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Continuous cultivation of microalgae in photobioreactors as a source of renewable energy: Current status and future challenges

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ABSTRACT

Microalgae are promising sustainable energy sources for biodiesel production due to their rapid photosynthesis growth rate and capacity to be cultivated in wastewater, seawater, or freshwater. Moreover, microalgae could complete the entire growth cycle *via* photosynthesis reactions that convert light energy into renewable energy. The closed photobioreactor, PBR is resistant to infection from uninhabited algae species and allows frequent monitoring of various factors such as temperature, light intensity, and pH during the cultivation phase. Thus, this study focuses on continuous cultivation technology which produces higher biomass productivity with sustainable energy-saving operation as compared to batch culture. High productivity of microalgae biomass tends to accumulate higher concentrations of lipid and carbohydrates composition which is essential for the production of biofuels. The energy balance of numerous microalgae-based biofuels was discussed, and it was discovered that the net-energy ratio was greater than 1, indicating that the process is both commercially feasible and environmentally friendly. This study also summarizes the most recent discoveries on continuous cultivation constraints through photobioreactors, PBRs as well as potential challenges to tackle in scaling up the continuous sustainable culture mechanism. The research gaps, market opportunities, and future development directions of continuous photobioreactor systems are discussed to explore future development opportunities. A continuous photobioreactor, architecture is recommended for a pilot-scale trial, as a cost-benefit comparison would be beneficial in commercializing the framework.

1. Introduction

The global population growth has significantly increased the demand for energy requirements. The current global energy structure relies approximately 85.5% on fossil fuels and many countries consume more fossil fuels than they can produce [1]. Continuous dependence on fossil fuel energy resources increases the emission of greenhouse gas which is harmful to the global environment in a long run. On the other hand, the limitation and depletion of fossil fuels have driven researchers to explore renewable energy sources such as geothermal energy, bioenergy, hydropower energy, and solar energy. One of the potential biofuel sources can be produced by microalgae, which is a promising substitute for fossil fuels. Microalgae belong to a group of photosynthetic microorganisms

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found in both terrestrial and aquatic environments. Microalgae are classified as either prokaryotic or eukaryotic, and are known as cyanobacteria (Chloroxybacteria and green algae) [2,3]. There are more than 50,000 species of microalgae, but only about 30,000 of the species have been investigated and analyzed [4]. Microalgae are preferred over other plant sources due to their high rates of photosynthesis which contribute to mitigating CO_2 emission, productive removal of nitrogen and phosphorus, and growth in wastewater/seawater with no competition with freshwater or arable land of agriculture [5]. During photosynthesis, algae transform both CO_2 and light energy into biomass that is rich in mineral components [6,7] which can be harvested for biofuels such as biodiesel, bioethanol, biomethane, and biohydrogen. In specific, the production of bio-diesel is initiated from the presence of lipid and protein content in the microalgae biomass *via* thermochemical/biochemical process, bioethanol from carbohydrates fermentation, biomethane by anaerobic digestion, and production of biohydrogen from photo-fermentation [8,9].

The growth rate of microalgae varies according to their specific cell characteristics based on their cultivation conditions such as autotrophic, heterotrophic, or mixotrophic [10]. Microalgae that depend on light sources to generate energy are classified under autotrophic cultivation, whereas heterotrophic organisms utilize organic carbon as a source of energy. A combination of both autotrophic and heterotrophic cultivation is categorized as mixotrophic. This allows the cultured microalgae to have both supplies of inorganic carbon and some organic carbon source which will contribute to a higher yield of microalgae [11]. The high yield of microalgae biomass consists of high lipid and carbohydrates composition which is essential to produce bioenergy. Microalgae cultivated under mixotrophic culture needs lower light intensity, resulting in lower energy consumption, as compared to autotrophic and heterotrophic culture [12]. Apart from the choices of microalgae species, its cultivation parameters such as temperature [13,14], pH [15] and salinity [16], light intensity [17], and nutrition [18] availability in culture medium have to be taken into consideration in the cultivation process. Cultivation of microalgae is the initial stage that improves the productivity of microalgae biomass, as it will be a sustainable source for biofuel production. The conversion of microalgae to biofuel begins with cultivation followed by harvesting, drying, and oil extraction [19]. Microalgae are photosynthetic microorganisms with basic growth needs (light, sugars, CO_2, nitrogen, phosphorus, and potassium) that could generate massive amounts of lipids, proteins, and carbohydrates in a short time [20]. . Thus, this review mainly focuses on the impact of culture medium and PBR design technologies on the enhancement of microalgae cultivation.

List of abbreviations

AI	Artificial intelligence
ANN	Artificial Neural Networks
BBM	Bold Basal Medium
BG-11	Broth is universal Medium
BRT	Biomass retention time
CO_2	Carbon dioxide
FBN	Feed Forward Back Propagation Neural Network
GHG	Greenhouse gases
HCO_3	Bicarbonate
HRT	Hydraulic retention time
HTL	Hydrothermal liquefaction
LED	Light Emitting Diode
MBR	Membrane photo-bioreactor
MPBR	Microalgae membrane photo-bioreactor
N_2	nitrogen
NER	Net energy ratio
N/P	Negative to positive electrode
NPK	Inorganic fertilizer code
PBR	Photobioreactor
PES	Polyethersulfone
PFC	Perfluorocarbon emulsions
RUF-DPBS	Resonant ultrasound field incorporated dynamic photo-bioreactor system
SRT	Sludge retention time
SS	Suspended Solid
UASB	Up-Flow Anaerobic Sludge Blanket Reactor
USD	United State Dollar
WWTP	Wastewater treatment plant

2. Benefits and industrial application of microalgae biomass

2.1. Economic analysis of cultivated microalgae

Microalgae biomass secrete biomolecules that can be further processed to commercial products such as bio-fuel, and high value-added products from other biotechnological fields like cosmetics, pharmaceuticals, chemicals, food, and feed [21]. This is possible because microalgae consist of a high number of proteins, lipids, and carbohydrates which could be processed to form a variety of products. The production of bio-diesel is initiated from the presence of lipid and protein content in the microalgae biomass and bioethanol from carbohydrates fermentation [22]. Gouveia et al. (2014) discovered that the introduction of a Photosynthetic Alga Microbial Fuel Cell (PAMFC) has the ability to produce bio-electricity and value-added pigments simultaneously [23]. PAMFC is controlled by the photosynthetic activity since power output and pigment production are directly affected by light intensity conditions. The production of bioelectricity will mostly be used for the harvesting process of microalgae culture instead of branching out to external sources. The benefits of microalgae biomass in the cosmetic industry are due to its capability in producing bioactive compounds. The potential uses are anti-aging creams, sunscreen, skin whitening, pigmentation, and moisturizing agents [24,25]. Microalgae such as *Arthrospira, Chlorella,* and *Nannochloropsis* are reported to be an essential source of proteins and polysaccharides which provide health benefits in terms of prebiotic application in the form of capsules and tablets [26].

Microalgae also produce pigments (carotenoids) that contain provitamin-A, which can be used as an additive in multivitamin supplements and also as a source for food coloring on butter, cheese, and margarine [27]. The vast application of microalgae-based products demands high biomass productivity with low energy and production cost which in turn makes the commodity to be more competitive in the market with other commercial fossil fuel-based products. Table 1 illustrates the market price of microalgae-based products for the past five years. It indicates that different microalgae species have a good market potential to be applied. Microalgae could also be a huge contribution to the environment as it possesses a greater effect of CO_2 reduction and that of N_2 in enhancing the generation of syngas evolved from the thermal degradation of microalgae (*M. aeruginosa)* [26]. Other function of microalgae includes as bio-fertilizers and soil conditioners for industrial usage. The microalgae species grouped in cyanobacteria strains have the ability to fix the atmospheric nitrogen which highly benefits plant growth especially for rice cultivation [18].

Table 1
Market price of microalgae-based products.

