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Selection of microalgae with potential for cultivation in surfactant-stabilized foam

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ABSTRACT

Recently, microalgal cultivation in liquid foams has been developed. Compared to the traditional systems, this concept is expected to offer advantages such as increased mass transfer and reduced biomass harvesting costs and water consumption. However, there is limited information, thus far, on the microalgal performance in foam-bed photobioreactors. Therefore, this study was aimed at comparing the foaming properties of six algal strains to identify the criteria that could be broadly employed for assessing the microalgal potential for cultivation in a foam-bed photobioreactor. The microalgal strains investigated were selected based on their different nature and potential uses. All the microalgal strains could not naturally produce stable foam, thus necessitating the use of a surfactant. To investigate the differences in the foaming properties of the selected microalgae, the natural surfactant, bovine serum albumin, was employed. Factors such as culture age, algal hydrophobicity, and biomass concentration differently influenced the key foaming properties (foamability, microalgal partitioning, and foam stability) depending on the microalgal strain. In conclusion, the assessment of the foaming properties of microalgal strains together with their inherent growth characteristics revealed large differences in the potentiality of microalgae to be cultivated in a foam-bed photobioreactor. In particular, among the microalgal strains tested, the commercial strains *Chlorella sorokiniana*, *Nannochloropsis gaditana*, and *Scenedesmus obliquus* showed the highest potentiality for cultivation in foam.

Overall, the following criteria could be broadly applied to select suitable microalgae for cultivation in a foam-bed photobioreactor: high or moderate foamability of the microalga-surfactant suspension, and microalgal partitioning, stability of the foam formed, and robustness and fast growth of the strains.

1. Introduction

To make microalgae-based production processes economically feasible, a variety of cultivation systems have been studied, including traditional raceway ponds and closed photobioreactors, in which algal cells are suspended in the liquid broth; and biofilm-based photobioreactors, where algal cells grow attached to a solid surface [1]. To produce algal products on a large scale, limitations such as high operational costs, including high water consumption and energy requirements, should be overcome [2]. Microalgal cultivation in surfactant-stabilized foams has been recently reported as a promising alternative to the conventional microalgal cultivation systems [3]. It presents several theoretical advantages that might overcome some of the main current limitations of microalgal bio-products production. For example, high water consumption in the algal cultivation process has been recognized as an important parameter; reduction in the water consumption results in decreased harvesting costs by avoiding the first step of biomass pre-concentration, commonly referred to as primary or bulk harvesting. Microalgal cultivation in foam could be directly connected to the secondary dewatering or thickening step of microalgal biomass harvesting achieved usually by energy-intensive methods as centrifugation or filtration. Altogether, it could be beneficial to cultivate microalgae in foam as it might reduce water and energy consumption. Microalgal cultivation in the foam can also improve mass transfer efficiency. Foam is basically a complex network of liquid channels surrounding gas bubbles. Due to the increased interfacial surface area, the transfer of CO_2/O_2 between the liquid (containing microalgal cells) and gas phases (initially enriched with CO_2) is

Abbreviations: BSA, Bovine serum albumin

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enhanced. Altogether, higher biomass concentrations can be achieved in the foam cultivation systems [3], which might result in higher productivities.

In a foam-bed photobioreactor, a variety of factors may impact the efficiency of the microalgal biomass cultivation process, which can be classified into the following two main groups: (i) factors related to the foam system configuration and operational parameters, and (ii) the characteristics of the microalgal strain used, and if needed, the properties of the surfactant employed and its interactions with the algal cells.

Regarding the foam-bed photobioreactor configuration, foam production and foam break-up systems have been recently found to be essential for an optimal reactor performance to ensure a sufficient CO_2 supply and to avoid oxygen build-up to inhibiting levels [3]. However, very little information is available in the literature on the effect of operational parameters in a foam-bed photobioreactor as extensive research has not been conducted on microalgal cultivation in liquid foam yet.

Microalgae have the ability to produce natural surfactants that might facilitate their cultivation in foam-bed photobioreactors; however, their production and effectiveness vary considerably among species [4]. The natural foamability of *Chlorella vulgaris* and *Chaetoceros* sp. has been already reported; such capability was used to harvest biomass on a small and large scale via foam flotation [5,6]. If natural foam production ability of a cultivated microalga is insufficient, a suitable combination of microalgal strains and surfactants may be required for successful algal cultivation in foam. The usage of surfactants has been so far reported in foam columns or flotation tanks as a part of the microalgal harvesting process [7,8] but only bovine serum albumin (BSA) has been reported to be useful for short-time cultivation of one microalgal species in foam [3]. Therefore, it is essential to assess the need for surfactant addition in algal biomass cultivation for each specific microalgal species.

Surfactants contain hydrophilic and hydrophobic groups, which interact with the generally negatively charged microalgal surface and gas bubbles, respectively. For a microalgal cell to migrate from the liquid suspension to the foam phase, it has to collide with a bubble and attach to it. However, the collision probability strongly depends on the particle size; and the algal hydrophobicity is a crucial factor for the attachment of the cells to the bubbles [9]. In general, surfactants can make the microalgal surface more hydrophobic, favoring the attachment of the cells to the bubbles, thus determining the flotation performance [7,8,10,11].

