Revista de Chimie
https://revistadechimie.ro
https://doi.org/10.37358/Rev.Chim.1949

Silver Adsorption on the Low Temperature Activated Alumina Grades. II. Antimicrobial Activity of the Silver Adsorbed on the Alumina Surface

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***Abstract**: The subject of this paper was the study of antimicrobial capacity of silver loaded on the low temperature activated alumina products and evaluation of these products as decontaminants in the water and waste waters decontamination treatments. The low temperature activated alumina products have been characterized as valuable adsorbents in previous papers. So, by reason the next step to investigate these silver loaded products and finding their performaces as antimicrobial agents was an alluring prospect. For this purpose, a common bacterian inoculus was choosen for experiments, and a penmisive method to measure the rate of inhibition was adopted. Experimental data have shown the dependence of the inhibition rate on the following parameters concerning the adsorbent properties: the thermal treatments, particle size dimension of adsorbent (low temperature activated alumina products), concentration of siver adsorbed on particle surface and density of bioreactive centers, representing the number of particles on unit volume of the liquid containing the bacterial cells. Also, the experiments lay out that the aluminum hydroxide calcined at 300 and 400ºC exibits the largest silver adsorbed concentration and the highest inhibition rate (close to 100%). Little dependence of inhibition rate on pH, in the interval 5.0 – 8.0, was observed. For application of depolluting agent in diferent technological processes, it is necesary to measure the minimum inhibitory concentration in therms of g of silver loaded on the low temperature activated alumina /L.*

***Keywords**: aluminium hydroxide, activated alumina, silver antimicrobial activity, silver adsorption/desorption*

1. Introduction

Ideas about silver toxicity and its antibacterial activity have been considered over some many centuries. Nevertheless, the knowledge about the interaction mechanism between living microorganisms and the entities carrying the silver ions, and eventually elaboration on new concepts, like release strategies of silver ions from the nanoparticles materials for the bacterial growth inhibition and total de-structuration, was quite a long way [1, 2]. Early studies, about mechanisms involved in the silver biological activity as free ions in solution, substantiated four main mechanisms of action from silver activation till the final toxic cells eradication.

First mechanism is grounded on the hypothesis of cell membrane destabilization by silver ions releasing into solution and by biding the sulphur from sulfhydryl groups of the proteins and enzymes

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lying out right on the bacterial cells surface [3]. This mechanism assumes the antimicrobial action of silver or of silver compounds is proportional with the concentration of the bioactive ion (Ag(I) released in solution and to its availability to interact with bacterial or fungal cell membranes [3, 4]. The silver ion is bio reactive and actually, is ready to interact with proteins, amino acid residues, free anions and eukaryotic cells membranes. Bacterial and probably fungal sensitivity to silver has to be relates to the levels of intracellular silver uptake in the microorganism cells, and also, to silver impact capability, which produces irreversibly damages in key enzyme systems. On the other hand, silver exhibits low toxicity in the human body and minimal risk is expected due to inhalation and ingestion through the dermal, urological or hematogenous routes. But, any chronic ingestion or inhalation of silver, which leads to deposition of silver as a metal/silver sulphide particle in the skin (argyria), eye (argyrosis) and in other organs can be easily removed by mild treatments [4]. A particular characteristic of the Ag (I) in water neutral solutions is its oligodynamic effect, defined by gradual increase in antimicrobial capacity of silver ions, as far as the dilution turns toward very low concentrations, as low as one part per million [5].

Second mechanism concerns the unexpected capacity of colloidal silver, as microscopic silver particles suspension, to reduce the duration and severity of many bacterial infections [6]. This type of activated silver does not tear down the bacteria directly, but set off a deactivation of enzymes responsible with respiration, metabolism and multiplication [7]. Also, these activated silver ions exhibit particular affinity for sulfhydryl groups and protein residues accumulated in cells membranes [8]. Because the reactivity of colloidal silver is associated to oxidation processes this mechanism is called "the production of reactive oxygen species (ROS)" Also, this mechanism is well described by the experimental data related to some effective antimicrobial therapies with colloidal silver against difficult-to-treat Gram-negative pathogens, where was observed that the silver disrupts multiple bacterial cellular processes, including the disulphide bond formation, as well the iron metabolism and homeostasis [9]. All these clear-cut feedbacks are associated with raises in yielding the reactive oxygen species (ROS) and respectively, with significant increases in membrane permeability for Gram-negative bacteria. Other examples of oxidation by ROS technology and enhancing the activities of a large row of antibiotics against Gram-negative bacteria are shown in the following papers [10, 11].

Third and fourth mechanisms of the silver antimicrobial activation did come across as a result of intensive researches, and only when the emergence of drug resistant pathogens was understood, as a serious threat to human health. Both mechanisms were substantiated by finding the ways to disrupt cells metabolism [12, 13] and the DNA structure, with further damaging the nucleic acids replicate structure and functions [14]. In both mechanisms, ligands and nanoparticles silver upheave the intensity and yields of biological processes. The third mechanism presumes the silver ions and their oxidative nanoparticles aggregates are enforcing significant changes in cellular respiratory and metabolic processes. On the contrary, in the fourth mechanism, the targets are DNA and RNA and further the size dimension and nature of nanoparticles control the extent of all changes [15-17]. An interesting comparison between the third and fourth mechanisms is given in the paper [18].

Recently, the control of the emerging antibiotic-resistant bacteria has become a serious public health problem. New and better antimicrobial agents are still to be developed to control specific targeted microorganisms. However, achieving a fine directed antimicrobial effect to a particular class of microorganism is still a major challenge for researchers in the domain of antimicrobial agents [19-21]. All the microorganisms are continuously interacting with different inorganic compounds. Sometimes, the interactions are essential for cells biological functions. Other times, these compounds are exerting inhibitory effects that limit the normal cells development or the effects are quite inoffensive. Aluminum is a metal without biological functions [22, 23]. The bioavailability of an inorganic compound is a measure of its interaction with cells biological systems, which produce measurable effects [24-27]. Thus, the inorganic ions that interact with bacteria belong to three classes: (a) a group of key ions (K^+, Mg^{2+}, SO_4^{2-}) and others vital for normal metabolism; (b) a group of ions, indispensable as micronutrients, which are toxic when are present in relatively high concentrations (Cu^{2+}, Zn^{2+}, Co^{2+}) and others; (c) a

group of mostly toxic ions with no known biological functions (Pb^{4+}, Hg^{2+}, Cd^{2+}, Ag^{+}, Al^{3+}) and other [28, 29].