Microalgae-based products	Microalgae Species	Price ($)	Reference
Biofuel (bioethanol)	*Chlorella vulgaris*	1.30–2.40 (per kg)	[28]
Biofuel (biodiesel)	*Chlorella vulgaris*	9.84–20.53 (per kg)	[29]
Biofuel (bio-hydrogen)	*Chlamydomonas reinhardtii*	14.22 (per MJ)	[30]
Biofuel (biogas)		1.16 (per MJ)	
Cosmetics & Pharmaceutical (skin care)	Zeaxanthin	10,000 (per kg)	[31]
Pigments (health food)	Astaxanthin	2500–7000 (per kg)	[32]
Proteins (animal feed)	*Chlorella vulgaris*	3.00 (per kg)	[33]

Note: Values expressed in USD according to currency value of the year of references publication; Market price varies according to the type of microalgae species.

2.2. Cultivation mode and growth conditions

As the demand for microalgae-based products increases in the market, there is also an urge for researchers to further investigate the cultivation process in a large-scale model. Open pond cultivation was widely used but studies show that there is a high risk of contamination and insufficient control of growth conditions, especially when it involves the influence of external atmospheric temperature and solar radiation [34]. Although open pond cultivation is cost-saving in terms of energy and materials [35], the application of a closed system PBR is preferred as it has control over the growth conditions and contamination [36]. The efficiency of batch-PBR is being studied vigorously to establish it for large-scale production [37]. It is reported that the major challenge faced in batch process cultivation is the requirement of a longer lead time for the cells to be matured after harvesting is done and also the high operational cost every time the process starts up [38]. Hence, a substitution to the batch operation will be a continuously fed cultivation process, which reduces the downtime for microalgae to mature and lead to quality production of microalgae biomass [39]. To commercially generate bioenergy (bio-fuels), te biomass productivity yield must be maintained at optimal conditions that provide a light intensity of 60–100 mol/m2/s with precise temperature control between 20 °C - 30 °C [40].

Besides, the steady-state operations in continuous mode also give a simpler and precise control of the CO_2 supply, light intensities, and fresh culture medium flowrate. This is an added advantage as the changes in parameters can be implemented on a large-scale microalgae biomass process without having longer downtime for the harvesting process [41]. Harvesting is a process that can be carried out using the cultured biomass by centrifugation, filtration/sedimentation, and coagulation/flocculation which requires high energy inputs [42]. The bio-flocculation method is considered an innovative approach that has the ability to reduce energy consumption of microalgae harvesting. Bio-flocculation is induced by extracellular polymer compounds such as polysaccharides and proteins which are derived from microalgae [43]. Studies have also proven that all flocculation methods strongly depend on the cell surface properties of microalgae (species, culture conditions, and growth phase) [44]. Thus, it is essential to initiate an efficient cultivation process for the production of a high-quality bioenergy production yield. Continuous mode cultivation would lead to cost-effective bioenergy processing by providing labor-saving and non-fouling advantages [45]. Apart from these, continuous cultivation can allow partial harvesting of the microalgae biomass during the cultivation process (exponential phase), which shortens the overall cultivation time (elimination of lag phase) and results in higher biomass production [45]. The future research development of continuous cultivation can be explored, especially on how it can affect the composition of the microalgae biomass which may be customized to suit the production of bioenergy.

2.3. Comparisons of open pond and PBR cultivation approaches

The construction of microalgae bioenergy refinery, economic factors such as installation and operating cost, oil content, microalgae yield, conversion tax, labor cost, and other overhead costs are factors to be considered in fabricating a PBR and process plant [46]. Table 2 compares the estimated biofuel production cost from microalgae with an ideal open pond and PBR system. This comparison is performed based on a batch mode cultivation approach for 300 days. It was observed that the open pond system is mostly associated with operating costs such as labor and utilities, while the PBR system is dominated by the capital cost of equipment and purchase tax. A cost reduction of about 50% could be achieved if the supply of CO_2, nutrients, and water is obtained at a lower cost (re-use of flue gas/wastewater) [47]. However, implementation of the PBR system is still considered the best alternative as the biomass productivity is higher compared to open pond cultivation [15].

In terms of environmental aspects, microalgae are considered a photosynthetic microorganism that can grow and produce biomass in rough environmental conditions [49]. Its basic requirement is an adequate supply of carbon dioxide, light, and nutrients. Hence, the culturing of microalgae reduces greenhouse gas emissions by consuming CO_2 which relatively contributes to a balance atmospheric air [50]. Biofuels produced by microalgae biomass releases less CO_2 (10%) and SO_2 gas (30%) compared to other commercial fuels [51]. A recent study also reported that the production process of biodiesel from microalgae biomass does not contain any hazardous compounds that will harm the atmospheric air quality [52]. This indicates that microalgae-based biofuel plays a significant role in reducing global warming by adsorbing CO_2 gas and also has the ability to consume carbon and nitrogen from industrial/domestic wastewater [53].

3. Effect of culture medium on the production of microalgae biomass and its relationship to bioenergy

3.1. Presence of culture medium on the growth of microalgae in batch, semi continuous and continuous cultivation

Nutrients present in the culture media will impact the growth and quality of microalgae biomass. Thus, the selection of culture media is significant to the overall cultivation cost contribution. The growth pattern of microalgae consists of four phases: lag, exponential, stationary, and death phase. The main function of the culture medium is to trigger the exponential phase once the microalgae adapt to the surrounding environment and to sustain their stationary phase. The presence of lipid and carbohydrates will be initiated and developed during the cultivation phase [54]. Thus, the behavior of microalgae during the stationary phase decides the quality of biomass and its capability for bioenergy production. The culture medium will be fed into PBRs in three types of mode, namely, batch, semi-continuous, or continuous cultivation mode [55]. In batch cultivation, the medium is injected into the culture at a single dose, where the culture medium will be monitored until its cell density reaches its maximum limit [56]. Once maximum growth is achieved, the culture will be harvested followed by the

Table 2
Comparisons of algae cultivation cost using open pond and PBR system [48].

Cultivation approach	Open Pond	PBR
Power	1.55–1.58	6.60–8.40
Labor	1.30–1.50	6.40–6.60
Carbon dioxide	0.40–1.30	6.20–6.40
Purchase Tax	1.58–1.60	8.40–9.80
Other cost (Installation)	1.60–1.80	9.80–10.20
Depreciation	0.25	6.00

Note: Unit cost = algae biomass production cost (USD/kg).

processing of products. For a continuous system, the cultivation medium will be fed into the PBR slowly in a continuous flow and once the cell density reaches its maximum limit, the matured cell will be withdrawn for harvesting while a fresh medium is fed into the PBR simultaneously [57] (see Fig. 1).

The inlet flow rate of fresh medium will be kept equal to the outlet flowrate of the matured microalgae to maintain a constant volumetric rate inside the PBR. However, in semi-continuous cultivation, the fresh culture medium will only be fed into the PBR after harvesting is completely done for the first cycle [58]. For semi-continuous cultivation, the fresh medium equivalent to the withdrawal volume will be replaced to expand the microalgae growth, whereas for batch cultivation the fresh culture mediums will be completely replaced starting a new cultivation cycle. Each time when harvesting occurs, there will be a downtime in the process that affects the overall growth of microalgae. Relatively for efficient large-scale bioenergy production to occur, continuous flow of medium is required as it provides a continuous supply of nutrients for the growth of microalgae and also reduces the inconsistency of lipid yield. Fig. 2 illustrates the comparison of the growth curves in batch, semi-continuous, and continuous cultivation. According to the growth patterns, the cultivation efficiency could sustain for a longer time in a continuous flow of culture medium during the cultivation process. Continuous biomass growth also means that there will be continuous lipid production that acts as an important source for the biodiesel process.

