Biomass Conversion and Biorefinery (2024) 14:7219–7236
https://doi.org/10.1007/s13399-022-02783-9

REVIEW ARTICLE

A concise review on the cultivation of microalgal biofilms for biofuel feedstock production

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Received: 5 February 2022 / Revised: 26 April 2022 / Accepted: 3 May 2022 / Published online: 17 May 2022

Abstract
The enormous capability of microalgae for biomass production and co-products has recently been widely researched from a range of research approaches. Microalgae biomass has been discovered as a suitable feedstock for biofuel generation in the third generation. Although they may easily be cultivated in the laboratory, commercial cultivation involves several important considerations, including design, expense, contamination risk, and hygiene. This paper reviews some conventional microalgal cultivation methods along with some harvesting techniques. A short note on the disadvantages of conventional microalgal biofilm cultivation and the need for advanced cultivation techniques are also listed. Further, it highlights some of the modern techniques used for the cultivation of biofilm-based microalgae. It also gives brief information on the various factors affecting the formation of microalgal biofilm. A detailed description of the application of microalgal biofilm concerning biofuel generation is also reviewed.

Keywords Microalgal biomass · Biofuel · Bioethanol · Biodiesel · Photobioreactor · Biohydrogen · Biomethane

1 Introduction

Microscopic organisms adhere to any solid surface or substratum to eventually colonize and form what is called biofilm. Biofilms are themselves life forms. Researchers tempt to focus on microalgae due to their efficacy in wastewater treatment and the pharmaceutical industry [1]. Because of their rapid growth rate and high biomass yield, microalgae biofuels are seen as a possible alternative energy source to replace limited fossil fuels. Microalgae can also accumulate lipid and carbohydrate within their cells, which can be

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turned into biodiesel and bioethanol, respectively [2]. Microalgae cultivation is necessary for biofilm production.

Conventional techniques involve dispersing the microalgae in a liquid medium [3]. These methods, however, suffer from various limitations, such as lower biomass yield, cumbersome harvesting of biomass and retrieval, increased cost of installation and functioning, and a greater necessity for water. The other major drawback of using conventional cultivation method involves difficulty in maintaining sterility and high chances of contamination of cultivated algae [4]. Moreover, these methods cannot be used for production of microalgae on large scale as it is difficult to grow specific algal cultures for extended periods with consistent quality. Hence, microalgal biofilms prove a pioneering strategy where microalgae are cultivated to produce biofilms[4]. This methodology, thus, overcomes the limitations of conventional frameworks of cultivation [5].

Biofilm-based cultivation of microalgae has garnered considerable attention as a viable platform for algal development as well as other uses like treating wastewater [6]. Conventional suspension systems have not yet to prove its economic viability, so algal biofilm cultivation technologies offer an alternative. One of the most significant benefits of algal biofilm systems is that algae may be harvested simply by scraping, avoiding the costly harvesting techniques employed in conventional suspension-based harvesting including centrifugation and flocculation [7]. Microalgae biofilm is an immobilized cultivation method that yields energy, as well as several environmental advantages, including bioenergy generation, nutrient retrieval, and carbon sequestration [8], while simultaneously improving the final yield of biomass and algal cell density [9, 10]. Microalgae also offer tolerance to growth stresses, higher cell density, and economic feasibility concerning harvesting and concentration [11, 12]. Another advantage of microalgae biofilm is their multi-layer construction involving horizontal, vertical, and rotating configuration, enhancing the yield per ground area and the efficacy of ground use [7, 13, 14]. Biofilm reactors are developed to treat wastewater by utilizing microalgae [15]. Mixotrophic cultivation is preferred because algae nurture in autotrophic and heterotrophic conditions when an appropriate amount of sunlight, inorganic and organic carbon compounds are supplied [16]. The main objective of this study is to analyze a new and innovative cultivation approach called a mixotrophic microalgae biofilm, for the economic cultivation of algal feedstock [17, 18].

Microalgal biofilms are investigated from both technological and ecological characteristics. Implementation of these biofilms in aquaculture, wastewater treatment, and upgrading of antifouling chemicals are a major scientific aspect in the current society [19, 20]. As every technology intends to have some negative impact, microalgal biofilm too suffers from fouling. However, knowledge in this aspect is still a mystery. Extended probes need to be more rivet on fouling including mitigation strategies in near future. The following study intends to revise the current literature regarding microalgal biofilms as a novel technique. Current investigative research aims to summarize the current state of known research data on the significant features and synergistic properties of biofilm formation in either marine or freshwater environments.

This extensive literature review primarily concentrates on the various conventional cultivation strategies of microalgal biofilms along with their advantages and disadvantages. Further, it highlights recent techniques used for the production of microalgal biofilms and their advantages over traditional techniques. It also describes different factors obstructing their applications, mixotrophic cultivation of microalgae with a detailed description of the requirements, protocol, and statistical analysis. Algal biomass yield, impacts of cell-surface characteristics upon the exploitation of microalgae biofilm, algal feedstock quality, and growth metabolism of microalgae biofilm in wastewater are a few other topics covered. The significance and implementation of microalgal biofilms with the scope of their efficiency in the future are reviewed in this paper.

2 Basics of microalgae

Microalgae are generally unicellular microorganisms with the potential to aggregate, which allows them to develop various cell structures including unicellular, colonial, and filamentous. Microalgae have recently attracted scientific interest after being identified as a raw material for the processing and manufacturing industries. These bacteria appear to have a variety of advantages. Fourth-generation biofuels, fertilizers, aquaculture feed, nutraceuticals, and wastewater management are some of their applications.

Due to the advantages listed below, microalgae production is preferred over other terrestrial plant cultivation.

a) Their chemical structure varies depending on the media in which they are grown. This is because they have large biomass biodiversity [21].
b) They do not compete for agricultural land with terrestrial plants because they do not require land for their cultivation [6].
c) Wastewater can be used to meet their nutritional and water requirements [22].
d) There are no seasonal restrictions on their cultivation, and certain species may persist in harsh environments. They double their biomass in a couple of hours, resulting in a significant increase in production [23].
e) They vitiate the environmental eco-dynamics less because they use fewer pesticides and fertilizers [24].
f) They could also provide the added benefit of phycoremediation by removing contaminants (like nitrogen, phos-

phate, and heavy metals) from liquid waste streams and CO_2 from the atmosphere [25].

g) Some microalgal species produce more bioenergy per square metre than traditional oil crops [26]. Microalgal species are regarded a viable feedstock for bioenergy generation due to their comparatively high lipid content. Because the lipids obtained from microalgae are chemically comparable to traditional vegetable oils, they have been demonstrated as a potential bioenergy source. For instance, in one of the studies, algal culture technologies such as raceway ponds, vertical tank reactors (VTR), and polybags were investigated for large production of algal consortia using carpet industry (CI) untreated wastewater,. Polybags (21.1 g m^{-2} day^{-1}) had the highest overall areal biomass yield, followed by VTR (8.1 g m^2 day^{-1}) and raceways (5.9 g m^{-2} day^{-1}). Using 20 and 30 L capacity polybags with triple row arrangement, a biomass productivity of 51 and 77 t ha^{-1} $year^{-1}$ may be attained, respectively. Proteins accounted for 53.8% of the biomass recovered from the algal consortium, while carbohydrates (15.7%) and lipids (5.3 %) were low. A polybag-grown consortium showed the capacity to produce 12,128 m^3 of biomethane per year [27]. Table 1 shows various algal species used for the production of bioenergy.

In the last few decades, there has been a lot of study towards making biofuel from microalgae biomass. Microalgae, being autotrophic organisms, synthesize organic macromolecules such as lipids and carbohydrates that can be processed into biofuel using solar energy and atmospheric CO_2. As a result, algae have the potential to supply a carbon-neutral fuel with hardly any inputs. Some microalgal species can be grown heterotrophically for increased productivity, however this necessitates the addition of carbon feed. Microalgae are of particular interest because some strains produce significant amounts of oil, primarily the glycerolipid triacylglycerol (TAG), which can be transesterified into biodiesel [33]. Microalgae's high oil generation means that much less land would be required to generate the same amount of biofuel as oil crops like soybean or oilseed [34]. Microalgae are also intriguing as some strains have the potential to create hydrogen, which might be employed as a fuel source. Furthermore, some microalgae, particularly *Botryococcus braunii*, can produce significant concentrations of unique triterpenic hydrocarbons that can be easily turned into fuels using traditional hydrocracking and distillation methods [35]. In conclusion, microalgae can produce 58,700 l of oil per hectare and 121,104 l of biodiesel per hectare, making them a promising alternative to conventional fossil fuels [36].

