Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2018.22780

123 (2018) 109–118
August

Silver-modified clinoptilolite-heulandite-rich tuff as microbicide agent in a column system for specific microorganisms and consortium from a deionized water suspension

V.E. Gonzaga-Galeana[a], I. De-La-Rosa-Gómez[a], M.T. Olguin[b,*]

[a]*Laboratorio de Investigación en Ingeniería Ambiental, Tecnológico Nacional de México/Instituto Tecnológico de Toluca, Av. Tecnológico s/n., Col. Agrícola Bellavista, C.P. 52149 Metepec, Estado de México, Mexico, emails: enrique.gonzaga@live.com.mx (V.E. Gonzaga-Galeana), kivodelarosa@yahoo.com (I. De-La-Rosa-Gómez)*
[b]*Departamento de Química, Instituto Nacional de Investigaciones Nucleares, Carretera México-Toluca s/n, La Marquesa, Ocoyoacac, Estado de México C.P. 52750, Mexico, Tel. +5553297200 Ext. 12265; email: teresa.olguin@inin.gob.mx*

Received 12 March 2018; Accepted 12 July 2018

ABSTRACT

The pathogen microorganism found in water causes several diseases in the human being and it is essential finding alternatives for water disinfection especially for regions which not count with purified water distribution systems. The natural zeolites modified with metals have been investigated for this purpose. However, few works have been considered a consortium of microorganisms and packed column systems. Therefore, the microbicide effect of silver-modified clinoptilolite-heulandite-rich tuff (ZGAg) on a specific microorganisms or microorganism consortium composed by *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* in a continuous system was investigated considering the characteristics of each microorganism, the complexity of microorganism systems (individual species or consortium), and the height of the natural zeolite bed (mass of the microbicide agent). The release of silver from the silver-modified clinoptilolite-heulandite-rich tuff after the disinfection processes in the continuous system was also considered in this work. The natural zeolite of 30 mesh particle size from Guerrero (Mexico) was modified with silver using an $AgNO_3$ solution. Unmodified and silver-modified natural zeolites were characterized by scanning electron microscopy, X-ray energy dispersive spectroscopy, and X-ray diffraction. Microbial cultures from the American Type Culture Collection were acquired for the disinfection experiments. The obtained disinfection breakthrough curves were analyzed to obtain different parameters (among them breakpoint, disinfected water volume, and disinfection process kinetics). It was found that the disinfected water volume was higher for *E. coli* than *S. aureus* and *C. albicans* for both 300 and 400 mg of ZGAg and the volume varied depending on the mass of the microbicide zeolitic material. The resistance of the microorganism to the microbicide zeolitic material was changed when the microorganisms are in a consortium. The disinfection experimental data were well fitted to a nonlinear logistic model and the mass of the ZGAg and the type of microorganism affect the t_{50} and k parameters.

Keywords: Silver-modified-clinoptilolite-heulandite; Disinfection; Continuous system; Microbial consortium

1. Introduction

A total of 3,928 km³ of freshwater are consumed around the world every year. Agriculture has been estimated to account for 44% (1,716 km³/year) of such consumption. The remaining 56% of the water (2,212 km³/year) is released into the environment as waste, for example, from municipal and industrial effluents and as wastewater from agriculture [1]. In 2015, 2,477 operating plants in Mexico processed 120.9 m³/s, 57% of the 212.0 m³/s of water collected by sewage systems, while the industry treated 70.5 m³/s of the

* Corresponding author.

wastewater; based on these data, The National Commission of Water (Mexico), CONAGUA, estimated that 19.8 m^3/s were reutilized directly (before discharge) and 88.1 m^3/s of treated wastewater were used indirectly (after discharge); such interchange of treated wastewaters, in which water of first-use is substituted, was estimated to be 5.1 m^3 [2]. Although sewerage coverage has increased and wastewater treatment performance levels have been upgraded in some countries [3], such improvements should take place simultaneously to avoid the increase of pollution loads. Probably this could explain the first conclusions of the Global Water Quality Monitoring Programme indicating that approximately one-third of all African, Latin American, and Asian fluvial courses are severely contaminated by pathogenic organisms (caused by human and animal excrement) consequently the health of millions of people is at risk [4]. Bacteria are the most abundant type of microorganisms responsible for water contamination [5]. Some of the most important opportunistic human pathogens found until now are *Cryptococcus neoformans* and *Stachybotrys chartarum*, as well as members of the *Acremonium*, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Candida*, *Chaetomium*, *Cladosporium*, *Exophiala*, *Fusarium*, *Mucor*, *Nectria*, *Paecilomyces*, *Penicillium*, *Phialophora*, *Phoma*, *Rhizopus*, *Rhodotorula*, *Sporothrix*, and *Scopulariopsis* [6]. Niemi et al. [7] reported the presence of fungi in 50%–100% of the water samples. These results were confirmed by Novak et al. [8], who found that 80% of tap water samples contained fungi and yeasts, most of them are potentially pathogenic.

Safe use of drinking water is achieved by supervising bacterial parameters of fecal contamination. These parameters are correlated with gastrointestinal diseases, although the cause of the illnesses is the viral agents resulting from fecal contamination [8]. Therefore, it is relevant to monitor microorganisms such as *Escherichia coli*, a bacteria widely used as a water quality indicator because its presence indicates recent contamination of drinking water due to wastewater or animal feces. Depending on its strain, *E. coli* may cause symptoms such as abdominal cramps, fever, vomit, diarrhea, and hemolytic uremic syndrome, among others [9]. Other bacteriological and viral agents of gastroenteritis cause a self-limited disease with symptoms similar to the flu, such as the case of intoxication by toxin-producing *Staphylococcus aureus* strains that induce nausea, vomit, and diarrhea. The survival capacity of *Staphylococcus* explains its presence when coliforms cannot be detected [10]. Besides these organisms, *Candida albicans* was also considered in this work because their virulence factors represent a potential health risk, especially in the case of immunocompromised individuals. Yamaguchi et al. [11] demonstrated that bottled mineral water was more contaminated than tap water; *Candida* spp. species identified were *Candida parapsilosis*, *Candida glabrata*, and *Candida albicans*, which indicate that bottled mineral water from water dispensers and tap water could represent a possible source of infection by filamentous fungi and yeasts. The fact that yeasts and filamentous fungi were found in samples in which no fecal or total coliforms were detected highlights the importance of reevaluating the criteria used to analyze drinking water for microorganism presence.

Different chemical agents have been used as disinfectants to eliminate biological contaminants from water. However, some of them carry inconvenient such as the case of trihalomethanes and chlorophenols which are by-products of disinfection processes using chlorine gas (Cl_2) and hypochlorite (ClO^-), respectively [12]. Large-scale use of ozone is expensive (O_3) for water disinfection [13], and its reaction to organic substances in water results in decreased concentrations of total organic carbon and the generation of undesirable and prejudicial by-products such as aldehydes, ketones, carboxylic acids, hydroxy acids, alcohols, and esters. Ultraviolet (UV) light is not suitable for residual disinfection: depth and organic matter dissolved in the water prevent the germicide activity of the radiation. The interaction between reactions results in inactivation, including biochemical reactions that can be affected by temperature during the irradiation period, but it is mainly during the post-irradiation period that reactivation processes are produced if sufficient conditions are available [14]. One of the essential factors that affect the yield of the UV reactor is the trajectory of the microorganisms flux because it is generally turbulent [15].

