Attached cultivation for improving the biomass productivity of *Spirulina platensis*

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**Highlights**
- *Spirulina platensis* was successfully cultivated in attached biofilm.
- Electrostatic flocking cloth is a practicable industrial substratum.
- Biomass productivity of 60 g m\(^{-2}\) d\(^{-1}\) was obtained in an outdoor bench.

**Abstract**

To improve cultivation efficiency for microalgae *Spirulina platensis* is related to increase its potential use as food source and as an effective alternative for CO\(_2\) fixation. The present work attempted to establish a technique, namely attached cultivation, for *S. platensis*. Laboratory experiments were made firstly to investigate optimal conditions on attached cultivation. The optimal conditions were found: 25 g m\(^{-2}\) for initial inoculum density using electrostatic flocking cloth as substrata, light intensity lower than 200 l mol m\(^{-2}\) s\(^{-1}\), CO\(_2\) enriched air flow (0.5%) at a superficial aeration rate of 0.0056 m s\(^{-1}\) in a NaHCO\(_3\)-free Zarrouk medium. An outdoor attached cultivation bench-scale bioreactor was built and a 10 d culture of *S. platensis* was carried out with daily harvesting. A high footprint areal biomass productivity of 60 g m\(^{-2}\) d\(^{-1}\) was obtained. The nutrition of *S. platensis* with attached cultivation is identical to that with conventional liquid cultivation.

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1. Introduction

In recent years, the global concern on greenhouse effect has driven a variety of attempts to reduce gas emissions by fixation of CO\(_2\). Microalgae have been thought to be an effective way to solve this problem because of their concomitant capacity to fix CO\(_2\) into algal biomass through photosynthesis and potentially produce biodiesel (Wijffels and Barbosa, 2010). In order to make this process economically feasible and environmentally sustainable, the premise is represented by the large-scale production of microalgae biomass efficiently at low cost (Larkum, 2010). *Spirulina* sp., one of the most popularly cultivated microalgae, has been widely used as a healthy food, forage and additive since the 1970s (Hu et al., 1998; Jimenez et al., 2003), due to its rich nutrients including proteins, polyunsaturated fatty acids, polysaccharides, carotenoids and vitamins (Belay et al., 1993; Madkour et al., 2012). Though the commercialized production of *Spirulina* sp., as healthy food and valued additives is profitable, more efficient cultivation technique...
in pursuit of lower cost is also currently an important direction, especially in order to enlarge the usage of *Spirulina* sp.

To date, the commercial production of *Spirulina* sp. is carried out almost exclusively in open ponds, which are easy to build and do not require particular control of some parameters such as illumination and temperature. However, the drawback of these systems is the low biomass productivity, less than 15 g m\(^{-2}\) d\(^{-1}\) ([Jimenez et al., 2003; De Bhowmick et al., 2014]). Furthermore, in open ponds, there were other drawbacks, such as the difficulty of reaching very high biomass concentration due to difficulty maintaining optimal cultivation parameters, the easiness of contamination and the serious water evaporation between ponds and surrounding environments ([Guterman et al., 1990]). Another cultivation way of *Spirulina* sp. is the use of a variety of enclosed photobioreactors (PBRs) including bubbling column, airlift reactor, tubular, panel and plastic bags ([Lee, 1986; Converti et al., 2006]). These culture systems showed a better control of nutrients, light exposition and carbon absorption, as well as contamination avoidance resulting in cultures more dense, a little higher biomass productivity and a better quality of products than those reached in open ponds. Conversely, PBRs are characterized by high cost for their construction and by difficulty in system maintenance ([Chen et al., 2011]).

In a word, *Spirulina* sp., certainly with other microalgae, is usually cultivated in water suspension, hence it suffered a fragility of the cell sheath and the filamentous morphology response to shear force due to stirring and bubbling in suspended cultivation ([Tomaselli et al., 1981]). However, beside of grown in water suspension, some microalgae are capable to grow as productive biofilms over substrata ([Wuertz et al., 2003]). This pattern of growth may be able to solve the technical defects in liquid suspension culture, and thus disinhbit biological limitation for culturing *Spirulina* sp. therein.

