Lipid accumulation and CO$_2$ utilization of two marine oil-rich microalgal strains in response to CO$_2$ aeration

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Abstract

Biological CO$_2$ sequestration by microalgae is a promising and environmentally friendly technology applied to sequester CO$_2$. The characteristics of neutral lipid accumulation by two marine oil-rich microalgal strains, namely, Isochrysis galbana and Nannochloropsis sp., through CO$_2$ enrichment cultivation were investigated in this study. The optimum culture conditions of the two microalgal strains are 10% CO$_2$ and f-medium. The maximum biomass productivity, total lipid content, maximum lipid productivity, carbon content, and CO$_2$ fixation ability of the two microalgal strains were obtained. The corresponding parameters of the two strains were as follows: ((142.42±4.58) g/(m$^2$·d), (4.99±1.80) g/(m$^2$·d)), ((39.95±0.77)%), (37.91±0.58)%), ((84.47±1.56) g/(m$^2$·d), (89.90±1.98) g/(m$^2$·d)), ((45.98±1.75)%), (46.88±2.01)%), and ((33.74±1.65) g/(m$^2$·d), (34.08±1.32) g/(m$^2$·d)). Results indicated that the two marine microalgal strains with high CO$_2$ fixation ability are potential strains for marine biodiesel development coupled with CO$_2$ emission reduction.

Key words: Isochrysis galbana, Nannochloropsis sp., CO$_2$ enrichment cultivation, neutral lipid, biodiesel, open raceway pond


1 Introduction

The CO$_2$ concentration in the atmosphere continuously increases; as a consequence, this increase significantly affects the global environment. Extensive CO$_2$ emissions from anthropogenic activities cause global warming (Ramanan et al., 2010). These emissions can be reduced through biological CO$_2$ sequestration by using photosynthetic microalgae because these organisms unlikely compete with food crops for arable land and fresh water resources (Kumar et al., 2010). Cultivated microalgae not only exhibit high CO$_2$ fixation ability but also produce significant amounts of renewable biomass for biofuels. These organisms also yield value-added products from biomass, such as proteins, fatty acids, and dietary supplements for humans, animals and fish (Pulz and Gross, 2004).

Studies on carbon sequestration based on microalgae, specifically the CO$_2$ tolerance of microalgae, have achieved certain progress. However, research objects mostly include freshwater algae, such as Scenedesmus sp., Spirulina platensis, and Chlorella sp., and the CO$_2$ tolerance of these algae ranges from 5% to 40% (Yang et al., 2011). Present research on carbon sequestration of marine microalgae is mainly concentrated on several species, such as Nannochloropsis sp., Dunaliella salina, Chlorella sp., and Phaeodactylum tricornutum (Salih, 2011). Results show that the growth rate and biomass accumulation are inhibited to a certain degree when the CO$_2$ concentration exceeds 5% (Lee and Tay, 1991), and the CO$_2$ emission concentration of power plants is approximately 10% (Bai et al., 2006). Therefore, carbon emission reduction based on marine microalgae must withstand 10% of CO$_2$. Meanwhile, research on lipid accumulation of these microalgae under carbon-rich conditions, especially the accumulation of neutral lipid, is rarely undertaken.

Biodiesel is formed by the fatty acid methyl esters derived from a transesterification reaction between triglycerides and methanol (Chisti, 2008). Neutral lipids or triglycerides, which are mainly found as storage lipids in microalgae, are essential for biodiesel production (Wang et al., 2010). The chemical and physical qualities of biodiesel are closely related to the properties of its parent oil. Therefore, the feasibility of microagal species as a biodiesel feedstock depends on the optimization of its biomass and neutral lipid content. To enhance the novel feedstock, re-
searchers should select suitable strains and CO₂ concentration for mass scale cultivation. Adverse environmental effects of high CO₂ emissions can be reduced by using biofuels. Biodiesel can be produced from microalgae in a small scale. However, large-scale biodiesel production by microalgae remains economically unviable (Liu et al., 2006; Xu et al., 2011). However, the characteristics of neutral lipid accumulation under CO₂-enriched cultivation in covered raceway ponds have yet to be reported. Therefore, microalgal cultivation under CO₂-enriched conditions in covered raceway ponds must be improved.

