Facile and Economical Functionalized Hay Biochar with Dairy Effluent for Adsorption of Tetracycline

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ABSTRACT: The present study reports a novel hay biochar functionalized with dairy effluent for enhanced tetracycline (TC) adsorption in the aqueous phase for the first time. The enrichment of hay (i.e., alfalfa) with dairy effluent led to significant accumulation of cationic metals during biochar production. The dairy effluent-functionalized alfalfa biochar (DEAF-BC) possessed strong crystallization (i.e., CaCO₃), functional groups (i.e., CO₃²⁻, C–O stretching), and high surface area (334 m²/g) related to TC adsorption. Therefore, DEAF-BC showed higher TC adsorption capacity (835.7 mg/g) than that of the alfalfa biochar (94.5 mg/g). The adsorption isotherm and kinetic results for the DEAF-BC were correlated with the Freundlich, pseudo-second-order, and intraparticle diffusion models for TC. For the TC adsorption onto DEAF-BC, the thermodynamic analysis implied a spontaneous and endothermic process. Possible mechanisms would include metal complexation, hydrogen bonding, van der Waals forces, and π–π interaction.

1. INTRODUCTION

Antibiotics including macrolides, sulfonamides, quinolones, and tetracyclines are broadly used in hospitals, pharmaceutical industries, livestock, and agriculture.¹–⁴ In particular, tetracycline (i.e., TC, hereafter), one of the most widely used antibiotics, has been increasingly applied for the treatment of infectious diseases in humans and live-stocks because of its effective inhibition of protein synthesis in microorganisms.¹,⁵,⁶ However, TC release to various water bodies causes contamination, toxicity, and antibiotic resistance affecting negatively human health and environments.⁷ Various technologies such as advanced oxidation, separation by a membrane, and biological treatment have been investigated for TC removal in water and wastewater.⁵,⁷ However, these methods showed several limitations including high costs associated with chemical and energy consumption, difficult maintenance, and slow and fluctuating removal of TC.⁹ Adsorption, among various methods, offers simple and effective remediation of TC at very dilute concentration in water with low operating costs.⁴,⁹ Moreover, the adsorption is a more attractive means due to no toxic intermediates and byproducts occurs in the process.¹⁰ Activated carbon (AC), graphene, and carbon nanotube (CNT) have shown high adsorption capacities of TC mainly because of their high surface area.¹,¹¹ Nevertheless, these materials have certain demerits including regeneration, high production, and disposal costs for the removal of TC. On the other hand, biochars (BCs), solid carbonaceous materials (CMs) after pyrolysis from varieties of biomass and solid wastes, showed high potential for TC adsorption in the aqueous phase (e.g., wastewater and water).¹²,¹⁴

Recently, many BCs derived from wood, sawdust, grass, sewage sludge, microalgae, and swine manure have been studied to eliminate TC in the aqueous phase.¹²,¹⁴,¹⁵–¹⁷ The previous studies indicated that the TC adsorption onto raw (un-modified) and activated biochars would be controlled by mainly hydrophobic and π–π interactions.¹,¹²,¹³ Since these interactions rely on the surface area of BC, the raw BCs from various feedstocks with limited surface areas provided low TC adsorption (Table 1).¹,¹²,¹³,¹⁴,¹⁵,¹⁶,¹⁷,¹⁸ Compared to raw BCs, the chemically and thermally activated BCs did show high adsorption of TC owing to their well-developed pore structures and high surface area (Table 1).¹,¹³,¹⁴,¹⁵,¹⁷,¹⁸ However, the use of activated biochar would suffer from high costs associated with high consumption of chemicals and energy for the production of activated biochar.¹⁵,¹⁶
Table 1. Adsorption Capacities of Various Carbonaceous Materials for TC in DI Water

