Title: Algal Biofilm Reactors for Integrated Wastewater Treatment and Biofuel Production: a review

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ABSTRACT

This review analyzes various algal biofilm reactors used for integrated wastewater treatment and biofuel production to overcome the current challenges for algal biofuel production. Various reactor configurations, support materials and operation strategies of algal biofilm reactors are discussed and compared with conventional suspended culture systems in terms of algal biomass productivity, nutrient removal, biomass harvest and biofuel production. The rotating biofilm reactor among various types of biofilm reactors was found to be a promising option to provide high biomass productivity and efficient utilization of nutrients in wastewater. Some materials such as stainless steel, nylon and natural fibers among various materials were found to be highly effective for supporting microalgal biofilm. To date mainly municipal wastewater has been integrated with algal bioreactors while only a few agricultural wastewater have been used for algal bioreactors due to inhibition of algal growth with high ammonium concentrations in animal manure and poor light delivery with high turbidity of animal manure. Overall, the algal biofilm reactors integrated with wastewater would have great potential for high productivity of algal biomass and efficient wastewater treatment if various conditions are optimized.

Keywords: Microalgae, algal biofuel, wastewater treatment, biofilm reactor, suspended system, harvest of algae

1. Introduction

Conversion of various biomass to cost-effective biofuels has been considered as a potential solution to replace current use of fossil fuels as fossil fuels become scarce and more expensive [1]. The first-generation feedstock using food crops brought serious competition between food and biofuel [1]. The second-generation biofuel using lignocellulosic biomass can avoid the competition between food and biofuel, but is limited by the high cost associated with pretreatment of lignocellulosic biomass for removal of lignin [2].

Microalgae is a very promising source for biofuel by using efficient photosynthesis for fixation of CO₂. Compared to terrestrial plants, microalgae show faster growth rate, with high efficiency (above 10%) exceeding that of terrestrial plants by a factor of 10-50 [3]. Under unfavorable environmental conditions and in marginal land, microalgae can grow and produce large amounts of lipid, which is used for biodiesel production [4]. In addition, microalgae can use various water sources while recycling nutrients from wastewater streams [5]. Thus, microalgae has distinct advantages such as non-competition with food crops over limited land, high biomass productivity, and high lipid content compared with the feedstock for first and second generation biofuels. In fact, areal productivity of algal biomass is much higher than first and second feedstocks and they also have high lipid content and growth rate [3]. They also require a smaller amount of water and land than first and second feedstocks for biofuels [6]. The possible biofuels from microalgae include biodiesel made from algal lipid content, methane by anaerobic digestion, and bioethanol from algal carbohydrates [7, 8]. The remaining biomass can be

used as a feed for animals and fish, and it can be used as the materials in bioplastics [9, 10].

However, commercial application of microalgal biofuel has been limited by its high operating costs associated with costs for substrate/nutrients, low productivity of algal biomass, and high energy consumption during algal cell harvest. For producing a microalgae biomass, the suspended systems include open and closed types. The open type culture systems like a raceway pond has several disadvantages due to contamination and evaporation problems. Also, it needs large surface area for photosynthesis. Closed bioreactors are not suitable for biofuel production since their operation costs are expensive. No matter what open or closed system, suspended systems consume massive costs for harvesting microalgae cells. Compared with suspended systems, biofilm reactors showed higher algal biomass productivity and easy harvest of algal biomass by scrapping [11].

Besides, algal biofuel production could be integrated with wastewater treatment to lower the overall costs. The Department of Energy's report showed that wastewater treatment should be coupled with the development of microalgae biofuel technologies for economical biofuel production [6]. Current wastewater effluent has high concentration of nitrogen and phosphate which often causes eutrophication and various harmful effects on ecosystems while changing the pH, decreasing dissolved oxygen and causing death of aquatic organisms. While elimination of these nutrient requires a huge amount chemicals and energy [12], adopting microalgae for wastewater treatment can solve the eutrophication problem and treat the water without toxic compounds. Furthermore, wastewater can be nutrients for microalgae to increase microalgae biomass with

wastewater treatment. Therefore, research combining microalgae production and wastewater treatment has received increasing attention [11].

