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# Nitrogen Removal from Wastewaters by Microalgae Without Consuming Organic Carbon Sources

**LEE, KWANGYONG AND CHOUL-GYUN LEE***

*Institute of Industrial Biotechnology, Department of Biotechnology, Inha University, Incheon 402-751, Korea*

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**Abstract** The possibility of microalgal nitrogen treatment was tested in wastewaters with a low carbon/nitrogen (C/N) ratio. *Chlorella kessleri* was cultured in the two different artificial wastewaters with nitrate as a nitrogen source: one contained glucose for an organic carbon source and the other without organic carbon sources. The growth rates of the two cultures were almost identical when the aeration rate was over 1 vvm. These results suggest that microalgae could successfully remove nitrogen from wastewater, as far as the mass transfer of $CO_2$ was not limited. Nitrate was successfully reduced to below 2 mg $NO_3^-$-N/ml from the initial nitrate concentration of 140 mg $NO_3^-$-N/ml in 10 days, even in the wastewater with no organic carbon source. Similar results were obtained when ammonium was used as the sole nitrogen source instead of nitrate. Higher concentrations of nitrogen of 140, 280, 560 and 1,400 mg/ml were also tested and similar amounts of nitrogen were removed by algal cultures without showing any substrate inhibition.

**Key words:** Nitrogen removal, low C/N ratio, *Chlorella kessleri*, wastewater treatment

Microalgae have vast industrial and economic potential [27] as valuable sources for pharmaceuticals, health foods, carotenoids [20, 25], dyes, fine chemicals, biofuels, and others [7, 8, 21]. The history of the commercial use of algal cultures with various applications covers about 50 years [4, 26]. Furthermore, they may be able to solve emerging environmental problems, such as the greenhouse effect [22, 23] and waste treatments [5, 19, 24, 30]. They can fix carbon dioxide by photosynthesis [17, 18, 29] and remove excess nutrients efficiently at a minimal cost [2, 9, 10, 22]. In addition, photosynthetically produced oxygen can relieve biological oxygen demand (BOD) in wastewater. Microalgae also have the ability to take up various kinds of nitrogen [11, 13], and to absorb heavy metals [12, 14] and phosphorus [1]. They can utilize various organic compounds - particularly eutrophic compounds containing nitrogen and phosphorus - for their carbon sources. As a result, many researchers have studied microalgae as a possible solution to environmental problems [6, 15, 16].

In general, both nitrogen and phosphorus are the major sources of eutrophication, therefore, high concentrations of nitrogen or phosphorus can cause algal blooms and other hazardous environmental problems [28]. Urban wastewaters with major eutrophic compounds have an adverse effect on the ecological system of water. In Korea, algal blooms increase every year and so do the environmental and ecological damages. However, most terminal facilities for wastewater treatments at present operate with only primary and secondary treatments, therefore, with insufficient nitrogen removal, especially when the C/N ratio is relatively low. So, a tertiary treatment system must be installed to prevent algal blooms, and other environmental and ecological impacts. Current biological wastewater treatment systems have a drawback in that the influent stream must have a specified ratio of carbon to nitrogen compounds, because the microorganisms in the wastewater treatment system have a fixed ratio of C/N. Korean urban sewage has a low C/N ratio and the traditional biological wastewater system cannot remove all the nitrogen from the Korean urban sewage. As a result, these biological systems cannot meet the government criteria if the C/N ratio was outside of a certain range.

This study is focused on applications for nitrogen removal in wastewaters with low C/N ratios, exploiting the photosynthetic ability of microalgae. When microalgal cells are cultured under photoautotrophic conditions, these cells can utilize molecular $CO_2$ from air or bicarbonate ions. Thus, microalgal cultures can remove excess nitrogen together with $CO_2$ while minimizing the reduction in chemical oxygen demand (COD) and total organic carbon (TOC). Thus, removal efficiencies under various aeration rates and with various concentrations of other organic

*Corresponding author
Phone: 82-32-860-7518; Fax: 82-32-872-4046;
E-mail: leecg@inha.ac.kr

carbon and/or nitrogen sources were tested, and the results obtained are reported herein.