In several studies, researchers recommend the use of zeolites, both natural and synthetic, to incorporate metallic ions (Ag, Cu, Zn, Hg, Sn, Pb, Bi, Cd, Cr, and Ti) to develop microbicide agents for water disinfection [16–25]. In few investigations have been considered the columns packed with silver-modified natural zeolites for water disinfection, and focus the attention only on one microorganism and not microorganisms in a consortium. Recently, Akhigbe et al. [26] simultaneously investigated the bactericide action of the silver-modified zeolite for *E. coli* and the capability of this material to remove Cd, Pb, and Zn from aqueous solutions in the presence of this microorganism using a column system. The authors found that the flow rate and the bed height effect on the column service life. Therefore, the aim of this paper was to evaluate the disinfection process by silver-modified clinoptilolite-heulandite-rich tuff packed column from continuous system, taking into account the behavior of specific microorganism and a consortium of microorganisms (*E. coli*, *S. aureus*, and *C. albicans*) suspended in deionized aqueous media at different height of the zeolite bed columns, which is also related with masses of the microbicide agent used for disinfection. The desorption (release) of silver from the silver-modified clinoptilolite-heulandite-rich tuff after the water disinfection processes was also investigated.

2. Materials and methods

2.1. Materials

The natural zeolite used in this work was collected in the state of Guerrero (ZG). The material was ground and sieved to obtain 30 mesh size particles. Reagents used were commercial analytical grade.

2.2. Treatment of natural zeolite

2.2.1. Zeolite treated using NaCl solution

The sodium form of zeolite was obtained by the procedure described by Rivera et al. [22]. For that purpose, 100 g of 30 mesh particle size ZG were weighted and added to 500 mL of a 1 M solution of NaCl for 12 h under reflux. This procedure was repeated twice. The solution was then decanted,

and solids were washed using deionized water until washing water was free of Cl^-, for which $AgNO_3$ was employed. Solids were finally dried at 85°C for 5 h. The result of this process was sodium-conditioned zeolite (ZGNa).

2.2.2. Zeolite treated using $AgNO_3$ solution

A 45 g sample of ZGNa was subjected to reflux using 500 mL of a 0.01 M solution of $AgNO_3$ for 12 h. This procedure was carried out twice. The solution was decanted, and solids materials were washed using deionized water until washing water was free of the excess of $AgNO_3$, verified by the use of NaCl solution considering the reaction: $AgNO_3 + NaCl \rightarrow AgCl\downarrow + NaNO_3$ in which the AgCl is an insoluble precipitate. The sample was then dried at 85°C for 5 h, which resulted in silver-conditioned zeolite (ZGAg). The procedure was carried out in the absence of visible light to prevent Ag photoreduction [22].

2.3. Zeolite characterization

2.3.1. Scanning electron microscopy and X-ray energy dispersive spectroscopy

The morphology and elemental composition of the different natural zeolites were determined using a JEOL JSM-6610LV scanning electron microscope (SEM) with an Oxford X-ray energy dispersive spectroscopy (EDS) system. Samples were mounted directly onto the holders, and 2,000× images were obtained at 20 kV. Five different regions of the zeolitic materials were analyzed at 500× to determine their elemental composition.

2.3.2. X-ray diffraction

Powder X-ray diffraction patterns of zeolite samples were obtained using a SIEMENS D5000 diffractometer coupled to a copper-anode X-ray tube. The setup was established at 4° and 60° for angle 2θ and step size of 0.05 s.

2.4. Microbicide effect

2.4.1. Microorganisms

E. coli (ATCC 25922), *S. aureus* (ATCC 6538), and *C. albicans* cells (ATCC 10232) were used as pathogenic microorganisms; cells were propagated aerobically in Luria-Bertani liquid medium, tryptic soy broth, and Sabouraud agar, respectively, at 37°C for 20 h in water bath (Lab-Line Shak-R-Bath). Cultures were centrifuged (Hettich Zentrifugen Universal 32R) at 10,000 rpm for 10 min at 4°C and washed twice with sterile deionized water to eliminate Cl^- ions present in the solution. Cells were resuspended in sterile distilled water and dilutions were repeated until a concentration of 1.0×10^7 colony forming units (CFU)/100 mL was achieved. The microbial consortium was prepared using the three microorganisms to emulate wastewater, although other associated components were not present [18].

2.4.2. Column system

A glass column with a diameter of 1 cm and a height of 40 cm was used for the experiments; 300 and 400 mg of ZGAg were packed into the column. These masses correspond to heights of 3 and 5 mm, respectively. A liquid suspension containing 10.0×10^7 CFU/100 mL of *E. coli*, *S. aureus*, and *C. albicans* cells was prepared. Experiments were conducted using each microorganism separately or in the consortium; for that purpose, solutions were passed through the column at a 10 mL/min flow rate. The same procedure was performed for reference using ZGNa.

2.4.3. Quantification of microorganisms

Aliquot parts of 1 mL of the sample were taken every hour for 24 h; aliquots were diluted using 30 mL of 0.01 M phosphate solution. These samples were filtered using a 0.45 μm membrane per APHA [27] method to quantify coliform bacteria. Membranes were kept at 35°C for 24 h in an incubator (Shel Lab LI5). Microorganism colonies were quantified using a SOL-BAT Q-20 counter.

2.5. Quantification of silver in the effluent

Other samples were taken to determine the concentration of silver in the effluent or silver desorbed from the ZGAg, as the same time as microorganism were collected. The pH of the aqueous solution was adjusted to a value of 3 to preserve the chemical species of the silver. A Perkin Elmer 3110 spectrophotometer using an Ag hollow cathode lamp at a 328.1 nm wavelength was employed to quantify silver. It is important to mention that the Ag^+ contained in the silver-modified clinoptilolite-heulandite-rich tuff is desorbed when the influent (suspended microorganisms in aqueous media) has troughed the packed column of the zeolitic material and this could be explained with the base on the following reaction:

ZGAg (Ag^+) + X^+ (cationic ions in the suspension of microorganisms in aqueous media) → ZGAg (X^+) + Ag^+ (Ag^+ in the suspension of microorganisms in aqueous media)

2.6. Modeling of breakthrough curves using nonlinear equations

To obtain parameters to describe the disinfection process in a continuous flow system the model used by Mthombeni et al. [28] to deactivate microbes in a drinking water column system was applied in this work.

When microorganisms (N_i) pass through a bed containing silver-modified zeolite, a number of microorganisms (N_t) are obtained at certain intervals (t); when N_t is plotted as a function of t, the resulting graphic is a sigmoid curve. The general form of the equation is as follows:

$$N_t = \frac{N_i}{\left(1+\exp^{-k\left(t-t_{50}\right)}\right)} \tag{1}$$

whose linear form is as follows:

$$\ln\left(\frac{N_t}{N_i - N_t}\right) = k\left(t - t_{50}\right) \tag{2}$$

where k (min^{-1}) is the rate constant, that is, a measure of the steepness of the slope of the breakthrough curve. The

necessary time to achieve median concentration (half N_i) of bacteria counts is named t_{50} (min).

