Bioresource Technology 244 (2017) 1245–1253

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Biofilm based attached cultivation technology for microalgal biorefineries—A review

Junfeng Wang [a], Wen Liu [a,b], Tianzhong Liu [a,*]

[a] Key Laboratory of Biofuels, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, Shandong 266101, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China

HIGHLIGHTS

- Progress in attached cultivation of microalgae in recent decade was summarized.
- Principles should be built for rational design of bioreactor structure.
- Studies on properties of cells and substratum materials are important.
- High biomass productivity lies on illumination and CO_2/O_2 transport properties.
- Engineering difficulties for enlarged cultivation was also discussed.

ARTICLE INFO

Article history:
Received 31 March 2017
Received in revised form 19 May 2017
Accepted 21 May 2017
Available online 22 May 2017

Keywords:
Attached cultivation
Biofilm
Bioreactor
Substratum material
Engineering

ABSTRACT

The attached cultivation for microalga has many superiorities over the conventional aqua-suspend methods, which make it a promising pathway to supply feedstock for microalgae based bio-refinery attempts. In this review, the current reports on bioreactor, application, modeling, substratum material and engineering aspects were summarized and the future research and developments should be focused on the following aspects: 1) Build principles and guidelines for rational structure design by studying the relationship of physiological properties with typical structures and light regimes; 2) Set up theory foundation of substratum material selection by studying the physic-chemical properties of algal cells and substratum materials; 3) Further understanding the mass transfer behaviors of both CO_2 and nutrients in biofilm for enhanced growth rate and products accumulation; 4) New equipment and machines for inoculation, harvesting and moisture keeping should be developed and integrated with bioreactor structure.

1. Introduction

Microalgae are huge group of oxygen releasing photosynthetic organisms that living in auto/mixotrophic life. Many of the microalgal species have been used for producing valuable bioactives since 1950's (Goodwin and Jamikorn, 1954). In recent decades, the requirements in renewable energy substitution and environmental protection have inspired the renaissance of researches on microalgae. High photosynthetic efficiency and derived high biomass productivity is the key to the large scale application of microalga, especially for bioenergy and environmental scenarios. According to the basic theory of photosynthesis, the upper limit of photosynthetic efficiency is 12.6% of full solar spectrum, corresponding to microalgal biomass productivity of 120–150 g m^{-2} d^{-1} (Tredici, 2010; Weyer et al., 2010; Zhu et al., 2008; Ooms et al., 2016). For the dominating cultivation methods of pen pond and photobioreactors, there are no exciting breakthroughs and improvements on biomass productivity after more than half century of research, testing and applied practice as the global averaged biomass productivity is still 10–30 gm^{-2} d^{-1} (Brennan and Owende, 2010; Mata et al., 2010). Many efforts are still putting on the improvement of these aqua-suspended methods (Gong and Bassi, 2016). However, due to the inherent property of huge water proportion in total volume (>95%, Olivieri et al., 2013), many disadvantages for these conventional cultivation methods seem hard to be overcome, including huge water requirement, energy costs, poor biomass productivity, difficulty in scale-up, easy contamination, etc., which dimmed the future of ongoing practices.

The attached cultivation is an alternative for the suspended ones. This cultivation method and related bioreactor is also called "biofilm cultivation" (Schnurr et al., 2013; Kesaano et al., 2015; Gao et al., 2015), "twin-layer" system (Shi et al., 2014; Schultze

* Corresponding author.
E-mail address: liutz@qibebt.ac.cn (T. Liu).

http://dx.doi.org/10.1016/j.biortech.2017.05.136
0960-8524/

et al., 2015; Kiperstok et al., 2017), aeroterrestrial (Chen et al., 2015; Katarzyna et al., 2015), "Porous Substrate Bioreactor" (PSBR, Podola et al., 2017), etc. Anyway, we will use the term "attached cultivation" in this review as it is more precisely presented the relationships between the microalgal cells and substratum. Meanwhile, this name is also adopted by many other researchers (Johnson and Wen, 2010; Gross et al., 2015b, 2016; Shen et al., 2016). In this method, high density microalgal cell paste is settled on the surface of artificial substratum material to form biofilm that is photosynthetically active. In this method aqua-medium and the biomass are largely separated, so that the disadvantages caused by huge water proportion could be largely neutralized, resulting merits including water saving, high biomass productivity and high efficient harvesting. In recent years, those designs are greatly improved which could be summarized as: 1) no more immersed under water (Podola et al., 2017) or encapsulated inside gel (Katarzyna et al., 2015), so that to make the algal surface to catch enough light, 2) balanced and sufficient nutrient supplement makes the biofilm unstressed. Liu et al. (2013) put this attached biofilm concept into the cultivation of microalgae aiming at the biomass production by arranging the biofilm in multiple planar matrix style and finally achieved a biomass productivity of 50–80 $gm^{-2}\,d^{-1}$, which is seven times higher than that of well operated open pond and represents the highest footprint area biomass productivity outdoors by now. The high biomass productivity makes this attached cultivation a promising method to overcome the feedstock shortage for microalgal industry (Zhang et al., 2016).

Since 2010, there is a burst of scientific and technological reports on attached cultivation of microalgae ranging from bioreactor structure, operation parameters, substratum materials, species, to outdoor application, basic physical-chemistry properties, etc. They are already well summarized in many recent reviews by the leading research groups in this area including Podola et al. (2017), Berner et al. (2015), Gross et al. (2015a), Hoh et al. (2016), Katarzyna et al. (2015), Kesaano and Sims (2014). Considering this fact, based on the authors' knowledge and experiences in bench and outdoor works, this review will focus on some of the points that have not been fully discussed before, give out commentaries on some representative researches and put forward perspectives on the future of research as well as application of attached cultivation.

2. Biofilms

The "biofilm" is the key character for the attached cultivation. A non-noticed point is there are two basic types of "biofilm" involved in attached cultivation. The first type is the biofilm consisted with pure and single species of microalga. For this biofilm, the attached cultivation is initialed from the concentrated algal biomass slurry that was pasted onto the supporting substratum material (Liu et al., 2013; Zhang et al., 2014, 2015; Cheng et al., 2013, 2014; Shi et al., 2014; Schultze et al., 2015; Kiperstok et al., 2017). In this case, the species composition of biofilm keep stable during cultivation and no obvious contamination by exotic organisms was observed. For example, in Zhang et al. (2015), the *Spirulina* was attached cultivated in a vertical system, the biomass was harvested everyday and lasted for 10 days with footprint biomass productivity of 46–70 $gm^{-2}\,d^{-1}$. Schultze et al. (2015) reported a similar attached system for *Halochlorella rubescens* that continued cultured outdoors for 80 days. The surface biomass density increased from ca. 5 gm^{-2}–125 gm^{-2} in 40 days and finally to ca.150 $g\,m^{-2}$ in day 80. For this type of attached cultivation, the biofilm are easily washed out at the incubation period. After that, a tight attachment between the algal cells and substratum material was produced to against the peel force caused by medium flow. The formation of stabilized algal biofilm is greatly contributed by microalgal secreted large molecular, e.g. extracellular polysaccharide substances (EPS) (Xiao and Zheng, 2016).

Another kind of biofilm is related with biofouling (Katarzyna et al., 2015), it may also start from pure microalgal biomass, however, during the mature process, the exotic bacterial, fungi, cyanobacterial, etc. are also settled inside the biofilm and finally, the biofilm become a balanced micro-ecosystem that consists many kinds of microorganism (Mieszkin et al., 2013). During this procedure, the EPS or some kind of secreted small proteins play important roles to combine the different components together (Ramanan et al., 2016; Mieszkin et al., 2013; Schnurr and Allen, 2015; Parnasa et al., 2016). The biofouling biofilm is a dynamic ecosystem that integrated with a changeable biomass components adapted to environment. The biomass productivity before biofilm maturity is usually poor, however, after the routine harvesting cycle is set up, the biomass productivity will increase a lot (Schnurr and Allen, 2015). Most of the attached cultivation reports on wastewater treatments with open system are using this kind of biofilm in the beginning (Boelee et al., 2011; Liu et al., 2016; Su et al., 2016), or the biofilm will changed to this sort with the cultivation went on for some time (Al-Mailem et al., 2014).

