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Application of Rice-Straw Biochar and Microorganisms in Nonylphenol Remediation: Adsorption-Biodegradation Coupling Relationship and Mechanism

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Abstract

Biochar adsorption presents a potential remediation method for the control of hydrophobic organic compounds (HOCs) pollution in the environment. It has been found that HOCs bound on biochar become less bioavailable, so speculations have been proposed that HOCs will persist for longer half-life periods in biochar-amended soil/sediment. To investigate how biochar application affects coupled adsorption-biodegradation, nonylphenol was selected as the target contaminant, and biochar derived from rice straw was applied as the adsorbent. The results showed that there was an optimal dosage of biochar in the presence of both adsorption and biodegradation for a given nonylphenol concentration, thus allowing the transformation of nonylphenol to be optimized. Approximately 47.6% of the nonylphenol was biodegraded in two days when 0.005 g biochar was added to 50 mg/L of nonylphenol, which was 125% higher than the relative quantity biodegraded without biochar, though the resistant desorption component of nonylphenol reached 87.1%. All adsorptive forms of nonylphenol (f_{rap}, f_{slow}, f_r) decreased gradually during the biodegradation experiment, and the resistant desorption fraction of nonylphenol (f_r) on biochar could also be biodegraded. It was concluded that an appropriate amount of biochar could stimulate biodegradation, not only illustrating that the dosage of biochar had an enormous influence on the half-life periods of HOCs but also alleviating concerns that enhanced HOCs binding by biochar may cause secondary pollution in biochar-modified environment.

Introduction

Compared with conventional carbonaceous materials such as commercial activated carbon, the primary advantages of biochar are its low cost and the diversity of biomass from which it can be produced [1]. Because of its strong adsorption capacity, biochar has attracted increasing interest in recent years for the immobilization of HOCs [2–4] and is a promising solution in remediation [5,6]. Therefore, investigating the influence of biochar on the migration and transformation of HOCs is important for HOCs pollution control and eco-environmental security.

In previous studies, it has been demonstrated that biochar application has significant negative effects on HOCs degradation [7,8]. On one hand, the polyaromatic recalcitrant matrix of biochar is highly stable, and biochar may remain in the environment for tens or hundreds of years [9,10]. On the other hand, biochar has been shown to reduce the concentration of aqueous HOCs by reactive surface adsorption or physical trapping within biochar pores [11,12]. For the above two reasons, the desorption efficiency of adsorbed HOCs is very slow [13,14], resulting in reduced biodegradability and poor bioavailability [15–17]. From the above findings, it was proposed that biochar application suppressed the transformation of HOCs, resulting in a longer half-life and persistence in the soil and/or sediment. For instance, Muter et al. investigated the influence of biochar on the persistence of the herbicide 4-chloro-2-methylphenoxyacetic acid (MCPA) in soils and found that after 37 days, the biodegradation rate was significantly reduced when 5.3% biochar was applied, dropping from 100% to 69.3% ($P < 0.01$) [17]. A similar phenomenon was also observed by Xin et al., who found that the transformation rate of 2,2',4,4'-tetrabromodiphenyl ether was decreased by 87.50–92.19% when 1% biochar was added to the soil [18].

Despite extensive data showing the reduced bioavailability of contaminants in the presence of biochar, other studies investigating HOCs biodegradation have indicated that biochar application promotes HOCs mineralization in the presence of abundant microorganisms [19–21]. Biochar is typically prepared at temperatures ranging from 300–700°C, thus biochar is rich in nutrient and trace elements (e.g., P、C、Na and Mo) for biota [22,23], which gives biochar the potential to have a positive impact on contaminant transformation [21] or no significant adverse effects [24]. Tong et al. found that 1% biochar accelerated PCP transformation from 12.5% to 60.7%, and 100% transformation rate was observed when a 5% dosage of biochar was added [21]. A possible explanation may be that the microorganisms were capable of adhering to or forming biofilms on biochar particles by releasing extracellular enzymes, which constitutes an important mechanism to overcome mass-transfer limitations in environmental remediation [25,26].

There is no consensus regarding the complex interactions resulting from biochar application due to variations in experimental time scale, HOCs concentration, biochar dosage etc. Therefore, it was speculated that there may be an appropriate biochar dosage range to enhance biodegradation rather than inhibit it, thus optimizing coupled adsorption-biodegradation for a specific concentration of HOCs. To verify these conjectures, nonylphenol, a representative HOCs with estrogenic effects, was selected as the target contaminant, while rice-straw-derived biochar was selected as an adsorbent. First, the effects of biochar dosage on the adsorption-biodegradation of various concentrations of nonylphenol were investigated. Second, changes in the adsorptive form of nonylphenol during biodegradation were investigated using Tenax desorption technology, and the biodegradation potential of the desorbed forms of nonylphenol was also calculated. Third, the coupled effects of biochar and microorganisms on the fate of HOCs were discussed.

Materials and Methods

Chemicals and materials

Nonylphenol (> 99% purity) was purchased from Aladdin (Shanghai, China) and prepared as a concentrated stock solution with acetonitrile. Tenax TA (60–80 mesh) was obtained from Supelco (Bellefonte, Pennsylvania, USA) and regenerated by ultrasonic washing with methanol, acetone and hexane in order [14]. Acetonitrile, methanol, acetone, hexane and dichloromethane (chromatographic grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Biochar was produced from rice straw following a procedure detailed in a previous study [14].

