IOSUD - Universitatea Politehnica Timişoara
Şcoala Doctorală de Studii Inginereşti

NEW MATERIALS WITH TARGETED ANTIMICROBIAL PROPERTIES DERIVED FROM NATURAL POLYMERS

Doctoral Thesis - Abstract
for obtaining the scientific title of doctor at
Polytechnic University of Timisoara
in the doctoral field of CHEMICAL ENGINEERING

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CONTENS

EXTENDED SUMMARY OF THE DOCTORAL THESIS

Biomaterials are considered the materials of the future, becoming a part of everyday life as a result of the applications they have in fields such as cosmetics, pharmacy, food industry, medicine, chemical industry, electronics, electrotechnics, etc. Their beneficial effect on the protection of the environment, but especially their antimicrobial role, has caught the attention of scientists. At the same time, it is absolutely necessary to know their physicochemical properties, such as shape, size, crystalline structure, solubility, or surface chemistry in order to establish the role of each property in the manifestation of the antimicrobial effect of the biomaterial.

The research and development of biopolymers has gained significant momentum, driven by "green chemistry" principles and sustainability principles that are increasingly adapted in industry. The characteristics of biopolymers, such as their biocompatibility, precisely engineered degradability rate, thermal processability, relatively high strength, low toxicity, controlled crystallinity, and hydrophilicity, have made biopolymers highly useful in biomedical applications.

In recent years, drug-resistant microorganisms have represented a serious problem for public health. Therefore, new strategies are needed to control the activity of bacteria and materials with directed properties may be a promising approach.

In the context of the resistance of microbial strains to most bactericidal agents known until now, the development of new antibacterial materials or at least with bacteriostatic properties, starting from biomaterials, has opened new opportunities for inhibiting microbial adhesion and limiting their transmission. In addition, in the current context, of the increasingly extensive use of biomass and bioenergy concepts, comparative study of some biopolymers such as cellulose and chitosan offers broad perspectives for their use, based on the scientific and engineering advances made in the field of biomaterials. Based on the structural similarity of the two biopolymers studied, the doctoral thesis aimed at the synthesis of new materials, by grafting/functionalisation by impregnation of some chemical compounds with pendant groups of N, P, or S, in this context having the role of extractants on surface of chitosan and cellulose.

The purpose of this doctoral thesis is to "develop the world of biomaterials" by modifying their properties so that the applications of the materials obtained have an antimicrobial effect.

It will be highlighted that the antimicrobial effect is directly dependent on the support:extractant ratio and, at the same time, "the effect is influenced by the interaction between the cationic component of the biocide and the anionic component of the bacterial cell membrane.

The doctoral thesis is structured in two parts, structured in 6 chapters and 149 pages.

Part I of the doctoral thesis presents the current state of knowledge in the field of synthesis, characterization and applications of new biomaterials intended to present significant microbiological properties.

The need to carry out this study starts from the desire to obtain biomaterials with directed properties, in order to improve their microbiological action and effectiveness. The doctoral thesis, by its content, required theoretical and experimental knowledge, having a deep interdisciplinary character through the use of knowledge of chemical and environmental engineering, biotechnology, microbiological technique, and modern methods of analysis.

Chitosan and cellulose represent the most abundant natural polymers of animal or plant origin, they are non-toxic and biodegradable, each carrying functional groups that give them biocompatibility and superior properties compared to natural precursors.

The two biopolymers studied, chitosan and cellulose, show structural symmetry, having the same β-glycosidic bonds, the main difference being the presence of primary amino groups in C2 in chitosan, instead of hydroxyl groups in the structure of cellulose (Figure 6.1).

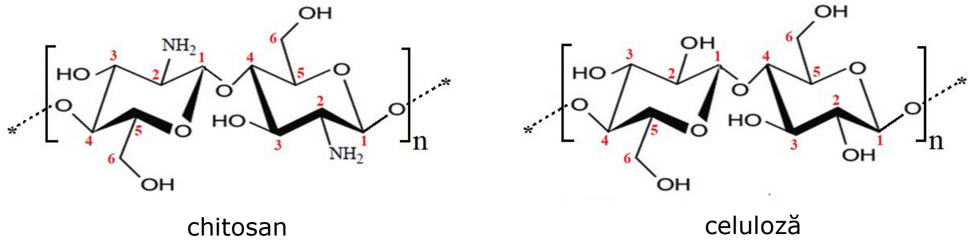

Figure 6.1. The structure of chitosan and cellulose

In the native state, both biopolymers studied have very few applications compared to the diversity of applications of their modified forms. When the basic structure of the two polymers is chemically modified, most of the native properties are improved, or new properties are imprinted, including a substantial antimicrobial effect.

Although biopolymers with an antimicrobial effect can be obtained by permanently modifying biopolymers through chemical reactions, in this doctoral thesis a functionalization technique by impregnation, the SIR (Solvent Impregnated Resin) method, was used, obtaining 135 new materials. These materials are obtained starting from two supports: cellulose (CE) and chitosan (CH), 6 environmentally friendly extracts: (i) quaternary ammonium salts (dodecyl-trimethyl-ammonium bromide (DDTMABr), tetradecyl-trimethyl bromide -ammonium (TDTMABr), hexadecyl-trimethyl ammonium chloride (HDTMACl), (ii) phosphonium salts (dodecyl-triphenyl phosphonium bromide (DDTPPBr), tri n-butyl-hexadecyl phosphonium bromide (HDTBPBr)) and (iii) sulfur salts (thiourea (Thio) or mercaptobenzothiazole (MBT)). Support:extractant mass ratios for all studied materials are: 1:0.012; 1:0.024; 1:0.05; 1:0.075 ; 1:0.1; 1:0.2; 1:0.3; 1:0.4; 1:0.5. Additionally, in the case of CH-DDTMABr, CH-TDTMABr, Ch-HDTMACl, since at the lowest functionalisation ratio CH: quaternary ammonium salts = 1:0.012 taken in the study, no bacterial growth was observed, materials were synthesised with these extractants at lower mass ratios, respectively 1:0.003; 1:0.006; 1:0.009.

To establish whether the material has bactericidal properties, we carried out some microbiological studies that would highlight the behavior of these materials in the presence of a consortium of bacteria obtained from the water taken from the Bega River. Microbiological studies were carried out using conventional cultivation techniques, following the development of microorganisms.

Highlighting the bactericidal effect was achieved by determining the total number of aerobic and facultatively anaerobic mesophilic bacteria, cultured at 37°C (total number of germs, NTG). The presence of aerobic and facultatively anaerobic heterotrophic bacteria was highlighted by seeding water or decimal dilutions through the incorporation process in a solid nutrient medium (nutritional agar), according to SR EN ISO 6222:2004.

The main purpose of colony counting is the possibility to detect variations in relation to the modification of the support:extractant ratios.

Results were expressed as the **number of colony-forming units per milliliter** (CFU/mL).

In the absence of colonies in seed boxes containing volumes of undiluted samples to be analysed, the result is expressed as undetectable in one millilitre.

If the inoculated boxes contain more than 300 colonies, the result is only expressed as an approximate number of colonies forming units. Later, to express the effectiveness of the material in microbial development, the results obtained were expressed in the form of the **inhibition rate of microbial growth.**

A schematic presentation for the method of obtaining and microbiological testing of the materials can be found in Figure 6.2.