3 Cultivation of microalgae

Microalgae biomass has been discovered as a suitable feedstock for biofuel generation in the third generation. Although they may easily be produced in the lab, commercial cultivation necessitated several important considerations, including design, cost, contamination risk, and cleanliness. Microalgae naturally develop in lakes, rivers, and oceans, but such ecosystems are unsuitable for large-scale extraction due to low biomass densities. Recently, 23.8 million wet tons of algae were farmed to meet commercial demand [37]. Organic and inorganic chemicals have been widely used as viable nutrient media for extensive microalgae cultivation. However, its use is hindered by its high cost and environmental hazards, which may make large production of microalgae biomass impractical. As a result, organic fertilizers and wastewater from the home and industrial runoffs have been offered as low-cost nutrient mediums for successful production.

Microalgal cultivation is categorized into five different metabolic pathways. Photoautotrophic cultivation is mainly based on the growth of photoautotrophs for the synthesis of biofuel. Photoautotrophs are organisms that generate organic compounds using light energy and inorganic carbon. Photosynthesis is carried out by all identified photoautotrophs. Plants, algae, and cyanobacteria are the best examples. A microdroplet photobioreactor is used for the culture of photoautotrophic algal cells [38]. Heterotrophic microalgae can thrive in the dark by consuming organic substances as the sources of carbon and energy [39]. Heterotrophic culture promotes lipid accumulation and would be advantageous in producing biodiesel. Mixotrophic cultivation is intriguing

Table 1 Microalgae commonly used for the production bioenergy

Sr. no.	Algal species	Cultivation technique used	Product	Reference
1.	*Ostreococcus tauri*	Photobioreactor	Oils	[28]
2.	*Dunaliella salina*	Brackish seawater ponds	β-Carotene	[29]
3.	*Chlamydomonas reinhardtii*	Photobioreactor	Oils, carbohydrates, hydrogen, and methane	[30]
4.	*Botryococcus braunii*	Photobioreactor	Triterpene oils	[31]
5.	*Synechocystis* and *Synecococcus*	Photobioreactor	Isoprenes, oils	[32]

because algae can develop by utilizing sunlight and inorganic or organic carbon under autotrophic and heterotrophic conditions simultaneously. Mixotrophic growth can enhance resource utilization and reduce issues related to light constraints, resulting in a faster growth rate and increased lipid concentration [16]. Photoheterotrophs are heterotrophic phototrophs that rely on light for energy but cannot live solely on carbon dioxide. As a result, they rely on environmental organic substances to meet their carbon needs [40].

Various cultivation techniques including traditional and modern methods are described in the paper below.

3.1 Conventional cultivation techniques

Open ponds and closed photobioreactors are the two basic types of microalgae cultivation methods [41].

An enclosed PBR can be best defined as a man-made closed vessel that helps microalgae cells to carry out photosynthesis upon exposure to light as an energy source. However, economic risks are evident as the construction and operating cost of a PBR is higher than the pond system. Microalgae cultivation in enclosed PBR, on contrary, requires less or no agricultural land. Microalgae can be grown in enclosed PBRs on nonarable soil with nutrients supplied by wastewater treatment [42]. The most well-known closed culture systems utilized on a commercial scale for microalgae cultivation are tubular and flat-panel PBRs. Tubular PBR is typically made up of horizontal, vertical, fence-like, inclined, or helix-shaped glass or plastic tubes arranged in horizontal, vertical, fence-like, inclined, or helix arrangements. The tubular solar array has been designed and configured to capture as much sunlight as possible. They are organized in a row, parallel to each other, and flat on the surface. These horizontal solar tubes can also be placed in a fence-like pattern to incorporate more tubes in a given area [43].

Closed algal cultures (photobioreactors) are coated with a transparent substance or housed within clear tubes and are not exposed to the atmosphere. Photobioreactors have the distinct benefit of not permitting water to evaporate (Fig. 1).

Microalgae growing in these types of systems have the added benefit of decreasing contamination hazards, limiting CO_2 emissions, providing repeatable cultivation conditions, and versatility in system designs. Photobioreactors, both closed and semi-closed, are primarily utilized to produce high-value algal products [41].

The separation of biomass from treated wastewater has been one of the primary challenges in the use of microalgae for wastewater treatment. As a result, a fixed system, such as the biofilm PBR, has been suggested as a more adaptable alternative to the standard suspension method. Microalgae cells adhere to supporting elements in the biofilm PBR, and wastewater is delivered via the biofilm, which decreases the nutrient content that reaches the microalgae. The most important component that influences the efficacy of biofilm PBR is the selection of supportive materials for microalgae adhesion. The supporting material can be divided into two categories: biomaterial and non-biomaterial, with the rougher the surface of the materials, the easier it is for microalgae to adhere. While choosing the supporting materials, other factors such as microalgae strain and wastewater properties must be taken into account. To prevent reactions between materials and compounds in particular types of wastewaters, control measures must be taken [44].

The principal disadvantage of closed photobioreactors is that they are less cost-effective than open ponds. Closed systems now have several disadvantages, including high infrastructure costs, energy (pumping and cooling) operation, and

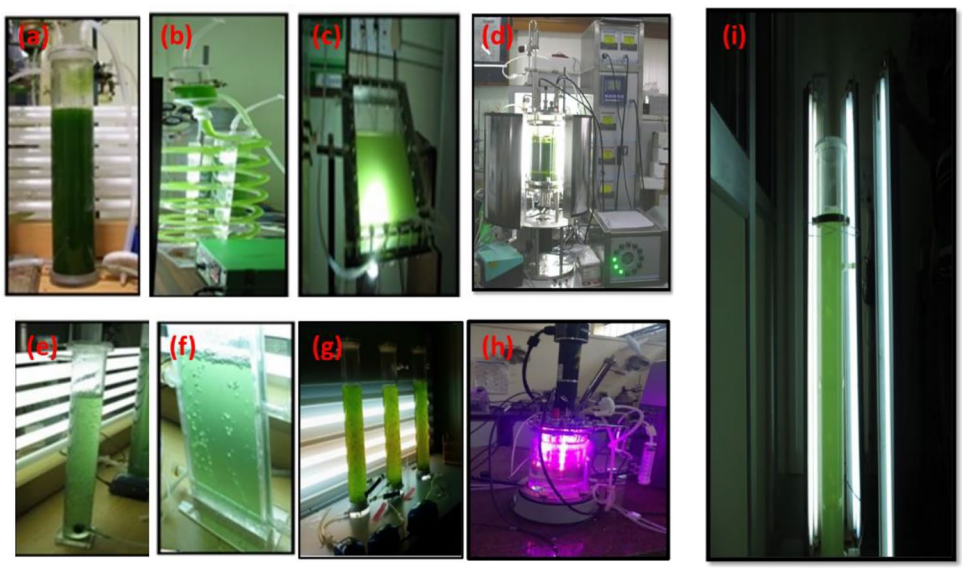

Fig. 1 Different PBRs. **a** Airlift reactor with orifice sparger. **b** Helical reactor. **c** Flat panel rocking reactor. **d** Bioengineering controlled reactor. **e** Bubble column reactor with orifice sparger. **f** Flat panel reactor. **g** Bubble column reactor with ring sparger. **h** Internal LED light illuminated controlled reactor. **i** Twenty-liter airlift reactor with orifice sparger. Courtesy: - Biohydrogen Lab, Indian Institute of Technology Kharagpur, West Bengal, India

maintenance [such as cleaning and sterilization, as well as scaling up challenges [45]. However, if these challenges can be addressed, these closed systems with controlled situations may be facilitated for commercial mass production of a greater number of microalgal species in a wider range of locations [46] (Table 2).

