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Microalgal biomass productivity and dominant species transition in a Korean mass cultivation system

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ABSTRACT

Mass cultivation of microalgae was carried out at a large-scale outdoor raceway facility in Korea from June 2014 to January 2017 and we analyzed the transition of dominant microalgal species and influence of zooplankton predation. Concurrently the microalgae yield, biomass characterization, and resource consumption results were examined from two identical 675.0 m^2 raceways with or without a semitransparent film cover to determine which model is a better fit for Korean geoclimatic conditions. Year-round cultivation was possible in the covered raceway, but the average seasonal productivities were lower than those of the non-covered one, probably due to the reduced solar radiation. The addition of a cover did not confer advantages for the production of microalgae, even though the production period was extended over sub-zero temperature winter seasons. Species transitions were observed throughout the cultivation period, and the most dominant microalgal genera found year round were *Acutodesmus* and *Pseudopediastrum*. In addition, an algal predator, *Vorticella*, was present during most of the cultivation periods. The seasonal water temperature fluctuation and presence of predators greatly affected the dominant microalgae and biomass productivity. The maximal productivity, 29.3 g dry weight (DW)/m^2-day with 15.0% lipid and a calorific value (CV) of 20.1 MJ/kg, was attained in the non-covered raceway during the summer of 2016. Overall, a yearly average productivity of 8.9 g DW/m^2-day was obtained from the raceways, and the biomass had an average lipid content of 12.8% and CV of 17.7 MJ/kg. The grazer-resistant microalgae were allowed to dominate rather than maintaining target strains and the results demonstrated the potential of naturally occurring microalgae as a biofuel source since the CVs of the biomass were close to those of terrestrial energy crops. Also, the mass cultivation of the indigenous isolates could be applied to wastewater treatment due to their high capacity to assimilate nutrients.

1. Introduction

Microalgae have received considerable attention since they are able to transform carbon dioxide (CO_2) into cellular biomolecules such as biofuels, antioxidant compounds, nutraceuticals, animal/fish feeds, and fertilizers via photosynthesis [4,13,34]. Numerous strains with appropriate characteristics for producing the above mentioned biochemicals have been isolated and/or developed [2,14,20]. There are two main types of large-scale cultivation systems for microalgal biomass: open pond raceways (OPRs) and closed photobioreactors (PBRs). Currently, the majority of microalgal biomass is produced in OPRs due to their good scalability and low capital and operating costs [19,32]. However, outdoor ponds are less suitable for the production of specific strains or products than PBRs due to the difficulties in controlling physiochemical (temperature, wind, rainfall, solar radiation, etc.) and biological factors (bacteria, predatory zooplankton, other algal species, etc.) [3,36]. Low productivity caused by these parameters is one of the main barriers that limits industrial-scale production in OPRs [6,28]. To overcome these challenges, it is necessary to develop more efficient and commercially viable cultivation systems specific to local conditions. In recognition of the current limitations, a pilot research plant was constructed in August 2013 and operated for almost 3 years to determine biomass productivity and species transition, and the cultivation data from the OPRs are presented. This study is a continuation of our previous work where we emphasized the potential of mass cultivation of indigenous microalgae as a source of biofuel under Korean weather conditions [15]. The

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aim of current study is to address the physical and ecological factors on the large quantity production of biomass since the success of the commercial-scale culture systems heavily relies on an understanding of the physiology of microalgae and predatory grazers as well as the operation and management of open pond raceways. The findings of the present work would be a valuable contribution to commercialization of microalgae-based industry in South Korea and regions with similar climate conditions as not many previous studies have explored the efficiency and productivity of outdoor mass cultivation.

2. Materials and methods

2.1. Pilot research plant construction

A freshwater microalgae mass cultivation facility was built in the central region of South Korea with an average of 2311 sunlight hours per year (data from 2014 to 2016) at Chilgok-gun Agricultural Technology Center (36° 02′ 18.91″N, 128° 22′ 57.71″E; Dongan-ri, Yakmok-myeon, Chilgok-gun, Gyeongsangbukdo, South Korea). To reduce the cost of construction, compacted earth ponds were built, and the earthen raceways were then lined with a 2 mm thick ultraviolet-resistant high density polyethylene membrane. Two identical 675.0 m^2 OPRs (OPRs #1 and #3) were constructed (Figs. 1 and 2), and a semitransparent film was used to cover OPR #1 in order to counter adverse weather conditions such as monsoon and freezing temperatures. These two raceways were compared for their biomass yield, dominant microalgae and predator species, and consumption of nutrients. In addition, a 526.5 m^2 OPR (uncovered, OPR #2) was constructed as an auxiliary raceway for seed culture preparation and biomass harvest processes. A dissolved air flotation (DAF) unit (Dongshin EnTech, Yangsan, South Korea) was installed to the automated microalgae harvesting system (Fig. 2).