| carbonaceous materials | surface area (m²/g) | Qₘₐₓ (mg/g) | references |
|------------------------|---------------------|--------------|------------|
| rice straw biochar      | 21.7                | 14.2         | Wang et al.²⁶ |
| swine manure biochar    | 10.6                | 8.1          |            |
| holm oak pruning biochar| 76.4                | 11.9         | Antón-Herrero et al.²³ |
| oak, eucalyptus and pine pruning mixture biochar | 30.4 | 15.2 |            |
| sewage sludge biochar   | 2.3                 | 15.2         | Yang et al.¹⁷ |
| Pinus taeda biochar      | 1.4                 | 29.4         | Jang et al.⁴  |
| Spirulina sp. biochar    | 2.6                 | 147.9        | Choi et al.² |
| maple leaves biochar     | 191.1               | 407.3        | Kim et al.²⁷ |
| sawdust biochar (iron and zinc coated) | 102 | 70.9 | Zhou et al.⁹ |
| chicken bone-based biochar (Fe₃O₄ coated) | 328.1 | 98.9 | Oladipo and Ilébajo²⁰ |
| rice straw biochar (g-MoS₂ decorated) | 176.8 | 249.5 | Zeng et al.¹⁴ |
| sewage sludge biochar (ferric activated) | 126.9 | 87.8 | Yang et al.¹⁷ |
| P. taeda biochar (NaOH activated) | 959.9 | 274.8 | Jang et al.⁴  |
| rice straw biochar (H₃PO₄ activated) | 372.2 | 552.0 | Chen et al.¹³ |
| swine manure biochar (H₃PO₄ activated) | 319 | 365.4 | Torrejón-Pérez et al.¹⁷² |
| beet pulp biochar (steam activated) | 821 | 288.3 | Herrero et al.¹⁹ |
| commercial activated carbon (steam activated coconut shell) | 1138 | 133.1 |            |
| commercial activated carbon (H₃PO₄ activated wood) | 1515 | 816.7 |            |
| alfalfa biochar (AF-BC) | 87 | 94.5 | this study |
| dairy effluent-functionalized alfalfa biochar (DEAF-BC; CaCO₃ coated) | 334 | 835.7 |            |

On the other hand, some BCs functionalized with metals (i.e., iron, zinc, molybdenum disulfide, and magnetite) revealed a notable increase of TC adsorption capacity compared with that of the raw BC.⁹,¹⁴,²⁰ Because the functionalization with these metals would accompany electrostatic attractions, hydrogen bonding, and metal–TC complexation for effective adsorption of TC. Thus, effective functionalization of BC with metals would provide high adsorption of TC in water. However, the current functionalization of BCs with these metals requires additional chemicals, energy, and facilities for wet impregnation of metals onto BCs followed by pyrolysis or calcination at high temperatures.⁹,¹⁴,²³ Therefore, this study presents the production, physicochemical characteristics, and application of a dairy effluent-functionalized alfalfa biochar (DEAF-BC) for effective TC removal in water.

As previously reported, alfalfa is well known as one of the most plentiful hays for feeding cows in the US.²⁴,²⁵ The dairy effluent containing various metal ions (i.e., calcium, magnesium, and iron) was collected from the 2nd lagoon at Southwestern Dairy Research Center belonging to Tarleton State University (Stephenville, TX). It was used as an economical source for metals used for functionalization of BCs. The metal ions in the dairy effluent were immobilized onto the surface of alfalfa feedstock when 10 g of crushed alfalfa and 100 mL of the dairy effluent were vigorously mixed. Then, the alfalfa feedstock containing various metals was pyrolyzed to make a metal-functionalized alfalfa-derived BC for TC adsorption in water at 750 °C for 1 h. Thus, the dairy effluent-functionalized alfalfa biochar (DEAF-BC) would be an effective and economical adsorption material for TC removal in the aqueous phase. To the best of the author’s knowledge, there were no researches using a hay biochar functionalized with dairy effluent for effective TC removal in water.

The objectives of the study are to (1) assess detailed TC adsorption behavior of the DEAF-BC based on its physicochemical characteristics, (2) evaluate the TC adsorption capacity of DEAF-BC compared to those of other BCs and commercial activated carbons, and (3) demonstrate possible working mechanisms contributed to TC adsorption onto the DEAF-BC.

2. RESULTS AND DISCUSSION

2.1. Physicochemical Characteristics of AF-BC and DEAF-BC. The scanning electron microscope (SEM) images in Figure 1 showed that DEAF-BC possessed the well-developed pore structure and multiple elements heterogeneously deposited on its surface compared to AF-BC. As Table 2 indicated, DEAF-BC had its pore volume (0.15 cm³/g) and surface area (334 m²/g), which were approximately 3–4 times higher than those of AF-BC (87 m²/g, 0.05 cm³/g) probably owing to its well-developed pore structure. The metal compositions in the dairy effluent supported higher content of Ca and Mg in DEAF-BC than those in AF-BC (Table 3). The energy-dispersive X-ray spectroscopy (EDX) results also indicated higher Ca (16.37%) of DEAF-BC than AF-BC (Table S1). The higher metal contents on the DEAF-BC could be attributed to the deposition of metals in the dairy effluent onto the AF feedstock, which was converted to DEAF-BC via the pyrolysis in this study. Please note that the dairy effluent applied for functionalization of BC in this study contained various metal ions (i.e., Mg, Ca, Na, K, and Fe).

