

DIVISÃO 3 - USO E MANEJO DO SOLO

Comissão 3.1 - Fertilidade do solo e nutrição de plantas

UREA COATED WITH OXIDIZED CHARCOAL REDUCES AMMONIA VOLATILIZATION⁽¹⁾

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SUMMARY

Urea is the most consumed nitrogen fertilizer in the world. However, its agronomic and economic efficiency is reduced by the volatilization of NH_3 , which can reach 78 % of the applied nitrogen. The coating of urea granules with acidic compounds obtained by charcoal oxidation has the potential to reduce the volatilization, due to the acidic character, the high buffering capacity and CEC. This work aimed to evaluate the effect of HNO_3 -oxidized carbon on the control of NH_3 volatilization. These compounds were obtained by oxidation of *Eucalyptus grandis* charcoal, produced at charring temperatures of 350 and 450 °C, with $4.5 \text{ mol L}^{-1} \text{ HNO}_3$. The charcoal was oxidized by solubilization in acidic or alkaline medium, similar to the procedure of soil organic matter fractionation (CHox350 and CHox450). CHox was characterized by C, H, O, N contents and their respective atomic relations, by the ratio E4 (absorbance 465 nm) by E6 (absorbance 665 nm), and by active acidity and total acidity (CEC). The inhibitory effect of CHox on the urease activity of *Canavalia ensiformis* was assessed *in vitro*. The NH_3 volatilization from urea was evaluated with and without coating of oxidized charcoal (U-CHox350 or U-CHox450) in a closed system with continuous air flow. The pH of both CHox was near 2.0, but the total acidity of CHox350 was higher, 72 % of which was attributed to carboxylic groups. The variation in the ionization constants of CHox350 was also greater. The low E4/E6 ratios characterize the high stability of the compounds in CHox. CHox did not inhibit the urease activity *in vitro*, although the maximum volatilization peak from U-CHox450 and U-CHox350 occurred 24 h after that observed for uncoated urea. The lowest volatilization rate was observed for U-CHox350 as well as a 43 % lower total amount of NH_3 volatilized than from uncoated urea.

Index terms: fertilizer, nitrogen, urease.

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RESUMO: VOLATILIZAÇÃO DE AMÔNIA DA UREIA REVESTIDA COM CARVÃO VEGETAL OXIDADO

*A ureia é o fertilizante nitrogenado mais consumido no mundo, porém a sua eficiência agrônômica e econômica é comprometida pela volatilização de NH₃, que pode chegar a 78 % do N aplicado. O revestimento da ureia com compostos obtidos pela oxidação ácida do carvão vegetal tem potencial para reduzir a volatilização, em razão do caráter ácido, do elevado poder-tampão de acidez e da elevada CTC. Este trabalho objetivou avaliar o efeito do carvão de eucalipto oxidado com HNO₃ no controle da volatilização de NH₃ a partir da ureia. Esses compostos foram obtidos a partir da oxidação de carvão de *Eucalyptus grandis* produzidos nas temperaturas de carbonização de 350 e 450 °C com HNO₃ 4,5 mol L⁻¹. Os carvões oxidados (CHox350 e CHox450) foram obtidos por meio da solubilização em meio ácido ou alcalino, semelhante ao procedimento de fracionamento da matéria orgânica do solo. Os CHox foram caracterizados por meio dos teores de C, H, O, N e respectivas relações atômicas, da relação E4 (absorvância 465 nm) / E6 (absorvância 665 nm), da acidez ativa e acidez total (CTC). Avaliou-se o potencial inibidor dos CHox na atividade *in vitro* da urease de *Canavalia ensiformis*. Em um sistema fechado com fluxo contínuo de ar, avaliou-se a volatilização de NH₃ a partir da ureia perolada sem e com o revestimento de carvão oxidado (U-CHox350 ou U-CHox450). Ambos os CHox mostraram pH próximo de 2,0, mas o CHox350 apresentou maior acidez total, sendo 72 % desta atribuída a grupos funcionais carboxílicos. O CHox350 também apresentou maior variação nas constantes de ionização. Os baixos valores das relações E4/E6 caracterizam a elevada estabilidade dos compostos nos CHox. Os CHox não inibiram a atividade da urease *in vitro*, no entanto o pico de máxima volatilização a partir da U-CHox350 e U-CHox450 ocorreu 24 h depois daquele verificado para a ureia sem revestimento. A U-CHox350 apresentou menor taxa de volatilização e redução de 43 % na quantidade total de NH₃ volatilizada, em relação à ureia sem revestimento.*

Termos de indexação: fertilizantes, nitrogênio, urease.

INTRODUCTION

The agronomic efficiency of urea is reduced by NH₃ volatilization losses, which can reach up to 78 % of the applied nitrogen (Lara Cabezas et al., 1997; Sengik & Kiehl, 1995a, b; Silva et al., 1995; Mattila, 1998). Volatilization is increased by raising the pH around the urea granules during urease hydrolysis (Kissel et al., 1988; Rodrigues & Kiehl, 1986; Ouyang et al., 1998). Given this context, alternatives must be found to improve the agronomic efficiency of urea, once it represents 52 % of the N fertilizer consumed in Brazil and the consumption is expected to triple by 2020 (Lopes et al., 2007; ANDA, 2011).

Mixing urea with humified organic material, for example with peat, reduced NH₃ volatilization (Siva et al., 1999). This was attributed to the acid character and to the high acidity, acid buffering capacity and cation exchange capacity (CEC). Consequently, a humic compound obtained by Trompowsky et al. (2005) by HNO₃ oxidation of eucalyptus charcoal could be a possibility as well. This compound is considered similar to humic acids for having been isolated by the extraction method of humic substances from the soil, according to Swift (1996), and having physicochemical characteristics similar to the humic acids extracted from soils rich in pyrogenic carbon. Particularly important is the acidic character with predominantly carboxylic and phenolic groups and, consequently, a

high CEC. However, the term "humic acid" is not appropriate, which would designate one of the components of soil organic matter, characterized by insolubility in pH ≤ 2.0.

