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Nitrobenzene degradation pathways and their interaction with sulfur and nitrogen transformations in horizontal subsurface flow constructed wetlands

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ABSTRACT

Nitrobenzene (NB) degradation pathways and their interaction with sulfur and nitrogen transformations in constructed wetlands are not fully understood. This study investigates the effectiveness of horizontal subsurface flow constructed wetlands (HSSFCWs) in NB biodegradation including the biodegradation pathways and microbial interactions. The investigation was based on two laboratory-scale wetlands planted with *Juncus effusus*. One wetland was intermittently aerated while the other was not. Data about NB degradation were collected at varying influent loading rates [35 and 140 mg/L] for 120-day period. From the results, both wetlands exhibited an overall performance of 99% NB removal. More so, intermittent aeration did not significantly improve overall performance but rather it enhanced the buffer capacity of NB degradation to shock influent loading. The result also demonstrates that NB mineralization account for about 96% and 4% for volatilization. Formation of ammonium from NB degradation increased the ammonium concentration in the pore water. In unaerated wetland, 84 mg/L of sulfide accumulated from complex microbial reaction involving sulfate as electron acceptor and NB as electron donor.

1. Introduction

Global industrialization and rapid social developments have led to the increase of untreated or partially treated industrial effluent discharged into the environment. To a large extent, the biggest quantities of such pollutants are organic compounds such as nitrobenzene (NB) (Lin et al., 2012; Mu et al., 2009). NB is a simple and an important nitroaromatic compound used in industry to produce dyes, pesticides, plastics, lubricating oil, synthetic rubber and intermediates in chemical syntheses (Mu et al., 2009; Wang et al., 2011; Kuscu and Sponza, 2007; Bell et al., 2003). The US Environmental Protection Agency based on known or suspected carcinogenicity, mutagenicity, teratogenicity, and high acute toxicity designate NB as a “priority pollutant” (Kulkarni and Chaudhari, 2007). Furthermore, because of its persistence in the environment NB poses potential public health risks and hazards (Yu-nan et al., 2009; Razo-Flores et al., 1997; Dickel et al., 1993; Majumder and Gupta, 2003).

In literature, several physical, chemical, and biological methods have been employed to treat NB wastewater (Huang et al., 2012a). In most reported cases, both physical and chemical methods are generally efficient with high removal rates that make them applicable for large-scale industrial wastewater treatment. However, their high-energy inputs and maintenance cost make them less attractive to small and medium rural-based industries. The limitation for biological methods is their high sensitivity to external environmental changes, which affects specific enzymes and various intermediate formation resulting in low rates of degradation (Wang et al., 2011; Kulkarni and Chaudhari, 2007; Razo-Flores et al., 1997). However, biological methods have merits of relatively low investment, eco-friendly, economical cost, and sustainability (Zhao et al., 2011). Some of these advantages allow in situ bioremediation of pollutants for rural-based industries. In addition, new formation of intermediate harmful products is low during biological conversions (Wang et al., 2011; Kulkarni and Chaudhari, 2007). For this reason, biological methods have attracted wide public acceptance (Dušek et al., 2008).

Constructed wetlands (CWs) are one of the biological methods that have gained popularity in remediating various wastewaters in most parts of the world in the last five decades. CWs, also known

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as *treatment wetlands*, are engineered systems that are designed and constructed to utilize naturally existing processes to remove different pollutants from wastewater streams (Faulwetter et al., 2009). The application of CWs as an effective, economical treatment alternative has been successfully extended to treat various wastewaters, because of their robustness, simplicity, flexibility, and low operational costs (Tanner et al., 2012; Trang et al., 2010). These wastewaters include municipal sewage (Hadad et al., 2006), domestic sewage (Brix et al., 2005), industrial effluent (Mitsch and Wise, 1998), agricultural effluent (Sun et al., 1999), urban runoff (Cooper et al., 1996), acid mine drainage (Nyquist and Greger, 2009; Mays and Edwards, 2001) and landfill leachate (Ramirez et al., 2005). Therefore, CWs have a great potential for use in wastewater remediation in developing countries especially in removing pollutants in rural-based industries.

From literature, however, the use of CWs to treat industrial wastewater pollutants is not well established, particularly for NB-contaminated effluents (Haarstad et al., 2012). Only few studies have examined the direct application of CWs to treat NB. Lin et al. (2012) and Lv et al. (2013) investigated the treatment of synthetic NB wastewater using laboratory-scale CWs. The results indicated the potential role of CWs in remediating NB-polluted wastewater, but mainly from the aspect of influent-effluent concentrations and overall performance.