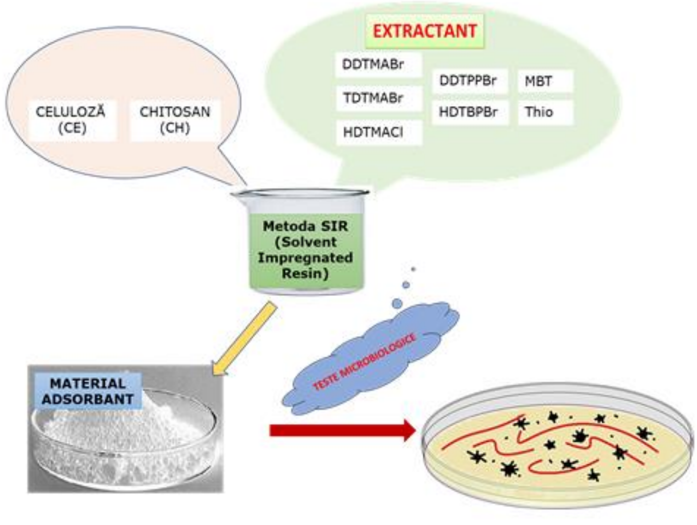

Figure 6.2. The method of obtaining and microbiological testing of materials

Materials obtained by functionalization, by impregnating cellulose (CE) or chitosan (CH) with a series of extractants, in the mass ratio support:extractant=10:1, were characterised by scanning electron microscopy, SEM, X-ray energy dispersion X, EDX and Fourier transform infrared spectroscopy, FT-IR to highlight the presence of the extractant (DDTMABr, TDTMABr, HDTMACl, DDTPPBr, HDTBPBr, MBT and Thio) on the surface of the support (CE or CH). The specific surface area, pore diameter, and total pore volume of the synthesized materials were also determined. So:

- In the case of the materials obtained by the functionalisation of cellulose, it can be observed from the SEM micrographs that, for all the materials obtained, the cellulose fibres are more agglomerated following the functionalization. It was observed that the materials obtained have many thinner fibers. However, no major structural differences were observed between cellulose fibres and materials obtained by functionalising cellulose with extractants.This morphological change is attributed to swelling and partial disintegration of the cellulosic fibers during the surface modification process, followed by the insertion of fragments with active groups present in the extractants, which cover the surface of the fibers.However, analogous micro-sized fibers with a rough and rod-like surface microstructure are revealed for the microcrystalline cellulose-based samples, demonstrating homogeneous surface functionalisation without large variations in surface morpho.
- In the case of the materials obtained by functionalisation, by impregnation of chitosan, it was found from the SEM images that the morphology of the materials obtained following the functionalization of chitosan does not change significantly compared to the support material (CH). It has a granular form.
- From the EDX spectra, specific peaks of the cellulose composition, namely C and O, or specific peaks of C, N and O, can be observed from the chitosan structure. At the same time, the characteristic EDX spectra of the materials synthesised by the functionalization of cellulose are presented, in which the specific peaks of the elements present in the composition of the cellulose, but also of the extractants, are observed. Thus, peaks specific to the extractants DDTMABr and TDTMABr appear, namely Br and N, or Cl. At the same time, specific P and Br peaks appear, elements present in the DDTPPBr and HDTBPBr extractants, or specific S and N peaks, elements present in the MBT and Thio extractants.
- From the FT-IR spectra it can be stated that both cellulose and chitosan were successfully functionalised with the 7 extractants studied.
- From the data regarding the specific surface area, the pore diameter and the total pore volume of CE and the materials obtained by CE functionalisation, it can be stated that: (i) the CE-DDTMABr surface area of the materials (0.002 nm) and CE-HDTMACl (0.082 nm) is lower than that of CE (0.091 nm), and that of the other materials is higher (between 0.168 nm and 5.429 nm); (ii) the pore diameter of the CE-DDTMABr materials (176 nm) is greater than that of CE (66.0 nm), and that of the other materials is smaller (between 3.08 nm and 36.5 nm); (iii) the total pore volume of the material CE- DDTMABr (6.278e-04 cm3/g) is lower than that of CE (2.563e-04 cm3/g), and that of the other materials is higher (between 2.335e- 04 cm3/g and 1.077e-03 cm3/g);
- From the data regarding the specific surface area, the pore diameter, and the total pore volume of CE and materials obtained by CH functionalisation, it can be stated that: (i) the surface area of the materials is smaller than that of CH; (ii) the pore diameter of the materials is roughly similar to that of CH; (iii) the total pore volume of the materials is smaller than that of CH.

6.1. The antibacterial effect of materials obtained by CE functionalisation on a heterotrophic inoculum

Regarding the antimicrobial potential of CE, it can be highlighted only through the materials obtained by its functionalisation, native CE having no significant antimicrobial effect (bacterial growth inhibition rate of 17.93%). Therefore, the use of this biopolymer to obtain materials with advanced properties is justified mainly by this increased antibacterial activity against pathogenic microorganisms.

We synthesised materials with CE support and three extractants, quaternary ammonium salts: DDTMABr, TDTMABr and HDTMACl; two extractants – phosphonium salts: DDTPPBr, HDTBPBr and two sulphur and nitrogen salts: MBT and Thio.

To establish the efficiency of the obtained materials, several CE:extractant (w:w) ratios were used, so that the efficiency of the functionalisation ratio could be followed. The ratios of support:extractant used were: 1:0.012; 1:0.025; 1:0.050; 1:0.075; 1:0.1; 1:0.2; 1:0.3; 1:0.4; 1:0.5. Subsequently, the materials obtained in these reports constituted solid materials for testing their efficiency, in the aspect of antimicrobial activity, performing microbiological tests.

The efficiency of the materials obtained by functionalising CE with quaternary ammonium salts, in terms of the number of bacterial colony-forming units developed, is presented in the figure. 6.3., respectively, the inhibition rate achieved by each material obtained after functionalisation is presented in Figure 6.4.

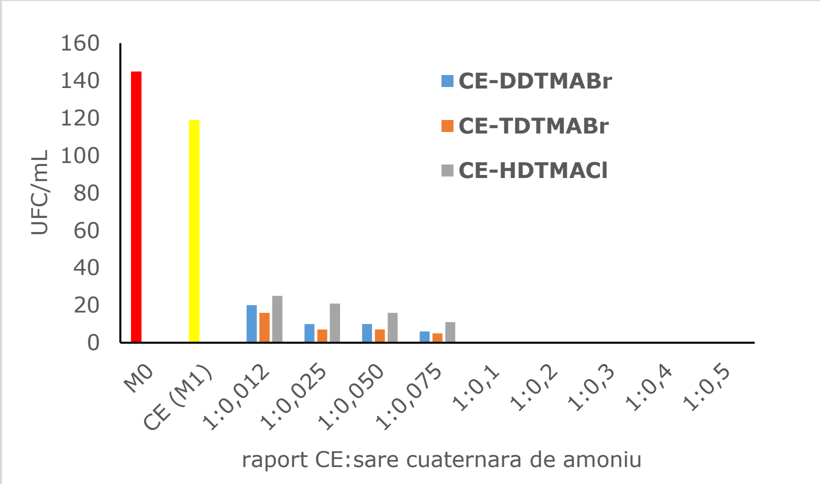

Figure 6.3. The number of bacterial colony-forming units developed in the case of materials obtained by functionalizing CE with quaternary ammonium salts.

