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Unraveling characteristics of nutrient removal and microbial community in a novel aerated landscape – Activated sludge ecological system

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HIGHLIGHTS

- Construct novel landscape-based biological systems for wastewater treatment.
- Achieve excellent efficiencies of nitrogen and phosphorus removal, high SND in LASeM.
- Unravel the effect of aeration on microbial community and operation performance.
- Reveal *Proteobacteria* and *Bacteroidetes*-dominant phyla as effective biodegraders.

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ABSTRACT

In this study, a novel landscape-activated sludge ecological system (LASeM) was constructed with the advantages of promising treatment, less land need and significant landscape services. Compared to literature, this study provided promising integrated wastewater treatment and landscape for wastewater treatment. This first-attempt study clearly deciphered interactive effect of aeration rate (AR) on nutrient removal and microbial community structure in LASeM. When AR was 0.016 $m^3\ h^{-1}$, the most appropriate removal of COD, NH_4^+-N and TP were 96%, 97% and 74% with the effluent of 14.3, 1.7 and 0.7 mg L^{-1}, respectively, which showed satisfactory capabilities for rural domestic wastewater treatment. According to clone library analysis, *Proteobacteria* (71%), *Bacteroidetes* (17%) were found to be the dominant bacterial phylums present in LASeM for biodegradation. In particular, the incorporation of plants altered the microbial community and strengthened capability for the nutrients removal likely due to synergistic interactions among species in the ecosystem.

1. Introduction

The removal of nitrogen and phosphorus from wastewater is one of top priority concern problems for conservation of aquatic ecosystem. In fact, the enrichment of surface water with nutrients from municipal wastewater treatment discharges even strongly affected water quality, leading to risk of eutrophication. Therefore, regulations of the nitrogen and phosphorus from wastewater discharge tended to be gradually stringent (Ahmed, 2012). Moreover, due to progressively increased awareness upon the environment, not only the regulations of water quality are more severe, but also social demands for living environment are also increasing.

To prevent significant oxygen depletion from eutrophication, aeration to promote excess microbial activities to degrade such “high-nutrient” bearing pollutant seemed to be inevitable. However, the considerable costs of energy consumption and operation and maintenance are apparently one of main concern problems for large-scale aeration system (Diego et al., 2008). That is, appropriate manipulating the aeration process would be crucial to minimize operating costs in wastewater treatment plants (WWTPs). As a matter of fact, optimization of biological nutrient removal processes through strategies of varying the aeration rate (AR) has been mentioned in literature (Amand and Carlsson, 2012; Zhang et al., 2014). For example, optimal aeration would be at oxygen peak-to-peak amplitude range between 0.7 and 1.8 mg L^{-1} via simulation, depending on the influent variation and ammonium level in the effluent (Amand and Carlsson, 2012). Zhang et al (2014) indicated that phosphorus removal deteriorated greatly, when AR dropped to approx. 20 L h^{-1}. The abilities of anaerobic phosphorus release and anoxic phosphorus uptake were both improved after increasing AR to 30 L h^{-1}. Ali et al (2011) studied the effect of organic and aeration rate (AR) on the removal performance in a lab-scale upflow aerated submerged fixed-film

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bioreactor, showing that the COD removal could stably maintain at airflow rate of 8 L min^{-1}. However, COD removal performance significantly decreased at aeration rate of 4 L min^{-1}. Such reduction of removal performance was very likely associated with evolution of "keystone degrader(s)" populations in the microbial community. In fact, a better understanding of microbial communities in WWTPs not only directly provided guidance in system design for stable operation, but also clearly suggested strategies of bioaugmentation and/or biostimulation for long-term.

To decipher such fluctuations of operation efficiency and microbial community during wastewater treatment, traditional culture-dependent techniques and microscopic observations were regularly used. Recently, to obtain detailed figures of the community, the second generation sequencing technologies so-called high-throughput sequencing technologies through polymerase and ligase-based in vitro sequencing via synthesis have been developed. They could elucidate the evolutionary characteristics of microbial communities more completely and accurately (Aksyonov et al., 2006). As Guadie et al. (2014) revealed via clone library analysis, *Proteobacteria* (59%), *Firmicutes* (12%) and *Bacteroidetes* (11%) were the dominant bacterial group in a novel fluidized bed reactor–membrane bioreactor system. The Shannon–Wiener indices were used as indicators to disclose performance of activated sludge for wastewater treatment (Hu et al., 2012) and metabolic functions of the microbial community in constructed wetlands (Chen et al., 2015; Button et al., 2015).