3. Results and discussion

3.1. Characterization

3.1.1. SEM and X-ray EDS

The ZG SEM image (Fig. 1(a)) shows that crystals have a hexagonal shape with well-defined beveled edges, which are characteristic of clinoptilolite-heulandite [29]. Similar results were observed for ZGNa and ZGAg (Figs. 1(b) and (c)). However, in the case of ZGAg, small particles were found on crystal surfaces, which correspond to the contrast points shown in Fig. 1(c). As will be discussed in the section on zeolitic material composition, these particles are enriched by Ag [30].

Significant element weight percentages measured in ZG were 46.52% ± 2.0%, 34.89% ± 2.16%, and 7.41% ± 1.57% for O, Si, and Al, respectively (Table 1). Besides other elements, Na, Mg, K, and Ca were detected. Given that K and Ca weight percentages were higher than the values for Mg and Na, the natural zeolite was of the potassium-calcium type. Fe contents in the natural zeolite could be due to an associated iron ore [31].

When ZG is treated with sodium to obtain ZGNa, Na increases 48-fold concerning its weight percentage as found in ZG, and Ca decreases significantly; therefore, the ensuing ion exchange reaction can explain the following behavior:

$$\mathrm{ZG(z)} + 2\mathrm{Na^+(s)} \leftrightarrow \mathrm{ZGNa\,(z)} + \mathrm{Ca^{2+}(s)}$$

where z and s represent the natural zeolite and the aqueous solution, respectively.

When ZGNa was placed in contact with the $AgNO_3$ solution, Na decreased noticeably, and a weight percentage of 12.93% of silver was found in ZGAg (Table 1). The ion exchange reaction possibly taking place is as follows:

$$\mathrm{ZGNa(z)} + \mathrm{Ag^+(s)} \leftrightarrow \mathrm{ZGAg\,(z)} + \mathrm{Na^+(s)}$$

This shows that Na^+ is displacing by Ag^+ from the exchange sites located in the clinoptilolite-heulandite crystalline network [18,22].

Table 1
Elemental analysis of natural zeolite from the state of Guerrero, before and after the modifications

Element	Wt.%		
	ZG	ZGNa	ZGAg
O	46.52 ± 2.02	49.17 ± 2.51	42.54 ± 1.62
Na	0.05 ± 0.04	2.40 ± 0.49	0.07 ± 0.04
Mg	0.89 ± 0.15	0.91 ± 0.26	0.70 ± 0.25
Al	7.41 ± 1.57	7.11 ± 1.39	6.74 ± 1.05
Si	34.89 ± 2.16	34.23 ± 0.59	31.53 ± 1.04
K	5.15 ± 1.10	3.79 ± 0.79	2.98 ± 1.04
Ca	2.27 ± 0.80	0.98 ± 0.90	0.56 ± 0.17
Fe	2.83 ± 0.31	1.43 ± 0.61	1.96 ± 0.72
Ag	ND	ND	12.93 ± 2.79

ND: not detected.

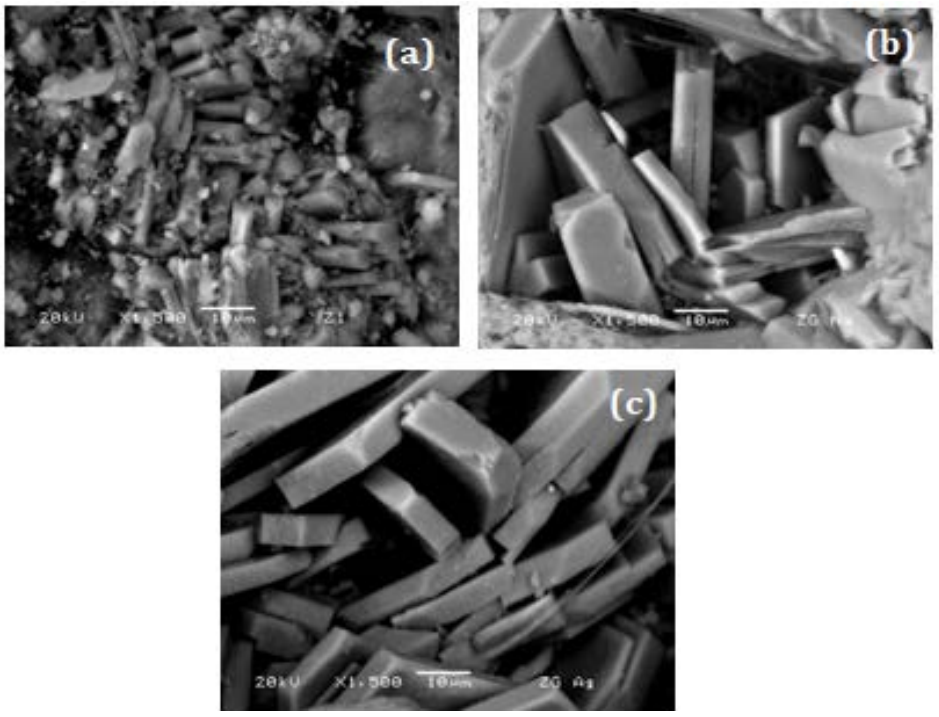

Fig. 1. SEM image of (a) ZG, (b) ZGNa, and (c) ZGAg.

3.1.2. X-ray diffraction

Fig. 2 shows that the crystal structures of ZGNa and ZGAg remained unchanged, due to no displacements of the reflexions were observed, when comparing ZGNa and ZGAg X-ray diffraction patterns with those of ZG, the only changes seen were in the intensity of the reflections along the diffraction pattern due to the replacement of ions in zeolite for Na^+ or Ag^+, depending on the treatment applied to the zeolitic material.

The X-ray diffraction patterns of the zeolite-rich tuff unmodified and silver-modified were compared with those of clinoptilolite (C), heulandite (H), and quartz (Q) in which powder diffraction files numbers are 00-039-1383 for clinoptilolite and 01-085-1843 for heulandite. The powder diffraction patterns of the samples were similar with those of the standards, which confirm that clinoptilolite, heulandite, or both are components of the zeolitic material. Quartz content in the zeolite-rich tuff was low with the base on the reflexion intensity at 26.7 2q (Miller's index 011), which was compared with the PDF number 03-065-0466, as can be seen in Fig. 2.

3.2. Microbicide effect

3.2.1. ZGAg mass of 300 mg

3.2.1.1. Specific microorganisms

Figs. 3–5 present breakthrough curves for *E. coli*, *S. aureus*, and *C. albicans* using ZGAg as well as silver desorption. Microorganisms *E. coli*, *S. aureus*, and *C. albicans* showed no growth for 5.5, 3, and 2 h, respectively.

Fig. 3 shows that the concentration of silver released in the effluent by ZGAg was 1.38 mg/L (0.0128 meq/L) at 1 h (corresponding to 600 mL of water), which had decreased down to 0.88 mg/L at 5.5 h. Below such silver concentration, *E. coli* cells recover their growth by 52% with respect to the initial microorganism concentration. It should be mentioned that the volume of disinfected water before the breakpoint was 3,300 mL. Mthombeni et al. [28] reported that the breakpoint in which bacteria appeared for the first time in the effluent water was reached under conditions similar to those observed in this work.

Akhigbe et al. [26] obtained similar results for *E. coli* and argued that they interrupted the process because the bed had become obstructed due to biofilm formation and disinfection efficiency had decreased.

