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ANTIBIOTICS IN AGROECOSYSTEMS: STATE OF THE SCIENCE

Sorption of Lincomycin by Manure-Derived Biochars from Water

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Abstract

The presence of antibiotics in agroecosystems raises concerns about the proliferation of antibiotic-resistant bacteria and adverse effects to human health. Soil amendment with biochars pyrolized from manures may be a win-win strategy for novel manure management and antibiotics abatement. In this study, lincomycin sorption by manure-derived biochars was examined using batch sorption experiments. Lincomycin sorption was characterized by two-stage kinetics with fast sorption reaching quasi-equilibrium in the first 2 d, followed by slow sorption over 180 d. The fast sorption was primarily attributed to surface adsorption, whereas the long-term slow sorption was controlled by slow diffusion of lincomycin into biochar pore structures. Two-day sorption experiments were performed to explore effects of biochar particle size, solid/water ratio, solution pH, and ionic strength. Lincomycin sorption to biochars was greater at solution pH 6.0 to 7.5 below the dissociation constant of lincomycin (7.6) than at pH 9.9 to 10.4 above its dissociation constant. The enhanced lincomycin sorption at lower pH likely resulted from electrostatic attraction between the positively charged lincomycin and the negatively charged biochar surfaces. This was corroborated by the observation that lincomycin sorption decreased with increasing ionic strength at lower pH (6.7) but remained constant at higher pH (10). The long-term lincomycin sequestration by biochars was largely due to pore diffusion plausibly independent of solution pH and ionic composition. Therefore, manure-derived biochars had lasting lincomycin sequestration capacity, implying that biochar soil amendment could significantly affect the distribution, transport, and bioavailability of lincomycin in agroecosystems.

Core Ideas

- Manure-derived biochars had lasting sequestration potential to lincomycin.
- Lincomycin sorption on manure-derived biochars followed two-phase kinetics.
- Long-term lincomycin sorption was controlled by slow diffusion into biochar pores.

ANTIBIOTICS are considered contaminants of emerging concerns, and increasing concentrations of antibiotics in agroecosystems could lead to the proliferation of antibiotic-resistant bacteria and potential adverse impacts on human health (Homem and Santos, 2011; Martinez, 2009; Rivera-Utrilla et al., 2013). The ubiquitous existence of antibiotics in agroecosystems has been linked to the widespread and imprudent use of veterinary antibiotics in animal feeding operations as nontherapeutic feed additives (Martinez, 2009). After administration to food animals, a large percentage of veterinary antibiotics are excreted into manure as parent compounds or bioactive metabolites and then released to the environment through manure land application (Kümmerer, 2009; Kuchta and Cessna, 2009; Zhu et al., 2013). Thus, animal manure has been considered a major source of antibiotics in agroecosystems, and manure-borne antibiotics will likely increase selective pressure on bacteria and facilitate the development of antibiotic resistance (Martinez, 2009; Rivera-Utrilla et al., 2013).

Lincomycin and combination antibiotics containing lincomycin are widely used in food animals for the treatment and control of diseases (e.g., dysentery and porcine proliferative enteropathies in pigs, necrotic enteritis in chicken, acute mastitis in dairy cattle, and contagious foot rot in sheep) as well as for growth promotion (Reeves, 2011). Resultant antibiotic-resistant bacteria often possess macrolide–lincosamide–streptogramin B cross-resistance (Reeves, 2011). This type of multidrug resistance poses an enormous threat to human and ecosystem health. Therefore, it is essential to reduce the release of lincomycin and other antibiotics to the environment and to reduce their mobility and bioavailability.

Lincomycin is frequently detected in the environment as a result of veterinary overuse, manure land application, and limited degradation of lincomycin (Andreozzi et al., 2006; Kolpin et al., 2002; Loftin et al., 2008; Watanabe et al., 2010). Lissemore et al. (2006) found lincomycin concentrations of 0.2 to 355 ng L^{-1} in 91% of 125 water samples collected from the Grand River in Canada, and agricultural husbandry was considered the major

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Abbreviations: SSA, specific surface area.

source. Kuchta and Cessna (2009) reported lincomycin concentrations of 0.008 to 0.84 μg L^{-1} in snowmelt runoff samples from agricultural land receiving liquid swine manure containing lincomycin concentrations of 2.5 to 240 μg L^{-1}. Although soil is an important filter media and sink for antibiotics, Watanabe et al. (2010) have indicated the high mobility of manure-borne lincomycin from soil to groundwater. Therefore, novel soil amendment is needed for enhancing sequestration of lincomycin and other antibiotics in agricultural soils and thus reducing their environmental risks.

Biochar as a soil amendment has received increasing attention because of its potential agronomical and environmental benefits, such as improving soil quality, carbon (C) sequestration, contaminant immobilization, and agricultural waste management (Beesley et al., 2011; Lehmann et al., 2011; Lehmann and Joseph, 2009). Biochars are C-rich porous materials typically produced from a variety of feedstock (e.g., manure, woody biomass, crop residues, and sewage sludge) under limited oxygen condition and at temperatures less than 700°C (Lehmann and Joseph, 2009). Depending on feedstock type and pyrolysis temperature, biochars can have vastly different chemical and physical properties (Enders et al., 2012; Lehmann et al., 2011; Morales et al., 2015). Previous studies have shown that biochars typically have strong sorption ability for organic contaminants (Beesley et al., 2011; Chen et al., 2012; Chun et al., 2004; Zhu et al., 2005). Because sorption of contaminants in soil often decreases their mobility and bioavailability (Beesley et al., 2010; Rhodes et al., 2008; Tolls, 2001), the addition of biochars to soils may play an important role in controlling transport and bioavailability of antibiotics in agroecosystems.

