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High physical properties of cashew nut shell biochars in the adsorbtion of mycotoxins

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Abderahim Ahmadou[1,2,4]*, Alfredo Napoli[3], Noel Durand[1,2] and Didier Montet[1,2]

[1]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), UMR QualiSud, 73 rue Jean-François Breton, 34398 Montpellier Cedex 5, France.
[2]Qualisud, Université de Montpellier, CIRAD, Montpellier SupAgro, Université d'Avignon, Université de La Réunion, Montpellier, France.
[3]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), UR Biowoeb, 73 rue Jean-François Breton, 34398 Montpellier Cedex 5, France.
[4]Institut Polytechnique Rural de Formation et Recherche Appliquée (IPR/IFRA) de Katibougou, Annexe de Dar-salam Bamako, Mali.

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ABSTRACT

Biochars were produced from cashew nut shell by pyrolysis on tubular oven at 400, 600 and 800°C. They were then analyzed and characterized (CHN, volatiles and pH); and were tested at different pH (4.15, 6.54 and 9.05) under different adsorption conditions (filtration and stirring) for their ability to capture aflatoxins and ochratoxin A (OTA). Above 25 mg of biochar in 5 mL of water-methanol mixture containing the mycotoxins (aflatoxins and OTA), all biochars adsorbed up to 100% of the aflatoxins at all pH (4.15, 6.54 and 9.05) and under all conditions (filtration or stirring) and pyrolysis temperature. Biochars also showed no effect on aflatoxins adsorption. Great differences were observed for the adsorption rates of OTA in function of the studied conditions. The adsorption efficiency of biochar for OTA increased with the increase of the pyrolysis temperature, which increases the specific surface area. The method used (filtration or stirring) had a strong influence on the adsorption rate, ranging from 29% by filtration up to 52% for 1000 mg of a biochar in 5 mL of water-methanol solution; and stirring increased the adsorption rate. In general, pH had less effect on the adsorption rate of OTA (2-5%). OTA best adsorption rate was observed for biochar produced at 800°C. The affinity between biochars and aflatoxins is very strong because at the same experimental conditions and equal masses, biochars adsorb more aflatoxins than OTA (5 times more).

INTRODUCTION

In developing countries, cereals which constitute the staple food of the population are susceptible to fungal infections which result in mycotoxin contamination due to poor agronomic and postharvest practices (Okello et al., 2016). Mycotoxins are toxic secondary metabolites produced by filamentous fungi contaminating various food and feed crops posing serious health risks for both human and animal (Wu, 2007). The Food and Agriculture Organization estimates that one quarter of the world's food crops are affected by mycotoxins (CRA, 2011).

Although hundreds of fungal toxins are known but only few of them play an important role in food safety (Shepard, 2008). Up to now, approximately 400 secondary metabolites with toxigenic potential, produced by more than 100 molds have been reported (Jard et al., 2011). Fungal toxins of most concern are produced by

*Corresponding author. E-mail: abderahim.ahmadou@cirad.fr.

species within the genera of *Aspergillus, Fusarium*, and *Penicillium*, which frequently contaminate major food crops in the field and during storage (Redy et al., 2010). Among all mycotoxins, aflatoxins, fumonisins, zearelenone, ochratoxin and deoxynivalenol are five major groups of mycotoxins that are the most toxic to mammals (Karlovsky et al., 2016).

Biochar is a pyrogenic black carbon produced by pyrolysis conversion of biomass feedstock, including agricultural and forest residuals, in an inert atmosphere. Biochar has attracted great attention because of its potential to help mitigate climate change and improve soil fertility (Lehmann et al., 2007). In addition, many researchers have found that biochar can be used as an alternative adsorbent to remove different kinds of contaminants, including heavy metals, nutrients, and pharmaceuticals, from aqueous solutions (Zhou et al., 2013).

The mechanisms by which biochar increases soil fertility are not fully understood. Research has demonstrated that biochar application to soil increases the soil organic carbon, improves water holding capacity and water release, and soil aeration, increases the cation exchange capacity, neutralizes the pH of acidic soils and improves the soil microbial ecology (Sohi et al., 2010).

In addition to these purported benefits, biochar largely consists of a long life carbon fraction in soils, which has been demonstrated to be very stable, with a half-life of over 1000 years in the soil (Lehmann et al., 2007; Kuzyakov et al., 2009; Joseph et al., 2010; Zimmerman, 2010). Biochar is mainly produced by slow pyrolysis process due to elevated yields obtained.

When biomass is pyrolyzed in the aim of producing biochars, slow pyrolysis with moderate temperature (350–800°C) is normally adopted (Qi et al., 2017a). Within this temperature range, most of biochars are carbonized from biomass feedstocks.

Therefore, the remaining organic phase of biochars normally consists of carbonized organic matter that is more aromatic and more stable and non-carbonized organic matter that is relatively more aliphatic and less stable (Joseph et al., 2010; Chen et al., 2008). Biochar labile organic carbon phase can be easily oxidized during the biotic and abiotic ageing processes while the oxidation of more aromatic phase takes place more slowly (Mukome et al., 2013). Hence, the stability of biochars can influence their composition and thereby the sorption capacity (Qi et al., 2017b).

In 2015, West Africa and South-Eastern Asia are almost producing the same quantity of Raw Cashew Nuts (RCN) with around 1.500.000 Metric Tons each that represent around 90% of the world production together. Among the main cashew production areas, West Africa is the most recent and dynamic in the world.

In Mali, the cashew sector has developed in recent years due to the Spanish program CTARS and through the introduction of new seed varieties. The Malian annual production of cashew nut is 95.000 tons (Rabany et al., 2015).

Cashew nut shells are available in abundance because they represent 73% of cashew nut mass. At present, cashew nut shells are discarded by local cashew nut processors. Some are burnt as a mean for waste management. The burning of these biomass resources has serious socio-environmental problems including greenhouse gas emission and accumulation of tars and soot on houses close to the factory, leading to complaints from neighbors (Singh et al., 2006).