3.2. Medium studies on microalgae cultivation using photo bioreactor in batch, semi-continuous and continuous mode

There are several modes of culture medium such as batch, semi-continuous, and continuous mode supply, which has a high influence on biomass productivity. The production of bioenergy partially depends on the raw material source and quality (biomass productivity). The flow and selection of culture medium play a significant role in manipulating biomass productivity as different nutrients consumed by microalgae can lead to the development of different compositions in their cells. Microalgae are largely found in saltwater (oceans), freshwater (river), and wastewater (industrial effluent) due to the abundance of nutrients [59]. However, the quality of the microalgae biomass could vary according to the medium used for cultivation. Table 3 lists the pros and cons of using saltwater, open pond (freshwater), and industrial wastewater as the culture media for microalgae cultivation. The three mediums are discussed as they do not constitute the use of inorganic (chemical) nutrients. It is also considered a cost-saving and environmentally friendly approach to microalgae growth. Regardless of the culture medium used, a closed cultivation method using PBRs was established to track microalgae growth and regulate cultivation parameters. Various types of commercial culture media have been used in a closed PBR system for both batch and continuous systems to study its efficiency for microalgae biomass production. Table 4 shows the comparisons on the amount of *Chlorella vulgaris* biomass produced for batch, semi-continuous, and continuous cultivation systems based on various types of culture medium.

According to the comparison Table 4, continuous cultivation biomass productivity was the highest compared to the other two cultivation modes even if a similar culture medium is used. The batch process implements downtime for reactor cleaning and startup between runs, which contributes to the increasing demand for labor, water, and chemicals [15]. Economically, this is not preferred as the amount of biomass produced is insufficient to cover the capital and overall operating cost. Another drawback of implementing batch cultivation to pilot scale is the period required for one batch to complete. These drawbacks can be possibly overcome by semi-continuous/continuous cultivation mode, as the biomass productivity will be achieved within a few hours to days of its cultivation and can be maintained for a longer period in an optimized state. This can also be further prolonged by manipulating the light intensity and nutrition availability during cultivation. The continuous flow of biomass production indicates that the presence of lipid and carbohydrate composition can be maintained at an optimum yield which leads to efficient biofuel production. Continuous cultivation mode also influences the reduction of downtime since the replacement of matured cell withdrawal will be done simultaneously with a fresh medium inlet. These mechanisms contribute to the continuous production of microalgae biomass in bulk quantity. Thus, continuous mode microalgae cultivation has a greater chance of being scaled up to pilot scale for biofuel production.

3.3. Recent technology advancement in cultivation medium for bioenergy production

Although many elements influence the development of microalgae cultivation, the culture medium is one of those that highly contributes to the overall microalgae biomass cultivation cost. The advancement of culture medium technology that could lower the economic costs without

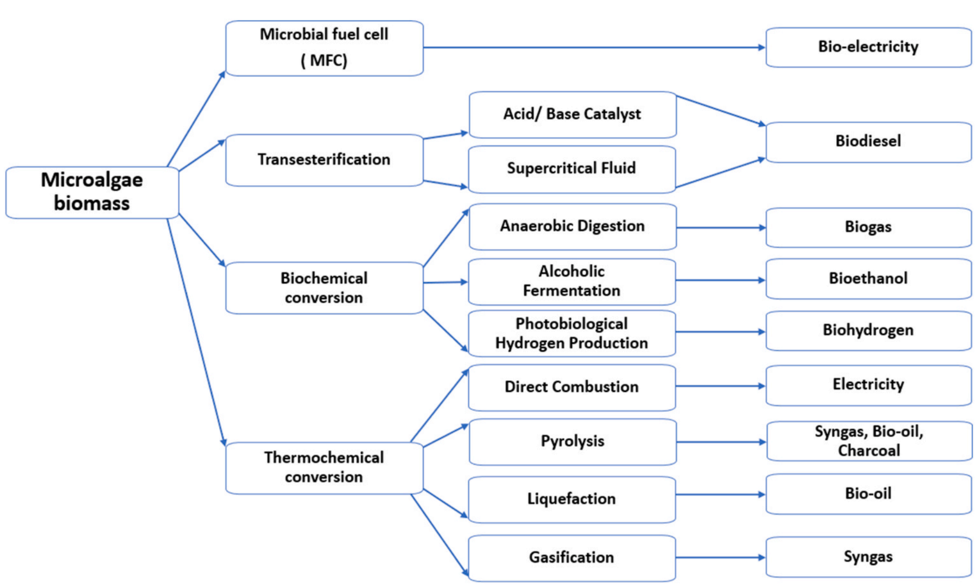

Fig. 1. Conversion of microalgae biomass to bio-energy production.

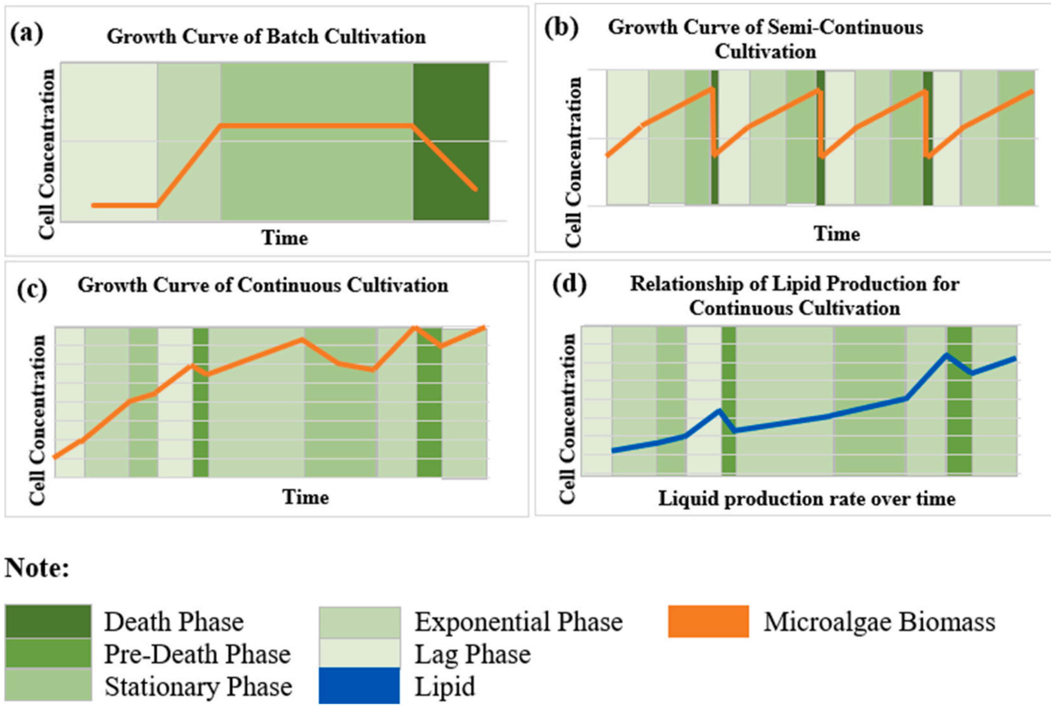

Fig. 2. Comparisons of the microalgae growth pattern in (a) batch, (b) semi-continuous and (c) continuous cultivation and (d) lipid production rate with biomass growth over time.

affecting the nutritional value of microalgae has been analyzed. Wang et al. (2013) developed a theoretical model that can recycle culture medium (nutrients) which is considered a sustainable approach during microalgae cultivation [76]. This is because nutrient recycling lowers the transportation cost of fresh medium supply and reduces waste effluent discharge from the cultivation process. Remaining water from the harvesting phase can also be recycled without affecting biomass productivity [71]. The results demonstrated the harvested water could be re-used back twice into the system with the addition of an adequate amount of nutrients, which also directly contributes to a higher lipid, protein, and carbohydrate composition for the production of biodiesel. The major advantage of recycling culture medium rather than using freshwater is that there is no additional requirement of electrical source for water pumping and treatment, making it an energy and cost-effective approach. However, digital monitorization (meta-analysis) is needed to examine the mixing ratio that is appropriate according to the nutrient and biomass productivity [77].

