Marine Pollution Bulletin 131 (2018) 142–150

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Nutrients removal and substrate enzyme activities in vertical subsurface flow constructed wetlands for mariculture wastewater treatment: Effects of ammonia nitrogen loading rates and salinity levels

Meng Li[a], Zhenlin Liang[a], Myriam D. Callier[b], Emmanuelle Roque d'orbcastel[c], Guoxiang Sun[d,f], Xiaona Ma[d,e], Xian Li[d,f], Shunkui Wang[g], Ying Liu[h,*], Xiefa Song[a,**]

[a] Fisheries College, Ocean University of China, Qingdao 266001, China
[b] Ifremer, UMR MARBEC (IRD, Ifremer, University of Montpellier, CNRS), Chemin de Maguelone, F-34250 Palavas-les-Flots, France
[c] Ifremer, UMR MARBEC (IRD, Ifremer, University of Montpellier, CNRS), Avenue Jean Monnet, CS 30171, F-34203 Sète, France
[d] Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[e] University of Chinese Academy of Sciences, Beijing 100049, China
[f] National & Local Joint Engineering Laboratory of Ecological Mariculture, Qingdao 266071, China
[g] Shandong Oriental Ocean Sci-Tech Co., Ltd., Yantai 264003, China
[h] Dalian Ocean University, Dalian 116023, China

ARTICLE INFO

Keywords:
Mariculture wastewater
Constructed wetland
Salicornia bigelovii
Enzyme activity
Nutrients removal

ABSTRACT

This study aims to investigate the effects of ammonia nitrogen loading rates and salinity levels on nutrients removal rates and substrate enzyme activities of constructed wetland (CW) microcosms planted with *Salicornia bigelovii* treating mariculture wastewater. Activities of urease (UA), dehydrogenase (DA), protease (PrA) and phosphatase (PA) were considered. Using principal component analysis (PCA), nutrient removal index (NRI) and enzyme activity index (EAI) were developed to evaluate the effects. The results revealed that increasing ammonia nitrogen loading rates had positive effects on nitrogen removal rates (i.e. NH_4-N and DIN) and enhanced substrate enzyme activities. Compared with low salinity (i.e. 15 and 22), high salinity levels (i.e. 29 and 36) enhanced nutrients removal rates, DA and UA, but weaken PA and PrA. In conclusion, CW microcosms with *Salicornia bigelovii* can be used for the removal of nutrients under a range of ammonia nitrogen loadings and high salinity levels.

1. Introduction

Recent estimates showed that the world food fish aquaculture production expanded at an average annual rate of 5.8% in the period 2005–2014, to 73.8 million tons (FAO, 2016). In recent years, there has been growing interest in the development of intensive land-based marine aquaculture, especially recirculating aquaculture systems (RAS) which could make seafood production more compatible with environmental sustainability (Martins et al., 2010). However, treatment of wastewater from marine aquaculture in an environmentally friendly way is crucial for sustainable intensification of aquaculture. In a study on a land-based Atlantic salmon RAS farm in China, for example, Sun et al. (2016) reported that only 36.5–47.8% nitrogen (N) and 20.4–38.6% phosphorus (P) in the feed distributed were digested by fish. Without proper treatment, the remaining nutrients in discharged saline wastewater can damage the physiology of farmed organisms and give rise to eutrophication in adjacent ecosystems, which would further destroy the balance of ecological structure (Hongfang et al., 2014; Buhmann et al., 2015; Hayes et al., 2017). Therefore, there is a significant need for treatment of saline wastewater from marine aquaculture facilities.