Recently, several studies revealed that the biofilm reactors surpassed the suspended reactors in regard to biomass productivity and wastewater treatment efficiency [13-15]. Current studies to develop algal biofilm reactors have included the use of secondary effluent from municipal and agricultural wastewater in various types of bioreactors and substrates [16-18]. Particularly the agricultural wastewater treatment by microalgae sometimes required dilution before treatment since it has high COD, nutrients, turbidity and dark color. Biomass productivity and total biomass are closely related to surface area, reactor design and supports. The porosity and roughness of supporting material can increase the surface area leading to high biomass productivity and wastewater treatment ability. Therefore, this review deals with limitations of current algal biofuel production, and summarizes various biofilm reactors integrated with wastewater treatment as viable solutions to overcome these limitations. Various types of current algal biofilm reactors and support material used for current algal biofilm reactors are comparatively investigated. In addition, algal biomass production combined with treatment of municipal and agricultural wastewater is also discussed.

2. Limitations of current algal bioreactors for biofuel production

Microalgae have distinct advantages such as non-competition with food crops over limited land, high biomass productivity, and high lipid content. Although microalgae have these advantages, the current biofuel from microalgae has not reached competitive prices. Compared to plant oils, microalgal oil is estimated to be 3-4 times more expensive

[19]. The limiting factors of biofuel production using microalgae are the cultivation and harvest steps [20]. Cultivation accounts for 40% of the cost and energy in microalgal biofuel production [21]. Major factors that affect cultivation are nutrient supply, land and water availability, gas transfer and exchange, photosynthetically active radiation (PAR) delivery and culture integrity. The harvest step takes 20-30% of the total microalgal biofuel cost [20].

2.1. Types of bioreactors

Most of the current algal bioreactors rely on suspended cultures, which can be categorized into open and closed systems. No matter which type is used, suspended culture systems need huge amount of energy to harvest algal cells and eliminate the water in downstream. The harvest costs also account for 20-30% of algal biofuel production [20]. The open pond culture system can be considered the simplest and most economical method among algae cultivation systems. Several types of open pond system including raceway pond, slope system and circular ponds have been developed [7, 22]. The advantage of open system is that the construction and operation cost is cheaper than those of closed system. However, its disadvantages can offset the advantages. The open pond system has low biomass productivity due to several limitations (typical biomass productivity of 4 to 21 g m⁻² d⁻¹) [6, 23]. The limitations include temperature fluctuation, low CO₂ transfer, limited light transmission and contamination with other organisms such as protozoa [20]. Evaporation is also disadvantageous because it can change the ionic strength. In contrast, closed culture systems (engineered photobioreactors) have been developed to overcome the limitations of open culture systems. There is less of a

contamination problem and it is easy to control light, temperature and CO₂. Thus it has higher productivity compared to the open system. For instance, tubular and flat panel photobioreactors (PBR) showed the areal biomass productivity of 13 to 47.7 g m⁻² d⁻¹ for the tubular PBR and 10.2 to 22.8 g m⁻² d⁻¹ for the flat panel PBR [24, 25]. However, due to high operation and construction costs, it is more suitable to produce high-value products such as β-carotene, astaxanthin, and C-phyococyanin [26].

2.2. Nutrient supply

For cultivating microalgae, major nutrients are carbon, nitrogen and phosphate. Carbon is important because it is the source of energy for many cellular events, such as metabolite production and reproduction and it is part of the physical structure of the cell. Carbon can be supplied in two ways: inorganic (i.e., CO₂) and organic (i.e., heterotrophic substrates) sources. Carbon uptake will be discussed in more detail in the gas transfer and exchange section.

Most microalgae dry mass is carbon (40-50%), followed by nitrogen (1%-10%) [27]. A lack of nitrogen and phosphate in algal medium can limit microalgae cultivation. Nitrogen contributes to the production of protein, chlorophyll, and amino acids. Low nitrogen concentration can cause decrease in cell growth and chlorophyll content while increasing lipid production [28]. On the other hand, phosphate is used for energy transfer, photosynthesis, DNA and RNA. Besides nitrogen and phosphate, micronutrients such as sulfur, calcium, sodium, potassium, magnesium, iron, manganese, nickel, zinc and cobalt are needed for effective microalgae cultivation. Domestic and agricultural wastewater contain these nutrients, so the cultivation of microalgae using wastewater as a nutrient

algae medium is a promising treatment method [29]. Lundquist suggested competitive microalgae biofuel production with wastewater treatment by showing diverse models [30]. In the microalgae cultivation, the typical optimal C:N:P ratio is 106:16:1, but it can vary depending on the algal strains [31]. For higher biomass productivity, wastewater can be reformulated to include modified nutrients which are either supplemented or diluted [18].