## MATERIALS AND METHODS

### Microorganism and Media

*Chlorella kessleri* (UTEX 398), a green eukaryotic photoautotrophic microorganism from the UTEX (The Culture Collection of algae at the University of Texas at Austin, TX, U.S.A.) was cultured in the N-8 media [31], which served as an artificial wastewater. The media contains nitrate and phosphate but no organic carbon. Removal rates of nitrogen and other eutrophic compounds were compared in the original N-8 media and in modified N-8 media supplemented with organic carbon sources. Cultures were maintained in 250-ml Erlenmeyer flasks with 100 ml of the media at 30°C and 200 rpm.

### Cell Concentration and Cell Size Distribution

Cell concentration and size distribution were measured by a Coulter Counter (Model Z2, Coulter Electronics, Inc., Hialeath, FL, U.S.A.). The Coulter method of sizing and counting particles is based on measurable changes in electrical resistance produced by nonconductive particles suspended in an electrolyte (ISOTON II, Coulter Electronics, Ltd., Hong Kong, China).

### Bubble Column Photobioreactors (BCPs)

BCPs consist of columns, light sources, and pumps for air injection. Columns were made of Pyrex tubes (650 mm of height, 35 mm of internal diameter). Fluorescent light tubes (FL20D, OSRAM, Korea) were used as light sources for photosynthesis. Two fluorescent lamps were placed at a distance of 20 mm from the photobioreactor columns. Light intensity was set to 200 µmol $m^{-2}$ $s^{-1}$ on the column surface. Light intensity was measured by a Quantum Sensor (Model LI-190SA, LI-COR, Lincoln, NE, U.S.A.) with a Datalogger (model LI-1400, LI-COR). Filtered air, containing 0.03% $CO_2$, was injected to the bottom of the glass columns at a constant flow rate. The temperature was kept at between 25 and 30°C. The pH was adjusted between 6.0 and 8.0, using KOH and HCl.

### Analysis

The concentrations of nitrate, ammonium, and total organic carbon (TOC) were measured after removing algal cells by centrifugation at 3,000 rpm for 10 min. Nitrate and ammonium concentrations were determined by an ion meter (Model 750P, Istek, Korea) with an ammonium ion selective electrode (ISE) and a nitrate ISE, respectively (Models NH41502-003B and NO31502-003B, respectively, Phoenix Electrode Co., Houston, TX, U.S.A.). Samples were diluted to the calibration curve range. TOC was measured by a TOC analyzer (TOC 5000, Shimadzu, Japan). After filtering through 0.45 µm filter, COD was measured according to the standard methods [3]. Excess potassium dichromate and organic compounds were oxidized with chromic and sulfuric acids at 150°C for 2 h. After oxidative digestion, remaining potassium dichromate was titrated with ferrous ammonium sulfate, followed by calculation of oxygen equivalent.

## RESULTS AND DISCUSSION

### The Effect of Aeration on Cell Growth and Nitrate Removal by BCPs

Carbon and nitrogen are necessary elements for algal growth and maintenance. Carbon and nitrogen contents of typical microalgae are 50% and 10%, respectively. Some algae, such as *Chlorella*, can take up both inorganic and organic forms of carbon. Some algae can grow only on $CO_2$ via photoautotrophic growth. Even if algae metabolize gaseous $CO_2$ from air for a carbon source, they still require a nitrogen source in the culture broth. This property of microalgae will allow them to selectively remove nitrogen from wastewaters without using organic carbon sources.

Two kinds of artificial wastewaters were prepared; one did not contain organic carbon sources, and the other contained 1.126 g/l of glucose as organic carbon. Inorganic carbon was supplied as $CO_2$ gas by blowing air into the culture broth. Organic carbon was supplied in the form of glucose. When the culture was inoculated at $10^7$ cell/ml and cultured with an aeration rate of 0.2 vvm (vol $vol^{-1}$ $min^{-1}$) in the two forms of wastewaters, faster growth was observed in wastewater with the organic carbon source

