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ORIGINAL RESEARCH PAPER

Aeration strategy for biofilm cultivation of the microalga *Scenedesmus dimorphus*

Chunli Ji · Junfeng Wang · Tianzhong Liu

Abstract

Objective Biofilm cultivation of microalgae may be useful for biofuel production. However, many aspects for this cultivation method have not been well assessed. Accordingly, aeration strategy for biofilm cultivation of *Scenedesmus dimorphus* has been explored.

Results Biomass, lipid and triacylglycerol (TAG) productivity in increased *S. dimorphus* as the CO_2 concentration increased within 0.038–0.5 % and kept constant with further increases. The biomass, lipid and TAG productivity increased with the speed increasing and an obvious threshold point was observed at 6.6 ml^{-2} min^{-1}. The lipid and TAG content was unaffected by the aeration rate.

Conclusions Both the CO_2 concentration as well as aeration speed affected the growth of *S. dimorphus* in biofilm cultivation. The optimized aeration strategy for biofilm cultivation was continuous air flow enriched with 1 % CO_2 (v/v) at 6.6 ml^{-2} min^{-1}.

Keywords Aeration · Biofilm cultivation · Biofuel · CO_2 supply · *Scenedesmus dimorphus* · Total lipid · Triacylglycerol · TAG

Abbreviations

A_{mini}	Minimum aeration speed
C_{CO_2}	Optimal CO_2 concentration for aeration
C_{End}	Carbon concentration in algal cells that has been cultivated in biofilm condition for 7 days
C_{Ini}	Carbon concentration in initial biomass
DW_{End}	Biomass concentration by the end of biofilm cultivation
DW_{Ini}	Biomass concentration at initial biofilm cultivation
DW_t	Biomass concentration of biofilm cultivation at day t
$P_{biomass}$	Biomass productivity
PBR	Photobioreactor
Q_{carbon}	Maximum net carbon element deposited inside the biofilm
T	Cultivation time
TAG	Triacylglycerol
V_{CO_2}	Minimum CO_2 gas volume that required for each chamber during cultivation
W_t	Total biomass of the algal disk sample at day t

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Introduction

Microalgae are a promising feedstock for biofuel production because of their high photosynthetic efficiency, high oil content as well as non-competition with food production (Liang et al. 2009; Wijffels and Barbosa 2010). Open ponds and closed photobioreactors (PBRs) dominate the cultivation methods through the open pond is considered as the only financially-affordable method for outdoor, large scale biofuel production due to its cheap and easy in building and operation (Sompech et al. 2012). The major drawback of the open pond is its low biomass productivity, which is only 10–25 g m^{-2} d^{-1} annually (Tredici et al. 1986; Lee 2001; Pulz 2001; Jimenez et al. 2003). This value is much lower than the estimated potentials of 150 g m^{-2} d^{-1} (Zhu et al. 2008; Tredici 2010) and would destroy most of the advantages of microalgae over higher plants in biofuel production.

Biofilm-based immobilized cultivation is a substitute for the aqua-suspended methods. We have found this method to have a high photosynthetic efficiency. Biomass productivity of 50–80 g m^{-2} d^{-1} is obtained outdoors with the oleaginous microalga, *Aucutodesmus,* which is 500–700 % higher than that of conventional open ponds (Liu et al. 2013). Besides the increased photosynthetic efficiency, the biofilm immobilized cultivation system also shows many other advantages including long duration, easy in contamination control, low overall energy consumption and water saving, etc. (Liu et al. 2013; Ji et al. 2014). In general, this biofilm cultivation method has exhibited huge potentials in improving the microalgal biofuel industry.

Due to the fundamental difference in the behavior of the algal cells (suspended vs. immobilized), the current knowledge and information on biotechnological aspects derived from conventional open pond or PBRs may not be well adapted to the biofilm conditions. For example, for suspended cultivation, aeration generally serves multiple functions including preventing the sedimentation of algal cells, removing the O_2, adjusting the pH and supplying the carbon source (CO_2). However, for biofilm cultivation, agitation is not necessary, O_2 removing and pH adjusting functions are substitutable by other methods and only the supply of CO_2 is meaningful. So, with this changed function list, what is the proper flow speed and CO_2 concentration? These are the questions we want to answer in this research.

