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Comparison of *Chlorella vulgaris* biomass productivity cultivated in biofilm and suspension from the aspect of light transmission and microalgae affinity to carbon dioxide

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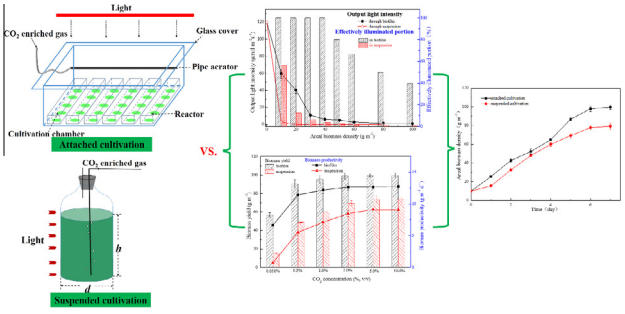
HIGHLIGHTS

- Biomass productivity of microalgae biofilm is 30.4% higher than that of suspension.
- Number of cells that illuminated in biofilm was 16 times higher than in suspension.
- The microalgae showed a higher affinity to CO_2 in attached cultivation system.
- CO_2 saturation points of biofilm and suspension were 1.5% and 4.5%, respectively.

GRAPHICAL ABSTRACT

Higher areal biomass density of *Chlorella vulgaris* was obtained in attached system than that in suspended system, due to its better light penetration performance and higher affinity to CO_2.



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ABSTRACT

To investigate light transmission and cells affinity to CO_2, *Chlorellavulgaris* was attached to microfiltration membrane that laid on the solidified BG11 medium compared to that in suspended cultivation mode in this study. The results showed that *C. vulgaris* showed a 30.4% higher biomass production (103 g m^{-2}) in attached than in suspend system. The upper layer of biofilm with a thickness of 41.31 μm (the corresponding areal density of 40 g m^{-2}) was effectively illuminated under light intensity of 120 μmol m^{-2} s^{-1} and more than 40% of the microalgal cells were in light even the areal density was high to 100 g m^{-2}. While only 2.5% of the cells were effectively illuminated in the suspended cultivation system. Furthermore, microalgae cells in biofilm showed a higher affinity to CO_2 compared with that in suspension, and CO_2 saturation point of microalgae cells in biofilm was 1.5% but 4.5% in suspension.

1. Introduction

With the progress of society and economy, global warming and energy crisis have become to global concerns. Microalgae cultivation is an effective way to simultaneously mitigate CO_2 emission and produce biodiesel to solve the environmental and energy issues due to its high growth rate and high lipid content (Chisti,

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2008; Makareviciene et al., 2013; Wijffels and Barbosa, 2010). The primary microalgae cultivation system is that microalgae cells are suspended in liquid medium. However, due to the limitation of slow CO_2 dissolution rate and poor light penetrability within microalgae suspension (Gross et al., 2013; Wang et al., 2013), the photosynthesis of suspended microalgal cells are seriously restricted. Consequently, microalgae biomass productivity is usually less than 30 g m^{-2} day^{-1} (Brennan and Owende, 2009), which is much lower than the theoretical maximum biomass productivity values of 120–150 g m^{-2} day^{-1} (Chisti, 2012; Tredici, 2010). Therefore, biomass yields in these systems were generally below 6 g L^{-1} (with water content of 99.4%), resulting in a 21% of the total energy consumption in the biofuel production process was contributed by dewatering (Davis et al., 2011). On the contrary, these disadvantages of suspended cultivation might be relieved dramatically if microalgae are cultivated with an attached mode (Gross et al., 2015; Johnson and Wen, 2010; Shen et al., 2014; Zhang et al., 2015).

Generally, microalgal cells were directly inoculated onto the surfaces of an adhering materials in high density and fed with nutrients in the attached cultivation mode, which could dramatically reduce microalgal dewatering costs (Blanken et al., 2014; Gross and Wen, 2014). Ozkan et al. (2012) inoculated microalgae *Botryococcusbraunii* onto a concrete surface. The microalgae biomass concentration was significantly increased up to 96.4 g L^{-1} with the lipid content being 26.8%. Moreover, microalgae biomass of biofilm was harvested easily from the concrete surface by gentle mechanical scraping with a squeegee (Ozkan et al., 2012), the energy requirement for dewatering process was decreased by 99.7%. And water requirement per unit of microalgal biomass production was decreased by 45% compared with that cultivated in the open pond. Liu et al. (2013) attached the high dense oleaginous microalgae *Scenedesmus obliquus* cells onto the filtration paper surface and cultivated outdoor. The biomass productivity achieved 50–80 g m^{-2} day^{-1}, which is 400–700% higher than that grown in aqueous suspension under the same environmental conditions. Even *B. braunii*, a species that grow very slow at ordinary times, still showed a high biomass productivity of 50 g m^{-2} day^{-1} when cultivated in the form of biofilm (Cheng et al., 2013).