Microalgae biomass is the right approach for commercial biofuel production as a dynamic approach, and the variety of ways to culture microalgae is of primary interest. Microalgae cultivation in open ponds using solar energy has been well-known for more than 60 years and can be performed in open or covered spaces, in natural waterways (lakes and lagoons), or man-made shallow basins [42]. A few open pond systems, such as natural, circular, raceway, and inclined systems, are accessible and are preferred for most commercial activities, owing to their convenience and cost-effective construction features, as well as higher production capacity than closed systems [51]. In terms of nutrient availability, runoff water from land areas is prevalent, but the strategy of integrating microalgae cultivation with sewage or wastewater treatment plants is now attracting interest from a variety of industries as a phytoremediation tool and a way to reduce upstream processing costs. Overall, the open pond system excels in terms of financial prospects and simplicity of scalability, even though the huge scale synthesis of microalgae is hampered by physical, chemical, and biological variables that must be addressed comprehensively before the technology can be implemented. Apart from the requirement of a large amount of space to set up an open pond system, microalgae resistance to such cultivating methods is triggered by changes in the surrounding and cultural circumstances, as well as unsupervised solar light intensity and temperature. Because sunlight can only reach a specific depth in pond water and thus microalgae exposure to sunlight is inconsistent, poor light intensity and dispersion has an impact on microalgae growth and cell density. The inability to maintain an optically dark zone is another shortcoming of an open pond system [52]. Because evaporation is used to cool the system, a lack of control over the culture temperature leads to excessive water evaporation and, as a result, CO_2 diffusion into the atmosphere. Another issue with open pond cultivation is the high sensitivity to contamination by foreign predators and the flourishing of heterotrophs that feed on the algae, making such a system only practicable for microalgae that can survive harsh environmental conditions. Although a large and growing body of literature papers on the long-term success of open pond cultivation systems, another disadvantage is the inadequate mixing mechanisms provided by the paddlewheel (in open raceway ponds) and pivoted agitator (in open circular ponds), which result in reduced mass transfer and ultimately reduced volumetric productivities [51].

The other conventional techniques used for the cultivation of microalgal species are algal turf scrubber (ATS) and hybrid cultivation systems (HCS). Water flows across a sloped surface in ATS, which encourages the development

Table 2 Advantages and disadvantages of conventional cultivation system

Sr. no.	Type of cultivation system	Advantages	Disadvantages	References
1.	Closed photobioreactor systems	Environmental factors are under control including pH, temperature, and intensity of light.	Designing bioreactor plants and harvesting biomass are difficult challenges.	[3, 47, 48]
		Ideal for a specific species of microalgae.	Due to the low biomass/water ratio, there is a high need for water.	
		Enough light is accessible.	High operating and energy costs and transportation.	
		Contamination is reduced to a minimum.	Biomass yield is low.	
2.	Open pond	Simple structure.	Biomass yield is lower in open systems than in closed systems.	
		Convenient to use.	Setting in a large land area	
		Low investment and operating expenses.	Based on the surrounding environment	
		Solar lighting is freely available.	Inadequate light system.	
		Combination operations with wastewater treatment are simple and convenient.	Contaminations are difficult to control, and biomass harvesting is challenging.	
3.	Algal turf scrubber (ATS)	Easy maintenance and low surveillance.	Considerable infrastructure is required	[49]
		Biomass is harvested from open cultivation systems.	Lower wastewater handling potential	
4.	Hybrid cultivation system	High productivity	High capital expenditure	[50]
		Low rate of contamination	Needs large infrastructure.	

of benthic, filamentous macroalgae, and periphytic microalgae, whereas HCS combines two or more systems in order to enhance microalgal productivity, lower cost, and less energy consumption [49, 50] (Fig. 2).

3.2 Harvesting of microalgae biomass

Many recent discoveries in upstream and downstream handling of microalgae biomass have also been shown to fulfil our critical energy requirements. Apart from analytical research on the production of biomass composition and oil production, the harvesting stage, which is the extraction of microalgae cells from broth, remains the principal constraint in microalgae biofuel generation. Because of the small size of microalgae cells, poor density, and colloidal stability, the current harvesting devices have a high investment cost and energy input to operate [53].

Harvesting microalgae biomass is typically a two-stage procedure including thickening to enhance the solid concentration of the microalgae culture and dewatering to yield larger separation efficiency at a cheaper price. Dewatering is the process of separating concentrated slurry from the broth by removing the supernatant or skimming the cells off the top. Nonetheless, based on the quantity of water footprint to be treated and the harvesting method used, any of the procedures motioned above can be applied. Gravitational sedimentation, floatation, electrical-based process, and flocculation are examples of concentrating procedures, whereas filtration and centrifugation are frequent dewatering processes used with microalgae broth[54].

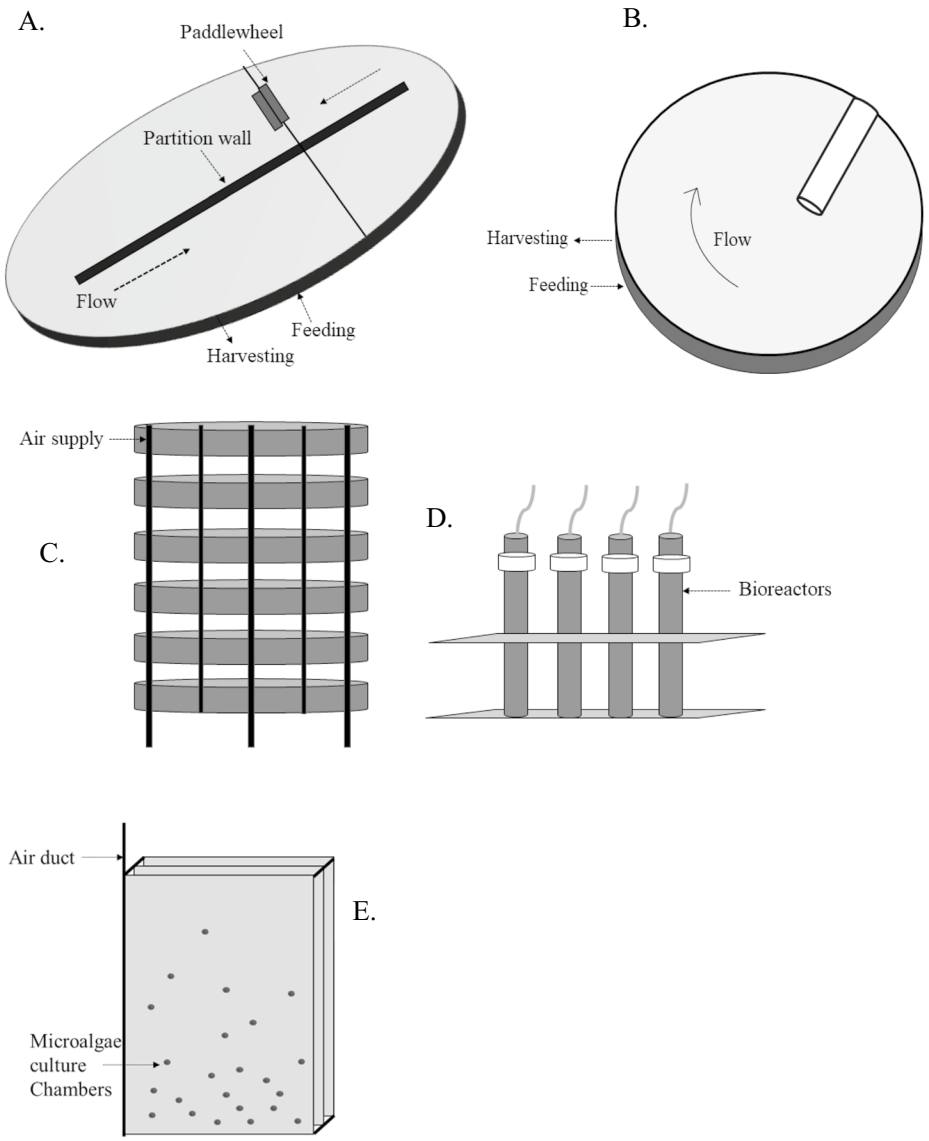

Fig. 2 Conventional microalgal cultivation systems. **A** Raceway pond. **B** Circular pond. **C** Helical PBR. **D** Vertical PBR. **E** Flat-plate PBR

3.2.1 Gravitational sedimentation

Gravitational sedimentation is the process of microalgae cells settling as a function of gravitational forces. Harvesting microalgae is a frequent practice since gravitational sedimentation is generally considered the simplest and most cost-effective approach compared to others. The rate of sedimentation is very selective, depending on the density and radial size of specific microalgae cells, with larger and denser cells settling rapidly than smaller and denser cells. This feature, however, is a limiting element of this procedure because it is time demanding, has a low recovery of microalgae biomass, and may result in biomass degeneration. If the sedimentation procedure is conducted in a sealed compartment, biological activity may reduce O_2 levels, resulting in biomass deterioration. However, using a lamella separator and a sedimentation tank can boost the extracting rate of this technology, and sedimentation tanks or settling ponds are commonly employed in sewage-based operations for biomass extraction [55].

