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Copper-impregnated ceramic membranes and their anti-microbial effect against *Escherichia coli*

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ABSTRACT

In this work tubular commercial α alumina and mullite (M1 and M2, respectively) ceramic membranes were impregnated with copper nanoparticles and characterized aiming at their use in the disinfection of waters. Copper nanoparticles were obtained by the dissolution of Cu $(NO_3)_2 \cdot 3H_2O$ in a poly (vinyl alcohol) solution (PVA) 5% (m/v) followed by chemical reduction of the copper ions into metal particles by the use of sodium borohydride. The colloidal copper dispersion was impregnated into the commercial membranes by dip-coating and then the impregnated membranes were calcinated at the temperature of 600°C for PVA removal. In order to assess the bactericidal efficiency of the prepared membranes microfiltration and inhibition tests were carried out using *Escherichia coli* as indicator microorganism. As a result, copper-containing membranes had bactericidal efficiencies, approximately, between 88.7 and >99.9%,and inhibition zone between 10 and 13 mm, approximately. On the other hand, copper less membranes resulted in low bacterial retention efficiencies, between 40.3 and 73.7%, and were devoid of any inhibition zone. The ceramic membranes without copper presented low efficiency due to their variation in pore size. The M1 membrane presented pores size between 0.1 and 3 µm, while the M2 membrane between 0.03 and 4 µm. According to the results, it was possible to confirm the importance of the presence of copper in the membranes. The results obtained in this work show that copper impregnation into tubular ceramic membranes was efficient and provided bactericidal effect.

Keywords: Membranes; Copper nanoparticles; Dip-coating; Microfiltration; *Escherichia coli*

1. Introduction

The contamination of water bodies and the deterioration of water quality is a public health concern. According to the World Health Organization (WHO), nearly 80% of diseases are caused by water contamination. WHO states that any water directed to human use should contain zero CFU (colony forming units) of *Escherichia coli* by 100 mL of sample. Countless chemical and physical processes are provided aiming at the microbiological decontamination of water, such as the use of ozone and free chlorine, ultraviolet radiation and filtration. However, some of these processes can bring undesired consequences to human beings, such as color changes, taste, visual aspect, insufficient efficiency and formation of carcinogenic chemical products [1,2].

Among the water process treatments, membrane separation processes (MSP) are considered as one of the most innovative technologies [3]. MSP are processes which are quick, economic, occupy shorter area space, being highly selective, flexible and compatible, most of the time with other treatment processes [4]. However, according to Sawada et al., (2012), one of the trickiest problems of membranes is permeability reduction throughout their use due to scale from organic matter contained in raw water and microorganisms which adhere to the microporous membrane or within the pores [5].

In the selection of materials for the manufacture of membranes, ceramic materials (alumina, zirconia, titania

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and silica) as compared with polymeric materials have advantages such as longer useful life, high mechanical strength and easy cleaning [6]. However, these materials are very costly to obtain and most of times are not selective in the withdrawal of low molecular weight particles.

Several studies present new materials aiming at the withdrawal and disinfection of water pathogenic microorganisms. The bactericidal effect is reached many times by employing bactericidal materials such as metallic nanoparticles (NPs), such as copper and silver. Table 1 lists the bibliographic search on the effect of some NPs on the inactivation of pathogenic microorganisms.

Copper as nanoparticles is being increasingly employed as antimicrobial agent by the textile industry, in hospital equipment, wood preservation and anti-scale paintings. The copper bactericidal effect for a wide range of gram-positive and gram-negative bacteria has been reported by various authors [10–12,15,16].

Copper nanoparticles (NPCu) require higher concentrations relative to silver nanoparticles (NPAg) to exhibit a bactericidal effect. The bactericidal capacity of NPs has shown that these release ions which cause pH and conductivity changes in a liquid medium. Cu^{2+} ions are able to disrupt bacterial cell membranes and their entry through the membranes interrupts the enzymatic function. The indirect effects exerted by the environment can also have impact on the efficacy of metal nanoparticles against microorganisms [15].

The study by Baek and An (2011) evaluated the bactericidal effect of CuO, NiO, ZnO and Sb_2O_3 nanoparticles against the *E. coli, Bacillus subtilis* and *S. aureus* microorganisms. This study concluded that CuO NPs have higher effect on *E. coli* bacterium than the other nanoparticles. This augmented effect exhibited by copper is related to higher interaction of copper ions with microorganisms. The effect of the metallic ions released by nanoparticles does not

Table 1
Toxic effect of different nanoparticles on different microorganisms

NPs	Microorganism	Diameter	Dosage	Mechanism of toxicity action	Comments	References
Ag	*E. coli*	3–18 nm SEM	1.4 mg·L^{-1}	Interaction with the cell wall, protein oxidation and cellular homeostasis disturbance	The Ag NPs interact in large proportions the smaller the particle size	[7]
Ag	*S. aureus*	20–150 nm TEM	0.107–0.535 mg·L^{-1}	Inactivation of the respiratory chain, rupture of the cell membrane, blocking of DNA replication	The Ag NPs interact more easily with gram-negative bacteria because of the thin layer of peptidoglycan	[8]
Ag	*S. aureus*	2.8–6.7 nm TEM	–	–	–	[9]
Cu	*S. aureus*	50–70 nm DLS	1.875–3.75 mg·L^{-1}	The copper ions in contact with the target interrupt the biochemical processes, inactivating enzymes	The copper NPs have similar mechanisms to silver NPs	[10]
Cu	*E. coli*	100–600 nm SEM	10 and 65 mg·g^{-1} of Cu/paper	The copper NPs cause irreversible damage to the bacterial membrane by increasing of the membrane permeability and destabilizing cells	The action of copper NPs can cause inactivation of microorganisms in a few minutes	[11]
Cu	*Pseudomonas spp., E. coli, Bacillus spp., Staphylococcus spp.*	9–32 nm SEM, TEM and XRD	–	The copper NPs cause the inactivation of proteins and enzymes, leading to cell death	The inactivation of proteins occurs due to the interaction of the nanoparticles with the sulfhydryl groups	[12]
ZnO	*E. coli*	15–70 nm TEM	210 mg·g^{-1} of chitosan/ bentonite	Bacterial affinity by electrostatic interaction, free radical generation and disturb of the permeability	The toxicity effect is lower on gram-positive bacteria due to the thicker peptidoglycan layer	[13]
TiO_2	*E. coli*	50 nm TEM	62.5 mg·L^{-1}	Decomposition of the outer bacterial membrane by ROS*	The TiO_2 NPs haven't toxicity in the absence of light	[14]

