MODIFIED ZEOLITIZED TUFFS IN CONTROL OF PATHOGENIC BACTERIA

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ABSTRACT

The antibacterial activity of modified natural zeolites (MNZs) containing (in wt%): 2.60 of Cu^{2+} (CuNZ), 0.52 of Ni^{2+} (NiNZ), 1.47 of Zn^{2+} (ZnNZ), 8.50 of benzalkonium ion - BC (BCNZ) or 8.50 of benzalkonium ion and 1.90 of acetylsalicylic acid - AS (BCASNZ) were tested against pathogenic bacteria: *Acinetobacter baumannii* belonging to the EU clone I and II, *Escherichia coli* and *Staphylococcus aureus.* The CuNZ, BCNZ and BCASNZ showed the bactericidal activity (100% of antibacterial activity) against both clones of *A. baumannii.* The BCNZ and BCASNZ showed the bactericidal activity against *E. coli* and *S. aureus.* For NiNZ and ZnNZ a slight antibacterial activity was found. The bactericidal activity of CuNZ, BCNZ and BCASNZ was due to the activity of Cu or BC cations loaded onto NZ and not to NZ itself. Among all tested MNZs, the BCNZ is a promising material for the control of pathogenic bacteria.

Key words: Antibacterial activity; Clinoptilolite; Disinfection; Heavy metals; Surfactant.

INTRODUCTION

Nowadays the emergence of pathogenic bacteria resistant to commonly used antibiotics is a worldwide problem in clinical medicine. Multi- and pandrug resistant bacteria persist in the environment for extended periods of time, representing the transmission route for occurrence of outbreaks in the hospital environment. The survival in environment is attributed to its resistance to antibiotics, disinfectants, drying and the ability to form biofilms on abiotic surfaces [1]. Pathogenic bacteria *A. baumannii*, *E. coli* and *S. aureus* are known causes of hospital-acquired infection. *A. baumannii* causes severe pneumonia, urinary tract and bloodstream infections. Pathogenic strains of *E. coli* cause food poisoning, gastroenteritis, urinary tract infections, and neonatal meningitis. Pathogenic strains of *S. aureus* cause food poisoning, skin infections, and respiratory disease.

Recently, the natural zeolitized tuffs (NZs) have been extensively studied as promising materials for hosting ions with antimicrobial activity [2-5]. The aim of this work was to study the antibacterial activity of the modified natural zeolites (MNZs) against pathogenic bacteria: *A. baumannii*, *E. coli* and *S. aureus*.

EXPERIMENTAL

The NZ containing 70 wt% of clinoptilolite was obtained from the sedimentary deposit Zlatokop, Serbia. The NZ of particle size 0.063-0.1 mm was firstly converted into the Na-rich form (NaNZ) in order to improve the clinoptilolite cation exchange capacity and then used for the preparation of MNZs. The MNZs were prepared by using 1.0 g of the NaNZ and 100 mL of 6 mM aqueous solution of XCl_2 (X=Cu, Ni, Zn) or benzalkonium chloride. The suspensions were shaken (25ºC/24h), separated by filtration and obtained products were dried. The benzalkonium modified zeolite was then treated for 4 h with an aqueous solution of acetylsalicylic acid (1 mg/mL). The MNZs contained (in wt%): 2.60 of Cu^{2+} (CuNZ), 0.52 of Ni^{2+} (NiNZ), 1.47 of Zn^{2+} (ZnNZ), 8.50 of benzalkonium ion (BCNZ) or 8.50 of

benzalkonium ion and 1.90 of acetylsalicylic acid (BCASNZ). The details of their characterization have been previously published [4,6]. The BCNZ and BCASNZ were tested for antibacterial activity without previous sterilization in order to avoid the disintegration of loaded organic cations. The CuNZ, NiNZ, ZnNZ and NZ were sterilized by autoclaving prior to the experiments.