Preparation of nonylphenol biodegradation inoculum

A mixed cultivation inoculum, able to utilize nonylphenol as a sole carbon source for growth, was isolated from sediments (0–10 cm depth) collected from the Qiantang River in Hangzhou, Zhejiang Province, China. A mineral salt medium was prepared containing (per liter) 4.0 g of K_2HPO_4, 4.0 g of NaH_2PO_4, 2.0 g of $(NH_4)_2SO_4$, 0.41 g of $MgSO_4{\cdot}7H_2O$, 0.01 g of anhydrous $CaCl_2$, 0.01 g of $MnSO_4{\cdot}H_2O$, and 0.01 g of $FeSO_4{\cdot}7H_2O$, pH was maintained at 7.0–7.2 using 1 M NaOH. A 25-g sediment sample was added to a 250-mL flask containing nonylphenol-spiked medium (90 mL medium with 10 mg/L nonylphenol). The flask was incubated at 30°C on a 150 rpm shaker in the dark for 7 days, after which 10 mL of the liquid was transferred to another flask with nonylphenol-spiked medium (20 mg/L) and incubated under the same conditions. Successive incubations in nonylphenol-spiked medium (30, 40, 50 mg/L) were performed until colonies showed evidence of nonylphenol degradation.

The culture was enriched in medium (1 L medium contained 5.0 g of NaCl, 10.0 g of tryptone, and 5.0 g of beef extract, pH was maintained at 7.0–7.2 using 1 M NaOH) at 30°C while shaking at 150 rpm. After 24 h incubation, the culture was centrifuged at 4000 g for 10 min. The supernatant was discarded, and the microorganisms were resuspended in mineral salt medium. This procedure was repeated four times to ensure a thorough removal of any residual nutrient substances. The OD_{600} was adjusted to 1.0, and the inoculum was stored in refrigerator at 4°C until adding to the reactor.

Pre-adsorption experiments

All sorption experiments were conducted in triplicate. The batch reactors consisted of 50-mL glass centrifuge vials with permeable silica gel stoppers to allow adequate oxygen. Each reactor contained 9 mL mineral salt medium with 0, 0.005, 0.01 or 0.10 g biochar, and all reactors were autoclaved twice. Nonylphenol stock solution with concentration of 1000 mg/L was added with 6, 30, 50 and 100 μL, respectively, to each vial to obtain final concentrations of 6, 30, 50, and 100 mg/L, and vials were then shaken at 150 rpm and 30°C in an orbital shaker for 24 h to reach adsorption equilibrium.

Nonylphenol biodegradation experiments

Nonylphenol biodegradation experiments were performed in triplicate. A 1-mL aliquot of prepared nonylphenol biodegradation inoculum was added to all reactors, and the reactors were shaken at 150 rpm on a horizontal shaker at 30°C for 0, 1, 2, 4, 6, 10 and 16 d in the dark. Both the aqueous and solid concentrations of nonylphenol were measured. The sum of these quantities was defined as the residual. The concentration suppression curve of nonylphenol was explored using equal biochar dosages and a series of nonylphenol concentrations, using an identical set-up except that the sampling time was 6 d. To quantify the loss of nonylphenol due to abiotic processes and systematic loss, sterilized reactors with 0.2 mg/mL sodium azide were operated in parallel.

Analysis of aqueous nonylphenol concentration. At each designated time, reactors were taken from the shaker and centrifuged at 4000 g for 10 min to separate the aqueous and solid phases. In this study, the aqueous phase means the freely dissolved form in the mineral salt medium, and the solid phase is the form sorbed by biochar and/or microorganisms. From the aqueous phase, 0.5-mL aliquots were transferred to a 2-mL plastic centrifuge tube, and methanol was added at a water/methanol volumetric ratio of 1:1 to dissolve nonylphenol. Samples were then analyzed by high performance liquid chromatography (Agilent 1100 series) using a previously reported method [14].

Analysis of solid phase nonylphenol concentration. The supernatant was discarded, after which a defined amount of anhydrous sodium sulfate was added to the precipitate, and

nonylphenol was extracted with 5 mL of organic extractant (methanol:methylbenzene = 6:1, v/v) by sonication for 40 min. The extracts were collected into nitrogen blowpipes. This procedure was repeated four consecutive times to ensure thorough extraction of the nonylphenol from the sodium sulfate. Eventually the collected solvent was nitrogen-flushed to 1 mL and analyzed by high performance liquid chromatography.

Desorption of residual nonylphenol after degradation

The experimental set-up was the same as in the biodegradation experiments mentioned above. At each designated time interval, vials were taken from the shaker and 0.2 mg/mL sodium azide was added to stop biodegradation. Then, 0.1 g of Tenax beads and another 20 mL of mineral salt medium were added to begin the desorption experiment. The subsequent steps have been detailed in a recent work [14]. Finally, the combined extracts from the Tenax beads were concentrated to 1 mL using nitrogen flow and analyzed by high performance liquid chromatography.

Data analysis

Concentration suppression curve of nonylphenol. The rate of nonylphenol biodegradation was plotted against time (t), and the Haldane model was fitted to the data, allowing the optimal concentration of nonylphenol to be calculated:

$$V = \frac{V_{\max}}{1 + {K_m}/{S} + {S}/{K_i}} \tag{1}$$

$$S_{\max} = \sqrt{K_m K_i} \tag{2}$$

where V represents the substrate reaction rate (i.e., the nonylphenol biodegradation rate in this study), V_{max} represents the maximum reaction rate, K_m represents the half rate constant, K_i represents the inhibition constant, S represents the substrate concentration, and S_{max} represents the optimal concentration.

