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Using resonant ultrasound field-incorporated dynamic photobioreactor system to enhance medium replacement process for concentrated microalgae cultivation in continuous mode

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ABSTRACT

Holding cells in the cultivation broth for subsequent medium replacement is often considered as one of the most difficult procedures during perfusion cell culture. Although a number of harvest approaches have been reported in the past decades, their drawbacks such as high cost, increased contamination, and/or labor consuming remain obstacles for practical application. To overcome these challenges, a novel resonant ultrasound field-incorporated dynamic photobioreactor system (RUF-DPBS) was developed in which the cell retention and medium replacement are carried out by acoustic radiation forces and gravity, respectively. Based on the collection efficiency of microalgae, the RUF operation was optimized by 1 MHz and output intensity of 8 W/cm^2 with circulating velocity of 2 mL/min whereby 93% of *Nannochloropsis oculata* in 30 mL can be collected within 2-h operation. Moreover, the cells cultured with RUF-DPBS in which the medium was changed every three days exhibited increased volumetric productivity that the yields of biomass, total lipid, and eicosapentaenoic acid of the *N. oculata* after 12-days cultivation significantly enhanced 2.6, 2.1, and 2.5 folds ($P < 0.05$ for each), respectively, as compared to the group without medium replacement. In summary, the semiautomatic RUF-DPBS offers a non-fouling, labor-efficient, and cost-effective means for high-density microalgae culture in continuous mode.

1. Introduction

Microalgae have long been recognized as promising biosources for live feed (Ju et al., 2009; Patil et al., 2007), pharmaceuticals (Kang et al., 2011; Mimouni et al., 2012), and alternative fuel (Chisti, 2007; Gouveia and Oliveira, 2009) since they can provide abundance of essential chemicals such as vitamins, polysaccharides, and fatty acids. To minimize the potential contamination and provide quality cell products, microalgae culture in closed setting (i.e., photobioreactor) is generally considered as a favorable approach since all the microalgae growth-related factors, such as light, pH, temperature, and microorganism can be strictly controlled. However, decrease of nutrient and/or accumulation of cellular metabolites in the culture medium including excretions and dissolved oxygen generated from the microalgal photosynthesis may severely inhibit cell growth (Weissman et al., 1988; Molina et al., 2001). Therefore, renewing the growth medium to maintain the balance and sufficiency of micro- and macronutrients in the living environment is crucial for massive microalgae cultivation.

Efficacy of cell collection plays the central role in the process of medium replacement since all the cells should be retained in the culture broth after the medium change. However, microalga is technically challenging to harvest due to its low density (0.3–5 g/L) and/or small size (2–20 μm). So far quite a few harvesting techniques including flotation,

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centrifugation, sedimentation, flocculation, filtration, as well as combinations of above techniques have been used in the field of microalgae culture (Gultom and Hu, 2013) and each method is selected based on the characteristics of the microalgae collected (Olaizola, 2003). However, since microalgae may contact with particular chemicals and/or filtration devices while they are manipulated by aforementioned harvest approach including flotation, flocculation, and filtration, an additional separation procedure is usually required for the collected cells and that may increase the risk of contamination, complexity of the process, and labor consuming. Furthermore, most of the methods described above are expensive due to consumption of intensive energy and/or costs of adjunctive materials such as membranes or flocculants. Therefore, developing an effective cell harvest approach with merits of cost- and labor-efficiency is certainly needed for concentrated microalgae culture.

Ultrasound has long been identified as a feasible tool for particle separation and that is so-called acoustic filtration (Shirgaonkar et al., 2004). The mechanism is that upon exposure to non-cavitation, MHz-frequency-ranged resonant ultrasound field (RUF), micrometer-sized particles (e.g., microalgae) can quickly move to the acoustic pressure nodes and aggregate due to effects of the primary (F_1) and secondary (i.e., Bjerknes force; F_2) acoustic radiation forces, leading to particle separation from the media as reported in the previous studies (Courtney et al., 2010; Hawkes et al., 1998).

$$F_1 = -\frac{\pi P_0{}^2 V \beta_0}{2\lambda} \times \left(\frac{5\rho_p - 2\rho_0}{2\rho_p + \rho_0} - \frac{\beta_p}{\beta_0}\right) \times \sin\left(\frac{4\pi z}{\lambda}\right) \quad (1)$$

$$F_2 = 4\pi R_0{}^6 \left[\frac{(\rho_p - \rho_0)^2 \left(3\cos^2\theta - 1\right)}{6\rho_0 d^4} v^2 - \frac{\omega^2 \rho_0 (\beta_p - \beta_0)^2}{9d^2} P^2\right] \quad (2)$$