For treating nutrients (i.e. N and P) in the saline wastewater, biological technologies are usually applied, such as sequencing batch reactors, submerged bio-filters, trickling filters, rotating biological contactors and fluidized bed reactors (Van Rijn, 1996; Zhao et al., 2016). However, these approaches are costly in terms of capital investment, energy consumption and maintenance requirements (Papenbrock and Turcios, 2014). Moreover, the difficulty in controlling effectively the diffuse pollution (non-point source pollution) is another problem using these conventional methods (Wu et al., 2013). Apart from these

* Correspondence to: Y. Liu, No. 52 at Heishijiao Street, Shahekou District, Dalian 116023, China.
** Correspondence to: X. Song, No. 5 at Yushan Road, Shinan District, Qingdao 266001, China.
E-mail addresses: yingliu@dlou.edu.cn (Y. Liu), yuchuan@ouc.edu.cn (X. Song).

https://doi.org/10.1016/j.marpolbul.2018.04.013
Received 22 December 2017; Received in revised form 3 March 2018; Accepted 7 April 2018

methods, the CW is environmental friendly, cost effective, work efficient and has aesthetic values (Wallace, 2008; Shpigel et al., 2013; Haiming et al., 2015). Therefore, it has received more attention in recent decades and has been successfully applied in treating various types of wastewater (e.g. aquaculture wastewater, domestic effluents and industrial sewage) (Lin et al., 2002; Justin and Zupančič, 2009; Guittonny-Philippe et al., 2015). As for the treatment of saline wastewater, especially mariculture wastewater with the salinity up to 30, the functions of hydrophytes and microorganisms in the CW would be influenced by salts (Liang et al., 2017). However, when planting halophytes or salt-tolerant plants (e.g. *Salicornia* spp., *Typha angustifolia* and *Canna indica*, etc.) and provided with identified influential factors, the CW would have the potential to remove efficiently the nutrients from saline wastewater.

Substrate enzyme activity (EA) is an effective way to describe the activity and quantity of microorganisms which play vital roles in nutrients removal (especially N removal) of CW (Margesin et al., 2000a; Margesin et al., 2000b; Liang et al., 2003). For instance, urease plays an important role in catalyzing the hydrolysis of urea into ammonia and carbon dioxide; dehydrogenase is an indicator of microbial activity in CW beds, considering its function in oxidizing organic matter by transferring electrons and protons from substrates to acceptors; protease is one kind of the N-cycling-related key enzymes, which hydrolyzes proteins to peptides and amino acids and supplies large amounts of available N; while phosphatase is used to catalyze the organic phosphate into inorganic phosphate (G.N. Zhang et al., 2013; Baddam et al., 2016). In summary, substrate enzyme activities offer an alternative to understand the status of microorganisms in the CW.

Few investigations have been conducted on how other factors, like influent nitrogen loads and salinity levels, influence the nutrients removal as well as the related substrate EA of CW treating marine aquaculture wastewater. In fact, nutrient concentrations and salinity in mariculture wastewater depend on various factors, like breeding species, culture methods and the quantity and quality of feed (Boeuf and Payan, 2001; McIntosh and Fitzsimmons, 2003). Therefore, considering the limited published information about the impacts of various factors on CW treating mariculture wastewater, the current study was conducted using *Salicornia bigelovii* as the wetland plants with the objectives of: (1) evaluating the nutrient removal rates and substrate EA (i.e. urease, dehydrogenase, protease and phosphatase) in CW at three influent total ammonia nitrogen loading rates; and (2) elucidating the effects of salinity levels on the nutrient removal and EA in CW. The information obtained from this study will promote our ability to use CW to remove nutrients from mariculture wastewater, and to lay a foundation to understand the functional performance of CW treating saline wastewater.

2. Materials and methods

2.1. Materials

Two experiments were carried out during the period August 11th, 2015 to October 22nd, 2015 under greenhouse conditions on an intensive recirculating Atlantic salmon farm (i.e. Oriental Ocean Ltd., located in Shandong Province, China). Experiment 1 is designed to evaluate the effects of different total ammonia nitrogen (TAN) loading rates (i.e. 0.7, 3.0, and 10.2 g TAN m^{-2} day^{-1}) on nutrients removal and substrate enzyme activities in CW microcosms. Experiment 2 aims to clarify the influences of four salinities on nutrients removal rates and substrate enzyme activities. Salinities ranged from 15 to 36 practical salinity scale (pss). For both of the two experiments, *Salicornia bigelovii* seedlings (about 2 g ind^{-1}) were purchased from Weifang, Shandong Province, China in May 2015. The *Salicornia bigelovii* went through a thirty-day salt acclimation to adapt to the salinity of local seawater (i.e. 29) before moved to the CWs.

