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

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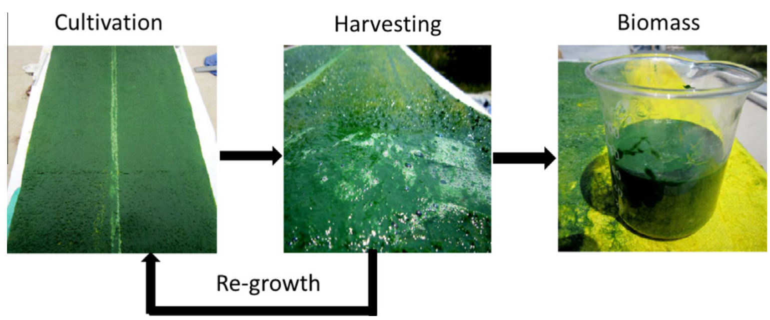
HIGHLIGHTS

- *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.

GRAPHICAL ABSTRACT



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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 μmol 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.

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

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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 difficultly 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 disinhibit biological limitation for culturing *Spirulina* sp. therein.

Recently, a novel technology for microalgal 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 *Aucutodesmus 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.

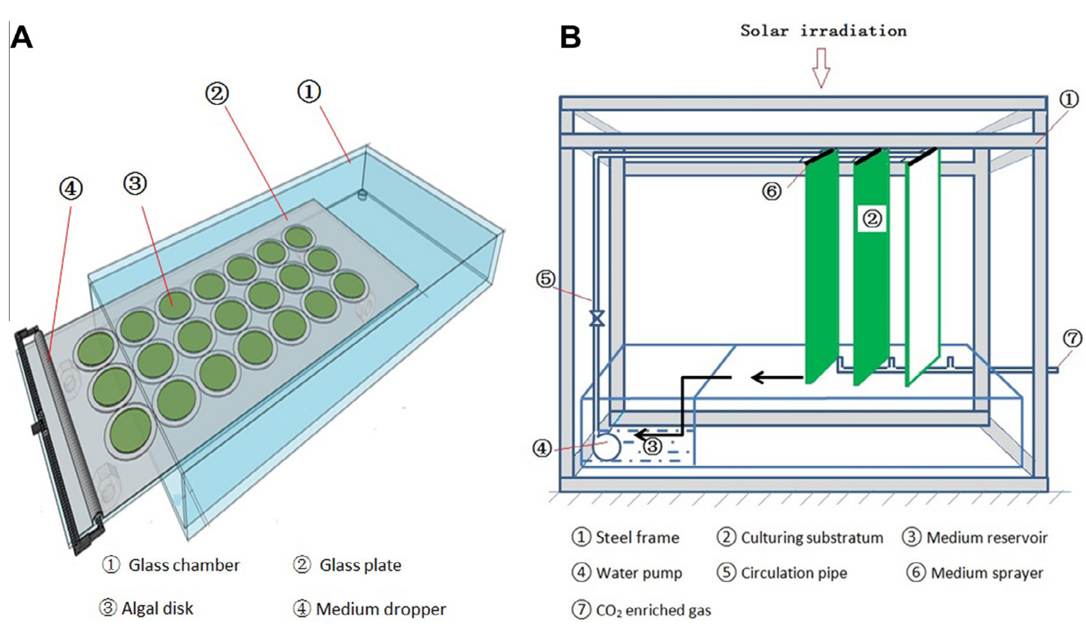

Fig. 1. Schematic diagram of the attached cultivation bioreactor for *S. platensis*. (A) Laboratory device. (B) Outdoor bench-scale bioreactor.