Organics removal, nitrogen removal and $N_2O$ emission in subsurface wastewater infiltration systems amended with/without biochar and sludge

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**A B S T R A C T**

Organics removal, nitrogen removal, $N_2O$ emission and nitrogen removal functional gene abundances in four subsurface wastewater infiltration systems (SWISs), named SWIS A (no intermittent aeration without biochar and sludge), SWIS B (no intermittent aeration with biochar and sludge), SWIS C (intermittent aeration without biochar and sludge), SWIS D (intermittent aeration with biochar and sludge) were investigated. Intermittent aeration enhanced chemical oxygen demand (COD), ammonia nitrogen ($NH_4^+$-N), total nitrogen (TN) removal and the abundances of nitrogen removal functional genes (amoA, nxrA, napA, narG, nirS, nirK, qnorB and nosZ) compared to non-aerated SWISs. High COD (95.4 ± 0.2%), $NH_4^+$-N (96.2 ± 0.6%), TN (86.4 ± 0.5%) removal efficiencies and low $N_2O$ emission rate (18.4 mg/(m$^2$ d)) were obtained simultaneously in intermittent aerated SWIS amended with biochar and sludge. The results suggested that intermittent aerated SWISs amended with biochar and sludge could be an effective and appropriate method for improving treatment performance and reducing $N_2O$ emission.

1. Introduction

Subsurface wastewater infiltration system (SWIS) as a land wastewater treatment has been extensively used to treat decentralized domestic wastewater (Pan et al., 2016a). SWISs have good removal performances for organics and phosphorus. However, limited nitrogen removal remains as a major challenge for conventional SWISs because of insufficient oxygen supply and lack of biodegradable organics (Li et al., 2011; Pan et al., 2016b).

Nitrification and denitrification are the most effective pathways for nitrogen removal (Wang et al., 2010). In the SWIS, nitrification requires aerobic conditions while denitrification occurs in anoxic or anaerobic environment, which could not be fulfilled simultaneously. Intermittent aeration could significantly enhance removal rates of organic pollutants and ammonia nitrogen ($NH_4^+$-N) (Pan et al., 2015, 2016a). Decomposition of organic matter takes place mainly in the upper part of the SWIS, which leads to carbon source lack in the lower part and low denitrification (Wang et al., 2010). Adding external carbon source to the water was the main solution to improve nitrogen removal (Wang et al., 2010; Li et al., 2011).

Recently, many researches have been interested in biochar or sludge as a new type of amendment for wastewater treatment (Zhou et al., 2017; Kizito et al., 2017). Biochar is a porous carbon-rich material produced by biomass pyrolysis in anoxic and anaerobic conditions (Sohi et al., 2010), which has high porosity, large surface area and cation exchange capacity. These characteristics are benefit for the pollutants adsorption and biofilm attachment ability, which can improve the pollutants degradation (Dalahmeh et al., 2012). Furthermore, the addition of biochar to wastewater treatment can improve effluent quality and reduce greenhouse gas emissions (Zhou et al., 2017). Sludge is a heterogeneous mixture of organic matter, micro-organisms, colloids and cations, which is the byproduct of wastewater treatment. Sludge treatment and reuse is a challenging issue for water industries. Lately, several investigations have successfully made sludge as a microbial inoculum in biological wastewater treatment, which can significantly improve chemical oxygen demand (COD) and total nitrogen (TN) removal (Li et al., 2011, 2013). According to Kadam et al. (2008), amending with sludge in soil filter system would produce high biomass concentration, which resulted in more rapid increase in pollutants removal. So far, very few literatures focus on the application of biochar and sludge substrate for enhancing treatment performance in intermittent aerated SWISs. Therefore, the main objective of this study was to evaluate the effects of intermittent aerated SWISs amended with biochar and sludge on organics removal, nitrogen removal and $N_2O$...
2. Material and methods

