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Influence of growth phase and nutrient source on fatty acid composition of *Isochrysis galbana* CCMP 1324 in a batch photoreactor

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Abstract

The marine microalgal strain *Isochrysis galbana* CCMP 1324 was cultured in Walne medium to examine the changes in lipid bodies and fatty acid composition, with special emphasis on different iron, nitrogen, and phosphorus sources associated changes in the growth kinetic parameters and fatty acid composition. All the experiments were performed at 20 °C, with the culture medium at pH 9.0, a specific rate of air supply of 1.0 $vv^{-1}\,m^{-1}$ and a continuous illumination of 68 $\mu Em^{-2}\,s^{-1}$. Lipid bodies in *I. galbana* CCMP 1324 were observed at different growth phase in Walne medium by light and transmission electron microscopy. Lipid granules from 0.4 to 2.0 μm were observed from exponential growth stage to stationary stage. The *I. galbana* CCMP 1324 had the highest contents of saturated and monounsaturated fatty acids (SFA + MUFA) as well as polyunsaturated fatty acids (PUFA) in the early stationary phase. The n-3/n-6 value reached a maximal value of 4.9 in the late stationary phase. The Walne medium could be a good iron, nitrogen and phosphorus sources to culture *I. galbana* to maximize PUFA production and harvesting the biomass at stationary phase may enable better yields in lipid and PUFA composition. The ratio of SFA + MUFA to PUFA was highest in NH_4NO_3 modified Walne culture and minimal in Walne culture. The n-3 PUFA was predominant in Walne culture. A compromise between the docosahexaenoic acid (DHA) production and growth kinetics can be achieved by using NH_4NO_3 as N-source in modified Walne medium, which provides the greatest value of DHA production (15.6% of total fatty acids) with the highest maximum specific growth rate (2.34 per day) even though the microalgal cell productivity (43.1 $mg\,l^{-1}$ per day) is not the highly attained. The approaches presented in this study could be employed for the design of pilot or full-scale photoreactor for PUFA commercial productions by *I. galbana* CCMP 1324.

Keywords: *Isochrysis galbana*; Growth phase; Nutrient source; Polyunsaturated fatty acids; Photoreactor

1. Introduction

Marine microalgae are widely used in aquaculture as a preferred natural feed for marine animals, in particular larval, juvenile mollusks, crustaceans and fish species [1]. The nutrition value of microalgae is related to their proximate biochemical composition, especially the lipid class and fatty acid compositions [2,3]. Microalgae have received increasing interest because of their ability to produce the polyunsaturated fatty acids (PUFA). To date, PUFA are mainly obtained from fish oil, which is relatively cheap raw material, but may not be an ideal source of n-3 PUFA due to their scarcity and odor, as well as geographical and seasonal variations in quality [4]. Hence, microalgae can be considered as a good alternative source of n-3 PUFA. In particular, the docosahexaenoic acid (DHA), one of n-3 PUFA, play an important role in human health, prevention of heart and circulatory disease and brain development in infancy [5–7]. Among marine microalgae, the golden-brown flagellate *Isochrysis galbana* (*I. galbana*) has good nutritive characteristics since it is rich in PUFA and used commonly as feed in bivalve hatcheries [8,9]. In addition, *I. galbana* also grows well in mass cultures, either indoors or outdoors [10].

I. galbana can significantly change with variations in culture conditions such as temperature, air flow rate, pH and light intensity. The chemical composition of *I. galbana* is also known to vary during their growth phase, particularly with respect to their

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lipid components [11,12]. At present, there are various culture media to provide the nutrients for the growth of *I. galbana*. In the production of *I. galbana* with certain desired characteristics, the composition of the culture medium is a fundamental factor. The relationship between the nutrients used and the composition of the microalgal cells is known [7]. In general, the major nutrients (nitrate, phosphate and silicate) affect the growth rate and the maximum level the production of *I. galbana*, but this growth is also limited by the availability of iron [13]. Modifications in culture medium such as nitrogen, phosphorus and iron affect the growth rate of microalgae, cellular composition, fatty acid profile of the lipid fraction, as well as the final yield of the *I. galbana* [9]. However, the influence of growth phase and nutrient source on fatty acid composition has not been clearly investigated. Moreover, previous reports have lacked detailed investigation of lipid granules for *I. galbana* in every growth phase using light and transmission electron microscopies, although Liu and Lin [4] observed lipid bodies formation and accumulation in *I. galbana*, especially as cells entered the stationary phase. Thus, manipulation of culture conditions and harvesting at a specific growth phase and nutrient source may change the lipid formation and PUFA composition of microalgal culture to be tailored for a specific industrial purpose.