Most bacteria have developed specific transport systems for essential ions. Usually, these pathways are free of toxic ions. Recently, some new researches are constantly developing new bioactive compounds or composites with antibacterial properties for protecting the pathways for essential ion transport. Parts of these investigations are aimed to enlarge the area of interest to new bioactive silver materials and nano-scale silver composites [30]. Silver is one of the precious metals with important and well-known industrial applications. At the same time, silver has an important antimicrobial capacity. The effectiveness of silver as an antimicrobial material depends largely on ways the silver is used for this purpose [31]. For this reason, the Ag (I) ions have to be locked in a porous matrix, to avoid dispersion or agglomeration of Ag (I) in the containing materials. In this paper, the matrix is the low temperature activated alumina products, described in previous papers [31-34]. Incorporation of the Ag (I) particles into different matrices can extend their usefulness, especially in terms of biological applications. Such materials could present new advantageous properties, as well as increases in the biocidal activity. One of the promising class of materials, which can be used as a silver-containing matrix, is the group of porous low temperature activated alumina oxides, shaped as particulated adsorbents [35, 36]. The use of silver based materials or the high silver loaded nanoparticles compounds, acting as antibacterial and antimicrobial agents has a powerful impact factor in the recent designed technologies for removal of pollutants from toxic natural or industrial sources [31]. Large surface areas covered by ionic silver or the small particle size from the porous silver coated materials are enhancing the efficiency of silver in all of its applications as antibacterial and antimicrobial agent. But, exceeding the endorsed limits of silver concentrations in the water industrial systems always results in well-known adverse effects over environment, with hazardous outcomes on both human and aquatic life. Hence, the use of silver loaded materials should be treated with serious awareness about the balance between their efficacy as antimicrobial agents in removal of pollutants and their potentially toxic nature. Thus, any efficient technology to remove particular pollutants using silver antimicrobial power should be doubled by a technology to recover silver as precious metal.

The present paper subject concerns the biological tests of low temperature activated alumina products loaded with Ag (I) ions at *p*H 5, which can release free ions in aqueous media at the same *p*H, at which the samples were silver loaded, or to other higher *p*H values. The accepted mechanism of silver adsorption is the first mechanism described above. Adsorbents are the low temperature alumina activated products at 260, 300 and 400ºC, with variable particle size distributions. Their properties and potential uses as adsorbents and support materials for silver were fully described in the papers [32-34]. The study encompasses a first stage well set out for the loading Ag (I) ions onto the adsorbents surface. For the experiments there were chosen: two non-thermal treated samples (GDAH-01-25 and GDAH-04-25) and two thermal treated samples at 300ºC and respectively, 400ºC (GDAH-04-300, GDAH-04-400) in order to compare, both the effects of thermal treatments and of the particle size dimension on the adsorptive and antimicrobial properties of this materials class. The second stage of this study was allocated entirely to the biological tests of the selected samples, in order to measure intensity of the antimicrobial effects and the ways to improve this effect. After final selection, the data will show the best results in terms of concentration of Ag (I) on adsorbent surface, thermal treatment of adsorbent and its particles size dimension.

2. Materials and methods

2.1. Samples materials

The raw samples of aluminium hydroxide were collected from the last test of the new production line at Alum SA Tulcea, Romania, which was built up by implementation of the project “Endow the Research and Development Department of SC ALUM SA Tulcea with independent and efficient research facilities to support the economic competitiveness and business development”, project cofounded by the European Regional Development Fund through the Competitiveness Operational

Program 2014–2020. Samples from the low temperature activated alumina products GDAH-01-25, GDAH-04-25, GDAH-04-300, GDAH-04-400 were prepared according to the protocol described in the previous papers [32-34]. Their particular properties, specific for adsorbents, are given in the Table 1. Further, the samples from these products were saturated at equilibrium with Ag(I), using a solution of $AgNO_3$ with variable concentration in Ag(I) at *p*H 5. After the silver loading saturation, these new samples were carefully washed with deionized water at *p*H 5 and, at last, dried at temperature 60-80ºC. These samples are the products with highest silver loaded in this experimental study (Table 1 and Table 2). Also, similar samples loaded up to smaller silver content and dried afterward, have been used for performing the biological tests (Table 2).

Table 1. Main properties of the low temperature activated alumina products

Properties	GDAH-01-25	GDAH-04-25	GDAH-04-300	GDAH-04-400
Particle's size, µm	0-150	0-10	0-10	0-10
BET specific surface, m^2/g	3.5419	9.4725	6.9195	238.6443
Average pore size, nm	4.68861	7.4804	3.7566	3.2303
Water adsorption capacity, %, 25ºC and 50% humidity	0.02	0.27	10.28	12.98
Maximum Ag(I) adsorption capacity at saturation, mg/g	4.9	6.2	10.18	9.67
Mineralogical phases	G+A	G+A	G+B+GA+A	GA+A
G-Gibbsie; B-Boehmite: GA- Gama-Alumina: A-Amorphous.				

Table 2. Silver loaded concentrations on to the sample's particle surface, expressed in mg/g

Silver concentration in the working solutions	GDAH-01-25	GDAH-04-25	GDAH-04-300	GDAH-04-400
Adsorption capacity when use 1 mg Ag(I)/L, mg/g	0.25	0.25	0.25	0.25
Adsorption capacity when use 3 mg Ag(I)/L, mg/g	0.75	0.75	0.75	0.75
Adsorption capacity when use 5 mg Ag(I)/L, mg/g	1.25	0.67	1.25	1.25
Adsorption capacity when use 7 mg Ag(I)/L, mg/g	1.42	0.87	1.51	1.59
Adsorption capacity when use 10 mg Ag(I)/L, mg/g	1.92	1.37	2.17	2.17
Adsorption capacity when use 20 mg Ag(I)/L, mg/g	2.39	3.33	3.75	3.57
Adsorption capacity when use 30 mg Ag(I)/L, mg/g	3.25	5.7	5.74	5.87
Adsorption capacity when use 40 mg Ag(I)/L, mg/g	4.9	6.1	7.81	7.93
Maximum adsorption capacity, when use 90 mg Ag(I)//L, mg/g	4.9	6.2	10.18	9.67

From the large categories of samples, presented in the papers [32, 33], the samples GDAH-01-25, GDAH-04-25, GDAH-04-300, GDAH-04-400 were chosen as representative for the biological tests, because these samples make difference between non-milled GDAH-01-25 sample and milled and classified sample GDAH-04-25, at any load of silver on their particles surface area. The samples GDAH-04-25, GDAH-04-300 and GDAH-04-400 make difference between heated and unheated samples, at any load of silver on their particles surface area. These differences let user to find easy the type of material is best fitted to his process (Table 1 and Table 2).

2.2. Silver adsorption capacity and the samples silver loading protocols

Determination protocol of the silver maximum adsorption capacity onto low temperature activated alumina products was presented in the paper [31]. In a similar way, the same protocol was used for loading smaller silver quantities onto samples of low temperature activated alumina products, with the

defined purpose to detect the critical rates of the biologic material cells inhibition for each of the above representative products (Table 2). Thus, the samples of 0.1g from the products (GDAH-01-25, GDAH-04-25, GDAH-04-300 and GDAH-04-400) were mixed with 25 mL solutions with variable contents in silver (from 1 mL/L up till 90 mg/L) at 25ºC and *p*H 5 for 60 min. At the end of silver loading time, the silver containing solutions were removed and the solid materials were washed and dried. Both the initial and final silver content in the working solutions for each sample were analyzed and the silver loaded quantities were computed in units of mg/g adsorbent. These data are given in the Table 2. For these experiments the silver concentrtion were measured with Varian SpectrAA 280 FS spectro-photometer.