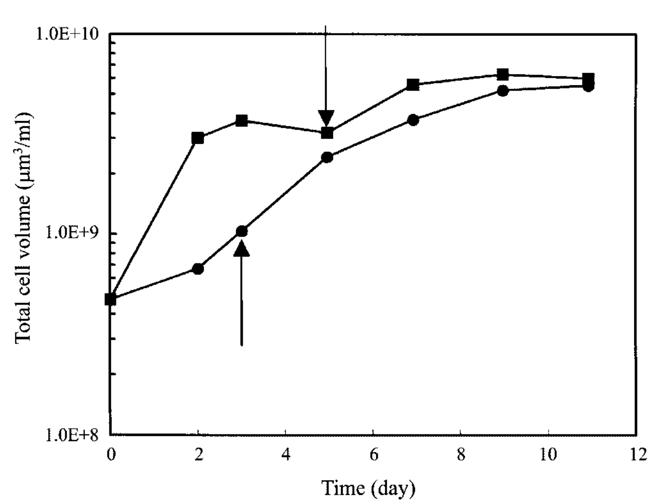


**Fig. 1.** Cell growth in BCPs with different aerations and carbon sources.
The arrows indicate the change in flow rate, from 0.2 to 1.0 vvm; wastewater without glucose, (-●-); glucose-containing wastewater, (-■-).

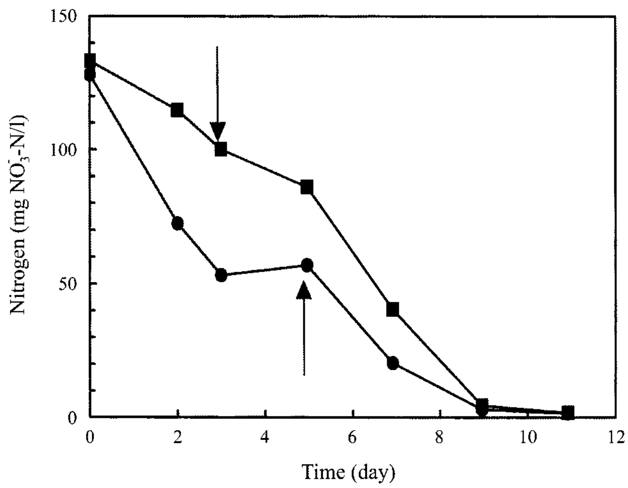


**Fig. 2.** The effect of aeration on nitrogen removal in BCPs.
The arrows indicate the change in flow rate, from 0.2 to 1.0 vvm; wastewater without glucose, (-●-); glucose-containing wastewater, (-■-).

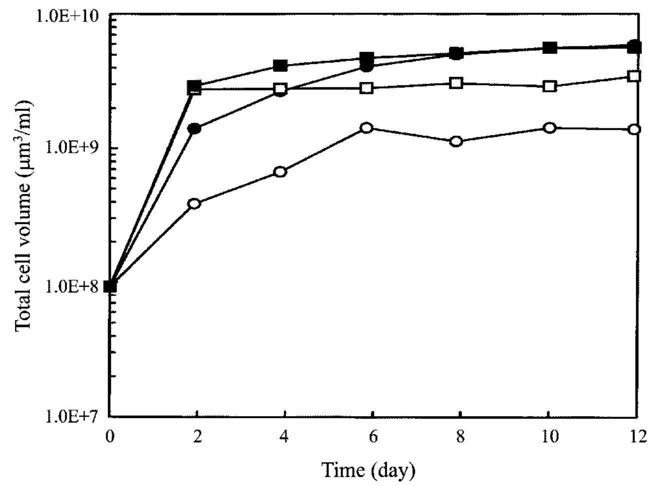


**Fig. 3.** The effect of aeration rate and carbon source on cell growth in BCPs.
Wastewater with 0.2 vvm aeration with no extra carbon source, (-○-); wastewater with 1.0 vvm aeration without any other carbon source, (-●-); addition of 1.125 g/l of glucose wastewater with 0.2 vvm aeration, (-□-); addition of 1.125 g/l of glucose wastewater with 1.0 vvm aeration, (-■-).

(Fig. 1). After two days, total organic carbon was reduced to about 200 mg/l from 650 mg/l (data not shown). This result suggests either that this algal strain preferred glucose as carbon source to inorganic $CO_2$ or that the $CO_2$ supply rate was not high enough to support the growth rate. The aeration rate was increased from 0.2 to 1.0 vvm on Day 3, to determine which of the two possibilities was correct. As can be seen in Fig. 1, the growth rate in the wastewater without the organic carbon source increased after increasing the aeration rate. Thus, an aeration rate of 0.2 vvm was not sufficient to transfer enough $CO_2$ for maximum growth. If enough $CO_2$ was supplied, the growth rate in the media with no organic carbon was comparable to that in the media with glucose. On the 10th day, the initial nitrogen of 130 mg/l was consumed in both types of the media (Fig. 2). The current biological wastewater treatment systems encounter a difficult situation when the C/N ratio is low, since the systems cannot remove all the incoming nitrogen. The results shown here (Figs. 1 and 2) can be an excellent solution to the problems for the conventional biological systems.