Materials and methods

Microalgae strain and inoculum preparation

Scenedesmus dimorphus was obtained from the Key laboratory of Biofuel (Qingdao, China) and maintained in BG11 medium (Stanier et al. 1971). To prepare the inoculum for biofilm cultivation, *S. dimorphus* was inoculated in glass columns (0.58 m × 5 cm inner diameter with 0.7 l working volume). Columns were illuminated by continuous fluorescent lamps at 100 μmol photons m^{-2} s^{-1}. The cultures were grown at 25 ± 1 °C and aerated continuously with CO_2 and enriched air flow (air:CO_2 = 1:0.02, v/v) at 0.25 vvm. Biomass was harvested at late growth phase (ca. 7 days after inoculation) to inoculate the biofilm cultivation.

The attached cultivation system

The attached culture cultivation system included a 0.4 m × 0.2 m glass plate (2 mm width) that was placed inside a 0.5 m × 0.25 m × 0.05 m glass chamber. The upper surface of the glass plate was covered by a layer of filter paper. The medium was dripped (0.06 l h^{-1}) on to the paper through a perforated nylon tube that placed on the upper brim of the glass plate between the filter paper and glass to wet the filter paper. Ten mg algal cells were evenly filtered onto a nitrate cellulose/cellulose acetate filter membranes (diam. = 50 mm, pore size = 0.45 μm) to form an algal disk with 10 ± 0.5 cm^2 footprint so that the inoculation concentration was ~10 g m^{-2}. Then 16 algal disks were placed onto the filter paper (Fig. 1). To keep the stability of the nutrient
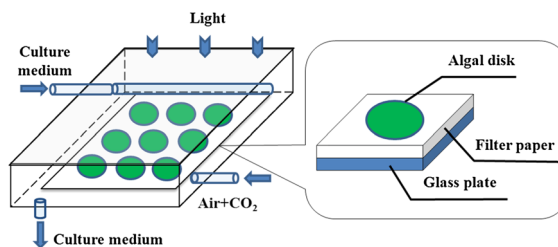

Fig. 1 Schematic diagram of the attached system

environment, the medium flowed through the filter paper was non-recycled. The chamber was illuminated with fluorescent lamps at 100 ± 5 μmol m^{-2} s^{-1}. The biofilm was kept at 25 ± 1 °C during cultivation. The CO_2 enriched compressed air was continuously injected into the glass chamber during cultivation.

Experimental design

The optimal CO_2 concentration (C_{CO_2}) was estimated by cultivating the algal biofilm under air flow that enriched with CO_2 concentration gradients of 0.038 % (air), 0.06, 0.1, 0.2, 0.5, 1, 2, 5 and 10 % (v/v), and the CO_2 concentration resulted in highest triacylglycerol (TAG) productivity was selected as C_{CO_2}. The aeration flow speed was 66.4 l m^{-2} min^{-1} for this experiment. The aeration speed was further optimized by aerating the chamber with speed gradients of 1.2, 2.8, 5.1, 6.6, 12.9, 26.1, 32.4, 66.4 l m^{-2} min^{-1}. According to stoichiometric calculation (Table 1), a flow speed of 0.8 l m^{-2} min^{-1} could supply enough carbon so that there were no problem of carbon shortage in case of a flow speed of 1.2 l m^{-2} min^{-1}. [The unit 'vvm' is not used in this research because it is only meaningful for aqueous-suspended cultivations like open pond and PBRs in which whole space inside the container is occupied by the algal broth, however, for the biofilm cultivation, the volume of the chamber was not a critical parameter that determine the growth of the biofilm because thickness of the biofilm is generally less than 200 micron (Liu et al. 2013), which is several magnitudes lower than that of the air space.]

The stoichiometric calculation on carbon element followed these procedures: Firstly, the final algal biomass of C_{CO_2} treatment, viz. 1 % CO_2 treatment (Fig. 2), were harvested and lyophilized to analyze the carbon concentration (C_{End}, %DW) with elemental analyzer (Elemental Vario EL III, GmbH, Germany). The carbon concentration in the initial biomass was also measured ($C_{Ini.}$, %DW). The initial and final biomass concentration of algal biofilm was marked as $DW_{Ini.}$ and DW_{End} (g m^{-2}), respectively. The maximum net carbon element (Q_{carbon}, mol m^{-2}) that deposited inside the biofilm was calculated as:

$$Q_{carbon} = \frac{(C_{End} \times DW_{End} - C_{Ini} \times DW_{Ini})}{12} \quad (1)$$

