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EVALUATION OF THE MICROBIOLOGIC PROPERTIES OF BENTONITE SUBMITTED TO IONIC EXCHANGE WITH Ag^+ AND TREATED IN DIFFERENT ACID ENVIRONMENTS

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Abstract

The various types of bentonite existent in nature present many application possibilities in industry. A significant number of industries, from the ceramic sector to the cosmetics, the market for the bentonite are of thousands of tons. In this context, the practice of study in the attempt to amplify its scope of application may present possibilities of application in new products or in new industrial sectors. One of the possibilities to achieve that is to endow this clay with bactericide properties, allowing it to be applied as a bactericide additive in whatever product that requires those particular properties. Antimicrobial materials are compounds which possess the ability to inhibit growth or kill determined microorganisms.

In this particular work the possibility of incorporating bactericide properties in bentonite was evaluated. After its characterization, the bentonite was submitted to treatments with different acid solutions with different times of immersion. The following were used as acid solutions: chloride acid, sulfuric acid and sodium carbonate. The treated bentonite was grinded and submitted to ionic exchange with silver nitrate in an environment at 430°C, after that it was prepared for the proper microbiologic tests. Standard Agar-Diffusion microbiologic tests and ICM - Inhibitory Concentration Minimum were applied for microbiologic characterization. The bacteria used in the microbiologic tests were the *Escherichia coli* (gram positive) and *Staphylococcus aureus* (gram negative). The microbiologic results showed it are possible to incorporate the bactericide active principle into the bentonite and that the bactericide effect is dependant of the acid solution employed in the acid treatment.

Keywords: ionic exchange, bactericide bentonite, silver nitrate, microbiological materials.

INTRODUCTION

Since the ancient times, it is known that human beings develop activities of great relevance when it comes to ceramic tiles, involving more specially clays. Clays have not only been used as domestic utensils, bricks and such. This class of materials also covers applications as paints and even cosmetics.

A clay mineral, the bentonite was determined by Roos and Shannom in 1926 as being a rock consisting of a montmorillonite (smectite) clay mineral (SANTOS, 1992). This clay mineral presents quite peculiar properties, which makes it a very versatile material. The reason for that is because the smectites possess a high capability of swelling, a high area of surface, tixotropy and capability of undergoing ionic exchange.

Many applications within bentonite have been studied by industries and laboratories. The ability of the bentonite to remove ions in different environments is object of studies and application by industries. Naseem et. al. studied the ability of the bentonite clay to remove Pb(II) from aqueous solutions and from nitric acid, hydrochloric acid and perchloric acid solutions at different optimized conditions of concentrations(2) and removal of Pb(II) and Ni(II) from aqueous solutions by sorption onto natural bentonite was investigated by Donat et. al.. Experiments were carried out as a function of particle size, the amount of bentonite, pH, concentration of metals, contact time, and temperature. Donat soggier that natural bentonite is suitable as a sorbent material for recovery and adsorption of metal ions from aqueous solutions(3). He HP at. al. realized studies involving electron paramagnetic resonance (EPR) and X-ray diffraction (XRD) in montmorillonite. These studies show that there are two ways for the adsorption of Cu (II) ion by montmorillonite: exchangeable and specific(4).

The capacity of the bentonite to adsorb some ionic specimens has been studied too. Adsorption of Cu(II) by raw bentonite (RB) and acid-activated bentonite (AAB) samples were investigated as a function of the initial Cu(II) concentration by Eren at. al.. Eren available the effect of structural charges on the reactivity of the edge groups was evidenced by the particular proton adsorption behavior of the bentonite samples and showed that the positions and shapes of the fundamental vibrations of the OH and Si-O groups were influenced by the adsorbed Cu(II) cations(5).

Aiming to enhance the range of application of the bentonite, this paper presents results of studies directed toward the incorporation of microbiologic properties to the bentonite. Specifically, procedures prior to the acid treatment of the bentonite in environments containing HCl and environments containing H_2SO_4 were evaluated, as well as its ability to incorporate bactericide properties. In the successive evaluations of the bactericide properties of the bentonite Agar diffusion and Inhibitory Concentration Minimum tests were employed. For microorganisms *Staphylococcus aureus* and *Escherichia coli* were used.

