Dynamics of nitrobenzene degradation and interactions with nitrogen transformations in laboratory-scale constructed wetlands

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HIGHLIGHTS

► Potential application of CWs for NB-containing wastewater treatment was proved.
► NB toxicity produced a negative impact on nitrogen transformations in CWs.
► Positive effect of plants for NB degradation enhancement was demonstrated.
► NB degradation by the release of ammonium with ring cleavage was indicated.

ARTICLE INFO

ABSTRACT

Three laboratory-scale CWs (i.e., tidal flow CW as well as planted and unplanted horizontal subsurface flow CWs) were set up to treat artificial nitrobenzene (NB) industry effluents in this study. An inflow NB load equal to or less than 70 mg/L achieved approximately 95% NB removal regardless of wetland type. When NB influent load increased to 160 mg/L, NB removal efficiency decreased to 57%, 46%, and 33% in planted and unplanted horizontal CWs as well as tidal flow CWs, respectively. Higher NB degradation efficiency in planted horizontal CW highlighted the positive effect of wetland plants. Moreover, strong inhibition of nitrogen removal was initiated in CWs with an increase of NB loads to 160 mg/L, which was probably caused by NB toxicity. The investigation indicated not only the potential application of treatment wetlands as a secondary ecological treatment system for NB-containing wastewater, but also the interactions with nitrogen transformations in CWs.

1. Introduction

The environmental and health effects of toxic and hard biodegradable industrial effluents have been an increasing concern (González-Bahamón et al., 2011). Industrial effluents originated from the production of various industrial products, such as dyes, pesticides, explosives, plastics, lubricating oils, drugs, synthetic rubber, and intermediates in chemical syntheses (Ye et al., 2004; Lin et al., 2012), often contains nitroaromatic compounds, such as nitrobenzene (NB). Annually, over 10 million lbs of nitrobenzene is estimated to be discharged into the environment (Haigler and Spain, 1991). The compound and its transformed products present high risks to the ecosystem and to human health, causing vomiting, skin and eye irritation, headache, liver damage, and anemia (Majumder and Gupta, 2003; Wen et al., 2012). Accordingly, many countries consider NB as a "priority pollutant" due to its known or suspected carcinogenicity, mutagenicity, teratogenicity, and high acute toxicity (Majumder and Gupta, 2003; Lin et al., 2012).

Current methods for the treatment of NB-containing industrial effluents mainly include ozonization (Tong et al., 2011), fenton oxidation (Jiang et al., 2011), electrochemical reduction (Liu et al., 2012), and ultrasonic irradiation (Zhao et al., 2009). However, the high operating costs and complex control requirements of these methods limit their successful application in economically undeveloped areas (Wang et al., 2010).

The use of constructed wetlands (CWs) as a cost-effective, extensive, and efficient alternative biological technology has increasingly become globally important for wastewater treatment (Brix, 1999; Vymazal and Cycling, 2005; Garcia et al., 2010). The application of CWs has been significantly extended from traditional secondary and tertiary domestic sewage systems to high-strength wastewater systems, such as agricultural effluents (Wood et al., 2007), landfill leachate (Yang and Tsai, 2011), contaminated groundwater (Braeckevelt et al., 2008; Hathaway et al., 2010), and industrial effluents (Stottmeister et al., 2003).

The degradation of NB could be performed in both oxidative and reductive pathways (Nishino and Spain, 1993; Spain, 1995). Under anaerobic conditions, NB can be reduced to aniline and then degraded and mineralized through a following aerobic process. Under

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aerobic conditions, NB degrades with the release of ammonium after ring cleavage, which seems to be more common among isolates from contaminated groundwater (He and Spain, 1997). Transformed compounds are then completely mineralized to CO_2 and H_2O (Dickel et al., 1993; Zheng et al., 2007). Inside the wetland bed exists a great number of specific macro- and micro-gradients of redox conditions, which enable the development of highly diverse microbial consortia capable of different redox reactions (Wu et al., 2012a). In particular, the environment near plant roots was found to be of high importance for the removal of contaminants because of the oxygen and organic carbon released from the roots of helophytes into surrounding rhizosphere zones (Wiessner et al., 2005). Several microbial transformations, such as nitrification, mineralization of organic carbon, and denitrification, have been reported to be performed simultaneously occur in a small spatial scale (Liesack et al., 2000; Bezbaruah and Zhang, 2004). However, according to the above known mechanisms of NB degradation, whether CWs are suitable for NB degradation as a low-cost and easily operated system has not been fully elucidated. Only a limited number of CWs studies on the interactions between NB degradation and ammonium removal are available so far.

In this study, experiments in tidal flow CW (TFCW) as well as planted and unplanted horizontal subsurface flow CWs (HSSFCWs) were conducted to investigate the possibilities of NB degradation in CWs. In addition, the nitrogen removal efficiency as well as the nitrification and denitrification intensity of wetlands under varying NB loads were examined to determine the impact of NB degradation on nitrogen transformations. Moreover, the effect of plants on NB degradation was also measured to compare the performance of NB degradation between planted and unplanted HSSFCWs.

2. Methods

2.1. Laboratory-scale wetlands

The HSSFCWs were set up in plastic containers (length, 100 cm; width, 15 cm; height, 50 cm), which were filled uniformly with approximately 100 kg of gravel (diameter, 2 mm to 6 mm; density, 1.67 g/cm^3; porosity, 35%) up to a height of 45 cm and a free pore water volume of 25 L. Inflow rate was 5 L/d. Water level was adjusted to 5 cm below the surface of the gravel bed. Sieves of perforated plastic were placed at a distance of 3 cm in front of the inflow and outflow of the gravel bed to create small inlet and outlet zones. These free liquid volumes were specially designed to ensure an equal distribution of inflow and laminar (plug) liquid flow through the gravel bed.