Regardless of the quaternary ammonium salt used for CE functionalisation, the materials obtained showed a total antibacterial effect starting with the CE: quaternary ammonium salt = 1:0.1.

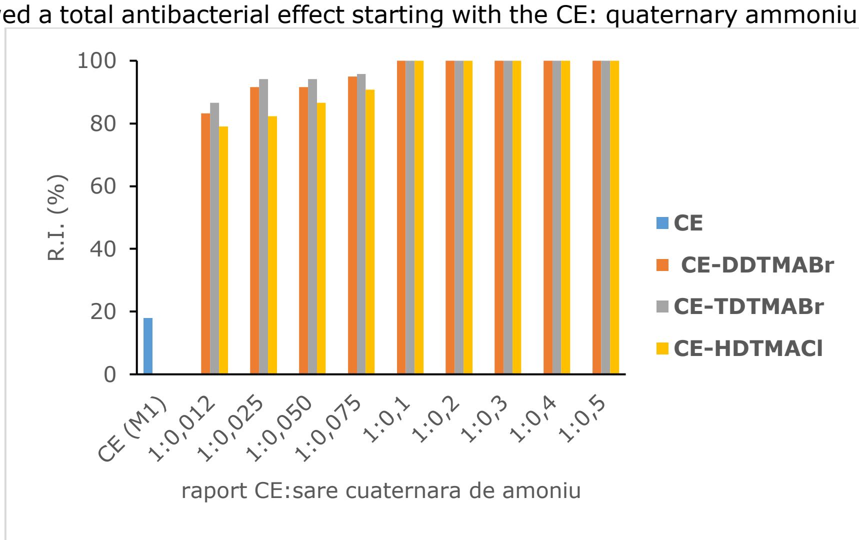

Figure 6.4. Bacterial inhibition rate, in the case of materials obtained by CE functionalization with quaternary ammonium salts

The inhibition rate of bacterial growth is maximal, starting with the functionalisation ratio CE:quaternary ammonium salt = 1: 0.1. Even in lower functionalization ratios, a bacterial growth inhibition rate between 78.99%, for the CE-HDTMACl material obtained at the ratio CE:HDTMACl= 1:0.012) and 95.80%, for the CE material was obtained -TDTMABr obtained at the ratio CE:TDTMABr = 1:0.5. At small CE: TDTMABr functionalisation ratios = 1:0.012 – 1:0.075, the CH-TDTMABr material showed slightly higher inhibition rate.

The inhibition rate of bacterial growth, when materials obtained by functionalising CE with quaternary ammonium salts were tested, increased in order TDTMABr > DDTMABr > HDTMACl. This aspect was correlated with the distance given by the length of the alkyl substituent arm, from the basic structure of the polymer (polymer backbone) to the positive charge given by the quaternary nitrogen.

The efficiency of the **materials obtained by functionalising CE with phosphonium salts**, in terms of the number of bacterial colony-forming units developed, is presented in the figure. 6.5., respectively, the inhibition rate achieved by each material obtained following CE functionalisation is presented in Figure 6.6.

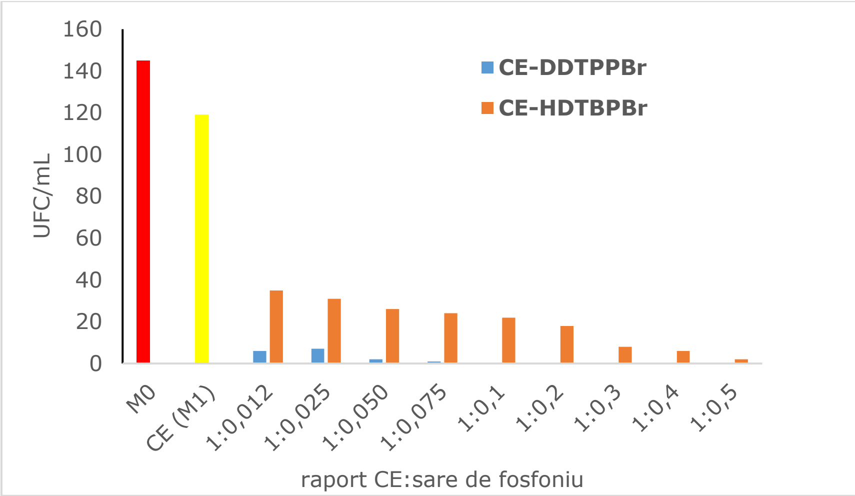

Figure 6.5. The number of units that form bacterial colonies in the case of materials obtained by functionalising CE with phosphonium salts.

Materials obtained by CE with phosphonium salts showed good and very good antimicrobial efficiency, as evidenced by the list of bacterial colony growth for CE-DDTPPBr material, starting with the functionalisation ratio of 1:0.1.

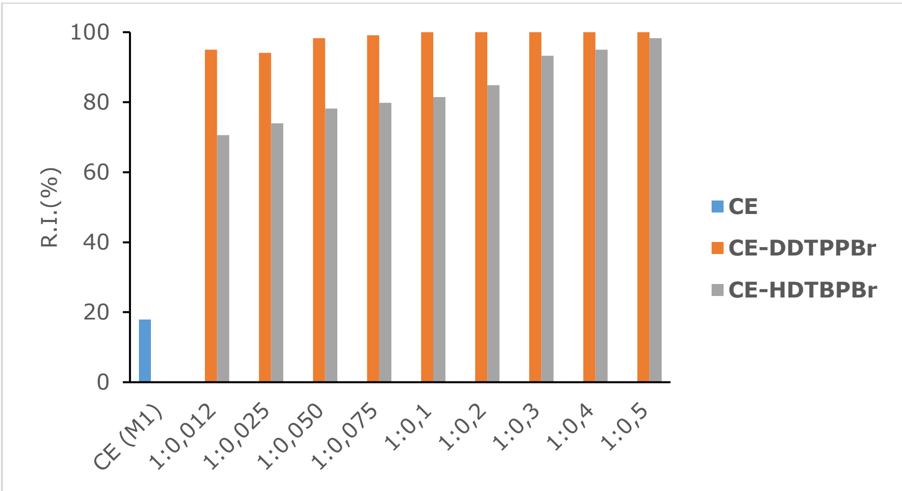

Figure 6.6. Bacterial inhibition rate, in the case of materials obtained by functionalising CE with phosphonium salts.

The bacterial growth inhibition rate for CE-HDTBPBr materials ranged between 70.59% in the functionalization ratio CE:HDTBPBr =1:0.012 and 98.32% at the functionalization ratio CE:HDTBPBr =1:0.5 . For the CE-DDTPPBr material, the variation in the microbial growth inhibition rate was in the range of 94.96%, at the functionalization ratio CE:DDTPPBr=1:0.012) and 100%, at the functionalization ratios CE:DDTPPBr =1:0.5), which indicates very good antimicrobial efficiency for this material.

