Biochar as sustained-release carbon source and carrier for microbial denitrification

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Abstract: The biochar used to improve the soil also affects the nitrogen cycle in the water after flowing into the waterbody. In this paper, the function of biochar prepared at different pyrolysis temperatures in the denitrification process in water is studied. Some substances on the surface of biochar can be used as a carbon source for microorganisms, but the carbon source provided by biochar is limited. With the increase of pyrolysis temperature, the effectiveness of biomass on the surface of biochar shows a downward trend. When the pyrolysis temperature is 300℃, the C element content on the surface of biochar is the highest, which is 1.76 $mg \cdot g^{-1}$, and the oxidizable part increases. Scanning electron microscopy (SEM) analysis confirmed that the surface of biochar was enriched with denitrifying bacteria and provided a usable carbon source.

1. Introduction

Since the industrial era, human activities have extremely released the total amount of reactive nitrogen (N) to the environment [1]. Although nitrogen element is the crucial nutrient for crops growth, it is estimated that over half of the nitrogen fertilizer added into farmland is transferred to air and water rather than uptaken by plants [2]. In recent years, there have been a series of major environmental problems such as the sharp increase in greenhouse gas nitrogen oxide emissions from farmland soil ecosystems and the eutrophication of water bodies caused by the leaching of nitrogen nutrients from farmland. [3]. For example, it can induce health problems such as cancer and methemoglobinemia [4], adverse environmental impacts such as eutrophication, increase the greenhouse effect [5], and damage sensitive crops [6]. Therefore, there is an urgent need to develop mitigation strategies to cope with the increase in nitrogen flux.

Biomass-derived carbon (a type of black carbon) is a class of highly aromatic refractory substances produced by pyrolysis of plant biomass at 300-700°C under the condition of complete or partial hypoxia [7]. The special physical and chemical properties of biochar, applied to the soil as a soil amendment, can change soil characteristics, retain nutrient elements, increase soil organic carbon content and its own adsorption, etc., thereby affects the biogeochemical cycle of the soil ecosystem. Biochar plays an important role in carbon sequestration and emission reduction in agricultural ecosystems, and has gradually become a research focus on the fields of soil science and environmental science [7]. The application of biochar is considered to be an emerging strategy to improve soil ecosystem services.

The actual effect of biochar will vary with the amount of application, climatic conditions, soil properties, crop type and residence time in the soil [7-8]. In addition, the inherent complexity of the soil nitrogen cycle has caused the addition of biochar to change various mechanisms of nitrogen conversion. This is why there are many inconsistencies in the literature about the effect of biochar on nitrogen flux, either increasing or decreasing, or having no effect at all [9]. Despite the above differences, many studies have pointed out that biochar can reduce NO_3^- leaching [10], biomass charcoal and nitrogen fertilizer not absorbed by crops are directly lost as compounds under the action of rainfall and irrigation water. Soluble NH_4^+-N and NO_3^--N are important factors that cause surface water eutrophication and groundwater pollution. However, the impact of this application on water quality parameters has not been widely discussed.

To study the effect of biochar on nitrogen cycles in water, the effect of pine biochar on microbial denitrification was investigated. The main objectives are to evaluate denitrification efficiency of biochar as carbon source and compare the effect of pyrolysis temperature on the utilization of biochar carbon source. The results of this study will provide new insights into the understanding of the impact of biochar on the nitrogen cycle of water bodies, and will facilitate future research on the conversion to biochar-related resources.

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2. Materials and methods

2.1. Material and microorganism

2.1.1. Preparation of biochar

The pinewood strips 1.5cm×1.5cm×15cm were collected from a local wood processing factory located in Chongqing, China. After washed by tap water several times and oven dried at 105°C for 24h, the feedstock was pyrolyzed 2h in a tubular furnace (KTF-5-12Q, Yixing Qianjing Co., Ltd., China) under nitrogen flow at setting temperatures. Then the residues (biochar)were broken and screened into particles of size at 40-80 mesh. To remove ash, the produced biochar was soaked with 1.0 mol·L^{-1} HCl for 6h and rinsed with distill water several time until constant solution pH. The biochar pyrolyzed at 300℃, 700℃ and 900℃ were designated as L300, L700 and L900, respectively. In addition, the biochar pyrolyzed at 700℃without removing ash was marked as B700.

