Ecological Engineering 94 (2016) 117–126

Contents lists available at ScienceDirect

Ecological Engineering

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

Nitrogen and phosphorus transformations and balance in a pond-ditch circulation system for rural polluted water treatment

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ARTICLE INFO

Article history:
Received 13 October 2015
Received in revised form 24 March 2016
Accepted 22 May 2016
Available online 6 June 2016

Keywords:
Pond-ditch circulation system (PDCS)
Rural wastewater
DGGE
Nitrogen removal
Phosphorus removal

ABSTRACT

A pond-ditch circulation system (PDCS), which located along the bank of Donghu Lake (Wuchang District, Wuhan, China) and can effectively remove nitrogen and phosphorus, was proved to be a promising solution for the restoration of a degraded rural water environment. However, little is known about the removal process of nitrogen and phosphorus in the PDCS and the contribution of various pathways to nitrogen and phosphorus removal. This study aims to investigate the mechanisms of nitrogen and phosphorus removal for the PDCS. The results showed that (1) for nitrogen removal in the PDCS, it effectively reduced the total nitrogen level within the first 20 days, where dissolved oxygen (DO) played a pivotal role in the process. Based on the mass balance approach, the plant uptake and sediment storage removal contributed to 20.5% of the total nitrogen input removal, whereas other pathways such as N_2 emission accounted for 79.5% of the nitrogen removal. These results indicated that nitrogen removal mainly depended on nitrification and denitrification. Meanwhile, in addition to microbial mechanisms, plant uptake and sediment storage were the major N transformation and removal pathways; in the static system, the pathways for nitrogen removal were similar with those observed in the PDCS system. (2) For phosphorus removal, microorganism processes coupled with plant uptake might be the major methods for phosphorus removal in the PDCS. In the static system, however, the phosphorus removal was mainly attributed to plant uptake.

1. Introduction

Domestic wastewater treatment in rural areas has become a major environmental issue in China due to the rapid economic development and population growth. What is disappointing is that the amount of domestic wastewater treated in China is only 11% for county towns and less than 1% for rural villages because of the limitation of rural domestic sewage collection systems and treatments (Wu et al., 2011). In most Chinese rural regions, non-point source pollution becomes a major reason for the deterioration of aquatic environments. More than 50% of the non-point source pollution is estimated from the direct discharge of rural sewage into surface water bodies (Yu et al., 2012). Agricultural wastewater and domestic sewage are the major sources of the nonpoint-source pollution, including nitrogen (N) and phosphorus (P), which lead to the eutrophication of water bodies (Li et al., 2009). Therefore, developing a cost-effective way to remove N and P from rural wastewater is urgently needed to improve water quality in rural areas.

Thus, increasing attention is being paid to wastewater treatment and disposal in rural areas of China. Considering the low level of economic development, the shortage of energy, and the lack of environmental technical staff in rural areas, the conventional centralized wastewater treatment technologies are generally not feasible for the treatment of rural domestic wastewater. Some decentralized treatment approaches have significantly improved water quality in many rural areas of China, such as the constructed wetlands (Calheiros et al., 2015; Wu et al., 2011), by an integrated step-feed biofilm process (Liang et al., 2010), tower vermifiltration (Kumar et al., 2015; Wang et al., 2011), and bio-ecological combined systems (Fan et al., 2013; Liang et al., 2009). We have developed a new, onsite decentralized system, which is called the pond-ditch circulation system (PDCS) (Ma et al., 2015a). This PDCS, which enhances the mobility of water, significantly improves water quality (especially by effectively removing N and P) in ecological systems. The optimal operating conditions (flow speed and circulation interval) of the PDCS were uncovered in the previous research (Ma et al., 2015b). However, little is known about the mechanism

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http://dx.doi.org/10.1016/j.ecoleng.2016.05.051
0925-8574/

for the reduction of N and P in the PDCS; therefore, further investigations are needed to elucidate the underlying mechanisms of N and P removal.

Previous studies have reported that soil microorganisms, important degraders of contaminants, play a significant role in nutrient removal from wastewater (Shehzadi et al., 2014; Zhang et al., 2015). Generally, in the removal of biological nitrogen, there are two major steps, nitrification and denitrification (Coban et al., 2015; Salvato et al., 2012). Specifically, ammonia is first converted into nitrite, which is further oxidized to nitrate during the nitrification step. During the denitrification process, nitrate is finally reduced to nitrogen gas. Microorganisms are largely responsible for phosphorus removal, i.e., polyphosphate accumulating organisms (PAOs), and usually exist in wastewater treatment systems (Zhang et al., 2013). Extracellular enzymes acting as a biological catalyst can drive the decomposition of organic matter (Liu et al., 2009). Meanwhile, the overall enzyme activity of soil is derived from the activity of both the accumulated enzymes and the proliferating microorganisms (Menon et al., 2013). Hence, extracellular enzymes were used as an index to evaluate the activity of microorganisms in substrates (Huang et al., 2012; Kolehmainen et al., 2009). These enzymes were examined to trace the degrading pathways for both nitrogen and phosphorus removal in this study.

