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Effect of Light/dark Cycles on Wastewater Treatments by Microalgae

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Abstract *Chlorella kessleri* was cultivated in artificial wastewater using diurnal illumination of 12 h light/12 h dark (L/D) cycles. The inoculum density was 10^5 cells/mL and the irradiance in light cycle was 45 μmol m^2 s^{-1} at the culture surface. As a control culture, another set of flasks was cultivated under continuous illumination. Regardless of the illumination scheme, the total organic carbon (TOC) and chemical oxygen demand (COD) was reduced below 20% of the initial concentration within a day. However, cell concentration under the L/D lighting scheme was lower than that under the continuous illuminating scheme. Thus the specific removal rate of organic carbon under L/D cycles was higher than that under continuous illumination.

This result suggested that *C. kessleri* grew chemoorganotrophically in the dark periods. After 3 days, nitrate was reduced to 136.5 and 154.1 mg NO_3^--N/L from 168.1 mg NO_3^--N/L under continuous illumination and under diurnal cycles, respectively. These results indicate nitrate removal efficiency under continuous light was better than that under diurnal cycles. High-density algal cultures using optimized photobioreactors with diurnal cycles will save energy and improve organic carbon sources removal.

Keywords: microalgae, nitrogen removal, diurnal cycle, wastewater

INTRODUCTION

Microalgae have a vast industrial and economic potential as valuable sources for pharmaceuticals, health foods, carotenoids, dyes, fine chemicals, biofuels, and other applications [1,2]. The history of the commercial use of algal cultures spans about 50 years resulting in various applications [3,4]. Furthermore, they may serve as a solution to emerging environmental problems such as greenhouse effect and waste treatments [5-7] because they can fix carbon dioxide by photosynthesis and remove excess nutrients efficiently at a minimal cost [8-11].

Moreover, photosynthetically produced oxygen can relieve biological oxygen demand (BOD) in wastewater. Microalgae also have the ability to absorb heavy metals and to reduce nitrogen and phosphorus levels [12]. They can also utilize various organic compounds – especially eutrophic compounds containing nitrogen and phosphorus – for their carbon sources. As a result, many researchers have been studying microalgae as a possible solution to environmental problems [13-15].

In general, phosphorus is often the limiting nutrient for algal blooms or algal growth in seas and lakes. However, both nitrogen and phosphorus are major sources of eutrophication and thus a high concentration of nitrogen or phosphorus can cause algal blooms and other hazardous environmental problems. Urban wastewaters having major eutrophic compounds cause adverse effects on the ecological system of water. In Korea, algal blooms increase every year and so does the environmental and ecological damage. But currently, most terminal facilities for wastewater operate only primary and secondary systems that have an insufficient removal of nitrogen. Consequently a tertiary treatment system must be installed to prevent algal blooms and other environmental and ecological sequelae.

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 systems must have a fixed carbon-to-nitrogen ratio (C/N ratio). Korean domestic sewage has a low C/N ratio and thus the traditional biological wastewater systems cannot remove all the nitrogen sources from domestic sewage. As a result, these biological systems cannot meet government criteria if the C/N ratio is outside of a certain range.

Microalgae have adapted to an intermittent light environment in nature for over 1 billion years. There are seasonal changes, periodic diurnal variations of the solar light and intermittence by streams and waves. The key to the efficient photosynthesis lies in their adaptation to these changing-light conditions. This paper suggests that microalgae will show effect of L/D cycles on the removal of eutrophic compounds.

The present study is focused on microalgal application in the treatment of wastewaters having a low C/N

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by exploiting the photosynthetic ability of microalgae. When microalgal cells are cultured under photoautotrophic conditions, they can utilize molecular CO_2 from air. Thus, microalgal cultures can remove excess nitrogen sources even though there are just trace amounts of total organic carbon (TOC).

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, USA) was cultured in N-8 media [16], which served as artificial wastewater. This 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 100 mL of media in 250-mL flasks at 30°C, 200 rpm. Light intensity was measured by a Quantum Sensor (model LI-190SA, LI-COR, Lincoln, NE, USA) with a Datalogger (model LI-1400, LI-COR).

Cell Concentration and Cell Size Distribution

The cell concentration and the size distribution were measured by a Coulter Counter (Model Z2, Coulter Electronics, Inc., Miami, FL, USA). 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).