Desorption data interpretation. The initial adsorbate concentration in the system was assumed to be S_0, and the residue after biodegradation time T was S_T. Kinetic Tenax desorption experiments were preformed after the nonylphenol biodegradation step, and the adsorbate concentration at desorption time t was S_t. Nonylphenol desorption data after biodegradation were fitted by the modified two-domain model using Origin 8.0 software [14].

$${S_t}/{S_T} = F_{rap}e^{-k_{rap}t} + F_{slow}e^{-k_{slow}t} + F_r \tag{3}$$

where F_{rap}, F_{slow} and F_r represent the rapid, slow and resistant fractions, respectively, and $F_{rap}+F_{slow}+F_r = 1$; k_{rap} and k_{slow} represent the kinetic constants (h^{-1}) of each domain.

By multiplying both sides of the equation by S_T/S_0,

$${S_t}/{S_0} = f_{rap}e^{-k_{rap}t} + f_{slow}e^{-k_{slow}t} + f_r \tag{4}$$

$$f_{rap} = F_{rap} \cdot \frac{S_T}{S_0} \quad (5)$$

$$f_{slow} = F_{slow} \cdot \frac{S_T}{S_0} \quad (6)$$

$$f_r = F_r \cdot \frac{S_T}{S_0} \quad (7)$$

where f_{rap}, f_{slow} and f_r represent the three fractions of the transmutative two-domain model beginning with biodegradation.

QA/QC

All experiments treatments were in triplicate, sterilized reactors with 0.2 mg/mL sodium azide were included as control. Pure acetonitrile: methanol (9:1, v/v), the mobile phase for HPLC analysis was analyzed in HPLC as solvent blank. From preliminary experiments, the average extraction recovery of nonylphenol samples was greater than 98% for biodegradation experiments. Statistical analysis of data was analyzed with Origin 8.0 and SPSS 11.0. Significance was assigned at $P \leq 0.05$.

Results and Discussion

Effect of biochar on nonylphenol biodegradation

Two mechanisms resulted in the disappearance of nonylphenol: abiotic dissipation and biotic degradation. Control reactors representing abiotic loss showed no significant reduction in nonylphenol ($P > 0.05$), indicating that biodegradation was responsible for the transformation of nonylphenol. For all experimental groups, nonylphenol underwent progressive reduction over 16 d of incubation, as shown in Fig 1.

To identify the role of biochar in nonylphenol biodegradation at various initial concentrations of pollutant, the nonylphenol concentrations were divided into low (C_0 = 6 and 30 mg/L) and high (C_0 = 50 and 100 mg/L) ranges. In the absence of biochar, biodegradation of nonylphenol was found to be generally efficient from an initial 6 mg/L to a final 0.574 mg/L, with a biodegradation rate of 90.4% over 16 d of incubation; this rate was similar to previously reported results [27–29]. Therefore, observations from this and other studies suggest that biodegradation may be an important method to govern HOCs such as nonylphenol [30,31]. However, the residual nonylphenol was significantly greater with biochar addition ($P < 0.01$), and the higher the dosage of biochar, the more the residual nonylphenol increased ($P < 0.01$). In particular, at a dosage of 0.1 g, almost half of the nonylphenol was not transformed, indicating that biochar application inhibited nonylphenol biodegradation at a low nonylphenol concentration. When more biochar was added, there was an enhanced negative effect on nonylphenol transformation. Similar phenomena have been observed in other studies as well [17,32]. Biochar is widely regarded as an effective adsorbent to adsorb a broad range of HOCs due to its large surface area and highly aromatic structure [11]. Nevertheless, this high capacity for sorption may decrease the bioavailability and reactivity of contaminants, resulting in poor biodegradability [16,33].

For the high nonylphenol concentrations (C_0 = 50 and 100 mg/L), residuals of 5.550 and 16.540 mg/L were measured in the absence of biochar, which were higher values than those observed for low nonylphenol concentrations. In addition, the high nonylphenol

