Ecological Engineering 67 (2014) 198–205

Contents lists available at ScienceDirect

Ecological Engineering

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

Microbial activity and community structure in two terrace-type wetlands constructed for the treatment of domestic wastewater

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ARTICLE INFO

Article history:
Received 17 September 2013
Received in revised form 15 February 2014
Accepted 31 March 2014
Available online 4 May 2014

Keywords:
Microbial activity
Microbial community
PCR-RFLP
Terrace-type wetlands constructed
Domestic wastewater

ABSTRACT

The aim of the current study was to investigate the assembly and function of microbial community structure two terrace-typed constructed wetlands (TCWs) designed to improve the quality of domestic wastewater in rural areas. Also, the performance of 1-year-old wetlands was investigated. The TCWs had a strong ability remove the COD, NH_4^+-N, TN and TP, and promotes higher pollutant removal rates relative to single stage CWs. The microbial numbers and fluorescein diacetate (FDA) hydrolysis varied among different levels in both wetlands, and associated with the category of the filter media. The microbial community composition of the biofilm formed on the surface of filter media was studied by PCR-amplified restriction fragment length polymorphism (PCR-RFLP) and sequence analysis of 16S rRNA genes. A total of eight bacterial communities were sampled from the TCWs. Clones of each library were selected randomly for PCR-RFLP analysis of rDNA fragments, and eventually 87 genotypes were identified by RFLP fingerprintings. These 87 genotypes were sequenced and their respective phylotype was identified through the Blast tool of NCBI (similarity 95–100%) and phylogenetic analyses. Among these phylotypes, *Proteobacteria* was the most abundant group. Sequence analyses revealed that 52.9% (46) of the clone sequences were similar to those of the uncultured bacteria in the environment. Moreover, the bacterial diversity and composition were clearly displayed in different wetland levels and filter media. The bamboo charcoal and wetland levels may be the main factors affecting the composition of the microbial community.

1. Introduction

The agricultural towns are predominated in central and western China. These towns have small populations and scattered living areas, and the wastewater is characterized by large fluctuations in the quality and quantity of water. Limited by the levels of economic development, traditional large-scale wastewater treatment plants are not the most appropriate options. In terms of the common bio-ecotech in domestic water treatment, biological contact oxidation, SBRs, constructed wetlands, stabilization basins and other combined processes were the most adopted to remove COD, nitrogen, phosphorus and other pollutants. However, there are some problems in the adaptation of these processes in distributed wastewater treatment, which were mainly reflected as the high cost of the infrastructural investment maintenance fee, sophisticated operation schedule and large power consumption (Matos et al., 2013). Therefore, ecological processes, such as constructed wetlands, have greater advantages to purify the distributed wastewater without requiring a complete wastewater disposal pipe network (Kivaisi, 2001; Zurita et al., 2009; Sims et al., 2013).

Constructed wetlands (CWs) are widely used as low-cost alternatives to conventional tertiary municipal wastewater treatments worldwide, which are often employed to remove SS, COD, N, P and other pollutants in wastewater (Kadlec and Wallace, 2008; Vymazal, 2010, 2011; Langergraber, 2013). While a variety of removal mechanisms including sedimentation, filtration, precipitation, volatilization, adsorption and plant uptake are well documented (Chazarenc et al., 2009). Although pollutant removal mechanism in CWs is variable and complicated, it has become common recognition that removal of most pollutants in treatment wetlands is due primarily to microbial activity (Vymazal, 2007; Faulwetter et al., 2009). Microbial assemblages can be found as a biofilm on substrate (filter media) and root surfaces (Faulwetter et al., 2009). Detailed knowledge about the microbial

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http://dx.doi.org/10.1016/j.ecoleng.2014.03.079
0925-8574/

assemblages is needed to understand and explain the CWs functioning (Calheiros et al., 2009). However, many parameters affect biofilm structure, especially wastewater characteristics, filter media or other environmental conditions (Kierek-Pearson and Karatan, 2005).

Several studies have investigated the effects of different wetland conditions on the community composition in constructed wetlands. Tarr et al. (2005) successfully assessed the microbial community structure in different layers of planted soil wetlands for domestic wastewater treatment. Iasur-Kruh et al. (2010) observed that depth was the main factor affecting both microbial community composition and microbial activity, such as vegetation and gravel type, compared with other parameters in wetland microcosms. In contrast, presence different filter media or vegetation did not significantly affect wetland microbial activity and community composition (Silyn-Roberts and Lewis, 2004; Osem et al., 2006). Also, biofilm community composition and activity of different wetland systems were influenced through organic matter (Nguyen, 2000) and surface properties (Ladd et al., 2004). Thus, not many experimental results pointing to the same direction and the connection between microbial community structure and wastewater nature and properties of the wetland remained an interesting but uncleared issue.

At present, CWs mainly designed for post-treatment of effluent wastewater from the sewage-treatment plants. And Most of CWs are single stage, the removal efficiency is relatively low. So, in order to improve the removal efficiency of domestic wastewater, and examine the dynamics of the microbial community and activity in the CW, we designed two terrace-type constructed wetlands (TCWs) which planted with local vegetation and used bamboo charcoal (a local biomass resource) as a filter media. The specific purpose of this investigation are (1) to propose a new type of constructed wetland: TCW, which was developed to be used in the wastewater treatment in hilly areas and (2) to assessed the effect of filter media and wetland levels on microbial community structure and activity in TCWs microcosms.