However, the mechanisms for the superiorities of attached cultivation method over suspended cultivation have been rarely studied. Microalgal cells assimilate CO_2 as carbon resource and utilizes light as energy source for photosynthesis, which makes CO_2 transfer and light delivery playing decisive roles on microalgal photosynthetic growth (Chisti, 2013; Ji et al., 2014a). Thus, it is important to reveal the differences in light delivery and CO_2 transfer caused by the change of water environment in microalgae biofilm. In this study, to demonstrate the reasons why microalgae biofilm could achieve higher productivity and photosynthetic efficiency, we explored the effects of light penetration and CO_2 concentration on *Chlorella vulgaris* cells growth. Besides, the effect of initial biofilm thickness on microalgal growth rate was investigated.

2. Materials and methods

2.1. Strains and media

The freshwater microalgae *Chlorella vulgaris* FACHB-31 (*C. vulgaris*) was purchased from Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). The inoculum was cultivated in the autotrophic nutrient medium BG11. *C. vulgaris* cells were pre-cultivated in a 500 mL glass serum bottle aerated with 10% CO_2 (v/v) (balanced with air) at 25 ± 1 °C under light intensity of 120 ± 5 μmol m^{-2} s^{-1} for 5 days before inoculation.

2.2. Attached cultivation system

Six cuboid boxes (300 × 60 × 50 mm, L × W × H) that made of polymethyl methacrylate (PMMA) were used as bioreactors for microalgae biofilm, which placed into a PMMA chamber (400 × 300 × 150 mm) covered by a glass plate (Fig. 1). Parallel fluorescent lambs were fixed above the PMMA chamber as light sources. Light intensity was adjusted by controlling the number of lambs. A pipe aerator was fixed on the back side of the PMMA chamber for CO_2 sparging. 1% (w/w) agar powder was dissolved into BG11 medium and heated to 121 °C and then poured into PMMA bioreactors cooling at room temperature until solidification. Although, agar powder is an organic carbon source, it cannot be digested by *C. vulgaris*, so cells in biofilm are still cultured photoautotrophically rather than mixotrophically (Remmal et al., 1993). The function of agar powder is to solidify the medium that could provide nutrients and maintain the wettability of the algal biofilm (Ji et al., 2014a). The solidified BG11 medium was used to supply nutrients and maintain the wettability for the microalgal biofilm. A filtration membrane (diameter: 50 mm, average pore size:0.45 μm) was attached onto the surface of the solidified BG11 medium served as adhering material for microalgal biofilm growth. V_0 mL of pre-cultivated microalgal suspension with a dry biomass concentration of C_0 g L^{-1} was evenly vacuum filtered onto a fibrous microfiltration membrane to form an initial microalgal biofilm with area of 0.0011 m^2. The initial inoculum density was set at 10.0 ± 0.1 g m^{-2} for all experiments, except for the effect experiments of initial inoculum density on growth, suspended microalgal cultivation in a cylindrical reactor (inner diameter: 80 mm) was used as the control.

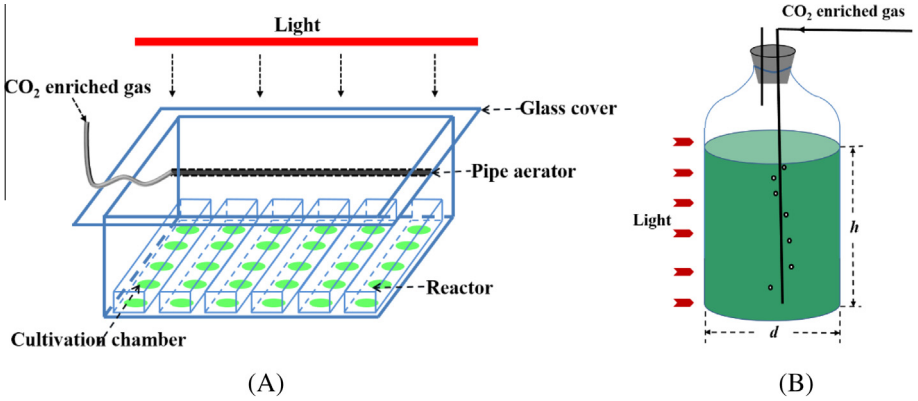


Fig. 1. Schematic of experimental setup of microalgal (A) attached biofilm cultivation and (B) suspended cultivation.

2.3. Determination of the light penetration in microalgal biofilm and suspension

The prepared microalgal suspensions were concentrated by centrifuging. Subsequently, the concentrated microalgal pellets were washed with distilled water and poured into a transparent PMMA cube (100 × 100 × 150 mm) to form biofilm with different areal biomass densities (10, 20, 30, 40, 50, 60, 70, 80 and 100 g m^{-2}). The transparent PMMA cubes were placed vertically under fluorescent lamps. The incident light intensity of 120 μmol m^{-2} s^{-1} was the light intensity after penetrating through the surface of the PMMA by adjusting the distance between the cube and fluorescent lambs. Photosynthetic quantum sensor (Li-250A, Li-Cor, Lincoln, NE, USA) was placed underneath each cube and tightly attached to the PMMA sheet to measure the penetrated photon flux density. While light penetration through microalgal suspension was determined by the light path in the suspension. And, in order to eliminate the influence of glass, the output light intensity of bioflim was added the light intensity loss that penetrating through PMMA.