The objective of this research was to study the adsorption of two main families of toxic mycotoxins (aflatoxins and ochratoxin A) by various biochars obtained from cashew nut shell produced in three pyrolysis conditions. The results indicate the correlations between the biochar properties and its adsorption capacity of mycotoxins.

MATERIALS AND METHODS

Biomass

The cashew nut shell is a co-product resulting from kernel extraction activity. It was purchased from "Agribusiness Mali" located in Bamako, Mali and conditioned in a room at 20°C and at an hygrometry of 66% HR for more than 72 h in order to have a homogeneous biomass; its moisture and elemental composition was measured before pyrolysis. Biomass (cashew nut shell) was pyrolyzed without prior grinding.

Biochar production technology

Pyrolysis experiments were conducted in a horizontal fixed-bed tubular reactor (Figure 1) in which the temperature was controlled using a PID controller as described by Bordoloi et al. (2015). 20 g of biomass samples were placed into the reactor at ambient temperature; the heating rate of the reactor was 5°C per min to rise the selected peak pyrolysis temperature of 400, 600 and 800°C, under constant flow rate of nitrogen at 30 L/min. Residence time into the reactor was fixed at 1 h after reaching the peak temperature. The cooling step of biochar is done by natural convection. Mass yield of biochar was calculated using the following equation (Niandou et al., 2013):

$$\text{Mass yield (\%)} = \frac{Wf}{Wo} \times 100$$

Where, Wf is the mass (g) of the dry biochars and Wo is the mass (g) of the dry biomass.

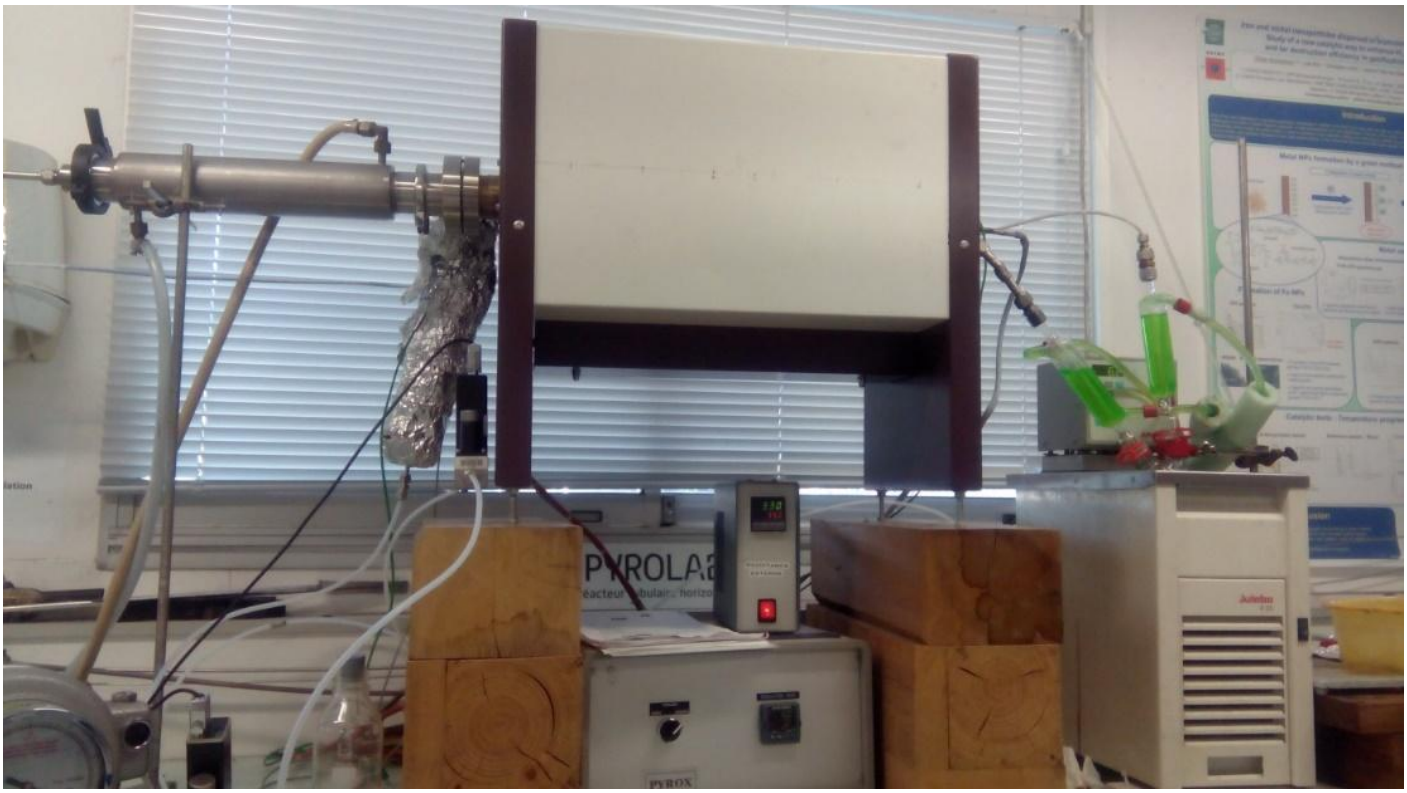

Figure 1. Biochar production equipment.

Biochar characterization

Before any analysis and tests, the different biochars were ground and sieved at 200 µm.

Immediate analysis

Moisture and ash contents analyses were done by heating the samples in oven air to 105°C for 24 h and to 710±10°C for 2 h, respectively, and weighing the residue. Volatiles analyses were done by heating the samples without air to 900°C for 7 min. Fixed carbon content was calculated by 1 – ash (wt.%) – volatile matter (wt.%). All measurements were conducted on the dry base.

