Hydrogen peroxide levels in biochar-based bioretention cells with three environmental conditions and its potential risks

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ABSTRACT

Recently, biochar with persistent free radicals has widely been applied to the removal of nutrient in bioretention cells. However, there is a lack of information on hydrogen peroxide (H_2O_2) levels in biochar-based bioretention cells with three environmental conditions and its potential risks. In this study, batch experiments were used to investigate H_2O_2 levels in biochar-based bioretention cells with different weight ratios of sand to biochar (S/B), hydraulic loading rate (HLR) and antecedent dry period (ADP). Potential risks of H_2O_2 on dominant community were also explored. Results showed that H_2O_2 levels in bioretention cells with 9:1, 8:2 and 7:3 of S/B were significantly high with the range from 0.024 to 0.044 mmol/L, and the production decreased with the increasing contents of biochar. Furthermore, H_2O_2 levels in three different heights of substrate layers and the bottom outlet enhanced with the rise of HLR, where the amplification of H_2O_2 in the middle layer was the largest, reaching 438.5% (ratio of H_2O_2 level at 2.0 m³/m²/d of HLR to the one at 0.4 m³/m²/d of HLR). In contrast, the long ADP was unfavorable to H_2O_2 levels in biochar-based bioretentes and Firmicutes), and the dosage of biochar could be the key. Altering these mentioned factors to achieve the regulation of microbial community structure in bioretention cells with biochar could be one of the promising approaches for the improvement of biological degradation function.

Keywords: Bioretention; Biochar; Substrate; Hydrogen peroxide; Microbial community

1. Introduction

In the recent years, urban impervious underlying surfaces increased with the accelerated urbanization, causing the more accumulation of stormwater runoff, and then leading easily to urban flood, riverbank erosion and aquatic habitat destruction. Meanwhile, many pollutants in urban stormwater runoff were found including suspended solids, organic matters, heavy metals, pathogens, etc. [1–4]. Bioretention cells, as one of the best management practices, have been widely used for the control of pollutants in stormwater runoff, like organic and inorganic chemicals [5,6] and pathogens [7]. Tirpak et al. [8] reported that removal mechanisms of chemical pollutants (such as nitrogen, phosphorus and organic matter) in bioretention cells were filtration, sedimentation, ion exchange, adsorption, biodegradation, etc. This could be satisfactory to capture particles and particle-associated pollutants through adsorption and precipitation. But, removal of dissolved pollutants

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(e.g., nitrogen) in stormwater runoff remained to be a challenge [9], which could be related to the weak biodegradation caused by low ratios of carbon to nitrogen (C/N) in stormwater runoff [2]. Thus, carbon sources could be a theoretical key for the enhancement of biodegradation in bioretention cells. At present, biochar was increasingly used as a filter medium and potential carbon source in bioretention cells for the decontamination of urban stormwater runoff, because of its high cation exchange capacity, rich pore structure and large specific surface area [10,11]. Furthermore, the modified biochar appeared to provide more treatment performance with effective dissolved nutrient removal and minimal by-product generation in various stormwater conditions [12,13].

On the other hand, there were a large number of persistent free radicals (PFRs) in biochar [14,15]. Many researchers have attempted to utilize biochar as a catalyst to generate reactive oxygen species (ROS) and sulfate radicals [16]. For example, Lyu et al. [17] found that biochar could react with dissolved oxygen (O_2) to induce hydroxyl radical (•OH) without the addition of oxidants. Du et al. [18] claimed that liquid water molecules could form energy barrier on free radical reaction by dissolving radicals or forming local hydrogen bond, causing 'OH radical to have a strong energetic tendency to form the new molecule, hydrogen peroxide (H₂O₂), in the singlet potential energy surface. This could be proved by the findings that the addition of biochar led to the increase of H₂O₂ levels in the rice root system [19]. Urban stormwater events were usually discontinuous, where stormwater runoff was characterized by its low strength and high O₂ content [20]. This could lead to high O₂ levels in stormwater bioretention cells. These facts indicated that a certain level of H₂O₂ could be present during the operation of biochar-based bioretention cells. Undoubtedly, H₂O₂ always played an extremely important role in water treatment. However, there was currently limited information on the different levels of H2O2 in biochar-based bioretention cells, which would be of great significance to the better application of biochar into bioretention cells.