Recently, a novel technology for microgal biofilm cultivation, namely attached cultivation has been announced ([Liu et al., 2013]). Since then, more and more attentions have been paid on applicability of this technology and developing derivative technologies. With such technologies, a diverse of algal strains have been cultivated ([Shen et al., 2014; Naumann et al., 2013; Blanken et al., 2014; Gross and Wen, 2014; Shi et al., 2014]). Among of which, the best biomass productivity was 80 g m\(^{-2}\) d\(^{-1}\), obtained with the oleaginous microalgae *Acutodesmus obliquus*, which was 700% higher than that obtained with conventional open ponds under the same climate and light conditions ([Liu et al., 2013]). Beside of high biomass productivity, potential in reducing harvesting-cost and power consumptions have been concluded ([Liu et al., 2013; Ji et al., 2014a; Ozkan et al., 2012]). The improvement of cultivation technology to enhance the biomass productivity and to reduce the cost associated to its production is beneficial for the expansion of this application. However, no attempts have been reported before on cultivation of *Spirulina* sp. with this technique.

The present work is to implement an attached cultivation technique for *Spirulina platensis*. The purpose lies in technological conditions of optimal cultivation, and the expansion of scale in outdoor environment. In order to reach this goal, firstly indoor experiments were carried out to clear the influence of cultivation conditions, such as initial inoculation density, light intensity, carbon supplementation by CO\(_2\) aeration and substrata materials, on the growth of attached *S. platensis* biofilm. Later, an outdoor attached cultivation bench bioreactor was constructed and a 10 d attached cultivation practice was carried out. Results have shown that the attached cultivation was effective to significantly improve the biomass productivity for *S. platensis*.

**2. Methods**

**2.1. Microalgae strain and culture medium**

The microalga *S. platensis* was purchased from the Institute of Oceanology, Chinese Academy of Sciences, PR China. The strain as inoculum for attached cultivation experiments was grown in a Zarrouk medium ([Zarrouk, 1966]). Bubbling glass columns (0.5 m long, 0.05 m in inner diameter with 0.007 m\(^{-3}\) working volume) were used, and aerated with 1% CO\(_2\) (v/v) enriched air at 0.25 vvm (volume/volume per minute), under a continuous illumination of 100 µmol m\(^{-2}\) s\(^{-1}\), and maintained a temperature of 25 ± 1 °C for 6 d.

**2.2. Attached cultivation**

**2.2.1. Indoor experiments**

The attached cultivation bioreactor used in indoor experiments, which is shown in Fig. 1A, was similar to that used by Liu et al.

![Fig. 1. Schematic diagram of the attached cultivation bioreactor for S. platensis. (A) Laboratory device. (B) Outdoor bench-scale bioreactor.](image-url)

was enriched compressed air (air:CO₂ = 1:0.01, v/v) was supplied into the reactor, with a superficial aeration rate of 0.0056 m s⁻¹, through a plastic pipe installed at the left bottom of the steel frame. The CO₂ concentration of aerated gas was doubled of the indoor values, due to consider the fact that the reactor was not well sealed and the cultivation space is far bigger than that occupied by the three EFCs.

The algal film was sampled by gently manual scraping of the substrata surface with a soft rubber blade from each surface of the clothes at 6:00 pm every day. The residual biomass was left behind to act as inoculums for the next day’s growth cycle. The harvested algal slurry was twice washed with distilled water, and then was oven-dried for biomass productivity measurement.

All the outdoor bench experimental cultivations were carried out in the campus of Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences (35°–35° N, 119°–30° E) during May 21 to 30, 2012. The photon flux density (PFD) was measured using a photometer (Li-250A, LI-COR Co., Ltd., USA) every hour from 6 am to 6 pm every day and the simple averaged value is calculated to present the PAR (photosynthetic active radiation). Meanwhile, the atmosphere temperature in the cultivation cavity was also recorded. A certain volume of distilled water was added to the medium reservoir to compensate the water loss by evaporation every day.

2.3. Growth analysis

The biomass productivity (P, g m⁻² d⁻¹) was measured with gravimetric method. The algal cells were totally washed off from the “algal disk” using distilled water. For outdoor experiments, algal cells were collected from the whole surface of each EFC for gravimetric measurement, using a same protocol as indoor experiments. The collected algal cells were then filtered on a pre-weighted (W₀, g) NC membrane (pore size 0.45 μm). The algal paste was washed twice with distilled water to remove all soluble nutrients. The membrane was then oven-dried for 12 h at 105 °C and then weighted (Wₚ, g) after cool down.

The biomass productivity based on unit area of the substrate after n days culture (Pₜ₋ₙ, g m⁻² d⁻¹) is calculated as shown in Eq. (1):

\[ P_{t-n} \ \text{g m}^{-2} \ \text{d}^{-1} = \frac{(W_A - W_0)}{(A_\text{t} \times n)} \]  (1)

where the Aₜ was 0.001 m² for indoor experiments, and was 0.2 m² for outdoor experiments. The surface area of EFC (1.0 m × 0.2 m).

