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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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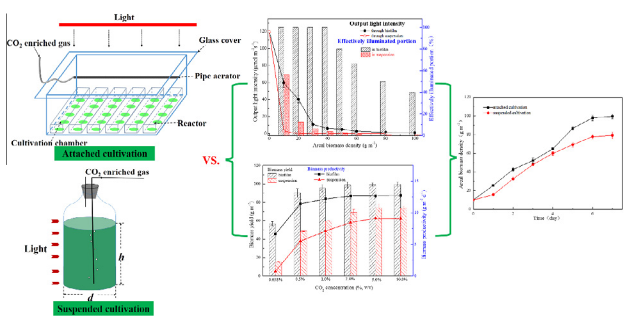
HIGHLIGHTS

- 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.

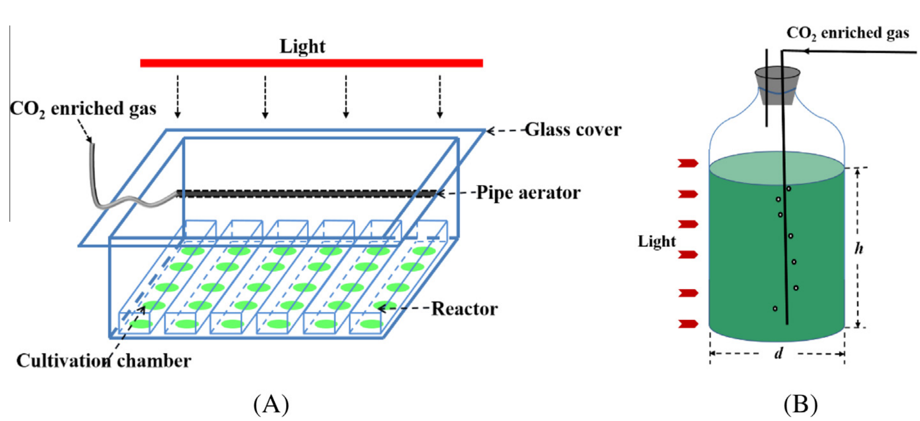

Fig. 1. Schematic of experimental setup of microalgal (A) attached biofilm cultivation and (B) suspended cultivation.