Two marine oil-rich microalgal strains, namely, *Isochrysis galbana* CCMM5001 and *Nannochloropsis* sp. CCMM7001, exhibit high environmental adaptability (Wei et al., 2015; Liu and Wang, 2014). The outdoor-cultured biomass of these microalgae can reach 26.4 g/(m²-d) (Liu et al., 2013) and 9.9 g/(m²-d) (Dobbioli et al., 2012), respectively. The lipid content of these marine microalgae is also high. Their lipid content is 7.0%-40.0% (Mata et al., 2010) and 22.7%-52.0% (Dobbioli et al., 2012; Moazami et al., 2012). The two microalgal strains can strongly tolerate CO₂ (>15%, v/v) (Chiu et al., 2009). The CO₂ concentrations of the two microalgal strains in industrial flue gases from power plants usually range from 10% to 15%, and these concentrations may provide a carbon source for large-scale microalgal cultivation. *Isochrysis galbana* and *Nannochloropsis* sp. were selected from marine microalgal strains with high biomass and high lipid contents (neutral lipid and total lipid) in our preliminary experiment. Mass cultivation coupled with CO₂ emission reduction was conducted in a covered raceway pond. This study provided a basis for CO₂ sequestration and biodiesel technology.

2 Materials and methods

2.1 Microalgal strains

*Isochrysis galbana* CCMM5001 and *Nannochloropsis* sp. CCMM7001 were provided by Han Xiaotian from the Institute of Oceanology, Chinese Academy of Science. These strains were maintained in f/2 medium at (20±1)°C with continuous illumination. The water surface height is 30 cm. The speed is 30 r/min (Moheliman and Borowitzka, 2007).

2.2 The open raceway pond

The open raceway pond was 200 cm×30 cm×40 cm in dimension, the water surface height is 30 cm. The speed is 30 r/min (Moheliman and Borowitzka, 2007).

2.3 Culture conditions

The two marine microalgal strains were grown in a 100 L photobioreactor, and inoculated in the exponential phase with an initial cell concentration of 2.12×10⁶ (tobioreactor, and inoculated in the exponential phase with an initial cell concentration of 2.12×10⁶). After growth, the biomass was separated from the medium by centrifugation at 10 000 g for 10 min, then freeze dried (DWₚ).

Dry weight was calculated by the equation:

\[ M = (DW₋ – DWₚ) / 0.01, \]

where \( M \) is the dry algae biomass (g/L).

Neutral lipid was determined with Nile red method (Chen et al., 2009, 2011). Unit volume of fluorescence value (excitation wavelength 480 nm, emission wavelength 580 nm) was measured to characterize the change of neutral lipid accumulation. Fluorescence value in single algal cell was calculated by the equation:

\[ FI = (Fₚ – F) / Y, \]

where \( FI \) is fluorescence value in single algal cell, \( Fₚ \) is the total of fluorescence value, \( F \) is fluorescence value in the medium, and \( Y \) is cell concentration (Wang et al., 2010).

2.5 Biomass productivity, total lipid and lipid productivity analysis

The biomass productivity was calculated by the equation:

\[ W = (m₋ – mₚ) / (tₚ – t₁) × 1000 × 0.3, \]

where \( W \) is biomass productivity (g/(m²-d)), \( m₋ \) is biomass dry weight (g/L) when the time is \( t₋ \), \( mₚ \) is biomass dry weight (g/L) when the time is \( tₚ \), \( t₁ \) is culture time (d).

Lipid productivity was calculated by the equation:

\[ L = WP, \]

where \( L \) is lipid productivity (g/(m²-d)), \( W \) is biomass productivity (g/(m²-d)), \( P \) is the content of lipid (%) (Huerlimann et al., 2010; Tang et al., 2011).

2.6 Total lipid and fatty acids analysis

Microalgae were harvested at the late exponential growth phase by centrifugation at 10 000 g for 5 min. Total lipid was extracted and determined by the modified method (Huerlimann et al., 2010; Tang et al., 2011). Fatty acids were extracted and determined by the method (Yang et al., 2013).

2.7 Carbon content and CO₂ fixation rate analysis

CO₂ fixation rate was calculated by the equation:

\[ F_{CO₂} = Cₚ × W × (Mₚ / Mₚ) / 100, \]

where \( F_{CO₂} \) is CO₂ fixation rate (g/(m²-d)), \( Cₚ \) is carbon content (g/g), \( W \) is the average of biomass productivity (g/(m²-d)), \( Mₚ \) is molecular weight of CO₂ (44 g/mol), \( Mₚ \) is molecular weight of carbon (12 g/mol) (Zhao et al., 2011).

2.8 Statistical analysis

All data were obtained by using at least three replicated biological samples. Experimental results were expressed as mean values±SD. Statistical analysis was performed using the SPSS11.5 statistical package. The statistical significance was achieved when \( p<0.05 \).