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# Differences between attached and suspended microalgal cells in ssPBR from the perspective of physiological properties

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ABSTRACT

Attached microalgae cultivation for the algae-based products is considered as a promising approach to simplify biomass recovery processes and reduce the cost. However, as an incipient research field, biomass accumulation is the mainly index for attached microalgal growth evaluation. To break through such limitations, physiological properties of attached microalgae (e.g. the oxygen evolving activity and the main organic composition of cells), which are important for microalgal growth evaluation but are still unclear in most studies, were studied using an attached microalgae culture system, i.e. suspended-solid phase photobioreactor (ssPBR) in this paper. As light, nutrients and other environmental conditions of attached microalgae were different from the suspended microalgae, physiological properties of attached microalgae also varied from the suspended ones. Besides the relatively lower biomass accumulation rate, attached microalgae also had a lower oxygen evolving activity (65% on average) comparing to suspended microalgae. The composition of microalgae changed towards accumulating more protein when suspended microalgae turned to attached status. The relative protein content of attached microalgae (50.1% ± 10.1%) was approximately 30% higher than the suspended algae (36.0% ± 16.1%) on average. The discovery of physiological properties of attached microalgae in this paper could help the production of high-protein microalgae-related products and explain some phenomenon during the production of microalgae-related products.

## 1. Introduction

Microalgae-based products have been applied in many aspects of our daily life. Some protein-rich microalgae such as *Spirulina* and some components of algae (e.g. chlorophyll and astaxanthin) have been used as value-added health food [1,2]. More importantly, microalgal biofuels have attracted worldwide attentions in the past few decades for their potential to replace petroleum and relieve global energy crisis [3,4]. Nevertheless, some blocks still exist in upstream microalgae biomass cultivation. Up till now, traditional means of microalgae cultivation is still suspended cultivation, where the small-sized microalgal cells are scattered in the culturing medium with overall solid content in the cultivation system less than 1%. This leads to energy and labor intensive processes (e.g. centrifugation and filtration) to recovery microalgal biomass from the liquid medium [5,6], which may contribute to up to 20–30% of the overall cost in microalgae-based bioenergy production procedures [7,8].

Aiming at simplifying biomass recovery operation and reducing the harvest cost, a number of attached microalgae cultivation systems have been proposed. Besides the suspended-solid phase photobioreactor (ssPBR) designed by our lab (Fig. 1) [9], other attached cultivation systems such as rotating algal biofilm reactor (RAB), parallel plate air lift (PPAL) reactor, revolving algal biofilm (RAB) have also been widely studied [10–12]. Within attached systems, microalgal cells grow crowdedly in the form of biofilms, rendering 20-fold more concentrated biomass than in traditional suspended systems [9]. Thereby, the cost of biomass harvest and dewatering per unit dry weight will be saved to a great extent owing to the greatly reduced culture volume.

As the microalgae cultivation is always combined with the wastewater treatment, biomass accumulation and nutrients removal/uptake are the most concerned indexes among previous studies. It was reported that the footprint growth rate of attached microalgae was fast and the removal rates of nitrogen and phosphorous were high [10,13]. Physiological properties, e.g. the oxygen revolving activity and organic composition, could indicate the growth status and the content of target products. However, the physiological properties of microalgal cells

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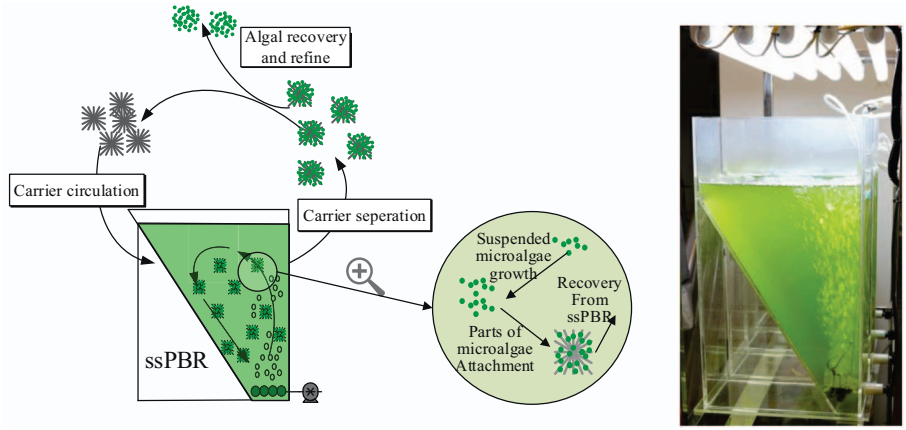


**Fig. 1.** The schematic diagram and photo of the ssPBR.

after transforming from liquid phase to solid phase have not been sufficiently studied. Considering the mass transfer of $CO_2$, $O_2$, nitrogen and phosphorous in the ambient environment of attached microalgae might be different from the suspended microalgae cultivation and the light transmission is blocked to the deep layer of microalgal biofilm [14], physiological properties of attached microalgae may be different from the suspended microalgae. In this paper, the physiological property of attached microalgae in solid phase in the ssPBR was studied and compared with the suspended microalgae.