Oxidation with nitric acid is used to increase the amount of oxygen fixed in activated carbon (Moreno-Castilla et al., 2000; Chen & Wu, 2004; Liu, et al., 2007; Troca-Torrado et al., 2011). Activated carbon has a high specific surface, large pore volume and oxygen-containing functional groups, such as carboxylic lactones and phenol (Boehm, 1994; Figueiredo et al., 1999; Shim et al., 2001; Chen & Wu 2004; Shafeeyan et al., 2010). Nitric-acid oxidation of activated carbon has been shown to be effective for the generation of these functional group (Figueiredo et al., 1999; Chen & Wu, 2004; Shafeeyan et al., 2010) and also of N-containing groups (Pels et al., 1995; Abe et al., 2000). These functional groups make activated carbon acidic or basic and are responsible for its adsorption capacity (Karanfil & Kilduff, 1999; Karanfil et al., 1999; Szymanski et al., 2002; Shafeeyan et al., 2010), while the acidic character defines the CEC (Boehm, 2002). Therefore, the compounds obtained by Trompowsky et al. (2005) are similar to oxidized activated carbon and could more appropriately be called oxidized charcoal (CHox).

Due to these characteristics, CHox can attenuate the increase in pH resulting from urea hydrolysis, which would reduce the urease activity, with a pH

optimum between 7 and 8 (Krajewska (2009) and favor the formation of NH_4^+ and its adsorption. The objective of this study was therefore to evaluate the effect of eucalyptus charcoal oxidized with HNO_3 on reducing NH_3 volatilization from urea.

MATERIAL AND METHODS

Production and acid oxidation of charcoal

To prepare the coal, wood blocks of *Eucalyptus grandis* were carbonized in a laboratory furnace for 8 h, at final carbonization temperatures of 350 or 450 °C, as proposed by Trompowsky et al. (2005). The coal was ground in an ultra-centrifugal mill, sieved through 74 μm mesh and oven-dried at 105 °C for 12 h.

Charcoal (CHox) was oxidized by the procedure described by Trompowsky et al. (2005) with some modifications. To this end, the mixture of 4.5 mol L^{-1} HNO_3 with charcoal, at a proportion of 22 g mL^{-1} , was heated to boiling and refluxed for 4 h. Subsequently, the mixture was left to stand at room temperature for approximately 15 h and then vacuum-filtered through a 0.45 μm nylon membrane (Millipore HAWP04700), discarding the filtrate (soluble material). The material retained in the filter was dissolved in 0.5 mol L^{-1} NaOH in a volume sufficient to elevate the pH to 12.0, left to stand for 20 h and then centrifuged at 3,345 g for 30 min. The supernatant was then vacuum-filtered through nylon mesh (0.45 μm L^{-1}), the pH of the filtrate adjusted to 2.0 with 5.6 mol L^{-1} H_2SO_4 and the solution maintained at room temperature for 20 h. It was then centrifuged at 3,345 g for 30 min, discarding the supernatant. The decanted material was dissolved again in 0.5 mol L^{-1} NaOH and the pH raised to 12 and left to stand for 1 h. Then the solution was acidified to pH 2.0 and allowed to stand for 20 h, after which it was centrifuged (3,345 g for 30 min), the supernatant discarded and the decanted material (CHox) transferred to a glass container and dried in a forced-air oven at 60 °C.

The CHox was dispersed in ultrapure water at a rate of 130 g L^{-1} and stored in cellophane bags and subjected to dialysis in a container with ultrapure water. The water was changed twice a day, until the increase in conductivity was no greater than 1 μS in the dialysis water 1 hour after the exchange (Benites et al., 2005). After dialysis, the CHox was dried in a forced-air oven at 60 °C, ground to obtain particle size < 149 μm , and stored in a desiccator. In this article, CHox obtained from coal carbonized at 350 and 450 °C is identified as CHox350 and CHox450, respectively.

Characterization of CHox

The contents of C, H and N were determined using an elemental analyzer (Perkin-Elmer 2400 Series II

CHNS/O Analyser). Oxygen was determined by the difference between the initial weight and the contents of C, H, N. The atomic ratios C/N, H/C and O/C were calculated.

The pH of the CHox suspensions was determined in deionized water or in 5 mmol L^{-1} CaCl_2 , at 1:10. To characterize the aromaticity of CHox, absorbance was determined at 465 (E4) and 665 nm (E6) and the ratio E4/E6 computed (Chen et al., 1977).

The total acidity that characterizes the cation exchange capacity (CEC) was determined according to Inbar et al. (1990), which differentiates the carboxylic from the phenolic functional groups. For this, a potentiometric titration system was used as described by Reis et al. (2010). A 100 mg sample of each CHox was solubilized in 5 mL of 100 mmol L^{-1} NaOH prepared in 100 mmol L^{-1} NaCl. The volume was increased to 24 mL with 100 mmol L^{-1} NaCl solution and the mixture maintained under shaking, adding 1 mol L^{-1} HCl to obtain a pH close to 2.0. The mixture was titrated with aliquots of 100 μL of 100 mmol L^{-1} NaOH solution in 100 mmol L^{-1} NaCl up to 15 mL, resulting in 151 pH values. The contents of the phenolic and carboxylic functional groups were calculated using the volume of titrant required to raise the pH from 3.0 to 8.0 and 8.0 to 10, respectively, considering that this pH range dissociates 50 % of the phenolic radicals (Bowles et al., 1989). The total acidity, or CEC, was calculated as the sum of the contents of carboxylic and phenolic functional groups. The different pKa values were identified by the second derivative of the titration curve, considering that the second derivative is zero for each inflection of the titration curve (Manunza et al., 1992; Masini et al. 1998).