3.2.2 Floatation

Floatation is considered a more constructive and comparatively rapid method than sedimentation. Due to the diminished surface charges on microalgae cells, floating can successfully extract microalgae cells with diameters ranging from 10–30 to 500 m [56]. It is frequently used in association with flocculation for a wide range of microalgae extraction in wastewater. It is a less-expensive technology based on a physiochemical gravity extraction activity in which gas bubbles move via a liquid-solid mixture, enabling microalgae to adhere to the gaseous bubbles and rise to the top. The suspended particle instability is the most important factor in influencing the productivity and effectiveness of this system, with higher air-particle contact corresponding to lesser instability. The particle size is critical in the flotation method, as the smaller the particle (ideally less than 500 m), the more likely it is to be raised to the top of the media by the bubbles [57].

3.2.3 Electrical-based technology

Electrical-based methods such as electrophoresis, electroflocculation (EF), and electro-flocculation-floatation have recently been demonstrated to be efficient microalgae harvesting methods. It is quick and suitable for a wide range of microalgae species, making this approach superior to others methods in the laboratory. Two metallic electrodes, one as a nonreactive anode and the other as a cathode, are used in electrolytic or electrophoresis [54]. Electrophoretic mobility is caused by negatively charged microalgae cells being drawn to a positively charged anode. As a result of charge neutralization (coagulation), microalgae cells aggregate to form flocs, which may deposit at the bottom of the tank or float on the surface, depending on density. Electro-flocculation, on the other hand, involves the introduction of reactive (sacrificial) electrodes into microalgae broth to generate metal flocculants that will stimulate flocculation through the different steps: (a) releasing metallic flocculants through electrolytic oxidation of sacrificial anodes, (b) destabilizing microalgae cell suspension, and (c) floc development as a coagulation act of the destabilized particles [42].

3.2.4 Flocculation

The introduction of flocculants to enhance microalgae cell aggregation for high-density floc production began to hit the dewatering trends three decades ago due to energy-intensive and expensive harvesting processes [58]. Because of the ionized functional groups on their surface and the adsorption of ions from organic matter, microalgae cells have primarily negative charges, generating cell–cell repulsion. To facilitate aggregation or floc development by coagulation, a stable microalgae cell suspension must be upset by adding flocculants. To address the most significant problems in the microalgae harvesting process, a variety of strategies have been assigned in flocculation studies involving chemical flocculation, as well as inexpensive and toxic-free methods employing flocculants or natural biomass-derived flocculants, or by modifying culture conditions [59].

3.2.5 Centrifugation

Centrifugation is a widely used method for recovering microalgae biomass, in which the broth is separated by centrifugal force. This approach is quick; thus, it is frequently favored over gravitational sedimentation, and it provides a high biomass extraction rate of up to 95% under ideal conditions [60]. Additionally, all microalgae strains may be centrifuged, and the apparatus is simple to clean with little potential for microbial contamination of the biomass. However, this method of harvesting can be costly because a large energy input is required for the maintenance and operation of the apparatus. The use of centrifugation to harvest microalgae cultures ranging from 0.04 to 4% dry weight costs 1.3 kW h m^{-3} of pond water on average [61]. Centrifugation is a suitable alternative for recovering high-value substances due to its hygienic operation, which will result in a high turnaround and profitability. The major drawback of this method is the risk of cell injury caused by high shear forces that allow microalgae intracellular components to leak into the culture broth.

3.2.6 Filtration

The use of membrane filtration to extract microalgae cells with smaller cell dimensions, such as *Scenedesmus*, *Dunaliella*, and *Chlorella* species, is a recent innovation in biomass extraction. Due to its simplicity and convenience of accessibility, conventional filtering is facilitated by microstrainers with a size of more than 70 mm, although many studies have revealed that flocculation should be performed before microstraining to flocculate smaller sized cells into larger flocs [42]. Microfiltration with a pore diameter of 100–10,000 nm and ultrafiltration with a pore diameter of 1–100 nm are frequently used forms of membrane filtration. Based on their field of application, several materials are examined to make membranes with various geometries such as compressed, tubular, multi-channelled, hollow, capillary, or spiral. For instance, polymer membranes have been proven to be successful in harvesting marine microalgae species including *Hasleaostrearia* and *Skeletonema costatum*, but they are challenged by hydrodynamic circumstances, microalgae properties, and cell concentration. Whereas, microalgae researchers have discovered that tangential flow filtration, which integrates high-rate filtration for harvesting cells, can harvest 70–89% of freshwater microalgae species such as *Stephanodiscus hantzschii*, *Cyclotella* sp., *Rhodomonasminuta*, and *S. astraea* [42].

4 Microalgal biofilms

Microalgal biofilms involve a range of formations made up of microalgae and bacteria, which are the main organisms that build them up. These biofilms were discovered on solid substrates that were sufficiently humidified, lighted, and capable of supplying nutrients to the microbes. Many additional species, such as bacteria, could be incorporated in microalgal biofilms (nonaxenic cultures), which play an important role in biofilm formation. Autotrophic biofilms made up of microalgae (including cyanobacteria) and heterotrophic microorganisms (fungi, bacteria, and protozoa) are also known as microalgal biofilms. The production of microalgal biofilms is regarded to be a difficult process that is poorly understood. It is also thought that the method of biofilm development and growth differs depending on the species involved. As a result, concluding the future approach for the various forms of current biofilms is challenging. The production of microalgal biofilms may be subdivided into two stages. To generate a conditioning film, the cells first attach to the solid substratum by adsorption in the first stage. This is typically a reversible step. Because of the EPSs synthesis, a second irreversible adhesion occurs in the second stage [4]. Various methods employed in microbial-based biofilm cultivation are described in the following.

4.1 Cultivation of microalgal biofilms

The relative analogous position of the culture medium and the microalgae on the cultivation medium divides biofilm cultivation systems into three basic classifications, namely, (i) constantly submerged systems, (ii) intermittently submerged systems, and (iii) perfused systems. Microalgae are immediately submerged under a layer of the medium in the first two types of system, either for all the period (constantly submerged systems) or only for the portion of the period (intermittently submerged systems). According to this categorization, these systems can be further classified into three types: (i) permanently immersed biofilms, which are entirely immersed in liquid culture medium; (ii) biofilms between two phases, which oscillates between gaseous and liquid phases; and (iii) permeated biofilm systems, which supply culture medium directly to the substratum [3]. Some of these experiments are summarized in Table 3. In terms of microalgae and medium configuration, this classification could be regarded as more comprehensive (Fig. 3).

4.1.1 Permanently immersed biofilms

A permanently immersed biofilm provides the cells with a constant supply of water and nourishment. Shen et al. constructed a simple structure to increase the lipid output of *Nannochloropsis oculata*. As supporting material, four layers of glass fiber–reinforced plastic was inserted in 500-mL beakers. A suitable liquid culture media has been utilized for filling up the beakers, covering the upper supporting material as well. Three criteria were evaluated to determine the production of oil: nitrogen deficit, high sunlight, and a combination of the two prior factors[62].

