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Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO_2

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ABSTRACT

In order to reduce the cost of the production of microalgae for biodiesel, the feasibility of using the mixture of seawater and municipal wastewater as culture medium and CO_2 from flue gas for the cultivation of marine microalgae was investigated in this study. Effects of different ratios of municipal wastewater and 15% CO_2 aeration on the growth of *Nannochloropsis* sp. were examined, and lipid accumulation of microalgae was also studied under nitrogen starvation and high light. It was found that optimal growth of microalgae occurred in 50% municipal wastewater, and the growth was further significantly enhanced by aeration with 15% CO_2. When *Nannochloropsis* sp. cells were transferred from the first growth phase to the second lipid accumulation phase under the combination of nitrogen deprivation and high light, both biomass and lipid production of *Nannochloropsis* sp. were significantly increased. After 12 days of the second-phase cultivation, the biomass concentration and total lipid content increased from 0.71 to 2.23 g L^{-1} and 33.8–59.9%, respectively. This study suggests that it is possible to utilize municipal wastewater to replace nutrients in seawater medium and use flue gas to provide CO_2 in the cultivation of oil-bearing marine microalgae for biodiesel.

1. Introduction

Microalgae are considered one of the most promising feedstocks for biodiesel production for the higher growth rates and oil productivity comparing to other oil crop plants [1–3]. However, the main drawback for economical biodiesel production from microalgae is the high cost of algal cultivation [4]. Microalgae contain approximately 50% carbon of dry weight; so, about 1.8 kg of CO_2 is required to generate 1 kg of algal biomass [5]. Furthermore, about 3726 kg water, 0.33 kg nitrogen and 0.71 kg phosphate are also needed to produce 1 kg microalgae-based biodiesel, if the freshwater is used without recycling [6]. Therefore, the huge consumption of water resources, inorganic nutrients (mainly nitrogen and phosphate) and CO_2 is costly for microalgal cultivation, which is a serious problem encountered in the mass culture of microalgae [7,8]. One possible solution for overcoming the high cost of microalgal cultivation is to replace freshwater with seawater or wastewater and to use flue gas as carbon source for culturing microalgae.

Marine microalgae have been considered as potential sources of renewable energy due to high lipid [9,10]. Furthermore, they can grow in brackish water or seawater, avoiding demand for fresh water. However, it is necessary to add nutrients into seawater to maintain a good condition for marine microalgae. It is well known that wastewater is a good nutrient source for microalgae [11]. Some studies have demonstrated the feasibility of utilization of wastewater as the nutrient source for the cultivation of marine microalgae [12,13]. In addition, the flue gases from the power plant contain 10–15% (v/v) CO_2, which may provide a carbon source for microalgal cultivation [14]. Therefore, if an integrated system of seawater, wastewater and flue gas is used to cultivate marine microalgae, the cost of water, nutrients and CO_2 needed in microalgal cultivation process should be decreased significantly; so, the microalgal energy industry should become more attractive due to its economic benefits in the form of low production costs as well as environmental benefits. However, until now little is known about this integrated system for cultivation of marine microalgae.

In addition, oil content of microalgae is an important parameter for biodiesel production. However, when only encounter the environmental stress (e.g., nutrient deprivation or high light intensity), some microalgae start to produce lipids [9,10,15]. One measure is to cultivate high concentration of algal biomass firstly and then induce lipid accumulation under nutrient stress.

This study firstly investigated the feasibility of the integrated process using the mixture of seawater and municipal wastewater and 15% CO_2 for the production of marine microalgae, and the effects of different ratios of municipal wastewater and 15% CO_2 aeration on growth of *Nannochloropsis* sp. were studied. Then the lipid

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accumulation of microalgae was tested by a two-phase culture process under high-light exposure and nitrogen deprivation.

2. Materials and methods

2.1. Microalgal strain and inoculum preparation

Nannochloropsis sp. was provided by Dr. Qiang Hu (Arizona State University at the Polytechnic Campus, USA). Microalgal inoculum were incubated in sterilized seawater with f/2 medium at 25 °C on an orbital shaker at 150 rpm under continuous illumination of 30 μmol m^{-2} s^{-1}. After reaching the late exponential phase, the microalgal cells were recovered by centrifugation and suspended in sterilized seawater before being used for inoculation.