Activated sludge processes (ASPs) are well-known systems of wastewater treatment with cost–effectiveness and environmental friendliness. Even they owned high-efficiency for biodegradation of organics, some disadvantages (e.g., biomass instabilities like sludge bulking and emersion, low tolerance to shock loadings, high aeration cost in large volume and not much operation flexibility) were still present. Moreover, odor-control to have minimal impact to neighborhood was one of the most challenging aspects of the wastewater treatment plant (Samantha et al., 2015). To overcome aforementioned disadvantages, novel ecological alternatives for WWT apparently should be provided. Moreover, to consider environmental friendliness and landscape renovation to neighborhood for sustainable development, an integrated fixed-bed activated sludge system with infrastructure of fixed-biofilms propagated on roots of landscape plant species in odorless, botanical garden-designed facility for wastewater treatment was applied. In addition, landscaping provided attractive natural appearance and tended to create recreational use areas to neighbor communities. Recently, the integrated fixed-biofilm and activated sludge reactor (IFAS) has been reported to remove autotrophic nitrogen (Veuillet et al., 2014). Here, interactive systems of activated sludge, ceramsites biofilm carrier with landscape plants (*Cyperus alternifolius*) were used to construct a newly developed landscape-activated sludge ecological system (LASeM) for ecologically friendly treatment of rural domestic wastewater.

Although literature revealed that some novel combination of ASP with other systems (e.g., fixed-biofilm activated sludge reactor) have been implemented at laboratory and pilot scale for efficient nutrient and organic matter removal (Zhang et al., 2015), transient dynamics of species evolution in the microbial community from landscape-activated sludge ecological system were still remained not disclosed. Inevitably, this led to difficulties for scale-up to process control and system optimization. Thus, this study tended to decipher the diversity and abundance of microbial communities in diverse compartments in LASeM for system analysis. Assessment upon the nutrient removal efficiency and microbial community structure at different ARs was carried out to reveal explanations of operation performance for process optimization.

2. Materials and methods

2.1. LASeM setup

Lab-scale experiments of landscape-activated sludge ecological system (LASeM) were carried out for feasibility study (refer to schematics in Fig. 1). The LASeM is an integrative device which consisted of two plexiglas cuboid containers of total volume of 55 L, where the smaller container nested within the bigger container. From the bottom up, the system could be divided into activated sludge area, ceramsite and plant root area and fresh water area for plant growth. The ceramsitess (height is 14 cm) with a diameter of 10–14 mm were placed in the smaller container with some connecting in the bottom with the bigger container. As nitrogen and phosphorus removal capabilities of *Cyperus alternifolius* were emphasized elsewhere (Cui et al., 2009, 2011), grass-like plant species *Cyperus alternifolius* was cultivated upon the ceramsites. A well-sparged and diffused aeration system was provided for sufficient oxygen distribution for microbial growth and hydraulic retention time (HRT) was specifically set at 48 h for feasibility testing. Unless some sludge was used for microbial analysis, chiefly the reactor was operated without sludge withdrawal. The influent flow was controlled via peristaltic pumps and effluent was overflowed to maintain constant volume. To guarantee safe water quality and operation stability, some fishes (*Cyprinus carpio haematopterus*) were raised in the chamber of treated wastewater for indicator purpose. The plants and fishes assembled in the system were simulated ecosystem services provided by landscape design of LASeM.

The LASeM was subsequently operated for at least 30 d to achieve stable steady-state conditions prior to treatability study. LASeM was then continuously operated for 112 d. During continuous operation, aeration rate was gradually decreased from 0.1 to 0.012 $m^3\ h^{-1}$ through 4 stages of step changes. To reveal the relative abundance and species diversity of the microbial community, samples of activated sludge, ceramsites biofilm and *Cyperus alternifolius* roots at different aeration rates were taken.

2.2. Wastewater characteristics

The synthetic wastewater was prepared according to rural domestic wastewater. It contained (mg L^{-1}) starch (278), glucose (278), potassium dihydrogen phosphate (13.16), ammonium sulfate (165), and sodium bicarbonate (111). Trace mineral solution containing (mg L^{-1}): calcium chloride (6), magnesium sulfate (66), manganese sulfate (6). Typical composition of the synthetic wastewater was COD (chemical oxygen demand) 320 mg L^{-1}, TN (total nitrogen) 35 mg L^{-1}, NH_4^+-N 35 mg L^{-1}, TP (total phosphorus) 3 mg L^{-1} (Fig. 3). Influent sodium bicarbonate was applied to provide as buffering solutions for nitrification process, the wastewater was kept at nearly neutral pH 7.0 ± 0.5.

2.3. Chemical analytical methods

All water samples were filtrated by 0.45 μm filter before analysis. The water sample were analyzed using standard analysis method of water and wastewater (APHA, 2005) for COD (APHA-5220C), TN(APHA-4500P-J), TP(APHA-4500N), NH_4^+-N (APHA-4500-NH_3), NO_2^--N (APHA-4500-NO_2^--B) and NO_3^--N (APHA-4500-NO_3^-). The turbidity was measured with spectrophotometric analyses of HACH-2100Q. The DO levels were measured with oxygen probe YSI550A. Volume loading was calculated with the formula (1) as follows:

$$\mathrm{Fr} = (C_1 - C_2) \times Q/1000V = (C_1 - C_2)/1000t, \quad (1)$$