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Development and Applications of Attached Growth System for Microalgae Biomass Production

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Abstract

With realizing the potential of algal biomass as a good natural resource for the harnessing of valuable bioproducts, algal biomass production has gained a lot of interest in recent years. However, due to some limitations such as low harvesting efficiency, higher nutrient supply, and high water requirement, the production of algal biomass is uneconomical. Over the past several years, researchers are continuously working on growing algae as a biofilm for easy microalgae harvesting, concentration of algal biomass to a great extent, and requiring less quantity of water as compared to other microalgae cultivation methods. Most of the documented studies have been carried out on either use of algal biomass for tertiary treatment of wastewater or cultivation and harvesting of algal biomass for biofuel production. Limited research studies have documented other applications of the algal biofilm system. The present review paper summarizes the current knowledge on various factors affecting microalgae growth, development of algal biofilm, and operation of algal biofilm systems to help properly understand and optimize these factors for better economics, more positive environmental impacts, and successful potential applications of the attached growth systems. The important factors include the structure of algal biofilms, EPS matrix, supporting materials, nutrient availability, environmental conditions, and biofilm thickness and harvesting frequency. The potential applications such as wastewater treatment, CO_2 sequestration, microalgae–microbial fuel cell, large-scale biomass production, and water quality improvement are also discussed.

Keywords Algal biofilm · Microalgae harvesting · Microalgae cultivation · Biofuel · CO_2 sequestration · Large-scale biomass production

Introduction

Algae are the photosynthetic microorganisms that require nutrients like nitrogen and phosphorous and the presence of light energy source for growth and reproduction. The water bodies rich in nitrogen and phosphorous stimulate the growth of algae that may cause taste, odor, turbidity, and clogging of filters in drinking water treatment plant. Some algae species produce toxins during their growth, and even after their death, dead cell decompositions release toxins and nutrients in water [1]. Thus, the presence of the algal and the nutrients in the water body adversely affect water quality and beneficial uses of surface water sources. Researchers are continuously working on developing techniques for removing nutrients and the microalgae from the surface water bodies. Traditional methods like ion exchange and adsorptions are neither very effective nor economical for nutrients removal, and therefore, an alternate approach is necessary to tackle the problem. Similarly, the removal of microalgae from the surface water bodies is also a challenging task. Methods like coagulation and flocculation, flotation, centrifugation, and filtration or a combination of techniques are generally used for harvesting microalgae from their cultural medium. Among these coagulation and flocculation are considered to be the most economical method for microalgae harvesting. The use of organic flocculants can effectively reduce microalgae and nutrients from the water column and helps in restoring water quality [2, 3]. Pugazhendhi et al. [4] also suggested harvesting toxin-free microalgae by flocculants as a cost-effective method for biomass recovery. Microalgae are rich in polyunsaturated fatty acids and can nourish the nutritional needs of the population [5]. Algae are the primary producers and their biomass can be a source of food, medicines, biofuels, and fertilizer. Algae can

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be also be used as a wastewater purifier and pollution controller by fixing CO_2. The algal products like nutraceuticals, fatty acids, stable isotopic biochemical, phycobiliproteins, and carotenoid add to its commercial importance [1, 6]. Mathimani et al. [7] has summarized the advancements in microalgae cultivation and thermochemical processing for rapid biofuel production from microalgal biomass. High-lipid producing algal strains with maximal triacylglycerol (TAG) content are preferred for sustainable biodiesel [8]. For biodiesel, a lipid is a necessary precursor that can be stimulated by growing microalgae in nitrogen deprivation conditions [9]. Microalgae are now emerging as a new energy feedstock with the ability to survive and grow in even extreme conditions. In spite of having the inherent potential of being a feedstock for biofuel, their commercialization is limited due to high cost and inefficient harvesting techniques. However, low biomass concentrations and biomass/liquid separation problem in microalgae growth culture hinder the industrial application of algal biomass [10]. Thus, an alternate approach is required to cultivate and harvest microalgal biomass to meet the microalgae-based biomass production challenges.

Many researchers have cultivated microalgae in various types of natural and engineered environments such as open raceway ponds and photobioreactors. The most problematic task in biofuel production is the cost-effective harvesting of microalgae. For mitigation of this problem, scientists have come up with a new technology known as the algal biofilm system. In the algal biofilm system, microalgae are allowed to grow on the surface of any support material and 10–20% concentrated algal biomass can be collected easily by just scraping the surface [11]. The scraping is done in such a way that a thin layer with some microbial cells is retained on the surface, and these cells can act as an inoculum for the next batch of biofilm growth.