Lee et al. (2014) used mesh-type materials in an open pond to assess the biofilm production of a microalgae consortium (such as Chlorella, Nitzschia, Scenedesmus, etc.). Their results were compared to another experiment in which microalgal species were suspended in an open pond. Even though most algae cultivation systems function in suspended culture, an adhered growth system has significant benefits over suspended systems. As the microalgal load increases in the suspended system, algal cultivation becomes light-limited; however, due to the more transparent water in connected systems, sunlight penetrates stronger and deeper. The connected system exhibited 2.8 times the biomass production and total lipid efficiency of the suspended system. The yield of biomass can be boosted further by optimizing the cultivation conditions. Furthermore, as mesh-type substrates with associated microalgae were simply eliminated from the culture and the leftover treated wastewater could be dispensed directly, algal biomass harvesting and dewatering were easily implemented and cheaper in associated systems. The palmitic acid (C16:0) content increased by 16% when

Table 3 Description of various experiments on microalgal-based biofilm cultivation systems

Type of culture system	Cultivation medium	Species	Productivity	References
A.Permanently immersed biofilm				
1. Attached culture system	Synthetic seawater	*Nannochloropsis oculate*	3.38–3.67 g m^{-2} day^{-1}	[62]
2. Membrane biofilm reactor	Synthetic culture medium	*Chlorella vulgaris*	9.27 g m^{-2} day^{-1}	[13]
B.Biofilms between two phases				
5.Attached algal culture system	Modified basal medium	*Scenedesmus dimorphus; Chlorella protothecoides; Chlorella vulgaris; Scenedesmus obliqnus; S. dimorphus; Chlorococcum* sp.	0.39 g m^{-2} day^{-1}	[64]
2. Laboratory-scale rotating algal biofilm (RAB) system	Bold's basal medium	*Chlorella vulgaris*	1.08 g m^{-2} day^{-1}	[68]
C.Permeated biofilm systems				
1. Multi-layer PBR	Modified basal medium	*Botryococcus braunii*	3.19 g m^{-2} day^{-1}	[72]
2. Biofilm PBR	BG11 medium	*Botryococcus braunii*	0.71 g m^{-2} day^{-1}	[10]

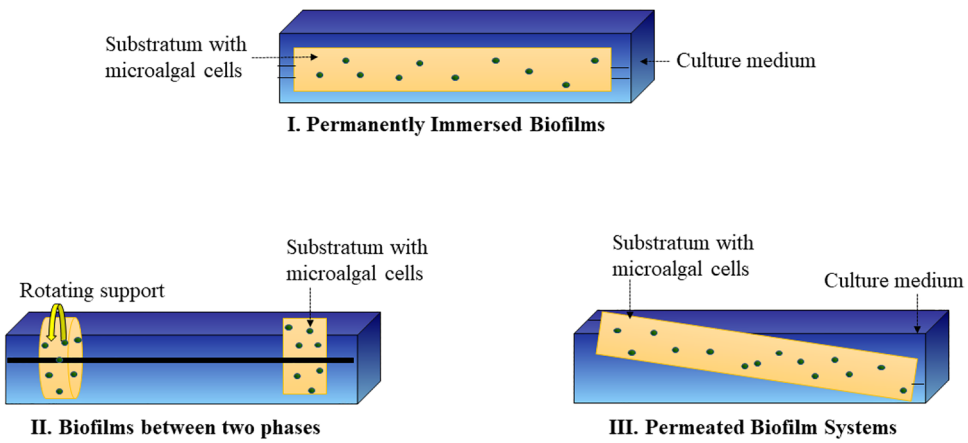

Fig. 3 Illustration of microalgal-based biofilm cultivation systems

the algal biomass was dewatered employing natural sunlight instead of the freeze-drying process. The other fatty acid compositions did not change appreciably. As a result, the algae growing system connected is a promising technology for bulk biodiesel synthesis [63].

Rincon et al. developed a mixotrophic algal biofilm reactor using glycerol and urea as carbon and nitrogen sources to quantify algal biomass produced. The algae were grown in a continuous mode membrane bioreactor with recycle under 50 µmol photons m^{-2} s^{-1} light irradiation. There was no CO_2 provided from outside sources. When 2 and 5 g L^{-1} initial glycerol concentrations were used, the productivities of algal biofilm were 9.27 ± 0.47 g DW m^{-2} day^{-1} and 12.64 ± 0.94 g DW m^{-2} day^{-1}, respectively. The biofilm reactor's design enabled *C. vulgaris* to develop with very little light and less water. The fatty acid profile of *Chlorella vulgaris* oil was found to be highly polyunsaturated. The biofilm growth mode influenced *C. vulgaris* metabolism, allowing the cell to fulfil its CO_2 requirements internally while producing a high oil output without nitrogen deficiency [64].

In one of the studies, biochar was used as a solid support for *Klebsormidium flaccidum* and *Anabaena cylindrica* cultures using BG11 culture medium, and their growth was compared to cultures that did not have solid support. Dry biomass, total carbon, and nitrogen contents in cultures of these microalgae with and without carbonaceous solid supports were assessed after 20 days of incubation with a 16:8 (light/dark) photoperiod. When compared to cultures

without biochar, the growth of *A. cylindrica* was increased by 80% in the presence of biochar [65].

In another study, under continuous illumination and increasing pretreatment (centrifuged) swine slurry loading rates, the biodegradation capacity of a unique enclosed tubular biofilm photobioreactor seeded with a *Chlorella sorokiniana* strain and an adapted activated sludge consortium was examined. This photobioreactor design achieved simultaneous and effective [66] phosphorous elimination efficiency of 94–100% and 70–90%, resp. Maximum total organic carbon (TOC), NH_4^+, and PO_4^{3-} elimination rates of 80 ± 5 g C m_r^{-3} day^{-1}, 89 ± 5 g N m_r^{-3} day^{-1}, and 13 ± 3 g P m_r^{-3} day^{-1}, resp., were documented at the maximum swine slurry concentrations (TOC of 1247 ± 62 mg L^{-1}, N–NH_4^+ of 656 ± 37 mg L^{-1}, P–PO_4^{3+} of 117 ± 19 mg L^{-1}, and 7 days of hydraulic retention time). The unconventional substrate diffusional pathways defined within the phototrophic biofilm enabled simultaneous denitrification/nitrification at the maximum swine slurry concentration rate while also protecting microalgae from any probable inhibition effect facilitated by the combination of high pH and high NH_3 concentrations. This biofilm-based photobioreactor also allowed for effective biomass retention (> 92% of the biomass created during pre-treatment swine slurry biodegradation) [66].

4.1.2 Biofilms between two phases (liquid phase and air phase)

A biofilm in between phases is one in which rotational or oscillatory activity is employed to ensure that the biofilm is periodically exposed to liquid and gas phases. This eliminates the need to mix the growth medium, but it raises concerns about the influence of shear stress on biofilm adherence.

Gross et al. created a rotating algal biofilm (RAB). Different mechanisms were investigated for their potential as adherence materials for *Chlorella vulgaris* cells in a plexiglass chamber on a rocker shaker. When one side of the triangle was immersed in the liquid medium, the other two sides were divulged to the atmosphere. Algal cultures were grown on a substance that shifted between a nutrient-rich liquid and a CO_2-rich gaseous phase. Scrapping biomass from the associated surface saved expenses on labor-intensive harvesting methods like centrifugation. Cotton sheets outperformed all other attachment materials in terms of algal growth, durability, and cost-effectiveness. Harvest frequency, rotation speed, and CO_2 levels were all modified on a lab-scale RAB system. The water content of algal biomass from the RAB system was equivalent to that of centrifuged biomass. When compared to a control open pond, an open pond raceway retrofitted with a pilot-scale RAB system produced significantly higher biomass productivity. Overall, the research indicates that the RAB system is an effective algal culture method that allows for easier biomass harvest and increased biomass productivity [7].

