Practical Design of Hybrid Photobioreactor System for Economic Feasibility and Environmental Sustainability

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Abstract

Modern photobioreactors for microalgae-based biofuel production face various budgetary challenges in comparison to its competitor design, the traditional open pond system. Herein, a novel design of a double-chamber hybrid photobioreactor is proposed, featuring low-cost components including a 3-W centrifugal pump and recyclable plastic modules for its solar flank, as well as derivatives from tubular and membrane photobioreactors. The hybrid photobioreactor exhibits reasonable hydrodynamic performance with a circulation time of 120 seconds and a mixing time of 38 seconds. A side-by-side runthrough of Chlorella vulgaris cultivation with the novel hybrid photobioreactor and a standard open pond system was done to compare growth rates and system performance under equal environmental conditions. Resulting measurements show that the hybrid photobioreactor produced a 34.87% comparatively greater amount of C. vulgaris by the tenth day of cultivation, demonstrating a significantly higher growth rate ($P < 0.05$).

In contrast, wet-sludge lipid extraction processes show that the C. vulgaris culture grown in the open pond system held a substantially larger lipid accumulation than that of the hybrid photobioreactor, although the difference was not statistically significant ($P > 0.05$). The pilot-scale photobioreactor not only costed as little as the open pond system but also incurred 88.6% less expenses compared to a similarly designed bioreactor made of clear PVC material. Consequently, this project demonstrates the candidacy of the proposed low-cost hybrid photobioreactor design for microalgal biofuel production.

Keywords: Microalgae, *Chlorella vulgaris*, biofuel, photobioreactor, recyclables.

Introduction

Biofuels have evolved significantly and become increasingly important nowadays due to a combination of environmental, economic and energy security considerations since vegetable oil was first used as fuel for engines in 1937[17]. The production of biofuels from plant or plant-like materials and organisms has the potential to stimulate economic growth as an industrial consequence and lower greenhouse gas emissions through the carbon sink effect[20]. In particular, chlorella-encapsulating microalgae have gained special attention as an important source of biofuels in recent years. This is mostly because microalgae have a highly effective upstream process in the biofuel production cycle attributable to their rapid growth rate[11].

Despite progress in the development of microalgae production facilities, there are still barriers in place that hinder the implementation of large-scale microalgae bioreactor systems. One critical challenge is the high expenditure required to build bioreactors for the propagation of microalgae at an industrial scale[2]. Also, continuous maintenance and nutrient replenishment are required during microalgae cultivation due to their fast growth rate, resulting in extra cost of capital and time[16]. Altogether, biofuels derived from microalgae are cost-wise less competitive than fuels derived from land-based crops.

As of now, there have been ongoing attempts to resolve the cost barrier of type III biofuels. Two major types of algae bioreactor systems have been developed in an effort to lower constructional and operational expenditures. The first is the open raceway bioreactor, a type of open pond system that utilizes an oval-shaped basin to culture algae[6]. This type of bioreactor is currently the most popular method for producing microalgae, with 80% of worldwide algal biomass harvested from this specific type of system[32]. Under circumstances in which algae quality and growth efficiency are prioritized over cost, the photobioreactor (PBR) is more typically used in place of open pond systems.

Unlike open pond systems, PBRs are closed to the external environment and harness greater control over internal environmental settings, such as temperature or pH. There are categorically five well known types of PBR configurations including gas-airlift, tubular, membrane, floating and hybrid bases. The gas-airlift PBR is an air-circulated bioreactor with a very straightforward design that relies upon upward-moving gas bubbles for its main source of culture circulation[7]. The tubular PBR typically consists of a series of transparent tubes through which light can penetrate, a main container, a pump and other apparatus that can be used to grow microalgae[32].

Despite its rigid construction, the tubular design allows for efficient pneumatic mixing and light exposure as the tubes are often arranged in a spiral or helical configuration to maximize the surface area exposed to light. The membrane PBR is another design that uses a membrane to separate the culture medium from microalgae[32]. This configuration is intended to allow growers to manage the growth

environment and prevent contamination. PBRs under this classification are typically for wastewater treatment and for the manufacture of high value compounds like pigments or nutraceuticals.

Following a similar budgetary level is the floating PBR, a water-submersible, self-contained variation of membrane/tubular systems, capable of operating in environmentally-unconstrained large bodies of water. Unlike other types, the growth conditions in the floating PBR are completely relegated to the outer environment with the interior system only being separated from the exterior by an impermeable plastic membrane. Ultimately, when the designs of various types of PBRs are taken into account to compensate for disadvantages and enhance virtues of both, the result is a hybrid PBR which can take on many forms.

Although PBRs have been explored as a more effective method for growing microalgae in comparison to open pond systems, their construction and management cost can be unsustainably high, even for research purposes[27]. For instance, NASA developed a floating PBR called OMEGA in 2010 for use in wastewater settings. While OMEGA was able to create an efficient small-scale algae production cycle, it was not able to meet the required energy and economic returns on investment and the project was later discontinued[4]. It is imperative for the scientific community to focus research on cutting the costs of PBRs, given the enormous potential for industrial applications of these systems.

Regarding the constructional and operational cost setbacks, the question lies in whether an effective PBR can be built for less than or similar to the cost of an open pond system. PBRs are commonly built of expensive materials such as PVC, rubber, vinyl and glass, with its cost constituting a major portion of the system's overall expenditures[13]. Therefore, research and development in cost-effective materials and construction methods could potentially make PBRs more accessible for industrial-scale applications. To address this, a promising alternative microalgae production unit has been proposed and tested in the present study: a hybrid tubular PBR system that runs on a low energy (3-W) centrifugal water pump with a total top-view working space of 91.4 x 86.36 cm and a max-capacity of 35 L. This practical design was accomplished through several novel approaches to sourcing construction materials and changing reactor configurations.

