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Cultivation and harvesting of microalgae in photobioreactor for biodiesel production and simultaneous nutrient removal

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ABSTRACT

Microalgae, *Chlorella vulgaris* and *Scenedesmus obliquus* were cultivated in a small scale vertical flat-plate photobioreactor (PBR) supplemented with municipal wastewater in order to achieve simultaneous wastewater treatment and biomass production for biofuel generation. Microalgal growth and nutrient removal including total nitrogen (TN), total phosphorus (TP), total inorganic carbon (TIC) and trace elements (Ca^{2+}, Na^{+}, Mg^{2+} and Zn^{2+}) were monitored during microalgae cultivation. *C. vulgaris* and *S. obliquus* showed optimal specific growth rates (μ_{opt}) of 1.39 and 1.41 day^{-1}, respectively, and the TN and TP were completely removed (>99%) from the wastewater within 8 days. Microalgal biomass in the PBR was harvested using a natural flocculant produced from *Moringa oleifera* seeds. The harvesting efficiency of *M. oleifera* was 81% for *C. vulgaris* and 92% for *S. obliquus*. The amounts of saturated, mono-unsaturated, and poly-unsaturated fatty acids in the harvested biomass accounted for 18.66%, 71.61% and 9.75% for *C. vulgaris* and 28.67%, 57.14% and 11.15% for *S. obliquus*, respectively. The accumulated fatty acids were suitable to produce high quality biodiesel with characteristics equivalent to crop seeds oil-derived biodiesel. This study demonstrates the potential of microalgae-based biodiesel production through the coupling of advanced wastewater treatment with microalgae cultivation for low-cost biomass production in a PBR.

1. Introduction

Global forecasts have indicated that, by 2050, the world population will require 70% more food, 50% more fuel, 50% more water, and a 50–80% reduction in carbon dioxide (CO_2) emissions to maintain social, political and climate security [1–3]. Microalgae have evolved the ability to tap into the vast energy resource of the sun and absorb atmospheric CO_2 to produce food, fuel and clean water [4]. They also have the potential to be cultivated in brackish and marine water or wastewater [5,6]. Microalgae systems must deliver both positive economic returns and positive energy return on energy invested (EROEI) for the commercial feasibility of biofuel technology, which requires continued improvement [7].

The bottlenecks for economical biofuel production using microalgae are technological and engineering barriers including the high cost of microalgae cultivation and harvesting [8]. Growing microalgae in domestic and/or municipal wastewater could minimize the energy input and reduce the cultivation cost, as wastewater is a readily available source of water, which has nutrients required for microalgae proliferation. The coupling of microalgae cultivation with wastewater treatment and recycling has recently been proposed as a cost effective strategy for microalgae-based biofuel production [9,10]. The application of different wastewater sources for microalgae cultivation has been well-established in batch studies [11–13]. Further investigation is needed for scaling up microalgae cultivation using wastewater in a photobioreactor (PBR) for the achievement of economical biomass production for biofuel generation. PBR studies on cultivation of microalgae have been reported using various types of synthetic media and wastewater [14,15–17]. However, microalgal recovery and the biochemical characteristics of biomass harvested from PBRs have not been extensively investigated [15,18–21].

The present study was focused on the combination of the wastewater treatment process with the cultivation of *Scenedesmus obliquus* and *Chlorella vulgaris* in a small scale vertical flat-plate

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PBR for nutrient removal and biomass production. *C. vulgaris* and *S. obliquus* were selected for this study based on their higher biomass productivity and lipid content [11]. These two microalgal species are promising candidates for biodiesel production and tolerant to the toxicity of wastewater [11,12]. *Moringa oleifera* seeds were applied as natural plant flocculant in order to harvest the microalgal biomass. This study investigates both cultivation and harvesting of microalgae through the coupling of advanced wastewater treatment with algal cultivation for low-cost biomass production in a PBR. The growth rate of both microalgae strains during the cultivation period along with the removal of total nitrogen (TN), total phosphors (TP), total inorganic carbon (TIC), and trace minerals (Ca^{2+}, Na^{+}, Mg^{2+} and Zn^{2+}) were monitored. The kinetic assessment of specific growth rate, doubling time and specific nutrient consumption of *C. vulgaris* and *S. obliquus* were evaluated. The fatty acid composition of the harvested microalgal biomass was also analyzed along with the estimation of biodiesel quality.