Analysis

The concentrations of phosphate, nitrogen TOC, and COD were measured after removing algal cells by centrifugation at 3,000 rpm for 10 min. Phosphate contents were measured by the vanadomolybdophosphoric acid colorimetric method [17]. In a dilute orthophosphate solution, ammonium molybdate reacts under acidic conditions to form a heteropoly molybdophosphoric acid. When vanadium reacts with molybdophosphoric acid, yellow vanadomolybdophosphoric acid is produced. The intensity of the yellow color is proportional to the phosphate concentration in the samples. Spectrophotometry was used at 400 nm for the quantification of phosphate.

Nitrate concentrations were determined using an ion meter (model 750P, ISTEK, Korea) with a nitrate ion selective electrode (NO31502-003B Phoenix Electrode Co, Huston, TX, USA). Samples were diluted to a precalibrated range before measurement.

TOC was measured by a TOC analyzer (TOC 5000, Shimadzu, Kyoto, Japan). Samples were filtered using a 0.45 μm filter before measuring TOC. Samples were combusted to CO_2 and H_2O. TOC value was calculated by the difference between total carbon concentration and inorganic carbon concentration.

COD was measured according to the Standard Methods [17]. Excess potassium dichromate and organic compounds were oxidized by chromic and sulfuric acid at 150°C for 2 h. After oxidative digestion, remaining potassium dichromate was titrated with ferrous ammonium sulfate. Then, oxygen equivalent was calculated.

RESULTS AND DISCUSSION

Cell Growth under Different Light Conditions

C. *kessleri* was cultured in flasks at 30°C and 300 rpm. Two sets of flasks were prepared: one was cultured under continuous light as a control set and the other was cycled between light and dark conditions (12:12 h). The artificial wastewater used was a modified N-8 media containing 12 mg PO_4^{3-}-P/L of phosphorus and 1.125 g/L of glucose.

The inoculum density was 10^5 cells/mL and the irradiance in the light cycle was 45 μmol m^{-2} s^{-1} at the culture surface. Fig. 1(a) shows that the cell concentration was increased to 1.6×10^7 cells/mL under continuous light in 3 days. However, the total cell concentration in flasks with L/D lighting scheme was increased to 9.1×10^6 cells/mL during the same period was only 57% of that under continuous illumination. The difference in cell concentration between the two lighting schemes was proportional to the duration of light exposure. The reduction in cell biomass under the L/D lighting scheme seemed to night biomass loss by respiration. The existence of dark periods could make difficult environmental conditions for autotrophic microalgae. This could explain the relatively bigger average cell size under L/D cycles, since cells would not divide under unfavorable conditions (Fig. 1(b)).

As shown in Fig. 1(c), the profiles of average chlorophyll concentration were almost identical, but that of the total chlorophyll concentration was quite different (Fig. 1(d)), because of a much higher cell concentration under the continuous light scheme. The specific growth rate under L/D lighting scheme was lower than that under the continuous light scheme (Fig. 2).

Consumption of Organic Carbons by Microalgae

Carbon is the most important element found in algal biomass and it constitutes over 50% in typical algal biomass [18]. Some algae like *Chlorella* can grow both autotrophically and heterotrophically [19]. However, microalgae cannot metabolize all the organic sources. Simple organic carbon sources such as acetate and glucose are usually preferred by microalgae.

When the cultures were grown in artificial wastewaters containing 1.125 g/L of glucose, organic carbon was