The impact of reusing culture medium on the microalgae (*Chlorella vulgaris*) biomass productivity, cell quality, and its sustainability was studied [78]. Based on the results obtained, it was documented that no remarkable impact on the *Chlorella vulgaris* growth was noticed even after 63 days of recycling and the productivity remained stable at around 550 mg/L/day. The possibility of combining a membrane bioreactor (MBR) and microalgae membrane photo-bioreactor (MPBR) in a novel configuration was explored. The studies revealed that integration of microalgae cultivation and pre-harvesting in an MPBR with a wastewater treatment plant (WWTP) offered a remarkable reduction in nutrient and primary harvesting costs as well as a reduction in nutrient removal cost in the WWTP [79]. Wang et al. (2018) examined two species of microalgae that were cultured *via* poultry wastewater in a two-stage cultivation system for algal biomass production. The ultrafiltration method was utilized to harvest the first species from the first cultivation stage followed by a re-cycled culture medium for the second species growth [80].

A novel resonant ultrasound field incorporated dynamic PBR system (RUF-DPBS) was developed where the medium replacement was carried semi-automatically *via* RUF media separation by gravity-driven as shown in Fig. 3(a) [45]. By analyzing the volumetric productivity, and eicosapentaenoic acid (EPA) after 12 days of continuous cultivation, it was concluded that the RUF-DPBS gives better results compared to the conventional separation methods and also gives a positive impact to the process with non-fouling, minimum shearing, cost-effective microalgae culture. The total lipid yield extracted from continuous operation was twice better than the conventional cultivation system [45]. Besides, the recycling of culture medium is co-related to the development of PBR design that contributes to a more sustainable and cost-effective process as shown in Fig. 3 (b). Strategies such as the substitution of commercial media to an alternative medium obtained at a lower price or waste medium with good nutritional values have been implemented. Agricultural fertilizer media was reported to be 8 times cheaper than the conventional medium, and organic fertilizer is feasible since it is widely available [81]. Agricultural medium has high phosphorus, nitrogen, urea, and iron (Fe) supply which contributes to the production of high lipid and carbohydrate extractives [82]. Sipaúba-Tavares et al. (2017) justify microalgae grown in an inorganic fertilizer (NPK, 20-5-20) medium developed higher biomass productivity than microalgae grown in commercial media culture [83].

3.4. Industrial feasibility of microalgae growth on the production of bioenergy

3.4.1. Factors that influences the microalgae cultivation

The selection of biomass used in the development of bio-products is also directly related to the environmental and economic aspects such as greenhouse gas emissions, energy, and structural costing [84]. The first-generation feedstock (*e.g.* wheat, corn, sugar beet) and second-generation feedstock (*e.g.* agriculture waste, firewood, perennial grass) were not suitable for the generation of bioenergy because they are needed as food supply [85] and require high capital investment on arable land space [86]. The characteristics of biomass are very important for the production of bioenergy as it gives flexibility to researchers in developing multiple ranges of products such as biochar, bio-oil,

Table 3
Pros and cons in using salt water, open pond and wastewater culture media on microalgae cultivation.

Culture Media	Pros	Cons
Salt Water (Oceans)	• Do not need large land allocation. Ideally, seawater can be pumped into the culture medium in the coastal region [59]. • Lower operating costs, as seawater is naturally available [60].	• Requires pre-treatment to remove unwanted compositions which can restrain the microalgae growth. • The pre-treatment processes demand high energy consumptions (NER <1) which leads to higher biofuel production costs [61]. • Rainfall may occur for d and such precipitation into a 20–30 cm depth reduces the salinity of algal culture; Evaporation on hot sunny d will increase water requirement on the algal growth [61].
Fresh Water (Open Pond)	• Lower construction and operating costs; easy to clean [35]. • Do not require high energy inputs for pre-treatment, as elements from the open pond can be fed as nutrients for the cultivation process [62].	• Large land requirement for a considerable biomass yield [62]. • Expose to environmental factors such as sunlight, atmospheric air, and solar heat that can cause contamination and a high risk of contamination [63]. • Poor dispersion of CO_2 to the environment [35].
Waste Water (Industrial/ Domestic effluent)	• Algae purifies the wastewater by using up its nutrients content for its growth [64]. Some contaminants are removed and this enables the wastewater to be recycled back or eases the wastewater treatment process. • Economically sustainable, as most local manufacturers generate wastewater in large amounts which can be utilized free of charge [65].	• Although the presence of nutrients favors microalgae growth exponentially, not all algae species have the ability to adapt in a very high nutrient condition [66]. • Most industrial wastewater consists of heavy metals and pathogens that can defect the development of algae [67], hence there is a need for a pre-treatment process before cultivation. This however leads to an increase in energy consumption and operating cost. • Large variations of wastewater in temperature and pH can hinder algal growth [68].

biogas [87].

Biomass and carbohydrates productivity can be maximized by optimizing the cultivation parameters such as temperature, pH, nitrogen, and carbon source concentration. The maximum biomass of 491 mg/L/day and carbohydrate productivity of 270 mg/L/day, was achieved at initial pH of 6.69, the temperature of 27.65 °C, glucose concentration (carbon source) of 3.33 g/L, and urea concentration (nitrogen source) of 126.77 mg/L [88]. The productivity of carbohydrates by microalgae biomass is essential for the production of bioenergy products such as bio-hydrogen, bio-ethanol, and bio-diesel [31]. By optimizing the cultivation strains, high lipid and calorific value could be achieved on the microalgae biomass which directly contributes to the increase in energy production. The optimization of cultivation growth may differ according to the carbon dioxide gas supply, light intensity, quality of nutrients medium, mixing speed, and the number of days of cultivation period. All these supplies have to be provided continuously during the cultivation period.

Huang et al. (2017) revealed that the light intensity of 60–100 μmol/m^2/s with temperature control between 25 °C - 45 °C provides the optimum microalgae growth for the production of bioenergy [89]. The selection of culture medium can vary but rich nitrogen and carbon nutrient source with an adequate amount of air supply could generate an optimum cultivation condition. The bio-methane yield obtained from pretreated microalgae biomass was much better than the raw microalgae biomass [90]. Hence, it can be derived that high lipid content along with high calorific value indicates an increase in bioenergy potential of the selected microalgae biomass. The idea of mixing/recycling culture mediums during the cultivation process could also contribute to the quality of the bioenergy produced. The mixture of biogas slurry and municipal wastewater for cultivating microalgae yielded a lipid content of 8% higher compared to that of classic BG11 medium [91]. Taking into consideration the benefits that microalgae-based biomass contributes to bioenergy production, pilot-scale investigations on microalgae cultivation and refinery have been initiated to cater to the production of renewable bioenergy [92].

3.4.2. Net energy ratio (NER) for microalgae-based biofuel production

The industrial feasibility of each microalgae biofuel process must be quantified on the metrics of net energy ratio (NER). NER is an important parameter that is used to access the energy consumed over energy produces and also the greenhouse gas (GHG) emissions [93]. A Net-energy ratio larger than 1 means there is a net gain in useable energy whereas a net-energy ratio smaller than one means there is an overall energy loss. Table 5, lists down some literature studies related to the energy balance of different microalgae-based biofuels that obtained more than 1 as a net-energy ratio. NER was calculated by dividing the output energy, E_{OUT} (energy contained in the final product of the

Table 4
Comparisons of *Chlorella vulgaris* biomass productivity for batch, semi continuous and continuous cultivation system.