The cultivation of microalgae can be either in a suspended or attached growth form. Several types of raceway ponds and photobioreactors are designed to cultivate suspended microalgae. In the attached growth system, benthic microalgae are attached on the surface of natural (soil, rocks, and plants) or artificial materials (porous materials, filters, and concrete surfaces, PVC, etc.) to form a thin biofilm of microalgae. In general, a thin biofilm of the attached biomass consists of benthic microalgae, cyanobacteria, and heterotrophic bacteria that are entrapped in the EPS produced by them [12]. Biofilm cultivation can be carried out either by submerging the supporting material constantly or intermittently and passing the nutrient solution through the porous substrata with biofilm growth on the outer side [13]. Choudhary et al. [14] had classified biomass cultivation systems as perforated biofilm systems, constantly submerged biofilm systems, intermittently submerged biofilm systems, horizontal support biofilm systems, vertical support biofilm systems, and flow cell/channel biofilm systems.

Most of the documented studies have been carried out on either use of algal biomass for tertiary treatment of wastewater or cultivation and harvesting of algal biomass for biofuel production. Limited information related to other applications of the algal biofilm system is documented in the literature. Various factors affecting microalgae growth, development of algal biofilm, and operation of algal biofilm systems need to be properly understood, and the present knowledge gaps need to be filled for better development of the algal biofilm system. The present review paper summarizes the current knowledge on the factors such as the structure of algal biofilms, EPS matrix, supporting material, nutrient availability, environmental conditions, biofilm thickness, and harvesting frequency that affect the growth of microalgae, development of algal biofilm, and operation of algal biofilm systems. The potential applications of algal biofilm systems such as wastewater treatment, CO_2 sequestration, microalgae–microbial fuel cell, large-scale biomass production, and water quality improvement are also discussed.

Structure of Algal Biofilm

Algal biofilm is a complex structure that consists of multispecies of heterotrophic and photoautotrophic prokaryotic and eukaryotic organisms living in symbiosis in a multilayered 3D structure [15]. The organisms include filamentous and unicellular macro and microalgae, cyanobacterial species, protozoa, flagellates, bacterial, and fungal cells. These organisms colonize according to their most favorable growth zones [15, 16]. Schnurr and Allen [17] explained the development of mixed culture algal biofilm through four different stages. According to Schnurr and Allen [17], the growth surface is first conditioned with extracellular polymeric substances (EPS) which are produced by the bacteria. These EPS matrix provide sites for various species of microalgal cells to colonize, multiply their population, and live in a symbiotic relationship with the other microbes present in the biofilm.

Development of Algal Biofilm

The production of algal biomass through an engineered biofilm system requires a good knowledge of the factors which govern the development of biofilm on the surface of a material. The limited information on the algal biofilm growth factors, standard operating procedures, and testing for biofilm systems create hindrances in developing an optimized biofilm growth system. The development and survival of the algal biofilm system depend on many factors such as EPS matrix, supporting material, algal and bacterial species combination, nutrient availability, environmental conditions (light, temperature, flow velocities), biofilm thickness, and harvesting

frequency [13, 18]. Earlier, Kesaano and Sims [18] have focussed on nutrient removal and algal biofilm growth conditions for wastewater treatment. Berner et al. [13] have focused on the design of biofilm cultivation system for the production of algal biomass, Schnurr and Allen [17] have reviewed the factors which affect algal biofilm growth and lipid production, and Choudhary et al. [19] have reviewed support material and harvesting frequency for algal biofuel production. The productivity of the algal biofilm systems may be reported as gram dry biomass per square meter of substrata surface per day ($g_{(dry\ biomass)}\ m^{-2}\ day^{-1}$) or as gram dry weight per square meter of footprint per day [13].

EPS Matrix

The EPS consists of high molecular weight compounds such as proteins, nucleic acids, lipids, polysaccharides, and humic acids. They may be produced either by cell secretion, cell lysis, shedding of cell surface material, and adsorption from the environment [20, 21]. The EPS matrix not only binds the microalgae to the growth surface but also provides absorbed nutrients and inorganic carbon from bacteria for the growth of microalgae [20, 22]. Thus, EPS forms a microenvironment to protect cells from environmental stress such as dehydration and fluctuations of pH, temperature, water or nutrient shortages, presence of biocides, and other antimicrobial agents and conditions [21, 23–25]. Although EPS is 99% water and collapses upon itself when dehydrated [26], it can compose up to 90% of the organic matter in some (bacterial) biofilms [21]. EPS production can be influenced by environmental stress and other factors such as growth surface, bacteria and microalgal species, and nutrient concentration [27–29]. Becker [29] and Shen et al. [28] reported an increase in EPS production when growth materials with good adhesion strength were used. Domozych et al. [27] and Shen et al. [28] observed that an increase in nutrient concentration especially nitrogen increased the EPS production in some species of diatom and green algae. Becker [29] and Shen et al. [28] suggested that EPS production by algae increases as their colonies get mature or aged. Domozych et al. [27] observed that temperature stress and mineral (calcium) accumulation also affect EPS production from algal cells. Compared to bacterial systems, the literature on EPS production and EPS matrices in axenic and mixed community algal biofilms is limited [30].