NH_3 volatilization from CHox-coated urea

The granular urea coated with CHox350 or CHox450 was evaluated for NH_3 volatilization. To promote adhesion to CHox, 4 mg g^{-1} of soybean oil was added to the urea granules. The mixture was performed in test tubes that were gently shaken for 15 min, so as not to break the granules. Thereafter, the greased urea was transferred to new tubes and 250 mg g^{-1} CHox350 (U-CHox350) or CHox450 (U-CHox450) added, corresponding to the predetermined maximum amount of carbon capable of adhering to the urea. The tubes were shaken again carefully for 15 min to allow a homogeneous coating of the urea granules. Urea coated with soybean oil only (U-oil) was also produced. The urea granules with and without the coatings (Figure 1) were stored in a desiccator until use.

The volatilization test was performed in the laboratory, using air-dried fine earth of a sample from the surface 10 cm of a Red-Yellow Ultisol under no-tillage. The soil had pH- H_2O 5,58 (1:10), 46 mg dm^{-3} P and 240 mg dm^{-3} K, both in Mehlich-1, 3.7 and 0.8 cmol_c dm^{-3} of Ca^{2+} and Mg^{2+} (KCl 1 mol L^{-1}),

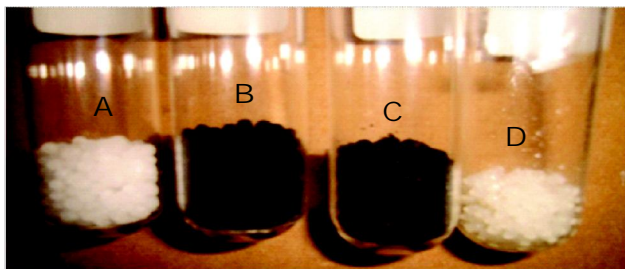


Figure 1. Granular urea without coating (A) and coated with HNO₃-oxidized charcoal from eucalyptus carbonized at 350 °C (B) or 450 °C (C) and coated with soybean oil (D).

respectively, 6.8 cmol_c dm⁻³ of H+Al (0.5 mol L⁻¹ calcium acetate at pH 7.0), 50 g kg⁻¹ of organic matter (Walkley-Black), 36.5 mg L⁻¹ remaining P, and V 43 %.

To measure NH₃ volatilization, a closed collection system with air circulation was used, consisting of incubation chambers (340 cm³ glass containers) connected to the NH₃ collector units (erlenmeyer flasks with 60 mL of 200 g L⁻¹ H₃BO₃). The system received a continuous flow of NH₃-free and moistened air, at approximately 250 mL min⁻¹, to renew the atmosphere in the chambers and carry the NH₃.

Three days before initiating the test, the soil was moistened to field capacity with deionized water and maintained under laboratory conditions (25 ± 2 °C) to reactivate the microbial activity. Then 100 cm³ of the soil was filled into the incubation chambers. The quantity of urea granules (Ug), of CHox-coated urea (U-CHox350 or U-CHox450) and U-oil to supply 10 mg N were applied to the soil surface in the incubation chambers. Chambers containing soil without urea application were also included. The chambers were immediately sealed and connected to the NH₃-collecting units and the air flow was established. The volatilized NH₃ was measured 24, 48, 72, 96, 120, 144, 192, and 240 h after closing the chambers. During this period, the soil humidity was monitored based on the change in weight of the chambers and, when necessary, water was injected through a rubber-sealed side hole. For each measurement, the collecting units were removed and replaced by others. The captured NH₃ was quantified by potentiometric titration to pH 4.5 with 1 mmol L⁻¹ HCl, by an automatic titrator (Mettler Toledo DL 15). The net amount of volatilized NH₃, i.e., urea-derived NH₃, was calculated by subtracting NH₃ volatilized from the soil without urea and was expressed in relation to the N dose applied. The accumulated amount of NH₃ volatilized in eight measurements was calculated.

The test was structured in split plots, where the plots corresponded to the four urea forms and the subplots corresponded to the times of measurement. The experimental design was completely randomized with five replications. Data were subjected to analysis

of variance and the cumulative amount of NH₃ volatilized was related to the time measured by means of the hyperbolic model: $\hat{y} = a + b_1/x$, where a is the maximum quantity volatilized and b_1 the volatilization rate. The means of the total amount of volatilized NH₃ were compared by the Tukey test at 10 %.

Effect of CHox on urease activity

The effect of CHox350 and CHox450 on urease activity *in vitro* was evaluated using Urease Type III from jack beans (*Canavalia ensiformis*) (Sigma No. U1500) with a specific activity of 40 U mg⁻¹. One enzyme unit (U) is the amount of enzyme required to produce 1 μmol min⁻¹ NH₃, at pH 7 and 25 °C. The other reagents used in the test had analytical purity grade.

In a previous study, a concentration of 1.0 g L⁻¹ urease and a period of 12 min for the reaction were found to ensure a linear response to enzyme activity. In the enzymatic assay, 0, 5, 50, 250, or 500 μL of solution with 2 g L⁻¹ of CHox350 or CHox450 prepared in 200 mmol L⁻¹ sodium phosphate buffer, at pH 7.4, were transferred to test tubes which contained concentrations of 0 (control), 50, 250 and 500 μL mL⁻¹ of the respective CHox. To standardize the volume, 1480, 1475, 1430, 1230, and 980 μL of sodium phosphate buffer was added and then 20 μL of 1.0 g L⁻¹ urease solution, also prepared in phosphate buffer sodium, resulting in 1.5 mL of the reaction mixture. The tubes with the reaction mixtures were preincubated at 25 °C for 5 min. Next, 500 μL of 40 mmol L⁻¹ urea prepared in sodium phosphate buffer was added and the tubes were incubated for 12 min at 25 °C. At the end of this time, NH₄⁺ produced in the reaction solution was quantified by spectrophotometric determination with hypochlorite - phenol using a wavelength of 636 nm (Witte & Medina-Escobar, 2001). Standard curves were produced with 0 - 10 μmol L⁻¹ NH₄Cl containing each of the respective CHox350 or CHox450 concentrations. The enzyme activity (μmol L⁻¹ NH₄⁺) was expressed in relation to the control. Each CHox represented an experiment set up in a randomized design with three replications. The data were subjected to analysis of variance.

