

# Advancements in Design and Development of Lab Scale Photobioreactors for Improved Microalgal Feedstock

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## HIGHLIGHTS

- Microalgal species act as a promising source of biofuel, bioethanol, and biohydrogen.
- In photobioreactors (PBRs), microorganisms use light and CO<sub>2</sub> for photosynthesis, which speeds up the production of biomass.
- The development of low-cost, high-efficiency microalgal growing systems is crucial for cost-competitiveness.

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## ABSTRACT

Recent studies on soil, air and water pollution revealed that algae plays prominent role in controlling pollution and are abundantly found in all known habitats where light and moisture are available. Micro-algae can easily be cultured in nature e.g in a pond, lake or in vitro conditions in labs. Photobioreactors (PBR) are a closed culturing system of microorganisms using light and CO<sub>2</sub> for photosynthesis to accelerate the rate of biomass production. Development of low-cost and high-efficiency microalgal cultivation systems is important for the cost-competitiveness of micro-algal high biomass production. Different types of PBRs have been designed, but still needs new developments. This paper includes a basic introduction of photobioreactor and their types, working parameters and criteria for lab-scale aquaculturing. The present review paper deals with recently developed innovations in PBR and also lists different types of closed laboratory-scale PBRs along with the difficulties faced in large-scale open over closed PBRs and their importance in the production of microalgal biomass.

**Keywords:** PBRs, Microalgae, Biodiesel, Bioethanol, Biohydrogen.

## GRAPHICAL ABSTRACT



## INTRODUCTION

Algae are a diverse group of photoautotrophs uses light to produce their food via a process of photosynthesis, capturing water and carbon dioxide from the atmosphere (73). During the process, light energy is converted into chemical energy and stored in various carbon forms (72). Their photosynthetic efficiency is 10-50 times higher than that of terrestrial plants and therefore is a promising source of CO<sub>2</sub> sequestration (75). They are abundantly found in all known habitats where light and moisture are available (30). They are considered a bio-factory and produce a wide range of biologically active micro and macro molecules, including carbohydrates, carotenoids, chlorophylls, enzymes, phycobilins, polysaccharides, proteins, vitamins and many other primary and secondary metabolites (63,65), which make them a unique and fascinating group of micro-organisms among other microbes and plants (47). Algae are being cultivated in large scale at a commercial level worldwide for the mass production of noble bioactive compounds for their application in various fields, including green energy (22,19,28).

Microalgal species are potential sources for biofuel, bioethanol and biohydrogen and are considered a promising source of third-generation feedstock for biofuel and biodiesel production (46). Due to this, a large number of biological researcher globally have chosen them as noble organisms for their study for different purposes (68, 67, 69, 71, 70).

Microalgae can be used as a potential tool for biofuel production to replace petroleum, but are limited by high production cost and variable efficiency (17). Compared to the first and second, third-generation feedstock has higher yields and high oil content and have advantage of growing in non-arable lands, non-potable water bodies such as wastewater, polluted water (86). Food security concerns have been raised by first-generation biofuels, which are economically produced from edible oils like sugarcane, maize and palm oil as well as vital food crops. As a result, lignocellulosic biomass, municipal wastes, agricultural residues, and non-edible oils have all been used to create second-generation biofuels (15).

"Bioreactor" refers to any apparatus or container used to perform one or more biological reactions in order to transform any material (i.e., a substrate) into a product. Utilizing bioreactors is essential for any production method based on biotechnology, including the transformation of one molecule into another, the generation of biomass or metabolites, or the breakdown of undesirable waste. Airlift devices, packed beds, fluidized beds, bubble columns, stirred tank reactors and PBRs are the main categories of bioreactor setups that are covered (10). PBR uses a light source to cultivate microorganism. These microorganism uses CO<sub>2</sub> and light for photosynthesis. Light source can be natural or artificial. They can be used to cultivate microalgae and macroalgae. Under an artificial environment, the rate of light and other nutrient concentrations can be controlled to accelerate the rate of biomass production. Nutrient-rich domestic wastewater, agricultural wastewater and industrial wastewater can be used to culture algae and other microorganisms. PBRs have advantages like controlled pH, temperature and nutrient conditions, less water evaporation, high rate of biomass production and easy algal harvesting.

PBR can be open like a natural lake or pond system or artificial raceways and closed like tubular PBR, spiral PBR, plate PBR and horizontal PBR, as shown in figure 1. Algal growth in open lakes and pond are the best example of an open system. Algae can grow in mono or mixed culture according to environmental and nutrient conditions. These algae best suited for the study of climate change and water pollution. But due to their dependence on the environment, the productivity is limited, and water evaporation is high. Open ponds have greater CO<sub>2</sub> storage capacity than tubular reactors because of their greater culture volume per square meter. However, after recarbonation, open ponds tend to desorb CO<sub>2</sub> to the atmosphere (86).

This review paper addresses the research gap by enlisting different types of closed PBR of lab scale and their importance in microalgal production. This paper includes a basic introduction of photobioreactor and their types, working parameters, criteria for lab-scale aquaculturing, and recent developed innovations in PBR. It also describes difficulties faced in large-scale open PBRs over lab-scale closed PBRs.

## MICROALGAE CULTURE SYSTEM

Microalgae culture system can be divided into two classes i.e., open system (natural and artificial) and closed system (PBR). Open systems represent a great variety of combinations, including natural ponds having a natural wind agitation system,

while artificial pond/ circular pond having a mixing process ensured by a central stirrer or raceway pond with a pedalled agitation system. On the other hand, closed systems represent a great variety of closed vessels such as PBRs where microalgae can be cultured in a controlled way by managing light, temperature, carbon dioxide and nutrients to facilitate photosynthesis. Different types of microalgae culture systems are described as under.

### 1. Raceway pond:

Raceways are artificial open systems most widely used due to low construction cost, ease to maintainance, low energy requirement and their ease of scalability. It can be constructed on a horizontal surface with a few meter heights. Raceways have an advantage over ponds or lake i.e we can control nutrient concentration and can produce a single culture. But, raceway ponds have limitation of controlling temperature, water evaporation, high contamination risk and low microalgal biomass concentration. During summers the evaporation rate is high, and to maintain the media it needs to be refilled continuously. On rainy days, it needs to be protected from extra nutrient addition through rain-water. It implies power consumption as it requires shear force pump, airlifts or paddled wheels (86).

