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Removal of nitrogen and phosphorus by *Chlorella sorokiniana* cultured heterotrophically in ammonia and nitrate

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ABSTRACT

The growth rate of biomass and removal characteristics of nitrogen and phosphorus were examined using nitrate and ammonia as nitrogen sources for batch cultures of the heterotrophic microalgae, *Chlorella sorokiniana*. The initial nitrogen concentrations examined were 10, 20, 40, 80 and 160 mg-N l^{-1}. The results show that a nitrate concentration of 80 mg-N l^{-1} as a nitrogen source provided the maximum growth rate and greatest removal of nitrogen and phosphorus, while the growth rate decreased with decreasing ammonia concentration. The maximum growth rate and removal amounts of nitrate and phosphorus were 0.48 d^{-1}, 32.6 mg-N and 5.9 mg-P, respectively, with 80 mg NO_3–N l^{-1}. With ammonia as a nitrogen source, the maximum growth rate and removal amounts of nitrogen and phosphorus were 0.6 d^{-1}, 79.2 mg-N and 5.9 mg-P, respectively, with 160 mg NH_3–N l^{-1}. When ammonia was used as a nitrogen source, removal rates of nitrogen and phosphorus were higher than those with nitrate. However, the pH decrease caused by ammonia affected the growth of *Chlorella sorokiniana*, resulting in a lower yield of biomass than when using nitrate as a nitrogen source.

1. Introduction

Since the early 1970s, studies on advanced wastewater treatment using microalgae have been performed in the USA and Germany (McGriff and McKinney, 1971; Osward et al., 1978; Shelef et al., 1978). Recently, microalgae used for advanced wastewater treatment include autotrophic microalgae that can grow using an inorganic carbon source and can perform photosynthesis (Wang et al., 2010; Cristina et al., 2011; Su et al., 2011).

Wastewater treatment using autotrophic microalgae makes it possible to fix nitrogen with a low C/N ratio, an economical removal of nitrogen and phosphorus. However, autotrophic microalgae are limited in achieving high wastewater treatment efficiency because of a low biomass yield due to insufficient light from the shading effect among microalgae (Muge et al., 2012). This problem can be solved by using a heterotrophic culture that has a rapid growth rate and an organic compound as a substrate and that can freely grow in spite of the shading effect because heterotrophic microalgae can grow under lightless conditions (Perez-Garcia et al., 2011). In addition, heterotrophic cultures have not only high biomass production compared to autotrophic cultures, but also high oil contents in vivo, resulting in high lipid production that is valuable in terms of bio-fuel (Vazhappilly and Chen, 1998; Xu et al., 2006; Liang et al., 2009).

Microalgae need carbon, nitrogen and phosphorus for growth, with nitrogen as the second most important component following carbon affecting microalgae growth (Shi et al., 1997). Microalgae metabolize by absorbing nitrate, ammonia and urea as nitrogen sources.

Ammonia concentration and pH are very important components of microalgae growth (Tam and Wong, 1996). Becker (1994) reported that ammonia as a single nitrogen source negatively affects microalgae growth because of sharply decreased pH, and a greater than 5 mM (85 ppm) ammonia concentration and pH greater than 8 toxically act on the growth of *Dunaliella* species. In addition, Przytocka-Jusiak (1976) reported that an ammonia concentration of 330 mg l^{-1} results in an about 50% growth inhibition for *Chlorella vulgaris*, and Matusiak (1976) reported that *C. vulgaris* does not grow at pH 8–9 or in an ammonia concentration of 700 mg l^{-1}. Shi et al. (2000) reported that an ammonia concentration of 84 mg l^{-1} in a culture of *Chlorella protothecodis* decreased the pH to 4, in which the logarithmic growth phase of the microalgae could not be observed. These results imply that, when ammonia is used as a nitrogen source, the ammonia concentration and pH are major factors affecting microalgae growth.

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When nitrate is used as a nitrogen source, the nitrogen concentration and pH affect microalgae growth. When *C. vulgaris* was cultured in heterotrophic conditions with nitrate as a nitrogen source in a pH range of 3–9, the optimal pH for growth was 7, while microalgae could not grow at pH 3 and presented a low growth rate at pH 9 (Mayo and Noike, 1996). In a study by Jeanfils et al. (1993), when *C. vulgaris* was cultured in a nitrate range of 0–1360 mg l^{-1}, a maximum biomass production of 72 mg l^{-1} was obtained, while biomass production decreased as concentration increased.

In the case of nitrate as a nitrogen source, microalgae absorb nitrate into the cell and then reduce it to nitrite by nitrate reductase, then continuously reducing the nitrite to ammonium by nitrite reductase and finally converting the ammonium to amino acids by glutamine synthetase and glutamate synthetase (Becker, 1994; Perez-Garcia et al., 2011). In contrast, in the case of ammonia as a nitrogen source, microalgae directly absorb ammonia into the cell, accumulate it in the form of amino acids and use the amino acids for metabolism (Yang et al., 2000). Therefore, ammonia as a nitrogen source is generally favorable for intake by microalgae (Kim et al., 2010; Perez-Garcia et al., 2011). Uptake rate and growth characteristics differ between nitrogen sources due to different nitrogen uptake mechanisms of microalgae.

Almost all previous studies have been performed with autotrophic microalgae, so studies on the growth characteristics of heterotrophic microalgae are insufficient. Studies about growth characteristics of biomass and removal of nitrogen and phosphorus depending on influent wastewater properties with heterotrophic microalgae for advanced wastewater treatment are especially limited. Therefore, in this study, the heterotrophic microalga *Chlorella sorokiniana* was selected for wastewater treatment, and the effects of microalgae growth on the removal of nitrogen, phosphorus and organic compounds in different nitrogen sources and concentrations were evaluated.

