Impact of pyrene on pollutant removal and microbial enzyme activities in bioretention systems

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Abstract. Bioretention system can effectively remove polycyclic aromatic hydrocarbons (PAHs) in urban surface runoff through adsorption. However, the accumulation of PAHs may have potential inhibitory effect on microbial growth and activity in the system, and thus influence the overall performance. In this study, laboratory-scale bioretention cells with three different filter media were constructed. Pyrene, a high-molecular-weight PAH with 4 benzene rings, was periodically introduced into the bioretention cells to evaluate its effect on purification of carbon, nitrogen and phosphorus, and related microbial enzyme activities. The results showed that the removal capability of chemical oxygen demand (COD) was significantly influenced by pyrene contamination, which was difficult to recover at high pyrene level of 90 mg/kg. Increased effluent total nitrogen (TN) concentration were observed in the bioretention cells with high pyrene content, while no significant change on effluent total phosphorus (TP) concentration was detected. The soil dehydrogenase enzyme activity decreased with the increase of pyrene level, which might contribute to the decreasing COD removal rate. The urease activities in the bioretention cells were obviously inhibited by the addition of pyrene, probably leading to the decreasing nitrogen removal capacity of the system. In summary, the bioretention cells containing coal ash and lava rock performed better and were more stable under pyrene contamination.

1. Introduction

As the rapid urbanization and industrialization, the non-point source (NPS) pollution associated with urban stormwater runoff have deleteriously affected the quality of receiving water bodies [1]. To address this issue, the concept of low impact development (LID) has been emerging in the 1990s, which aims to utilize decentralized and small-scale source control techniques for stormwater management [2]. Bioretention system is a typical LID practice that captures and store stormwater runoff and passes it through a filter bed of engineered soil media. Various pollutants, including nutrients, organic compounds and heavy metals, can be removed by bioretention system through filtration, sorption, vegetative uptake and microbial mineralization [3].

Polycyclic aromatic hydrocarbons (PAHs), as a class of organic compounds with two or more benzene rings, are ubiquitous in the urban environment and suspected to induce carcinogenic, teratogenic and mutagenic effects [4]. Many studies found that PAHs are important components of petroleum hydrocarbon pollutants in urban stormwater runoff, contributing about a quarter of the total PAH load to aquatic ecosystems. Typically, bioretention system was considered as an effective process for removing PAHs. Hong *et al* [5] observed that approximately 90% of naphthalene, a 2 ring PAH, was adsorbed in a bench-scale bioretention system and biodegraded within a short period. LeFevre *et al* confirmed that adsorption, with the assistance of microorganisms and plants, was the dominant naphthalene removal mechanism in bioretention system [6]. However, the high-molecular-weight PAHs (HMW PAHs) are very difficult to be biodegraded due to their high hydrophobicity, low water solubility and stable molecular structure, and therefore more likely to persist in the system. The concentration of HMW PAHs in the system could potentially rise to hazardous levels to inhibit the activities of the functional microorganisms related to removal of other organic compounds and nutrients. Therefore, the accumulation of HMW PAHs may lead to the performance deterioration of the bioretention system and raise concerns for the quality of receiving waters.

In this study, laboratory-scale bioretention cells with three different filter media were constructed. The systems were periodically spiked with pyrene, a 4 ring PAH, over a 150-day period and the removal performance of carbon, nitrogen and phosphorus, as well as the key microbial enzyme activities, was evaluated to investigate the impact of accumulation of HMW PAHs.

2. Materials and methods

2.1. Bioretention column apparatus

Six Plexiglas columns with the same specifications of 10 cm in inner diameter and 1 m in height were filled with three groups of filter media (figure 1). From top to bottom, the structural configuration of the column was aquifer layer (12 cm), artificial media layer (78 cm), and gravel drainage layer (10 cm). The artificial media layer in column S1 and S2 were filled with 3:1 mixture of topsoil and sand (as Media S). In column SC1 and SC2, coal ash was mixed 1:1 with Media S (as Media SC), while lava rock, coal ash and Media S were mixed in a ratio of 1:1:1 (as Media SLC) in column SLC1 and SLC2. All the filter media were obtained locally in Xi'an City, Shaanxi, China. Table 1 shows that the filter media in all three bioretention systems have similar physicochemical properties of pH, total carbon (TC), total nitrogen (TN) and total phosphorus (TP), while the total organic carbon (TOC) and organic matter (OM) content in Media SC are relatively lower than the others.