Figure 1. SEM images of AF-BC (A) and DEAF-BC (B).

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ZnCl₂, and KOH during pyrolysis. As listed in Table 3, dairy 
peak (2 BC (Figure 2). This result also supported that the DEAF-
BC possessed high contents of Ca and Mg including CaCO₃ 
while only moderate peak for CaCO₃ from the surface of AF-
BC. Furthermore, the C, H, O, and N of AF-BC and DEAF-BC 
were vigorously soaked onto the AF feedstock and 
accumulated on the surface of DEAF-BC during the pyrolysis.

The higher surface area of DEAF-BC than that of AF-BC could 
be explained by a couple of hypotheses. The specific 
surface area and porosity of biochar could be enhanced when 
biochar is activated by activating agents including FeCl₃, 
ZnCl₂, and KOH during pyrolysis resulting in the increased surface 
degradation of lignocellulosic biomass and activation of 
Fe, Zn, and K contents than AF, which could enhance 
adsorption onto DEAF-BC clearly indicated the TC adsorption onto 
the AF feedstock (Tables 2 and 3).

Table 3. Metal Compositions of Dairy Effluent, AF, DEAF, AF-BC, and DEAF-BC

| various sources | N (mol/mol) | P (mol/mol) | K (mol/mol) | Ca (mol/mol) | Mg (mol/mol) | Fe (mol/mol) | Na (mol/mol) | Zn (mol/mol) | Cu (mol/mol) | Mn (mol/mol) |
|----------------|-------------|-------------|-------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| AF             | 2.81        | 0.24        | 2.18        | 1.23         | 0.34         | 0.02         | 0.41         | 0.001        | 0.001        | 0.002        |
| DEAF           | 3.28        | 0.48        | 3.51        | 2.37         | 0.75         | 0.16         | 0.85         | 0.006        | 0.005        | 0.007        |
| AF-BC          | 3.23        | 1.11        | 0.56        | 5.22         | 1.83         | 0.21         | 0.41         | 0.004        | 0.003        | 0.012        |
| DEAF-BC        | 3.01        | 1.54        | 0.52        | 9.26         | 2.91         | 0.29         | 0.49         | 0.005        | 0.005        | 0.018        |
| dairy effluent | 1.48        | 0.21        | 6.76        | 2.26         | 1.57         | 0.04         | 2.76         | 0.006        | 0.011        | 0.008        |

AF: alfalfa feedstock, DEAF: dairy effluent-functionalized alfalfa feedstock, AF-BC: alfalfa-derived biochar, DEAF-BC: dairy effluent-functionalized alfalfa biochar. The dairy effluent was collected from the dairy farm at Tarleton State University (Stephenville, TX).

The higher surface area of DEAF-BC than that of AF-BC could be explained by a couple of hypotheses. The specific surface area and porosity of biochar could be enhanced when biochar is activated by activating agents including FeCl₃, ZnCl₂, and KOH during pyrolysis.

The C, H, O, and N of AF-BC and DEAF-BC were also used for calculating their polarity ((N + O)/C), hydrophilicity (O/ C), and aromaticity (H/C) (Table 2). Since DEAF-BC had higher (N + O)/C, H/C, and O/C than AF-BC, it was suggested that DEAF-BC had less aromaticity and higher hydrophilicity than AF-BC. This would be likely due to the higher metal elements (particularly, Ca and Mg) and lower carbon contents at the surface of DEAF-BC. Furthermore, the X-ray diffraction (XRD) analysis showed the highly intensive peak (θ = 29, 39, 43, 47, and 48) for CaCO₃ and moderate peak (θ = 33) for MgCO₃ from the surface of DEAF-BC, while only moderate peak for CaCO₃ from the surface of AF-BC (Figure 2). This result also supported that the DEAF-BC possessed high contents of Ca and Mg including CaCO₃ and MgCO₃ when it was made via pyrolysis of dairy effluent-enriched alfalfa feedstock. These results indicate owing to various rich metal ions (e.g., Ca, Mg, Na, K, and Fe) in the dairy effluent, as shown in Table 3. In this study, the TC adsorption onto DEAF-BC could be attributed to the Ca and Mg (including CaCO₃ and MgCO₃) actively reacting with TC. Please note that CaCO₃ showed high TC adsorption capacity (364 mg TC/g powder) under the same experimental conditions applied for TC adsorption onto DEAF-BC in this study (0.1 L of 100 mg/L TC, 0.01 g CaCO₃, pH 6). As the previous literatures reported, TC may be adsorbed on the surface of biochar by cation exchange such as Ca²⁺ and Mg²⁺ and formation of the insoluble chelates with cations because TC structure contains various binding sites.

