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Reduction of oxygen inhibition effect for microalgal growth using fluoroalkylated methoxy polyethylene glycol-stabilized perfluorocarbon nano-oxygen carriers

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ABSTRACT

Oxygen accumulation in the medium has long been considered as one of the major limitations for closed and mass cultivation of microalgae. Although the dissolved oxygen may be removed via air sparging-based photobioreactor technology, disadvantages such as high cost, increased contamination, and severe cell damage caused by hydrodynamic stress remain obstacles for microalgal culture. To overcome the effect of oxygen inhibition on microalgal growth, a strategy of using perfluorocarbon nanoemulsions (PNEs) as oxygen scavengers was explored. Our results showed that the PNEs prepared by fluoroalkylated methoxy polyethylene glycol (MPEG; MW = 2000) and perfluorooctyl bromide (PFOB) with a volume ratio of 9:1 (PNEs-2000/PFOB (9:1)) exhibited the highest stability and capacity of deoxygenation under the photobioreactor operation. With the aid of 1% (v/v) PNEs-2000/PFOB (9:1), the *Nannochloropsis oculata* successfully multiplied in the hyperoxygenated environment where the growth rate of 0.467 day^{-1} was comparable to the normal breeding with neither PNEs nor oxygen injection (0.498 day^{-1}), and the biomass production was five-fold higher than the O_2-treated group without PNEs after seven-days cultivation. In summary, the developed PNEs-2000/PFOB (9:1) enabled to efficiently remove the dissolved oxygen in the photobioreactor that provided a feasible means for mass culture of microalgae in closed setting.

1. Introduction

Microalgae have been widely recognized as promising biosources for live feed [1,2], pharmaceuticals [3,4], and fuel [5,6] due to the abundance of essential chemicals that they produce, such as carotenoids, vitamins, polysaccharides, and polyunsaturated fatty acids. To provide a high quality of microalgal bioproducts and/or the ability to freely handle microalgae species that are prone to contamination, cell culture in the closed setting (i.e., photobioreactor) is generally considered to be a favorable approach because most of the parameters relative to microalgal growth, such as light, pH, salinity, and the prevention of microorganism growth, can be strictly controlled. However, oxygen generated from microalgal photosynthesis will accumulate and quickly reach a supersaturated concentration in the closed culture system that could severely impact cell growth.

Dissolved oxygen (DO) built up in the photobioreactor is one of the most detrimental factors for enclosed microalgal cultivation. Although this issue is not commonly addressed in small-scale cultivation because the used gas transfer apparatuses enable to provide sufficient air/CO_2 supply to promptly remove the DO out of the culture environment, it does seriously hinder the microalgal growth in many large-scale/industrial culture systems [7,8]. It has been demonstrated that the accumulated DO may cause damage to the photosynthetic apparatus, membrane or components of microalgal cells, thereby leading to diminished cell growth rate and/or collapse of cultivation [9–11]. In fact, photosynthesis in many microalgae species is tremendously inhibited when the DO concentration is over the level at air saturation (i.e., 0.225 mM at 20 °C) [12,13], even though the concentration of CO_2 is maintained at elevated levels [13]. The DO may further become toxic to most microalgae when its concentration is higher than 1.1 mM [14,15]. Conventionally the

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Nomenclature

A	absorbance (O.D)
C	cell concentration (cells/mL)
DO	dissolved oxygen
I	intensity of light (μmol photons/m^2/s)
K_La^{ac}	the mass accumulation rate coefficient (h^{-1})
PFC	perfluorocarbon
PFOB	perfluorooctyl bromide
PNEs	perfluorocarbon nanoemulsions
PNEs-S/PFOB	(x:y) perfluorocarbon nanoemulsions prepared by surfactant S and PFOB with volume ratio of water:perfluorocarbon = x:y
R_F-MPEG	fluoroalkylated methoxy polyethylene glycol
t	time (day)
V	volume (mL)
μ	specific growth rate (day^{-1})

DO is eliminated by using a degasser (i.e., gas exchange unit) [16,17] or by vigorously sparging air into the medium as is performed in the airlift/bubble-column photobioreactors [18,19]. However, several concerning drawbacks associated with these methods include a high cost, an increase in contamination, and an insufficient deoxygenation due to a low oxygen transfer efficiency [20]. Furthermore, use of aerated devices may cause cell rupture due to the hydrodynamic stress generated from the entrance of the high-speed gas, the break-up of the bubbles, and/or bubble formation at the sparger [21–25]. Therefore, developing an efficient and moderate approach of DO removal for the culture of valuable microalgae in a large-scale, closed setting is a pivotal issue that needs to be addressed.