2. Materials and methods

2.1. Microalgae species and wastewater sampling

Chlorella vulgaris (*C. vulgaris*) and *Scenedesmus obliquus* (*S. obliquus*) isolated from freshwater in Wonju, South Korea, (GenBank Accession No. FR751187 and GU732418, respectively) were used in this study. Municipal wastewater collected from a wastewater treatment plant in Wonju, South Korea, was used as the culture media (Fig. 1A). The wastewater sample was filtered using a 0.045-μm hollow fiber membrane module in order to remove the microorganisms and fine suspended particles (Fig. 1B–D). The chemical properties of the filtered wastewater were analyzed (Table 1). The TN and TP were measured using a Hach Kit, (Ce-Max UV–Visible Spectrophotometer, Qvis 5000H, South Korea) by chromotrophic acid and acid persulfate methods, respectively. Total inorganic carbon (TIC) was measured with a Shimadzu TOC-V_{CPH} analyzer (Tokyo, Japan). Metal ion concentrations were analyzed using an ELAN DRC II inductively coupled plasma-mass spectrophotometer (PerkinElmer Sciex, USA) [11]. The solution pH was measured using an Orion 5-Star pH/ORP/Conductivity/DO Meter (Thermo Scientific, USA).

2.2. PBR start-up

Four rectangular, vertical flat-plate PBRs (650 × 600 × 200 mm) constructed using transparent poly acrylic plastic material, with a total volume of 60 L each were used (Supplementary Fig. 1). The PBR was washed with deionized water containing 0.04% NaOCl and 0.2% NaOH for disinfection, followed by three washes with sterile deionized water [22]. The hollow fiber membrane module was directly connected to the PBRs system and each PBR was loaded with 40 L of filtered municipal wastewater. A red light-emitting diode (LED) strip was attached on the outer wall of the photobioreactors. Red light has been reported to enhance microalgae growth [19,23]. The PBRs were located indoor, where the temperature was maintained at 25 ± 2 °C under continuous illumination by red fluorescent light for 10 days. The cultures were

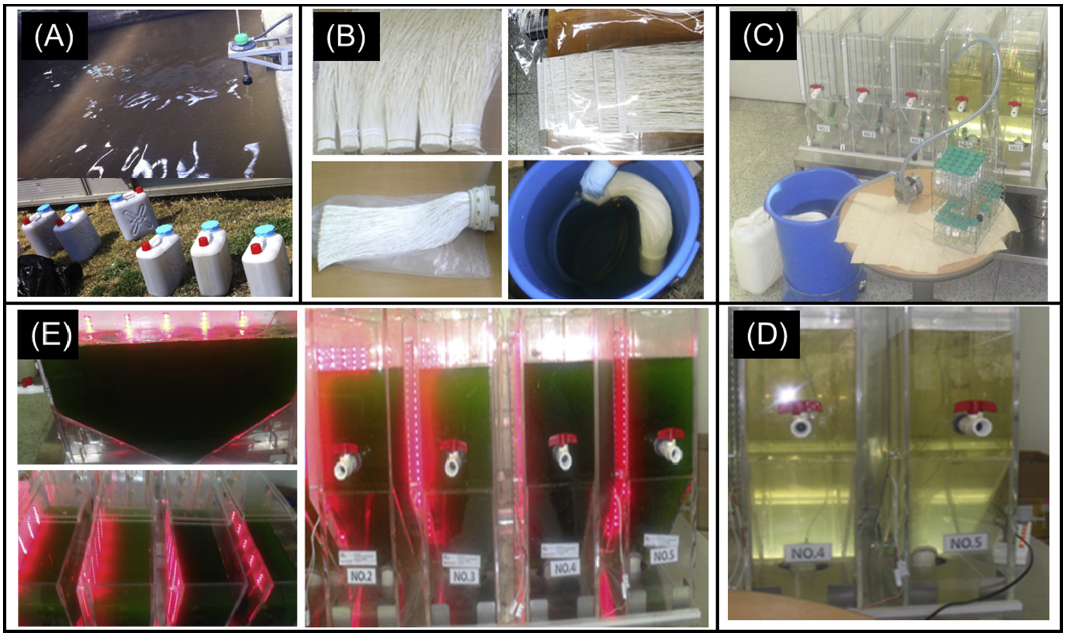

Fig. 1. Coupling of the wastewater treatment process with microalgae cultivation in a photobioreactor for nutrient removal and biomass production: (A) sampling of wastewater, (B) preparation of the hallow fiber membrane, (C and D) filtration process for the wastewater and (E) microalgae after 10 days of cultivation.

Table 1
Chemical properties of municipal wastewater after filtration using a hollow fiber membrane.

Parameter	Value	Parameter	Value
pH	7.39	Calcium (Ca^{2+}, mg L^{-1})	35.33 ± 0.06
Total nitrogen (TN, mg L^{-1})	12.25 ± 1.5	Sodium (Na^{+}, mg L^{-1})	56.48 ± 0.71
Total phosphorus (TP, mg L^{-1})	1.80 ± 0.07	Magnesium (Mg^{2+}, mg L^{-1})	5.49 ± 0.01
Total inorganic carbon (TIC, mg L^{-1})	32.35 ± 1.06	Zinc (Zn^{2+}, mg L^{-1})	0.13 ± 0.00

Results are mean of triplicates experiments showing the mean and standard deviation.