2.1.2. Denitrifying microorganism

The denitrifying microorganism comes from the activated sludge in the SBR reactor (Sequencing Batch Reactor Activated Sludge Process).

2.1.3. Devices

The test device is composed of a number of 500mL conical flask and a thermostatic water bath oscillator. The conical bottle mouth is sealed with a rubber plug inserted with a glass exhaust pipe. The glass pipe is connected with a rubber hose and inserted into the water to remove nitrogen from the conical bottle and maintain a hypoxic environment. Three 500mL conical flasks were taken and denoted as R1, R2 and R3, respectively. In R1 reactor, 50g biochar of B700 was added, and in addition the activated sludge and nitrate solution (potassium nitrate and potassium dihydrogen phosphate, N:P=5:1) were also added. The sludge and nitrate solution were prepared by adding were 300mL in total, the concentration of sludge was and nitrate nitrogen was 1000mg·L^{-1} and 60-70mg·L^{-1}, respectively. Only nitrate solution and activated sludge of the same volume in as R1 reactor were added in reactor R2. The R3 reactor was added with 300 mL of distilled water and 50g of biochar of L700. The Put three reactors (R1, R2, R3) were placed in a water bath oscillator cultivation at constant temperature water bath oscillator cultivation, and oscillator control speed control in was 180 r·min^{-1}, water bath temperature control in 30°C.

In addition, three conical flasks of 500mL volume were labeled as L3, L7 and L9. 50g of biochar L300, L700 and L900 were added into each reactor, and then activated sludge and nitrogen solution (Potassium nitrate KNO_3; Potassium phosphate monobasic KH_2PO_4; ratio N:P= 5:1) were also added.

2.2. Test method

2.2.1. Determination of COD in Biochar Hot Alkali Extract

2.50g of biochar were placed into a 50mL Erlenmeyer flask, 25mL of 0.5mol·L^{-1}NaOH was added, and then it was placed in a water bath at 100 °C in a constant temperature for 2 h. After cooling at room temperature, it was acidified with sulfuric acid, calibrated with a 1000 mL volumetric flask, and filtered through a 0.45μm organic phase filter to determine COD.

2.2.2. Determination of oxidizable part on biochar surface

0.30g of biochar put into a 250 mL Erlenmeyer flask, and accurately add 0.4 mol·L^{-1} of $K_2Cr_2O_7$ standard solution and 10 mL of concentrated H_2SO_4 with a pipette, and shake well to ensure homogenous distribution of biochar in $K_2Cr_2O_7$ solution**.**

2.2.3. Adsorption of biochar

0.50g preweighted biochar (L300, L700 and L900) was added in a 50mL centrifuge tube. And then 25mL nitrate nitrogen solution with the concentration of 20mg·L^{-1}, 40mg·L^{-1}, 60mg·L^{-1}, 80mg·L^{-1}, 100mg·L^{-1} and 120mg·L^{-1} were added into the 50mL centrifuge tube, respectively. The above 50mL tubes were placed into the constant temperature at 30℃ using water bath oscillator at 180r·min^{-1} for adsorption reaction. After shaking for 48 hours, the solution was filtered with a 0.45μm membrane. The filtrate was diluted and the nitrate nitrogen concentration was measured. The experiments were conducted duplicated and the mean value was recorded. The adsorption isotherms were fitted by Langmuir and Freundlich models, which the equations were as followed:

$$\text{Langmuir} \qquad q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \qquad (1)$$

$$\text{Freundlich} \qquad q_e = K_F C_e^{\frac{1}{n}} \qquad (2)$$

where q_m is the Langmuir maximum sorption capacity (mg g^{-1}), K_L is the Langmuir constant related to sorption energy (L mg^{-1}), K_F is the Freundlich affinity coefficient ($mg^{(1-n)}$ L^n g^{-1}), and n is the Freundlich linearity constant.

