Adsorption of bovine serum albumin and urease by biochar

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Abstract. The application of biochar to soil improvement inevitably affects free soil enzymes. However, there is little information on the interaction of soil enzymes with biochar to our knowledge. We thus investigated the adsorption of bovine serum albumin (BSA) and urease onto two biochars from giant reed pyrolyzed at 300 and 600 °C (BCF300 and BCF600). The adsorption amount of BSA and urease on BCF300 and BCF600 was up to 45.6-209 mg/g and 75.3-808 mg/g, respectively, suggesting that the two proteins could be adsorbed onto the biochars effectively. The sorption rate of BSA and urease significantly decreased as the protein concentration increased, suggesting that their adsorption was nonlinear. For the same initial concentration (50 or 200 mg/L), the adsorption amount of BSA on the biochars was lower, only 25.9-60.5% of that of urease. The high specific surface area and hydrophobicity of the biochars may play important roles in the immobilization of the proteins by biochars. These findings will be helpful for better understanding the effects of biochar adding on the soil enzymes.

1. Introduction
Biochar is a porous carbonaceous material derived from pyrolysis of biomass (e.g., agricultural and forestry residues, poultry manure and municipal sludge) in an anaerobic or anoxic condition [1]. The properties of biochar including alkalinity, specific surface area, pore structure, elemental composition and functional groups are diversified due to different feedstocks and pyrolysis conditions, especially pyrolysis temperature [2]. Biochar has been described as a soil amendment which can be stored in soil for hundreds or even thousands of years to achieve carbon sequestration [3], and can also improve microbial activity and soil fertility, decrease soil nutrient leaching, reduce bioavailability and phytotoxicity of heavy metals and organic pollutants in soils [4].

Soil enzymes participate in nutrients cycling and energy metabolism in soil, determining the intensities and directions of soil biochemical reaction process (e.g. soil organic matter formation or decomposition, plant productivity and resource supply), which plays important roles in soil ecosystems [5]. Urease, one of soil hydrolases, is involved in purine metabolism and ornithine cycle [6]. Urea, as a widely used nitrogen (N) fertilizer added into soil, can be easily degraded to ammonia via urease catalysis [7]. Application of biochar can adsorb NH₄⁺, reduce NH₃ volatilization and N₂O emission, as well as increase plant uptake of N [8]. Lu et al. reported that the addition of eucalyptus-derived and poultry litter-derived biochar to a cropland area caused different effects on β-glucosidase, β-glucosaminidase, urease and phosphomonooesterase activities [9]. Demisie et al. also reported that 0.5%, 1% and 2.0% oak wood biochar and bamboo biochar significantly improved the urease activity in a degraded red soil. [10]. It is thus clear that biochar will inevitably interact with soil enzymes, but this interaction is overlooked.
The objectives of this study are to: (1) examine the effect of solid to liquid ratio on the sorption rate of BSA by the biochars; (2) study the adsorption kinetics of BSA and urease on the biochars. Bovine serum albumin (BSA) as a model protein due to its high structure stability and relatively clear nature was selected to be a reference adsorbate. This work will provide valuable data about environmental behavior of urease in the biochar-amended soils.

2. Materials and methods

2.1. Materials
The biochars were produced from giant reed (Arundo donax L.) at 300 and 600 °C as described by Zheng et al. [10], respectively, and sieved through a 0.125 mm sieve for further characterization and experiments. A washing treatment was employed to remove excess minerals from the biochars. Briefly, the ground biochars were repeatedly soaked in 1 M HCl solution 4 times at a ratio of 1:20 (w/v), and then treated with 1 M HCl-HF solution for the same procedure. These biochar samples were thoroughly washed with distilled water several times until their pH values retained stable, and then oven-dried at 80 °C to get the ash-free biochars. The deashed biochars were hereafter named as BCFX, where X represents temperature. Bovine serum albumin (BSA) (V900933) and urease (U1500) were both purchased from Sigma-Aldrich.

2.2. Stability assays of BSA
BSA stock solution (5000 mg/L) was prepared in a acetate buffer solution (NaAc-HAc, 0.1M, pH 5.0±0.1) containing 200 mg/L sodium azide (NaN₃). The experiments were conducted in 8-mL glass vials with Teflon-lined screw caps. The BSA stock solution was diluted to 50 mg/L and 200 mg/L. The samples were kept in dark and shaken at 150 rpm at 25 °C for 120 h and sampled periodically. The vials were centrifuged at 3500 rpm for 10 min, and the absorbance of the supernatant was measured at 280 nm by a UV spectrophotometer (Lambda 35, PerkinElmer, USA) [11]. All the samples were run in triplicates.