2.1. Experimental systems and procedures

Four lab-scale SWISs made of plexiglass vertical tubes (120 cm in height and 50 cm in diameter) were performed in parallel (SWIS A: no intermittent aeration without biochar and sludge; SWIS B: no intermittent aeration with biochar and sludge; SWIS C: intermittent aeration without biochar and sludge; SWIS D: intermittent aeration with biochar and sludge). Distributing pipe was installed in 50 cm depth below the surface in each infiltration system. The schematic diagram of four SWISs is shown in Fig. 1. Matrix sampling ports were installed at 50, 80 and 110 cm from the top of the SWISs. 10 cm of deep gravel (10–20 mm, diameter) was prepared at the bottom to support infiltration system and evenly distributed the treated water. The treated wastewater was collected at the bottom of each system near the outlet. Aerated SWISs were composed of aerated units which consisted of air compressors, air tubes and micro-bubble diffusers in the depth of 40 cm. Micro-bubble diffuser and distributing pipe were surrounded by gravel (10–20 mm, diameter) to protect clogging and diffuse air.

SWIS A and C were filled with 80% brown soil and 20% coal slag by weight ratio. Infiltration beds of SWIS B and D were divided into two layers. The upper was 70 cm of 80% brown soil and 20% coal slag and the lower was 40 cm of 80% brown soil, 10% sludge and 10% biochar by weight ratio. The brown soil collected from the top 20 cm from Shenyang Ecological Station consisted of 31.3 ± 0.3 g/kg of total organics, 159.2 ± 2.1 m²/kg of surface area. The coal slag purchased from a local market, 4–8 mm in diameter was used to improve the permeability and absorption area of the matrix. Sludge collected from the sludge-dewatered unit of wastewater treatment plant, air dried after being centrifuged. Corn straw was carbonized under anaerobic conditions with a 15 h slow pyrolysis, at a temperature ramp of 10 °C/min to maximum temperature of 500 °C. The resultant biochar materials were crushed using a bench scale hammer mill and sieved to a particle size range of 2 mm. The particles were subsequently washed with distilled water to remove ash, fine particles and dust. The matrix components were mixed in a blender five times with 15 min/time to ensure the uniformity.

Aerated SWISs were intermittently aerated for 4 h (The aeration was between 0:00–1:00, 6:00–7:00, 12:00–13:00 and 18:00–19:00) at an airflow rate of 2.0 L/min every day. Domestic wastewater was pre-treated in a septic tank prior to being fed into each SWIS continuously with hydraulic loading rate of 0.08 m³/(m² d). The ranges of wastewater pretreatment were COD 179.7–264.2 mg/L, NH4+-N 30.2–41.6 mg/L, TN 35.5–43.5 mg/L, total phosphorus (TP) 2.8–6.3 mg/L, pH 6.9–7.3. All SWISs were operated 50 days before sampling to allow systems mature.

2.2. Sampling and analysis

Water samples were collected from the influent tank and from the outlet of each system every 5 days, respectively. COD, NH4+-N and TN were analyzed according to standard methods (APHA, 2003). Gas samples were collected at the same time of the day between 9:00 AM and 11:00 AM after enclosure every 5 days by closed static chambers. N2O concentration was analyzed by Agilent 6890N gas chromatography. N2O emission rate was calculated according to Nakano et al. (1995). N2O conversion ratio is the quality percentage of nitrogen convert to N2O occupied in TN. Matrix samples were collected from sampling ports after each experiment which were stored in an ice incubator. Soil DNA kits (Omega, D5625-01) were used to extract and purify the total genomic DNA from the samples. Extracted genomic DNAs were detected by 1% agarose gel electrophoresis, and preserved at −20 °C freezer until using. Quantitative analysis was made for nitrogen removal functional gene abundances by qPCR. The details about steps of methods were described in previous study (Pan et al., 2017). Statistical checks were made at significant differences of 0.05 using SPSS 12.0 (n = 20).