Supporting Material

Due to the detrimental effects of algal biofilm on support materials, most of the studies were focused on controlling or subsiding algal biofilm growth to protect the materials [31]. Algal biofilm being ubiquitous in nature had provided an idea to researchers for using it as an alternate option for the production of algal biomass. The material used for supporting biofilm growth should be inexpensive, reusable, easily available, and easy to handle. Various materials used for biofilm growth include polycarbonate [32–37], polyvinyl chloride [38–40], concrete [41], plastic scrubber screen [42], polyethylene [43–53], stainless steel [28, 54–56], glass-reinforced plastic [28, 57], glass fiber filter [58], cellulose acetate/nitrate filter [59–61], paper filter [62, 63], nylon [64], chromatography filter [65], polyethylene woven geotextile [66, 67], electrostatic flocking cloth (EFC) [68], nonwoven spunbond fabric [69], polystyrene [49], cotton [12, 70–72], and canvas [73].

Many researchers have studied the material properties like surface tension, hydrophobicity, polar surface energies, and surface micropatterning [18]. Finlay et al. [74], Ozkan and Berberoglu [75] and Sekar et al. [55] have concluded that hydrophobic surfaces may be ideal support surfaces for growing microalgal biofilm. However, Genin et al. [76] and Irving et al. [77] were found less or no correlation of hydrophobicity with the biofilm growth. Cao et al. [78] and Sekar et al. [55] have demonstrated an increase in cell attachment on the surface by micropatterning and increasing surface roughness. On the other hand, Irving et al. [77] and Blanken et al. [54] observed no long-term effect of change in the roughness of the surface on the biofilm growth. Schnurr and Allen [18] have reviewed the effects of material properties on algal biofilm growth and concluded that the surface properties may affect the cell attachment or cell colonization, but once confluence is reached, the surface roughness does not affect the biofilm biomass growth.

Jhonson and Wen [49] tested different types of foam, cardboard, fabric, and sponge. Among various materials, cardboard lost its rigidity after the growth and harvest cycle. The sponge and polyurethane foam had microalgae growth in pores and harvesting of microalgae was difficult. Polystyrene foam was found to be best with a biomass yield of 25.65 $g_{(DW)}/m^2$. Christenson and Sims [70] used nylon, polypropylene, cotton, acrylic, jute, and polyester in a cord and sheet form. Cotton cording was found to be an effective substratum with biomass productivity of 20–31 g m^{-2} d^{-1} after 12–20 days of operation.

The productivity of the biofilm on the surface of various materials has shown large variations due to the varying species selection, nutrient availability, and growth conditions.

Nutrient Availability

Microalgae require N and P as key nutrients for its growth. A Redfield ratio N:P of 16:1 by moles, in general, is considered a balanced supply of nutrients [79]. The N:P ratio regulates the dominance of planktonic communities by blue-green algae or cyanobacteria [80]. Dodds and Smith [81] reported that both N and P and benthic algal biomass were statistically linked

together, and high TN:TP ratio produced more algal biomass per unit P in the water column.

Earlier many researchers had worked on increasing the lipid content of the algal biomass and observed that the application of certain environmental stresses can trigger lipid accumulation response in the microalgae. Among the environmental stresses, nutrient starvation has been applied as the most common stress to control and increase the yield of lipids before harvesting the biomass [82, 83]. Ho et al. [84] observed that *Scenedesmus obliquus* produced maximum lipid and carbohydrate content of 22.40 and 46.65% for 5-day and 1-day N-starvation, respectively. Schnurr et al. [85] studied lipid accumulation through nutrient starvation and suggested that optimizing lipid yields through starvation of biofilms was not suitable.

Various algal species differ in their nutrient requirement but macronutrients such as nitrogen, phosphorus, and carbon are essential for all microalgae species [86]. The micronutrients Mo, K, Co, Fe, Mg, Mn, B, and Zn are also required in trace amounts. All macro as well as micronutrients influence many enzymatic activities associated with the growth of algal cells [87]. Urea is a commonly used source of inorganic nitrogen and glycerol, and acetates or CO_2 are added as a source of carbon for the growth of algae culture [88]. The nutrient availability generally affects carbohydrates and lipid content in microalgae [89].

Environmental Conditions (Light, Temperature, Flow Velocity, CO_2)

Light

The sun is the ultimate source of energy, which provides light to the microalgae for carrying out photosynthesis. The duration and intensity of light directly affect the photosynthesis and biochemical composition of microalgae [90]. Usually, the light of wavelengths between 400 and 700 nm is used in the photosynthetic process. This range of wavelength is commonly called photosynthetic active radiation (PAR) [91]. In the attached growth system, biofilm is typically under some depth of water column and previously attached cells are shaded by newly attached cells within the biofilms. Therefore, light available to the cells in a deeper underwater column and within the biofilm is limited. Thus, to deal with this problem, ambient PAR should be provided in order to achieve maximum output. PAR adjustment can be accomplished using the Beer-Lambert equation and an extinction coefficient [92, 93]. Low light intensity in shaded parts of biofilm or high light intensity in upper parts of biofilm can inhibit the growth of the microalga [19, 94–97]. Liehr et al. [93] estimated 90 and 700 $\mu E/m^2/s$ as an optimum value of light required for the proper growth of biofilm. The biofilm growth becomes light-limited below this range and photo inhibited above this range. Thus, optimal light intensity needs to be determined experimentally in each case to maximize CO_2 assimilation with a minimum rate of photorespiration and as little photoinhibition as possible [97]. The design of the biofilm system should have the optimized light intensity for the proper growth of biofilm. However, certain microalgae have the ability to optimize the light intensity to prevent photodamage.

