Ecological Engineering 84 (2015) 38–45

Contents lists available at ScienceDirect

Ecological Engineering

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

Effects of plant biomass on bacterial community structure in constructed wetlands used for tertiary wastewater treatment

Yi Chen [a,b], Yue Wen [a,*], Zhiru Tang [a], Jingang Huang [c], Qi Zhou [a], Jan Vymazal [b]

[a] Key Laboratory of Yangtze Water Environment of Ministry of the State Education, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, PR China
[b] Department of Applied Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, 16521 Prague 6, Czech Republic
[c] Institute of Environmental Science and Engineering, Hangzhou Dianzi University, Hangzhou 310018, PR China

ARTICLE INFO

Article history:
Received 16 February 2015
Received in revised form 17 June 2015
Accepted 27 July 2015
Available online 14 August 2015

Keywords:
Subsurface flow constructed wetlands (SSF CWs)
Tertiary treatment stage
Plant biomass
Bacterial community structure
454 high-throughput pyrosequencing

ABSTRACT

Constructed wetlands (CWs) have been used successfully to treat municipal wastewater. However, few studies have focused on the microbial community in CWs used for tertiary wastewater treatment. In this study, 454 high-throughput pyrosequencing was applied to analyze the bacterial community in unplanted, planted, and litter loaded CWs. Results showed that both plants and litter increased the diversity and abundance of the microbial community in CWs. The effects of plant biomass on the bacterial community at the phylum level were not pronounced, but the compositions were significantly affected at the class and genus levels. Specific comparison down to the genus level revealed that the relative abundances of nitrifier, denitrifier and sulfate-reducing bacteria were positively correlated with the removal efficiencies of ammonia, nitrate and sulfate observed. Highly functional organization (>70%) of the bacterial communities indicates that only a small group of species played a dominant role in CWs. The presence of plants and litter significantly affected the bacterial composition via alteration of carbon content (%) and pH values in the gravel matrix. The observation in this study provides useful information about the effects of plant biomass on the bacterial community structure, which plays an important role in the tertiary wastewater treatment process in CWs.

1. Introduction

Constructed wetlands (CWs) are widely used to treat different types of wastewater due to their simple operation, small energy requirements, and low implementation costs. Given that CWs can efficiently treat wastewater effluent with low concentrations of organics and eliminate emerging contaminants (Vymazal, 2011a), they are frequently used to polish the effluent from wastewater treatment plants (WWTPs) (Chen et al., 2014).

Microorganisms are widely considered to be the main force driving the treatment processes in CWs, as they can mineralize organic matter under both aerobic and anaerobic conditions (Truu et al., 2009). Plants are another indispensable component of CWs. Although their effects on the treatment process in CWs are variable, most studies have shown that systems with plants have higher removal efficiency of organics and nutrients than systems without plants (Vymazal, 2011b). Additionally, interactions between plants and bacteria were recently reported to accelerate the degradation of some refractory organic contaminants (Toyama et al., 2011). Therefore, it is important to investigate how plants affect the microbial ecology in CWs in order to understand the complex processes occurring in these ecosystems and to optimize design parameters and improve treatment performance of CWs.

Several studies have focused on the influence of plants on microbial community structure in CWs. Most of these studies reported that plants had little or no effect on the community structure or abundance of the overall bacteria (Ahn et al., 2007; Baptista et al., 2008) or particular functional groups, such as the ammonia oxidizing bacteria (Gorra et al., 2007), sulfate reducing bacteria (Baptista et al., 2008), and methane oxidizing communities (DeJournett et al., 2007). However, several studies revealed that the presence of plants had a significant effect on the species richness and the structure of bacterial communities in CWs (Calheiros et al., 2009, 2010). To date, studies conducted to assess how plants affect the microbial community structure in CWs have used conventional molecular biology methods (e.g., PCR–DGGE and FISH). The PCR–DGGE method enables detection of abundant microbes, but it cannot

* Corresponding author at: Room 301, Mingjing Building, School of Environmental Science and Engineering, Tongji University, Shanghai 200092, PR China.
E-mail address: weny@tongji.edu.cn (Y. Wen).

http://dx.doi.org/10.1016/j.ecoleng.2015.07.013
0925-8574/

efficiently detect species present in low abundance. The FISH method is often limited by the unfitness of probes or primers, and the specific probes used cannot provide an overall profile of the microbial community. Thus, a significant portion of the microbial community remains unidentified when traditional molecular biology methods are used (Ibekwe et al., 2007; Truu et al., 2009). Therefore, more sensitive and comprehensive detection is needed to assess the impact of plants on microbial community structure and composition in CWs.

High-throughput DNA pyrosequencing developed by Roche 454 Life Science is an analytical method that can produce a large amount of DNA data through a massively parallel sequencing-by-synthesis approach (Margulies et al., 2005), and thousands of operational taxonomic units (OTUs) can be identified to assess the microbial diversity in various environmental samples (Ye et al., 2011). Recently, pyrosequencing was shown to provide a sufficient number of 16S rRNA sequences for assessment of full taxonomic diversity of microbes in CWs (Ligi et al., 2013; Ansola et al., 2014). However, few studies have rigorously evaluated the effect of plant biomass on bacterial communities in CWs using the pyrosequencing method. Previous studies have shown that changes in soil properties (i.e., nitrogen (N), phosphorous (P), and pH) could lead to shifts in bacterial composition (Wobus et al., 2003; Lauber et al., 2009; Dong and Reddy, 2010), suggesting that plants might indirectly impact the bacterial community via alteration of soil properties. However, little is known about the relationship among plants, soil properties, and the bacterial community in CWs. Additionally, how microbial communities self-organize and function over the course of long-term operation of CWs is unknown. Consequently, the objectives of this study were to: (1) characterize and compare the bacterial communities in the absence or presence of plants and litter; (2) predict the impacts of plant biomass on bacterial community composition through path analysis linking soil chemistry with bacterial community.