2.4. Determination of microalgal growth

The biofilm was washed down from the supporting membrane followed by centrifuging and drying to constant weight at 105 °C to calculate the areal density of biofilm (X, g m^{-2}). The biomass concentration of microalgae suspension was determined by dry biomass weight per liter culture (g L^{-1}), which could be transformed to areal density corresponding to the incident light surface area (m^2). Thus, the biofilm growth rate (RX, g m^{-2} day^{-1}) was calculated by Eq. (1):

$$RX = (X_n - X_0)/n \quad (1)$$

where X_n and X_0 are the biofilm areal density on the substrata sample at day n and day 0 (initial inoculation density), respectively, and n represents the time of cultivation (days).

The elemental composition of the *C. vulgaris* was analyzed using elemental analyzer (vario MACRO cube, Elementar Analysensysteme GmbH Ltd., Germany).

Chlorophyll content was determined according to the methods described by Wellburn (Wellburn, 1994). The chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b) concentrations (mg L^{-1}) were calculated by Eqs. (2) and (3):

$$Chl_a = 12.21OD_{663nm} - 2.81OD_{646nm} \quad (2)$$

$$Chl_b = 20.13OD_{646nm} - 5.03OD_{663nm} \quad (3)$$

2.5. Determination of the thickness of biofilm

The thickness of biofilm at different cultivation days was measured by means of a surface profiler (Veeco Dektak150, Veeco Instruments Inc., Plainview, NY, USA) and the corresponding biofilm areal density was also measured (refer to the 'Determination of microalgal growth' section). Accordingly, the correlation of the thickness (d, μm) of the biofilm with the areal density (X, g m^{-2}) was calculated by Eq. (4):

$$d = 1.03 \times X + 2.2 \quad (4)$$

For later experiments, the thickness (μm) of biofilm during cultivation was estimated by this equation. According to experiments, this equation could be applied in the density range of 0–120 g m^{-2} for *C. vulgaris* grown with the attached cultivation mode (R^2 = 0.996).

3. Results and discussion

3.1. Comparison of microalgae C. vulgaris growth and light penetration between attached and suspended cultivation

The areal densities of microalgal biomass that cultivated in the form of biofilm and suspension are shown in Fig. 2. After 7 days cultivation, areal density of *C. vulgaris* cultivated in attached system achieved a stable value of 103 g m^{-2}, which is 30.4% higher than that in the suspended system. Liu et al. (2013) also found that microalgae in attached cultivation showed higher productivity than that in suspended cultivation. A biomass productivity of 50–80 g m^{-2} day^{-1} was obtained for *S. obliquus*, corresponding with the photosynthetic efficiency of 5.2–8.3% (total solar radiation). These values are approximately 60% higher than those in traditional suspended cultivation.

The light penetration capacity in *C. vulgaris* biofilm and suspension were measured, as shown in Fig. 3. Light intensity attenuated remarkably with the increase of *C. vulgaris* areal biomass density both in biofilm and suspension (Fig. 3A). Similar results had been reported by Tredici (2010). The output light intensity of the suspension rapidly decreased from 120 to 3 μmol m^{-2} s^{-1} with the areal biomass density of microalgae suspension increasing from 0 to 10 g m^{-2}. Nevertheless, output light intensity from the 10 g m^{-2} biofilm was 59 μmol m^{-2} s^{-1}. When the biofilm biomass density increased to 40 g m^{-2}, the output light intensity was still 6.06 μmol m^{-2} s^{-1}. Light penetrating though biofilm seem to be easier with respect to penetrating in microalgal suspension. That maybe one of the reasons why *C. vulgaris* accumulated more biomass with attached cultivation than in suspended cultivation.

The effect of different incident light intensity on light penetration though microalgal biofilm is shown in Fig. 3B. When 120 μmol m^{-2} s^{-1} of the incident light intensity penetrating though 10 g m^{-2} of biofilm, the light attenuated by 50%, corresponding to the output light intensity of 60 μmol m^{-2} s^{-1}. With the incident light intensity increased from 80 to 240 μmol m^{-2} s^{-1}, the output light intensity increased from 7.8 to 18.6 μmol m^{-2} s^{-1} under a biofilm density of 30 g m^{-2}. While the light intensity higher than 80 μmol m^{-2} s^{-1} could penetrate the biofilm with density inferior to 40 g m^{-2}. However, if the biofilm density exceeded 50 g m^{-2}, even as high as 240 μmol m^{-2} s^{-1} of light could not penetrate throughout the microalgal biofilm. That indicated that light

