



Silica-based bioactive solids obtained from modified diatomaceous earth to be used as antimicrobial filler material



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ABSTRACT

It is well known that microbiological spoilage in indoor surfaces has a negative impact on human health. Antimicrobial functionalized materials are intensely studied. The present work seeks the synthesis of eco-friendly and cheap bioactive hybrid filler from diatomaceous earth (DE). The activation method used proved to be efficient to enhance the amount of quaternary ammonium groups supported by DE which was corroborated by spectroscopic methods and the thermogravimetry analysis. Zeta potential measurements revealed a bilayer arrangement of the ammonium groups on the functionalized solids and the bioassays showed their antimicrobial activity.

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1. Introduction

Complex mixture of microorganisms, toxins, allergens, volatile microbial organic compounds and other chemicals are known to affect the health of people living or working in dwellings and buildings [1]. Therefore, new materials need to be developed to control and prevent microbial contamination [2]. The association of an antimicrobial agent in an organic or inorganic matrix to be used in paints and coatings is an interesting way to achieve the objective mentioned above [3].

The present work value is the application of an economic, eco-friendly and abundant natural resource like the diatomaceous earth (DE) as interesting framework, whereby an active molecule could be associated to be used as part of a material or a protective coating as a filler. DE is a common name for the unconsolidated sediment of the amorphous silica (opal, $\text{SiO}_2 \cdot n\text{H}_2\text{O}$) which constitutes cell walls of dead diatoms [4]. The bioactive agent selected to be used in this work was a quaternary ammonium salt (QAS), hexadecyltrimethylammonium bromide ($[\text{CH}_3(\text{CH}_2)_{15}\text{N}(\text{CH}_3)_3]\text{Br}$), a cationic surfactant. The aliphatic QAS are highly soluble and have shown to be safe and their degradation products did not cause genotoxic effects [5].

The aim of this research was to obtain a functionalized natural silica-based material from diatomaceous earth and a QAS to be

used as bioactive additive. The raw DE and the modified ones obtained were characterized by scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR), zeta potential (ZP), differential thermal analysis (TGA) and X-ray diffraction (XRD). The assessment of antimicrobial activity was made by diffusion agar method with fungal (isolated from biodeteriorated coatings) and bacterial (importance in nosocomial infections) strains.

2. Material and methods

2.1. Alkaline activation

The DE used in the present work comes from Río Negro, Argentina. The main components of the fresh material are 75.6% SiO_2 , 14.1% Al_2O_3 , 4.2% Fe_2O_3 , 3.6% MgO and 2.5% CaO . Ten grams of DE was treated with 200 ml of NaOH solution 2.2 M [4]. The procedure was carried out at 100 °C on a stirrer/hot plate for 2 h, keeping the volume of liquid stable. Afterward, the sample solution was also filtered and washed with distilled water (DW) to remove residues and lowering the pH to 9. The resulting solid (DEa) was finally dried at 100 °C.

2.2. Obtaining of the modified DE

The DEa was mixed in 100 mL of DW with the QAS in a relation 1:1 by weight respectively. It continued stirring for 24 h at room temperature. Then, the solids obtained were centrifuged, washed with DW and dried at 55 °C. The same procedure was performed

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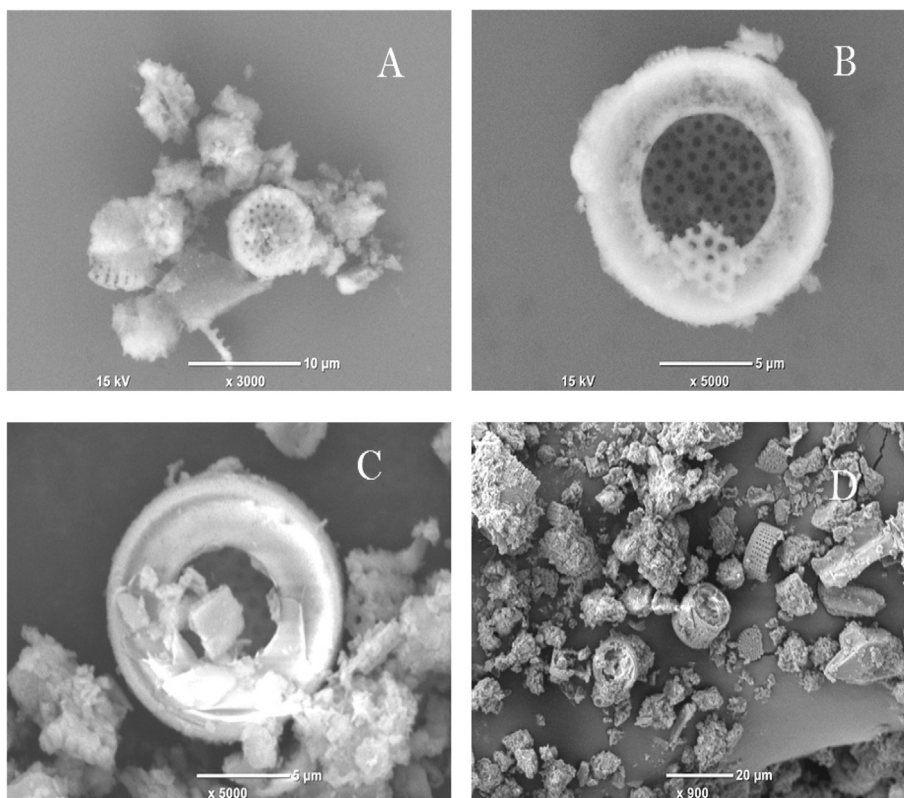