Culture Medium	Cultivation Method	Biomass productivity of *Chlorella vulgaris* sp. (g/L/day)			Reference
		Batch	Semi- Continuous	Continuous	
BG11 medium	Cylindrical PBR	0.20 ± 0.05*a	N/A	N/A	[69]
Modified Kolkwitz		0.19 ± 0.03*a	0.30 ± 0.03*d	N/A	
BBM	Culture tubes	114.21 ± 0.85*b	N/A	N/A	[70]
Modified BG-11		70.83 ± 0.83*b	N/A	N/A	
Modified *Spirulina*		57.92 ± 2.32*b	N/A	N/A	
BG11 medium	Flat Outdoor PBR	60.46 ± 0.42*c	82.40 ± 3.27*e	N/A	[71]
BBM	Cylindrical PBR	0.06 ± 0.03*b	0.07 ± 0.03 *f	N/A	[72]
	Glass PBR vessel	N/A	N/A	57.00 ± 1.20*g 110.00 ± 2.30*g 126.00 ± 0.80*g	[73]
BG11 medium	Automated bioreactor	84.48 ± 1.80*a	N/A	92.70 ± 1.80*h	[74]
	Membrane PBR	7.30 ± 0.42*a	N/A	42.60 ± 0.42*i	[75]

*a6th day (Stationary phase). *bData on 12th day. *cData on 15th day. *dData on 21st day. *eData on 20th day. *fData on 32nd day. *gData on 55th day. *hData on 13th day. *iData on 16th day.

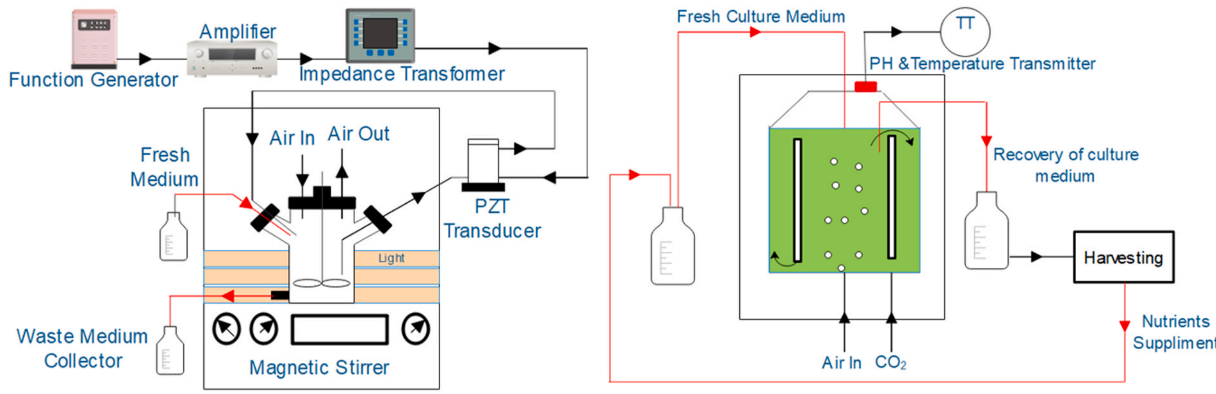

Fig. 3. Schematic diagram of (a) RUF-DPBS PBR system (b) PBR design with recycling of culture medium.

Table 5
Net-Energy Ratio, NER ≥1 for microalgae biofuel production.

Microalgae based biofuels with NER≥ 1	Description	References
Biogas and Bio-oil	• The energy requirements for biomass cultivation, harvesting, and dewatering. • The thermochemical results showed the range of energy efficiency for gasification to be 16.80% (char), 56.40% (bio-oil), and 34.10 (gas); pyrolysis: 14.60% (char), 55.90% (bio-oil) and 59.90% (gas); and finally for torrefaction to be 14.40% (char), 38.90% (bio-oil), and 14.30% (gas).	[95]
Biogas and Biodiesel	• Nutrient recycle approach plays an integral role in achieving favorable net-energy ratio and greenhouse gases, GHGs. • The increase in lipid content increases the NER of the system by 20% due to a decrease in the amount of mass going to the anaerobic digester.	[96]
Biodiesel	• Three processes are evaluated which are hydrothermal liquefaction (HTL), oil secretion, and alkane secretion. • Biodiesel from HTL generates a net energy ratio of 1.99; wet lipid extraction at 1.66. • HTL can be considered as an alternative to wet lipid extraction.	[97]
Bio-oil	• The study includes cultivation, harvesting, cell pre-treatments (cell disruption, drying, grinding), lipid extraction, transesterification, gasification and hydrothermal liquefaction with bio-oil stabilization, and hydro processing. • Positive energy balances with a NER value of 1.11 (open raceway ponds) and 1.14 (closed PBRs).	[98]
Biogas	• An up-flow anaerobic sludge blanket, UASB reactor followed by a high-rate algal pond in terms of sewage treatment efficiency and biogas production. • The system showed a positive energy balance, with 70–180% more energy produced than consumed throughout the year.	[99]
Bio- Methanol (transportation fuel)	• Microalgae cultivation and treatment, transport of dry microalgae, methanol conversion, transport of methanol, methanol combustion are included in the evaluation. • It is reported that the energy conversion efficiency of fuel methanol is 1.24.	[100]

system) by the Cumulative Input Energy, E_{IN} of the production process as shown in Equation (1) [94].

$$NER = E_{OUT} \Big/ \sum E_{IN} \tag{1}$$

The scenario of microalgae-to-energy with NER ≥1 will provide energy for the whole manufacturing chain, with a significant reduction in GHG. The energy obtained will be during the direct microalgae biomass combustion process an analysis comparing NER value for three separate energy generation routes from microalgae biomass for transportation purposes; 1) biodiesel and electricity production from biogas, 2) biodiesel and electricity production from biomass combustion and 3) electricity production from biomass combustion alone. Hence, it was concluded that microalgae lipid extraction is an energy-intensive process with most NER for the third route (direct combustion of microalgae biomass for the production of electricity) [101,102]. This demonstrated that microalgae-to-energy technology could become economically viable and environmentally sustainable. The energy production from microalgae biomass has achieved a favorable energy balance analysis. The influence of country electricity grids on GHG emission and NER over the microalgae life cycle analysis were decisive compared to fossil fuels [103]. Thus, there are large opportunities for the commercialization of microalgae-based products.

3.5. *Challenges faced in pilot scale microalgae cultivation process*

The commencement of a pilot-scale plant for mass production of microalgae comes with several challenges, one being the effects that affect the growth of biomass such as light modification (LED), nutrients (flow rate and timing), harvesting rate, and temperature control [104]. In terms of energy analysis, it is proven that most powers are consumed for optimization of light distribution and proper culture mixing as it involves both mechanical and electrical energies regardless of all sorts of PBR designs [105]. Apart from these, the operation of continuous data monitoring PID (proportional–integral–derivative) controller also demands energy input. The use of Easy JAVA Simulations, which initiate the learning on manipulation of an essential variable to optimize a PBR design for efficient microalgae growth [106]. This approach can be utilized for a large-scale PBR design facility to avoid any sort of energy wastage.

A pilot-scale study on continuous recycling of growth medium for mass culture was performed in an open raceway mixed ponds under increasing salinity. After analysing the data for 5 months, it was concluded that microalgae (*Tetraselmis MUR 233*) can be grown continuously in recycled medium without any reduction in biomass productivity with minimal freshwater input [107]. Peak productivity of 37.50 ± 3.10 $g/m^2/day$ was achieved, and it is claimed to be cost-efficient by minimizing the usage of freshwater and nutrient supply. Experts had a rough time extending the development of microalgae in the continuous phase as the concentration of nutrients in the recycled culture medium varied with time which also leads to an inconsistency of biomass.

In Japan, a pilot plant of 1 ha open pond (1000 cm^2 x 10 ponds) implemented continuous mode cultivation using flue gases and wastewater as nutrient sources simultaneously. The production capacity was documented for 350 days of operation and 15 days off for yearly maintenance and cleaning [108]. In terms of economic feasibility, the sensitivity analysis was performed based on the microalgae biomass productivity and it was observed that the process cost reduction could reach down to 100 Japanese yen per litre for 50,000 mg L/m^2 of biomass productivity with 50% of lipid yield percentage of dry weight [108]. However, the challenges faced during the continuous operation of microalgae operation were the build-up of suspended solids (SS) in the wastewater that leads to an unbalance N/P ratio, low biomass density, the requirement of pre-treatment/sedimentation pond [109].