RESULTS AND DISCUSSION

The yield of CHox350 and CHox450 was 257 and 541 g kg⁻¹, respectively. Trompowsky et al. (2005) also derived higher amounts of humic acid-like compounds from coal produced at 450 °C than at 350 °C. According to these authors, carbonization at 350 °C may have resulted in a material with a low degree of polycyclic condensation, facilitating its degradation into smaller molecules during acid oxidation, equivalent to the fulvic acids. Consequently, solubility would be higher, which was evidenced by

the darker and lower amount of filtered residue. The higher yield of CHox450, on the other hand, can be attributed to the higher condensation polycyclic and resistance to nitrate oxidation (Trompowsky et al., 2005).

CHox consists essentially of C, H, N and O, representing 99.9 % of both CHox350 and CHox450 (Table 1). C, H and O are derived from the plant material itself in the charcoal production when the N is incorporated into CHox during nitric acid oxidation since the plant N is volatilized during carbonization (Trompowsky et al., 2005). The atomic relations indicate differences in the composition of the two CHox types. The C:N ratio of CHox350 was slightly lower, suggesting that during oxidation a greater amount of N was incorporated into the carbon structure (Table 1). The lower H:C and O:C ratios observed in CHox450 indicate a greater proportion of aromatic or unsaturated structures, associated with a higher condensation degree of the coal produced at 450 °C, explaining the higher yield.

The E4/E6 ratios of CHox (Table 1) were lower than the values found for soil-extracted humic acids, varying between 4.7 and 7.0 (Chen et al., 1977; Ceretta et al., 2008). Pimenta et al. (2009) reported E4/E6 ratios between 6.8 and 9.1 and between 5.5 and 6.7 for humic acid species derived from charcoal of semi-arid species, carbonized at 350 and 450 °C, respectively. Since this ratio decreases with the humification degree of organic matter, due to the higher aromaticity which stabilizes the humic acids (Kononova, 1966), the conclusion was drawn that CHox is highly stable, in particular CHox350, with the lowest E4/E6 ratio.

The active acidity of CHox was high, and CHox350

was slightly more acidic (Table 1). The small difference between pH in water and CaCl_2 5 mmol L⁻¹ shows the electroneutrality of CHox. The total acidity that determines the CEC was higher in CHox350, due to the higher levels of both carboxylic and phenolic functional groups (Table 1). The carboxylic groups accounted for 72 and 75 % of the CEC of CHox350 and CHox450, respectively. The shape of the titration curve (Figure 2a) reflects the greater number of ionizable functional groups in CHox350, but does not show the inflection points, due to the proximity of the pKa values. The second derivative of the titration curve for each inflection is zero and shows the greater range of pKa values for the functional groups in CHox350 (Figure 2b). These functional groups are the most abundant ionizable sites in the humic acids (Masini et al., 1998) and they determine the acid character, CEC and acid-buffering capacity. Ionizable functional groups, e.g., carboxylic and phenolic compounds, were also observed by Trompowsky et al. (2005) in humic acids obtained from charcoal based on infrared spectrum analysis and ¹³C nuclear magnetic resonance, aside from N-containing functional groups introduced by nitric acid oxidation. Despite the lower yield, the physicochemical characteristics of CHox350 are more appropriate to control NH₃ volatilization from urea hydrolysis.

Volatilization test

The coated urea, both with CHox350 and CHox450 showed 36 % of N due to the dilution in oil (0.03 %) and CHox (25 %) attached to the granules.

The urea coatings of both CHox types delayed the NH₃-volatilization peak by 24 h (Figure 3). However, only the U-CHox350 altered the volatilization kinetics, reducing the volatilization rate, evidenced by a lower *b1* coefficient of the hyperbolic model and a lower accumulation of volatilized NH₃ (Figure 4 and Table 2). The amount of NH₃ volatilized from U-CHox350 was 43 % lower than from uncoated urea.

It was observed that the U-CHox granules were still visible on the soil up to 10 h after application, while the urea without coating had disappeared after 3 h. Thus, the delay in peak volatilization can be attributed to slower urea dissolution, due to the hydrophobic character of CHox.

According to the results of enzymatic kinetics *in vitro*, the lower volatilization from U-CHox350 cannot be attributed to urease inhibition, since its activity was not reduced by CHox350 or CHox450 (Table 3). The small increase in urease activity with CHox application can be attributed to the time spent on the addition of the phenol-containing solution to inactivate the enzyme, since this analytical procedure was applied from the control treatment to the highest CHox concentration, thus extending the urease activity by a few seconds. In soil, however, the acidity caused by CHox could have some effect on the urease activity, mainly in the

Table 1. Chemical properties of HNO₃ - oxidized plant charcoal produced from eucalyptus carbonized at 350 °C (CHox350) or 450 °C (CHox450)

Property	CHox350	CHox450
C (g kg ⁻¹)	629	672
H (g kg ⁻¹)	36.9	35.0
N (g kg ⁻¹)	31.4	32.2
O (g kg ⁻¹)	302	260
C:N	23.40	24.33
H:C	0.70	0.62
O:C	0.36	0.29
pH (water)	1.82	2.02
pH (CaCl ₂ 5 mmol L ⁻¹)	1.80	2.00
E4 = Abs 465 nm	1.075	0.989
E6 = Abs 665 nm	0.474	0.316
E4/E6	2.27	3.13
Carboxylic groups (cmol _c kg ⁻¹)	320	250
Phenolic groups (cmol _c kg ⁻¹)	120	80
Total CEC ¹ (cmol _c kg ⁻¹)	440	330

¹ total acidity.