The performance of bioretention cells was restricted to environmental conditions including key design and operational parameters [21]. Increasing literatures were found to study the mixture of sand and biochar for pollutant removal in bioretention cells [13,22,23]. In theory, the more biochar means the higher potential H₂O₂ levels in bioretention cells, but it could also result in the compost to exhaustion of attachment sites on biochar [24], which might cut down the number of PFRs and further impact the potential H₂O₂ levels. Hydraulic loading rate (HLR) was a critical variable in nutrient removal in bioretention cells [25,26]. Lopez-Ponnada et al. [9] found that the lowest HLR provided the longest hydraulic retention time (HRT) in the internal water storage zone, which indicated that the HLR could result in the change of water treatment amount and could further alter the total air (O_2) amount that is contacting with biochar in bioretention cells. This might contribute to impact the production of H₂O₂. Similarly, antecedent dry period (ADP) also played an important role in influencing the treatment performance of bioretention cells. Different ADPs could change spatial distribution and abundance of nitrogen functional genes [27], which could be due to the aerobic conditions

developed during the ADP [22]. Oxidation–reduction potential (ORP) can reflect states for both redox and aeration in the soil. Previous study found that the NH⁺₄–N removal in the bioretention cell with *Lythrum salicaria* L. was the highest (88.1%), which was consistent with ORP in the bioretention cell [28]. However, it is not clear whether current design and operation could impact H₂O₂ levels in biochar-based bioretention cells. Meanwhile, it has been agreed widely that the oxidation disinfection effect of H₂O₂ is not selective, which might have a negative impact on the function of microbial flora in bioretention cells [29]. Nevertheless, the potential risks of H₂O₂ were still not well understood in stormwater bioretention cells.

Therefore, in this study, a mixture with sand and biochar was selected as the substrate to investigate the potential H_2O_2 levels in biochar-based bioretention cells with different weight ratios of sand to biochar (S/B), HLR and ADP. The potential risks of H_2O_2 on dominant community were also explored in biochar-based bioretention cells. To the authors' best knowledge, this is the first study looking into H_2O_2 levels in biochar-based bioretention cells and its potential risks, while it mainly focused on substrate, HLR and ADP design. This study could contribute to guiding the modification of bioretention cells and addressing the challenges of emerging pollutants in stormwater runoff. Further studies are suggested to investigate the impact of other key variables, for example, plants and characteristics of rainfall events.

2. Materials and methods

2.1. Chemicals

The main chemicals were obtained from Nanjing Jiaodeng Science Equipment Co., Ltd., (China) and Nanjing Wanqing Chemical Glassware Instrument Co., Ltd., (China), including sulfuric acid (purity 99.5%), soluble starch (purity > 99%), ammonium chloride (NH₄Cl, purity > 99%), and potassium dihydrogen phosphate (purity 99%). The stock solutions of chemicals were prepared by dissolving the corresponding compound into DI water to yield the desired concentrations for chemical oxygen demand (COD), ammonia nitrogen and total phosphorus (simulated stormwater quality). The conventional pollutant concentrations of simulated stormwater included suspended solid (SS) (150 mg/L, surface soils located in Nanjing University of Information Science and Technology (NUIST), China, were sterilized using the autoclave for 15 min at 121°C), COD (30 mg/L, in terms of biochemically degradable concentration in runoff), NH⁺₄-N (2 mg/L as N), and TP (1 mg/L as P).

2.2. Experimental setup and operation

2.2.1. Experimental setup

Pilot-scale bioretention cells (Fig. 1) were comprised of 12 kinds of cells with three replication (Table 1), consisting of weight ratios of sand (particle size, 0.5 ± 0.2 mm; organic matter, 4.31 g/kg; TN, 2.06 g/kg; TP₂O₅, 0.09 g/kg) to biochar (particle size, 2.0 mm; C, 771.5 g/kg; N, 210.0 g/kg; K, 0.350 g/kg from the market), HLR and ADP. Each bioretention cell was made using the DN300 PVC pipe with 6 mm thickness and 120 cm height, where three embedded electrodes were



Fig. 1. Pilot-scale bioretention cell.

