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Synthesis of Silver-Treated Bentonite: Evaluation of its Antibacterial Properties

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In this study, bentonite intercalated with Ag^+ was prepared in order to develop an antibacterial material. Raw clay samples of bentonite from Quatro Barras-Brazil were submitted to cationic exchange with silver ions, at environment temperature. The modified clays were tested for antimicrobial activity against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeroginosa* by microbiological test (Kirby-Bauer's Diffusion Disk Method). Results so far present a strong antimicrobial activity of the modified Ag-bentonite, which considerably inhibited the growth of ordinary microorganisms, including Gram-positive and Gram-negative bacteria.

1. Introduction

Bentonitic clays are commonly used in a wide range of industrial applications, mainly due to the very peculiar properties of its clay minerals such as high cationic exchange capacity, thixotropy, swelling and surface area, among others. Some studies explore these features in the formation of new materials, combining these qualities with its characteristic of intercalation with diverse chemical species of interest (such as organic molecules and metallic ions) in its interlayer space, with the most different purposes.

The montmorillonite layer structure has pulled its use as antimicrobial and antifungal carrier. Researches have shown that montmorillonites intercalated with Ag^+ and other bacteriostatic specimens provide a strong antimicrobial activity to the clay (Oya *et al*., 1991; Ohashi and Oya, 1992; Zhou *et al*., 2004; Hu and Xia, 2006; Frost *et al*., 2006). In order to amplify the clay's adsorption power and surface area, some of the studies in this area employ acidic mediums and/or high temperatures. Such routine can obviously improve the antimicrobial activity of a bentonite, but with a direct effect on the structure of the montmorillonites, which certainly affects their essential properties. To aggravate this problem, silver cations are very reactive and easily transformed into elementary silver by the high Ag^+ reduction power to Ag^0 due to the poor stability of silver ions in aqueous solution after exposure to light or heat (Zhou *et al*., 2004). This compromises greatly the microbiologic performance, once only ionic silver reacts over microorganisms. To prevent that, some researches propose the use of chelates to stabilize the silver ions, but at more complexity and cost to the method (Ohashi and Oya, 1992).

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The objective of this study is to prepare a modified Ag-bentonite, using a Brazilian clay at environmental temperature and without any special conditions, and investigate if this material has a similar potential compared to the ones obtained by procedures described in the literature.

1.1 Bentonite

Bentonites are clays formed mainly by montmorillonite, a clay mineral from the smectite group, which physical properties are established by this clay mineral. The remarkable adsorption properties and cationic exchange capacity, for example, originate from its structure configuration (Figure 1): two tetrahedral layers of silica linked by one octahedral layer of alumina constitute the basic crystalline arrangement. Each one are separated by an interlayer space, where exchangeable cations (Na^+, Ca^{2+}, Mg^{2+}) are present to balance an apparent negative charge of the structure superficies, due to isomorphic substitutions of less-charged cations in the octahedral sheet (Keller-Besrest *et al*., 1994).

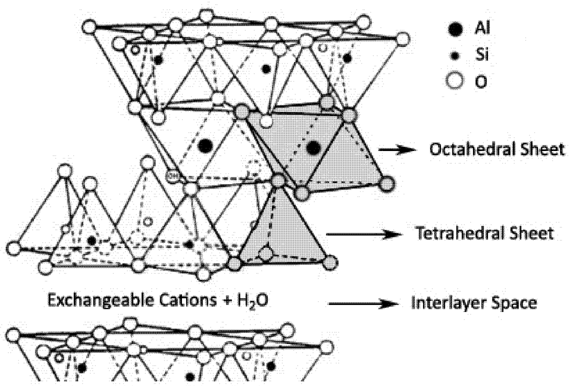

Figure 1: Crystalline structure of montmorillonites

These characteristics allow the application of montmorillonite as a support for silver ions, by promoting its simple exchange with the cations in the interlayer space.