## 2. Materials and Methods

### 2.1. Microalgae Species and Carriers

*Scenedesmus*. LX1 (Collection No. CGMCC 3036 in China General Microbiological Culture Collection Center) was cultured in BG11 medium for further use in this study [15]. Each carrier used in this study was made into pom-pom with the diameter of 2.5 cm by 20 strings of cotton, linen or mohair.

### 2.2. Experiment Setup

*Scenedesmus*. LX1 was cultured in 10 L suspended-solid phase photobioreactors (ssPBR) under different conditions for 17 batches. Just as the other open attached microalgae culture systems [10–12], the culture medium and carriers were not sterilized before putting into ssPBR. Because of the strong antibacterial capability of *Scenedesmus*. LX, the bacterial density in ssPBR was kept at $10^4$ cells/mL during the whole culture period according to previous study [16]. The size of ssPBR was 30 cm length × 15 cm width × 60 cm height. More details about ssPBR were described in our previous studies [9]. Throughout microalgae cultivation, light intensity was controlled at around 150 μmol photons·$m^{-2}$·$s^{-1}$ with light/dark ratio of 14 h/10 h, other culture conditions were shown in Table 1. When suspended microalgae density in liquid phase reached $10^6$ cells/mL, 30–60 solid carriers were put into each ssPBR for microalgae attachment with the packing density of 2–4 g/L. During the entire cultivation process, some of the suspended microalgae attached onto solid carriers, while the rest remained suspended in liquid phase. * It was proved that the dosage of carriers were not too large to inhibit the light transfer in liquid phase and the growth of suspended microalgae subsequently according to the precious study [9]. After a period of accumulation of attached microalgae biomass, the carriers were captured and pulled out of the culture system. At the same time, the attached microalgae were recovered from the liquid phase along with the carriers. Fresh carriers should be put into culture system, and the remained suspended microalgae in liquid phase could be attached and growth on the surface of carriers (Fig. 1). The concentrated microalgae solution could be obtained by squeezing the carriers or just get the algal-related products by hydrothermal liquefaction of the mixture of carrier and attached microalgae.

30 mL culture medium with suspended microalgae and 2 carriers with attached microalgae as the samples were taken out randomly for indexes detection each two days. The carriers were marked in case of retake as samples and put back into ssPBR to aviod the disturbance of taking samples.

### 2.3. Analytical Methods

Dry weight of suspended microalgae in liquid phase was determined by the difference in dry weight of filter paper before and after microalgae filtration [17]. The microalgae density was counted by hemocytometer under the optical microscope. The microalgae attached on the carriers were resuspended in deionized water by slightly stirring and finally formed 40 mL resuspension. Then, the resuspended solution was used for the further measurement. The dry weight and microalgae density of attached microalgae were measured indirectly by the resuspension and calculated based on the whole volume of ssPBR other than the volume of carriers for the easily comparison with suspended microalgae. All tests were carried out in triplicate ($n = 3$) and *t*-test was done to find out the consistency between different groups by the software SPSS (IBM SPSS Statistics 19).

Productivity of microalgae was calculated by the formula below:

$$P = \frac{X \times V}{T \times A}$$

P: productivity of microalgae, g·$m^{-2}$·$d^{-1}$;X: biomass concentration of microalgae, g·$L^{-1}$;V: the volume of ssPBR, L;T: the culture time of microalgae in ssPBR;A: the surface area of ssPBR, $m^2$.

Oxygen evolving activity indicates the photosynthesis activities of microalgae. The Oxygen evolving activity of both suspended and attached microalgae were tested by an oxygen electrode chamber DW2 (Hansatech, England). By detecting the electrical signal changes, the $O_2$ concentration in microalgae-containing solution could be monitored. This method is very sensitive and convenient to determine the photosynthesis rate of microalgae under a series of illumination intensities. Then the oxygen evolving activity curve was drawn to describe the relationship of oxygen evolving activity and illumination intensity. The X value, where the oxygen evolving activity curve intersects with X axis, is the light compensation point (i.e. $I_c$). The X value, where the oxygen evolving activity curve reaches to the peak, is the light saturation point (i.e. $I_s$). The light compensation point (i.e. $I_c$) and light saturation point (i.e. $I_s$), mean the minimum light intensity that microalgae start to accumulate organic matters and the minimum light intensity that the oxygen evolving activity of microalgae stops increasing, respectively.