*ROS: Reactive Oxygen Species.

depend only on the metal used, but also on the microorganisms which is exposed [16].

Copper toxicity on bacteria depends on the combination of several factors such as temperature, aeration, pH, NP concentration and bacteria concentration. The increase in temperature and aeration and pH reduction diminishes copper agglomeration and raises its toxicity. Reduced agglomeration favors more surfaces available for interaction with bacterial membranes and copper ions solubilization, leading to higher toxicity. Still, metallic and ionic copper forms yield hydroxyl radicals which damage essential proteins and DNA [17,18].

In this context, the development of materials which would render possible the separation of water impurities while providing bactericidal effect with low manufacturing cost and physical and chemical characteristics inherent to the treating process is seen as a promising alternative to water treatment. On the basis of this context, this work aims at preparing and characterizing tubular ceramic membranes impregnated with NPCu and the application of the proposed materials for the microbial inactivation of *E. coli* – contaminated waters. To attain these goals inhibition and microfiltration tests were carried out.

2. Experimental

2.1. Materials

Tubular commercial alumina (α-Al_2O_3) (M1) and mullite (Al_2O_3-SO_2) (M2) ceramic membranes were supplied by Tecnicer Tecnologia Cerâmica Ltd a (São Carlos – SP, Brazil). These materials are of tubular and microporous structure, having average pore size between 0.40 ± 0.17 µm and 0.60 ± 0.17 µm, for M1 and M2, respectively. Table 2 lists the information on the dimensions of the membranes used in the present work.

The reagents used in the synthesis of the nanoparticles of this work were poly(vinyl alcohol) (PVA, Vetec, MM = 89 kDa), trihydrated copper nitrate ($Cu(NO_3)_2 \cdot 3H_2O$, Sigma Aldrich) and sodium borohydride ($NaBH_4$, Merck). For the preparation of colloidal dispersions ultra pure water was used (Milli-Q/Millipore system).

For the microbiological analyses was used, as culture medium for growing the *E. coli* microorganism,the medium Broth nutrient (Merck) and agar nutrient (HIMEA). Further, sodium chloride (NaCl) (Merck) (for dilutions and plating) and sodium hypochlorite (3% v/v) to carry out the disinfection of the microfiltration system before and after the microfiltration process were used.

Table 2
Dimensions of the ceramic membranes used in this study

Dimensions (cm)	M1	M2
Length	19.6	19.6
Wall thickness	0.16 ± 0.04	0.15 ± 0.05
Internal diameter	0.80 ± 0.01	0.81 ± 0.03
Area (cm^2)	49.25 ± 0.61	50.30 ± 2.31

M1-Aluminae M2-Mullite.

2.2. Obtention of copper nanoparticles

The obtention of NPCu was performed in an aqueous medium starting with the chemical reduction of Cu^{2+}, using as reducing agent sodium borohydride. To stabilize the NPCu a dispersion of PVA was used. The procedure for obtaining NPCu can be viewed in Fig. 1.

For obtaining copper nanoparticles at first 5 g PVA were dissolved in 80 mL water under constant magnetic agitation and at the approximate temperature of 80°C. Thereafter, 10 mL of a trihydrated copper II nitrate solution of 1.07 mol·L^{-1} concentration was prepared and then this solution was added drop wise (1 drop per second) to the PVA solution, previously prepared and cooled to the approximate temperature of 23°C. After the admixture of these two solutions, 10 mL solution $NaBH_4$ of 185 mmol·L^{-1} concentration was prepared and added drop wise (1 drop by second) to the copper II nitrate solution and PVA under constant agitation. At the end of this procedure the color of the dispersion was dark red. After complete reduction, the solution was stored in a refrigerator (at 5°C and in the absence of light) for further characterization.

The estimated copper nitrate concentration at the end of the procedure was approximately 25.75 g·L^{-1}. The concentration of copper present in solution was determined by atomic absorption spectrometry in an atomic absorption

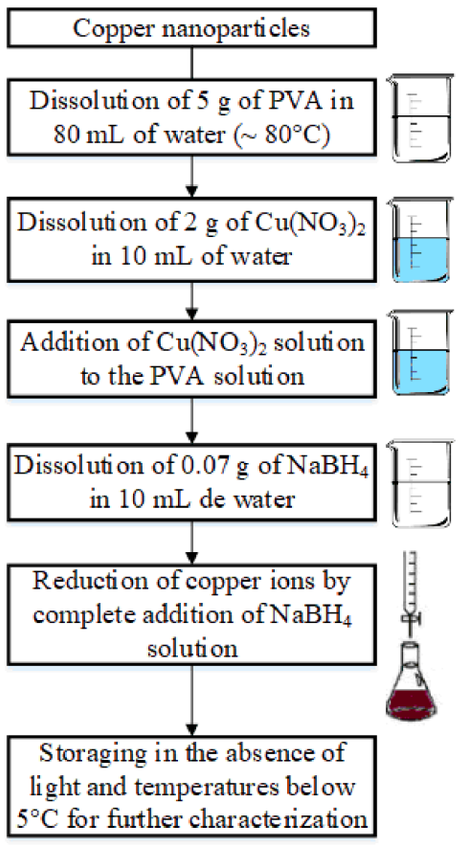

Fig. 1. Procedures developed to obtain copper nanoparticles.

spectrometer (AANALYST 200) at the Laboratory of Environmental Analyses and Researches (LAPAM) of the University of Caxias do Sul (UCS) (RS/Brazil).