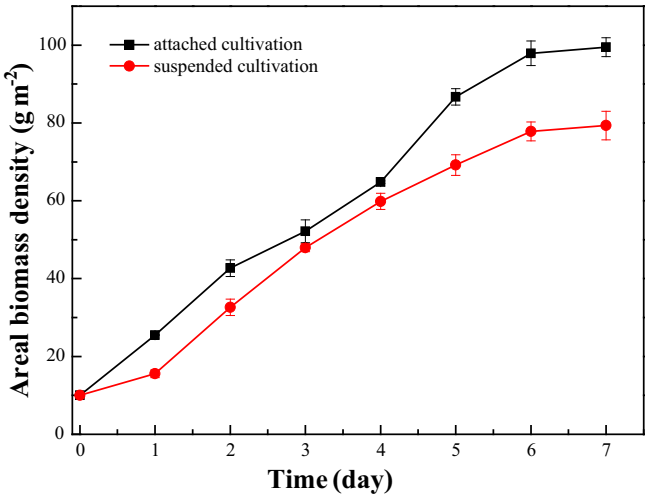


Fig. 2. Comparison of areal biomass density of *C. vulgaris* between attached cultivation and suspended cultivation. Microalgae were cultivated with a continuous light intensity of 120 ± 3 μmol m^{-2} s^{-1} by aerating with 10% CO_2 (v/v) at 0.01 vvm.

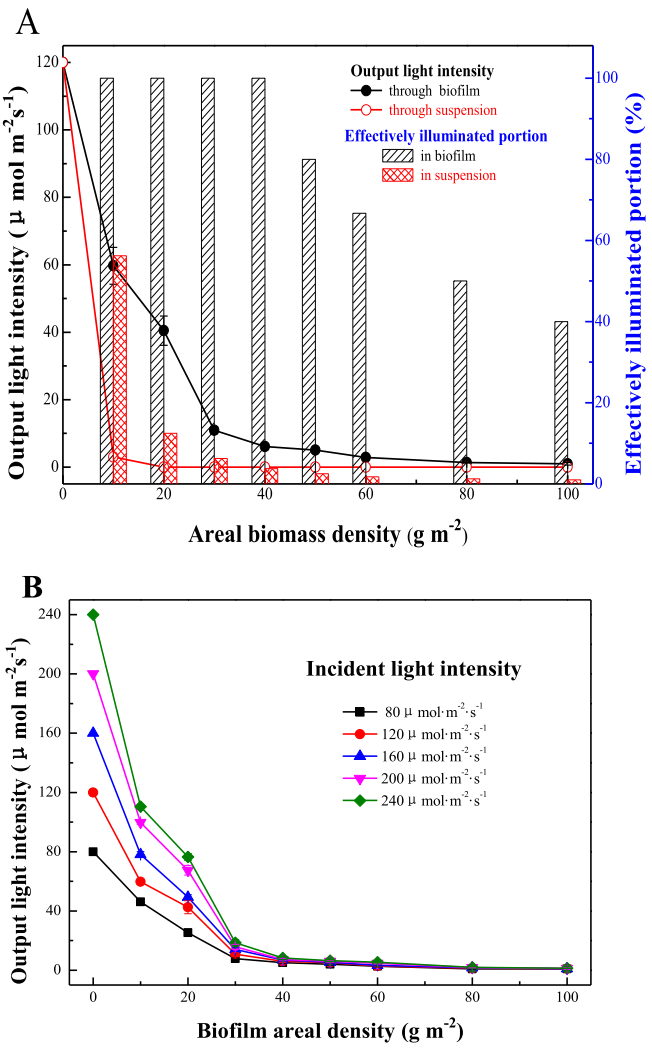


Fig. 3. Light penetration capacity in *C. vulgaris* biofilm and suspension. (A) Comparison of light penetration capacity in microalgal biofilm and suspension (incident light intensity: 120 μmol m^{-2} s^{-1}). (B) Effect of different incident light intensity on light penetration in microalgal biofilm.

could only penetrate the biofilm at a density of 40 g m^{-2}. Fortunately, microalgal biofilm density in attached cultivation is generally lower than 100 g m^{-2} (Liu et al., 2013), which ensured that at least 40% of the microalgal biofilm could in light and microalgae cells could maintain activity and grow better.

On the contrary, with the increase of biomass density, effectively light penetration (depth from the surface to the light compensation point) decreased rapidly in microalgal suspension (Table 1). Even when 120 μmol m^{-2} s^{-1} of the light intensity penetrating though microalgal suspension at densities lower than 10 g m^{-2}, the output light intensity was only 3 μmol m^{-2} s^{-1}, which is lower than the light compensation point (LCP) of *C. vulgaris*. For *C. vulgaris*, LCP is 6 ± 0.5 μmol m^{-2} s^{-1} (Shiraiwa and Miyachi, 1983), where the microalgae photosynthetic accumulation rate equilibrates with the respiration rate (Chisti, 2012). While the output light intensity though biofilm could reach up to 59.72 μmol m^{-2} s^{-1} with the same areal biomass density of 10 g m^{-2}, and even though 40 g m^{-2} of biofilm, the output light intensity was still 6.06 μmol m^{-2} s^{-1} higher than value of LCP. In attached biofilm, effectively illuminated portion was as high as 100% with areal biomass density lower than 40 g m^{-2}. Almost all the upper 40 g m^{-2} (corresponding with a biofilm thickness of 41.31 ± 3.73 μm, measured by Dektak 150 surface profilometer, Veeco, USA) of microalgal cells, which accounted for much than 40% of the biofilm even when the areal biomass density was 100 g m^{-2} were always effectively illuminated and could maintain a higher photosynthetic active rate. This higher percentage of effectively illuminated portion of biofilm might be one of the reasons why the biomass productivity and photosynthetic efficiency of microalgae cultivated in attached biofilm was higher than that in suspension.

