# ON THE ANTIBACTERIAL ACTIVITY OF METAL-EXCHANGED ZEOLITES

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## ABSTRACT

The antibacterial activity of metal-exchanged clinoptilolite (M-Z; M - Cu, Zn, Ni, Ag) and zeolite A (M-A) toward Gram- positive (S*taphylococcus aureus*) and Gram-negative (*Acinetobacter baumannii* and *Escherichia coli)* bacteria was investigated in different water media. The cations released from the zeolites are mainly found to be responsible for the antibacterial action. According to the results, the antibacterial action of the examined zeolites decreases in the following order: Ag-Z, Ag-A > Cu-Z, Cu-A > Zn-Z, Zn-A > Ni-Z, Ni-A toward all examined bacterial types. Mechanism of the bactericidal activity of silver ions toward pathogenic multidrug resistant Gram-negative *Acinetobacter baumannii* has been proposed on a molecular level using the X-ray near-edge structure (XANES) spectroscopy. Silver ions bind prevalently the amine (>NH) and hydroxyl (-OH) groups belonging to DNA inside bacterial cells and to the sites in the outer cell membrane of *A. baumannii*. Considering the bactericidal activity of Ag-Z a novel antibacterial polymer composite, based on Ag-Z and poly(vinyl chloride) was prepared.

Keywords: zeolites, metal, antibacterial activity, multidrug resistant, mechanism of antibacterial activity.

## INTRODUCTION

In the last decade the resistance of the pathogenic bacterial strains to commonly used antibiotic presents the worldwide problem. Among a broad range of pathogens, the infections are mostly caused by *A. baumannii*, *E. coli* and S. *aureus*. These bacteria cause different diseases of urinary, respiratory and blood tract, especially in hospitals and intensive care units. Zeolites (natural and synthetic ones) enriched with the heavy metal ions have been considered as promising alternative disinfectants enabling slowly realise of the heavy metal ions with antimicrobial effect. The aim of this study was to investigate the antibacterial activity of two types of zeolites enriched with transition metal ions (Cu, Zn, Ni, Ag) and their potential application in materials for medical use. Both zeolites are available and low cost.

## EXPERIMENTAL

### *Preparation of zeolite samples*

Zeolite from Serbia (Vranjska Banja deposit, Z) and zeolite A (Ventron, Italy, A) were used in the experiments. The transition metal-exchanged zeolites were prepared by a simple ion-exchange procedure using 6mM solution of $MSO_4$ (M - Cu, Zn or Ni) or $AgNO_3$ and a solid/liquid weght ratio of 1:100. All M-A and M-Z samples contained 0.25 mmol M/g. Before antimicrobial test the samples were sterilized by autoclaving (121 ºC, 20 min). Ag-containing samples were sterilized in a dry sterilizer for 24 h at 60 ºC. No microbial contamination was observed during the experiments.

*Bacterial stains*

Gram-negative *E. coli* (DSM 498), isolates of *E. coli* from the waterborne in Serbia, *A. baumannii* (EU clone I and II; ST145 and RUH 134) and Gram-positive *S. aureus* (DSM 799) were used in the experiments.

*Preparation of Ag-Z/poly(vinyl chloride) composite*

A series of composites containing 1-15 wt.% of Ag-Z was prepared by the previously published procedure[1]. The samples were denoted as Ag-ZN where the N shows the weight percentage of Ag-Z in the composite (N=1-15).

*Antibacterial tests*

Approx. 1.0 g of M-Z or M-A was suspended into the 100 $cm^3$ of different water media: real effluent water from a secondary stage of the biological wastewater treatment in Zagreb, from Sava lake (Belgrade), Luria Bertani (LB) medium, synthetic and commercial water Jana. The antibacterial activity toward all *E. coli* and *S. aureus* was examined in all of these suspensions. Test with *A. baumannii* was performed in a sterile 0.85 % NaCl solution. The antibacterial activity of the prepared composites toward *A. baumannii* ST145 was studied by suspending about 0.05 g of each composite into the 100 $cm^3$ of phosphate buffer (PBS). The experiments were performed at 36±1 ºC with mixing suspensions at 150 rpm. The antibacterial activity was determined after 1 and 24 h and the number of bacteria was determined as colony forming units (CFU) per $cm^{-2}$ or $cm^{-3}$. All experiments were done in triplicate. The number of CFU was logarithmically transformed and the antibacterial activity was given as percentage reduction of log CFU in comparing with corresponding control.