In this study, we proposed an innovative win-win strategy for novel manure management and antibiotics abatement by land application of biochars produced from pyrolysis of manures. Using manure as feedstock to produce biochars could have a number of potential benefits, such as inactivation of microbial pathogens and degradation of antibiotics in manure from the thermal treatment (i.e., 300–700°C). Then the manure-derived biochars could be either directly applied to the land or mixed with manures before spreading to reduce the mobility and bioavailability of antibiotics. To develop better strategies of biochar soil amendment for antibiotics sequestration, it is important to understand the behaviors and underlying mechanisms of antibiotics sorption by manure-derived biochars. Therefore, the aim of this study was to assess the potential of biochar soil amendment for in situ sequestration of antibiotics in agroecosystems through sorption studies. Lincomycin was chosen as a model compound to investigate the sorption of antibiotics by manure-derived biochars from water due to its prevalence, high mobility, and limited degradation in the environment. The sorption kinetics and quasi-equilibrium sorption of lincomycin were evaluated to elucidate the underlying lincomycin sorption mechanisms by biochars.

Materials and Methods

Biochar Samples and Characterization

Biochar samples used in this study were produced by slow pyrolysis of oven-dried manure feedstock at 600°C (Daisy Reactor, Best Energies Inc.). A detailed description of the biochar production can be found in Enders et al. (2012) and Rajkovich et al. (2012). The produced biochars were ground and passed through sieves to obtain the fractions of 150 to 850, 75 to 150, and <75 μm and then stored in glass vials before use. These biochars were designated according to feedstock type and pyrolysis temperature as BM600 (bull manure with sawdust pyrolized at 600°C), DM600 (dairy manure with rice hulls pyrolized at 600°C), PM600 (poultry manure with sawdust pyrolized at 600°C), and AM600 (anaerobically digested dairy manure pyrolized at 600°C).

The four manure-derived biochars have previously been well characterized (Enders et al., 2012; Rajkovich et al., 2012). Volatile matter, fixed C, and ash content of biochar samples were determined by the modified ASTM D1762-84 method. Carbon and nitrogen (N) content were determined by a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd.). Hydrogen (H) content was determined by a Hekatech HT Oxygen Analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd.). Oxygen content was calculated by subtracting C, N, H, and ash content from total mass. Cation exchange capacity and exchangeable cation concentrations were determined by the ammonium acetate exchange method. A more detailed biochar characterization can be found in Enders et al. (2012) and Rajkovich et al. (2012).

Specific surface area (SSA) of biochar samples was determined from five-point CO_2 adsorption isotherms using the Brunauer–Emmett–Teller method by a Micromeritics Tristar 3020 analyzer (Micromeritics) at Pacific Surface Science Inc. Zeta potential of the biochars was measured by Malvern Zetasizer Nano-ZS equipped with a MPT-2 autotitrator (Malvern Instruments). The isoelectric point of biochars was determined by measuring the zeta potential of biochars as a function of solution pH as described in the supplemental material. To monitor the change of surface properties and surface morphology of biochar particles, zeta potential measurement and scanning electron microscopy (JEOL JSM-7500F) were performed for biochar samples of 75 to 150 μm prepared from biochar suspensions of 1- and 180-d water exposure.

Sorption Experiments

Chemicals and Experimental Setup

Lincomycin hydrochloride (purity ≥90%) was purchased from Sigma-Aldrich. The chemical structure and properties of lincomycin are summarized in Supplemental Table S1. Deionized water was used for all the solution preparations. Amber borosilicate glass vials covered with aluminum foil were used in the experiments to prevent photodegradation of lincomycin. Batch sorption experiments were conducted in duplicate at room temperature (23 ± 1°C). Before the sorption experiments, lincomycin solution was preadjusted to pH of 3 or 10 by 0.1 mol L^{-1} HCl or NaOH solution. Solution pH was not controlled during the sorption experiments, but the final solution pH was recorded. To prevent a competitive effect of other salts during lincomycin sorption, no electrolyte was added to control ionic strength unless otherwise noted. The biochar fraction of 75 to 150 μm and the solid/water ratio of 1 g L^{-1} were used, unless mentioned otherwise, to achieve the removal efficiency of lincomycin at about 20% after 2-d equilibration to better study sorption kinetics over a longer period. More details of the experimental protocols are given below.

Sorption Kinetics

Sorption kinetics experiments were performed to evaluate kinetic sorption rates and the equilibration time required for the subsequent sorption isotherm experiments. Additionally, sorption kinetics can be used to probe underlying sorption mechanisms (Chen et al., 2012) and are highly complementary to equilibrium sorption isotherm data. Eight milligrams of each biochar with particle size of 75 to 150 μm were added to amber borosilicate glass vials containing 8 mL lincomycin of a concentration of 1000 μg L^{-1} at pH 10. The vials were placed on an end-over-end shaker (Glas-Col) and shaken at 30 rpm for 1 h to 180 d. At predetermined times, a subset of the sample vials was withdrawn, and 2 mL of the suspensions drawn from the vials were filtered through a 0.45-μm syringe filter with mixed cellulose esters membrane (Millipore). During filtration, the first 1 mL of filtrate was discarded, and the remaining 1 mL of filtrate was collected to avoid the loss of lincomycin to the filter. Lincomycin concentrations in the filtrate were determined by a Shimadzu Prominence high-performance liquid chromatograph coupled to an Applied Biosystems Sciex 3200 triple quadrupole mass spectrometer as described in the supplemental material. The data from the biochar-free control experiments indicated a negligible loss of lincomycin via degradation throughout the experiments (Supplemental Fig. S1). The filtrates in the 180-d samples were examined using the precursor ion scan mode by liquid chromatography/tandem mass spectrometry. Based on Calza et al. (2012), no degradation candidates of lincomycin were detected (Supplemental Fig. S2), suggesting that the disappearance of lincomycin from the aqueous phase was caused by sorption onto biochars instead of degradation. Therefore, the sorbed lincomycin concentration on the biochars was determined by the difference between initial and final lincomycin concentrations in the aqueous phase.