2. Materials and methods

2.1. Wetland design and operation

Two types of wetland systems, designed for the post-treatment of effluent wastewater, were constructed through the simulation of terraced fields in the hilly land on the campus of Jinggangshan University. The two systems were terrace-typed constructed wetlands with vertical subsurface flow (VTCW) and horizontal subsurface flow (HTCW) with four levels. Constructed wetland engineers designed the variability of the wetland levels, as detailed in Fig. 1. These systems contained different filter media. The first level of the constructed wetlands contained gravel with a grain size of 10 mm, the second level contained gravel with a grain size of 5 mm, the third level contained a mixture of gravel (5 and 10 mm) in the same proportions, and the fourth level contained gravel (5 mm) and bamboo charcoal mixtures in the same proportions. In addition, all wetland systems were planted with a mixture of *Canna sp.*, *Typha angustifolia* and *Phragmites australis* (Fig. 1). These wetland systems were operated from September 1, 2011 to August 31, 2012. The influent wastewater from a storage reservoir was pumped into the first layer through a peristaltic pump at an inflow rate of 5–7 L/h (BT100-100M, Longer, China). The hydraulic loading rate was approximately 1000–2000 mm/day. The influent and effluent water of each level were sampled once a week to determine the treatment performance of the wetlands (Zhang, 2007). The parameters COD, NH_4^+-N, TN and TP were tested according to the Methods for Examination of Water and Wastewater (APHA, 1998), and the characteristics of the influent water qualities during sampling periods are listed in Table 1.

Table 1
The wastewater characteristics of TCWs influent.

Items	Range	Average	Discharge standard
pH	7.1–7.4	7.26	6–9
COD (mg/L)	68.1–124	93.5	50
NH_4^+–N (mg/L)	6.8–20.4	12.7	5 (8)
TN (mg/L)	9.31–44.54	22.6	15
TP (mg/L)	0.64–3.07	1.55	0.5

2.1.1. Sampling

After a 12-month operation period, filter media samples were collected from the systems using a cylindrical corer. The media surfaces (2–15 cm) were divided into quadrants, and the sampling points within each quadrant were randomly selected. Triplicate media samples were obtained per quadrant, combined in a polyethylene bag and transported to the lab in a cooler filled with ice. In the laboratory, the samples from each level were manually homogenized and designated V_1 (VTCW, level 1), V_2 (VTCW, level 2), V_3 (VTCW, level 3), and V_4 (VTCW, level 4) and H_1 (HTCW, level 1), H_2 (HTCW, level 2), H_3 (HTCW, level 3), and H_4 (HTCW, level 4). From each homogenized sample, nine copy subsamples of 5 g were obtained and placed in sterile 50 mL tubes for use in subsequent analyses.

2.1.2. Microbial numbers and activity

The total numbers of culturable bacteria and fungi were determined as colony forming units (CFUs) on agar plates using dilution plate methods. Briefly, 5 g of media with biofilm was completely washed using 10 mL of sterilized double-distilled water, and 0.1 mL of each serial dilution of the sample suspension was spread over an agar (2%) plate with beef extract peptone medium for culturing bacteria and Martin's medium for culturing fungi. Beef extract peptone medium was composed of 0.05%beef extract, 0.05%peptone, 0.03%NaCl (w/v) and 0.05%fungicidine, pH7.0. Martin's medium was composed of 1%glucose, 0.5%peptone, 0.1%K_2HPO_4, 0.05%$MgSO_4{\cdot}7H_2O$, 0.003% (w/v) Rose Benga and 0.003%streptomycin, pH7.0. Six continuous 10-fold dilutions of each sample were prepared starting with 9.0 mL of sterilized phosphate-buffered saline and 1.0 mL of sample suspension. All plates were incubated at 30 °C in the dark until colonies appeared (2 days for bacteria and 3 days for fungi).

The general microbial hydrolytic activity was measured through the hydrolysis of fluorescein diacetate (FDA) (Iasur-Kruh et al., 2010). Briefly, 5 g of media with biofilm was incubated at 30 °C with a phosphate buffer (0.5 mM, pH7.6) and 100 μL of FDA (2 mg/mL). After 3 h of incubation, the samples were centrifuged (2 min, 13,400 × *g*), and the supernatant was analyzed using a spectrophotometer (494 nm).

2.1.3. DNA extraction

Approximately 5 g of filter media with biofilm was used to isolate DNA according to the methods of Zhou et al. (1996), with slight modifications. The extracted DNA was dissolved in 50 mL of TE (10 mM Tris–HCl and 1 mM Na_2EDTA, pH 8.0), and confirmed on 1% agarose gel electrophoresis.

2.1.4. PCR amplification of 16S rDNA and cloning

Two rounds of PCR (nested-PCR) were performed. The first round was performed in a final volume of 25 μL using 1 μL (about 1 ng) extracted DNA as the template, 0.5 μL each of the primers