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Full Length Research Paper

Growth characteristics and nutrient removal properties of the freshwater cyanobacterium *Synechococcus* sp. PCC7942 under different kinds of nitrogen sources

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Microalgae are promising candidates for the nutrient removal in tertiary treatment of domestic wastewater. In this study, batch experiments were carried out to investigate the effects of five different kinds of nitrogen sources ($NaNO_3$, urea, $NaNO_2$, NH_4Cl and glycine) on the growth characteristics and nutrient removal properties of a freshwater cyanobacterium, *Synechococcus* sp. PCC7942. Among the nitrogen sources, the order of specific growth rate (*r*) of the microalga was NH_4-N>NO_3-N>NO_2-N>urea-N>glycine-N, while that of chlorophyll yield coefficients was glycine-N>NO_3-N>NO_2-N>NH_4-N>urea-N. With nitrate, nitrite and glycine as nitrogen source, total nitrogen (TN) and total phosphorus (TP) could be removed efficiently. However, final cell density and nutrient removal efficiency were relatively low when urea or ammonium was used as nitrogen source.

Key words: *Synechococcus* sp., growth characteristics, chlorophyll a content, nutrient removal property, nitrogen source.

INTRODUCTION

Nitrogen and phosphorous discharged through agricultural sewage and industrial effluent are the major contributors to ecological eutrophication (Liu et al., 2010; Abe et al., 2002). Though primary and secondary treatment of wastewater help in settling down of solid materials and removal of organic matter, secondary effluents from wastewater treatment plants still contain higher nitrogen and phosphorous contents in the form of nitrate, nitrite, ammonia/ammonium, and phosphorus (Abe et al., 2002).

Many studies demonstrated that cultivating microalgae for tertiary wastewater treatment could reduce nutrient costs for microalgal cultivation and preserve precious freshwater resources (Li et al., 2010; Khan and Yoshida, 2008). Thus, microalgae have received much attention for the nutrient removal in recent years, including *Scenedesmus* (Li et al., 2010), *Chlorella* (Sánchez et al., 2001), *Haematococcus plurialis* (Kang et al., 2006) and *Spirulina* (Olguín, 2003). *Synechococcus* sp. PCC7942 is a unicellular cyanobacterium in freshwater, which preferentially uses inorganic nitrogen for growth (Luque et al., 1994). However, the influences of different nitrogen sources on nutrient removal properties of *Synechococcus* sp. PCC7942 have seldom been reported. The aim of this work was to study the effects of different nitrogen sources on growth characteristics, chlorophyll a content and nutrient removal properties of *Synechococcus* sp. PCC7942.

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MATERIALS AND METHODS

Microorganism

A unicellular cyanobacterium, *Synechococcus* sp. PCC7942, was used in this study and obtained from Prof. Xiaowen Zhang (Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences). The stock culture and inoculum were grown in BG11 medium (Stanier et al., 1971). The inoculum was pre-cultured aseptically in 500 ml Erlenmeyer flasks with 200 ml of BG11 medium, which were placed in a 28ºC illumination incubator

(Jiangnan Instrument Factory, Ningbo, China) for 5 days under 12 h light/12 h dark and 40 μE m^{-2} s^{-1}.

Culture conditions

Cultures were also grown in 500 ml Erlenmeyer flasks containing 200 ml of modified BG11 medium with 15 mg L^{-1} total nitrogen (TN) and 1.3 mg L^{-1} total phosphorus (TP) (simulating the typical concentration of nitrogen and phosphorus in secondary effluents) (Li et al., 2010). In all experiments, $K_2HPO_4 \cdot 3H_2O$ (9.6 mg L^{-1}) was used as phosphorus source. Besides nitrogen and phosphorus, the composition of other elements was the same as BG11 medium. The flasks were shaken three times every day during cultivation and pre-cultivation.