2.3 Gas transfer and photosynthetically active radiation (PAR) delivery

For efficient gas exchange, CO₂ supply and O₂ elimination should be accompanied. Dissolved carbon exists in different forms: CO₂, bicarbonate (HCO₃⁻) or carbonate (CO₃²⁻). However, microalgae can use CO₂ well, bicarbonate often, and carbonate hardly. Due to the C:N:P ratio and its low solubility, CO₂ should be injected continuously for biomass production and wastewater treatment. Efficient transfer of carbon dioxide in several types of bioreactors leads to high biomass productivity [32, 33] while the elimination of O₂ is also important to produce algal biomass. Because O₂ can hinder photosynthesis, the development of PBR should be considered for O₂ elimination. For instance, the transfer and exchange of CO₂ and O₂ can be easily solved by employing a gas-liquid contactor such as rotating biological contactor (RBC). Zeevalkink et al. [34] showed that RBC has higher gas exchange efficiency than other bioreactors using surface aerators, diffuser aerators, or trickling filters. Blanken et al. [35] used the RBC in microalgae production and showed high biomass productivity of 20.1 g m⁻² day⁻¹ mainly due to the effective gas transfer and exchange.

Light delivery has been studied as an important operating factor in algal culture in terms of light intensity, quality and cycle [36-39]. Microalgae cells absorb almost all of

the photons they receive, although they cannot use them all, and they dissipate any excess light source [40, 41]. Thus, the inner cells of the bioreactor cannot receive enough light for growth because the light cannot penetrate cells. Over the thickness of the culture, the algal cell cannot obtain adequate light for photosynthesis [42], and it is commonly observed that algal growth depends on light intensity. This is the light limitation in algal growth [27]. On the other hand, excessive light intensity causes reduction of the cell growth and productivity because of the photoinhibition phenomenon [43]. Utilizing light efficiently and preventing photoinhibition could be accomplished by reactor design, efficient culture mixing, and intermittent light supplementation [44-48].

2.4. Harvest of algal cells

Separating the algae and culture broth is a big hurdle for algal biofuel production because of tiny size (i.e., 3–30 μ m for eukaryotic algae, 0.2–2 μ m for cyanobacteria) and strong negative charge of algal cells [20]. For these reasons, 20-30% of the total cost of algal biofuel production is used for harvesting the algal cells, and this process can be the limiting step in the downstream of algal biofuel production [20]. The harvesting and dewatering technologies that have been researched include mechanical, chemical, and electrical methods. The most common method for algal cell harvest is centrifugation, which separates using difference of density [20]. However, the cost is high and the strong gravity forces can affect cell structure [49]. Filtration methods can cause membrane clogging with high cell density. On the other hand, tangential flow filtration can separate algae and water effectively [50]. However, this consumes a huge amount of energy and operating costs due to membrane fouling [51, 52]. Sedimentation by gravity is

cheap, but it takes a long time for settlement. Furthermore, it is hard to separate at low cell count due to poor settling [53]. Dissolved air flotation (DAF) is commonly used for wastewater treatment for removing floating matter. As air is injected, the algae attach to the surface of the air, and then can be harvested using a skimmer. However, it needs pretreatment, so it is not suitable for mass harvest. Chemical flocculation can be used for diverse strains [54] using inorganic or organic coagulants. Due to the negative charge of microalgae cells, they can be turned into flocs [52] using aluminum sulfate and ferric chloride that have a positive charge. However, it is sensitive to pH, makes a large amount of sludge, and the algae can be affected by chemicals such as ammonium and iron salt. Organic coagulants such as chitosan and cationic starch are large polymers that have a neutral charge [53, 55], but are expensive for mass harvest [56, 57]. Negatively charged algal cells can also be separated by electric field [58, 59] which is a safe alternative without addition of any chemicals while consuming high electric energy and not being suitable for mass cultivation and harvest [52].

3. Algal biofilm bioreactors as an alternative platform for algal biomass production and wastewater treatment

Section 2.1 describes the limitations of current algal bioreactors (mostly suspended algal culture) such as open pond systems and closed bioreactors. Section 3 deals with current algal biofilm reactors to overcome current limitations of suspended culture systems such as high energy consumption and costs [60]. According to a DOE report, algal biofilm technology can be introduced as a promising system that can increase culture density while requiring less water and land [61]. Microalgae cells

produce an extracellular polymeric substance (EPS), consisting of polysaccharides, proteins, nucleic acids, and phospholipids which helps to make the biofilm [62]. In this section, suspended and biofilm bioreactors will be compared in terms of biomass productivity, waste removal efficiency and harvest. In the following sections, various types of biofilm reactors and supports will be addressed when the biofilm reactors are integrated with wastewater treatment.