2.2. Culture media and aeration gases

The f/2 medium contained (mg L^{-1}) $NaNO_3$, 75; $NaH_2PO_4{\cdot}H_2O$, 5; $Na_2EDTA{\cdot}H_2O$, 4.16; $FeCl_3{\cdot}6H_2O$, 3.15; $CuSO_4{\cdot}5H_2O$, 0.01; $ZnSO_4{\cdot}7H_2O$, 0.022; $CoCl_2{\cdot}6H_2O$, 0.01; $MnCl_2{\cdot}4H_2O$, 0.18; $Na_2MoO_4{\cdot}2H_2O$, 0.006; Vitamin B_{12}, 0.0005; Vitamin B_1, 0.1; and Biotin, 0.0005 [16]. The seawater (SW) used in the study was obtained from Shilaoren Beach (Qingdao, China). The municipal wastewater (MW) was collected from Qingdao Tuandao Sewage Treatment Plant (Qingdao, China). The characteristics of the seawater and municipal wastewater were shown in Table 1. The seawater and municipal wastewater were filtered through 0.45 μm membrane prior to use. The aeration gases used in the study were the compressed gases (CO_2/N_2 = 15/85) and the compressed air.

2.3. Cultivation of microalgae

Nannochloropsis sp. was cultured in a cylindrical glass photobioreactor (30 cm length, 8 cm diameter) with 1 L of working volume at 26 ± 1 °C. All cultures, unless stated, were grown under a light intensity of 70 μmol m^{-2} s^{-1} with continuous illumination. The illumination of these photobioreactors was artificial daylight from Philips TLD 36 W/54 straight fluorescent tubes (120 cm long) mounted on a cladding.

2.3.1. Cultivation of microalgae with municipal wastewater and seawater

Nannochloropsis sp. was grown in the mixture of municipal wastewater (MW) and seawater (SW). The ratios of MW in SW were 10%, 30%, 50%, 80% and 100% (v/v). The culture grown in SW with f/2 nutrients was served as the control. All cultures were stationary culture without aeration.

2.3.2. Cultivation of microalgae with 15% CO_2

After *Nannochloropsis* sp. was cultured with the SW containing 50% MW for 24 h, the cultures were aerated with filtered compressed gases (15% CO_2) and the control cultures were bubbled with the filtered compressed air. The gases were continuously bubbled through the cultures at the rate of 0.1 L min^{-1}.

2.3.3. Lipid accumulation of microalgae in the two-phase cultivation process

Nannochloropsis sp. was cultured in the SW containing 50% MW for 6 days, under a light intensity of 70 μmol m^{-2} s^{-1} and 15% CO_2 (phase I). Then, the lipid accumulation phase (phase II) was established by changing the culture conditions. For nitrogen-deprived and high light treatment (HL-N), the cells were centrifuged and washed with sterilized seawater and then the cells were transferred to the nitrogen-free f/2 medium and simultaneously exposed to high light (150 μmol m^{-2} s^{-1}). For high light treatment (HL), the cells were directly exposed to high light (150 μmol m^{-2} s^{-1}) after phase I. The control culture with low light (70 μmol m^{-2} s^{-1}) in the single-phase cultivation was kept under same conditions as described above during the whole culture time.

2.4. Analysis

2.4.1. Cell growth

The biomass concentration of microalgae was measured spectrophotometrically from the optical density of the culture. The regression equation is:

$$X = 0.3373 \times OD_{750} + 0.0085 (R^2 = 0.9911) \quad (1)$$

where X (g L^{-1}) is the dry weight, OD_{750} is the absorbance of suspension at 750 nm. Chlorophyll a content was photocolorimetrically determined as described by Porra et al. [17]. The specific growth rate (μ, d^{-1}) was calculated according to the equation:

$$\mu = \frac{\ln(X_t - X_0)}{t - t_0} \quad (2)$$

where X_t and X_0 are the concentrations of biomass at the end and the beginning of the exponential phase, respectively, and $t{-}t_0$ is the duration of the exponential phase.

2.4.2. Chlorophyll fluorescence measurements

PS II photochemical activity was indicated by the maximum PS II quantum yield (F_v/F_m) which was measured using a Dual-PAM-100 measuring system. The fluorescence parameters are defined as follows: F_0, the initial level of chlorophyll fluorescence; F_v, variable chlorophyll fluorescence; and F_m, maximum chlorophyll fluorescence. After 15 min of incubation in the dark, the algal cell suspension (1 ml) was placed into the measurement chamber.

Table 1
Characteristics of the natural seawater and the filtered municipal wastewater used in the study (n.d. = not determined).

	Natural seawater	Municipal wastewater
pH	8.0	7.5
Total carbon (TC, mg L^{-1})	2.7	113.5
Total organic carbon (TOC, mg L^{-1})	1.9	59.7
Total inorganic carbon (TIC, mg L^{-1})	0.8	53.8
Total nitrogen (TN, mg L^{-1})	n.d.	110.2
Ammonia nitrogen (NH_4–N, mg L^{-1})	n.d.	92.0
Nitrate-nitrogen (NO_3–N, mg L^{-1})	n.d.	3.9
Nitrite-nitrogen (NO_2–N, mg L^{-1})	n.d.	n.d.
Total phosphate (TP, mg L^{-1})	0.1	5.3
Chemical oxygen demand (COD, mg L^{-1})	120	n.d.
Salinity (‰)	37	0