3. Results and discussion

3.1. COD and nitrogen removal

Fig. 2 shows the influent, effluent water qualities and removal efficiencies of four SWISs. COD removal efficiencies of SWIS C (94.4 ± 0.4%) and D (95.4 ± 0.2%) were best, which were much higher than that of SWIS A (85.8 ± 0.8%) and B (81.8 ± 1.6%). COD removal efficiencies in intermittent aerated SWIS C and D were significantly higher than those in non-aerated SWIS A and B with/without biochar and sludge (P < .05). This was largely attributed to intensify oxygen supply generated by intermittent aeration in SWIS C and D, which was in accordance with previous studies (Pan et al., 2015, 2016a). In a SWIS, organic matter was absorbed by the soil, and then broken down by aerobic and anaerobic microbial processes. The aerobic heterotrophic bacteria played an important role in the aerobic degradation of organic matter (Pan et al., 2013b). Ong et al. (2010) and Wu et al. (2015) reported that sufficient oxygen supply would greatly elevate the performance of aerobic biochemical oxidation. Disadvantageous aerobic environment always limited organic matter degradation (Pan et al., 2016b). COD concentration in the effluent of SWIS A (39.6 ± 2.9 mg/L) was higher than that of SWIS B (30.9 ± 2.6 mg/L). Biochar has a highly porous structure and large surface area which provides enough space for microbial growth. Sludge has a high biomass concentration. Therefore, biochar was effective in the adsorption of COD and microorganism of sludge enhanced the degradation of COD. Former studies concluded biochar played an important role for the reduction of COD by adsorption (Kadlec and Wallace, 2008; De Rozari et al., 2015; Zhou et al., 2017) and matrix amended with sludge could improve COD removal in a SWIS (Li et al., 2011, 2013). COD concentration in the effluent of SWIS C was 12.1 ± 0.9 mg/L, which was similar to that of SWIS D (10.3 ± 1.3 mg/L). Although biochar had adsorption capacity of COD in SWIS D, the rate of aerobic degradation was faster than that of adsorption.

It is widely accepted that nitrification could occur with aerobic
conditions (Albuquerque et al., 2009; Li et al., 2011). Most conventional SWISs fail to achieve an efficient nitrification as a result of disadvantageous anoxic or anaerobic conditions (Fan et al., 2013b). SWIS C and SWIS D achieved NH$_4^+$-N removal efficiencies of 93.1 ± 0.4% and 96.2 ± 0.6%, respectively, while low NH$_4^+$-N removal efficiencies in SWIS A (69.2 ± 1.8%) and SWIS B (72.2 ± 1.8%) were observed. NH$_4^+$-N removal efficiencies in intermittent aerated SWIS C and D were significantly higher than those in non-aerated SWIS A and B with/ without biochar and sludge (P < .05). In SWIS C and D, nitrification was improved with NH$_4^+$-N effluent concentration of 2.6 ± 0.3 mg/L and 1.4 ± 0.3 mg/L, respectively, which was mainly due to sufficient oxygen supply by intermittent aeration. Former study also found that more nitrifying bacteria involved in nitrogen removal were detected in intermittent aerated SWISs than non-aerated SWISs (Fan et al., 2013b; Pan et al., 2015). Nitrification was inhibited in SWIS A and B with NH$_4^+$-N effluent concentration of 11.4 ± 0.7 mg/L and 10.3 ± 0.6 mg/L, respectively. The poor nitrification was mainly due to insufficient oxygen supply. NH$_4^+$-N removal efficiencies of SWISs with biochar and sludge were higher than those without biochar and sludge in intermittent aerated systems or in no intermittent aerated systems. Biochar adsored NH$_4^+$-N, so the absorption of biochar reduced NH$_4^+$-N in SWISs. The reason was that the rate of nitrification was higher than NH$_4^+$-N absorption rate (Zhou et al., 2017).

TN removal efficiencies were 46.2 ± 2.2% for SWIS A, 55.6 ± 2.5% for SWIS B, 72.9 ± 0.8% for SWIS C and 86.4 ± 0.5% for SWIS D. Low TN removal efficiencies in SWIS A and B were mainly attributed to poor nitrification and there were insufficient NO$_3^-$-N for denitrification. TN concentration in the effluent of SWIS A (21.3 ± 1.5 mg/L) was higher than that in SWIS B (17.6 ± 1.5 mg/L). In SWIS C and D, intermittent aeration supplied sufficient dissolved oxygen (DO) which improved nitrification and denitrification simultaneously. TN concentration in the effluent of SWIS C (10.8 ± 0.8 mg/L) was higher than that of SWIS D (5.4 ± 0.4 mg/L). TN removal efficiencies of SWIS D were significantly higher than those without biochar and sludge in intermittent aerated systems or in no intermittent aerated systems (P < .05). Intermittent aeration well developed aerobic conditions in upper matrix and anoxic or anaerobic conditions in the subsequent in one cycle simultaneously which facilitated nitrification in the previous studies (Pan et al., 2015, 2016a). After effective nitrification under aerobic conditions, the NO$_3^-$-N and NO$_2^-$-N as electron accepters could not be removed permanently unless sufficient organic carbon was supplied as electron donor (Fan et al., 2013a). Carbon deficiency then became the key limiting factor for TN removal in aerated reactors (Wang et al., 2010; Li et al., 2011). Many studies reported that biochar could retain nitrogen and then facilitate denitrification (Ding et al., 2016; Zhou et al., 2017). Sludge provided denitrifying bacteria. In addition, biochar and sludge as carbon rich materials were also potential carbon source which could facilitate denitrification.