3.1 Biofilm reactors surpassing suspended reactors for biomass production, harvest and nutrient removal

Recently, several research groups have studied microalgae cultivation using wastewater focusing on biofilm bioreactors, wastewater types and harvesting methods. Several studies indicated that the biofilm bioreactors have much higher biomass productivity at the same conditions as the suspended systems as listed in Table 1[13-15].

Christenson and Sims [13] treated the secondary effluent municipal wastewater using the rotating algae biofilm reactor (RABR) in the lab and pilot scale. The RABR showed the biomass productivity of $31 \text{ g m}^{-2} \text{ d}^{-1}$ which was much higher than the suspended system (biomass productivity of $7.4 \text{ g m}^{-2} \text{ d}^{-1}$). The fatty acid methyl esters (FAME) concentration in the algal biomass cultivated by the RABR was also higher than the suspended system: $2\text{-}2.3 \text{ g m}^{-2} \text{ d}^{-1}$ and $1.0\text{-}1.2 \text{ g m}^{-2} \text{ d}^{-1}$ respectively [13]. Lee et al. [14] also used secondary effluent from municipal wastewater treatment plants, and had similar results as Christenson et al.'s research [13]. The biomass productivity from the algal biofilm reactor reached 9.1 (average) and $13.5 \text{ g m}^{-2} \text{ d}^{-1}$ (max) while the suspended system showed the biomass productivity of $3.2 - 6.1 \text{ g m}^{-2} \text{ d}^{-1}$. This clearly indicates that

the biomass productivity of the biofilm reactor was 2.8 times higher than that of the suspended system. The biofilm's total lipid was estimated to be $3.7 \text{ g m}^{-2} \text{ d}^{-1}$ (max) and $1.9 \text{ g m}^{-2} \text{ d}^{-1}$ (average), however, the suspended system's total lipid was limited to $1.2 \text{ g m}^{-2} \text{ d}^{-1}$ (max) and $0.7 \text{ g m}^{-2} \text{ d}^{-1}$ (average). Thus, the biofilm bioreactors led to the biomass and total lipid productivity which were higher than the suspended system [13].

Johnson and Wen [15] treated dairy manure wastewater using the algal biofilm reactor. The biomass productivity in the biofilm reactor and the suspended system were $2.57 \text{ g m}^{-2} \text{ d}^{-1}$ and $0.127 \text{ g L}^{-1} \text{ d}^{-1}$. Although these productivities are expressed in different units, the experiment was carried out in the same volume such that the absolute amount can be compared [15]. According to Gross et al. [8] using *Chlorella vulgaris* (UTEX #265) and the synthetic algal medium for cultivation, the biomass productivity of the biofilm reactor and the suspended system reached $14.1 \text{ g m}^{-2} \text{ d}^{-1}$ and $3.5 \text{ g m}^{-2} \text{ d}^{-1}$ respectively in the pilot scale.

In terms of nutrient removal, the algal biofilm reactors showed higher sequestration ability than those by the suspended culture system at the same operating conditions and reactor size. Christenson and Sims [13] reported that the biofilm reactor sequestered phosphate six times higher than the suspension culture system when the municipal secondary effluent was fed to the algal bioreactors. Other studies also confirmed that the algal cell harvest in the biofilm reactors can be much more efficient than the suspended systems [14, 15]. The other harvest method, centrifugation, which consumes massive energy, has a recovery rate of more than 90 % [52, 63]. Also, Johnson et al. [15] showed similar data that there is no large difference in water content (1.6%) between scrapping from biofilm and centrifuge [15]. Compared to other harvest methods

(tangential filtrations [52], sedimentation [63], dissolved air floatation [52, 63]), scrapping the biofilm has been shown as a possibly economical harvest method due to lower energy consumption.

Ozkan et al. [64] compared raceway ponds, flat-plate photobioreactor (PBR), tubular PBR and algal biofilm PBR in terms of algal biomass production and energy efficiency when *Botryococcus braunii* was cultivated in various bioreactors. The biofilm PBR provided highest algal biomass concentration (96 g L^{-1}) followed by the flat PBR (2.7 g L^{-1}), tubular PBR (1 g L^{-1}) and raceway pond (0.35 g L^{-1}) [64]. The areal algal biomass productivity of the algal biofilm PBR ($0.71 \text{ g m}^{-2} \text{ d}^{-1}$) was behind the raceway ponds ($11 \text{ g m}^{-2} \text{ d}^{-1}$), flat ($27 \text{ g m}^{-2} \text{ d}^{-1}$) and tubular PBRs ($25 \text{ g m}^{-2} \text{ d}^{-1}$). However, the energy input to produce one kg algal biomass (MJ/kg biomass) for the algal biofilm PBR was lower than other bioreactors (4.7 for algal biofilm PBR compared with 9.2 for raceway ponds, 17 for flat PBR and 386 for tubular PBR). Compared to the raceway ponds, the biofilm PBR reduced the consumption of water for production of 1 kg of algal biomass by 45% and lowered the energy for dewatering algal biomass by 99.7% [64]. Furthermore, the NER (total energy production per energy input, including harvest and dewatering process) for the biofilm PBR was much higher than other bioreactors indicating the algal biofilm reactor could produce high concentration of algal biomass for biofuel with lower energy consumption. It also supports that the algal biofilm reactor would lead to cost-effective production of algal biomass since it showed significant reduction of energy used for cultivation, harvest and dewatering of algal biomass [65].