2. Materials and methods

2.1. Design and operation of the SSF CW

Six sequencing batch SSF CW microcosms (3 sets × 2 replicates), each with a bulk volume of 0.045 m^3 (length: 0.3 m, width: 0.3 m, height: 0.5 m) and a pore volume of 12 L, were set up in this study. Three types of systems were established: unplanted control microcosms (W_C, no plants), planted microcosms (W_P, 40 plants/m^2, *Typha latifolia*), and litter-added microcosms (W_L, 100 g cattail litter) (Fig. S2). All of the wetland microcosms were filled with gravel (diameter of 8–13 mm, porosity of 0.4) collected from a quarry, and the plants in W_P microcosms were originated from the water courses in the vicinity of the laboratory. Details about the microcosm design and gravel characteristics are provided in the previous studies (Wen et al., 2010; Chen et al., 2014, 2015).

These wetland microcosms were in operation for 5 years to treat secondary effluent in an air-conditioned greenhouse (Table 1 shows characteristics of the influent). The six microcosms were operated as batch systems, which were filled with municipal secondary effluent at the start of each batch and were gravity drained within 1 h prior to introduction of the next batch. Each batch lasted for 5 days, and there were 18 batches in each stage (90 days) and a total of 20 stages (1800 days).

At the beginning of each stage, 100 g of cattail litter (*T. latifolia*, 1–2 cm pieces) were homogeneously mixed with gravel and filled to a height of 40 cm in the W_L microcosms in order to simulate the decomposition of plant litter over time in wetlands. The sources, collection and preparation of the cattail litter have been described in the previous study (Chen et al., 2015). Water samples were collected at a depth of 20 cm from the center sampling pipe of each CW during every batch, and the analyses of COD, sulfate, ammonia, nitrate, nitrite, total nitrogen and total phosphorus were performed according to standard methods (APHA, 1998).

2.2. Gravel matrix sampling and analyses

Samples were collected on day 1324 when the bacterial community was assumed to be stable in the CWs. For each CW, four different depths (S1, 15 cm; S2, 25 cm; S3, 35 cm; S4, 45 cm) of gravel and sixteen samples within each depth were collected (Fig. S1). Thus, 64 samples were collected from each CW and combined for chemistry analysis. The carbon (C) and N concentrations in organic matter coated around gravel were measured using an elemental analyzer (N A 2500), total P (TP) was determined as $P—PO_4^{3-}$ after persulfate digestion at 120 °C for 35 min, and pH and ORP were measured using a multi-parameter probe (sensION1, HACH).

2.3. DNA extraction and PCR amplification

The gravel/litter was collected at the depths of 5, 20 and 40 cm, and the samples from the three depths were combined for biofilm collection and DNA extraction. The attached biofilm was extracted by shaking gravel/litter samples at 225 rpm for 3 h in sterile glass bottles, and the precipitate was collected in bottles after centrifuging twice at 5000 g for 20 min. Microbial DNA was extracted from the gravel using the EZNA® Soil DNA Kit (OMEGA Bio-Tek).

To construct gene libraries, DNA from the CWs was amplified by PCR using primer set 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 533R (5′-TTACCGCGGCTGCTGGCAC-3′) for the V1–V3 region of the 16S rRNA gene. The composition of the PCR mixture and detailed thermocycling steps are described in the Supporting Information. The fused forward primer included a 10-nucleotide barcode inserted between the Life Sciences primer A and the 8F primer. The barcodes were used to sort multiple samples in a single 454 GS-FLX run.

2.4. 454 high-throughput 16S rRNA gene pyrosequencing and biodiversity analysis

High-throughput 454 GS-FLX pyrosequencing of the 16S rRNA gene was conducted according to standard protocols (Margulies et al., 2005). The qualified sequences were clustered into OTUs with a 95% similarity level, produced rarefaction curves, calculated species richness estimators (Chao1), the Shannon diversity index, and Good's coverage, and conducted principal component analysis (PCA) using the MOTHUR program. BLAST analysis of taxonomic classification down to the phylum, class, and genus levels was conducted using MOTHUR via the SILVA 106 database with a set confidence threshold of 80%. A Venn diagram with shared and unique OTUs was used to compare the similarity and difference among the three communities. Functional organization indices (F_O) in different CWs were calculated using Pareto–Lorenz evenness distribution curves (Wittebolle et al., 2008). Path analysis was applied as a visual framework for identifying the correlations among plant biomass, gravel chemistry, and bacterial community composition using AMOS version 21 (SPSS, IBM). An analysis of variance (ANOVA) was used to test the significance of results, and $p < 0.05$ was considered to be statistically significant. Details about pyrosequencing and statistical analysis are provided in the Supporting Information.