### 2. Tubular PBR:

Tubular PBR has an interconnected parallel tube system made up of plastic or glass tubes arranged horizontally or vertically. It is connected with the central utilities installation with a pump, sensors, nutrients and CO<sub>2</sub>. A pump circulates the microalgal culture through the tube, where a central tank collects and recycles it. They are helpful in large-scale production of microalgae (49). As a closed system, they offer better control over growth conditions and lead to high purity. They are used for the cultivation of *Chlorella vulgaris* and *Arthrospira platensis* for human nutrition and animal feed. They are also used for the production of compounds like astaxanthin, omega-3 fatty acids and phycobillins for food supplements and cosmetics.

### 3. Spiral PBR:

Spiral PBR system has a layout of springs which increase the surface area to volume ratio, ensuring that more cells have access to light as culture circulates. The combination of turbulence and the closed concept allows a clean operation and a high operational availability. It is a three-dimensional structure enabling maximum light efficiency and reducing shade. It does not require a large space to be installed. This technique enables the temperature of the bioreactor to be controlled without external cooling and can be used in different climate zones, which is another critical hurdle on the path to the industrial production of microalgae. The experimental spiral PBR's key benefit includes (1) a large ratio of culture volume to surface area combined with optimal light penetration depth; (2) simple temperature and contaminant control; (3) efficient fresh air and CO<sub>2</sub> spatial distribution; (4) improved CO<sub>2</sub> transfer through a large interface surface between fresh air and culture liquid medium; and (5) a novel automated flow-through sensor that continuously monitors cell concentration. (3).

### 4. Plate PBR:

Plate PBR uses less expensive material than tubular PBR. Instead of tubular system plate PBR has disposable bag equipped with gas spared at the bottom of plastic bag for aeration and a heat exchanger.

The bag can be easily replaced when convent, excessive fouling or contamination being the most common factors requiring a replacement. (82).

**5. Horizontal PBR:**

Horizontal PBR solves the problem of even distribution of incidence light. Tubular system is placed horizontally and hence larger light quantity can be exploited in order to improve photoconversion efficiency. The mixing is done by rotary pump.

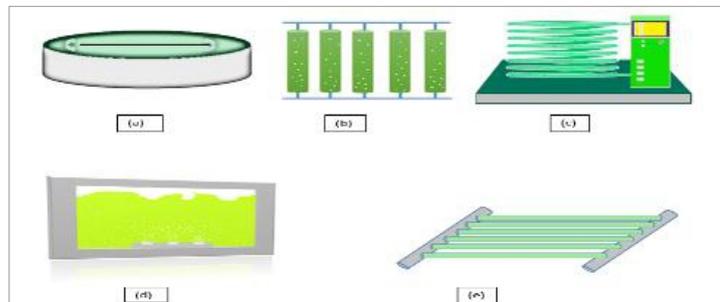


Figure 1: Icon diagrams of different types of PBR (a) Raceway pond (b) Tubular PBR (c) Spiral PBR (d) Flate plate PBR (e) Horizontal PBR.

**WORKING PRINCIPLE OF PBR**

For large scale production of algae at commercial scale the strain of algae should be selected on some specific criteria like algal life cycle, growth rate, productivity of the desired products, genetic stability, nutritional requirement and tolerance to shear stress (74).

Key parameters influencing the performance of the PBR, include light, mixing, mass transfer, temperature, pH and capital and operational costs. The lifespan and the costs of cleaning and temperature control should also be emphasized for commercial exploitation. A typical PBR is a three-phase system that includes the culture medium as the liquid phase, the cells as the solid phase and CO<sub>2</sub> enriched air as the gas phase in auto-phototrophic cultivation. As a unique feature, the light in a PBR is a superimposed radiation field that is usually referred to as a fourth phase. (8).

Factors affecting the growth of microalgae in PBR can be divided under design and installation parameter, environmental parameter and operational parameter as described in figure 2 (13). Out of several major factors light source and CO<sub>2</sub> are major influencing factors which directly affect photosynthesis and hence the rate of cell growth and biomass. Other factors like pH and temperature indirectly affects the growth by altering optimum conditions for cellular functions which include photosynthesis (14). Factors affecting the growth of microalgae are mentioned below:

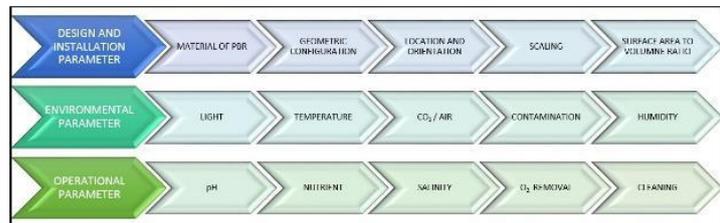


Figure 2: Factors affecting the growth of microalgae in PBR.

**1. Light:**

The growth of microalgae is equally dependent on the type and intensity of the light. Three phases can be distinguished in the relationship between light intensity and the photoautotrophic growth of microalgae: the light limitation phase, the light saturation phase, and the light inhibition phase (as shown in figure 3). Light in the cultivation system must be distributed equally during the saturation period, but this is made difficult by the shading effect. The effect of shade grows in tandem with the concentration of cell and biofilm growth on the reactor vessel's surface (5). Cells can be regularly mixed to get around this. One investigation showed how light intensity affected the levels of Mg, pH, NADPH, and lipids (41). The available light sources are discharge lamps (such mercury, xenon, or fluorescent lamps), light-emitting diodes (LEDs), lasers, and incandescent lamps (tungsten or halogen lamps). Light intensity above the saturation point is dangerous because it damages photosystems and leads to photo-oxidation. We refer to this stage as the inhibition phase.

