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Growth and uptake kinetics of nitrate and phosphate by benthic microalgae for phytoremediation of eutrophic coastal sediments

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HIGHLIGHTS

- We investigated the uptake rates of nitrate and phosphate by 4 benthic microalgae.
- The uptake rate were high in the order of blue, mixed, red, yellow wavelength.
- The *Nitzschia* sp. showed the highest specific uptake rates under all wavelengths.
- The *Nitzschia* sp. may be a useful species for phytoremediation.

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ABSTRACT

In the present study, the effect of monochromatic light (blue, yellow and red) and mixed wavelength on the nutrient uptake and growth kinetics of benthic microalgae *Achnanthes* sp., *Amphora* sp., *Navicula* sp. and *Nitzschia* sp. were investigated. The maximum uptake rate (ρ_{max}) for nitrate and phosphate obtained by short-term experiments were high in the order of blue, mixed, red, yellow wavelength, and among the 4 benthic microalgae, *Nitzschia* sp. was the highest ρ_{max}. The half-saturation constant (*Ks*) was higher than other taxon. The specific maximum growth rate (μ'_{max}) and minimum cell quota (q_0) for the nitrogen and phosphorus-limited condition, *Nitzschia* sp. showed the highest μ'_{max} and q_0 values among the 4 benthic microalgae. These results suggest that the benthic microalgae are adapted to high nutrient concentration. In particular, *Nitzschia* sp., which have a higher capability of storage and uptake, may be a useful species for phytoremediation.

1. Introduction

In the greater part of the bay in South Sea of Korea, domestic sewage and industrial wastewater, as well as self-pollution from aquaculture activity, are increasing organic pollution of the sedimentary environment in the coastal areas. The change in sediment quality of these environments is extremely limited compared with seawater because the material exchange in sediment is only accomplished by means of pore water. In particular, because of the low exchange rates in the semi-closed bay, decomposition of accumulated organic matter leads to anoxic conditions at the sediment–water interface. These conditions, in turn, lead to problems such as a decrease in microbial activity, loss in biodiversity, and the accumulation of toxic gasses (*i.e.* ammonia and hydrogen sulfide). To remediation of the eutrophic coastal sediments, physical methods (dredging and aeration), chemical methods (additional yellow loess, slag and oyster shell) and biological methods (microbial activity) have been used (Murphy and Prepas, 1990; Karim et al., 2003; Yamamoto et al., 2008; Asaoka and Yamamoto, 2010).

In Korea, the dredge is considered to be the most practical method, and it is used throughout the enclosed eutrophic bay. However, it need huge budgets as paid the 42 billion won for a 5 year (1 dollar = *ca.* 1300 won) in Masan Bay, where is one of eutrophic coastal area of Korea. Moreover, the method generated secondary problems such as silt diffusion, prohibition of agricultural work, treatment of contaminated dredged sediment and the huge budget. Therefore, ideal remediation methods should be eco-friendly methods as phytoremediation, which enough leads to self-purification system with natural environment, and is not a surgical operation such as dredging.

Light is the important energy inducing photosynthesis of microalgae. The spectral quality of light is known to affect many aspects of the physiology: pigment composition, photosynthesis, chemical composition, growth rate, and ion transport (Wallen and Geen, 1971; Kowallik, 1987; Oh et al., 2008; Das et al., 2011). Some researchers have investigated the effects of monochromatic light on growth in several microalgae (Sánchez-Saavedra and Voltolina,

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1994; Oh et al., 2006, 2008; Wang et al., 2007; Das et al., 2011). Some diatoms (*e.g. Chaetoceros* sp. and *Skeletonema costatum*), blue-green algae (*e.g. Spirulina platensis*), and green algae (*e.g. Chlorella* sp. and *Nannochloropsis* sp.) have been grown under all wavelengths (blue, yellow, red), and the wavelength for optimal growth was shown differ by species (Sánchez-Saavedra and Voltolina, 1994; Wang et al., 2007; Oh et al., 2008; Das et al., 2011). On the other hand, the growth of some dinoflagellates (*e.g. Cochlodinium polykrikoides* and *Heterocapsa circularisquama*) was inhibited under wavelengths from green to yellow wavelength (Oh et al., 2006, 2008). Thus, the growth of microalgae under monochromatic light might be species-specific or taxon-specific.

To remediation of eutrophic coastal sediments with no light due to high suspended solid, Oh et al. (2007) suggested that phytoremediation using benthic microalgae (BMA) and light emitting diode (LED). If the wavelength of light, which BMA could grow but not harmful algae, is able to illuminate the eutrophic coastal sediments, BMA may be able to remediate because of oxygen production and nutrient uptake during photosynthesis of BMA. In this study, we investigated the effects of monochromatic light from LEDs on the uptake and growth kinetics of nitrate and phosphate of the BMA, which are dominant in eutrophic coastal sediments. Also, based on these results, we discussed the possibility of phytoremediation using BMA and LED.