First, the tubular section was made with recyclable plastic bottles instead of PVC or vinyl pipes, lowering the initial construction costs. Second, the main container section was constructed on the basis of the interior of a membrane PBR.

This design would help evenly disperse algae and pneumatically circulate the system in the area where cycled algae reenter the system. This imitation was intended to address the PBR's issue that microalgae would accumulate on top of each other in an enclosed environment without the direct incorporation of a gas-airlift mechanism[30].

Third, the designed hybrid PBR was built around a 3-W energy pump, requiring little energy to run the entire PBR. Its low wattage enables the PBR to be placed in many different locations with the addition of solar panels as its main source of energy. It is hypothesized that the aspects of this pilot-design allow the hybrid PBR to retain the production virtues of different types of PBRs, as well as the cost-effective trait of the open pond bioreactor.

Material and Methods

Microalgae Source and Culture Media: The species of *C. vulgaris* microalgae was chosen in this study for its high cellular concentration of lipids[14] and industrial practicality of natural tolerance to non-potable water[12]. The nutrient medium used for the *C. vulgaris* culture was a 1:1000 dilution of concentrated F/2 vitamin solution (Aqua Algae Co., OH) into distilled water as suggested by Guillard et al[8]. The *C. vulgaris* culture was obtained from the Carolina Biological Supply Company and was inoculated in a small indoor gas-airlift PBR filled with 0.5 L of the nutrient medium at room temperature. The culture was lit upon with a soft-white and fluorescent bulb positioned 10-cm above the surface of the nutrient medium.

Regarding the light intensity at approximately water level, the surface reached a light energy of 310 PPFD (Photosynthetic Photon Flux Density), as measured with an illuminance meter. An additional amount of nutrient medium — 10% by original volume — was replenished daily to the PBR until its container held 4 L of the microalgae culture. Microalgae growth was monitored daily through measurement of cellular optical densities at 750-nm wavelength (OD750) using a UV-visible spectrophotometer as described in an early study by Santos-Ballardo et al[26]. The microalgae culture was used for subsequent experiments when samples reached an OD750 of 0.8.

Optimization of Nutrient Supply: Two preliminary experiments were carried out indoors to identify an effective method for nutrient supplementation during cultivation of the *C. vulgaris* microalgae in the bioreactor systems. In the first experiment, microalgae growth without replenishment of additional nutrients was examined daily by OD measurement. The growth of microalgae in such conditions would give insight into the optimal ratio of biomass production to the nutrient supplement as described in the below subdivision.

The second experiment utilized the results from the first to check whether the population of microalgae would increase exponentially which would be indicated by the presence of a linear trend between time and OD. A reasonable rate of growth[26] would then be used to justify the existing microalgae fertilization procedure that will be used later for the cultivation experiments.

Cultivation of *C. vulgaris* without Nutrient Replenishment: This experiment began with inoculation of 10 mL *C. vulgaris* culture from the indoor bioreactor as described above into 30 mL of distilled water, plus 40 µL of the concentrated F/2 vitamin solution in a 50 mL glass beaker. The mixture was cultured at room temperature with plastic wrap covering the beaker to prevent evaporation. The culture was constantly stirred with an air diffuser at a low flow rate and illuminated under a 100-W fluorescent light lamp. The aerator and lamp were plugged into an outlet timer set to a 15-hour on-cycle from 6:00 to 21:00. The timer setting would prevent microalgal culture from entering into a state of photoinhibition in response to an extended light exposure[9].

The microalgal growth was examined daily by optical measurement of cellular density using a spectrophotometer as described above. The OD750 data collected over time was later used to determine the best nutrient supply conditions for growing *C. vulgaris*. To do this, an exponential regression was first used to identify the day before the largest increase in residual values, which was deemed to have the optimal biomass to nutrient ratio. Using a absorbance/cellular count chart[26], an optimal ratio of the F/2 vitamin solution to biomass production was then calculated.

***C. vulgaris* Cultivation with Optimized Nutrient Supplementation:** With the optimal ratio calculated above, another batch of *C. vulgaris* culture was set up with the same procedure as the first experiment, except nutrients which were replenished every three days. The amount of F/2 addition would change every time based upon the *C. vulgaris* biomass present in the culture at the moment of replenishment. Culture OD was monitored daily and the recorded data were statistically compared with those from the first experiment to evaluate how the optimized nutrient supplementation impacted on the microalgae exponential growth. The significance of the impact would determine whether the current rate of nutrient application would be sufficient for the subsequent PBR cultivation studies.

Construction and Operation of Open Pond Bioreactor: An open pond bioreactor was built to serve as a side-by-side control system for bias-free evaluation of the hybrid tubular PBR. The open pond cultivator's main reactor was a recycled foam container (31.8 x 23.9 x 20.0 cm inner measurements and 3.2 cm wall thickness). The inside bottom of the container was covered with a hard plastic cutout and sealed at the edges with silicone sealant. 3-W Pulaco air diffuser was attached to the bottom center with waterproof tape and push pins. The foam box cover was cut to make a sizable rectangular frame with a border size of 2.5 cm. Parafilm slices (31.8 x 10.2 cm) were lined up side by side, stretched over the foam cover frame and linked together by a layer of duct tape.

According to previous studies, light intensity between 26–400 PPFD would be ideal for algal cultivation[19]. Herein, the semi-transparent configuration was made to reduce the direct sunlight intensity (≥ 2000 PPFD) to prevent the early photoinhibition in algae cultivation. The constructed bioreactor was set at a flat 0° gradient on top of an adjusted wooden platform on a bright hillside with no overhead obstructions.