2.3. Characterization of copper nanoparticles

Powder NPCu samples were analyzed in a Shimadzu, model XRD-6000 X-ray diffractometer with CuKα radiation and λ =1.5406 Å. To obtain the powder samples the copper colloidal dispersions were centrifuged in a Bio Eng BE-5000 centrifuge at 4,000 rpm for 30 min. After centrifugation samples were washed with commercial acetone and dried in an oven for 24 h at approximately 70°C.

Images of the NPCu were obtained in a Transmission Electronic Microscope (TEM) JEOLJEM-1200 Ex II. The equipment was operated at the voltage of 80 kV. The copper colloidal dispersions samples were diluted (1:10) and treated in an ultrasound system for 10 min for better nanoparticle dispersion. Thereafter, a drop of the NPCu dispersion was deposited on a copper grid with Formvar (300 mesh) film and left under vacuum at the temperature of 23°C for 24 h.

The NPCu-containing dispersions were analyzed in a Termo Spectronic (UV-Visible) ultraviolet Genesys spectrophotometer 10 mV, at the wavelength range situated between 250 and 800 nm using a poly (methyl methacrylate) cell of 10 mm optical path.

The copper nanoparticles size distribution was assessed in a NANO-flex® 180° DLS equipment. For the analyses, the operation range of 0.8–6500 nm was used. Samples of copper colloidal dispersions were diluted (1:10) and treated in an ultrasound system for 1 min aiming at dispersing the nanoparticles.

2.4. Obtention of copper-recovered ceramic membranes

Fig. 2 shows the process utilized for copper nanoparticles impregnation in the interior of the commercial ceramic membranes.

NPCu were impregnated into the M1 and M2 ceramic membranes by the dip-coating method. To this end, copper colloidal dispersions were added to the interior of the ceramic membranes, one end of which was closed. In order to avoid nanoparticles oxidation by atmospheric air during the dip-coating process the other end of the ceramic tube also remained closed.

The impregnation process lasted 2 h and at the end of this period the excess solution contained inside the membranes was removed and drying in an oven was carried out for 40 min at approximately 70°C. The purpose of drying was to promote adherence of PVA-containing metallic nanoparticles to the interior of the ceramic membranes. The impregnation process was repeated two more times, the total impregnation period being 8 h. Finally, the copper-recovered ceramic membranes were thermally treated at 600°C for 4 h for PVA removal.

2.5. Characterization of copper-recovered ceramic membranes

The presence of copper on the internal surface of the ceramic membranes was assessed by scanning electronic

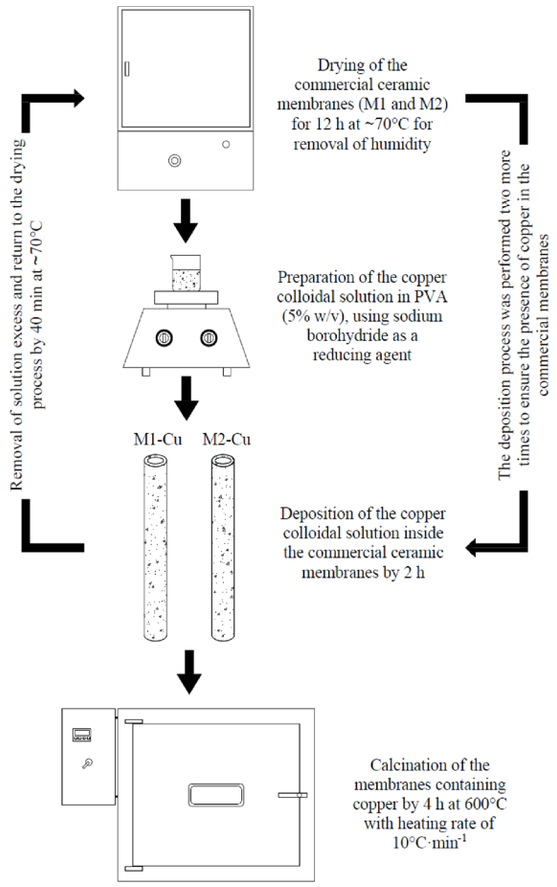

Fig. 2. Procedures for obtaining ceramic membranes impregnated with copper. Where: M1-Cu is copper-containing alumina membrane and M2-Cu is copper-containing mullite membrane.

microscopy (SEM) in a TESCAN model MIRA3 field emission scanning electronic microscope (FE-SEM) coupled to an EDS detector. In this same instrument the presence of copper was assessed by energy dispersion spectroscopy. Membranes were fractured and metalized by cathodic pulverization with a fine gold layer.

In order to assess the approximate concentration of metallic copper in the ceramic membranes an atomic absorption spectrometer (AANALYST 200) was used. In order to carry out the analysis, the copper-containing ceramic material samples were macerated in a mortar/pestle. The copper amount released in liquid medium from the ceramic membranes was analyzed in an inductively coupled plasma optical emission spectrometry (ICP-OES) equipment. According to the methodology of Lv et al. the samples of copper-containing ceramic membranes were added to 50 mL of ultra pure water and immediately after, ultrasound was performed for 15 min [19].