2.3. Solid to liquid ratio experiment
The BSA stock solution was diluted to 50-1000 mg/L using the acetate buffer solution. BCF300 (5 mg) or BCF600 (2 mg) was added into 8-mL vials containing 7.5 mL BSA solution with different concentrations (50, 100, 500 and 1000 mg/L). All the vials were shaken at 150 rpm for 24 h at 25 °C. After centrifuging at 3500 rpm for 10 min, the BSA concentrations in the supernatants were determined at 280 nm by Lambda 35. The adsorption rate (%) was calculated as follows:

$$ R = \frac{C_0 - C_24}{C_0} \times 100\% $$ (1)

Where $R$ is adsorption rate (%), and $C_0$ and $C_24$ (mg/L) is concentration of BSA at 0 and 24 h in solutions, respectively.

2.4. Adsorption kinetics of BSA and urease on the biochars
The BSA or urease stock solution was prepared and diluted to 50 and 200 mg/L using the acetate buffer solution in 2.2. A 5-mg BCF300 or 2-mg BCF600 were added into 8-mL vials with the BSA or urease solution at two initial concentration (50 and 200 mg/L). All the vials were shaken at 150 rpm at 25 °C, and sampled at different time (8-120 h) and centrifuged at 3500 rpm for 10 min to examine the BSA or urease concentration. The urease concentration was determined by Coomassie brilliant blue method [12]. The adsorption capacity ($Q_t$) was calculated as follows:

$$ Q_t = \frac{C_0 - C_t}{m} \times V $$ (2)

Where $Q_t$ is adsorption amount (mg/g), $C_0$ and $C_t$ is protein concentration (mg/L) at time 0 and t, respectively, $m$ is biochar amount (g), $V$ is solution volume (L).

2.5. Data analysis
Microsoft office excel (version 2013) was used for data analysis. Significant differences were tested using least significant difference (LSD) \((P < 0.05)\) by Statistical Product and Service Solutions (version 20.0).

3. Results and discussion
The absorbance values of BSA solution after shaking for different times are shown in Figure 1. Whether low or high concentration of the BSA solutions, there was no significant difference for the absorbance within 120 h, implying that BSA can sustain structure stability in the tested buffer solution for 120 h.

![Figure 1 Absorbance of BSA solution after shaking for different time.](image1)

![Figure 2 Adsorption rate of BSA by the biochars. Lowercase letters represent the significant difference of the BSA adsorption on the same biochar among the different BSA concentration. For a given BSA concentration, asterisk represents the significant difference of the adsorption rate of BSA between BCF300 and BCF600 \((n = 3, P < 0.05)\).](image2)

The adsorption rate decreased with increasing BSA concentration for both BCF300 and BCF600 (Figure 2). For instance, the adsorption rates of BCF300 and BCF600 reduced to 9.66% and 6.67% from 79.0% and 62.7% when the BSA concentration increased from 50 mg/L to 1000 mg/L, respectively. This indicated that the adsorption efficiency of BSA by the biochars greatly depended on the adsorbent dose.
BSA and urease were effectively sorbed by BCF300 and BCF600, and the sorption reached up to equilibrium at 24 h and 36 h (Figure 3), respectively. The adsorption capacities of the two adsorbates were different. For example, when the initial concentration was 200 mg/L, the sorption amount of BSA on BCF300 was 88.5 mg/g, significantly lower than that of urease (337 mg/g), implying that urease was easier to be adsorbed by biochar (Figure 3). That may depend on the differences of structure and properties of the tested protein. Furthermore, the adsorption amount of BSA and urease on BCF600 was both higher than that on BCF300, indicating that BCF600 had stronger affinity than BCF300. In our previous report, H/C ratio of BCF300 and BCF600 was 0.82 and 0.34, respectively, and the specific surface area was 2.72 and 50 m²/g, respectively [9]. Therefore, the greater sorption of BSA and urease on BCF600 is likely related to its higher surface area and hydrophobicity. Hitherto, there is no information about the interaction mechanisms between soil enzymes and biochar to our knowledge. Further investigations therefore should be carried out to explore the mechanisms of protein uptake by biochar.

4. Conclusions
The results suggested that BSA could retain structure stability in a acetate buffer solution within 120 h, and be effectively immobilized onto the deashed biochars. The adsorption of BSA and urease on the biochars reached to equilibrium at 24 h and 36 h, respectively. BCF600 exhibited stronger adsorption capacity for both BSA and urease than BCF300. And urease exhibited better affinity onto the biochars than BSA. In addition, the high hydrophobicity and specific surface area may be possible mechanisms for BSA and urease adsorption on the biochars.

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