Fourier transform infrared (FTIR) analysis of AF-BC and DEAF-BC before and after the adsorption of TC showed the weak spectra attributed to carbonates (CO₃²⁻, 875 cm⁻¹), C=O stretching (vibration of alcoholic groups, 1049 cm⁻¹), C=H bending (aliphatic hydrocarbon, 1430 cm⁻¹), and tetracycline (–NH₂ at A ring, 1520 cm⁻¹, C=O stretching at C ring, 1580 cm⁻¹) from both DEAF-BC and AF-BC (Figure 3). The weak spectra in DEAF-BC and AF-BC were mainly due to the significant loss of functional groups (i.e., mainly CO₃²⁻, C=O stretching, C=O stretching at C ring, and –OH) in the dairy effluent-enriched alfalfa feedstock and alfalfa feedstock via pyrolysis at high temperature (i.e., 750 °C in this study) as reported by the previous studies. However, the increase of the peak at 1580 cm⁻¹ in DEAF-BC after the TC adsorption onto DEAF-BC clearly indicated the TC adsorption onto the DEAF-BC. Additionally, the reduction of oxygen-containing

Figure 2. XRD patterns of AF-BC and DEAF-BC (a: SiO₂, b: CaCO₃, and c: MgCO₃).
Conducted in triplicate and the error bars represent the 95% confidence interval.100 mL, 100 mg/L TC, 0.01 g BC, 22°C, 5 days. The experiment was conducted in triplicate and the error bars represent the 95% confidence interval.

2.2. Influences of Initial Solution pH on TC Adsorption onto AF-BC and DEAF-BC. TC adsorption capacities of AF-BC (7–94 mg TC/g BC) and DEAF-BC (57–573 mg TC/g BC) were evaluated at each initial solution pH (pH 3–8) (Figure 4). These results may be owing to the low TC adsorption capacity at pH <5 and >7 (Figure 4) although it had much lower TC adsorption capacities than those of DEAF-BC. Similar to DEAF-BC, the highest adsorption capacity (94 mg TC/g BC) of AF-BC at pH 6 would be elucidated by mainly nonelectrostatic interactions such as hydrogen bonding, hydrophobic, and π−π interactions.

2.3. Adsorption Kinetic Studies of TC. The dynamic adsorption of TC onto DEAF-BC was investigated under two different initial concentrations of TC to understand possible mechanisms and rate-limiting steps (Figure 5 and Table S3). The elovich, pseudo-first-order (PFO), and pseudo-second-order (PSO) models were widely applied for adsorption of the adsorbate from a liquid solution. PSO ($R^2 = 0.967$) from the adsorption kinetic data was the better-fitted model compared with the PFO ($R^2 = 0.959$) and Elovich ($R^2 = 0.927$) models owing to PSO possessed higher correlation with TC adsorption. The two-compartment kinetic model depicts a continuous distribution with two parameters in compartments. The adsorption kinetic data were also found to be well fitted to the two-compartment kinetic model, indicating that the TC adsorption onto DEAF-BC followed two dominant processes (Figure 5 and Table S3). The higher mass fraction of slow adsorption ($F_{slow}$) revealed compared with that of fast adsorption, indicating that the slow adsorption phase was the predominant process. Similar to the results by Zhou et al.,5 the adsorptive behavior of TC onto Fe/Zn-biochar was also found. In addition, the adsorptive process for TC could be controlled by intraparticle or liquid film diffusion or both combined.32 The intraparticle diffusion (IPD) showed a better fit model for the TC adsorption kinetic than that the liquid film diffusion. In this study, all of the intercepts ($K_i = 17.980, K_i = 11.597, K_3 = 4.622, C_i = 44.5$) were positive from IPD, implying that swift adsorption took place.33 Moreover, three-stage multilinear plots and positive $C_i$ value of experimental results showed that multiple stages would affect the adsorptive performance. It is generally known that the first phase is associated with the external mass transfer resistance, but the second and third phases are because of the IPD model. As $K_1 > K_2 > K_3$ is indicating the IPD model, the order rate constant of DEAF-BC with resistance was associated with TC adsorption. Instantaneous or external surface adsorption in the first step and gradual adsorption in the second step were related between DEAF-BC and TC (Figure 5 and Table S3).