Total elemental analysis

The total content of C, N and H were determined using a dry combustion method using Variomacrocube CHN analyzer. The O + S content was determined by subtracting the ash and C, N and H contents from the total mass of the sample.

pH determination

The pH of biochar was determined according to Novak et al. (2009) and Cheng and Lehmann (2009). Two grams of biochar (ground and sieved at 200 µm) were shaken with 40 mL distilled water for 30 min. This suspension was allowed to stand for 10 min before measuring the pH with a pH electrode pH Lab (Mettler Toledo).

Determination of specific surface area

Carbon dioxide adsorption isotherms were recorded on a 3FLEX Physisorption instrument (Micromeritics) at 273 K between 0 and 1 atm (that is, relative pressure between 0 and 0.028). The microporous specific surface areas of biochars were determined by applying the Langmuir model. Microporous volumes were determined at a relative pressure of c.a. 0.028.

Mycotoxins adsorption experimental procedure

The mycotoxins (aflatoxins and OTA) adsorption onto the biochar depended on biochar intrinsic properties,

modalities, and contact duration between biochar and mycotoxins. In this sense, two different contact methods have been considered. Both methods simulate short or long biochar-mycotoxin exposure time.

The three biochars produced at 400,600 and 800°C were previously ground and sieved at 200 µm. Contact between biochar and mycotoxins was done by a method called filtration method (short contact time) or by contact by stirring (stirring method – long time contact) described below.

Preparation of water-methanol mixture containing mycotoxins

Generally, pH plays an important role during mycotoxins adsorption because animal feed additives firstly gather in stomach where the pH value is below 3.5 and then pass through the intestine where the pH value is 6.5. pH has also an important effect on soil quality and crops yield.

Three different pH solutions were tested to measure the effect of pH on the adsorption of mycotoxins by biochars. To get an acidic pH, the pH of the water was decreased to 4.15 by using 1 M HCl solution. To get a basic pH, pH of water was increased to 9.05 by using 1 M NaOH solution. A 50/50 water-methanol solution (w/w) was prepared to solubilize mycotoxins standards (aflatoxins and OTA).

The first adsorption tests were carried out at 20 ng/mL for each mycotoxin and the results obtained showed a major difference in the adsorption rates depending on the modalities and the mycotoxin used. This reason led us to modify the mycotoxin concentration as follow: A solution of a pure mycotoxin standard was added to the 50/50 ultrapure water-methanol solution (w/w) to obtain a concentration of 38 ng/mL for OTA and 180 ng/mL for total aflatoxins. The mycotoxin standard was purchased from R-Biopharm France.

Filtration method

This method was chosen because it was close to natural conditions. It consists of covering the bottom of a funnel with hydrophilic cotton on which it was deposited constant masses of biochar 25, 100, 175, 250, 500 and 1000 mg. Subsequently, 5 mL of the 50/50 water-methanol solution (w/w) containing the mycotoxin at the above-mentioned concentration was filtered on the biochar during 1 to 2 min. The filtrate was recovered and filtered using a syringe and then analyzed by HPLC. The difference between the initial and final concentration was the amount of mycotoxin captured by the biochar. Each test was done in triplicate. Adsorption rate was calculated using the following equation:

$$\text{Adsorption rate (\%)} = 100 - \frac{MFC}{MIC} \times 100$$

Where, MFC is mycotoxin final concentration and MIC is mycotoxin initial concentration.

Stirring method

This adsorption method under improved experimental conditions allowed to quantify the effect of contact time and stirring mechanical force on the mycotoxin adsorption. It consisted of placing each mass of biochar (mentioned above) and 5 mL of 50/50 water/methanol solution (w/w) in a 50 mL falcon tube. The whole solution was stirred at 500 vibrations per min for 45 min. Each test was performed in triplicate.

Mycotoxins analysis

Ochratoxin A: OTA was quantified by HPLC with a fluorescence detector Shimadzu RF 20A, Japan (Nakajima et al., 1997). The operating conditions were as follows: injection volume of 100 µL; C18 reverse-phase HPLC column, uptisphere type, ODS, 5 µm particle size, 5 ODB, 250 × 4.6 mm, with identical pre-column, thermostatically controlled at 35°C; isocratic flow rate of 1 mL/min (mobile phase: methanol/water/acetic acid, 69/30/1); excitation wavelength of 333 nm and emission wavelength of 460 nm. The contents were calculated from a calibration curve established from an OTA standard (1 µg/mL; ref PD 226 R. Biopharm Rhône Ltd, Glasgow, UK).

Aflatoxins: Aflatoxins were quantified by HPLC with a fluorescence detector (Shimadzu RF 20A, Japan) (Nakajima et al., 1997) after post column derivatization with an electrochemical system (Kobra Cell™ R. Biopharm Rhône Ltd, Glasgow, UK). The operating conditions were as follows: injection volume of 100 µL; C18 reverse-phase HPLC column, Uptisphere type, ODS, 5 µm particle size, 5 ODB, 250 × 4.6 mm, with identical pre-column, thermostatically controlled at 40°C; isocratic flow rate of 0.8 mL/min (mobile phase: water/methanol, 55/45 with 350 µL Nitric acid 4 M and 119 mg/L potassium bromide); excitation wavelength of 362 nm and emission wavelength of 425 nm. The contents were calculated from a calibration curve established from an aflatoxins mix standard (ref TSL-108 R. Biopharm Rhône Ltd, Glasgow, UK).

RESULTS AND DISCUSSION

Characterization of the biomass sample

The moisture content of the cashew nut shell after

Table 1. Properties of biochar at different temperatures.

Sample	Pyrolysis temperature (°C)	Elemental composition			VM (%)	FC	Ash (%)	pH	Biochar yield (%)
		C (%)	H (%)	N (%)					
Cashew nut shell	400	70.04	3.65	0.61	21.90	69.85	8.25	10.61	25.02
	600	83.51	2.06	0.59	14.08	77.39	8.53	9.83	23.13
	800	87.42	0.85	0.89	10.22	77.13	12.65	9.81	21.54

VM, Volatile matters; **FC,** fixed carbon.

stabilization in climatic room was 5.35% at 20°C and the standard deviation was 0.28. Its Carbon, Hydrogen and Nitrogen contents were respectively 50.04, 3.89 and 0.42%. This material is suitable for biochar production.