2.6. Inhibition tests

In order to carry out the inhibition tests, 100 µL of autoclaved distilled water containing *E. coli* (~10^6 UFC·mL^{-1}) cells were added over the plates and uniformly spread. Then,

samples of the M1, M2, M1-Cu and M2-Cu membranes were placed on the solidified agar gel, as well as on the neat PVA films and aluminum foil. PVA film and aluminum foil were tested as control.

Aluminum foil was used as surface for impregnating PVA dispersions containing the NPCu. This method was used since PVA is solubilized in the presence of humidity and by temperature increase, rendering difficult visualization on the plates. Finally, plates were incubated at 35°C for 24 h for later analysis of the inhibited bacterial zone.

2.7. Microfiltration tests

Experiments were carried out on the copper-impregnated and non-impregnated ceramics in a laminar flow exhaust hood which had been sterilized with ultraviolet radiation for 15 min to avoid possible external contaminations.

Tests related to the (*E. coli*) microorganism were carried out for 1 h under transmembrane pressures of 50, 100, 150 and 200 kPa. During the microfiltration tests feed and permeate samples were collected every 20 min in order to perform plating and cell counting tests.

The method used for counting bacteria was the spread-plate. *Escherichia coli* was selected as an indicator of fecal contamination of water(~10^6 UFC·mL^1).The nutrient Broth and agar nutrient were used as the growth medium. Was collected samples 1 mL of permeated and feed. The bacterial suspension collected of *E. coli* was diluted to 10^{-1}–10^{-6} in 10 mL distilled water. This process was performed in triplicates and for all pressures under study.

3. Results and discussion

3.1. Copper nanoparticles

Atomic absorption spectrometry results led to copper concentration in solution of 7.97 g·L^{-1}. This value is close to the theoretical value (8.7 g·L^{-1}).

Scanning of the copper colloidal dispersion had an absorption band around 265 nm. As for the NPCu, surface plasma resonance values depended on nano particle shape (triangular prisms, elongated particles, cylinders and spheres). However, the lack of homogeneity in size and shape of the samples and the absence of control on inter particle distances resulted in enlarged surface plasma resonance values, suggesting that NPCu do not constitute an ideal plasma material as compared with NPAg [20].

According to Dhand et al. and Tiwari et al. metallic nanoparticles exhibit an optical phenomenon called uncommon surface resonance plasma due to the collective cumulated oscillation of conduction electrons at the metal surface in phase with the incident electromagnetic radiation [8,20]. This property is a function of the particle kind, size and shape as well as of the chemical environment [20,21].

Fig. 3 shows DRX patterns of NPCu tested on the basis of the copper powder formed. DRX for the illustrated sample had diffraction peaks located at 2θ = 36.3°, 42.2°, 52.4°, 61.2°, 73.5° and 77.2°, corresponding to the CFC copper

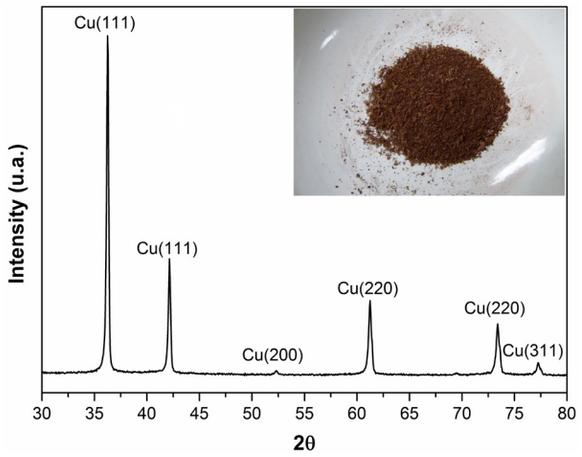

Fig. 3. Images of powder and diffractogram obtained by XRD of copper nanoparticles.

crystalline phase. This result corroborates the existence of metallic copper. The DRX pattern result was compared with the ICSD n° 43493 crystallographic chart.

Fig. 4 a depicts the TEM images of the NPCu. TEM of the copper nanoparticles colloidal dispersion shows spherical shaped nanoparticles of average diameter around 100 nm. In Fig. 4b the distribution histogram for NPCu obtained by TEM images can be seen. According to the histogram, NPCu have average diameter of 95 ± 20.54 nm.

DLS analyses of NPCu size indicated average size of 15.01 ± 3.58 nm, with maximum intensity peak at 12.77 nm, Fig. 4c. The difference between values shown by the methods employed in the assessment of copper particle sizes can be attributed to the period involved in the analysis. The light scattering method provides quick results (a few minutes after obtention of copper colloidal dispersions). On the contrary, for TEM results around 48 h are consumed between sample preparation and image obtention. For this reason, over time and by atmospheric air contact copper nanoparticles tend to agglomerate and form larger particles, which influence the difference exhibited for particle size results among the methods employed [22,23].

3.2. Copper-impregnated ceramic membranes

The presence of copper in M1 and M2 membranes can be observed in Figs. 5 and 6. It can be evidenced that copper may have recovered the analyzed material. Copper dispersion on the membrane M1 surface was not good, Fig. 5. On the other hand, as can be seen in Fig. 6, membrane M2 surface exhibited good copper particle dispersion. Besides, the presence of copper can be confirmed by atomic absorption spectrometry data. The amount of copper was approximately 1 g copper by kg of ceramic material for M1 and approximately 0.7 g copper per kg of ceramic material for M2.

