Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2019.24712

170 (2019) 75–79
December

Antibacterial activity of metal-containing clinoptilolite in natural seawater

Jelena Dikić[a], Jasna Hrenović[b], Goran Durn[c], Ana Kovačić[d], Nevenka Rajić[e,*]

[a]*Innovation Centre of the Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia, Tel. +381 11 3303804; email: jmilenkovic@tmf.bg.ac.rs*
[b]*Faculty of Science, University of Zagreb, Zagreb, Croatia, Tel. +38516189700; email: jasna.hrenovic@biol.pmf.hr*
[c]*Faculty of Mining, Geology and Petroleum Engineering, University of Zagreb, Zagreb, Croatia, Tel. +38515535793; email: gdurn@rgn.hr*
[d]*Institute of Public Health of Split and Dalmatia County, Split, Croatia, Tel. +38521401109; email: ana.kovacic19@gmail.com*
[e]*Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia, Tel. +381113303623; Fax: +381113370387; email: nena@tmf.bg.ac.rs*

Received 5 April 2019; Accepted 14 July 2019

ABSTRACT

Antibacterial activity of a natural zeolite, clinoptilolite (Z), enriched with about 0.4–0.5 mmol of Ag (Ag–Z), Cu (Cu–Z) or Zn (Zn–Z) was tested toward two strains of Gram-negative bacteria – *Escherichia coli* and *Acinetobacter baumannii* – in natural seawater from Adriatic Sea (Croatia). The Ag–Z and Cu–Z exhibited bactericidal activity toward *E. coli* and *A. baumannii* after 3 and 6 h of exposure, respectively. The Zn–Z showed antibacterial activity toward both strains, but lacking the bactericidal effect even after 24 h of exposure. All studied samples released metal cations in seawater. Concentration of Ag and Zn leached from Ag–Z and Zn–Z was higher than maximum allowable concentrations (MAC) for drinking water, whereas concentration of Cu was below MAC. Due to high concentration of Na^+ in seawater (about 1.2 g Na^+ dm^{-3}) an ion-exchange process can be responsible for the leaching of the studied cations. The lowest extent of leaching from Cu–Z was ascribed to different position and geometry of sites occupied by Cu(II) in the clinoptilolite lattice. The antibacterial activity of M–Z was attributed to the metal cations leached from the zeolite lattice into seawater. The obtained results indicate that Cu–Z can be considered as a promising disinfectant for treatment of seawater.

Keywords: *Escherichia coli*; *Acinetobacter baumannii*; Natural zeolite; Silver; Copper; Zinc; Bactericidal activity

1. Introduction

Gram-negative bacteria *Escherichia coli* and *Acinetobacter baumannii* are the leading human pathogens of XXI century causing the hospital-acquired infections [1] because of their resistance to the different types of antibiotics. These bacteria are also found in natural environment influenced by human waste [2] causing public health concern.

E. coli is a well known coliform bacterium, and its presence in water is traditionally used as an indicator of faecal contamination. For emergent human pathogen *A. baumannii* has been found to survive in natural environment for prolonged time. It was found in treated urban wastewaters [3] as well as in seawater where it survived for 50 d [4].

Chlorination is the most widespread disinfection method for treatment of seawater due its low price. The main disadvantage of this method is the formation of harmful chloro-organic byproducts [5]. Namely, natural organic matters (NOM) are generally present in water body and increase of NOM in the last decade causes many problems in water treatment processes [6]. Ozone is more effective, however its solubility in water is significantly lower than that of chlorine

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* Corresponding author.

and also this disinfectant should be used in higher concentrations that makes the situation difficult for maintaining. Moreover, ozone is also unsuitable for seawater treatment since its effectiveness alters in the presence of bromide ions [7]. Other methods based on advanced oxidation processes such as microwave irradiation, UV radiation or Fenton oxidation are effective but their application is usually associated with high operational costs [7]. In this regard, use of safe and environmental friendly antimicrobial agents has been considered a promising method for disinfection treatment not only of freshwater but also of seawater.

Due to their abundance and low cost, natural zeolites have attracted a great attention in drinking water and wastewater treatments. The significant characteristic of zeolites is their ability to exchange cations present in the lattice voids with external medium as well as a large surface area. Recently, it has been found that they exhibit significant antibacterial effect toward different pathogenic strains of bacteria [8–10]. Several transition metal-containing zeolites possess bactericidal activity toward a broad range of Gram-negative and Gram-positive bacteria. The strongest activity has been reported for Ag-containing zeolites [11,12], but Cu-containing zeolite showed also the bactericidal effect [10].