The reactor was filled with distilled water (12 L) and high concentration F/2 vitamin solution (12 mL) and inoculated with *C. vulgaris* culture (1 L) from the gas-airlift PBR as described above. Microalgae culture OD was checked daily for 10 days with the help of a spectrophotometer and F/2 nutrient was replenished every three days using a transfer pipette based on the optimal biomass/nutrient ratio.

Construction and Operation of Hybrid Tubular PBR: The configuration of this hybrid PBR consisted of two functioning parts: a clear plastic tubular array and a modified foam container embedded with a low-energy cost centrifugal pump. The clear plastic tubular array was mainly constructed with twenty empty plastic bottles (23.5 cm L x 5.7 cm D each), which had been collected as recyclables for the purpose of this study. To build the tubular array, the plastic bottle subunits were first prepared for each array column (Figure 1a).

The center subunit (Bottle #3) was made by cutting a 2.54 cm bottle from the ends of both sides. The extended subunits (Bottle #2 and #4) were made by cutting bottles 1.3 cm from both sides. The end subunits (Bottle #1 and #5) were made by cutting bottles 2.54 cm only from the bottom, leaving the cap side intact. An array column unit was then assembled by inserting each subunit into the ends of the others according to the illustrated arrangement (Figure 1a; 1b). To ensure leak-proof connections, each cut end of bottles #1, #3 and #5 were immersed 2 cm into flex seal (a liquid rubber-based elastomer produced by Swift Response Co) before fitting together.

The points of connection with Flex Seal were further cured partially with a hot glue gun to prevent streaking and excess materials were wiped off. This construction process was repeated three more times to assemble the remaining array columns. The cap ends of columns were linked with pliable vinyl pipes (35.6 cm L x 2.5 cm OD) to make the array as illustrated (Figure 1c). Again, flex seal was used to ensure leak-proof connections between the vinyl pipes and the cap ends. The vinyl pipes were also used to connect the array with the centrifugal water pump in the main container (Figure 1c). The length of each tubular column was approximately 91.4 cm, with 15.2 cm distance in between.

The main container of the hybrid PBR was based on the same type of foam container as the open pond bioreactor, with inside changes made to meet the system's architecture. First, a large gradual circular depression was made in the container bottom in order to fix the 3-W centrifugal water pump. The bottom was covered with hard plastic cutout and

sealed at the edges with silicone sealant to prevent leakage. The water pump was placed in the center of the circular depression and secured with waterproof tape and push pins. Second, the foam box cover was altered by making a small hole for the pump tubing and a larger slanted hole for fitting a small plastic funnel cone and cutting a corner for the pump's electrical cord (Figure 1e). A small plastic funnel cone was fit onto the slanted hole and secured with a glue gun. The funnuel was placed directly underneath the opening of the end of the tube array.

A small plastic bag with scattered pinholes was attached to the bottom of the foam box lid with an opening to the internal funnel hole. This contraption would allow algae medium from the funnel hole to drain into the plastic bag where the medium would spray out through small holes, aerating the medium and circulating algal biomass without the use of a gas diffuser. To connect the main container to the plastic array, a small hole was made on a bottle cap to insert with a vinyl pump tubing (~ 90 cm long) and the connection was secured with the flex seal. This part served as an adapter to connect the tubular columns and the water pump.

The constructed tubular system was set up at the same location as the open pond bioreactor. In order to form an effective flow loop during the microalgae cultivation, the main container's position was adjusted so that one end of the tubular array was connected to the water pump and the other end connected to a stream of microalgae culture directly into the funnel. The 3-W water pump was set to a slightly lower power setting — 2-W — to maintain flow cycles and prevent overflowing. Despite low energy input, the pump was able to circulate liquid within 2 minutes to avoid oxygen toxicity in the sealed tubes.