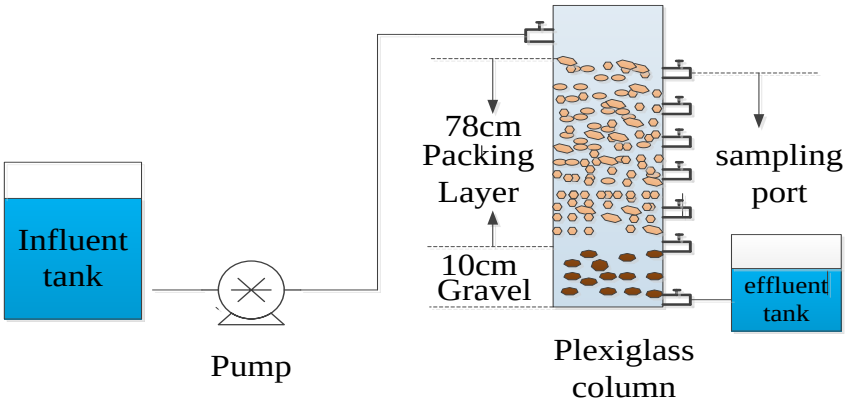

Figure 1. Schematic diagram of bioretention system.

Table 1. Physicochemical properties of filter media.

Media	pH	TC (%)	TOC (%)	OM (%)	TN (g/kg)	TP (g/kg)
S	7.84±0.03	37.4±3.2	1.53±0.17	2.64±0.29	0.53±0.06	0.13±0.02
SC	7.46±0.19	38.5±1.4	0.83±0.47	1.42±0.82	0.49±0.06	0.12±0.01
SLC	7.92±0.04	42.2±2.0	1.28±0.21	2.20±0.36	0.56±0.07	0.13±0.05

2.2. Bioretention column experiments

In this study, a synthetic runoff solution was made up to provide controlled input conditions according to the water quality monitoring data in Xi'an City. The main pollutant concentrations in the synthetic stormwater are presented in table 2. Chicago design storm was used to simulate a 120-min duration storm with 2-year return period. The catchment/infiltration area was set at 10:1, and the rainfall peak coefficient was set at 0.35. The calculated average and maximum rainfall intensities were 0.23 mm/min and 1.13 mm/min, respectively. Once per 10 days, total of 2.15 L synthetic stormwater was added to each column to simulate a storm. Due to the low water solubility of PAHs, acetone is commonly used as a solvent for spiking of soil with PAHs for experimental purposes [7]. Upon initiation of the experiment (Phase I, Day 0-40), 100 mL of acetone containing 5 g/L pyrene (Sigma-Aldrich) was introduced into column S2, SC2 and SLC2. Acetone was allowed to evaporate, and then the systems were idle for 10 days for microbes to acclimate the PAH shock. Subsequent identical pyrene spikes occurred once per 40 days for a total of 3 spikes (Days 40, 80, 120) during the 150-day experiment and thus the experiment was proceed in Phase II (Day 41-80), Phase III (Day 81-120) and Phase IV (Day 121-150). Pyrene was chosen as the model compound since it was found as one of the dominant HMW PAH components in stormwater samples [8]. Column S1, SC1 and SLC1 were treated as control group without addition of PAHs.

Table 2. Synthetic stormwater runoff characteristics.