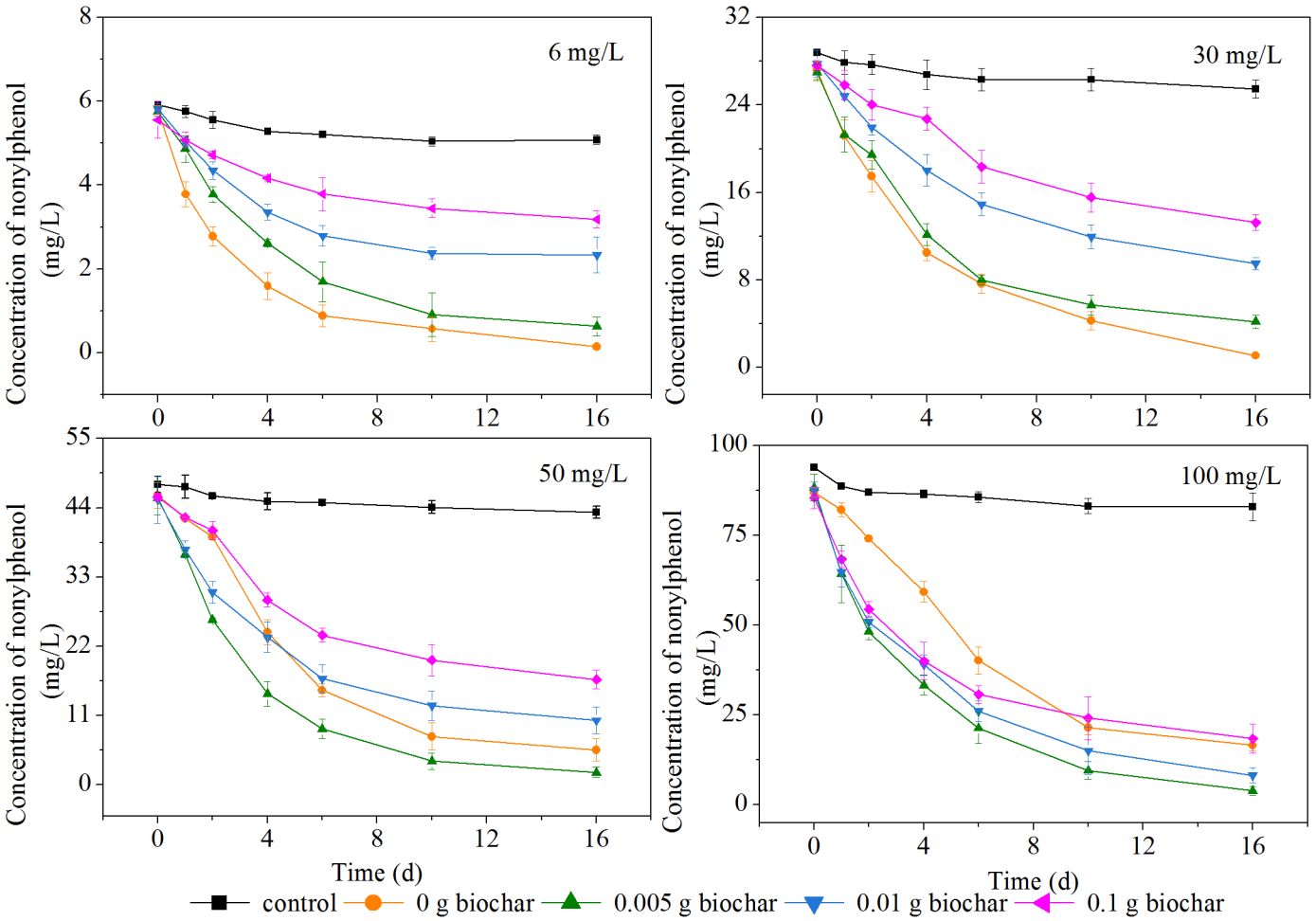

Fig 1. Changes in nonylphenol concentration with different biochar dosages. The degradation kinetics of different concentrations of nonylphenol (6, 30, 50 and 100 mg/L) at various biochar dosages (0, 0.005, 0.01 and 0.1 g).

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concentrations had a slow initial phase of nonylphenol biodegradation, yielding 13.2% nonylphenol after the first two days, compared with 50.3% at low concentration; this may be due to the biotoxicity of nonylphenol [34], or it may be that enzyme saturation kinetics limited biodegradation at the higher concentrations used in these experiments [35]. Nonylphenol was degraded more at a biochar dosage of 0.005 g than in the absence of biochar, throughout the entire incubation period; for instance, 47.6 and 51.8% (C_0 = 50 and 100 mg/L) nonylphenol was transformed in the initial two days, 125 and 99.2% higher than in respective experiments without biochar, indicating that biochar accelerated nonylphenol transformation. Interestingly, with the addition of more biochar, nonylphenol biodegradation was reduced. The results at both high and low nonylphenol concentrations were similar: degradation was inhibited at greater biochar dosage.

To further elucidate the relationship between adsorption and biodegradation of nonylphenol, the ratios of nonylphenol biodegradation rates with and without biochar were calculated to obtain relative rates of biodegradation, as shown in Fig 2. The relative rates of nonylphenol biodegradation were significantly affected by nonylphenol concentration ($P < 0.01$). For a 0.005 g biochar dosage, the relative rates varied between the low (C_0 = 6 and 30 mg/L) and high (C_0 = 50 and 100 mg/L) nonylphenol concentrations, with values less than 1.0 for low