When compared to other enhancing SWISs, average COD, NH$_4^+$-N and TN removal efficiencies of intermittent aerated SWIS amended with biochar and sludge in this study were higher than those in intermittent aerated SWIS without biochar and sludge (91.4% for COD, 95.6% for NH$_4^+$-N, 81.6% for TN, Pan et al., 2015), in sludge amended SWIS (92.3% for COD, 90.0% for NH$_4^+$-N, 78.6% for TN, Li et al., 2013), and in bioaugmentation SWIS (83.8% for COD, 75.0% for NH$_4^+$-N, 61% for TN, Zou et al., 2009).

3.2. N$_2$O emission and functional genes abundances involved in nitrogen removal

Fig. 3(a) shows N$_2$O conversion ratio and emission rate of four SWISs. N$_2$O is the byproduct of incomplete nitrification and intermediate product of incomplete denitrification in SWISs (Kong et al., 2014; Zhou et al., 2017). Average N$_2$O emission rates were 38.1 mg/(m$^2$ d) for SWIS A, 33.4 mg/(m$^2$ d) for SWIS B, 24.7 mg/(m$^2$ d) for SWIS C and 18.4 mg/(m$^2$ d) for SWIS D. Average N$_2$O conversion ratios of four SWISs (0.37–0.76%) were consistent with previous studies (Johansson et al., 2003; Li et al., 2017; Jiang et al., 2017). N$_2$O emission rate in conventional SWIS was more than 2 times higher than that in intermittent aerated SWIS without biochar and sludge (91.4% for COD, 95.6% for NH$_4^+$-N, 81.6% for TN, Pan et al., 2015), in sludge amended SWIS (92.3% for COD, 90.0% for NH$_4^+$-N, 78.6% for TN, Li et al., 2013), and in bioaugmentation SWIS (83.8% for COD, 75.0% for NH$_4^+$-N, 61% for TN, Zou et al., 2009).

Ammonia monoxygenase (amoA), nitrite oxidoreductase (nxrA), periplasmic nitrate reductase (napA), membrane-bound nitrate reductase (narG), nitrite reductase (nirK/nirS), nitric oxide reductase (norB) and nitrous oxide reductase (nosZ) are functional genes...
More NO$_3^-$ as the substrate of anaerobic denitrification enhanced the abundances of six genes in intermittent aerated SWISs (Jiang et al., 2017). In the same depth, the abundances of amoA, nxrA, napA, narG, nirS, nirK, qnorB and nosZ genes of SWISs with biochar and sludge were higher than those without biochar and sludge in intermittent aerated systems or in no intermittent aerated systems, which could further explain higher NH$_4^+$-N and TN removal efficiencies in biochar and sludge amended SWISs compared with the SWISs without biochar and sludge.

4. Conclusion

High COD, NH$_4^+$-N, TN removal efficiencies of 95.4 ± 0.2%, 96.2 ± 0.6%, 86.4 ± 0.5% and low N$_2$O emission rate of 18.4 mg/(m$^2$ d) were obtained in intermittent aerated SWIS amended with biochar and sludge. Intermittent aeration enhanced the abundances of nitrogen removal functional genes (amoA, nxrA, napA, narG, nirS, nirK, qnorB and nosZ) compared with no-aerated SWISs. The results from this study indicated that intermittent aeration SWISs amended with biochar and sludge could be an effective strategy for achieving high COD, NH$_4^+$-N, TN removal and low N$_2$O emission.

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