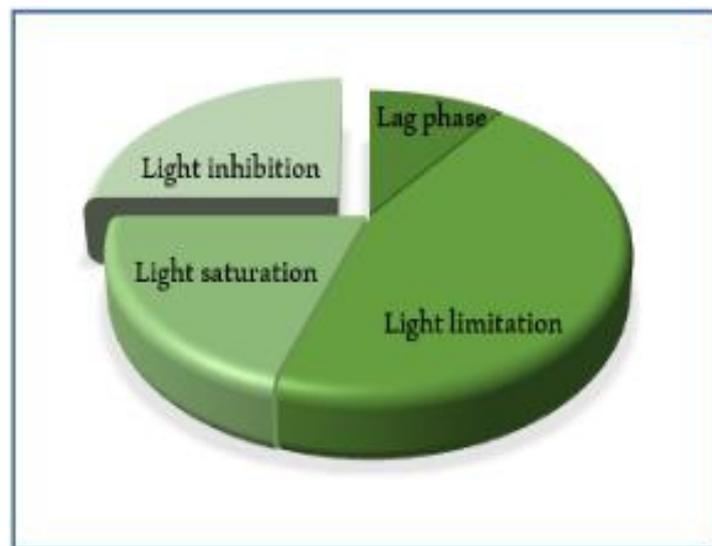


Figure 3: Light intensity stages for cell growth

**2. CO<sub>2</sub>:**

It is possible to cultivate microalgae utilising a variety of carbon sources, including carbon dioxide, methanol, acetate, glucose, and other organic substances, in photoautotrophic heterotrophic and mixotrophic environments (90). In photoautotrophic environments, microalgae obtain their carbon supply from inorganic carbons such carbon dioxide or bicarbonates during photosynthesis (61). In the presence or absence of light, microalgae that are heterotrophic directly consume organic carbon (12). The majority of microalgae exhibit 10- 50 fold greater rates of cell growth and CO<sub>2</sub> fixation than terrestrial plants, indicating yet another benefit of directly converting photoautotrophic microalgal development. Thus, it makes more sense to manufacture microalgae-based biofuels through the photoautotrophic development of microalgae from an economic and environmental protection standpoint. Another idea, though, is to use organic carbon sources (such sugars) obtained from biomass to grow microalgae under heterotrophic conditions and then turn them into biofuels.

Because the heterotrophic development of microalgae is typically quicker than the autotrophic growth, this could significantly increase the output of biofuels (6). In PBR, the medium needs to be supplemented with more CO<sub>2</sub> in order to support the algae's rapid development. This extra CO<sub>2</sub> is provided as enhanced CO<sub>2</sub> gas mixture bubbles in enclosed PBRs. A CO<sub>2</sub> concentration of more than 1% (by volume) inhibits the growth of algae. Algae that had acclimated to low partial pressure grew less quickly when exposed to a gas with a high CO<sub>2</sub> partial pressure (40). CO<sub>2</sub> dissolution rate in relation to reactor temperature, pressure, and bubble contact time (77). It is possible to buy CO<sub>2</sub> that has been produced commercially by burning fossil fuels. Through pretreatment and utilisation of waste biomass resources, such as feedstock crops, organic carbon can be generated from raw biomass. One by-product of the sugar industry that has been studied as a substrate for microalgal culture is waste molasses, which is rich in simple sugars. *Chlorella protothecoides*, with a biomass, oil content, and oil output of 70.9 g/L, 57.6%, and 40.8 g/L, respectively, could efficiently use hydrolysed waste molasses as a carbon source. Furthermore, employing molasses as the feedstock resulted in a 50% decrease in the cost of producing biofuels (92).

### 3. Contaminants:

In nature, algae are typically found in mixed cultures and can be infected by bacteria, fungi, insects, or predatory algal species. However, in order to produce algae on a commercial scale or for scientific purposes, it is necessary to isolate them. Industrialists typically choose extremophilic microalgae for production because they can endure harsh environments that predators cannot. Because close systems, like PBRs, shield the algae from the outside world, they help prevent contamination, whereas open systems are more vulnerable to invasion by predatory organisms.

### 4. Temperature:

The environment has a significant impact on how quickly microalgae develop. Temperature and growth rate exhibit a bell-shaped curve, meaning that an initial rise in temperature promotes a higher growth rate, but further increases result in a drop phase. A study found that increasing the temperature from 10°C to 25°C initially increased the pace at which biomass was produced in *Scenedesmus* sp. LX1, but that increasing the temperature further led to a falling phase (89). Since it is challenging to control this feature in open PBR, locked PBR are generally considerably simpler to work with (57).

### 5. pH:

The measure of the water's acidity or basicity is called pH. Variations in it may have an impact on the growth and production of the intended product. For the growth of microalgae, the range 7-9 is ideal (2, 23). pH may have an impact on a variety of biological processes in microalgae culture, including ion uptake, cell growth, and metabolism (36). The pH is raised by the CO<sub>2</sub> that is used in photosynthesis. The most readily available form of dissolved inorganic carbon (DIC) is CO<sub>3</sub><sup>2-</sup>; beyond 8, CO<sub>2</sub> is the most readily available form; below 7, CO<sub>2</sub> is more prevalent than HCO<sub>3</sub><sup>-</sup>; and from 7 to 10, HCO<sub>3</sub><sup>-</sup> is more prevalent than CO<sub>2</sub> (20).

### 6. Nutrients:

Among the macronutrients required for microalgae growth are phosphorus and nitrogen.

Trace metals including Fe, Mg, B, Mo, K, Co, Zn, and Mb are necessary for the growth of microalgae. Changes in morphology and physiology can result from excess or insufficiency of certain nutrients, which can also disrupt metabolic pathways (45). The synthesis of proteins and other metabolites depends on nitrogen, a macronutrient. Insufficient nitrogen causes an increase in the synthesis of lipids (42), while algae with high protein content that are utilised as dietary supplements need more nitrogen. These sources of nitrogen include urea and amino acids, or they might be inorganic and come in the form of nitrate, nitrite, and ammonium (43).

### 7. O<sub>2</sub> removal:

The process of photosynthesis produces oxygen in exchange for CO<sub>2</sub> fixation, but too much of it can lead to the creation of free oxygen reactive species, which can harm cells through oxidative stress. The O<sub>2</sub> content in the medium shouldn't be higher than 400% in relation to the air saturation level. The concentration of O<sub>2</sub> in the medium might increase during the dark phase of photosynthesis. Because of photorespiration or the Wurburg effect, certain algae are unable to tolerate such high concentrations for longer than two to three hours. This issue can be resolved by increasing turbulence or cycling fresh air to remove O<sub>2</sub> (77).

### 8. Salinity:

The variety of habitats found in microalgae is widely recognised. Freshwater, saltwater, subterranean, terrestrial, and buildings can all contain them. High salinity environments, or 1.7 M, are home to marine species. Additionally, it was found that when an additional 0.5 or 1 M NaCl was added to the culture medium at the conclusion of the exponential phase of growth, *D. tertiolecta* ATCC 30929 showed an increase in lipid content of up to 70% from 60% (with 0.5 M NaCl) (79). Saturated and polyunsaturated fatty acid concentrations (91), as well as the extractability of hydrocarbons (18), can all be impacted by salinity. Additionally, salinity can negatively affect microalgae growth rate and photosynthetic efficiency (25).

### 9. Cleaning:

Microalgae growth rate is impacted by periodic PBR cleaning. The likelihood of contamination and biofilm growth on the wall is decreased. Easier cleaning can be achieved by structural reforms during design, such as fewer bends, larger internal dimensions for easier washing, and smooth walls (85).