These two kinds of biofilm should be used for different purpose. The pure algal biofilm is more suitable for the applications that demand high biomass productivity, or purified high-value bioproducts, e.g. lipid derived bio-energy, algal pigments and CO_2 mitigation, etc. While the biofouling biofilm is more appropriate used in wastewater treatment because there are both autotrophic and heterotrophic components in matured biofilm eco-system, which is inclined to fully utilized the nutrient and the pollutions in wastewater (Boelee et al., 2011; Katarzyna et al., 2015; Kesaano and Sims, 2014).

3. Species

Many microalgal species can be been successfully cultivated with attached methods, which had been summarized in review papers by Berner et al. (2015), Gross et al. (2015a) and the cited article there in. Liu et al. (2013) proved that 5 different microalgal species that screened from different habitats including fresh water and sea water, from different taxonomic order including cyanobacteria, green alga, yellow alga, etc. could be cultivated with attached methods. Though it could not be told that all of the microalgal species could be cultivated with this way, since "microalgae" contains 100,000+ of species (Guiry, 2012), and the currently tested species were only a small portion, however, at least it is reasonable to suppose that those screened microalgae which can form colony on agar plates could also grow with attached cultivation method because there is not essential differences between agar and other substratum material if appropriate nutrients, CO_2 and light were provided. Among all of the successfully attach cultivated species, some are especially interesting, including:

Haematococcus. It is a species that could accumulate high content of astaxanthin, the most powerful antioxidant in nature (Terao, 1989). The *Haematococcus* has been cultivation for more than decades around world including Israel, Czech, Australia, Japan, Hawaii, China, etc. mainly with open pond before and with tubing PBRs in recent years. The first attached cultivation of *Haematococcus* is reported by Zhang et al. (2014) using horizon bioreactor with pure *Haematococcus* biofilm supported by cellulous nitrate membrane under indoor controlled conditions, the productivity is ca. 7 $gm^{-2}\,d^{-1}$. Then, similar works are followed by Wan et al. (2014) and Kiperstok et al. (2017). In Yin et al. (2015), the horizon bioreactor used by Zhang et al. (2014) is further improved by narrowing glass chamber and greatly reduced air flow, with which the water footprint is reduced 90% and the final biomass and astaxanthin productivity reaches 5 $gm^{-2}\,d^{-1}$ and 124 $mg\,m^{-2}\,d^{-1}$ respectively. The main bottleneck for indoor cultivation with suspend cultivation mode, e.g. low biomass productivity and high light requirement for red stage, could be totally overcome with attached cultivation. With the continuous progressed LED technology especially in different wavelength and intermittent light supply model (Kim et al., 2006; Katsuda et al., 2008), economical profits for attached cultivation under indoor condition could be greatly improved.

Botryococcus. This algal species is featured in its ability to synthesis hydrocarbons that can be converted into oxygen free biofuels. However, the biomass accumulation rate for *Botryococcus* in suspended cultivation is very slow especially in the lag phase, some of which will last for over 1 month (Metzger and Largeau, 2005; Banerjee et al., 2002; Cheng et al., 2013, 2014). With the attached method, Liu et al. (2013) and Cheng et al. (2013, 2014) proved that this species could growth as fast as other quick growth species like *Scenedesmus*, *viz.* 10 $gm^{-2}\,d^{-1}$ at 100–150 $\mu mol\,m^{-2}\,s^{-1}$ light intensity without notable lag phase. Comparison research on the difference of attached and suspended cultivation methods may reveal the secret that hampered the growth rate at lag phase of suspended cultivation. Another attracting property of this species is the synthesized hydrocarbons are secreted out of the cell to form a shell layer around the cells. This property may provide continuous production system that coupled the separation of hydrocarbon with cultivation. Griehl et al. (2015) reported such a "continuous milking bioreactor" based on suspended cultivation, however, it was supposed to be higher efficient in hydrocarbon productivity and lower costs for attached way because of the lower water content as well as the separation of cell and aqua-medium. Similar system could also be built for microalgal species that could secrete aimed by-products outside the cells. For example, *Porphyridium cruentum*, a species that could secrete polysaccharide outside the cell (Lutzu et al., 2017).

Filamentous microalgae. The multiple cell filamentous microalgae represented a group of species that are easiest to be cultivated with attached method. For unicellular stains, the initial attachment on the material surface is not strong enough to resist the repeal force by the flow of medium. So, most of the attached cultivations for such unicellular stains had to be complemented in biofouling film type. The twin-layer structure could be considered as a compromised to remain good attachment as well as pure biofilm (Shi et al., 2014; Schultze et al., 2015; Kiperstok et al., 2017). In the design, the algal cells are directly attached to a layer of material with micron level pores that prevent the direct contact of algal cells with the flow medium. Meanwhile, the nutrients are absorbed by the cells through capillary effects (Hamano et al., 2017). Notably, filamentous microalgae are much easier to well attach on substratum surface than unicellular strains. Zhang et al. (2015) reported *Spirulina* attached cultivation with single layer of flocking cloth. Further work with *Spirulina* in a 200 m^2 pilot attached cultivation system with towels as substratum material also validated the feasibility (Fig. 1B, unpublished results). Meanwhile, the successful cultivation of *Spirulina* by attached cultivation enlightened a *novo* strategy for strain screening. The traditional pathway for screening microalgal strain is firstly to study the physiological properties of a pool of strains and selected ones with interested property, e.g. high content of oil and pigment, and then the selected strains are cultivated in large scale to evaluate the application potential. However, with this method, a common problem is the selected strain is performed far below the anticipation when transferred outdoors, without any value for large scale cultivation. An alternative screen strategy is to reverse the step chain by firstly monitoring the performance under large scale cultivation and then selecting the better performed ones for further detailed physiological study. With this strategy, the long-time neglected filamentous yellow microalga *Tribonema minus* is recently re-discovered as a potential candidate for biofuel production and high valuable by-products of palmitoleic acid (Wang et al., 2013, 2016).

4. Bioreactor structure and light dilution strategies

In this review, "bioreactor structure" specifically refers to the arrangement style of biofilm. The bioreactor structure is directly related with the light regimes of the biofilms and could be considered as the results of light dilution strategies. Under direct full sunshine, the maximum photosynthetic flux density is generally over 2000 $\mu mol\,m^{-2}\,s^{-1}$, which is far beyond the photosynthetic light saturation point of 100–150 $\mu mol\,m^{-2}\,s^{-1}$ for common algal species (Liu et al., 2013). However, in attached cultivation, this intensive light can be diluted. One of the light dilution strategy is putting the algal biofilms in vertical multiple planar array, the top incident light scatter on the curtain surface along the light pathway through the gap between adjacent planar. As the results, the cultivation surface is enlarged several times compared with footprint area. The dilution rate is 2 × planar height then divided by gap width (Liu et al., 2013). Another light dilution strategy is intermittently illuminating the biofilm with appropriate frequency to produce time-averaged irradiation around 100–150 $\mu mol\,m^{-2}\,s^{-1}$, so that more area than footprint could receive proper illumination in a fixed total time (Toninelli et al., 2016; Gross and Wen, 2014). With these two basic light dilution strategies or mixture of them, the structure for attached cultivation could be designed in much more diversity than that of suspended PBRs. Some of the reported bioreactor structures were listed in Fig. 1. In the review of Berner et al. (2015), the structure and characters of the current biofilm systems was intensively generalized and they were divided the biofilm cultivation systems into 3 types, *viz.* constantly submerged under water, intermittently submerged system and perfused system. If these structure designs were re-grouped according to light dilution strategy, Fig. 1A and B adopted surface enlarge, the triangular style in Fig. 1E adopted time dilution, and Fig. 1D, F–J adopted both of two strategies. However, no matter what kind of strategy is used or what structure employed, all bioreactor designs by now have faced some critical problems when practice in large scale. For example, the stationary vertical planar matrix style that has been adapted by many researches (Liu et al., 2013; Schultze et al., 2015; Johnson et al., 2015) has unevenly distribution of light intensity along the pathway, algal cells located near the openings of the bioreactor will receive more light and grow faster, while cells at far end will receive less light and the biomass increases slowly. The difference in light regimes will lead to different physiological changes if the cultivation lasted for long time. For example, the cells near the light incoming window will changed color to yellow and brown, indicating loose of chlorophyll and accumulation of TAG. This unevenly distribution of light and types of cells will surely bring troubles for producing high value by-products like TAG for biodiesel. An alternative design is to make the attached surface move along the light path from bottom to top which ensures all of the algal biofilm experience same duration of low light and high light (Gross et al., 2013). This method adopts mixed light dilution strategy. However, when designing an attached bioreactor that involved time light dilution or intermittent illumination style, the light-dark frequency as well as duty cycle (time ratio of light vs. cycle) must be considered carefully. As Toninelli et al. (2016) has reported that at duty cycle of 0.33, the photosynthetic efficiency (PE) is the lowest with intermittent cycle time of 30 s, corresponding to frequency of 0.03 Hz (Fig. 2A). The authors' recently research also confirmed this phenomenon and from the figure of frequency vs. duty cycle vs. pho-

