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Development of a Glassy Material Doped with Zn^{+2} for the Treatment of Contaminated Water by Bacteria *Escherichia coli* and *Staphylococcus aureus*

Desenvolvimento de um material de vidro dopado com Zn^{+2} para o tratamento de água contaminada com bactérias *Escherichia coli* e *Staphylococcus aureus*.

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Abstract

Being something of fundamental importance for the maintenance of health life in the planet, conservation programs and water reuse are good tools for the preservation and promotion of a sustainable environment. It has already been studied that the application of antibacterial materials in water treatment could be an efficient method to promote the reuse of this natural resource. It is known that, through the effect oligodynamic, some metals are able to promote death of some microorganisms and/or to prevent their proliferation. This research aimed to study the use of a glassy matrix doped with zinc ions on the treatment of water contaminated with Escherichia coli and Staphylococcus aureus bacteria. To evaluate the antimicrobial activity and the release of zinc ions, the compound was submitted to tests of Minimum Inhibitory Concentration (MIC), Atomic Absorption (AA) and X-ray Fluorescence (XRF). It has been concluded that the matrix doped with zinc ions is an excellent alternative for the treatment of contaminated water, whereas for the highest concentration tested, there was a reduction of approximately 100% in bacterial growth. It was checked that the compound is nontoxic, which further enhances the application of this compound.

Keywords: *Glass particle doped with Zn^{+2}. Water treatment. Glass particle as filter element.*

Resumo

Os programas de conservação e reuso de água são boas ferramentas para a preservação e a promoção de um ambiente sustentável. É conhecido que a aplicação de materiais antibacterianos no tratamento de água pode ser um método eficiente para promover a reutilização deste recurso natural. Sabe-se que, através do efeito oligodinâmico, alguns metais são capazes de promover a morte de alguns microrganismos e/ou evitar a sua proliferação. Este estudo teve como objetivo estudar o uso de uma matriz vítrea dopada com íons de zinco no tratamento de água contaminada com bactérias Escherichia coli e Staphylococcus aureus. Para avaliar a atividade antimicrobiana e a liberação de íons de zinco, o composto foi submetido a testes de Concentração Inibitória Mínima (MIC), análises de Absorção Atômica (AA) e fluorescência de raios X (FRX). Concluiu-se que a matriz dopada com íons de zinco é uma alternativa excelente para o tratamento de água contaminada, que, para a concentração mais elevada testada, houve uma redução de cerca de 100% do crescimento bacteriano. Foi verificado que o composto é não tóxico, o que aumenta ainda mais a possibilidade de aplicação deste composto.

Palavras-chave: *Partículas de vidro dopadas com Zn^{+2}. Tratamento de água. Partículas de vidro como elemento de filtro.*

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1 Introduction

Water quality has been the subject of several scientific studies, because it is something of fundamental importance for the maintenance of health life in our planet. Programs of conservation and reuse of water have been carried out in order to promote the development of a sustainable environment (MATSUMURA & MIERZWA, 2008).

Some methods of disinfecting water have already been used, as chlorination, ozonization and ultraviolet irradiation processes. Among these methods, chlorination is the most widely used method because of its practicality and efficiency (TORTORA et al., 2005).

However, the development of new methods involving the application of special materials with active components can promote an outstanding innovation. The most promising materials are based on some metals, which present the oligodynamic effect, promoting the death of microorganisms and preventing their proliferation. The oligodynamic property is a toxic effect of metal ions on cells, algae, fungi, bacteria and viruses, even in relatively low concentrations. This antimicrobial effect is observed for mercury, silver, copper, iron, zinc, gold, aluminum and other metals ions, especially of heavy metals (CHAKRAVARTI et al., 2005; TIEN et al., 2008). When incorporated to other materials the biocidal efficiency is dependent on the ability to release such ionic species (MATSUMURA & MIERZWA, 2008).

Although the exact mechanism of antimicrobial action of these elements has already been studied, the oligodynamic effect is still unknown. Some data suggest that they denature proteins in prokaryotic cells of microorganisms, making them become inactive (AHNA et al., 2009; BRAYNER et al., 2006). Other authors suggest that there is an interaction with phosphorus ions present in the DNA, resulting in inactivation of DNA replication. Another theory also suggests that, the described ions, react with sulfur-containing proteins leading to inhibition of enzymes and affecting their functions (RAVISHANKAR & JAMUNA, 2011)

Thus, this work has as main objective the applying a glassy matrix compound doped with Zn^{2+} ions in wastewater treatment and evaluate the effectiveness of this method for promoting the decontamination of water from bacteria *Escherichia coli* and *Staphylococcus aureus*. The compound showed efficiency close to 100% in decontamination of water, when applied in high concentration.

