Agricultural residues-based activated carbons impregnated with chitosan nanoparticles by hydrothermal treatment

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Abstract
Much research related to the production of materials with high-quality adsorption capacity from agricultural residues has been done. However, the adsorbents themselves do not have the antibacterial capacity, an essential property in different water treatment applications. Hydrothermal treatment is an environmentally friendly approach to impregnate nanoparticles over a substrate. This paper aims to obtain and characterize activated carbon made of corn cob and red mombin seed impregnated with chitosan via the hydrothermal treatment to be tested as an antibacterial material for aqueous phase applications. The FESEM micrographics, RAMAN spectroscopy, and FTIR-ATR analyses confirmed the presence of chitosan nanoparticles (NPs) over the surface of the activated carbons. The impregnation of Chitosan NPs affected the pore size distribution in the activated carbon, but not total pore surface area or pore volume. The impregnation of chitosan increases the antibacterial capacity against *Escherichia coli* and *Shigella flexneri*.

Keywords: agricultural biomass, chitosan, antibacterial capacity, fecal bacteria

1. Introduction
Adsorption with activated carbon is commercially the most used water treatment method because of its efficiency and relatively low cost. In that sense, it is still attracting the possibility to find new sources of raw materials for activated carbon production to replace the everyday use of coal. Agricultural residues have been used as raw materials for activated carbon production with adequate characteristics for the adsorption of pollutants from water [1-3], mainly based on their high specific surface area and their micro-mesoporous structure. Based on their properties, the use of agro-wastes as raw materials for this aim is an attractive alternative from the economic and environmental point of view.
Chitosan is another adsorbent obtained via deacetylation of chitin, the structural component of mollusks, insects, crustaceans, fungi, algae, and marine invertebrates. Chitosan is usually obtained from aquaculture and fishing industry residues. Chitosan has been successfully used as an adsorbent in aqueous solutions [4]. Chitosan as an adsorbent offers some advantages such as amine and hydroxyl groups in its chemical structure capable of sorb cations and anions through several mechanisms: chelation, ion exchange, or ion-pair formation [5]. However, as adsorbent offers disadvantages such as difficulty to recover during an actual application in the water treatment, its solubility in acid solutions, low mechanical strength, and low surface area [6], another unique feature of the chitosan is its well-known antibacterial activity over a broad range of bacteria mainly gram-negative [7].

In that sense, the impregnation of chitosan onto activated carbon would permit to obtain a material with specific features from both materials, basically the high surface area, mechanical strength, thermostability and insolubility of the activated carbons; and, on the other hand, the antibacterial capacity, and the incorporation of new active sites of the chitosan.

The primary source of fecal bacteria as a water contaminant is sewage which in rural and urban areas in developing countries is disposed into the rivers without treatment; with the sanitary problems that this supposes [8].

The present work aimed to produce and characterize activated carbons made of corncob and red mombin seed impregnated with chitosan via hydrothermal treatment for preliminary antibacterial tests against *Escherichia coli* and *Shigella flexneri*.

2. Experimental

2.1. Chemicals

ZnCl$_2$ (Emsure ACS, ISO, Reag. Ph Eur - Merck) and Chitosan (Fluka, viscosity $\geq$ 400 mPa.s) were used as received.

2.2. Production of activated carbon

Activated carbons were prepared from residual biomass of corncob and red mombin seed (coded as CC and RMS, respectively). Dried, ground and sieved raw materials (0.5 – 1 mm particle size) were mixed with ZnCl$_2$ (ratio 1/1, w/w) and put into a metallic reactor to be carbonized at 600 °C. The heating rate was 10 °C/min to reach the final carbonization temperature. After two hours, the samples were cooled down and were washed with an acid solution of HCl (0.15 N). The samples were exhaustively washed with distilled water. Finally, the materials were dried overnight at 80 °C and sieved to get 0.5 – 1 mm particle size. These samples of activated carbon were codified such as CC and RMS for corncob and red mombin seed, respectively.

A mixture of 50 mL of chitosan solution (1% in 1% acetic acid solution) and 1 g of activated carbon was stirred and heated at 100 °C for one hour in a closed steel reactor. Chitosan was impregnated onto the activated carbons using the wet impregnation method assisted with pressure. The pressure increased until 20 psig during the process. Then the impregnated AC was filtered, rinsed with distilled water, and dried at 40 °C. The samples code for activated carbons impregnated with chitosan were CC-Ch and RMS-Ch.