**Removal of Nitrate-N by Using BCPs**

Two forms of artificial wastewaters with low C/N ratio were used for microalgal nitrate removal experiments: one with glucose as the organic carbon source and the other with no organic carbon source but with aeration of atmospheric air at 0.2 or 1.0 vvm. Total cell volume, which was calculated by multiplying the cell concentration and average cell size, was increased to $6.5\times10^9$ $\mu m^3$/cell in the artificial wastewater without glucose but with aeration of 1.0 vvm. A similar growth pattern was observed in the other type of wastewater that contained glucose (Fig. 3). As shown in Fig. 3, the growth profiles show almost identical patterns regardless of the existence of organic carbon sources, when the aeration rate was high (1.0 vvm). Over 98% of initial 127 mg $NO_3$-N/l was removed after 10 days of culture in both BCPs with 1.0 vvm aeration (Fig. 4). These results proved that microalgae can selectively remove nitrogen without using any organic carbon sources.

**Removal of Ammonium-N by Using BCPs**

Various forms of nitrogen, such as nitrate and ammonium, in urban wastewaters can be taken up by most algae.

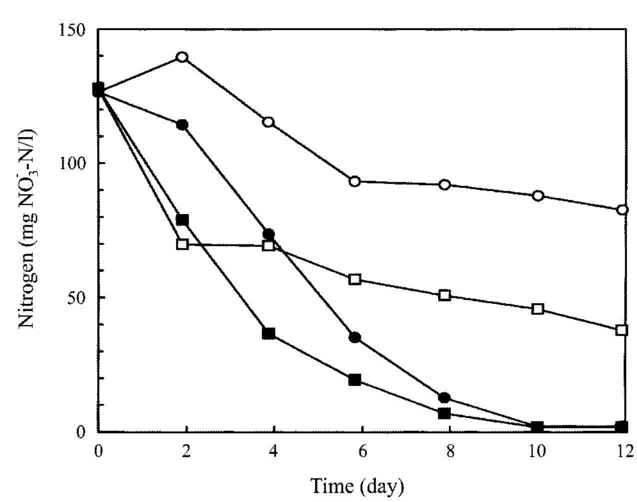


**Fig. 4.** The effect of aeration rate and carbon source on nitrogen removal in BCPs.
Wastewater with 0.2 vvm aeration with no extra carbon source, (-○-); wastewater with 1.0 vvm aeration without any other carbon source, (-●-); addition of 1.125 g/l of glucose wastewater with 0.2 vvm aeration, (-□-); addition of 1.125 g/l of glucose wastewater with 1.0 vvm aeration, (-■-).

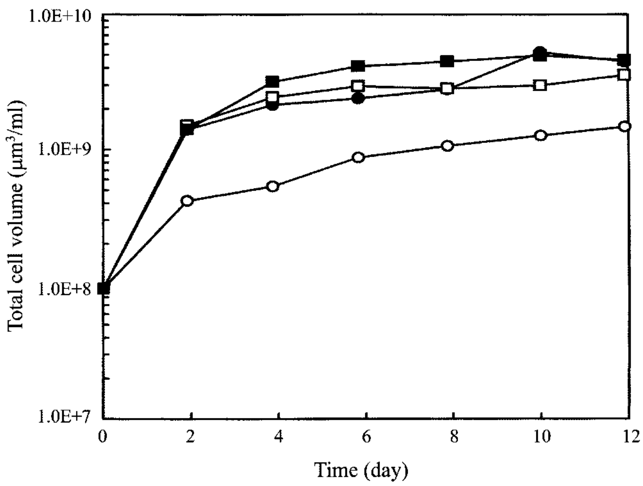


**Fig. 5.** Cell growth with ammonium as N source in BCPs.
Addition of 0.53 g $NH_4Cl$/ml ammonium (instead of nitrate in the original wastewater) with 0.2 vvm aeration, (-○-); addition of 0.53 g $NH_4Cl$/ml ammonium with 1.0 vvm aeration, (-●-); addition of 0.53 g $NH_4Cl$/ml ammonium in glucose containing wastewater with 0.2 vvm aeration, (-□-); addition of 0.53 g $NH_4Cl$/ml ammonium in glucose containing wastewater with 1.0 vvm aeration, (-■-).