Experimental set up

After pre-cultivation, algal inoculums reached the exponential phase of growth. A volume of 40 ml algal inoculums were collected by centrifugation (4000 g, 4℃, 15 min), and the deposited algal cells were washed with 15 mg L^{-1} $NaHCO_3$ solution twice. The algal cells were re-suspended in 1.0 ml $NaHCO_3$ solution and inoculated into the growth medium. The cultivation conditions were described above.

The effects of five kinds of nitrogen sources, $NaNO_3$ (91.1 mg L^{-1}), urea (32.2 mg L^{-1}), $NaNO_2$ (73.9 mg L^{-1}), NH_4Cl (57.3 mg L^{-1}) and Glycine (80.4 mg L^{-1}) on algal growth characteristics, chlorophyll a content and nutrient removal properties were studied.

Microalgal growth analysis

Microalgal cell density was determined turbidometrically at 680 nm using a spectrophotometer (UV1800, Mapada Instruments Company, Shanghai, China) every 2 days. According to our previous study, the relationship between the microalgal cell density (D, cells ml^{-1}) and optical density of microalgal culture at 680 nm (OD_{680}) was shown in Equation 1:

$D=1.827\times10^{8}OD_{680}$, R=0.9916 (1)

Furthermore, logistic equation, a classical model in describing the relationship between microorganism's growth rate and cell density in limited environmental conditions (Liu, 1999), was used to analyze microalgal growth in this study and shown in Equation 2:

$$D=\frac{K}{1+e^{a-rt}} \quad (2)$$

where D (cells ml^{-1}) is the microalgal cell density at time t (d); K (cells ml^{-1}) is the carrying capacity (Shen and Shi, 2002; Lampert and Sommer, 2007), that is, the maximum microalgal cell density reached in the culture; a is a constant; r (d^{-1}) is the specific growth rate.

Chlorophyll a content determination

Triplicate 5 ml of well-blended cultures were centrifuged at 4000 g for 15 min to discard the supernatants. And the pellets were homogenized with 80% acetone for chlorophyll a extraction. The mixtures were vigorously shaken using a vibrator, and then placed in a refrigerator in the dark at 4℃ for 24 h. The extracted samples were centrifuged at 10,000 rpm for 5 min to remove the pellets. Supernatants were transferred into 1×1 cm glass cuvettes, and measured for chl a content at 663 and 646 nm using a spectrophotometer. All absorbance values were corrected using the 80% acetone as control. The concentration of chl a was calculated by the following equation (Wang, 2006) (Equation 3):

Chl a (mg L^{-1}): $C_a=12.21A_{663}-2.81A_{646}$ (3)

Analysis of nutrient removal properties

Samples withdrawn from flasks were centrifuged at 4000 g to separate microalgae, and then the supernatants were filtered through a Millipore filter paper (pore size of 0.22 μm) for the measurement of TN and TP according to the Monitoring Method of Water and Wastewater. All tests were carried out in triplicate (n = 3).

In batch kinetics, the initial substrate removal rates, R_i, and yield coefficients, Y_i, were used to analyze the nutrient removal properties of *Synechococcus* sp. PCC7942 under different kinds of nitrogen sources. The rate of the removal of a substrate of interest, R_i, (i=phosphate-P, Nitrate-N, Nitrite-N, Ammonium-N or Organic-N), was calculated by the following equation (Wang and Lan, 2011) (Equation 4):

$$R_i=-\frac{S_{0,i}-S_i}{t_0-t} \quad (4)$$

where R_i (mg L^{-1} d^{-1}) represents the substrate removal rate; $S_{0,i}$ (mg L^{-1}) is the initial concentration of substrate i, and S_i ((mg L^{-1}) is the corresponding substrate concentration at time t (d), which is the time at which concentration of the substance did not change significantly.

Based on the concentration of chl a, yield coefficients for nitrogen sources and phosphorus sources were calculated by using Equations 5 and 6, respectively (Aslan and Kapdan, 2006).