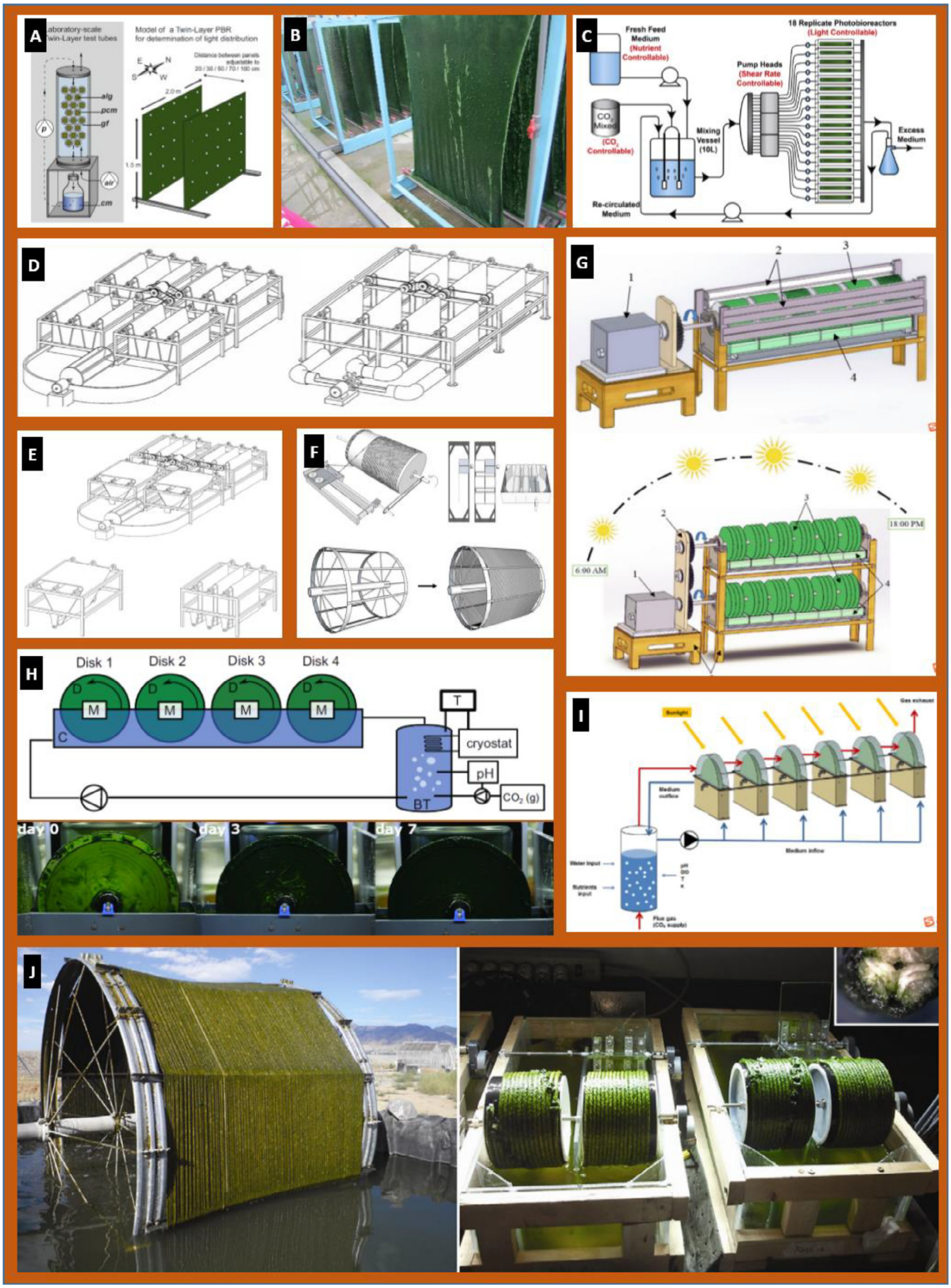

Fig. 1. Attached cultivation bioreactors for microalgae. A: Schultze et al., 2015; B: the 200 m^2 pilot cultivation set for *Spirulina* at authors' Lab; C: Schnurr et al., 2013; D: Gross et al., 2015b; E: Gross and Wen, 2014; F: Christenson and Sims, 2012; G: Shen et al., 2016; H: Blanken et al., 2014; I: Sebestyén et al., 2016; J: Bernstein et al., 2014.

tosynthetic efficiency, there is apparent "PE pit" when both of the frequency and duty cycle were low level (Fig. 2B, unpublished data). Indeed, we have noticed some of the current rotating design are failed to escape the PE pit which might be the reason for the extreme low productivity, e.g. max. 8.51 $gm^{-2}\,d^{-1}$ for triangular type (Gross and Wen, 2014). The physiology underlined this phenomena is the photosynthesis will experience photoinhibition under fluctuating light at some specific frequency (Tikkanen et al., 2012; Kono and Terashima, 2014; Sejima et al., 2014). In general, most of the current researches on bioreactor are only simple describing and performance testing of a specific structure. However, the basic principles that guided the designs are severely lack-