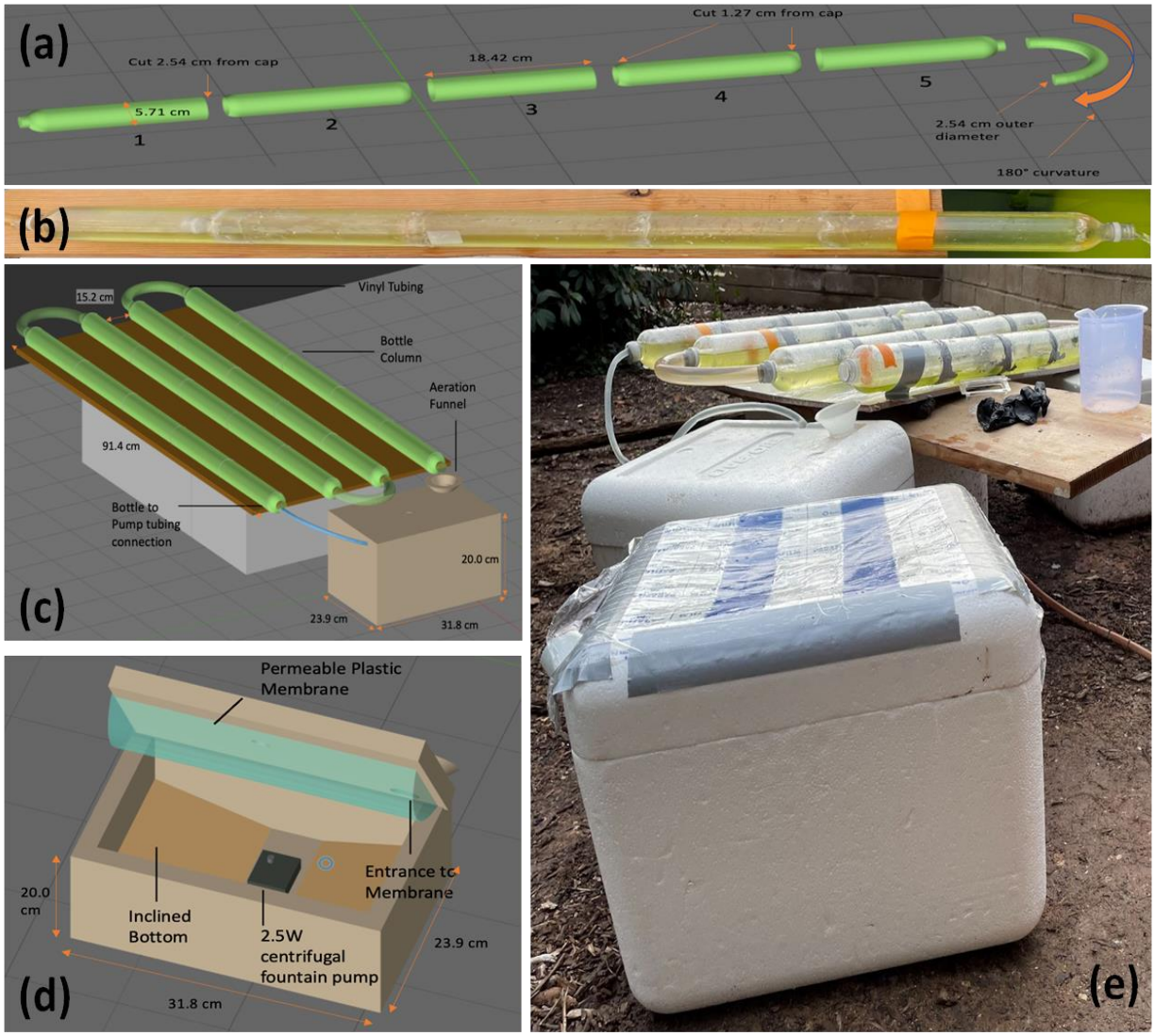

Figure 1: Construction of the hybrid Photobioreactor (PBR) as compared to the open pond system. (a) Blueprint illustrating arrangement of plastic bottle cutouts in a single PBR array column. (b) Construction of the single operating plastic bottle array column. Clear silicone sealant was used to secure the connections between bottle cutouts. (c) Diagram illustrating the finished array part of the Hybrid PBR. (d) Diagram illustrating the interior of the Hybrid PBR main container part. (e) Operation of the constructed Hybrid PBR and open pond system in an outdoor setting.

Similarly, the main container was filled with distilled water (12 L) and high concentration F/2 vitamin solution (12 mL) and inoculated with *C. vulgaris* culture (1 L) from the gas-airlift PBR as described above. The total photo-reaction surface area of the system was calculated to be 91.4 x 86.36 cm with a maximum liquid capacity of 35 L. The optical density of the microalgae culture was checked daily for at least 10 days using spectrophotometer and F/2 nutrient was replenished every three days using a transfer pipette based on the optimal biomass/nutrient ratio.

Lipid Buildup Analysis: Lipid extraction was carried out using a previously established procedure by Patel et al[23] to comparatively evaluate the potential of biofuel productivity in the developed hybrid PBR. In brief, post 10 days of cultivation, the *C. vulgaris* cultures (100 mL) were harvested from each bioreactor and the microalgae sludges were collected after centrifugation for 6 min at 3400 rpm, followed by careful removal of the supernatant water. The remaining algae sludge was weighed and transferred into a round bulb flask. A 1:1 volume ratio of methanol to algae sludge was added to the flask. The mixture was mixed by vortexing for 30 sec, followed by setting at room temperature on an orbital shaker 120 seconds at 210 rpm.

Afterwards, a 1:1 ratio of hexane to the algae sludge was added, followed by the same procedure of vortexing and setting on the orbital shaker for separation of the supernatant extract. The top-layer supernatant extracts in the bulb flask were then completely transferred to a small glass beaker. The solvents were removed by bathing the glass beaker in boiling water for 10 min. The weight of the lipid remnants was recorded for statistical analysis.

Statistical Data Analyses: All the experiments were initially conducted in December 2022 and then repeated in January 2023 in Claremont, California. The data generated in triplicate from these independent experiments were subjected to the student t-test with significant differences determined at the *P* value of 0.05 and the Prism (version 8.0) from GraphPad was used to generate high quality figures after the data analyses.

Results

The ambient conditions at the project site during the period of this study were derived from WeatherSpark's climate database. The daytime period lasted around 10.4 hours and the daily range of the outdoor temperature fluctuated between 5°C and 17.8°C. The sunlight intensity varied greatly at different times throughout the day, ranging from 200 to 2000 PPFD (Table 1).

Exploration of Nutrient Supply Strategy in Microalgae Growth: This preparatory experiment was conducted to explore an ideal nutrient/biomass ratio that could be applied on a larger scale to achieve high-density algae growth. For the course of this experiment, the growth medium inside the gas-airlift PBR averaged a temperature of 19.5°C according to the daily records at 16:00 PM PST. This temperature was not the best but still a favorable one promoting high growth rate of *C. vulgaris* culture[15]. As described above, the optimal quantity of nutrient per biomass would be on the day just before the largest increase in the residual value from an exponential regression.

Table 1
Outdoor daylight spectrum at 6500K in the project site of this study

Military Time	PPFD
8:30	336.03
10:30	1203.34
12:30	1942.20
14:30	1459.73
16:30	201.31

Note: The data were taken on December 8th of 2022 and the project site was in Claremont, CA with a latitude of 34.13° and longitude of -117.74°.