Effects of Biochar Particle Size and Solid/Water Ratio

We investigated the effects of biochar particle size and solid/water ratio to determine the desired experimental conditions for the sorption isotherm experiments. To examine the effect of solid/water ratio, 4, 8, 40, and 80 mg of biochar samples were added to 8 mL lincomycin (1000 μg L^{-1}) at pH 10 to achieve solid/water ratios of 0.5, 1, 5, and 10 g L^{-1}. Additionally, to examine the effect of biochar particle size, 8 mg of biochars with particle size of <75, 75 to 150, or 150 to 850 μm were mixed with 8 mL lincomycin of 1000 μg L^{-1} at pH 10. The suspensions were then shaken at 30 rpm for 2 d and filtered, and the lincomycin concentration in the filtrate was determined. The other sorption experimental procedures were identical to the protocols previously described.

Effects of Solution pH and Ionic Strength

We investigated lincomycin sorption mechanisms for the tested biochars through manipulating the interactions between lincomycin and biochar surfaces by changing solution pH and ionic strength. To determine the sorption isotherm, 8 mg of biochars were mixed with 8 mL lincomycin working solution at initial concentration of 100, 250, 500, 750, or 1000 μg L^{-1} in the absence of NaCl addition. To examine ionic strength effects, 8 mg of biochars were mixed with 8 mL of lincomycin working solution (1000 μg L^{-1}) with the addition of 0, 0.01, 0.05, or 0.1 mol L^{-1} NaCl. These experiments were conducted at initial solution pH of 3 and 10. Due to the alkalinity of the biochar samples, the final pH often increased to about 6.7 ± 0.5 for the lower pH tests and remained unchanged for the higher pH tests (about 10.0 ± 0.2). The other procedures were similar to those previously described.

Mathematical Modeling

The linear forms of pseudo–first-order (Eq. [1]), pseudo–second-order (Eq. [2]), and intraparticle diffusion (Eq. [3]) kinetic model given below were used to fit the experimental data (Ho and McKay, 1999; Wu et al., 2009):

$$\ln\left(q_t - q_e\right) = \ln q_e - k_1 t \qquad [1]$$

$$\frac{t}{q_t} = \frac{1}{q_e} t + \frac{1}{k_2 q_e^2} \qquad [2]$$

$$q_t = k_i t^{0.5} + C \qquad [3]$$

where q_e (μg g^{-1}) is the sorbed lincomycin concentration in the solid phase at equilibrium, q_t (μg g^{-1}) is the sorbed lincomycin concentration at time t, k_1 (d^{-1}) is the pseudo–first-order rate constant, k_2 (g μg $^{-1}$ d^{-1}) is the pseudo–second-order rate constant, k_i (μg g^{-1} d$^{-0.5}$) is the intraparticle diffusion rate constant, and C (μg g^{-1}) reflects the boundary layer effect.

The Langmuir (Eq. [4]) and Freundlich (Eq. [5]) isotherm models below were fitted to the experimental data:

$$\frac{C_e}{q_e} = \frac{1}{q_{max}} C_e + \frac{1}{k_L q_{max}} \qquad [4]$$

$$\ln q_e = \frac{1}{n} \ln C_e + \ln K_F \qquad [5]$$

where C_e (μg L^{-1}) is the equilibrium lincomycin concentration in the solution, q_{max} (μg g^{-1}) is the maximum lincomycin sorption capacity, K_L (L μg $^{-1}$) is the Langmuir constant, and K_F (μg$^{(n-1)}$ g^{-1} L^n) and $1/n$ are the Freundlich constants. The goodness of fit to the models was evaluated by RMSE and R^2.

Results and Discussion

Properties of Manure-Derived Biochars

Physicochemical properties of the four manure-derived biochars are shown in Table 1. Biochars BM600, DM600, and AM600 had high C content (62.8–76.0%), whereas PM600 had a relatively lower C content (28.7%). Conversely, BM600, DM600, and AM600 had relatively lower ash content (10.6–18.8%), whereas PM600 had a greater ash content (55.8%). The low atomic ratios of H/C, O/C, and (O + N)/C indicate that the biochars were highly carbonized, less hydrophilic, and low in polar surface functional group content (Chen et al., 2012).

The SSAs of BM600, DM600, and AM600 (183–237 $m^2\ g^{-1}$) were higher than that of PM600 (47 $m^2\ g^{-1}$) and were positively related to the C content ($R^2 = 0.996$), suggesting that the CO_2–SSA of these biochars was mainly a result of the C matrix (Sun et al., 2013).

Zeta potential measurements indicate that the manure-derived biochars carried net negative surface charge within a wide pH range (Supplemental Fig. S3). The negative zeta potential remained nearly constant between pH 6 and 10. Within this pH range, the zeta potential of BM600, DM600, and AM600 was around −56 to −64 mV, which was more negative than that of PM600 (i.e., about −36 mV). Below pH 6, the zeta potential became progressively less negative with decreasing pH, and the isoelectric point was found to be approximately 1.9 to 2.2. Finally, a complex porous structure of the biochars was revealed by the scanning electron microscopy images, and the pore size spanned widely from the nanometer level to the micrometer level (Fig. 1). The macroporous structure was likely preserved from the original feedstock structure, and the nanoporous structure was possibly formed during pyrolysis (Lehmann and Joseph, 2009).