### 10. Designing and installation:

The surface area to volume ratio is a significant determinant of PBR effectiveness. Additionally, a material with high transparency, like glass, should be employed. Although glass is durable, clear, and has a long lifespan, maintaining it is expensive. The substitutes for glass are acrylic PVC, polyvinylchloride (PVC), polyethylene, and Plexiglas. However, oxidation might cause them to lose their transparency over time. An additional factor to take into account is the running cost, which covers the price of cleaning, maintenance to stop leaks, temperature and pH controls, and auxiliary energy for mixing and gas transfer.

### SELECTION CRITERIA FOR LAB SCALE AQUACULTURING

In order to employ a sufficient culture volume to enable regular culture sample extractions for measuring a number of crucial parameters during the experiments, the ideal culture

compartment size should be sufficiently large enough to be appropriate for a variety of experimental setups. For microalgae production, the reactor should have optimal features such as an even light route and effective gas exchange. Multiple sensors and electrodes should be able to be inserted into the bioreactor in order to log and regulate various cultivation parameters. The reactor's design should occupy less bench area and include an easy-to-use, reasonably priced temperature control system. In order to reduce stress in breakable materials like glass generated by contact with tougher materials like metals, the materials should be sufficiently sturdy to withstand heavy use, taking into consideration features like the strength of the threads of ports and openings. Simple, repeatable sample extractions from culture should be possible with this methodology. The ability to autoclave the system is a crucial feature for monoculture tests conducted on a lab scale. A control system for regulating various parameters, including temperature, pH, agitation, CO<sub>2</sub> supply, and optical density (OD), as well as the ability to log parameters like dissolved oxygen (DO) and generated gas, should be incorporated into the PBR system. (76).

Materials used for the various PBR components should be evaluated based on standards like the following (76):

- Autoclavability and heat resistance
- Oxidation and corrosion resistance
- Possible negative impacts on the microalgae cultures
- Weight durability
- Market availability
- Transparency when required

## RECENT DEVELOPED INNOVATIONS

### 1. Electrochemical Oxidative PBR

Electrochemical oxidative (EO) PBR is a model formed by combining EO system and bubble column PBR (BPBR) system as described in figure 4. It uses distillery wastewater (DWW) as conductive medium due to its high concentration of ionic substance and reduce its COD (Chemical oxygen demand) and colour. It makes nitrogen and phosphorus available to the biological system to increase its productivity. In the sequential process, the algal biomass generated can replace 6% of the total energy used for EO. Therefore, consecutive EO-BPBR treatment can recover 32% of the energy used for EO. EO-PBR model was developed (26) and used Ti-RuO<sub>2</sub> anodes and then supplied the EO-DWW for culturing of microalgae present in BPB. Anode made up of Ti-PbO<sub>2</sub>, Ti-SbO<sub>5</sub> - SnO<sub>2</sub>, BDD can also be used for EO of DWW (58,78,1.)

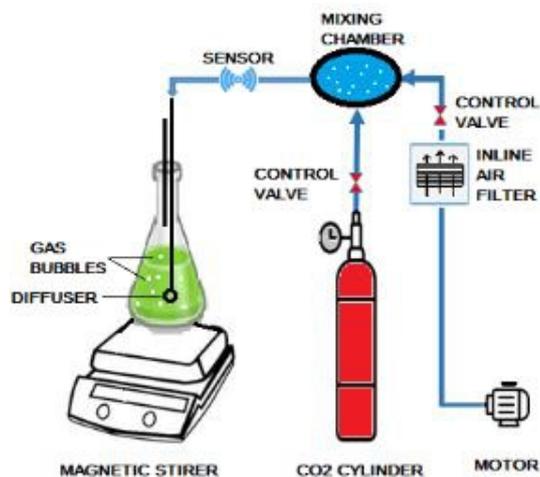


Figure 4: Bench scale bubble column algal photobioreactor with CO<sub>2</sub> supply.

### 2. Airlift PBR

A tubular PBR was designed which used airlift pump to circulate the culture. This airlift pump helped to amalgamate fluid mechanics principle and study the effect of irradiance, flow velocity and dilution rate on the growth of culture (49). It is illustrated in figure 5. It was observed that during maximum irradiance photoinhibition occur and lead to decrease in photosynthetic activity. Low flow velocity can cause degradation of culture but above the threshold velocity it directly affects bio productivity. The effect of oxygen concentration was studied in both outdoor and indoor conditions. It was observed, the outdoor setup has low productivity due to high illumination and photooxidation.

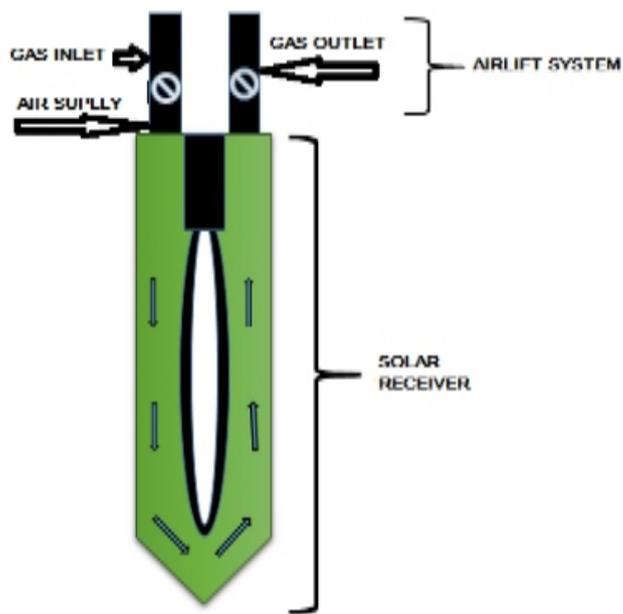


Figure 5: Airlift system.

### 3. Microfluidic PBRs

Microfluidic PBRs are recent option for studying microalgae. These chips consist of several culture compartments and connected with a fluidic channel, to allow the run-in of microalgae as well as nutrients. Usually built with polydimethylsiloxane (PDMS) layers, in which single colonies are trapped in an array. The dimension of each layer is around 2-3 cm long, 7-8 cm wide and 3 mm thick as shown in figure 6 (37). It allows the preparation of high-throughput experiments, since several different conditions can be studied at once in a small space e.g. effect of light cycle and light intensity variability in microalgae cultivation.

