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Photobioreactors for production of biofuels from microalgae: a concise review

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Abstract

Microalgal strains are potential cell factories capable of producing valuable biochemicals including biofuels. Photobioreactors are closed systems capable of producing large quantities of microalgae and high yields of biofuel under optimal operating conditions, namely, light, temperature and pH. The design configurations of these systems are horizontal or serpentine tube, flat plate, bubble column and stirred tank of which tubular and flat plate bioreactors show promising results in biofuel production. However, the separation of algal biomass from the treated wastewater poses a major challenge in the use of algae for wastewater treatment. To overcome this problem, biofilm-based photobioreactor, an immobilized algal cultivation reactor, has emerged as a promising strategy. In the present study, we discuss the different types of photobioreactors, the distinct advantages of using these reactors over the open pond technology, the microalgal growth dynamics, reaction kinetics, diffusional limitations, and challenges faced during reactor scale-up. The review finally tries to provide a perspective on how further developments can be made in this reactor technology for setting up an economical, controllable and efficient method of microalgae cultivation and biofuel generation.

Keywords: microalgae; photobioreactor; kinetics; diffusional limitations

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1. Introduction

In modern science developments in microalgal culture technology have played a role to a considerable extent. They have been producing various types of chemicals and bulk products from microalgae. Bio polyesters, lipids, carbohydrates, proteins, pigments, colors, and antioxidants, are all known as several biological derivatives [1-4]. They are all viable options for clean energy extraction and bioremediation [5]; recognized as feedstock for third-generation renewable biofuels and energy (e.g., biodiesel, bioethanol, biobutanol, biohydrogen, etc.). CO_2 can biofixate or attenuate bioelectricity [6-9], and clear the flue of hazardous substances such as wastewater [10] treatment, nitric, and sulfur oxides gas [11,12]. Microalgae do in fact hold a superior to their terrestrial plants, they have tremendous potential as cell factories.

The global energy demand for microalga has been steadily enhanced, and the yearly production rate is estimated at ca. 20,000 tons [4,13]. Nevertheless, the full potential of microalgae is still constrained by the existence of still less expensive alternatives in the market.

Photoautotrophic cultivation is currently the most widely used mode for producing microalgae, and the only workable strategy at present to obtain biomass at a large scale [5,14]; this is because sunlight is a free, renewable, and clean source of energy. Used to grow microalgae, there are different cultivation methods, depending on the desired product and available species. They result in higher costs. Open systems, such as circular and raceway ponds, are the most used industrial devices and account for 90% of the overall microalga annual production [15]. For the high rate of demand and interest in microalgae, closed cultivation systems known as photobioreactors (PBRs) have been developed. While open systems are relatively cheap and easy to operate, PBRs tend to be more complex and expensive. Open systems used for microalgae cultivation have certain drawbacks such as poor mass transfer rate and high-water evaporation, instability of culture conditions, susceptibility to contamination, and need for a large land surface [5,12,16]. On the other hand, a closed photobioreactor offers better control over the growth environment, including nutrients, temperature, pH, and lighting. This allows for the cultivation of single species of microalgae for longer periods, reducing the risk of external contamination [8,17]. Several types of photobioreactors have been developed to isolate and cultivate green microalgae. These reactors are used at small and pilot scales, in laboratories as well as outdoors [14,18,19]. The conventional closed PBR configurations include a few standard designs [20], including flat-plate [21-23], tubular (horizontal) [14,21,22,24], and column-type [10,11,17]. The column-type reactors are further divided into stirred tank-type PBR and aerated columns (bubble column or airlift). Scaling up capital costs presents major difficulties [25]. The choice of PBR geometry and operational methods depends on the intrinsic features of the microalga selected (in terms of energy demand and growth kinetics), the intended bio-compounds or by-products, and local conditions [16,26].