2.3. Biological materials

Agar dilution assay was chosen as standard test for evaluate the inhibition rate of silver loaded on the low temperature activated alumina products from Table 2.

Microbial culture media was prepared from Tryptone yeast extract agar purchased from VWR International, Belgium, Cod 84906.0500 lot 1485 in compliance with ISO Standard 11133:2014. The composition of the bacterial culture media was: Tryptone 6 g/L, Yeast extract 3 g/L and Agar 15 g/L. After sterilization, the *p*H was checked and adjusted to 7.2 ±0.2 at 25°C.

Bacterial inoculum was an easy fresh available water, collected from river Bega (Timisoara) early morning during each day when the expriments were performed. The bacterial inoculum is an local environmental heterotrophic type, containing aerobic and anaerobic bacteria, or facultatively anaerobic bacteria, which can be Gram-positive or Gram-negative. The composition of the heterotrophic inoculum is not clearly defined. In each experiment, the bacterial concentration is measured and calibrated with a densitometer McFarland, type DEN-1, product of Grant Instruments Ltd. Cambridge, United Kingdom. Actually, the usual working bacterial concentration was 1 to 2 x 10^8 CFU / mL (0.5 McFarland).

2.4. Samples inoculation protocol and inhibition rates

In order to evaluate the potential antimicrobial effect of alumina-based materials loaded with silver at well-chosen concentrations (non-heat treated and heat treated materials with different granulation, prepared at 25, 300 and 400°C), there were prepared the following starting samples: GDAH-01-25, GDAH-04-25, GDAH-04-300 and GDAH-04-400 (Table 2).

In the tables and figures dealing with the bacterial growth rate inhibition, depending on the nature of the adsorbent and the degree of loading with silver, the samples will be named as follows:

- GDAH-01-25(M0), GDAH-04-25(M0), GDAH-04-300(M0), GDAH-04-400(M0), standing for blanks in all experiments, and

- GDAH-01-25(Ag), GDAH-04-25(Ag), GDAH-04-300(Ag), GDAH-04-400(Ag), standing for samples loaded with silver from different working silver solutions with variable content in silver, as they are presented in Tables 3 and 4.

Each group of 4 samples, differentiated by the above given names, were loaded with silver from working solutions of various concentrations, expressed in mg/L, as they were being presented in Table 2. In Table 3, each sample bears a paranthesis indicating the concentration of silver adsorbed on its particles surface in m/g. Also, in Table 3, the paranthesis (Ag) are filfilled with the real concentration of silver loaded on particles surface in mg/g, under experimental conditions, denoted by the Table 2.

The inoculation protacol encloses the following well known steps found in similar analysis of the potential antimicrobial effect of common or new materials: a) Cleaning and sterilization the Petri dishes; b) Drying and weighting the silver loaded samples, 0.1 g; c) Preparation and sterilization of the microbial culture media; d) Preparation the blanks for each series of experiments, including also, the blancs for microbian heterotrof inoculus with a concentration of 1-2x10^8 CFU/mL; e) Preparation plates for all the samples from Table 2 - 5, by adding 15 mL microbial culture media at *p*H 7.2 ± 0.2, weighted 0.1g sample and 1 mL inoculum containing 1-2x10^8 CFU and carefully mixing the blend acording ISO 6222 and ISO 8199:2018; f) Holding the samples for 15 min resting to coagulatio ; g) Transfering the samples after coagulation into incubator and holding them at 37°C for 48 h.

At the end of the last stage of protocol, the Petri dishes were read with an automatic colony counter Flash&Go, YUL Instruments, Spain. The entire experiment was repeted for 3 times and the final results were calculated from the mediated values.

Eficiency of the bacterial inhibition process on the functionalized low temperature activated alumina samples was expressed as the rate of inhibition of microbial growth [37] and computed as the ratio between the number of colony-forming units on the silver loaded Ag samples and the control samples M(0), expressed as a percentage ratio, according to the equation:

$$\text{Growth inhibitiom (\%)} = (1 - CFU_{test}/CFU_{control}) \times 100$$

where $CFU_{control}$ is the number of colonies on the Petri dish control (M0) and CFU_{test} is number of colonies on the Petri dishes with each of the silver loaded samples from Tables 2 - 5.

2.5. Equipment

Studies of silver adsorption at constant temperature were performed using a water bath with thermoregulatorand agitation, type JULABO, model SW 23, class I from JULABO GmbH, Germany, 2008. Silver concentration in the fluid samples was determined spectrophotometrically, using a Varian SpectrAA 280 FS, Atomic Absorption Spectrometer equipment, Agilent Technologies Australia Ltd., 1997. For the determination of initial colonies number in inoculated samples was used a McFarland densitometer, type DEN-1, product of Grant Instruments Ltd. Cambridge, United Kingdom. For determination of the number of bacterial colonies grown on Petri dishes after 48 h of incubation was used a Flash & Go's automatic colony counter, produced by YUL Instruments Barcelona Spain, 2012. The results are expressed by the number of counted colonies in the unit of volume, corresponding to the surface of the counted plate and the final results are expressed in colonies / mL.

3. Results and discussions

Samples of silver loaded products on low temperature activated alumina, prepared for the biological test, are described broadly in the Tables 1 and 2. Selection of the samples from Table 2 was chiefly made in order to control all particular parameters describing each sample of alumina (activated products and silver carrier), e.g., particle size dimension, predominant mineral phase, the product thermal treatments, and also, to control the particular silver concentrations in each product from Table 2. These individual tested samples are distinct materials resulting from the grinding and classification on the basis of particle size dimension of the thermally activated alumina products, and also, from these products calcination temperatures. Thus, the low temperature activated alumina products are the adsorbents, and the silver loaded in variable concentrations on the surface of adsorbents particles is the biological reactive material. Actually, the low temperature alumina activated products, processed in some specific ways, have significant antimicrobial properties against many species of bacteria [38, 39]. Also, the L-cysteine has relevant effects in potentiating the different antimicrobial classes of products, including both ionic silver and silver containing bactericides [40, 41]. All samples from Table 2 were tested according to the protocol described in section 2.4. This protocol allows calculation of the inhibition rate from experimental data, as a measure of the antimicrobial activity of the silver adsorbed on the low temperature activated alumina particle surface. Also, the protocol permits the variation in the pH and adding of potentiating products, as well as variable silver concentrations and quantities of silver loaded products on low temperature activated alumina in the culture media.

Table 3 and Figure 1 show the results of experiments on bacterial inoculum inhibition rates, according to the tests described in section 2.3, at *p*H 7.