## RESULTS AND DISCUSSION

Cu-Z and Zn-Z show good antibacterial activity in water media toward all examined strains of *E. coli* and toward *S. aureus* DSM 799 (Tables 1 and 2). Both zeolite samples reduced growth of *E. coli* DSM 498 for more than 90% and *S. aureus* for more than 80% after 24 h in real and synthetic water. Ag-Z and Ag-A have bactericidal effect toward the isolates of *E. coli* after 30 minutes in studied media (not shown) whereas Cu-Z, Zn-Z and Cu-A were bactericidal after 24 h (Table 2).

The antibacterial activity of Ni-Z toward bacteria *E. coli* DSM 498 and *S. aureus* DSM 799 is negligible. *S. aureus* exhibits a higher resistance than *E. coli* DSM 498 toward all investigated zeolites in the real and synthetic water (Table 1) which could be attributed to different chemistry of the cell wall of Gram-positive and Gram-negative bacteria. Moreover, all examined M-Z are slightly active in LB medium which is attributed to the fact that LB medium is enriched with nutrients (the results are not shown).

Table 1. Reduction (%) in the numbers of *E. coli* DSM 498 and *S. aureus* DSM 799 after 1 and 24 h in different water media containing M-Z (10 g $dm^{-3}$). Initial bacterial number, CFU $cm^{-3}$: $t_o$ (*E. coli*)= $1.1x10^7$, $t_o$ (*S. aureus*) = $9.9x10^6$.

| Bacteria | Medium | Cu-Z | | Zn-Z | | Ni-Z | |
|---|---|---|---|---|---|---|---|
| | | Reduction (%) for different time (h) | | | | | |
| | | 1 | 24 | 1 | 24 | 1 | 24 |
| *E. coli* | Real effluent | 40.1 | 93.5 | 8.09 | 95.1 | 1.74 | 18.5 |
| DSM 498 | Synthetic water | 19.4 | 94.9 | 6.27 | 93.9 | 1.38 | 19.3 |
| *S. aureus* | Real effluent | 55.7 | 86.8 | 2.79 | 82.1 | 1.18 | 9.74 |
| DSM 799 | Synthetic water | 42.8 | 87.3 | 3.31 | 82.3 | 1.28 | 10.13 |

Table 2. Reduction (%) in the numbers of *E. coli* isolates I and II after 1 and 24 h in different water media containing zeolite samples (1 g $dm^{-3}$). Initial bacterial number, CFU $cm^{-3}$: $t_o$(*Isolate I*) =$2.0x10^7$, $t_o$ (*Isolate II*) = $2.2x10^7$.

| *E. coli* | Medium | Cu-Z | | Zn-Z | | Cu-A | | Zn-A | |
|---|---|---|---|---|---|---|---|---|---|
| | | Reduction (%) for different time (h) | | | | | | | |
| | | 1 | 24 | 1 | 24 | 1 | 24 | 1 | 24 |
| Isolate | Real effluent | 73.6 | 100 | 40.3 | 100 | 60.7 | 100 | 1.37 | 1.68 |
| I | Commercial water | 84.5 | 100 | 82.1 | 100 | 83.6 | 100 | -0.31 | 26.4 |
| Isolate | Real effluent | 60.2 | 100 | 10.0 | 100 | 51.1 | 100 | 3.09 | 6.68 |
| II | Commercial water | 64.0 | 100 | 26.7 | 100 | 62.0 | 100 | 1.95 | 14.0 |

Table 3. Reduction (%) of *A. baumannii* EU clone I and II after 1 and 24 h of contact with M-Z (1 g $dm^{-3}$) in comparing with corresponding control and minimal bactericidal concentration (MBC) of the zeolites. Initial bacterial number, CFU $cm^{-3}$: $t_o$ (EU I)=$8.8x10^6$, $t_o$ (EU II)=$1.4x10^7$.