Wastewater disinfection processes have been mainly focused to the disinfection of freshwaters. Generally, literature data on this issue are very scarce [13]. Accordingly, the main objective of this study was to examine applicability of zeolite-based disinfectants in disinfection of real sea water. In our previous work, we have found that Zn–, Ni–, Cu– and Ag-containing zeolites exhibit antibacterial or bactericidal performance toward different Gram-positive and Gram-negative bacteria in freshwaters [8–10] and in this study we investigated their antibacterial performance in seawater.

2. Experimental setup

2.1. Preparation of zeolite samples

The zeolitic tuff used in this work was obtained from the sedimentary deposit Zlatokop, Serbia. The composition of the tuff was determined by the Rietveld method using the Topas Academic v.4 software [14]. The mineral composition of tuff is as follows (wt.%) 77.8 – clinoptilolite, 12.5 – feldspar (anorthite) and 9.7 – quartz. The cation exchange capacity was determined by a standard method [15] and it was 227 mmol M^+/100 g. The particle size used in this work was within the range 63–125 μm, which was shown to be optimum by the previous investigations [8–10].

Prior to metal loading, the zeolite was converted into the Na-rich form by an ion-exchange reaction using a water solution of $NaNO_3$ (2.0 mol dm^{-3}) and a solid/liquid mass ratio 1:100. The suspension was left under magnetic stirring at 70°C for 48 h. Then, the solid was recovered by filtration and the product (Na–Z) was dried at 105°C in an oven, overnight to a constant mass.

M–Z (M=Ag, Cu or Zn) samples were prepared by mixing the Na–Z and the corresponding metal solutions in a solid/liquid mass ratio of 1:100, with shaking in a water bath at 25°C. The initial concentration of the solutions was 400 mg Ag or Cu dm^{-3} and 600 mg Zn dm^{-3}. After 24 h, the suspensions were separated by filtration and the products were washed and dried at the 60°C overnight to a constant mass. The M content in M–Z samples was determined by AAS (Varian, Spectra 55B) and it was 0.38, 0.38 and 0.48 mmol of M per g of Z for Ag, Cu and Zn, respectively.

2.2. Antibacterial activity test

The antibacterial activity of Ag, Cu and Zn–Z was tested against two Gram-negative bacteria: *Escherichia coli* strain DSM no. 498 obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, and *Acinetobacter baumannii* strain ATCC no. 19606 obtained from the American Type Culture Collection. The antibacterial activity was investigated in real seawater from Adriatic Sea (Split, Croatia). The surface sample of seawater was collected at the ferry port (43°30′12.8″N 16°26′32.7″E) on August 26th 2018. The sample was collected in Schott bottle and sterilized by autoclaving. The chemical analysis of seawater and commercial spring water was performed by DIONEX ICS-90 ion chromatography system (ICS-90) (Thermo Fisher Scientific, Sunnyvale, CA).

First, bacteria were pre-grown on the nutrient agar for 16 h, at 37°C in order to obtain the bacteria in log-phase of growth. The bacterial biomass was then suspended into the sterile 0.085% NaCl solutions. A 1.0 cm^3 of the suspended biomass of *E. coli* or *A. baumannii* was inoculated into the Schott's bottles containing 100 cm^3 of seawater. The initial number of bacteria was around 10^6–10^7 CFU (CFU-colony forming units). In each bottle, a 0.1 g of M–Z or Na–Z was added. The control bottles were left without the zeolite. The seawater and the zeolites before the testing were autoclaved (121°C/20 min). All bottles were sealed up and incubated for 24 h in water bath at 37°C with shaking at 105 rpm.

The number of viable cells was determined after 1, 3, 6 and 24 h. A 1.0 cm^3 of suspension from bottles was serially diluted (10^{-1}–10^{-8}) in sterile 0.085% solution of NaCl and 0.1 cm^3 was inoculated into the nutrient agar. The plates were incubated for 24 h at 37°C and after that the colonies were counted and the number of bacteria was reported as CFU cm^{-3}.

2.3. Statistical analyses

The absolute numbers of bacteria were logarithmically transformed. Percentage of reduction of viable bacteria was calculated as (log CFU cm^{-3} zeolite/log CFU cm^{-3} control) × 100; where log CFU cm^{-3} zeolite is bacterial concentration at designated time in experiment with addition of zeolite, and CFU cm^{-3} control is bacterial concentration at designated time in corresponding positive control (without addition of zeolite).

Statistical analysis was carried out using Statistica 13.3 (TIBCO Software Inc., Palo Alto, California, USA). For pairwise comparisons, the ordinary *t*-test for independent samples was used with the applied significance level of $p < 0.05$.

