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Original Research

Effects of H_2O_2-modified Rice Husk-Derived Biochar on Nitrification and N_2O Emission from Water by an Isolated Heterotrophic Nitrifying Bacterium

Chaoxu Wang[1, 2]*, Yuankun Li[1], Zinan Wang[1], Jing Ren[1]

[1]College of Environmental Science and Engineering, Taiyuan University of Technology, Jinzhong 030600, China
[2]Innovation Center for Postgraduate Education in Municipal Engineering of Shanxi Province, Jinzhong 030600, China

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Abstract

In order to explore the effect of biochar on nitrification and N_2O emission from water, the indoor incubation experiment was carried out after incorporating the pristine biochar (BC) or H_2O_2-modified rice husk-derived biochar (20% H_2O_2-BC and 30% H_2O_2-BC) combined with the screened heterotrophic nitrifying bacterium into the simulated wastewater. The dynamic changes of inorganic nitrogen contents, pH, bacterial amount, and N_2O emission of the incubation system were analyzed. Results showed that compared with BC, the amount of acidic oxygen-containing functional group of H_2O_2-modified biochars increased by 24.24%~26.98%, while the amount of alkaline oxygen-containing functional group, pH, and the specific capacitance significantly decreased by 22.16%~27.29%, 2.54~2.68, and 15.90%~30.94%, respectively. H_2O_2-modified biochars significantly inhibited nitrification driven by heterotrophic nitrifying bacterium compared with BC. Moreover, the 16-h cumulative N_2O emission of the treatments with 20% H_2O_2-BC or 30% H_2O_2-BC addition (0.0516 and 0.0525 μg N_2O/100 mL solution, respectively) were significantly higher than that with BC addition (0.0355 μg N_2O/100 mL solution). Correlation analysis showed that the cumulative N_2O emission of the treatments with 20% H_2O_2-BC or 30% H_2O_2-BC addition was significantly positively correlated with NH_2OH concentration in incubation system (r = 0.455 and 0.497, respectively), and showed no significant correlation relationship with NO_2^--N (p<0.05).

Keywords: biochar, H_2O_2-modification, nitrification, N_2O emission

*e-mail: cxwang127@126.com

Introduction

Ammonia nitrogen is an important pollutant that causes water body black and odorous. Ammonia nitrogen pollution control has always been a hot spot in environmental studies. Ammonia oxidation is the rate-limiting step of nitrification and the main pathway of ammonia transformation [1]. N_2O is a potent greenhouse gas and can destroy the ozone layer [2]. The main production pathways of N_2O during nitrification are hydroxylamine (NH_2OH) oxidation and NO_2^- reduction [3-4].

Biochar, a carbon-rich material, prepared by pyrolysis of the plant biomass and other organic waste under the condition of oxygen free and high temperature (300~800ºC) [5-6]. Biochar was characterized by a highly aromatic carbon structure, alkalinity (pH>7), and large specific surface area [7]. It has been widely used in environmental remediation and agricultural waste recycling [8]. The effects of biochar on soil nitrification and N_2O emission has been paid much more attention. The promotion and inhibition effect of biochar on soil N_2O emission coexisted, and the potential mechanism was also unclear [9-12]. However, there were few studies focusing on the effect of biochar on nitrification and N_2O emission from water. Considering that water has better homogeneity than soil, we speculated that the research in water could be more conducive to reveal the underlying mechanisms.

As the carrier of electron transfer, biochar is beneficial to redox reactions, and such characteristic of biochar has been reported by many other studies [13-14]. However, it was rarely reported that the effect of biochar's electrochemical property on nitrification and N_2O emission driven by heterotrophic nitrifying bacterium (HNB). H_2O_2-modification can increase the amounts of carboxyl group and acidic oxygen-containing functional group of biochar [15-16] and decrease the electrical conductivity and the specific capacitance of biochar [14,17]. Moreover, considering that H_2O_2-modification could induce less exogenous substances into the incubation system, the H_2O_2 oxidation method was used to prepare the modified rice husk-derived biochar.

