# Antibacterial Activity of Chitosan-Based Materials

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**Abstract**

Chitosan is a naturally occurring polymer with powerful antimicrobial properties against bacteria, yeasts and molds. The present work consists in developing chitosan-based materials and also in examining their *in-vitro* antimicrobial activities on commensal and pathogenic bacterial strains that are most often associated to a great extent with the microbiological deterioration of food. The bactericidal activity of these materials was investigated against two Gram-negative bacteria, i.e.

*E.coli ATCC25022* and *Pseudomonas*, and two Gram-positive bacteria, i.e. *Bacillus ATCC29522* and *Staphlococcus ATCC29422*. The agar well diffusion method was used for the antibacterial study.

The results found showed that Gram-positive bacteria were more sensitive than Gram-negative ones to the action of chitosan. In addition, it turned out that the clay/chitosan material showed a large inhibition zone diameter (Q = 41 mm) on the strain of *Escherichia coli ATCC 25022*.

**Keywords**: Clay; Chitosan; Composite; Antibacterial activity

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## Introduction

It is well known that bacterial infections are generally caused by different microorganisms and are the source of the most fatal diseases and the most widespread epidemics [1]. No denies that many antibiotics have been developed to treat them so far. However, their misuse and overuse was found to be responsible for the appearance of the bacterial multi-resistance to antibiotics. Today the control of bacterial infections is becoming more and more complex because many bacteria have developed some resistance to most antibiotics, which has engendered a considerable health problem throughout the world [2].

Over the last few years, naturally occurring polymers have generated significant interest in various sectors of chemical technology. Among these polymers, mention should be made of those derived from biomass, and in particular polysaccharides, which have undergone remarkable development, due particularly to their highly positive image of being biocompatible and biodegradable materials which present no danger to health and life environment [1-2]. One of this series of polymersis chitosan that is obtained by deacetylation of chitin which itself is extracted from crustacean shells.

It is widely admitted that the preparation of new biodegradable and bioactive materials that are composed of polysaccharide polymers and reinforced with clays previously modified through the intercalation with molecules known for their biological activity constitutes today a topical area of research [3-4].

Furthermore, the highly important hydrophobic and organophilic properties of clays have attracted the interest of a large number of researchers throughout the world [5]. The modification of clay by the chitosan biopolymer is a technique that is increasingly used in the recovery and elimination of pollutants present in industrial effluents. It should be noted that the clay exhibits good antibacterial activity [6 - 7].

It has been revealed that chitosan exhibits remarkable antimicrobial activity against different groups of microorganisms such as bacteria, viruses, fungi, yeasts and molds (Juneja et al., 2006) [8-10]. In general, molds and yeasts are the most sensitive to chitosan, and then comes the bacteria.

The antimicrobial activity of chitosan can be influenced by many factors, including the biological origin of chitosan, the percentage of chitosan deacetylation (the more chitosan is deacetylated, the stronger its antimicrobial activity), the degree of polymerization or molecular weight (the higher the concentration of chitosan polymers, the better the antimicrobial activity is observed), as well as the type of targeted organisms. However, the response of the microorganism depends on the chemical structure of the chitosan used as well as on the environmental conditions during the interaction (Cuero, 1999) [11-14].

Several research works that have previously been carried out on the mode of action of chitosan have provided a number of hypotheses on the different possible interactions between bacteria and chitosan, and more generally on the antimicrobial role of this polymer.

In this context, the present study aims primarily to study the antibacterial activity of materials synthesized from clay and chitosan.

## 1. Materials And Methods

### 1.1. Experimental device

The synthesis of the composite materials was carried out according to three protocols as described below:

In the first 5g clay suspension was prepared under stirring. Next, viscous chitosan marbles were added dropwise. After decantation, the solid phase was recovered and dried in the oven for 24 hours.

In the second way the chitosan-based solution previously prepared was then mixed with the clay suspension prepared with NaCl (1M) by adopting the same procedure as that of protocol one the resulting product was then recovered after decantation, dried and ground.

And in the three way a solution containing clay and sodium thiosulphate (1M) was prepared, to which the chitosan solution was slowly added, drop by drop. The suspension obtained was then dried, ground and stored.

During this study, we used three types of materials: clay/chitosan, clay/sodium, and clay/sodium/thiosulfate.