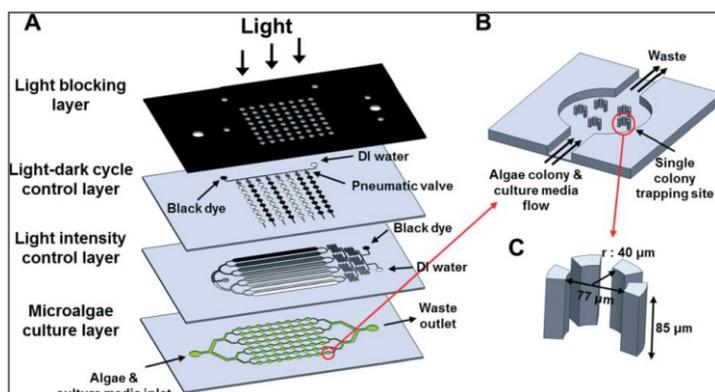


Figure 6: The high-throughput microfluidic microalgal PBR array. (A) The platform was composed of four layers- a light blocking layer, a microfluidic light-dark cycle control layer, a microfluidic light intensity control layer, and a microalgae culture layer. (B) Enlarged view of a single culture compartment having five single-colony trapping sites. (C) A single-colony trapping site composed of four micropillars. (37).

#### 4. Rocking PBR

Traditional PBR design were energy and money consuming and to upgrade these designs they require further more expensive technique. To deal with this and inspired by BICCAPS (bicarbonate-based integrated carbon capture and algae production system) strategy (7,8) a model was developed, (95) which used natural force like gravity for mixing on a sea saw rocker by placing simple plastic bags at the ends of sea saw as PBR chamber as shown in figure 7. It helped to reduce electricity usage and still stay more productive. These plastic bags were covered with transparent cover on top with holes in it to prevent evaporation and oxygen release. Effect of factors like PBR chamber depth, rate of rocking cycle and light intensity was observed on bioproductivity, pH fluctuation and DO accumulation. It was concluded that model was proven to be easy scaling- up, feasible, driven by nature force and less expensive. As a hybrid design of PBR and open pond, it is essentially a horizontal PBR, which combines the benefits of both, such as low manufacturing costs, closed systems, short easy scaling-up and light path. (16).

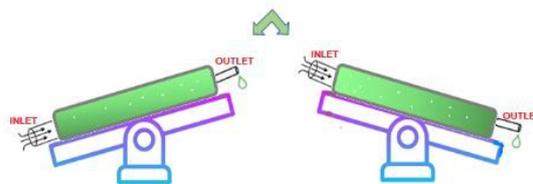


Figure 7: Rocking PBR.

#### 5. Insulated Glazed Photovoltaic Flat PBR

The large scale practical application of the lean management methodologies examined here is practicability, final product market value, system life and commercial feasibility. Combining existing and emerging technologies for enhanced light management a model was developed (53) that can be helpful to increase algal biomass and have low manufacturing cost and having less negative impact on environment e.g. and insulated glazed photovoltaic flat PBR (IGU PBR) (figure 8). Photosynthetically useful PAR is used by algae for its growth while UV and IR wavelength are useful for electricity generation. This type of PBR has advantages like productivity and overall low coat of cooling and energy usage. Table 1 lists some advantages and disadvantages of IGU PBR.

Table 1: Techniques used in IGU PBR (53)

Sr. no	TECHNOLOGIES	ADVANTAGE	LIMITATION
1.	LED lights	Controlled emission spectrum	Expensive
2.	Spectral shifting	Improved PAR	Scattering and internal lose
3.	Plasmonic waveguiding	Improved PAR	Reabsorption

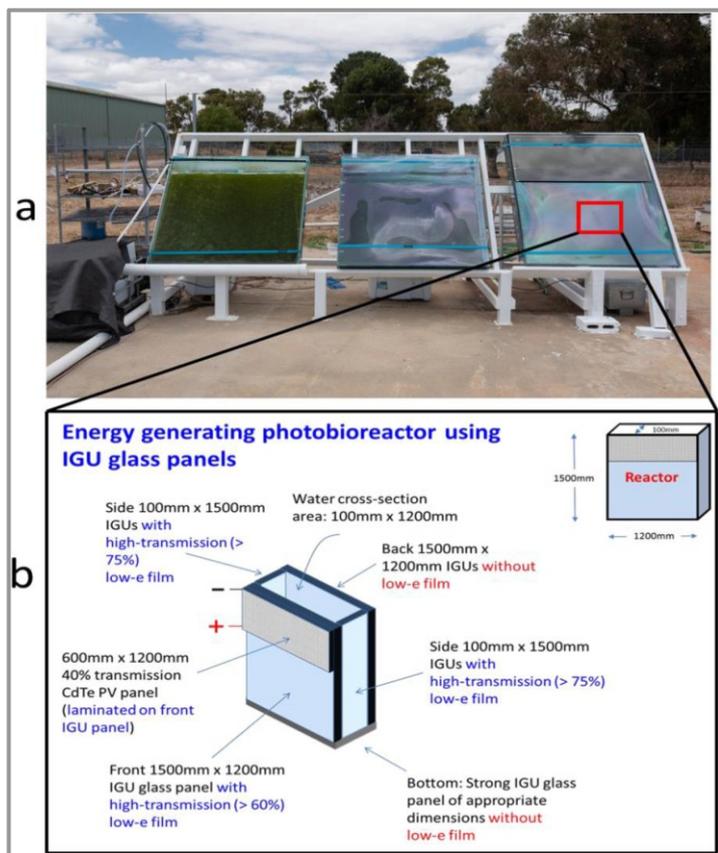


Figure 8: Microalgae cultivation flat plate PBR. (a) Image of the PBR in operation at the Algae R&D Centre, Murdoch University, Australia. Left to right: passive evaporative cooling (PEC), infrared reflecting film (IRF) and insulated-glazed photovoltaic (IGP) photobioreactors. (b) Schematic showing construction details of the IGP PBR. (53).

#### 6. Hybrid Anaerobic Baffled Reactor PBR

This model was developed by integrating Hybrid Anaerobic Baffled Reactor (HABR) with PBR (PBR) for producing algal biofuel and waste water treatment as shown in figure 9.

Wastewater being rich in organic and ionic content is used for generating feedstock with the help of HABR for cultivating biofuel rich microalgae in PBR unit (35). This type of photobioreactor has two layouts i.e HABR layout and PBR layout. HABR layout has seven chambered anaerobic chamber either insulated or uninsulated where 1<sup>st</sup> chamber – Settling chamber, 2<sup>nd</sup> - 5<sup>th</sup> chamber- Hanging baffled reactor, 6<sup>th</sup> & 7<sup>th</sup> chamber – Floated filter media chamber. HABR gets inoculated with waste water passing through settling chamber and then the following chambers. Floated filter media chamber gets filled with shredded soft drink lids to avoid clogging during treatment process. Insulation was done by applying 2 inch thick foam layer of polyurathane at room temperature (27- 25°C). PBR set up was constructed using clear soft drink bottles upto a desired height. Conical head of bottle was placed at bottom of the set up so that after cultivating microalgae gravity settling can help in harvesting.