2. Material and methods

Glass particles used in this study, as well as the compound matrix, have a high percentage of sodium and average size of 10 micrometers (MENDES et al., 2012). Therefore, this material was submitted to a doping process in which the ionic species of zinc were incorporated to the compound, according methodology defined by Fiori et al. (2009). The ionic species of Zn^{+2} were incorporated by the ionic exchange process between sodium species in the glass particles and the ionic zinc present in the ionic reaction medium, that containing 23.0 g of glass particle, 3.00 g of $NaNO_3$ and 1.5 g of $Zn(NO_3)_2$. The composition of glass particle is presented in the Table 1.

To check the antimicrobial effect of the glass particle matrices, were used water solutions with known concentrations of *Escherichia coli* and *Staphylococcus aureus* and LB culture medium. The concentration of each bacterium was evaluated by the analysis of intensity of a specific peak with absorption in 600 nm and was performed on Shimadzu UV-1800 spectrophotometer. The bacteria concentration were adjusted according to the *MacFarland* scale. The microbiological test used was the "minimum inhibitory concentration" (MIC), which is the lowest concentration of compound capable of inhibiting bacterial growth (phenomenon observed by reading the absorbance at 600 nm) after incubation at 37 °C for 24 hours (NCCLS, 2003; TORTORA et al., 2005).

The ability of releasing zinc ions by antimicrobial compound was evaluated by atomic absorption technique. The samples were immersed in deionized water at concentrations of 0.005, 0.05 to 0.5 g/ml for 48 hours. All concentrations were tested in triplicate. After 48 hours the solution was filtered to extract the glass particles and subjected to analysis (atomic absorption spectrometry flame and graphite furnace - Varian brand, model AA240FS).

The glass particle extracted from the solutions after 48 hours of immersion were submitted to chemical analysis by x-ray fluorescence (XRF: X-Ray Spectrometer dispersion wavelength - WDXRF - Philips model PW 2400) and atomic absorption (atomic Absorption Spectrometer Unicam brand, model SOLAR 969). This procedure allowed evaluating the percentage of zinc species present in the glass matrix of the additive before and after immersion in distilled water solutions.

Table 1 - Composition of the glass particle utilized as glassy matrix

Compound	Al_2O_3 (wt. %)	SiO_2 (wt. %)	Li_2O (wt. %)	NaO_2 (wt. %)
Glass particles	3.0	72.0	5.0	20.0

3. Results and discussions

Fiori et al. (2009) demonstrated that the percentage of ionic species in the reaction medium affect the amount of the ionic species incorporated into glassy matrix. The work developed with ionic silver species proved that with a percentage of 6.45 wt% of $AgNO_3$ in the ionic exchange medium it is possible to incorporate about 20 mg/L of Ag^+ in the glass particle structure (FIORI et al., 2009). So, it is expected that for the same percentage of $(ZnNO_3)_2$ in the reaction medium the amount of ionic zinc incorporated in the glass particles will be similar.

The glass particles are soluble in water. So, after its immersion in contaminated waters it is expected that the ionic species of Zn^{+2} will be released for the medium, providing death or inactivation of bacteria. The Table 2 shows that the concentration of ionic zinc in water solutions after immersion of different percentages of glass particles by 48 hours, determined by atomic absorption techniques. The solutions containing 0.005 g/ml of antimicrobial glass (solution 1) not showed the presence of ionic zinc specimens after 48 hours of immersion, according the resolution limits of the absorption atomic spectrometer. The solutions containing 0.05 g/mL of glass (solution 1) released 0.35 g/mL of ionic zinc and the solution containing 0.5 g/mL (solution 2), 1.70 g/mL. The glass particle doped with ionic zinc species are used as source of Zn^{+2} for the solutions and it is expected that increasing the amount of glass will promote the increase of the amount ionic zinc in the solution.

Figure 1 and Figure 2 show the microbiological results obtained with the minimum inhibitory concentration test (MIC) for the water solutions contaminated with *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 25923) and treated with different concentration of glass particles by 48 hours.