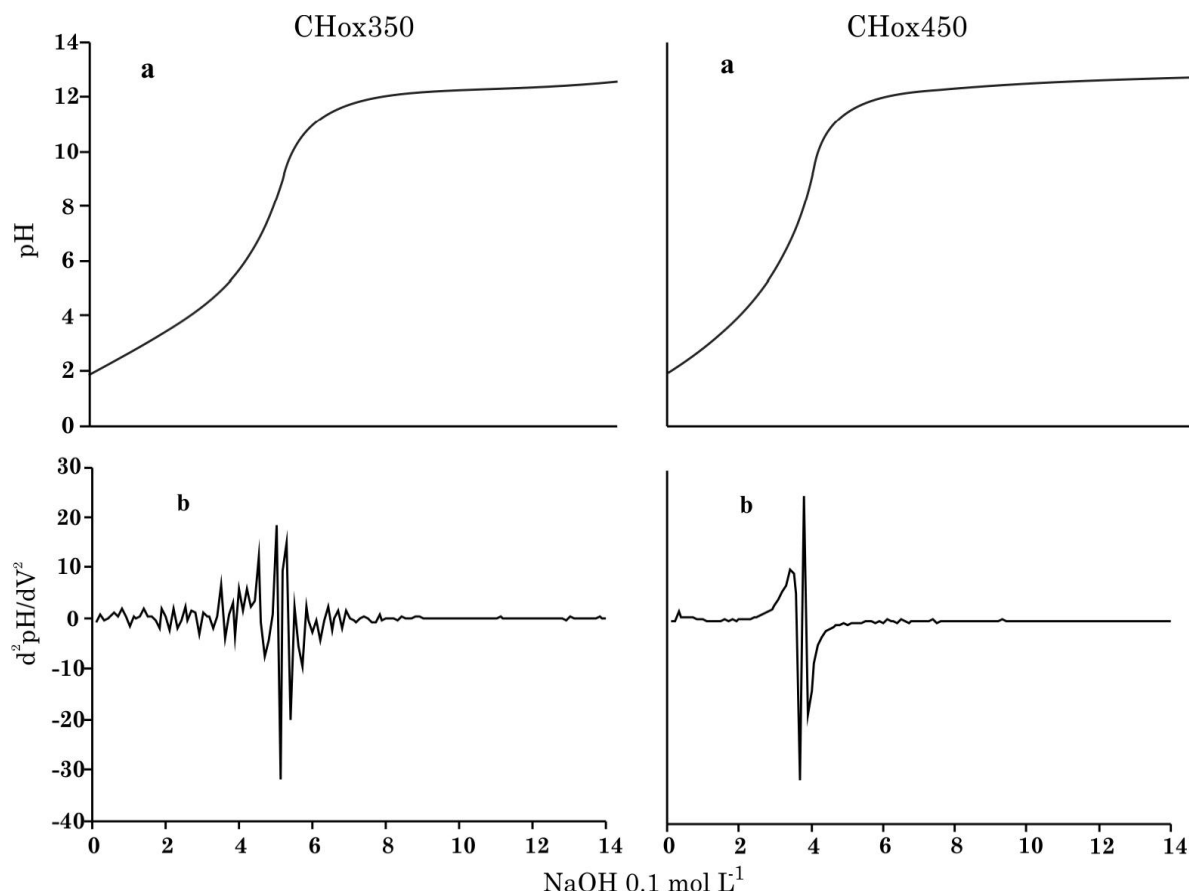


Figure 2: Variation in pH of the suspension of HNO₃ - oxidized charcoal produced from eucalyptus carbonized at 350 °C (CHox350) or 450 °C (CHox450), according to the volume of 0.1 mol L⁻¹ NaOH added at titration, characterizing the titration curves (a) and the second derivative of the titration curves (b).

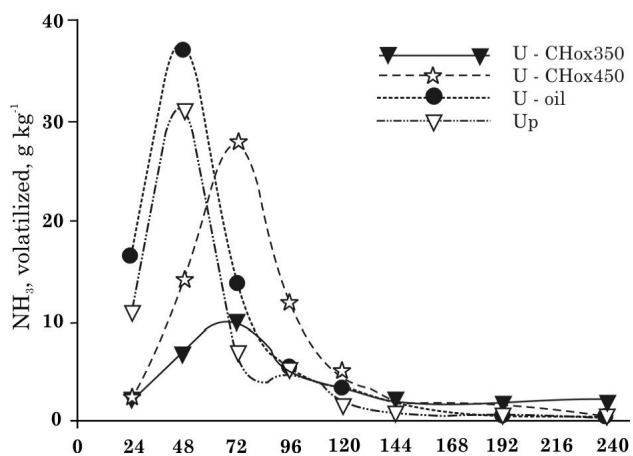


Figure 3. Content of volatilized N-NH₃ in a closed system with air circulation to capture NH₃ compared to the N rate applied to the soil (g kg⁻¹) as granular urea without coating (U_g) and coated with plant oil (U-oil) or with HNO₃-oxidized charcoal produced from eucalyptus carbonized at 350 °C (U-CHox350) or 450 °C (U-CHox450).