2. Methods

2.1. Strains and culture conditions

BMAs *Achnanthes* sp., *Amphora* sp., *Navicula* sp. and *Nitzschia* sp. were isolated from the surface sediments of the Sujeong Bay in Korea (N35°7′17″, E128°36′01″) in April, 2010. The isolates were rinsed repeatedly sterile seawater during the logarithmic growth phase, and were treated with the antibiotic mixture AM9 for axenic cultures. This procedure was repeated until axenic conditions could be confirmed using fluorochrome 4′,6-diamidino-2-phenylindole (DAPI) staining. Stock cultures were maintained in East Sea open water (N36°24′94″, E130°42′06″) enriched with f/2 medium (Guillard and Ryther, 1962), and containing 0.001 μM selenium (as H_2SeO_3) as suggested by Doblin et al. (1999). All media were adjusted to pH 8.2 using HCl or NaOH, and sterilized by membrane filter (Sterivex-GS, 0.22 μm filter, Millipore, USA). Temperature, salinity, and light conditions for stock cultures and all experiments were 20 °C, 30 psu, and 100 μmol/m^2/s (measured with QSL-2101, Biospherical Instruments, USA) under cool-white fluorescent lamps with an illumination cycle of 12 h:12 h light/dark cycle. Prior to the experiments, all equipment and glassware were treated with approximately 30% HCl, and then thoroughly rinsed with distilled water and autoclaved at 202 kPa for 20 min.

2.2. Short-term uptake experiment (time-course experiment)

To evaluate the relationship between nutrient uptake rate and ambient nutrient concentration, short-term uptake experiments should be carried out during of constant uptake rate (Harrison et al., 1989). According to Harrison et al. (1989), time-course experiments were conducted prior to the uptake experiments to determine the time period for the later experiments. In order to nitrogen-deficient cells, 4 BMAs were incubated with artificial seawater L1 medium (Keller et al., 1987) containing no nitrogen sources for 5 days. After 5 days in nitrogen free L1 medium, direct counting confirmed failure to grow, indicating nitrogen deficiency. Nitrogen-deficient cells of 4 BMAs were inoculated approximately 3×10^3–1×10^4 cells/ml, and 20 μM of nitrate was added. After adding nitrate, the nitrate concentrations were monitored over time at 0, 10, 20, 30, 45, 60, 90, 120, 180 and 240 min. For phosphorus, a similar procedure was applied using phosphate free L1 medium for 5 days and the amount of phosphate added was 3 μM and the concentrations monitored at 0, 10, 20, 30, 45, 60, 90, 120, 180 and 240 min. Experiments were carried out all experiments were 20 °C, 30 psu, and 100 μmol/m^2/s. To assess the effects of different wavelengths, light sources were used fluorescent lamp (mixed wavelength), blue LED (450 nm), yellow LED (590 nm), and red LED (650 nm). The concentrations of nitrate and phosphate were kept after filtration through GF/C filter (1.2 μm pore size, Whatman, USA), and were analyzed by the method of Strickland and Parsons (1972).

2.3. Short-term uptake experiment (uptake experiment)

Short-term nutrient uptake experiments were conducted as follows. Nitrogen- or phosphorus-starved cultures were distributed into 14 flasks and diluted with 150 ml of either nitrogen or phosphate free L1 medium. The cell density was approximately 1×10^4–5×10^4 cells/ml. For the nitrate uptake experiments, nitrate was added to give final concentrations of 1, 3, 5, 10, 20, 50, and 100 μM. For the phosphate uptake experiments, phosphate was added to give final concentrations of 0.5, 1, 2, 5, 10, 15, and 20 μM. All experiments were conducted in duplicate. The initial and final nutrient concentrations were determined by the method described above. Just after finishing the uptake experiment, cells were counted for a small portion of the unfiltered samples under a microscope (TE-2000, Nikon, Japan). The uptake rate (ρ) was calculated from the difference between the initial and the final concentrations for each nutrient. The results were fitted to the Michaelis–Menten equation:

$$\rho = \rho_{\max} \frac{S}{Ks + S} \quad (1)$$

where $\rho_{\max}$ is the maximum uptake rate (pmol/cell/h), S is the ambient nutrient concentration (μM) and Ks is the half-saturation constant (μM). The uptake kinetic parameters were estimated using the non-linear least squares method (Abe, 1985).

2.4. Semi-continuous growth experiment

An aliquot of culture containing approximately 1×10^4 cells/ml of exponentially growing 4 BMAs were distributed into 14 flasks containing 150 ml nitrogen-limited L1 medium. Nitrogen-limited semi-continuous cultures were conducted by replacing a portion of the medium once a day at 10:00 AM, with different dilution rates (D = 0.10–0.60/day). When steady state was achieved, cell numbers and nitrate concentration in the medium were measured. At steady state, there was less than 5% variation in cell numbers on successive days. A similar semi-continuous method with the same dilution rates were applied for the phosphorus-limited medium. All experiments were conducted in duplicate. For the results of the semi-continuous experiments, the following relationship can be applied between the specific growth rate (μ, /day) and the dilution rate (D, /day) under the steady-state condition (Tilman and kilham, 1976; Nakamura, 1985):

$$\mu = \ln(1 - D) \quad (2)$$

Cell quota (Q) of each nutrient was calculated from the difference between the nutrient concentration of the medium added (S_0) and the nutrient concentration of the medium in the culture vessel (S) before the addition of fresh medium divided by the cell density (N):

$$Q = \frac{S_0 - S}{N} \quad (3)$$