Two-phase Sorption Kinetics

Sorption of lincomycin by the biochars was characterized as a two-phase sorption kinetics (Fig. 2). Although the physicochemical properties varied greatly among the four biochars, the sorption kinetics showed similar patterns. In the initial sorption phase, the sorbed lincomycin concentration on the biochars increased rapidly during the first several hours of the first day and then gradually reached a first-stage sorption plateau after 2 d (Fig. 2a). In the longer-term sorption phase, the sorbed lincomycin concentration continuously increased and did not reach equilibrium by the end of the experimental period (i.e., 180 d) (Fig. 2b). Typically, the initial fast sorption phase primarily results from surface adsorption between sorbate and sorbent surfaces that often occurs almost instantaneously or at a rapid rate, whereas the second slow sorption phase was caused by diffusion of sorbate into sorbent pore structures that occurs at a much slower rate (i.e., pore-diffusion process) (Pignatello and Xing, 1996). Because the biochars have abundant surface sorption sites and pore structures, we believed that lincomycin sorption by the biochars was governed by both processes (fast surface adsorption followed by slow pore diffusion). In addition, only 25 to 34% of the applied lincomycin was removed

Table 1. Selected physical and chemical properties of manure-derived biochars.

Properties†	Biochar‡			
	BM600	DM600	AM600	PM600
Proximate analyses, %§				
Volatile matter	30.0	30.7	39.4	44.2
Fixed carbon	59.4	56.6	41.7	0
Ash	10.6	12.6	18.8	55.8
Elemental analyses, %§				
C	76.0	75.2	62.8	28.7
H	1.8	2.0	nd¶	0.4
O	14.3	11.6	nd	14.3
N	0.80	1.3	2.2	0.9
Atomic ratios				
H/C	0.28	0.32	nd	0.17
O/C	0.14	0.12	nd	0.37
(O + N)/C	0.15	0.13	nd	0.40
CEC, $mmol_c\ kg^{-1}$§	336	97	151	59
Exchangeable cation, $mmol_c\ kg^{-1}$§				
Ca	88.3	90.2	291	1098
Mg	67.7	15.6	164	126
K	464	60.5	413	464
Na	54.4	62.8	257	71.8
SSA, $m^2\ g^{-1}$				
<75 μm	nd	nd	nd	nd
75–150 μm	237	221	187	47
150–850 μm	250	229	187	37
pH_{IEP}	1.9	2.0	2.0	2.2
Zeta potential, mV				
1-d	−60.4 ± 1.5	−64.1 ± 0.4	−55.6 ± 3.5	−36.3 ± 2.5
180-d	−61.5 ± 3.1	−60.8 ± 0.9	−56.4 ± 1.2	−35.0 ± 1.0

† CEC, cation exchange capacity; SSA, specific surface area; pH_{IEP}, isoelectric point. Zeta potential was measured at pH 10.

‡ AM600, anaerobically digested dairy manure pyrolized at 600°C; BM600, bull manure with sawdust pyrolized at 600°C; DM600, dairy manure with rice hulls pyrolized at 600°C; PM600, poultry manure with sawdust pyrolized at 600°C.

§ Data from Enders et al. (2012) and Rajkovich et al. (2012).

¶ Not determined.

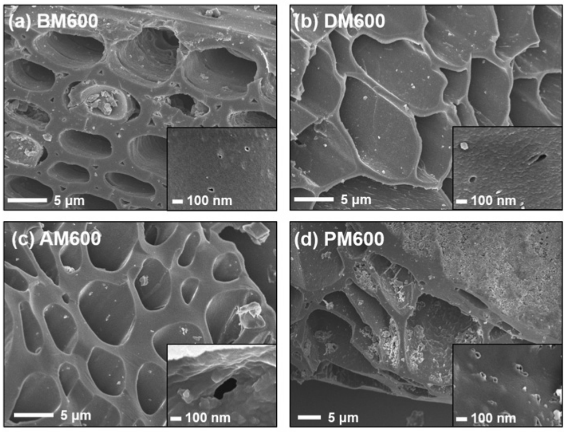

Fig. 1. Scanning electron microscopy images of manure-derived biochars prepared from biochar suspensions of 1-d water exposure. AM600, anaerobically digested dairy manure pyrolized at 600°C; BM600, bull manure with sawdust pyrolized at 600°C; DM600, dairy manure with rice hulls pyrolized at 600°C; PM600, poultry manure with sawdust pyrolized at 600°C.

from solution after 2 d, but 92 to 99% of that was removed after 180 d. This result indicated the large lincomycin sequestration potential of biochars and the predominant role of the pore diffusion process in lincomycin sorption over the long term. Indeed, the intraparticle diffusion model fitted the sorption kinetic data well, whereas the pseudo–first-order and pseudo–second-order kinetic models were less satisfactory in fitting the sorption kinetics, as indicated by greater RMSE values (Supplemental Table S2). This kind of two-phase sorption kinetics is consistent with the sorption of other organic compounds by biochars (Chen et al., 2012; Kasozi et al., 2010). For example, Kasozi et al. (2010) studied the sorption of catechol on biochars and showed a similar sorption kinetics trend, in which around 59% of total sorption occurred within the first few days and then reached sorption equilibrium after 14 d.