Characterization of biochar

Biochar yield and ash content

As shown in Table 1, biochar yield decreased logically with increasing pyrolysis temperature. It may be due to the conversion of compounds such as cellulose, hemicellulose and lignin into carboneous products, water and CO_2. Biochar yield decreased from 25.02% for the biochar produced at 400°C to 21.54% for the biochar produced at 800°C (Table 1).

Ash content increased with the increase of temperatures from 8.85% at 400°C to 12.65% at 800°C (Table 1). This was due to the increase in the relative abundance of the minerals that were stable during carbonization (Bordoloi et al., 2015). In the other side, lignocellulosic biomass basic components are hemicellulose, cellulose and lignin.

Researchers have already confirmed that lignin starts decomposing at low temperatures (160–170°C) and continues to decompose at low rate until approx. 900°C. Hemicellulose is the second component to start decomposing, followed by cellulose, in a narrow temperature interval from about 200 to 400°C. This is the interval in which the main decomposition takes place and accounts for the greatest decomposition in the biomass pyrolysis process consisting of degradation reactions. Beyond 400°C, the most important reaction leads to the aromatization process (Fisher et al., 2002).

Elemental and volatile matters

The results of the proximate, ultimate analyses of the biochars are summarized in Table 1. They revealed that carbon content increased with pyrolysis temperature. The biochar produced at 800°C had a highest carbon content, that is, 87.42% and biochar produced at 400°C had the lowest carbon content (70.04%).

In contrast to carbon content, the volatiles matter logically decreased with increasing pyrolysis temperatures from 400 to 800°C. Recovered volatiles were highest with 21.90% in biochar produced at 400°C and 10.22% for biochar produced at 800°C.

pH evolution

Biochar solution pH varied from pH 10.61 (400°C) to pH 9.81 (800°C). Our biochars were strongly alkaline. Previous literature reported biochar pH values (without further processing of biochars) between pH 4 and pH 12, with typical values being above pH 7. Zhuo et al. (2013) found biochar pH levels between 8.8 and 10.8, depending of biomass feedstock type. The biochars with alkaline pH are often used to increase the pH of acid soils.

Specific surface area

The Langmuir surface area of cashew nut shell biochars was strongly affected by production temperature. The specific surface area of biochar pyrolyzed at 400°C reaches 151 m^2/g, climbs to 250 m^2/g at 600°C and dramatically increases to 306 m^2/g for the biochar produced at 800°C. The increased surface area suggests that micropores in biochar gradually developed with increased production temperature in 400–800°C, which was also observed in previous studies (Yang et al., 2015).

For the three biochars produced at 400, 600 and 800°C the micropore volumes are respectively 0.05, 0.09 and 0.11 cm^3/g (Table 2). The dramatic increase in surface area from 400 to 800°C was due to the decomposition of lignin and quick release of H_2 and CH_4, which generates significant densities of micropores (Zhao et al., 2017a)

Mycotoxin adsorption

Ochratoxin A adsorption by filtration method

The adsorption rate varied according to the biochar mass used and pyrolysis temperatures (Figure 2). The average

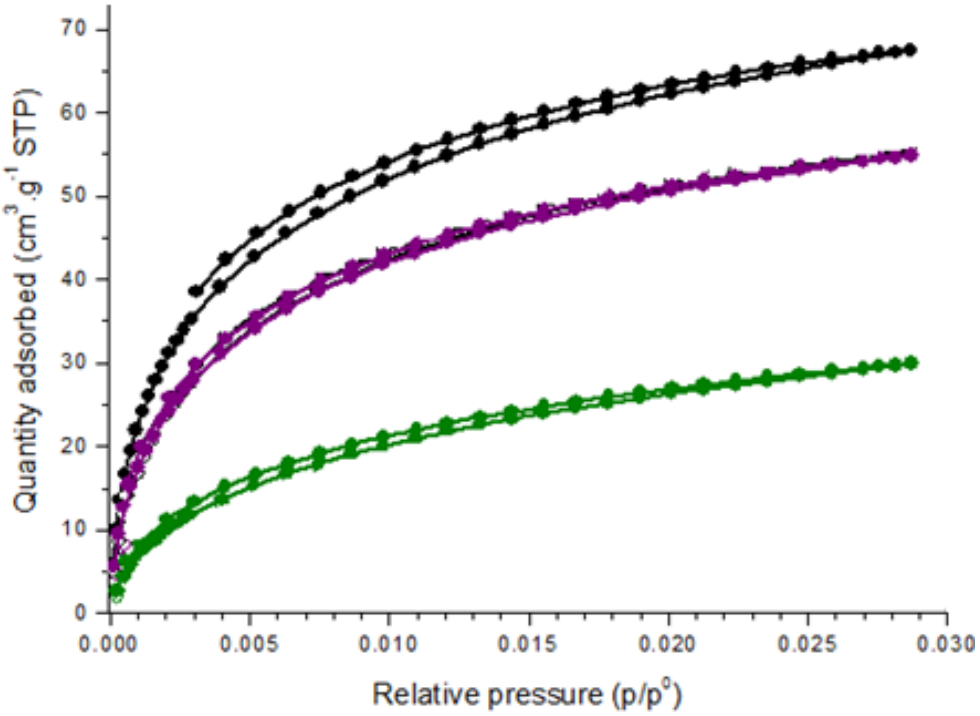

Figure 2. Influence of pyrolysis temperature on CO_2 adsorption by different biochars.

Table 2. Porous characteristics of biochars.

