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A-type zeolite containing Ag^+/Zn^{2+} as inorganic antifungal for waterborne coating formulations

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ABSTRACT

The biocide cations Ag^+ and Zn^{2+} were hosted in the cavities of an ordered aluminosiliceous framework. Starting from sodium A-type zeolite (NaA), LTA containing Ag^+ (AgA), Zn^{2+} (ZnA) and Ag^+/Zn (AgZnA) at different cation exchanged levels was obtained and its antifungal properties were evaluated. To determine the minimum inhibitory concentration (MIC) of the exchanged zeolites against *Aspergillus niger*, $[Ag^+]$ and $[Zn^{2+}]$ values ranging from $50 < [Ag^+] < 1000\ mg\ L^{-1}$ to $650 < [Zn^{2+}] < 2000\ mg\ L^{-1}$, respectively, were used for NaA, and for AgZnA: $30 < Ag^+ < 250\ mg\ L^{-1}$. The zeolite sample having $[Ag^+] = 100\ mg\ L^{-1}$, $[Zn^{2+}] = 90\ mg\ L^{-1}$ produces a growth inhibition comparable to that achieved with $230\ mg\ L^{-1}$ of Ag^{+1} (MIC value obtained for the single cation). The antifungal activity of these products after incorporation in waterborne coating formulations was also determined. Results indicate that Ag^+ and Zn^{2+} supported on A-type zeolite could be a beneficial tool for the development of waterborne coatings with a longer protection against microbiological attack when compared to traditional organic biocides.

1. Introduction

Coatings are usually susceptible to deterioration by a number of different microorganisms (bacteria, fungi or algae), so additives such as biocidal products are essential for the coating formulation. The results of microorganism colonization are the loss of several properties of the product that reduce its performance generating a negative economic impact and potential health risks [1,2].

For coatings in liquid state, the microbiological colonization produces product separation (sedimentation of solids) by the attack of the rheological additives such as cellulose-based thickeners. Problems associated with malodor, discoloration, pH drift and loss of viscosity are also a consequence of microbiological colonization. Furthermore, in the case of dry coating films applied on a surface, factors such as moisture, ventilation, lighting, and film roughness produce modifications in microbiological growth [3]. The presence of microorganisms on the coating surface is related to the degradation of the film (loss of mechanical and esthetic properties), and also to the spread of microbiological contamination [4]. While the addition of biocides to the coating film contributes to remedying the above-mentioned drawbacks, the substances most commonly used are essentially toxic. The environmental impact of their release requires careful monitoring and control [5] because most products used in the coating industry are based on isothiazolinones or formaldehyde. Also, some of these biocides exhibit poor efficiency since they have low mobility as a result of high molecular weight and are unable to effectively reset on the coating surface. Other disadvantages are the decrease of the killing power by aging, pH sensitivity, low thermal and alkali stability, etc. [3].

At present, studies focus on finding biocide compounds with no environmental impact, since some antimicrobial products (harmful synthetic chemicals or volatile organic substances) could be potentially toxic and dangerous [5]. Salts derived from metals have been recently studied, focusing on the metals from the standpoint of nanotechnology [6]. Other developments in the field of coatings and modified surfaces can include the incorporation of a polymer matrix containing biocides to provide controlled toxicant release. The general process consists of a rather complex methodology in which active substances are introduced into a matrix providing a further release of these active agents, depending on the specific requirements of the substrate on which the matrix is deposited [7–10]. Recent studies incorporate photocatalytic anatase as biocide additive for thin film formulations. The photocatalytic reaction produces O_2^- and OH^-, both highly reactive species involved in the degradation of microorganisms [11].

Concerning metal cations with biocidal properties, silver seems to be very effective in killing microorganisms. Many researchers have studied the role of silver in its possible forms, namely metallic silver, silver nanoparticles, bifunctional nanoparticles, silver compounds, etc., and also the silver support in several matrices (nanocomposites, natural zeolites, polymers) [12–18].

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Nanoparticles are usually considered more effective than the other silver compounds (metallic silver, silver sulfadiazine, silver nitrate) at the same concentrations [19]. Generally, MIC values for gram-negative and gram-positive bacteria ranged between 40 and 300 $\mu g\,mL^{-1}$ [20–23]. In the case of fungus, TEM analysis on *Candida albicans* species showed that Ag^+ released from silver nanoparticles affected the mycelia by attacking their membranes and consequently disrupting the membrane potential. Additionally, TEM analysis confirms significant changes to cell membranes, which are recognized by the formation of pits on their surfaces, and finally, result in the formation of pores and cell death [24,25]. In another study, the antifungal activity of silver nanocomposites against *Aspergillus niger* was determined at different experimental conditions [26].