Temperature

Temperature plays a critical role in the growth of microalgae by influencing the biochemical processes, including photosynthesis, in the algal cells. Controlling temperature is virtually impossible as temperature varies seasonally and within the day. Each species has its own optimal growth temperature. Usually, increasing temperature to the optimum range exponentially increases the algal growth, but an increase or decrease in the temperature beyond the optimal range retards or even stops algae growth and activity [98]. The optimum temperature range for most microalgal species is 20–30 °C [99, 100]. The thermophile algae such as *Anacystis nidulans* and *Chaetoceros* can sustain high temperatures up to 40 °C [101]. Grobbelaor and Soeder [102] and Atkinson et al. [103] observed that temperature affects the rate of respiration, and therefore, the system used for the cultivation of algal biofilm should have average temperature within the optimum range for microalgae. At low temperatures, photosynthesis is affected by low carbon assimilation activity, whereas at too high temperature, the destruction of photosynthetic proteins reduces photosynthesis and algal cell size [103]. Many researchers have observed higher productivity in summers as compared to the productivity in winters. Moller et al. [104] used temperature as a stress treatment to induce the production of valuable metabolites. Converti et al. [105] produced more carbohydrates and lipids from *Chlorella vulgaris* by growing it at 25 °C compared to grow at 30 °C.

Flow velocity

For firm attachment of the biofilm to the substrate surface, the rate of attachment should be greater than the washout rate. These processes are generally influenced by the velocity of the culture medium. The culture medium should have sufficient velocity so that the nutrients can be supplied regularly to the biofilm and helps in the washout of the by-products [106]. However, an increase in velocity increases the mechanical stress on the biofilm and results in wearing of biofilm [107]. Jhonson and Wen [49] reported that at the static status of growth media algal cells only settled on the surface of supporting material without firm attachment to the material. Also, benthic algae were found in the lotic

environment. Thus, they used a rocking mechanism that mimicked a surge or wave effect. Earlier, the rocking mechanism was used by Pizarro et al. [108] also.

CO_2

Microalgae require CO_2 as a carbon source for their growth. Insufficient CO_2 supply can be a limiting factor of productivity. CO_2 must be feed continuously during daylight hours, and it can be monitored by pH measurements. Mukherjee and Moroney [109] suggested that utilization of CO_2 by microalgae for respiration is safe, and it is advantageous to the ecosystem. Also, microalgae grown on CO_2 emitted from power plants can boost the sustainable development of the planet [110–113]. CO_2 concentration 0.038–10% is considered to be optimal for most microalgal species. Chiu et al. [114] observed maximum biomass production at 2.5% CO_2 for microalgae *Chlorella* sp. The maximum biomass production of *Scenedesmus obliquus* and *Chlorella kessleri* was observed at 6% CO_2 [115]. Some studies have shown a negative effect of higher CO_2 concentration (i.e., more than 5%) on the growth of *Chlorella* sp. [116–118]. However, some microalgae can grow under high CO_2 concentration level (10–15%), but the carbon fixation and biomass production rates are less than those under lower CO_2 concentrations. Very few microalgal species are able to tolerate extremely high CO_2 levels. The *Chlorella* sp. KR-1 and *Chlorella* sp. ZY-1 are able to tolerate up to 70% CO_2 and *Chlorella* sp. T-1 can tolerate up to 100% CO_2.

Biofilm Thickness and Harvesting Frequency

Biofilm development takes place by the attachment of microalgae cells on the surface of the support material. The microalgae cells grow in layers to form a biofilm on the support surface. Excess overlapping of cells results in limitation of light, nutrients, and gas exchange, thus, an appropriate thickness of biofilm is necessary [119]. A regrowth study conducted by Jhonson and Wen [49] concluded that initially grown algal biofilm had less biomass yield and biomass productivity than the regrown algal biofilm at a specific time of harvesting. The biomass productivity decreased with an increase in the harvesting period from 6 to 15 days, and the highest biomass productivity was between 3 and 4 $gm^{-2}d^{-1}$ for both initial and regrown biofilms. Boelee et al. [66] harvested biofilm at 2, 4, 7, and 20 days and observed the highest biomass productivity of 7 $gm^{-2}d^{-1}$ on the 7th day.