Since microalgae can take up CO_2 in the atmosphere, a pilot-scale microalgae cultivation for biomass production using exhaust gas from thermal power plants was conducted using a multi-stage PBR. The direct injection of exhaust gas is the most suitable condition as compared to the injection of an unutilized gas from the previous PBR. This investigation was also carried out in both batch and semi-continuous mode. It was reported that the best results were obtained at multi-step reactor cultivation where the PBRs were performing semi-continuous mode cultivation by connecting the systems in series [110]. The batch growth took place for 10 days with biomass of 0.50 g/L, whereas the semi-continuous growth occurred for 24 days producing 0.57 g/L of microalgae concentration. Table 6 lists down the top four challenges that need to be considered when a cultivation process is scaled up to a pilot-plant. Although a promising number of microalgae are cultivated by a semi-continuous approach, the challenges faced in pilot-scale study were the control of external influence such as temperature and operating cost in producing electricity-based light intensity. Apart from the development of culture medium-based technologies, the technical PBR designs are also equally important to initiate an efficient microalgae cultivation process.

Large scale microalgae biomass production also leads to the demand for frequent monitoring of temperature and pH of the culture medium and daily sampling for microalgae productivity which analyses the product consistency [111]. Moreover, the ASTM (American Society for Testing Materials) published biodiesel standards, and the microalgae biomass-based biodiesel are obligated to have similar properties to the standard biodiesel before market distribution [112]. Microalgal biodiesel has a high cetane number and several other beneficial properties, thus the downstream process must be maintained at all times. Besides, the separation of algae biomass from excessive water for harvesting remains a hurdle industrial scale. There are several harvesting methods such as chemical-based (chemical flocculation), mechanical-based (centrifugation process), and electrical-based (electric field repulsion between cells) [44]. The challenge relies on selecting an optimized separation method in terms of energy and cost-saving. Other downstream challenges will be the storing of bio-diesel as fuel that is susceptible to bacterial oxidation which could cause internal corrosion of the storage tanks. Hence, a high-quality large-scale anti-corrosion storage tank will be required in the case of large-scale production [113]. The proposed solution will be an immediate conversion of bio-diesel to energy supply, which can be supplied back for the cultivation process. By doing so, a positive NER can be initiated.

Table 6
Possible challenge to consider in a large-scale cultivation process.

Challenges	Description
Selection of material for PBR tube	• Transparent tubes impose riskier conditions in terms of contamination. • Plastic and glass materials are difficult to sterilize in large volumes from bacteria, viruses, and fungi.
Geographic region	• Requirement on a wide land ground space with a continuous large amount of CO_2 gas, nutrients (wastewater/seawater) source, and water supply.
Economical	• The balance of operating cost (electricity, energy) with microalgae-based bioenergy selling price has to make a profit. • A techno-economic analysis is compulsory taking into consideration the breakeven time.
Process control devices	• Development of equipment specifically for handling large volume microalgae broth for biomass growth monitors, contamination detector, and parameters controller will be necessary.

4. Recent technical studies of PBR operations

4.1. Novel PBR operations for bioenergy production

A novel flat-panel PBR concept was developed with a liquid foam bed that allows cost reduction in continuous microalgae growth for the production of biofuel as shown in Fig. 4 (a). Fresh bicarbonate (HCO_3)-enriched medium was continuously pumped from the bottom of the reactor to the top layer of the foam column throughout this process. Gravity will allow the fresh medium to drain down into the foam until it hit the peak. This framework controls the distribution of algae in the PBR, allowing an effective foam mixing [114]. Flat-panel PBRs do not demand high energy input as the gravity force plays its role during the system operation. Janoska et al. (2018) concluded that liquid foam-bed technology requires 0.23 kJ of energy for the production of 1 g dry biomass and the total harvesting energy was only 8.5% of conventional flat-plate PBR design (0.23 vs 2.72 kJ/g) [114]. Increased gas transferability and a reduction in liquid volume beneath the foam column could boost biomass productivity while lowering energy consumption for bioenergy production [115].

Vargas et al. (2017) conducted a pilot-scale study on the combination of continuous flat plate PBRs in series by allowing constant circulation throughout its cultivation and recirculation of the last unit back to the first unit mixed with some fresh medium [116]. The challenge faced in this operation was in the maintenance of mass balance and interactions between the light intensity, growth kinetics with mass transfer. A mathematical model was developed by splitting linear and non-linear equations to ease the analysis. In short, the overall series PBRs continuous design provides better results compared to a single unit of PBR system in terms of biofuel purity as the biomass produced from series PBR has very little biomass contamination. Another new design of PBR was also introduced equipped with a membrane, as shown in Fig. 4(b) [75]. Membranes may act as a filtration tool that purifies the culture medium before entering into the cultivation system, and by doing so the pre-treatment energy and cost consumption can be reduced. The implementation of MPBR design allows independent control of hydraulic retention time (HRT) and biomass retention time (BRT) which benefit the cultivation process by producing higher biomass productivity and nutrients removal rates [117]. Singh et al. (2012) demonstrated that MPBR utilizing Hydrophilic PES membrane-type could remove 50% of ammonium, 75% of nitrite, 35% of nitrate, and 60% of phosphorus under continuous culture (23 days) [118]. An investigation of the influence of sludge retention time (SRT) on biomass productivity using MPBR observed that the biomass productivity was improved when SRT decreases from 350 days to 2 days [119]. In addition, Solmaz et al. (2019) also prove that the optimal microalgae production was achieved at 0.12 g/L day^{-1} for an SRT of 3 days with constant 24 h HRT in an MPBR (hollow fiber membrane) [120]. Thus, SRT can be considered as a tool to optimize biomass productivity, especially for continuous PBR systems.

Velea et al. (2014) has developed a new hybrid PBR by combining the concept of flat PBR panel with enriched CO_2 gaseous transfer [121]. The combination has reinforced the surface to volume ratio of the PBR and the installation of bubble columns in series for CO_2 supply which has

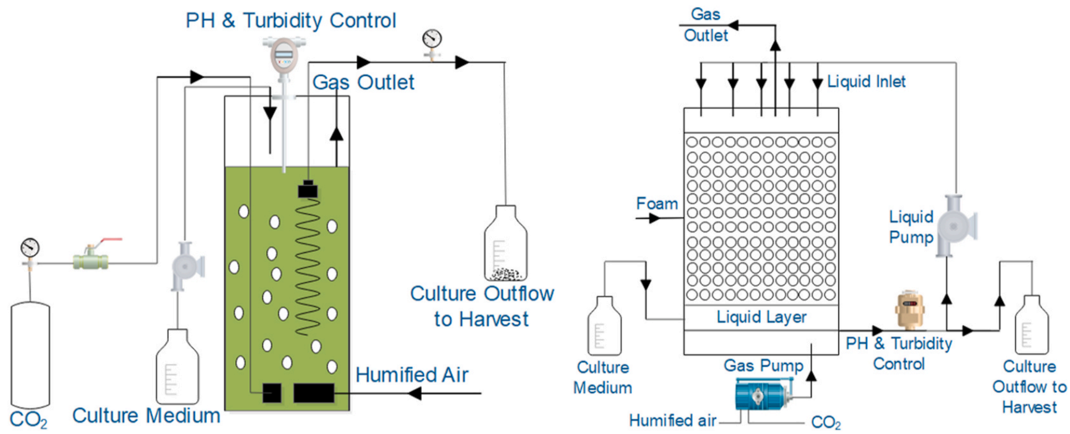

Fig. 4. Illustration of the PBR Design (a) Flat Panel (b) Hollow-fibre microfiltration membrane.

achieved high biomass productivity. Since the installation is done in series, there will be extra control devices such as gas flow control, pH indicator, turbidity sensor, pumps, and lighting source compared to flat-panel and MPBR systems. Hence it was reported that the requirement of energy inputs and cost can be compensated with the efficiency of biomass productivity According to the performance analysis based on biomass productivity, a hybrid PBR could achieve three times higher biomass concentration (lipid and carbohydrate composition) than the standard open flat plate PBR [121]. The kinetic data also proves that hybrid type PBR tends to mitigate the effects of CO_2 by reducing the emission of flue gas. Bahadur et al. (2013) developed a continuous solarized airlift PBR design to investigate the growth ability of microalgae in a simulated condition [122]. The hydrodynamics properties of the PBR could influence biomass productivity and liquid circulation velocity. The hybrid PBR is better than the conventional design PBR as the novelty of the design is the application of both LEDs and fluorescence tube light [123]. This is because solar energy does not provide a consistent amount of heat and light sources throughout the cultivation phase. However, artificial light may demand a continuous supply of electrical energy which costs lower than the installment of solar panels. Briassoulis et al. (2010) studied the continuous production of *Nannochloropsis* sp. at various color light beams (LED) using a helical-tubular PBR. The study mainly focused on investigating the relationship between the ratios of culture volume to the surface area while optimizing the light penetration into the culture medium [124]. It was observed that green light beams with low voltage produce cell concentrations at their maximum compared to that shown by red, blue, and infrared light beams. Moreover, the green wall flat panel increases its lipid productivity by 60% *via* microalgae biomass grown in nitrogen deprived medium [124].