The microbiological properties of zinc have also been studied. Zinc oxide nanoparticles have shown high antimicrobial selective properties with minimal effect on human beings. ZnO has been tested against different bacteria: *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Salmonella typhimurium and Staphylococcus aureus* [27,28]. It is also considered that the generation of peroxide by ZnO inhibits bacterial growth [29].

Additionally, zeolites are crystalline aluminosilicate compounds that are classified according to common features of the framework structures. Particularly, the NaA zeolite is a specific arrangement in which the unit cell contains 24 tetrahedra, 12 AlO_4, and 12 SiO_4. When fully hydrated, there are 27 water molecules per unit cell, and there is also one sodium cation for each aluminum present. These sodium ions are rather loosely held, so one of the main uses of this material is based on its cation exchange properties.

In this paper, samples of NaA containing different quantities of Ag^+, Zn^{2+} or Ag^+ and Zn^{2+} were prepared by cation exchange in order to evaluate their antifungal properties. The biocide cations Ag^+ and Zn^{2+} were hosted in the cavities of the ordered aluminosiliceous framework. Minimum inhibitory concentration values corresponding to the exchanged zeolites and the antifungal activity of these products against *A. niger* after incorporation in waterborne coating formulations were determined.

2. Materials and methods

2.1. Preparation of cation-exchanged zeolites

2.1.1. Materials

NaA, synthetized in our laboratory, with a theoretical ion exchange capacity of 7.04 $mEq\,g^{-1}$ (unhydrated base) and of 5.48 $mEq\,g^{-1}$ (hydrated base) was used. Zeolite crystals were prepared by batch hydrothermal crystallization. The batch experiment was carried out in a closed polypropylene container, at 365 K, without stirring. The raw materials used were NaOH (Carlo Erba, analytical reagent), commercial sodium aluminate [Al_2O_3 (36.5%), Na_2O (29.6%), and H_2O (33.9%)], soluble water/glass with SiO_2/Na_2O) 3.18 (w/w) (density equal to 1.36 $g\,mL^{-1}$), and distilled water. The product was characterized by X-ray diffraction. The diffraction pattern was obtained in a Philips PW 1732/10 equipment using Cu Kα radiation and a Ni filter, at a rate of 2°/min. The diffraction diagram of the zeolite sample was identified by comparison with those detailed in the literature [30]. Particle size and morphology were observed by scanning electron microscopy (SEM), by means of a Philips 505 microscope, using samples coated with a thin layer of Au.

The solutions for the cation exchange runs were prepared with $AgNO_3$ (Carlo Erba, p.a.), $Zn(NO_3)_2$ (Carlo Erba, p.a) and optionally NH_4NO_3 (Carlo Erba, p.a), using non-mineralized water as solvent.

2.1.2. Ion exchange experiments

Ion exchange runs were carried out contacting, under stirring, 1 g of NaA with 1 L of the corresponding exchange solution. After a contact time of 3 h at 25 °C, the solid was separated from the liquid by filtration. The solid phases were dried in an oven at 60° and stabilized at room temperature and 35% relative humidity.

The liquid phases were analyzed by atomic absorption spectrometry (AAS) with a Varian AA220 model double beam spectrophotometer. The chemical composition of the four zeolite products obtained, NaA, Ag-exchanged NaA (AgA), Zn-exchanged NaA (ZnA), Ag/Zn-exchanged NaA (AgZnA), and the corresponding solutions used for the exchange runs are listed in Table 1.

2.2. Antifungal assays

2.2.1. Minimum inhibitory concentration (MIC)

To evaluate the performance of AgA, ZnA and AgZnA against *A. niger*, the minimum inhibitory concentration method was used. MIC is defined as the minimum concentration at which the agent takes the fungal multiplication rate to zero. This value was determined by the agar dilution method [31]. The *A. niger* fungus used for these studies was isolated from a painted wall of the San Francisco de Asis Church, La Plata, Argentina [32]. The *A. niger* strain is registered as No. LPSc 1153 at the Instituto Spegazzini, Argentina.