When the algal bioreactor for biofuel production is integrated with wastewater treatment, it can save costs of nutrients for algal cultivation and contribute to nutrient

removal in wastewater. The US Environmental Protection Agency [66] reported current nutrient removal process as a tertiary treatment at municipal wastewater treatment plants to reduce total nitrogen level to 5 mg L^{-1} and total phosphorus level to 1 mg L^{-1} . It also indicated that the nutrient removal process costed \$ 2.48 per gpd (gallon of wastewater per day) for the capital cost and \$ 477 per MG (million gallon of wastewater) [66]. If the algal bioreactors utilize wastewater and effectively remove nutrients in wastewater, it would lead to saving the costs associated with nutrient removal in wastewater.

3.2 Configuration of Biofilm Reactors

Various types of biofilm reactors have been developed and researched for biofuel and wastewater treatment. There is a huge difference in biomass productivity and wastewater treatment efficiency depending on the types of bioreactors. Light transmission, CO_2 supply and O_2 removal are the major considerable factors to design the biofilm reactor because microalgal cells rely on photosynthesis for their growth and metabolism.

Firstly, the biofilm reactors used for municipal wastewater treatment include horizontal, vertical, flow cell, rotating, and rocker bioreactors (Table 2). The horizontal biofilm reactor has the advantage of effective absorption of light for photosynthesis. However, its surface area has to be large in order to increase biomass productivity and wastewater treatment efficiency. According to Posadas et al. and Ozkan et al. [64, 67], the horizontal biofilm reactors provided $0.5\text{-}3.1 \text{ g m}^{-2} \text{ d}^{-1}$ biomass productivity using a microalgae consortium and $0.71 \text{ g m}^{-2} \text{ d}^{-1}$ biomass productivity using *Botryococcus braunii* (LB572).

On the other hand, the flow lane biofilm reactor, which used flowed-over polycarbonate slides, showed a biomass productivity of $2.9 \text{ g m}^{-2} \text{ d}^{-1}$ using mixed microalgae [68]. Irving and Allen [69] showed higher biomass productivity $5.5 \text{ g m}^{-2} \text{ d}^{-1}$ utilizing *Scenedesmus obliquus* and *Chlorella vulgaris*. Boelee et al. [70] tested a flow cell with the PVC plastic sheet as a biofilm reactor. The biomass productivity of the mixed microalgae cells was $2.1\text{-}7.7 \text{ g m}^{-2} \text{ d}^{-1}$ [70].

One of the vertical biofilm reactors is a twin layer vertical reactor. The two-layer vertical reactor helps to deliver nutrients to the microalgae. Shi et al. [71, 72] cultivated a mixture of *Chlorella vulgaris* strain SAG 211-11b and *Scenedesmus rubescens* M2630 and *Halochlorella rubescens* using two-layer vertical reactors and achieved a biomass productivity of $6\text{-}12 \text{ g m}^{-2} \text{ d}^{-1}$. The other type of vertical biofilm reactor is the vertical bioreactor containing multiple glass plates with algal disks which revealed a biomass productivity of $50 \text{ to } 80 \text{ g m}^{-2} \text{ d}^{-1}$ [73]. The next biofilm reactor is the rocker bioreactor. Using a rocker, Johnson and Wen mixed a horizontal culture, and the *Chlorella* showed $2.57 \text{ g m}^{-2} \text{ day}^{-1}$ biomass productivity using dairy wastewater [15]. With another rocker system, based on previous research, Gross produced $3.51 \text{ g m}^{-2} \text{ day}^{-1}$ using BBM media and *Chlorella vulgaris* (UTEX #265) [8].