Because lincomycin sorption continued to increase over 180 d, it is important to evaluate the potential change of surface properties and surface morphology of biochar particles due to long-term water exposure. There was no significant difference in zeta potentials of biochars on exposure to water for 1 and 180 d (Table 1), implying that surface functional groups of biochar particles on average did not change significantly during the experiment. However, a close examination of biochar scanning electron microscopy images revealed that biochar surfaces became more eroded and cracked over time (Fig. 3). The results implied that fine biochar fragments, minerals such as silica, and soluble elements were potentially detached or dissolved from biochar particle surface after long-term water exposure (Mukherjee and Zimmerman, 2013; Wu et al., 2015; Xiao et al., 2014). In addition, biochar surface roughness might have increased with subsequent changes to its pore structure.

Based on the kinetic sorption results, the short-term (i.e., 2-d) sorption experiments could be used to characterize lincomycin adsorption to the external surfaces of biochars, whereas the long-term (i.e., 180-d) sorption experiments could be used to characterize lincomycin sorption to biochar interior spaces only accessible via pore diffusion. Because the underlying mechanisms during short-term surface adsorption have not been previously elucidated, 2-d sorption experiments were performed to better understand interactions between lincomycin and biochar external surfaces.

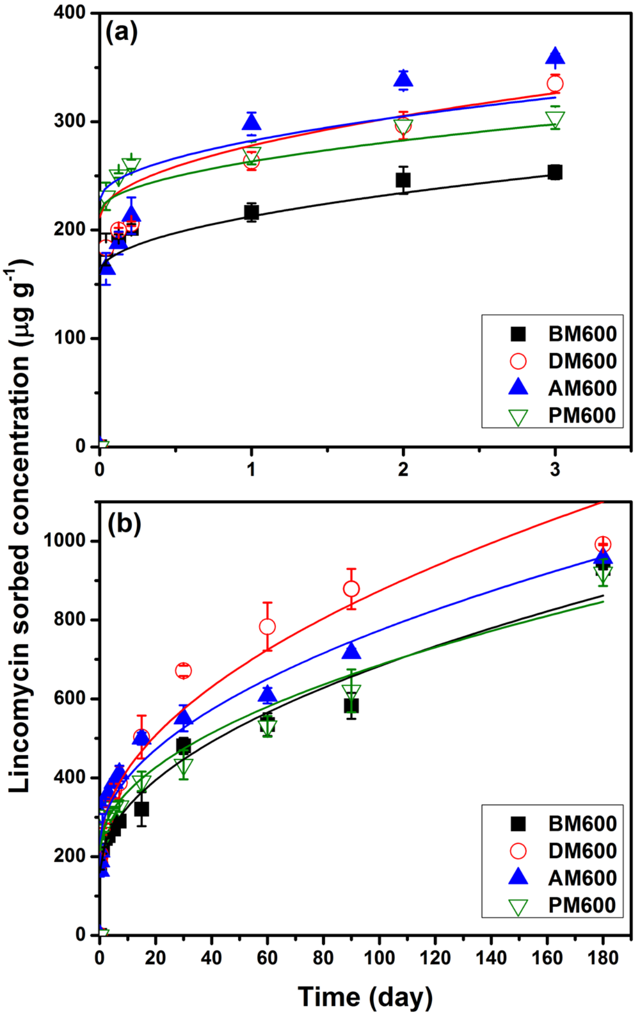

Fig. 2. (a) Short-term and (b) long-term lincomycin sorption kinetics on manure-derived biochars. The solid lines were fitted with the intraparticle diffusion model. AM600, anaerobically digested dairy manure pyrolized at 600°C; BM600, bull manure with sawdust pyrolized at 600°C; DM600, dairy manure with rice hulls pyrolized at 600°C; PM600, poultry manure with sawdust pyrolized at 600°C.

Effects of Biochar Particle Size and Solid/Water Ratio

Biochar particle size and solid/water ratio significantly affected lincomycin sorption processes. When the biochar particle size decreased from 150 to 850 µm to <75 µm, the sorbed lincomycin concentration increased from 182 to 291 $\mu g\ g^{-1}$ to 403 to 463 $\mu g\ g^{-1}$ (Fig. 4a). The increased 2-d sorption with smaller biochar particle sizes was likely due to increased external biochar surfaces easily accessible by lincomycin at smaller particle sizes. Because the SSA values for biochars of larger and smaller particle size were similar (Table 1), the Brunauer–Emmett–Teller SSA might not be an accurate index for biochar external surface areas. As expected, with increasing solid/water ratio, the lincomycin removal efficiencies increased from 16.8 to 24.0% to 89.7 to 92.8%, but the sorbed lincomycin concentration decreased from 348 to 497 $\mu g\ g^{-1}$ to 92.8 to 95.8 $\mu g\ g^{-1}$ (Fig. 4b). These results are in accordance with the sorption of other organic contaminants by carbonaceous materials, including biochars (Chai et al., 2012; Raposo et al., 2009; Zheng et al., 2010). For example,