Based on the optical density data curve, the day with the highest exponential regression residual in the first five days is day 2 and therefore the day with the optimal algae growth is day 1. According to the chart of OD750 and cellular count[26], the optimal microalgae growth on day 1 corresponded to a density of 3.0 x 10^9 *C. vulgaris* cells per 1 L of culture medium. Thus, in order to promote *C. vulgaris* to grow optimally over time, the nutrient could be replenished with a strategy of adding 1 mL of the concentrated F/2 solution for every freshly produced 3.0 x 10^9 microalgal cells in 1 L of the culture medium and a typical feeding interval of three days.

Microalgae Growth with Optimized Nutrient Supply: It was observed that *C. vulgaris* culture was able to maintain a significantly much higher growth activity over time ($P < 0.05$) under the optimized condition in relation to the situation without continuous supplement of F/2 nutrient (Figure 2), indicating that the identified ratio in the above experiment could be further established as an effective factor in promoting microalgae cultivation.

It was noticeable that the algae started to clump and sink after 15 days in the absence of nutrient replenishment; declining growth occurred. In contrast, the algae still visibly stayed afloat and culture biomass continued to grow after 15 days of growth with the allotted nutrient replenishment. In comparison to the growth rate of microalgae reported previously by Cristina et al[3], it suggests that the nutrient/biomass ratio identified in this study is practically supportive in providing *C. vulgaris* culture with a favorable development environment.

Comparison of Biomass Productivity: The optical density measurements showed that the hybrid PBR significantly outperformed the open pond system for the growth of *C. vulgaris* culture during the period of this side-by-side comparative study ($P < 0.05$).

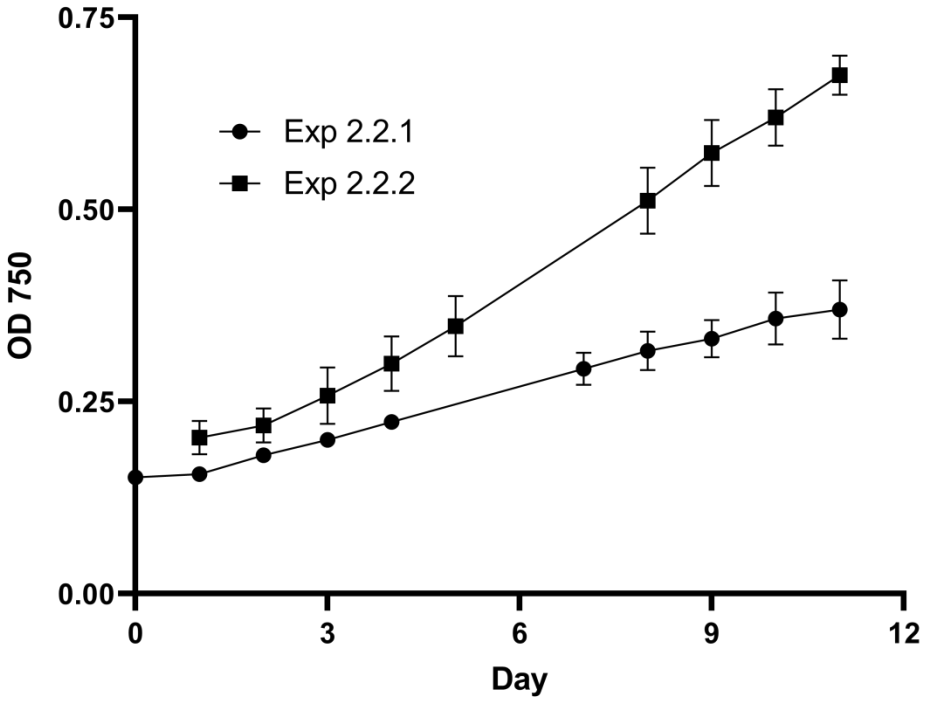

Figure 2: Comparison of *C. vulgaris* growth under distinct conditions of nutrient supplement. This comparative experiment was performed to establish an effective strategy of nutrient replenishment during the microalgae cultivation. The microalgae growth was monitored through daily measurements of the culture's optical density at 750nm (OD750). The nutrient in the first trials was not added further after the initial setup but was replenished accordingly every three days in the second experiment based on an identified ratio factor. The OD750 data were analyzed using one-tailed paired parametric t-test and the *P* value was calculated to be 0.0028 between the two groups. All data points are shown as the Mean ± SEM from three repeated experiments.