Recently the rotating biofilm reactor has been intensively studied and has been previously used for wastewater treatment. Using *Chlorella sorokiniana*, an RBC (rotating biological contactor) supplemented with synthetic medium containing urea, $20 \text{ g m}^{-2} \text{ d}^{-1}$ biomass productivity was observed [35]. In the case of utilizing the municipal wastewater, a pilot rotating algal biofilm reactor (RABR) showed $31 \text{ g m}^{-2} \text{ d}^{-1}$ using mixed culture [13].

Compared to municipal wastewater, a few algal biofilm reactors have been studied to treat agricultural wastewater and animal manure effluents. So far algal turf scrubbers (ATS) have been intensively researched as an economical and promising bioreactor to increase wastewater treatment and algal biomass production [16-18].

3.3 Biofilm supports for algal biofilm

For better cell attachment, effects of various supports on algal biomass production have been researched. The surface of the supports can affect algal biomass productivity because it increases the surface area for algal growth. The pores and roughness of the surface can also protect against hydraulic shear forces [74]. Several studies have tried to find adequate materials for biofilm development.

Cotton was used as a support material for rotating algal biofilm reactors using municipal wastewater effluent and BBM (Bold's basal medium) which provided an algal biomass productivity ranging $3.5\text{-}5.5\text{ g m}^{-2}\text{ d}^{-1}$ (lab) and $14\text{-}31\text{ g m}^{-2}\text{ d}^{-1}$ (pilot) [8, 13]. Lee et al. [14] compared the algal biomass productivity from plates made of polycarbonate and polyethylene and mesh made of nylon and stainless steel. Among these supports, nylon and stainless steel exhibited the highest biomass productivity, and nylon was selected for further experiments as an economical compromise. Besides, Johnson and Wen [15] found polystyrene foam was found to have the best productivity among various synthetic polymers (polystyrene foam, cardboard, polyethylene landscape fabric, loofah sponge, polyurethane foam, and nylon sponge).

Cao et al. [75] compared stainless steel sheet and stainless steel sheet treated with a micro scale laser. The laser-treated sheet had more surface area and showed higher

attachment of *Scenedesmus dimorphus* cells [75]. Similar to Cao's study, Blanken et al. [35] tested the roughness of the steel for algal biomass productivity. The rough steel mesh showed highest algal biomass productivity $21 \text{ g m}^{-2} \text{ d}^{-1}$ over the fine steel mesh ($18 \text{ g m}^{-2} \text{ d}^{-1}$) and the polycarbonate ($14.8 \text{ g m}^{-2} \text{ d}^{-1}$).

In addition to the synthetic polymer, plastic and steel, cotton rope showed a high biomass productivity of mixed algae compared with polyester and acrylic supports [11] indicating that natural polymers would be better for cultivating microalgae than synthetic polymers. Similar results were reported by Gross et al. [8] that cotton supports (cotton duct, cotton rag, cotton denim, and cotton corduroy) showed high algal biomass productivity. Sekar et al. [76] also investigated the affinity of *Chlorella vulgaris*, *Nitzschia amphibia*, and *Chroococcus minutes* species on various support material which showed more attachment of the microalgae onto the hydrophobic materials such as titanium, perspex, and stainless steel. Copper, aluminum and brass did not show good attachment ability due to their toxicity compared with stainless steel [76].

Therefore, these findings can support that roughness, porosity, hydrophobic property and biological affinity of support material can determine algal biomass productivity although support material needs to be tested depending on specific microalgae strains.

3.4. Integrated algal biomass production and wastewater treatment in algal biofilm reactors

Wastewater contains high N and P concentration (10-100 mg/L in municipal wastewater, more than 1000 mg/L in agricultural effluents) [42]. Release of excessive N

and P would cause interruption of aquatic ecosystems and eutrophication if N and P in wastewater are not properly treated [43–48]. However, wastewater containing high N and P can be used as an excellent nutrient with low costs for microalgal cultivation. Microalgae can uptake nutrients from wastewater and produce algal biomass for biofuel production [49–54].

Recently, there have been attempts to alleviate the high costs for microalgae cultivation by coupling with wastewater and waste treatment [77, 78]. Although microalgal cells can grow on and utilize nutrients in municipal and animal wastewater, the published studies focused on mostly municipal wastewater [13, 14, 67-69, 71, 72]. Among the wastewater treatment steps in municipal wastewater treatment plants, the microalgal processes were often designed as the tertiary treatment to remove nutrients (nitrogen, phosphate) from the effluent from the secondary treatment (i.e., activated sludge process) [13, 14, 67-69, 71, 72] .