Table 1 Design conditions of pilot-scale bioretention cells

Kinds of cells	S/B	HLR (m ³ /m ² /d)	ADP (d)	Pore volume (L)	Infiltration rate (mm/h)
A1	9:1	2.0	2	12.0	402.9
A2	8:2	2.0	2	13.4	519.1
A3	7:3	2.0	2	14.0	588.2
A4	6:4	2.0	2	14.5	620.7
A5	9:1	1.6	2	12.8	400.2
A6	9:1	1.2	2	12.8	410.2
A7	9:1	0.8	2	12.7	398.2
A8	9:1	0.4	2	12.1	410.6
A9	9:1	2.0	3	12.4	405.5
A10	9:1	2.0	4	12.3	406.4
A11	9:1	2.0	5	12.6	403.6
A12	9:1	2.0	7	12.4	409.5
Control	Without biochar	2.0	2	11.1	327.6

set at 50 mm (upper), 250 mm (middle), and 450 mm (bottom) of substrate layer, as well as three different reserved pipes. Cells with only sand in the substrate layer were used as the control (Table 1).

2.2.2. Experimental operation

The simulated stormwater (25 L, 11.1–14.5 L pore volumes) was pumped into each bioretention cell using peristaltic pumps (BT100-1L, China) with different levels of HLR (Table 1), following similar methods as described in the previous literature [7]. Sterilized polyethylene bottles were used to collect the outflow sample (0.5 L) and water sample (0.5 L) at each substrate layer through the reserved pipes using the peristaltic pump from the bioretention cell. There were varying ADPs with 2, 3, 4, 5, and 7 d, based on the previous studies [28,29]. The experimental operations of 12 kinds of cells were all 10 times, and ORP was determined through three embedded electrodes.

After that, the control was classified into three kinds of cells: including no addition of H_2O_2 and the addition of two different H_2O_2 (the levels were based on the above experiments), for the evaluation of the potential risks of H_2O_2 on dominant community in biochar-based bioretention cells. The experimental operations were in line with those mentioned in Table 1. The substrate samples at upper, middle, and bottom of substrate layer in each cell were collected through the reserved pipes by stainless steel sampler (after sterilization) to determine the microbial communities in substrate layers, once in every 2 weeks. Microbial communities in substrate layers were obtained using the mixture of substrate from three different substrate layers.

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2.3. Analysis

In this study, H₂O₂ was the target ROS, because of the rapid quenching of OH radical and its relationship with H₂O₂ [30]. The collected water samples were analyzed to obtain the H₂O₂ concentration by potassium titanium oxalate spectrophotometry at the maximum wavelength of 400 nm (Shimadzu, Model UV3600). ORP levels were detected through soil redox potential detector (SU-ORP, Beijing Allied Weive Technology Co., Ltd., China) at 0, 3, 6, 12, 24, 48, 72, 96, and 120 h after the end of each experimental operation, and the average of ORP (10 operation times, 3 replication cells) for each kind of cells was used for analysis in this study. The PCR primers, 341 F (5'-CCTAYGGGRBGCASCAG-3') and 806 R (5'-GGACTACNN GGGTATCTAAT-3'), ligated to index sequence and adapter sequence were used to amplify the V3-V4 region of the 16S rDNA gene of bacteria in the substrate samples. PCR products were analyzed by high-throughput sequencing using Illumina MiSeq platform (PE 300) in Anhui Microanaly Gene Technology Co., Ltd., China. All experiments were carried out in triplicate, and the average of three replicate experiments was calculated and used for data interpretation. Data analysis graphing was conducted by Origin (version 8) and SPSS (version 11.5).