2.2. Operational conditions

The *Coelastrella* culture grown in small-scale raceways (SCRs) was inoculated at 10% (v/v) into the OPRs, and microalgal species succession and zooplankton contamination were regularly monitored by morphological and molecular analyses. Commercially available water soluble fertilizers, Eco-Sol (N-P-K: 25-9-18, Dongbu Farm Hannong, Ulsan, South Korea) and monopotassium phosphate (N-P-K: 0-52-34, Sang Rok Chemical, Daegu, South Korea), were applied to each OPR at concentrations of 15.0–30.0 mg total nitrogen (TN)/L and 3.0–6.0 mg total phosphorus (TP)/L, respectively. To encourage circulation of the microalgae and nutrients, paddle wheels were used to generate water flow at a velocity of 0.25 m/s. Continuous microalgae cultivation was performed throughout the operation. When collecting biomass, approximately two thirds of the algal culture was dewatered and harvested as paste by the DAF system (Fig. 3), and each ORP was replaced with the same amount of underground water. The remaining third of the culture was re-used as inoculum and appropriate amounts of fertilizers were supplied. During the daylight hours, around 10.0 L/min CO_2 was aerated into the both OPRs through a Venturi system (Fig. 2) to maximize microalgae biomass productivity. OPR #3 was shut down during winter seasons because of the freezing of the paddle wheel, and these three months were excluded from the overall average biomass productivity calculation. In addition, from September 2015 to November 2015, the facility was out of operation due to repair work.

2.3. OPR monitoring

Microalgal culture samples were collected at three different points from the OPRs every three days and inspected at 1000 × magnification using a light microscope (Nikon Eclipse E100 Biological Microscope, Tokyo, Japan). Optical density was measured at 680 nm by spectrophotometer (X-ma 1200 V, Human, Seoul, South Korea), and dry weight was calculated to monitor the microalgal growth of each raceway and decide the harvest points. Dominant predator species were also monitored via light microscope observation. TN and TP concentrations of the samples were analyzed using HS-TN(CA)-L and HS-TP-L water test kits (Humas, Daejeon, South Korea). In addition, dissolved oxygen, pH, and temperature data were recorded every 3 h using a water quality meter (WQC-24, DKK-TOA, Tokyo, Japan) and used to obtain information on water-quality conditions within the OPRs.

2.4. Isolation of dominant microalgae and their identification

Representative microalgal species were isolated, identified, and added to our laboratory culture collection. Microalgae (1.5 mL) from the OPRs were centrifuged at 3000*g* for 15 min (Centrifuge 5424, Eppendorf, Germany) to separate the contaminants from the culture, and the resulting pellets were streaked on BG-11 agar [27] supplemented with meropenem (20 μg/mL). Plates were incubated at 25 °C under a light:dark cycle (16:8 h) and single colonies were aseptically transferred onto fresh BG-11 plates to obtain axenic algal cultures. Once axenic culture was established they were named as strains KNUA036 through 040. Molecular identification of each isolate was then performed using NS1/NS8 and ITS1/ITS4 primer pairs [16]. All the microalgae isolates were identified to the genus level (Table 1). DNA sequence data have been deposited in GenBank (accession numbers are KT883906-KT883913, KY654753, and KY655002).

2.5. Harvest of microalgal biomass

When the OPR cultures entered late exponential or early stationary growth phase, biomass was dewatered with 17% polyaluminum

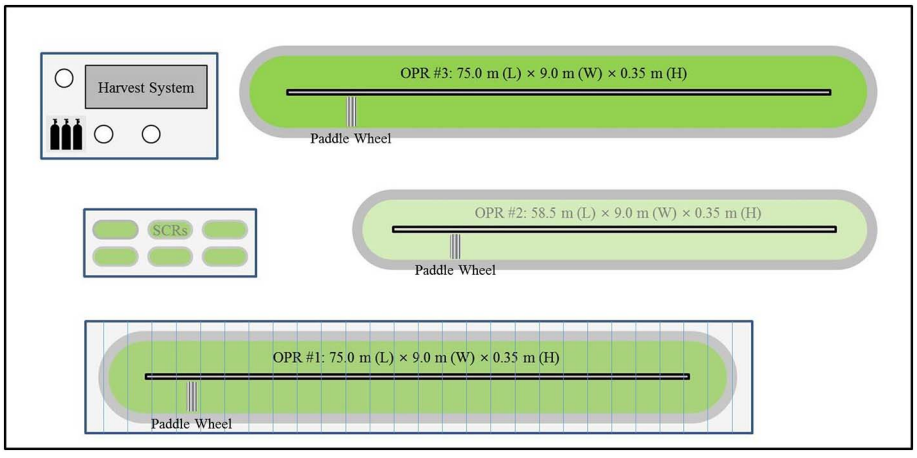

Fig. 1. Schematic diagram of the pilot research plant for the production of microalgae biomass. OPR: open pond raceway; SCR: small-scale raceway.