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Seawater desalination concentrate for cultivation of *Dunaliella salina* with floating photobioreactor to produce β-carotene

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ABSTRACT

Using seawater desalination concentrate to produce microalgae biomass enriched with high value products is a promising way to improve the profitability of desalination industry, but this is seriously limited by enormous land requirement for microalgae farming, as well as high cost of microalgae cultivation. In this study, low cost floating photobioreactor was adopted to cultivate *Dunaliella salina* to produce β-carotene, using seawater desalination concentrate as culture medium. With carbonate precipitate pretreatment, 97.6% Ca^{2+} and 60.3% Mg^{2+} were removed from seawater desalination concentrate. Then, this carbonate enriched medium was bubbled with CO_2, to generate bicarbonate, and this increased total inorganic carbon to 0.254 mol L^{-1}. Cultivation with this medium resulted in biomass production without difference from optimized concentrated artificial seawater medium. Pilot scale cultivation of *D. salina* with floating PBR on ocean showed that 300 g algal biomass containing 14.3 g β-carotene can be produced from 1 m^3 desalination concentrate. This amount of β-carotene enriched biomass has a value more than $ 40, and would significantly improve the profitability of desalination industry.

1. Introduction

Supply of fresh water has become a worldwide issue due to serious water shortages [1]. Desalination of seawaters has been established as a promising alternative for freshwater source, and it has been widely applied across the world [2]. However, its commercial application is still limited by high cost. Even best reverse osmosis technology is used [2], the cost of desalinized water is still at least two times higher than tap water. Simultaneously, desalination also produce enormous amount of desalination concentrate wastewater, which could cause serious environmental problems if not properly treated [3]. To improve profitability of desalination industry, an alternative approach is to produce value added products from seawater desalination concentrate (SDC). A potential way is to culture microalgae with SDC [4], since their biomass are enriched with high value products such as proteins, polysaccharide and pigments [5], and they have higher growth rate than terrestrial plants [6].

A challenge to utilize SDC for microalgae cultivation is its high salinity, which may inhibit growth of most algal species [7,8]. *Dunaliella salina*, however, has the ability to grow even in saturated salt water. *D. salina* is known as an excellent producer of β-carotene, which is a natural pigment and antioxidant, with wide application as nutraceuticals, food supplement, and feed additive [9]. Salinity of 1.5–3 M NaCl is usually used in cultivation of *Dunaliella* [10]. In traditional cultivation process, large amount of salts is supplemented into seawater medium, and this cost is higher than a third of its total production cost [11], which could be saved by utilization of SDC.

Although cultivating *Dunaliella* with desalination concentrate to produce high value β-carotene is a promising idea [12,13], a practical example of commercial application is still absent. A major hurdle is that enormous land is required for microalgae cultivation [14], since open pond, as the most common used cultivation system, usually has very low productivity, and requires large area of land. Desalination plants are usually built on coast, which has limited land space for algae farming. Also, open pond is vulnerable to algae predators, invading algae species, fungi and bacteria, as well as rain and dust. This leads to a very unstable production. Although cultivation with closed photobioreactor can well address these problems, all current available PBR

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systems have very high production cost, and leads to limited profitability in production of low value products [15], such as microalgal biofuels. Thus, development algae farm with low cost and without land requirement is in urgent demand.

To address the problem of land requirement, floating offshore microalgae farm [16–19] is a promising choice. A cultivation system named as Bicarbonate-based Carbon Capture and Algal Production System on Ocean (BICCAPSO) was developed in our previous study [20,21]. Compared with other microalgae culture system, BICCAPSO would have a much lower production cost, since it systematically reduced the cost of PBR manufacturing, operation, and maintenance [15,22]. With this system, it is promising to develop cost-effective microalgae culture process with desalination concentrate. Thus, this study investigated the cultivation of *D. salina* with BICCAPSO using SDC as the culture medium. Firstly, SDC was pretreated with sodium carbonate to remove excessive calcium and magnesium ions before cultivation. Growth of *D. salina* cultivated with pretreated SDC was tested in laboratory scale and further in an offshore BICCAPSO PBR with a volume of 75 L. Finally, potential to improve the profitability of desalination industry with carotene production from Dunaliella culture was preliminarily evaluated.

2. Material and methods

2.1. Microalgae strain and culture medium

Strain of *D. Salina* CCAP 19/18 was purchased from the Culture Collection of Algae and Protozoa in Scotland. The algae strain was maintained in the modified artificial seawater medium according to ATCC 1174DA medium [23], which contains: NaCl, 87.75 g L^{-1}; $NaHCO_3$, 8.4 g L^{-1} KNO_3, 0.5 g L^{-1}; $K_2HPO_4{\cdot}3H_2O$, 0.08 g L^{-1}; $CaCl_2$, 0.111 g L^{-1}; $MgCl_2{\cdot}6H_2O$, 0.507 g L^{-1}; $MgSO_4{\cdot}7H_2O$, 0.123 g L^{-1}; $FeCl_3{\cdot}6H_2O$, 0.0006 g L^{-1}; and 1 ml A5 per L, which contains $MoCl_2{\cdot}4H_2O$, 1.979 g L^{-1}; H_3BO_3, 3.092 g L^{-1}; $NaMo_4{\cdot}2H_2O$, 0.484 g L^{-1}; $ZnSO_4{\cdot}7H_2O$, 0.23 g L^{-1}; $NaVO_3$, 0.183 g L^{-1} $CoCl_2{\cdot}6H_2O$, 0.048 g L^{-1}; $CuSO_4{\cdot}5H_2O$, 0.2 g L^{-1}. Also, cultivation with this medium was used as the control for latter experiments in this study.