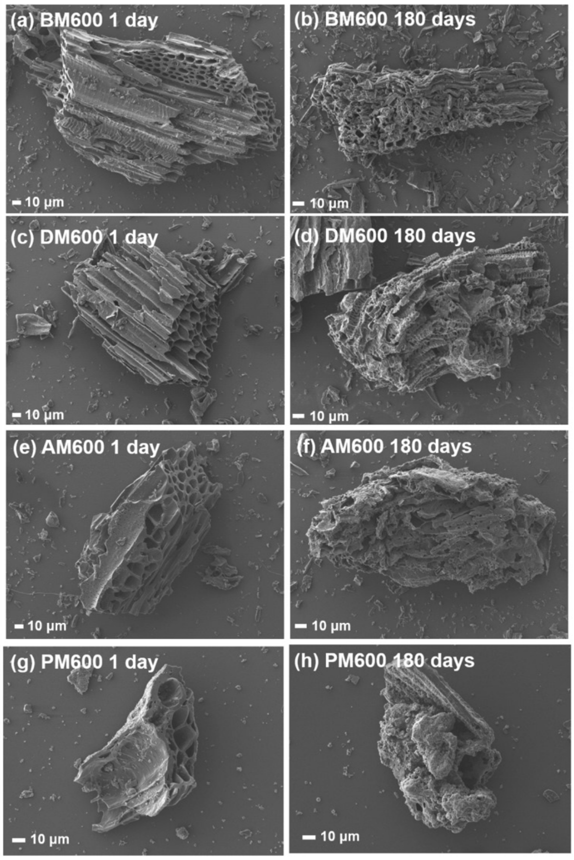

Fig. 3. Scanning electron microscopy images of manure-derived biochars after 1-d and 180-d water exposure. AM600, anaerobically digested dairy manure pyrolized at 600°C; BM600, bull manure with sawdust pyrolized at 600°C; DM600, dairy manure with rice hulls pyrolized at 600°C; PM600, poultry manure with sawdust pyrolized at 600°C.

Zheng et al. (2010) reported that the sorption of atrazine and simazine on biochars were greater and faster at smaller particle size. In addition, they observed increased removal efficiency and decreased sorption capacity of biochars for both pesticides with increasing solid/water ratio (Zheng et al., 2010).

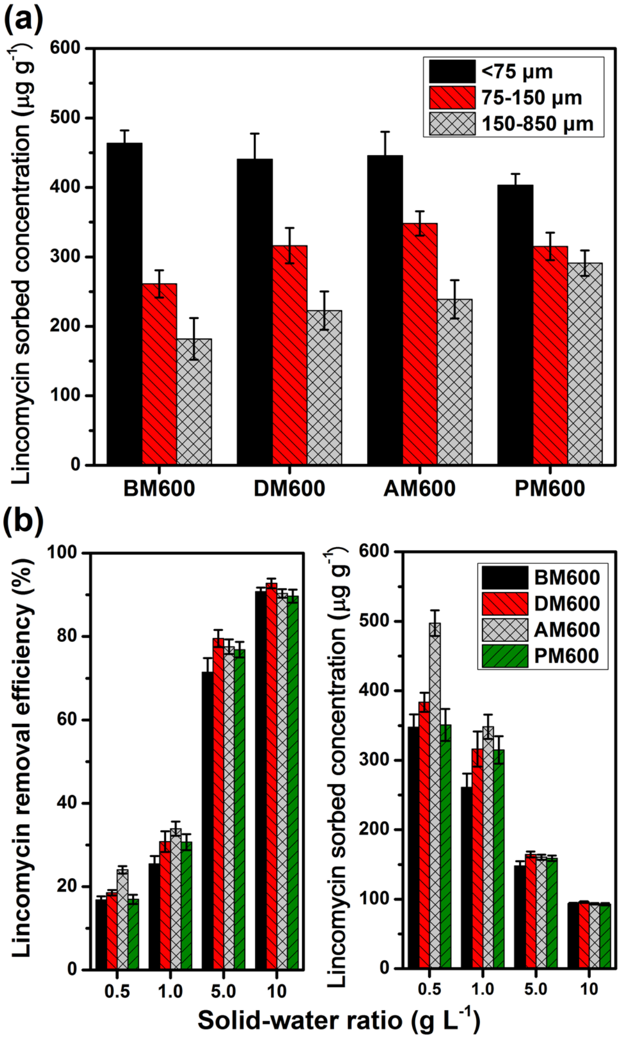

Fig. 4. Sorption of lincomycin on manure-derived biochars with varying particle sizes (a) and solid/water ratios (b). AM600, anaerobically digested dairy manure pyrolized at 600°C; BM600, bull manure with sawdust pyrolized at 600°C; DM600, dairy manure with rice hulls pyrolized at 600°C; PM600, poultry manure with sawdust pyrolized at 600°C.

Effect of Solution pH and Ionic Strength

Two-day sorption isotherms of lincomycin on the biochars at two different pH values are shown in Fig. 5. A nonlinear, concave-downward (L-type) shape was observed for all sorption isotherms. Because surface adsorption is considered the dominant process within 2 d, a nonlinear sorption isotherm was expected because the availability of active surface sorption sites was limited, and thus the sorption became progressively suppressed with increasing lincomycin loading. The fitted isotherm parameters for the Langmuir and Freundlich models are shown in Supplemental Table S3. The isotherm data were better fitted to the Langmuir model, supported by the lower RMSE values, than to the Freundlich model. However, given the heterogeneous nature of biochar surfaces, the Langmuir model can only be considered as an empirical fitting equation carrying no mechanistic meaning. In fact, the Langmuir model has been frequently used in studying sorption of environmental contaminants by natural geosorbents because it provides the empirical maximum sorption capacities that allow for evaluating the contaminant sequestration potential of natural geosorbents such as soils (Zhang et al., 2009).

