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Microalgae cultivation using an aquaculture wastewater as growth medium for biomass and biofuel production

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Abstract

Microalgae as a main feedstock has attracted much attention in recent years but is still not economically feasible due to high algal culture cost. The objective of this study was to develop a comprehensive eco-friendly technology for cultivating microalgae *Platymonas subcordiformis* using aquaculture wastewater as growth medium for biomass and biofuel production. *Platymonas subcordiformis* was grown in pretreated flounder aquaculture wastewaters taken from different stages. Each of wastewater contained different levels of nutrients. The biomass yield of microalgae and associated nitrogen and phosphorous removal were investigated. The results showed that algal cell density increased 8.9 times than the initial level. *Platymonas subcordiformi*s removed nitrogen and phosphorus from wastewater with an average removal efficiency of 87%–95% for nitrogen and 98%–99% for phosphorus. It was feasible to couple the removal of nitrogen and phosphorus from wastewater to algal biomass and biofuel production. However, further studies are required to make this technologies economically viable for algae biofuel production.

Key words: microalgae; wastewater treatment; nitrogen and phosphorus removal; biomass

Introduction

Microalgae as biofuel feedstock have gained much attention in recent years because of their high growth rate and oil content (Sheehan et al., 1998; Chisti, 2007). However, algal biofuel production has not been commercialized mainly due to high algal culture and harvest cost (Gouveia and Oliveira, 2007; Singh and Gu, 2010).

Recent studies demonstrated that microalgae have the potential for removing nitrogen and phosphorus from wastewater. These nutrients can be incorporated into algae cell biomass and subsequently removed from the wastewater. The cultivation of algae in wastewater offers the combined advantages of treating the wastewater and simultaneously producing algal biomass, which can further be exploited for valuable products (Lam and Lee, 2012; Christenson and Sims, 2011).

In our earlier work, a marine green alga, *Platymonas subcordiformis*, was shown to photobiologically evolve H_2 under the regulation of carbonylcyanide m-chlorophenylhrazone (Guan et al., 2004a, 2004b; Ran et al., 2006; Guo et al., 2008a, 2008b). To reduce the costs of algal biomass and biofuel production and wastewater treatment, the objective of this study is to develop a comprehensive eco-friendly technology for cultivating microalgae by the coupling the removal of nitrogen and phosphorus from wastewater to algal biomass and biofuel production.

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1 Materials and methods

1.1 Culture of *Platymonas subcordiformis*

The marine green algae, *P. subcordiformis* (wild-type) was grown at 25°C in an autoclaved natural seawater medium, supplemented with micronutrients with initial pH 8.0 as described earlier (Guo et al., 2008b). The cultures were illuminated from two sides with cool-white fluorescent lamps which provided an average light intensity of 50 μE/(m^2·sec) under 14 hr:10 hr light:dark cycle.

1.2 Flounder aquaculture wastewater pretreatment

Flounder aquaculture wastewater was obtained from a flounder aquaculture fishery in Dalian municipality, Liaoning Province, China. The pretreated flounder aquaculture wastewater was taken from different stages. Flounder aquaculture wastewater was filtered through a membrane filter (0.45 μm) prior to autoclaving. Therefore, only the soluble fraction of nitrogen and phosphorus was considered in the present study.

1.3 Kinetics of wastewater treatment by the cells

P. subcordiformis cultures were used to inoculate 300 mL of the pretreated wastewater in 500 mL Erlenmeyer flasks. The initial cell density was 5×10^5 cells/mL. The cultures were incubated as described above and shaken four times daily (Guo et al., 2008b). Culture density was measured by an ultra-plane hemacytometer after fixing the cells with formaldehyde.

Aliquots of *P. subcordiformis* were centrifuged at 250 g for 2 min from Erlenmeyer flasks throughout the experiment. All samples of the supernatant were collected and stored in sterile polypropylene tube at –80°C.

1.4 Analytical methods

Ammonium, nitrite, nitrate and phosphate were measured spectrophotometrically (UV BlueStar; Labtech) using the methods for the Chinese ocean monitoring standard for seawater quality (GB 12763.4-91). Ammonium was determined by the hypobromite oxidation method. Nitrite was measured by a diazotization-coupling reaction method. Nitrate was determined with the zinc cadmium reduction method. Phosphate was determined by Molybdenum Blue method.