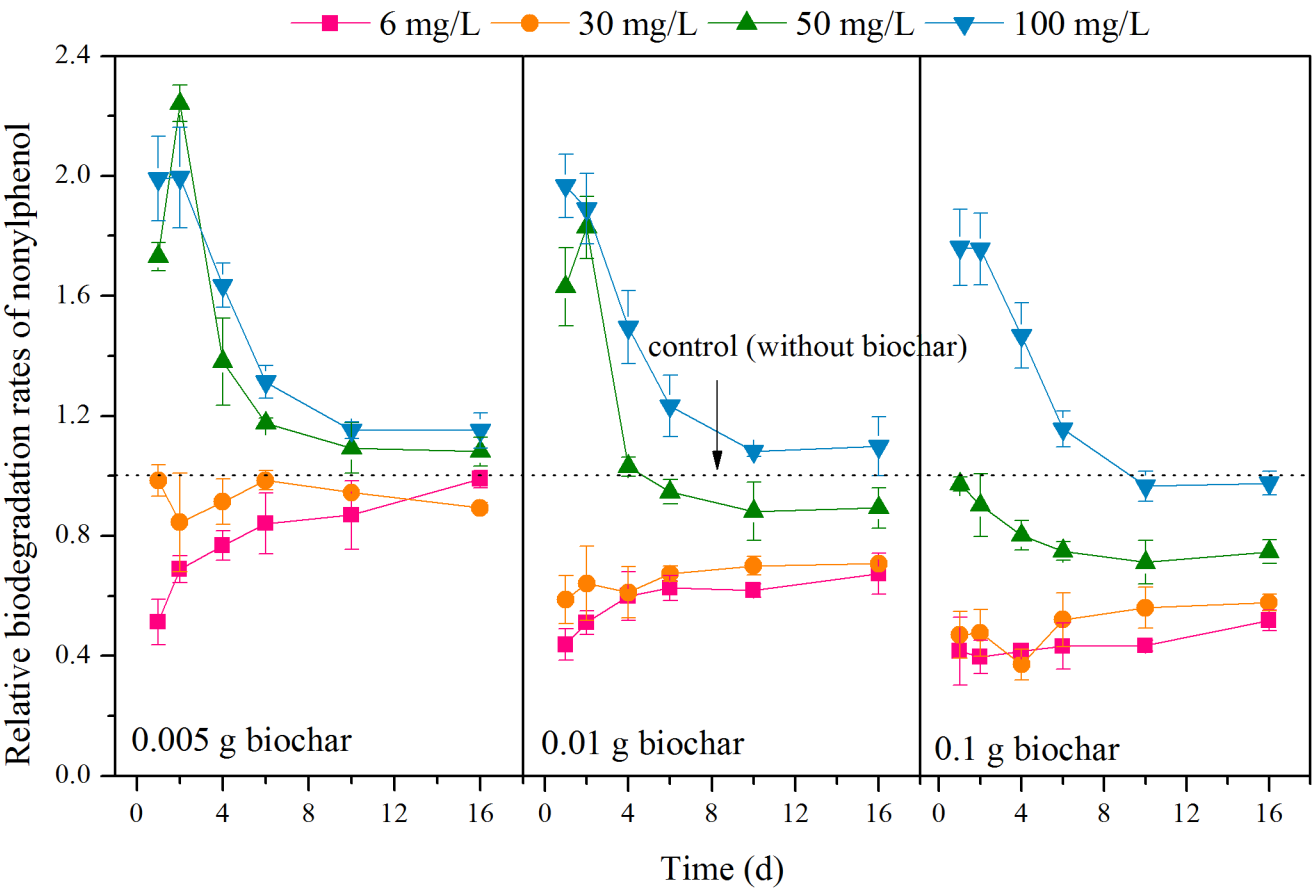

Fig 2. Relative rates of nonylphenol biodegradation with different biochar dosages. The ratios of nonylphenol biodegradation rates with and without biochar were calculated to obtain relative rates of biodegradation.

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concentrations, showing a negative effect on biodegradation, and values greater than 1.0 for high concentrations, showing a positive effect. When the biochar dosage increased to 0.01 g, the negative effect persisted and became more pronounced at low concentrations ($0.01 < P < 0.05$). At the high nonylphenol concentration of 100 mg/L, the relative rate of biodegradation was still above 1.0, but at 50 mg/L, the rate decreased below 1.0 after 4 days, with a residual of 26.195 mg/L. At a 0.1 g biochar dosage, all relative rates were below 1.0, except at a nonylphenol concentration of 100 mg/L, for which the relative rate dropped below 1.0 after 10 days.

Overall, there was an appropriate biochar dosage range for optimal biodegradation at each given nonylphenol concentration: less than 0.01 g for 50 mg/L and 0.005–0.01 g for 100 mg/L. The promotion effect of biochar gradually slowed down as nonylphenol was degraded.

Mechanism of biochar's effect on nonylphenol biodegradation

To explore the mechanism of biochar's effect on nonylphenol biodegradation at various concentrations, the change of aqueous ($C_{aqueous}$) and solid phase (C_{solid}) concentration were investigated during incubation (S1 and S2 Figs). In general, $C_{aqueous}$ and C_{solid} both underwent processive reduction during incubation, similar to total nonlyphenol changing in Fig 1. On one hand, $C_{aqueous}$ was much lower than the initial nonylphenol concentration without biochar addition, suggesting that the majority of nonylphenol was adsorbed to microorganisms, namely C_{solid}, but did not significantly suppress biodegradation because the biological adhesion

was unstable [35]. On the other hand, $C_{aqueous}$ was evidently reduced in all biochar dosage treatments ($P < 0.01$) and became less available for microorganisms [32], leading to reduced biodegradability. Besides, C_{solid} was relatively greater at low initial concentrations (C_0 = 6 and 30 mg/L) but getting lower at high initial concentrations (C_0 = 50 and 100 mg/L) with biochar dosage getting larger, confirming the results suggested above that biochar addition played a inhibition role at low concentration of nonlyphenol, but showed promotion at high nonlyphenol concentrations. Furthermore, $C_{aqueous}$ was higher at high nonylphenol concentrations than at low concentrations and thus may have acute toxicity against microorganisms [34], resulting in sluggish initial degradation.

Concentration suppression curves of nonylphenol with 4 biochar dosages are shown in Fig 3. The optimal concentration in the absence of biochar was approximately 5.395 mg/L, so the degree of biodegradation at low nonylphenol concentrations (C_0 = 6 and 30 mg/L) was slightly more complete than at higher concentrations (C_0 = 50 and 100 mg/L). With the addition of biochar, all optimal concentrations gradually increased to 54.23, 175.22 and 332.35 mg/L for 0.005, 0.01 and 0.1 g biochar dosage, respectively. Among the low concentrations, biodegradation rates followed the progression $V_0 > V_{0.005} > V_{0.01} > V_{0.1}$, suggesting that nonylphenol transformation was inhibited by biochar addition. In addition, $V_{0.005}$ reached a maximum at high nonylphenol concentration ($V_{0.005} > V_0 > V_{0.01} > V_{0.1}$ for C_0 = 50 mg/L, $V_{0.005} \approx V_{0.1} > V_0 > V_{0.1}$ for C_0 = 100 mg/L), in accordance with results shown above. The reverse is also plausible if an