In this present work, we studied the optimal cultivation condition for *I. galbana* CCMP 1324 in a batch photoreactor, observing the variation of lipid bodies during four growth phases, comparing nutrient source and analyzing the influence of each on growth kinetics as well as on fatty acid composition and DHA content of the cell produced.

2. Materials and methods

2.1. Microalgae

The experiments were performed with the marine microalgae, *I. galbana* CCMP 1324, obtained from the Provosoli-Guillard National Center for Culture of Marine Phytoplankton, USA. The microalgal cells were harvested at four different points in the growth curve: during the exponential growth phase, linear growth phase, early stationary phase and the late stationary phase [14]. Samples are always collected at the same moment in the different phase for comparison of the experimental results. Microalgal cells were harvested on Day 1, 4.75, 7.5 and 10 by centrifugation using a Hitachi Model SCR20BA superspeed refrigerated centrifuge. Samples were lyophilized and stored at −80 °C prior to chemical analysis [15].

2.2. Experimental apparatus

The culture apparatus of *I. galbana* CCMP 1324 used in this study is shown in Fig. 1. Intensity of light can be changed by adjusting the distance between the light source and illuminated surface. The culture medium in marine water was autoclaved in an autoclavable polycarbonate container at 120 °C for 45 min. Then the culture medium was transferred through autoclaved tygon tubing into feed flask [16]. Two-litre Roux flask containing 1600 ml culture medium with inoculum was then placed in an orbital shaker incubator (5304R, Firstek Scientific, Taiwan). To avoid flocculation of microalgae, the culture medium was agitated gently by bubbling air and the flow meter was used to control the air flow rate. Air was humidified before entering the culture vessels by incorporating a humidifier [17]. The air supply was sterilized by filtration through cellulose-nitrate membranes with a pore size of 0.2 μm. The apparatus designed here was used to find the optimal culture conditions and determine the growth kinetics of the microalgal cells.

2.3. Culture media

The Walne culture medium used in cultivation for *I. galbana* CCMP 1324 included nutrient solution, vitamin solution and trace metal solution. The nutrient solution contained (per litre): urea, 60.0 mg; $NaH_2PO_4{\cdot}2H_2O$, 20.0 mg; $NaNO_3$, 0.1 g; Na_2EDTA, 45.0 mg; H_3BO_3, 33.6 mg; $MnCl_2{\cdot}4H_2O$, 0.36 mg; $FeCl_3{\cdot}6H_2O$, 1.30 mg; $NaSiC_3$, 6.6 mg. The vitamin solution contained (per litre): vitamin B_{12}, 0.001 mg; vitamin B_1, 0.02 mg. The trace metal solution contained (per litre): $ZnSO_4{\cdot}7H_2O$, 4.4 g; $CoCl_2{\cdot}6H_2O$, 2 g; $(NH_4)_6\,Mo_7O_{24}{\cdot}4H_2O$, 0.9 g; $CuSO_4{\cdot}5H_2O$, 2 g. In order to compare the influence of EDTA-Fe(III) and malic acid-Fe(III) on the growth of *I. galbana* CCMP 1324, the malic acid was used to substitute the Na_2EDTA to form chelating compound of iron in culture

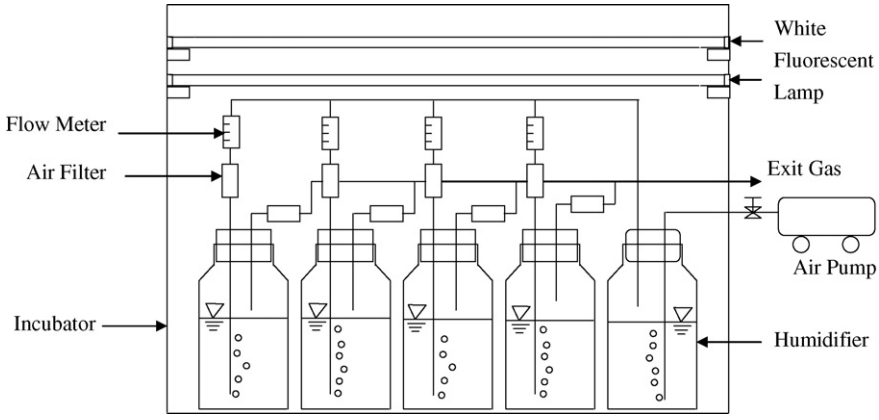

Fig. 1. Schematic diagram of apparatus for *I. galbana* CCMP 1324 culture.