The interaction between the ceramic materials and copper is entirely physical. The use of PVA was crucial to promote adherence of copper to membranes. The M1 mem-

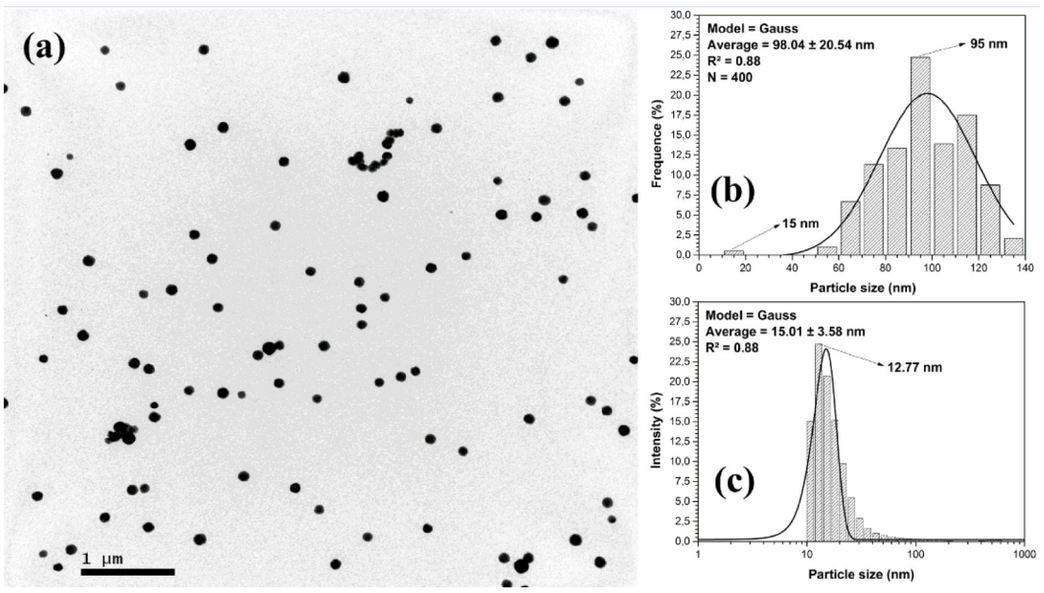

Fig. 4. (a) images of copper nanoparticles obtained by transmission electron microscopy, (b) size of copper nanoparticles obtained by transmission electron microscopy, where N is the sample number and (c) size of copper nanoparticles obtained by dynamic light scattering (c).

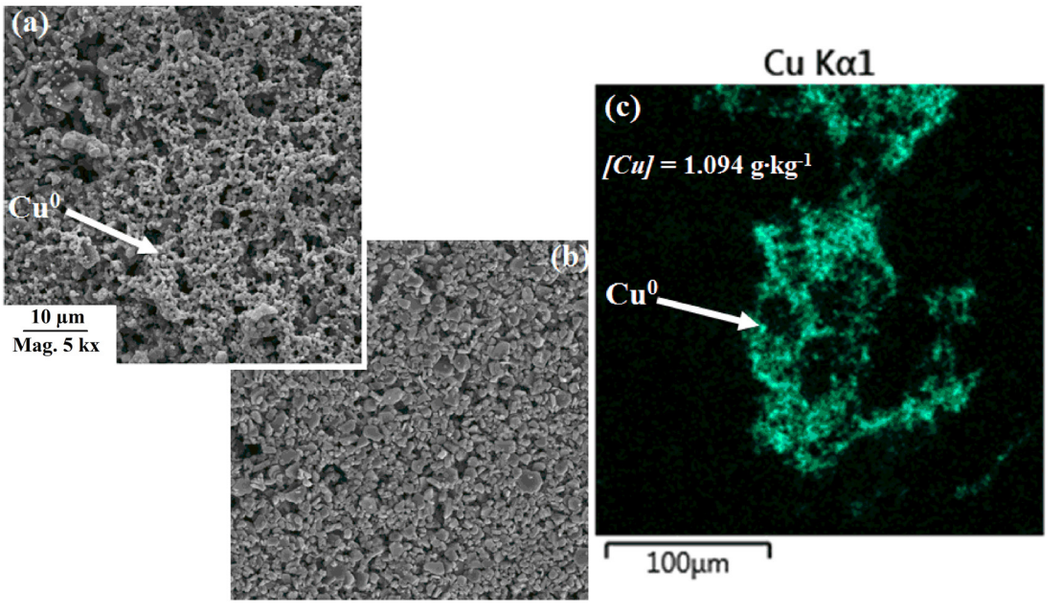

Fig. 5. Images of the mapping of alumina ceramic membrane composition containing copper. (a) With copper, (b) without copper and (c) mapping of composition.

brane presented less roughness when compared to membrane M2, which can justify a greater agglomeration of nanoparticles.

3.3. Inhibition zone tests

Table 3 lists the results for inhibition zone tests exhibited by control materials and by M1 and M2 ceramic membranes into the structure of which copper was impregnated. It can be observed that no influence of PVA, aluminum foil and ceramic materials was exerted on *E.coli* growth. These results demonstrated that in the absence of copper these materials do not bear any bactericidal effect. On the other hand, by impregnating these materials with copper they exhibit a significant inhibition zone [24,25]. The fact that no occurrence of bacterial growth was observed around