Algal biomass production and wastewater treatment using suspended microalgae have been widely studied [79, 80]. *Chlorella vulgaris* removed over 90% of N content and 80% of P content in the effluent from the primary treatment of municipal wastewater [79, 80]. They showed high waste removal and high cell productivity (leading to high lipid production) because of rich organic and inorganic sources in wastewater [81]. Particularly many microalgae have mixotrophic growth capacity which can utilize organic (i.e., glucose, acetate, BOD) and inorganic (i.e., CO₂, carbonate) carbon sources. Mixotrophic algal growth has several advantages such as fast growth, lower requirement of light intensity and fast nutrient removal. The mixotrophic growth of *Chlorella* sp. in a

pilot-scale photobioreactor using organic-rich wastewater (centrate wastewater) showed biomass productivity of $34.6 \text{ g m}^{-2} \text{ d}^{-1}$ [82].

The algal biofilm reactors integrated with wastewater treatment have been limited to several bioreactor types and municipal wastewater as summarized in Table 3. As the tertiary treatment process, the rotating algal biofilm reactor (RABR) using mixed culture showed an excellent algal biomass production ($31 \text{ g m}^{-2} \text{ d}^{-1}$) [13]. Lee et al. [14] cultivated attached algae in a raceway pond including various microalgae to have effective algal biomass production ($13.5 \text{ g m}^{-2} \text{ d}^{-1}$). Compared with the RABR, the horizontal biofilm reactors (i.e., flow lane and chamber biofilm reactors) using municipal wastewater effluents showed less algal biomass production, but led to high C, N and P removal from the wastewater (carbon removal > 91%, nitrogen removal > 70%, phosphorus removal > 85%) [67, 68]. Shi et al. [71, 72] developed both vertical and twin layer biofilm reactors and high algal biomass production ($\sim 6.3 \text{ g g m}^{-2} \text{ d}^{-1}$) when the municipal wastewater effluent was fed to the reactor. The twin layer biofilm bioreactor provided 70-99% removal of nitrogen and phosphorus [72].

Compared with municipal wastewater, there have been limited studies about algal bioreactors using animal manure and wastewater (please see Table 3). The limitations of utilization of animal manure wastewater are inhibition of algal growth with high ammonium concentrations and poor light delivery due to high turbidity. *Botryococcus braunii* showed high growth rate, lipid content and removal of nutrients (80% of the initial nitrogen content) from swine manure wastewater [83]. Johnson and Wen [15] used the algal biofilm reactor using dairy wastewater to cultivate *Chlorella sp.* and remove nitrogen and phosphorus. The algal biofilm reactor using polystyrene foam showed high

biomass yield (25.65 g/m^3) and fatty acid production (2.31 g/m^3) as well as high nitrogen and phosphorus removal (61-79% for total nitrogen, 62-93% for total phosphorus). Kebede-Westhead et al. [17] operated the lab-scale Algal Turf Scrubber (ATS) as a biofilm reactor using swine wastewater which demonstrated high algal biomass production ($7.1 - 9.4 \text{ g m}^{-2} \text{ d}^{-1}$) and nutrient removal (40-98% removal for total nitrogen, 41-76% removal for total phosphorus) at various loading rates of N and P in the wastewater. Similarly Mulbry et al. [18] used the pilot scale Algal Turf Scrubber (ATS) to produced high algal biomass ($2.5-25 \text{ g m}^{-2} \text{ d}^{-1}$) and high nutrient removal (70-90% removal for total nitrogen, 50-80% removal for total phosphorus) when dairy wastewater was used as a substrate. Currently dilution of the swine and dairy manure wastewater with municipal wastewater or seawater was found to enhance the algal biomass production by diluting the ammonium concentration and the turbidity in the manure wastewater [84].

4. Conclusions

Current microalgal biofuel production relying on suspended algal culture has been limited by its high operating costs associated with costs for substrate/nutrients, low productivity of algal biomass, and high energy-consuming harvest of algal cells. Various types of algal biofilm reactors integrated with wastewater treatment were developed to overcome current limitation of algal biofuel production. To date the horizontal, vertical, flow cell, and rotating biofilm reactors have been studied to produce algal biomass for biofuel and remove nutrients from wastewater. However, the rotating biofilm reactor among various types of biofilm reactors was found to be a promising option to provide

high biomass productivity and efficient utilization of nutrients in wastewater. Although a variety of materials have been used as the supports for algal biofilm, stainless steel, nylon and natural fibers were found to be highly effective support materials for microalgal biofilm. So far the integration of algal bioreactor and wastewater has been limited to municipal wastewater while a few agricultural wastewater have been used for algal bioreactors due to inhibition of algal growth with high ammonium concentrations and poor light delivery with high turbidity of animal manure. To enhance the algal biomass production using animal wastewater, dilution of the swine and dairy manure wastewater with municipal wastewater or seawater was also studied. Overall, algal biofilm reactors integrated with wastewater would have high potential for high productivity of algal biomass for biofuel and efficient wastewater treatment if various conditions are optimized.