2.4. Adsorption Isotherm Studies and Thermodynamic Analysis. Temkin, Freundlich, and Langmuir models were applied to figure the TC adsorption capacity of DEAF-BC in water (Figure 6 and Table S4).4,6,13,34 In this study, Freundlich revealed the best-fitted isotherm model based on the highest $R^2 (>0.95)$ among three isotherm models, indicating that a multilayer adsorption on a heterogeneous surface of biochar would occur.7 The parameter ($n_f$) from the Freundlich model for DEAF-BC implied that the TC adsorption was favorable (i.e., ranging from 1 to 10 $n_f$ value). However, both Langmuir and Temkin models showed poor fitting with the adsorption results.

Tang et al.6 and Jang et al. reported Freundlich as the best-fitted isotherm model for multilayer TC adsorption onto biochars produced from municipal sewage sludge and P. taeda.

![Figure 3. FTIR spectrum of AF-BC and DEAF-BC before and after reaction (a: CO$_3^{2-}$, b: C=O stretching, c: C-H bending, d: amino group (–NH$_2$) in ring A of TC, e: C=O stretching in C ring of TC).](image)

![Figure 4. pH-dependent TC species and effects of initial solution pH on the adsorption of TC onto AF-BC and DEAF-BC. [Conditions] 100 mL, 100 mg/L TC, 0.01 g BC, 22°C, 5 days. The experiment was conducted in triplicate and the error bars represent the 95% confidence interval.](image)
Similar to the previous literatures, the DEAF-BC also possessed the \( \frac{1}{n} \) value (0.702) less than 1 in the Freundlich model, indicating surface heterogeneity (see the Supporting Data).

Table 1 summarizes the maximum TC adsorption capacities \( (Q_m) \) of various biochars (pristine, functionalized, and activated biochars) and commercial activated carbons from the literatures, which were compared with those of AF and DEAF-BC. The DEAF-BC (835.7 mg/g) from this study possessed higher \( Q_m \) for TC than those of various pristine BCs (8.1–407.9 mg/g), functionalized BCs (98.9–102 mg/g), and activated BCs (87.8–552.0 mg/g). In addition, the DEAF-BC had the comparable \( Q_m \) with the commercial activated carbon (816.7 mg/g).

The thermodynamic analysis was made from the TC adsorption onto DEAF-BC at various temperature (20, 40, and 60 °C) to understand the adsorption mechanisms (Figure S2). Table 4 shows the thermodynamic parameters including enthalpy (\( \Delta H^0 \)), entropy (\( \Delta S^0 \)), and Gibbs free energy (\( \Delta G^0 \)) from the TC adsorption onto DEAF-BC at 20–60 °C. The positive \( \Delta H^0 \) value for the TC adsorption onto DEAF-BC (2.63 kJ/mol) implied that the endothermic adsorption process of TC, which also supports the enhanced adsorption of TC with increasing temperature, governed. The \( \Delta H^0 \) value was below 40 kJ/mol, implying that the adsorption process was dominated by physisorption.

Similar to the previous literatures, the DEAF-BC also possessed the \( 1/n_1 \) value (0.702) less than 1 in the Freundlich model, indicating surface heterogeneity (see the Supporting Data).