Fig. 1. SEM micrograph of: (A and B) raw diatomaceous earth (DE), (C) DE activated and (D) activated with QAS (DEaQ).

with DE (without activation). The obtained products were denoted DEQ and DEaQ, respectively.

To quantificate the QAS associated, quaternary ammonium groups were extracted with 50 mL of CH_3COONa 2 M solution per gram of treated DE by continuous stirring for 24 h. Then the solid was separated by centrifugation and QAS was quantified in the supernatant. The concentration was obtained spectrophotometrically at 645 nm based on the effect of quaternary ammonium groups on the Al^{3+} with the dye Chrome Azurol S. The measurements were performed in a spectrophotometer Spectrum (SP 2000 UV).

2.3. Characterization

The observation of the surface appearance of the samples was made by Scanning electron microscopy (SEM) and was performed using a field emission gun scanning electron microscope JCM-6000.

The Fourier transform infrared spectroscopy (FTIR) spectra of the DE, QAS and the modified DE were obtained using the potassium bromide disk technique and a Perkin-Elmer Spectrum One FTIR spectrometer. To determine the thermal degradation thermogravimetry analysis (TGA) tests were performed using a Rigaku Thermo plus EVO instrument. Samples of 10 mg were placed in an aluminum pan and heated from 30 to 1000 °C, at a scanning rate of 10 °C/min in air atmosphere with a gas flow rate of 40 mL/min.

X-ray diffraction (XRD) was employed to assess the crystallinity changes between samples. A Philips 3020 equipment was used to record XRD patterns (001 reflection), in the range of $3 \leq 2\theta \leq 70^\circ$. The operating conditions were: 40 kV and 30 mA, Cu $K\alpha$ radiation, Ni filter, a step width of 0.02°, and 2.0 s/step counting time.

Zeta potential (ZP) measurements to obtain the charges at the surface of the mineral particles were carried out with a Zeta Potential Analyser 90 Plus/Bi-MAS Multi Angle Particle Sizing (Brookhaven Instruments Corporation) at constant ionic strength of 10^{-3} M KCl. Samples were prepared at several pH values and pH was equilibrate over night by addition of concentrated HCl or KOH solutions dropwise.

2.4. Antimicrobial activity

The bioactivity of the modified DE and controls were assessed by the agar-diffusion method. The microorganisms used were *Chaetomium globosum*, *Alternaria alternata*, *Escherichia coli* ATCC 11229 (Gram-negative) and *Staphylococcus aureus*, ATCC 6538 (Gram-positive). The concentration was adjusted to 10^5 spores/mL to the fungal species and 10^6 CFU/mL to bacterial strains. Then 15 mL of the corresponding sterilized melted media, RB based agar to fungi and R3 Agar to bacterial strains were inoculated with the microbial suspensions correspondingly. Wells were made in seeded agar plates and each one was filled with 20 mg of the tested material. All the plates were incubated at 25 °C and 37 °C for fungal and bacterial strains respectively and finally inhibition zones diameters were measured.