To evaluate the MIC of the hosted cations, different metal concentrations were obtained by adding different weights of AgA, ZnA and AgZnA to an agar medium with salts (AGM). In the case of AgA, [Ag^+] values of 50, 100, 200, 250, 300, 500, 600, 800 and 1000 $mg\,L^{-1}$ were used; for ZnA, [Zn^{2+}]: 650, 800, 950, 1100, 1300, 1500, 1750 and 2000 $mg\,L^{-1}$; and for AgZnA: 30, 40, 50, 100, 150, 200 and 250 $mg\,L^{-1}$ for Ag^+ (the corresponding values of Zn^{+2} were 27, 36, 45, 90, 135, 180, 225 $mg\,L^{-1}$). The ranges of values selected correspond to effective biocide concentrations as described in the bibliography [33]. To ensure sterilization, zeolite powders were exposed to UV radiation.

To obtain the AGM agar medium, the following salts were added: KH_2PO_4 (Anedra) 0.7 $g\,L^{-1}$, $MgSO_4{\cdot}7H_2O$ (Anedra) 0.7 $g\,L^{-1}$, NH_4NO_3 (Anedra) 1 g, NaCl (Anedra) 0.005 $g\,L^{-1}$, $Fe_2SO_4{\cdot}7H_2O$ (Mallinckrodt Chemical Work) 0.002 $g\,L^{-1}$, $MnSO_4{\cdot}H_2O$ (Mallinckrodt Chemical Work), 0.001 g, K_2HPO_4 (Anedra) 0.7 g, $ZnSO_4{\cdot}7H_2O$ (Merck) 0.002 g. In all cases, stirring ensured a homogeneous distribution of the medium.

AGM Petri dishes supplemented with zeolite powder in the aforementioned concentrations were inoculated with 1 mL of the freshly prepared *A. niger* spore suspension in order to maintain an initial concentration of 2.51×10^6 spores mL^{-1} and then incubated for 10 days at 30 °C. After that, the colony diameter was measured.

Control Petri dishes with *A. niger* culture in AGM and in AGM with maltose addition (AGMM, pure maltose 1-hydrate, Biopack, 0.3 g per 10 mL) were prepared. All the experiments were carried out in triplicate.

The results of this assay allowed obtaining the Ag^{+1}, Zn^{+2} and Ag^{+1}/Zn^{+2} concentrations supported in zeolite matrices required to inhibit microbiological growth.

2.3. Evaluation of metal-exchanged zeolites as inorganic antifungal in waterborne coatings

2.3.1. Formulation and manufacture of waterborne coatings

A white waterborne coating was prepared according to the following formulation by weight: 37.6% distilled water, 1.5% rheological additive (Delanta), 0.1% ammonia (Ladco), 0.1% antifoaming agent (Miscela), 0.4% hexametaphosphate (Marpaq), 0.5% dispersant (Spech Chem), 0.1% surfactant (Marpaq), 10.7% titanium dioxide (Marpaq), 9.8% micronized calcium carbonate (Marpaq), 5.3% magnesium silicate (Marpaq), 20.8% precipitated calcium

Table 1
Ag^+, Zn^{2+} concentrations in the prepared materials and compositions of the corresponding aqueous solutions used for cation exchange.

Sample	$[Ag^+]$ (%w/w)	Zn^{2+} (%w/w)	$[Ag^+]$ in the exchange solution (mol L^{-1})	$[Zn^{2+}]$ in the exchange solution (mol L^{-1})	$[NH_4^+]$ in the exchange solution (mol L^{-1})
NaA	–	–	–	–	–
AgA	17.3	–	25	0.1	–
ZnA	–	5.6	25	–	0.05
AgZnA	7.95	7.16	60	0.36	1.66

Table 2
Biocide concentrations in coatings.

Coating	Zeolite type	$[Ag^+]$ (mg L^{-1})	$[Zn^{2+}]$ (mg L^{-1})	Commercial biocide[a] (mg L^{-1})
C1	AgA	500	–	–
C2	AgA	600	–	–
C3	AgA	800	–	–
C4	AgA	1000	–	–
C5	AgA	1200	–	–
C6	AgZnA	65	50	–
C7	AgZnA	97.5	75	–
C8	AgZnA	130	100	–
C9	AgZnA	260	200	–
C10	AgZnA	520	400	–
C11[a]	–	–	–	2000
C12	Control without any biocide			

[a] 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (CIT/MIT) and 2-octyl-4-isothiazolin-3-one (OIT).
CIT/MIT: 4/1 in volume.
CIT-MIT/OIT: 3/2 in weight.
CIT-MIT-OIT: 0.15% in formulation weight.

carbonate (Marpaq), 13.0% acrylic emulsion (Spech Chem), 0.1% ethylene (Ladco). To obtain an adequate dispersion, a high-speed disperser, Combimill model, was used. The specific weight was 1.4 g cm^{-3}.