Regardless of the functionalization ratio, the CE-DDTPPBr material exhibited better bacterial growth inhibition rate compared to the CE-HDTBPBr material. This aspect was correlated with the difference in hydrophobicity given especially by the different quaternization substituents (triphenyl and tributyl). Although the two extractants, DDTPPBr and HDTBPBr also have different chain lengths of the alkyl substituent (dodecyl and hexadecyl) which distance the positive charge of the active group from the basic structure of CE, the contribution of hydrophobicity is not essential, compared to that imprinted by the quaternization substituent.

The efficiency of the **materials obtained by functionalising CE with sulfur salts**, in terms of units forming the number of bacterial colonies developed, is presented in Figure 6.7, respectively, the inhibition rate achieved by each material obtained after functionalisation is presented in Figure 6.8.

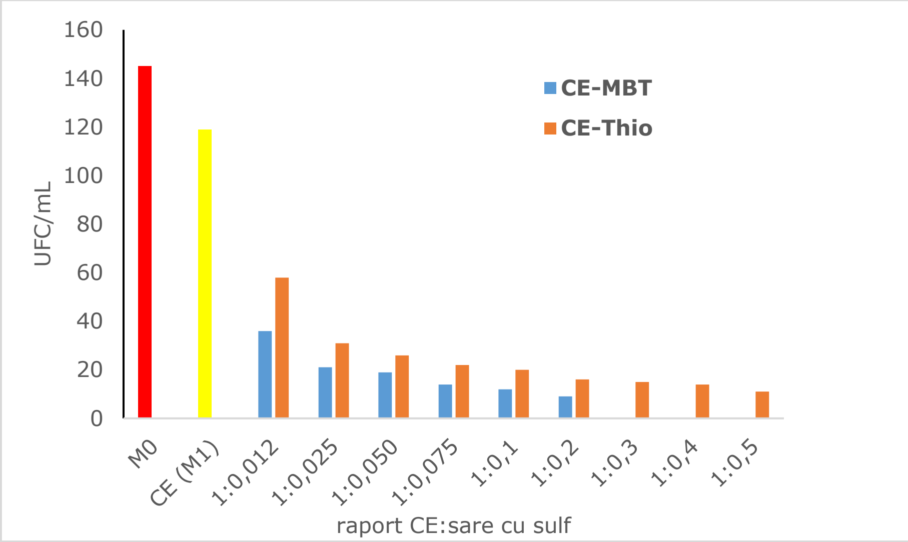

Figure 6.7. The number of bacterial colony-forming units developed in the case of materials obtained by functionalising CE with sulfur salts

With regard to the materials obtained by functionalising CE with sulfur salts, the CE-MBT material showed a better antimicrobial effect. Regardless of the functionalisation ratio, the CE-Thio material allowed the development of a larger number of bacterial colonies, compared to CE-MBT material, for which, once the functionalization ratio CE: MBT = 1:0.3 was reached, it was no longer was observed microbial growth. This aspect has been correlated with the achievement of a hydrophobic/hydrophilic balance, which appears to be necessary to achieve a full antimicrobial effect.

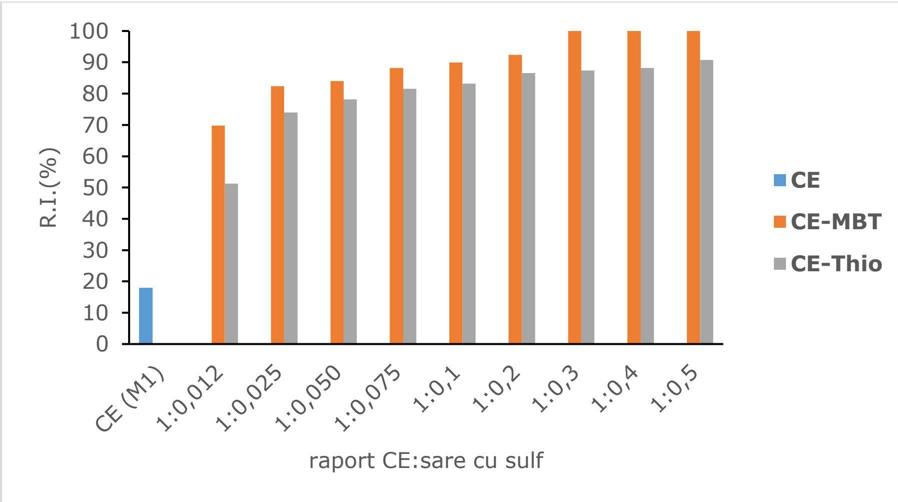

Figure 6.8. Bacterial inhibition rate, in the case of materials obtained by functionalising CE with sulfur salts.

The inhibition rate of bacterial growth for the materials obtained by functionalising CE with sulfur salts varies between 51.26% for the CE-Thio material in the functionalization ratio CE:Thio=1:0.012) and 100% for the CE-MBT material at CE ratios :MBT of functionalization greater than 1:0.3).

For the inhibition rate of CE-Thio material, since the bacterial growth was in the range of 51.26%-90.76%, corresponding to a 50-fold increase in the functionalization ratio, we can consider that these materials have a bacteriostatic effect, which implies an inhibition of the development of bacteria, without necessarily killing them.

Consequently, due to the abundance of hydroxyl groups on the CE surface, it is possible to easily modify the CE surface with a series of chemical groups (N, P, or S), which imprint specific properties on the newly formed derivative, with varied applications: high-performance biodegradable materials, biomedical engineering, pharmaceutical industry, as an antimicrobial agent, catalysis, textile industry, flocculant in water treatment, etc.

6.2. The antibacterial effect of materials obtained by CH functionalisation on a heterotrophic inoculum

Although CH has, through the amino group present in the structure, intrinsic antibacterial activity (proved by the bacterial growth inhibition rate of 41.38%), by modifying native CH, its derivatives with remarkable antimicrobial properties can be obtained, such as the materials obtained by functionalisation by impregnation of CH with quaternary ammonium salts or phosphonium salts, where the maximum rate of bacterial inhibition was reached.

They were synthesised by CH functionalization with the same extractants, materials at different functionalisation ratios, in order to follow the efficiency of the materials in terms of the manifestation of antimicrobial activity.

Thus, when the **materials obtained by functionalising CH with quaternary ammonium salts**, CH-DDTMABr, CH-TDTMABr, and CH-HDTMACl, were tested, bacterial growth was observed only at ratios of 1:0.003 to 1:0.009, in all others functional reports bacterial growth was not observed (Figure 6.9).