Table 4. Thermodynamic Parameters for TC Adsorption onto DEAF-BC. [Conditions] 100 mL, 10–100 mg/L TC, 0.01 g BC, pH = 6, 22 °C, 4 Days

| temperature °C | \( Q_e \) (mg/g) | \( C_e \) (mg/L) | \( Q_e/C_e \) (K_eq) | \( \Delta G \) (kJ/mol) | \( \Delta H \) (kJ/mol) | \( \Delta S \) (kJ/mol) |
|----------------|-----------------|-----------------|---------------------|-------------------------|------------------------|------------------------|
| 20             | 835.74          | 53.47           | 15.62               | -6.85                   | 2.63                   | 0.03                   |
| 40             | 930.00          | 56.08           | 16.58               | -7.47                   | 2.63                   | 0.03                   |
| 60             | 1057.62         | 59.38           | 17.80               | -7.96                   | 2.63                   | 0.03                   |

\( Q_e \): the amount of TC adsorbed per unit mass of biochar at equilibrium (mg/g). \( C_e \): liquid-phase concentration of TC at equilibrium (mg/L). K_eq: equilibrium constant.
adsorption occurred through physisorption, as suggested by the previous studies. The positive entropy ($\Delta S^\circ$) values suggested the increased randomness at the interface of DEAF-BC and water during the adsorption of TC onto DEAF-BC. The negative Gibbs free-energy ($\Delta G^\circ$) values indicated that the process was spontaneous for TC adsorption. It also supported that the spontaneity increases at a higher temperature.

2.5. Possible Working Mechanisms for TC Adsorption onto DEAF-BC. SEM-EDX has shown the abundant calcium (16%) on the DEAF-BC surface (see Table S1). It is the notable mechanism that complex formation could occur between the metal ions (i.e., Ca$^{2+}$ and Mg$^{2+}$) and TC. Thus, the surface complexation on the DEAF-BC surface may contribute to the adsorption of TC as one of the possible mechanisms. According to the XRD results, it revealed that DEAF-BC possessed much more CaCO$_3$ with AF-BC (Figure 2). The high content of CaCO$_3$ was made from the attachment of high concentration of calcium ions in dairy effluent onto the alfalfa feedstock followed by the pyrolysis. Thus, the Ca–TC complexation on the surface of DEAF-BC could be made via the binding of Ca onto functional groups of TC as one of the major working mechanisms, which associated with adsorption of TC onto DEAF-BC. The $Q_v$ values of pure CaCO$_3$ for TC (364 mg TC/g powder) under the conditions also supported the role of CaCO$_3$ at the surface of DEAF-BC for the formation of Ca–TC complexation contributing to the high TC adsorption capacity of DEAF-BC. There were negligible changes of FTIR spectra for AF-BC after the adsorption of TC in this study (Figure 3). These results indicated that there were negligible or weak interactions between the functional groups of AF-BC and TC during the adsorption. In contrast, there were clear changes of FTIR results (the peaks at 875, 1049, 1430, 1520, and 1580 cm$^{-1}$) for DEAF-BC after the adsorption (Figure 3). The changes of the FTIR spectrum in DEAF-BC were relevant to the changes of several functional groups such as aliphatic hydrocarbon (C–H bending, 1430 cm$^{-1}$), vibration of alcoholic groups (C–O stretching, 1049 cm$^{-1}$), and carbonates (CO$_3^{2−}$, 875 cm$^{-1}$) in DEAF-BC, suggesting that the functional groups of DEAF-BC participated in the TC adsorption. Particularly, the loss of C–H (1430 cm$^{-1}$) and CO$_3^{2−}$ (875 cm$^{-1}$) groups in DEAF-BC after the adsorption of TC indicated that these oxygen-containing functional groups as H-bond acceptors would favorably make hydroxide bonding with TC. The increase of FTIR peaks in amino group (–NH$_2$, 1520 cm$^{-1}$) and C==O stretching groups in TC (1580 cm$^{-1}$) in DEAF-BC also confirmed the TC adsorption onto DEAF-BC (Figure 3).

In addition, as Figure 4 shows the effects of initial pH on TC adsorption onto DEAF-BC, the high adsorption capacity of TC (511–573 mg TC/g BC) at pH 5–7 implied that the high TC adsorption onto DEAF-BC was driven by van der Waals forces and π–π interaction based on the surface area and hydrophobic (O/C) of DEAF-BC (please see Section 2.2). Similar to DEAF-BC, the microalgae, rice straw, and swine manurederived biochars also revealed the effective TC adsorption via van der Waals, hydrogen bonding, and π–π interactions. The TC adsorption onto DEAF-BC could be attributed to the Ca and Mg (including CaCO$_3$ and MgCO$_3$) actively reacting with TC in the present study. Please note that CaCO$_3$ showed high adsorption capacity (364 mg of TC/g powder) under the same experimental conditions applied for the TC adsorption onto DEAF-BC in this study (0.1 L of 100 mg/L TC, 0.01 g of CaCO$_3$, pH 6) (data not shown). As the previous literatures reported, TC may be adsorbed on the surface of biochar via cation exchange (i.e., Mg$^{2+}$ and Ca$^{2+}$) and formation of the insoluble chelates with cations because the TC structure contains various binding sites.