2.3. Measurement

The determination of ammonia nitrogen was carried out by Nessler's reagent colorimetry. The determination of nitrate nitrogen was carried out by ultraviolet spectrophotometry. The determination of nitrite nitrogen was carried out by spectrophotometry [11]. The elemental composition of biochar was determined by CHNS-O elemental analyzer (CHNS-O CLASSIC 4024). Biochar was scanned by electron microscopy using HITACHI S-3400N scanning electron microscope.

3. Results and Discussion

3.1. Feasibility study of biochar as a microbial carbon source

The changes in nitrate nitrogen concentration in three reactors R1, R2 and R3 (Fig. 1). After the biochar was inoculated into the sludge in the R1 reactor, the nitrate concentration decreased slowly at the beginning. Further, from the 5th day, the nitrate nitrogen concentration began to decrease sharply, it warranted that the microorganisms in the reactor gradually improved the adaptation period and entered the logarithmic growth phase. The rate of denitrification was constantly accelerated, and reaching the peak at the 10th day, and then gradually decreased. By the 12th day, the nitrate removal rate in the reactor reached 80.5%.

Only the activated sludge and nitrate nitrogen existed in the R2 reactor. The nitrate nitrogen concentration increased rapidly in the first day and reached equilibrium in the second day. Subsequently, the nitrate nitrogen concentration increased slightly, indicating that the biochar could be released a certain amount of nitrate nitrogen. The reason may be that, in the presence of only activated sludge and nitrate nitrogen, the microorganisms couldn't be denitrified due to the lack of a living carbon source, and the microorganisms undergo endogenous respiration, resulting in an increase in concentration of nitrate nitrogen in the reactor.

There is only distilled water and biochar put in the R3 reactor and nitrate has a process that rises first and then remains stable, which is due to the dissolution of part of the nitrate nitrogen in the carbon-biochar. The concentrations of nitrate nitrogen in three reactors were combined, which indicated that the R1 reactor had an anaerobic denitrification reaction, and the biochar could provide a carbon source for denitrification bacteria.

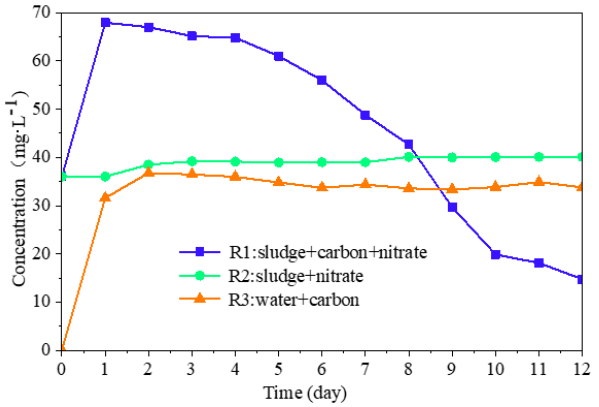


Fig.1. Nitrate nitrogen concentration in the R1, R2 and R3 reactor

The nitrate nitrogen and ammonia nitrogen in the R1 reactor are almost zero, which indicates that the nitrate nitrogen in the reactor is not converted to other nitrogen-containing compounds. However, it is reduced to N_2 by the denitrification reaction, and the biochar acts as a carbon source, providing electrons for the denitrification bacteria in denitrification reaction, and the reaction in the reactor is iso-oxidative denitrification (Fig. 2).