The TFCW column was constructed from a Perspex tube (18 cm diameter by 150 cm height). Aggregate (particle size, 10 mm to 30 mm) was filled to a depth of 36 cm as a bottom drain layer with an average porosity value of 50%. The main filter layer of gravel (particle size, 2–6 mm) was filled to a depth of 92 cm with an average porosity value of 35%. Tidal operation was generated by a peristaltic pump and an automatic drain valve controlled by a timer. The flood and drain cycle was set to take place every 2 h (1.5 h flooded and 0.5 h drained). Effluent was recycled to influent with a ratio of 11:1, which provided the system with a hydraulic loading rate of 12 L/d.

Three systems were wrapped with black plastic cloth on the column sidewall to prevent periphyton formation. The planted HSSFCW and TFCW beds were planted with *Juncus effusus*. Experimental CWs were placed in a greenhouse and operated under defined environmental conditions simulating an average summer day under moderate climatic conditions. Temperature was adjusted to 22 °C from 6 am to 9 pm and to 16 °C from 9 pm to

6 am to simulate daytime and nighttime, respectively. Lamps (Phillips, Master SON-PIA 400W, Shanghai, China) were switched on at daytime as an additional artificial light source when natural illumination fell below 60 klx.

2.2. Experimental conditions

Modified artificial wastewater containing NB, ammonium, chemical oxygen demand (COD), and phosphate concentrations was used in this study. Influent artificial wastewater was prepared by using $C_6H_{12}O_6$, NH_4Cl, and $K_2HPO_4 \cdot 3 H_2O$ dissolved in deionized water according to the required concentrations (Table 1). Moreover, the aqueous solution of NB was prepared by dissolving NB in deionized water. In order to investigate the maximum capacity of NB degradation and its interactions with nitrogen transformations in different CWs, the experiment ran continuously under four different phases of A, B, C, and D with influent NB load gradually increasing from 5 mg/L to 20, 70, and 160 mg/L, respectively.

Table 1 presents the corresponding influent loads. In all cases, a trace mineral solution (Wu et al., 2012b) containing EDTA-Na (0.100 g/L), $FeSO_4 \cdot 7 H_2O$ (0.100 g/L), $MnCl_2 \cdot 4 H_2O$ (0.100 g/L), $CoCl_2 \cdot 5 H_2O$ (0.170 g/L), $CaCl_2 \cdot 6 H_2O$ (0.100 g/L), $ZnCl_2$ (0.100 g/L), $CuCl_2 \cdot 5 H_2O$ (0.020 g/L), $NiCl_2 \cdot 6 H_2O$ (0.030 g/L), H_3BO_3 (0.010 g/L), $Na_2MoO_4 \cdot 2 H_2O$ (0.010 g/L), and H_2SeO_3 (0.001 g/L) was added to the artificial wastewater (1 ml/L).

The experiment was conducted from April to December 2012, for approximately 200 d. Before the experiment, the three wetlands were continuously fed with synthesized domestic wastewater for four months to allow plants and biofilms to develop well in the bed. Synthesized domestic wastewater was composed by $C_6H_{12}O_6$ (290 mg/L), NH_4Cl (115 mg/L), $K_2HPO_4 \cdot 3 H_2O$ (36 mg/L) and the trace mineral solution (1 ml/L).

2.3. Sampling and analysis

Water samples were collected from the influent and effluent of the beds. The pH value, redox potential (Eh), and dissolved oxygen (DO) concentration were immediately measured by using a portable Orion 5-Star DO/pH/Eh meter with a DO electrode (ORION, 086030MD), pH electrode (ORION, 9172BNWP), and Eh electrode (ORION, 9179BNMD). Nitrate-nitrogen ($NO_3^- - N$) was analyzed by using continuous flow colorimetry (SEAL Auto-Analyzer 3, British). After corresponding standard pretreatment and regent addition according to *Standard Methods* (AWWA and WEF, 1998), concentrations of ammonium-nitrogen (4500-NH_3 F, phenate method), nitrite-nitrogen (4500-NO_2^- B, colorimetric method), and COD_{cr} (method 8000) were measured through a Hach DR5000 Colorimeter according to its standard calibration and operation.

NB concentration was analyzed by using gas chromatography (Shimadzu, GC-2010 Plus, Kyoto, Japan) with HP-5 Type chromatographic column; length, width, and height of 30 m, 0.25 mm, and 0.25 μm, respectively; electron capture detector; monitoring tem-

Table 1
Operation parameters in experimental phases A, B, and C (in mg/L).

Parameter	HSSFCW (planted)	HSSFCW (unplanted)	TFCW
$NH_4^+ - N$	20 ± 3	20 ± 3	20 ± 3
$PO_4^{3-} - P$	5 ± 1	5 ± 1	5 ± 1
COD	200 ± 26	200 ± 26	200 ± 26
Nitrobenzene			
Phase A	5 ± 1	5 ± 1	5 ± 1
Phase B	25 ± 5	25 ± 5	25 ± 5
Phase C	70 ± 16	70 ± 16	70 ± 16
Phase D	160 ± 13	160 ± 13	160 ± 13