Based on the preparation and characterization of 20% or 30% H_2O_2-modified rice husk-derived biochars, the pristine and modified biochars were mixed with the simulated wastewater containing the screened HNB, *Pseudomonas putida* strain-N3, respectively. The dynamic changes of inorganic nitrogen concentrations, pH, bacterial amount, and N_2O emission during incubation were determined. The study aimed to explore the effects of pristine and H_2O_2-modified biochars on nitrification and N_2O emission from water and the potential mechanisms.

Materials and Methods

Biochar Preparation and Characterization

The rice husk-derived biochar (BC) used in the experiment was purchased from Dalian Jiucheng Products Co. Ltd., which was passed through a 100-mesh sieve before use. The biochar has a pH of 7.98±0.05, a point of zero charge (pH_{pzc}) of 7.13±0.09, an electrical conductivity of 48.80±1.31 mS/m, a specific capacitance of 5.85±0.29 F/g, an acidic oxygen-containing functional group of 0.656±0.012 mmol/g, and an alkaline oxygen-containing functional group of 0.546±0.013 mmol/g. H_2O_2-modified BC was prepared as follows: 20% or 30% H_2O_2 (250 mL) was added into the Erlenmeyer flask which was pre-filled with 5 g BC. After shaking for 12 h (25ºC, 170 r/min), the mixture was filtered and rinsed with deionized water until the pH of the filtrate achieved a constant. Finally, the biochars were dried and stored for later use, which were recorded as 20% H_2O_2-BC and 30% H_2O_2-BC, respectively.

The pH of biochar (biochar-water ratio 1 g:15 mL) was measured by a pH meter (Mettler Toledo Delta 320). The pH_{pzc} was measured by the titration method. The types of functional groups on the surface of biochar were measured by the Fourier transform infrared spectrometer (PerkinElmer Spectrum Two) and the scanning range is 400~4000 cm^{-1}. The oxygen-containing functional group amount was determined by the Boehm titration method [18]. The cyclic voltammetry curve of biochar was tested by the electrochemical workstation (CHI 760E), and the following formula was used to calculate the specific capacitance (F/g) of biochar [19].

$$C = \frac{I}{f \cdot m} \tag{1}$$

Where, I is the average cathodic current, A; f is the scan rate, V/s; and m is the mass of biochar on the glassy carbon electrode, g.

Incubation Experiment of HNB with Biochar Addition

In order to explore the effect of biochar on nitrification by the enriched and screened HNB, 0.3 g biochar (BC, 20% H_2O_2-BC or 30% H_2O_2-BC) and HNB cell collected from 15-mL liquid culture medium were added into a 50-mL centrifuge tube containing 30-mL simulated wastewater (Table 1) [20], which was incubated in the dark for 48 h (25ºC, 170 r/min). The three treatments were designated as HNB+BC, HNB+20% H_2O_2-BC and HNB+30% H_2O_2-BC, respectively. All samples were run in triplicate.

Table 1. Composition of the simulated wastewater.

Composition	Concentration (mg/L)
NH_4Cl	38.2
Glucose	275
$MgSO_4 \cdot 7H_2O$	25
$FeSO_4 \cdot 7H_2O$	2.5
$MnSO_4$	2.5
NaCl	125
K_2HPO_4	52
pH	7.0

HNB cell was prepared as the following procedures. The pre-saved HNB strain (*Pseudomonas putida* strain-N3, MN602471) [21] was selected from the slant culture medium, and then it was inoculated into 400 mL LB liquid culture medium (g/L, tryptone 10, yeast extract 5, NaCl 10) and cultivated to OD_{600} = 1.0 (25ºC, 170 r/min). After dividing the liquid culture medium into 15 or 50 mL, respectively, the HNB cell was collected by centrifuging the liquid culture medium followed by washing the centrifugated deposit with sterile water twice.

The water samples were taken at 1/6, 0.5, 1, 2, 5, 8, 16, 24, and 48 h during incubation, respectively. After filtering through a 0.45-μm membrane, the concentrations of NH_4^+-N, NO_2^--N, and NO_3^--N were determined by the colorimetric method at the wavelength of 420, 220/275, and 540 nm, respectively [22]. NH_2OH concentration was measured according to the method reported by Tian [23]. The pH and HNB amount (mL^{-1}) in the water samples were also measured.