Table 3. Rates of inhibition at *p*H 7. Samples from the Table 2

Samples,	GDAH-01-25 (M0)	GDAH-04-25 (M0)	GDAH-04-300 (M0)	GDAH-04-400 (M0)
Rate of inhibition, %	22.6	36.8	54.8	46.5
Samples,	GDAH-01-25-(Ag 0.25)	GDAH-04-25- (Ag 0.25)	GDAH-04-300 (Ag 0.25)	GDAH-04-400 (Ag 0.25)
Rate of inhibition, %	27.1	38.1	43.2	40.6
Samples,	GDAH-01-25-(Ag 0.75)	GDAH-04-25 (Ag 0.75)	GDAH-04-300 (Ag 0.75)	GDAH-04-400 (Ag 0.75)
Rate of inhibition, %	32.9	36.1	48.4	38.7
Samples,	GDAH-01-25-(Ag 1.25)	GDAH-04-25 (Ag 0.67)	GDAH-04-300 (Ag 1.25)	GDAH-04-400 (Ag 1.25)
Rate of inhibition, %	41.9	29.0	56.8	34.2
Samples,	GDAH-01-25-(Ag 1.42)	GDAH-04-25 (Ag 0.87)	GDAH-04-300 (Ag 1.51)	GDAH-04-400 (Ag 1.59)
Rate of inhibition, %	40.6	31.0	54.8	36.8
Samples,	GDAH-01-25-((Ag 1.92)	GDAH-04-25 (Ag 1.37)	GDAH-04-300 (Ag 2.17)	GDAH-04-400 (Ag 2.17)
Rate of inhibition, %	43.9	43.2	54.2	46.5
Samples,	GDAH-01-25-(Ag 2.39)	GDAH-04-25 (Ag 3.33)	GDAH-04-300 (Ag 3.75)	GDAH-04-400 (Ag 3.57)
Rate of inhibition, %	45.8	39.4	52.9	51.0
Samples, 30 mg/L Ag	GDAH-01-25-(Ag 3.25)	GDAH-04-25 (Ag 5.7)	GDAH-04-300 (Ag 5.74)	GDAH-04-400 (Ag 5.87)
Rate of inhibition, %	51.6	42.6	56.1	51.6
Samples,	GDAH-01-25-(Ag 4.9)	GDAH-04-25 (Ag 6.1)	GDAH-04-300 (Ag 7.81)	GDAH-04-400 (Ag 7.93)
Rate of inhibition, %	56.1	46.5	57.4	54.2
Samples (maximum adsorption capacity),	GDAH-01-25-(Ag 4.9)	GDAH-04-25 (Ag 6.2)	GDAH-04-300 (Ag 10.18)	GDAH-04-400 (Ag 9.67)
Rate of inhibition, %	34.2	49.7	48.4	51.0

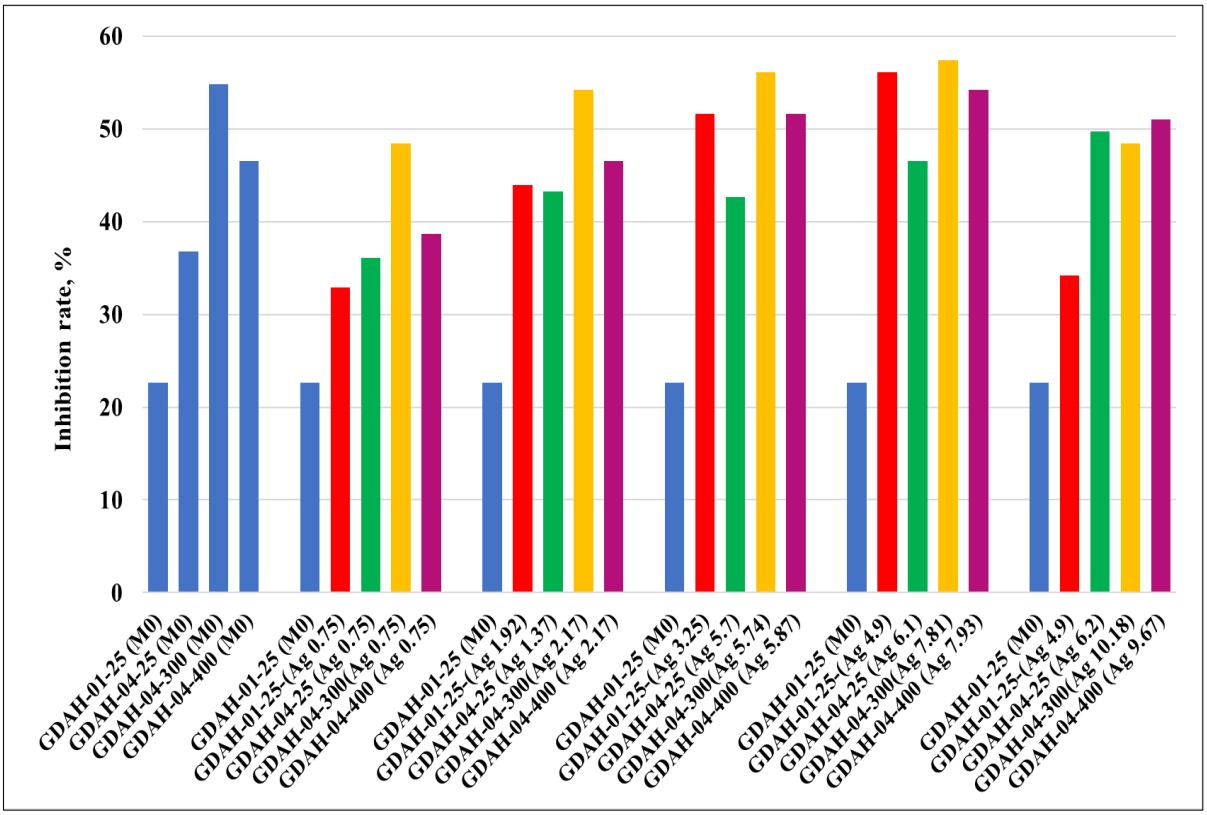

Figure 1. Rates of inhibition at *p*H 7. Sample from Table 3

All groups of samples in Figure 1 and Table 3 show the inhibition rates of cells growth at different concentrations of silver in the activated alumina (from 0 mg Ag/g activated alumina to 10.18 mg Ag / g activated alumina) in the all 4 samples that make object of this investigation: GDAH-01-25, GDAH-04-25, GDAH-04-300 and GDAH-04-400.

The data for blank samples show normal differences between each sample absorption rates. Thus, sample GDAH-01-25 (M0), representing non-calcined and unclassified aluminum hydroxide, has the lowest inhibition rate (20%). The classification of aluminum hydroxide and the selection of the fraction of less than 10 microns (GDAH-04-25 (M0)), leads to an increase in the inhibition rate up to approximately 37%. Samples thermally activated at 300 and 400°C with the particle size less than 10 microns, GDAH-04-300 (M0) and GDAH-04-400 (M0) have reached the highest inhibition rates, due to continuous increases in content of the amorphous-alumina, boehmit and gamma-alumina in the both samples composition (Table 3 and Figure 1). The lower values of the inhibition rates in the GDAH-04 - 400 (M0) sample are due exclusively to the specific surfaces decrease in thermally activated samples at 400ºC.