Artificial wastewater was used in this study. Wastewater was made of uneaten feed and feces collected from Atlantic salmon RAS following the steps described by Zhang et al. (2011). Uneaten feed and feces of salmon were dried with oven (105 °C for about 48 h); after ground into powders, they were mixed with seawater (i.e., 250 g powders in 1 L seawater), and then moved into a closed container for 7 days to carry out the anaerobic fermentation; finally, the "mother liquid" was produced after filtered through bolting-sild of 100 sieve number.

All the six sets of systems (i.e. ①, ②, ③, ④, ⑤ and ⑥) consisting of 18 CW microcosms were depicted in Fig. 1. For each set of the system, artificial wastewater was stored in the cylindrical barrel (diameter, 90 cm; height, 67 cm), and then pumped up to three parallel CW microcosms (30 cm length × 30 cm width × 30 cm height) using peristaltic pumps. The inflow rate of each microcosm was maintained at 100 ml min^{-1} in continuous mode. After purified by the CW, wastewater flew back to the cylindrical barrel from the standpipe aside by gravity. Empty and re-fill the cylindrical with artificial wastewater every 18 days. During the experimental period, each CW was planted with twelve individual *Salicornia bigelovii*.

A total of 18 vertical subsurface flow CW microcosms (30 cm length × 30 cm width × 30 cm height) made of polypropylene random (PPR) frames were established as shown in Fig. 1(B). The CW microcosms were placed on a stainless steel stand above the cylindrical barrels. From the bottom, each CW was placed with an 8-cm depth layer using clean, graded smooth cobblestone (with an equivalent diameter of 3–5 cm). The cobblestone was overlaid with a sheet of plastic mesh (0.2 mm^2 pore size) to separate with a 10-cm depth layer of cleaned haydites (with an equivalent dimeter of 0.5–0.8 cm). Then the first layer of haydites was covered with another plastic mesh (0.074 mm^2 pore size) and a second 12-cm layer of smaller haydites (with an equivalent diameter of 0.3–0.5 cm).

2.2. Experimental design

Both the two experiments were conducted for 72 days. In Experiment 1, three sets of CW systems were used (i.e. ①, ②, ③). Three trials were performed simultaneously at different TAN influent loading rates (i.e. 0.7, 3.0, and 10.2 g TAN m^{-2} day^{-1}) through controlling correspondingly the influent TAN concentrations (i.e. 0.44 ± 0.26, 1.90 ± 0.44 and 6.39 ± 0.88 mg l^{-1}). The three trials were denoted by low loading rate (LLR), medium loading rate (MLR) and high loading rate (HLR) respectively by increasing sequences. There were three replicated CW microcosms for each TAN loading rate treatment. Before the experiment, the CWs were fed in batches with seawater for 60 days for the natural-occurring microorganisms to adapted to the CW system.

In Experiment 2, four sets of CW systems were used (i.e. ④, ⑤, ② and ⑥). Four trials were performed simultaneously under different salinity levels including 15, 22, 29 and 36. The first three salinity levels were set by mixing the local seawater with freshwater in different proportions (i.e. 52% seawater + 48% fresh water, 76% seawater + 24% fresh water and 100% seawater, respectively); and the salinity of 36 was achieved by adding salt and other elements into the seawater. There were three replicated CW microcosms for each salinity treatment. Before the experiment, the CWs were fed in batches with local seawater for 60 days for the natural-occurring microorganisms to adapt to the CW system.

For both experiments, *Salicornia* were collected, balanced and then dried at 65 °C (to consistent weight) with oven at the beginning and in the end. These samples were used to measure element contents (i.e. N and P). For each CW microcosm, 100 ml water samples were collected from the inlet and outlet ten times over the experiment. Samples were analyzed for NH_4-N, NO_2-N, NO_3-N and PO_4-P. Meanwhile, water temperature and pH values were recorded. On Day 9, Day 32, Day 55, Day 72, 100 g substrate samples were taken to determine the substrate enzyme activities. All the methods for measurement are described later.