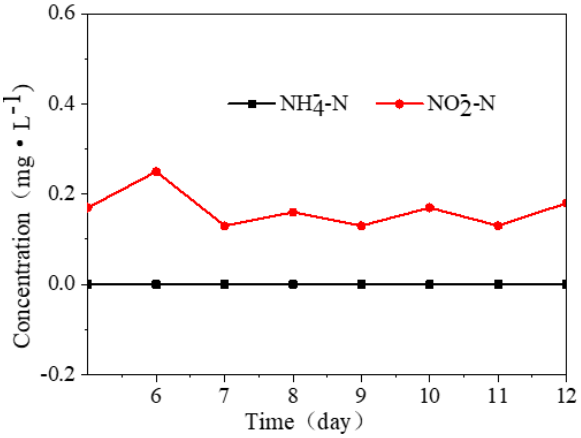


Fig.2. Nitrite nitrogen and ammonia nitrogen concentration in the R1 reactor

3.2. Denitrification performance analysis of biochar as carbon source

Biochar was added into the reactor of R1, and continued the operation up to 12 days, when a nitrate nitrogen solution ($1.8g \cdot L^{-1}$) was added into the reactor to restore the nitrate nitrogen concentration. The solution volume in the reactor was returned to the initial state, and kept in continuous running. During a 6-day continuous operation, the nitrate nitrogen concentration and the solution volume were added. At the end of each cycle for 7 days, the concentrations of nitrate nitrogen decreased in the reactors over the whole 7 cycles (Fig. 3).

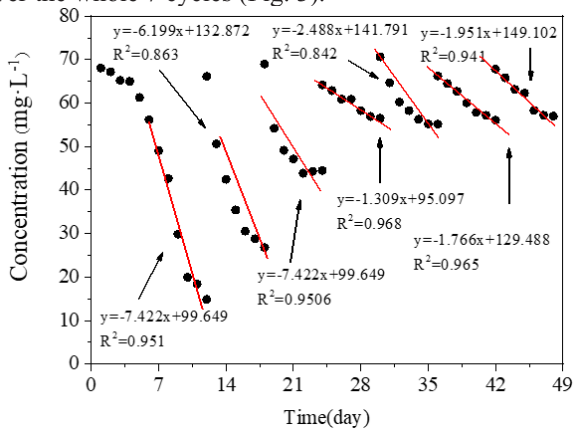


Fig.3. Nitrate nitrogen in each reactor in continuous operation cycle

As carbon sources, the denitrification rate of biochar is obviously lower, which indicate that the availability of biocarbon is poor. From the reaction kinetic equation of denitrification reaction in each cycle, it has seen that the denitrification reaction in the reactor is a zero-stage. Thus, the carbon source of biochar in the reactor is the limiting factor for denitrification and metabolism of denitrification bacteria.

3.3. Effect of pyrolysis temperature on availability of biochar carbon source

After inoculating the sludge into the reactors L300, L700 and L900, the nitrate nitrogen concentration in the three reactors changed (Fig. 4). When denitrifying bacteria used

biochar as a carbon source to reduce nitrate to N_2. The total amount of nitrate nitrogen reduced is the sum of the initial amount and the added amount of nitrate nitrogen in the reactor minus the amount of nitrate adsorbed by biochar.

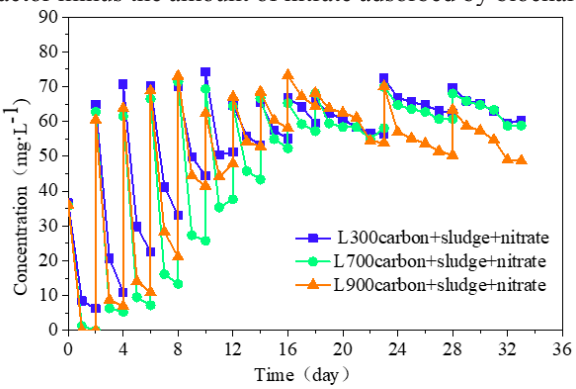


Fig.4. Nitrate nitrogen concentration with different biochar

In order to determine the adsorption capacity of biochar for nitrate nitrogen, the adsorption isotherm fitting was carried out for nitrate nitrogen solutions with initial concentrations of 20 mg· L^{-1}, 40 mg· L^{-1}, 60 mg· L^{-1}, 80 mg· L^{-1}, 100 mg· L^{-1}, and 120 mg· L^{-1} for three kinds of biochar (L300, L700, and L900) (Table 1). The adsorption isotherm of nitrate nitrogen by biochar L300 accords with Freundlich model, and the adsorption isotherm are reversible. The adsorption of nitrate nitrogen by biochar L700 and L900 confirms the better fitness of Langmuir isotherm model, and the adsorption is mainly monomolecular adsorption (Data not shown).