With the development of molecular microbiological techniques, denaturing gradient gel electrophoresis (DGGE) analysis of PCR-amplified 16 S rDNA has been widely used in environmental microbiology and recognized to give an acceptable view on the diversity and dominant species of microbial communities (Niu et al., 2011; Wu et al., 2014). In this study, to elucidate the mechanisms of N and P removal for the PDCS, the following are investigated: (1) the changes of different forms of N and P in water; (2) the contents of total N and P in substrate, water, and plant for pre- and post-circulation; (3) the variation of extracellular enzyme activity; (4) the association between environment variables and extracellular enzyme activity; (5) the diversity and dominant species of microbial communities in the substrate.

2. Materials and methods

2.1. Experimental systems

The settings of the PDCS are the same as previously described (Ma et al., 2015a,b) (Fig. S1, Table S1). Briefly, each PDCS was composed of a water-distribution apparatus, two ponds and one ditch. The sizes of two ponds are as follows (cm, length × width × height): 60 × 40 × 50 (pond 1) and 50 × 30 × 40 (pond 2), respectively. The trapezoidal ditch measured 35 × 25 × 30 cm with a length of 80 cm. Circulation modes for two PDCSs (S1 and S2) in this experiment are static and circulating every other 4 h (3.6 L/h) (Ma et al., 2015b), respectively. Meanwhile, the location of the sampling sites (A–D) is the same as previously described (Ma et al., 2015a,b) (Fig. S2). Moreover, the initial physic-chemical characteristics of the overlying water for this experiment are shown in Table S2.

2.2. Physicochemical analysis

The water samples were collected nine times from pond 1, the ditch and pond 2 between 8:30 and 9:30 AM on days 1, 4, 7, 11, 15, 20, 30, 40, and 50, respectively. Detailed determination methods for the water samples followed previous studies (Ma et al., 2015a,b)

2.3. Determination of nitrogen and phosphorus in the soil and macrophytes

The substrate samples pre- and post-circulation were air-dried and ground to pass through a 100-mesh sieve. The concentration of TN in the sediments was determined by alkaline potassium persulfate digestion (Smart et al., 1983) and analyzed by the UV spectrophotometric method (State EPA of China, 2002). To measure the concentrations of TP, the sediments was first heated at 450 °C for 3 h, extracted by 20 mL of 3.5 M HCl for 16 h, and then determined using the ascorbic acid method (State EPA of China, 2002). The contents of TN/TP in the sediment were expressed as mg TN/TP g^{-1} dry weight of the sediment.

The plant samples (pre- and post-circulation) were cleaned, dried at 80 °C for 48 h, weighed, and then ground to pass through a 100-mesh sieve. The dried plant samples were first digested by sulfuric acid (Martin et al., 1999) and analyzed for TN and TP contents using the Kjeldahl method (Jones, 1991) and the molybdenum blue method (Xie et al., 2013), respectively. Moreover, the plant samples (pre-circulation) were used as control. The final concentrations of TN/TP in the plants were expressed as mg TN/TP g^{-1} dry weight of plant.

2.4. Assays of microbial enzyme activity

The soil samples of pond 1, the ditch and pond 2 were collected with the water samples on days 1, 7, 15, 30, 40. Firstly, in the control/PDCS system, these 15 fresh soil samples were separately thoroughly mixed, and then immediately analyzed for the activity of three extracellular enzymes: urease, phosphatase, and nitrate reductase, which were assayed by the buffer method (Klose and Tabatabai, 2000), the colorimetric method (Jackson and Vallaire, 2009), and the colorimetric method (Singh and Kumar, 2008), respectively. Final enzyme activities were expressed as μg of substrate consumed by h^{-1} g^{-1} dry weight of the sediment. Results were averages of the duplicated assays and analyses and expressed on an oven-dry basis. Moisture was determined after being dried at 105.8 °C for 48 h.

2.5. DNA extraction, PCR amplification, and DGGE analysis

The filters containing microbes were cut into small pieces with a sterile scalpel and then bacterial genomic DNA was extracted using a Bacterial DNA kit (Omega) following the standard protocol purification on E.Z.N.A Gel Extraction Kit (Omega, USA) was then completed. The purified DNA was used as a template to amplify the V3 region of the 16 S rDNA gene (from 1055 bp to 1406 bp) with PCR primers pair GC-338f and 518r (Dong and Reddy, 2010). Specific separations of PCR amplifications (approximately 230 bp) were performed by DGGE by using a Biorad DCode apparatus (BioRad, Richmond, CA), as previously described (Hu et al., 2009; Muyzer et al., 1993).

2.6. DNA sequencing and phylogenetic tree

The sequences of the 16 S rRNA gene fragment clones were determined by Wuhan Tsingke BioTech Company and analyzed using the BLAST program at the internet site of NCBI (National Center for Biotechnology Information) (http://www.ncbi.nlm.nih.gov/blast). The sequence distance matrix for all pair wise sequence combinations was analyzed using MEGA 5.1 with the neighbor-joining method of phylogenetic tree construction with 1000 bootstrap replicates. The banding patterns of DGGE profiles were analyzed using the Quantity One V4.62 Software (Bio-Rad). The Shannon-Wiener index Hí was calculated following the reference procedure (Mota et al., 2005).

2.7. Statistical analysis

Differences in the environmental variables and the enzyme activities between the two systems were tested with one-way