2 Results and discussion

2.1 Removal of nitrogen from flounder aquaculture wastewater

Samples of wastewater were collected from different stages of flounder aquaculture fishery in Dalian, China to assess the potential of algae cultivation. The characteristics of wastewater in terms of nitrogen and phosphorus are shown in **Table 1**.

Figure 1 depicts the time course of removal nitrogen when the microalgae cultivated in flounder aquaculture wastewater taken from the initial stage and the final stage. The removal rates of NH_4^+-N, NO_2^--N, NO_3^--N and total nitrogen reached 0.12, 0.04, 0.35 and 0.51 mg/(L·day) in the 4-day culture, respectively. The concentration of total nitrogen was reduced from 2.39 to 0.32 mg/L (87% removal efficiency), while the removal rates of NO_3^--N reached 3.35 mg/(L·day) in the 14-day culture and the concentration of nitrogen was reduced from 47.8 to 0.82 mg/L (98% removal efficiency), respectively.

2.2 Removal of phosphate from flounder aquaculture wastewater

The time course of removal phosphate is shown in **Fig. 2** when the microalgae cultivated in flounder aquaculture wastewater taken from the initial stage and the final stage. As shown **Fig. 2a**, the concentration of PO_4^{3-}-P was drastically reduced from 0.213 to 0.005 mg/L (95% removal efficiency) in the 4-day culture and from 8.8 to 0.05 mg/L (99% removal efficiency) in the 14-day culture. The removal rate of PO_4^{3-}-P reached 0.05 mg/(L·day) in the 4-day culture and 0.63 mg/(L·day) in the 14-day culture. The removal of phosphorous was probably due to both algal metabolic uptake and phosphate precipitation (Chinnasamy, 2010a).

2.3 Algae growth in wastewater media

The effects of wastewater nutrient on the growth of *P. subcordiformis* are shown in **Fig. 3**. The growth was rapid in the beginning and remained constant after 4 days. The biomass of algae was 1.5 times in the 4-day culture and 8.9 times in the 14-day culture higher than the initial level. The result indicated that phosphate and nitrate are taken

Table 1 Composition of wastewater obtained from in flounder aquaculture fishery

	NH_4^+-N (mg/L)	NO_2^--N (mg/L)	NO_3^--N (mg/L)	PO_4^{3-}-P (mg/L)
Initial stage	0.529	0.164	1.697	0.213
Final stage	n.d.	n.d.	47.8	8.87

n.d.: Not dertermined.

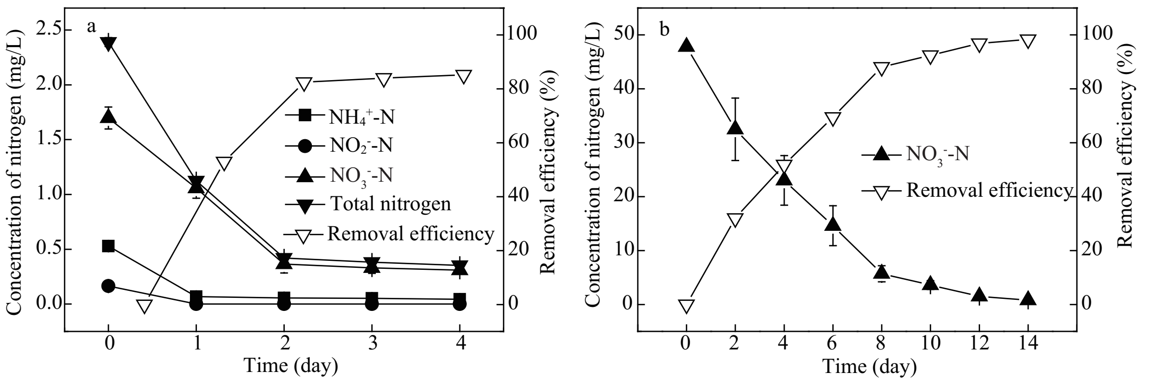

Fig. 1 Changes in nitrogen concentration and removal efficiency of *Platymonas subcordiformis* cultivated in flounder aquaculture wastewater taken from the initial stage (a) and the final stage (b). Error bars represent the standard deviation of replicate (n = 3) sediment samples.