3. Results and discussion

3.1. Characterization

The microstructure of the raw DE is showed in Fig. 1(A and B), micrographs present a regularly porous arrangement with radial symmetry (disc-like). Activation with NaOH caused partial loss of

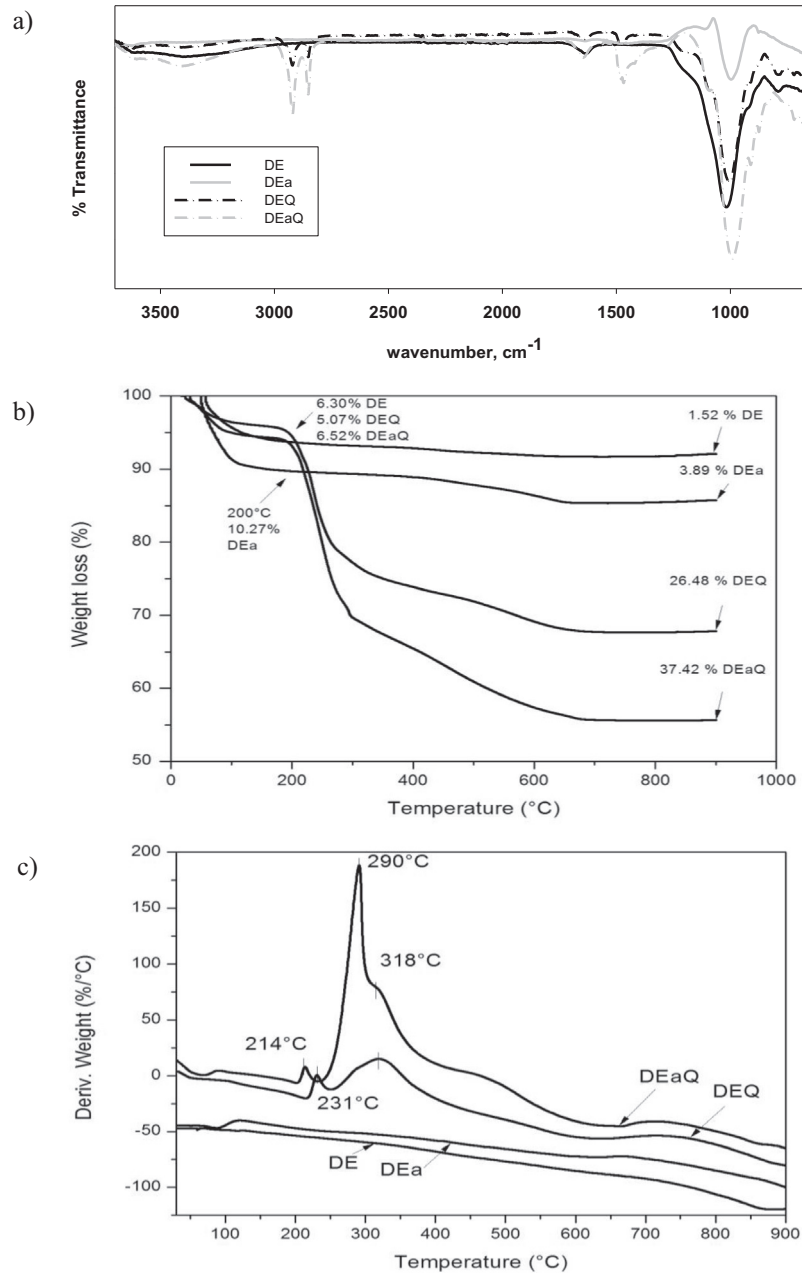


Fig. 2. (a) FTIR spectrum and (b-c) TG–DTA curves of: raw diatomaceous earth (DE), with QAS (DEQ), activated DEa and activated with QAS (DEaQ).

the skeletal structure, but some structures with original geometry can be observed in Fig. 1(C). This shows that the alkaline treatment only generates reactive sites without totally loss of original structure. The DEa hybrid with QAS presents no significant variation of the morphology, Fig. 1(D).

The amount of the incorporated QAS to the samples of DE and DEa obtained by spectrophotometry was found to be 3 and 7 mg/g to DEQ and DEaQ respectively. This shows that the activated DE would be more effective to support quaternary ammonium groups.

FTIR spectra in the Fig. 2(a) show that both DEQ and DEaQ present peaks at 2920, 2850 and 1480 cm^{-1} which correspond to the ammonium groups. These are more intense when corresponds with the activated DE. Besides, all the samples presented one intense peak around 1000 cm^{-1} that corresponds with silicon compounds.

Fig. 2(b and c) illustrates the TG analysis and the DTA curves of the raw and modified diatomite. Two distinct phases of mass loss during the heating process can be distinguished for the raw and modified samples respectively in the Fig. 2(b). The first phase appear as a combined steep slope of the TG curve in 30–200 $^{\circ}\text{C}$ range with weight loss of 6.3% and 10.27% for DE and DEa, respectively. These correspond to loss of physically bonded water. Different weight loss to DEa should be explained by increase of hydrophilicity by appearance of $\text{Na}_2\text{O} \cdot n\text{SiO}_2$ groups on the surface. The second step observed between 200 $^{\circ}\text{C}$ and 900 $^{\circ}\text{C}$ were assigned to the dehydroxylation of some Si-OH groups on the external surface with a mass loss of 1.52% and 3.89% to DE and DEa, respectively.