In Eq. 1, 12 means the mol mass of carbon element (g mol^{-1}), respectively. The minimum CO_2 gas volume (V_{CO2}, l m^{-2}) that required for each m^2 biofilm during cultivation could be calculated according to the ideal gas law:

$$V_{CO_2} = \frac{Q_{carbion} \times 8.314 \times 297.15}{100} \quad (2)$$

In Eq. 2, the Figs. 8.314, 297.15 and 100 represented the ideal gas constant, temperature in Kelvin and gas pressure in kilo Pascals, respectively. The minimum aeration speed (A_{mini}, ml m^{-2} min^{-1}) was calculated as:

$$A_{mini} = \frac{V_{CO_2} \times 1000}{C_{CO_2} \times 7 \times 24 \times 60} \quad (3)$$

It should be noted that the carbon content in medium including Na_2CO_3 (one of the medium component and could be used as carbon resource) and secreted organic molecules if any, is not considered when calculating the A_{mini}.

As shown in Table 1, the aeration speed should be at least 0.8 ml^{-2} min^{-1} (to give a CO_2 concentration of 1 %) to supply sufficient carbon to the biofilm. Lower speeds will result in carbon deficiency. Accordingly, we set the 1.2 ml^{-2} min^{-1} as the lower limit of the aeration.

Table 1 Stoichiometric calculation of carbon element that deposited in algal biofilm

Parameters	Value	
Initial biomass (DW_{Ini}, g m^{-2})	10.7	
End biomass (DW_{End}, g m^{-2})	77.9	
Initial C content (C_{Ini}, % DW)	51.3	
End C content (C_{End}, % DW)	56.2	
Net C gain (Q_{carbon}, mol m^{-2})	3.19	Refer Eq. 1
Minimum CO_2 requirement (V_{CO2}, l m^{-2})	77.5	Refer Eq. 2
Aeration CO_2 concentration (C_{CO_2}, %)	1	Refer Fig. 1
Minimum aeration speed (A_{mini}, l m^{-2} min^{-1})	0.8	Refer Eq. 3

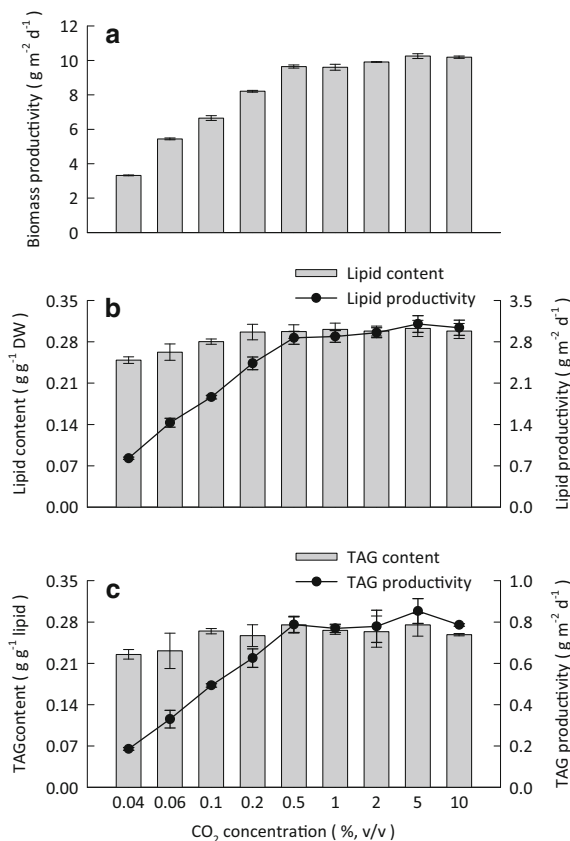The carbon source in medium, e.g. Na_2CO_3, was not considered in calculation

Fig. 2 The effects of CO_2 concentrations on the growth and lipid accumulation of the attached *Scenedesmus dimorphus*. The chamber was continuously illuminated with white fluorescent lamps at an intensity of 100 ± 5 μmol m^{-2} s^{-1}. The temperature of the algal biofilm was 25 ± 1 °C measured by an infrared thermometer. The compressed air (0.1 MPa) enriched with different concentrations of CO_2 was injected into the chamber with aeration speed of 1063 ml min^{-1}. Biomass productivity results were average $\pm$ standard derivation of nine replications (three measurements for each of three independent experiments); Lipid and TAG content results were average $\pm$ standard derivation of three replications (one measurement for each of three independent experiments). The culture medium was non-circulated, so that the nutrient concentrations around the algal cells kept constant during the cultivation

Growth analysis

Biomass concentration and productivity were measured gravimetrically. Algal disks of each treatment were sampled after 7 days cultivation. The algal cells on each membrane were detached by de-ionized water and then filtered on to pre-weighed 0.45 μm GF/C filter membrane (Whatman, England). The membrane with biomass was oven dried to constant weight at 105 °C for 12 h. The weight difference of before-and-after filtration of the GF/C membrane was considered as the total biomass of the algal sample (W_t, g) and the biomass concentration (DW_t, g m^{-2}) was calculated as:

$$DW_t = \frac{W_t}{0.001} \tag{4}$$

in which 0.001 represented the footprint area (m^2) of the attached algal biomass.