2.2. Seawater desalination concentrate and its pretreatment

Seawater desalination concentrate was kindly supplied by China National Petroleum Corporation, Dalian branch. The total inorganic carbon in SDC was determined with a Shimadzu TOC-5000A (Shimadzu, Japan). NO_3^- was measured with colorimetric method [24]. Contents of Ca, Fe and Mg in the SDC were determined with an inductively coupled plasma spectrometer (PerkinElmer, USA). The conductivity and total dissolved solids were measured with a conductivity meter (Kedida, China) and the pH was measured with a pH meter (Mettler, China).

Seawater desalination concentrate was firstly treated with sodium carbonate to remove Ca^{2+} and Mg^{2+}. The treatment process was shown in Fig. 1. Sodium carbonate with a concentration of 0.2 mol L^{-1} was slowly added to SDC with intensive mixing for 10 min. The milky suspension was subsequently allowed to settle for 60 min. After this, the supernatant was filtered through 0.22 μm hydrophilic membranes to another container, while the bottom precipitation was discarded. Then, this medium was bubbled with CO_2 for pH adjustment (until pH decreased to 8.0) and carbon replenishment. Thereafter, the same amount of nutrients as artificial seawater medium were added to the supernatant, which include KNO_3, 0.5 g L^{-1}; $K_2HPO_4{\cdot}3H_2O$, 0.08 g L^{-1}; $FeCl_3{\cdot}6H_2O$, 0.0006 g L^{-1}; and 1 ml A5 per L, but except for NaCl, $CaCl_2$, $MgCl_2{\cdot}6H_2O$, $MgSO_4{\cdot}7H_2O$ and sodium bicarbonate.

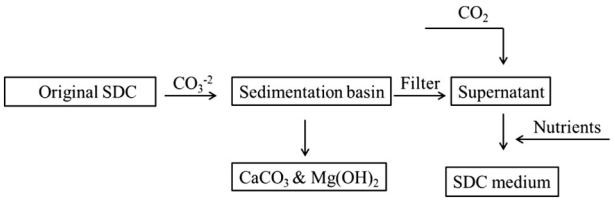

Fig. 1. Diagram of Seawater Desalination Concentrate (SDC) pretreatment and medium preparation.

2.3. Algae cultivation and experiment procedures

In BICCAPSO, the carbon source was supplied as bicarbonate, but the concentration of bicarbonate has significant effect on microalgae growth [25]. Thus, bicarbonate concentration in artificial medium was firstly investigated, and the concentrations were 10, 30, 50, 100, 200 and 300 mmol L^{-1}. To test the feasibility of using SDC for algae cultivation, the original SDC and the pretreated SDC with nutrients supplemented (SDC medium) were used as experimental media, and artificial seawater medium with optimized bicarbonate concentration was used as the control. Experiments were carried out in 1000 mL Erlenmeyer flasks with 300 mL of medium at 50 rpm and continual illumination of 115.4 μmol m^{-2} s^{-1}, and the temperature was controlled at 25 °C. Biomass productivity ($P_{biomass}$) and specific growth rate (μ) was calculated as

$$P_{Biomass}(g\ L^{-1}\ d^{-1}) = \frac{DCW_2 - DCW_1}{t_2 - t_1} \quad (1)$$

$$\mu = \frac{\ln(DCW_2/DCW_1)}{t_2 - t_1} \quad (2)$$

where $P_{Biomass}$ is biomass productivity (mg L^{-1} d^{-1}), μ is specific growth rate (d^{-1}), DCW_2 and DCW_1 are the dry cell weight (DCW) at time t_2 and t_1 respectively.

For determination of DCW, triplicates of 40 mL samples were firstly acidified with hydrochloric acid before centrifuge, then washed twice and re-suspended in 40 ml ammonium bicarbonate solution with 1.0 mol L^{-1}. Then, the algae pellets were dried overnight at 105 °C until constant weight. DCW was calculated by subtracting empty dish weight from total weight of dish and dried biomass.

2.4. Outdoor cultivation

For outdoor cultures in ocean, floating PBR with surface area of 1.0 m^2 was used (Fig. 2), and this PBR was constructed according to our previous study [20]. Briefly, the frame and arch structure was constructed with inflatable tube, and they were covered and sealed with anti-fogging PVC, but with sampling port and gas exchange port left. Beckets connected on inflated tube was used to anchor the PBR in ocean. Air is blown into inflatable tube through a valve to keep a firm structure. Two cultures in floating PBRs were conducted, one with artificial sea water, set as control, and the other with SDC medium. Both floating PBRs were anchored on the ocean field of Lingshui Bay at Dalian City (38°87′N, 121°55′E) in China. The depth of these two cultures was 7.5 cm, with a cultivation volume of 75 L. These two outdoor

Fig. 2. Photograph of the used floating PBR (1 m^2).