Applications of Attached Growth System

Wastewater Treatment System

Microalgae require nutrients for their growth; thus, they can be used for nutrient removal in tertiary treatment of wastewater. Earlier, mostly algal biofilms were used for the removal of nutrients from the wastewater [28]. Sometimes nutrients were supplied externally to meet molar stoichiometric ratios of carbon, nitrogen, and phosphorous (C:N:P) for successful algal biofilm growth [19]. Removal of nutrients is due to microalgae uptake, by precipitation of P with calcium and magnesium ions and NH_3 volatilization at elevated pH [39, 120, 121]. The high costs of wastewater treatment can be offset by incorporating microalgae to treat wastewater and obtaining useful bioproducts. The bioproducts can be used as a source of revenue for wastewater treatment infrastructure and energy requirements for wastewater treatment operations. Thus, the integration of wastewater treatment and bioproduct generation offers sustainability benefits in terms of economic, environmental, and industrial aspects.

Treating wastewater by using an algal biofilm system is a simple energy effective technique for the removal of nitrogen and phosphorus, followed by easy harvesting of the algal biomass. Macronutrients such as Na, Mg, Ca, and K; micronutrients such as Mo, Mn, B, Co, Fe, and Zn; and other trace elements are readily available in the wastewater. However, available nutrient concentrations are dependent on the wastewater source [70, 122].

Researchers have successfully grown algal biofilm using dairy, swine, and municipal wastewaters with biomass yield ranging from 0.5 to 31 $gm^{-2}\ d^{-1}$ [14, 19]. Biomass yield and nutrient uptake are directly proportional to nutrient loading rates. However, this relation ceases when maximum nutrient uptake capacity is reached [38]. Nutrient loading rates from 0.11 to 4.53 g N $m^{-2}\ d^{-1}$ and 0.01 to 0.58 g P $m^{-2}\ d^{-1}$ have been investigated for algal biofilm growth systems [50, 53]. Mulbry et al. [47] reported 70–90% of nutrient recovery with nutrient loading rates up to 1 g TN $m^{-2}\ d^{-1}$ and 0.15 g TP $m^{-2}\ d^{-1}$, for N and P, respectively; however, at higher loading rates, nutrient recovery decreased to 50–80%. Boelee et al. [38] increased the phosphorous loading rate by decreasing N:P ratio from 23:1 to 11:1 and observed reduced biomass N:P ratios. The reduction in biomass N:P ratio might be due to the luxury uptake of P. Frequent harvesting of algal biomass is of utmost importance as increased thickness of biofilms limits the transportation of nutrients and light penetration, ultimately leading to sloughing of biofilms [123]. The exponential growth phase of microalgae growth has shown the highest nutrient removal capacity in algal biofilms [124]. The use of algal biofilm for wastewater treatment successfully removes 30–100% nutrient from the wastewater with N and P removal

as 0.07–14.1 g N m^{-2} d^{-1} and 0.013–2.1 g P m^{-2} d^{-1}, respectively [50, 70, 125, 126].

The challenging part of using wastewater for algal biofilm growth is the contamination of produced biomass due to various bacteria, viruses, or heavy metals. The contaminated biomass may be toxic with low application potential. If the nutrient removal capacity is sufficient to meet the need for wastewater treatment, then the algal biofilm system is a cost- and energy-efficient system for tertiary treatment of wastewater. More research is required for understanding biological and physiological processes involved in algal biofilm formation, to help improve the sustainability and predictability of these systems with proper modeling [124].

CO_2 Sequestration

Microalgae have the potential for capturing and storing CO_2 [127–129]. Photosynthesis in microalgae directly fixes carbon into their cells using water and CO_2 in the presence of sunlight. The CO_2 sequestration using microalgae is effective and promising as it utilizes CO_2 and produces valuable products as compared to other CO_2 capture and storage techniques. Mirón et al. [130] and Huang and Tan [131] estimated that approximately about 50% of carbon by dry weight is present in microbial biomass. Herzog and Golomb [132] reported that about 1.6–2 g of CO_2 is captured per gram of algal biomass produced. The microalgae are considered to be the most productive biological systems that consume CO_2 and generate biomass. Further, as compared to terrestrial plants, CO_2 sequestration via algae is 1–2 times higher. The CO_2 capture efficiencies can be increased by harvesting 100% of the produced algal biomass, and it may be used as a potential carbon sink to remove excess CO_2 from the atmosphere [133].

Klinthong et al. [134] explained the different inorganic carbon assimilation pathways used by microalgae, i.e., either by direct CO_2 assimilation or by direct transport of bicarbonate via plasmatic membrane or by using bicarbonate induced by enzyme carbonic anhydrase. Cyanobacteria and eukaryotic algae utilize bicarbonates as a source of carbon for their growth [135, 136]. The bicarbonates are the most dominant (> 50%) available in the form of CO_2 when the pH of the algal growth system is between 6.4 and 10.3. The bicarbonate gets dehydrated, either spontaneously or by carbonic anhydrase to produce CO_2, which is ultimately captured by algal biomass.