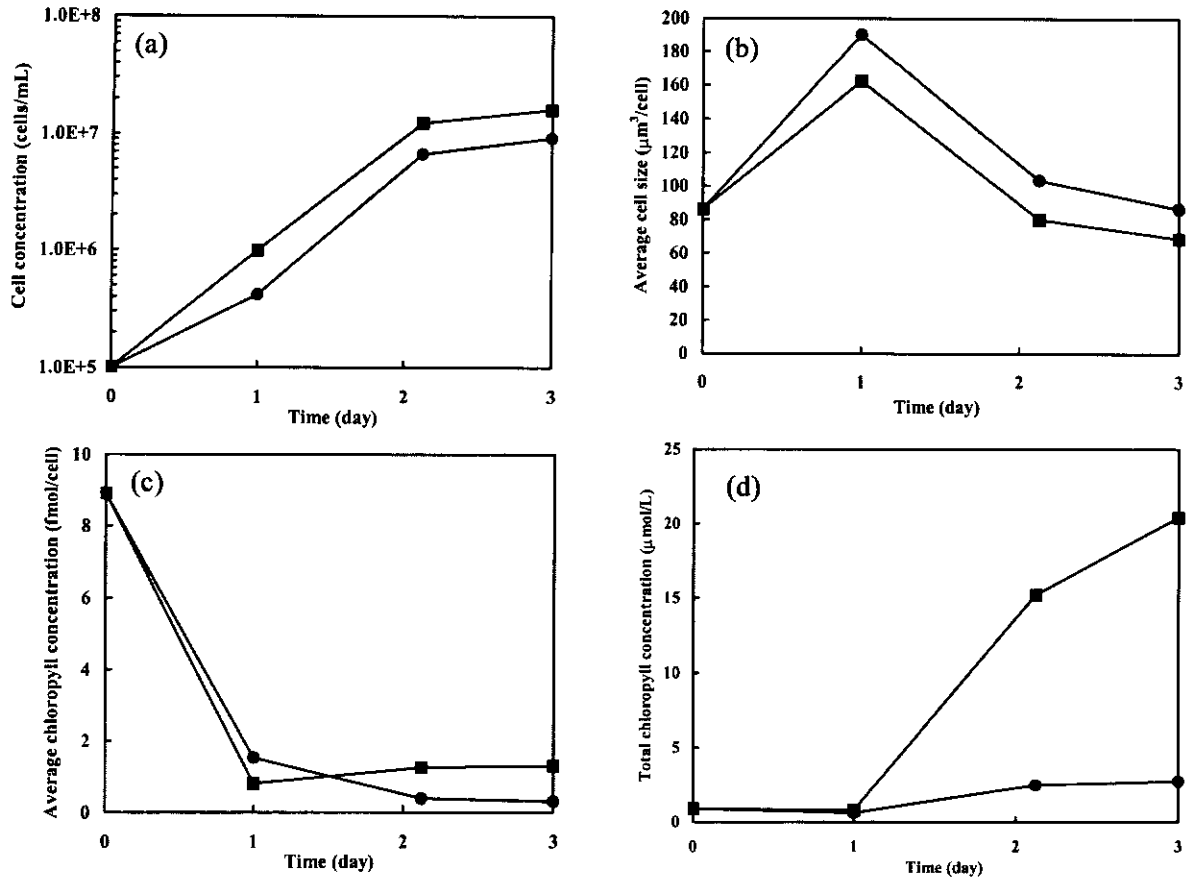

Fig. 1. The effect of diurnal cycles on (a) cell concentration profiles; (b) changes in average cell size; (c) average chlorophyll concentration per cell; and (d) total chlorophyll concentration; continuous illumination, (■); L/D cycles, (●).

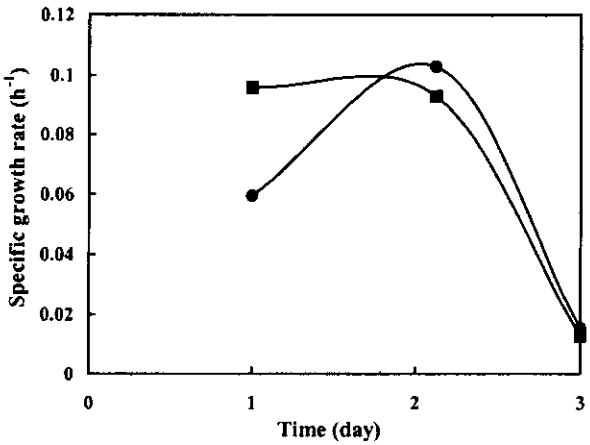

Fig. 2. Specific growth rate; continuous illumination, (■); L/D cycles, (●).

depleted drastically within the first day of culture. The results are summarized in Table 1. In continuous illumination and diurnal cycles, TOC was reduced to 81.4 and 71.5 from 600.2 mg/L, respectively. The COD removal rate exhibited a similar trend. The results clearly showed that organic carbon could be reduced in a relatively short period by the microalgae. However, the cell concentration under L/D cycles was lower than that under continuous illumination. Thus the specific removal rate of organic carbon under L/D cycles was higher than that under continuous illumination culture. This result suggested that *C. kessleri* grew chemoorganotrophically during dark periods.

