Bioresource Technology 102 (2011) 5639–5644

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Biomass production and nitrogen and phosphorus removal by the green alga *Neochloris oleoabundans* in simulated wastewater and secondary municipal wastewater effluent

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ARTICLE INFO

Article history:
Received 24 March 2010
Received in revised form 9 February 2011
Accepted 11 February 2011
Available online 17 February 2011

Keywords:
Secondary wastewater effluent
N/P removal
Biomass production
Microalgae
CO_2 sequestration

ABSTRACT

Biomass productivity of 350 mg DCW L^{-1} day^{-1} with a final biomass concentration of 3.15 g DCW L^{-1} was obtained with *Neochloris oleoabundans* grown in artificial wastewater at sodium nitrate and phosphate concentrations of 140 and 47 mg L^{-1}, respectively, with undetectable levels of residual N and P in effluents. In secondary municipal wastewater effluents enriched with 70 mg N L^{-1}, the alga achieved a final biomass concentration of 2.1 g DCW L^{-1} and a biomass productivity of 233.3 mg DCW L^{-1} day^{-1}. While N removal was very sensitive to N:P ratio, P removal was independent of N:P ratio in the tested range. These results indicate that *N. oleoabundans* could potentially be employed for combined biofuel production and wastewater treatment.

1. Introduction

Microalgae are promising candidates for large scale global biofuel production because of their high photosynthetic efficiency. Whereas a land plant such as switchgrass has a photosynthetic efficiency of less than 0.5% (Lewis and Nocera, 2006), that of microalgae can reach 20% or higher (Richmond, 2000; Huntley and Redalje, 2007). Nevertheless, the fundamental challenge in microalgal biofuel production remains its relatively high costs of production. Different strategies have been proposed to improve the cost-effectiveness of microalgal biofuel production (Li et al., 2008a), which include: cultivation using secondary wastewater effluents, farming using seawater (Huntley and Redalje, 2007), farming on land unsuitable for land-based agriculture, integration of microalgal biofuel production with flue gas CO_2 sequestration, production of high-value novel bioproducts, design of advanced photobioreactors (Lehr and Posten, 2009), and use of optimized media (Wang and Lan, 2011) and costeffective downstream processing technologies. Cultivating microalgae for tertiary wastewater treatment to generate low nitrogen (N) low phosphorus (P) effluents, could reduce nutrient costs for microalgal cultivation and preserving precious freshwater resources. *Neochloris oleoabundans*, a fast growing tri-glyceride-producing microalgal species (Tornabene et al., 1983) that can accumulate up to 40% lipid on a dry biomass basis at a lipid productivity of 0.133 kg m^{-3} day^{-1} (Li et al., 2008b; Pruvost et al., 2009), has been shown to be a promising candidate for biofuel production. It is therefore of interest to investigate the potential of this species for integrated wastewater treatment and biofuel production. In this study, we first measured the effects of temperature on cell growth of *N. oleoabundans* and then investigated the cell growth and N/P removal in artificial and secondary municipal wastewater effluents containing different levels of sodium nitrate and phosphate. The study thus provides valuable guidelines for industrial designs aiming at combined microalgal biofuel production and wastewater treatment using *N. oleoabundans* and other microalgal species.

2. Methods

2.1. Microalga strain, media and algal cultivation

N. oleoabundans OU2 was purchased from the UTEX Culture Collection of Algae, Texas (UTEX #1185) and grown in artificial wastewater containing (mg L^{-1}): $MgSO_4$ (37), $FeCl_3$ (3), $CaCl_2{\cdot}2H_2O$ (25), NaCl (0.025), $NaNO_3$ (850), KH_2PO_4 (75), K_2HPO_4 (175), EDTA-Fe (1.642), H_3BO_3 (2.860), $MnCl_2{\cdot}4H_2O$ (1.810), $ZnSO_4{\cdot}7H_2O$ (0.220), $CuSO_4{\cdot}5H_2O$ (0.079), and $(NH_4)_6MO_7O_{24}{\cdot}4H_2O$ (0.039). To study the effect of nitrate concentration on cell growth and N removal, artificial wastewater containing $NaNO_3$ at concentrations of 45, 70, 140, 218 mg N-NO_3^- L^{-1}, which corresponded to the N:P ratios of 0.42, 0.65, 1.33, and 2.02, respectively, were used. A relatively