2. Photobioreactors

Due to the limitations of open pond systems, extensive research has been carried out to find an alternative. Photobioreactors have emerged as a feasible option for cultivating algae due to their high rate of production and generation of quality

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algal cells. A photobioreactor is an enclosed vessel that uses light and CO2 to facilitate the rapid propagation of the cells (Tan et al., 2018). compared to open pond cultivation, photobioreactors require less space. Various types of photobioreactors have been developed such as tubular, bubble, flat, horizontal, foil, and porous photobioreactors. Among them, tubular and flat panel photobioreactors are the most widely used and efficient closed culture systems for the commercial cultivation of microalgae (Suparmaniam et al., 2019). Fig.-1 Represents the Design and applications of photobioreactors.

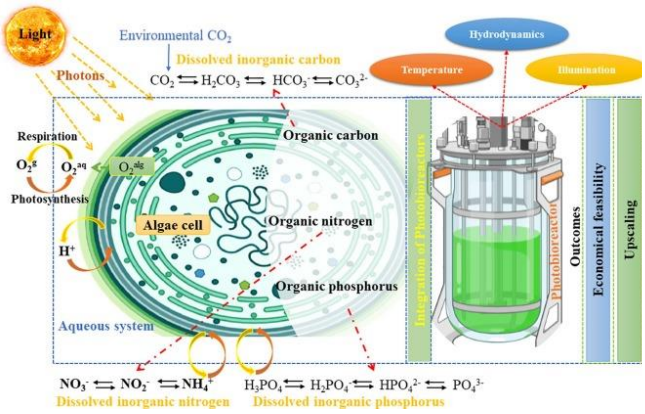

Fig. 1 Applications of photobioreactor

3. *Different types of PBRs & their Design Configuration:*

3.1 Flat-plate PBR:

The flat plate photobioreactor (FP- PBR) has been used since the 1950s and can be used indoors and outdoors [26]. However, there has been a high risk of photoinhibition, especially in outdoor cultures or at the early stage of growth. When exposed to high levels of light, cells become inhibited due to light over-saturation, which can severely affect photosynthesis and cellular metabolism. This can ultimately lead to the collapse of the culture [40]. The FP-PBR can be oriented vertically or inclined at a tilt angle to maximize incident light and increase biomass productivity. Agitation can be provided through either a pump lift over-driven or airlift method, with baffles included to improve mixing efficiency. Oxygen buildup is usually not a problem since there is an effective open gas disengagement system, except when a vertical alveolar panel is used [43]. A design of scalable airlift flat panel photobioreactor for microalgae cultivation is shown in Fig.- 2.

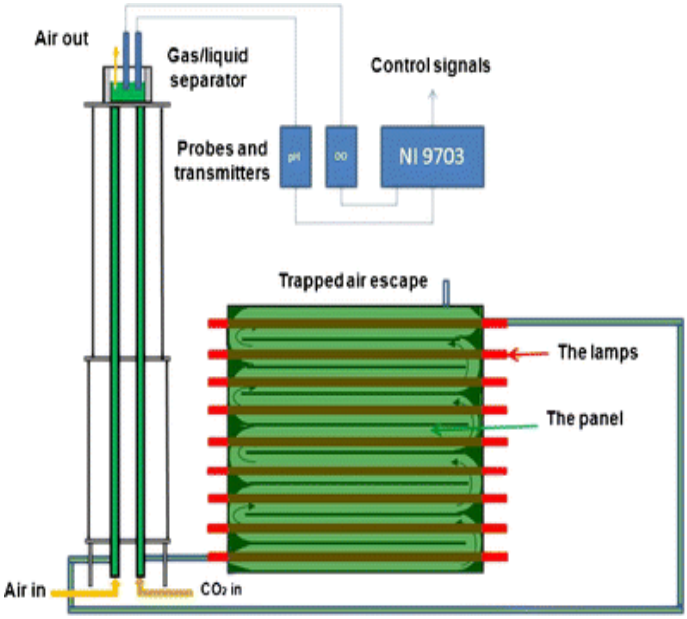

Fig.- 2 Airlift flat panel photobioreactor

3.2 Biofilm-based photobioreactor

A biofilm-based photo bioreactor is a specialised system designed to cultivate photosynthetic microorganisms, such as algae and cyanobacteria, in the form of biofilms. A biofilm is a complex, three-dimensional community of microorganisms that adhere to surfaces and grow together in a matrix of extracellular polymeric substances. In the context of photobioreactors the microorganisms grow on the solid substrate or surface and form a biofilm structure while being exposed to light for photosynthesis.