Effect of Light Conditions on Nitrate Removal

Microalgae contain 5-10% nitrogen [18] and they can utilize nitrate as a nitrogen source for their growth. In order to study the illumination effects on nitrogen removal in wastewater, the nitrate concentration profiles were obtained under the two different lighting schemes. Under continuous illumination, nitrogen was reduced to 136.5 from 168.1 mg NO_3^--N/L in 3 days. In contrast, nitrate was reduced to 154.1 mg NO_3^--N/L at diurnal cycles of light in 3 days (Fig. 3). In other words, the amounts of nitrate removed were 31.6 and 14.0 mg NO_3^--N/L, under the continuous lighting scheme and the L/D lighting scheme, respectively. During the same

Table 1. Cell concentration, TOC, and COD at day 1 under different illumination schemes

Illumination	Cell concentration (cells/mL)	TOC (mg/L)	TOC removal %	Removed TOC/ Increased cell (mg/cell)	COD (mg/L)	COD removal %	Removed COD/ Increased cell (mg/cell)
Continuous	9.9×10^5	81.4	86.4	5.7×10^{-4}	89.2	83.3	5.0×10^{-4}
L/D cycles	4.7×10^5	71.5	88.1	1.4×10^{-3}	72.2	86.5	1.3×10^{-3}

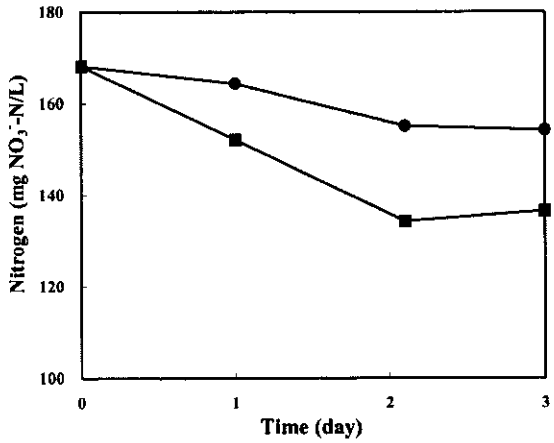

Fig. 3. The effect of lighting schemes on nitrate removal; continuous illumination, (■); L/D cycles, (●).

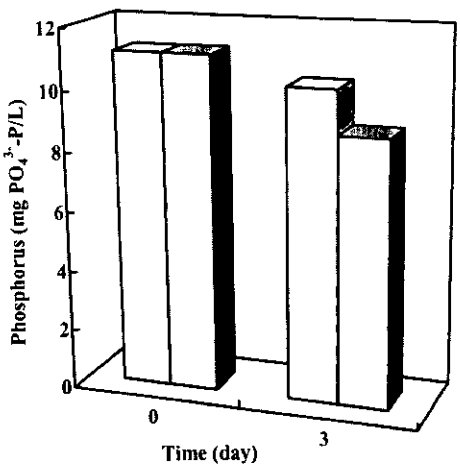

Fig. 4. The effect of lighting schemes on phosphorus removal; white bar presents phosphorus removal under continuous illumination; gray bar is under L/D cycles.

period, the cell concentration was increased to about 9.1×10^6 and 1.6×10^7 cells/mL under the L/D lighting scheme and the continuous lighting scheme, respectively. Previous results showed that the average cell size was depended on surrounding environment. Therefore, a direct comparison of cell concentration cannot properly represent cell growth under different conditions. To compare the cell growth under different lighting conditions, the total cell volume was used, which could be calculated by multiplying the cell concentration (cells/mL) and the average cell size (μm^3/cell). Then, algal biomass (dry cell weight) could be calculated from the total cell volume using a simple conversion factor, assuming a constant density of algal cells. Considering nitrogen content of algal biomass of 5-10% (about 15-30 mg of nitrogen is required for 300 mg dry cell weight), the amounts of nitrogen removal in the two sets of experiments were expected to be 13.6-27.1 mg-N/L in the continuous lighting scheme and 9.8-19.5 mg-N/L in the L/D lighting scheme. The actual amounts of nitrogen removal from experiments were 31.6 and 14.0 mg-N/L under continuous and L/D lighting scheme, respectively. The difference between the expected and the measured nitrogen amount under continuous illumination suggested that light can stimulate nitrogen consumption. Nitrogen containing compounds such as ATP and NADPH are produced actively when microalgae undergo photosynthesis, or when the cells are illuminated.

Effect of Light Conditions on Phosphorus Removal

Fig. 4 shows phosphorus removal by microalgae at day 3. The cultures under L/D cycles showed a slightly better removal efficiency of phosphate-P than that under continuous light. But the phosphorus removal ability under both lighting schemes was not efficient, because the amount of removed phosphate were only 8-20% of the initial amounts. More studies are necessary to elucidate the mechanisms of phosphorus metabolism such as phosphate transport, phosphate hydrolysis, and ATP formation from phosphate in algae. Many more parameters, such as light intensity and duration, chemical effects, temperature, and pH, must be examined to elucidate the effect of phosphate metabolism in an algal wastewater treatment system.