EXPERIMENTAL

Bentonite preparation

In the experimental procedures of this paper the bactericide effects incorporated to the bentonite submitted to the process of ionic exchange containing silver nitrate ($AgNO_3$) were evaluated.

Samples of both raw bentonite and bentonite submitted to chemical acid treatment were submitted to the ionic exchange. The bentonite was chemically treated in a solution containing H_2SO_4 (4 N) and in a solution containing HCl (8 N). The procedures for the chemical treatments in acid solution consisted of mixing the bentonite in chemical solution at a temperature of 90 °C and keep it under magnetic agitation for 2 hours. Next, the sample was removed and dried for 24 hours under 55 °C.

Ionic Exchange in Ag^+ environment

The raw bentonite and the one submitted to chemical treatment were submitted the process of ionic exchange in environments containing $AgNO_3$. In the procedure a ionic environment containing $NaNO_3$ and $AgNO_3$. The bentonite was added to the ionic environment and heated to 435 °C for 4 hours. This process was conducted using 23 g of bentonite, 3 g of $NaNO_3$ and 0.5 g of $AgNO_3$.

Once the ionic exchange was completed, the bentonite was left submerse and resting for 24 hours in distillated water. This step had as primary objective to dissociate the compounds that weren’t incorporated to the bentonite’s structure. After the dissolution the bentonite was dried in a vacuum system and then grinded again, to guarantee the initial grain distribution.

Microbiological tests

The samples which underwent the process of ionic exchange were submitted to microbiologic tests with the objective of evaluating their respective bactericide effects. The tests used were the Agar Diffusion and Inhibitory Concentration Minimum. The evaluation of the bactericide activity *in vitro* was made through the method of Agar-Diffusion. The sensitivity of Gram-positive (*Staphylococcus aureus* ATCC 25923 - *SA*) and Gram-negative bacteria (*Escherichia coli* ATCC - *EC*) were analyzed.

For the tests two small amounts of the test material were placed in each Petri dish. The dishes were incubated at (37.0 ± 0.5) °C for 24 hours. The analysis was made through the measurement of the inhibitory halos starting at the samples border and going until reaching the closest colony. The Inhibitory Concentration Minimum tests were made according to the methodology BY MacFarland scale(9).

All stages of the microbiologic characterization were made in the laboratories of the *Core of Antimicrobial Materials’ Study and Development at the Universidade do Extremo Sul Catarinense/UNESC.*

RESULTS AND DISCUSSIONS

Figure 1 presents Agar Diffusion microbiologic results for the raw bentonite without being submitted to ionic exchange. The results show the absence of bactericide and bacteriostatic principles of the mineral, for both types of bacteria, *EC*, Figure 1a; *SA*, Figure 1b.

Figures 2a and 2b present microbiologic results for the bactericide bentonite without acid treatment. Figures 2c and 2d, for the bactericide bentonite treated in HCl acid solution; Figures 2e and 2f for the one treated in H_2SO_4 solution. The results indicate the presence of bactericide action for both bacteria, and that the bentonite treated with HCl has a greater effect.

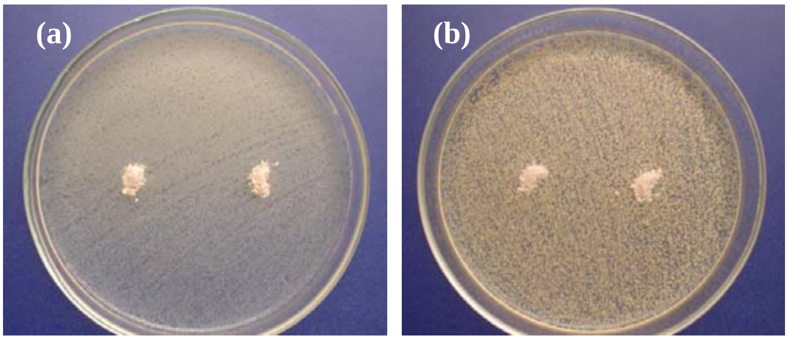

Figure 1 – Microbiologic tests applying the Agar-Diffusion method. (a) Raw *EC*, (b) Raw *SA*.