$(\text{chl a})_f-(\text{chl a})_i=Y_N(S_{N,0}-S_{N,f})$ (5)

or

$(\text{chl a})_f-(\text{chl a})_i=Y_P(S_{P,0}-S_{P,f})$ (6)

where $(\text{chl a})_f$ is the final chl a concentration (mg L^{-1}), $(\text{chl a})_i$ is the initial chl a concentration (mg L^{-1}) at the beginning of the experiments; Y_N (mg chla mg^{-1} N) and Y_P (mg chla mg^{-1} P) are the yield coefficients; $S_{N,0}$, $S_{P,0}$ and $S_{N,f}$, $S_{P,f}$ are the initial and the final concentrations of nitrogen sources and phosphorus sources (mg L^{-1}), respectively.

Statistical analyses

SPSS PASW Statistics 18 software was used for statistical analyses. The mean values, confidence intervals and standard deviation values of the triplicates for each treatment were calculated. The effects caused by different nitrogen sources on the growth characteristics, chlorophyll a content and nutrient removal properties were evaluated by one-way analysis of variance (ANOVA) at $P<0.05$.

RESULTS AND DISCUSSION

Microalgal growth characteristics

Nitrogen is an important nutrient for the production of

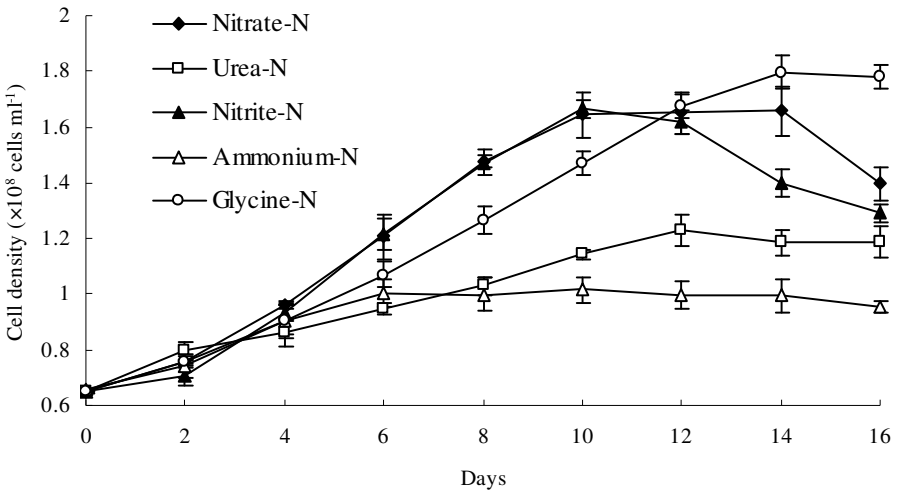

Figure 1. Growth profiles of *Synechococcus* sp. PCC7942 grown in different nitrogen sources. Points represent means of three replicates (n=3); *error bars* represent standard deviations.

microalgal biomass. The nitrogen content in microalgal biomass can range from 1% to more than 10%, which is dependent upon the amount, availability and type of nitrogen source (Vieira et al., 2001; Markou and Georgakakis, 2011). In this study, the effects of different kinds of nitrogen sources on cell density and growth characteristics of *Synechococcus* sp. PCC7942 were compared and analyzed.

The growth profiles of *Synechococcus* sp. PCC7942 with different nitrogen sources were shown in Figure 1. The specific growth rates (r) with nitrate, urea, nitrite, ammonium and glycine as sole N source obtained from logistic nonlinear regression analysis were 0.311±0.056, 0.242±0.029, 0.290±0.073, 0.410±0.075, and 0.241±0.027 d^{-1}, respectively. There was a small but statistically significant difference between ammonium-N and other nitrogen sources. This indicated that *Synechococcus* sp. PCC7942 grew fastest with ammonium as nitrogen source at beginning; however, much higher final cell density was obtained in the medium containing other nitrogen source than ammonium. This may be due to the assimilation of ammonium decreasing the medium pH value. Our previous study showed that the microalgal growth was inhibited under acidic pH conditions (data not shown). The low microalgal cell density obtained in medium containing ammonium was probably attributed to pH value decrease in the medium.