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doi:10.1016/j.biortech.2011.02.054

Table 1
Major components of secondary municipal wastewater (ROPEC wastewater-treatment plant, Ottawa, ON, Canada) and piggery wastewater (An et al., 2003).

Component	Municipal	Piggery
$N\text{-}NH_4^+$	12.3	4
$N\text{-}NH_3^-$	10	788
$P\text{-}PO_4^{3-}$	3–6	40
COD	340–560	284

high phosphate concentration of 108 mg $P\text{-}PO_4^{3-}$ L^{-1}, which exceeded the phosphate requirement of this strain, was used to avoid phosphate limitation. Furthermore, artificial wastewaters containing 140 mg-N L^{-1} sodium nitrate and varied phosphate concentrations of 5.3, 9.5, 14.8, 28.0, and 47.0 mg $P\text{-}PO_4^{3-}$ L^{-1}, which corresponded to the N:P ratios of 26.4, 14.7, 9.5, 5.0, and 3.0, respectively, were used to investigate the effects of phosphate concentration and N:P ratio on cell growth, nitrate removal, and phosphate removal when nitrate was supplied in excess. Finally, growth was also studied in secondary municipal wastewater effluent provided by the ROPEC wastewater-treatment plant (Ottawa, ON, Canada). Effluent was filtered through Whatman Grade GF/C Glass Microfiber Filters (1.2 μm) to remove solids. The properties of the secondary municipal wastewater are listed in Table 1. To investigate the feasibility of using municipal wastewater for *N. oleoabundans* cultivation and the ability of the green alga in N/P removal, experiments were carried out in enriched municipal wastewater containing $NaNO_3$ in the concentration of 0, 14, 35, 70, and 105 mg $N\text{-}NO_3^-$ L^{-1}. The pH of fresh medium was adjusted to 6.8 before autoclaving. *N. oleoabundans* was cultivated in a system described previously (Li et al., 2008b) in 500-ml cylinder flasks with 400-ml working volumes. Agitation was provided by a magnetic stirrer. The culture was bubbled continuously with air enriched with 5% CO_2 at a flow rate of 0.75 vvm. Cultivation bottles were located inside a cubic chamber which was equipped with nine fluorescent tube lamps (F18T8, 1 × 26 inches, 18 W, light output1280 Lumens). Temperature inside the chamber was controlled by forced circulation of ambient air using two fans located on two sides of the chamber.

2.2. Analytical methods

Biomass concentration was determined turbidometrically at 600 nm using a spectrophotometer (GENESYS 10 UV, Thermo Electron Co., USA). Samples were diluted to give an OD600 reading between 0.2 and 0.4. The OD600 reading was multiplied with a pre-determined conversion factor of 0.4 to obtain the dry cell weight (DCW g L^{-1}).

Nitrate concentrations were determined by HPLC (Jovanovic et al., 2007) with a system (Agilent Technologies Inc., CA, USA) loaded with an Anion IC-PAKTM column (50 × 4.6 mm, 10 μm particle size, Waters, Millipore, USA). The flow rate of the mobile phase was controlled at 1.2 ml min^{-1} and the UV detector set at 220 nm. The mobile phase (pH 8.5) was composed of borate buffer/gluconate concentrate, methanol, acetonitrile, and deionized water at a ratio of 2:12:12:74 (v/v/v/v). The borate buffer/gluconate concentrate was composed of 0.07 M sodium gluconate, 0.3 M H_3BO_3, 0.1 M $Na_2B_4O_7$ and 3.8 M glycerol in deionized water. The injection volume was 50 μl. Ammonia concentration was determined according to Standard Method 4500D (APHA, 1992) using an Orion ammonia electrode (model 95-12) connected to a Fisher Accumet® model 750 pH/ion meter (Fisher Sci., Ottawa, ON). PO_4^{3-} was determined colorimetrically (APHA, 1992). All the analyses were carried out with culture supernatants obtained after centrifugation at 1500*g* for 15 min.