Various anthropogenic sources of CO_2 may be used in algae cultivation but varying CO_2 concentrations, presence of other contaminating substances, high effluent gas temperature, and volume of the flue gas will affect the design of CO_2-delivery systems. Effluent gases from power plants usually have high CO_2 concentrations, ranging from 10 to 20%, along with a significant amount of nitrous and sulfur oxides. The use of these flue gases into algal ponds has shown increased algal biomass yields by as much as threefold, but at a high energy cost [137]. The studies have shown that compared to the equivalent concentration of pure CO_2, the direct use of effluent gasses into algal ponds increased the biomass yield by 30% [138]. The use of flue gases results in photorespiration which reduces the photosynthetic carbon fixation efficiency by 20 to 30% [139]. Fortuitously, the merit of using flue gas for algal growth is much higher as compared to the reduction in biomass yield due to photorespiration [140]. In particular, the ability of microalgae cells to absorb CO_2 suggests microalgae cultivation as an attractive alternative for CO_2 sequestration from fossil fuel power plant gas effluents to facilitate the reduction of greenhouse gas emissions [111].

Microalgae-Microbial Fuel cells

Microalgae–microbial fuel cell (mMFC) is a device which contains microalgae in the cathodic chamber and organic feed in the anodic chamber. The organic matter is oxidized producing CO_2 at the anode. The CO_2 is used by microalgae for respiration at the cathode to produce oxygen. O_2 acts as an electron acceptor which receives excess protons and electrons from the anode chamber. *Chlorella* vulgaris release oxygen to the suspension, in the cathodic chamber [141–144]. Lee et al. [145] explained MMFC functioning with the help of following reactions at the anode and the cathode.

At anode:

$$\text{Organics} \rightarrow CO_2 + H^+(\text{to cathode}) + e^-(\text{external circuit}) \quad (1)$$

At cathode:

$$O_2 + H^+ \text{ (from cathode)} + e^- \text{ (external circuit)} \rightarrow H_2O \quad (2)$$

Overall reaction:

$$\text{Organics} + O_2 \rightarrow CO_2 + H_2O + \text{external power} \quad (3)$$

Microalgae use sunlight to convert CO_2 into biomass and release oxygen to the water in the cathode chamber as per reaction (4). The microalgae biomass can be used in the anode chamber as a substrate or in the cathode chamber to produce oxygen and fix CO_2 simultaneously. The use of biomass generated in the cathode chamber can be used as feed in the anode chamber for a zero-carbon discharge scheme [145]. The cathodic chamber can have microalgae either in a suspended or in an attached form.

$$CO_2 + H_2O + \text{light} \rightarrow \text{biomass} + O_2 \quad (4)$$

The photosynthetic microorganisms, i.e., microalgae are capable of converting solar energy to electrical energy via biological metabolic activity [146]. They sequestrate CO_2 from the air and remove nitrogen from the waters [142, 147]. Some studies investigated the use of live green algae growing in the cathode chamber and dead microalgae biomass

as a substrate for anodic biofilm. This leads to the possible use of the biomass generated as feed (organics) in a zero-carbon discharge scheme [148–150].

The overall performance of photosynthetic algal microbial fuel cells (PAMFCs) depends on factors such as pH, temperature, substrate type, organic loading rate, photosynthetic efficiency, and designing of PAMFC/MFC (i.e, electrode and membrane) [151]. The EPS produced by algae allows adhesion of microalgae cells to each other for developing a healthier biofilm. The researchers have to explore and develop new strategies to produce a healthier biofilm for more efficient PAMFCs.

Large-Scale Biomass Production

Researchers have estimated that algae produce 2–10 times more biomass per unit land area than terrestrial systems [95, 138, 152]. The higher biomass yields are due to the high photosynthetic efficiency of algae than that of the terrestrial plants [152, 153]. The extraction and processing of biofuel from supportive structures of the plant (stems and roots) are laborious and can be energy-intensive as compared to extraction from microalgae cells. Moreover, microalgae can be harvested at any time round the year, but for terrestrial crops, harvesting is allowed only in their respective seasons. The accumulation of the lipids can be altered by altering the growth conditions (e.g., low nitrogen) or in the presence of supplemental reductants (e.g., sugar, glycerol). Researchers have reported that microalgae can produce up to 60% of lipids per gram of dry weight [152, 154]. Microalgae growth factors like temperature, pH, nutrients, CO_2 concentrations, light quantity, and quality can be monitored and optimized for maximum algal biomass and oil yields. Lee et al. [155], Johnson and Wen [49], and Economou et al. [156] compared microalgae growth for biomass yield and lipid content in attached and suspended growth systems. Lee et al. [155] reported that the biomass yield and lipid content were 2.8-times higher in the case of the attached growth system. Johnson and Wen [49] observed oil productivity ranging from 0.06 to 0.23 g/m^2 day for *Chlorella sp*. Similar oil productivity was reported for filamentous cyanobacterium *Limnothrix sp*. growing in the attached growth system [156]. Ho et al. [84] observed that nitrogen starvation in *Scenedesmus obliquus* triggered the accumulation of lipid and carbohydrate producing 840.6, 140.4, and 383.4 mg L^{-1} d^{-1} of biomass, lipid, and carbohydrate, respectively. They also reported that increase in light intensity from 60 to 180 μmol m^{-2} s^{-1} significantly increased the three major fatty acids (namely, palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1)) from 14.96 to 19.80%, 1.68 to 9.08%, and 11.61 to 16.41%, respectively. Similarly, Guiheneuf et al. [157] observed enhancement of three major fatty acids contents of microalga *Pavlova lutheri* under a high light intensity of 340 m^{-2} s^{-1}. For large-scale algae biomass production, coupling algae cultivation with wastewater treatment proves to be sustainable to treat wastewater and curtails the cost of commercial chemicals needed for growing algal biomass. Pittman et al. [158] had stated that the production of biofuels from algal biomass can be economically viable if the algae cultivation is done with the use of wastewater. However, the use of raw wastewater for large-scale algae biofuels production is uneconomical as it is hindered due to low gas exchange, low light transability, and poor environmental control. Lundquist et al. [159] analyzed different scenarios for cultivating algae using wastewater and concluded that only those cases that stressed on wastewater treatment were able to produce cost-competitive biofuels.