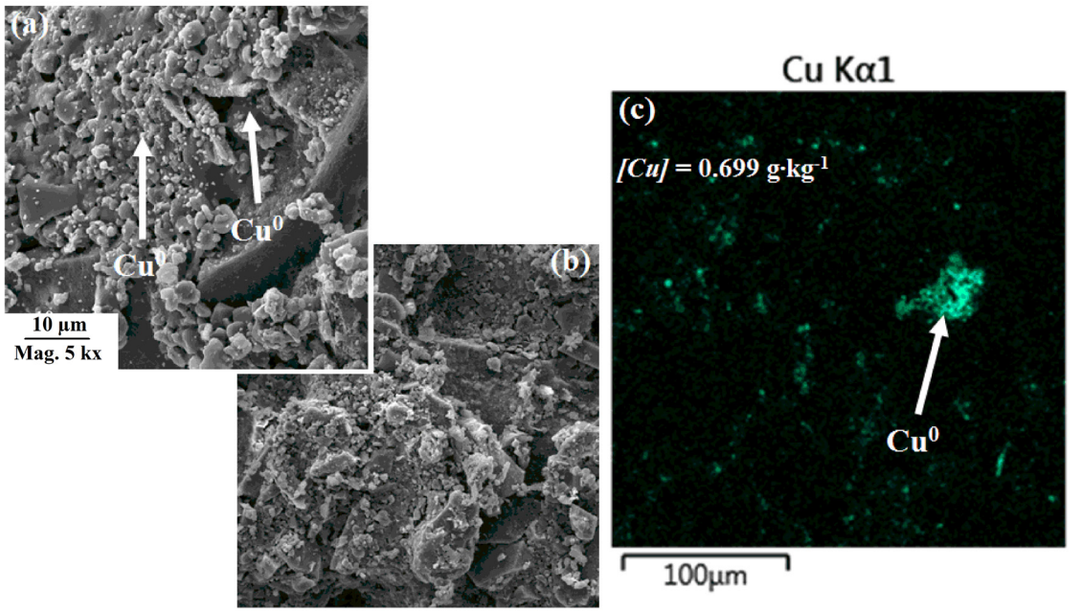

Fig. 6. Images of the mapping of mullite ceramic membrane composition containing copper. (a) With copper, (b) without copper and (c) mapping of composition.

Table 3
Results of inhibition zone tests presented by materials with and without copper

Materials	Inhibition zone (mm)	Standard deviation (mm)
PVA film with copper in aluminum paper	23.67	3.21
M1*	0.00	0
M2*	0.00	0
M1-Cu	11.67	1.52
M2-Cu	10.00	1
PVA*	0.00	0
Aluminum paper*	0.00	0

* Controls.

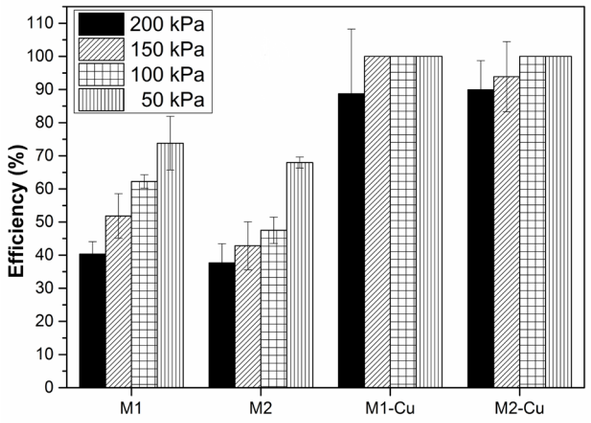

Fig. 7. Efficiency results of commercial ceramic membranes with and without impregnation of copper.

the copper-containing materials confirms the antibacterial property of the nanoparticles present on M1 and M2.

These results show that the slow release of copper ions provided efficient antibacterial activity. It is important to stress that the choice of the *E. coli* microorganism as object of investigation of microbiological contamination does not exclude similar experiments which should be conducted on other bacteria, such as *S. aureus* [11,19].

3.4. Microfiltration tests

Fig. 7 shows results of the efficiency of microfiltration tests shown by commercial ceramic membranes (M1 and M2) and by the copper-impregnated commercial ceramic membranes (M1-Cu and M2-Cu). As can be seen, ceramics impregnated with copper in their microporous structure exhibited bactericidal potential.

Commercial membranes M1 and M2 have shown bacteria retention efficiency, approximately, between 40.3 and 73.7%, respectively. On the other hand, by copper-impregnating membranes, they exhibited, besides retention, bactericidal capacity between 88.7 and <99.9%, approximately. These results corroborate the efficacy of the nanoparticles impregnation process on the internal surface of commercial ceramic membranes.

Membranes M1 and M2 did not show any bactericidal character and they presented low efficient in retaining bacteria. The combating against *E. coli* with the membranes occurred only by a physical factor, although the

alumina and mullite materials did not exhibit bactericidal influence or inhibition. For these membranes a filtration mechanism carried out by physical exclusion of particles exceeding the material porosity was observed [4]. Therefore, bacteria are constantly being retained and re-circulated as process feed and the amount retained in the pores and on the membranes surface can be associated to the reduction of bacteria in the feed throughout the experiments and to the permeated material. Inefficiency data exhibited by commercial membranes M1 and M2 could be interpreted as being due to the heterogeneity of the membranes pore size object of this study. In other words, the M1 membrane presented pore sizes ranging from 0.1 to 3 μm, and the M2 membrane presented pore sizes between 0.03 and 4 μm. These results corroborate the associated mechanism and the low efficiency of the membranes under study.

In the study by Dankovich and Smith (2014) the bactericidal efficiency was from 61 to 97%, using copper particles impregnated into absorbent papers. It could be observed that the copper efficiency against *E. coli* is related to the amount of bacteria and the amount of particles. For a bacteria amount of $5x10^6$ the efficiency observed was of 87 and 97%, for a copper amount of between 10 and 65 $mg{\cdot}g^{-1}$ of absorbent paper, respectively. And, for an amount of bacteria of $4x10^9$ an efficiency of 61 and 92%, for a copper amount of 10 and 65 $mg{\cdot}g^{-1}$ of absorbent paper, respectively was observed. The higher the amount of bacteria in liquid medium the lower will be the toxicity of the copper particles. On the other hand, the higher the amount of copper which contacts the microorganism the higher will be its toxicity [11].

In this work, efficiency >99.9% with the copper-impregnated M1 and M2 membranes was reached. These results are comparable to those of the literature [11,26,27].

