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The effects of wavelength and wavelength mixing ratios on microalgae growth and nitrogen, phosphorus removal using *Scenedesmus* sp. for wastewater treatment

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HIGHLIGHTS

- Effects of multiple wavelengths on wastewater treatment by microalgae were evaluated.
- Mixed red and blue light increased algae production rate rather than white light.
- N removal rate could be increased using wavelength mixing with red and blue light.
- Blue light was effective on phosphorus removal.
- Mixing light was effective in terms of electric consumption compared to white light.

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ABSTRACT

In wastewater treatment using microalgae, the effects of wavelength and wavelength mixing ratio on microalgae growth and removal of nitrogen and phosphorus were evaluated using LEDs (white light, 670 nm, 450 nm, and 525 nm). Microalgae production rates were enhanced by a maximum of 45% with 400–700 nm white light compared to that of a single wavelength. The phosphorus removal rate was as high as 90% with blue light. When red light and blue light were mixed and supplied, the microalgae production rate was about 50% higher than the rate of the culture with white light. Nitrogen and phosphorus removal rates were as high as approximately 15 mg/L/day at a wavelength mixing ratio of 7 (red light):3 (blue light) and 2.1 mg/L/day at a wavelength mixing ratio of 5 (red light):5 (blue light).

1. Introduction

Microalgae are used in the medical field and in the areas of dye and protein production. Vigorous studies are on-going to apply microalgae for bio-fuel production and wastewater treatment (Wang and Lan, 2011; Brennan and Owende, 2010; Spolaore et al., 2006).

A general wastewater treatment system supplies air to aerobic microbes via aeration to remove organic matter in the wastewater. Approximately 1 kW h of electricity is required to supply the air needed for 1 kg of BOD (biochemical oxygen demand) removal (Oswald, 2003). Providing aeration is around 40% of the total electricity cost in wastewater treatment plants (MOE, 2010), so aeration is a disadvantage due to increased operating costs. In addition, complex processes of the nitrification process oxidizing NH_4^+ to NO_3^-, the denitrification process reducing NO_3^- to N_2 for nitrogen removal and the aerobic–anaerobic process for phosphorus removal are required in general wastewater treatment plants.

In contrast, wastewater treatment technology using microalgae has a variety of benefits compared to existing wastewater treatment technology. Organic matter, nitrogen, and phosphorus can be simultaneously removed in a reactor; indeed, high removal efficiency of nutrients such as nitrogen and phosphorus is expected (Aslan and Kapdan, 2006; Perez-Garcia et al., 2011; Sancho et al., 1999; Shi et al., 2007). In addition, oxygen produced from photosynthesis process by microalgae is used as a source for aerobic microbes, thereby reducing aeration costs. Autophototrophic microalgae cannot only remove nitrogen and phosphorus, but can fix CO_2 by using the CO_2 as a carbon source. Typically 1.8 tons of CO_2 are required to produce 1 ton of algae (Oilgae, 2010).

At present, studies on the utilization of sludge produced from general wastewater treatment systems are on-going, but sludge use suffers from low efficiency (Liu et al., 2012). On the other hand,

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microalgae biomass can be used in various ways such as bio-fuel production and feedstock because the microalgae contain about 40–50% carbohydrate, lipid and protein contents in vivo (Singh and Gu, 2010).

Photoautotrophic microalgae absorb light energy (photons) and convert it to chemical energy such as ATP and NADP. These reactions occur in photosystems in the microalgae and the absorption of light energy occurs by chlorophyll pigments and carotenoid that compose a photosystem antenna complex in the photosystem. The absorbed light energy is sent to the reaction center and is converted to chemical energy for photosynthesis (Richmond, 2003).

Generally, microalgae use light of wavelengths from 400 to 700 nm for photosynthesis. The wavelengths absorbed by microalgae differ depending on the species. For instance, green microalgae absorb light energy for photosynthesis through chlorophylls as a major pigment absorbing light energy in the range of 450–475 nm and 630–675 nm and carotenoids as an accessory pigment absorbing light energy of 400–550 nm (Richmond, 2003).

Several studies reported that the growth of microalgae is different depending on wavelength. Red light (600–700 nm) and blue light (400–500 nm) stimulate the growth of microalgae, and growth rates and lipid content of the microalgae differ with light intensity (Chen et al., 2011; Cheirsilp and Torpee, 2012; Wang et al., 2007; Das et al., 2011).

Almost all previous studies focused on the growth of microalgae and on providing nutrients for growth using wastewater rather than on nutrient treatment in the wastewater (Christenson and Sims, 2011). A study on nitrogen and phosphorus removal in wastewater depending on wavelength and mixing ratios is insufficient.

In this study, when multiple wavelengths were applied for photoautotrophic microalgae in wastewater treatment, effects of the wavelength and mixing ratio on microalgae growth and removal of nitrogen and phosphorus in the wastewater were investigated.