For DEQ and DEaQ mass loss 5.07% and 6.52% respectively, from 30 to 200 $^{\circ}\text{C}$ corresponded to the dehydration. The mass loss observed within the second step (200–900 $^{\circ}\text{C}$) attributed to the

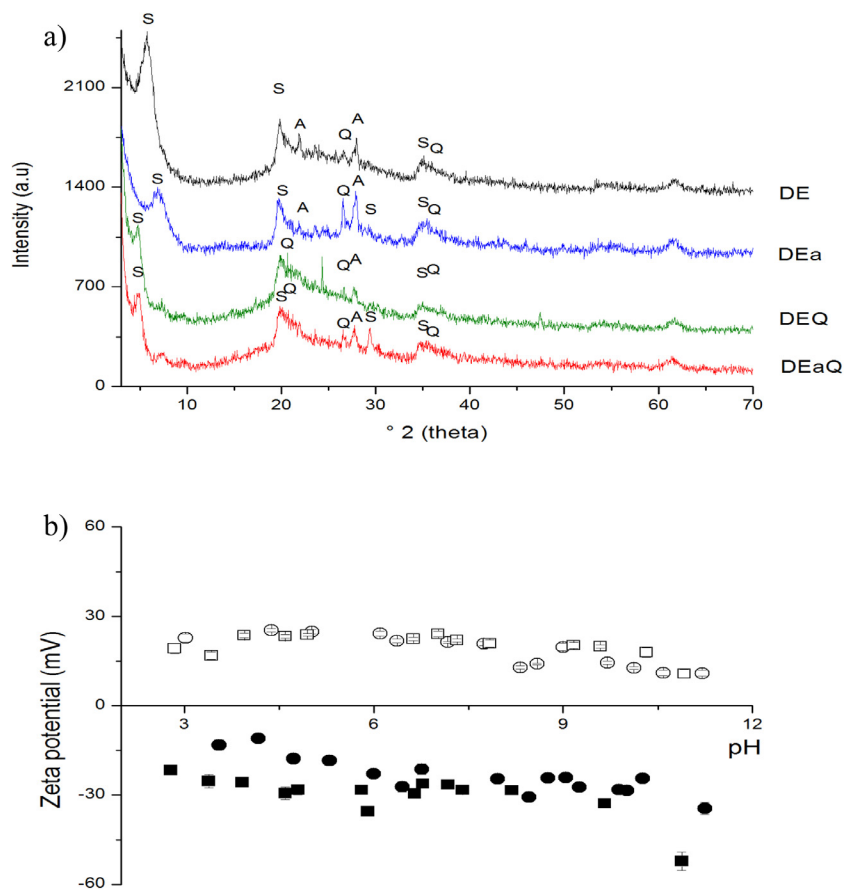


Fig. 3. (a) XRD patterns (A) Albite, (Q) quartz, (S) Smectite and (b) Zeta potential in function of pH for: (■) DE, (□) DEQ, (●) DEa and (○) DEaQ.

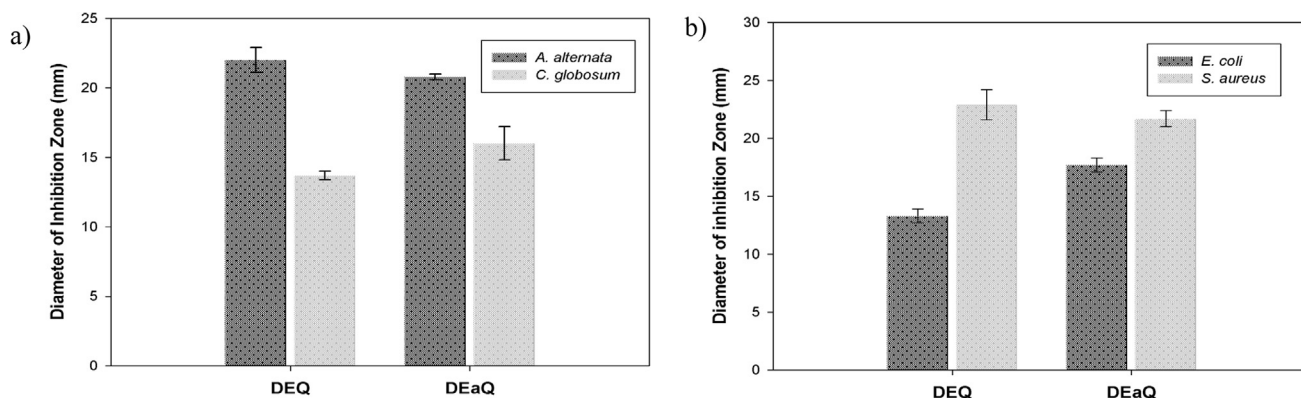


Fig. 4. Diameters of inhibition zone by agar diffusion tests against fungal (a) and bacterial (b) strains with DE samples with QAS (DEQ) and activated with QAS (DEaQ). The raw DE and DEa no presented inhibition zones in any case.