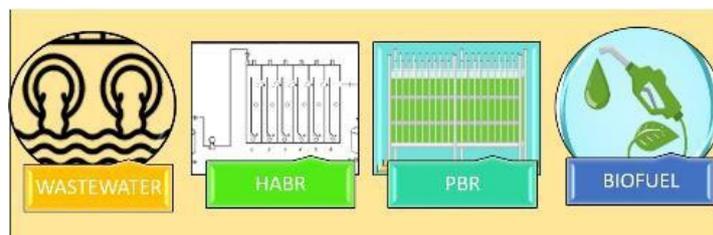


Figure 9: Illustration of algal biofuel production by HABR-PBR system.

#### 7. Quorum Quenching (QQ) Membrane PBR

The technique of Quorum Quenching (QQ) was implemented in Membrane PBR (MPBR) as a strategy to mitigate fouling issues (21). Figure 10 is describing this model. The QQ activity of the beads was assessed by using N-octanoyl homoserine lactone (C8-HSL) as a representative signal molecule (8). With the help of QQ beads, antifouling ability was analysis on membrane surface. It's affecting factors were transmembrane pressure (TMP), extracellular polymeric substance (EPS), algal species and its bloom morphology.

According to the findings, the TMP of the experimental MPBR (MPBR-QQ) was only 448 mbar and 676 mbar, respectively, while the TMP of the control MPBR (MPBR-C) reached 818 mbar and 912 mbar on the operation hours of 35 and 170 indicating 54% and 26% reduction respectively. The TMP reductions at both time points suggest that the experimental MPBR-QQ may offer a viable alternative to conventional membrane systems, especially in applications where lower TMP and longer operational lifetimes are crucial for sustainability and cost-effectiveness. All of these results point to a more effective membrane fouling control performance from the experimental MPBR-QQ, which could result in lower operating costs and a longer membrane lifespan.

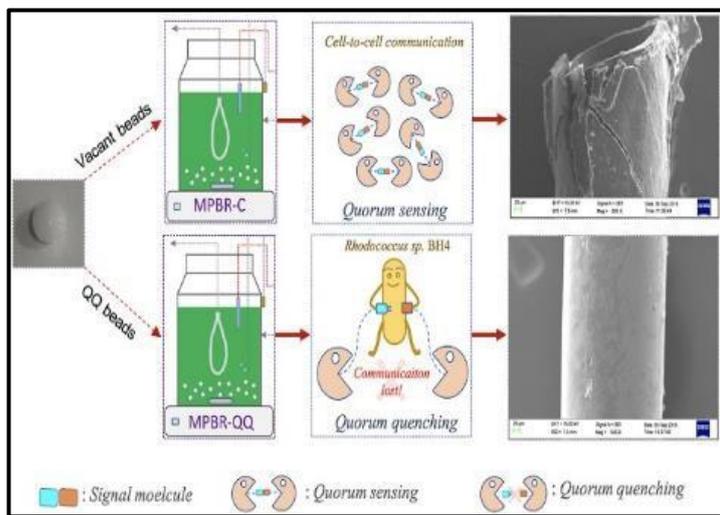


Figure 10: Quorum quenching strategy for biofouling control in membrane PBR (21)

8. Internet of Things PBR

Integration of Internet of Things (IoT) with different type of PBR designs can help to up-scale the results as compared to manual data. IoT is set-up that helps to connect mechanical or digital machine and other devices and transfer data with or without human to human or human to computer interaction.

Table1: Comparative analysis of innovations in PBR design.

Sr.No	Description of PBR	Type of PBR	Advantages	Drawback	Reference
1	The Off shore Membrane Enclosures for Growing Algae (OMEGA) system.	Floating PBR	Use organic carbon. Heterotrophic growth more than autotrophic growth.	Bio-fouling leading to low light transmission rate.	Wiley, 2013.
2	The large scale off shore PBR with simultaneous microalgae cultivation and wastewater treatment processes.	Floating PBR	Use organic carbon. Heterotrophic growth more than autotrophic growth.	Bio-fouling on top surface. Low photosynthetic efficiency.	Novoveska et al., 2016.
3	The floating plastic bottle PBR.	Floating PBR	Low cost of manufacturing.	Low productivity.	Naqqiuddin et al., 2014.
4	The floating polyethylene (PE) container PBR.	Floating PBR	Has application potential with high productivity.	Difficult to scale-up these PBRs. Bio-fouling.	Masakazu et al., 2015.
5	Floating vertical panels.	Floating PBR	Low energy requirement for cooling.	Bio-fouling and scaling up problem.	Solix biofuels, USA
6	The horizontal PBR with an aeration device.	Floating PBR	Bubbling CO <sub>2</sub> works for mixing. Short light path leads to high productivity.	Face scaling up problem.	Dogaris et al., 2015.
7	The permeable floating culture system.	Floating PBR	Use bicarbonate and other ion from seawater.	Low productivity. Scaling up problem.	Kim et al., 2016
8	The floating open pond.	Floating PBR	Use bicarbonate and other ion from seawater.	Need large area. Expensive.	Park et al., 2018.
9	The enclosed rotating floating PBR powered by flowing water.	Floating PBR	Cost efficient, energy saving, controlled temperature, uniform light distribution, simple design, easy maintenance, easy mixing.	Highly dependent on flow of water.	Huang et al., 2016.
10	The floating horizontal PBR without aeration or an agitation device.	Floating PBR	Low cost of manufacturing.	Transparency of film reduces with time affecting productivity.	Zhu et al., 2018.
11	Electrochemical oxidative (EO) PBR is a model formed by combing EO system and bubble column PBR (BPBR) system	Electrochemical oxidative (EO) PBR	The algal biomass generated can replace 6% of the total energy used for EO. Therefore, consecutive EO-BPBR treatment can recover 32% of the energy used for EO.	Nitrogen and phosphorus concentration of conductive medium vary every single time.	Prasad & Srivastava, 2009; Susree et al., 2013 ; Alvarez-Pugliese et al., 2011

With the help of AutoCAD numerous PBR designs can be drawn and increase the execution efficiency of design as illustrated in figure 11. It helps to monitor parameters like pH, temperature, light, liquid level and other things whether we are physically present in lab or not. An IoT used water proof sensors carried through Arduino NodeMCU board linked with smartphone application Blynk (81).