1.2 Antimicrobial material

Antimicrobial materials have the ability of inhibiting the growth or even killing some kinds of microorganisms. This property is very important at certain industry segments, normally those who need large purity and hygiene, along with a partial or complete removal of noxious microorganisms. Therefore, the search for products with antimicrobial properties has gain special importance on that kind of processes, like in raw materials directed to cosmetics and pharmaceuticals, hospital and veterinary products and utensils, food industry and animal feeding, among others (Li *et al*., 2002).

To attribute antibacterial properties to an inert material, it is necessary to treat it with metallic ions with bacteriostatic nature, such as silver, copper and zinc. It is shown that those ions can inhibit birth and growth, or even kill harmful microbes, by altering the metabolisms of bacterium and fungus.

Inorganic antibacterial products are usually in the form of a composite, in which the metallic ions are impregnated in or covered on a carrier (Li *et al*., 2002). Silver ions are extensively used in the synthesis of antibacterial agents because is a very effective specimen in killing bacteria, at relatively low concentrations, and with a low toxicity. In water treatment and food industry (in products such as active packaging), for instance, silver as antimicrobial agent is considered safer and relatively inert, and consequently, the most appropriate for such applications (Oliveira and Oliveira, 2004).

2. Materials and Methods

Samples of clay from Quatro Barras, in the region of Parana, Brazil, were characterized mineralogical and chemically as a policationic bentonite, by the presence of montmorillonite with amounts of quartz, albite, kaolinite and orthoclase. The cationic exchange capacity was determined by methylene blue adsorption as 42.5 meq/100g and the Foster's swelling index was of 2.0 ml/g. Such low values indicate the lack of sodium cations in the interlayer space, which can compromise the cationic exchange.

2.1 Method

To obtain an antibacterial material, 20 grams of bentonite (powders of 75µm, at maximum) were suspended in distilled water and mixed with a 10% (w/w) $AgNO_3$ solution. The suspension was kept stirring for 2 hours, in order to provide the best environment for the exchange of Na^+ from the clay minerals for Ag^+ from the saline solution. After the product was dried at 60°C and grounded in a ball mill to sizes less than 200 mesh.

2.2 Microbiological tests

Diffusion Disk Method (Kirby-Bauer) was used to evaluate the antimicrobial activity of the clay materials impregnated with silver. *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeroginosa* (ATCC 27853) were inoculated in Mueller Hinton, MacConkey and Cetrimide agar, respectively. Bacterial cultures were grown at 37°C for 24 hours. After incubation, each culture was diluted in sterile saline solution (0.9% NaCl) according to 0.5 MacFarland scale, in order to obtain a bacterial density of 1.0 x 10^8 cfu/mL approximately. A sample of this suspension was inoculated in Petri dishes containing Mueller Hinton, MacConkey and Cetrimide Agar (depending on the microorganism) homogeneously. A sample of 10 mg of each clay material (natural and silver-modified) was deposited in disk forms aseptically over the inoculated medium superficies. The dishes were incubated at 37°C for 24 hours. After incubation, the presence of inhibition halo round the material was verified.

3. Results and Discussion

Table 1 shows results of antimicrobial tests. As shown in this table, raw bentonite has no antimicrobial activity. The modified clay, in opposition, showed antimicrobial activity against all bacteria.

Table 1: Antimicrobial activity of natural and modified bentonite

Material	Antimicrobial Activity		
	S. aureus	*E. coli*	*P. aeroginosa*
Natural Clay	–	–	–
Ag^{+} Modified Clay	+	+	+

+ effective; – ineffective

The halo formation can be seen by Figures 2, 3 and 4. When the bacterium is inhibited from growth, a darker ring is evidenced around the disk formed by the clay sample. If no limits between the sample and the culture can be distinguished visually, there is an indication of no antibacterial activity.

As shown in Figures 2, 3 and 4 there is not the presence of halo for natural bentonites, which attest that raw clays show no antimicrobial activity for any of the bacterium tested. These control tests demonstrate that the original bentonite does not have any antibacterial properties. On the other hand, the clearly occurrence of halo round the clays treated with silver indicates an efficient antibacterial performance over all bacterium utilized.