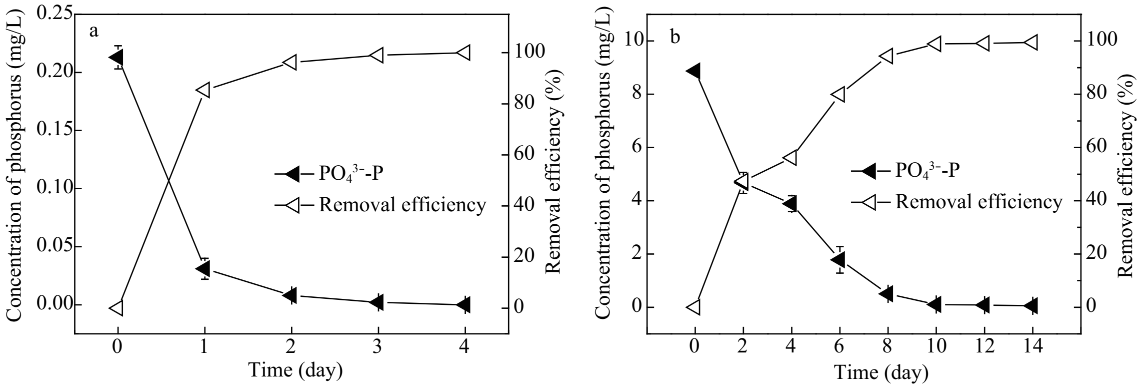

Fig. 2 Changes in phosphorus concentration and removal efficiency of *Platymonas subcordiformis* cultivated in flounder aquaculture wastewater taken from the intial stage (a) and the final stage (b). Error bars represent the standard deviation of replicate (n = 3) sediment samples.

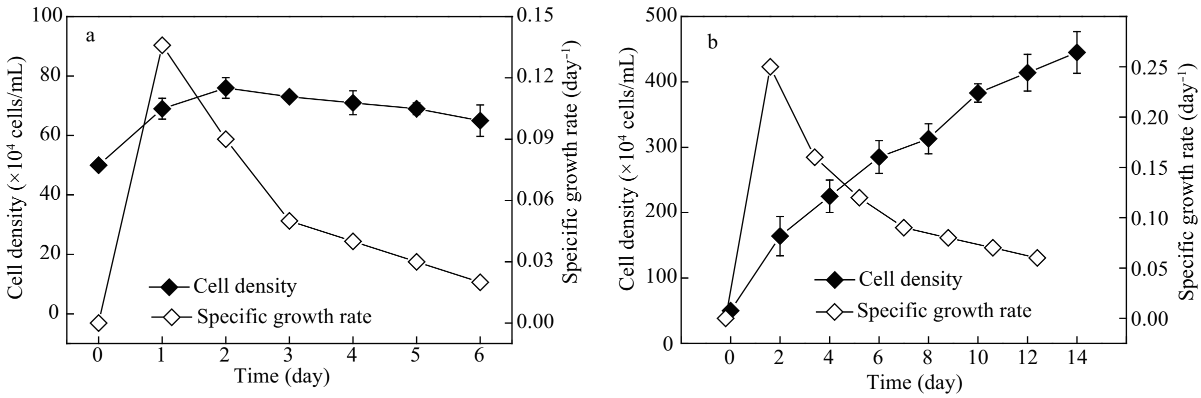

Fig. 3 Growth curve and specific growth rate of *Platymonas subcordiformis* cultivated in flounder aquaculture wastewater taken from the intial stage (a) and the final stage (b). Error bars represent the standard deviation of replicate (n = 3) sediment samples.

up metabolically by the microalgae and incorporated into their biomass.

Nitrogen and phosphate were two main components in wastewater, which could support algae growth. During the N and P removal, N/P ratio may not be optimized for the best performance of *P. subcordiformis*. Therefore, less removal of initial N and P loading could be the lower growth rate caused by the limited light transmission so that not enough algae cells absorbed N and P used to produce the biomass (Chinnasamy, 2010b).

3 Conclusions

In conclusion, it was feasible to couple the removal of nitrogen and phosphorus from wastewater to algal biomass and biofuel production. The marine alga, *Platymonas subcordiformis* could remove 87%–95% nitrogen and 98%–99% phosphorus in the flounder aquaculture wastewater. The biomass of algae was 8.9 times higher than the initial level. However further studies are required to make this technologies economically viable for algae biofuel production.

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