Oxygen gas produced as a by-product of the cultivation process could inhibit the growth of microalgae, especially on an industrial scale. Sawdonet al., (2015) developed an internal deoxygenated tubular PBR to cultivate microalgae [125]. The internal deoxygenated tubular PBR allowed perfluorocarbon emulsions (PFC), where the supply of CO_2 will be slowly pumped into the reactor while the removal of accumulated oxygen occurs simultaneously. The study concluded that the PFC function has reduced the oxygen concentration from 47% to 5% and give a positive impact on the microalgae growth rate with only 9 days of cultivation. Apart from these, soft frame designed PBR was invented on lab scale to investigate its biomass productivity as well as its energy and cost-efficiency [126]. The novelty of this design is that water and nutrients were supplied by the capillary action. The proposed design does not demand energy for CO_2 enriched air supply and mixing of culture medium where most energy could be saved. Jones et al. (2017) investigated a wave PBR for its mixing and mass transfer of the microalgae during the cultivation process and its effect on the microalgae biomass productivity [127]. The wave PBR demonstrated a sustainable and positive net-energy ratio (NER $\geq$1) result in terms of energy consumption (power input for tank's stirrer to the calorific value of microalgae biomass (lipid) [127].

4.2. Studies on the feasibility of pilot scale continuous PBR system

A preliminary lab-scale PBR design cannot justify its feasibility hence pilot-scale studies are required to carefully assess the designs. A pilot study provides a detailed insight into the design in terms of time, cost, energy, and sustainability, which can be useful for future enhancement. Park et al. (2019) conducted a feasibility study for biomass productivity by combining five PBR in series [110]. The overall biomass productivity and lipid extraction content was achieved as expected, although there was an inconsistency of CO_2 supply during the growing phase on a large scale. Thus, it was reported that microalgae-based biomass is a suitable feedstock for biodiesel production by absorbing CO_2 from thermal power plants during the cultivation process in on large scale. Furthermore, Gases et al. (2018) studied the sizing and design of the cultivation process equipment were fabricated to identify a practical physical evaluation of production feasibility cost with the aid of process simulation software [108]. By performing energy balance on a large scale, its economic analysis could be carried out and concluded that technology improvement is also a factor to be considered in achieving the operational feasibility and profitability process. This is because, simulation of the belt filter press drying method utilizes less energy for drying compared to the centrifuge or flocculation method. Ketheesan et al. (2012) investigated a 23 L airlift-driven raceway reactor to study its feasibility in both batch and continuous mode of microalgae cultivation [128]. The feasibility of this study was determined based on the biomass productivity achieved with energy-efficient productivity. The results obtained stated that airlift-driven PBR could produce 0.60–0.69 dry g/W/day which is higher than other conventional PBR designs reported. This power output can be transmitted for the biofuel production process.

One of the benefits of pilot-scale studies is their capacity to amplify the impact of minor variable changes in a big process. This is because the development of algae was at its best in a 1 g/L of glycerol in wastewater compared to the all the other combinations up to 15 g/L [129]. This helps the researchers to further investigate the effect of glycerol on nutritional removal efficiency for large-scale wastewater mediums. Microalgae species (*Graesiella* sp) were tested at the lab scale, and concluded that laboratory screening alone is not sufficient to identify the culturing ability [130]. The study was proceeded further using 10 L and 30 L tanks of bubbled column PBR and it was discovered that *Graesiella* sp could adapt to the pilot scale and still produce 12.03 g/m^2/day of biomass. Min et al. (2011) investigated a novel system that uses a viscous wastewater stream to produce microalgae biomass [131]. This is specifically to prevent sedimentation, which has a significant impact on lab-scale outcomes. Hence with the direct flow of the culture medium, it was noticed that the activation of bacteria in the thick medium is vigorous and interruptions the growth of microalgae. Although CO_2 is injected to depress the bacteria, biomass productivity and nutrient removal in the organic carbon-rich medium were not as expected. It also impacted the lipid and protein content in the biomass produced at the end of the growth phase which impacts the biodiesel yield as well. Thus, the system demands a pre-treatment phase before the harvesting and extraction process occurs. It can be concluded that large scale implementation could benefit researchers in knowing the obstacles that could occur before introducing a cultivation process concept into the industrial scale.

4.3. Consideration and technical challenges of designing PBR for continuous mode bioenergy production in large scale

This segment covers the design operation of PBR which is essential as it plays a major role in the efficiency of microalgae biomass production. The interaction between fluid mechanics, radiation exposure, species transport, and biomass growth kinetics has to be considered during the design of PBR [132]. Arabian et al. (2017) investigate the relationship between the diameter and number of spargers to the fluid dynamics performance of PBR [133]. The study concluded that by increasing the diameter and number of spargers of PBR, the fluid velocity would be increased over time, and a reduction in dead zone percent could be achieved. Additionally, there will be no extra energy required for extra spargers to rotate within the PBR. Thus, the diameter and number of spargers can be manipulated for an efficient cultivation process without increasing the overall energy input. Fluid dynamics cannot be neglected during cultivation especially after the CO_2 is injected into the PBR as it will create an impact due to the flow pattern inclusive of bubbles [134]. Many industrial researchers face technical difficulty in scaling up their lab-scale discovery especially when the biomass yield obtain *via* lab-scale could not be achieved on a large scale. The dimensional factor of design during scale-up is not directly proportional as it also involves biological processes. Hence, the Buckingham π-theorem was proposed to be applied at the pilot scale. The theorem is typically used by industries to perform the scale-up of their process and Paladino., et al. (2019) concluded that it could also be used for microalgae growth in PBRs due to its generalization qualities [135].

Apart from these, light and heat exposure during cultivation also play an important role in producing optimal algae growth. Hence, a continuous feedstock PBR with an arrangement that controls the heat flux from solar thermal radiation was designed [136]. It was noticed that the algae cultivation media temperature is affected during the daylight by analyzing the model and data obtained. The data obtained justifies that the cultivation medium temperature distribution decreases by increasing the width and medium height of the PBR. In terms of light distribution, an indoor PBR is always preferable considering that light can be exposed to the cultivation process both day and night. A modified pyramid-shaped PBR was studied using two sources of light distribution on both inside and outside the reactor [137] with blue, red, and white LED lights. It was also recorded that the internal light had a significant effect on photosynthesis to occur which leads to better biomass production than the external light source. The research also discovers that external LED sources consume 33% more electrical energy compared to internally installed LED lights, and the LED light colors do not have a significant impact on energy consumption. Thus, an internal immersed LED light PBR design was proposed [138]. This technology improves the light distribution and can achieve efficient light energy transfer *via* the surface to volume ratio with minimal heat generation. Hence, it was concluded that the higher surface illuminated, the higher the generation of cell concentration inside the PBR, which also increased the volumetric productivity of microalgae. The development of PBR microalgae cultivation is still in the evolution phase, thus there are several challenges faced in large-scale cultivation which will be discussed in the following section.