2.3. Calculations

The rate of the removal of a substrate of interest, R_i, (i = phosphate-P, Nitrate-N, or Ammonia-N), was calculated by the following equation:

$$R_i = \frac{S_{0,i} - S_i}{t_0 - t} \quad (1)$$

where $S_{0,i}$ is the initial concentration of substrate i, S_i the corresponding substrate concentration at time *t*.

3. Results and discussion

3.1. Effect of temperature on algae growth in artificial wastewater

As shown in Fig. 1, the growth profiles of *N. oleoabundans* were very similar between 25 and 30 °C, with a slightly higher biomass concentration of 3.0 g L^{-1} obtained at 30 °C at the end of the 240 h cultivation period. The biomass concentrations obtained in the same period were 2.8 and 2.5 g L^{-1} at 28 and 25 °C, respectively. However, cell growth was significantly reduced at 32 °C. The stationary phase at 32 °C started at approximately 96 h of cultivation and the maximum biomass concentration was 1.5 g L^{-1}, only 50% of what was obtained at 30 °C. It is worth mentioning that algal growth curves at all the four different temperatures were mostly linear before the stationary phase was reached, which was due to light limitation when cell density reached a certain level (Li et al., 2008b). These results suggest that the optimal growth temperature of *N. oleoabundans* was between 25 and 30 °C and significant inhibition to cell growth was observed at 32 °C. Examination of the growth profiles shown in Fig. 1 reveals that the difference in biomass concentrations at different cultivation temperatures was due to the lowered growth rate at 32 °C and the greatly reduced linear growth period, which decreased from between 172 and 216 h in the optimal temperature range (i.e., 25–30 °C) to 96 h at 32 °C. The drastic effect of a small temperature deviation from its optimal range on microalgae is not unique. For instance, it was reported that an increase in the temperature to above 28 °C will cause a sudden drop in the concentration of proteins and polyunsaturated fatty acids (PUFAs) in *Phaeodactylum tricornutum* due to metabolic stress (Bitaube Perez et al., 2008). The determination of optimal temperature range provides valuable guidelines for selection of regions of cultivation and the design of cultivation systems should this strain be employed for commercial biofuel

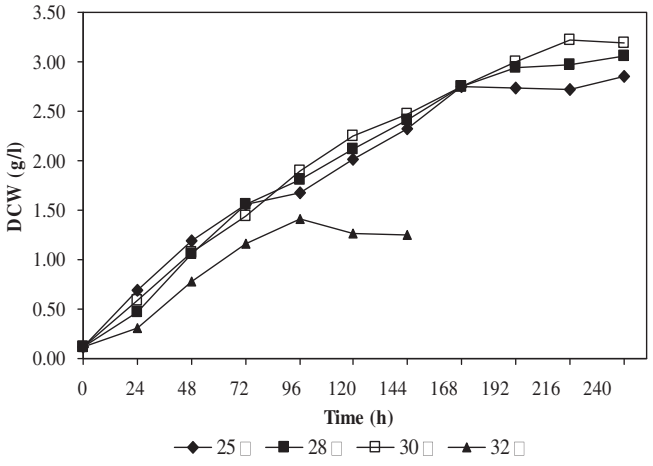

Fig. 1. Growth curves of *N. oleoabundans* at different temperatures (DCW: abbreviation of dry cell weight).