The biomass productivity based on footprint area of the bioreactor after n days culture (Pₕ₋ₙ, g m⁻² d⁻¹) is calculated as shown in Eq. (2).

\[ P_{h-n} \ \text{g m}^{-2} \ \text{d}^{-1} = \frac{(W_A - W_0)}{(A_\text{h} \times n)} \]  (2)

where \( \sum \) indicated the summary weight of algal cells from all four cultivation sides of EFC, Aₕ was the footprint area occupied by the four cultivation surfaces (0.2 m × 0.4 m), which could also be taken as the incidence light area.

2.4. Nutrient analysis

In order to carry out nutrient analysis, microalgae were harvested and first filtered and washed with plenty of de-ionized water. Later, the harvested biomass was lyophilized, then sealed and finally stored in the dark at ~20 °C before measurement.

Carbohydrates were determined colorimetrically (Cary50, Varain, USA) with the use of phenol-sulfuric acid (Dubois et al., 1956 and glucose as standard.

Protein content of S. platensis was analyzed by the Kjeldhal method (AOAC, 1984), using 6.25 as a conversion factor.
Total lipids were estimated gravimetrically according to the method of Chen et al. (2012), later modified by Guo et al. (2014).

3. Results and discussions

3.1. The influence of initial inoculation density

Fig. 2 reported the changes of the biomass productivity of *S. platensis* biofilm with different initial inoculum densities. It can be found the biomass productivity, both at the first day and the third day, increased till to the maximum values of 12 and 8.03 g m$^{-2}$ d$^{-1}$, respectively, with 7–11 g m$^{-2}$ as initial inoculum density, then the biomass productivity declined. It indicates that this range represents the appropriate initial inoculation density as reported for similar results obtained by other researches. The biomass productivity of *Pseudochlorococcum* sp. increased with the increasing of initial inoculation density, and the maximum biomass productivity of 6.0 g m$^{-2}$ d$^{-1}$ occurred at the initial inoculation density of 3–5 g m$^{-2}$ (Ji et al., 2014a). A higher initial inoculation density of 8–10 g m$^{-2}$ was reported to be appropriate for *Aucutodesmus obliquus* (Ji et al., 2014b). These results showed how different microalgae species have different optimal inoculation density even if the difference is not too big when using a same NC membrane substrata.

The influence of initial inoculation density reflects the penetration path of light to the biofilm. Light can penetrate the algal biofilm to a few dozens of microns with an irradiation intensity of 100 μmol m$^{-2}$ s$^{-1}$ as in this case (Ji et al., 2014a). When the initial inoculation density is very low, less algal cells (or a thinner biofilm) than those that can be penetrated by the light are involved in the photosynthesis. A thicker biofilm with the appropriate initial inoculation density means that more algal cells participate to the growth. However, it should be considered that when a high inoculum is used, the algal cells at the top layer of the substrata surface can be easily penetrated by the light, while the algal cells residing on the lower strata could lose their biomass due to respirations and disable to capture enough light for the biomass accumulation.

It also can be found that the cultivation on the first day had higher biomass productivity than the averaged value during the 3 d of cultivation. Data and error bars represent means ± SD of three repeats.

3.2. The influence of photon flux density (PFD)

The PFD is reported to significantly affects both biomass productivity and the metabolic activity of *Spirulina* sp. (Soletto et al., 2005; Ajayan and Selvaraju, 2011; Bezerra et al., 2012). The relationship between PFD and biomass productivity of *S. platensis* in biofilm cultivation was plotted in Fig. 3. The biomass productivity linearly increased as the PFD rose. The maximum biomass productivity value of 18 and 14 g m$^{-2}$ d$^{-1}$ has been achieved on day 1 and day 3, respectively with a PFD of 200 μmol m$^{-2}$ s$^{-1}$. Beyond this light intensity, the biomass productivity did not increased anymore. This value of PFD can be taken as a critical point for photosynthetic efficiency or, roughly considered as the light saturation point (LSP) for *S. platensis* grown in a biofilm. The maximum biomass productivity of *S. platensis* achieved in the present work are almost equivalent to those achieved on mass cultivation scale in open ponds (Jimenez et al., 2003) under solar illumination. It is also worth mentioning that the biomass productivity was also higher than those achieved by other microalgae species, including *Aucutodesmus obliquus* and *Pseudochlorococcum* sp., when grown in attached cultivation. The light intensity at LSP of attached *S. platensis* biofilm was higher than other microalgae species including *A. oblique* (150 μmol m$^{-2}$ s$^{-1}$) (Liu et al., 2013), *Pseudochlorococcum* sp. (100 μmol m$^{-2}$ s$^{-1}$) (Ji et al., 2014a) indicating that *S. platensis* is more resistant to strong irradiation.