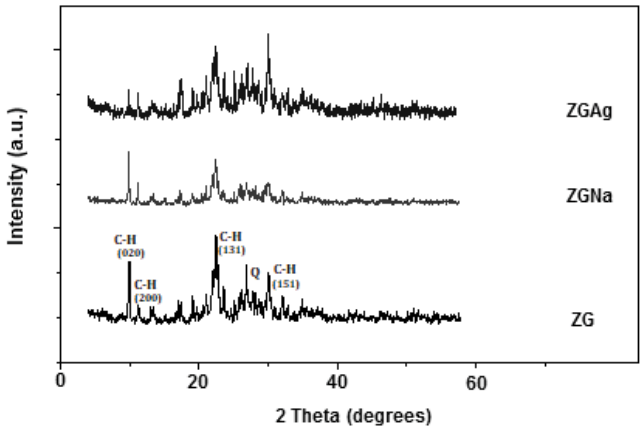

Fig. 2. X-ray diffraction patterns of ZG, ZGNa, and ZGAg. The C-H and Q, corresponding to the reflexions of clinoptilolite, heulandite, and quartz.

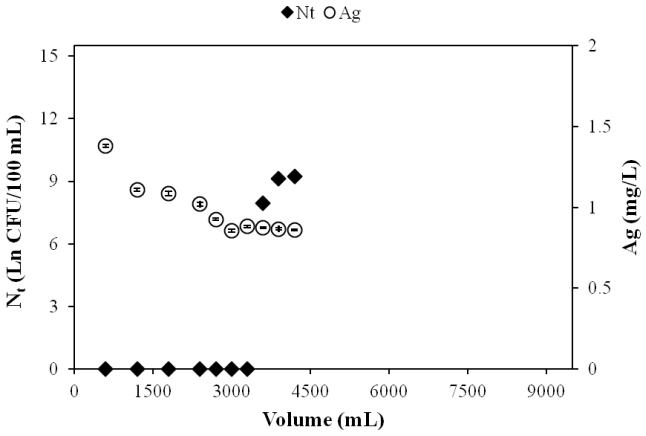

Fig. 3. Silver desorption and breakthrough curve of water disinfection using 300 mg of ZGAg with *E. coli*.

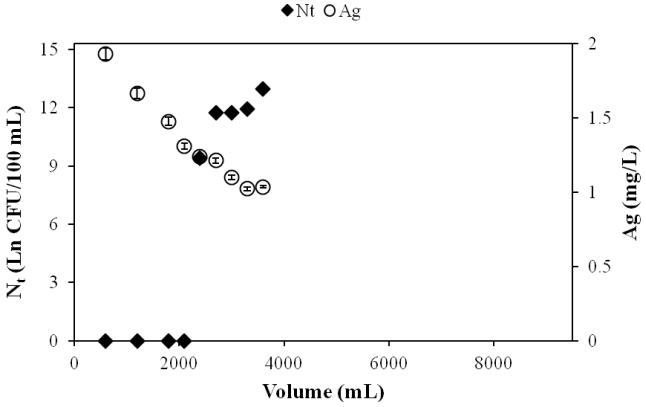

Fig. 4. Silver desorption and breakthrough curve of water disinfection using 300 mg of ZGAg with *S. aureus*.

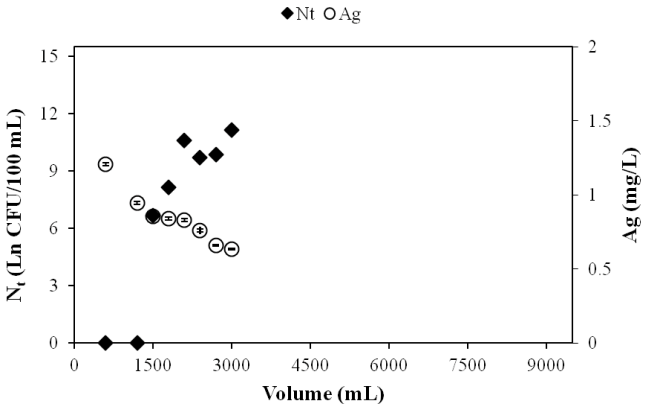

Fig. 5. Silver desorption and breakthrough curve of water disinfection using 300 mg of ZGAg with *C. albicans*.

In the case of *S. aureus*, disinfection after the continuous flow process using 300 mg of ZGAg was 1,800 mL; therefore, disinfection was 1.8 times higher for *E. coli* than for *S. aureus*. This result can be explained considering that *S. aureus* develops higher resistance to the microbicide activity of ZGAg as compared with *E. coli*, probably due to structural and physiological differences between the two microorganisms. Fig. 4 shows that *S. aureus* survival is zero for silver concentrations from 1.93 to 1.48 mg/L (0.0179 to 0.0137 meq/L) in the water. After that point, silver concentration decreased gradually down to a level of 1.24 mg/L (0.0115 meq/L), and the *S. aureus* population increased by 58% concerning the initial number of colonies.

Akhigbe et al. [26] state that biofilm formation during the disinfection process, as well as in the process published by Monds and O'Toole [32], is unique in biology because it involves the coordinated activity of several relatively small prokaryote genomes, as opposed to the larger eukaryote genomes necessary for the formation of a functional multicellular community. The formation process begins with the attachment of planktonic cells to surfaces [33]. Bacterial biofilm is affected by environmental conditions in the network, among them are hydraulic forces, disinfection regime, and materials, as well as intrinsic cell characteristics such as hydrophobicity, surface charge, polysaccharide production, and cell motility [34,35]. *S. aureus* presents features that result in biofilm formation. This microorganism regulates the process as a response to a cell–cell signaling mechanism termed quorum sensing (QS). In *Staphylococcus*, the QS system is called accessory gene regulator (agr), and its role is to regulate processes including virulence factors, antibiotic production, and biofilm formation; biofilm formation is used by the microorganism to adhere to and colonize new sites and to protect itself from phagocytosis and antibiotics [36]. It is thus reasonable to suppose that *S. aureus* resistance is partly due to the formation of biofilm on zeolite surfaces.

C. albicans presented the highest resistance to ZGAg microbicide activity (for a mass of 300 mg) of the three selected microorganisms. Some CFU/100 mL were observed in the effluent up to a volume of 1,200 mL (Fig. 5); this volume was 2.75 and 1.5 times lower with respect to the disinfected volume of *E. coli* and *S. aureus*, respectively. When the silver concentration was within the interval from 1.21 to 0.85 mg/L (0.0112 to 0.0078 meq/L), *C. albicans* mortality was 100%. However, when the silver concentration was lower than 0.85 mg/L, *C. albicans* increased 46% with respect to its initial population.