The initial amount of *E. coli* in the feed was approximately 10^6 $UFC{\cdot}mL^{-1}$. Based on the results of the bactericidal capacity of the prepared membranes, two possible antimicrobial mechanisms can be ascertained: (1) bacteria were killed directly by copper ions (Cu^{2+}) released from the M1-Cu and M2-Cu ceramic membranes. Copper ions were adhered to the negatively charged cell wall, altering cell wall permeability. This action, together with protein denaturation induces cell lysis and consequently death. Copper antibacterial activity is also related to its ability to modify DNA replication mechanisms, as well as to cause changes in cell size and cytoplasmic content, cell membranes and outer layers of more sensitive cells. In the second mechanism (2), bacteria which flow outside microporous ceramics are contaminated by copper ions but can still survive. However, they cannot grow in colonies of plates containing the solid growth medium since copper ions affect the cell ability to replicate. Once within the cell, ions intervene in bacterial growth [11,28].

Therefore, broadly, bacterial death and inhibition could be due to the bactericidal and/or bacteriostatic effect of copper ions released from metallic copper-impregnated M1 and M2 membranes.

3.5. Copper release in liquid medium

Data related to copper release in liquid medium can be visualized in Table 4. It can be observed that the amount released is equivalent to less than 0.002% of the amount present in commercial ceramic membranes.

Table 4
Results of copper release in the water. The samples of ceramic material were submitted to the ultra sonication (for 15 min) in ultra pure water

Membrane	Copper quantity ($\mu g{\cdot}kg^{-1}$)*
M1-Cu	4.1
M2-Cu	15.8

*The copper release is in microgram of the metal per kilogram of ceramic material sample.

According to WHO, the maximum copper concentration in water for human consumption is 2 $mg{\cdot}L^{-1}$. Otherwise, copper could cause bitter taste in water and stain kitchenware and clothing [29]. According to the Administrative Rule 2.914 of the Ministry of Health (a Brazilian agency), dated 2011, the maximum copper concentration allowed for human consumption water is 2 $mg{\cdot}L^{-1}$ [30]. Data obtained by ICP-OES indicated copper concentration of 0.00016 and 0.00062 $mg{\cdot}L^{-1}$ for M1-Cu e M2-Cu respectively. Still for the sake of comparison, Resolution 430 of CONAMA (Brazil), states as standard for liquid effluents discharge into hydric bodies a copper concentration of 1 $mg{\cdot}L^{-1}$ [31].

Therefore, based on the data obtained, it can be stated that copper is being released in liquid medium slowly and in low amounts, which can mean that metals are adhered to the ceramic material.

4. Conclusion

Copper nanoparticles obtained by chemical reduction had absorption bands at 265 nm. X-rays diffractograms exhibited high crystallinity nanoparticles and ascertained the existence of metallic copper. SEM and DLS data exhibited nano metric scale particles. As a result, copper average particle size was around 55 nm. Bacteriological results obtained in the present study point to the fact that the impregnation method developed was effective. Microfiltration tests pointed to bactericidal efficiencies for copper-containing membranes between 88.7 and >99.9%, approximately. On the other hand, copper less membranes resulted into lower efficiencies, between 40.3 and 73.7%, approximately. This low retention efficiency of bacteria by membranes (M1 and M2) can be attributed to variation in pore size, that permitted the passage of bacteria *E. coli*. And, the interaction of copper with cell wall was the factor related to the increased efficiency of copper-containing membranes. In order to corroborate the disinfection efficiency exhibited by copper-containing membranes experiments were run aiming at evaluating the inhibition zone of these materials. These experiments were considered as satisfactory, indicating that the materials not only cause *E. coli* death but also inhibit its growth. M1-Cu and M2-Cu membranes had inhibition diameter of 10 and 13 mm. Finally, results obtained in this work

substantiate the use of copper in ceramic materials aiming at imparting bactericidal effect to these materials.