2.3. Characterization of activated carbons

Textural, morphological, and structural properties of the samples with and without impregnation were conducted. Textural parameters were calculated over the information of the nitrogen physisorption isotherms performed by a Gemini VII 2390 Surface Area Analyzer (Micromeritics, USA). The specific surface area ($S_{BET}$) was calculated by the Brunauer–Emmett–Teller theory using the values of relative pressure ($p/p_0$) in the range between 0.05–0.25. The total pore volume ($V_{tot}$) was determined using the nitrogen adsorption data at the maximum value of $p/p_0$ (~0.9900) from the nitrogen adsorption branch. Mesoporous surface area ($S_{meso}$) and microporous volume ($V_{micro}$) were calculated by the $t$-plot method.

The pH of point of zero charges ($pH_{PZC}$) of the samples, including the commercial Chitosan, was determined based on the pH drift method [9]. A 0.01 M solution of KNO$_3$ was prepared and bubbled
with nitrogen to avoid the CO$_2$ effect; then, the solution was divided into eight 250 mL flasks, each with 50 mL of the solution. The pH level was adjusted in each flask to reach values between 3 and 10. 0.1 g of the activated carbon samples were put into each flask, and they were shaken for 48 hours to reach equilibrium. After, the activated carbon particles were filtered, and the final pH was measured. The pH$_{PZC}$ was calculated from the interception between the curves pH$_{initial}$ vs. pH$_{final}$ and pH$_{pH_{init}}$ vs. pH$_{pH_{init}}$. These calculations were done twice, and the average was reported.

FESEM Micrographics of the samples were taken by field emission electronic microscopy Zeiss Ultra plus at different magnifications. The samples were coated with platinum before the analyses.

The Raman spectra of the materials were obtained using a high-resolution confocal µ-Raman system Horiba Jobin-Yvon LabRAM HR800, where the analysis area and depth can be limited to 1 µm and 2 µm, respectively. The system is equipped with a 488 nm (visible) laser source.

A diffractometer Rigaku Miniflex II Desktop was used to obtain the samples' X-ray diffraction (XRD) and explain their structural properties. The diffractometer was operated at 30 KV and 20 mA, with CuKα radiation and 3 deg/min scanning velocity.

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Fourier transformed infrared spectroscopy with attenuated transmitted reflectance (FTIR-ATR) was conducted to determine the surface functional groups in the produced samples, with and without impregnation. The equipment used was a spectrophotometer IRTracer 100 Shimadzu (Kioto, Japan) with the Attenuated Total Reflectance.

2.4. Antibacterial capacity

The antibacterial capacity of the samples was tested against *Escherichia coli* (ATCC 25922) and *Shigella flexneri* (ATCC 12022). The bacteria were cultured in Luria broth at 36 °C +/- 1 for 24 hours. Then, an aliquot of culture was suspended in physiological saline solution (10 mL) until reaching an approximate concentration of tube 0.5 of the Mac Farland nephelometer. After adding 0.1 g of the sample to the suspension, it was stirred for 2 hours at room temperature. Subsequently, 0.1 mL of the suspension was taken between 0 – 140 min and then diluted. The diluted solution was seeded with 15 to 20 mL of Muller-Hinton agar and incubated at 37 ° C for 24 hours [10-12]. The elimination of bacteria was calculated and plotted as a function of time.

3. Results and discussion

3.1. Material characterization

FESEM images of both samples (Figure 1) clearly show the impregnated chitosan on the activated carbon surface. It is possible to identify that chitosan nanoparticles (NPs) are impregnated in the internal channels of the activated carbons. However, the morphology of the impregnated chitosan NPs over both samples of activated carbon is different. The chitosan NPs over the activated carbon made of corn cob (Figure 1a, b) have an irregular tubular shape. On the other hand, over the activated carbon made of red mombin seed, the morphology of chitosan NPs have irregular shape (Figure 1d). Another difference is that NPs in the case of RMS activated carbon are almost flat, and in the case of CC, NPs are tubular with different height. CC present porosity inside the more significant pore, indicating of the well-developed pore structure in this activated carbon. In this sense, chitosan NPs could get inside the CC.