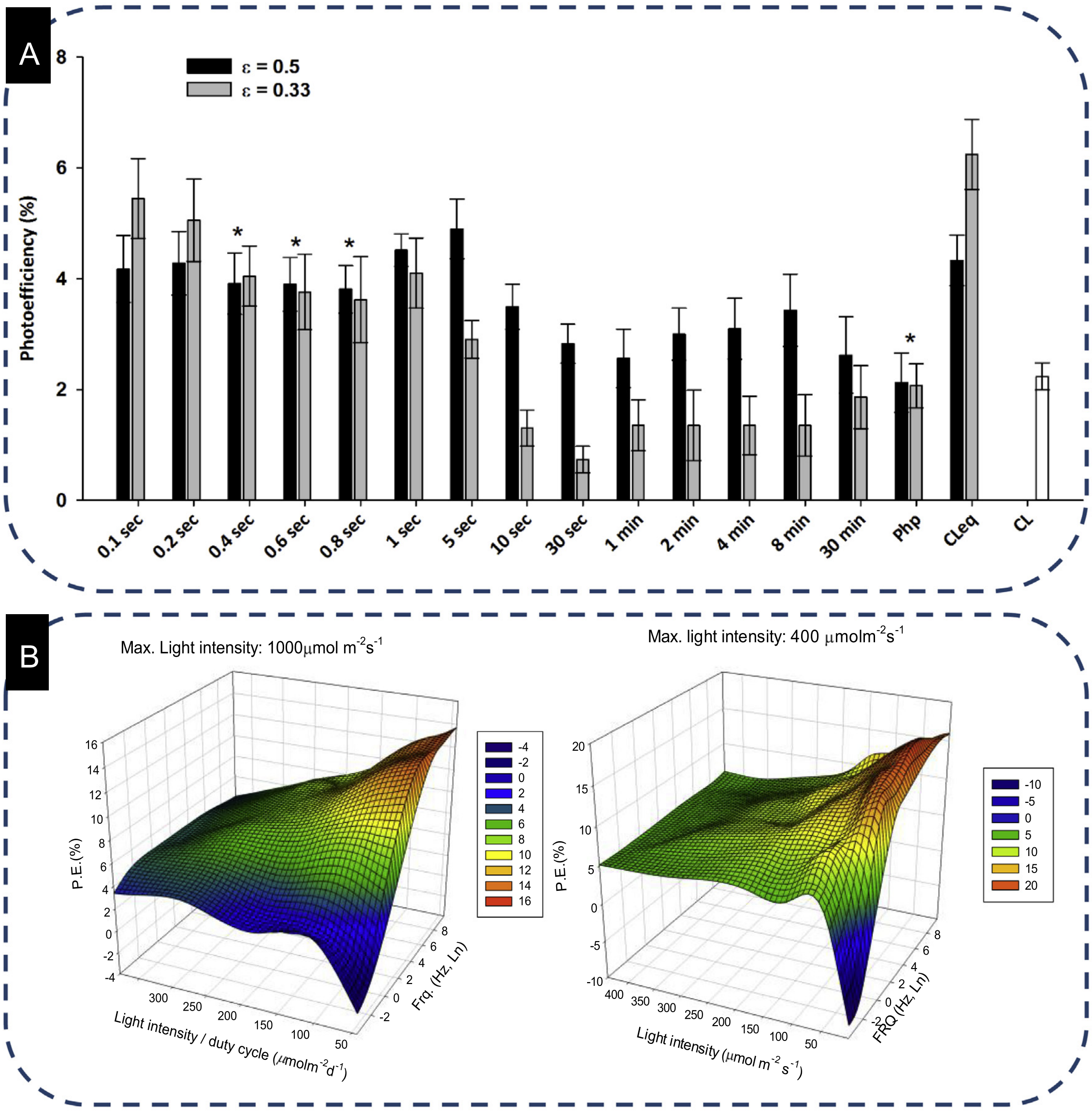

Fig. 2. The relationship of photosynthetic efficiency *vs.* illumination frequency and duty cycle. A: adapted from Toninelli et al. (2016); B: unpublished data from authors' recent research on flashing light effect.

ing. The studies on the relationships of photosynthetic physiology of attached biofilm with the structure are basis for rational design of attached bioreactors. Anyway, some of the structure designs deserve to be special introduced here because they solved specific difficulties in attached cultivation. One of the major problem for commercial level attach cultivation bioreactor is the difficulty in harvesting. The high efficient in harvest does not necessary meant the harvesting itself is an easy procedure. The discontinued cultivation surface make the harvesting is labor and time consumption. In the design of Christenson and Sims (2012) as well as in Bernstein et al. (2014), a rotating drum bioreactor was introduced. The most interesting part of this design is the cultivation surface is composited with a continuous long cotton rope. With this smart design, the harvesting of biofilm became very easy by continuous rolling the rope. Similar design is also seen in vertical conveyer belt design by Gross and Wen, 2014; Gross et al., 2015b, in which multiple module were connected by single conveyer belt, so that the harvesting could be much easier compared with other designs with independent biofilm module (Schultze et al., 2015; Liu et al., 2013; Shen et al., 2016; Blanken et al., 2014; Sebestyén et al., 2016; Johnson et al., 2015).

5. Basis for high biomass productivity

As has been pointed out in the "introduction" part, the outdoor applications of attached cultivation are potentially exhibited higher biomass productivity as well as photosynthetic efficiency than that of well operated open ponds and conventional PBRs. For example, Liu et al. (2013) reported a footprint biomass productivity of 50–80% $g\,m^{-2}\,d^{-1}$ for green microalga *Scenedesmus* that attached cultivated with vertical planar matrix bioreactor with 10x light dilution rate. Similar results were also reported by Schultze et al. (2015) with twin-layer system, they predicted a footprint biomass productivity of 50 $g\,m^{-2}\,d^{-1}$ for *Halochlorella*. Shen et al. (2016) reported a biomass productivity of ca. 55 $g\,m^{-2}$ - d^{-1} for rotating drum bioreactor. Gross et al. (2015b) reported a footprint biomass productivity of 46.8 $g\,m^{-2}\,d^{-1}$ using a rotating bioreactor. However, the biomass productivity for open pond and suspended PBRs are only 10–30 g m^{-2} d^{-1}. High biomass productivity is the basis for its applications at industrial level, especially for biofuels production and environmental related. Researches on modeling and kinetic analysis of the attached cultivation processes has revealed the reasons of the high biomass productivity in some extent, which is probably related with the following facts:

1) More portion of cells are effectively illuminated for attached cultivation compared with suspended method. Wang et al. (2015) firstly reported the light penetration depth inside the biofilm by rebuilding the biofilm on the top of a thin "indicator" microalgal layer. During the attached cultivation, the cellular level chlorophyll content decreased continuously and the effective light penetration depth became deeper and deeper with the cultivation time. From day 1 to day 10, the effectively illuminated cell portion is always 100%, meanwhile, for the well operated 30 cm depth open pond, maximum biomass concentration only reached 0.5 g L^{-1} in 15 days, and only ca. 30% percent of cells gained enough light, *viz*. only 45 g m^{-2} biomass was effectively illuminated. If combined the results of Wang et al. (2015) with Liu et al. (2013) in case of 10× of light dilution, at day 10 of cultivation, ca. 1 kg biomass is effectively illuminated at each square meter of footprint area for the attached cultivation. Similar results are also reported by Li et al. (2016) who found the light can effected illuminating a biofilm depth of 200 μm. Huang et al. (2016) found the effectively penetration depth is ca. 40 μm. The illuminated cells portion is 16 times higher in biofilm cultivation than that of in aqua-suspended pond.
2) CO_2 and O_2 transfer. For attached cultivation, the aqua layer between the microalgal cells and ambient gas phase is greatly reduced to a thin liquid film, so that the transportation of CO_2 from gas bulk to be taken by algal cells and the O_2 release from cell to gas bulk are much easier. Ji et al. (2017) set up a CO_2 transfer model that associated with biomass increase kinetics for attached cultivated *Scenedesmus dimorphus* by monitoring the medium pH changing. They found the CO_2 concentration, other than aeration rate or medium flow rate, dominates the biomass accumulation of microalgal biofilm. By analyzing the effects of CO_2 concentration on the growth of biofilm as well as suspended cultivation, Huang et al. (2016) concluded that the biofilm cultivation of microalgal has higher CO_2 affinity than suspended methods. Li et al. (2016) used a micro-sensor to measure the oxygen inside the biofilm. They found the oxygen exchange is very fast at the surface of the biofilm. However, inside the biofilm depth of 50–150 μm, the oxygen concentration is built up to high level. The optimal CO_2 concentration for biofilm is dominated by the light availability. Under high light the optimal CO_2 concentration is higher, and *vice versa* (Schnurr et al., 2016; Li et al., 2016). In their another research, Li et al. (2016) set up a model that could predict the biomass accumulation property of PSBR system, and with this model they also suggested there were extracellular carbonic anhydrase inside the biofilm that accelerate the formation of dissolved inorganic carbon (DIC).
3) Nutrients. The study on transportation of assimilation of inorganic nutrient ions in biofilm cultivation is still scarce. Murphy and Berberoglu (2014) set up a serial mathematic model that describing the relationship of biofilm growth with light intensity as well as phosphate supply. However, their model was only simulated based on un-optimized cultivation bioreactor as well as operation condition, so their predicted results might be misleading.