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References

[1] A.B. Pandit, J.K. Kumar, Drinking Water Disinfection Techniques, CRC Press, London, 2013.
[2] Metcalf e Eddy / AECOM, G. Tchobanoglous, H.D. Stensel, R. Tsuchihashi, F. Burton, Wastewater engineering: treatment and resource recovery. 5th ed. McGraw-Hill Education, 2014.
[3] B. Basile, A. Cassano, N.K. Rastogi, Advances in membrane technologies for water treatment: Materials, Processes and Applications. Elsevier Science Publishers Ltd, 2015.
[4] L.K. Wang, J.P. Chen, Y-T. Hung, N.K. Shammas, Handbook of Environmental Engineering: Membrane and Desalination Technologies, Volume 13, Springer Science Publishers Ltd., 2011.
[5] I. Sawada, R. Fachrul, T. Ito, Y. Ohmukai, T. Maruyama, H. Matsuyama, Development of a hydrophilic polymer membrane containing silver nanoparticles with both organic anti fouling and antibacterial properties, J. Membr. Sci., 387–388 (2012) 1–6.
[6] K. Scott, Handbook of industrial membranes, 2th ed,, Elsevier Science Publishers Ltd., 1998.
[7] S. Gurunathan, Biologically synthesized silver nanoparticles enhances antibiotic activity against Gram-negative bacteria, J. Ind. Eng. Chem. 29 (2015) 217–226.
[8] V. Dhand, L. Soumya, S. Bharadwaj, S. Chakra, D. Bhatt, B. Sreedhar, Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity, Mater. Sci. Eng., C 58 (2016) 36–43.
[9] A.C. Schneid, M.B. Pereira, F. Horowitz, R.S. Mauler, C.R. Matte, M.P. Klein, P.F. Hertz, T.M.H. Costa, E.W. Menezes, E.V. Benvenutti, Silver nano particle thin films deposited on glass surface using an ionic silsesquioxane as stabilizer and as cross linking agent, J. Braz. Chem. Soc., 26 (2015) 1004–1012.
[10] T. Kruk, K. Szczepanowicz, J. Stefańska, R.P. Socha, P. Warszyński, Synthesis and antimicrobial activity of mono disperse copper nanoparticles, Colloids Surf. B. Bio interfaces, 128 (2015) 17–22.
[11] T.A. Dankovich, J.A. Smith, Incorporation of copper nanoparticles into paper for point-of-use water purification, Water Res. 63 (2014) 245–251.
[12] K.M. Rajesha, B. Ajitha, Y.A.K. Reddy, Y. Suneetha, P.S. Reddy, Synthesis of copper nanoparticles and role of pH on particle size control, Mater. Today: Proc. 3 (2016) 1985–1991.
[13] S.C. Motshekga, S.S. Ray, M.S. Onyango, M.N.B. Momba, Preparation and antibacterial activity of chitosan-based nano composites containing bentonite-supported silver and zinc oxide nanoparticles for water disinfection, Appl. Clay Sci., 114 (2015) 330–339.
[14] R. Ahmad, M. Mohsin, T. Ahmad, M. Sardar, Alpha amylase assisted synthesis of TiO_2 nanoparticles: Structural characterization and application as antibacterial agents, J. Hazard. Mater., 11 (2015) 171–177.
[15] G. Ren, D. Hu, E.W.C. Cheng, M.A Vargas-Reus, P. Reip, R.P. Allaker, Characterisation of copper oxide nanoparticles for antimicrobial applications, Int. J. Antimicrob. Agents, 33 (2009) 587–590.
[16] Y.W. Baek, Y.J. An, Microbial toxicity of metal oxide nanoparticles (CuO, NiO, ZnO, and Sb_2O_3) to Escherichia coli, Bacillus subtilis and Streptococcus aureus, Sci Total Environ., 409 (2011) 1603–1608.
[17] M.J. Hajipour, K.M. Fromm, A.A. Ashkarran, D.J. Aberasturi, I.R. Larramendi, T. Rojo, V. Serpooshan, W.J. Parak, M. Mahmoudi, Antibacterial properties of nanoparticles, Trends Biotechnol., 30 (2012) 499–511.
[18] I.A. Minigalieva, B.A. Katsnelson, V.G. Panov, L.I. Privalova, A.N. Varaksin, V.B. Gurvich, M.P. Sutunkova, V.Y. Shur, E.V. Shishkina, I.E. Valamina, I.V. Zubarev, O.H. Makeyev, E.Y. Meshtcheryakova, S.V. Klinova, In vivo toxicity of copper oxide, lead oxide and zinc oxide nanoparticles acting in different combinations and its attenuation with a complex of innocuous bio-protectors, Toxicology 380 (2017) 72–93.
[19] Y. Lv, H. Liu, Z. Wang, S. Liu, L. Hao, Y. Sang, D. Liu, J. Wang, R.I. Boughton, Silver nano particle-decorated porous ceramic composite for water treatment, J. Membr. Sci., 331 (2009) 50–56.
[20] A.D. Tiwari, A.K. Mishra, S.B. Mishra, A.T. Kuvarega, B.B. Mamba, Stabilisation of silver and copper nanoparticles in a chemically modified chitosan matrix, Carbohydr. Polym., 92 (2013) 1402–1407.
[21] I.O. Ali, Synthesis and characterization of Ag^0/PVA nanoparticles via photo- and chemical reduction methods for antibacterial study, Colloids Surfaces A Physicochem. Eng. Asp., 436 (2013) 922–929.
[22] M. Abdulla-Al-Mamun, Y. Kusumoto, M. Muruganandham, Simple new synthesis of copper nanoparticles in water/acetonitrile mixed solvent and their characterization, Mater. Lett., 63 (2009) 2007–2009.
[23] B. Bagchi, S. Dey, S. Bhandary, S. Das, A. Bhattacharya, R. Basu, P. Nandy, Antimicrobial efficacy and biocompatibility study of copper nano particle adsorbed mullite aggregates, Mater. Sci. Eng., C 32 (2012) 1897–1905.
[24] U. Rasool, S. Hemalatha, Marine endophytic actinomycetes assisted synthesis of copper nanoparticles (CuNPs): Characterization and antibacterial efficacy against human pathogens, Mater. Lett., 194 (2017) 176–180.
[25] R. Javed, M. Ahmed, I. ulHaq, S. Nisa, M. Zia, PVP and PEG doped CuO nanoparticles are more biologically active: Antibacterial, antioxidant, antidiabetic and cytotoxic perspective, Mater. Sci. Eng., C 79 (2017) 108–115.
[26] R. Katwal, H. Kaur, G. Sharma, M. Naushad, D. Pathania, Electrochemical synthesized copper oxide nanoparticles for enhanced photo catalytic and antimicrobial activity, J. Ind. Eng. Ch., 31 (2015) 173–184.
[27] R.E. Morsi, A.M. Alsabagh, S.A. Nasr, M.M. Zaki, Multi functional nano composites of chitosan, silver nanoparticles, copper nanoparticles and carbon nano tubes for water treatment: Antimicrobial characteristics, Int. J. Bio. Macr., 97 (2017) 264–269.
[28] M.S. Islam, C. Larimer, A. Ojha, I. Nettleship, Antimycobacterial efficacy of silver nanoparticles as deposited on porous membrane filters, Mater. Sci. Eng., C 33 (2013) 4575–4581.
[29] World Health Organization. Guidelines for Drinking-water Quality, 4th ed., WHO Library Cataloguing-in-Publication Data, 2011.
[30] Brazil, Ministério da Saúde, Portaria 2.914, (2011) 1-16. http://bvsms.saude.gov.br/bvs/saudelegis/gm/2011/prt2914_12_12_2011.html
[31] Brazil, Conselho Nacional do Meio Ambiente – CONAMA, Resolução 430, (2011) 1-9. http://www.mma.gov.br/port/conama/legiabre.cfm?codlegi=646.