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Evaluation of 27 different biochars for potential sequestration of antibiotic residues in food animal production environments

Shannon M. Mitchell [a,b], Murugan Subbiah [b], Jeffrey L. Ullman [c], Craig Frear [a], Douglas R. Call [b,*]

[a] Department of Biological Systems Engineering, Washington State University, PO Box 646120, Pullman, WA 99164-6120, USA
[b] Paul G. Allen School for Global Animal Health, Washington State University, PO Box 647090, Pullman, WA 99164-7090, USA
[c] Department of Agricultural and Biological Engineering, University of Florida, PO Box 110570, Gainesville, FL 32611-0570, USA

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ABSTRACT

The goal of this project was to determine if biochar can be used to sequester antibiotic residues in the environment. Slurries of different biochars ($n = 27$) and water were evaluated for their capacity to adsorb two relatively hydrophilic veterinary antibiotics, florfenicol and ceftiofur. Freely available antibiotic was quantified using HPLC–UV and a bioassay. Biochars prepared at higher pyrolysis temperatures (>500 °C) adsorbed the antibiotics with greater efficiency compared with lower preparation temperatures ($P < 0.005$). Florfenicol was adsorbed (< 99.9%) by six different biochars while ceftiofur was adsorbed by these and nine additional biochars (> 99.98% and > 99.9%, respectively). Florfenicol was sorbed by four biochars (>99.94%) in the presence of soil; however, the sorption performance decreased for two biochars when calf urine and feces were added with the soil. The effect of the biochar proportions on florfenicol sorption in soil–urine–feces slurries were tested with two distinct pinewood biochars, yielding Freundlich sorption coefficients of 2160 and 312 L kg^{-1}. Pinewood biochar and potentially other types of biochar are excellent candidates for sequestering antibiotic residues in soil–urine–feces environments.

Introduction

Food-animal producers are increasingly under pressure to limit antibiotic use and limit environmental contamination from antibiotic residues. Evidence exists that antibiotic residues in calf urine can amplify populations of antibiotic resistant bacteria in animal pen soil, and these amplified populations can be subsequently transferred to calves [1,2]. Presumably, this increases the probability of transmission to the food chain. If excreted antibiotics can be sequestered by the addition of a low-cost soil or bedding amendment, this might make a significant impact on the population dynamics of antibiotic resistant microbes on farms.

Biochar is a potential amendment that could be used for this purpose. Biochar is a charcoal-like substance that is produced by pyrolysis. This involves treating a biomass such as wood with moderate to high temperatures (350–1000 °C) in the presence of minimal oxygen (<2%) [3]. This treatment converts the biomass ("feedstock") into a material that has a high surface area and that has both positively and negatively charged surfaces that promote adsorption [4–7]. Biochar produced at higher temperatures generally has greater surface area and has fewer negatively charged binding sites making it more hydrophobic [8]. Conversely, biochar produced at moderate temperatures (<500 °C) is composed of more negatively charged binding sites and tends to be more hydrophilic. In addition to their adsorptive characteristics, biochars are being studied for remediation applications [9] and as a soil amendment that can contribute to retention of moisture and nutrients thereby improving plant growth through enhanced soil microbial ecology [10].

Antibiotic sorption to biochar may vary greatly based on the antibiotic properties such as molecular mass, water solubility, hydrophobicity, and acid dissociation constant [11]. Sorption can also vary based on the biochar properties including surface area,

Abbreviations: ASTM, American Society for Testing and Materials; BET, Brunauer–Emmett–Teller; HPLC–UV, high performance liquid chromatography with ultraviolet detection; IBI, International Biochar Initiative; LB, Luria-Bertani Lennox broth; MIC, minimum inhibitory concentration; UF, University of Florida; WSU, Washington State University.

* Corresponding author at: Paul G. Allen School for Global Animal Health, 240 SE Ott Road, Room 331, Washington State University, Pullman, WA 99164-7090, USA. Tel.: +1 509 335 6313; fax: +1 509 335 6328.
E-mail addresses: shannon.mitchell@email.wsu.edu (S.M. Mitchell), msubbiah@vetmed.wsu.edu (M. Subbiah), jullman@ufl.edu (J.L. Ullman), cfrear@wsu.edu (C. Frear), drcall@wsu.edu (D.R. Call).

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surface charge, and porosity [7,12]. Environmental conditions such as pH and solution ionic strength can also affect antibiotic sorption to biochar [13]. Biochar sorption data are available for antibiotics that have a high affinity for adsorption to soils including tetracyclines, macrolides, and fluoroquinolones. Yao et al. [14] demonstrated that fluoroquinolones can be removed using wastewater sludge biochars, and Jeong et al. [15] found that a macrolide antibiotic was rapidly sorbed by biochar; sorption in this case being greatest with biochar that was produced using a hardwood compared to a softwood. Another study found effects from pH and ionic strength on oxytetracycline sorption to biochar [13]. Importantly, antibiotics with high soil distribution coefficients (K_d) such as tetracyclines, fluoroquinolones, and macrolides sorb to soil particles and remain bound to top soils [16]. Consequently, additional effort to sequester these may not be necessary [17]. Relatively hydrophilic sulfonamide antibiotics (e.g., sulfamethazine and sulfamethoxazole) have been studied in regard to sorption to biochar [18,19]. A study showed that biochar made at 600 °C was more efficient at sorbing sulfamethoxazole compared to biochar made at 300 °C [19]. To date there is no literature on amphenicol or beta-lactam antibiotic sorption to biochar.