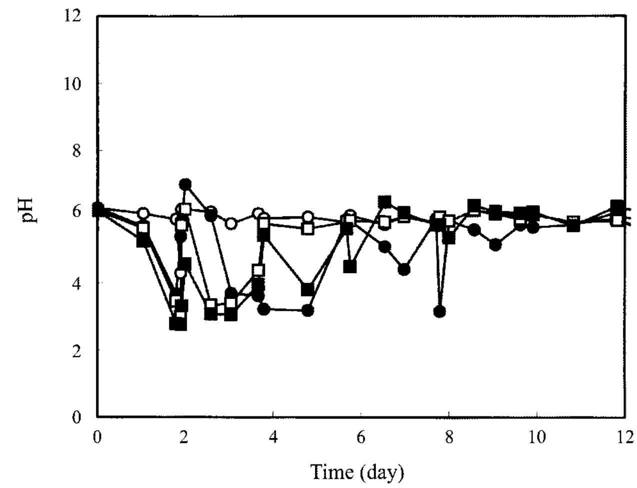


**Fig. 7.** pH variation during cell growth with ammonium-N contained media in BCPs.
Addition of 0.53 g $NH_4Cl$/ml ammonium (instead of nitrate in the original wastewater) with 0.2 vvm aeration, (-○-); addition of 0.53 g $NH_4Cl$/ml ammonium with 1.0 vvm aeration, (-●-); addition of 0.53 g $NH_4Cl$/ml ammonium in glucose containing wastewater with 0.2 vvm aeration, (-□-); addition of 0.53 g $NH_4Cl$/l ammonium in glucose containing wastewater with 1.0 vvm aeration, (-■-).

Therefore, another experiment was set up to check the removal ability of ammonium-N by microalgae. The media containing ammonium and equivalent molar nitrogen were prepared. The removal rate of ammonium-N was comparable to that of nitrate-N from the previous experiments, as shown in Figs. 5 and 6. However, the overall nitrogen removal efficiency at Day 12 was lower (85% removed at 1.0 vvm) than that of wastewater containing nitrate (98.6% removed with 1.0 vvm). This was rather expected, since large amounts of ammonium produced protons, reducing the pH (Fig. 7). The low pH condition must have had a harmful effect on algal growth and thus nitrogen consumption. More detailed study is necessary to verify the exact cause of slower growth in ammonium-containing media.

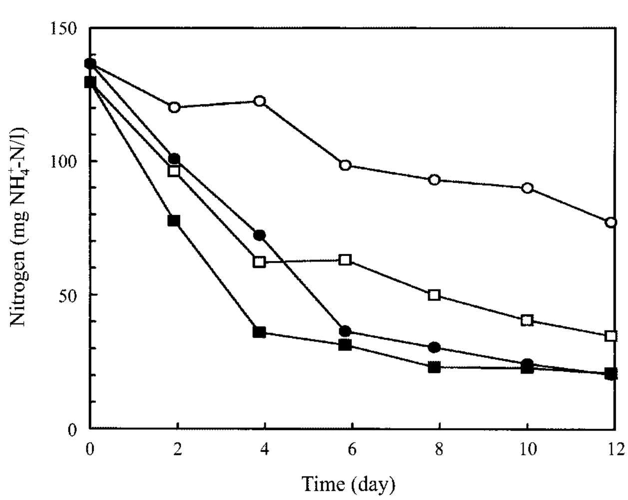


**Fig. 6.** Removal of ammonium by using BCPs.
Addition of 0.53 g $NH_4Cl$/ml ammonium (instead of nitrate in the original wastewater) with 0.2 vvm aeration, (-○-); addition of 0.53 g $NH_4Cl$/ml ammonium with 1.0 vvm aeration, (-●-); addition of 0.53 g $NH_4Cl$/ml ammonium in glucose containing wastewater with 0.2 vvm aeration, (-□-); addition of 0.53 g $NH_4Cl$/ml ammonium in glucose containing wastewater with 1.0 vvm aeration, (-■-).

