Journal of Environmental Management 163 (2015) 254–261

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Investigation into ammonia stress on *Cyperus alternifolius* and its impact on nutrient removal in microcosm experiments

Wendong Tao [a, *], Jianqiu Han [a, b], Hanyan Li [a]

[a] Department of Environmental Resources Engineering, College of Environmental Science and Forestry, State University of New York, 1 Forestry Drive, Syracuse, NY 13210, USA
[b] Department of Ecological Technology and Engineering, Shanghai Institute of Technology, 100 Haiquan Road, Fengxian District, Shanghai 201418, China

ARTICLE INFO

Article history:
Received 28 April 2015
Received in revised form
13 August 2015
Accepted 20 August 2015
Available online 30 August 2015

Keywords:
Ammonia toxicity
Constructed wetland
Cyperus alternifolius
Nutrient uptake
Plant physiology
Umbrella sedge

ABSTRACT

Ammonia stress on plants has been investigated at discrete ammonia concentrations in constructed wetlands. This study introduced a Gaussian model to simulate the kinetics of ammonia stress and investigated reversible and irreversible ammonia stress on *Cyperus alternifolius* in wetland-like microcosms. Ammonia stress on plant weight increase and oxygen release potential started at weekly ammonia concentrations of 27 and 28 mg N/L, reached 50% inhibition at 178 and 158 mg N/L, and resulted in lethal effects at 311 and 303 mg N/L, respectively. The stress of one-time ammonia concentrations up to 400 mg N/L could be reversible. Ammonia concentrations constantly above 219 mg N/L exerted irreversible stress. In the microcosms with ammonia concentrations above the 50% inhibition levels, plants played a minor role in nitrogen removal. Nitrogen removal performance was not affected considerably by ammonia stress. Orthophosphate removal was suppressed by ammonia stress due to less plant uptake. Design and operation of constructed wetlands should consider wastewater ammonia concentration so that the integrity of constructed wetland ecosystems can be maintained.

1. Introduction

Constructed wetlands have been used to remove nutrients from a variety of wastewater (Vymazal, 2007). Wetland plants not only take up nitrogen and phosphorus, but are integral parts of wetland ecosystems also. When constructed wetlands are used to treat ammonia-rich wastewater such as livestock wastewater, wetland plants may become stressed or even die due to ammonia toxicity. Stressed plants and death of plants could subsequently affect treatment performance and degrade ecosystem services. Although ammonia is a nutrient at low concentrations, it can be toxic when plants are cultured with ammonia as the exclusive nitrogen source (Britto and Kronzucker, 2002). To avoid toxicity of ammonia to plants and microorganisms, ammonia-rich wastewater is often diluted before loading it to constructed wetlands (He et al., 2012; Hill et al., 1997).

There are variations in ammonia tolerance amongst wetland species (Li et al., 2011; Britto and Kronzucker, 2002; Clarke and Baldwin, 2002; Hill et al., 1997). Cyperaceae is an ammonia-tolerant plant family (Britto and Kronzucker, 2002). *Cyperus* species such as *Cyperus alternifolius* have been planted in more than 20 free water surface constructed wetlands in North America and Africa (Vymazal, 2013). Commonly known as umbrella sedge, *C. alternifolius* has leafless triangular stems that arise from a network of woody rhizomes and grow to a height of 0.6–1.8 m. *C. alternifolius* had greater tolerance to ammonia-rich wastewater than *Typha latifolia*, *Phragmites australis*, and *Cyperus prolifer* in free water surface wetlands (He et al., 2012; Tao et al., 2012, 2011). *C. alternifolius* has been planted in a full-scale constructed wetland system for landfill leachate treatment in Singapore and pilot-scale subsurface flow wetlands for sewage treatment in China (Sim et al., 2013; Chan et al., 2008). *Cyperus involucratus*, a subspecies of *C. alternifolius*, has also been planted in pilot-scale free water surface wetlands for municipal wastewater treatment in Australia (Greenway and Woolley, 1999; Greenway, 1997). However, ammonia stress on *C. alternifolius* in treatment wetlands has not been quantified yet.

Symptoms of ammonia toxicity include visible changes such as death, foliage chlorosis, and shorter roots as well as other physiological changes such as growth suppression and oxidative stress (Li et al., 2011; Jampeetong and Brix, 2009; Wang et al., 2008; Britto

* Corresponding author. 1 Forestry Drive, Baker 402, Syracuse, NY 13210, USA.
E-mail address: wtao@esf.edu (W. Tao).

http://dx.doi.org/10.1016/j.jenvman.2015.08.028
0301-4797/

and Kronzucker, 2002). Several studies (Li et al., 2011; Clarke and Baldwin, 2002; Hill et al., 1997) have investigated the thresholds of ammonia concentration at which emergent plants such as *Sagittaria latifolia*, *P. australis*, *T. angustifolia*, and *T. latifolia* are stressed, by comparing plant biomass growth, tissue morphology, and other physiological parameters when treatment wetlands are continuously or semi-continuously fed (every two days) with wastewater at different ammonia concentrations (0–400 mg/L). The thresholds of ammonia stress have been determined arbitrarily based on experiments with discrete ammonia concentrations and with little kinetics discussion (Li et al., 2011; Clarke and Baldwin, 2002; Hill et al., 1997).