N_2O Emission Determination

In order to explore the effect of biochar on N_2O emission during nitrification driven by the selected HNB, 1.0 g biochar (BC, 20% H_2O_2-BC or 30% H_2O_2-BC) and HNB cell collected from 50-mL liquid culture medium were added into a 280-mL culture flask containing 100-mL simulated wastewater (Table 1). The mouth of the culture flask was sealed with a rubber stopper, and a three-way valve with good air tightness was installed on the rubber stopper for gas sample collection. The 9 culture flasks (3 treatments × 3 replicates) were incubated in the dark (25ºC, 170 r/min). 20-mL gas sample was collected with a syringe through the three-way valve after injecting 20-mL air into the headspace of the culture flask with three times of push-and-pull at 1/6, 0.5, 1, 2, 5, 8, and 16 h, respectively. N_2O concentration was determined by a gas chromatograph (SP-3420A), and the cumulative N_2O emission was calculated as μg N_2O/100 mL solution.

Data Analysis

Microsoft Excel 2010 was used for data calculation and statistics. Origin 8.5 was used for graphing and equation fitting. SPSS Statistics 22 (IBM, New York, USA) was used for variance analysis and multiple comparison (One-way ANOVA).

Results and Discussion

Effect of H_2O_2-modification on the Properties of Rice Husk-Derived Biochar

Compared with BC, the carboxyl and acidic oxygen-containing functional group amounts of 20% H_2O_2-BC and 30% H_2O_2-BC significantly increased, while the amount of alkaline oxygen-containing functional group, pH, point of zero charge (pH_{pzc}), electrical conductivity, and the specific capacitance of 20% H_2O_2-BC and 30% H_2O_2-BC significantly decreased ($p<0.05$) (Table 2).

The reason for the phenomenon that the pH and pH_{pzc} of H_2O_2-modified biochars were significantly lower than BC was that H_2O_2 is weakly acidic, which can neutralize the alkaline oxygen-containing functional group and enhance the acidic oxygen-containing functional group growth on the surface of biochar. In particular, the significantly increased carboxyl could lower the pH_{pzc} of biochar. The oxidation of biochar by H_2O_2 destroyed the aromatic ring structure of biochar and weakened its aromaticity [13,16]. Therefore, the conductivity of 20% H_2O_2-BC and 30% H_2O_2-BC decreased by 87.40% and 88.01% compared with BC, respectively. Moreover, the specific capacitance of biochar was also significantly impaired after H_2O_2-modification. The results were consistent with the characteristics of the biochars prepared at different pyrolysis temperatures reported by Chen et al. [17].

Fourier transform infrared (FTIR) spectrum can distinguish the type of functional group on the surface of biochar. The FTIR spectra of BC, 20% H_2O_2-BC, and 30% H_2O_2-BC were shown in Fig. 1. The characteristic absorption peaks of the three kinds of biochar were almost the same, which occurred at the wavenumbers of 3432, 1605, 1105, and 800 cm^{-1}, indicating the presence of -OH, C=O, C-O, and C-H functional groups, respectively. Compared with BC, the absorption intensity of 30% H_2O_2-BC at all the wavenumbers were enhanced, indicating the amount of -OH, C=O, C-O, and C-H increased after 30% H_2O_2-modification.

H_2O_2-modified Biochars Inhibited Nitrification Driven by HNB

NH_4^+-N concentration of the incubation system decreased during 0~16 h, while NH_2OH, NO_2^--N, and NO_3^--N concentrations increased during 0~8 h. Moreover, the pH showed a decreasing trend during the whole incubation period (0~48 h). All the results

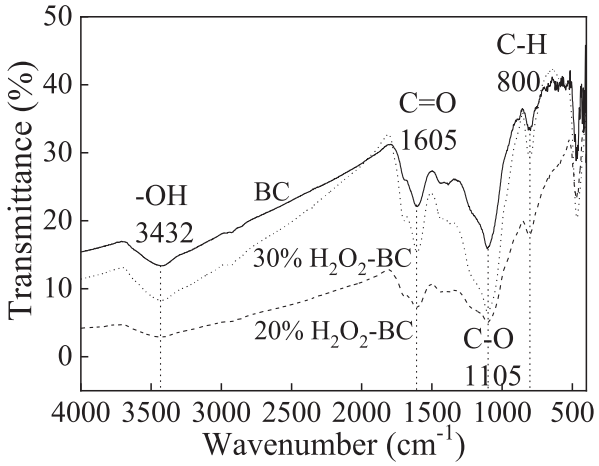

Fig. 1. Fourier transform infrared spectrum of rice husk-derived biochar before and after H_2O_2-modification.