	Biochar 800°C	Biochar 600°C	Biochar 400°C
Specific surface area (m^2/g) [#]	306	250	151
Micropore volume (cm^3/g) [§]	0.11	0.09	0.05

[#], Langmuir theory; [§], Micropore volume determined at p/p0 0,028.

adsorption rate of OTA by 25 mg of the three biochars was 20% (400, 600 and 800°C) at pH 6.54 (Figure 3). There was an increasing in the adsorption rate of OTA directly linked to the biochar mass used. The OTA adsorption rates for 1000 mg of biochars produced at 400, 600 and 800°C were respectively 33.65, 39.45 and 52.73%. A significant change was observed with biochar mass increases, the adsorption rate for 25 mg biochar changed following the pyrolysis temperature, thus the adsorption rate was 4.53% for a biochar produced at 400°C, 9.57% for a biochar produced at 600°C and 45% for a biochar produced at 800°C. With 1000 mg of biochar at 400, 600, 800°C the adsorption rates were respectively as follows 29.00, 47.77 and 50.40%. The best adsorption rate was obtained by the biochar produced at 800°C.

Ochratoxin adsorption by stirring method

The adsorption rates were affected positively by the effect of stirring (Figure 4). This was due to the action of the mechanical stirring force and the duration of the contact time. There was also an increase in the adsorption rate depending on the mass of biochars used and from 500 mg of biochar, OTA adsorption rate becomes constant.

For a better observation of pH effect, OTA adsorption experiments using 1000 mg of the three different biochars (400, 600 and 800°C) were compared at three pH conditions, and the result was depicted in Figure 5. The adsorption capacity at pH 9.05 was lower than that at pH 4.15 and 6.54. The biochar produced at 400°C was the main affected by the change of pH; because at 400°C

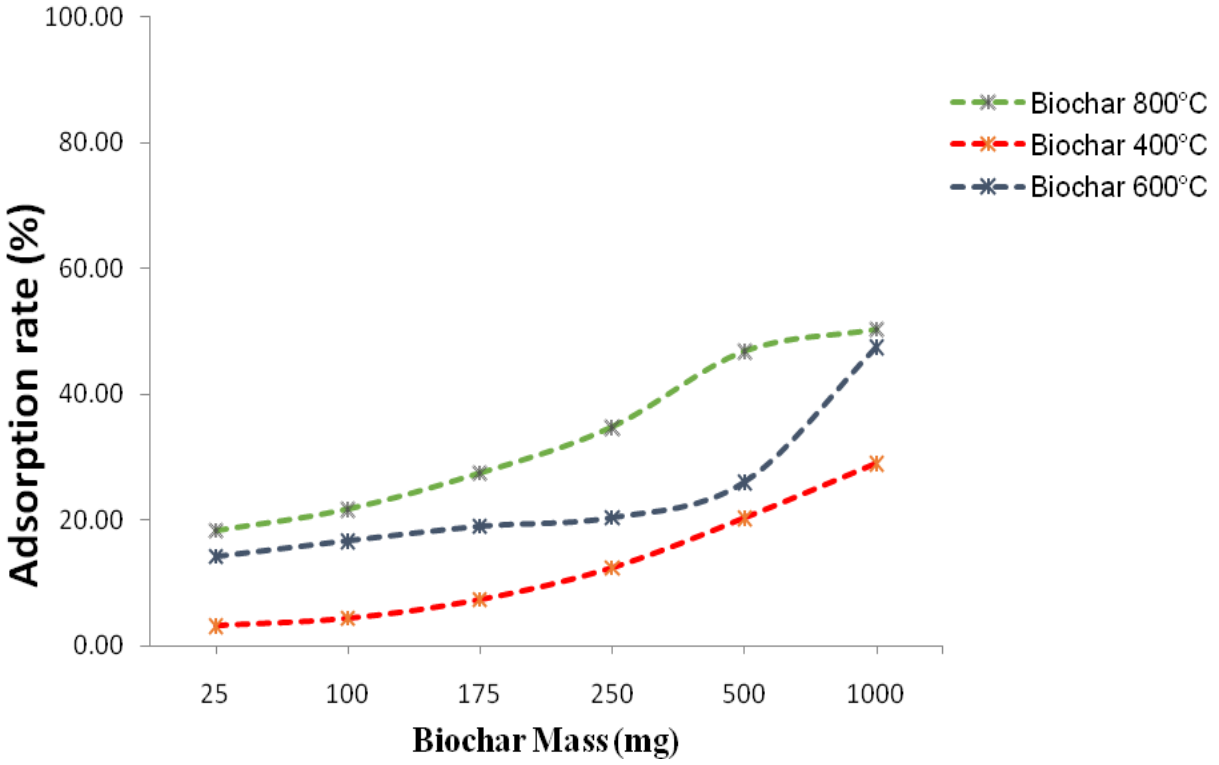

Figure 3. Ochratoxin A adsorption by filtration method using three different biochars (400, 600 and 800°C) at pH 6.54.