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3.3 Tubular PBR:

Transparent tubing, either made of glass or polyethylene is used to create conservative tubular PBRs. The most common configuration is a serpentine loop arranged in a single plane, which is usually displayed horizontally and is a popular choice for outdoor mass culturing [27]. Apart from the tube arrangement, tubular PBRs differ in tube length and diameter, flow velocity, form of recirculation, and geometric shape of the light receiver. The tubes used are typically between 0.1 and 0.6 cm in diameter [28], while their lengths can be several hundred meters long. The tube length is determined by the photosynthesis activity and the distance between liquid degassing points [29].

Tubular photobioreactors (PBRs) are considered as solar collectors since they use microalgae flow through a large surface area exposed to sunlight. The "lens effect" or "focusing effect" ensures that the incident light is evenly distributed, as it flows radially and is diluted along the circumference, which focuses it on the axis of the tube. This effect reduces the mutual shading and increases radiation intensity, which in turn reduces photo-inhibition [30]. One of the significant advantages of this configuration is the high surface-to-volume ratio, which is particularly suitable for efficient light harvesting while minimizing photo-inhibition [31-33].

3.4 Vertical column PBR:

Vertical column PBRs can be categorized into stirred-tank vessels and aerated columns, such as bubble columns or airlifts. the central regions of these types of reactors typically appear as dark or dimly lit environments, limiting cell exposure to light along the axis, which can negatively affect photosynthetic efficiency and microalga biomass production and productivity [34,35]. In general, the relatively low ratio of surface area to volume (A/V ratio) hampers scale-up [15,36]. For a better understanding of the advantages and drawbacks of both stirred tank and aerated column PBRs, please refer to [Table 1].

3.5 Stirred-tank type PBR:

Stirred tank PBRs are commercial bioreactors that are commonly made of steel, glass, or organic glass. They are often used in the industry to produce fine chemicals or pharmaceutical products. These systems are particularly useful for the heterotrophic growth of microalgae using appropriate organic carbon sources [26]. The main advantage of this apparatus is the precision, control accuracy of every operating parameter, and the minimization of contamination by heat sterilization. By using wall transparent arrangements, they can also be used for phototrophic cultivation, photomixotrophic, and photoheterotrophic modes if an external light source is provided (e.g., fluorescent lamp, LED, or even sunlight). Although it has a quite low A/V ratio, this configuration is useful for optimization processes at a laboratory scale (indoors) [37]. Fig.3 shows Façade integrated photobioreactors for building energy efficiency.

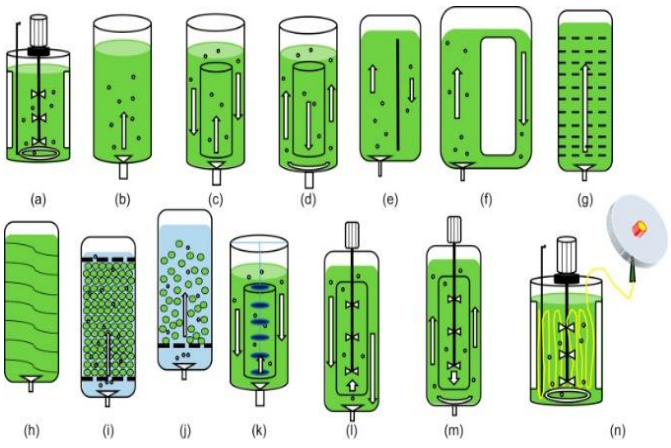

Fig.- 3 Façade integrated photobioreactor

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3.6 Aerated columns-PBR:

A common type of photobioreactor is the aerated column-PBR, which is typically made of transparent glass or plastic and has a vertical cylinder shape. To avoid shading effects caused by a high cell concentration, smaller radii have been suggested. However, the core regions of these reactors are often dimly lit or dark, which limits the exposure of cells to light along the axis and reduces photosynthesis efficiency [33]. Scale-up problems can also occur due to the relatively low AA/V ratio [6,38]. Aerated columns can be classified as bubble columns or airlift reactors, depending on their mode of liquid motion [39]. In both cases, agitation, gas, or CO2 is sparged at the bottom of the PBR to provide agitation and mixing, which ensures good overall mixing and sufficient gas transfer rates [36,40].

4. Designing Equation of Photobioreactor:

Designing a photobioreactor requires considering several factors, such as reactor geometry, the availability of light, temperature regulation, and the requirements of the microorganisms or algae you plan to grow. There I no single "design equation" for a photobioreactor since the design will be influenced by your specific objectives and limitations. However, some equations are commonly used in the design and operation of photobioreactors:

4.1 Light Intensity and Distribution:

4.1.1 Beer-Lambert Law:

This law is used to calculate light attenuation through a culture medium, which is important for determining the required light intensity.

$$\log\left(\frac{I0}{I}\right) = A = \in lC$$

- A is the absorbance
- ε is the molar attenuation coefficient or absorptivity of the attenuating species
- ℓ is the optical path length
- C is the concentration of the attenuating species

4.1.2 Mass Transfer and Oxygen Transfer:

Oxygen Transfer Rate (OTR): OTR is crucial for microorganism growth. It can be calculated using equations like oxygen transfer coefficient (Kla) and Henry's law.

Kla Equation: This equation relates the volumetric mass transfer coefficient (Kla) to the reactor design and operating conditions.

4.1.3 Hydrodynamic and Mixing Considerations:

Reynold's Number (Re): It is used to assess the flow regime inside the reactor, whether it's laminar or turbulent.

Mixing time: This is a measure of the time required to achieve good mixing in the reactor. It can be estimated based on the reactor geometry and agitation rate.

4.1.4 Temperature Control:

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Heat Transfer Equations: Depending on the design, heat transfer equations can be used to determine the cooling or heating requirements for maintaining the desired temperature.

4.1.5 Biomass growth kinetics:

Monod Equation: This equation describes the specific growth rate of microorganisms as a function of substrate concentration.

$$\mu = \mu_{max} * ([S])/(K_s + [S])$$

where: μ is the specific growth rate.

- μ_{max} is the maximum specific growth rate.
- S is the substrate concentration.
- Ks is the half-saturation constant.

Reactor Sizing:

- You will need to perform mass and energy balances to size the reactor appropriately based on your target biomass production.

Light Source Design:

- Depending on the type of light source (natural sunlight, artificial light), you'll need to design the lighting system to provide adequate and controlled light intensity