3.2. Effect of incident light intensity on microalgal biofilm growth

The microalgal biofilm growth rate on the first day (day 1) increased almost linearly from 8.8 to 30.5 g m^{-2} d^{-1} with the increase of light intensity from 40 to 280 μmol m^{-2} s^{-1} (Fig. 4A). While, the biofilm growth rate on the third day was maintained to be almost the same and the biofilm density was about 60 g m^{-2} if light intensity higher than 120 μmol m^{-2} s^{-1}.

Chlorophyll is the main light-capturing molecule for photosynthetic organism (Green and Durnford, 1996). In this study, the content of chlorophyll on day 1 with different light intensity was measured and values have been expressed as both a function of the dry biomass and the cultivated surface area. With light intensity increasing from 40 to 280 μmol m^{-2} s^{-1}, the dry mass-based chlorophyll content decreased from 3.62% to 2.03%. Although the dry mass-basedchlorophyll content decreased, the areal chlorophyll content increased due to the increase of microalgal biomass from 726.48 to 1037.37 mg m^{-2}, which indicates the more chlorophyll the higher biofilm growth rate was obtained (Fig. 4A). Date of day 3 was plotted in Fig. 4B. The dry mass-based chlorophyll content decreased with the increase of light intensity. In addition, the dry mass-based chlorophyll content of day 3 was lower than that of day 1, which indicates the dry mass-based chlorophyll content decreased as microalgae growth. The areal chlorophyll density of day 3 increased by a small margin from 1077.25 to 1218.52 mg m^{-2} with incident light intensity increasing from 40 to 120 μmol m^{-2} s^{-1} and then decreased to 1148.36 mg m^{-2} with incident light intensity of 280 μmol m^{-2} s^{-1}. The areal chlorophyll density directly determined the amount of light energy captured by chlorophyll for microalgal photosynthetic growth. Therefore the biofilm growth rate was maintained to be almost the same with light intensity over 120 μmol m^{-2} s^{-1} (Fig. 4A).

3.3. Effect of CO_2 supply on C. vulgaris biofilm growth

3.3.1. Effect of CO_2 flow rate on growth of C. vulgaris biofilm

Pure CO_2 were aerated into the cultivation chamber supplying carbon source for microalgal cells growth. With the increase of flow rate, biofilm growth rate decreased resulting in lower biomass accumulation (Fig. 5A). Obviously, more CO_2 supply did not mean to a higher biomass accumulation. CO_2 molecules transferred into microalgal biofilm for photosynthetic growth by gas Knudsen diffusion. According to Knudsen diffusion, molecular concentration is the primary factor affecting diffusion flux (Yang et al., 2016). Thus, the higher CO_2 molecular concentration in the chamber, the more CO_2 transferred into the biofilm. Particularly, 300 mL min^{-1} pure CO_2 was aerated into the cultivation chamber, so that sufficient carbon source was supplied for microalgal growth. Nevertheless, 100% CO_2 adverse to microalgal growth in biofilm. Similar results had been reported in suspended cultivation method due to the acid environment which is detrimental to microalgal cells (Lee and Tay, 1991). Therefore, CO_2 with moderate concentration need to be aerated into the cultivation chamber to maintain enough CO_2 molecule diffused into microalgal biofilm.

Table 1
Light distribution in *C. vulgaris* biofilm andsuspension with incident light intensity of 120 μmol m^{-2} s^{-1}. The whole depth of microalgal suspension was 8 cm which was just the diameter of serum bottle.

Biomass density (g m^{-2})	Output light intensity (μmol m^{-2} s^{-1})		Effective light penetration[a]		Effectively illuminated portion (%)[b]	
	Biofilm	Suspension	Biofilm (μm)	Suspension (cm)	Biofilm	Suspension
10	59.72	3	41.31	4.5	100	56.25
30	10.89	0	41.31	<1	100	12.5
40	6.06	0	41.31	<0.75	100	9.38
50	5.25	0	41.31	<0.5	80	5.25
80	1.32	0	41.31	<0.25	50	3.13
100	0	0	41.31	<0.2	40	2.5

[a] Effective light penetration was defined as the depth (cm) of suspension and thickness (μm) of biofilm where the output light intensity was equal to LCP.
[b] Effectively illuminated portion was defined as the percentage of microalgal cells being illuminated by light intensity which is higher than the value of *C. vulgaris* light compensation point.