Some studies have examined the anaerobic and aerobic biodegradation pathways for NB (Kulkarni and Chaudhari, 2007; Razo-Flores et al., 1997; Huang et al., 2012a). From these studies, during anaerobic degradation, NB is reduced to aniline and then aniline is mineralized into carbon dioxide (CO_2) and water. In aerobic pathway complete mineralization is achieved by bacterial action on the nitroaromatic compounds as a source of carbon, nitrogen, and energy (Kulkarni and Chaudhari, 2007). More so, the specific macro and micro gradients of redox conditions in the rooted zone of CWs enable a highly diverse development of the microbial consortia that are capable of different redox reactions (Bezbaruah and Zhang, 2004; Liesack et al., 2000). Although, all these processes play a part in pollutant removal and toxicity reduction, what remains unclear is their contribution and interaction in NB degradation. For better utilization of CWs in NB removal, the study on NB biodegradation pathways in CWs should be expedited. This understanding will enable the deployment of CWs for use in decentralized NB wastewater treatment in rural-based industries that are located in areas where centralized methods are impractical to implement.

In order to address a missing gap this study focuses on the following objectives: (1) performance evaluation of horizontal subsurface flow constructed wetlands (HSSFCWs) in NB degradation and their response to shock inflow loading under conditions of with or without intermittent aeration, (2) investigation into the coexisting NB biodegradation pathways and their interaction with other microbe-mediated processes such as sulfur and nitrogen cycles in HSSFCWs.

2. Materials and methods

2.1. Experimental setup

The experimental laboratory-scale HSSFCWs, planted with *Juncus effusus*, were located at Bioenergy Engineering and Low Carbon Technology Laboratory, China Agricultural University, China. The system consisted of two plastic containers (100 cm length, 15 cm width, and 50 cm height), filled up to 45 cm with gravel (2–6 mm diameter, 1.67 g/cm^3 density, and 35% porosity) leaving a free pore water volume of about 25 L. In order to prevent algal development during use, the exterior walls of the wetlands were covered with black polythene. Flow zones of 3 cm width in front of inlet and outlet were provided to create laminar flow and to ensure even distribution of flow through the gravel bed. After vibrant growth of the plants, wetlands were supplied with low NB synthesized wastewater for four months to acclimatize microorganisms and for the biofilm in the wetlands to develop well. Synthesized wastewater was continuously delivered to wetlands via peristaltic pumps at the rate of 5 L/d and hydraulic loading rate of 3.33 cm/d, and the water depth in the bed was maintained at 40 cm.

The first experimental HSSFCW was not aerated, whereas the second one was actively and intermittently aerated through a perforated pipe placed at the bottom of the bed. The perforated pipe was connected to an adjustable air pump (model ACO-6603, China), which was actuated using a microcomputer switch timer to pump air for a duration of 1 h every after 1 h. The airflow rate was set at 120 L/h. This air flow rate was taken after carrying out pretest on the aeration system to get an optimal air supply to the wetland that would not exert high pressure leading to unnecessary disturbance to the microbial community in the wetland matrix. The process of intermittent aeration was designed to ensure a mixture of aerobic and anaerobic conditions in the bed.

The synthetic influent wastewater was used to reduce variability in the experiment. The influent wastewater was prepared by first dissolving analytical grade NB in deionized water with methanol and then in tap water, which contained NH_4Cl and $K_2HPO_4{\cdot}H_2O$, according to the concentration as indicated in Table 1. The averaged influent sulfate concentration in the whole experiment was 74 ± 21 mg/L, and the mean influent COD was 80 ± 24 mg/L and 149 ± 25.8 mg/L for phases I and II, respectively. Mineral trace solution contained EDTA-NA (0.100 g/L), $FeSO_4{\cdot}7H_2O$ (0.100 g/L), $MnCl_2{\cdot}4H_2O$ (0.100 g/L), $CoCl_2{\cdot}5H_2O$ (0.170 g/L), $CaCl_2{\cdot}6H_2O$ (0.100 g/L), $ZnCl_2$ (0.100 g/L), $CuCl_2{\cdot}5H_2O$ (0.020 g/L), $NiCl_2{\cdot}6H_2O$ (0.030 g/L), H_3BO_3 (0.010 g/L), $Na_2MoO_4{\cdot}2H_2O$ (0.010 g/L), and H_2SeO_3 (0.001 g/L). This solution was added to the artificial wastewater (1 mL/L). NB was carefully regulated to prevent it from getting into the open environment.

2.2. System operation and management

To ensure proper operation, the system was carefully set as required. Influent delivery pipes and storage tanks were cleaned on a 3-day interval to remove biofilms and avoid delivery system blockage. Flow rate was set and checked on a daily basis, and necessary adjustments were made to ensure consistent inflow in both wetlands. The experiment in phase I lasted for 60 days to investigate the removal performance and fate of NB at the influent concentration of 35 mg/L. The second experimental phase lasted the following 60 days at the influent concentration of 140 mg/L to investigate the effect of shock loading on NB removal. In total the study period was 120 days. The inflow NB concentration of 35 mg/L and 140 mg/L were selected based on the reported prevalent concentration in industrial effluent wastewater (Patil et al., 2011; Jameson et al., 2002).

2.3. Sampling and analysis

To understand the process conditions in the system, water samples were obtained every 2 days at three main points: the inlet,

Table 1
Wastewater influent composition.

Parameter	Average (mg/L)
NB	35 (phase I)/140 (phase II)
PO_4–P	5
NH_4–N	16