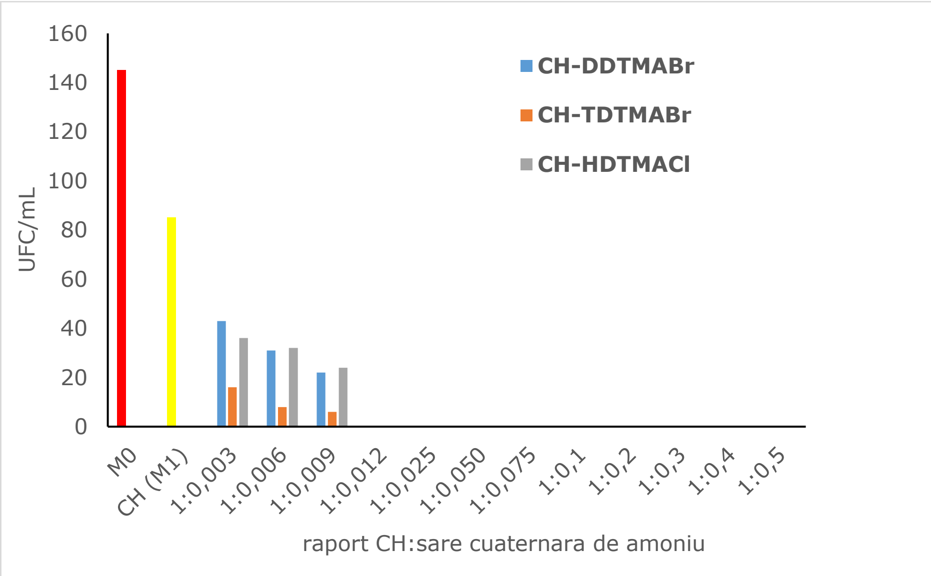

Figure 6.9. The number of units that form bacterial colonies developed in the case of materials obtained by functionalising CH with quaternary ammonium salts

Consequently, the inhibition rate of bacterial growth for materials obtained by functionalizing Ch quaternary ammonium compounds was maximal for ratios of 1:0.012 to 1:0.5.

At lower functionalisation ratios CH:quaternary ammonium salt, the inhibition rate of bacterial growth varied between 49.41%, for the material CH-DDTMABr obtained in the ratio CH:DDTMABr=1:0.012 and 92.94% for the material CH -TDTMABr obtained at the ratio = 1:0.009 (Figure 6.10).

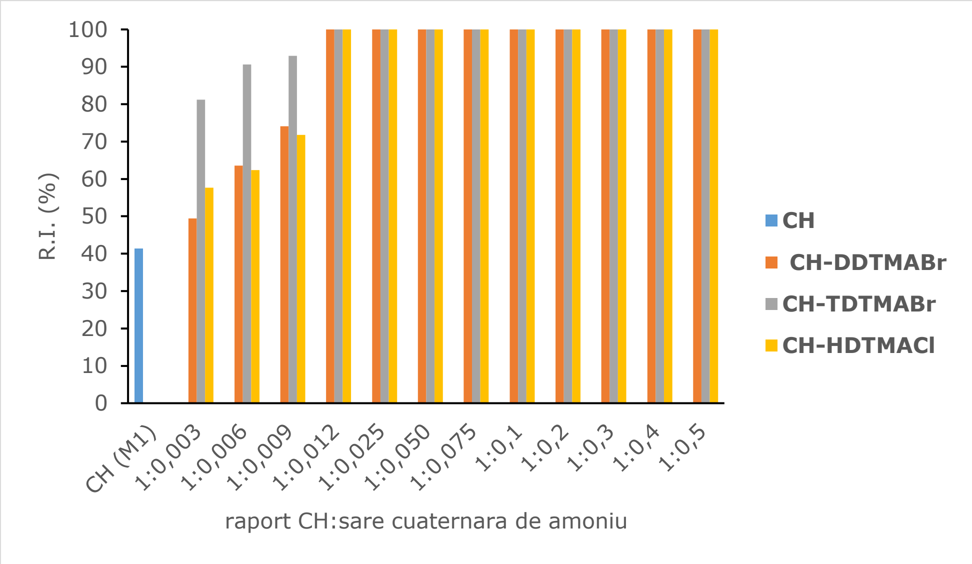

Figure 6.10. Bacterial inhibition rate, in the case of materials obtained by functionalising CH with quaternary ammonium salts

At low functionalisation ratios, for the materials CH-quaternary ammonium salt materials, the antibacterial effect was correlated with the length of the alkyl substituent of each extractant, and respectively with the distance from the quaternary nitrogen to the chitosan basic skeleton, given by the conformation of this arm.

In the case of the **materials obtained by functionalising CH with phosphonium salts**, CH-HDTBPBr and CH-DDTPPBr, at functionalisation ratios between 1:0.012 and 1:0.5, bacterial growth was observed in the CH-HDTBPBr material, while the CH-DDTPPBr material did not show bacterial growth (Figure 6.11).

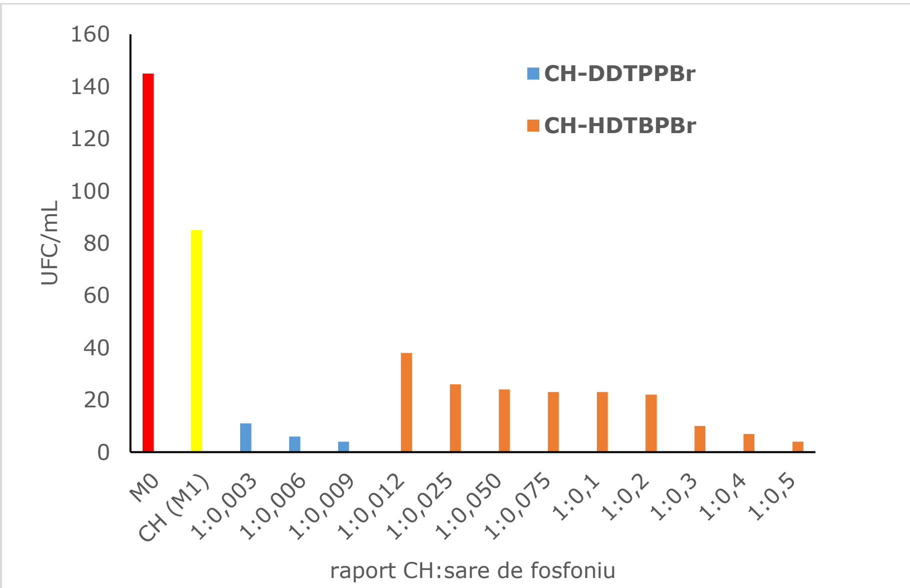

Figure 6.11. The number of units that form bacterial colonies developed in the case of materials obtained by functionalising CH with phosphonium salts

To observe from which functionalization ratio the antibacterial effect is manifested in the case of CH-DDTPPBr materials, we synthesised the ratios 1:0.003 to 1:0.009 and it was confirmed that the total bactericidal effect, in the case of these materials, is obtained from the mass ratio 1:0.012 (Figure 6.12.).