Parameter	Source	Value (mg/L, except pH)
pH	HCl or NaOH	7.0
COD	Glucose	300
Ammonium	NH_4Cl	4 (as N)
Nitrate	$NaNO_3$	4 (as N)
Organic nitrogen	Urea	4 (as N)
Phosphorus	KH_2PO_4	3 (as P)
Total dissolved solids	$CaCl_2$	120

2.3. Sample collection and determination

During the experiment, water samples were collected from 6 columns every 10 days and analyzed immediately. To evaluate the removal of organic substances in bioretention systems, chemical oxygen demand (COD) was measured by a HACH DR6000 (HACH, Loveland, Colorado, USA). Nitrogen species, including TN, total dissolved nitrogen (TDN), NH_3-N, NO_2-N and NO_3-N, were determined by using San++ continuous flow analyzer (Skalar, Netherlands). Particulate organic nitrogen (PON) was calculated as the difference between TN and TDN, and dissolved organic nitrogen (DON) was calculated by subtracting NH_3-N, NO_2-N and NO_3-N from TDN. Phosphorus species, including TP, dissolved phosphorus (DP), and soluble reactive phosphorus (SRP) were measured by a HACH IL500 TP analyzer (HACH, Loveland, Colorado, USA). Particulate phosphorus (PP) was calculated as the difference between TP and DP, and dissolved organic phosphorus (DOP) was calculated by subtracting SRP from DP. To assess the pollutant removal performance under different conditions, the concentration removal rate (RC) and mass load reduction rate (RL) for each column were calculated by:

$$R_C = (C_{in} - C_{out})/C_{in} \times 100\% \quad (1)$$

$$R_L = (C_{in}V_{in} - C_{out}V_{out})/C_{in}V_{in} \times 100\% \quad (2)$$

where C_{in} and C_{out} are the pollutant concentrations (mg/L) in influent and effluent; V_{in} and V_{out} are the flow (L) of influent and effluent.

Media samples were collected from the bioretention cell at Days 40, 80, 120 and 150 to investigate the changes in microbial enzyme activities. Samples were taken from the six sampling ports vertically along the column, mixed well and then analyzed immediately in case of enzyme inactivation. The activities of dehydrogenase, sucrase, urease and catalase were determined by a Synergy H1 microplate reader (BioTek, Winooski, Vermont, USA) with a set of enzymatic assay kits (Solarbio, Beijing, China).

3. Results and discussion

3.1. Effect of pyrene on organic matter removal performance

Table 3. Purification effects of the bioretention columns in different phases (mean value).

Column	Phase	COD		TN		TP	
		R_C (%)	R_L (%)	R_C (%)	R_L (%)	R_C (%)	R_L (%)
S1	Ⅰ	59	80	69	80	78	78
	Ⅱ	63	83	77	83	85	86
	Ⅲ	72	82	77	82	84	84
	Ⅳ	77	80	77	80	83	83
S2	Ⅰ	72	78	67	78	80	78
	Ⅱ	57	74	73	74	83	82
	Ⅲ	46	72	71	72	83	82
	Ⅳ	20	71	70	71	83	81
SC1	Ⅰ	71	84	73	84	79	80
	Ⅱ	72	89	85	89	81	81
	Ⅲ	70	88	85	88	83	82
	Ⅳ	68	88	83	88	83	82
SC2	Ⅰ	67	86	73	86	86	86
	Ⅱ	36	77	74	77	89	88
	Ⅲ	34	76	73	76	84	83
	Ⅳ	30	73	71	73	82	81
SLC1	Ⅰ	78	81	78	81	82	78
	Ⅱ	56	90	83	90	89	88
	Ⅲ	54	92	84	92	88	88
	Ⅳ	53	90	83	90	87	86
SLC2	Ⅰ	63	85	76	85	85	85
	Ⅱ	7	85	71	85	88	88
	Ⅲ	14	77	70	77	86	85
	Ⅳ	31	73	69	73	84	83

Note: total nitrogen (TN), total phosphorus (TP).

The pollutant removal performance of the bioretention systems in different phases are summarized in table 3. During Phase I, all the bioretention systems achieved stable operation in a short start-up period,

with COD concentration removal rates ranging from 81% to 90%, and COD load reduction rates ranging from 83% to 91%. After initial exposure to ~30mg/kg of pyrene (Phase II), the load reduction for COD in column S2, SC2 and SLC2 decreased to 42%, 35% and 48%, respectively. Then the performance gradually recovered and the COD load reduction rate reached to a level slightly lower than that in control. With the increase of pyrene loading (Phase III and IV), however, the removal capability of COD were significantly influenced and recovered very slowly. At the end of the experiment, COD load reduction rates in all the pyrene-contaminated bioretention systems were 33% to 65% of those in control. The column with Media SLC exhibited relatively a higher tolerance to HMW PAHs.