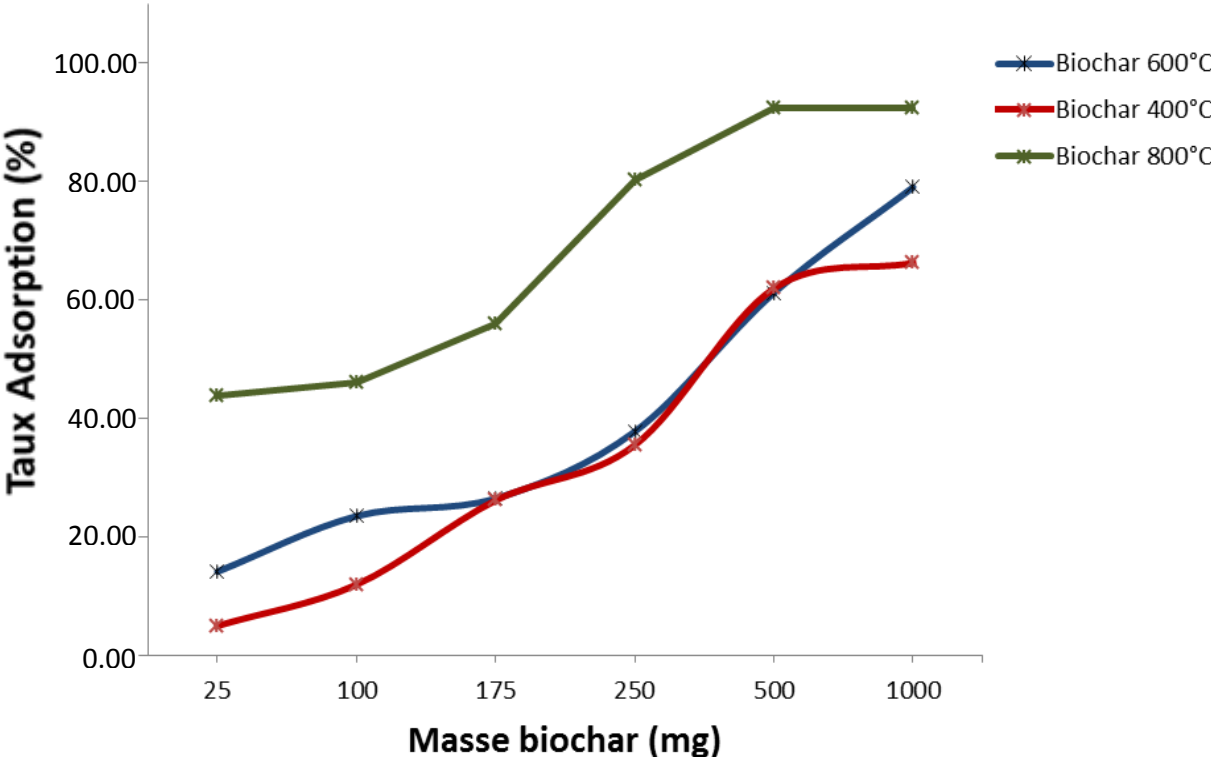

Figure 4. Ochratoxin A adsorption results by stirring method using three different biochars (400, 600 and 800°C).

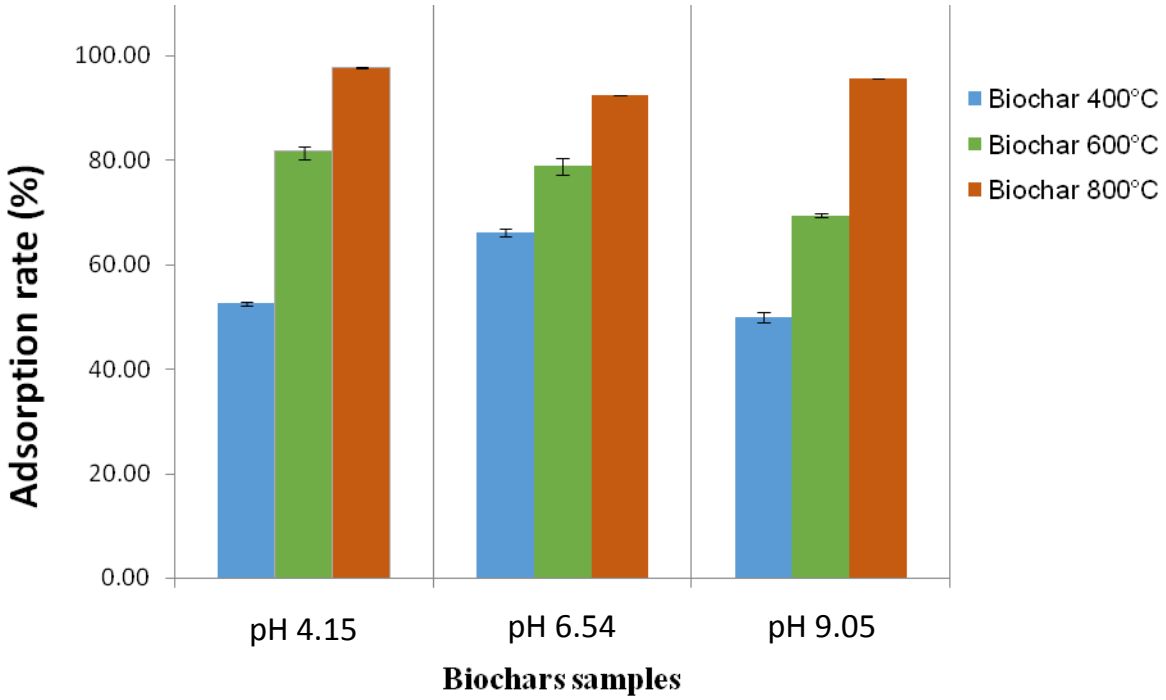

Figure 5. Ochratoxin A adsorption results by stirring method using three different biochars (400, 600 and 800°C) under different pH conditions.

the thermal decomposition of organic matter is incomplete and there may be traces of elements that may have pH-sensitive functional groups.

The best adsorption rate was obtained at pH 6.54. In general, for the three biochars tested, a slight pH effect can be observed and the best adsorption rates were obtained at pH 4.15 and 6.54. The pyrolysis temperature remained the most important parameter having an effect on OTA adsorption by the different biochars.

Aflatoxins adsorption by filtration method

The 3 biochars produced at 400, 600 and 800°C had the same adsorption capacities for aflatoxins (Figure 6). The pyrolysis temperature of biochar had not an important effect on aflatoxins adsorption. It was found that 1000 mg of each biochar adsorbed the totality of the aflatoxins (180 ng/mg).

By reducing the biochar mass from 1000 mg to 25 mg, the adsorption rate decreased from 99% to 73%. The study of pH effect on aflatoxins was carried out by using 25 mg of each biochar. The aflatoxins adsorption assays of the three biochars at three different pH (4.15, 6.54 and 9.05) by filtration method showed that the adsorption rate varied between 73 and 78% with an average adsorption rate of 75%. The pH does not appear as important on aflatoxins adsorption by the different biochars (Figure 7).