3.2.1.2. Microorganism consortium

The microorganism consortium composed by *E. coli*, *S. aureus*, and *C. albicans* showed a different behavior after treatment with the same mass of 300 mg of ZGAg in comparison with each microorganism species investigated separately. In this case, no CFU was detected at 0.83 h (which corresponds to a volume of 498 mL) for *E. coli* and *S. aureus*, and no growth was observed in *C. albicans* until 4 h (2,400 mL) (Fig. 6). *E. coli* and *S. aureus* were equally resistant to ZGAg microbicide activity. However, *C. albicans* was more labile than *E. coli.*

The different behavior displayed by the microbial consortium as a result of the disinfection process is probably due

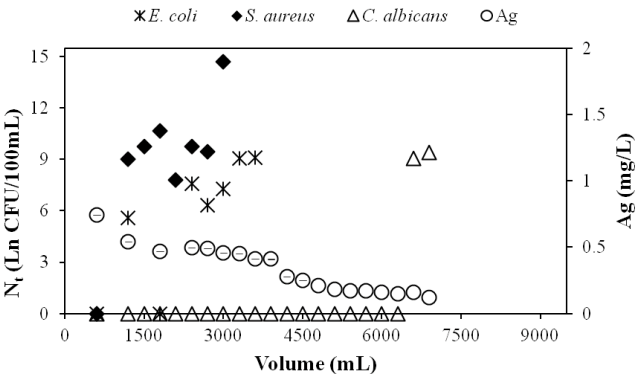

Fig. 6. Silver desorption and breakthrough curve of water disinfection using 300 mg of ZGAg with the microbial consortium (*E. coli–S. aureus–C. albicans*).

to biofilm formation [33], which is favored in cells presenting a high degree of adhesion due to polysaccharide production [34,35]. The biochemical biofilm formation process known as QS plays an important role in the initial fixation of cells on surfaces and in controlling biofilm growth. As already mentioned, QS systems are also involved in polysaccharide synthesis, microbial adherence, cell division, and cell motility [37,38].

3.2.2. ZGAg mass of 400 mg

3.2.2.1. Specific microorganisms

No *E. coli* CFU were detected until 7.5 h when the zeolite bed was increased from 300 to 400 mg, and the resulting disinfected water volume was 4,500 mL (Fig. 7), 1.36 times the volume obtained using 300 mg of zeolitic material. Initial silver concentration in the effluent during the disinfection period varied from 1.10 to 0.71 mg/L. After 7.5 h, the silver concentration decreases slightly to 0.60 mg/L, and *E. coli* cells grow 76% with respect to their initial concentration.

Therefore, a comparison of both disinfection processes shows that using a higher 400 mg bed of ZGAg results in longer column useful life and better performance than when using a 300 mg bed. Similar results were obtained by

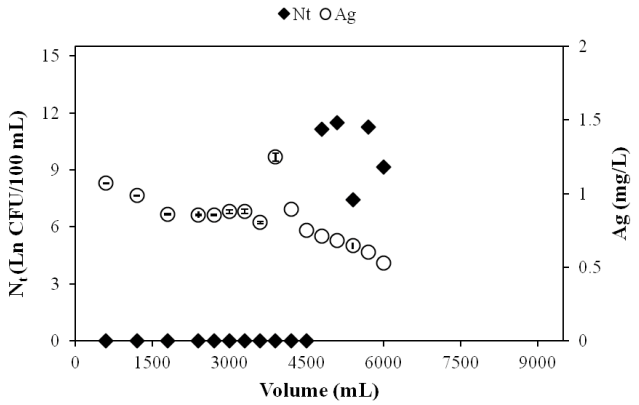

Fig. 7. Silver desorption and breakthrough curve of water disinfection using 400 mg of ZGAg with *E. coli*.

Mthombeni et al. [28], who used resin pearls modified with silver nanoparticles and Akhigbe et al. [26], who used 1, 2, and 5 mg of silver-modified zeolite.

Fig. 8 shows that the concentration of silver is initially higher when disinfecting water by microbicide activity of 400 mg ZGAg than when using a 300 mg bed. These results indicate that the amount of ZGAg released in the effluent does not necessarily depend on the initial mass of zeolitic material used to pack the column. Gonzaga [39] reported similar results from research using masses of 100 and 200 mg of silver-treated natural zeolite from the state of Guerrero.

Using 400 mg of ZGAg with *S. aureus* resulted in a 2.16 increase in the volume of disinfected water with respect to the 300 mg bed, which confirms the effect obtained from the increase in bed mass described by Akhigbe et al. [26] and Mthombeni et al. [28].

In the case of *C. albicans*, the disinfected water volume was 7,200 mL at 12 h. No significant differences in the silver concentration of effluents were observed as a result of using 300 or 400 mg ZGAg masses; the concentrations were 1.21 and 1.29 mg/L, respectively (Fig. 9).

The volume of disinfected water obtained by using 400 mg of ZGAg was six times larger than the volume achieved by using 300 mg. This disinfected water volume was obtained within a silver concentration interval between 1.29 and 0.53 mg/L. The microbicide activity of ZGAg against *C. albicans* was observed at silver concentrations as low as 0.53 mg/L.

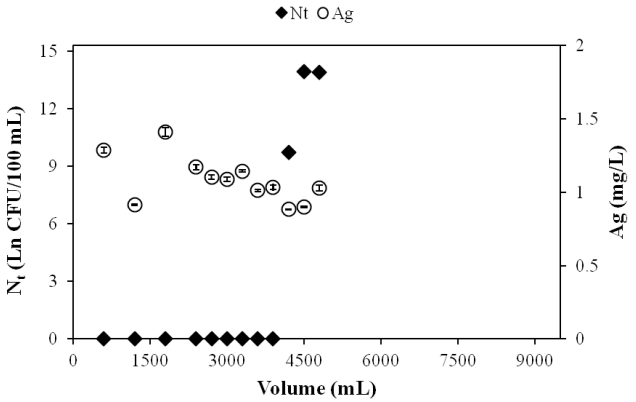

Fig. 8. Silver desorption and breakthrough curve of water disinfection using 400 mg of ZGAg with *S. aureus*.

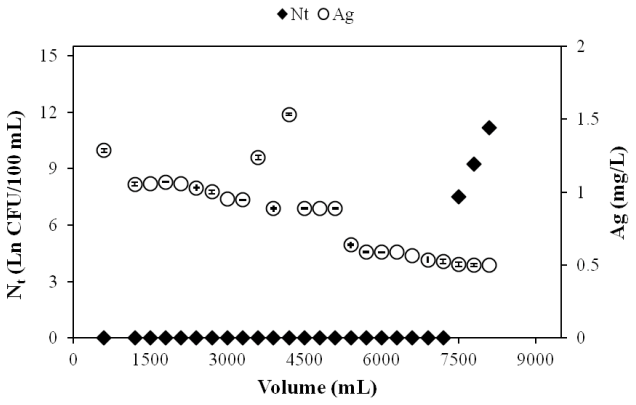

Fig. 9. Silver desorption and breakthrough curve of water disinfection using 400 mg of ZGAg with *C. albicans*.

3.2.2.2. Microorganism consortium

The results using a 400 mg mass of ZGAg resulted in differences in the disinfection process; the volume of disinfected water for *E. coli* and *S. aureus* decreased by 7% and 23%, respectively, as opposed to *C. albicans*, whose water volume increased by 4%. Therefore, *C. albicans* presents less resistance against the microbicide activity of ZGAg than *E. coli* and *S. aureus* when it is a member of the microbial consortium *E. coli–S. aureus–C. albicans* (Fig. 10).