6. Open or closed? Considerations on water footprint and contamination

The huge water footprint is a detriment to the spread of microalgal industrial. Around the world, some of the most suitable places for microalgal cultivation are badly lacking of water, e.g. Israel, Arizona, central Australia, west China, etc. The saving in water footprint is an inherent merit for attached cultivation due to the separation of algal biomass with the majority of aqua-medium (Ozkan et al., 2012; Ji et al., 2014b). Yin et al. (2015) has proved the water requirement for producing red *Haematococcus* biomass could be reduced 90%. This huge water saving could only achieved in closed system, however, if it was open, the water loss due to evaporation will increase dramatically, much higher than conventional open pond. For example, Gross et al. (2015b) found that the water loss is 10 L m^{-2} d^{-1} of footprint area for the attached cultivation. It is almost 10× of open pond. While if the attached cultivation was carried out in a closed environment, the increase of temperature by the accumulation of solar heat may exceed the tolerance of microalgae and then destroy the cultivation. Under this circumstance, cooling system should be employed to the closed system. Anyway, compared with suspended cultivation, the energy requirement for cooling down the closed biofilm system should be much lower because the specific heat capacity for air is much lower than that of water. Furthermore, the attached cultivation provide possibilities that maintain the temperature of biomass by cooling or heating the cultivation surface only, without caring about the space between the biofilm. If it was technological realized, the energy consumption for temperature control would be reduced furthermore. The temperature increase as well as huge water evaporation for close biofilm system also suggested new applications of the attached cultivation. For example, *novo* waste water or seawater purify process that combined with microalgal biomass production (Fig. 3).

The contamination by protozoa, fungi, bacterial and unwanted microalgae species are widely seen and it is the major reason of cultivation failure. The contamination has been studied intensively in suspended cultivation. For attached cultivation, the risk of contamination should also be paid attention to because all of the biofilm surface is directly contacted with ambient air. By now, few researches on contamination and contamination control technology for attached cultivation have been reported. If contamination occurred, the treatments by changing the physical conditions of the surface, like temperature, pH, light intensity, UV light, CO_2 concentration are optional. Another effective way is to applying pesticides. The regular dosage should be largely reduced for attached cultivation because of the reduced medium volume.

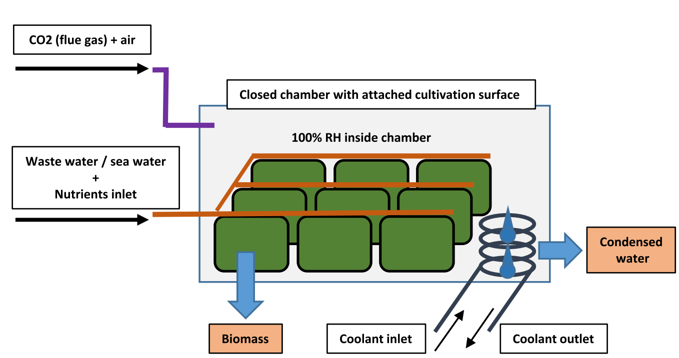

Fig. 3. Proposed novo microalgal attached cultivation system that combined with water purification.

7. Engineering difficulties for scaling up

Though the attached cultivation has many important advantages over conventional suspended methods, most of them has not been achieved outdoors. By now, the pioneer large scale applications of attached cultivation are in wastewater treatments (Liu et al., 2016; Shi et al., 2014; Kesaano and Sims, 2014). The major hurdles that hampered the industrialization of attached cultivation include:

Substratum material: The ideal supporting material for large scale cultivation should be cheap, durable, porous, non-toxic, proper water retention ability. However currently most of the used substratum materials are from market, such as polycarbonate (Bruno et al., 2012), plastic foam (Johnson and Wen, 2010), stainless steel plate (Blanken et al., 2014), micro-filter membrane (Liu et al., 2013; Cheng et al., 2013, 2014; Ji et al., 2014b, 2015), agar (Ji et al., 2014a), concrete slab (Ozkan et al., 2012), cotton rope (Christenson and Sims, 2012; Bernstein et al., 2014), newspaper (Naumann et al., 2013), printing paper (Benstein et al., 2014), etc. The choice of substratum is totally by experience or by evaluation from limited types of materials. It should be noted that the attachment of microalgae on substratum is influenced by many factors including the algal properties (e.g. surface charge, hydrophile, geometric structure, cell wall composition, secretion, etc.), substratum properties (e.g. surface charge, hydrophile, surface structure, etc.), medium composition and the hydrodynamic conditions. Understanding the attachment behavior and mechanism of microalgae on substratum is the basis for materials selecting. Ozkan and Berberoglu (2013) made an attempt to elucidate the interactions between cell to substratum and cell to cell of microalgae by theoretical XDLVO approach and found acid–base mechanism dominated the interaction of cells with substrata as well as cells with cells, and the hydrophobic/hydrophilic of both algal cell and substratum played important role in the adhesion. Gross et al. (2016) studied the physic-chemical properties of more than 30 candidate substratum materials and found the nylon and polypropylene mesh with 0.50–1.25 mm openings are best materials for initial attachment and long-term biomass productivity. Genin et al. (2014) studied the growth of multiple microalgal strains that attached to various substratum materials and find the biomass productivity of microalgal biofilm is strongly related with the polar surface energy of the material. Xia et al. (2016) studied the surface properties of 6 algal species and found all of the tested species are hydrophobic and intended to be electron donors. Ji et al. (2014a) reported that *Pseudochlorococcum* grows faster when attached to hydrophilic substrata. After the well understand of such adhesion mechanism, it is expected to construct a relationship of adhesion strength between algal and substratum properties and then provide strategies for rational substratum selection from market or modification (Chen et al., 2014; Naumann et al., 2013; Zheng et al., 2016).

Machinery operation procedure: Some procedures of the attached cultivation are lacking standard automatic implements, such as inoculating, harvesting and moisture keeping. The inoculation process for attached cultivation includes spray (Johnson et al., 2015) and filtration (Liu et al., 2013). However, all of these methods are labor and time consuming processes. Similar situation is also found in harvesting process, by now the biomass is mainly harvested by scraping and squeezing (Johnson et al., 2015). In large scale, some other forces could also be resorted to, including high pressure air stream, pressured water stream, etc. To keep the surface in wet is not difficulty problem in lab scale. It is realized generally by irrigating from the top, and the medium flow down to the bottom of the supporting material so that the attached biofilm is wetted