To investigate the antifungal activity, coatings containing metal-exchanged zeolites (AgA and AgZnA type) were prepared (Samples C1–C10). Two additional control coatings were prepared: (1) coating C11, containing a commercial biocide mixture based on isothiazolinones and (2) sample C12: coating without biocide (Table 2).

In order to get film samples, the prepared coatings were applied on one side of a glass surface (76.2 mm × 25.4 mm) using film applicator (film thickness: 150 μm) and cured at 30 °C for 7 days.

Two series of samples C1–C12 were obtained: one corresponding to cured coating samples (Series 1) and the other corresponding to cured coating samples subjected to weathering in UV apparatus (Series 2). All assays were performed in triplicate. The weathering test was intended to simulate deterioration caused by water as dew, concomitant effects of natural UV radiation and then to estimate the sensibility of biocides (commercial type and based on metal-exchanged zeolites) to leaching. The objective was to determine whether the performance of the biocide is maintained.

Series 1 was sterilized with UV radiation before beginning the microbiological test. Series 2 was aged in a QUV Weathering Tester (ASTM D4587) under the following conditions: 1000 h exposure to a 340 nm wave-length light at 40 °C with condensation cycles and then sterilized with UV radiation before the microbiological test.

The loaded carriers biocidal activities were compared with that obtained for the free biocide. The biocide efficacies of Series 1 and Series 2 were determined by a rapid screening test of microbiological growth inhibition [5]. Each coated glass was placed on an AGM Petri dish seeded with the fungus *A. niger*. The fungal growth was observed after incubation for 20 days in a stove at 30 °C. For each sample, the hyphae advance on a selected fraction of the coated surface (60 mm × 25.4 mm) was estimated by optical microscopy. The colonized surface (%) was quantified as [surface covered by hyphae (mm^2)/1524 mm^2)] × 100.

2.4. Diffusion method

The diffusion method was carried out by placing one dry cured coating drop (1 mL) containing AgZnA in Sabouraud agar (AGMS, Biokar Diagnostics) [34]. A suspension of *A. niger* spores was used to evaluate the diffusional antifungal activity of the dry coating drop. The fungus was grown aerobically for 10 days at 30 °C in agar maltose-peptone medium: agar 20 g (Britania), maltose 30 g (Biopack) and meat-peptone 5 g (Britania) in 1000 mL of distilled water. Then, an aliquot of this culture was aseptically transferred to an Erlenmeyer flask with sterile distilled water and the *A. niger* suspension was appropriately diluted (0.3×10^6 spores mL^{-1}). The number of spores was estimated in a Neubauer chamber. Due to the small size of *A. niger* spores (3–4 μm in diameter), the greatest magnification and count using smaller grids were employed.

AGMS medium (10 mL) was placed in sterile Petri dishes. Five dishes were prepared. Once solidified, the spore suspension (1 mL per dish) was homogeneously distributed onto each dish surface. Then, one dry coating drop was placed in the center of each dish. Before the test, the drops were exposed to UV radiation to ensure sterilization. The coatings selected for obtaining the drops are listed in Table 3.

3. Results and discussion

3.1. Zeolite characterization

DRX studies conducted on the solid obtained at the end of the hydrothermal synthesis show the diffraction peaks corresponding to pure NaA zeolite (Fig. 1). The morphology and crystal size of the sample observed by SEM can be seen in Fig. 2, where one can observe a typical morphology that characterizes NaA, consisting of cubes of about 0.5 μm in size.

3.2. Antifungal activity of exchanged zeolites. MIC values

Fungal growth measurements in the different specimens prepared are shown in Fig. 3.

In the case of adding AgA to AGM, the growth of *A. niger* colonies diminishes with increasing Ag^+ concentration up to 200 mg L^{-1}. Colony growth reductions of 92.26% for $[Ag^+]$ = 50 mg L^{-1}, 93.62% for 100 mg L^{-1} and 95.52% for 200 mg L^{-1}, with respect to AGMM culture (fungus in maltose without biocide), were observed. Due to these results, tests using $[Ag^+]$ values of 210, 220, 230 and 240 mg L^{-1} were performed, obtaining no growth for $[Ag^+] \geq 230$ mg L^{-1}. Then, this value was defined as MIC. In fact, values greater than 230 mg L^{-1} of Ag^+ inhibit the production of viable

Table 3
Dry coating drops composition.