The main problematic part of the industrial production of microalgae biomass is the harvesting of microalgae from its growth medium. The microalgae growth mediums typically have low cell density in the range of 0.3–0.5 g/L, against at least 300–400 g/L cell density required for industrial use [160]. Singh and Patidar [1] have discussed about the various techniques available for microalgae harvesting including coagulation and flocculation, flotation, centrifugation, and filtration or a combination of various techniques. All harvesting methods have some pros and cons, but one thing is common in all methods, i.e., concentrating microalgae suspension by at least 100 times is an energy-intensive process. The attached growth system has an advantage over the other harvesting techniques in terms of energy requirements. As compared to standard suspended culture systems, attached cultivation systems have many advantages including higher biomass production, feasibility for large-scale production, better light and nutrient distribution within the system, less water requirement, and proper operational control [161]. Lee et al. [155] compared microalgae growth in the attached and suspended growth systems and reported that the biomass yield and lipid content were 2.8-times higher in the case of the attached growth system. Also, the harvesting process was easy and cheaper for the attached growth system as compared to the complex and more expensive suspended growth system.

Water Quality Improvement

The surface runoff and other activities contribute a variety of pollutants including nutrients, nitrogen, and phosphorous in the surface freshwater bodies such as lakes, ponds, and rivers. The presence of high nitrogen and phosphorous concentration results in eutrophication of surface freshwater bodies with excessive growth of algae. Various control and remediation methods such as dilution and flushing, sediment dredging, aeration, aquatic and phytoremediation, herbicides, and algaecide have been used regularly over the years [162]. The research on attached algal communities traditionally called "periphyton" with special interest directed on the community structure and primary productivity in streams and rivers was

conducted intensively from the 1960s [163]. Sládečková et al. [164] used laboratory and continuous flow in situ models with artificial substrata made of a fine mesh plastic screen for the periphytic growth and demonstrated marked removal of nitrate and phosphate from enriched influents in drinking water reservoirs. Vymazal [165] observed maximum ammonium and orthophosphate removal efficiency of 80% and 70%, respectively, for an artificial stream made of wood with nylon screens as a substratum for periphyton growth. Algal turf scrubbing is reported as a novel technology for the treatment of agricultural runoff and eutrophic lake water [44]. Adey et al. [42] involved algal turf scrubbing (ATS) technology for the treatment of agricultural runoff and reported mean dry biomass production of 15–27 g/m^2/day using source water with TP concentrations of 0.012–0.148 ppm. The TP removal rates during the spring period ranged from 104 to 139 mgTP/m^2/day. Wu et al. [166] improved the quality of eutrophic waters by using algae-bacterium biofilm developed over the artificial aquatic mat made from a kind of mend macromolecule material. They observed TP, TN, and NH_4^+-N removal of 49.25, 94.97, and 70.15%, respectively, from eutrophic water. The transparency and DO of water were also increased. Ma et al. [167] cultivated algal biofilm as an alternative to control eutrophication and observed TN and TP removal of 93.8 and 79%, respectively, during 5-day treatment. The dried algal production ranged 3.7–7.2 g m^{-2} d^{-1}. Delp [168] reported a significant change in dissolved nitrate, phosphate, and ammonia in eutrophic freshwater pond water using *Chlorella sp.* biofilms grown on a pea gravels in an algal biofilm filtration system and results of the study suggested biofilms as a moderately effective potential treatment option for highly eutrophic ponds. So far, limited studies have been carried out and documented from the perspective of water quality improvement in surface freshwaters due to nutrients and microalgae removal by employing microalgae-based attached growth systems. More studies involving selected microalgae species based on their ability to reduce nutrients and novel support materials are necessary to economically improve the water quality of surface freshwaters as well as recovering valuable microalgal biomass for the production of biofuels and bioproducts.