C. kessleri Cultures in Flasks for Nitrogen Removal under Continuous Light

Microalgae need carbon, nitrogen, phosphorus and other micronutrients to grow. They can fix carbon dioxide from air through photosynthesis while taking up nitrogen and phosphorus from wastewaters.

In order to prove the nitrogen removal in typical wastewaters, where the nitrate concentration is much

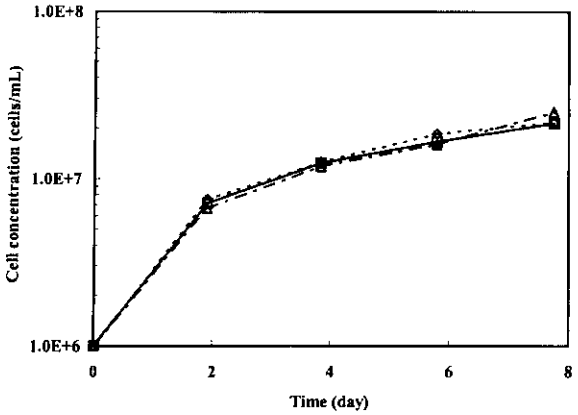

Fig. 5. Cell growth in flasks at 30°C, 200 rpm; 140 mg NO_3^--N/mL in artificial wastewater, (■); NO_3^- concentration was 50% of the wastewater, (◆); NO_3^- concentration was 10% of the wastewater, (▲).

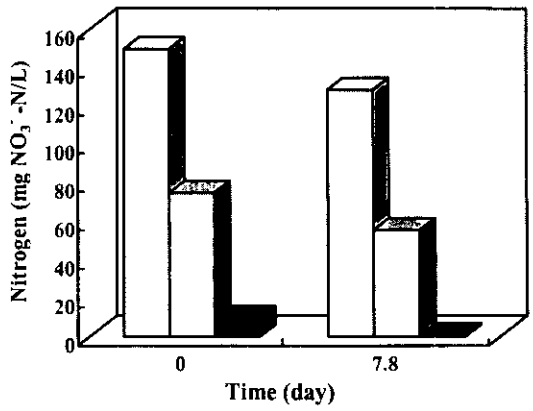

Fig. 6. Removal of nitrate in flasks at 30°C, 200 rpm; 140 mg NO_3^--N/mL in artificial wastewater, white bar, NO_3^- concentration was 50% of the wastewater, gray bar, NO_3^- concentration was 10% of the wastewater, black bar.

lower, media with lower nitrate contents were tested. *C. kessleri* were cultured in 250-mL Erlenmyer flasks in a shaking incubator at 30°C, 200 rpm with a working volume of 100 mL. The intensity of the continuous light was 45 μmol m^{-2} s^{-1} at the culture surface. Artificial wastewater contained various concentrations of nitrogen, 10 to 140 mg-N/L, from but without organic carbon sources.

Fig. 5 shows the cell growth under different nitrogen concentrations. The initial nitrogen concentration was 149.9, 74.7 and 9.7 mg NO_3^--N/mL, respectively. Although the initial nitrogen concentrations were quite different, the final cell concentrations were about the same, about 2×10^7 cells/mL. The results suggested that cell growthmay be limited by other factors such as CO_2 mass transfer, mixing, light intensity, *etc.* These results show that there was no growth inhibition by a high concentration of nitrate and that microalgae are promising candidates for domestic wastewater treatment with a low C/N ratio.

CONCLUSION

Chlorella kessleri was cultivated in artificial wastewater under two different lighting schemes: continuous illumination and diurnal illumination. Regardless of the illumination scheme, the total organic carbon and chemical oxygen demand was reduced below 20% of the initial concentration within a day. As expected, the cell concentration under the L/D lighting scheme was lower than that under the continuous illuminating scheme. However, introduction of L/D cycles gives better efficiency in the removal of organic carbon and phosphrous per increased biomass, while nitrate removal efficiency under the continuous light is slightly higher than that under L/D cycles. *C. kessleri* could grow chemoorganotrophically during the dark periods since they can metabolize the organic carbons for their growth without photosynthesis. These results prove that there is a great potential using microalgae powerful, environmentally friendly wastewater treatment method, particularly for eutrophic compound-rich wastewaters having a low C/N ratio. High-density algal cultures using optimized photobioreactors with diurnal cycles can also save energy and enhance the removal efficiency of organic carbon sources.

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