## Nitrogen Removal on High Concentration of Nitrate in Wastewaters

Wastewaters from certain industrial sectors, such as textile manufacturers or plating plants, contain very high concentrations of nitrogen. Consequently, it is necessary to confirm that microalgae can tolerate extremely high concentrations of nitrogen, in order to apply microalgal waste treatment systems to the wastewaters with high concentrations of nitrogen. As shown in Fig. 8, cell growths in artificial wastewaters, including various concentrations of nitrogen ranging from 140 to 1,400 mg-N/l, were almost identical, suggesting that the microalgal strain used here could tolerate 1,400 mg-N/l of nitrogen. In this experiment, an interesting trend was observed. As seen in Fig. 8, the final cell concentration tended to be lower as the initial nitrogen concentration increased: the final cell concentration in the original wastewater was the highest ($6.6\times10^7$ cell/ml) and that in the wastewater with 10 times higher nitrogen was the lowest ($3.3$–$5.4\times10^7$ cell/ml). However, as shown in Fig. 9, the trends of the average cell size showed the opposite, as the average cell size was directly proportional to the nitrogen contents of the wastewater. In the wastewater containing 10 times more nitrogen than the

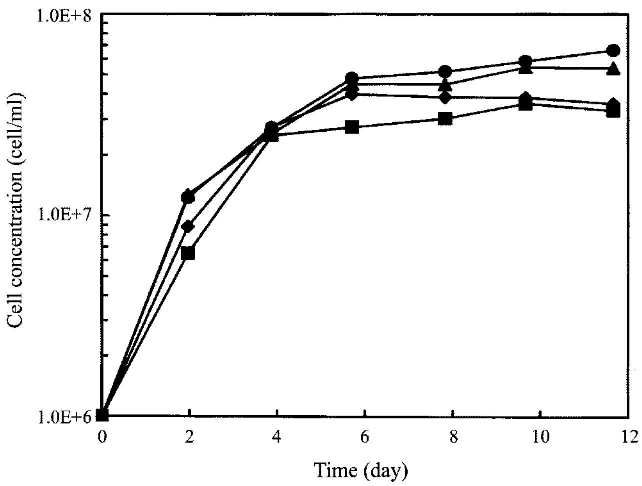


**Fig. 8.** The effect of various nitrate concentrations on cell growth with 1.0 vvm aeration.
The original wastewater, (-●-); wastewater with 2 times higher concentration of nitrate, (-▲-); wastewater with 5 times higher concentration of nitrate, (-◆-); wastewater with 10 times higher concentration of nitrate, (-■-).

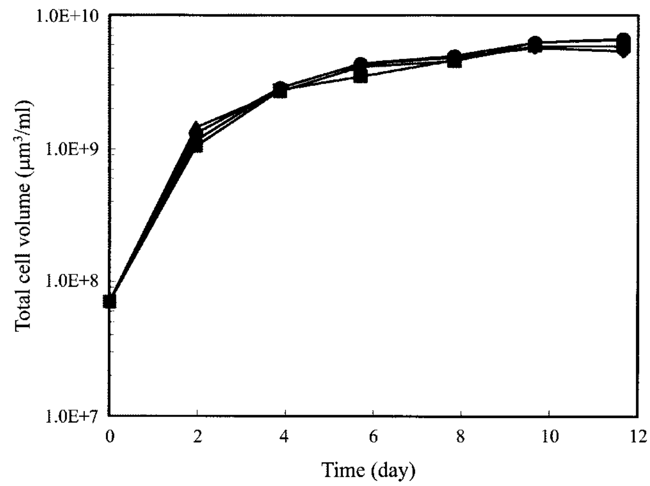


**Fig. 10.** The effect of various nitrate concentrations on total cell volume by using BCPs at 1.0 vvm aeration.
The original wastewater, (-●-); wastewater with 2 times higher concentration of nitrate, (-▲-); wastewater with 5 times higher concentration of nitrate, (-◆-); wastewater with 10 times higher concentration of nitrate, (-■-).

original artificial wastewater, the cell concentration was 50% more than that in the original wastewater. The average cell size in high nitrogen media was 150 $\mu m^3$/cell, which was 50% bigger than the cells in the original wastewater (Fig. 9). The exact reason is not known, but a high concentration of nitrogen might suppress cell division or promote cell aggregation. Due to the higher average cell volume, the total cell volume showed similar growth patterns, regardless of initial nitrogen concentration (Fig. 10). Nitrogen concentration profiles are shown in Fig. 11. There were large errors in reading nitrate-N, because the concentration in the wastewater containing 10 times higher nitrogen fluctuated. However, it was clear that the amount of nitrogen removal was directly proportional to the increase of total biomass. These results are potentially very promising, since microalgal cultures can successfully remove nitrogen even in very high concentrations of nitrogen (1,400 mg-N/l) without dilution or any other pretreatments. As a result, microalgal cultures can be applied to the wastewaters with low C/N ratios to selectively remove nitrogen independent of carbon contents.