The biomass productivity ($P_{biomass}$, g m^{-2} d^{-1}) was calculated as:

$$P_{biomass} = \frac{DW_t - DW_0}{t} \tag{5}$$

in which DW_0 was the biomass concentration of day 0 and t represented the cultivation time, viz. 7 days.

Lipid content and lipid composition analysis

Cells were collected by centrifugation at $10{,}000 \times g$ for 5 min. The sediment was lyophilized and ~ 50 mg dry cells were used to analyse the total lipid and TAG content according to the method of Chen et al. (2012).

Results and discussion

The biomass productivity increased as the CO_2 concentration increased within 0.038–0.5 % and kept constant with further increases of CO_2 concentration (Fig. 2a). Lipid and TAG productivities had similar trends with that of biomass productivity (Fig. 2b, c) and both were slightly lower at CO_2 concentrations below 0.5 % than those over 0.5 % (Fig. 2b, c). The effects of aeration speed on biomass and lipid variations are shown in Fig. 3. Biomass, lipid and TAG productivity increased with increasing speed and an obvious threshold point was observed at 6.6 l m^{-2} min^{-1} (Fig. 3a, b, c). The lipid and TAG content were constant under different aeration speeds (Fig. 3b, c). Accordingly, the optimized aeration strategy for this biofilm cultivation is suggested as: CO_2 concentration at 1 % and aeration speed of 6.6 l m^{-2} min^{-1}.

Carbon transportation is the main difference between aqua-suspended and biofilm attached cultivation. For suspended cultivation, CO_2 dissolves firstly in aqueous medium and then is absorbed by
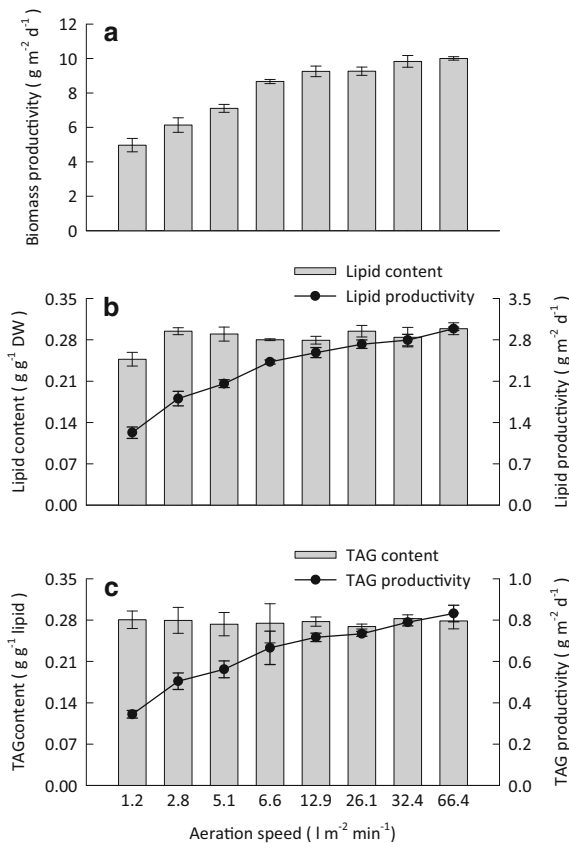
Fig. 3 The effects of aeration speed on the growth and lipid accumulation of the attached *S. obliquus*. The chamber was continuously illuminated with white fluorescent lamps at an intensity of 100 ± 5 μmol m^{-2} s^{-1}. The temperature of the algal biofilm was 25 ± 1 °C measured by an infrared thermometer. The compressed air (0.1 MPa) enriched with 1 % of CO_2 (v/v) was injected into the chamber with different aeration speed. Biomass productivity results were average $\pm$ standard derivation of nine replications (three measurements for each of three independent experiments); Lipid and TAG content results were average $\pm$ standard derivation of three replications (one measurement for each of three independent experiments). The culture medium was non-circulated, so that the nutrient concentrations around the algal cells kept constant during the cultivation