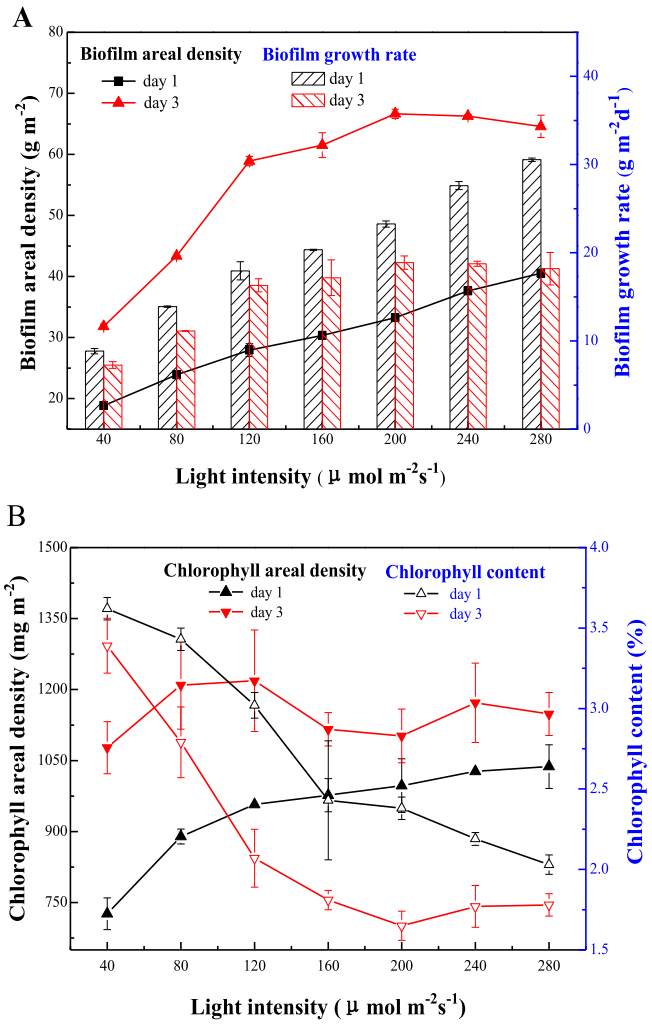
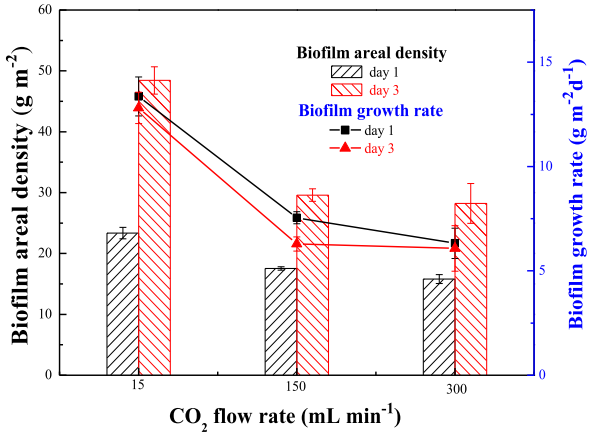


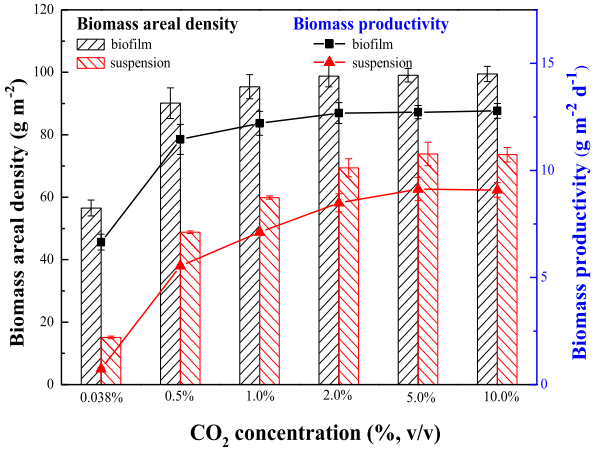
Fig. 4. Effect of light intensity on growth of *C. vulgaris* biofilm. (A) Influence of light intensity on biofilm areal density and growth rate. The microalgal biofilm was cultivated with 10% CO_2 (v/v) at 0.01 vvm for 3 days at an initial inoculum density of 10.0 ± 0.1 g m^{-2}. (B) The areal and dry mass-based chlorophyll contents. The biofilm growth rate of day 3 was the average growth rate that cultivated in 3 days.