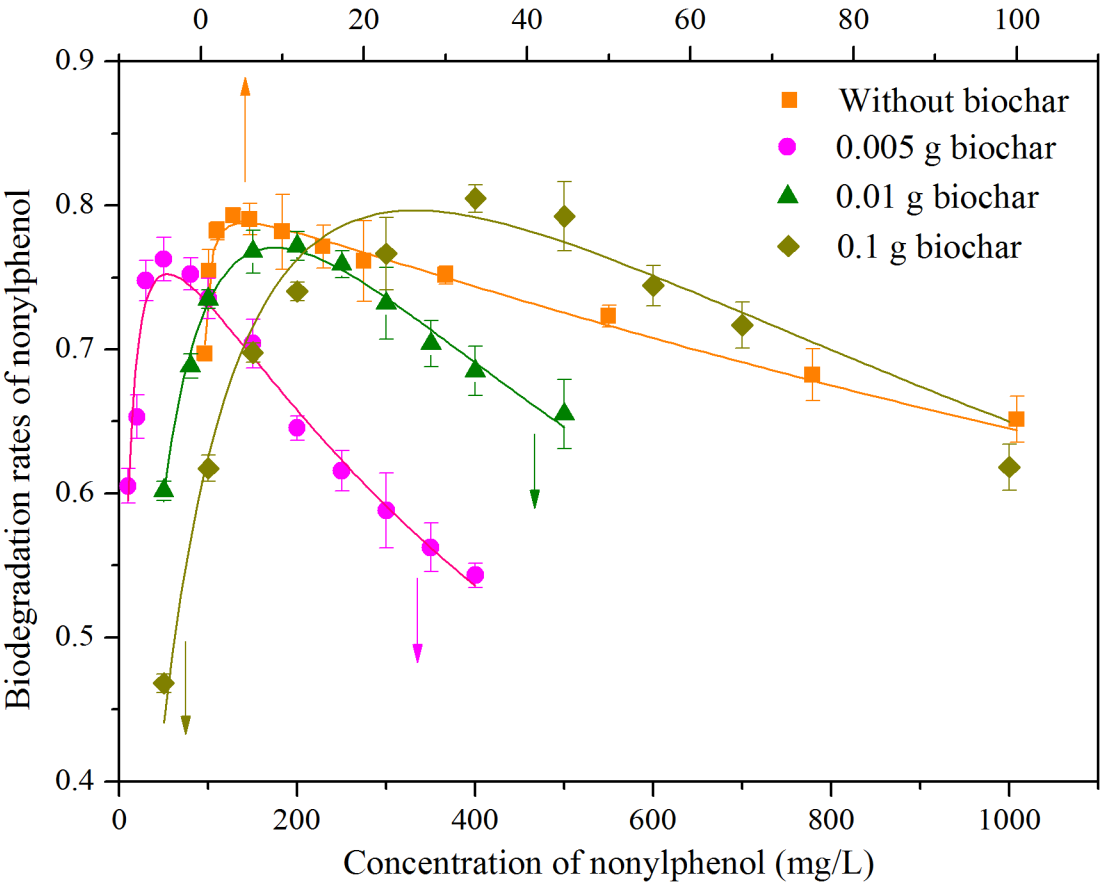

Fig 3. Concentration suppression curve of nonylphenol at different biochar dosages. The degradation rates of a series of nonlyphenol concentrations at 4 biochar dosages (0, 0.005, 0.01 and 0.1 g).

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applied nonylphenol concentration range corresponds with a specific biochar dosage. Therefore, the higher the nonylphenol concentration was, the more biochar required for remediation.

Effects of biochar on form of sorbed nonylphenol

Previous studies have demonstrated that less than 40% of strongly immobilized nonylphenol could be released from sediment during 16 days of desorption [14]. Our results, however, showed rates of nonylphenol biodegradation up to 47.0–96.4% in the presence of biochar, implying that the resistant fractions were unexpectedly bioavailable. Based on the above results, desorption kinetics of residual nonylphenol after biodegradation, as well as changes in form of sorbed nonylphenol, including the rapid, slow and resistant fractions, were investigated.

Statistical analysis revealed that the transmutative two-domain model provided a good fit for all biodegradation residual data (R^2 range 0.952–0.999). The changes in form of sorbed nonylphenol are shown in Fig 4.

Across experimental conditions, the desorption fractions followed the progression $f_r > f_{rap} \approx f_{slow}$ and were generally in order of 10^{-2} to 10^{-1}, 10^{-2} and 10^{-3} to 10^{-2}, respectively. Among these results, f_r was significantly greater than the other two fractions ($P < 0.01$), suggesting that f_r dominated in residual nonylphenol [14,36]. A slow decrease occurred in f_{rap}, f_{slow} and f_r over the course of biodegradation experiments, representing the transformation of all three fractions. The values of f_r ranged from 0.852±0.0050 to 0.149±0.031, 0.782±0.0046 to 0.103±0.016, 0.788±0.053 to 0.0110±0.019, and 0.804±0.064 to 0.0120±0.011 for each nonylphenol concentration, respectively, with the addition of 0.005 g biochar; f_r underwent a significant decrease ($P < 0.01$), illustrating the biodegradability of f_r. Furthermore, the minimal reduction of f_r at higher biochar dosage suggested the inhibition of the bioavailability of f_r.