Table 2. Properties of rice husk-derived biochar before and after H_2O_2-modification.

	pH	pH_{pzc}	(mmol/g)					Electrical conductivity (mS/m)	Specific capacitance (F/g)
			Carboxyl	Lactone	Phenolic hydroxyl	Acidic oxygen-containing functional group	Alkaline oxygen-containing functional group		
BC	7.98±0.05a	7.13±0.09a	0.595±0.016a	0.025±0.013a	0.036±0.015a	0.656±0.012a	0.546±0.013a	48.80±1.31a	5.85±0.29a
20% H_2O_2-BC	5.44±0.08b	4.46±0.08b	0.763±0.011b	0.013±0.012a	0.039±0.006a	0.815±0.006b	0.425±0.012b	6.15±0.10b	4.92±0.03b
30% H_2O_2-BC	5.30±0.13b	4.22±0.07c	0.760±0.013b	0.026±0.014a	0.048±0.009a	0.833±0.005c	0.397±0.005c	5.85±0.12b	4.04±0.02c

Note: data in the same column followed by the same letter have no significant difference at $p<0.05$; mean±standard deviation ($n = 3$); pH_{pzc}: point of zero charge.

indicated that microbial nitrification occurred with different extent during 0~8 h. However, NO_3^--N concentration of the incubation system decreased, while NO_2^--N and NH_4^+-N concentrations increased during 16~48 h, which might be resulted from dissimilatory nitrate reduction to ammonium caused by the inadequate dissolved oxygen in the latter phase of incubation.

NH_4^+-N concentrations of the treatments HNB+20% H_2O_2-BC and HNB+30% H_2O_2-BC (decreased from 9.70 to 1.47 mg/L and from 9.70 to 1.49 mg/L, respectively) were higher than that of the treatment HNB+BC (decreased from 9.92 to 0.47 mg/L) during 0~16 h. At the same time, NO_3^--N concentrations of the treatments HNB+20% H_2O_2-BC and HNB+30% H_2O_2-BC (increased from 0.24 to 1.96 mg/L and from 0.24 to 2.20 mg/L, respectively) were lower than that of the treatment HNB+BC (increased from 0.21 to 2.27 mg/L) during 0~8 h. In addition, the pHs of the treatments HNB+20% H_2O_2-BC and HNB+30% H_2O_2-BC were obviously lower than that of the treatment HNB+BC during the whole incubation period, and the amounts of HNB were also lower than that of the treatment HNB+BC during 16~48 h. The results indicated that H_2O_2-modified rice husk-derived biochars inhibited microbial nitrification of the incubation system (Fig. 2).

Why H_2O_2-modified rice husk-derived biochars inhibited microbial nitrification? Firstly, compared with BC, the pHs of 20% H_2O_2-BC and 30% H_2O_2-BC decreased by 2.54 and 2.68, respectively. Correspondingly, the pHs of the treatments HNB+20% H_2O_2-BC and HNB+30% H_2O_2-BC decreased by 0.47 and 0.54, respectively after 1/6 h incubation. Considering nitrification consumes alkalinity and 2 mol H^+ will be produced for every 1 mol NH_4^+ oxidation, the relatively lower pH of the incubation system was not conducive to nitrification.