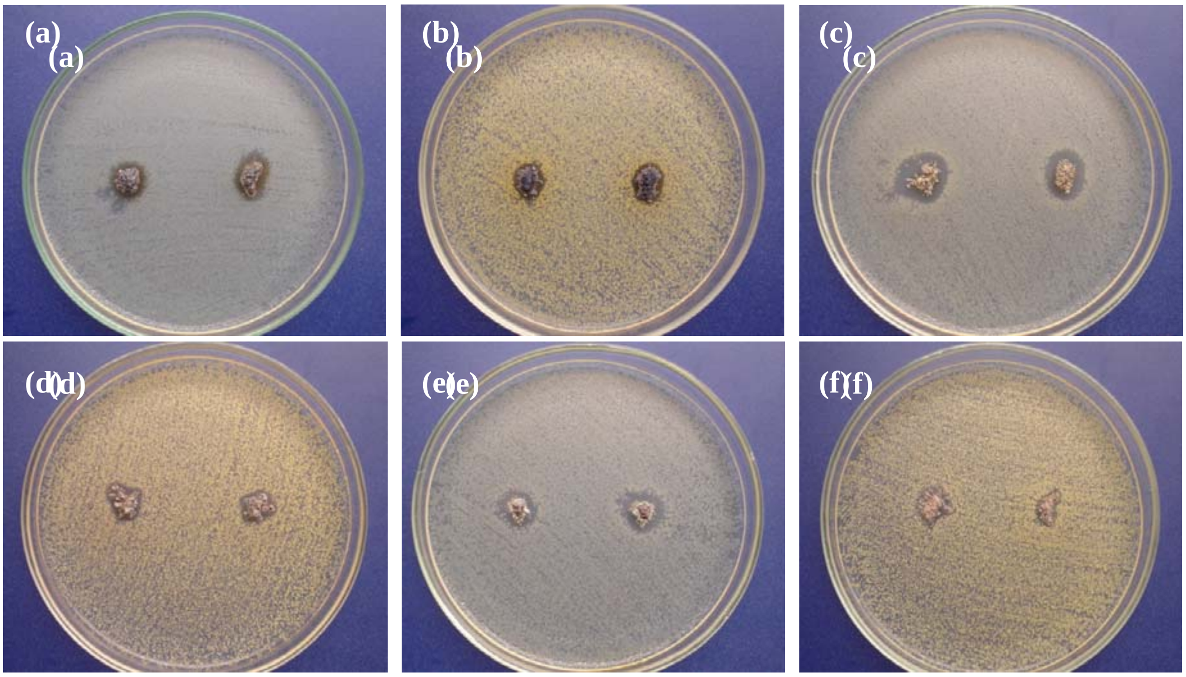

Figure 2 – Microbiologic tests applying the Agar-Diffusion method. (a) Raw bentonite + $AgNO_3$ - *EC* bacteria, (b) Raw bentonite + $AgNO_3$ - *SA* bactéria, (c) Bentonite (HCl) + $AgNO_3$ - *EC* bactéria, (d) Bentonite (HCl) + $AgNO_3$ - *SA* bactéria, (e) Bentonite (H_2SO_4) + $AgNO_3$ - *EC* bacteria e (f) Bentonite (H_2SO_4) + $AgNO_3$ - *SA* bacteria.

Figure 3 presents a comparison between the bentonite submitted to ionic exchange without acid treatment and the ones pre-treated in acid environments and then submitted to ionic exchange, regarding the bactericide effect evaluated by the Agar Diffusion microbiologic test. The results indicate the treatment in environment containing HCl provides the bentonite with better microbiologic properties, compared to treatments in acid environments containing H_2SO_4 for both types of bacteria, *SA* (Figure 3a) and *EC* (Figure 3b).

The greater effect presented by the bentonite treated with HCl may be an indication that the treatment with hydrochloridric acid favors with Ag^+ the ionic exchange mechanisms of the bentonite, promoting consequently the incorporation of a higher concentration of ionic silver in the bentonite structure. Since the bactericide effect is associated with the action of the silver ions in the cellular structure of the bacteria the bactericide principle becomes more significant for the mineral because it incorporated a greater number of these ionic species during the ionic exchange procedures.