Acknowledgement

This work was supported by U.S. Department of Agriculture (Project number: HAW05024-H).

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Table 1
Comparison of algal biomass production in algal biofilm reactors and suspended culture systems.

Wastewater	Treatment	Biomass production in biofilm (g m ⁻² d ⁻¹)	Biomass production in suspended culture (g m ⁻² d ⁻¹)	Scale	HRT/SRT (d)	Microalgae species	Reference
Municipal	Tertiary treatment	31 (pilot)	7.4 (pilot)	Pilot (8000L)	HRT: 2 SRT: 12-20	Mixed culture	[13]
	Tertiary treatment	13.5	6.1	8000 L 13.5 m ²	HRT: 18	<i>Scenedesmus, Chlorella, Pediastrum, Nitzschia, Cosmarium</i> , filamentous microalgae	[14]
Dairy	Dairy manure wastewater	2.57	1.87	Lab (14L, 0.2L)	HRT: 10-15	<i>Chlorella</i> sp	[15]
Synthetic	BBM (Bold's basal medium)	14	3.51	Pilot	HRT: 5	<i>Chlorella vulgaris</i> UTEX #265	[8]

Table 2

Comparison of algal biofilm reactors at various supports and operating conditions in terms of algal biomass productivity

Bioreactor	Media / wastewater	Supports/Dimension	Biomass production (g m ⁻² day ⁻¹)	HRT (d)	Microalgae species	Reference
horizontal	Municipal	5 mm thick PVC foam	0.5-3.1	10	microalgal consortium	[67]
	BG-11 medium	Media recirculation, 8 mm thick concrete slab (0.275 m ²)	0.71	35	<i>B. braunii</i> (LB572)	[64]
flow lane	Municipal	Flow-lane Incubator with polycarbonate slides (76×25×1mm)	2.9	30	mixed culture	[68]
	Municipal	Flow chamber (2.5 x 2.5 x 0.5 cm)	5.5	9-10	<i>Scenedesmus obliquus</i> <i>Chlorella vulgaris</i>	[69]
	Synthetic wastewater	Flow cell with 1 mm PVC plastic (0.018 m ²)	2.1-7.7	9-25	microalgal consortium	[70]
Vertical	Municipal	Twin-layer system, nitrocellulose	1.32	9	<i>Chlorella vulgaris</i> SAG 211-11b, <i>Scenedesmus rubescens</i> M2630	[71]
	Municipal	Twin layer photobioreactors (1 m x 1 m)	6.3	8	<i>Halochlorella rubescens</i>	[72]
	BG-11	Multiple glass plates with algal disks	50-80	10	<i>Scenedesmus obliquus</i>	[73]
Rotating biological contactor	M8	1.0 mm (L) x 0.4 mm (W) x 0.7 mm (H)	20	7	<i>Chlorella sorokiniana</i>	[35]
	Municipal	Cotton rope around aluminum wheels, bench-medium-pilot (8, 535, 8000L)	5.5 (bench) 31 (pilot)	12 -20	mixed culture	[13]

Table 3
Algal biofilm reactors integrated with wastewater

wastewater	Biofilm reactors	Biomass production (g m ⁻² day ⁻¹)	Microalgae species	Reference
Municipal	Rotating biofilm reactor	31	mixed culture	[13]
Municipal	Attached cultivation in a raceway pond	13.5	<i>Scenedesmus</i> , <i>Chlorella</i> , <i>Pediastrum</i> , <i>Nitzschia</i> , <i>Cosmarium</i> , filamentous microalgae	[14]
Municipal	Horizontal biofilm reactor	0.5-3.1	microalgal consortium	[67]
Municipal	Flow lane biofilm reactor	2.9	mixed culture	[68]
Municipal	Vertical biofilm reactor	1.32	<i>Chlorella vulgaris</i> SAG 211-11b, <i>Scenedesmus rubescens</i> M2630	[71]
Municipal	Twin layer biofilm reactor	6.3	<i>Halochlorella rubescens</i>	[72]
Dairy manure	Biofilm reactor using polystyrene foam	2.57	<i>Chlorella</i> sp	[15]
Swine manure	Lab scale ATS	7.1-9.4	Microalgal consortium	[17]
Raw and anaerobically digested dairy manure	Pilot scale ATS	2.5 -25	Microalgal consortium	[18]