C. albicans was found to be the most sensitive microorganism to the microbicide effect of silver using either 300 or 400 mg of ZGAg, followed by *E. coli*, and finally *S. aureus*, which was the most resistant microorganism in the consortium, as already stated. An increase in ZGAg mass results in an increase of disinfected water volume [26,28,39]. The behavior of this process is probably a result of biofilm formed by bacteria in the structure of ZGAg, especially *S. aureus*, due to its capacity to synthesize large amounts of extracellular polysaccharides due to its agr activator [36]; for their part, Mei-Hiu et al. [40] found a QS detection response taking place as bacterial colony density increases, which results in the synthesis of biosurfactants by bacteria, which weaken water surface tension and allow bacteria to propagate rapidly, thus increasing the resistance to the microbicide effect of Ag. On the other hand, the already formed biofilm is used by *E. coli*; monocultures have been demonstrated to adhere easily to surfaces, and laboratory scale focused on biofilms in drinking water have reported the incorporation of *E. coli* into the matrix [41–43] and concluded that *E. coli* had grown into the biofilm. Moreover, two independent biofilm culture studies focusing on water distribution systems quantified *E. coli* in the systems and estimated that the microorganism represented 0.1% of the total biofilm microbial community [44,45]. For its part, *C. albicans* produces different QS detection molecules involved in biological processes, morphological changes, or biofilm formation; tryptophol is one of the most important of these molecules [46]. Biofilm structures

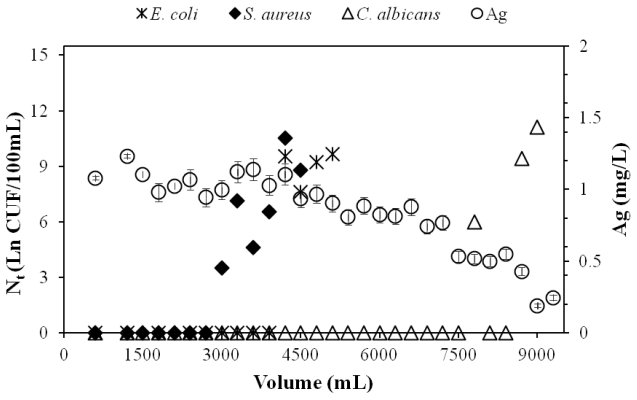

Fig. 10. Silver desorption and breakthrough curve of water disinfection using 400 mg of ZGAg with the microbial consortium (*E. coli–S. aureus–C. albicans*).

may vary depending on growth conditions; in the case of *C. albicans*, biofilms take 24–48 h to achieve maturity [47,48]. This result suggests that *C. albicans* lacks enough adhesion capacity to form a biofilm on the ZGAg structure, and that any biofilm actually forming would lack the necessary time to achieve maturity and provide resistance to this microorganism against the microbicide effect of Ag.

Biofilm cells can be between 10 and 1,000 times more resistant than planktonic cells against a large number of broad-spectrum antibiotics (ampicillin, streptomycin, tetracycline, gentamicin, etc.) as well as oxidizing biocides such as chlorine, iodine, and ozone [49,50]. Different relationships between fungi and bacteria could be based on various compositions of separate species in water systems, different methods, or different biological mechanisms affecting such relationships [6]. Frequently, fungi are secondary colonists of pre-established bacterial films [51,52].

In general, the silver released from the silver-modified clinoptilolite-heulandite-rich tuff after the disinfection processes in a column system is found in the range of 0.4–1.3 mg/L. The observed values are higher than the maximum secondary level established by the Environmental Protection Agency (USA), which is 0.1 mg/L.

3.3. Modeling of breakthrough curves using nonlinear equations

Experimental data were fit to the logistic growth model described in Section 2.5. The kinetic constant and the time in which microbial colony concentration is half the initial concentration (t_{50}) are shown in Table 2.

Both the t_{50} parameter and k reveal the effect of the microbicide agent (ZGAg) on microorganisms responsible for water contamination [53]. The t_{50} increases as antimicrobial material mass increases [28]. However, the k decreases around 10% for *E. coli* using 400 mg in comparison with 300 mg and this behavior is opposite for *S. aureus* and *C. albicans*. The k increases 6.7 times for *S. aureus* and 1.2 times for *C. albicans* when the mass of ZGAg increases from 300 to 400 mg. As shown in the table, when using masses of 300 mg of ZGAg for specific microorganism disinfection processes, the highest velocity constant is observed in *E. coli*, followed by *C. albicans* and finally *S. aureus*. The time (t_{50}) microorganisms take to reach half their initial concentration is higher for bacteria in comparison with yeast. This behavior changes when the mass is increased from 300 to 400 mg of ZGAg because the t_{50} parameter is higher and the k value is lower for yeasts than for bacteria.

In the microbial consortium, k values show that when using a 300 mg mass of ZGAg, the microorganism with the highest velocity constant is *C. albicans*, followed by *E. coli* and finally by *S. aureus*. Similar results were obtained when the mass was increased from 300 to 400 mg of ZGAg. Table 3 also shows that the t_{50} parameter is higher for yeasts than for bacteria; *S. aureus* reaches half its initial concentration in the shortest time among microorganisms of its type.

3.4. Disinfection mechanism

The silver (Ag^+) released (desorbed) from the silver-modified materials as well as the Ag nanoparticles deposited on the surface could interact with the microorganisms to disrupt their metabolism and kill them [20,54,55]. In the case of this paper, the authors modified the clinoptilolite-heulandite-rich tuff with taking in account the ion exchange properties of the zeolitic material assuming that the Ag^+ occupied the ion exchange sites on the clinoptilolite or heulandite network. Therefore, the Ag^+ domain over the other silver chemical species under the experimental conditions of this work and the disinfection mechanism could be carried out when the Ag^+ has been released from the zeolitic material and the cell wall

Table 2
Parameters of the logistic model describing water disinfection for specific microorganisms in a column system (flux at 10 mL/min)

Microorganism	Mass (mg)					
	300			400		
	t_{50} (min)	k (min^{-1})	r^2	t_{50} (min)	k (min^{-1})	r^2
E. coli	354.7	0.36	0.99	741.5	0.32	0.99
S. aureus	226.6	0.09	0.99	418.6	0.61	1
C. albicans	144.3	0.15	0.95	744.3	0.18	0.99

Table 3
Parameters of the logistic model describing water disinfection for test microorganisms in the consortium in a column system (flux at 10 mL/min)

Microbial consortium	Mass (mg)					
	300			400		
	t_{50} (min)	k (min^{-1})	r^2	t_{50} (min)	k (min^{-1})	r^2
E. coli	216.3	0.12	0.93	405.05	0.18	0.99
S. aureus	95.1	0.08	0.83	328.43	0.03	0.88
C. albicans	640.5	1.28	0.99	867.94	0.82	0.82

is damaged and as a consequence their internal components. In this context, Akhigbe et al. [26] consider that some bacterial cells death by the action of silver ions released from the silver-modified zeolite named this mechanism as liquid phase disinfection, and also mention that some cells are attached to the particles when they are in contact with silver ions from the zeolite matrix named this mechanism as solid phase disinfection. In general, the mechanisms of interactions of Ag-heulandite-clinoptilolite-microorganisms are not well understood.

4. Conclusions

Clinoptilolite-heulandite is a major component of natural zeolite from the state of Guerrero. Its crystalline structure remained unchanged after contact with a solution of NaCl and $AgNO_3$ used to obtain sodium and silver zeolitic material.

ZGNa failed to present microbicidal activity against *E. coli*, *S. aureus*, and *C. albicans* in a continuous flow system.

When using a mass of 300 mg of ZGAg, the microorganism showing highest sensitivity to its microbicidal effect is *E. coli*; when the mass was increased to 400 mg, the most sensitive microorganism is *C. albicans*.

S. aureus presents a different behavior, and it shows the highest resistance to the microbicide effect of ZGAg independently of the mass in the packed column.

C. albicans is the most sensitive microorganism to the microbicide action when the microbial consortium formed by *E. coli*, *S. aureus*, and *C. albicans* are in contact with ZGAg.