3. Results and discussion

3.1. Hydrogen peroxide levels in bioretention cells

3.1.1. Impact of weight ratios of sand to biochar

 H_2O_2 levels detected in samples from three different heights of substrate layers and the bottom outlet decreased with the reduction of S/B (Fig. 2), which indicated that the increasing contents of biochar in bioretention cells could hinder the production of H_2O_2 . The levels of H_2O_2 in the bioretention cells with 9:1, 8:2, and 7:3 of S/B were significantly high with the range from 0.024 to 0.044 mmol/L, while the one in the bioretention pond with 6:4 of S/B was low and



Fig. 2. H_2O_2 concentration for three substrate layers and bottom outlet of the used bioretention cells with different S/B (HLR 2.0 m³/m²/d and ADP 2 d).

similar to that in the control. It was well known that H_2O_2 could inactivate microorganisms. Meanwhile, the addition of biochar provided carbon sources for the denitrification in bioretention cells [31]. This suggested that biochar played a major role as the carbon source in bioretention cells with 6:4 of S/B. On the other hand, levels of H_2O_2 generated in bioretention cells with 9:1 of S/B showed a gradually increasing trend from upper to bottom of the substrate layer. However, for bioretention cells with 8:2 and 7:3 of S/B, H_2O_2 levels in the substrate layer were low at the middle and high at the bottom and top. H_2O_2 concentration was the highest (0.044 mmol/L) at the bottom of substrate layer for bioretention cells with 9:1 of S/B, which was consistent with the maximum (0.045 mmol/L) at the bottom outlet of this kind of bioretention cells.

Biochar used in this study was prepared using commercial coconut shell at high temperature (600°C). Odinga et al. [32] found that PFRs on biochar surface could produce H_2O_2 in the presence of oxygen, while Chen et al. [33] reported that H_2O_2 could be activated by biochar, and then decomposed into hydroxyl radicals. This implied that the content of O_2 in the bioretention cells might be insufficient to deal with the superfluous biochar. Based on the previous literature [18], hydroxyl radicals in water could theoretically combine with each other to produce H_2O_2 , but this phenomenon was not actually found in this study, which might be related to the participation of easily quenched hydroxyl radicals [30] in pollutant degradation processes in bioretention cells.

3.1.2. Impact of different hydraulic loading rates

Results from Fig. 3 showed that H_2O_2 concentration in samples from three different heights of substrate layers and the bottom outlet increased with the increase of HLR. Therefore, the amplification of H_2O_2 in the middle layer was maximum, reaching 438.5% (ratio of H_2O_2 level at 2.0 m³/ m²/d of HLR to the one at 0.4 m³/m²/d of HLR), and that in the bottom layer was minimum, reaching 278.9%. Moreover,



Fig. 3. H_2O_2 concentration for three substrate layers and bottom outlet of the used bioretention cells with different HLR (S/B 9:1 and ADP 2 d).

 H_2O_2 levels in the top layer and bottom outlet at 2.0 m³/m²/d of HLR were both about 350% of the one at 0.4 m³/m²/d of HLR. H_2O_2 concentrations in the top layer, middle layer and at bottom outlet had a significant linear relationship with HLR (correlation coefficients greater than 0.95, p < 0.005). This was similar to the findings that the nitrogen removal demonstrated a positive linear relationship with increasing HRT under steady-state conditions of underdrain [34].

It was found that a minimum of three water molecules were needed to bind two free radicals in order to separate them from falling into a barrierless zone on the potential energy surface leading to recombination reaction [18]. Furthermore, two free radicals at and near the water microdroplet interface recombined to form H₂O₂ [35]. This indicated that large inflow would lead to more hydroxyl radicals bound to generate more H₂O₂ in bioretention cells under the high HLR. Inversely, the lower HLR provided the longer HRT in the conventional and modified bioretention cells [9]. Decezaro et al. [36] reported that the highest oxygen transfer rate was found for the lowest HLR in vertical flow constructed wetlands. This implied that there could be deficient O₂ levels at low HLR in bioretention cells, which further resulted in the low H₂O₂ levels. Low HLR would also lead to the long humidity period in bioretention cells. The previous literature claimed that the high humidity could inhibit the formation of PFRs or lead to the rapid attenuation of PFRs [37,38], which might accelerate the attenuation of PFRs under low HLR, and further lead to low H₂O₂ production, another possible reason for low H₂O₂ levels at low HLR. In addition, there was no obvious linear relationship between H₂O₂ concentration and HLR with the correlation coefficient less than 0.8. This might be due to the large external interference from the close contact between top layer and air.

