Bioresource Technology 196 (2015) 668–676

Contents lists available at ScienceDirect

Bioresource Technology

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

Trophic mode conversion and nitrogen deprivation of microalgae for high ammonium removal from synthetic wastewater

Jinghan Wang [a,b], Wenguang Zhou [a,c,*], Haizhen Yang [b], Feng Wang [b], Roger Ruan [a,c,*]

[a] Center for Biorefining, Bioproducts and Biosystems Engineering Department, University of Minnesota, Saint Paul, United States
[b] Research Institute of Environmental Planning and Management, College of Environmental Science & Engineering, Tongji University, Shanghai, China
[c] School of Resources, Environmental & Chemical Engineering and MOE Biomass Engineering Research Center, Nanchang University, Nanchang, China

HIGHLIGHTS

- One promising locally isolated microalgal strain UMN266 was selected.
- Development of a three-stage process for high ammonium removal from wastewater.
- The maximal NH_4^+-N removal rates are 12.4 mg/L/d for 80 mg/L and 19.1 g/L/d for 160 mg/L.
- The three-stage process strategy provided a novel NH_4^+-N-rich wastewater removal approach.

ARTICLE INFO

Article history:
Received 18 June 2015
Received in revised form 30 July 2015
Accepted 3 August 2015
Available online 21 August 2015

Keywords:
Trophic mode conversion
Nitrogen deprivation
Ammonium removal
Microalgae
Nitrogen starvation

ABSTRACT

In this study, a well-controlled three-stage process was proposed for high ammonium removal from synthetic wastewater using selected promising microalgal strain UMN266. Three trophic modes (photoautotrophy, heterotrophy, and mixotrophy), two N sufficiency conditions (N sufficient and N deprived), two inoculum modes (photoautotrophic and heterotrophic), and different NH_4^+-N concentrations were compared to investigate the effect of trophic mode conversion and N deprivation on high NH_4^+-N removal by UMN266. Results showed that photoautotrophic inoculum with trophic mode conversion from heterotrophy to photoautotrophy and N deprivation in Stage 2 turned was the optimum plan for NH_4^+-N removal, and average removal rates were 12.4 and 19.1 mg/L/d with initial NH_4^+-N of 80 and 160 mg/L in Stage 3. Mechanism investigations based on algal biomass carbon (C) and N content, cellular composition, and starch content confirmed the above optimum plan and potential of UMN266 as bioethanol feedstock.

1. Introduction

Ammonium (NH_4^+-N) removal is one of the most challenging tasks in worldwide wastewater treatment, since various agro-industrial sections generate volumes of sewage with high NH_4^+-N concentrations, such as livestock wastewater, landfill leachate, and fertilizer wastewater, causing eutrophication, odor, groundwater contamination, soil degradation, etc. (Wiszniowski et al., 2006; Cai et al., 2013). NH_4^+-N could be removed through multiple physicochemical and biological ways, among which biological methods have been of great interest and widely studied in the past few decades (Parker et al., 1975; Lau et al., 1995; Posadas et al., 2013). Activated sludge is the most widely adopted biological treatment process, removing NH_4^+-N through nitrification–denitrification, requiring high cost in aeration, but with NH_4^+-N removal seldom satisfactory for direct discharge (Godos et al., 2009). To overcome this, microalgae-based systems were introduced (Ogbonna et al., 2000; Ji et al., 2012). As the most readily uptake form of nitrogen source for microalgae (Cai et al., 2013), NH_4^+-N could be better removed in microalgae-based systems than in bacteria-based activated sludge systems (Posadas et al., 2013). Principles of NH_4^+-N removal in these systems are (1) O_2 produced through algal photosynthesis facilitates nitrification–denitrification; (2) pH increase caused by photosynthesis stimulates ammonia (NH_3) volatilization; (3) intensified NH_3 volatilization with system agitation (known as air-stripping effect); (4) uptake of NH_4^+-N by microalgal cells. Assimilation of NH_4^+-N by algal cells constitutes only a fraction of overall NH_4^+-N removal in such systems (Voltolina et al., 1998; Godos et al., 2009), leading to volume NH_3 discharge and possible air pollution. To monitor the net NH_4^+-N removal by algal assimilation, a well-controlled process using synthetic wastewater