3. CONCLUSIONS

The enrichment of alfalfa hay with dairy effluent led to significant accumulation of cationic metals during biochar production. The dairy effluent-functionalized alfalfa biochar (DEAF-BC) possessed strong crystallization (i.e., CaCO$_3$), functional groups (i.e., CO$_3^{2−}$, C–O stretching), and high surface area (334 m$^2$/g) related to effective TC adsorption in aqueous solution. The DEAF-BC revealed higher TC adsorption capacity than those of AF-BC, other raw, and activated biochars. The adsorption isotherm and kinetic results for the DEAF-BC fitted to Freundlich, intraparticle diffusion,
and pseudo-second-order models for TC well. The thermodynamic analysis indicated that the TC adsorption onto DEAF-BC would be spontaneous and an endothermic process. Possible working mechanisms contributed to TC adsorption onto DEAF-BC would include hydrogen bonding, metal complexation, van der Waals forces, and π−π interaction. Future studies are needed to investigate optimization of pyrolysis conditions and adsorption process and cost-effective regeneration of DEAF for practical application. Furthermore, the feasibility of DEAF for other emerging contaminants such as cyanotoxins and per- and polyfluoroalkyl substances (PFAS) will be investigated.

4. MATERIALS AND METHODS

4.1. Reagent and Feedstock for Biochar Preparation. Tetracycline (analytical grade) was obtained from Sigma-Aldrich. Alfalfa (Medicago sativa L.) used for BC preparation in this study was obtained from a hay store (Stephenville, TX).

4.2. Biochar Production. DEAF-BC was produced via the pyrolysis of alfalfa feedstock enriched with the real dairy effluent containing various metal ions. The alfalfa (AF, hereafter) was crushed to sieve size 8 × 35 (2360–500 μm). The actual dairy effluent was collected from the 2nd lagoon at the Southwestern Dairy Research Center belonging to Tarleton State University (Tarleton dairy farm, Stephenville, TX). Please see the elemental compositions of dairy effluent in Table 3. The dairy effluent was centrifuged at 3500 rpm for 20 min and then filtered through a 110 mm filter paper (Whatman No. 1) to remove suspended solids. Ten grams of crushed AF was gently stirred with 100 mL of the dairy effluent in 250 mL glass beakers for 2 days for an economical immobilization of metals. The metal ions in the dairy effluent immobilized into alfalfa feedstock before pyrolysis may be potential pretreatment compared to the general metal coating onto biochar after pyrolysis. Then, the dairy effluent-enriched AF was dried at 80 °C for overnight before pyrolysis. Ten grams of AF feedstock (as a control) and the dairy effluent-enriched AF were loaded into a pyrolysis reactor (Multi Position Tube Furnace GSL-1100X-S, MTI Co., Richmond, CA) to make AF-BC as the pristine BC and DEAF-BC as the functionalized BC. The pyrolysis conditions included the pyrolysis temperature of 750 °C for 1 h reaction with a heating rate of 10 °C/min under oxygen-free conditions by flushing nitrogen at 2 L/min. The biochars were designated to AF-BC (alfalfa-derived biochar) and DEAF-BC (dairy effluent-functionalized alfalfa biochar). The AF-BC and DEAF-BC were ground and then sieved to below 140 mesh (<106 μm). Five grams of AF-BC and DEAF-BC were washed using distilled water (500 mL) two times before the adsorption experiments.