3.1.3. Impact of different ADP

Soberg et al. [39] reported that outflow concentrations of Nox-N and TN from standard bioretention columns increased with increasing length of ADP. Similarly, Alam et al. [40] found that nutrient outflow concentration was found to have a positive correlation with ADP. However, profiles of H₂O₂ in this study showed another case. Results from Fig. 4 indicated that H₂O₂ levels in samples from three different heights of substrate layers and the bottom outlet gradually decreased with the increase of ADP. There was a linear relationship between ADP and H₂O₂ concentration in the bottom and upper layers and at bottom outlet ($R^2 > 0.92$, p < 0.01), signifying that the long ADP was unfavorable to H₂O₂ levels in the biochar-based bioretention cells. This agreed with that longer ADP and submerged zone could help alleviate outflow NO₂ leaching [41]. Meanwhile, H₂O₂ levels in the bottom and middle layers decreased fast with close to 50% of the amplitude when ADP less than 4 d, while the decline in the bottom layer was the maximum with 76% after 7 d of ADP. In addition, under the condition of different ADP, there was similar order of magnitude of H₂O₂ concentrations in different substrate layers and at bottom outlet with the fluctuation in the range of 0.011-0.045 mmol/L.

Changes in top-down environmental conditions (like ORP) of substrate layers with different ADP should be responsible for H_2O_2 profiles. High ORP (greater than

100 mV) represented aerobic environment while low ORP (less than-100 mV) reflected anaerobic environment [42]. In this study, both upper (50 mm) and bottom layers (450 mm) were the aerobic environments for different interval periods (Fig. 5), which was in line with that the maximum redox gradient was between the surface and the bottom of the bed for continuous planted wetlands (407.7 ± 73.8 mV) [43]. There were decreasing trends of ORP levels for both upper and bottom layers with the increase of interval periods, and the decrease of ORP in the upper layer after 24 h of the operation was most rapid from 747.8 mV to 158.4 mV, which might be related to the decrease of substrate moisture [44]. For the middle layer (250 mm), the aerobic environment could be detected before 6 h of interval periods, and the hypoxia environment appeared obviously with ORP less than 92 mV after 12 h of interval periods. The mentioned distribution of ORP was consistent with the profiles of H₂O₂



Fig. 4. H_2O_2 concentration for three different substrate layers and bottom outlet of bioretention cells with different antecedent dry period.



Fig. 5. Change of ORP in different substrate layers with interval time.

levels with different ADP, implying that ADP could impact H₂O, levels through changing ORP.

3.2. Potential risks of hydroxyl radicals on dominant community

3.2.1. Phylum level

There were top 8 dominant microflora (accounting for more than 1%) found at the beginning of operations with the relative proportion of the total sequence exceeded 81.5% (Fig. 6), including Proteobacteria, Firmicutes, Acidobacteria, Bacteroidetes, Actinobacteria, Chloroflexi, Verrucomicrobia, Nitrospirae. Therefore, characteristics for Proteobacteria, Firmicutes, Acidobacteria and Bacteroidetes could be found from the previous literature [29], as well as their functions. Actinobacteria had the denitrification function [45], indicating that the denitrifying bacteria could obtain energy for the denitrification, which leads to nitrate removal by bioretention cells. Nitrospirae belonged to the nitrifying bacteria [46], while both Chloroflexi and Verrucomicrobia could degrade organic matters [47].

However, the dominant microflora changed in different magnitudes after the operation for bioretention cells with the addition of different H₂O₂ levels. After two weeks of the operation, there were still top 8 dominant microflora, including Proteobacteria, Firmicutes, Acidobacteria, Bacteroidetes, Actinobacteria, Chloroflexi, Verrucomicrobia, Gemmatimonadetes. Ratios (K) of their relative abundances in different columns to the corresponding one in the control were obviously varying. For the columns with the addition of 0.01 mmol/L H_2O_2 , K of Firmicutes was the largest with 1.41, and the second were the one for both Chloroflexi and Nitrospirae, with 1.12 and 1.21, respectively, suggesting that 0.01 mmol/L H₂O₂ would not impact their dominant microflora in the used bioretention cells. For the columns with the addition of 0.04 mmol/L H₂O₂, K values of three mentioned dominant microflora were higher than 1.1 but slightly less than the one under the condition of 0.01 mmol/L H2O2. Moreover, K values of Bacteroidetes was highest with 1.47. After four weeks of the operation, top 7 dominant microflora could be found in the control: Proteobacteria, Firmicutes, Acidobacteria, Bacteroidetes, Actinobacteria, Chloroflexi,



Fig. 6. Changes of dominant bacteria at phylum level in bioretention cells.