Results showed that the glass particles doped with Zn^{+2} can be used for the treatment of contaminated water by bacteria. It is evident that the increase of the concentration of glass particles in the solution favor the increase of the concentration of Zn^{+2}, so is expected an increase of the antimicrobial effect in solutions.

For the concentration of 0.005 g/ml of glass particles there was a reduction of 37 % of the concentration of *Escherichia coli*. For the bacteria *Staphylococcus aureus* the reduction is much more evident, since that, for the same concentration, the percentage of reduction was of about 90%. The difference on the microbiological response between the two bacteria occurs because *Escherichia coli* is a gram-negative bacterium, with a cellular structure

Table 2 - Amount of ionic zinc in water solution after the immersion of the doped glass by 48 hours

Water solutions	Glass (g/mL)	(g/mL)	σ (g/mL)
*Solution 1 (0.005 g/ml)	0.00 g	0.00	± 0.00
Solution 1(0.05 g/mL)	0.50 g	0.35	± 0.07
Solution 2 (0.5 g/mL)	0.05 g	1.70	± 1.44

*According the resolution limits of the absorption atomic spectrometer

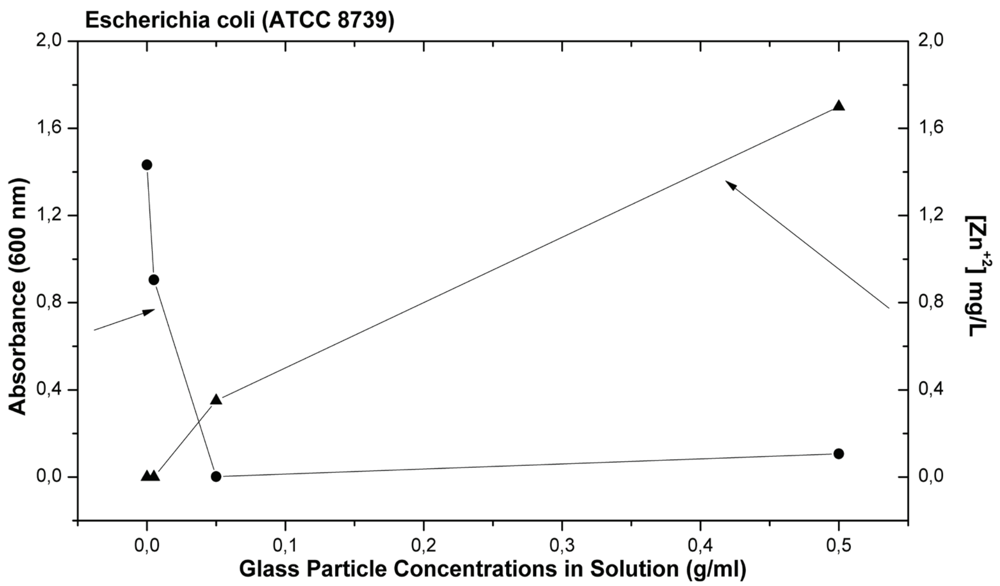

Figure 1 - Microbiological results obtained with the minimum inhibitory concentration test