The experimental data are well fitted to a nonlinear logistic model using parameters to describe a disinfection process in a column system; the values of breakthrough curve meantime (t_{50}) increases as ZGAg mass increases as well. The k parameter varied depending on the mass of microbial agent in contact with microorganisms and the type of them (bacteria or yet).

Concerning the microbial consortium, nonlinear logistic model fitting and parameters t_{50} and k were different with respect to the data obtained for specific microorganisms, which confirms the existence of an interaction between microorganisms and the Ag from ZGAg.

The silver desorbed (released) from the silver-modified clinoptilolite-heulandite-rich tuffs is higher than the maximum secondary level established by the Environmental Protection Agency (USA) when the breakpoint is reached in all investigated systems.

Acknowledgments

The authors thank the financial support given by CONACyT Project 254665 and T.N.M. 5888.16-P. The authors also thank the technical support from Laboratory of Investigation on Environmental Engineering (LIIA). E. Gonzaga thanks CONACyT for scholarship number 426509/265594.

References

[1] WWAP (United Nations World Water Assessment Programme), The United Nations World Water Development Report 2017: Wastewater, The Untapped Resource, UNESCO, Paris, 2017.

[2] CONAGUA, National Commission of Water, Statistics of water from Mexico [Estadísticas del agua en México], Planation General Subdirection, Mexico, 2016.

[3] UNICEF/WHO (United Nations International Children´s Emergency Fund and World Health Organization), Progress on Sanitation and Drinking Water-2015 Update and MDG Assessment, WHO Library Cataloguing in Publication Data, New York, 2015.

[4] UNEP (United Nations Environment Programme), UNEP Frontiers 2016 Report: Emerging Issues of Environmental Concern, Nairobi, 2016.

[5] J.G. Tortora, R.B. Funke, L.F. Case, Introduction to Microbiology (Introducción a la microbiología), 9th ed., Editorial Médica Panamericana, Buenos Aires, Argentina, 2009.

[6] P. Sonigo, A. De Toni, K. Reilly, A review of fungi in drinking water and the implications for human health, Defra, 33 (2011) 1–107.

[7] R.M. Niemi, S. Knuth, K. Lundström, Actinomycetes and fungi in surface waters and in potable water, Appl. Environ. Microbiol., 43 (1982) 378–388.

[8] B.M. Novak, P. Zalar, B. Ženko, S. Džeroski, N. Gunde-Cimerman, Yeasts and yeast-like fungi in tap water and groundwater, and their transmission to household appliances, Fungal Ecol., 20 (2016) 30–39.

[9] S.L. Miles, R.G. Sinclair, M.R. Riley, I.L. Pepper, Evaluation of select sensors for real-time monitoring of *Escherichia coli* in water distribution systems, Appl. Environ. Microbiol., 77 (2011) 2813–2816.

[10] M.W. LeChevallier, R.J. Seidler, *Staphylococcus aureus* in rural drinking water, Appl. Environ. Microbiol., 39 (1980) 739–742.

[11] M. Yamaguchi, R. de Cássia Pontello Rampazzo, S. Yamada-Ogatta, C. Nakamura, T. Ueda-Nakamura, B. Prado Dias Filho, Yeasts and filamentous fungi in bottled mineral water and tap water from municipal supplies, Braz. Arch. Biol. Technol., 50 (2007) 1–9.

[12] S.M.T. Olmedo, Byproducts of water disinfection by the use of chlorine compounds. Effects on health [Subproductos de la desinfección del agua por el empleo de compuestos de cloro. Efectos sobre la salud], Hig. Sanid. Ambient., 8 (2008) 335–342.

[13] D.C.G. Gordillo, Technical and Economical Research on Waste Water Disinfection by Oxidation Methods [Investigación técnica y económica sobre desinfección de aguas residuales por sistema de oxidación], Doctor thesis, Higher Technical School of Civil, Canal and Port Engineers, Madrid, Spain, 2013.

[14] I. Salcedo, J.A. Andrade, J.M. Quiroga, E. Nebot, Photoreactivation and dark repair in UV-treated microorganisms: effect of temperature, Appl. Environ. Microbiol., 73 (2007) 1594–1600.

[15] X. Zhao, S.M. Alpert, J.J. Ducoste, Assessing the impact of upstream hydraulics on the dose distribution of ultraviolet reactors using fluorescence microspheres and computational fluid dynamics, Environ. Eng. Sci., 26 (2009) 947–959.

[16] A.C. Burrola, Kinetic of the Disinfection Process of Water Using Natural Clinoptilolite Exchanged with Metallic Ions as Microbicide [Cinética del proceso de desinfección del agua al utilizar clinoptilolita natural intercambiada con iones metálicos como microbicida], Master thesis, Autonomous University of the State of Mexico, Mexico, 2004.

[17] G.I. De la Rosa, Behavior of the Zeolitic Rocks Modified with Silver for the Disinfection Process of Municipal Waste Water [Comportamiento de rocas zeoliticas acondicionadas con plata, en el proceso de desinfección de agua residual de origen municipal], Doctor thesis, Technological Institute of Toluca, Mexico, 2007.

[18] G.I. De la Rosa, M.T. Olguín, D. Alcántara, Silver-modified Mexican clinoptilolite-rich tuffs with various particle sizes as antimicrobial agents against *Escherichia coli*, J. Mex. Chem. Soc., 54 (2010) 139–142.

[19] R. Guerra, E. Lima, M. Viniegra, A. Guzmán, V. Lara, Growth of *Escherichia coli* and *Salmonella typhi* inhibited by fractal silver nanoparticles supported on zeolites, Microporous Mesoporous Mater., 147 (2012) 267–273.