Verrucomicrobia. *K* values of Bacteroidetes, Actinobacteria and Verrucomicrobia were more than 1.1 for the columns with the addition of 0.01 mmol/L H_2O_2 , while that for Firmicutes, Acidobacteria and Verrucomicrobia was larger than 1.1 for the columns with the addition of 0.04 mmol/L H_2O_2 . These results reflected that H_2O_2 produced in bioretention cells with biochar either promoted or hindered the nitrification and denitrification process, and the dosage of biochar could be one of the key factors, which was confirmed by the relationship of nitrogen removal and biochar proportions [48].

3.2.2. Genus level

The top 11 dominant microflora (accounting for more than 1%) at the genus level were detected at the beginning of operations (Fig. 7) including: Bacillus, Gp6, Pseudomonas, Geobacter, Gp16, Effusibacillus, Arthrobacter, Nitrospira, Oxalophagus, Tumebacillus, Cystobacter. After two weeks of the operation, there were still top 11 dominant microflora in the control, but the species had a weak change. Bacillus, Gp6, Pseudomonas, Geobacter, Gp16 and Effusibacillus were always being the dominant microflora. Similarly, Bacillus, Gp6, Pseudomonas, Geobacter, Gp16 and Oxalophagus in the control were always being the dominant microflora after four weeks of the operation, where only 10 different kinds of dominant microflora could be found. The species and the relative abundances of the dominant microflora fluctuated significantly during the operation of bioretention cells with the addition of different H₂O₂ levels. For the columns with the addition of 0.01 mmol/L H_2O_2 , K values for both Effusibacillus and Gp6 were in the range of 1.0-1.1, indicating that 0.01 mmol/L H₂O₂ would not impact their dominant microflora in the used bioretention cells. When the addition of H2O2 was 0.01 mmol/L, K values of Bacillus, Clostridium sensu stricto, Pseudomonas, Geobacter and Effusibacillus were larger than 1.1. However, ratios of their relative abundances in the columns with the addition of 0.01 mmol/L H₂O₂ to the corresponding one in the control were less than 1.0 after four weeks of the operation. K values for both Bacillus and Gp16 were more than 1.0 when the addition of 0.04 mmol/L H₂O₂. This implied that the produced H₂O₂ had a significantly negative influence on microbial population structure



Fig. 7. Changes of dominant bacteria at genus level in bioretention cells.

with the change of operation time. However, the process of producing H_2O_2 by PFRs on the surface of biochar could be irreversible based on the single-electron transfer process [15], which indicated that the amount of PFRs on the surface of biochar tended to decrease during the operation. It could be inferred that the influence of H_2O_2 on microbial population structure was mainly reflected in the early stage of the operation. But, the proportion of biochar, HLR and ADP determined H_2O_2 levels based on the results in this study. Therefore, altering these mentioned factors to achieve the regulation of microbial community structure in bioretention cells with biochar could be one of the promising approaches for the improvement of biological degradation function.

4. Conclusions

In this study, H₂O₂ levels in bioretention cells with 9:1, 8:2 and 7:3 of S/B were significantly high with the range from 0.024 to 0.044 mmol/L, while the one in the bioretention cell with 6:4 of S/B was very low, this indicated that the increasing ratio of biochar added into bioretention cells could hinder the production of H2O2. H2O2 levels at three different heights of substrate layers and the bottom outlet increased with the increase of HLR, where the amplification of H₂O₂ in the middle layer was the largest, reaching 438.5% (ratio of H₂O₂ level at 2.0 m³/m²/d of HLR to the one at 0.4 m³/m²/d of HLR). This was the opposite profiles with the change of ADP, signifying that the long ADP was unfavorable to H₂O₂ levels in the biochar-based bioretention cells. H₂O₂ produced in bioretention cells with biochar resulted in the change of dominant microflora (like Bacteroidetes and Firmicutes), and the dosage of biochar could be one of the key factors. Altering these mentioned factors to achieve the regulation of microbial community structure in the bioretention cells with biochar could be one of the promising approaches for the improvement of biological degradation function.

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