Chitosan NPs impregnated over corn cob activated carbon present particle size between ~100 – 300 nm. However, it is not discarded that there are Chitosan with slower size inside the pores.
Figure 1. FESEM images of the samples impregnated with chitosan, CC-Ch (a,b), RMS-Ch (c,d).

The shape of the nitrogen adsorption isotherms of the samples (figure 2) revealed that the activated carbons with and without impregnation are mainly microporous materials (isotherm type I according to IUPAC) [13]. Thus, all samples have a microporous-mesoporous structure. However, the presence of a hysteresis loop indicates a mesoporous structure as well.

Figure 2. Nitrogen adsorption isotherms at -77 °C of the produced samples.

Values of total specific surface area ($S_{BET}$), net pore volume ($V_{net}$), microporous volume ($V_{micro}$), and mesoporous surface area ($S_{meso}$) of the samples are shown in Table 1. $S_{BET}$ of bare and impregnated ACs are not significantly different, nor the $V_{net}$. However, the pore size distribution is affected by chitosan impregnation. In the case of RMS activated carbon, the impregnation slightly reduces the mesopores volume. Nevertheless, in the case of CC-activated carbon, the chitosan impregnation significantly increases $S_{meso}$ and decreases $V_{micro}$.
Textural properties of the bare ACs (CC and RMS) are different. CC has a higher $S_{BET}$ and it is a significantly more microporous structure. This micropores structure is affected by the impregnation. Still, the mesoporosity increases because of the incorporation of the Chitosan NPs in the shape of high tubes (as we observed in the FESEM pictures). It is not happened in the case of RMS, because the Chitosan NPs are almost flat. The microporous structure is decreased in the case of CC-activated carbon because chitosan NPs could partially block this region. In the case of RMS activated carbon, chitosan NPs might not access this region and not significantly affect it.

| Sample  | $S_{BET}$ (m$^2$/g) | $V_{net}$ (cm$^3$/g) | $V_{micro}$ (cm$^3$/g) | $S_{meso}$ (m$^2$/g) | pH$_{PZC}$ |
|---------|---------------------|----------------------|------------------------|---------------------|-----------|
| CC      | 1247.4              | 0.74                 | 0.51                   | 380.9               | 6.9       |
| CC-Ch   | 1215.2              | 0.72                 | 0.32                   | 673.1               | 4.8       |
| RMS     | 1194.7              | 0.70                 | 0.32                   | 661.4               | 6.8       |
| RMS-Ch  | 1168.9              | 0.69                 | 0.34                   | 586.4               | 5.3       |

The values of the pH$_{PZC}$ of the samples are shown in Table 1 as well. Both barely activated carbons present higher pH$_{PZC}$ than those of the impregnated activated carbons. The impregnation of chitosan in the activated carbons causes a decrease in the pH$_{PZC}$ of the activated carbons. It means that after impregnating Chitosan over the agro wastes based-activated carbons, the samples increase the amount of acidic functional groups, or the basic functionalities present in the activated carbon are occupied during the impregnation or both processes.

Figure 3 shows the Raman spectra of the analyzed samples. Two centered bands were identified for all samples, impregnated and without impregnation. One band, close to 1600 cm$^{-1}$, corresponds to G-band, and another one, close to 1350 cm$^{-1}$, corresponds to D-band. G-band is labeled as a graphitic band, while D-band is labeled as a disorder band. Both bands are typical for activated carbons [14] and describe the amorphous character of the prepared samples dominated by nanocrystalline forms. The ratio of the intensity of D-band ($I_D$) to the G-band ($I_G$) indicates that the samples impregnated with chitosan ($I_D/I_G$ equal to 0.95 and 0.92 for CC-Ch and RMS-Ch, respectively) present more disordered structure than the samples without impregnation ($I_D/I_G$ equal to 0.90 and 0.76 for CC and RMS respectively). The presence of chitosan over the activated carbon causes disorder in its structure.

Additional pick in the CC-Ch at 1103, 808 and 571 correspond to $\nu$(COC), NH$_{4}^+$ + C=O + CH$_3$ and $\nu$(CH) rings, respectively [15, 16]. All these peaks are because of the presence of chitosan NPs over the CC-activated carbon surface.