3.3.2. Effect of CO_2 concentration on microalgae growth in biofilm and suspension

As shown in Fig. 5B, the increase of CO_2 concentration to the gaseous phase of the biofilm system did not further improve the biomass yield and productivity. This was probably due to the improvement of the CO_2 transfer efficiency to microalgal cells. As a result, even at a low CO_2 concentration level (0.038%), the microalgal cells can still assimilate enough CO_2 from the gaseous phase. In addition, high concentrations of CO_2 did not benefit microalgal growth due to acidic environment described previously. In contrary, CO_2 concentration significantly affected growth of suspended microalgal cells. It has been shown that when atmospheric air was used as the carbon source (Ji et al., 2014b), the suspended microalgal cells were barely able to accumulate biomass. A higher



(A) Effect of different pure CO_2 flow rates



(B) Effects of CO_2 concentration on biomass production in 7 days

Fig. 5. Effect of different CO_2 flow rates (A) and concentration (B) on growth of *C. vulgaris* in biofilm and suspension under 120 ± 3 μmol m^{-2} s^{-1} of continuous illumination.

Table 2
Effect of CO_2 concentration on the carbon and nitrogen content of biomass.

CO_2 Conc.	0.038%	1%	5%	10%	100%	10%
Cultivation mode	Attached					Suspended
C content (%)	49.42	53.23	52.21	52.71	52.28	49.79
N content (%)	6.42	6.72	6.99	6.83	7.17	8.47
H content (%)	6.59	7.30	7.12	7.36	7.51	6.72
C/N ratio	7.70	7.91	7.46	7.71	7.30	5.86

CO_2 concentration dramatically prompted microalgae growth due to the higher dissolved inorganic carbon in suspension which was the main carbon source for suspended microalgal cells (Chang et al., 2016). It can be found that there was a critical value of CO_2 concentration of 1.5%, below which the growth of biofilm was inhibited by carbon deficiency, and above which there was no positive effect on the growth anymore but a lower utilization efficiency of CO_2. This value was defined as CO_2 saturation point for *C. vulgaris* grown in biofilm. While this value for suspended microalgae growth was 4.5%. The results suggested there was significant influence of CO_2 concentration on growth of suspended microalgal cells due to mass transfer resistance from gas–liquid interface to microalgal cells (Zhao et al., 2015). In a suspended microalgae cultivation system, CO_2 has to overcome the gas–liquid interface to transfer into the liquid medium for microalgal cell utilization. The unique design of the biofilm bioreactor is that the microalgal cells can directly contact gaseous CO_2 when exposed to the gas phase. Different from the suspended cultivation method of microalgae which requires dissolved inorganic carbon resource in open ponds or a variety of PBRs, the microalgal attached cultivation mode provided another way of carbon supply for microalgal photoconversion by transfer through gas–solid contact. Instead of dissolving CO_2 in the liquid, the attached cultivation system brings microalgal cells to the CO_2 rich gaseous phase for more efficient mass transfer, thus, the CO_2 utilization efficiency can be improved (Gross et al., 2013). Generally speaking, CO_2 molecule was easier to diffuse into cells in attached cultivation system than that in suspended system, due to lower transfer resistance between gas–solid (CO_2-cells) than gas–liquid-solid (CO_2-medium-cells).

The carbon (C) and nitrogen (N) content of dry microalgal biomass and C/N ratio are shown in Table 2. With the CO_2 concentration increasing from 0.038% to 1%, C content of microalgal biomass increased obviously from 49.42% to 53.23% when cultivated in attached mode. However, with the flow-up increase of CO_2 concentration, even the pure CO_2, the carbon content did not increase any more keeping almost the same level with that of 1%. The C content of biomass cultivated in suspended cultivation method with 10% CO_2 (v/v) was just 49.79%, almost the same as that of biomass cultivated in biofilm with 0.038% CO_2 (v/v). Results suggest that attached cultivation mode benefits carbon assimilation of microalgae, which results from CO_2 molecule directly contacts the microalgal cells. While, N content had no significant change with CO_2 concentration increasing in attached cultivation. However, N content of biomass of suspension was higher than that of attached biofilm. Nitrogen source for microalgal growth was dissolved in medium. This can be attributed to the fact that suspended microalgal cells surrounded by medium could assimilate more nitrogen than microalgal cells in attached biofilm by soaking medium from agar gel. Partial microalgal cells were exposed in the air in attached cultivation method, which induced lipid synthesis (data not shown) (Shiratake et al., 2013).

3.4. Effect of initial inoculum density on microalgal growth

The changes in areal biomass density and growth rate at different initial inoculum densities after 1 day of attached cultivation are shown in Fig. 6. Both biomass areal density and growth rate were quickly increased when the initial inoculum density increased from 2.5 to 12.5 g m^{-2}, and the maximum growth rate of about 27.85 g m^{-2} day^{-1} was obtained at an initial inoculum density of 12.5 g m^{-2}. When initial inoculum density was further increased to 25 g m^{-2}, the growth rate decreased. Actually, the multiplication of microalgal cells, which thickens the microalgal biofilm, is mostly driven by the amount of absorbed light photons. The penetration path of light in the dense microalgal biofilm is very limited. In previous section the light penetration path of *C. vulgaris* biofilm under 120 μmol m^{-2} s^{-1} illumination intensity was only about 41.31 ± 3.73 μm, which corresponded to the areal biomass density of ca. 40 g m^{-2}. This means that only the top layer of about 40 g m^{-2} could be effectively illuminated for photosynthetic growth. As seen, when the initial inoculum density was