Nevertheless, it should be noticed that the light intensity at LSP of attached *S. platensis* biofilm was much lower than outdoor solar irradiation (1000–2000 μmol m$^{-2}$ s$^{-1}$). This means that if attached *S. platensis* biofilm was directly exposed under the blazing sun, photoinhibition on algal growth would be very serious, and even the biofilm would be bleached. Therefore, light dilution, as proposed by Liu et al. (2013), is required to avoid the photo inhibition and make full use of solar radiation for biomass synthesis. An attempt to cultivate *S. platensis* in a bench-scale bioreactor with a 10 times light dilution was made and the results will be illustrated in the Section 3.5.
The biomass productivity is also gradually reduced with the prolonging of cultivation time. It may be attributed to the increased thickness of algal biofilm by growth as stated above.

### 3.3. The influence of CO2 concentration and aeration rate

Currently, bicarbonate is used as carbon source for most of the *S. platensis* cultivation in open ponds. However *S. platensis* can also use dissolved CO2, which can come in various forms, from pure CO2 to industrial gaseous effluents, a more biocompatible and economic alternative. For attached cultivation, the direct contact between aerated gaseous CO2 and algal biofilm has been validated to effectively provide carbon for algal growth (Liu et al., 2013; Ji et al., 2014a). Similarly, the concentration of CO2 and aeration rate of the aerated gas have critical influence on the attached cultivation. The effect of CO2 concentration on the biomass productivity of attached *S. platensis* biofilm with NaHCO3 free Zarrouk medium is illustrated in Fig. 4A. When the CO2 concentration increased from 0.038% (natural atmosphere CO2 concentration) to 0.5%, the biomass productivity quickly increased. Beyond 0.5% of CO2, the biomass productivity slightly reduced. It means that a CO2 concentration of about 0.5% represented the optimum. The same trend has also been found with *Pseudochlorococcum* sp. attached cultivation but in that case the critical CO2 concentration was 1.0% (Ji et al., 2014a).

Fig. 4B demonstrates that the effect of superficial aeration rate on the biomass productivity of attached *S. platensis* biofilm. The biomass productivity increased to the maximum value when superficial aeration rate rose to 0.0056 m s\(^{-1}\). After that, stronger aeration intensity caused a dramatical reduction of the biofilm growth. It should be considered that *Spirulina* sp. is a basophilic microalga and consequently prefers high pH environment. Therefore, an inappropriate aeration condition with too high flow rate and content of CO2 may faintly acidify the contact region between the wet biofilm and the gaseous phase and then inhibit the growth of algal cells (Langley et al., 2012). Thus, an optimal aeration condition is needed in order to balance the benefits related to CO2 supplementation and the risk of physiological stress due to the acidification.

### 3.4. The influence of substrata

In addition of NC membrane, four industrial materials were also used as substrata. The growth of algal biofilm on both EFC and FS seems more active than that on the surface of both SFT and FC, to form varying sizes of visible bubbles on the biofilm due to oxygen-release by photosynthesis. The biomass productivity of algal cells on these four substrata is shown in Fig. 5. It can be seen that EFC has the highest biomass productivity, the FS and SFT have a slight lower biomass productivity, while the FC is the worst.

It was reported that the properties of substrata; in particular, the hydrophilicity/hydrophobicity and the surface structure of substrata, play important roles on growth of algal attached biofilm (Singh, 2011; Cui and Yuan, 2013). The EFC has hydrophilic fluffs on the cloth surface with tiny pores, the FS is made of hydrophilic PVC material, rich of tiny labyrinth pores and shows excellent liquid holding capacity. The SFT is woven by polyester superfine which also has strong hydrophilicity. However, the FC, made of coarse nylon fibers and with big pore size, shows hydrophobicity and bad ability in water retention. In the present work, the three substrata (EFC, FS and SFT) showed better ability than FC on attached cultivation of *S. platensis*, demonstrates that hydrophilic material with tiny pore size and good liquid holding capacity is preferable for the attachment cultivation.

Compared with four industrial substrata, the biomass productivity on NC membrane is slightly higher. Furthermore, the initial inoculums density has to be higher for industrial substrata

![Fig. 4. Effects of CO2 concentration (A) and superficial aeration rate (B) on biomass productivity of attached *S. platensis* with NaHCO3-free Zarrouk medium. Culture conditions: CO2 enriched air (0.5–5%), 100 μmol m\(^{-2}\) s\(^{-1}\) light intensity, and room temperature. Data and error bars represent means ± SD of three repeats.](image1)

![Fig. 5. Effect of industrial substrata on biomass productivity of attached *S. platensis*.](image2)
CO₂ enriched air flow (air:CO₂ = 1:0.005, v:v) at a superficial aeration rate of 0.0056 m s⁻¹ in a NaHCO₃-free Zarrouk medium.