It is possible to notice in the X-ray spectra (Figure 3) that both impregnated activated carbons show two asymmetric bands corresponded to 2$\theta$=21.52° and 2$\theta$=43° for CC-Ch and 2$\theta$=21.36° and 2$\theta$=42.48° for RMS-Ch. The same bands are identified in the activated carbon made of corncob, but they are not apparent in the activated carbon made of red mombin seed. Those asymmetric bands could be assigned to disordered graphite in plane 002 and plane ten respectively, which is typical for the structure of activated carbon (2$\theta$=24° and 2$\theta$=42°) [17].

Additional picks that correspond to ZnO (Z) and SiO$_2$ (Q) are possible to identify in the X-ray spectra of the activated carbons without impregnation (CC and RMS). In the case of RMS, those picks are more intensive than those of CC indicated a higher amount of those impurities over the AC. ZnO impurities evolved from the chemical activator ZnO$_2$ during the carbonization process in the activated carbon production, and SiO$_2$ is evolved from the usual composition of the raw materials.

However, those picks are not present in the spectra of the impregnated activated carbons. This fact supposes that in the impregnation process, which occurs in acid media, high temperature and pressure conditions ZnO and SiO$_2$ are released from the activated carbon surface to the impregnation solution—comparing both activated carbons without impregnation, the sample RMS content more amount of ZnO than the sample CC.
Figure 3 RAMAN (a,b) and XRD (c,d) spectra of the produced samples.

The FTIR-ATR spectra of the activated carbons with and without impregnation and the commercial chitosan sample are shown in Figure 4. The similarities and differences between the spectra are identified in Table 2. Comparing commercial Chitosan and activated carbons impregnated with Chitosan, some situation can be found: i) the pick close to 2885 cm$^{-1}$ that suggest the presence of C-H groups, ii) the presence of the pick in 1645 cm$^{-1}$ for Chitosan and in 1687 cm$^{-1}$ for both impregnated activated carbons, which is assigned to the group amide I; however, the picks assigned for amide I and II only are present in the chitosan spectrum, iii) Picks assigned to saccharide structure is present as well only in the chitosan spectrum, not in the impregnated activated carbon spectra, iv) only the chitosan spectrum present broadband centered in 3372 cm$^{-1}$ corresponding to N-H and O-H groups.

There are three kinds of groups that are present in the spectra of activated carbon with and without impregnation, but not in the chitosan spectrum: i) the picks assigned to aliphatic groups (CH, CH$_2$ or CH$_3$) between 2100 – 1990 cm$^{-1}$; ii) the presence of broadband centered in 1548 cm$^{-1}$ which correspond to C-O of carboxyl groups; and iii) the presence of picks around 870 – 880 that could correspond to C-H, O-H, COO or C-O-C. Those three kinds of groups are common in activated carbons activated with ZnCl$_2$ [18].
Figure 4. FTIR-ATR spectra of the produced samples, with and without chitosan impregnation. Comparison to bare chitosan spectra.

Table 2. Functional groups according to FTIR-ATR analysis of the produced samples and commercial chitosan.

| Chitosan | CC    | CC-Ch  | RMS    | RMS-Ch  | Possible Functional groups | References                                      |
|----------|-------|--------|--------|---------|----------------------------|-------------------------------------------------|
| Broad band 3372 | ---   | ---    | ---    | ---     | N-H, O-H                   | Branca, D’Angelo, Crupi, Khouzami, Rifici, Ruello and Wanderlingh [19] |
| 2885     | ---   | 2885   | ---    | 2883    | C-H                        | Branca, D’Angelo, Crupi, Khouzami, Rifici, Ruello and Wanderlingh [19] |
| ---      | 2100, 1992 | 2104, 1992 | 2098, 1992 | 2100, 1992 | Aliphatic $\text{--CH, ---CH}_2\text{--CH}_3$ | Angin [18]                                      |
| 1645, 1590, 1317 | ---   | 1685   | ---    | ---     | amide I, amide II, amide III | Branca, D’Angelo, Crupi, Khouzami, Rifici, Ruello and Wanderlingh [19] |
| ---      | Broad band 1548 | Broad band 1548 | Broad band 1548 | Broad band 1548 | C-O carboxyl groups | Mohammadi, Karimi, Afzali and Mansouri [20] |
| 1147, 1060, 1024, 1024, 891 | ---   | ---    | ---    | ---     | Chitosan saccharide structure C-O-C | Branca, D’Angelo, Crupi, Khouzami, Rifici, Ruello and Wanderlingh [19] |
| ---      | 875   | 873    | 879    | 879     | C-H, O-H, COO, C-O-C       | Luan and Yang (2005)                              |
The impregnation of chitosan over the activated carbon was effective at the experimental conditions. The high temperature and the pressure had a positive effect on the impregnation of the chitosan via two mechanisms: i) the improvement of the mass transfer of the chitosan solution through the pore structure caused by the viscosity reduction of the chitosan solution and the additional driving force generated by the pressure; ii) the reduction of the molecular weight of the chitosan caused by the exposition of chitosan to high temperature in acid media [21] that allow the chitosan molecules to access deeper inside the porosity and to have small particle size.