Dry coating drops	Coating
D1	C8
D2	C9
D3	C10
D4	C11
D5	C12

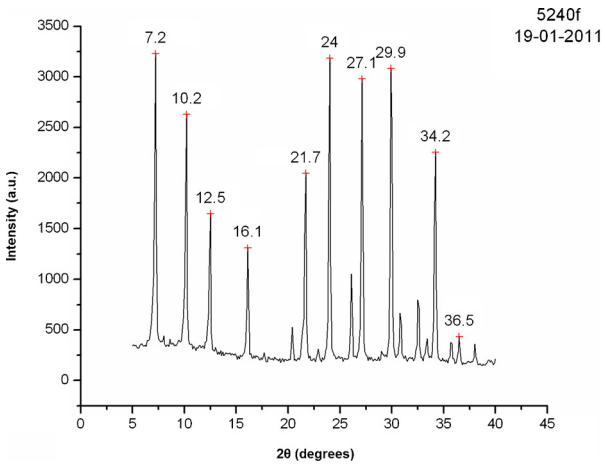

Fig. 1. X-ray diffraction patterns for the NaA zeolite obtained by hydrothermal synthesis.

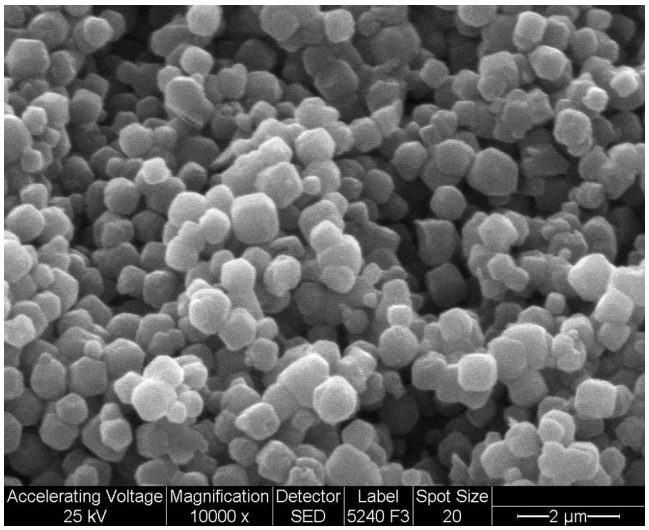

Fig. 2. SEM micrographs of NaA zeolite obtained by batch hydrothermal crystallization (magnification ×10,000).

mycelium and spores. These results are in agreement with similar studies showing the antimicrobial activity of Ag^+ [18,35]. For ZnA, the increase in $[Zn^{2+}]$ also produces a minor colony growth (Fig. 3). No *A. niger* growth was observed for $[Zn^{2+}] \geq 1250\ mg\ L^{-1}$ (MIC).

In previous studies, higher MIC values have been reported for zinc compared to those found for silver [23]. Metal nanoparticles

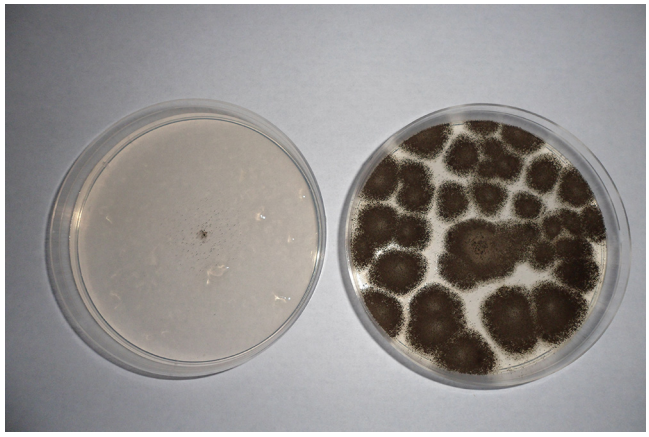

Fig. 4. Left: *A. niger* growth with 100 mg L^{-1} Ag^{+1}–90 mg L^{-1} Zn^{2+} (10 days, 30 °C). Right: *A. niger* growth with 30 mg L^{-1} Ag^{+1}–27 mg L^{-1} Zn^{2+} (10 days, 30 °C).

tested against *Streptococcus mutans* showed an average MIC of $4.86 \pm 2.71\ \mu g\ mL^{-1}$ for silver and $500 \pm 306.18\ \mu g\ mL^{-1}$ for zinc, i.e. a hundred times greater for the divalent ion [36].