In Table 3 and Figure 1, the other groups of samples, in which the values of the inhibition rate are due to variations of the same parameters: particle size, aluminum hydroxide activation temperature and the effect generated by the variation of silver concentrations at the adsorbent particles surface shows that dependency is similar to the blank sample group, significant data for the specific contribution of each parameter to the increase of the cells growth inhibition rate. However, the total inhibition effect remains somewhere between 55 and 60%, and the contribution of silver loaded at any concentration on particles surface seems to be very poor or at least blocked, before the end of inhibition process.

Similar results have been obtained raising or lowering the *p*H of the sample in the testing protocol over a rational interval of *p*H variation 5.0- 8.0, to keep up with the standard design of experiment. The experiments performed with the above-described culture medium and the same bacterial inoculum at *p*H values lying in interval 5.0-8.0 turned out similar results as those given in Table 3 and Figure 1.

When the final results of biological tests exhibit incomplete inhibition or poor oxidation of the pollutant cells, or the entire process is too slow, some suitable potentiating additives from various compatible classes with oxidizing agents might be used beside the main inhibitors. The L-cysteine is one of the enhancers of biological activity when using inhibitors with acceptable contents in alumina and silver [40-43].

In Table 4 and Figure 2 are given the results of last series of experiments. When compared these new data with rates of inhibition in the previous tests, it is easy to observe that all the results are certainly far away from expectations.

Table 4. Rates of inhibition at *p*H 7, Samples from Table 2, modified by adding L-cysteine, 1g/L

Samples,	GDAH-01-25 (M0)	GDAH-04-25 (M0)	GDAH-04-300 (M0)	GDAH-04-400 (M0)
Rate of inhibition, %	23.6	33.6	46.4	44.3
Samples,	GDAH-01-25- (Ag 0.25)	GDAH-04-25- (Ag 0.25)	GDAH-04-300 (Ag 0.25)	GDAH-04-400 (Ag 0.25)
Rate of inhibition, %	24.3	34.3	37.1	34.3
Samples,	GDAH-01-25- (Ag 0.75)	GDAH-04-25 (Ag 0.75)	GDAH-04-300 (Ag 0.75)	GDAH-04-400 (Ag 0.75)
Rate of inhibition, %	22.9	28.6	40.0	35.0
Samples,	GDAH-01-25- (Ag 1.25)	GDAH-04-25 (Ag 0.67)	GDAH-04-300 (Ag 1.25)	GDAH-04-400 (Ag 1.25)
Rate of inhibition, %	26.4	24.3	46.4	31.4
Samples,	GDAH-01-25- (Ag 1.42)	GDAH-04-25 (Ag 0.87)	GDAH-04-300 (Ag 1.51)	GDAH-04-400 (Ag 1.59)
Rate of inhibition, %	28.6	23.6	40.0	27.9
Samples,	GDAH-01-25- ((Ag 1.92)	GDAH-04-25 (Ag 1.37)	GDAH-04-300 (Ag 2.17)	GDAH-04-400 (Ag 2.17)

Rate of inhibition, %	31.4	32.1	46.4	40.0
Samples,	GDAH-01-25-(Ag 2.39)	GDAH-04-25 (Ag 3.33)	GDAH-04-300 (Ag 3.75)	GDAH-04-400 (Ag 3.57)
Rate of inhibition, %	40.7	42.1	47.9	45.7
Samples,	GDAH-01-25-(Ag 3.25)	GDAH-04-25 (Ag 5.7)	GDAH-04-300 (Ag 5.74)	GDAH-04-400 (Ag 5.87)
Rate of inhibition, %	45.7	45.0	51.4	46.4
Samples,	GDAH-01-25-(Ag 4.9)	GDAH-04-25 (Ag 6.1)	GDAH-04-300 (Ag 7.81)	GDAH-04-400 (Ag 7.93)
Rate of inhibition, %	51.4	48.6	52.9	49.3
Samples (maximum adsoption capacity),	GDAH-01-25-(Ag 4.9)	GDAH-04-25 (Ag 6.2)	GDAH-04-300 (Ag 10.18)	GDAH-04-400 A(g 9.67)
Rate of inhibition, %	35.7	44.3	48.6	45.7

The poor results might be an outcome of a missing parameter which has to be essentially reconsidered for all samples. Reanalyzing the literature data and the entire experiment together with subsequent acquired data, it was found that minute concentrations of ionic silver were required usually for reaching high inhibition rates, just only when ionic silver is released as a free ion in the liquid phase.

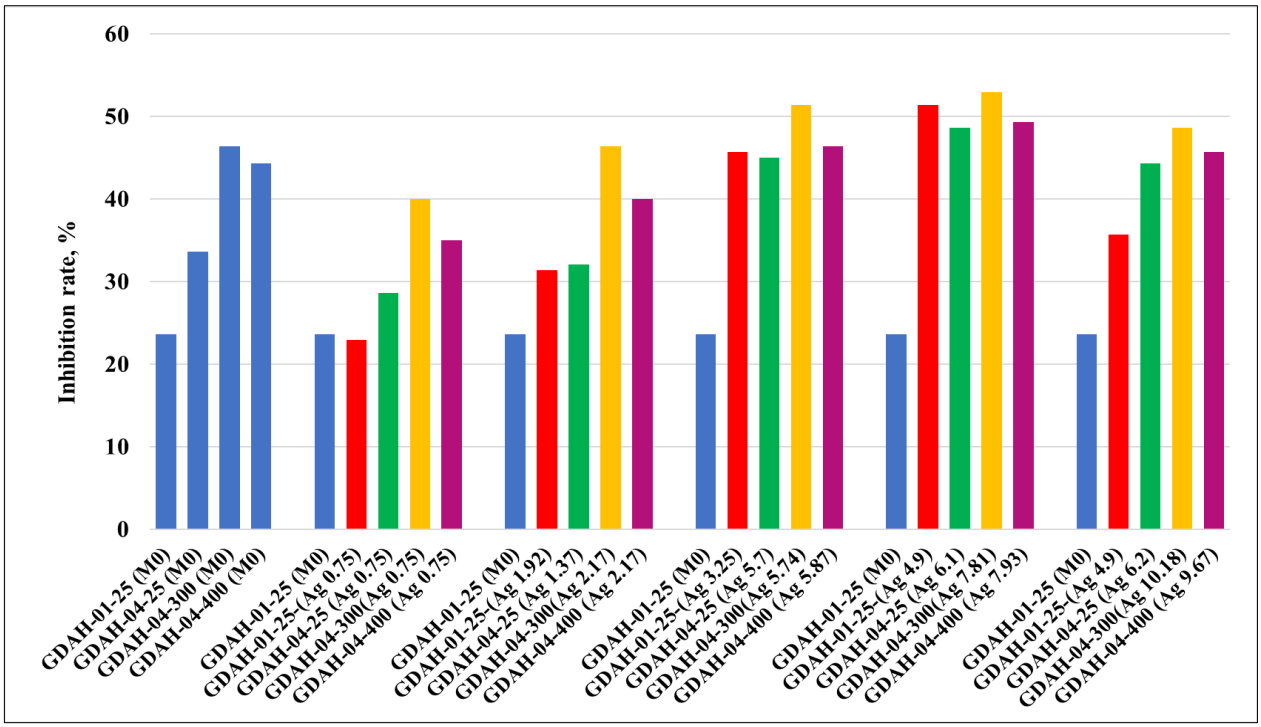

Figure 2. Rates of inhibition at *p*H 7, Samples from Table 2, potentiated by adding L- cysteine, 1 g/L

In the case of low temperature activated alumina as carrier for the loaded silver, the silver ion is much stronger bonded by chemical adsorption, and consequently, much more particulate material is required for keep up with the critical concentration of released ions in the liquid phase. According to the above arguments, the doses of silver loaded low temperature activated alumina in the biological tests described in section 2.4, were multiplied by 2, 4 and 8 times, as is shown in Table 5.