### 1.2. Experimental device for the antibacterial activity

The present research work was conducted in the biology laboratory under the supervision of Dr. A. AMMAM.

This study showed that the antibacterial agents of the composite materials thus synthesized act on bacteria at the molecular level by disrupting some of their essential functions which mainly depend on the following four factors:

- The weather

- The antibacterial agent concentration

- The physico-chemical conditions of the environment

- The number and condition of bacteria.

**-Culture media**

For the purpose of studying the antibacterial activity of the composite materials prepared, it was found useful to use nutrient broth as an enrichment medium for bacterial strains such as *E. Coli*, *Bacillus*, and *Streptococcus*. The bacterial strains were then subcultured on media of nutrient agar (NA) or Sabouraud agar in order to obtain young colonies. With regard to the antibacterial activity, the Mueller - Hinton agar and *potato dextrose agar* media were used while adopting the solid medium diffusion method. For this, 20 ML of each supercooled medium were distributed in Petri dishes as shown in fig1, 90 mm in diameter, in order to obtain a thickness of 4 mm. Before solidification of the agar, the dishes were placed on a flat surface in order to ensure good uniformity of the agar surface.

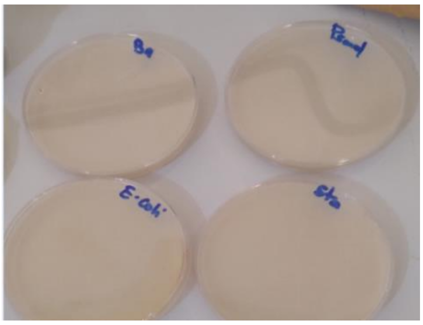

**Figure 1: Culture medium in Petri dishes**

**- Inoculum preparation**

To prepare the inoculum, 1 to 2 well isolated and perfectly identical bacterial colonies were taken from the young colonies, using a platinum loop. Afterwards, they were emulsified in a tube containing 5 ml of physiological water. The resulting mixture was then subjected to vortex-mixing. Note that the density of the inoculum was adjusted to 0.5 McFarland. Appropriate dilutions were made with physiological water in order to obtain a bacterial suspension at CFU/ml.

**Diffusion method**

The agar medium diffusion method is a qualitative technique that can be used to determine the sensitivity of microorganisms to the substance to be tested, and known to be antimicrobial. This method is based on the diffusion capacity of the antimicrobial solution inside a Petri dish from wells made in a solid nutrient medium (Mueller-Hinton agar or PDA) previously inoculated with a microbial culture.

**a) Seeding**

One to two milliliters of each bacterial suspension, prepared beforehand, were distributed in Petri dishes in which we poured the Mueller-Hinton agar for the bacterial strains, or the *potato dextrose* agar (PDA) for the fungal strains. Note that the bacterial suspension used was homogenized in all directions so as to cover the entire surface of the agar dish. It was then left in contact with the agar for 1 min, and then the excess suspension was removed using a sterile Pasteur pipette supplied with a *pear*-shaped rubber. The agar plates thus seeded were left for 15 min at laboratory temperature.

**b) Material deposits**

A Pasteur pipette was briefly passed through the Bunsen burner flame to be used later on. Then, some wells were made on the surface covered with the Mueller-Hinton agar. The different materials produced were deposited in their respective wells. The agar dishes thus prepared were maintained at laboratory temperature for 15 min in order to allow pre-diffusion. Then the bacterial strains were incubated at 7°C for 24 hours.

**c) - Reading the results**

The diameters of the inhibition zones around the wells were determined using a caliper or a ruler.

## 2. Results And Discussion

1. Characterization of composite materials

1.1 X-ray fluorescence analysis

The chemical analysis by X-ray fluorescence was applied to determine the chemical composition of the prepared composite materials. The results obtained from this analysis are summarized in Table 1. The findings indicate that the chemical compositions of clay have undergone a highly remarkable modification, which confirms that there has been intercalation of clay into chitosan, sodium and thiosulfate.