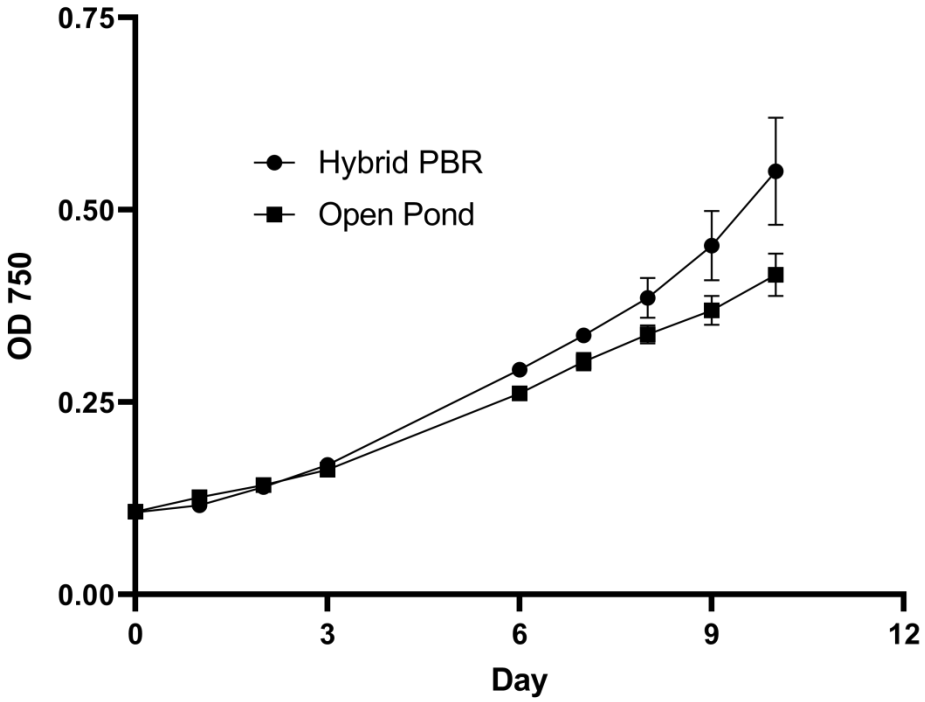

Figure 3: Comparison of *C. vulgaris* cultivation in the constructed hybrid PBR and open pond system. The microalgae growth was monitored through daily measurements of the culture's optical density at 750nm (OD750). The OD750 data were analyzed using one-tailed paired parametric t-test and the *P* value was calculated to be 0.0262 between the two groups. The data points are presented as Mean ± SEM from three repeated experiments.

The absorbance value of the *C. vulgaris* culture in the hybrid PBR had already begun to overtake that in the open pond system by day 2 although the latter started off with a slight advantage over the former (Figure 3). The lower optical density of the PBR in the first few days of the experiment may have been caused by the algae's transition from a low to high turbulence cultivation system. By day 10, the *C. vulgaris* culture in the hybrid PBR had a significantly greater optical density, averagely 0.375 AU higher compared to the open pond system.

It is worth noting that the growth rates of both outdoor systems are relatively slower in comparison to the indoor gas-airlift PBR. The growth-optimized culture in the indoor experiment was OD750 0.08 higher than the hybrid PBR's on day 10. This difference in absorbance values is possibly due to the increased variability in weather and temperature with setting the hybrid PBR in an outdoor environment.

For instance, on day 1, the temperature of the algae medium in the hybrid PBR was 12.5°C, significantly lower than 30.0°C found in the gas-airlift PBR. Also, the weather was cloudy for the majority of the days, leading to decreased photosynthetic productivity.

The optical density curve suggests an exponential development in the *C. vulgaris* culture according to the logarithmic relationship between biomass and absorbance[26]. Two linear regression equations were projected for predicting absorbance with day based on the replicate data: y (absorbance in PBR) = 0.0427x + 0.0651 with R^2 = 0.8937; y (absorbance in Open Pond) = 0.0312x + 0.0880 with R^2 = 0.9606. With the slopes of these equations, it is calculated that culture from the hybrid PBR is 32.39% more optically dense than culture from the open pond system from day 10.

Based on the biomass/absorbance chart by Santos-Ballardo et al[26], the cellular count of *C. vulgaris* on Day 10th was calculated to be 1.10E+7 ± 1.13E+6 and 1.50E+7 ± 3.61E+6 per microliter in the open pond and hybrid PBR respectively.

Thus, the microalgae in the hybrid PBR would hold an average 34.87% biomass advantage on day 10th. This advantage was visually discernible as the culture *C. vulgaris* in the hybrid PBR was conspicuously darker than that from the open pond system on day 10.

Comparative Analysis of Lipid Buildup: Typical lipid extraction procedures were performed to quantify lipid content of *C. vulgaris* culture in the hybrid PBR as compared to that in the open pond system. Out of the 100 mL microalgae culture on day 10, the open pond system accumulated a total lipid mass of 22.40 ± 10.79 mg, while the hybrid PBR had 19.13 ± 9.62 mg (Figure 4). The lipid content of the *C. vulgaris* culture from the open pond system was averagely 17.08% higher than the culture from the hybrid PBR although the difference was not statistically significant ($P > 0.05$). This discrepancy in lipid content might be explained through the *C. vulgaris* cellular mechanisms.

It has been known that microalgae like *C. vulgaris* tend to accumulate lipids under the metabolic stress[21]. It was possible that the higher concentration of lipids accumulated in the open pond culture might have been spurred by existing/increasing metabolic stress inside the system whether from reduced light levels inside the reactor or from competition brought on by algae accumulating on top of each other.