Another study was conducted to investigate an attached culture technique to cultivate the microalgae *Chlorella* sp. by using dairy manure wastewater as a growing medium for the biodiesel feedstock. Polystyrene foam produced a firm adhesion, high biomass output (25.65 g m^{-2}, dry basis), and high fatty acid output (2.31 g m^{-2}) among the other supporting tested materials for algal adhesion. Scraping was used to extract the biomass adhered to the surface of the supporting material; the leftover colonies formed on the surface functioned as inoculum for regrowth. Because of the downtime spared for first algal attachment, algae regeneration on the colony-established surface led to a higher biomass production than initial growth on a fresh surface. The 10-day regrowth culture yielded a strong biodiesel generation capability, by yielding 2.59 g/m^2 of fatty acid methyl esters and a productivity rate of 0.26 g m^{-2} day^{-1}. Depending on the cell cultures, the adhered algal culture recovered 61–79% nitrogen and 62–93% total phosphorus from dairy contaminated water. The attached growth system biomass had a moisture content of 93.75%, which was similar to the suspended culture system biomass. With respect to biomass production, biodiesel generation potential, the efficiency of harvesting biomass, and physical resilience for reuse, the adhered algal culture system with polystyrene foam as a supporting material performed well [67].

Gross et al. aimed to develop a rotating algal biofilm (RAB) growing method that microalgae producers may use to collect biomass more easily. Algal cells were grown on a substance that alternated between a nutrient-rich liquid and a CO_2-rich gaseous phase. Scraping biomass from the connected surface was cost effective on labor-intensive harvesting methods like centrifugation. Cotton sheet outperformed all other adhesive materials in terms of algal growth, endurance, and economic viability. Harvest frequency, rotation speed, and CO_2 levels were all modified on a lab-scale RAB system. The water content of algal biomass from the RAB system was equivalent to that of centrifuged biomass. When compared to a control open pond, an open pond raceway modified with a pilot-scale RAB system produced significantly higher biomass yield (3.51 ± 0.48 g m^{-2} day^{-1}). Overall, the research indicates that the RAB system is an effective algal culture method that allows for easier biomass harvest and increased biomass yield [68].

Orandi et al. developed a photo-rotating biological contactor (PRBC). A microbial consortium characterized by green microalgae (*Ulothrix* sp.) was used to develop the biofilm. For the biofilm formation, 16 polyvinyl chloride disks with roughened surfaces were used in the creation of the PRBC. The disk was inserted in a shaft and submerged in a plexiglass tank by 40%. The shaft was connected to a motor that controlled the disks' spinning speed. The liquid

media (multi-ion synthesized simulated AMD) was delivered to the disk via the plexiglass tank, which was connected to a feed container. The characteristics needed to produce an algal-microbial biofilm for heavy metal elimination are also reviewed, notably nutritional requirements and rotating speed. The PRBC was tested using synthesized AMD and contaminated water with a multi-ion and acidic content (including 18 elements and a pH of 3.5–0.5), from which the bacterial consortium was obtained. Over 60 days of the batch process, the biofilm was successfully established on the PRBC's disk consortium. Over 10 weeks, the PRBC was operated continuously with a 24-h hydraulic residence time. Additionally, a weekly examination of water revealed that the algal-microbial biofilm was capable of removing 20–50% of the major metals in the sequence Cu > Ni > Mn > Zn > Sb > Se > Co > Al [69].

Chlorella sorokiniana microalgal biofilms were developed with high productivity and photosynthetic efficiency during simulated day-night cycles. There were no changes in light use performance when comparing day-night and continuous illumination. This means that the sugar ingested overnight is for the synthesis of new functional biomass as well as maintenance-related respiration. Highest yields and photosynthetic performances were calculated using a model of microalgal biofilm growth. Experiments were used to create, calibrate, and test a light-limited microalgal biofilm development model that took into consideration both diurnal carbon partitioning and maintenance under prolonged dark conditions. Maintenance-related respiration was lowered when there were long periods of darkness. Depending on simulations using the validated biofilm growth model, it has been determined that biofilm growth has a better photosynthetic efficiency than suspension growth. This is due to the fact that the biofilm's dark zones have a reduced maintenance rate than the dark zones of suspension cultures that are constantly mixed with the photic zone [70].

In another experiment, a rotating biological contactor (RBC)-based photobioreactor was used as a development platform for microalgae biomass cultured in biofilm in this investigation. Microalgae develop in biofilm over vertical rotating disks partially immersed in a growth media in the photobioreactor known as Algadisk. Evaluation of the Algadisk photobioreactor's potential was carried out by considering the influences of disk roughness, disk rotation speed, and CO_2 concentration. An efficiency of 20.1 ±0.7 g per m^2 disk surface per day was achieved in the lab-scale Algadisk reactor, with a biomass yield on the light of 0.9 ± 0.04 g dry weight biomass per mol photons. The impact of different disks rotation speeds on biofilm formation and substrate diffusion into the biofilm were modest. Without re-inoculation of the Algadisk, productivity could be maintained for 21 weeks. Extreme circumstances, such as pH 9–10, temperatures exceeding 40 °C, and low CO_2 concentrations, reduced productivity. However, when proper cultivation conditions were restored, maximum productivity was gradually restored [71].

Shen et al. investigated at how microalgae biofilms formed in different growth conditions. Initially, the adherence of six different freshwater algae species was compared. Because of the high adhesion biomass productivity (ABP) and adhesion rate attained, *Chlorococcum* sp. was selected. Further, the adherence of *Chlorococcum* sp. to nine regularly used supporting materials was assessed, and glass fiber-reinforced plastic was found to be the best substrata. Finally, a second-order polynomial model was developed based on response surface methodology experiments to investigate the influence of culture period, initial total nitrogen concentration (ITNC) in manure wastewater, pH, and culture volume of the growth chamber on *Chlorococcum* sp. adhesion using glass fiber-reinforced plastic. The highest ABP was anticipated to be 4.26 g m^{-2} day^{-1} under optimal culture conditions, which included an 11-day culture period, an ITNC of 70 mg L^{-1}, a pH of 8, and a culture volume of 340 mL [72].

4.1.3 Permeated biofilm systems

A permeated biofilm uses capillary pressure to wick growth media through the solid support it is associated with. This method does not involve mixing or motion, but it does involve excerption towards a porous and hydrophilic substratum.

Botryococcus braunii, a green alga, was grown as a biofilm. The novel algae biofilm photobioreactor described in an experiment was able to produce a direct algal harvest volume of 96.4 kg m^{-3}, which is more than 35 times higher than the highest previously reported direct harvest density, making downstream process integration simpler and less energy-intensive. Furthermore, the system had a net energy ratio of 6.00, compared to 1.06 for open ponds. In addition, the light to biomass energy conversion was 2.02%, which was equivalent to planktonic systems [10].

Microalgae cells developed on the interface of vertical artificial supporting material to produce algal biofilm in an attached culture method. The first dubbed the "single layer vertical plate," was made out of a glass plate that was implanted in a glass chamber. While one plate surface was lighted, the other was coated with filter paper and microalgal cells. To facilitate high photosynthetic efficiency, several algal biofilms were stacked in an array to dilute sun irradiation. The experimental findings demonstrated that this attachment approach can grow a wide variety of microalgae species. *Scenedesmus obliquus* had biomass productivity of 50–80 g m^{-2} day^{-1} in the open air, equivalent to a photosynthetic efficiency of 5.2–8.3% (total solar radiation). This attached approach has several potential benefits over traditional open ponds, including water conservation, harvesting,

contaminant management, and scale-up. The attached culture is a promising approach for producing microalgae biofuels at a reasonable cost [73].

Y. Shen along with his co-workers discovered the relationships between culture conditions, EPS, microalgal biofilm formation, and lipid accumulation in microalgal biofilms. *Botryococcus braunii*, a freshwater alga, was cultured in multi-layer photobioreactors with various culture medium and substrates. The results showed that culture period, nutrition, and substrate all had an impact on EPS formation. Increasing EPS production may help biofilms grow faster. However, as compared to total EPS, the EPS components, specifically proteins and polysaccharide, had a greater impact on biofilm development, with protein being more important than polysaccharide. The biomass yield was found to be 3.19 g m^{-2} day^{-1} [74].