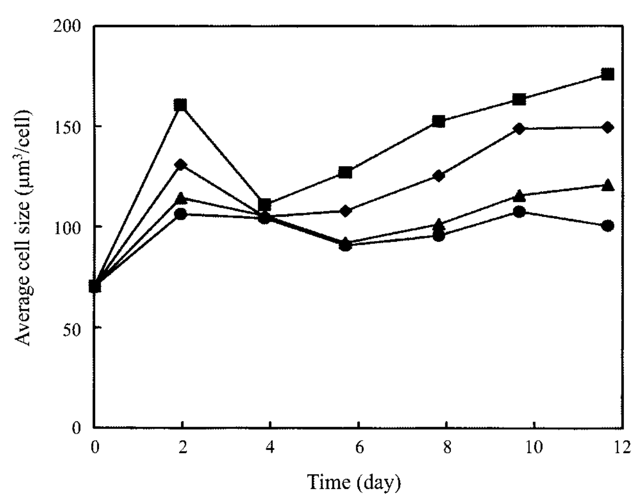


**Fig. 9.** The effect of various nitrate concentrations on average cell size by using BCPs at 1.0 vvm aeration.
The original wastewater, (-●-); wastewater with 2 times higher concentration of nitrate, (-▲-); wastewater with 5 times higher concentration of nitrate, (-◆-); wastewater with 10 times higher concentration of nitrate, (-■-).

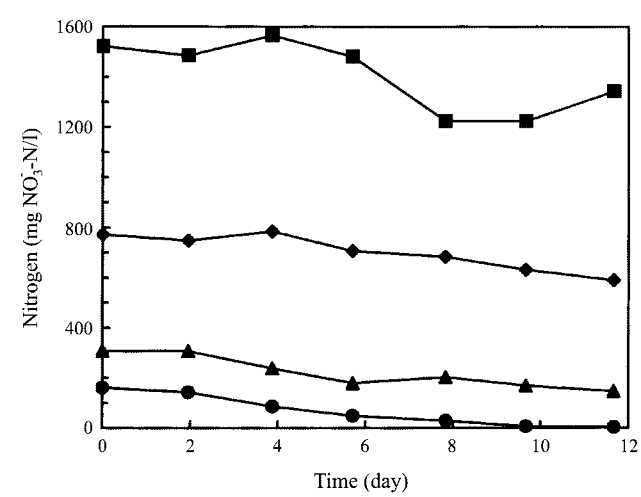


**Fig. 11.** Nitrate removal under various nitrogen concentrations by using BCPs at 1.0 vvm aeration.
The original wastewater, (-●-); wastewater with 2 times higher concentration of nitrate, (-▲-); wastewater with 5 times higher concentration of nitrate, (-◆-); wastewater with 10 times higher concentration of nitrate, (-■-).

## CONCLUSIONS

An encouraging result was obtained for wastewater treatments using microalgal bubble column photobioreactors. When the aeration rate was over 1 vvm, microalgae could effectively treat wastewaters with low or close to zero C/N ratios. Even in the wastewaters with no organic carbon sources, 98.6% and 81.1% of the initial nitrogen were removed by Day 10 (from the initial value of 127.9 to 1.8 mg $NO_3^-$-N/l and from 129.6 to 24.5 mg $NH_4^+$-N/l, respectively). The results showed that microalgal cultures could be applied to the treatment of wastewaters with low C/N ratio. Also, microalgae could efficiently remove nitrogen, even in high nitrogen concentrations over 1,400 mg-N/l. The volumetric growth in high concentrations of nitrogen over 1,400 mg-N/l was comparable to that in low nitrogen concentration wastewater. These results prove the possibility of implementing microalgal wastewater treatment systems to the wastewaters with very high concentration of nitrogen, such as the textile industrial wastewater.

The removal of nitrogen without supplement of organic carbon sources promises a bright future of the biological wastewater treatments, where the insufficient ability of nitrogen removal has long been a major problem. In conclusion, a high-density algal culture is a very effective means of treating high nitrogen content wastewaters with low C/N ratios. More detailed studies are necessary to make this process economical for the future of economic and safe waste treatment systems.

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