The dissociation constant (p*K*a) of lincomycin is 7.6 (Supplemental Table S1). Hence, the lincomycin in aqueous solution would exist predominantly as cationic species at pH values much lower than 7.6 and as neutral species at pH values much greater than 7.6. The lincomycin sorption of all four biochars was greater at pH 6.0 to 7.3 than at pH 9.9 to 10.4 (Fig. 5). Because biochar particles were negatively charged at these two pH levels (Supplemental Fig. S3), the enhanced lincomycin sorption at lower pH likely resulted from electrostatic attraction between positively charged lincomycin and negatively charged biochar surfaces (Tan et al., 2015), similar to the observations for the sorption of tetracycline (Liao et al., 2013). To further investigate the possibility of electrostatic interactions (i.e., cation exchange and cation–π bonding) as lincomycin sorption mechanisms, the sorption experiments were conducted under different ionic strength at two pH levels (i.e., below and above 7.6).

The effects of solution pH and ionic strength were interactive (Fig. 6). With increasing ionic strength, lincomycin

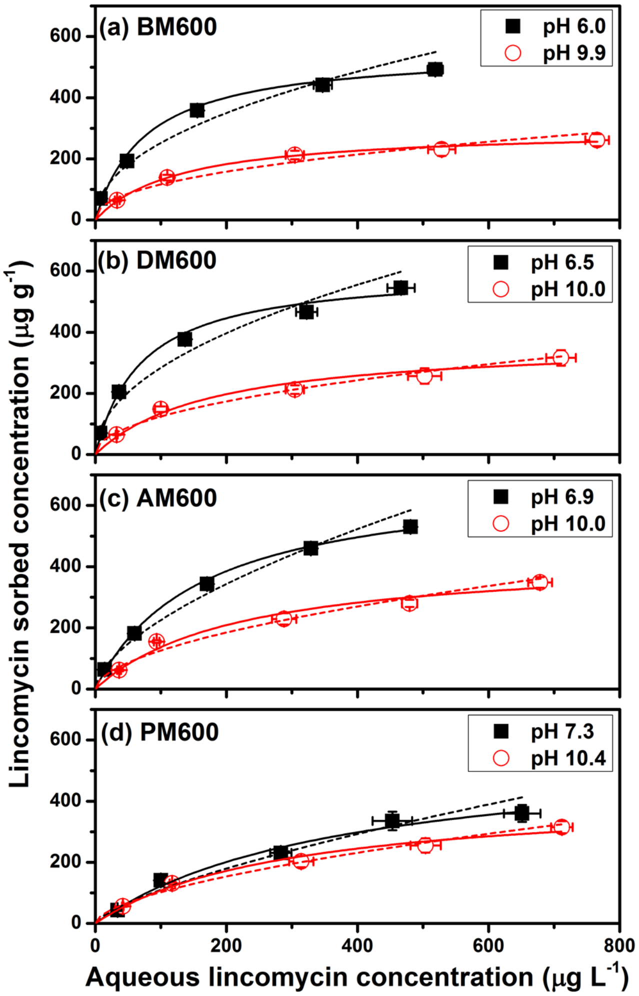

Fig. 5. Observed and fitted sorption isotherms of lincomycin on biochars at solution pH 6.0 to 7.3 and pH 9.9 to 10.4. The solid lines were fitted with the Langmuir model, and the dashed lines were fitted with the Freundlich model. AM600, anaerobically digested dairy manure pyrolized at 600°C; BM600, bull manure with sawdust pyrolized at 600°C; DM600, dairy manure with rice hulls pyrolized at 600°C; PM600, poultry manure with sawdust pyrolized at 600°C.

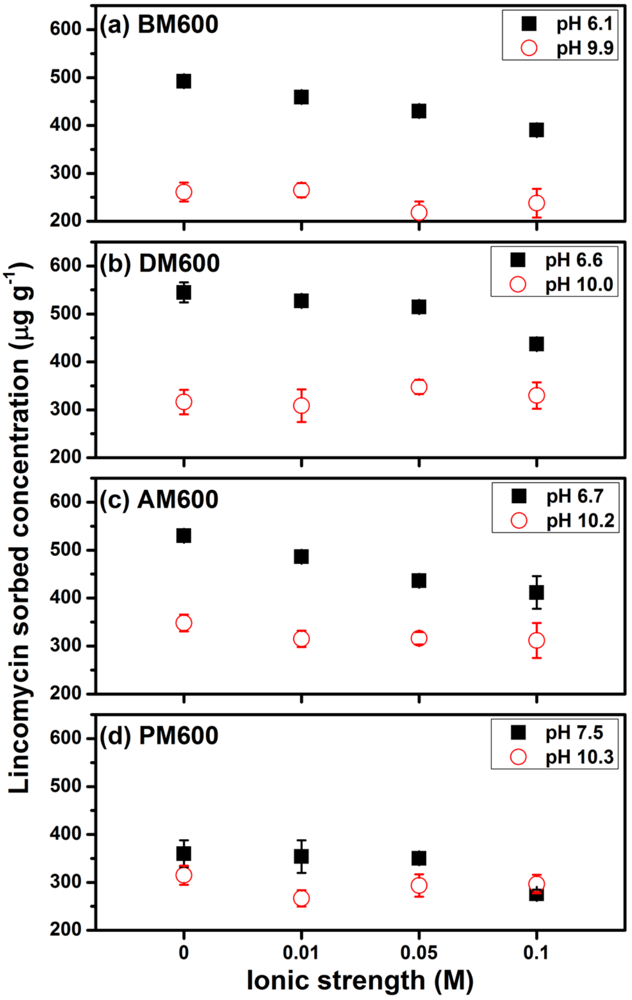

Fig. 6. Sorption of lincomycin on manure-derived biochars at solution pH of 6.1 to 7.5 and 9.9 to 10.3 under varying ionic strength. AM600, anaerobically digested dairy manure pyrolized at 600°C; BM600, bull manure with sawdust pyrolized at 600°C; DM600, dairy manure with rice hulls pyrolized at 600°C; PM600, poultry manure with sawdust pyrolized at 600°C.