Table 1 shows microbiologic tests using inhibitory concentration minimum methodology comparing the bactericide effect incorporated in the bentonite pre-treated with HCl and with H_2SO_4. The Inhibitory Concentration Minimum microbiologic tests show the raw bentonite doesn't present bactericide properties, while the ones submitted to ionic exchange in environments containing silver present such effect. The results indicate a greater bactericide capacity for the bactericide bentonite pre-treated in acid environment containing HCl for both types of bacteria. Considering the tests with *SA* bacteria, the bactericide Inhibitory Concentration Minimum for the bentonite pre-treated with HCl is of 0.022 g, while for the one pre-treated with H_2SO_4 is 0.038 g. For the *EC* bacteria the bactericide Inhibitory Concentration Minimum is 0.012 g for the bentonite pre-treated with HCl and of 0,038 g for the one pre-treated with H_2SO_4.

The results of inhibitory concentration minimum show a greater bactericide effect for bacteria of the *SA* type. Such effect is expected, since the *SA* bacteria are gram-positive and present less bactericide resistance when compared to *EC*, gram-negative. However this effect is observed only for the bentonite pre-treated with HCl. It is pointed out that the results are in accordance with the results presented by the Agar Diffusion tests.

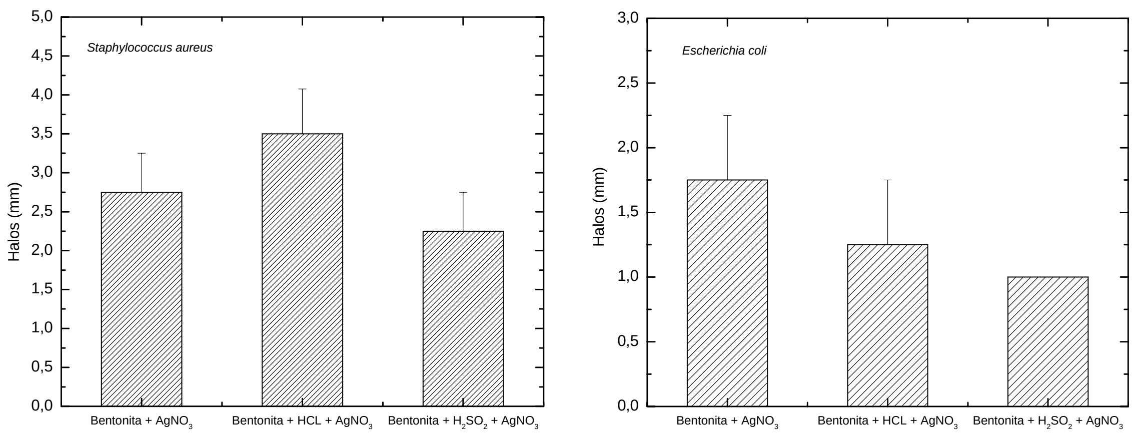

Figure 3 - comparison between Agar Diffusion microbiologic test to bentonite submitted to ionic exchange without acid treatment and the ones pre-treated in acid environments. (a) Bacteria *Staphylococcus aureus* e (b) bacteria *Escherichia coli*.

Table 1 – Microbiologic tests applying inhibitory concentration minimum method.

Staphylococcus aureus							
treatments	0,346g	0,200g	0,115g	0,066g	0,038g	0,022g	0,012g
Raw	*	*	*	*	*	*	*
HCl	---	---	---	---	---	*	*
H_2SO_4	---	---	---	---	*	*	*
Escherichia coli							
treatments	0,346g	0,200g	0,115g	0,066g	0,038g	0,022g	0,012g
Raw	*	*	*	*	*	*	*
HCl	---	---	---	---	---	---	*
H_2SO_4	---	---	---	---	*	*	*

* no inhibition --- inhibition

CONCLUSIONS

The proceedings adopted for the obtention of bentonite with bactericide properties showed to be satisfactory. The Agar Diffusion and Inhibitory Concentration Minimum microbiologic tests indicated the presence of significant bactericide effects for the bentonite submitted to ionic exchange in environment containing ionic silver species. They also indicated that the bentonite submitted to pre-treatments in acid environments containing HCl present better bactericide properties compared to the ones submitted to treatments in acid environments containing H_2SO_4.

Generally, it was noticed that there are possibilities of aggregating microbiologic properties to the bentonite. More detailed studies may indicate optimization routes for these properties and detail the mechanisms associated to these properties and the acid activation mechanisms of the mineral.

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