200
100
0
0
20
40
60
80
100
120
Time (h)
D
pH
9
8
7
6
5
0
20
40
60
80
100
120
Time (h)

Figure 5.tif

Addition of a 3D printed paddlewheel in a flat panel photobioreactor increases growth and photosynthetic activity of algae by improving mixing.



Paddlewheel Graphical Abstract.png