Table 2. Total amount of volatilized N-NH₃ in 240 h, with the respective standard deviation from the mean in a closed system with air circulation to capture NH₃, in relation to the N applied to the soil (g kg⁻¹) as granular urea without coating (U_g), coated with plant oil (U-oil) or coated with HNO₃-oxidized charcoal, produced with carbonized eucalyptus at 350 °C (U-CHox350) or 450 °C (U-CHox450)

Treatment	Volatilized N-NH ₃
	g kg ⁻¹ of N-NH ₃
U _g	57.90 ± 5.08 b
U-oil	79.19 ± 20.77 b
U-CHox350	33.25 ± 4.66 a
U-CHox450	65.84 ± 7.74 b

Means followed by the same letter did not differ statistically at 10 % by the Tukey test.

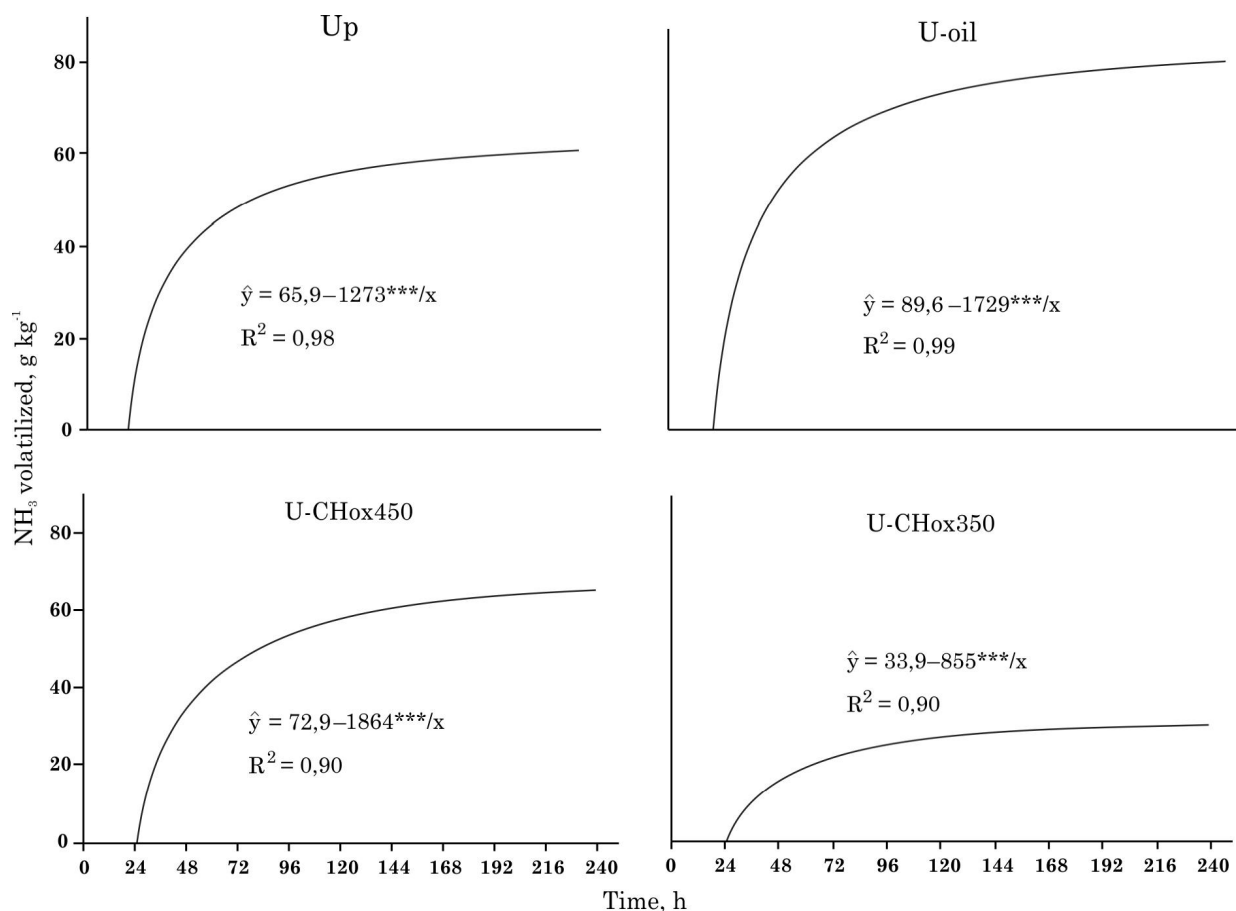


Figure 4. Accumulated content of volatilized N-NH₃ in a closed system with air circulation to capture NH₃ compared to the N rate applied to the soil (g kg⁻¹) as granular urea without coating (Ug) and coated with plant oil (U-oil) or with HNO₃-oxidized charcoal produced from eucalyptus carbonized at 350 °C (U-CHox350) or 450 °C (U-CHox450). *** Significant at 1 % probability.

Table 3. Activity of urease from *Canavalia ensiformes* in vitro with respective average standard error relative to the control (zero), according to the concentration of charcoal oxidized with HNO₃ produced from eucalyptus charcoal carbonized at 350 (CHox350) or 450 °C (CHox450) in solution of the enzymatic reaction

Concentration of oxidized charcoal		Relative activity
mg L ⁻¹		%
	CHox350	
0		100 ± 2.03
5		101 ± 1.81
50		109 ± 4.64
250		110 ± 4.27
500		110 ± 4.27
	CHox450	
0		100 ± 6.44
5		103 ± 8.69
50		104 ± 3.90
250		113 ± 3.91
500		115 ± 1.81

first hours, since its optimum pH is between 7 and 8 (Krajewska, 2009). This fact could not be verified in the enzymatic analysis due to the buffering of the reaction medium of pH 7.4. Moreover, the time of pre-incubation of 5 min of CHox with the enzyme may also have been insufficient, since You & Zhou (2008) used a pre-incubation period of 1 h to measure the inhibition capacity of Cu complexes.