Table 5. Rates of inhibition. Samples selected from the Table 2, modified by multiplication of the mass doses of low temperature activated silver loaded alumina products

Samples	Inhibition rate, %			
	Standard sample mass x 1	Standard sample mass x 2	Standard sample mass x 4	Standard sample mass x 8
GDAH-01-25-(M0)	22.6	28.57	40.26	50.6

GDAH-04-300-(M0)	54.8	48.05	55.84	66.2
GDAH-04-300-(Ag1.51)	54.8	58.44	61.04	62.3
GDAH-04-300-(Ag5.74)	56.0	77.92	81.82	81.8
GDAH-04-300-(Ag7.81)	57.4	80.52	84.42	85.7
GDAH-04-300-(Ag10.18)	48.4	85.71	92.21	100.0

Data from Table 5 and Figure 3 are fully reflecting the real dependence of bacterial cells growth inhibition on the silver content added in as the silver loaded on low temperature activated alumina products.

The selected samples are representing: the raw aluminum hydroxide - GDAH-01-25-(M0), non-heated and non-milled (blank sample), the aluminum hydroxide fraction below 10 microns - GDAH-04-300-(M0), milled, classified and heated to 300ºC (blank sample), carrying no silver, and the rest of samples milled, classified and heated to 300ºC, containing variable concentrations of loaded silver (from 1.51 mg/g of carrier up till 10.18 mg/g of carrier). The carrier is the low temperature alumina activated products. On the horizontal lines in the Table 5, can be read effects of the carrier mass on the inhibition rate, and on the vertical lines, can be read effects of the silver charge of each carrier on the inhibition rate.

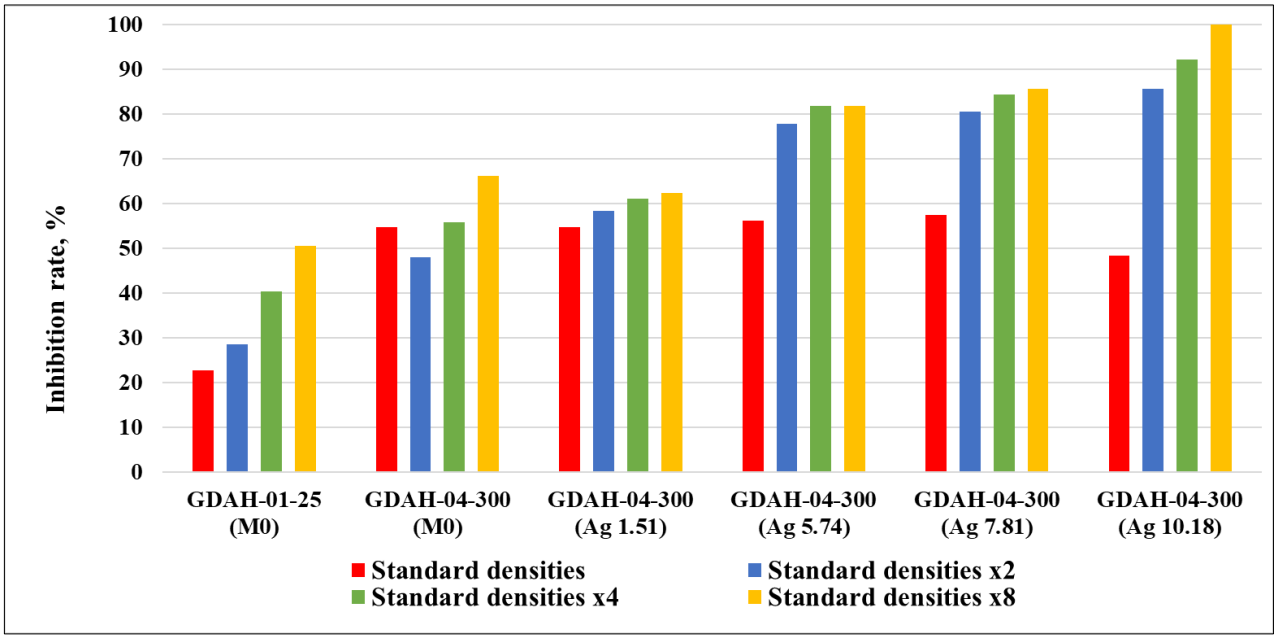

Figure 3. Rates of inhibition at *p*H 7, Samples from Table 5

The first group of bars in Figure 3 stands for blank sample - GDAH-01-25-(M0) – carrying no silver, but is carrying different mass quanties of raw aluminum hydroxide (0.1, 0.2, 0.4 and 0.8 g per sample). This group of bars shows the dependence of inhibition rate on mass of carrier at zero content of silver. The second group of bars is refering to the second blank sample - GDAH-04-300-(M0) – and have the same significance as first group in the second blank sample analysis. The rest of groups of bars are sustaining the dependence of the inhibition rate on the variable mass of carrier, each one bearing a given silver concentration.

From Figure 3 data, it can be concluded that when the sample GDAH-04-300-(Ag10.18) (the highest concentration in silver) is used as depolluting agent, the highest rate of bacterian cells growth inhibition will be certainly reached. Accordinly, the future investigations concerning the use of low temperature activate alumina products silver loaded as bacterial growth inhibitors should started with the computation of minimum inhibitor conccentration (MIC) for silver loaded samples on the basis of the above described data.

4. Conclusions

This paper is the first tentative to evaluate the silver loaded low temperature activated alumina products, manufactures at Vimetco Alum SA Tulcea, Romania, as bactericide agents for treating polluted waters and waste waters. Standard agar diffusion tests were chosen for their simplicity and efficient quick measuring the rate of bacterial growth inhibition. The first rows of tests demonstrated that low temperature activated alumina products have themselves a small capacity to inhibit bacterial growth and the rate of inhibition is dependent on particle size dimension (activation by milling) and also, by the thermal treatments and intensity of thermal treatments (activation by changing the ratios between new born alumina mineral phases, and changes in particles specific surface and other physical properties). Also, these tests surprisingly revealed low rates of inhibition for all samples of silver loaded low temperature activated alumina products, even if the dependence of inhibition rates on particle size dimension and on thermal treatments remanded still visible. Repeating the entire row of experiments and using the L-cysteine as possible additive has no significant effects on the measured inhibition rates of bacterial growth. Because the above experiments, as they were performed, exhibit rational dependences on the mains parameters involved in efficiency control in all experiments, it was concluded that mass concentrations ratio silver/bacterial inoculum should modified in the experiment protocol. The last row of experiments demonstrated that for reaching the maximum rate of inhibition is necessary to raise the mass concentrations ratio silver/bacterial inoculum above critical values, specific for each silver loaded low temperature activated alumina product.