Secondly, biochar can promote electron transfer between the substrate and microorganisms as a redox active mediator, which was called “electron shuttle” [14,

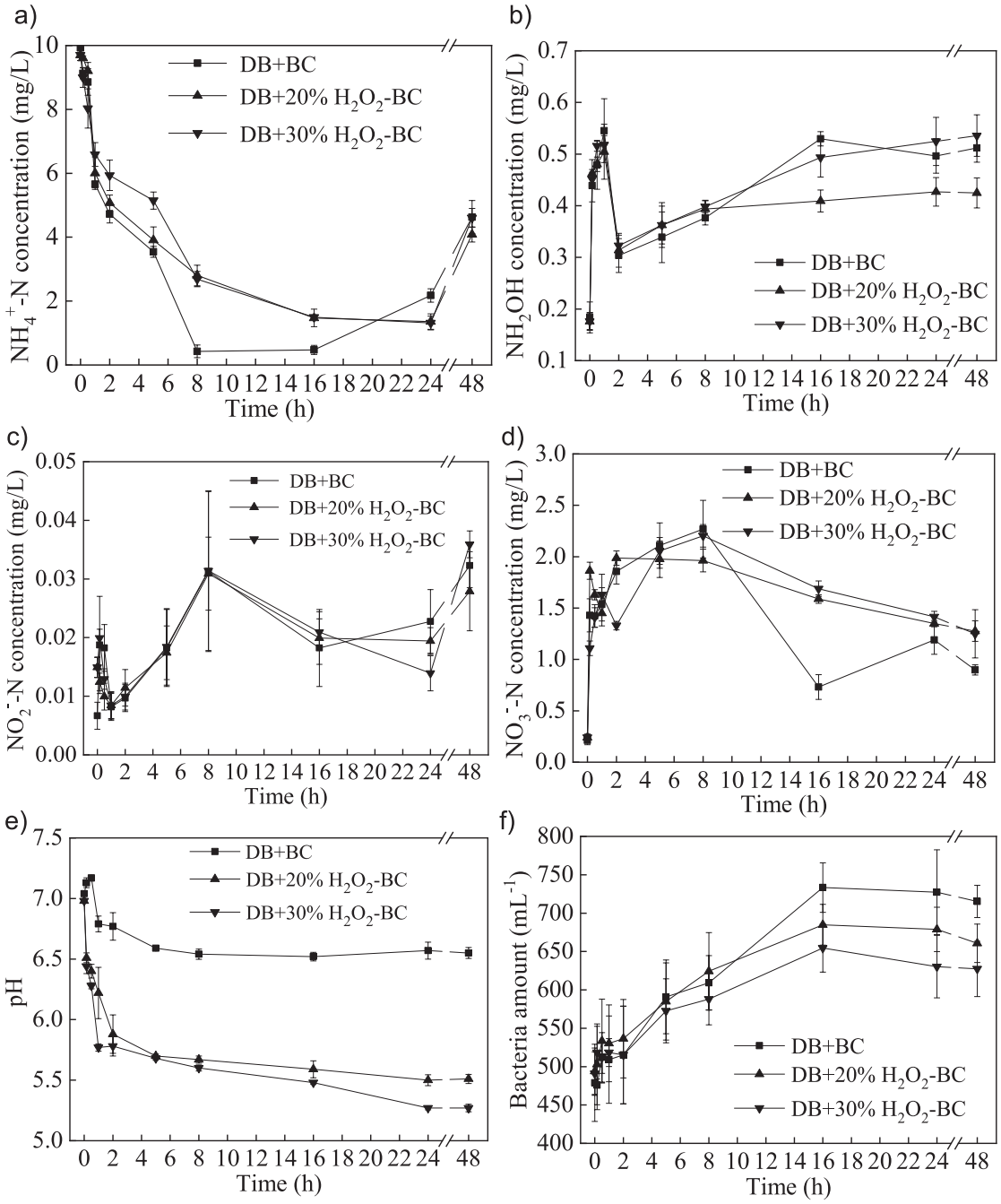

Fig. 2. Dynamic changes of NH_4^+-N a), NH_2OH b), NO_2^--N c), NO_3^--N d) concentrations, pH e), and bacteria amount f) in the incubation system.

17, 24]. Compared with BC, the specific capacitances of 20% H_2O_2-BC and 30% H_2O_2-BC decreased by 15.90% and 30.94%, respectively, which weakened the ability of biochar as an electron shuttle and was not conducive to the electron transfer between NH_4^+-N and the screened HNB, thereby inhibiting nitrification.