Aflatoxins adsorption by stirring method

By stirring, there was a total adsorption of aflatoxins by all biochars. The adsorption rate was 100% with all biochars masses used even at 25 mg (Figure 8).The pH and temperature of pyrolysis had not effects on aflatoxins adsorption by the different biochars.

The adsorption of the totality of aflatoxins can be explained by a high availability of specific surface area; and stirring promotes contact between biochar adsorption sites and the aflatoxins molecules. Phillips et al. (1995) suggests that one adsorbed AFB1 molecule occupied about 1.38 nm^2 surface area; or the cashew nut shell biochars specific surface areas are between 151 and 306 m^2/g as presented in Table 2 which is dramatically larger than the required specific surface area for aflatoxins sorption; which could explain why the pyrolysis temperature has few effects on biochars adsorption capacity and biochars could adsorb more aflatoxins because the adsorption limits are not reached.

Conclusions

Results of this research demonstrated that the laboratory-made biochars prepared from cashew nut shell could adsorb OTA and aflatoxins. Sorption studies showed that biochars adsorbed aflatoxins more efficiently (near 100%

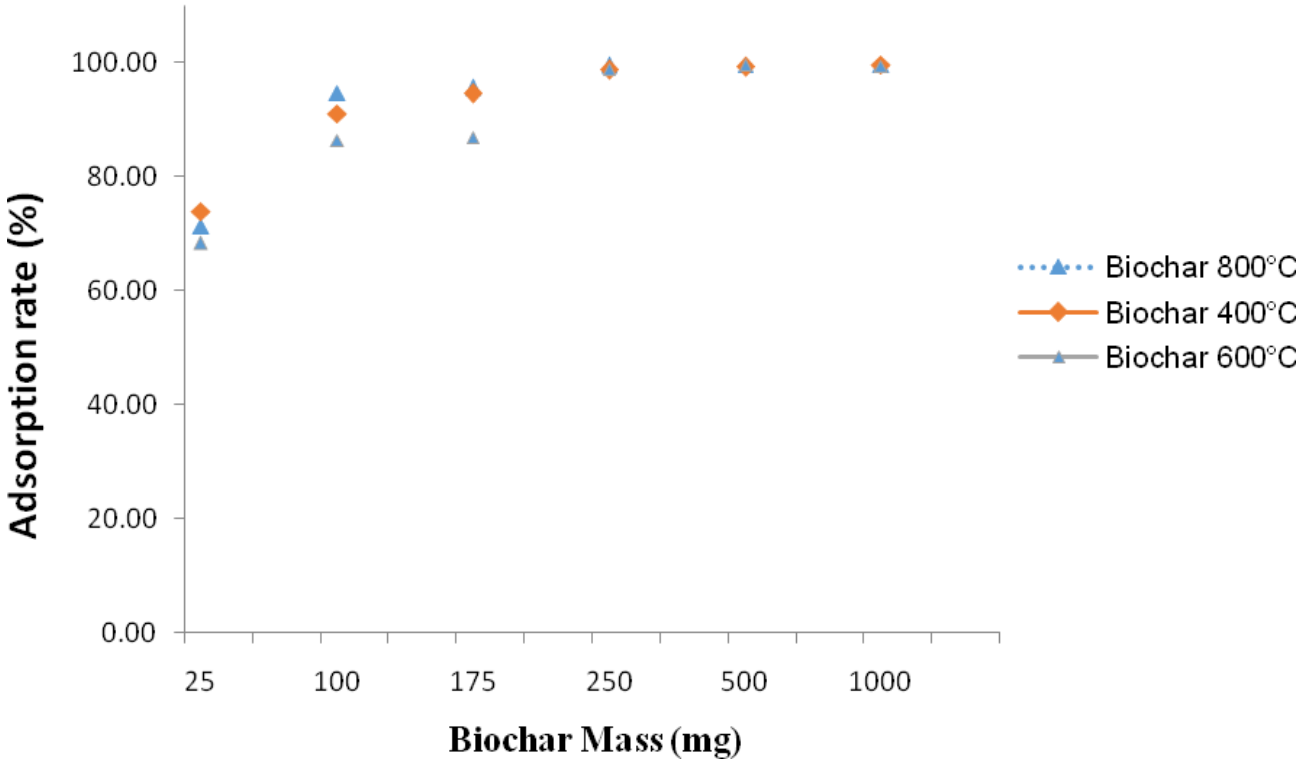

Figure 6. Aflatoxins adsorption test results by filtration method using three different biochars (400, 600 and 800°C).