Acknowledgments: This study was made possible by the implementation of the "Endow the Research and Development Department of SC ALUM SA Tulcea with independent and efficient research facilities to support the economic competitiveness and business development" project, which was co-funded by the European Regional Development Fund through the Competitiveness Operational Program of 2014–2020. Under this project, the following were purchased and commissioned: "Independent equipment/ installation for research and development of the technology for wet aluminum hydroxide classification", "Independent equipment/installation for research and development of technology for obtaining the dried aluminum hydroxide", and "Independent equipment/installation for research and development of the technology for grinding and screening the dried aluminum hydroxide".

References

1.CLEMENT, J.L., Penelope S. JARRET., P.S., Antibacterial silver, Met Based Drugs. 1(5-6): 1994; 467-482, doi: 10.1155/MBD.1994.467.
2. XU, Z., ZHANG, C., WANG, X., LIU, D., ACS Appl. Bio Mater. 4(5), 2021, 3985-3999, ttps://doi.org/10.1021/acsabm.0c01485.
3.LANSDOWN, A.B.G., Silver in health care: antimicrobial effects and safety in use, Curr. Probl. Dermatol. 33, 2006, 17- 34. doi:0.1159/000093928.
4.FENG, Q.L., WU, J., CHEN, G.Q., CUI, F.Z., KIM, T.N., KIM, J.O., A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J. Biomed. Mater. Res. 52, 2000, 662–668. doi:10.1002/1097-636(20001215)52:4<662: aid-jbm10>3.0.co;2-3
5.BARANOWSKI Z., Colloidal Silver: The Natural Antibiotic Alternative. Healing Wisdom Publications; New York, NY, USA: 1995. 1-18.
6.TRAN P., HUYNH E., HAMOOD A., DE SOUZA A., MEHTA D., MOELLER K., MORGAN M., REID T.W., The ability of a colloidal silver gel wound dressing to kill bacteria in vitro and in vivo. J. Wound Care. 26(4), 2017, S16-S23. doi: 10.12968/jowc.2017.26.Sup4.S16.
7.BARRAS F., AUSSEL L., EZRATY B., Silver and Antibiotic, New Facts to an Old Story. Antibiotics. 7(3), 2018, 79. doi: 10.3390/antibiotics7030079.
8.LANSDOWN A.B., Silver in Health Care: Antimicrobial Effects and Safety in Use. Curr. Probl. Dermatol., 33, 2006, 17-34. doi: 10.1159/000093928.

9.MORONES-RAMIREZ J.R., WINKLER J.A., SPINA C.S., COLLINS J.J., Silver enhances antibiotic activity against gram-negative bacteria. Sci. Transl. Med. 2013, 5(190, doi: 10.1126/scitranslmed.3006276.
10.GU M., IMLAY J.A., The SoxRS response of Escherichia coli is directly activated by redox-cycling drugs rather than by superoxide. Mol. Microbiol. 79, 2011, 1136-1150. doi: 10.1111/j.1365-2958.2010.07520.x.
11.VILA DOMÍNGUEZ, A., AYERBE ALGABA, R., MIRÓ CANTURRI, A., RODRÍGUEZ VILLODRES, Á., SMANI, Y., Antibacterial Activity of Colloidal Silver against Gram-Negative and Gram-Positive Bacteria, Antibacterial Activity of Colloidal Silver against Gram-Negative, Antibiotics 9(1), 2020, 36. doi: 10.3390/antibiotics9010036.
12.WANG, H., WANG, M., YANG, X., XU, X., HAO, Q., YAN, A., HU, M., LOBINSKI, R., LI, H., SUN, H., Antimicrobial silver targets glyceraldehyde-3-phosphate dehydrogenase in glycolysis of E. coli, Chem Sci. 10(30): 2019, 7193-7199.
13. PETICA, A., GAVRILIU, S., LUNGU, M.V., BURUNTEA, N., PANZARU, C., Colloidal silver solutions with antimicrobial properties. Mater. Sci. Eng. B 2008, 152, 22-27. doi: 10.1016/j.mseb.2008.06.021.
14. SYED, A.A., SABYA, S.D., AYESHA, K., MOHAMMED, T.A., MOHD. S., SAQUI, H., AMAT, K N., Bactericidal activity of silver nanoparticles: A mechanistic review. Materials Science Energy Technol. 3, 2020, 756 -769. doi: 10.1016/j.mset.2020.09.002.
15. PAL, S., TAK, Y.K., SONG, J.M., Does the antibacterial activity of silver nanoparticles depends on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli, Appl. Environ. Microbiol. 73 (2007) 1712-1720.
16.CHOI, O., DENG, K.K., KIM, N.J., ROSS, L., SURAMPALLI, R.Y., HU, Z., The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth, Water Res. 42 (2008) 3066-3074.
17.RAFFI, M., HUSSAIN, F., BHATTI, T., AKHTER, J., HAMEED, A., HASAN, M., Antibacterial characterization of silver nanoparticles against E. coli ATCC-15224, J. Mater. Sci. Technol. 24, 2008, 192-196. https://www.researchgate.net/publication/221720622.
18.PLATANIA, V., KALDELI-KEROU, A., KARAMANIDOU, T., KOUKI, M., TSOUKNIDAS, A., CHATZINIKOLAIDOU, M., Antibacterial Effect of Colloidal Suspensions Varying in Silver Nanoparticles and Ions Concentrations. Nanomaterials 2022, 12, 31. doi: 10.3390/ nano12010031.
19.ROQUE-RUIZ, J.H., CASTILLO-RAMÍREZ, D., RUÍZ-BALTAZAR, Á.J., ESPINOSA-CRISTÓBAL, L.F., REYES-LÓPEZ, S.Y., Preparation of Silver-Doped Alumina Spherical Beads with Antimicrobial Properties, J. Nanomat., 2018, Article ID 7127843, 13 pages. doi:10.1155/2018/7127843.
20.LÓPEZ-ESPARZA, J., ESPINOSA-CRISTÓBAL, L.F., DONOHUECORNEJO, A., REYES-LÓPEZ, S. Y., Antimicrobial activity of silver nanoparticles in polycaprolactone nanofibers against gram-positive and gram-negative bacteria, Ind. Eng. Chem. Res., 55(49), 2016, 2532- 2538. doi: 10.1021/acs.iecr.6b02300.
21.CIOBANU, C.S., ICONARU, S.L., CHIFIRIUC, M.C., COSTESCU, A., LE COUSTUMER, P. PREDOI, D., Synthesis and antimicrobial activity of silver-doped hydroxyapatite nanoparticles, Bio-Med Research International, 2013, Article ID 916218, 10 pages. doi: 0.1155/2013/916218
22. PIFIA, R.G., CERVANTES, C., Microbial interactions with aluminium, BioMetals, 9(3), 1996, 311-316. doi:10.1007/BF00817932.
23.EXLEY, C., BIRCHALL, J.D., The cellular toxicity of aluminium, J. Theor. Bio., 159(1), 1992, 83-98. doi: 10.1016/s0022-5193(05)80769-6.
24.ROMERO, J.L., GRANDE BURGOS, M.J., PÉREZ-PULIDO, R., GÁLVEZ, A., LUCAS, R., Resistance to Antibiotics, Biocides, Preservatives and Metals in Bacteria Isolated from Seafoods: Co-Selection of Strains Resistant or Tolerant to Different Classes of Compounds., Front. Microbiol., 8, 2017, Article 1650. doi: 10.3389/fmicb.2017.01.