partial decomposition of QAS which reflects considerable complexity of the thermal decomposition/combustion of the organic components. The exothermic peaks at 231/318 °C and 214/290/318 °C of DEQ and DEaQ respectively, corroborate these results on the DTA curves in the Fig. 2(c). Further increase of the temperature of 290–500 °C caused the complete decomposition of QAS. A similar behavior in the DEaQ was observed but with a higher total mass loss (~40%).

X-ray diffractograms of DE, DEa, DEQ, and DEaQ samples are presented in Fig. 3(a). The XRD pattern of DE is characteristic of one broad reflection centered at approximately 22.5°, which is in

good agreement with that of the reference opal-A structure of amorphous hydrated silica and traces of minerals such as albite (A) quartz (Q) and smectite (S). The XRD pattern of DEa was similar to that of DE, indicating that the NaOH etching did not produce an important effect in the diatomite crystallographic mineral structure. Diffraction peaks at $2\theta = 20.8^\circ$, 26.6° and 36.5° correspond to quartz, at $2\theta = 22.0^\circ$, 27.8° and 28.0° correspond to albite and those at $2\theta = 5.8^\circ$, 19.8° and 29.1° correspond to minerals clays, probably smectite type. Precisely speaking, the smectites undergo swelling and dehydration and this is evidenced at peak d (001). The added surfactant produced a decrease at d (001) in the case of DEQ and

DEaQ. Smectites generate an intermediate increase in the interlamellar space approximately 3 and 5.8 nm for DE and DEa, respectively.

The ZP values of the different samples as a function of pH are shown in Fig. 3(b). The results indicated that surfaces of the raw and activated diatomite possess a net of negative electrostatic charges in the pH range from 2 to 12. The bilayer arrangement formed on the basis of hydrophobic (chain–chain) interactions should be completed, with some positive ammonium groups oriented out of the surface. This arrangement was evident in the positive charge ZP of both samples DEQ and DEaQ.

3.2. Antimicrobial activity

The diffusion test results are presented in Fig. 4(a and b), data is expressed as mean \pm SD of three experiments. Raw DE and activated DE no showed antimicrobial activity. DE modified with QAS, DEQ and DEaQ, presented clear inhibition zones around the samples against all test microorganisms. *A. alternata* and *S. aureus* were the most susceptible with higher diameters of inhibition zones compared to the other ones. The results were similar to DEQ and DEaQ.

4. Conclusions

Diatomaceous earth can be modified with quaternary ammonium groups to obtain hybrid materials that could be used as antimicrobial agents. The activating method shows to be more effective to achieve the stated objective with a concentration of quaternary ammonium salt of more than twice compared to the other one.

The present work show the feasibility of use modified diatomaceous earth as antimicrobial filler material. Therefore, the exploitation of local deposits of diatomaceous earth results promising due to the important added value of its applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.matlet.2017.01.144>.

References

- [1] World Health Organization (WHO), Guidelines for indoor air quality: dampness and mould, <<http://www.who.int/indoorair/publications/7989289041683/en/>>, 2009. (accessed 05.09.16).
- [2] F. Siedenbiedel, J.C. Tiller, Antimicrobial polymers in solution and on surfaces: overview and functional principles, *Polymers* 4 (2012) 46–71.
- [3] G. Sørensen, A.L. Nielsen, M. Pedersen, S. Poulsen, H. Nissen, M. Poulsen, S.D. Nygaard, Controlled release of biocide from silica microparticles in wood paint, *Prog. Org. Coat.* 68 (2010) 299–306.
- [4] W.T. Tsai, K.J. Hsien, J.M. Yang, Silica adsorbent prepared from spent diatomaceous earth and its application to removal of dye from aqueous solution, *Colloid Interface Sci.* 275 (2004) 428–433.
- [5] E. Grabinska-Sota, Genotoxicity and biodegradation of quaternary ammonium salts in aquatic environments, *J. Hazard. Mater.* 195 (2011) 182–187.