Clinical isolates of Gram-negative bacteria *A. baumannii* were collected during two different outbreaks, first from 2002-2007 (EU clone I) and second from 2009-2010 (EU clone II) in Clinical Hospital Center Split, Croatia. Gram-negative bacteria *E. coli* (strain DSM no. 498) and Gram-positive bacteria *S. aureus* (strain DSM no. 799) were obtained from the Deutsche Sammlung von Microorganismen und Zellkulturen GmbH.

For experiments with *A. baumannii* into the bacterial suspension in the sterile 0.85% NaCl solution a 1 g/L of each MNZ was added. For experiments with *E. coli* and *S. aureus* into the bacterial suspension in the sterile Luria Bertani medium (composition in g/L: tryptone 10, yeast extract 5, NaCl 10) a 10 g/L of each MNZ was added. Serial dilutions of content were made according to the standard method [7]. The control tubes were left without addition of the zeolites. The tubes were shaken during 24 h at 36.0 °C. To confirm the bactericidal action of examined cations which were loaded onto MNZs, the experiments with $CuSO_4$ x $5H_2O$, BC or AS were set up in the same way as described above. To confirm the absence of the antibacterial activity of NZ, the experimental tubes containing the highest tested concentration of the NZ were performed. The number of bacteria was determined after 1 and 24 h of contact on the nutrient agar plates and reported as colony forming units, CFU/mL. The numbers of CFU were logarithmically transformed. The antibacterial activity of the MNZs was expressed as the percent reduction of log CFU as compared to the corresponding control and minimum bactericidal concentration (MBC) was determined after 24 h of contact. In tubes showing MBC the leaching of cations from MNZs was determined by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

The CuNZ, BCNZ and BCASNZ showed the bactericidal activity (100% of antibacterial activity) against both clones of *A. baumannii* (Table 1, 2). *A. baumannii* EU clone II was more sensitive to CuNZ (MBC 125 mg/L) than isolate from EU clone I (MBC 250 mg/L). The EU clone I was more sensitive to BCNZ and BCASNZ (MBC 250 mg/L) than EU clone II (MBC 500 mg/L). For NiNZ and ZnNZ a slight antibacterial activity with no MBC was found.

The BCNZ and BCASNZ showed the bactericidal activity against *E. coli* (Table 3) and *S. aureus* (Table 4). The *S. aureus* was more sensitive to BCNZ and BCASNZ (MBC 1,000 mg/L) than *E. coli* (MBC 5,000 mg/L). For CuNZ, NiNZ and ZnNZ a slight antibacterial activity with no MBC was found.

Table 1. Percent reduction in the numbers of *A. baumannii* EU clone I after 1 or 24h of contact with modified zeolites (1g/L) as compared to the corresponding control and MBC of modified zeolites. Mean values of triplicate measurements are expressed. t_0 *A. baumannii* EU clone I (CFU/mL) = $8.8x10^6$; *significantly different as compared to corresponding control.

Zeolite	Reduction 1h (%)	Reduction 24h (%)	MBC (mg/L)
CuNZ	100.0*	100.0*	250
NiNZ	5.0*	9.0*	>1,000
ZnNZ	13.0*	28.5*	>1,000
BCNZ	100.0*	100.0*	250
BCASNZ	67.9*	100.0*	250

Table 2. Percent reduction in the numbers of *A. baumannii* EU clone II after 1 or 24h of contact with modified zeolites (1g/L) as compared to the corresponding control and MBC of modified zeolites. Mean values of triplicate measurements are expressed. t_0 *A. baumannii* EU clone II (CFU/mL) = $1.4x10^7$; *significantly different as compared to corresponding control.

Zeolite	Reduction 1h (%)	Reduction 24h (%)	MBC (mg/L)
CuNZ	100.0*	100.0*	125
NiNZ	11.8*	23.8*	>1,000
ZnNZ	10.5*	22.0*	>1,000
BCNZ	100.0*	100.0*	500
BCASNZ	94.3*	100.0*	500

Table 3. Percent reduction in the numbers of *E. coli* after 1 or 24h of contact with modified zeolites (10g/L) as compared to the corresponding control and MBC of modified zeolites. Mean values of triplicate measurements are expressed. t_0 *E. coli* (CFU/mL) = $1.1x10^7$; *significantly different as compared to corresponding control.