Thirdly, the nature of biochar surface charge is related to both the pH_{pzc} of biochar and the original pH of the incubation solution [23]. The pH_{pzc} of BC (7.13) was higher than the original pH of the simulated wastewater (7.00), making its surface positively charged. However, the pH_{pzc} of 20% H_2O_2-BC and 30% H_2O_2-BC (4.46 and 4.22, respectively) were lower than the original pH of the simulated wastewater, making its surface negatively charged. Given that the pH_{pzc} of most bacteria was 3~4 [25], which was lower than the original pH of the simulated wastewater, the surface of bacteria would be negatively charged. Therefore, the positively charged BC could adsorb microorganisms in the incubation system by electrostatic attraction. Moreover, biochar possessed abundant bioavailable carbon, and its porous structure could provide a suitable habitat for the growth of HNB [26], thus being in favor of NH_4^+-N degradation. However, the electrostatic repulsion between the negatively charged

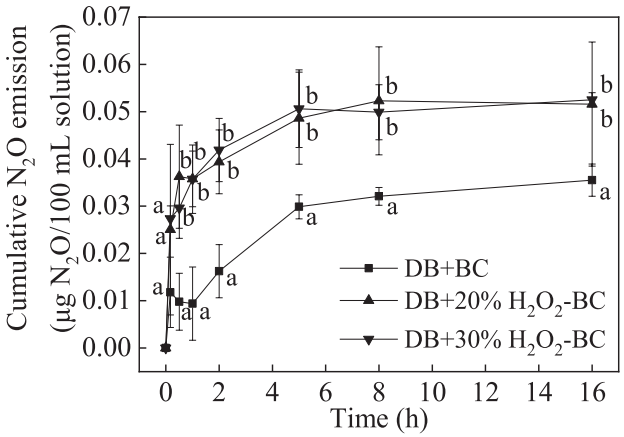

Fig. 3. Dynamic change of cumulative N_2O emission from the incubation system. Data marked with the same letter at each time point indicate no significant difference between treatments at $p<0.05$.

20% H_2O_2-BC/30% H_2O_2-BC and the negatively charged microorganisms was not conducive to microorganism attachment to the surface of biochar and their growth would be negatively affected. Consequently, compared with the treatment with BC addition, HNB amount of the treatments with H_2O_2-modified biochar addition was obviously lower. Nitrifiers are the main players of the microbial transformation of ammonia to nitrate, and their quality affects the progress of nitrification directly [27].

H_2O_2-modified Biochars Promoted N_2O Emission during Nitrification

The cumulative N_2O emission in the headspace of culture flask increased sharply during the 0~5 h of incubation while it increased moderately during the 5~16 h. The cumulative N_2O emissions of the treatments HNB+BC, HNB+20% H_2O_2-BC, and HNB+30% H_2O_2-BC during 0~5 h were 0.0299, 0.0486, and 0.0506 μg N_2O/100 mL solution, respectively, which accounted for 84.12%, 94.21%, and 96.42% of the cumulative N_2O emission during the whole incubation period, respectively.

The cumulative N_2O emissions of the treatments HNB+20% H_2O_2-BC and HNB+30% H_2O_2-BC during 0.5~16 h were significantly higher than that of the treatment HNB+BC, while there was no significant difference between the treatments with H_2O_2-modified biochar additions ($p<0.05$). The cumulative N_2O emission of the treatments HNB+20% H_2O_2-BC and HNB+30% H_2O_2-BC during 0~16 h were 0.0516 and 0.0525 μg N_2O/100 mL solution, respectively, which were 1.45 and 1.48 times that of the treatment HNB+BC (Fig. 3).

Under the condition of relatively high dissolved oxygen concentration, the main pathway of N_2O production during nitrification was NH_2OH oxidation [28]. Liu et al. [29] also reported that N_2O production had closer relationship with the activity of hydroxylamine oxidase under aerobic conditions, who found that dissolved oxygen was sufficient at the initial stage of incubation, and NH_4^+-N was rapidly oxidized to NH_2OH, resulting in NH_2OH accumulation. Further, NH_2OH was oxidized to N_2O under the catalysis of hydroxylamine oxidase. In our study, NH_2OH concentrations of the treatments with H_2O_2-modified biochar addition were higher than that of control during the incubation period of 0~8 h. Correspondingly, the cumulative N_2O emission of the treatments with H_2O_2-modified biochar addition was also significantly higher than that of control during the incubation period of 0.5~16 h. Therefore, we speculated the reason that H_2O_2-modified biochars promoted N_2O emission during nitrification could be related to NH_2OH oxidation.