In one of the studies, the adhesion of *Scenedesmus obliquus* and *Nitzschia palea* cells on a glass substrate was tested using a semi-continuous system consisting of 12 rectangular flat plates put in parallel. For each species, linear growth curves were determined until nutrient depletion occurred, at which time development terminated and/or biofilms sloughed off their substratum. Nutrient deprivation did not affect neutral lipid contents in any of the biofilms, but it steadily increased their lipid concentrations when they have grown in suspension. *N. palea* and *S. obliquus* had biomass productivities of 2.8 and 2.1 g m^{-2} day^{-1} and lipid productivities of 0.45 and 0.18 g m^{-2} day^{-1}, respectively. The findings imply that starvation of biofilms for lipid production is not the desired strategy for algae biofilm biofuel production systems, but that lipid production rates are comparable to traditional terrestrial biofuel sources [74].

Attached culture involves the growth of concentrated cells on the substratum, which differs from suspended culture in terms of light adsorption mechanisms. For the adherent culture of the thermotolerant microalga *Desmodesmus* sp. F51, an energy-saving biofilm reactor based on the capillary effect was initially introduced. The impacts of light-related techniques on lipid production (including light intensity, photoperiod, and light-switching tactics) were studied. The amount of light required for biomass or lipid accumulation changed dramatically at different phases of growth, most likely due to increased biofilm thickness. At day 8, a maximum biofilm/lipid production of 241.67/53.62 g m^{-2} was attained by adjusting the light intensity from 700 to 1134 mol m^{-2} s^{-1} in Strategy I. A similar procedure was followed in Strategy II, with the exception of a transition to a light intensity of 938 mol m^{-2} s^{-1} on day 5, and a maximum biofilm/lipid production of 223.58/66.65 g m^{-2} on day 8. The operation of light-switching was found to be a successful approach for supplying the right light for each growth stage of immobilized cells, as well as advantageous for biofilm/lipid synthesis [26].

Similarly, to incubate *B. braunii* FACHB 357, a unique approach of "attached cultivation" was used. Early in the cultivation process, high biomass productivity of 6.5 g m^{-2} day^{-1} was attained in a single layer connected system. The biomass, lipid, and hydrocarbon productivity rates were 5.5, 2.34, and 1.06 g m^{-2} day^{-1} on day 10. Under nitrogen deficiency, both lipid and hydrocarbon content was increased, although the hydrocarbon profile remained nearly similar, while oleic acid (18:1) content increased and linolenic acid (18:3) level dropped. Under continuous illumination of 500 mol m^{-2} s^{-1}, a multi-layer photobioreactor produced biomass productivity of 49.1 g m^{-2} day^{-1} and photosynthetic efficiency of 14.9% [75].

5 Various factors affecting microalgal biofilms

The development of biofilm and its morphology is influenced by a variety of factors and relations among organisms that promote colonization and growth (Table 4). Some of the significant factors affecting the growth of biofilms are discussed herein.

Table 4 Comparison of various factors influencing the development of microalgae biofilm along with their biomass yield

Microalgal species	Culture system	Light intensity (μmol m^{-2} s^{-1})	CO_2 (% v v^{-1})	Temperature	Flow velocity (L min^{-1})	Productivities (g m^{-2} day^{-1})	References
Nitzschia palea	Horizontal flat plate PBR	160	2	25	23.3×10^{-3}	2.8	[4, 81]
Chlorella vulgaris	Algal biofilm membrane PBR (BMPBR)	642	4	N/A	0.07	4.3	
Chlorella sp.	Horizontal flat plate PBR	110–120	0.04	20	-	2.6	
Scenedesmus obliquus	Vertical flat plate PBR	300	2	30	$(6.59–21.5) \times 10^{-3}$	15	

5.1 Selecting a suitable strain of microalgae

Different species of microalgae behave differently in terms of characteristics and growth. Some strains might favor solid substratum for their development. Few others prefer to grow in suspensions or liquid media [74]. *Nitzschia palea* is a highly adherent microalga that yields greater biomass and can develop strong biofilms compared to *Scenedesmus obliquus*. Few other strains of microalgae cannot develop a single-species biofilm. Hence, they have to be accompanied by such species which show high adherence. Under axenic conditions, *Chlorella vulgaris* exhibits low initial adhesion compared to *S. obliquus*, as observed by Irving and Allen [76].

5.2 Impact of nutrient requirements

In the initial stage of growth, the efficacy of nutrient uptake is quite less as a consequence of the ineffective establishment of the biofilm. When the growth ascends, nutrient uptake also rises. At the death stage of growth, this efficacy diminishes as the biofilm drops its integrity due to shedding [77]. The accumulation of lipids in the microalgae is hindered by nutrient exhaustion. When the nitrogen content is raised, the synthesis of EPS from green algae and diatoms becomes proficient. In contrast to bacteria, the accumulation of photosynthetic biomass in a microalgal biofilm increases when the nitrogen and phosphorus levels are raised in the surrounding medium.

5.3 Accessibility of light

Availability of light is the major factor for any microalgal species to develop biofilms. When it reaches the light saturation point, microalgae undergo a linear pattern of growth with elevating the intensity of light. Availability of light is crucial to the attachment of biofilm and for the production and accumulation of EPS. However, highly intensified light in the topmost region of biofilm addressed as photoinhibition and minimized light in the bottom region, photolimitation, obstructs the development of biofilm.

Hill and Larsen [78] classified shaded, unshaded and UV biofilms and the influence of light intensity. The content of microalgae biofilm in the shaded region was found to be dissimilar to that of unshaded and UV regions. Hultberg et al. investigated the impact of colors or corresponding wavelengths on the development of biofilm in *Chlorella vulgaris*. The conclusion was that the white, blue, and purple lights formed more biofilm at a faster pace in contrast to red, yellow, and green lights [79].

5.4 Fluctuating temperatures

Elevation of temperature to an optimum value led to greater metabolism and a higher yield of biomass. Rising temperature also enhances the function of enzymes by which organic substrates are consumed by bacteria. Like other factors, temperature also affects microalgal growth by changing the values. Also, the appropriate temperature shifts from one microalgal species to other. Mesophilic microalgae, for example, grows best at an optimal temperature of 20 to 25 °C. Microalgal biofilms are vulnerable to instabilities in temperature compared to suspension cultures due to lesser amounts of water. Water also serves as a buffer for temperature [10].

5.5 The pH of the microenvironment

By now we have an understanding that a biofilm itself is an ecological aspect on a microscale. It has its environmental requirements like light, temperature, and pH, which are dissimilar to the surrounding medium or environment. Throughout the biofilm, different regions adapt to the different pH. The adhesion of microalgal cells is highly dependent on the pH to form. When the pH is less, the separation of amino and carboxyl groups is improved and subdued, respectively. This causes the negative surface charge of microalgae to diminish and the microalgal attachment is boosted. The optimum pH values for the development of microalgae are in the range of 6 to 9 [64].

5.6 Carbon dioxide and carbon levels

Carbon is a crucial element for the metabolism of any photosynthetic microalgal species. Aqueous CO_2 and HCO_3^- present in wastewater supply the required amount of carbon to the microalgae. Atmospheric CO_2 and the CO_2 released from the bacterial consumption of organic carbon, are also offered to the microalgae. In phototrophic growth, CO_2 is the sole source of primary carbon. When the uptake of CO_2 exceeds its supply, the yield of microalgae plummets due to a lack of carbon [71]. When CO_2 content is high the yield is maximized.

5.7 Perfect substratum for adhesion

Glass, polystyrene foam, muslin cheesecloth, polyurethane foam, vermiculite, jute, polyester, cardboard, polylactic acid, fiberglass, cotton duct, and cotton rope are the various materials used for the adhesion of biofilm. The suitable material for this purpose is estimated by its robustness, renewability, expense, and the extent of adhesion [68]. The structure and morphology of these materials determine the attachment of biofilm. Researchers portrayed that polystyrene foam is the

efficient material for the biofilm formed by *Chlorella* sp., concerning physical features, biomass yield, metabolism, and growth.

5.8 Flow velocity

The development of biofilm is influenced by the flow of the liquid phase, in which the biofilm is submerged and provide nourishment to the microorganisms [21]. The circulation of liquid should be in such a way that nourishment is provided properly to the microalgae and wastes are eliminated from them. Hence, the velocity of circulation is significant. Nonetheless, shear stress on the biofilm is a consequence of greater velocity. The width of biofilm reduces as a result of cell detachment caused by the turbulency of liquid. To conclude, a lower velocity of liquid flow is required for the adhesion of cells in the initial phase of growth. For subsequent growth, a greater flow velocity is favored so that the microalgae are replenished with adequate nutrients.