Table 1 Isotherms parameters for nitrate nitrogen onto biochar

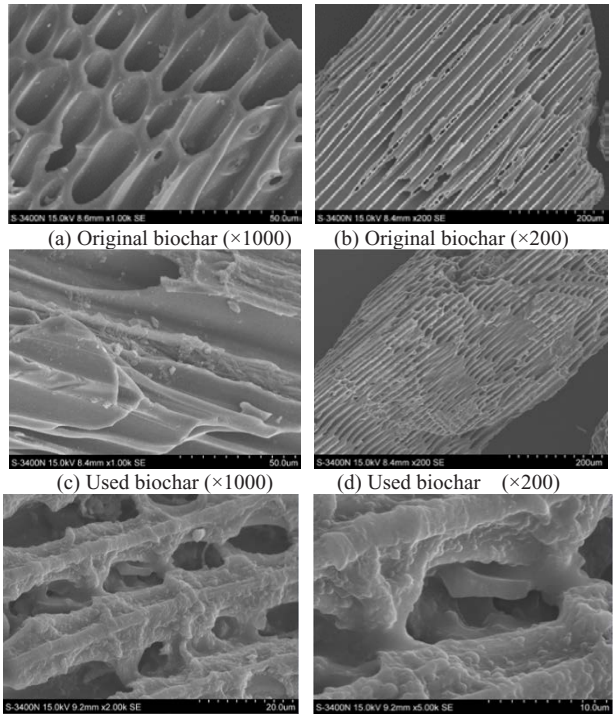
Biochar	Langmuir model			Freundlich model		
	Q_m	K_L	R2	1/n	K_F	R2
L300	0.7537	0.022	0.972	0.4695	0.060	0.989
L700	1.4455	0.4474	0.971	0.3642	4.3558	0.958
L900	1.4695	0.055	0.976	0.3314	0.2821	0.955

Three reactors with L300, L700, and L900 were operated for total of 33 days after inoculation of sludge, and the total amount of biocarbon used as denitrification carbon source was converted from the total nitrate nitrogen of bacterial denitrification to C single carbon source. With the increase of pyrolysis temperature of biochar, the total amount of C as a carbon source decreased in 33 days. It indicates that the availability of biocarbon as a carbon source show a decreasing trend with the increase of preparation temperature, which is also consistent with the aromatic nature of biocarbon [12-13]. Thus, it indicated that the carbon source on the surface of biochar may be mainly cellulose, hemicellulose and other chain alkanes.

3.4. Biochar characterization

3.4.1. Surface property

The biochar of L700 in reactor R1 was selected after being utilized by microorganisms, and the unutilized biochar L700 was scanned by SEM (Fig. 5). It revealed that the surface structure of the biochar is relatively smooth which is not used by microorganisms. In contrast, the surface of the biochar after being utilized by microorganisms become rough, which confirms that some substances on the surface of the biochar provide denitrifying bacteria. In general, after the biochar is used by microorganisms, the main structure change is not obvious, indicating that the carbon source materials available on the surface are limited.



(a) Original biochar (×1000) (b) Original biochar (×200)
(c) Used biochar (×1000) (d) Used biochar (×200)
(e) Biofilm on the biochar (×2000) (f) Biofilm on the biochar (×5000)

Fig.5. SEM photos of biochar surface

Visible cellulose structure can be seen in a band-like distribution, and the texture damage before and after the reaction is not obvious, which contributes to the adhesion of microorganisms. At the same time, it displayed that the microbes on the surface of biochar are mainly cocci, and there was no Vibrio, indicating that cocci are dominant populations. Overall, the biofilm on the surface of biochar is very thin, which should be related to the difficulty of using surface materials. This is similar to the denitrification reaction of biodegradable polymer (PBS) [14] and biodegradable polycaprolactone (PCL) [15] as solid carbon sources, but different carbon sources lead to different microbial composition and biofilm thickness.