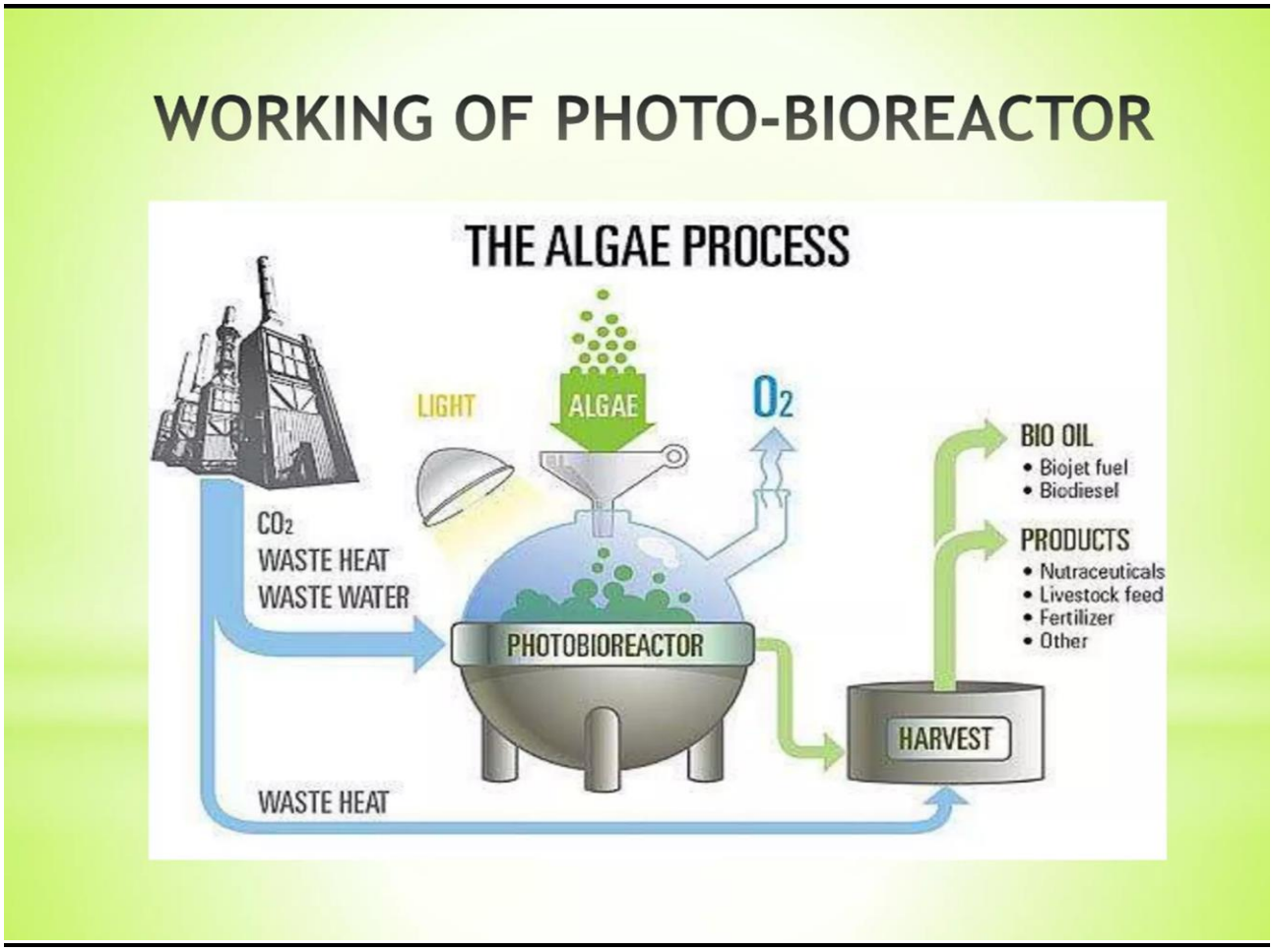

Fig.- 4 Working of photobioreactors

Figure 4 Represents the working of Photo-Bioreactors

6. Comparison between most common enclosed photobioreactor configuration for microalga cultivation system. (Adapted from [35,37,40]):

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Types of photobioreactor	Advantages	Limitations	Applicability/observations
Flat-plate	-High area-to-surface ratio -Large, illuminated surface area. -Good light path -Moderate biomass yields	-Expensive construction materials -Easily subjected to photo-inhibition -Hard temperature control -Scale-up problems	-Suitable for outdoor and indoor -Application to algal strains with high lipid content
Tubular (horizontal)	-High S/V ratio -Effective in the capture of solar radiation -Relatively low cost to build	-Poor mass transfer -High risk of pH gradient and O2 build-up -Risk of photo-inhibition or photo-oxidation -Risk of overheating -High land surface area required	-Well suited for cultivation outdoors -Well suited for industrial cultivation of most common microalgae species
Column (vertical) Stirred tank	-Precise monitoring of each culture parameter -Used for optimization studies. -Cheap and compact -Low maintenance cost	-Low area-to-volume ratio -Poor efficiency in light conversion -Low productivity	-Ideal for producing added value compounds. -Cultivation of biomass for wastewater treatment -Limited to heterotrophic microalgae
Aerated columns (bubble columns and airlift)	-Good mixing -Efficient CO2 supply and O2 removal -Low fouling -Low land requirements	-Risk of high shear stress on cultures -Photo-inhibition problems -Small illumination area -Deficient scale-up	-Unstable for microalgae prone to flotation and/ or species highly sensitive to shear stress