Effects of different nitrogen sources on chlorophyll a content

As the main objective of this study was to access growth characteristics and nutrient removal properties of *Synechococcus* sp. PCC7942, the effects of different nitrogen sources on chlorophyll a content were evaluated and compared with $NaNO_3$, urea, $NaNO_2$, NH_4Cl and glycine as sole nitrogen source, respectively. These could eventually be translated into biomass productivity and nutrient removal rate.

As shown in Figure 2, the nitrogen source greatly influences the chl a concentrations in *Synechococcus* sp. PCC7942. The final chl a concentration was highest in the medium containing glycine. Moreover, the yield coefficients were 0.211 (2.398 mg chl a mg^{-1} P), 0.093 (1.265 mg chl a mg^{-1} P), 0.172 (1.969 mg chl a mg^{-1} P), 0.164 (1.348 mg chl a mg^{-1} P), and 0.213 mg chl a mg^{-1} N (2.421 mg chl a mg^{-1} P) for nitrate, urea, nitrite, ammonium, and glycine, respectively. Thus, nitrate and glycine are the preferable nitrogen sources for the cultivation of *Synechococcus* sp. PCC7942.

Nutrient removal properties

The changes in TN and TP with time during the 16-day batch culture were shown in Figure 3a and 3b, respectively. Total nitrogen dropped from 15 mg L^{-1} to 0.32, 1.22, 0.84, 4.35, and 0.62 mg L^{-1} in centrates after *Synechococcus* sp. PCC7942 cultivation with nitrate, urea, nitrite, ammonium, and glycine as sole nitrogen sources, respectively. In addition, the initial substrate removal rates of TN, R_N, were 0.896, 0.842, 0.877, 0.644, and 0.898 mg L^{-1} d^{-1} for nitrate, urea, nitrite, ammonium, and glycine, respectively. The low TN removal efficiencies with ammonium as the nitrogen source were related to microalgal growth.

Total phosphorus was drastically reduced from 1.3 mg L^{-1} to 0.03 mg L^{-1} during the first 12 days of cultivation with nitrate, nitrite, ammonium, and glycine. Meanwhile, TP dropped to 0.36 mg L^{-1} in urea and stayed at similar

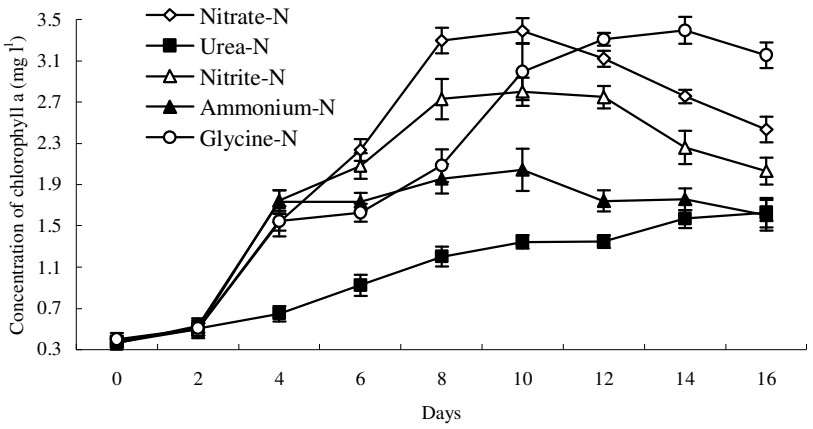

Figure 2. Time courses for chlorophyll a concentration of *Synechococcus* sp. PCC7942 grown in different nitrogen sources. Points represent means of three replicates (n=3); *error bars* represent standard deviations.