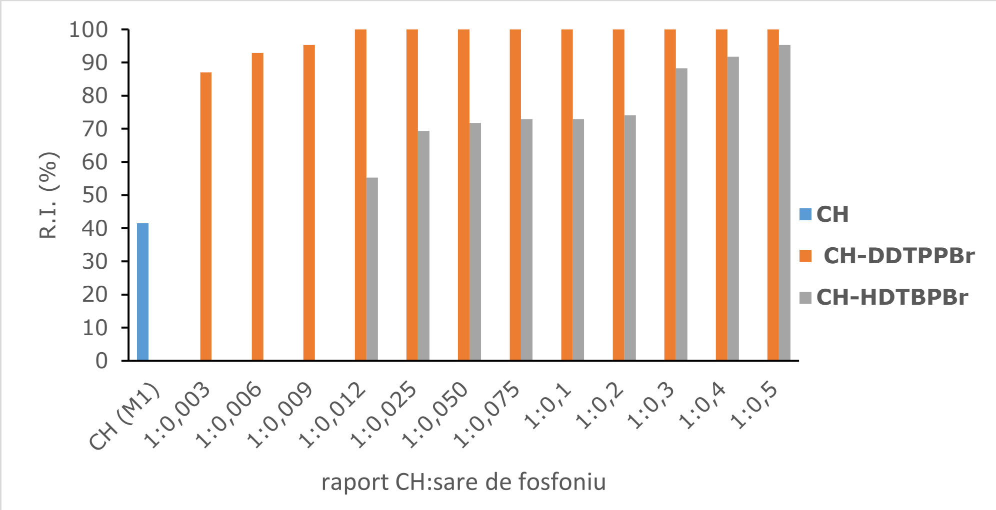

Figure 6.12. Bacterial inhibition rate, in the case of materials obtained by functionalising CH with phosphonium salts

The bacterial growth inhibition rate for the CH-DDTPPBr material ranged between 87.06% (CH:DDTPPBr ratio=1:0.003) and 95.29% (CH:DDTPPBr ratio = 1:0.009), implying a very good antibacterial activity even when using a very small amount of extractant.

The very good results, in terms of the manifestation of the antibacterial effect, were justified by the contribution of the phenyl substituent in the case of the CH-DDTPPBr material, compared to the butyl substituent in the case of the CH-HDTBPBr material.

This proves the extremely important role played by the hydrophilic/hydrophobic balance in the manifestation of the bactericidal effect, upon interaction with the bacterial cell.

For the **materials obtained by functionalising CH with sulfur salts**, the results are similar to those obtained by functionalizing CE with sulphur salts. The CH-MBT material has a better antibacterial effect, comparable to the CH-Thio material, regardless of the functionalization ratio (Figure 6.13).

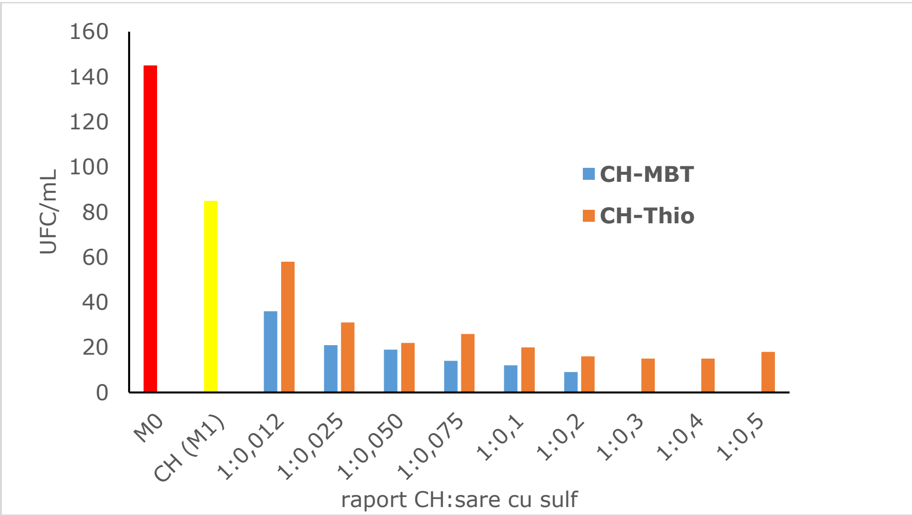

Figure 6.13. The number of bacterial colony-forming units developed in the case of materials obtained by functionalising CH with sulfur salts

The inhibition rate of bacterial growth increased proportionally with the increase in the functionalization ratio (Figure 6.14.), reaching the maximum value (100%) for the CH-MBT material starting with the functionalisation ratio CH:MBT=1:0.3.

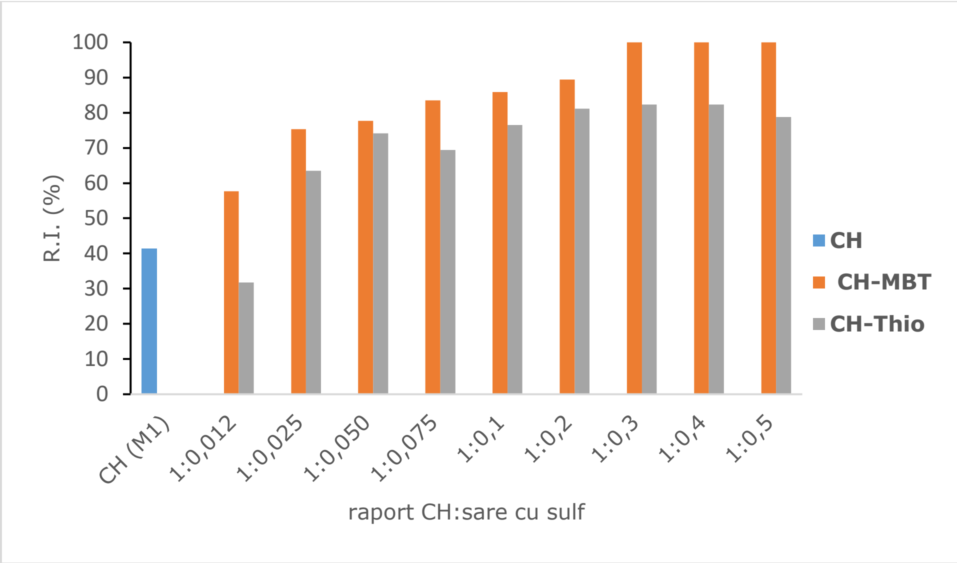

Figure 6.14. Bacterial inhibition rate, in the case of materials obtained by functionalising CH with sulfur salts

At the functionalisation ratio CH:Thio=1:0.012 for the CH-Thio material a bacterial growth inhibition rate of 31.76% was obtained, while in the case of the same material, for the functionalization ratio CH:Thio=1: 0.5 the inhibition rate of 78.82% was reached. Based on these results, although the functionalization ratio was increased 50 times, the inhibition rate only increased 2.5 times, which leads us to consider that this material, CH-Thio, has rather a bacteriostatic effect and not bactericidal.

Due to the presence of the –NH2 group, which acts as a strong nucleophile, CH can be functionalised with a wide range of active/pendent groups to modulate its structure for specific applications. This structural versatility gives modified chitosan multiple applications: in the food industry thanks to the protective films that can be obtained and later applied to preserve products, in medicine and pharmacy as an antibacterial or antifungal agent, in tissue engineering and wound healing, in industry medicines, in the textile industry, in the treatment of waste water, etc.

Therefore, the particularity of CH is given by the presence of the primary amino group, which gives it a high degree of reactivity, while in the structure of cellulose, the presence of three hydroxyl groups in each monomer unit allows the formation of strong intramolecular and intermolecular H bonds.

6.3. Antimicrobial effect of materials reference strains. Comparative presentation.

The two biopolymers studied, CE and CH, although they have similar molecular structures, with the same β-glycosidic bonds between the monomer units, in terms of the chemical nature of the biopolymers studied, cellulose is a neutral or anionic polysaccharide, while chitosan is a neutral or cationic. For this reason, even if the basic structure of the biopolymers is similar, they showed differentiated antibacterial effects depending on the extractant with which the functionalisation was carried out and the type of microbe on which they were tested.