2. Materials and methods

2.1. Microalga strain and culture conditions

The strain of *C. sorokiniana* (UTEX 1670) was obtained from University of Texas, Austin. The strain was maintained in liquid proteose medium. The proteose medium consisted of the following components (mM): $NaNO_3$ (2.94), $CaCl_2 \cdot 2H_2O$ (0.17), $MgSO_4 \cdot 7H_2O$ (0.3), K_2HPO_4 (0.43), KH_2PO_4 (1.29), NaCl (0.43) and 1 g l^{-1} of proteose peptone. Ten grams per liter of glucose was added to the medium as an organic carbon source for the heterotrophic culture. For the stock culture, *C. sorokiniana* cultured in solid medium were inoculated with 200 mL of the proteose medium in a 250 mL cell culture flask. The flask was then incubated at 25 °C using a rotary shaker and agitated at 140 rpm in dark conditions. The pH of the medium was adjusted to 7.0 after autoclaving.

2.2. Experimental procedure

Batch experiments were performed for seven days to evaluate the growth characteristics of *C. sorokiniana* and the removal of organic carbon, nitrogen and phosphorus according to nitrogen source and concentration. Proteose medium was added into a 1 l Erlenmeyer flask, and the working volume was 600 ml. *C. sorokiniana* at 1.0 OD_{540} were inoculated into the solution at a proportion as great as 10% (v/v), about 50 mg l^{-1} of initial biomass concentration. Glucose as an organic carbon and phosphorus in the form of KH_2PO_4 and K_2HPO_4 were injected as 5 g l^{-1} and 10 mg-P l^{-1}, respectively. Nitrogen concentrations were set at 10, 20, 40, 80 and 160 mg-N l^{-1} using $NaNO_3$ and NH_4Cl as nitrogen sources. The initial pH before inoculating *C. sorokiniana* was 7, and the cell culture flasks were operated under dark conditions at a shaking speed of 140 rpm at 25 °C. The pH was adjusted to 7 every day using 10% HCl and 1 M NaOH.

2.3. Analytical method

Liquid samples of 10 mL were filtered through a 0.47-μm GF-C filter, and the filtered paper was dried for 2 h at 105 °C. Biomass was calculated from the dry weight, and the filtrate was used for measuring glucose, nitrate, ammonia and total phosphorus. The glucose concentration was analyzed using a UV spectrometer (HS3300, Humas Co.) via the DNS method. The nitrate, ammonia and total phosphorus were measured using a water analyzer (HS 3300, Humas, Korea).

3. Results and discussion

3.1. Effects of nitrogen source on pH and algal growth

Fig. 1(a) and (b) show the results of *C. sorokiniana* cultured in heterotrophic conditions on the pH variation by nitrogen source and concentration. Generally, heterotrophic microalgae oxidize organic compounds and produce carbon dioxide, resulting in pH decreases, while pH variation is dependent on the nitrogen source (Shi et al., 2000; Park et al., 2010). In this study, when ammonia was

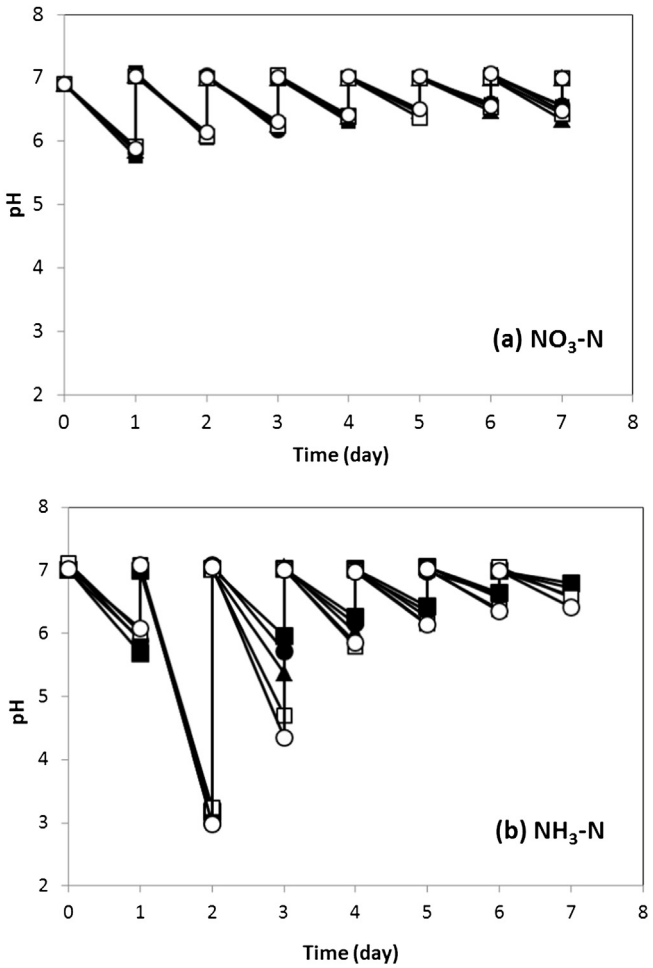

Fig. 1. Cultivation of *C. sorokiniana* under heterotrophic conditions, the pH variations in (a) nitrate and (b) ammonia concentrations: (■) 10 mg-N l^{-1}; (●) 20 mg-N l^{-1}; (▲) 40 mg-N l^{-1}; (□) 80 mg-N l^{-1}; (○) 160 mg-N l^{-1}.