Modeling biodegradable contributions from various desorption fractions

The results of correlation among degradable fractions during desorption demonstrated that the slopes for the degraded fraction and f_{rap}+f_{slow} exceeded 1.0, indicating that apart from the rapid and slow fractions, there must be an additional contribution to biodegradation, namely f_r.

Based on the above analysis, assumptions were put forward: First, all three fractions (rapid, slow and resistant) contributed independently to the total biodegradation, with independent biodegradation coefficients. Second, the rapid fraction could be degraded completely, and its coefficient was regarded as 1 ($BD_{rap} = 1$). Therefore, the overall biodegradable fractions may be described by the following relationships:

$$BD = BD_{rap}f_{rap} + BD_{slow}f_{slow} + BD_{r}f_{r} \quad (8)$$

BD represents the total biodegradable fraction, namely the rate of nonylphenol biodegradation in each period, and BD_{rap}, BD_{slow} and BD_r represent the coefficients of the rapid, slow and resistant desorption fractions contributing to degradation. f_{rap}, f_{slow} and f_r together with BD at each designated time interval were then subjected to statistical analysis using multiple linear regression to obtain the regression coefficients BD_{rap}, BD_{slow} and BD_r.

The calculated coefficients BD_{rap}, BD_{slow} and BD_r at various desorption intervals are shown in Table 1. Data from all treatments were closely fitted by the model, and the independent variables explained 0.567–0.999 of the variations in the regression. All fitted BD_{slow} and BD_r values were above 0, confirming the bioavailability of the slow and resistant fractions. Specifically, the values of BD_{slow} ranged from 0.439±0.235 to 1.155±0.174, with most values either above 0.5 or very close to 1.0, suggesting good bioavailability of the slow desorption fraction. BD_r decreased to 0.01 upon increasing the biochar dosage to 0.1 g. The reduction of BD_r was due to intense suppression of bioavailability by biochar application [16]. In contrast, there was no apparent

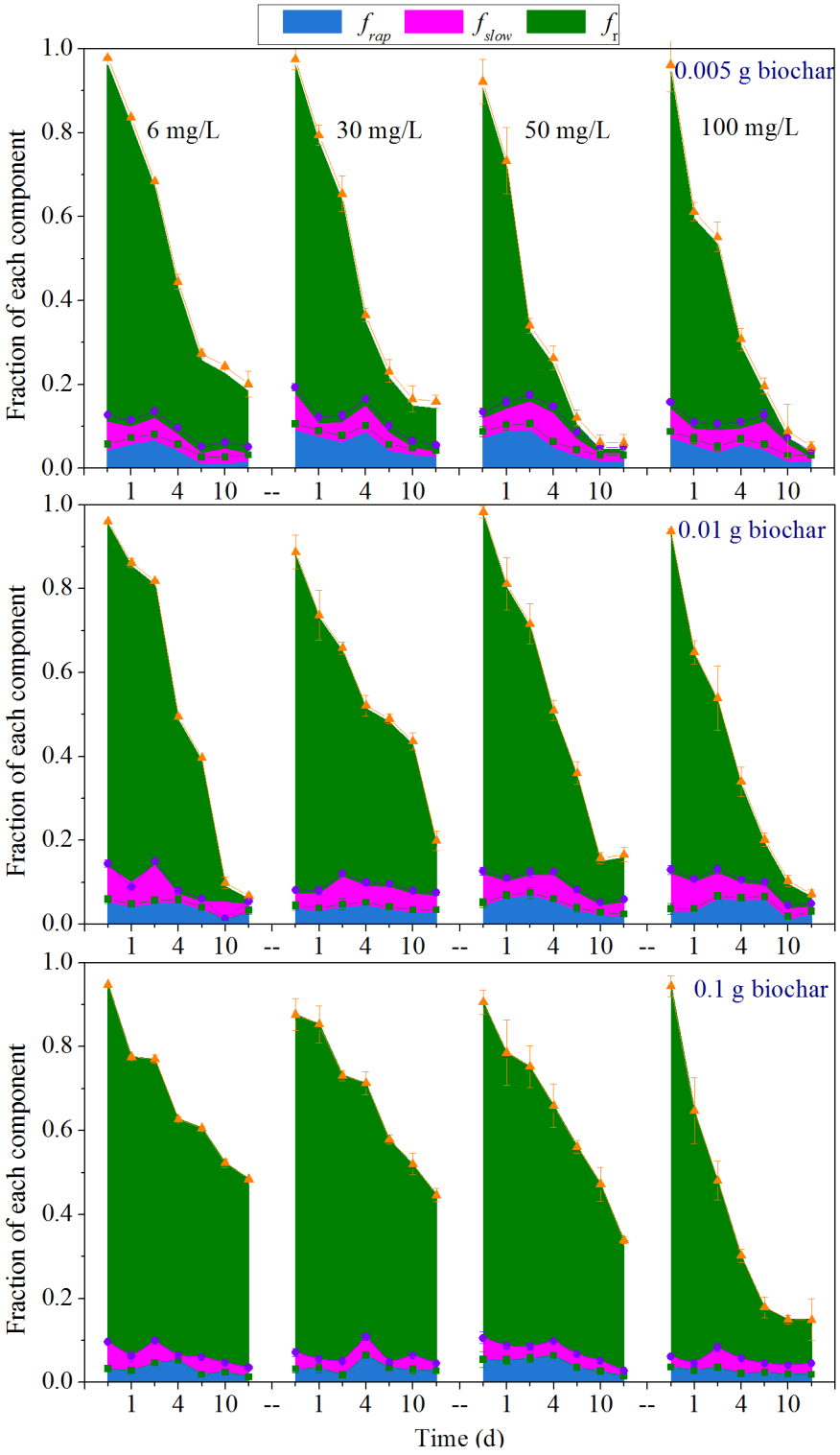

Fig 4. Fraction of each component during nonylphenol desorption kinetics experiments, fitted with transmutative modified two-domain model. Desorption data were fitted with a transmutative modified two-domain model, and each component gradually decreased over 16 d incubation time.