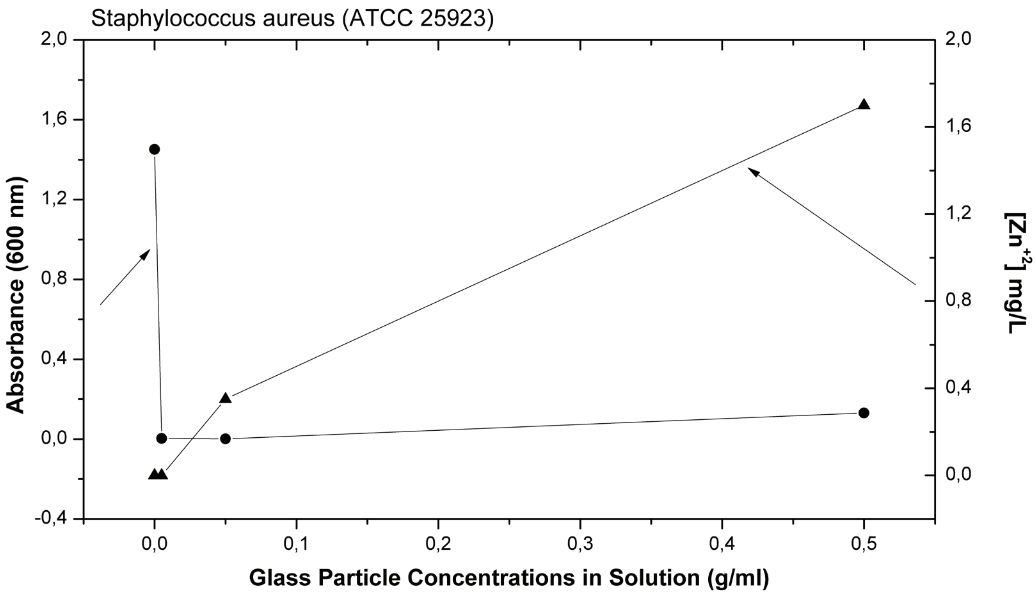

Figure 2 - Microbiological results obtained with the minimum inhibitory concentration test (MIC) for the water solutions contaminated with *Staphylococcus aureus* (ATCC 25923) treated with different concentrations of glass particles by 48 h

much more organized than *Staphylococcus aureus*, which is gram-positive. The *Staphylococcus aureus* bacteria are more susceptible to the action of antimicrobial materials, explaining the results obtained (TRABULSI et al., 1999).

Concentrations above 0.05 g/mL were sufficiently to kill both bacteria. For *Escherichia coli* almost 96 % of the bacteria were eliminated and for the *Staphylococcus aureus* approximately 95 % were killed. The results show that concentrations above 0.05 g/L of glass particle do not promote significant modification on the antimicrobial effects in water solutions, so the minimum inhibitory concentration of glass particle doped with ionic zinc, for both types of bacteria evaluated, was 0.05 g/ml.

According to the results of atomic absorption, the solutions with concentration of 0.05 g/ml showed release rate of zinc ions of (0.35 ± 0.07) mg/L, while the solutions with concentration of 0.5 mg/L showed a rate of (1.70 ± 1.44) mg/L. Microbiological results demonstrate that water solutions containing higher amounts of glass particle have higher antimicrobial effect. Thus, it is possible to relate the antimicrobial actions directly with the percentage of ionic zinc in solution and with the percentage of the glass particle in solution.

Table 3 presents the results of chemical analysis by x-ray fluorescence and atomic absorption for the glass doped particles extracted from the water solutions after 48 hours of immersion, highlighting sodium and zinc.

Results indicate that the percentage of zinc and sodium species of glass particles employed with 0.05 g/ml and 0.5 g/ml are almost equal after its use in water solution. In both conditions the same amount of sodium and zinc are observed in the glass particles after the immersion for 48 hours. It is possible to verify lower amounts of zinc in the structure of the glass than that observed previously in glass particle in nature. The reminiscent zinc percentage in glass particle after its immersion is 7.13 wt. % and 9.70 wt. % for solution 1 and solution 2, respectively. These values are practically equal, considering that standard deviation is high for the experiment involving the solution 2.

X-ray fluorescence and atomic absorption analysis showed that glass particles still have zinc species in its structure after immersion by 48 hours in the water solutions. This result is an important characteristic and

Table 3 - Chemical Analysis by X-ray fluorescence and atomic absorption of the antimicrobial glass doped after immersion for 48 hours in deionized water

Water solutions	**Na_2O (wt%)**	**ZnO (wt%)**	**SUM (wt%)**
Solution 1 (0.05 g/mL)	18.97	7.13	26.10
Solution 2 (0.5 g/mL)	16.37	9.70	26.07

demonstrates that the glass particles have the ability to release ionic zinc in solution for longer times, maintaining the water protected. The glass particle doped with Zn^{+2} can be applied for treatment of contaminated water and to maintain protected by bacteria.

These results are positive indicative of use of this glassy material as agent for purification of water contaminated with gram positive and gram negative bacteria. The emphasis on use of zinc ions is mainly in the fact that these ions are already present in the soil, assisting the growth of plants, water and as part of our metabolism. It is also considered non-toxic at the concentrations tested, which further strengthens its application.

4 Conclusions

Results showed that glass particle doped with ionic zinc is an efficient alternative for the treatment of contaminated water with Escherichia *coli* and Staphylococcus *aureus* bacteria. The concentrations from 0.05 g/ml promote the reduction of over 95 % for both bacteria in 48 hours. However, at concentrations from 0.005 g/ml the 85% of the bacteria *Staphylococcus aureus* is already eliminated. For conclusive evaluations new studies are required with longer periods of water treatment and more detailed studies about the release rate of ionic species by varying this additive concentrations.

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