During the impregnation of the chitosan over activated carbons is possible to identify to facts: i) chitosan is protonated in the acid solution and is charged positively; and ii) since the $pH_{PZC}$ levels of the bare activated carbons are 6.8 and 6.9 and the $pH$ of the solution is in the acid region ($3 – 4$), the activated carbons have a positive net charge [22]. Although it could be a drawback for the impregnation, it is well known that activated carbons present an amphoteric behavior [23] and contain positive and negative active sites (see Figure 4 and Table 2). Therefore, the impregnation was succeeded.

### 3.2. Antibacterial capacity

*Escherichia coli* and *Shigella flexneri* were selected in this study because both are enterobacteria, Gram-negative bacilli and are usually in urban wastewater. The samples of activated carbons impregnated with Chitosan could eliminate *Escherichia coli* and *Shigella flexneri* bacteria (Figure 5a, b). RMS-Ch eliminated both bacteria more efficiently than the sample CC-Ch, being *Shigella flexneri* more sensitive than *Escherichia coli*. All curves reach a maximum value of elimination and then stay almost constant. The presence of Chitosan and nanoparticles gives the adsorbents the antibacterial capacity [24, 25].

The bare activated carbon made of red mombin seeds exhibited antibacterial properties at the level of the impregnated activated carbons. It is based on the presence of ZnO on the surface that has antibacterial and antifungal activity [26]. The inhibition of bacterial growth observed may be due to the formation of a cationic bond with zinc and the amino group, which, by joining the negative part of the cell wall and the membrane of the microbial cell, damages the exchange of nutrients with the medium [25], and loss of plasma content [27], including the toxicity produced by the increase of reactive oxygen species (ROS) [28], causing a decrease in microbial growth.

The bare activated carbon made of corncob does not exhibit antibacterial capacity. The curve with an increasing linear tendency for eliminating both bacteria by this sample results from the accumulation of the bacteria on the surface of activated carbon. However, it is necessary to mention that Chitosan, be it from shrimp or squid feathers, may present less efficacy when functionalized with high activated carbon content, as demonstrated by Muangchinda et. al. [29] with the elimination of diesel oil. This might be happened with the RMS-Ch.

As demonstrated by Tang et al. [30], activated charcoal alone does not inhibit the growth of *E. coli* and *Staphylococcus aureus* bacteria. However, when functionalized with Chitosan, these bacteria were partially inhibited. And when the activated carbon is functionalized with AgNPs, there is no microbial growth. Therefore, the antibacterial activity is improved through the functionalization of the AgNPs in the activated carbon/Chitosan, similar to that obtained in the present work. In this way, the use of nanomaterials, which exhibit attributes such as solid adsorption, superior redox, and photocatalytic activity, potentially eliminate water-borne pathogens [31].
Figure 5. The antibacterial capacity of the investigated material against *Escherichia coli* (a,b) and *Shigella flexneri* strains (c,d).

4. Conclusions
Activated carbon prepared from corn cob and red mombin seed was successfully impregnated with chitosan via wet impregnation assisted by pressure (20 psig) and heat (100°C). The FESEM micrographics and FTIR-ATR analyses confirmed the presence of chitosan over the surface of the activated carbons. Chitosan is homogenously distributed with particles sizes between 100 – 300 nm and donut morphology. The activated carbon sample impregnated with Chitosan presents fewer impurities and more structure disorder than the bare samples. The combination of chitosan and activated carbon produces a material with higher antibacterial effectiveness than bare samples.

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