Ammonia concentration in wetland water decreases over time in batch operation and varies in field operation of continuously-fed constructed wetlands. Event-driven constructed wetlands such as those for treatment of combined sewer overflows are subject to frequent alternation of high and low ammonia concentrations (Tao et al., 2014; Kadlec and Wallace, 2009). Clarke and Baldwin (2002) found that ammonia concentrations in excess of 200 mg/L inhibited growth of *Juncus effusus* and *T. latifolia* after several weeks, whereas shorter periods of elevated ammonia concentrations did not appear to affect plant growth. Through short periods of laboratory experiments (4–8 d), Wang et al. (2008) found that the toxic effects of ammonia and oxidative stress on submerged species *Vallisneria natans* (Lour.) H. Hara were greater over longer exposure and at higher ammonia concentrations in the range from 1.4 to 39 mg N/L. The stress levels found in short periods of experiments may be reversible in long-term field operation due to fluctuation in ammonia concentration, especially in event-driven constructed wetlands.

The objectives of this study were to develop a kinetic model for simulation of ammonia stress and determine both reversible and irreversible levels of ammonia stress on *C. alternifolius*. Quantifying ammonia stress on wetland plants will provide guidance on design and operation of constructed wetlands for treatment of ammonia-rich wastewater. When carbon turnover increases as a result of nitrogen assimilation, demands for phosphorus and other micronutrients would increase (Brun et al., 2002). Therefore, this study investigated ammonia stress along with nutrient removal efficiency in microcosm experiments treating ammonia-rich dairy wastewater.

2. Materials and methods

2.1. Experiments identifying ammonia stress

Eight wetland-like microcosms, W1–W8, were built with polyvinyl chloride pipes (each 450 mm tall, inner diameter of 155 mm) and packed with 130 mm of marble chips (effective chip size 8.1 mm). Three plugs of *C. alternifolius* pre-cultured with tap water were washed and put on the marble chips of each microcosm except for one control (W8) without plants (Fig. 1). Each plug had approximately 3 shoots with viable roots and 20–40 mm of rhizome cuttings. The microcosms were operated with dairy wastewater in 5-week batch mode in a greenhouse. The batch experiments were replicated three times. A 400-W high-pressure sodium (HPS) white grow light supplemented lighting on a 12-h light 12-h dark cycle in the second batch experiments in January and February. Each microcosm received 4 L of dairy wastewater at the beginning of each batch. Drinking water was added twice a week (0–0.32 L each time) to compensate for water loss due to evapotranspiration. Dairy wastewater for Microcosm W1 was 20× diluted filtrate of anaerobically digested dairy manure (Xia et al., 2012), which had ammonia concentration targeted at 30 mg N/L. Dairy wastewater for W2–W8 had different amounts of ammonium chloride dissolved in the diluted filtrate, targeting ammonia concentrations at 50, 100, 150, 200, 300, 400, and 200 mg N/L, respectively.

Fig. 1. Ammonia stress on *C. alternifolius* demonstrated by eight microcosms (W1–W8) after one week of batch operation with dairy wastewater at different initial ammonia concentrations, i.e., 33, 51, 94, 147, 193, 293, 400, and 194 mg N/L in W1–W8, respectively. W1–W7 with *C. alternifolius* and W8 without plants.

Water samples were collected on a weekly basis after taking measurements of pH, dissolved oxygen (DO), and temperature with a portable pH meter and an YSI ProODO handheld DO meter. Water samples were analyzed colorimetrically for total ammonia, nitrate, and nitrite with a QuickChem 8500 series flow injection autoanalyzer (Lachat Instruments, Loveland, CO), following the flow injection analysis methods (APHA et al., 2012). Orthophosphate concentration was determined with a DR2800 spectrophotometer (Hach Company, Loveland, CO), following the molybdovanadate method (APHA et al., 2012). Water samples (10–40 mL each) were digested with 4 mL of concentrated sulfuric acid and 10 mL of 50% hydrogen peroxide using a Digesdahl digestion apparatus (Hach Company, Loveland, CO). Concentration of total Kjeldahl nitrogen (TKN) in the digests was determined with the autoanalyzer. Total phosphorus (TP) concentration in the digests was determined with the spectrophotometer using the molybdovanadate method.

After collecting water samples each week, the plants were washed with drinking water, blotted with absorbent towels, and measured for fresh weight (ww). Cumulative plant weight increase was calculated to reveal plant biomass growth over time. The percentage of weekly increase in fresh weight was calculated to represent plant growth rate. The plants were then put into N_2-purged dairy wastewater in cylindrical tanks to determine oxygen release potential as given in Equation (1), which was written in the principle of DO mass balance. Oxygen release from vascular structure of plants is a diffusion process. It is stimulated by increasing external oxygen demand (Sorrell et al., 1993). The rate of oxygen release in this study was determined by DO concentration increase over 2 h of incubation with initial DO concentration at approximately 0.5 mg/L, thus representing oxygen release potential.

$$OR_{plant} = \left[\left(DO_{plant,2} - DO_{plant,0}\right) - \left(DO_{control,2} - DO_{control,0}\right)\right] \times V/W/2 \quad (1)$$

where OR_{plant} = oxygen release potential, mg/g (ww)/h; $DO_{plant,0}$, $DO_{plant,2}$ = DO concentrations measured initially and after two hours in N_2-purged wastewater with plants, mg/L; $DO_{control,0}$, $DO_{control,2}$ = DO concentrations measured initially and after two