* Corresponding authors at: Center for Biorefining, Bioproducts and Biosystems Engineering Department, University of Minnesota, Saint Paul, United States. Tel.: +1 612 625 1710; fax: +1 612 624 3005.
E-mail addresses: zhouw@umn.edu (W. Zhou), ruanx001@umn.edu (R. Ruan).

http://dx.doi.org/10.1016/j.biortech.2015.08.036
0960-8524/

needs to be adopted to rule out influences of nitrification–denitrification, elevated pH, and NH_3 volatilization.

Effects of nitrogen starvation/deprivation on microalgal growth have been widely studied, mostly with the purpose of biofuel production (Hsieh and Wu, 2009; Wang et al., 2013; Griffiths et al., 2014). Generally, under N starvation/deprivation, synthesis of N-rich constituents necessary for cell growth and division is restrained significantly, leading to reduced or even ceased algal growth. Meanwhile, metabolic pathway of carbon assimilation diverts from protein synthesis to lipid or carbohydrate production as carbon and energy storage (Griffiths et al., 2014). While previous research focused on how N starvation/deprivation affects biomass composition and algal growth, few studied the response of algal growth and nutrient uptake after recovering from N starvation/deprivation. Tendency to uptake more NH_4^+-N after recovery from N starvation/deprivation has been reported among higher plants and marine phytoplankton (Dortch et al., 1982; Lee and Rudge, 1986), but not yet among freshwater microalgae.

Studies showed that higher inoculation of microalgae into wastewater favors NH_4^+-N removal, owing much to better shock load adjustment (Lau et al., 1995). To achieve higher inoculation, more algal biomass should be readily obtained. Among the three major modes of microalgae cultivation, i.e., photoautotrophy (with light as energy source and inorganic carbon as carbon source), heterotrophy (with organic compounds as both energy source and carbon source), and mixotrophy (able to use both light and organic compounds as energy source, and both inorganic and organic carbon as carbon source), heterotrophy and mixotrophy generally display much higher biomass yield than traditional photoautotrophy (Wang et al., 2014). Thus, application of heterotrophically or mixotrophically cultivated microalgae as "seeds" should enhance NH_4^+-N removal.

Constituents such as proteins, nucleic acids, and chlorophylls, are the N pools of microalgal cells, most of which are photosynthesis related therefore light dependent. Our hypothesis is that the trophic mode conversion from heterotrophy or mixotrophy to photoautotrophy would trigger more NH_4^+-N uptake, since exclusive photoautotrophic growth would contribute to the synthesis of more light dependent constituents. This paper therefore, aims to investigate high concentrations of NH_4^+-N removal in a well-controlled three-stage process using synthetic wastewater. Stage 1 aims to heterotrophically or mixotrophically accumulate as much as possible algal biomass, followed by 48 h N deprivation in Stage 2, and photoautotrophic cultivation with high NH_4^+-N concentrations in Stage 3. One indigenous freshwater strain tolerant of high NH_4^+-N concentration and could grow well in heterotrophy was selected and identified out of ten microalgal strains. Three trophic modes, two N sufficiency conditions in Stage 2, and different NH_4^+-N concentrations in Stage 3 were compared, to investigate the effects of trophic mode conversion, N deprivation and NH_4^+-N concentrations on NH_4^+-N removal thus NH_4^+-N-rich wastewater treatment. Photoautotrophic and heterotrophic modes of inoculation were also compared to investigate the effect of inoculum trophic mode on NH_4^+-N removal. This paper could serve as a first detailed study about the influence of N deprivation and trophic mode conversion on NH_4^+-N removal by locally isolated freshwater microalgae, offering useful information for NH_4^+-N-rich wastewater treatment.