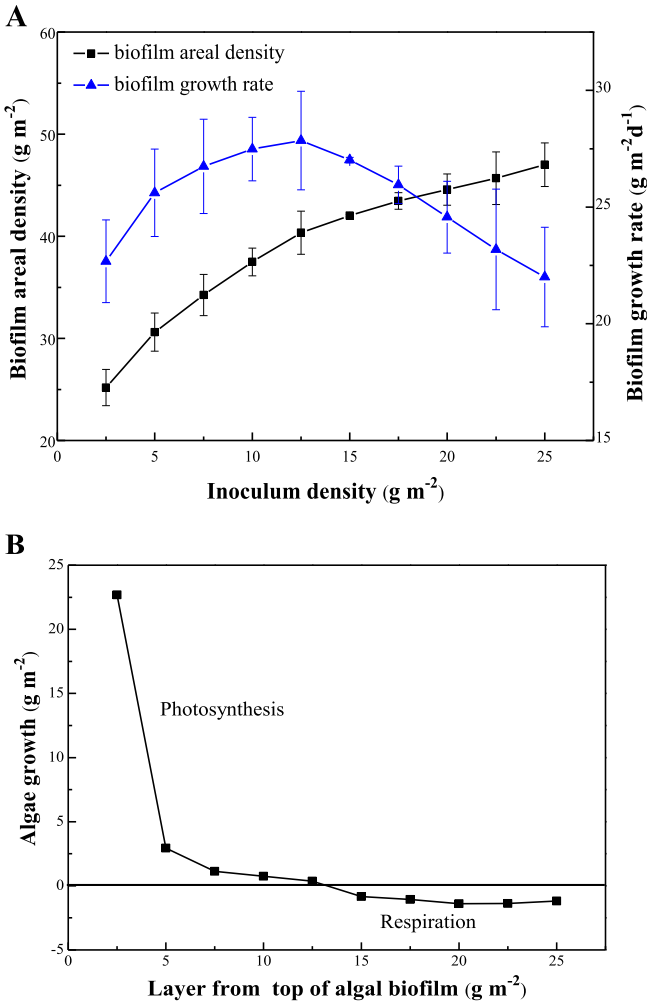


Fig. 6. Effect of initial inoculum density on the growth of *C. vulgaris* biofilm that cultivated 1 day under 120 ± 3 μmol m^{-2} s^{-1} continuous illumination by aerating with 10% CO_2 (v/v) at 0.01 vvm.

12.5 g m^{-2}, which was considered as the optical inoculum density, areal biomass density after 1 day culture was 40.35 g m^{-2} in accordance with effective light penetration. As shown in Fig. 6B, the microalgal biofilm was separated into different layers from the top to the bottom, 2.5 g m^{-2} for each layer by initial inoculum density. Algae growth of different biofilm layer was calculated by the following equations:

$$AG_{2.5} = RX_{2.5}; \quad AG_5 = RX_5 - RX_{2.5}; \quad AG_{7.5} = RX_{7.5} - RX_5 \ldots AG_{25} = RX_{25} - RX_{22.5}$$

where $AG_{2.5}$, AG_5, $AG_{7.5}$ ..., AG_{25} represent algae growth of different biofilm layer and $RX_{2.5}$, RX_5, $RX_{7.5}$, $RX_{22.5}$, RX_{25} represent growth rate of biofilm with initial inoculum density 2.5, 5, 7.5, 22.5 and 25 g m^{-2}, respectively. Therefore, the top 2.5 g m^{-2} layer photosynthesized growth 22.68 g m^{-2} and the layer 12.5 g m^{-2} dramatically decreased to 0.36 g m^{-2}. Light intensity attenuated with light penetrating downward in the algal biofilm, algae photosynthesis weakened. Then, the layer 15–25 g m^{-2} transformed into respiration consumption. Thus, an initial inoculum denser than the optimal value, for instance 15–25 g m^{-2}, would result in a more significant effect of consumption by respiration of those microalgal cells below the effectively illuminated top layer, giving rise to a reduction of the average growth rate (Fig. 6B). On the other hand, the growth rate of microalgal biofilm was gradually decreased during cultivation (as shown in Fig. 4). It may also result from the accumulated thickness of biofilm.

4. Conclusions

40–100% of the microalgal cells in the biofilm were effectively illuminated at light intensity of 120 μmol m^{-2} s^{-1} with the areal density from 10 g m^{-2} to 100 g m^{-2}. By contrast, the effectively illuminated portion of cells in microalgae suspension was only 56.25%. Besides, microalgae cells that in biofilm seem to be more amiable to CO_2 than that in microalgae suspension. The enhanced CO_2 and light transmission in biofilm improved light energy converting efficiency and biomass productivity. Still, further study should be done on CO_2 transfer in biofilm to reveal its growth advantage.

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