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Table 1. Parameters obtained from fitting the measured biodegradation factor (*BD*) values to the multivariate model (Eq (8)).

Nonylphenol concentration (mg/L)	Biochar (g)	Fitted parameters			
		BD_{rap}	BD_{slow}	BD_r	R^2
6	0.005	1	0.619±1.154	0.122±0.0993	0.862
	0.01	1	0.772±0.396	0.0513±0.0395	0.943
	0.1	1	0.635±0.0941	0.0106±0.00583	0.992
30	0.005	1	0.920±1.284	0.0669±0.132	0.567
	0.01	1	0.975±0.0900	0.0312±0.00845	0.996
	0.1	1	0.972±1.015	0.0179±0.0493	0.670
50	0.005	1	0.980±0.420	0.130±0.00522	0.999
	0.01	1	0.913±0.0569	0.0527±0.00539	0.998
	0.1	1	1.001±0.352	0.0213±0.0201	0.994
100	0.005	1	0.980±0.0420	0.130±0.00522	0.998
	0.01	1	1.037±0.114	0.117±0.00937	0.996
	0.1	1	1.155±0.174	0.131±0.0106	0.991

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decrease in BD_r for the highest nonylphenol concentration (C_0 = 100 mg/L), with all values exceeding 0.1, indicating the favorable biodegradability of the resistant fraction. Thus, given that higher nonylphenol concentrations are more easily degradable and that nonylphenol generates carbon and energy sources, the conditions were optimized for microbial growth, with the results demonstrating strong biological processes [29,30]. Based on the estimated BD_{rap}, BD_{slow} and BD_r, which were influenced by both nonylphenol concentration and biochar dosage, the rapid and slow fractions had favorable degradability, while the resistant fraction was only partly degraded. Nevertheless, the contributions of f_r could not be neglected due to the dominance of this parameter.

From the joint assessment of biodegradation and desorption, the following two conjectures were proposed: First, free aqueous phase nonylphenol was preferentially degraded [15], breaking the equilibrium between adsorption and desorption, promoting desorption of nonylphenol from biochar and converting the desorbed fractions into bioavailable form. Second, the microorganisms and nonylphenol were both adsorbed to biochar, allowing processes such as biofilm formation and the release of extracellular enzymes to contribute directly to the biodegradation of adsorbed nonylphenol [25,26]. As shown in Fig 4, f_r was still the major component in the system after 16 days of desorption, suggesting a limited extent of desorption for the resistant fraction [14]; thus, it was reasonable to conclude that the reduction of f_r was predominantly due to direct degradation by microorganisms.

Coupled effects of biochar and microorganisms on the fate of HOCs

Typically, the application of biochar is coupled with microorganisms during HOCs remediation, resulting in a complex system in which adsorption-desorption of HOCs and microorganisms to biochar, abundant microbes and biofilms, and biodegradation of HOCs exist simultaneously. According to the results above, adsorbed HOCs were consumed gradually until becoming completely mineralized [21]. Most importantly, the effects of various biochar dosages vary, and the appropriate biochar dosage was capable of accelerating HOCs transformation. Contradictory results following biochar application in previous reports were attributed to the complex impact of HOCs concentration, biochar dosage and experimental time scale. At sites polluted with low concentrations of HOCs, biochar addition is inadvisable due to its inhibition of bioavailability; nonetheless, long-term biofilm formation may allow efficient in-situ

treatment of HOCs contamination. Biochar plays a beneficial role in the transformation of high HOCs concentrations in the short term, but can inhibit transformation when HOCs are degraded gradually, similar to the effects at low HOC concentrations.

Conclusion

In this study, nonylphenol was selected as a target contaminant and rice-straw biochar as an adsorbent to investigate the effects and mechanism of biochar dosage on nonylphenol biodegradation. The results showed that the optimal biochar dosage varied depending on the concentration of nonylphenol. Furthermore, three form of sorbed nonylphenol (f_r, f_{rap} and f_{slow}) decreased gradually, and the results of model quantification suggested that the slow and resistant fractions were subject to biodegradation dependent on the nonylphenol concentration and biochar dosage. Above all, biochar dosage is a very important factor that should be taken into account when biochar is applied to environmental remediation.

Supporting Information

S1 Fig. Changes in aqueous concentration of nonylphenol at 4 biochar dosages. The aqueous concentration of nonylphenol (6, 30, 50 and 100 mg/L) at each sampling time underwent a gradual reduction for 4 biochar dosages (0, 0.005, 0.01 and 0.1 g).
(TIF)

S2 Fig. Changes in solid concentration of nonylphenol at 4 biochar dosages. The solid concentration of nonylphenol (6, 30, 50 and 100 mg/L) at each sampling time underwent a gradual reduction for 4 biochar dosages (0, 0.005, 0.01 and 0.1 g).
(TIF)

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Author Contributions

Conceived and designed the experiments: LPL BLH. Performed the experiments: LDY LXW. Analyzed the data: LDY LPL. Contributed reagents/materials/analysis tools: GHC YFH. Wrote the paper: LDY LPL GHC.

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