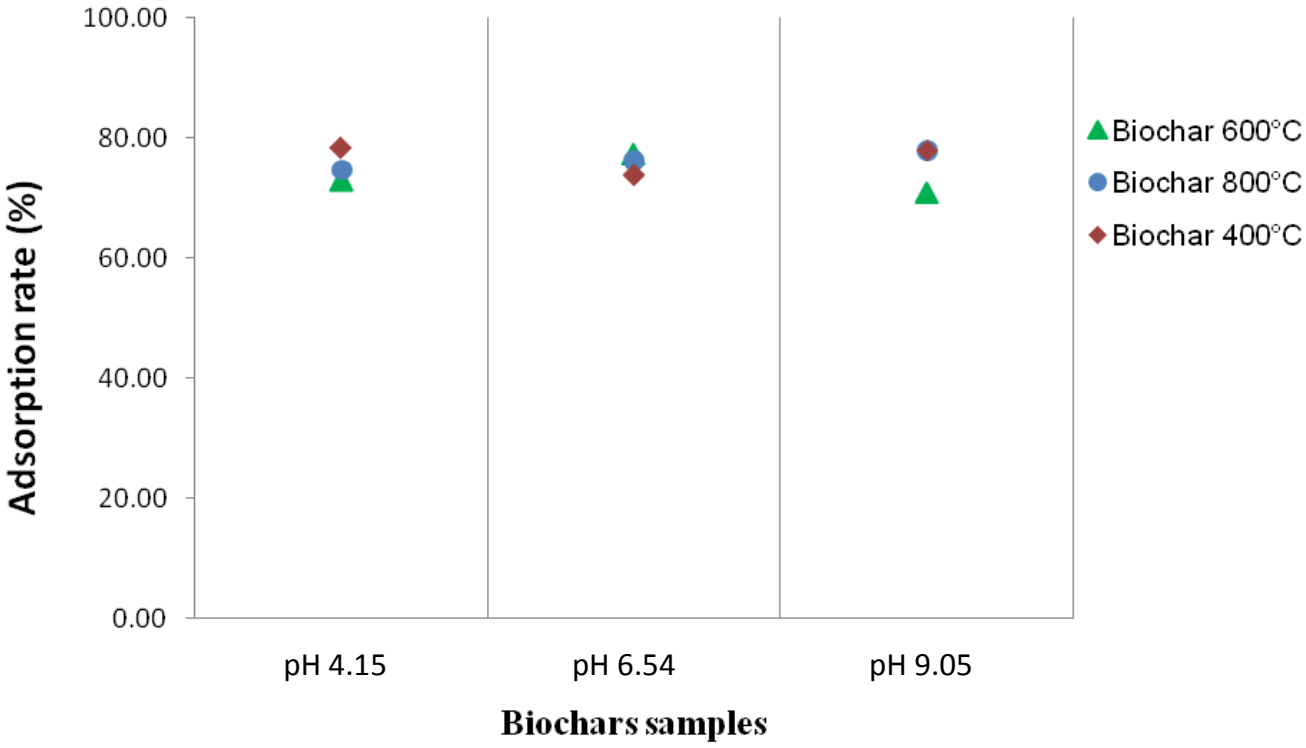

Figure 7. Aflatoxins adsorption results by filtration method using three different biochars (400, 600 and 800°C) under different pH conditions.

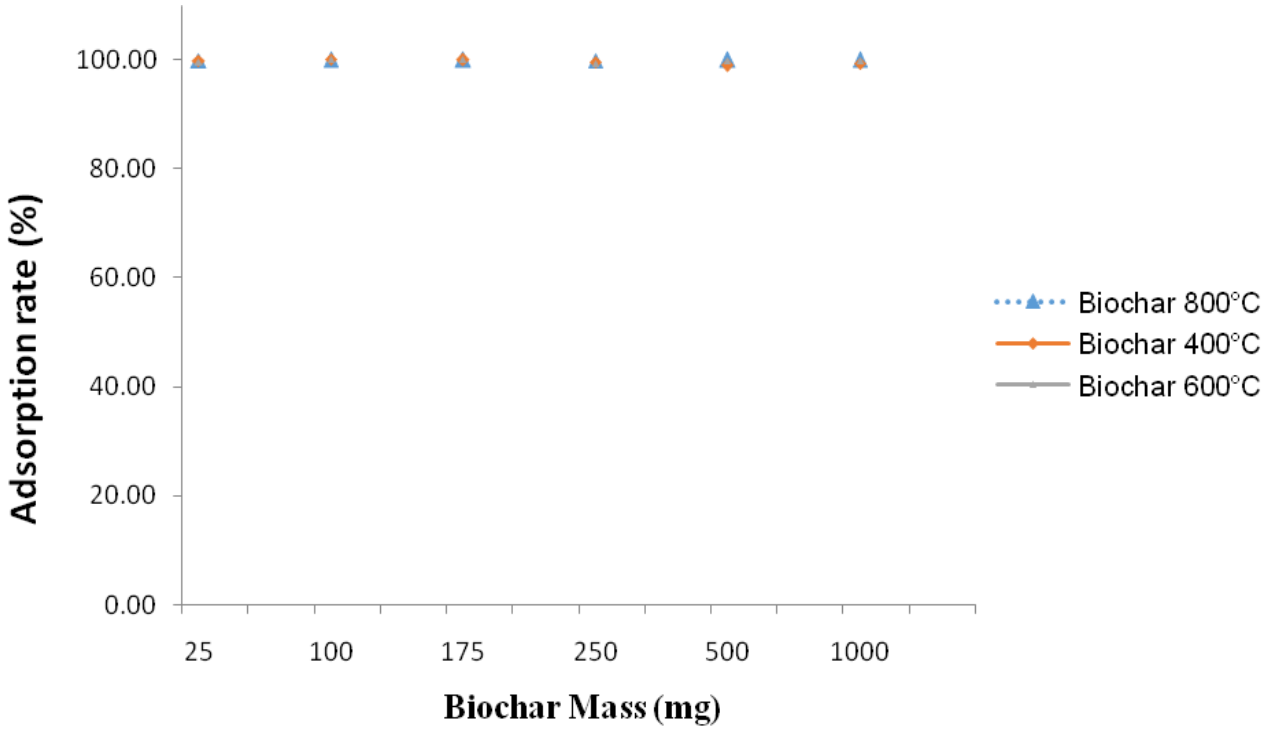

Figure 8. Aflatoxins adsorption test results by stirring method using three different biochars (400, 600 and 800°C).

in our conditions) than OTA. Pyrolysis temperature, biochar mass used, pH and adsorption conditions (filtration/stirring) were the most important parameters for OTA sorption.

For OTA, an increase in the pyrolysis temperature caused a great increase in the adsorption rate while pH has a weak effect on sorption (2-5%). Biochar produced at 800°C adsorbed almost 98% of the OTA by stirring at pH 4.15 while its rate was 52.7% by filtration. Above to 25 mg of any biochar, adsorption of aflatoxins was 100% and it was thus impossible to quantify the effect of all other parameters (pyrolysis temperature, stirring or filtration, pH, biochar mass). All biochars have the same aflatoxin adsorption rates regardless of the conditions. Biochar adsorbs more aflatoxins compared to OTA. It means that the molecules of aflatoxins had a better affinity for all biochars than OTA.

Future studies should focus more in detail on the physico-chemical mechanisms of OTA and aflatoxins sorption and study of adsorption equilibrium and kinetics.

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