In the microbiological test for ZnAgA, the MIC value was obtained for the sample containing 100 mg L^{-1} Ag^{+1}–90 mg L^{-1} Zn^{+2} (Figs. 3 and 4). This result suggests a synergic effect due to the presence of both cations. Comparing the MIC value obtained using AgA to the one corresponding to AgZnA, higher inhibitory effects are achieved at lower $[Ag^+]$ values when Zn^{2+} is included. The sample having $[Ag^+] = 100\ mg\ L^{-1}$, $[Zn^{2+}] = 90\ mg\ L^{-1}$ produces a growth inhibition comparable to that achieved with 230 mg L^{-1} of Ag^{+1}.

These results are similar to values previously reported by Kawahara et al. [33]. These authors measured the antibacterial effect of a commercial product based on silver-zeolite and other cations, the MIC being in the range 4.8–38.4 $\mu g\ mL^{-1}$ Ag^+ for bacteria under anaerobic conditions.

Furthermore, in previous work it was observed that the MIC of a commercial silver A zeolite containing a Ag percentage about its maximum exchange capacity ($[Ag^{+1}] = 38\%$ (w/w)) was 125 mg L^{-1} [37]. In this commercial zeolite, the silver ions occupy almost all the exchange positions and silver is completely available in its biologically active ionic form. On the contrary, in the AgA prepared in this work, the exchange level is about 50% ($[Ag^{+1}] = 17.5\%$ (w/w)), presenting a corresponding lower MIC value. Additionally, the silver ions in the Ag/Zn zeolite are sharing the exchange positions with Zn^{2+} and NH_4^+ cations, resulting in a different antifungal activity.

It is known that the inhibition is also dependent on the nature and concentration of the microorganism species tested [22]. It is

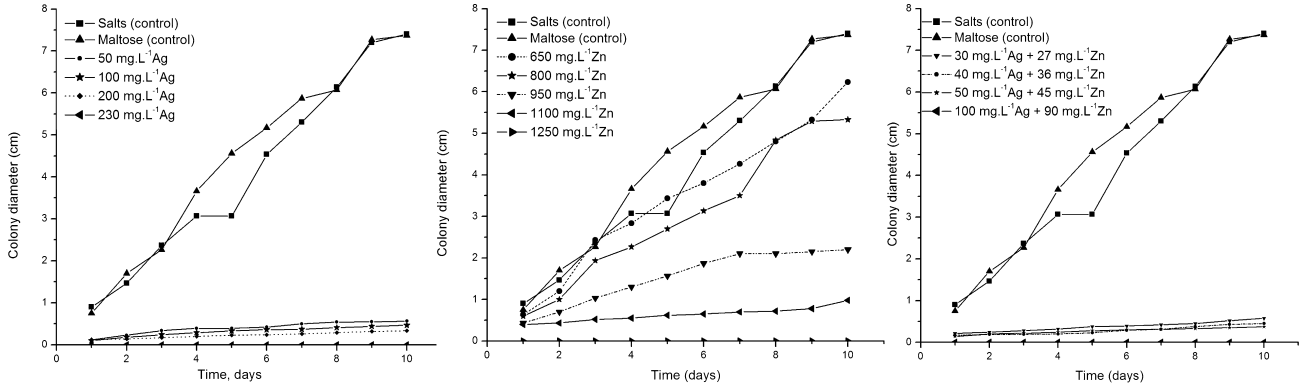

Fig. 3. *A. niger* colony growth mean value[1] in AGM with several metal-exchanged zeolite concentrations (cm). Left: AgA, center: ZnA, right: ZnAgA. [1]Three Petri dishes (10 mL).

estimated that the suitability of the silver ions for exerting an effective biocide action is determined by the complexity of the organism to be treated. Fungi, organisms having eukaryotic cells, exhibit increased resistance to toxic substances compared to bacteria (prokaryotic cells) [16]. The present study demonstrates that the concentration values of biocide cations needed for complete inhibition of *A. niger* growth are slightly higher than those found for bacteria. Our results can be explained based on the fact that *A. niger* belongs to a group of more complex organisms.