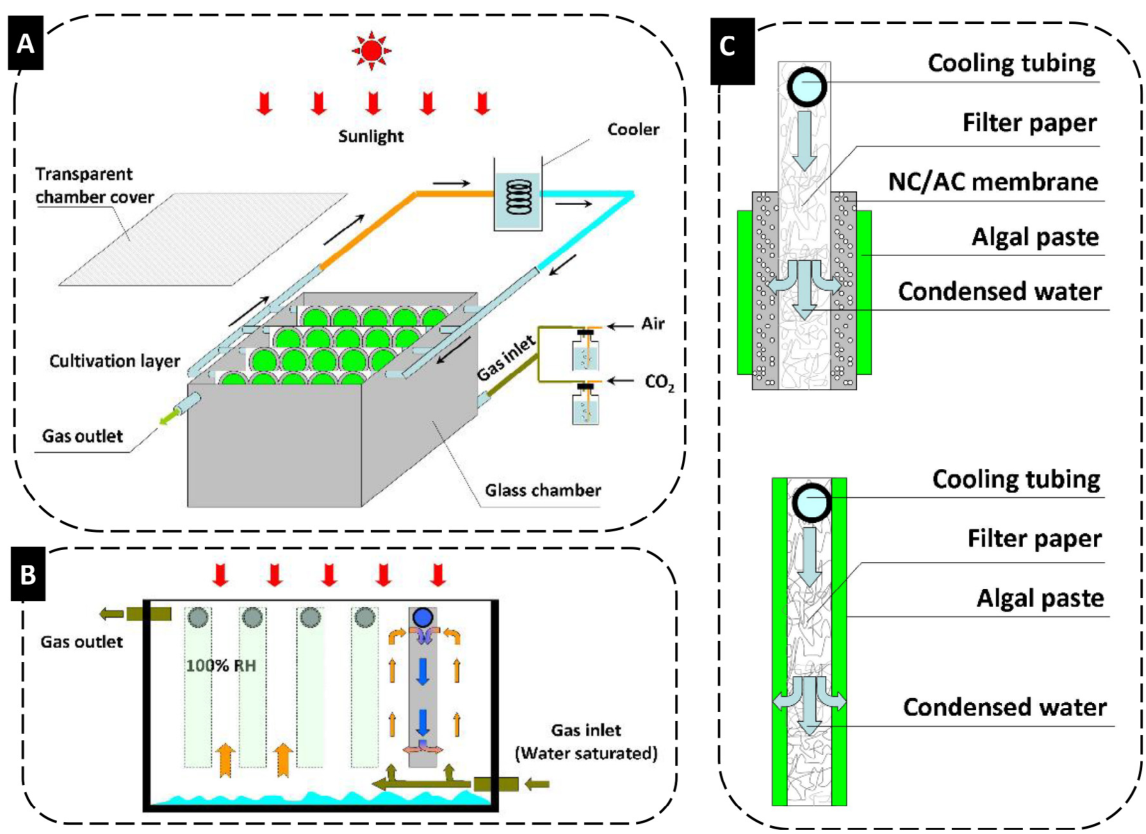

Fig. 4. Proposed irrigating system using condensing principle for attached cultivation A: the schematic of whole system; B: details inside the chamber; C: supposed liquid behavior inside the biofilm.

directly or by capillary effects. However, when enlarged to commercial scale, the irrigating from top meets great troubles, mainly the uneven distributed moisture through the entire surface. In close system, other methods then top irrigating could be adapted to keep moisture, e.g. misting or condensing (Fig. 4). In general, all of the procedures from inoculation to harvesting are needed to be re-designed to realize machinery and automatic operation so that to reduce the human labor.

Costs: The "costs" for this attached cultivation technology could be discussed from two aspects: feedstock cost and building cost. Due to the lack of large scale routinely operated system, reliable costs analyzes as well as life cycle assessments for attached cultivation by now are still scarce. For microalgae based biofuel production with open pond system, over 70% of the whole production cost is consumed to get dry biomass feedstock (feedstock cost), mainly including growth, harvesting and dewatering processes (Moreno-Garcai et al., 2017). For attached cultivation, because of the segregation of biomass with liquid medium, the cost for mixing and stirring could be totally saved. Meanwhile, the harvesting costs would be largely reduced because the biomass density of biofilms could reached to 100–200 g L^{-1}, which is 2–3 orders of magnitude higher than that in suspended culture (Podola et al., 2017). However, as has been pointed out in the former section, it must be emphasized that these cost saving potential can only be realized when the related procedures were operated in highly machinery automatic, otherwise the huge investment in human labor will destroy all of the feasibilities. The building costs for attached cultivation bioreactor system should be higher than open pond because it contains sophisticated structures. However, it should be cheaper than kinds of suspended PBRs because the building material for cultivation surface is not limited to glass or plastics anymore. The attached substratum material contributes most of the investment (Podola et al., 2017). Some kinds of textile made of petroleum-based chemical poly-fibers are considered as the cheapest material.

8. Perspectives

The attached cultivation enlightened an alternative pathway to solve the problem of efficient production of microalgae biomass for industrial application. Many researches on the feasibility of microalgae attached cultivation have been carried out and dozens of photobioreactor structures have been reported during the past decade, however the physiological behavior of microalgae when attached as biofilm is far to be elucidated, and almost all the reported reactor systems do not satisfy the requirements simultaneously in efficient cultivation, convenient inoculation and harvesting and easy scale-up for mass cultivation. More work should be paid on the following aspects: 1) Bioreactor structure, which is the key to the success of large scale outdoor applications. More research should be focused to building principles and guidelines for rational structure design by studying the relationship of physiological properties with typical structures and light regimes; 2) Selection of supporting substratum material. The physic-chemical properties of algal cells and substratum as well as their interactions are fundamental scientific questions deserved further in-depth studies. The answers to these questions will set up the theory foundation of substratum material selection; 3) Further researches on the physiology behaviors of attached algal cells and the mass transfer of both CO_2 and nutrients inside biofilm will be helpful to build efficient strategy that enhancing growth rate and products accumulation; 4) New equipment and machines for inoculation, harvesting and moisture keeping should be developed and integrated with bioreactor structure.

9. Conclusions

Progresses on bioreactor, applications, physiology, substratum material and engineering aspects of the attached cultivation for microalga were summarized. Future research and developments should be focused on rational structure design, substratum material selection, mass transfer and machinery automation.

Acknowledgements

This work was supported by the National Natural Science Foundation of China [grant number 31572637]; and National Key Research and Development Program of China [grant number 2016YFB0601002].

References

Al-Mailem, D., Kansour, M., Radwan, S., 2014. Bioremediation of hydrocarbons contaminating sewage effluent using man-made biofilms: effects of some variables. Appl. Biochem. Biotechnol. 174, 1736–1751.

Banerjee, A., Sharma, R., Chisti, Y., Banerjee, U.C., 2002. *Botryococcus braumii*: a renewable source of hydrocarbons and other chemicals. Crit. Rev. Biotechnol. 22, 245–279.

Benstein, R., Cebi, Z., Podola, B., Melkonian, M., 2014. Immobilized growth of the peridinin-producing marine *Dinoflagellate Symbiodinium* in a simple biofilm photobioreactor. Mar. Biotechnol. 16, 621–628.

Berner, F., Heimann, K., Sheehan, M., 2015. Microalgal biofilms for biomass production. J. Appl. Phycol. 27, 1793–1804.

Bernstein, H., Kesaano, M., Moll, K., Smith, T., Gerlach, R., Carlson, R., Miller, C., Peyton, B., Cooksey, K., Gardner, R., Sims, R., 2014. Direct measurement and characterization of active photosynthesis zones inside wastewater remediating and biofuel producing microalgal biofilms. Bioresour. Technol. 156, 206–215.

Blanken, W., Janssen, M., Cuaresma, M., Libor, Z., Bhaiji, T., Wiffels, R., 2014. Biofilm growth of *Chlorella sorokiniana* in a rotating biological contactor based photobioreactor. Biotechnol. Bioeng. 111, 2436–2445.

Boelee, N., Temmink, H., Janssen, M., Buisman, C., Wijffels, R., 2011. Nitrogen and phosphorus removal from municipal wastewater effluent using microalgal biofilms. Water Res. 45, 5925–5933.

Brennan, L., Owende, P., 2010. Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. Renewable Sustainable Energy Rev. 14, 557–577.

Bruno, L., Di Pippo, F., Antonaroli, S., Gismondi, A., Valentini, C., Albertano, P., 2012. Characterization of biofilm.-forming cyanobacteria for biomass and lipid production. J. Appl. Microbiol. 113, 1052–1064.

Chen, X., Liu, T., Wang, Q., 2014. The growth of *Scenedesmus* sp. attachment on different materials surface. Microb. Cell Fact. 13, 142.

Chen, L., Zhang, L., Zhang, W., Liu, T., 2015. Comparative analysis of growth and carotenoid accumulation of *Trentepohlia arborum* in aerial, subaerial, and aquatic cultivation. J. Appl. Phycol. 27, 1079–1087.