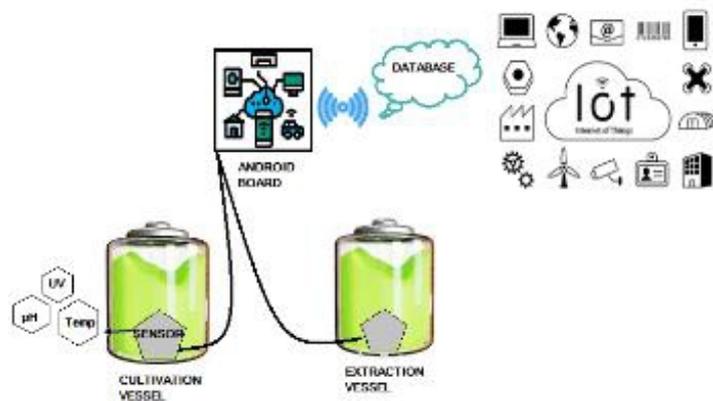


Figure 11: Illustration of PBR setup with IoT.

9. Rotating floating PBR

RFP model was developed by placing the simple PBR in open flowing water attached with a paddle wheel (24). Waves generated by flowing water turn the paddle wheel and thus causing the rotation of PBR. The waves generated can be due to flowing stream, river, tidal waves or open raceway pond. It has advantages like cost efficient, energy saving, controlled temperature, uniform light distribution, simple design, easy maintenance, easy mixing and many more. Many researchers have tried different type of updates with floating PBR to make them economical and productive. Figure 12 shows open raceway pond present in CCS University, Meerut.

12	It uses airlift pump to circulate the culture.	Airlift PBR	Study the effect of irradiance, flow velocity and dilution rate on the growth of culture on culture growth.	Decrease in photosynthetic activity due to high illumination and photooxidation.	Molina et al., 2001
13	Consist of several culture compartments and connected with a fluidic channel.	Microfluidic PBR	Different conditions can be studied at once in a small space e.g. effect of light cycle and light intensity variability in microalgae cultivation.	Expensive and require skilled person to handle.	Kim et al., 2014
14	Uses natural force like gravity for mixing on a sea saw rocker.	Rocking PBR	Driven by natural force and is less expensive.	Easy scaling- up	Zhu et al., 2017, Dogaris et al., 2015
15	Useful PAR is used by algae for its growth.	Insulated glazed photovoltaic flat PBR	Increase algal biomass and have low manufacturing cost.	Expensive	Nwoba et al., 2020
16	Used for mitigating fouling issues by using N-octanoyl homoserine lactone.	Quorum Quenching Membrane PBR	Mitigate fouling issues, cost-effective.	Affected by transmembrane pressure (TMP), extracellular polymeric substance (EPS), algal species and its bloom morphology	Güneş & Taşkan., 2022.
17	Integration of IoT (Internet of Things) with different type of PBR designs.	Internet of Things PBR	Helps to connect mechanical or digital machine and other devices and transfer data with or without human to human or human to computer interaction	Can be expensive due to involvement of software and digital devices.	Tham, P.E et al., 2022.

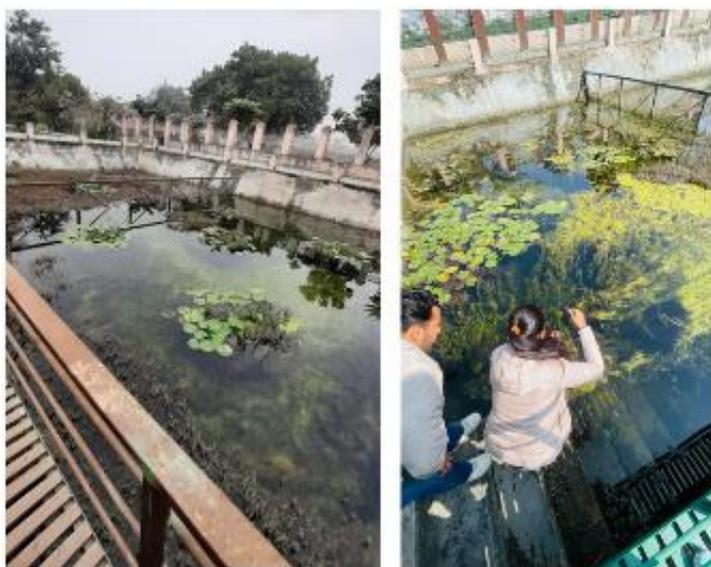


Figure 12: Open raceway pond, CCS University, Meerut.

**10. Thermosiphon PBR**

A novel passive heat control technology for growing photosynthetic microorganisms like algae is the thermosiphon PBR. It works on the basis of thermosiphon circulation, which does not require energy-intensive pumps since temperature variations within the bioreactor create natural convection currents. The culture medium is effectively circulated throughout the bioreactor using this technique, guaranteeing ideal light exposure and temperature control for photosynthesis. Thermal and hydrodynamic performance of the prototype reactor based on the thermosiphon phenomenon and employing computational fluid dynamics (CFD) was examined (11). Photobioreactors develop temperature- induced density shifts as an outcome of light absorption. A radiative transport equation that integrates experimentally measured spectrum irradiance and attenuation characteristics was used to describe the uneven volumetric sensible heating from absorption of light, which is predominantly mediated by microbial cells in the reactor. The buoyancy-driven convection in the thermosiphon PBR (TPBR) has been explained via the boussinesq approximation as well as by empirical and analytical heat transfer coefficients. Figure 14 describes a diagrammatic illustration of TPBR.

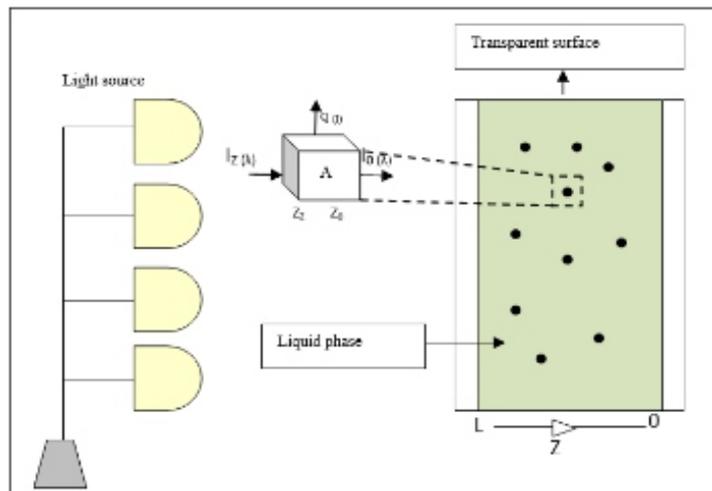


Figure 13: Diagrammatic representation of the TBPR's lighting. The front surface ( $z=0$ ), to the rear surface ( $z=L$ ), with  $A$ ,  $q(z)$ ,  $I_z(z)$ ,  $I_{0(z)}$  being the irradiated area, incident radiant illumination, radiant illumination at any point  $z$  and volumetric sensible heat generated at that point,  $z$  within the riser respectively.