Thus, the lower volatilization from U-CHox350 (Table 2) can be attributed to the higher total acidity (Table 1) and the presence of carboxylic functional groups with a greater variety of ionization potential for CHox350 (Figure 2), which increased the buffering capacity. The acid character and buffering restrict the initial urease activity and the elevation of pH resulting from urea hydrolysis while the ionization of functional groups promotes the adsorption of NH₄⁺ formed. This hypothesis is supported by the fact that when the volatilization peak was reached, 30 % of the total NH₃ of U-CHox350 had been lost by volatilization, while for

the uncoated urea, U-oil and U-CHox450, losses were 55, 47 and 42 %, respectively.

CONCLUSIONS

1. The CHox350 and CHox450 obtained by solubilization in acidic or alkaline medium of charcoal produced at final carbonization temperature of 350 °C and 450 °C, respectively, do not alter the urease activity determined *in vitro* with a medium buffered at pH 7.00 .

2. NH₃ volatilization from granular urea coated with CHox350 is 43 % lower than from uncoated granular urea.

3. NH₃ volatilization from urea coated with CHox350 is lower because of its higher acidity and higher buffering capacity.

LITERATURE CITED

- ABE, M.; KAWASHIMA, K.; KOZAWA, K.; SAKAI, H. & KANEKO, K. Amination of activated carbon and adsorption characteristics of its aminated surface. *Langmuir*, 16:5059-5063, 2000.
- ASSOCIAÇÃO NACIONAL PARA DIFUSÃO DE ADUBOS - ANDA. Setor de Fertilizantes. Anuário Estatístico 2010. São Paulo, ANDA, 2011. 178p.
- BENITES, V.M.; MENDONÇA, E.S.; SCHAEFER, C.E.G.R.; NOVOTNY, E.H.; REIS, E.L. & KER, J.C. Properties of black soil humic acids from high altitude rocky complexes in Brazil. *Geoderma*, 127:104-113, 2005.
- BOEHM, H.P. Some aspects of the surface chemistry of carbon blacks and other carbons. *Carbon*, 32:759-769, 1994.
- BOEHM, H.P. Surface oxides on carbon and their analysis: a critical assessment. *Carbon*, 40:145-149, 2002.
- BOWLES, E.C.; ANTWEILER, R.C. & MACCARTHY, P. Acid-base titration and hydrolysis of fulvic acid from the Suwannee River. In: AVERETT, R.C., ed. *Humic substances in the Suwannee River, Georgia, Interactions, properties, and proposed structures*. Denver, U.S. Geological Survey, 1989. p.205-230.
- CERETTA, C.A.; BAYER, C.; DICK, D.P.; MARTIN-NETO, L. & COLNAGO, L.A. Métodos espectroscópicos. In: SANTOS, G.A.; SILVA, L.S.; CANELLAS, L.P. & CAMARGO, F.A.O. eds. *Fundamentos da material orgânica do solo: ecossistemas tropicais & subtropicais*. 2.ed. Porto Alegre, Metropole, 2008. p.277-290.
- CHEN, J.P. & WU, S. Acid/Base-Treated activated carbons: Characterization of functional groups and metal adsorptive properties. *Langmuir*, 20:2233-2242, 2004.
- CHEN, Y.; SENESI, N. & SCHNITZER, M. Information provided on humic substances by E4/E6 ratios. *Soil Sci. Soc. Am. J.*, 41:352-358, 1977.
- FIGUEIREDO, J.L.; PEREIRA, M.F.R.; FREITAS, M.M.A. & ORFÃO, J.J.M. Modification of the surface chemistry of activated carbons. *Carbon*, 37:1379-1389, 1999.
- INBAR, Y.; CHEN, Y. & HADAR, Y. Humic substances formed during the composting of organic matter. *Soil Sci. Soc. Am. J.*, 54:1316-1323, 1990.
- KARANFIL, T. & KILDUFF, J.E. Role of granular activated carbon surface chemistry on the adsorption of organic compounds. 1. Priority pollutants. *Environ. Sci. Technol.*, 33:3217-3224, 1999.
- KARANFIL, T.; KITIS, M.; KILDUFF, J.E. & WIGTON, A. Role of granular activated carbon surface chemistry on the adsorption of organic compounds. 2. Natural organic matter. *Environ. Sci. Technol.*, 33:3225-3233, 1999.
- KISSEL, D.E.; CABRERA, M.L. & FERGUSON, R.B. Reactions of ammonia and urea hydrolysis products with soil. *Soil Sci. Soc. Am. J.*, 52:1793-1796, 1988.
- KONONOVA, M.M. *Soil organic matter*. Oxford, Pergamon Press, 1966. 450p.
- KRAJEWSKA, B. Ureases I. Functional, catalytic and kinetic properties: A review. *J. Molec. Catalysis B: Enzymatic*, 59:9-21, 2009.
- LARA CABEZAS, W.A.R.; KORNDORFER, G.H. & MOTTA, S.A. Volatilização de nitrogênio da amônia na cultura de milho: I. Efeito da irrigação e substituição parcial da uréia por sulfato de amônio. *R. Bras. Ci. Solo*, 21:481-487, 1997.
- LIU, S.X.; CHEN, X.; CHEN, X.Y.; LIU, Z.F. & WANG, H.L. Activated carbon with excellent chromium(VI) adsorption performance prepared by acid-base surface modification. *J. Haz. Mat.*, 141:315-319, 2007.
- LOPES, A.S.; BASTOS, A.R.R. & DAHER, E. Fertilizantes nitrogenados no Brasil: Um problema de escassez. LOCAL, International Plant Nutrition Institute - Brazil, 2007. (Informações Agronômicas 120)
- MANUNZA, B.; GESSA, C.; DEIANA, S. & RAUSA, R. A normal distribution model for the titration curves of humic acids. *J. Soil Sci.*, 43:127-131, 1992.
- MATTILA, P.K. Ammonia volatilization from cattle slurry applied to grassland as affected by slurry treatment and application technique – first year results. *Nutr. Cycl. Agroecos.*, 51:47-50, 1998.
- MASINI, J.C.; ABATE, G.; LIMA, E.C.; HAHN, L.C.; NAKAMURA, M.S.; LICHTIG, J. & NAGATOMY, H.R. Comparison of methodologies for determination of carboxylic and phenolic in humic acids. *Anal. Chim. Acta*, 364:223-233, 1998.
- MORENO-CASTILLA, C.; LÓPEZ-RAMÓN, M.V. & CARRASCO-MARÍN, F. Changes in surface chemistry of activated carbons by wet oxidation. *Carbon*, 38:1995-2001, 2000.