3.2. Effect of pyrene on nitrogen removal performance

Table 4 summarizes the effluent concentration of N species in bioretention systems at different phases. During Phase I, the TN removal performance in all bioretention systems gradually improved with the time. At the end of Phase I, all the bioretention systems exhibited promising treatment performance, with TN concentration removal rates ranging from 77% to 88%, and TN load reduction rates ranging from 84% to 91%. The load reduction for TN decreased after spiked with pyrene in Phase I, while the performance in column SLC2 gradually recovered in the subsequent experiment. In the late Phase IV, the TN load reduction rates in the bioretention systems contaminated by pyrene were lower than those in control, ranging from 72% to 77%.

The effluent N is dominated by NO_3-N (49%), DON (26%) and NH_3-N (17%). After entering into the system, DON could be captured by filter media, and then be mineralized to NH_3-N by microorganisms via ammonification. Urea was used as the DON component in the synthetic runoff, which could be readily degraded by most heterotrophic microorganisms. The NH_3-N ions are positively charged and could be consequently adsorbed to the filter media with high cation exchange capacity (CEC) and negatively charged surface. Microorganisms attached on the media could then incorporate NH_3-N into organic biomass or convert it to NO_2-N and NO_3-N via nitrification, and biologically regenerate the ion exchange sites on the media. Therefore, many studies reported effective NH_3-N removal performance in bioretention systems [9,10]. In the anaerobic/anoxic conditions, NO_3-N can be reduced to N_2O and N_2 via denitrification processes, or be utilized by some organisms as electron acceptor and converted to NO_2-N and NH_3-N via dissimilatory nitrate reduction to ammonium (DNRA) process. With the absence of an anaerobic zone in the system, the NO_3-N from influent or produced via nitrification could be adsorbed to the media but could not be transform into N_2, resulting in a relatively poor NO_3-N removal performance in this study. After the exposure to pyrene, the effluent concentration of DON and NH_3-N largely increased, which may be related to the inhibition of microbial activities and release of soluble microbial products (SMPs) influenced by PAH pollution [11].

Table 4. Effluent concentration of N species in bioretention columns (mean value, unit: mg/L).

N species	Phase	Column					
		S1	S2	SC1	SC2	SLC1	SLC2
NH_3-N	Ⅰ	0.47	1.08	0.40	0.39	0.25	0.33
	Ⅱ	0.37	0.76	0.34	0.59	0.40	0.56
	Ⅲ	0.39	0.81	0.39	0.59	0.41	0.64
	Ⅳ	0.41	0.71	0.44	0.68	0.45	0.66
NO_3-N	Ⅰ	1.90	2.18	1.91	1.85	1.54	1.23
	Ⅱ	1.52	1.61	0.82	1.50	0.83	1.86
	Ⅲ	1.47	1.67	0.82	1.60	0.74	1.87
	Ⅳ	1.44	1.74	0.83	1.71	0.78	1.89
NO_2-N	Ⅰ	0.09	0.06	0.06	0.06	0.05	0.06

	Ⅱ	0.06	0.05	0.05	0.05	0.03	0.06
	Ⅲ	0.05	0.06	0.05	0.05	0.03	0.06
	Ⅳ	0.05	0.06	0.05	0.81	0.04	0.06
DON	Ⅰ	1.16	1.08	0.71	0.81	0.68	1.11
	Ⅱ	0.68	0.76	0.44	0.73	0.64	0.76
	Ⅲ	0.67	0.81	0.47	0.75	0.63	0.85
	Ⅳ	0.68	0.82	0.51	0.81	0.63	0.87
PON	Ⅰ	0.17	0.15	0.11	0.14	0.09	0.12
	Ⅱ	0.15	0.22	0.12	0.21	0.08	0.18
	Ⅲ	0.14	0.24	0.12	0.22	0.09	0.20
	Ⅳ	0.15	0.24	0.15	0.22	0.10	0.22