In addition, NO_2^--N concentration of all the treatments during incubation was very low, which was an order of magnitude lower than NH_2OH concentration. Yu et al. [30] also indicated that NO_2^- hardly accumulated during nitrification because NH_2OH did not transformed to NO_2^- but transformed to N_2O and N_2 and released from the incubation system. Therefore, the reason that H_2O_2-modified biochars promoted N_2O emission during nitrification could not be related to NO_2^- reduction.

Correlation Analysis between the Cumulative N_2O Emission and NH_2OH, NO_2^--N Concentration, and pH of the Incubation System

In order to explore the influence of biochar on N_2O emission during nitrification and the potential mechanisms further, the correlation relationship

Table 3. Pearson correlation coefficients between the cumulative N_2O emission and NH_2OH, NO_2^--N concentration, and pH of the incubation system during the 48-h incubation period.

	NH_2OH (mg/L)	NO_2^--N (mg/L)	pH	Data range
Cumulative N_2O emission (μg N_2O/100 mL solution)	0.280	0.576**	-0.795***	HNB+BC (n = 24)
	0.455*	0.353	-0.565**	HNB+20% H_2O_2-BC (n = 24)
	0.497*	0.259	-0.654**	HNB+30% H_2O_2-BC (n = 24)
	0.358**	0.337**	-0.860***	All three treatments (n = 72)

Note: *, **, and ***denoted the significant difference at 0.05, 0.01, and 0.001 level, respectively.

between the cumulative N_2O emission and the concentrations of the intermediate products of nitrification, NH_2OH and NO_2^--N, was analyzed. The results showed that the cumulative N_2O emission was significantly positively correlated with NH_2OH concentration both in the treatments HNB+20% H_2O_2-BC and HNB+30% H_2O_2-BC, and the correlation coefficients were 0.455 and 0.497, respectively, while there was no significant correlation relationship with NO_2^--N ($p<0.05$). The correlation analysis based on the data of all the three treatments showed that the cumulative N_2O emission was significantly positively correlated with both NH_2OH and NO_2^--N concentration, and the correlation coefficient with NH_2OH (0.358) was higher than that with NO_2^--N (0.337). Furthermore, there was a significant negative correlation between the cumulative N_2O emission and the pH of incubation system ($p<0.01$), indicating that the cumulative N_2O emission was affected by pH greatly (Table 3).

Conclusions

Compared with BC, the amount of acidic oxygen-containing functional group of H_2O_2-modified biochars significantly increased by 24.24%~26.98%, while the amount of alkaline oxygen-containing functional group, pH, and the specific capacitance significantly decreased by 22.16%~27.29%, 2.54~2.68, and 15.90%~30.94%, respectively. Compared with BC, H_2O_2-modified biochars inhibited nitrification driven by the isolated heterotrophic nitrifying bacterium (HNB), *Pseudomonas putida* strain-N3. The main reasons were that H_2O_2-modified biochars with relatively low pH made the pH of the incubation system lower, and the decreased specific capacitance of H_2O_2-modified biochars weakened the function of biochar as an electron shuttle, which was not conducive to the proliferation of heterotrophic nitrifying bacteria and nitrification. Compared with the treatment with BC addition, the cumulative N_2O emission of the treatments with H_2O_2-modified biochar addition increased significantly. Correlation analysis showed that the cumulative N_2O emission of the treatments HNB+20% H_2O_2-BC and HNB+30% H_2O_2-BC was significantly positively correlated with NH_2OH concentration in incubation system (r = 0.455 and 0.497, respectively), and showed no significant correlation relationship with NO_2^--N ($p<0.05$). The study will provide a theoretical reference for the application of biochar in wastewater treatment.

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Conflict of Interest

The authors declare no conflict of interest.

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