**11. Miniature Algal Raceway Setup (MARS)**

Inspired from raceway pond and open pond we developed a miniature setup to cultivate algae in small scape at Department of Botany , CCS University, Meerut as described in figure 14. This small, hand-made model displays a raceway pond system, which is a popular aquaculture technique for raising fish or other aquatic creatures. The model, which was made of sturdy plastic trays, depicts the distinctive layout and flow dynamics of a raceway pond on a smaller size. A continuous, moving water system that replicates natural water conditions while guaranteeing ideal oxygenation and waste disposal is frequently provided by the raceway pond. This model has several interconnected trays set up to mimic water flow and the way aquatic life is raised in such systems. Plastic trays provide a lightweight, adaptable structure that is perfect for presentations, teaching, or as a decorative piece. The design provides a straightforward and understandable method of comprehending the fundamentals of raceway pond engineering by emphasising important components such the water entry, flow channels, and overflow systems. It's the ideal illustration of how aquaculture systems effectively promote environmentally friendly fish farming methods while utilising the least amount of space and resources possible.



Figure 14: Miniature Algal Raceway Setup (MARS) , Department of Botany, CCS University, Meerut.

## ECONOMIC ISSUES AND LIMITATIONS OF LARGE-SCALE PBRs

With their regulated settings that increase biomass productivity, large-scale PBRs (PBRs) offer a viable path towards sustainable microalgae cultivation. However, a number of obstacles prevent them from being economically viable. Depending on the design and materials chosen, the initial capital investment required to build PBRs can be significant, frequently exceeding €0.5 million per hectare (51). Energy-intensive requirements for temperature regulation, lighting, and mixing, particularly in closed systems intended to avoid contamination, significantly increase operational costs (9). Additionally, light attenuation problems limit the scalability of PBRs; as culture depth increases, light penetration decreases, which impacts photosynthetic efficiency (48). Surface area-maximizing designs are therefore required, which unintentionally increase land consumption and related expenses. Microalgal biomass harvesting presents additional financial difficulties because low cell densities in cultures need processing huge amounts of water, which raises the cost of dewatering and drying (60). Operational costs are further increased by the need for strict sterilization procedures and monitoring due to the potential of biofouling organisms and contamination (84). Even while PBR technology is being developed to address these problems—such as creating vertical and thin-layer systems to improve light utilization—economic viability is still a major obstacle to widespread use (56). As a result, resolving these financial constraints is essential to the economic and sustainable use of PBRs in microalgae farming.

## IMPORTANCE OF LAB-SCALE PBRs

The development of algal biotechnology and bioengineering is greatly aided by lab-scale PBRs (PBRs), which provide a controlled environment for the investigation and production of microalgae. Researchers can improve growth conditions, examine physiological reactions, and examine the effects of environmental factors including temperature, light intensity, carbon dioxide content, and nutrition availability using these small-scale systems (84). The dependability and reproducibility of experimental results are guaranteed by the contamination-free environment that lab-scale PBRs offer in contrast to open pond systems (83). Design versatility allows for customized experiments to meet particular species and goals. Examples of these are flat-panel, tubular, bubble column, and airlift PBRs (56). They are essential for screening high-value metabolites, such as proteins, lipids, pigments, and antioxidants, under various stress situations, and their use is not restricted to biomass productivity (59). Prior to scaling up, lab-scale PBRs enable early-stage research, enabling parameter fine-tuning and risk mitigation (62). Additionally, they supply vital information for modelling and simulation projects, which aid in the optimization of system design and the prediction of large-scale behaviour (48).

According to Wijffels et al. (2013), these reactors are crucial for metabolic engineering research because they allow genetically changed strains to be assessed under specific circumstances. Additionally, they support small-scale, manageable wastewater treatment trials and environmental applications such as CO<sub>2</sub> sequestration (60). The ability to automate lab-scale PBRs using sensors for pH, dissolved oxygen, light, and nutrition levels improves the precision of experiments (4). In classrooms, lab-scale PBRs are useful resources for teaching and illustrating the fundamentals of photosynthesis, microbiology, and bioprocess engineering. They are affordable and accessible to a variety of labs and institutions because of their small size (39). In general, laboratory-scale PBRs are essential for research, innovation, and knowledge creation, all of which advance commercial-scale application and long-term solutions in microalgal biotechnology.

## FUTURE OUTLOOK:

Commercial biofuel production requires the perfect balance of system and process technology improvements, as well as realistic economic viability. It is clear that adding enabling technologies like genetic engineering and nanomaterials might greatly boost the production of biofuel. The latter has a noticeable impact on the productivity of microalgae-based biofuel production. Numerous microalgal species were investigated, and the production of biofuel and harvesting efficiencies were enhanced by the use of nanoparticles. Furthermore, transcriptome analyses were presented because of their function in microalgal species' metabolite production. Techno-economic and environmental factors are the primary obstacles to the manufacturing of biofuel. Although cultivating algae in an isolated setting may assist with reducing environmental difficulties, pretreatment processes' energy and cost breakeven values need to be lowered because they are far from optimal. Further research and development, beginning with the cultivation of micro algae and the biofuel production process, can help overcome these obstacles and arrive at a commercially viable, affordable alternative. (54).

## CONCLUSION:

The latest developments in PBRs for increasing the biomass output of biofuel derived from microalgae, as well as the challenges and restrictions associated with their usage, were the main topics of this review. It is clear that the use of enabling technologies could greatly boost the productivity of biofuels. Aquaculturing of microalgae in PBRs has been done for long time, but it is still challenging. Issues like high cost, labour-intensive, technology, space, maintenance and much more. PBR configuration also gets affected by environmental factors like light and temperature conditions, pH and others. In order to model cultural systems and forecast their output, the outcomes of meticulously regulated circumstances are crucial. This review paper aims to list different types of PBR dealing separately with the above-mentioned issues, hoping to provide a brief vision to researchers for designing a comprehensive setup of PBR that addresses these issues.

## AUTHORS' CONTRIBUTION:

All authors have contributed equally to the writing of the original draft and the review and editing of the manuscript. The authors approved the final version of the submitted manuscript.

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