3.4.2. Changes of COD in extracts

After the biochar is pyrolyzed, the residual organic matter such as cellulose is dissolved under the action of a strong alkali, and the extract contains monosaccharides produced by cellulose degradation and further degraded products and other substances [12]. These substances determine the COD of the biochar extract. The COD of the biochar extract after biochar was utilized by microorganisms increased obviously, and also decreased with the increase of the biochar preparation temperature. This indicate that

as the preparation temperature increases, the organic components such as cellulose remaining after the pyrolysis biochar will gradually decrease (Fig.6). However, in the process in which the biochar surface material is utilized by microorganisms, some organic components such as cellulose and hemicellulose are decomposed by microorganisms, and organic components such as cellulose remaining on the surface are increased, thereby increasing COD in the extract.

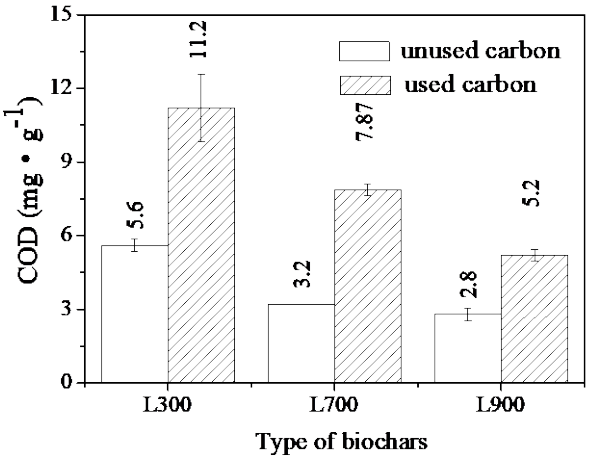


Fig.6 Changes of COD of extract solution

3.4.3. Changes in the oxidizable part of the surface

The biochar composition has changed before and after microbial denitrification process as shown in Table 2. The ratio of H/C of biochar decreased with increasing preparation temperature, indicating that the degree of biochar carbonization and aromaticity increase with increasing preparation temperature. This also verifies that the total amount of C and the rate of denitrification provided by biochar as a carbon source. Conversely, the increase in the H/C ratio of biochar after microbial utilization indicated that the degree of carbonization and aromaticity of biochar after microbial utilization was lower than that of biochar, which suggest biochar providing electrons in the process of microbial denitrification process.

The content of oxidized part of the surface of biochar increased with the increase of preparation temperature (Data not shown). When the preparation temperature increased from 700 °C to 900 °C, the amount of oxidized surface of biochar increased rapidly. It confirmed that the surface regularity of the biochar increased with preparation temperature increasing. However, the total amount of carbon source was reduced. It suggested that the increase in the oxidizable portion of the surface on the biomass carbon only enhanced the reduction of the surface of the biomass carbon, but did not increase the availability of microorganisms. The result was also consistent with the results of elemental analysis of biochar.

Table 2 Elemental analysis of biochar carriers

Pyrolytic temp.	Usage Condition	Element mass fraction (%)					Atomic ratio
		C	H	O	N	S	H/C
300°C	Before	79.07	1.49	18.13	1.02	0.29	0.226
	After	79.09	1.84	17.31	1.15	0.61	0.280
700°C	Before	85.18	0.67	12.58	1.11	0.26	0.094
	After	83.62	1.24	13.49	1.24	0.41	0.179
900°C	Before	89.18	1.13	7.88	1.11	0.7	0.152
	After	87.5	1.14	9.88	1.17	0.31	0.156

4. Conclusions

Biochar is an incompletely carbonized material that can be used as an available carbon source for microbial denitrification. The pyrolysis temperature affects the availability of carbon source in biochar, which the availability of biochar carbon decreases with increasing pyrolytic temperature. Biochar provides electrons and better carrier for biofilms attachment to facilitate the metabolism of microorganism denitrification. The infrared spectrum and scanning electron microscopy analysis revealed the feasibility of biochar as both solid carbon source and support for denitrification.

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