| Bacteria | Sample | Reduction (%) for different time (h) | | MBC (mg $dm^{-3}$) |
|---|---|---|---|---|
| | | 1 | 24 | |
| EU I | Cu-Z | 100 | 100 | 250 |
| | Zn-Z | 13.0 | 28.5 | >1000 |
| | Ni-Z | 5.0 | 9.0 | >1000 |
| | Ag-Z | 100 | 100 | 500 |
| EU II | Cu-Z | 100 | 100 | 125 |
| | Zn-Z | 10.5 | 22.0 | >1000 |
| | Ni-Z | 11.8 | 23.8 | >1000 |
| | Ag-Z | 100 | 100 | 62,5 |

Cu-Z and Ag-Z show bactericidal activity toward multidrug resistant *A. baumannii* EU I and EU II after 1 h of contact. Considering MBC values, EU II is more sensitive than EU I toward Cu-and Ag-Z (Table 3). The activity of Zn-Z and Ni-Z is negligible.

A possible role of the released M cations was examined by measuring of the concentration of M in water media after 24 h in all studied systems. Metal concentration was up to 0.5 mg M $dm^{-3}$ indicating that the released metals could be responsible for the antibacterial activity.

Bactericidal action of silver ions towards *A. baumannii* was studied on molecular level using the XANES analysis. The results indicated that the bactericidal action of silver ions can be predominantly ascribed to the silver bonds to nitrogen from amine (>NH) and oxygen from

hydroxyl (-OH) groups. This groups are present in amino acids or DNA inside the bacterial cells but also to the sites in the outer cell membrane of *A. baumannii*[2].

Due to the bactericidal efficiency of Ag-Z, Ag-Z/PVC composites were also prepared. They are active toward *A. baumannii* ST145 for all examined amounts of Ag-Z in the composites (Figure 1). Coating of the composites with D-Tyrosine enhanced the efficiency of Ag-Z1/PVC which showed the bactericidal action after 24 h of contact. Since the rheological properties of PVC were unaffected by Ag-Z[1], novel composite presents a perspective material for use in medical purposes (catheters, endotracheal tubes, etc.).

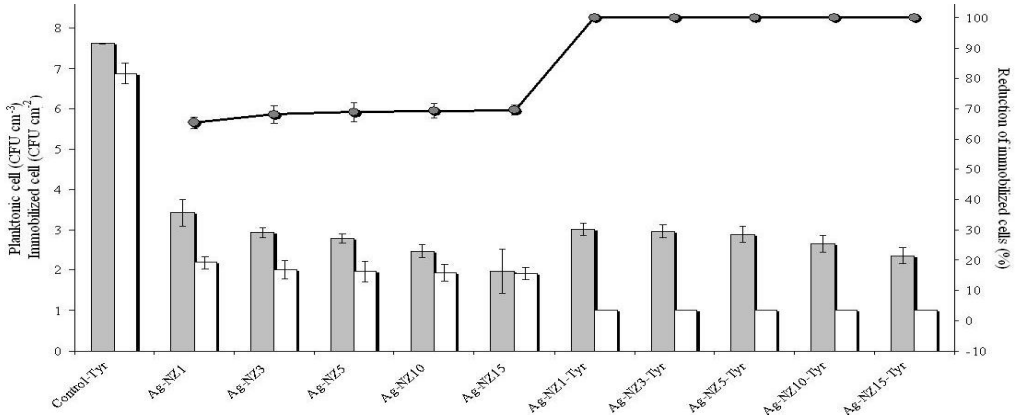


Figure 1. Reduction (%) of *A. baumannii* ST145 after 24 h of contact with composites Ag-Z/PVC (1 g $dm^{-3}$) in comparing with corresponding control. Initial bacterial number, CFU $cm^{-3}$: $t_o$ (*A. baumannii*)=$8.8x10^6$ (▭ Planktonic cell; ▭ Immobilized cell; ⟶ Reduction of immobilized cells).

## CONCLUSION

Natural zeolite - clinoptilolite and zeolite A enriched with Cu, Zn or Ag are perspective as antibacterial agents. The activity of Ag-Z and Ag-A is highest which could be explained by a high antibacterial efficiency of silver ions. Their bactericidal effect towards *A. baumannii* is ascribed to bonding of silver ions to DNA inside the cells and to the sites in outer cell membrane. Ag-Z shows the bactericidal effect towards *A. baumannii* when is added to PVC indicating that the composite could find application as antibacterial material.

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