3.5. Cultivation of S. platensis in outdoor bench-scale bioreactor

In order to practice attached cultivation of S. platensis in a bigger scale, outdoor cultivation experiments in a bench-scale bioreactor, consisted of three pieces of 0.2 m² EFC (Fig. 1B), were carried out in early summer of 2012 in Qingdao. Most parameters for attached cultivation, such as substrata, initial inoculum density, superficial aeration rate of CO₂, and culture medium, were following the optimum ones obtained from indoor experiments. The CO₂ concentration of the aerated gas was doubled of the indoor one, due to considering the fact that the outdoor bioreactor was not well sealed and the cultivation space is far bigger than that occupied by the three EFC. Although the temperature and the PFD were not adjusted to the optimum, these two parameters were recorded during the whole outdoor cultivation period and plotted in Fig. 6A. The averaged PFD was around 1100–1200 μmol m⁻² s⁻¹ and the temperature was quite steady around 24–32 °C in daytime, except for cloudy days on May 24 and 30.

The biomass productivity of algal biofilm was shown in Fig. 6. The biomass productivity based on unit area of the substrate (Pₓ), recorded on the four cultivated surfaces of EFC in different dates, ranged 6–7 g m⁻² d⁻¹ (Fig. 6B). This value showed a little variation, due to unstable temperature and PFD during outdoor cultivation. The variation of Pₓ, on the other hand, is probably due to the difference on the amount of harvested algal pastes and on the amount of retained algal cells after manual harvesting, which may influence the next day's growth as aforementioned in the Section 3.1.

The biomass productivities based on the footprint area of the bench-scale bioreactor (Pᵧ) ranged 46–70 g m⁻² d⁻¹ (Fig. 6C), with a maximum of 70 g m⁻² d⁻¹ on May 27, during whole outdoor experiment period. The minimum Pᵧ is 46 g m⁻² d⁻¹, occurred on May 24, when both PFD and temperature reach the lowest during experiment period.

When culturing Spirulina sp. in water suspension, particularly in open pond, the Pᵧ was usually less than 15 g m⁻² d⁻¹ (Jimenez et al., 2003; De Bhownick et al., 2014). The value obtained from present outdoor attached cultivation is much higher than that recorded from open pond and other PBRs, with the exception for the results by Hu et al. (1998), which was obtained using an extreme thin flat panel under strong illumination.

Comparing with the Pᵧ from other microalgae species cultured as biofilm, the Pᵧ from S. platensis is lower than 80 g m⁻² d⁻¹ from A. obliquus (Liu et al., 2013), but is much higher than 7.7 g m⁻² d⁻¹ from Halochlorella rubescens (Shi et al., 2014) and 20.1 g m⁻² d⁻¹ from Chlorella Sorokiniana (Blanken et al., 2014). Nevertheless, all these values are far less than theoretical value of microalgae, ca. 120 g m⁻² d⁻¹ (Tredici, 2010).

3.6. Protein, carbohydrate and lipid contents of S. platensis

The contents of protein, carbohydrate and lipid in S. platensis biomass from attached cultivation and conventional liquid suspension cultivation are listed in Table 1. It can be found that the protein, carbohydrate and lipid contents of S. platensis are around 50%, 12% and 10% respectively, when grown in attached cultivation for 1 and 3 d cultivation. This protein content of S. platensis showed a little variation, ranged 41–56%, during whole outdoor cultivation. These values are very close to those values from conventional liquid suspension cultivation. Also, the nutrient component of S. platensis are very close to work of Fedekar et al. (2012), who reported the protein, carbohydrate and lipid contents of 52.9%, 13.2% and 7.5% respectively, in liquid cultivation of Zarrouk...
medium. It indicates that the attached cultivation technique could produce high nutritive product.

4. Conclusions

An attached cultivation technique was introduced to cultivate *S. platensis* in indoor and outdoor environment. The optimum conditions for attached cultivation of *S. platensis* were firstly carried out from indoor experiments. Attached cultivation of *S. platensis* was successfully implemented in an outdoor bench-scale bioreactor thereafter. A high biomass productivity of 60 g m\(^{-2}\) d\(^{-1}\) for *S. platensis* was obtained therein. The nutrition of *S. platensis* from attached cultivation technique is same as that from conventional technique. The results demonstrate that the attached cultivation technique is a promising way to greatly improve production of high nutritive *S. platensis*.

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