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7. General parameters affecting PBR performance:

The performance of a photobioreactor is influenced by a variety of factors, including physicochemical parameters like temperature, pH, dissolved oxygen, CO2 availability, shearing, and nutrient availability, as well as physical and operational factors. Light requirements surface area to volume ratio, mixing and agitation patterns, exchange rates of Co2 and O2, nutrients provisions and renewal temperature and pH control, the quality of construction material, and biofouling all play a crucial role in the proper operation of a PBR. Some of these parameters interact with one another, which makes designing an effective PBR as a cultivation system a complicated task [41].

7.1 Temperature:

Temperature control is a significant operational parameter in PBR performance; it greatly influences the growth rate of microalgae [42]. The efficiency of photosynthesis depends on a balance between light and temperature [43]. Microalgae hold an optimum temperature interval – that should be sought a priori. This is essential to promote effective light harnessing and CO2 biofixation, and thus reach high biomass productivity. Optimum temperatures typically range within 20–24 ◦C. Nevertheless, most microalgae can tolerate temperatures between 16 and 35 ◦C [44-46].

7.2 pH:

Maintaining an optimal pH level is crucial for the performance of photobioreactors (PBRs). Any deviation from the optimum range can severely impact the microalgal cell's ability to absorb CO2 and other essential nutrients like iron, thereby affecting overall the health of the culture [47]. The pH level is primarily controlled by balancing the supply and mass transfer of the CO2 in the liquid phase and the uptake by the microalgal cells. The total dissolved inorganic carbon (DIC) in the medium plays a significant role in controlling the pH level. If the DIC appears mainly in the form of CO2, it can cause acidic pH shifts (<7). Conversely, frequent alkaline pH shifts occur when the main form of DIC is carbonate (i.e., CO3 2-) [40].

7.3 Light and surface area-to-volume ratio

Achieving effective photoautotrophic cultivation of microalgae requires an efficient light supply and the appropriate wavelength range. During photosynthesis, most microalgae process energy within the 400–700 nm range, also known as photosynthetically active radiation (PAR). Solar light is often used as a cost-effective source of energy for microalgae growth in common outdoor mass cultivation systems.

The Surface area-to-volume ratio (S/V ratio) plays a vital role in the performance of PBRs. The distribution of light over the PBR surface depends on the total transparent surface area available, in general, which in turn is affected by the S/V ratio. Generally, the higher the S/V ratio, the higher the percentage of light presenting the PBR surface, leading to an improvement in photosynthetic efficiency, and higher productivity of biomass and metabolite productivities [16,20,48].

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7.4 Mixing and agitation

In microalga cultivation systems, the cells are typically suspended in the broth medium. As a result, mixing is a crucial factor that helps to ensure that the culture is homogenized and to prevent the cells from settling or clumping together on the walls of[the PBRs, particularly in horizontal tubular PBRs [36]. Proper mixing also helps to distribute the nutrients evenly and reduces temperature and pH gradients. If mixing is poor, the nutrients and pH may accumulate in undesired gradients, leading to biofouling on the walls of the PBRs, as well as an increase in oxygen levels in the medium [49]. To improve mixing, baffles or static mixtures can be used inside the reactors [43,50].

7.5 Gas exchange

The ability to exchange gasses (such as removing O2 and adding CO2) is an important feature of PBRs for cultivating microalgae [45]. However, the process of delivering CO2 is limited by mass transfer, which can be a problem for open systems due to the low atmospheric pressure of CO2. This means that microalgae cannot directly use gaseous CO2, but instead require it to be dissolved in a liquid phase for enhanced mass transfer rates [51].