Studies on the biocide capacity of Ag^+ ions have proposed several mechanisms of action to explain the inhibitory effect. Some researchers have reported that the positive charge of silver ion is fundamental for antimicrobial activity. The electrostatic attraction between the negatively charged cell membrane and the positive charge of Ag^+ interferes with the permeability of the membrane [38–40]. Moreover, the action mechanism for Zn^{2+} ions was determined to be the same as that described for Ag^+. The antibacterial activity is based on the release of Zn^{2+} ions that can damage the cell membrane and interact with intracellular contents. As mentioned for silver cations, the antimicrobial activity improved with an increasing content of zinc ions [41,42].

Additionally, the antibacterial activity of the cations hosted in zeolite structures could depend on their location inside the zeolitic cages. Metallic ions occupying the exchange positions could be electrostatically attracted by the cell and subsequently released from the inorganic aluminosilicate framework. Then, the metal ions interact with the cell, disrupting the biochemical activity and causing cell death. Moreover, the present results show that it is possible to attain similar inhibitory effects against *A. niger* fungus at lower cation exchange levels when a fraction of silver is partially replaced by zinc ($[Ag^+] + [Zn^{2+}] < [Ag^+]$).

3.3. Antifungal activity of exchanged A zeolites in coatings

Fig. 5 shows fungal growth percentages measured on the samples pertaining to Series 1 and Series 2. As an example, Fig. 6 shows the C11, C2 and C9 waterborne coatings in *A. niger* culture.

For both series, the microbiological tests done on coatings formulated with different concentrations of $[Ag^+]$ allow confirming that coating films containing a higher silver concentration show higher biocide activities. For Series 1, for levels of $[Ag^+]$ in the coating of 600 mg L^{-1} (C2), it can be observed that the performance was similar to that obtained with the commercial biocide (C11, 2000 mg L^{-1}). The biocide amount incorporated in C2 is almost enough to inhibit *A. niger* growth (about 3% of colonized area). Furthermore, for coating C2, containing $[Ag^+] = 600$ mg L^{-1}, the microbiological inhibition obtained was similar to that of C9, where $[Ag^+] + [Zn^{2+}]$ were 260 mg L^{-1} and 200 mg L^{-1}, respectively. For C5 ($[Ag^+] = 1200$ mg L^{-1}) and C10 ($[Ag^+] + [Zn^{2+}] = 520$ mg L^{-1} and 400 mg L^{-1}, respectively), *A. niger* growth was not observed. These concentrations prevented the advance of the fungus hyphae on the coated surface. These results confirm the synergistic effect produced by the addition of both cations.

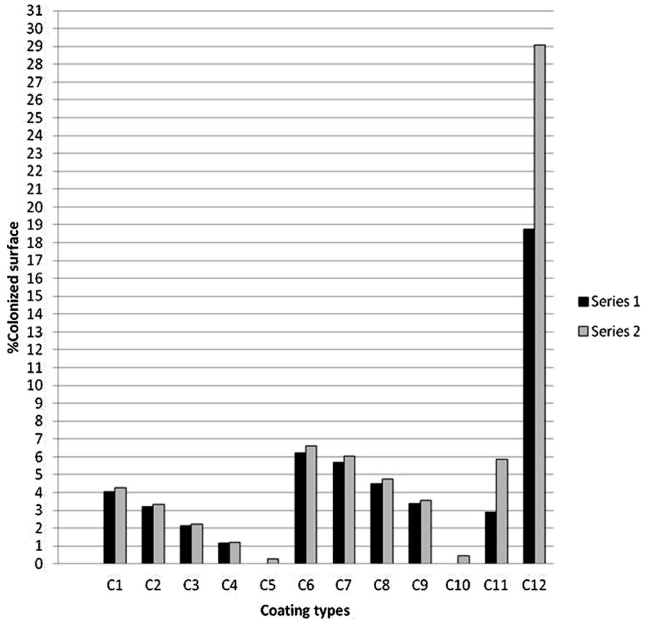

Fig. 5. *A. niger* growth in Series 1 and Series 2.

On the other hand, Zn^{2+} incorporation improves the visual appearance of the coating films. For formulations containing only silver it was observed that high concentrations of silver cation generated a darkening of the coating film due to silver photo-oxidation (Fig. 6, center). Consequently, zinc addition allows decreasing the amount of silver in the film by 57%, providing the same inhibitory effect. In this case, defects in coloring were not observed, the color and the hiding power being similar to those obtained using the commercial biocide.