4.3. Biochar Characterization. The elemental compositions in the AF-BC and DEAF-BC were analyzed by a PerkinElmer 2400 elemental analyzer (Series II, MA). The volatile and fixed carbon and ash content were also measured according to D1762-84 and D5142-02a, also known as the ASTM standard.31,41,42 The metal compositions in AF-BC and DEAF-BC were analyzed by Spectro Radiial Modula ICP (Marlborough, MA). The morphology and elements on the AF- and DEAF-BC surface were analyzed with a scanning electron microscope (SEM) and energy-dispersive X-ray spectroscopy (EDS) (Hitachi S-4800, Hitachi Co., Japan). Rigaku MiniFlex II (Rigaku, Japan) was used for determination of X-ray diffraction (XRD) patterns of AF-BC and DEAF-BC at a range from 2 to 72° with 0.02° (2θ) at 30 kV and 15 mA. The measurements of the Brunauer–Emmett–Teller (BET) specific surface area of AF-BC and DEAF-BC were performed under N2 adsorption at 77 K with Micromeritics Gemini VII 2390p (Norcross). The pHpzc (pH at point of zero charge) of AF-BC and DEAF-BC were measured by the same procedures previously described.2,3 Please see the Supporting Information for the detailed protocols of characterization.

4.4. Batch Adsorption of Tetracycline (TC) onto AF-BC and DEAF-BC. TC adsorption capacities of AF-BC and DEAF-BC were estimated by stirring 0.01 g of biochar and 0.1 L of 100 mg/L TC in DI water at pH 6 for 5 days.2,27 The TC concentrations in DI water before and after the adsorption experiments were analyzed by a Shimadzu LC-2030C HPLC (Torrance, CA) with a Phenomenex Aeris Peptide XB-C18 column (150 × 4.6 mm², 3.6 μm) and a photodiode array (PDA) detector at 355 nm wavelength. For the elution, a mixture of HPLC-grade water with 0.1% HPLC-grade formic acid and methanol (67:33, v/v) was used under isocratic run and quantified using linear regression of TC. The TC adsorption capacities of AF- and DEAF-BC were determined by eq 1.

\[ Q = \frac{\text{(initial TC concn} - \text{final TC concn}) \times \text{volume of water}}{\text{amount of biochar}} \]  

4.5. Adsorption Kinetic and Isotherm Modeling of DEAF-BC. The adsorption kinetic studies were performed in 250 mL glass flasks containing 0.01 g of DEAF-BC in 0.1 L of 100 mg/L TC solution at pH 6 (the optimum pH determined from Section 2.2) and various temperatures (20, 40, and 60 °C). The samples were taken at a regular interval for 4 days. The adsorption isotherm studies were also performed by stirring 0.01 g of DEAF-BC and 0.1 L of 10–100 mg/L TC solution in 250 mL glass flasks for 5 days. The six adsorption kinetic models including elovich, pseudo-first-order, pseudo-second-order, liquid film diffusion, two-compartment order,
and intraparticle diffusion models were used to fit the kinetic data in this study. In addition, three isotherm models including Freundlich, Langmuir, and Temkin models were applied for fitting the isotherm data in this study. The kinetic and isotherm model equations are summarized in Table S6. In addition, the thermodynamic parameters (i.e., change in enthalpy ($\Delta H^0$), entropy ($\Delta S^0$), and Gibbs's free energy ($\Delta G^0$)) for the TC adsorption onto DEAF-BC have been calculated using eqs 2 and eq 14:

$$\Delta G^0 = \Delta H^0 - T \Delta S^0 \quad (2)$$

$$\log \left( \frac{q_e}{C_e} \right) = \frac{\Delta S^0}{2.303R} + \frac{-\Delta H^0}{2.303RT} \quad (3)$$

where $q_e$ is the adsorbed TC amount per unit mass of DEAF-BC (mg/g), $C_e$ is the TC concentration at equilibrium (mg/L), $R$ is the gas constant (8.314 J/K mol), and $T$ is the temperature (K).

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c01099.

Characterization of biochar; pH$_{pzc}$ of AF-BC and DEAF-BC; adsorption of TC onto DEAF-BC at various temperatures; EDX results of AF-BC and DEAF-BC; surface charge of DEAF-BC and TC adsorption at various solution pH; adsorption isotherm parameters of TC onto DEAF-BC; adsorption kinetic parameters of TC onto DEAF-BC; cations in leachate of DEAF-BC at various pH; adsorption kinetic and isotherm models (PDF)

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Y.-K.C. conducted all of the experiments and wrote the original manuscript. R.S. and Y.-K.C. conducted the analysis of biochar. E.K. provided the concept and experimental design for this research work and revised the manuscript.

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