**Table 1: X-ray fluorescence analysis of composite materials**

| Componds | Clay(%) | C.CHI (%) | C. S CH (%) | C. T CHI (%) |
|---|---|---|---|---|
| $Na_2O$ | 1.860 | 1.840 | 9.130 | 12.474 |

| | | | | |
|---|---|---|---|---|
| MgO | 3.767 | 3.470 | 2.823 | 1.959 |
| $Al2O_3$ | 15.606 | 15.388 | 15.022 | 9.992 |
| $SiO_2$ | 57.006 | 55.725 | 55.123 | 36.140 |
| $SO_3$ | 0.216 | 0.021 | 0.012 | 10.190 |
| $K_2O$ | 1.401 | 1.150 | 1.155 | 0.861 |
| CaO | 0.637 | 2.536 | 0.044 | 0.007 |
| $Fe_2O_3$ | 2.204 | 2.364 | 2.010 | 1.438 |
| $Mn_2O_3$ | 0.028 | 0.022 | 0.020 | 0.011 |
| $P_2O_5$ | 0.042 | 0.028 | 0.034 | 0.024 |
| TiO* | 0.160 | 0.150 | 0.147 | 0.103 |
| SrO | 0.014 | 0.014 | 0.002 | 0.001 |
| $SiO_2/Al_2O_3$ | 3.65 | 3.62 | 3.66 | 3.61 |

**1.2 X-ray diffraction analysis**

This analysis showed that the composite materials (C.CHI, C.S.CHI and C.T.CHI) certainly include chitosan.

Figures 2, 3, and 4 clearly show that, regarding the three modified clays, for angle 2 theta = 5.828°, d =15.15 Å for clay/chitosan (C.CHI), for angle 2 theta = 6.73 °, d = 13.13 Å for clay/sodium/chitosan (C.S.CHI), and finally for angle 2 theta = 7.54°, d = 11.47 Å for clay/thiosulfate/chitosan (C.T.CHI). These three situations correspond to the basal reflection d001. The order of change of these values indicates that the interfoliar spaces of the three modified clays are probably occupied by chitosan in the case of C.CHI, C.S.CHI and C.T.CHI.

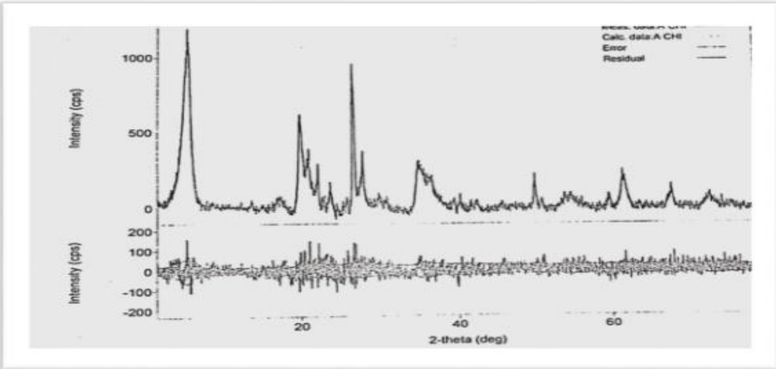


**Figure 2: X-ray diffractogram of clay/chitosan**

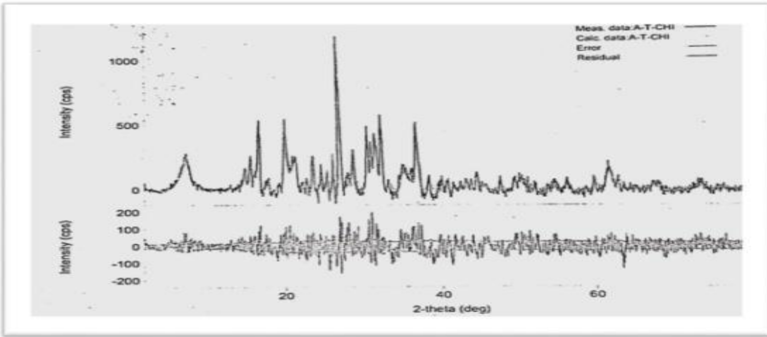


**Figure 3: X-ray diffractogram of clay/thiosulfate/chitosan**

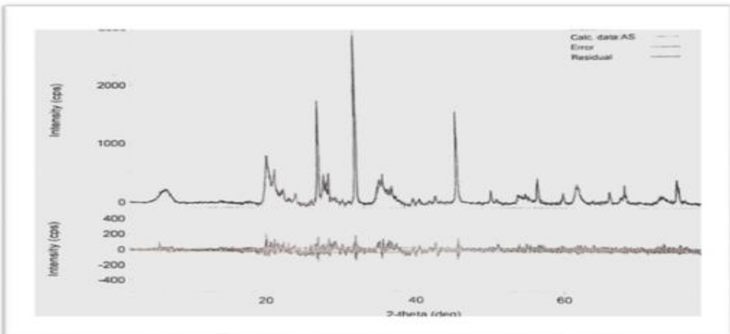


**Figure 4: X-ray diffractogram of clay/sodium.**

### 3. Infrared spectroscopic analysis

This analysis showed the presence of bands characteristic of chitosan in the composite materials thus synthesized. These bands are found at:

- 1635.5 $cm^{-1}$: average band characteristic of $NH_2$ groups.
- 870 $cm^{-1}$: band characteristic of a cyclic compound.

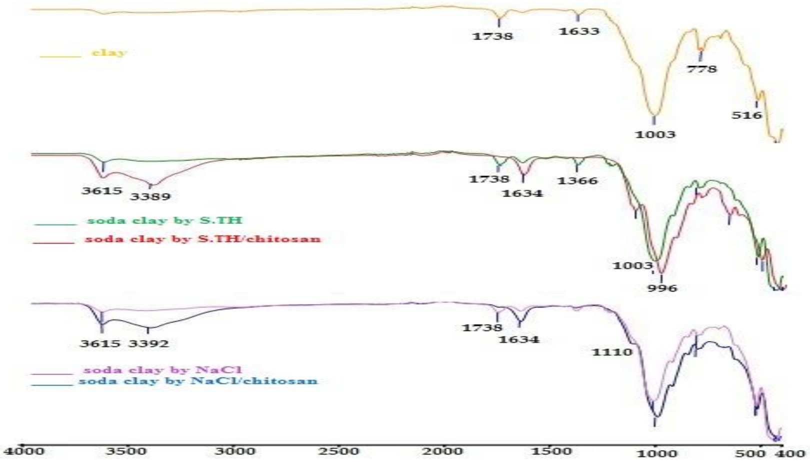


**Figure 5: Infrared spectrum of chitosan and thiosulfate soda clay**

The three analysis techniques (X-ray fluorescence - X-ray diffraction - Infrared spectroscopy) confirmed the presence of chitosan in the synthesized materials. The antibacterial activities of these materials were then tested against four microbial strains.

### 3. Results of antibacterial activity

The following figures 6,7,8and 9 show photos of Petri dishes used for testing the inhibition zones of the clay/chitosan (CC), clay/thiosulphate/chitosan (CTC), clay/soda/chitosan (CSC) materials against bacterial stains. The results of the observation are grouped below. One should know that:

1 refers to CC

2 refers to CTC

3 refers to CS

**Figure 6: Inhibitory zone tests of adsorbent materials against *Staphlococcus***

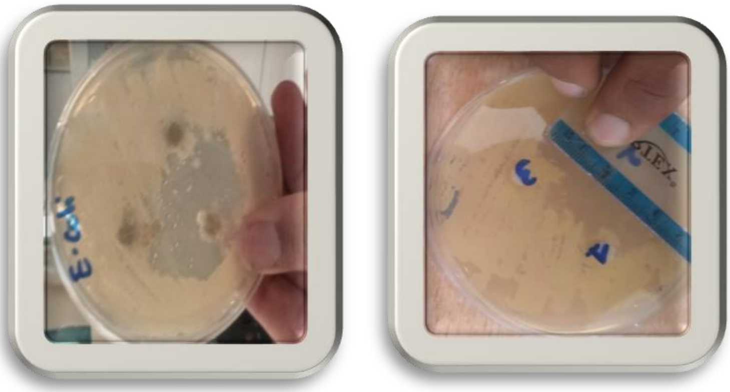


**Figure 7: Inhibition zone tests of adsorbent materials against *Echerichia. col***

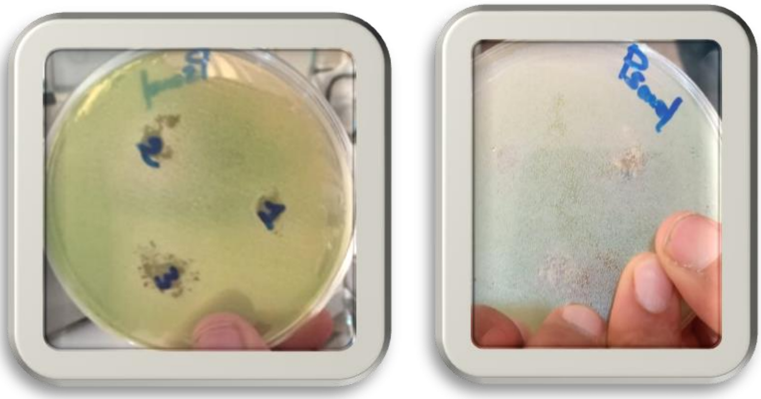

**Figure 8: Inhibition zone tests of adsorbent materials against *Pseudomonas 25922***

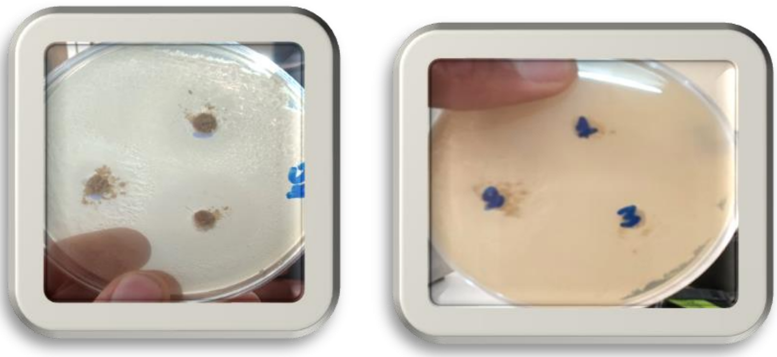

**Figure 9: Inhibitory zone tests of adsorbent materials against *Bacillus subtilis*.**

Table 2 given below shows the results of the evaluation of the antimicrobial activity of the different adsorbent materials, i.e. CC, CTC, and CSC, evaluated by the well diffusion method by measuring the inhibition zone diameters, as shown in Figures 8, 9, 10, and 11.

**Table 2: Results of the evaluation of the antibacterial activity**

| | Bacterialstrains | | | |
|---|---|---|---|---|
| | Gram negative | | Gram positive | |
| | *E. coli* *ATCC25022* | *Pseudomonas* | *Bacillus* *ATCC29522* | *Staphlococcus* *ATCC29422* |
| CC | 41 mm | NA | 33.7 mm | NA |
| CTC | NA | 18 mm | NA | NA |
| CSC | NA | 18 mm | 20 mm | 22 |

- For diameter >15 mm, there is good antibacterial activity
- For diameter <15 mm, antibacterial activity not active (NA)

Based on the interpretation of the results of the antibacterial activity against Gram-negative (*E.coli ATCC 25922* and *Pseudomonas 25922*) bacteria and Gram-positive (*Bacillus ATCC 25922* and *Streptococcus ATCC 25922*) bacteria, it can be concluded that:

The antibacterial activity of clay/chitosan (CC) is better than those of clay/thiosulphate/chitosan (CTC) and clay/soda/chitosan (CSC), which means that chitosan possesses a very high antibacterial activity due to the presence of the amine and hydroxyl groups which give it excellent antimicrobial activity against various groups of microorganisms, such as bacteria, viruses, fungi, yeasts and molds (Juneja et al., 2006), on the one hand, and the addition of bentonite which reinforces the structure of chitosan and increases its stability, on the other [3]. Therefore, chitosan or composite chitosan/clay readily adheres to microbial cells because the positively charged amines interact with the negative charges on the cell membrane surface to release intracellular constituents and inhibit the Escherichiacoli bacteria by almost 100 %. In addition, the combination (chitosan-montmorillonite) may be useful as a functional material for the adsorption of dyes, and can be employed in antibacterial applications [2-4].

## Conclusion

In conclusion, it can be said that the main objectives of the study have been achieved. The synthesized materials were characterized by different analysis techniques, namely X-ray fluorescence, IR analysis and XRD technique.

The assessment of the antibacterial activity of the chitosan-based composite materials, which were synthesized in this work, was carried out by means of the well diffusion method. The results obtained showed that Gram-positive bacteria were more sensitive than Gram-negative ones to the action of chitosan. The chemical compound (clay/chitosan) showed a significant inhibition diameter (41 mm) on the strain of *E. coli ATCC25022*. This high antibacterial activity of the composite (clay/chitosan) is the result of the presence of the amine and hydroxyl groups. These findings corroborate the importance of chitosan which exhibits a significant antimicrobial power.

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