the algal cells (van den Hende et al. 2012), while for the attached system, because the thickness of water film covers the biofilm is very thin, the diffusion of CO_2 to the cells is much easier (Ji et al. 2014). However, the CO_2 concentration has a similar effect on biomass accumulation for these two cultivation system, viz. higher CO_2 concentration stimulates growth (Fig. 2a, b; de Morais and Costa 2007; Chiu et al. 2008) but when the concentration exceeds certain

limit, more CO_2 will not generate further biomass accumulation (Tang et al. 2011). Superfluous CO_2 supplement is of no consequence for biomass and lipid accumulation. The constant lipid and TAG content under different aeration speed (Fig. 3b, c) indicated the aeration speed did not affect the carbon uptake inside the algal cells. The increased biomass productivity with increased aeration speed (Fig. 3a) might due to the O_2-removing rate being higher under higher aeration speed. From Figs. 2 and 3, we can tell that the CO_2 concentration and aeration speed affected the biomass accumulation more readily than lipid and TAG concentrations. This phenomenon indicated the CO_2 and aeration environment mainly affect the carbon absorption procession rather than the carbon and energy portioning process.

Before this research, we proposed a most 'ideal' tactic to supply CO_2 to this biofilm system, viz. seal a volume of CO_2 (or air/CO_2 mixture) inside the chamber without any auxiliary power input to drive the air flow. However, after this research we found this 'ideal' tactic might not be practical because the flow of the aeration gas seemed to be an essential requirement for the success of biofilm cultivation (Fig. 3a). This might be because the gas flow stream could take away the O_2 produced by biofilm but it could also keep the CO_2 environment constant around the biofilm so that to facilitate the photosynthesis.

References

Chen L, Liu T, Zhang W, Chen X, Wang J (2012) Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. Bioresour Technol 111:208–214

Chiu SY, Kao CY, Chen CH, Kuan TC, Ong SC, Lin CS (2008) Reduction of CO_2 by a high-density culture of *Chlorella sp.* in a semicontinuous photobioreactor. Bioresour Technol 99:3389–3396

de Morais MG, Costa JAV (2007) Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbondioxide. Energy Convers Manag 48:2169–2173

Ji C, Wang J, Zhang W, Liu J, Wang H, Gao L, Liu T (2014) An applicable nitrogen supply strategy for attached cultivation of *Aucutodesmus obliquus*. J Appl Phycol 26:173–180

Jimenez C, Cossio BR, Niell FX (2003) Relationship between physicochemical variables and productivity in open ponds for the production of *Spirulina*: a predictive model of algal yield. Aquaculture 221:331–345

Lee YK (2001) Microalgal mass culture systems and methods: their limitation and potential. J Appl Phycol 13:307–315

Liang Y, Sarkany N, Cui Y (2009) Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. Biotechnol Lett 31:1043–1049

Liu T, Wang J, Hu Q, Cheng P, Ji B, Liu J, Chen Y, Zhang W, Chen X, Chen L (2013) Attached cultivation technology of microalgae for efficient biomass feedstock production. Bioresour Technol 127:216–222

Pulz O (2001) Photobioreactors: production systems for phototrophic microorganisms. Appl Microbiol Biotechnol 57:287–293

Sompech S, Srion A, Nuntiya A (2012) Synthesis of perovskite-type lanthanum cobalt oxide powders by mechanochemical activation method. Scienceasia 38:102–107

Stanier R, Kunisawa R, Mandel M, Cohen-Bazire G (1971) Purification and properties of unicellular blue-green algae (order *Chroococcales*). Bacteriol Rev 35:171–205

Tang D, Han W, Li P, Miao X, Zhong J (2011) CO_2 biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO_2 levels. Bioresour Technol 102:3071–3076

Tredici MR (2010) Photobiology of microalgae mass cultures: understanding the tools for the next green revolution. Biofuels 1:143–162

Tredici MR, Papuzzo T, Tomaselli L (1986) Outdoor mass-culture of *Spirulina-maxima* in sea-water. Appl Microbiol Biotechnol 24:47–50

Van den Hende S, Vervaeren H, Boon N (2012) Flue gas compounds and microalgae: (Bio-)chemical interactions leading to biotechnological opportunities. Biotechnol Adv 30:1405–1424

Wijffels RH, Barbosa MJ (2010) An outlook on microalgal biofuels. Science 329:796–799

Zhu XG, Long SP, Ort DR (2008) What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? Curr Opin Biotechnol 19:153–159