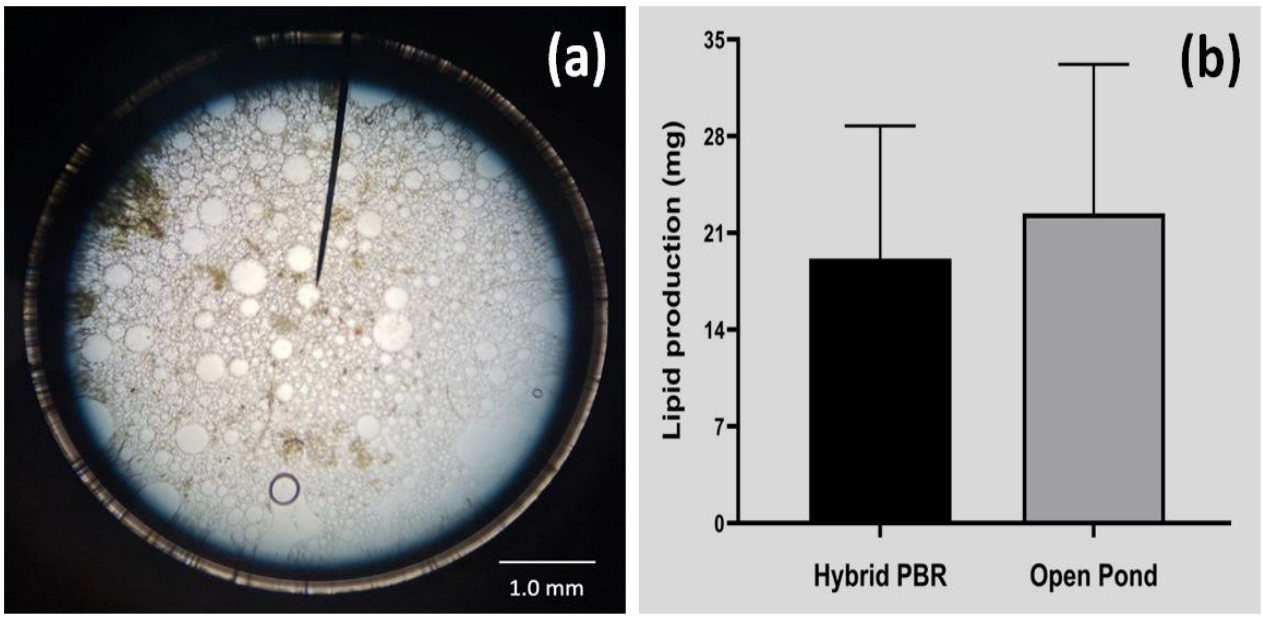

Figure 4: Lipid production from the *C. vulgaris* culture. (a) Lipid and hexane emulsion examined under a 40x compound microscope. (b) The lipid content produced per 100 mL of the microalgae cultures from the hybrid PBR and open pond system on the day 10th of the outdoor cultivation

Table 2
Configuration specification of open pond and hybrid PBR systems in this study

Cost of Specification	PBR this study	Open Pond	Vinyl-Tube PBR
Foam box (31.8x23.9x20.0 cm)	Recyclable	Recyclable	Recyclable
3-W centrifugal water pump (Amazon)	$11.59	N/A	$11.59
Plastic bottle (6.4x6.4x23.5 cm)	Recyclable	N/A	N/A
Aquarium Aerator (Petco)	N/A	$11.09	N/A
Flex Seal Liquid (Lowes)	$5.46	N/A	$5.46
Parafilm (Flinnsci)	N/A	$0.72	N/A
Black duck tape (Homedepot)	$0.71	$0.71	$0.71
Plastic wrap (Walmart)	$0.003	N/A	$0.003
Large foam blocks	Recyclable	N/A	Recyclable
Small Plastic Funnels (Walmart)	Recyclable	N/A	Recyclable
PVC tube (Grainger)	$6.12	N/A	$6.12
Flat plastic board	Recyclable	Recyclable	Recyclable
Large Wooden Board Laminate	Recyclable	N/A	Recyclable
Clear PVC pipe			$84.82
Assembling time	4 hr	1 hr	4 hr
Expenditure w/o recyclables			
Total Practical expenditure	$12.30	$12.52	$108.70

Discussion

The primary barrier of algae-based biofuel industry involves the economic feasibility of production systems. As the primary source of commercially-produced microalgae biomass in the world, modern open pond bioreactors have continually been limited by multiple factors such as surface adhesion, CO_2 accumulation etc. that PBRs were able to control[22]. In this regard, priority of the present study has been given on designing a cost-effective hybrid PBR while taking into account the constraints of existing bioreactor systems. The configuration of the hybrid PBR in this study has demonstrated an integrative approach to the construction of microalgae farms without the incorporation of expensive materials (Table 2).

It has illustrated the practicality of utilizing upcycled plastic bottles at a time when the price of vinyl and rubber is increasing, as well as the versatility of design is needed for operating a PBR on an industrial scale.

The deployment of this system on a larger scale would be substantially less expensive than other existing types of bioreactors due to the significantly lower cost of construction and operation by using recyclables. It is apparent that clear PVC tubing is much more expensive ($84.82 for 2.66 m of tubes for the same capacity as the one designed in this study) than recycling plastic bottles. Further, utilization of less energy-guzzling pumps is another plus in this design — an annual electrical cost of $3 if run continuously.

In addition, instead of operating under energy-intensive artificial illumination, the hybrid PBR utilizes natural sunlight for photosynthetic functions, optimizing energy profit and allowing for greater scalability. The development of the hybrid PBR in this study has considered and integrated the virtues of previous designs of PBR and open pond systems.

In addition to cost reduction, reuse of the recyclables is another good fit for this project in terms of environmental sustainability. Nowadays, most plastic bottles are made with polyethylene terephthalate (PET), a material that is 100% recyclable but generally better suited to tolerate UV rays over long periods of time[10]. PET is also regarded as being safer for food packaging in comparison to PVC which has partial toxicity when unprocessed[10]. Given these facts, PET bottles are considered to be a preferable material for the construction of the solar tubes in this project.

Further, the PET plastic bottles are much more bendable due to their lesser wall thickness which is advantageous in constructing a PBR array column without the need of an intermediate connector. Regarding their watertightness, the PET bottle sections were able to hold even without the use of sealing elastomers during test runs. Besides, there are no essential differences between the center sections of the 2.54 cm inner-diameter PET plastic bottles and clear PVC tubing of similar dimensions during PBR operation, except for the blue tint of PVC that renders passing sunlight more dim[29].

The operation of the hybrid PBR and the control open pond system at the same location allowed a bias-free comparison of their performance regardless of environmental variables. By day 10 of cultivation, the hybrid PBR biomass sample was 31.50% more optically dense than the open pond's sample and held 34.87% more algal cells. There might be several reasons linked to this outcome. First, the combined surface area of the solar tubes in the hybrid PBR was much larger than the top area of the open pond, amounting to

greater exposure to sunlight. Secondly, the enhanced aeration mechanism of the hybrid PBR allowed for more equal culture distribution, compared to the open pond system, which used a singular gas-diffuser as its main source of culture agitation.