- OUYANG, D.; MACKENZIE, A.F. & FAN, M. Phytotoxicity of banded urea amended with triple superphosphate and potassium chloride. *Agron. J.*, 90:734-739, 1998.
- PELS, J.R.; KAPTEIJN, F.; MOULIJN, J.A.; ZHU, Q. & THOMAS, K.M. Evolution of nitrogen functionalities in carbonaceous materials during pyrolysis. *Carbon*, 33:1641-1653, 1995.
- PIMENTA, A.S.; SANTANA, J.A.S.; ANJOS, R.M.; BENITES, V.M. & ARAÚJO, S.O. Caracterização de ácidos húmicos produzidos a partir de carvão vegetal de duas espécies florestais do semi-árido: Jurema Preta (*Mimosa tenuiflora*) e Pereiro (*Aspidosperma pyrifolium*). *R. Verde*, 4:1-11, 2009.
- REIS, C.; FONSECA, R.A.D.; REIS, E.L.; REIS, C.; BRIGHENTI, C.R.G. & MATIAS, A. Programa de ajuste multiparamétrico de curvas de titulação potenciométricas de ácidos húmicos. *R. Bras. Ci. Solo*, 34:569-573, 2010.
- RODRIGUES, M.B. & KIEHL, J.C. Volatilização de amônia após o emprego de uréia em diferentes doses e modos de aplicação. *R. Bras. Ci. Solo*, 10:37-43, 1986.
- SENGIK, E. & KIEHL, J.C. Controle da volatilização de amônia em terra tratada com uréia e turfa pelo emprego de sais inorgânicos. *R. Bras. Ci. Solo*, 19:455-461, 1995a.
- SENGIK, E. & KIEHL, J.C. Efeito de resíduos orgânicos e do fosfato monocalcico na volatilização de amônia em terra tratada com uréia. *R. Bras. Ci. Solo*, 19:321-426, 1995b.
- SHAFEEYAN, M.S.; DAUD, W.M.A.W.; HOUSHMAND, A. & SHAMIRI, A. A review on surface modification of activated carbon for carbon dioxide adsorption. *J. Anal. Appl. Pyrol.*, 89:143-151, 2010.
- SHIM, J.-W.; PARK, S.J. & RYU, S.-K. Effect of modification with HNO₃ and NaOH on metal adsorption by pitch-based activated carbon fibers. *Carbon*, 39:1635-1642, 2001.
- SILVA, A.J.; LIMA JÚNIOR, M.A.; FERREIRA, N.C.M. & FRAGA, V.S. Perdas de amônia por volatilização proveniente da uréia aplicada a solos dos trópicos úmidos. *R. Bras. Ci. Solo*, 19:141-144, 1995.
- SIVA, K.B.; AMINUDDIN, H.; HUSNI, M.H.A. & MANAS, A.R. Ammonia volatilization from urea as affected by tropical-based palm oil palm effluent (pome) and peat. *Comm. Soil Sci. Plant Anal.*, 30:785-804, 1999.
- SZYMANSKI, G.S.; KARPINSKI, Z.; BINIAK, S. & SWIATKOWSKI, A. The effect of the gradual thermal decomposition of surface oxygen species on the chemical and catalytic properties of oxidized activated carbon. *Carbon*, 40:2627-2639, 2002.
- SWIFT, R.S. Organic matter characterization. In: SPARKS, D.L., ed. *Methods of soil analysis. Part 3. Chemical methods*. Madison, Soil Science Society of America, 1996. p.1018-1020. (Soil Sci. Soc. Am. Book Series, 5)
- TROCA-TORRADO, C.; ALEXANDRE-FRANCO, M.; FERNÁNDEZ-GONZÁLEZ, C.; ALFARO-DOMÍNGUEZ, M. & GÓMEZ-SERRANO, V. Development of adsorbents from used tire rubber: Their use in the adsorption of organic and inorganic solutes in aqueous solution. *Fuel Proc. Technol.*, 92:206-212, 2011.
- TROMPOWSKY, P.M.; BENITES, V.M.; MADARI, B.E.; PIMENTA, A.S.; HOCKADAY, W.C. & HATCHER, P.G. Characterization of humic like substances obtained by chemical oxidation of eucalyptus charcoal. *Organic Geochem.*, 36:1480-1489, 2005.
- YOU, Z.L. & ZHOU, P. Synthesis, characterization and crystal structures of a pair of azido-bridged polynuclear Schiff base copper(II) complexes with urease inhibitory activity. *Transition Met. Chem.*, 33:453-457, 2008.
- WITTE, C.-P. & MEDINA-ESCOBAR, N. In-gel detection of urease with nitroblue tetrazolium and quantification of the enzyme from different crop plants using the indophenol reaction. *Anal. Biochem.*, 290:102-107, 2001.