2. Methods

2.1. Algal strains and growth medium

10 strains were used in the study, 8 of which were isolated in local water bodies, named as UMN230, UMN244, UMN258, UMN266, UMN268, UMN270, UMN271, UMN285 (Zhou et al., 2011); 2 other strains, UTEX78 and UTEX2714, were purchased from the Culture Collection of Algae at the University of Texas.

Algal strains were cultivated in autoclaved synthetic wastewater according to Perez-Garcia et al. (2010) with some modifications (mg/L): NaCl, 7; $CaCl_2$, 4; $MgSO_4{\cdot}7H_2O$, 2; K_2HPO_4, 21.7; KH_2PO_4, 8.5; Na_2HPO_4, 25; NH_4Cl, 63 for photoautotrophic stock culture and 315 for heterotrophic stock culture; H_3BO_3, 0.57; $MnCl_2{\cdot}4H_2O$, 0.25; $ZnSO_4{\cdot}7H_2O$, 1.1; $FeSO_4{\cdot}7H_2O$, 0.25; $CoCl_2{\cdot}6H_2O$, 0.08; $Na_2MoO_4{\cdot}2H_2O$, 0.015, and Na_2EDTA, 2.5. HEPES was added as buffer, on the basis of 5 mM for photoautotrophic stock culture (Shi et al., 2007). pH was controlled at 7.0. 10 g/L glucose was added as organic carbon source for heterotrophic stock culture. For heterotrophic stock culture, algal cells in photoautotrophic stock culture were first inoculated onto glucose supplied sterile agar plates, cultivated in dark for 3–4 days, after then microalgal cells on the agar plates were inoculated onto another batch of glucose supplied sterile agar plates, and cultivated in dark for 3–4 days. This step was repeated 3 times to accomplish full adaptation from photoautotrophic to heterotrophic cultivation. Then, algal cells on the plates were inoculated into autoclaved heterotrophic synthetic wastewater, cultivated in dark and kept as heterotrophic stock culture. The stock culture was transferred to fresh autoclaved heterotrophic synthetic wastewater every 3 days, and to prevent bacterial contamination, microscopic examination was conducted before each transfer.

Algal strains were inoculated 10% ($V_{inoculation}/V_{wastewater}$) into 100 mL synthetic wastewater in 250 mL Erlenmeyer flasks. The flasks were placed on a shaker with 100 rpm rotation speed. The cultures were kept at 25 ± 2 °C under continuous cool-white fluorescent light with intensity of 70 μmol/(m^2 s).

2.2. Synthetic wastewater

To rule out influences of nitrification–denitrification, elevated pH, and NH_3 volatilization on NH_4^+-N removal, synthetic wastewater was autoclaved throughout this study. The recipe was based on growth medium with some modifications (mg/L): NH_4Cl 252, 504, 755 to get NH_4^+-N concentrations of 80, 160, and 240 mg/L; a mixture of 190 mg/L $NaHCO_3$ and Na_2CO_3 (C concentration) was added when inorganic carbon sources were included. HEPES was added as buffer and pH was controlled at 7.0.

2.3. Strain selection and identification

2.3.1. Strain selection

10 strains of microalgae were heterotrophically cultivated in 250 mL flasks with 100 mL synthetic wastewater. Strains with significantly higher biomass yield were chosen for further selection using a two-step strategy. In the first step, strains were heterotrophically cultivated for 3 days; then heterotrophically cultivated but deprived of N for 48 h in the second step. Algal biomass was collected through centrifuge and was washed once with N deprived synthetic wastewater before transferred to the second step. Optimum strain based mainly on biomass productivity after step-2 was selected for further investigation. All cultures were carried out in duplicate. The optimum strain was kept in both photoautotrophic and heterotrophic stock cultures.

2.3.2. Strain identification

Genomic DNA of algal cells was extracted using Qiagen DNeasy® Plant Mini Kit (Qiagen, Germantown, MD, USA) according to manufacturer's instruction with minor modification. Around 100 mg algal paste was placed in a 2.0 mL centrifuge tube after being washed with autoclaved distilled water for 3 times. 200 μL AP1 Buffer and 4 μL RNase A was added and vortexed for 2 min,