Adequate agitation should be enough to prevent microalgae from congregating in parts of the main container. Thirdly, the hybrid PBR's aeration system consisted of a freefall from the end of the tubular section into a tunnel that leads to a hole-ridden membrane (functioning like a Plinko board), whereas the open pond had a gas diffuser placed in the bottom of the main container. The complexity of the hybrid PBR in contrast may have contributed to a higher gas concentration in the water, which is more optimal for *C. vulgaris* growth. In combination, these design improvements over the open pond system may have caused the hybrid PBR to outperform in terms of biomass production.

In terms of the lipid content, it seems that the open pond system has more buildup than the hybrid PBR. It is known that metabolic stress propagates the lipid accumulation in microalgae[28]. A study by Yang et al[31] reported that nutrient starvation was capable of increasing lipid concentrations in *Chlamydomonas reinhardtii* up to 93%. An experiment by Rai et al[25] demonstrated that five days of nitrogen starvation could increase neutral lipid content to 50.43% of the dry cell weight. However, the growth curve in this study (Figure 3) indicates that nutrients in the open pond had not been depleted throughout the 10 days of the experiment and thus starvation-induced stress did not take place within the system yet.

Presumably, a higher concentration of lipids with a lower rate of algae reproduction may imply that lipid buildup is negatively connected with growth rate even when nutritional levels are sufficient. There exists a variety of standardized methods that could be used to bolster lipid buildup by inducing metabolic stresses into reactor systems based on previous studies[25,31]. Regarding the hybrid photobioreactor in this study, some feasible ways to increase lipid concentration before harvesting could be setting the aquarium pump to a lower level, replacing the F/2 vitamin solution with a nitrogen-free solution, or diffusing relatively high concentrations of carbon dioxide into the algal medium.

It needs to be pointed out that the hybrid bioreactor designed in this study has also inherited a downside that is endemic to all outdoor PBR systems. It is notable that the microalgae growth in the outdoor bioreactors was apparently slower than the indoor cultures (Figure 2; Figure 3). This presumes that environmental climate can severely affect the overall performance of the hybrid PBR although the solar tubes have been designed to optimize distribution of the light and heat. An optimal circumstance of operation is considered to be a brightly-lit outdoor area with a PPFD over 1000 $\mu molm^{-2}s^{-1}$ and water temperature at 28°C[33,34] and insufficient light or heat can lead to lower growth rates and lower lipid productivity[1]. This has limited the use of natural sunshine as the primary source of light and heat in colder climates, necessitating the need of additional equipment for optimal PBR productivity.

Remarkably, this study has been designed to rule out the environmental impact on the evaluation of the created hybrid system. As a preemptive measure against confounding performance with external variables instead of design superiority, the performance of the hybrid PBR in this study was side-by-side compared to the control open pond operating at the same location. This allowed unbiased comparison of the microalgae growth and lipid production in the two systems regardless of medium temperature, solar irradiation and other environmental factors. At the current moment, however, it is impossible to directly compare the energy output of the hybrid PBR with other existing systems besides the open pond, due to the absence of a remotely equal scale of algae production and time given.

The low density of the *C. vulgaris* solution at the beginning of the cultivation experiments also prohibits an accurate estimation of product output. The hybrid PBR significantly outperforms the open pond system in terms of biomass and density on an equal cost basis whose results can be amplified by the implementation of further lipid-accumulation techniques including nitrogen starvation and induction of metabolic stress.

Prospectively, there might be other ways to further improve the functionalities of the hybrid PBR prototype based on the design in this study. The operating pump's low power consumption may allow the system to be coupled with a solar panel (typically $5) to become off-grid - a desirable feature for long term projects. Sewage sludge, a cheaper alternative to F/2 vitamin solution, can be tested for use as a main source of nutrient in PBR to reduce cost[18]. An even more aggressive design could be proposed by stacking multiple layers of solar tubes to increase liquid capacity and light distribution in PBR with consideration of plastic bottles' adaptability and null cost. A membrane can be attached to an electrocoagulation system on one side of the PBR main container to maximize the algae harvesting efficiency as proposed by Pishgar et al[24]. Anyhow, an even more improved PBR system would be expected hereafter.

Conclusion

This study has shown that it is feasible to build and operate a functioning hybrid PBR system for economical biofuel production at the similar cost of an open pond system. Design of the hybrid PBR in this project is initially a practical take on predominant versions that have low market competitiveness and improvements over open pond systems. The outcome has confirmed that construction of a PBR does not have to inherently involve expensive materials like glass or vinyl to achieve a growth level higher than that of an open pond system, making PBRs more accessible and affordable for small-scale applications.

The usage of recycled materials in the PBR demonstrates a commitment to environmental sustainability, a commonly-positively perceived trait[5]. The construction of more hybrid PBR units with plastic bottles and recycled materials would consequently reduce waste, leading to a reduction in environmental impact. While the growth of *C. vulgaris* in both bioreactors has been optimized with a nutrient appliance strategy, the hybrid PBR had led in biomass production but delayed lipid accumulation over the open pond system. The lower biomass but higher lipid content might suggest that quality of life for *C. vulgaris* in the open pond system has been possibly exposed to adverse conditions such as increased metabolic stress.

From another point of view, it is reasonable to assume that the microalgae culture in the hybrid PBR would also prevail ultimately in terms of lipid accumulation, attributing to its higher growth rate, more biomass production and eventual exposure to metabolic stress. The present pilot-size design is considered as one unit, suitable and economically viable for small-mid size growers who want to cultivate microalgae with minimal cost. One upside of this system is that due to the affordability of each PBR unit, multiple units can be arranged to grow algae separately. The current design of the PBR solar tubes also allows them to be easily combined to create a larger single unit. Ultimately, there are many opportunities for this prototype to scale up. Future studies should consider a long-term monitoring of the hybrid PBR system and to evaluate the reduction of operating costs, energy input and environmental impact for an optimized larger-scale employment towards commercial algal biofuel production.

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