5. Overall challenge faced in large scale biomass production processes

Many promising studies and technologies are being developed to produce a sustainable method for cultivating microalgae that can enhance biomass production and energy output. However, the shift towards large-scale studies is ongoing and yet to be established due to the high cultivation cost in a closed system. Recently, the development of an efficient bioreactor design was suggested to improve the profitability of large-scale cultivation with a high yield of bioenergy production [139]. The optimization in a PBR design in terms of cost efficiency especially for large scale will be mainly on the light regime, selection of cultivation medium, and material used for the wall construction [140]. Another major challenge to consider during the scaling up of the cultivation process is the selection of closed or open system configuration. The drawback in an open system is the difficulty in having an accurate control of CO_2 supply, and light source distribution although it is available naturally which makes the system to be cost and energy-saving [141]. For a closed system, contamination, and water loss through evaporation can be avoided and also provide excellent control of light, oxygen, and temperature control by compensating the capital investment [142]. However, there will be an additional cost demand to supply the electrical energy required to keep control of the cultivation process.

The concept of using microalgae biomass as a source for bio-energy production is considered to be ecological. However, the implementation of its process flow is also essential to make it an environmentally friendly approach. The cost factor of energy also plays a role in making bioenergy production worthwhile. The cost of biofuel production from microalgae currently outweighs the cost of petroleum fuel. Despite the environmental advantages, it is difficult to encourage industries to adapt this renewable source for fuel production as profits are ranked as a priority in any business model. This hurdle can be reduced with the implementation of a biorefinery concept, which allows the production of more than one bioenergy source and other bioproducts. This along with the cost savings in wastewater treatments or carbon sequestration will provide producers with more options to cut costs and possibly generate a better profit margin from biofuel. It was reported that the CO_2 supply for the cultivation to occur can be used as a carbon source, which not only lowers the operating cost but also contributes to the benefit of CO_2 mitigation [143]. Apart from these, water supply for continuous cultivation using PBR can also be a challenge to overcome in large-scale operations. Martins et al. (2018) reported that 60% of the water consumption is needed during the cultivation process in a closed pilot-scale multi-tubular PBR as it is associated with electricity, nutrients, and cleaning agent [144].

Energy consumption plays a major challenge factor in large-scale cultivation operations, especially for liquid and gas pumping, artificial light, and spinning of spargers continuously throughout the process which promotes the use of microalgae cells to generate bioelectricity [107]. For continuous wastewater treatment and electricity generation, a device containing a sequential anode-cathode configuration microbial fuel cell and a PBR was developed [145]. The effluent from cathode was used for microalgae cultivation, effluent of anode was enriched with electro-active microbes that generate bioelectricity which will be transmitted as power *via* a sequential microbial fuel cell. Thus, it is proven that bioelectricity produced from microalgae can be re-used back into the PBR system to initiate a NER $\geq$1.

The cost of total algae biomass is 30% greater than the cost of lignocellulosic biomass, due to the increase in operating costs [78]. Hence, placing the PBR outdoor will be a good idea to reduce energy consumption [146]. The sunlight exposure would provide light intensity naturally to the microalgae culture where artificial light and heating are not required, enabling the possibility of achieving positive energy efficiency compared to indoor PBR. However, due to the uncertain weather conditions, an indoor PBR could be preferred under different circumstances. For indoor PBR, optimizing the position of the light is significant to model it to an energy-efficient system [147]. LED lights are favored because they can produce sufficient photon fluxes of a specific wavelength which can cut out unnecessary spectrum segments and be switched off periodically. The focus towards optimizing the LED light efficiency in terms of energy savings is increasing. Several studies have been conducted to study the effect of various LED light wavelengths in terms of energy efficiency by immersing it into the culture medium [137,148].

6. Future work and prospects

The process of microalgae cultivation is widely studied but there is still plenty of room for improvement especially when it comes to the design of a PBR. A greenhouse construction of solar PBR that could be in build on a rooftop of a laboratory was proposed [149]. However, further study is required in terms of consistency throughout the year to implement at an industrial or household scale. The availability of sunlight may differ according to the contingency weather or various regions. Combinations of the bioremediation process with microalgae production also give an enhancement in the wastewater treatment technology and also produce biomass with higher energy content [7]. This core idea can be further developed by identifying the optimized combination for both wastewater treatment technology and cultivation of microalgae to cater for bioenergy production. Apart from this, the geometrical shape of the PBR can also contribute to the overall microalgae cultivation efficiency [150]. One of the factors to consider when exploring PBR design is its space limitation that affects how the culture can be supplied and collected from the system. An octagon-shaped PBR has demonstrated a positive result in terms of microalgae biomass productivity *via* continuous process cultivation [151]. This design has also been established at an industrial scale with a 25,000 L capacity that produces 15,000 to 40, 000 tonnes of biomass annually. The structure of the design can be further developed such that the microalgae will contain more desired compounds for bioenergy and biofuel production.

Nevertheless, monitoring of microalgae cultivation in PBR can be digitalized by integrating colour acquisition with artificial intelligence, AI. Artificial neural networks (ANN) were used to track algal density precisely using a single fluorescence emission spectrum measurement and back-propagation ANN Optimized by Genetic Algorithms (GA-BP model) [152]. The model was validated by estimating the microalgae cell concentration using samples from various growth batches. Future research can be conducted in terms of developing colour acquisition model studies that can predict the microalgae cell concentration growth by analysing the colour thickness of its culture. Moreover, the relationship of culture medium concentration and biomass growth can be modelled using a feed-forward back propagation neural network (FBN) [153]. As a result, the impact of multivitamin supply in the culture medium can be predicted in advance, making the PBR control mechanism for the cultivation process efficient. It is suggested to develop, a data-driven response model for all the other cultivation conditions such as pH, light intensity, CO_2: air source, nitrate concentration that allows an outdoor large scale PBR operation. Computer vision and AI algorithms have the ability to enhance the efficiency of cultivation and conversion of microalgae by reducing the number of experiments and conditions optimization.

7. Conclusion

This review discusses the energy recovery technologies designed for microalgae cultivation using a PBR system. The limitation in fossil fuel sources has initiated the studies of microalgae-based products especially in the production of bioenergy. The microalgae cultivation process can simultaneously reduce greenhouse gas emissions by consuming CO_2 and produce valuable bioproducts and bioenergy, which relatively contributes towards a balanced ecosystem. Biofuels produced by microalgae biomass release about 10% less CO_2 and 30% less SO_2 gas compared to other current commercial fuels. Thus, various design of the continuous flow PBRs was evaluated to find the suitable design that incorporates energy and cost-saving elements while producing high biomass yield. Higher yield of microalgae biomass will also gather more lipid and carbohydrates composition which is essential for the production of bioenergy. However, to proceed with large-scale processing, the continuous production of microalgae biomass may show complexity in the process due to several limiting factors.

The selection of culture mediums source and breakdown of the operating energy cost such as electricity, water, and light supply are a few of the limiting factors discussed. Pilot-scale studies that can produce 50% of lipid yield percentage of dry weight face difficulty in operating a continuous cultivation process due to the build-up of suspended solid from its untreated culture medium. These issues were only notable during the large-scale operation and the discoveries have been reviewed. With the recent expansion of artificial intelligence research, the wide-ranging operating condition and data from the cultivation process can be analyzed quantitatively. Future smart factory digitalization advancements would undoubtedly speed up the cultivation process for immense biofuel production. The role of culture medium and PBR designs are essential to developed a cost and energy-saving microalgae cultivation technology for bioenergy and bioproducts generation.

Credit author statement

Angela Paul Peter: Conceptualization, Critical Thinking, Writing - original draft. Apurav Krishna Koyande: Investigation, Visualization. Kit Wayne Chew: Conceptualization, review & editing. Shih-Hsin Ho: Review & Editing. Wei-Hsin Chen Review & editing. Jo-Shu Chang: Review & editing. Rambabu K: Review & Editing Fawzi Banat: Review & Editing. Pau Loke Show: Validation, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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