Zeolite	Reduction 1h (%)	Reduction 24h (%)	MBC (mg/L)
CuNZ	5.5*	5.5*	>10,000
NiNZ	1.4*	3.2*	>10,000
ZnNZ	4.9*	5.1*	>10,000
BCNZ	100.0*	100.0*	5,000
BCASNZ	100.0*	100.0*	5,000

Table 4. Percent reduction in the numbers of *S. aureus* after 1 or 24h of contact with modified zeolites (10 g/L) as compared to the corresponding control and MBC of modified zeolites. Mean values of triplicate measurements are expressed. t_0 *S. aureus* (CFU/mL) = $9.9x10^6$; *significantly different as compared to corresponding control.

Zeolite	Reduction 1h (%)	Reduction 24h (%)	MBC (mg/L)
CuNZ	0.4	10.7*	>10,000
NiNZ	0.3	4.4*	>10,000
ZnNZ	1.6	12.9*	>10,000
BCNZ	100.0*	100.0*	1,000
BCASNZ	100.0*	100.0*	1,000

The bactericidal activity of CuNZ, BCNZ and BCASNZ was due to the activity of Cu or BC cations loaded onto NZ (Table 5) and not to the NZ itself. The AS showed a slight antibacterial activity against the tested bacteria. The change of pH was not the reason for the bactericidal activity of MNZ found in aerobic conditions, since the difference of control and experimental tubes was not higher than 0.8 pH units. By one 24 h exposure a relatively low leaching of Cu (4.1-7.9 wt% of the cation loaded onto NZ) was observed at MBC. The leaching of BC at MBC was about the half (48.4-52.9 wt%) of the BC loaded onto NZ. However, the time needed to obtain MBC with BCNZ was 1 h. These suggest the possibility of reuse of MNZs for the reduction of pathogenic bacteria in water media.

Table 5. Bactericidal activity of Cu, BC and AS (at concentration loaded onto NZ showing the MBC) and no antibacterial activity of NZ (at the highest tested concentration) after 24 h of contact as compared to the corresponding control.

Reduction (%)	*A. baumannii* EU I	*A. baumannii* EU II	*E. coli*	*S. aureus*
Cu	100	100	6.2	12.3
BC	100	100	100	100
AS	13.4	15.0	0.3	0.5
NZ	-1.3	-1.9	-1.1	-1.8

CONCLUSION

The MNZs showed differences in antibacterial activity on the level of species and subspecies of bacteria. The CuNZ was efficient in reduction of *A. baumannii*, while not for *E. coli* and *S. aureus*. The BCNZ and BCASNZ were efficient in reduction of all three tested bacterial species. NiNZ and ZnNZ acted slightly antibacterial. The BCNZ is a promising material for the control of pathogenic bacteria. The obtained results open a possibility for further clinical investigations and application of the MNZ as coatings or filters for use in hospitals.

REFERENCES

[1] I. Roca, P. Espinal, X. Vila-Farres, and J. Vila, *Front Microbiol*., 2012, **3**, 148.

[2] J. Hrenovic, J. Milenkovic, T. Ivankovic, and N. Rajic, *J Hazard. Mater.*, 2012, **201-202**, 260-264.

[3] J. Hrenovic, M. Rozic, L. Sekovanic, and A. Anic-Vucinic, *J. Hazard. Mat.*, 2008, **156,** 576-582.

[4] S. Jevtic, S. Grujic, J. Hrenovic, and N. Rajic, *Microporous & Mesoporous Mater*., 2012, **159**, 30-35.

[5] J. Hrenovic, J. Milenkovic, I. Goic-Barisic, and N. Rajic, *Microporous & Mesoporous Mater*., 2013, **169**, 148-152.

[6] D. Stojakovic, J. Milenkovic, N. Daneu, and N. Rajic, *Clay Clay Min.*, 2011, **59**, 277-285.

[7] Clinical and Laboratory Standards Institute, *Performance standards for antimicrobial susceptibility testing*, 17th informational supplement, M100-S17. CLSI, Wayne, PA, 2007.