where P_0 is the peak pressure amplitude of the ultrasonic standing wave; λ is sound wavelength; V is the volume of the particles; z represents the propagating distance of the ultrasonic wave which is perpendicular to the pressure nodal planes; ρ_p and β_p are the density and compressibility, respectively, of the particles and ρ_0 and β_0 denote the density and compressibility, respectively, of the surrounding bulk phase; R_0 is the radius of the particle; d is the distance between particles; θ is the angle between the centerline of the particle and the propagating direction of the sound wave; ω is the angular velocity; v denotes the particle velocity; and p is the amplitude of acoustic pressure at the pressure nodes. As compared to the conventional methodologies of cell harvest, the RUF-mediated approach is featured with non-fouling and no chemicals involved, and currently an ultrasound-mediated technique, which is so-called BioSep (Applikon) has been built for cell retention. However, cells collected through the BioSep may be permanently lost if they cannot be captured by ultrasound any time during the harvest process according to the design of the apparatus and that is unfavorable for long operation.

In this study, a newly designed RUF-associated cell culture device which is so-called RUF-incorporated dynamic photobioreactor system (RUF-DPBS) is developed for high-density microalgae cultivation. We first optimized the RUF operation parameters and then evaluated the efficacy of RUF-DPBS with defined settings on the microalgae culture based on the productivities of cellular biomass and lipids as compared with that obtained from the cells with and without medium change through centrifugation. In terms of the use of microalgae, *Nannochloropsis oculata* (*N. oculata*) was selected as the model microorganism in this study because of its high content (>50 wt%) of eicosapentaenoic acid (EPA; C20:5n-3) (Guschina and Harwood, 2006) which is highly valuable in healthcare application (Song and Zhao, 2007; Sublette et al., 2011; Watanabe et al., 2011).

Table 1 – Constituents of Walne's medium.

	Chemical	Concentration
Seawater	–	–
Trace metals	$ZnCl_2$	15.4 nM
	$CoCl_2{\cdot}6H2O$	8.4 nM
	$(NH_4)_6MO_7O_{24}{\cdot}4H_2O$	0.73 nM
	$CuSO_4{\cdot}5H_2O$	8 nM
Vitamins	Cyanocobalamin	0.74 pM
	Thiamine	3.3 pM
	Biotin	0.08 pM
Nutrients	$FeCl_3{\cdot}6H_2O$	0.48 μM
	$MnCl_2{\cdot}4H_2O$	0.18 μM
	H_3BO_3	54 μM
	Na_2EDTA	12 μM
	$NaH_2PO_4{\cdot}2H_2O$	12.8 μM

2. Materials and methods

2.1. *Microalgae culture*

The *N. oculata* was purchased from Taiwan Fisheries Research Institute (Tung-Kang, Taiwan R.O.C.) and was regularly cultured with Walne's medium consisting of autoclaved seawater and filter-sterilized trace metals, vitamins, and nutrients as listed in Table 1. Maintenance and propagation of *N. oculata* were performed in an aerated 1L-Erlenmeyer flask in which the cells were continuously illuminated by fluorescent lamps with intensity of 80 μE/m^2/s under 25 ± 2 °C and 100-rpm stirring. A day before the experiment, defined amount of cells were transferred to designated flasks for the experimental use, and the culture condition in the duration of the experiment was identical to the settings performed in the maintenance mode. In this study, the concentration of *N. oculata* was measured by UV–vis spectrometry at 691 nm based on Beer–Lambert's law (Fig. 1).

2.2. *Setup of RUF-DPBS*

The RUF-DPBS is consisting of (1) a culture chamber, (2) an acoustic chamber, (3) two medium reservoirs, and (4) three ultrasonic devices as schemed in Fig. 2. In this study, the aerated three-neck spinner flask is employed as the culture chamber (i.e., photobioreactor). The acoustic chamber is a closed and transparent polystyrene cylinder with 13-mm inner diameter, 18-mm height, and 0.5-mm thickness as shown in Fig. 2 (inset photograph II). The transducer (Olympus, Tokyo, Japan) is made of a lead zirconate titanate (PZT) disc with 13-mm diameter and 1-mm thickness, and is mounted on the bottom of the acoustic chamber as illustrated in Fig. 2. The 1-MHz oscillation signal with 50% duty cycle and defined output intensity is generated from a function generator and is transferred through an amplifier and an impedance matching transformer before reaching the transducer. The containers for the fresh and used culture medium are located on the top and beneath of the three-neck spinner flask, respectively, by which the delivery of waste and/or fresh medium is driven by gravity. Prior to the medium replacement, the cells-containing medium is continuously injected into the acoustic chamber for conduction of RUF-mediated cell harvest. After the cell collection is accomplished, the RUF is turned off and followed by draining used medium and filling fresh medium in subsequence towards the three-neck spinner flask. All the collected microalgae in the acoustic chamber are then flushed with the