Cheng, P., Ji, B., Gao, L., Zhang, W., Wang, J., Liu, T., 2013. The growth, lipid and hydrocarbon production of *Botryococcus braunii* with attached cultivation. Bioresour. Technol. 138, 95–100.

Cheng, P., Wang, J., Liu, T., 2014. Effects of nitrogen source and nitrogen supply model on the growth and hydrocarbon accumulation of immobilized biofilm cultivation of *B. braunii*. Bioresour. Technol. 166, 527–533.

Christenson, L., Sims, R., 2012. Rotating algal biofilm reactor and spool harvester for wastewater treatment with biofuels byproducts. Biotechnol. Bioeng. 109, 1674–1684.

Gao, F., Yang, Z., Li, C., Zeng, G., Ma, D., Zhou, L., 2015. A novel algal biofilm membrane photobioreactor for attached microalgae growth and nutrients removal from secondary effluent. Bioresour. Technol. 179, 8–12.

Genin, S., Aitchison, J., Allen, D., 2014. Design of algal film photobioreactors: material surface energy effects on algal film productivity, colonization and lipid content. Bioresour. Technol. 155, 136–143.

Gong, M., Bassi, A., 2016. Carotenoids from microalgae: a review of recent developments. Biotechnol. Adv. 34, 1396–1412.

Goodwin, T., Jamikorn, M., 1954. Studies in carotenogenesis. 2. Carotenoid synthesis in the alga *Haematococcus pluvialis*. Biochem. J. 57, 376–381.

Griehl, C., Kleinert, C., Griehl, C., Bieler, S., 2015. Design of a continuous milking bioreactor for non-destructive hydrocarbon extraction from *Botryococcus braunii*. J. Appl. Phycol. 27, 1833–1843.

Gross, M., Wen, Z., 2014. Yearlong evaluation of performance and durability of a pilot-scale revolving algal biofilm (RAB) cultivation system. Bioresour. Technol. 171, 50–58.

Gross, M., Henry, W., Michael, C., Wan, Z., 2013. Development of a rotating algal biofilm growth system for attached microalgae growth with *in situ* biomass harvest. Bioresour. Technol. 150, 195–201.

Gross, M., Jarboe, D., Wen, Z., 2015a. Biofilm-based algal cultivation systems. Appl. Microbiol. Biotechnol. 99, 5781–5789.