25.MARTINEZ-BUSSENIUS, C., NAVARRO, C.A., JEREZ, C.A., Microbial Biotechnology, 10(2), 2017, 279-295. doi:10.1111/1751-7915.12450.
26.IMRON, M.F., KURNIAWAN, S.B., ABDULLAH, S.R.S., Resistance of bacteria isolated from leachate to heavy metals and the removal of Hg by Pseudomonas aeruginosa strain FZ-2 at different salinity levels in a batch biosorption system. Sustain. Environ. Res. 31, 2021, 14. doi: 10.1186/s42834-021-00088-6.
27.KHAN, A.M., BAKAR, N.K.A., BAKAR. A.F.A., ASHRAF M.A., Chemical speciation and bioavailability of rare earth elements (REEs) in the ecosystem: a review. Environ. Sci. Pollut. Res. Int. 24(29), 2017, 22764-22789. doi: 10.1007/s11356-016-7427-1.
28. VAIDYA. M., MCBAIN A.J., BUTLER, J. A., BANKS, C.F., WHITEHEAD, K.A., Antimicrobial Efficacy and Synergy of Metal Ions against Enterococcus faecium, Klebsiella pneumoniae and Acinetobacter baumannii in Planktonic and Biofilm Phenotypes, Sci Rep. 7. 2017, 5911. doi: 10.1038/s41598-017-05976-9.
29. KAHIL, K., WEINER, S., ADDADI. L., GAL. A., Ion Pathways in Biomineralization: Perspectives on Uptake, Transport, and Deposition of Calcium, Carbonate, and Phosphate, J. Am. Chem. Soc. 143 (50), 2021, 21100-21112. doi: 10.1021/jacs.1c09174.
30. SONG, X. GUNAWAN, P., JIANG, R., LEONG, SSJ., WANG, K., XU, R., Surface activated carbon nanospheres for fast adsorption of silver ions from aqueous solutions. J. Hazard Mater., 30(194), 2011,162-168, doi: 10.1016/j.jhazmat.2011.07.076.
31.DOBRA, G., GARCIA-GRANDA, S., NEGREA, A., CIOPEC, M., NEMEȘ, N., NEGREA, P., DUTEAN, N., ILIEV, S., COTET, l., BOIANGIU, A., FILIPESCU, L., Silver adsorption on the low temperature activated alumina grades. I. Adsorption capacity and kinetics. *Rev. Chim,* **73**(2), 2022, 16-32
32.VASILE, B.S., DOBRA, G., ILIEV, S., COTET, L., NEACSU, I.A., NICOARA, A.I., SURDU, V.A., BOIANGIU, A., FILIPESCU, L., Thermally Activated $Al(OH)_3$ - part I-Morphology and Porosity Evaluation, Ceramics 4(2), 2021, 265-277. doi: 10.3390/ceramics4020021.
33.VASILE, B.S., ILIEV, S., COTET, L., NEACSU, I.A., NICOARA, A.I., SURDU, V.A., BOIANGIU, A., FILIPESCU, L., Thermally-Activated $Al(OH)_3$ part II - effect of different thermal treatments, Ceramics, 4 (4), 2021, 564-575. doi: 10.3390/ceramics404004022.
34. DOBRA, G., ILIEV, S., COTET, L., BOIANGIU, A., VASILE, B.S., NEACSU, I.A., NICOARA, A.I., SURDU, V.A., FILIPESCU, L., Technical properties and uses of the aluminium hydroxide, dried, milled and classified, J. Sib. Fed. Univ. Eng. Technol. (under press).
35.GORBUNOVA, M., LEMKINA, L., LEBEDEVA, I., KISELKOV, D., CHEKANOVA, L., Synthesis and potential applications of silver-porous aluminium oxide nanocomposites as prospective antiseptics and bactericides. Journal of Materials Science: Materials in Medicine 28(3), 2017, 1-8. doi: 10.1007/s10856-016-5841-z.
36.PASCU, B., NEGREA, A., CIOPEC, M., DAVIDESCU, C., M., NEGREA, P., GHERMAN, V.D., DUTEANU, N., New Generation of Antibacterial Products Based on Colloidal Silver, Materials 13(7), 2020, 1578. doi: 10.3390/ma13071578.
37.HOGEKAMP, L., HOGEKAMP, S.H., STAHL, M.R., Experimental setup and image processing method for automatic enumeration of bacterial colonies on agar plates. PLoS ONE 15(6), 2020, e0232869. doi: 10.1371/journal.pone.0232869.
38.MANYASREEM D., KIRANMAYI, P., RAVI KUMAR, R.V.S.S.N., Synthesis, characterization and antibacterial activity of aluminium oxide nanoparticles, Intern, J, Pharm, Pharm, Sci, 10(1), 32-35. doi: 10.22159/ijpps.2018v10i1.20636.
39.OLUFUNMISO, O., ADEOYE-ISIJOLA, M., BABASOL, B.A., OSOPALE, B.A., COOPOOSAMY, R., In vitro Effects of Magnesium-Aluminium Hydroxide (Maalox) on the Anti-bacterial Activity of Ciprofloxacin against Clinical Bacterial Isolates, Journal of Pure and Applied Micro-biology 12(2), 2018, 623-632. doi:10.22207/JPAM.12.2.20.

40.BERGLIN, E.H., EDLUND, M.B., NYBERG, G.K., CARLSSON, J., Potentiation by L-cysteine of the bactericidal effect of hydrogen peroxide in Escherichia coli. J. Bacteriol, 152(1),1982, 81-88. doi: 10.1128/jb.152.1.81-88.1982.
41.PERNI, S., HAKALA, V., PROKOPOVICH, P., Biogenic synthesis of antimicrobial silver nanoparticles capped with L-cysteine. Colloids and Surfaces A: Physicochemical and Engineering Aspects 460, 2014, 219-224. doi: 10.1016/j.colsurfa.2013.09.034.
42.LIU, Y., YANG, K., JIA, Y., SHI, J., TONG, Z., WANG, Z., Cysteine potentiates bactericidal antibiotics activity against gram-negative bacterial persisters, Infect; Drug Resist. 13, 2020, 2593-2599. doi: 10.2147/IDR.S263225.

Manuscript received: 4.04.2022