3.3. Effect of pyrene on phosphorus removal performance

Table 5 summarizes the effluent concentration of SRP in bioretention systems at different phases. During Phase I, the average TP load reduction rate in all bioretention systems increased from 72% to 87% with the time. There is no significant difference observed between TP removal performances in PAH-contaminated systems as compared to the controls. Even in Phase IV with the highest pyrene level caused by accumulation, the average concentration removal rate and load reduction rate of TP were still 81% and 83%, respectively. The P species in the effluent is dominated by SRP (98%), while the amount of PP and DOP could be neglected. Since adsorption and precipitation are regarded as the major P retention mechanisms while microbial uptake only account for a small part, the accumulation of HMW PAHs in the bioretention media would have little effect on the overall TP removal performance.

Table 5. Effluent concentration of SRP in bioretention columns (mean value, unit: mg/L).

Phase	Column					
	S1	S2	SC1	SC2	SLC1	SLC2
Ⅰ	0.68	0.63	0.65	0.41	0.56	0.45
Ⅱ	0.46	0.51	0.59	0.33	0.35	0.36
Ⅲ	0.48	0.53	0.51	0.50	0.38	0.43
Ⅳ	0.51	0.52	0.51	0.54	0.40	0.49

3.4. Effect of pyrene on microbial enzyme activities

Enzymatic activity of soil dehydrogenases was used as an indicator of overall soil microbial activity, as well as an indicator of contamination of the environment with petroleum products [12]. As shown in table 6, the relative activities of soil dehydrogenases in bioretention systems decreased with the increase of PAH level, indicating the inhibitive influence of pyrene on the metabolism of aerobic microorganisms. The hydroxylases (e.g., sucrase and urease) can hydrolyze the macromolecules, such as polysaccharides and proteins, to form the smaller molecules that are easily absorbed by microorganisms and to accelerate the carbon and nitrogen cycles within the soil ecosystem [13]. The results showed that addition of pyrene had negative impacts on the activities of both sucrose and urease. Especially when the pyrene contents increased to higher levels (60~90 mg/kg), urease activities were almost completely suppressed, which may be one of the reasons for the poor TN treatment performance. Catalase is a representative heme enzyme that can split hydrogen peroxide into molecular oxygen and water and thus prevent cells from damage by reactive oxygen species (ROS) [14]. Strong catalase activity suggests the presence of many soil microorganisms, which play a significant role in the decomposition of organic matter and humus formation [15]. It was observed that the catalase activity showed a declining trend correlated with higher dose of pyrene, which should be related to the toxicity caused by pyrene exposure.

Table 6. Effects of pyrene on the relative activities of enzymes (unit: % of control).

Enzyme	Phase	Column		
		S2	SC2	SLC2
Dehydrogenase	Ⅰ	98.48	109.51	105.33
	Ⅱ	80.00	102.94	48.27
	Ⅲ	74.99	104.76	57.89
	Ⅳ	37.11	81.00	40.22
Sucrase	Ⅰ	98.41	92.76	95.31
	Ⅱ	87.09	55.11	38.25
	Ⅲ	95.18	62.40	44.64
	Ⅳ	73.46	59.76	44.91
Urease	Ⅰ	106.25	92.59	116.63
	Ⅱ	15.12	48.42	27.47
	Ⅲ	5.23	17.59	16.39
	Ⅳ	4.11	4.84	12.08
Catalase	Ⅰ	106.85	101.98	107.98
	Ⅱ	90.46	107.89	73.18
	Ⅲ	61.15	78.25	48.58
	Ⅳ	56.06	62.85	50.40

4. Conclusions

The accumulation of HMW PAHs in the bioretention systems had significant effects on COD and TN removal performance, while little effect on TP removal performance. After the exposure to high level of pyrene, the effluent concentration of COD, DON and NH_3-N largely increased, resulting in the loss of recovery ability in the system, which might be related to the inhibition of microbial activities and the release of SMPs. Among all enzymes measured in this study, urease is the most sensitive one, which could be used as an indicator of PAH contamination. According to the results, coal ash and lava rock could be applied as the recommended media in the bioretention systems with a potential risk of HMW PAH accumulation.

Acknowledgments

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