After aging (Series 2), an increase in the % colonized surface of coatings was obtained for all the samples analyzed. Weathering produces film structure deterioration, i.e. reactions of primary valence bond breakage and those producing chemical changes during the weathering test, increasing the water permeability of the film. It is known that polar groups generated as a result of photochemical reactions of aging facilitate water permeability. Furthermore, the coating weathering causes an increase in the concentration of the surfactant at the surface, produced by its migration toward the film–air interface. Thus aliphatic hydrophobic tails remain on the surface of the paint. These two mentioned facts increase the supply of needed nutrients promoting the growth of fungi [43]. Aged films containing metal-exchanged zeolite

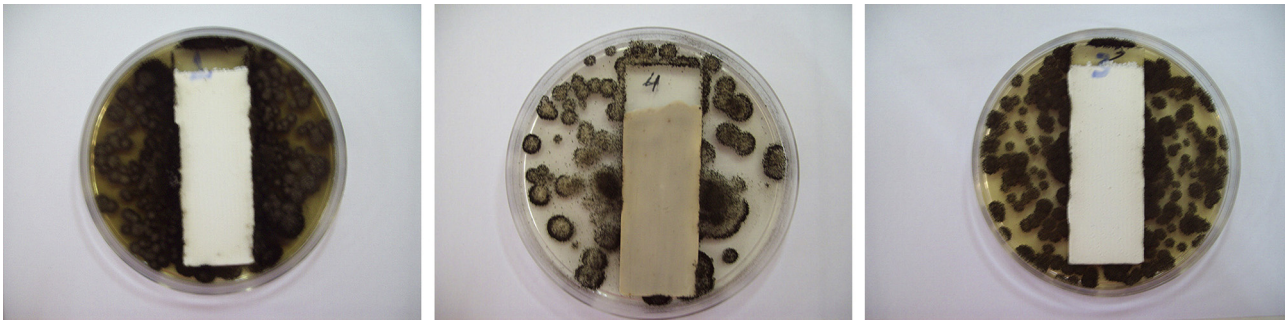

Fig. 6. Left: Commercial waterborne coating in *A. niger* culture. Center: Coating with Ag+ zeolite in *A. niger* culture, (600 mg L^{-1} Ag^+). Right: Coating with Ag^+ zeolite in *A. niger* culture, (260 mg L^{-1} Ag^+ + 200 mg L^{-1} Zn^{2+}).

showed an increase of about 0.3–5% compared with the percentages observed for Series 1. Sample C11, containing commercial biocide, showed an increase of colonized surface of 121%. The antifungal activity provided by the commercial biocide after the weathering test is undoubtedly poorer compared with the values obtained for the metal-exchanged zeolite. On these bases, a considerably slower biocide release from the zeolite-containing coatings compared to an ordinary formulation with CIT/MIT-OIT freely dispersed biocides could be inferred. The CIT/MIT-OIT increased solubility and stability decrease above 40 °C would be other causes of leaching of commercial biocide when the film was aged under irradiation. It is important to mention that the level incorporated in the coating formulation of CIT/MIT-OIT was 2000 mg L^{-1} (MIC = 5 mg L^{-1} for *A. niger*), while the recommended level for CIT/MIT is about 10–30 mg L^{-1} taking into account its skin sensitizing potential [3,44]. Furthermore, after the weathering test the coating C12 formulated without biocide also increased the colonized surface (55%) (Fig. 5). The results of this study provided sufficient evidence to support the observation that paint films containing the loaded carriers retained more biocidal activity after leaching than those containing free biocides.

3.4. Diffusion measurements

Concerning the mobility of cationic biocides, the microbiological tests performed by the diffusion method allowed concluding that cations supported in zeolite matrices effectively possess sufficient mobility to migrate to the dried coating film interface. This property is very important as it ensure the membrane attack of the microorganisms under study. The results showed that the inhibitory zone (i.e. the no growth area of *A. niger* around the dry coating film) is enlarged as increased concentrations of Ag^+ and Zn^{2+} are used. However, small inhibition radii were generally observed, showing a low leaching tendency of cationic biocides.

4. Conclusions

Biocidal cations such as Ag^+ and Zn^{2+} supported on zeolites might be a beneficial tool for the development of coatings with a longer protection against microbiological attack. The replacement of a fraction of silver by zinc cations in the zeolitic material offers two advantages: firstly, to design waterborne coating formulations with identical performance and lower cost than those obtained when only monovalent silver cations are employed; secondly, the esthetic properties could be maintained. It is also important to mention that the materials prepared could be considered for the substitution of many of the traditionally used organic biocides, which according to environmental regulations should be replaced by low-toxicity and environmentally friendly alternatives.

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