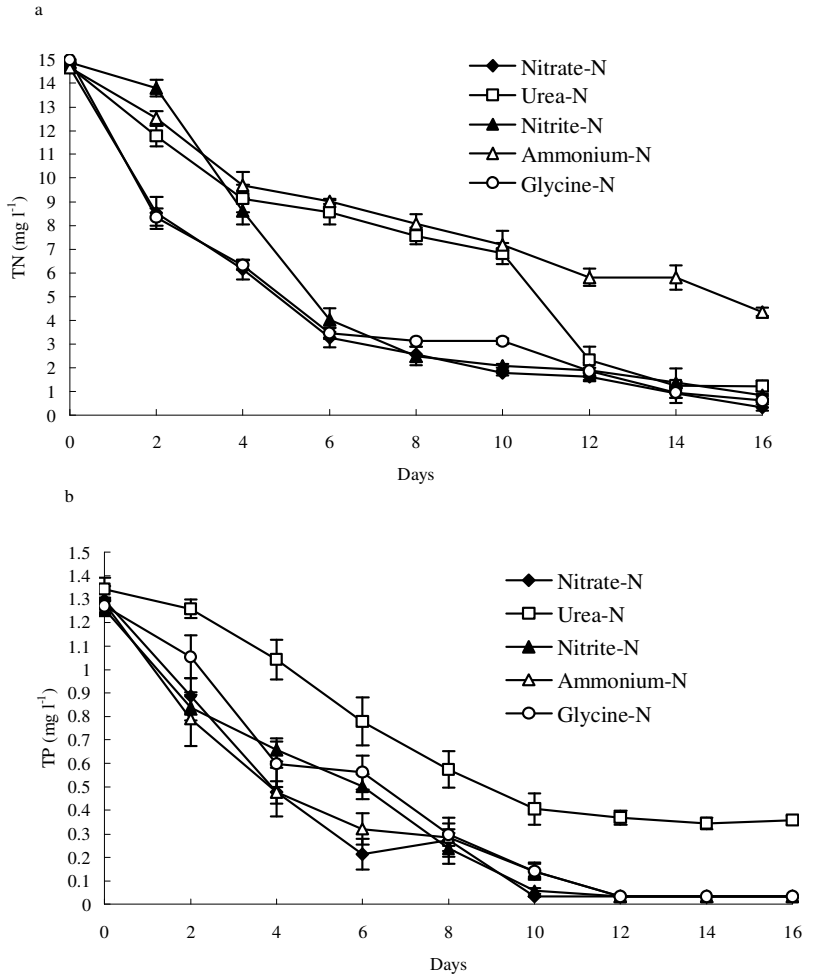

Figure 3. Changes of TN and TP with time during the 16-day batch culture of *Synechococcus* sp. PCC7942. (a) Total nitrogen removal profiles; (b) Total phosphorus removal profiles. Points represent means of three replicates (n=3); *error bars* represent standard deviations. TN and TP: abbreviation of total nitrogen and total phosphorus, respectively.

levels until the end of experiments. The initial substrate removal rates of TP, R_P during the 16-day batch experiments were 0.126, 0.081, 0.102, 0.104, and 0.103 mg L^{-1} d^{-1} for nitrate, urea, nitrite, ammonium, and glycine, respectively.

Conclusion

Synechococcus sp. PCC7942 assimilate nitrate, nitrite, and glycine well, unlike urea and ammonium. TN could be removed by 97.8, 94.3, and 95.9% with nitrate, nitrite, and glycine as the nitrogen sources, respectively; whereas TP can be removed by up to nearly 100%. *Synechococcus* sp. PCC7942 grew fastest (specific growth rate 0.410±0.075 d^{-1}) with ammonium as the nitrogen source. However, the stable phase of algal growth and nutrient removal were inhibited by the acidic pH caused by the H^+ released by the algal culture through the utilization of NH_4-N.

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