Need of Techno-Economic Analysis (TEA) and Life Cycle Analysis (LCA)

Techno-economic analysis (TEA) and life cycle analysis (LCA) help in the identification of specific problematic components in a system and in developing innovating techniques with reduced energy and environmental footprint. The microalgal biomass production on a large scale involves several processes such as cultivation, harvesting, drying, and downstream processing. These processes involved in microalgae biomass production have environmental footprints based on the inputs in terms of energy and materials. The TEA and LCA are essential for developing a sustainable technology for large-scale microalgae production with the ultimate goal of zero net energy and zero waste generation. In recent years, researchers have shown keen interest in identifying the hotspots in microalgal based biofuels and bioproduct production through TEA and LCA [169–176]. The integrated economic and ecological assessment helps in arriving at a cost-effective sustainable method of large-scale microalgae biomass production for biofuels and bioproduct recovery.

Xin et al. [171] evaluated the wastewater-based algal biofuel production and estimated $2.23/gal as the selling price of the produced biofuel. While conducting TEA, they considered wastewater treatment, foul gas emissions, biofuel production costs, and coproduct utilization. Juneja and Murthy [172] conducted TEA and LCA to evaluate the production of renewable diesel (RD) from algae via the hydrothermal liquefaction (HTL) process and estimated $6.62/gal as the cost of the RD production. They concluded that the cost of renewable diesel production depends on the size of the plant and the lipid content of algae. The total greenhouse gas emitted and the total fossil energy used were − 110 kg CO_2 equivalent and 241.6 MJ equivalent, respectively, per 1000 MJ of energy produced by the RD. Barlow et al. [173] also evaluated the production of RD via HTL of biomass from a rotating algal biofilm reactor. The TEA has shown that biomass feedstock costs regulate the minimum fuel selling price. The LCA results have shown that the optimization of the system reduced the minimum fuel selling price from $104.31 to $11.90/gal, global warming potential from 80 to − 44 g CO_2 equivalent MJ^{-1} and net energy ratio from 1.65 to 0.33. Kang et al. [174] performed TEA to evaluate the economics of lipid production from different microalgae species and observed that the total lipid production costs were significantly different for different microalgae species. The total production costs were ranged from $6.4 to $8.3/kg lipid, and costs for each processing stage were different depending on the microalgae species. Yadav et al. [175] conducted a LCA on biomass production and CO_2 sequestration under different conditions. The semicontinuous cultivation mode resulted in 3.5 times more biomass production with reduced greenhouse gas emissions (GHG) and other impacts by roughly 45–50% as compared to the batch regime. Porcelli et al. [169] conducted LCA on using different sources of CO_2 for cultivating the microalgae and observed that the semiindustrial production of *P. tricornutum* using waste gas containing CO_2 in place of synthetic CO_2 was better particularly for GHG emission reduction. Moreover, slightly higher productivity was also observed in the cultivation stage for waste gas CO_2 source. Sensitivity analysis also confirmed that algal productivity could improve the environmental performances by up to 20–25%.

Researchers have intensively worked on many technological issues and mainly focused on biomass quantity, lipid content, and nutrient removals. Processing of algae to the final product requires dewatering of algae after their harvesting and it is energy-intensive step. TEA and LCA studies have highlighted that cultivating and harvesting stages in the production of algal biofuels and bioproducts significantly affect the production cost and environmental impacts [169–176]. More studies involving advance harvesting techniques and energy-efficient unit operations are required for developing a cost-effective and sustainable technology for large-scale microalgal biomass production.

Conclusions

The algal biofilm-based attached growth systems have potential applications in wastewater treatment, CO_2 sequestration, microalgae–microbial fuel cell, large-scale biomass production, and water quality improvement, but inherent variability in associated growth conditions hinders its large-scale implementation. The techno-economic and life cycle analyses have shown that cultivation and harvesting stages account for a large proportion of production cost and environmental impacts in the production of algal biofuels and bioproducts. Thus, various factors affecting microalgae growth, development of algal biofilm, and operation of algal biofilm systems need to be properly understood and optimized for better economics, more positive environmental impacts, and successful potential applications with significant increase in algae biofilm biomass productivities as well as biochemical concentrations in the produced algal biomass. Growing specific microalgae cultures in surface freshwaters, carefully characterized wastewater and/or effluent gases help in water quality improvement, CO_2 sequestration, treating wastewater, and/or effluent gases to mitigate the problems associated with the greenhouse gas emissions and global warming and producing contamination-free algal biomass for various beneficial applications. The recovery of valuable biofuels and bioproducts from the algal biomass will add economic and sustainability benefits to water quality improvement, wastewater, and/or effluent gas treatment operations. Microalgae–microbial fuel cell technology simultaneously works on wastewater treatment, bioelectricity production, and resource recovery.

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Compliance with ethical standards

Statement of informed consent No conflicts, informed consent, and human or animal rights applicable.

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