8. Process of Microalgae to Biofuel Production:

8.1 Harvesting:

The development of a cost-effective microalgal biodiesel industry requires the use of minimum energy harvesting techniques. However, the small size of microalgal cells, negative charges on their surface, their density near water, and their suspension in dilute media present some hindrances in the development of a low cost harvesting approach (Muhammad et al., 2020). There are many harvesting techniques available, including centrifugation, filtration, flocculation, electrophoresis, gravitational sedimentation, and flotation. Centrifugation is the most widely used technique on a laboratory scale, but it is highly energy-demanding and may lead to cell damage, thus not being applicable to large-scale biodiesel production (Morais Junior et al., 2020).

8.2 Lipid extraction:

To produce biodiesel, it is essential to extract lipids from the cells. However, the extraction process requires a suitable technique. For optimal lipid extraction, pretreatment of harvested and dried cells is necessary. This pretreatment involves mechanical disruption of cells using mortar and pestle, bead mills, ultra-sonication, etc (Zheng et al., 2011; Greenly and Tester, 2015; Rivera et al., 2018). Alternatively, methods such as microwaving, acid/based treatment, and enzyme treatment can be used to break down cell structure (Zuorro et al., 2016). Additionally, pulsed electric field (PEF) and high voltage electric discharge (HVED) are efficient approaches for cell lysis.

8.3 Transesterification:

The transesterification reaction is a process where one mole of triglyceride and three moles of alcohol react to produce a simple ester (biodiesel) in the presence of a catalyst. This method is widely accepted as one of the best approaches for biodiesel production (Tabatabaei et al., 2019). The catalyst plays a crucial role in determining the ease of converting triglycerides to ester (Pathak et al., 2018; Changmai et al., 2020).

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9. Determination of algal growth rate kinetics

The algal growth rate was determined by measuring the growth of microalgae using a spectrophotometer. Briefly, samples were withdrawn, and the algal growth was measured by recording the absorbance at 680 nm. For the determination of biomass, the algal suspension was centrifuged at 15,000 rpm for 10 min and dried at 55 °C for 60 min in a hot air oven. The relation between the growth rate and the biomass was estimated using a liner regression equation (eq:2) and specific growth rate (eq:3) (Tamil Selvan et al. 2020):

$$\mathbf{Y = 0.8754X - 0.3645} \quad (2)$$

where, X—optical density at 680 nm and N—dry biomass weight (gmL−1)

$$\boldsymbol{\mu = \frac{\ln(N_1 - N_0)}{t_1 - t_0}} \quad (3)$$

Were, μ—Specific growth rate and Ln—Linear regression.

9.1 Determination of CO2 utilization kinetics:

The CO2 biosorption ability and the biofixation efficiency rate (BCO2) of the selected microalgal algal species were calculated using the modified methodology of De Morais and Costa (2007). The bio fixation efficiency rate, percentage of CO2 removal, and consumption rate were calculated as mentioned below: The BCO2 (Eq:4) and CO2 removal (%) (Eq:5) were estimated based on the equations, given as the determination of bio fixation efficiency rate:

$$\boldsymbol{B_{CO2} = X_C * P(\frac{Z_{CO2}}{Z_C})} \quad \mathbf{(4)}$$

where, Xc % of carbon content from the given microalgal cell, P is the biomass productivity expressed in terms of mg mL−1d−1, ZC is the molecular weight Carbon (C) and ZCO2 is the molecular weight carbon dioxide (CO2). The determination of CO2 removal (%):

$$\boldsymbol{R_{CO2} = \left(\frac{total\ CO_2 biofixed(V)}{total\ CO_2 input(V)}\right) * 100} \quad \mathbf{(5)}$$

where RCO2 is carbon dioxide (CO2) removal in terms of percentage and V is the volume of CO2.