Thus, the response of the studied bacteria was followed by the action of the materials obtained by functionalising cellulose, respectively chitosan, with nitrogen, phosphorus, or sulfur groups, having a different chemical structure. In all cases, the synthesised material had improved antibacterial effect in comparison to the native biopolymer.

Microbiological control tests were performed using a bacterial suspension of approximately $1x10^8$ CFU / ml of reference microbial strains (ATCC). To highlight the effect on Gram-negative bacteria, materials inoculated with Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were studied, while the effect of the materials on Gram-positive bacteria was followed on the strain of Staphylococcus aureus ATCC 25923. The efficiency of these materials, in terms of antifungal activity, was tested in Candida albicans ATCC 10231.

Unfunctionalized CE showed slightly weaker bactericidal effect in Gram-positive bacteria (Figure 6.15) compared to Gram-negative ones (Figure 6.16 and Figure 6.17), while native chitosan showed slightly better bactericidal effect on Gram-negative bacteria (Figure 6.16 and Figure 6.17).

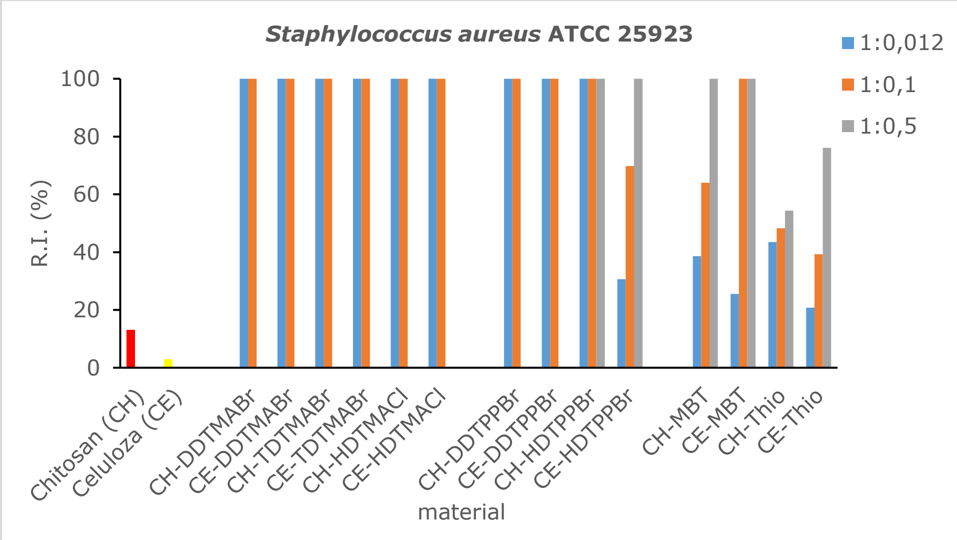

Figure 6.15. Bacterial growth inhibition rate of materials in Staphylococcus aureus ATCC 25923

Regarding the effect of materials on the Pseudomonas aeruginosa species, all materials obtained by functionalizing CE or CH with tested quaternary ammonium salts showed a bactericidal effect, correlated with the amount of extractant used for functionalisation (Figure 6.16.).

The maximum inhibition rate was not reached in any ratio studied, probably requiring a much higher amount of biocide. The outer membrane of the bacterial cell in the species Pseudomonas aeruginosa is an important selective barrier that causes a reduced adsorption of the biocide in the cell due to the reduced susceptibility of this species to biocidal agents.

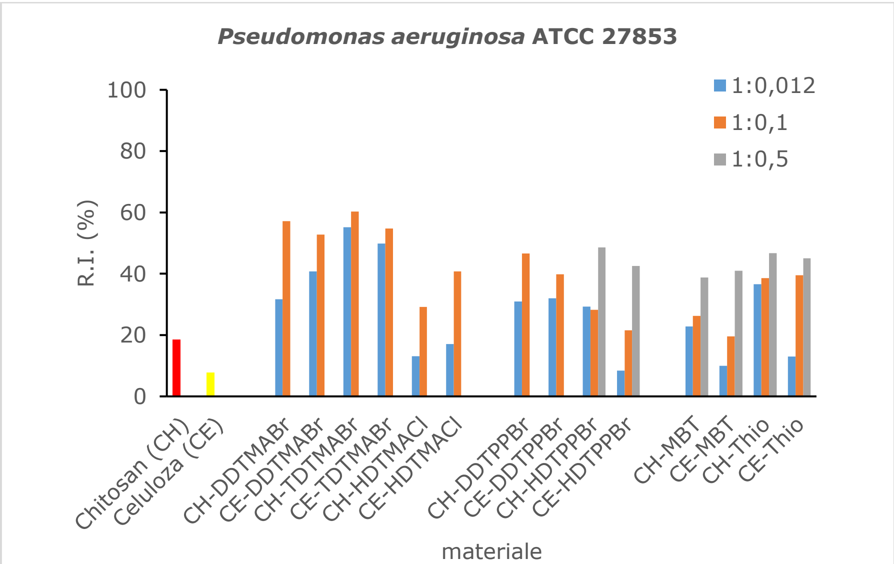

Figure 6.16. Bacterial growth inhibition rate of materials in Pseudomonas aeruginosa ATCC 27853

The materials obtained by functionalising CH with quaternary ammonium salts showed very good antimicrobial activity for all microorganisms examined (except Pseudomonas aeruginosa), a bacterium known for its resistance to most common antibiotics.

All materials obtained by the functionalisation with quaternary ammonium salts had a higher antibacterial activity, in general, against Staphylococcus aureus than against Escherichia coli species (Figure 6.15 and Figure 6.17).

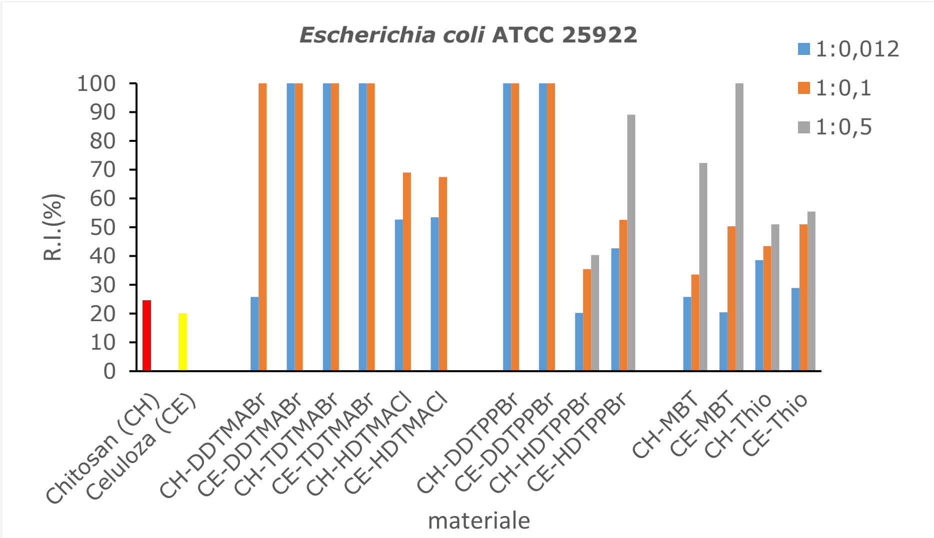

Figure 6.17. Bacterial growth inhibition rate of materials in Escherichia coli ATCC 25922

All materials obtained by CE functionalisation with quaternary ammonium salts used (DDTMABr, TDTMABr, HDTMACl), showed very good antibacterial activity against the Staphylococcus aureus and Candida albicans species, regardless of the functionalisation ratio of the support material (Figure 6.15 and Figure 6.18).