[20] J. Hrenovic, J. Milenkovic, T. Ivankovica, N. Rajic, Antibacterial activity of heavy metal-loaded natural zeolite, J. Hazard. Mater., 201–202 (2012) 260–264.
[21] J. Hrenovic, J. Milenkovic, N. Daneu, K.R. Matonickin, N. Rajic, Antimicrobial activity of metal oxide nanoparticles supported onto natural clinoptilolite, Chemosphere, 88 (2012) 1103–1107.
[22] G.M. Rivera, M.T. Olguín, S.I. García, D. Alcántara, F.G. Rodríguez, Silver supported on natural Mexican zeolite as an antibacterial material, Microporous Mesoporous Mater., 39 (2000) 431–444.
[23] C. Rosabal, F.G. Rodríguez, N. Bogdanchikova, P. Bosch, M. Alvaros, V.H. Lara, Comparative study of natural and synthetic clinoptilolites containing silver in different states, Microporous Mesoporous Mater., 86 (2005) 249–255.
[24] L.G. Rossainz-Castro, I. De-La-Rosa-Gómez, M.T. Olguín, D. Alcántara-Díaz, Comparison between silver- and copper-modified zeolite-rich tuffs as microbicide agents for *Escherichia coli* and *Candida albicans*, J. Environ. Manage., 183 (2016) 763–770.
[25] A. Top, S. Ülkü, Silver, zinc and cooper exchange in a Na-clinoptilolite and resulting effect on antibacterial activity, Appl. Clay Sci., 27 (2003) 13–19.
[26] L. Akhigbe, S. Ouki, D. Saroj, Disinfection and removal performance for *Escherichia coli* and heavy metals by silver-modified zeolite in a fixed bed column, Chem. Eng. J., 295 (2016) 92–98.
[27] APHA, AWWA, WEF, Standard Methods for Examination of Water and Wastewater, 22nd ed., American Public Health Association, Washington, 2012.
[28] N.H. Mthombeni, L. Mpenyana-Monyatsi, M.S. Onyango, M.N.B. Momba, Breakthrough analysis for water disinfection using silver nanoparticles coated resin beads in fixed-bed column, J. Hazard. Mater., 217–218 (2012) 133–140.
[29] F. Mumpton, O. Clayton, Morphology of zeolites in sedimentary rocks by scanning electron microscopy, Clays Clay Miner., 24 (1976) 1–23.
[30] G.M. Rivera, Antibacterial Property of the Clinoptilolite Exchanged with Silver Against *Escherichia coli* and *Streptococcus faecalis* [Propiedad Antibacteriana de la Clinoptilolita intercambiada con plata, frente a *Escherichia coli* y *Streptococcus faecalis*], Master thesis, Autonomous University of the State of Mexico, Mexico, 1999.
[31] D.W. Breck, Zeolite Molecular Sieves: Structure, Chemistry, and Use, Wiley-Interscience Publication Book, New York, 1974.
[32] R.D. Monds, G.A. O'Toole, The developmental model of microbial biofilms: ten years of a paradigm up for review, Trends Microbiol., 17 (2009) 73–87.
[33] L.C. Simoes, M. Simoes, R. Oliveira, M.J. Vieira, Potential of the adhesion of bacteria isolated from drinking water to materials, J. Basic Microbiol., 47 (2007) 174–183.
[34] B. Li, B.E. Logan, Bacterial adhesion to glass and metal-oxide surfaces, Colloids Surf., B, 36 (2004) 81–90.
[35] L.C. Simoes, M. Simoes, M.J. Vieira, Influence of the diversity of bacterial isolates from drinking water on resistance of biofilms to disinfection, Appl. Environ. Microbiol., 76 (2010) 6673–6679.
[36] E. Cervantes-García, R. García-González, P.M. Salazar-Schettino, General characteristics of *Staphylococcus aureus* [Características generales del *Staphylococcus aureus*], Rev. Latinoamer. Patol. Clin., 61 (2014) 28–40.
[37] I. Douterelo, J.B. Boxall, P. Deines, R. Sekar, K.E. Fish, C.A. Biggs, Methodological approaches for studying the microbial ecology of drinking water distribution systems, Water Res., 65 (2014) 134–156.
[38] V. Lazar, Quorum sensing in biofilms-How to destroy the bacterial citadels or their cohesion/power? Anaerobe, 17 (2011) 280–285.
[39] G.V.E. Gonzaga, Interaction of the Ammonium Ion with Silver Modified Natural Zeolite and Its Effect on the Disinfection of Contaminated Water with a Consortium of Microorganisms Gram (+) and Gram (–) [Interacción del ion amonio con zeolita natural acondicionada con plata y su efecto sobre la desinfección de agua contaminada frente a un consorcio de microorganismos Gram (+) y Gram (–)], Master thesis, Technological Institute of Toluca, Mexico, 2013.
[40] L. Mei-Hiu, K. Wan-Ju, L. Chao-Chin, Y. Meng-Wei, Modulation of *Staphylococcus aureus* spreading by water, Sci. Rep., 6 (2016) 25233.
[41] C.L. Abberton, L. Bereschenko, P. van der Wielen, C.J. Smith, Survival, biofilm formation, and growth potential of environmental and enteric *Escherichia coli* in drinking water microcosms, Appl. Environ. Microbiol., 82 (2016) 5320–5331.
[42] S. Fass, M.L. Dincher, D.J. Reasoner, D. Gatel, J.C. Block, Fate of *Escherichia coli* experimentally injected in a drinking water distribution pilot system, Water Res., 30 (1996) 2215–2221.
[43] M.M. Williams, E.B. Braun-Howland, Growth of *Escherichia coli* in model distribution system biofilms exposed to hypochlorous acid or monochloramine, Appl. Environ. Microbiol., 69 (2003) 5463–5471.
[44] T. Juhna, D. Birzniece, S. Larsson, D. Zulenkovs, A. Sharipo, N.F. Azevedo, F. Menard-Szczebara, S. Castagnet, C. Feliers, C.W. Keevil, Detection of *Escherichia coli* in biofilms from pipe samples and coupons in drinking water distribution networks, Appl. Environ. Microbiol., 73 (2007) 7456–7464.
[45] L. Mezule, S. Larsson, T. Juhna, Application of DVC-FISH method in tracking *Escherichia coli* in drinking water distribution networks, Drinking Water Eng. Sci., 6 (2013) 25–31.
[46] R.Y.A. Hassan, R.O. El-Attar, H.N.A. Hassan, M.A. Ahmed, E. Khaled, Carbon nanotube-based electrochemical biosensors for determination of *Candida albicans*'s quorum sensing molecule, Sens. Actuators, B, 244 (2017) 565–570.
[47] Y. Kaneko, S. Miyagawa, O. Takeda, M. Hakariya, S. Matsumoto, H. Ohno, Y. Miyazakia, Real-time microscopic observation of *Candida* biofilm development and effects due to micafungin and fluconazole, Antimicrob. Agents Chemother., 57 (2013) 2226–2230.
[48] P. Uppuluri, A.K. Chaturvedi, A. Srinivasan, M. Banerjee, A.K. Ramasubramaniam, J.R. Köhler, D. Kadosh, J.L. Lopez-Ribot, Dispersion as an important step in the *Candida albicans* biofilm developmental cycle, PLoS Pathog., 6 (2010) 1–13.
[49] P. Muller, B. Guggenheim, P.R. Schmidlin, Efficacy of gasiform ozone and photodynamic therapy on a multispecies oral biofilm in vitro, Eur. J. Oral Sci., 115 (2007) 77–80.
[50] P.S. Stewart, J.W. Costerton, Antibiotic resistance of bacteria in biofilms. Lancet, 358 (2001) 135–138.
[51] G. Kinsey, R. Paterson, J. Kelley, Filamentous Fungi in Water Systems, D. Mara, N. Horan, Eds., Handbook of Water and Wastewater Microbiology, Academic Press, London, 2003.
[52] R.R.M. Paterson, N. Lima, Fungal Contamination of Drinking Water, J. Lehr, J. Keeley, J. Lehr, III. T.B. Kingery, Eds., Water Encyclopedia, John Wiley and Sons, New Jersey, 2005.
[53] C.L.G. Rossainz, Microbicidal Activity of Clinoptilolite Modified with Silver or Copper Against a Microbial Consortium (coliforms and yeasts) in the Presence of Organic Components Associated with Municipal Wastewater [Actividad microbicida de la clinoptilolita modificada con plata o cobre frente a un consorcio microbiano (coliformes y levaduras) en presencia de componentes orgánicos asociados al agua residual municipal], Doctor thesis, Technological Institute of Toluca, Mexico, 2017.
[54] D. Jiraroj, S. Tungasmita, D.N. Tungasmita, Silver ions and silver nanoparticles in zeolite A composites for antibacterial activity, Powder Technol., 264 (2014) 418–422.
[55] P. Lalueza, M. Monzó, M. Arruebo, J. Santamaría, Bactericidal effects of different silver-containing materials, Mater. Res. Bull., 46 (2011) 2070–2076.