9.2 Determination of heavy metals biosorption capacity:

To study the biosorption ability of heavy metals using microalgae, 50 mL of the effluent treated with algae was filtered using a nylon Millipore membrane filter. The filtered microalgal cells were collected and utilized for biosorption capacity studies using atomic adsorption spectroscopy. The kinetics studies of heavy metals biosorption using microalgae were evaluated by kinetic models such as the Langmuir and Freundlich model (Tamil Selvan et al. 2020) as mentioned below.

For the Langmuir model, the biosorption ability was calculated using the following equation:

$$\boldsymbol{\frac{C_e}{q_e} = \left(\frac{1}{bq_{max}}\right) + \left(\frac{C_e}{q_{max}}\right)} \quad \mathbf{(6)}$$

where q_e is Algal biosorption capacity at equilibrium (mg g−1), Ce is the Concentration of metals at equilibrium (mg L−1),q_{max} is Maximum

biosorption capacity (mg g−1), and is Langmuir constant (L g−1).

For the Freundlich model, the biosorption ability was calculated using the following equation:

$$q_e = K_F * C_e^{1/n} \quad (7)$$

where K_F is the Freundlich constant,1/n is Adsorption intensity, q_e is Algal biosorption capacity at equilibrium (mg g−1) and C_e is the Concentration of metals at equilibrium (mg L−1).

10. Challenges and Opportunities:

10.1 Biofouling:

Biofouling is a major issue in PBRs, especially in closed ones. This happens when cells aggregate and stick to the inner walls of the system, the biomass concentration decreases, and the system's performance is negatively affected [52]. Choosing the right construction material and geometry is crucial to prevent biofouling. Designs like flat plate PBRs with a more cuboidal shape and are easier to clean and maintain than tubular reactors. PBRs that have continuous turbulent regimes due to random stirring or gas bubbling are less prone to biofouling. Additionally, fluid radial flow patterns like those cylindrical vessels achieved via rotation stirring are better than axial flow only [54].

Many unconventional designs for photobioreactors (PBRs) have been suggested to overcome the major challenges of classical configurations. These modifications have improved light conduction, hydrodynamic patterns, mass transfer, and controllability. However, there are still several obstacles to be addressed.

PBR configurations designed to reduce light path (and increase the surface area to volume ratio) show promising advances in improving light distribution inside the system, which is a major issue when dealing with high-density microalgae culture. However, optical fibers and light guide devices in column vessels and compact systems are difficult to scale in terms of both cost-effectiveness and long-term performance [36,42].

LED-based lighting technologies have become important in novel PBRs and supporting technologies have experienced significant advancement in recent years. Enhancing hydrodynamics with tailor-made internal flow patterns and developing effective flashing light effects and light/dark cycles have proven useful in improving microalgae biomass productivity. Active mixing has been found to enhance microalgae performance when exposed to intercalated illumination and dark cycles [41,53]. Many low-cost plastics favour the transmittance of light into the microalga cultures, yet photo-limitation may be induced. Less expensive, yet more fragile materials are more susceptible to leakage and contamination. They can undergo photo-degradation when exposed to UV-radiation (combined with exposure to high temperatures) [32], thus compromising plastic optical properties and eventually impairing regular microalgae growth and performance.

Overall, the development of novel PBRs still faces several challenges as unconventional configurations for microalgae cultivation, especially in view of the high construction and operational costs incurred in scaling up. Nevertheless, investigation and development in this field offer a major opportunity to combine empirical experience and theoretical fundamentals in attempting to produce economically more

feasible and environmentally sustainable systems [54].

10. Conclusion:

We've developed a profitable and sustainable biodiesel production method using carefully selected microalgae species. Hybrid bioreactors are effective for mass-producing algae, and combining different types of bioreactors may help develop appropriate bioreactors for mass algal culture. To produce large quantities of algal biomass, we suggest using photobioreactors with photonics and biotechnologies. However, more economic assessments are needed to compete with petroleum-derived fuels.

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