The current study investigated the degree of antibiotic sorption to biochar for two hydrophilic antibiotics, ceftiofur and florfenicol. Ceftiofur (beta-lactam class) and florfenicol (amphenicol class) are used to treat bacterial infections in food-producing animals [20]. They are administrated by injection and the bulk of the parent and metabolized compounds are excreted via urine. Ceftiofur is a third-generation cephalosporin that is used to treat infections in cattle. It is excreted mostly as a bioactive metabolite (desfuroylceftiofur) in urine (~70%) and feces (~30%) [21]. Florfenicol is an important and potent antibiotic used to treat infections in cattle; approximately 52% is excreted in cattle urine and feces [22]. In soils, these compounds remain mostly bioavailable because they are not completely sorbed to soil particles and are not otherwise transformed completely within 24 h [17]. Ceftiofur dissipation in soil is dependent on the soil physicochemical and biological properties. Approximately 35–60% of ceftiofur dissipated in soil-water slurry after 24 h (unpublished data). In the same soils, less than 15% of florfenicol sorbed to soils after 24 h (unpublished data). The objectives of this study were to (1) test ceftiofur and florfenicol sorption to a diverse panel of biochars, (2) test the effect of soil and soil with calf urine and feces on florfenicol adsorption to biochar, and (3) estimate the amount of biochar needed to effectively remove florfenicol in soil–urine–feces slurry.

Materials and methods

Materials

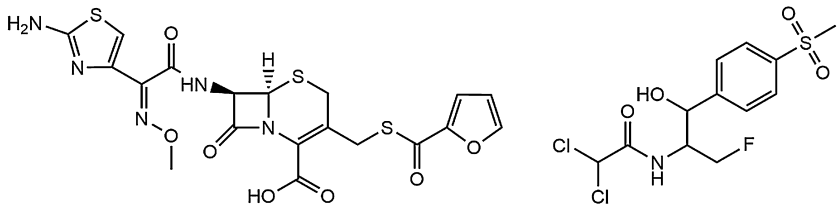
Materials used for this study included the following: purified calcium chloride pellets (J.T. Baker, Phillipsburg, NJ, USA), A.C.S reagent potassium phosphate monobasic (J.T. Baker, Phillipsburg, NJ, USA), HPLC-grade methanol (J.T. Baker, Center Valley, PA, USA), and Difco™ Luria-Bertani (LB) Lennox broth (Becton, Dickinson and Co., Sparks, MD). 1× and 2× concentrations of LB were made by adding 20 and 40 g, respectively, of LB broth powder to sterile nanopure water in a glass bottle which was autoclaved for 15 min. Nanopure water (18.0 MΩ cm) was obtained from a Barnstead E-pure system (Dubuque, IA, USA). Two bacterial strains were used in this study, *Escherichia coli* K-12 (MIC = 0.5 mg L^{-1} for ceftiofur, MIC = 8 mg L^{-1} for florfenicol) and nalidixic acid resistant (nal^R) bla_{CMY-2} positive *E. coli* H4H, which is multidrug resistant including to ceftiofur and florfenicol (MIC = 8 mg L^{-1} for ceftiofur, MIC = 16 mg L^{-1} for florfenicol) [23]. The antibiotics ceftiofur hydrochloride (32422) and florfenicol (F1427) were analytical grade from Sigma–Aldrich (St. Louis, MO, USA). They are non-volatile and stable in water at ambient conditions. The antibiotic octanol–water partition coefficient (Log K_{ow}), water solubility, and acid dissociation constant (pK_a) are shown in Fig. 1 along with the antibiotic chemical structures.

Three sets of biochars, comprising a total of 27 samples, were made available by large/small commercial collaborators and two universities, Washington State University (WSU) and University of Florida (UF). Pyrolysis at WSU used feedstocks readily available in the Pacific, Northwest, U.S. – pinewood, pine bark, hybrid poplar wood, and anaerobically digested dairy fiber. Pyrolysis at UF used feedstocks available in the southeast (U.S.) – hickory wood, bamboo, peanut hull, and Brazilian pepper. Commercial collaborators provided biochars from pinewood, mixed wood and cherry pit feedstock, with each pyrolized at various temperature and moisture operating conditions.

WSU and UF pyrolysis conditions

WSU samples were treated using a bench-scale pyrolysis reactor, with all samples dried (60–80 °C), ground using a pioneer mill (Model 400 HD, Bliss Industries, Inc.) to 2 mm or less, and dried again (105 °C) prior to entry into the reactor. A standard tube furnace (Lindberg Blue M TF55030A-1, Thermo Scientific) was used as the heating unit. The other parts of the reactor were made from stainless steel and built and assembled at WSU (Bioenergy and Bioproducts Engineering Laboratory, Pullman, WA, USA). Pyrolysis conditions were: 1 atm of pressure, gas flow rate 275–300 mL min^{-1} for cold N_2 gas and 550–600 mL min^{-1} for hot gas, heating rate approx. 100 °C min^{-1}, varying temperatures 350, 450, and 600 °C, and gas/vapor residence time of 30 min. After pyrolysis, chars were cooled under N_2 gas until the solids reached room temperature. These chars were not rinsed with water because there was no evidence of oil or other undesirable characteristics.

UF samples were also prepared using a bench-scale reactor, and feedstocks were dried, ground using a hammermill, and dried



Ceftiofur
Log K_{ow} = 1.6
Water sol. = 23 mg/L
pK_a = 3.7

Florfenicol
Log K_{ow} = -0.12
Water sol. = 1,320 mg/L
pK_a = 9.0

Fig. 1. Ceftiofur and florfenicol chemical structures and physical properties.