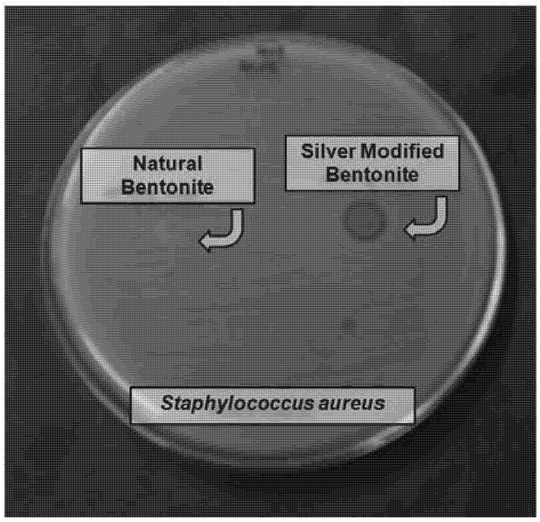

Figure 2: Antimicrobial activity of natural and modified bentonite for S. aureus.

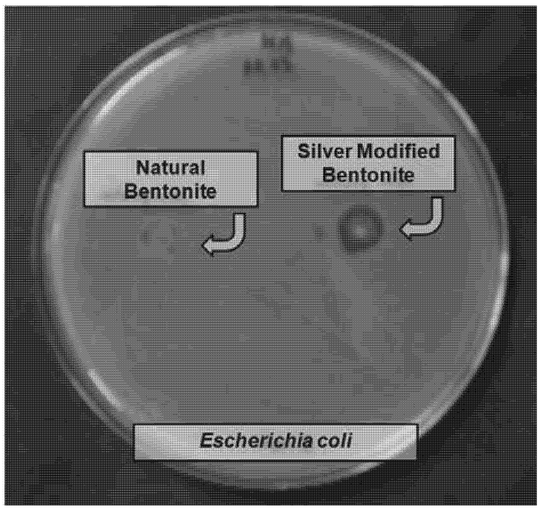

Figure 3: Antimicrobial activity of natural and modified bentonite for E. coli.

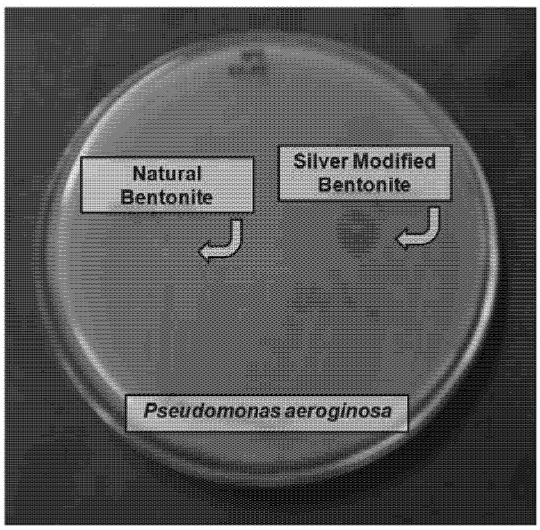

Figure 4: Antimicrobial activity of natural and modified bentonite for P. aeroginosa.

4. Conclusions

In this study, antibacterial material was synthesized using raw bentonite and silver nitrate. The methodology was based only at the cationic exchange between the ionic specimens involved, at environment temperature.

Antibacterial activity tests against *S. aureus*, *E. coli* and *P. aeroginosa* showed that the raw bentonite do not present any bacteriostatic ability. In contrast, the halos evidenced by the tests applied to the silver-doped bentonite show that it has excellent antibacterial activity.

The results indicate that bentonites can be beneficiated by the simple methodology proposed, resulting on a promising antimicrobial material with good efficiency against Gram-positive and Gram-negative bacteria.

The positive antibacterial responses endorse a continuation on this research, focusing on the mechanism of Ag^{+} diffusion on this bentonite, a quantitative study of Ag+ reduction to metallic silver and the possible applications of such synthesized material.

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