5.9 Relationship with other microorganisms

Usually, bacteria are thought to be a contaminant to microalgal growth. However, new research has proven this fact wrong. Microalgae–bacteria association has become vital in algal biotechnology since it enhances the development of microalgae and the flocculation process [80]. Biofilm formation by the microalgae is enhanced by the existence of bacteria in the wastewater. O_2 released by the microalgae is utilized by the bacteria which oxidizes NH_4 and organic substratum. In response, the bacteria supplement its partner with CO_2, released by respiration, which is crucial for photosynthesis. When the colonization and diversity of bacteria increases, greater amounts of carbon become evident to the microalgae and several microalgal cells can adhere to the solid substratum.

6 Applications and recent technologies for microalgal biofilms

6.1 Algae biomass as a potential source of biofuel

Microalgal biofilms have been highlighted as a possible source of sustainable biomass energy, with both biomass and oil contents suitable for the generation of biodiesel [82]. Microalgal biomass has emerged as a promising fuel for bioenergy production in recent decades. It is now being actively researched for the production of liquid (bioethanol, biodiesel) and gaseous (biomethane, biohydrogen) fuels. Because they take up soluble inorganic C to develop, biofilms are reported to absorb solar energy and fix CO_2 at a rate 5–20 times greater and have higher biomass output. Microalgae are fast-growing photosynthetic organisms that have the capacity to convert 9–10% of solar energy into biomass, with a theoretical production of 77 g biomass m^{-2} per day [83].

Microalgal biofilms can be employed to yield sustainable biofuels for transportation and jet fuel in a variety of methods, with anaerobic digestion producing methane being the most fundamental application [52]. Microalgal biomass, particularly *Chlamydomonas reinhardtii*, has been attracting great emphasis in the photobiological synthesis of biohydrogen as a promising substitute of green energy, in addition to being a significant possibility for biodiesel synthesis.

Manufacturing biodiesel from algae is acknowledged as the most effective method of producing biofuels, and it also seems to be the only existing sustainable source of oil capable of meeting the world's transportation fuel requirements [84]. The key benefits of microalgal biofilms for biodiesel production are that they:

- Possess a greater efficacy of photon conversion
- Can be harvested in batches throughout the year, ensuring a consistent and stable supply of oil
- Can employ salt and wastewater channels, lowering the amount of freshwater used
- Can combine the generation of CO_2-neutral fuels with CO_2 sequestration
- Produce biofuels that are nontoxic and extremely biodegradable

Christenson and Sims used the rotating algae biofilm reactor (RABR) to treat secondary effluent municipal wastewater. The biomass yield measured by the RABR was 31 g m^{-2} day^{-1}. The algal biofilm reactor was used by Johnson and Wen [67] to treat dairy manure effluent. In the biofilm reactor and the suspended system, biomass yield was 2.57 g m^{-2} day^{-1} and 0.127 g L^{-1} day^{-1}, respectively. When the algal bioreactor for the synthesis of biofuel collaborates with wastewater treatment, the expenses of nutrients for the algal growth reduces [85].

6.2 Other applications

A major application of microalgal biofilms after biodiesel production is the treatment of wastewater. They are also used to treat synthetic wastewater. Lipid production in biofilm is also observed. Other derivatives may be generated based on the microalgal biomass content. The idea of using biomass as a fertilizer is appealing, but it can only be done if there are no heavy metals or other refractory substances in the wastewater that microalgae can accumulate [86]. Another option is anaerobic digestion for biogas synthesis, though autotrophic N and P elimination or retrieval will be required afterwards. The CO_2 released during digestion might be used

to supplement the CO_2 feed in the microalgal biofilm. Biofilm can be used for a variety of purposes, including [87]:

- The estimation of the proportion of microalgal species that serve as bio-flocculation
- The purification of some wastewaters using high efficacy and low-energy techniques
- As renewable supplements with optimal concentrations for the generation of biofuel including biodiesel and biohydrogen

7 Future prospects

Biofilms are micro-environmental species of bacteria, algae, fungi, and protists that develop on various substrates by cell adhesion. Microalgae have recently piqued the scientific community's interest due to their numerous potentials in areas such as wastewater treatment and the pharmaceutical industry. Microalgae biofilm is an immobilized cultivation method that yields energy as well as several environmental advantages including bioenergy generation, nutrient retrieval, and carbon sequestration, while simultaneously improving the final yield of biomass and algal cell density. Mixotrophic cultivation is mostly preferred because algae nurture in both autotrophic and heterotrophic conditions when an appropriate amount of sunlight, inorganic and organic carbon compounds are supplied[2]. Choice of the most suitable microalgal strain, nutrient availability, intensity of light, temperature, pH, supply of CO_2, the substrate for adhesion, the rate of flow of liquid in the medium and the existence of other microscopic organisms are the major parameters influencing the formation of biofilm.

Microalgal biofilm has a wide range of applications, especially in the treatment of wastewater and the production of biofuel, biohydrogen and a potential alternative for third-generation biodiesel production. The implication of biofilm as fertilizers are also under research. Even though microalgae can be cultivated in a laboratory, commercial production requires configuration, expense, contamination factor, and maintenance to be considered. Raceway pond is the favorable choice to cultivate microalgae for commercial production of biofuel [1].The experimental results have been characterized as quite promising.

Compared to suspension-based/conventional systems, which have acquired considerable funding and resources over the last several decades, algal biofilm systems are still under development. Future research on algal biofilm systems should concentrate on (a) identifying the best strains and materials for optimal formation of biofilm and development, (b) investigating the value of specialty compounds like EPS in biofilm-derived biomass, (c) CO_2 transfer and light penetration within the biofilm, (d) long-term pilot and demonstration-scale research, and (e) the economics and sustainable development of algal biofilm systems. Algal biofilm systems will emerge into commercially viable options for algal cultivation as we gain a deeper grasp of the above important areas [4].

8 Conclusion

Microalgal biofilms are a contentious issue in science right nowadays because of its wide-scale application in wastewater treatment, biomass production and biomass recovery. The experimental outcomes have been regarded as promising. These systems, which are mostly used in laboratories, provide useful information, but several connections between elements and how they influence microalgal biofilm development remain uncertain. Although some efforts have been made, it is important for such systems to be evaluated on a broader scale and in real world situations. The evaluation of such a system's performance is hampered by a variety of variables. It is required to examine them using the same construction and operation baselines in order to determine their potential.

Acknowledgements Authors duly acknowledge the grant received from Sharda University seed grant project (SUSF2001/01). Authors duly acknowledge the grant received from the Life Sciences Research Board, Defense Research and Development Organization (DRDO) (File No. LSRB/81/48222/LSRB-368/BTB/2020) and Department of Science and Technology (File No. CRD/2018/000022) for the accomplishment of this work. Kavindra Kumar Kesari and Janne Ruokolainen thanking Aalto University for providing an open access support.

Author contribution Conceptualization: Soumya Pandit, Dolly Wattal Dhar, and Dipankar Ghosh; writing—original draft preparation: Sanchita Bipin Patwardhan, Srijoni Banerjee, and Dibyajit Lahiri; writing—review and editing: Rina Rani Ray, Moupriya Nag, Sanket Joshi, Kavindra Kumar Kesari; review—editing: Piyush K Gupta, Janne Ruokolainen, and Rina Rani Ray; supervision: Soumya Pandit and Kavindra Kumar Kesari. All authors have read and agreed to the published version of the manuscript.

Funding Open Access funding provided by Aalto University. Authors duly acknowledge the grant received from Sharda University seed grant project (SUSF2001/01). Authors duly acknowledge the grant received from the Life Sciences Research Board, Defense Research and Development Organization (DRDO) (File No. LSRB/81/48222/LSRB-368/BTB/2020) and Department of Science and Technology (File No. CRD/2018/000022) for the accomplishment of this work.

Declarations

Conflict of interest The authors declare no competing interests.

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