Another factor affecting the algal flotation efficiency is the culture age. The charge density of algal cell surfaces, which can influence their interactions with the surfactant, varies significantly throughout the growth phases [7,12]. Moreover, biomass composition tremendously changes during the cultivation period. For example, the excretion of molecules with foaming properties, such as proteins or exopolysaccharides, may be enhanced during the stationary phase of growth [13–15]. Proteins are the most frequent surface active biomolecules. In addition to proteins, polysaccharides can improve foam stability by increasing the bulk viscosity, which decreases the rate of disproportion and drainage [16].

Ionic strength is another key parameter that influences foam flotation processes. High salinity prevents bubble coalescence [17], which possibly leads to superior foam stability. Conversely, several studies on freshwater microalgae harvesting have indicated that the inner salt concentration negatively affects algal flotation performance [7,10]. This suggests that the potential of freshwater microalgal strains for cultivation in foam might differ from that of marine strains.

Cell concentration may also play a role in the culture foaming properties. For traditional photobioreactors, the viscosity increment due to biomass concentration increase is considered insignificant in terms of the fluid mechanics [18]. However, in a foam-bed photobioreactor, a higher biomass concentration can be achieved and the excreted surfactant-like molecules could be accumulated further. Hence, the viscosity increase may play a major role in microalgal cultivation in foam. As mentioned above, the higher the viscosity, the higher the foam stability [16]. On the contrary, an increased viscosity lowers the diffusion of surface-active compounds to the air/water interface, affecting the foamability [19]. Moreover, algal cells can be considered as particles that possibly function as foam stabilizers or destabilizers according to their characteristics.

Although most of the principles that may affect the foaming properties of algal suspensions are partially understood, the specific characteristics of different algal strains are expected to influence algal-enriched foam formation and its characteristics. Therefore, this study was aimed at defining the criteria to clearly assess the suitability of a given microalgal species to be cultivated in foam. Hence, the following parameters were proposed to be considered: microalgal foamability (natural and mediated by a surfactant), algal partitioning, foam stability, and microalgal growth characteristics. Several microalgal strains (particularly different and most of them of commercial importance) were used to compare the identified parameters. Moreover, microalgal cell surface hydrophobicity was studied along the different growth phases and was correlated to the microalgal partitioning. The effect of medium salinity, culture age and biomass concentration on foaming properties was also investigated because they could influence the formation of algal-enriched, stable foam.

2. Materials and methods

2.1. Cultivation of algae

Scenedesmus obliquus and *Nannochloropsis gaditana* were kindly provided by Fitoplancton Marino S.L. (Cádiz, Spain). *Botryococcus braunii* var. Showa was obtained from University of California, Berkeley, Herbarium. *B. braunii* CCALA–778 was obtained from Culture Collection of Autotrophic Organisms, Trebon, the Czech Republic. *Chlorella sorokiniana* CCAP 211/8K and *Neochloris oleoabundans* UTEX 1185 were obtained from UTEX culture collection. All the microalgal strains were cultivated in 1L-Roux flasks at 25 °C, continuously illuminated at 110 μmol photons $m^{-2} s^{-1}$ with fluorescent lamps, and bubbled with air containing 2.5% (v/v) CO_2 as the unique carbon source. The following culture media were employed: (1) modified BG11 [20] for *S. obliquus*; (2) modified F2 medium [21] without vitamins and containing 0.2 g L^{-1} of $Na_2HPO_4{\cdot}2\ H_2O$ and 0.75 g L^{-1} of $NaNO_3$ for *N. gaditana* and *N. oleoabundans;* (3) modified M–8 medium [22] for *C. sorokiniana* and (4) modified Chu medium [23] without citric acid and vitamins for both *B. braunii* strains.

2.2. Foamability tests

Natural foamability was assessed for all the six algal strains in their corresponding culture media using culture samples from linear and stationary phase cultures. Moreover, BSA-mediated foamability of different culture media without microalgae was tested at different BSA concentrations, ranging from 0.02 to 0.1 g L^{-1}, to evaluate the influence of culture media salinity and to identify the optimal BSA concentration for the assays with algal samples supplemented with BSA. Furthermore, the effect of biomass concentration on foamability was evaluated in BSA-mediated foamability assays with the algal samples. To study the effect of biomass concentration, three-fold concentrated biomass samples were used and centrifuged at 3000 × *g* for 5 min (Eppendorf 5702, Germany). Considering the optimal pH for growth of the different strains was proximate to neutrality, and pH might influence the foaming properties, the pH of all the samples was adjusted to 7.0 before performing the tests to avoid its influence.

The foam production system consisted of a one-liter glass graduated cylinder with 2 air stone diffusers placed at the bottom. The corresponding suspension (200 mL; algal broth, culture media supplemented