2. Methods

2.1. Microalgae strain and culture conditions

The strain of *Scenedesmus* sp. obtained from KORDI (Korea Ocean Research & Development Institute) and cultured using Bold's Basal Medium (BBM) consisted of $NaNO_3$ 250 mg/L, $MgSO_4 \cdot 7H_2O$ 75 mg/L, NaCl 25 mg/L, K_2HPO_4 75 mg/L, KH_2PO_4 175 mg/L, $CaCl_2 \cdot 2H_2O$ 25 mg/L, H_3BO_3 114 mg/L, 1 mL of a trace elements solution ($ZnSO_4 \cdot 7H_2O$ 8.82 mg/L, $MnCl_2 \cdot 4H_2O$ 1.44 mg/L, MoO_3 0.71 mg/L, $CuSO_4 \cdot 5H_2O$ 1.57 mg/L, $Co(NO_3)_2 \cdot 6H_2O$ 0.49 mg/L), 1 mL of EDTA stock ($EDTANa_2$ 50 mg/L, KOH 31 mg/L), and 1 mL of Fe solution ($FeSO_4 \cdot 7H_2O$ 4.98 mg/L and H_2SO_4 1 mL) in a 250 mL cell culture flask. Temperature was maintained at 25 ± 1 °C, and light with a photosynthetic photon flux density (PPFD) of 100 μmol/m^2/s was used.

2.2. Batch experiment-1: effects of wavelength

To evaluate nitrogen and phosphorus removal efficiencies and the growth of microalgae by wavelength, light-emitting diodes (LEDs) were used because it is possible to control wavelengths. Blue light (400–500 nm, with a peak at 450 nm), green light (470–580 nm, with a peak at 525 nm, and red light (600–700 nm, with a peak at 670 nm) were used. White light (400–700 nm) was used as a control group.

BBM and pre-cultured *Scenedesmus* sp. were mixed at a 9:1 ratio in a 1 L Erlenmeyer flask, with a working volume of 600 mL. The concentration of microalgae was 0.3 OD (optical density), and the initial pH was adjusted to 8. Inorganic carbon for growth of microalgae was supplied at 1 vvm (aeration volume/medium volume/minute) using an air pump and air stone. Batch tests were performed for 6 days at 25 ± 1 °C and 100 μmol/m^2/s of PPFD for each wavelength.

Table 1
Mixing ratios of red LED and blue LED.

PPFD	Wavelength mixing ratio	
	Red light (wavelength range 600–700 nm, peak wavelength 670 nm)	Blue light (wavelength range 400–500 nm, peak wavelength 450 nm)
100 μmol/m^2/s	9	1
	7	3
	5	5
	3	7
	1	9

2.3. Batch experiment-2: effects of wavelength mixing ratio

To evaluate nitrogen and phosphorus removal efficiencies and the growth of microalgae by wavelength mixing ratio based on light intensity, Blue light (400–500 nm, with a peak at 450 nm) and red light (600–700 nm, with a peak at 670 nm) were used. The evaluated mixing ratios are presented in Table 1. Green light was eliminated for the experiment due to large electric power consumption and low microalgae production rate. Batch tests were performed for 6 days, and other operating conditions were the same as those in batch experiment-1.

2.4. Analysis

Suspended solids (SS) were measured to determine microalgae growth (APHA-AWA-WEF, 2005), and OD was analyzed using a spectrophotometer (X-ma 2000, Humas crop, Korea) at 660 nm. TC (total carbon), TOC (total organic carbon), and TIC (total inorganic carbon) were measured with a total organic carbon analyzer (TOC-Vcsn, Shimadzu, Japan). T-N and T-P were analyzed using a water analyzer (HS 3100, Humas, Korea) after pre-treatment using an exclusive water analysis reactor (HS R-200, Humas, Korea). The pH was measured with a pH meter (pH-200L, Istek, Korea), and electric power consumption for each LED was computed by measuring the voltage and current using a digital multimeter (M-3860D, Metex Corporation, Korea).

3. Results and discussion

3.1. Effects of wavelength on microalgae production and nutrient removal by Scenedesmus sp.

3.1.1. Microalgae production rate and CO_2 fixation

The production rate of *Scenedesmus* sp. and the CO_2 fixation rate were evaluated at different wavelengths using green, blue, and red light. The results were compared with those of white light. Microalgae production was about 45% higher with white light compared to green, and the corresponding microalgae production rate for green light was about 64 mg/L/day. The microalgae production rate was highest in white light, followed in order by red, blue, and green light. Therefore, white light providing both 450–475 nm and 630–675 nm, the ranges used for photosynthesis by microalgae, is more appropriate for the growth of microalgae than providing a single wavelength. The CO_2 fixation rate in all types of light was 1.8–1.9 mg CO_2/g microalgae and was similar to literature values (Oilgae, 2010) (see Fig. 1).

3.1.2. Nitrogen and phosphorus removal

Nitrogen and phosphorus removal efficiencies by *Scenedesmus* sp. were evaluated depending on wavelength. The removed nitro-