Perfluorocarbon (PFC), a fluorine-substituted derivative of hydrocarbons, can dissolve large respiratory and other non-polar gases as compared to water [26], exhibiting a great potential for use as an oxygen transporter. PFCs have been widely used in medical applications to deliver oxygen to mammalian tissues and/or remove metabolically waste as so-called blood substitutes [27,28]. Additionally, using PFCs as oxygen carriers to enhance the efficiency of microbial, plant or animal cell growth has also been extensively reported in the past two decades [29–31]. Previously, we have demonstrated the feasibility of using PFC liquids in two-phase PFC/medium forms to enhance the growth of microalgae in a closed and hyperoxygenated spinner flask [32]. However, since PFCs are virtually insoluble with aqueous solutions, use of PFCs in the manner of emulsion will be more appropriate for serving as the oxygen carriers because they can disperse in the culture medium and provide a higher contact area for oxygen adsorption. To the best of our knowledge, the application of PFC emulsions for microalgal culture is scarce and only recently reported by Sawdon and Peng [33], who applied PFC emulsions prepared by FC-77 and Pluronic F-68 surfactants to facilitate cultivation of microalgae *Chlorella vulgaris* in a tubular photobioreactor setting. However, several critical issues, such as the capacity of oxygen transfer and the stability of the PFC emulsions while operated in the dynamic photobioreactor were not referred in the literature yet, indicating that more efforts are certainly needed to recognize this methodology.

In this study, we aimed to explore the potential of using PFC nanoemulsions (PNEs) as oxygen carriers to reduce the effect of oxygen inhibition for closed microalgal cultivation. Because the PNEs typically move along with the culture medium, which is usually treated by agitation and/or circulation in the photobioreactor, the stability of the emulsion particles under the dynamic operation will play a key role in their applicability. To strengthen their robustness, the PNEs used in this study were stabilized by fluoroalkylated methoxy polyethylene glycol (R_F-MPEG), a type of fluorosurfactant where strong interactions between the PFC and the fluorophilic moieties of the surfactant were anticipated to enhance the integrity of the emulsions and offer a high degree of stability to the PNEs accordingly. In terms of the cellular experiment, *Nannochloropsis oculata* (*N. oculata*), a marine unicellular microalga with high economic value due to its high content (>50%, w/w) of eicosapentaenoic acid (EPA, C20:5n3) [34] was used as the model cellular organism in this study. Furthermore, to demonstrate the positive effectiveness of R_F-MPEG on the stability of PNEs, the emulsions prepared by Pluronic F-68, one of the most commonly used surfactants for PFC emulsification [35], was employed as a control throughout the study. The feasibility of using R_F-MPEG-stabilized PNEs as oxygen scavengers to reduce the effect of oxygen inhibition for *N. oculata* growth was comprehensively investigated by using a closed stirred photobioreactor as the model setting.

Table 1
Composition of Walne's medium.

	Constituent	Concentration
Seawater	–	–
Trace metal solution	$ZnCl_2$	15.4 nM
	$CoCl_2 \cdot 6H_2O$	8.4 nM
	$(NH_4)_6MO_7O_{24} \cdot 4H_2O$	0.73 nM
	$CuSO_4 \cdot 5H_2O$	8 nM
Vitamin solution	Cyanocobalamin	0.74 pM
	Thiamine	3.3 pM
	Biotin	0.08 pM
Nutrient solution	$FeCl_3 \cdot 6H_2O$	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. Microalgal culture

The *N. oculata* was purchased from the Taiwan Fisheries Research Institute (Tung-Kang, Taiwan R.O.C.) and maintained in Walne's medium (pH 8) consisting of autoclaved seawater with filter-sterilized trace metals, vitamins, and nutrients as listed in Table 1. Maintenance and propagation of the cultures were performed in a 1 L-Erlenmeyer flask with magnetic stirring at 100 rpm in which filtered air was continuously supplied. The cells were constantly illuminated by daylight fluorescent lamps with a light intensity of 80 μmol photons/m^2/s at a room temperature of 25 ± 2 °C throughout the study.

2.2. Synthesis and characterization of R_F-MPEG surfactants

The R_F-MPEG surfactants utilized in this study were fabricated by the amination of MPEG-2000 and MPEG-5000 (MW = 2000 and 5000, respectively, Sigma, St. Louis, MO) and followed by a nucleophilic substitution reaction of amino-MPEG as previously reported [36]. In brief, 10 g of MPEG was first dissolved in 150 mL of toluene containing 500 μL of pyridine in a 250 mL, three-necked, round bottom flask. Pre-distilled thionyl chloride (Sigma) was then added dropwise into the three-necked flask in which the molar ratio of MPEG to thionyl chloride was 2:3, and the mixture was heated at 65 °C for 4 h. Following the filtration of pyridine hydrochloride and evaporation of the solvent in a vacuum at 85 °C, the residue was dissolved in 100 mL of dichloromethane and dried with anhydrous potassium carbonate. The residue was then re-suspended in 150 mL of ethanol with an excess of ammonia and heated at 70 °C for 24 h.