sorption decreased by 10.5 to 23.3% at lower solution pH (6.1–7.5) but remained essentially unchanged at higher solution pH (9.9–10.3). Likely, sorption competition occurred between the background electrolytes of Na^+ and positively charged lincomycin at lower solution pH (pH < pKa); conversely, this competition effect would not occur between Na^+ and neutral species of lincomycin at higher solution pH (pH > > pKa). Although the four biochars had the same trend for pH and ionic strength effects, the lincomycin sorption capacity of PM600 was lower. This may due to the less negative surface charge of PM600 (Supplemental Fig. S3) and the smaller cationic fraction of lincomycin at the final solution pH (7.3 or 7.5) of PM600 close to the p*K*a (7.6).

The above-mentioned observations suggest that electrostatic interaction was involved in lincomycin sorption on biochars when solution pH was below the p*K*a of lincomycin. Nonetheless, an appreciable amount of lincomycin could still be adsorbed on the biochars at high solution pH. The Langmuir maximum sorption capacity (q_{max}) at pH 9.9 to 10.4 was 54 to 74% of that at pH 6.5 to 7.3 for the four biochars (Supplemental Table S3). For the higher pH at which lincomycin exists as neutral species, electrostatic interaction was unlikely to play a role. Therefore, nonelectrostatic interactions were also involved in lincomycin sorption on biochars. Some of the nonelectrostatic interactions proposed in the past include hydrophobic partition, π–π electron donor–acceptor interaction, hydrogen bonding, and van der Waals forces (Ji et al., 2009; Ji et al., 2011; Liao et al., 2013). Due to the high water solubility and low log K_{ow} value of lincomycin (Supplemental Table S1), the hydrophobic partition should not be significant in this study. Moreover, due to the lack of aromatic ring structures and π-electron–acceptor functional groups of lincomycin, the π–π electron donor–acceptor interaction between the lincomycin and the graphite-like biochar surface should not exist. Hence, considering the functional groups, molecular size, and molecular structure of lincomycin, it seems reasonable to infer that the nonelectrostatic interactions may include hydrogen bonding and van der Waals forces. Nonetheless, the proposed mechanisms need to be validated by direct evidence in future studies.

Implications

These findings have interesting implications in the application of biochars for sequestration of antibiotics in soils. Although natural soils rarely have pH values of 10, the results of lincomycin sorption at higher pH were useful for estimating the contribution of nonelectrostatic sorption expected to be operative at all pH ranges. Because the electrostatic interaction at lower pH is prone to competition and inhibition from other cations present in soil water (Wang et al., 2009; Wang et al., 2012), nonelectrostatic sorption appears to be a more useful index of antibiotics' sequestration capacity of biochars, independent of solution pH and ionic strength effect. Moreover, given that lincomycin sorption only decreased by 10.5 to 23.3% at the maximum when ionic strength increased to 0.1 mol L^{-1} NaCl (Fig. 6), representing the higher end of typical ionic strength in soil water (2×10^{-4} to 0.18 mol L^{-1}) (Harter and Naidu, 2001), the competition from monovalent electrolytes in soil water would be limited. The effect of divalent cations such as Ca^{2+} and Mg^{2+} should be further studied. Nonetheless, the presence of Ca^{2+} and Mg^{2+} in the background solution is expected due to dissolution from the unwashed biochars, as suggested by the exchangeable cation concentrations of the biochars (Table 1). Competition with these divalent cations should be manifested to a certain extent by the sorption results at lower pH. In any case, the decrease in lincomycin sorption due to potential competition with other cations should be less than 26 to 46% for the four biochars, assuming an unlikely case of complete suppression of electrostatic interaction. The remaining lincomycin sorption would be due to the nonelectrostatic interactions, such as hydrogen bonding and van der Waals force.

Finally, the above discussion was based on the short-term sorption results, excluding the long-term pore diffusion. Over the long term, the pore diffusion could result in 2.8 to 3.8 times greater lincomycin sorption than that over the short term (Fig. 2). We hypothesize that the pore diffusion would be minimally influenced by solution pH and by the ionic composition of soil water. Future studies should examine the potential effects of organic molecules competing for the filling of biochar pores.

Biochars tested in this study had lower lincomycin sorption than humic acids and smectite clays (Ding et al., 2013; Wang et al., 2009). However, lincomycin sorption to humic acids and smectite clays could be severely affected by solution pH and ionic composition, and pore diffusion might not be important (Ding et al., 2013; Wang et al., 2009). Moreover, biochars had much greater lincomycin sorption than whole soils (Wang et al., 2012). Therefore, manure-derived biochars may be attractive geosorbents used for in situ long-term sequestration of antibiotics via soil amendment. After being applied to agricultural soils, biochars may provide not only quick immobilization of antibiotics in the short term by surface adsorption but also a lasting antibiotics sequestration over the long term through pore diffusion, thus decreasing the bioavailability and mobility of antibiotics in agroecosystems.

Supplemental Material

Supplemental material is available online for this article, including supplemental methods; physicochemical properties of lincomycin (Supplemental Table S1); fitted parameters of kinetic, Langmuir, and Freundlich models (Supplemental Tables S2 and S3); aqueous-phase lincomycin concentration kinetics (Supplemental Fig. S1); liquid chromatography/tandem mass spectrometry precursor ion scan spectra of sorption solution (Supplemental Fig. S2); and zeta potential versus solution pH for biochars (Supplemental Fig. S3).

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