This effect is due to the similar behaviour of the cell wall from the S.aureus species to the fungal cell of the Candida albicans species.

The material adheres strongly to the bacterial cell wall and the fungal cell membrane, damaging their structure and preventing the exchange of nutrients necessary for microbial development, which subsequently leads to cell death of the cell and the achievement of the antimicrobial effect.

Another important role in the permeability of the cell membrane is the amount of fatty acids present in its structure, which is later correlated with the degree of penetration of the toxic element into the cell.

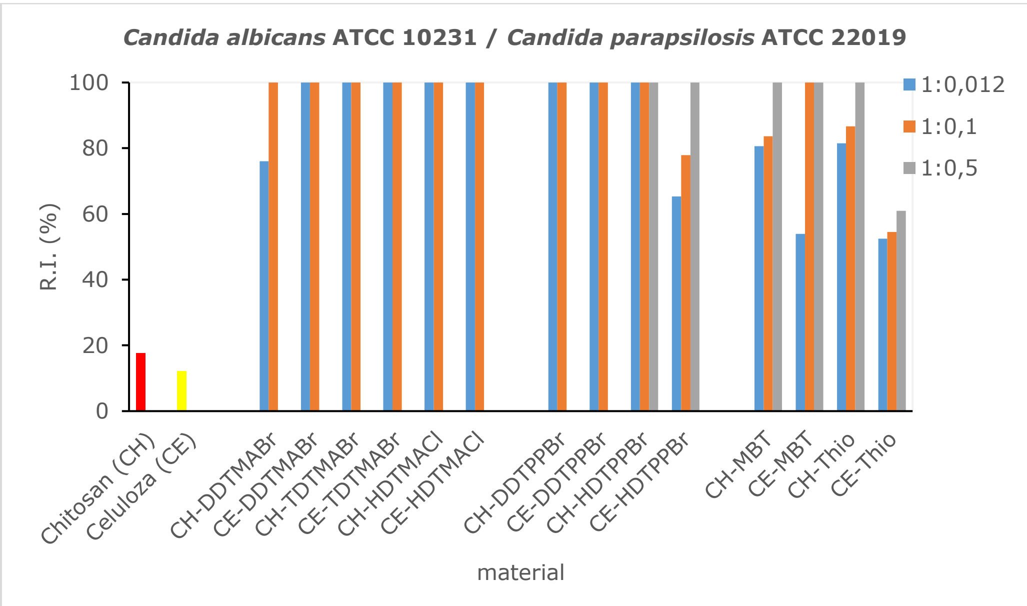

Figure 6.18. The rate of inhibition of bacterial growth of the materials on Candida albicans ATCC 10231 (materials obtained by CE functionalisation) / Candida parapsilosis ATCC 22019 (materials obtained by CH functionalization)

The materials obtained by functionalising CE with quaternary ammonium salts and phosphonium salts showed substantial antibacterial capacity against Staphylococcus aureus, Escherichia coli, Candida albicans and Pseudomonas aeruginosa species even at very low concentrations of antimicrobial agents immobilized on the surface. Growth inhibition of

Staphylococcus aureus, Escherichia coli, and Candida albicans strains was achieved using CE-DDTMABr, CE-TDTMABr, and CE-DDTPPBr materials, regardless of the functionalisation ratio tested.

Materials obtained by functionalising CE with sulfur salts generally showed a bacteriostatic or fungistatic effect. An exception was in the case of CH-MBT material when the maximum inhibition rate in Staphylococus aureus and Candida albicans species was reached at functionalization ratios starting with the CH:MBT = 1:0.3.

In the case of materials obtained by CH functionalisation with sulfur salts, the antibacterial/antifungal activity against Staphylococcus aureus and Candida parapsilosis species is less evident at low functionalization ratios and increases with increasing CH: sulphur salt ratio.

In the case of the Gram-negative bacteria tested, the effect of the CH-Thio material is rather a bacteriostatic one than a bactericidal one, motivated by the inhibition rate of approximately the same order of magnitude, at a 50-fold change in the CH:Thio functionalisation ratio.

Hydrophobicity, which correlates well with the bioactivity of chemicals, is a very important characteristic, and the different hydrophobic behaviour of the materials obtained by CH functionalisation plays an important role in their biological activity mechanisms, regardless of whether it is antibacterial or antifungal activity.

In the case of materials obtained by functionalising biopolymers with quaternary ammonium salts, the antibacterial activity depended on the length of the alkyl chain that distances the pendant group of the quaternary salt from the basic structure of the biopolymer.

In the case of materials obtained by functionalising biopolymers with phosphonium salts, the antibacterial activity was strongly influenced by the hydrophobic character of the pendant group (phenyl compared to butyl), to which the distance given by the alkyl substituent of the quaternary salt (dodecyl compared to hexadecyl).

Taking into account the potential of the materials obtained by functionalising CH with phosphonium salts, to be used to obtain biocompatible materials for various medical technologies, it is certain that the use of CH-DDTPPBr and CH-HDTPPPr materials had very good results on the Staphylococcus aureus species , which represents a desideratum in obtaining biomaterials with biomedical applications.

The materials obtained by functionalising biopolymers with the extractant DDTPPBr, i.e. CE-DDTPPBr and CH-DDTPPBr, have a total bactericidal effect on Gram-positive bacteria and the Escherichia coli strain. The materials obtained by functionalizing CE and CH with the HDTBPBr extractant, i.e. CE-HDTBPBr and CH-HDTBPBr, have a maximum bactericidal effect only on Gram-positive bacteria.

The interaction of the cationic components in the basic structure of the polymers with the negatively charged components present in the bacterial cytoplasmic membranes is considered a crucial step in the manifestation of the antibacterial effect.

In terms of hydrophobicity, when we compare these two extractants used to functionalise the support material, the hypothesis is supported that by increasing the hydrophobicity of cationic biocides, they become able to interact with the cytoplasmic membrane.

The results obtained confirm that the materials obtained by functionalising CE or CH with DDTPPBr, which are more hydrophobic, have a better antibacterial action.

Although a multitude of materials can be synthesised from natural or synthetic precursors by various chemical methods, the functionalisation of biodegradable biopolymers with materials known as bactericidal agents is a preferred functionalization method in response to environmental laws and management issues. subsequent reaction secondary products, which give materials with improved properties and wide applicability.

The original research presented in this doctoral thesis was realised through the publication and/or communication of 6 scientific works on journals indexed in the Web of Science. Three of the articles are graded Q1 and the other three articles are Q2. Also, 1 bachelor's thesis and 1 dissertation thesis were completed; The sum of the impact factors is 27.5. H-index-3.

It should be mentioned that the paper with the title: *Factors Influencing the Antibacterial Activity of Chitosan and Chitosan Modified by Functionalization*, ***of which I am the first author, published in the*** *International Journal of Molecular Sciences*, ***has 58 citations after 2 years of publication.***

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