Gross, M., Mascarenhas, V., Wen, Z., 2015b. Evaluating algal growth performance and water use efficiency of pilot-scale revolving algal biofilm (RAB) culture systems. Biotechnol. Bioeng. 112, 2040–2050.
Gross, M., Zhao, X., Mascarenhas, V., Wen, Z., 2016. Effects of the surface physico-chemical properties and the surface textures on the initial colonization and the attached growth in algal biofilm. Biotechnol. Biofuels 9, 38.
Guiry, M., 2012. How many species of algae are there? J. Phycol. 48, 1057–1063.
Hamano, H., Nakamura, S., Hayakawa, J., Miyashita, H., Harayama, S., 2017. Biofilm-based photobioreactor absorbing water and nutrients by capillary action. Bioresour. Technol. 223, 307–311.
Hoh, D., Watson, S., Kan, E., 2016. Algal biofilm reactors for integrated wastewater treatment and biofuel production: a review. Chem. Eng. J. 287, 466–473.
Huang, Y., Xiong, W., Liao, Q., Fu, Q., Xia, A., Zhu, X., Sun, Y., 2016. Comparison of *Chlorella vulgaris* biomass productivity cultivated in biofilm and suspension from the aspect of light transmission and microalgae affinity to carbon dioxide. Bioresour. Technol. 222, 367–373.
Ji, B., Zhang, W., Zhang, N., Wang, J., Lutzu, G., Liu, T., 2014a. Biofilm cultivation of the oleaginous microalgae *Pseudochlorococcum* sp. Bioprocess. Biosyst. Eng. 37, 1369–1375.
Ji, C., Wang, J., Zhang, W., Liu, J., Wang, H., Gao, L., Liu, T., 2014b. An applicable nitrogen supply strategy for attached cultivation of *Aucutodesmus obliquus*. J. Appl. Phycol. 26, 173–180.
Ji, C., Wang, J., Liu, T., 2015. Aeration strategy for biofilm cultivation of the microalga *Scenedesmus dimorphus*. Biotechnol. Lett. 37, 1953–1958.
Ji, C., Wang, J., Li, R., Liu, T., 2017. Modeling of carbon dioxide mass transfer behavior in attached cultivation photobioreactor using the analysis of the pH profiles. Bioprocess. Biosyst. Eng. http://dx.doi.org/10.1007/s00449-017-1770-6.
Johnson, M., Wen, Z., 2010. Development of an attached microalgal growth system for biofuel production. Appl. Microbiol. Biotechnol. 85, 523–534.
Johnson, M., Villani, T., Azmat, A., Simon, J., Both, A., 2015. Evaluation of algal biomass production on vertical aeroponic substrates. Algal Res. 10, 240–248.
Katarzyna, L., Sai, G., Singh, O., 2015. Non-enclosure methods for non-suspended microalgae cultivation: literature review and research needs. Renewable Sustainable Energy Rev. 42, 1418–1427.
Katsuda, T., Shiraish, H., Ishizu, N., Ranjbar, R., Katoh, S., 2008. Effect of light intensity and frequency of flashing light from blue light emitting diodes on astaxanthin production by *Haematococcus pluvialis*. J. Biosic. Bioeng. 105, 216–220.
Kesaano, M., Sims, R., 2014. Algal biofilm based technology for wastewater treatment. Algal Res. 5, 231–240.
Kesaano, M., Gardner, R., Moll, K., Lauchnor, E., Gerlach, R., Peyton, B., Sims, R., 2015. Dissolved inorganic carbon enhanced growth, nutrient uptake, and lipid accumulation in wastewater grown microalgal biofilms. Bioresour. Technol. 180, 7–15.
Kim, Z., Kim, S., Lee, C., 2006. Enhanced production of astaxanthin by flashing light using *Haematococcus pluvialis*. Enzyme Microb. Technol. 39, 414–419.
Kiperstok, A., Sebestyen, P., Podala, B., Melkoniam, M., 2017. Biofilm cultivation of *Haematococcus pluvialis* enables a highly productive one-phase process for astaxanthin production using high light intensities. Algal Res. 21, 213–222.
Kono, M., Terashima, I., 2014. Long-term and short-term responses of the photosynthetic electron transport to fluctuating light. J. Photochem. Photobiol. B 137, 89–99.
Li, T., Piltz, B., Podola, B., Dron, A., de Beer, D., Melkonian, M., 2016. Microscale profiling of photosynthesis-related variables in highly productive biofilm photobioreactor. Biotechnol. Bioeng. 113, 1046–1055.
Liu, T., Wang, J., Hu, Q., Cheng, P., Ji, B., Liu, J., Chen, Y., Zhang, W., Chen, X., Chen, L., Gao, L., Ji, C., Wang, H., 2013. Attached cultivation technology of microalgae for efficient biomass feedstock production. Bioresour. Technol. 127, 216–222.
Liu, J., Danneels, B., Vanormelingen, P., Vyverman, W., 2016. Nutrient removal from horticultural wastewater by benthic filamentous algae *Klebsormidium* sp., *Stigeoclonium* spp. And their communities: from laboratory flask to outdoor Algal Turf Scrubber (ATS). Water Res. 92, 61–68.
Lutzu, G., Zhang, L., Zhang, Z., Liu, T., 2017. Feasibility of attached cultivation for polysaccharides production by *Porphyridum cruentum*. Bioprocess. Biosyst. Eng. 40, 73–83.
Mata, T.M., Martins, A., Caetano, N., 2010. Microalgae for biodiesel production and other applications: a review. Renewable Sustainable Energy Rev. 14, 217–232.
Metzger, P., Largeau, C., 2005. *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. Appl. Microbiol. Biotechnol. 66, 486–496.
Mieszkin, S., Callow, M., Callow, J., 2013. Interactions between microbial biofilms and marine fouling algae: a mini review. Biofouling 29, 1097–1113.
Moreno-Garcai, L., Adjalle, K., Barnabe, S., Raghavan, G., 2017. Microalgae biomass production for a biorefinery system: recent advances and the way towards sustainability. Renewable Sustainable Energy Rev. 76, 493–506.
Murphy, T., Berberoglu, H., 2014. Flux balancing of light and nutrients in a biofilm photobioreactor for maximizing photosynthetic productivity. Biotechnol. Progr. 30, 348–359.
Naumann, T., Çebi, Z., Podola, B., Melkonian, M., 2013. Growing microalgae as aquaculture feeds on twin-layers: a novel solid-state photobioreactor. J. Appl. Phycol. 25, 1413–1420.
Olivieri, G., Salatino, P., Marzocchella, A., 2013. Advances in photobioreactors for intensive microalgal production: configurations, operating strategies and applications. J. Chem. Technol. Biotechnol. 89, 178–195.
Ooms, M., Dinh, C., Sargent, E., Sinton, D., 2016. Photon management for augmented photosynthesis. Nat. Commun. 7, 12699.
Ozkan, A., Berberoglu, H., 2013. Cell to substratum and cell to cell interactions of microalgae. Colloids Surf. B 112, 302–309.
Ozkan, A., Kinney, K., Katz, L., Berberoglu, H., 2012. Reduction of water and energy requirement of algae cultivation using an algae biofilm photobioreactor. Bioresour. Technol. 114, 542–548.
Parnasa, R., Nagar, E., Sendersky, E., Reich, Z., Simkovsky, R., Golden, S., Schwarz, R., 2016. Small secreted proteins enable biofilm development in the cyanobacterium *Synechococcus elongates*. Sci. Rep. 6, 32209.
Podola, B., Li, T., Melkonian, M., 2017. Porous substrate bioreactors: a paradigm shift in microalgal biotechnology? Trends Biotechnol. 35, 2.
Ramanan, R., Kim, B., Cho, D., Oh, H., Kim, H., 2016. Algae-bacteria interactions: evolution, ecology and emerging applications. Biotechnol. Adv. 34, 14–29.
Schnurr, P., Allen, D., 2015. Factors affecting algae biofilm growth and lipid production: a review. Renewable Sustainable Energy Rev. 52, 418–429.
Schnurr, P., Espie, G., Allen, D., 2013. Algae biofilm growth and the potential to stimulate lipid accumulation through nutrient starvation. Bioresour. Technol. 136, 337–344.
Schnurr, P., Molenda, O., Edwards, E., Espie, G., Allen, D., 2016. Improved biomass productivity in algal biofilms through synergistic interactions between photon flux density and carbon dioxide concentration. Bioresour. Technol. 219, 72–79.
Schultze, L., Simon, M., Li, T., Langenbach, D., Podola, B., 2015. High light and carbon dioxide optimize surface productivity in a twin-layer biofilm photobioreactor. Algal Res. 8, 37–44.
Sebestyén, P., Blanken, W., Bacsa, I., Tóth, G., Martin, A., Bhaiji, T., Dergez, Á., Kesserű, P., Koós, Á., Kiss, I., 2016. Upscale of a laboratory rotating disk biofilm reactor and evaluation of its performance over a half-year operation period in outdoor conditions. Algal Res. 18, 266–272.
Sejima, T., Takagi, D., Fukayama, H., Makino, A., Miyake, C., 2014. Repetitive short-pulse light mainly inactivates photosystem I in sunflower leaves. Plant Cell Physiol. 56, 1184–1193.
Shen, Y., Zhu, W., Chen, C., Nie, Y., Lin, X., 2016. Biofilm formation in attached microalgal reactors. Bioprocess. Biosyst. Eng. 39, 1281–1288.
Shi, J., Podola, B., Melkonian, M., 2014. Application of a prototype-scale twin-layer photobioreactor for effective N and P removal from different process stages of municipal wastewater by immobilized microalgae. Bioresour. Technol. 154, 260–266.
Su, Y., Mennerich, A., Urban, B., 2016. The long-term effects of wall attached microalgal biofilm on algae-based wastewater treatment. Bioresour. Technol. 218, 1249–1252.
Terao, J., 1989. Antioxidant activity of beta-carotene-related carotenoids in solution. Lipids 24, 659–661.
Tikkanen, M., Grieco, M., Nurmi, M., Suorsa, M., Aro, E.-M., 2012. Regulation of the photosynthetic apparatus under fluctuating growth light. Philos. Trans. R. Soc. London, Ser. B 367, 3486–3493.
Toninelli, A., Wang, J., Liu, M., Wu, H., Liu, T., 2016. *Scenedesmus dimorphus* biofilm: photoefficiency and biomass production under intermittent lighting. Sci. Rep. 6, 32305.
Tredici, M., 2010. Photobiology of microalgae mass cultures: understanding the tools for the next green revolution. Biofuels 1, 143–162.
Wan, M., Hou, D., Li, Y., Fan, J., Huang, J., Liang, S., Wang, W., Pan, R., Wang, J., Li, S., 2014. The effective photoinduction of *Haematococcus pluvialis* for accumulating astaxanthin with attached cultivation. Bioresour. Technol. 163, 26–32.
Wang, H., Gao, L., Chen, L., Guo, F., Liu, T., 2013. Integration process of biodiesel production from filamentous oleaginous microalgae *Tribonema minus*. Bioresour. Technol. 142, 39–44.
Wang, J., Liu, J., Liu, T., 2015. The difference in effective light penetration may explain the superiority in photosynthetic efficiency of attached cultivation over the conventional open pond for microalgae. Biotechnol. Biofuels 8, 49.
Wang, H., Gao, L., Zhou, W., Liu, T., 2016. Growth and palmitoleic acid accumulation of filamentous oleaginous microalgae *Tribonema minus* at varying temperatures and light regimes. Bioprocess. Biosyst. Eng. 39, 1589–1595.
Weyer, K., Bush, D., Darzins, A., Willson, B., 2010. Theoretical maximum algal oil production. Bioenergy Res. 3, 204–213.
Xia, L., Li, H., Song, S., 2016. Cell surface characterization of some oleaginous green algae. J. Appl. Phycol. 28, 2323–2332.
Xiao, R., Zheng, Y., 2016. Overview of microalgal extracellular polymeric substances (EPS) and their applications. Biotechnol. Adv. 34, 1225–1244.
Yin, S., Wang, J., Chen, L., Liu, T., 2015. The water footprint of biofilm cultivation of *Haematococcus pluvialis* is greatly decreased by using sealed narrow chambers combined with slow aeration rate. Biotechnol. Lett. 37, 1819–1827.
Zhang, W., Wang, J., Wang, J., Liu, T., 2014. Attached cultivation of *Haematococcus pluvialis* for astaxanthin. Bioresour. Technol. 158, 329–335.
Zhang, L., Chen, L., Wang, J., Chen, Y., Gao, X., Zhang, Z., Liu, T., 2015. Attached cultivation for improving the biomass productivity of *Spirulina Platensis*. Bioresour. Technol. 181, 136–142.
Zhang, T., Hu, H., Wu, Y., Zhuang, L., Xu, X., Wang, X., Dao, G., 2016. Promising solutions to solve the bottlenecks in the large-scale cultivation of microalgae for biomass/bioenergy production. Renewable Sustainable Energy Rev. 60, 1602–1614.
Zheng, Y., Huang, Y., Liao, Q., Zhu, X., Fu, Q., Xia, A., 2016. Effects of wettability on the growth of *Scenedesmus obliquus* biofilm attached on glass surface coated with polytetrafluoroethylene emulsion. Int. J. Hydrogen Energy 41, 21728–21735.
Zhu, X., Long, S., Ort, D., 2008. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? Curr. Opin. Biotechnol. 19, 153–159.