The percentages of reduction of viable *E. coli* or *A. baumannii* in spring and seawater after designated time of contact was compared (designated as in Table 1). Also, the percentages of reduction of viable *E. coli* in spring or seawater after designated time of contact were compared to the reduction of *A. baumannii* (designated as b in Table 1).

Table 1
Percentage of reduction of *E. coli* and *A. baumannii* after different times of exposure to M–Z in spring and seawater, as compared with positive control (without zeolite)

Zeolite sample	*E. coli*							
	Spring water				Seawater			
	1 h	3 h	6 h	24 h	1 h	3 h	6 h	24 h
Na–Z	1 ± 2	0 ± 0	2 ± 1	0 ± 2	–3 ± 2	5 ± 4	0 ± 0^{a}	-9 ± 1^{a}
Ag–Z	100 ± 0	100 ± 0	100 ± 0	100 ± 0	14 ± 1^{a}	100 ± 0	100 ± 0	100 ± 0
Cu–Z	67 ± 1	100 ± 0	100 ± 0	100 ± 0	22 ± 0^{a}	100 ± 0	100 ± 0	100 ± 0
Zn–Z	11 ± 0	50 ± 0	55 ± 1	59 ± 0	2 ± 1^{a}	3 ± 1^{a}	6 ± 0^{a}	45 ± 1^{a}
Zeolite sample	*A. baumannii*							
	Spring water				Seawater			
	1 h	3 h	6 h	24 h	1 h	3 h	6 h	24 h
Na–Z	1 ± 0	1 ± 1	0 ± 0	0 ± 0	-1 ± 1^{a}	–1 ± 4	$3 \pm 1^{a,b}$	$3 \pm 1^{a,b}$
Ag–Z	65 ± 0^{b}	100 ± 0	100 ± 0	100 ± 0	$6 \pm 1^{a,b}$	$84 \pm 3^{a,b}$	100 ± 0	100 ± 0
Cu–Z	27 ± 1^{b}	75 ± 0^{b}	100 ± 0	100 ± 0	$20 \pm 0^{a,b}$	$67 \pm 2^{a,b}$	100 ± 0	100 ± 0
Zn–Z	3 ± 1^{b}	3 ± 1^{b}	14 ± 1^{b}	56 ± 4	2 ± 0	3 ± 3	6 ± 0^{a}	47 ± 1^{a}

[a] Significantly lower activity than in spring water.
[b] Significantly lower activity than against *E. coli*.
Note: Initial number of bacteria in experiments averaged 7.5 ± 0.2 log CFU cm^{-3}. Average values of a triplicate measurement and standard deviation are presented.

3. Results and discussion

All M–Z samples showed the antibacterial activity toward *E. coli* DSM 498 and *a. baumannii* ATCC 19606 in real seawater (Table 1). It is evident that Ag– and Cu–Z showed bactericidal activity toward *E. coli* after only 3 h of contact, and toward *A. baumannii* after 6 h of contact. For Zn–Z, reduction of both strains for about 50% was found after 24 h.

For all M–Z, samples a consistent significantly lower antibacterial activity was observed in seawater as compared with spring water, with significantly lower activity against *A. baumannii* as compared with *E. coli*. Na–Z does not exhibit activity neither to the *E. coli* nor to *A. baumannii* in both water media. A slight variation of bacterial numbers observed for both media can be explained by a slight multiplication of bacteria due to a low concentration of nutrients.

For all studied M–Z, significantly lower antibacterial activity was observed in seawater than in spring water after 1 h of exposure. Also, significantly lower activity against *A. baumannii* is evident in comparison with *E. coli* (Table 1). Bactericidal activity of Ag–Z evident in spring water after 1 and after 3 h toward *E. coli* and *A. baumannii*, respectively, is slightly postponed in seawater (3 and 6 h for *E. coli* and *A. baumannii*, respectively). A lower activity against *A. baumannii* in comparison with *E. coli* is in accord with the fact that bacteria of the genus *Acinetobacter* are preferably immobilized onto natural zeolite in significantly higher numbers than *E. coli* [16].

Cu–Z showed an antibacterial activity toward *E. coli* after 1 h in both media but this strain is more resistant in seawater (reduction of 22% and 67% is evident for sea and spring water, respectively). *A. baumannii* exhibited similar effect (20% and 27% in sea and spring water, respectively). The bactericidal effect in both media was achieved after 3 h for *E. coli* and after 6 h for *A. baumannii*.

The activity of Zn–Z toward both strains in both media after 1 h is negligible. After 24 h the activity for both strains in both media is similar (*A. baumannii*: 47% and 56% in sea and spring water, respectively; *E. coli*: 45% and 59% for sea and spring water, respectively).

In order to get an insight into mechanism of antibacterial activity, leaching experiments were conducted and the concentrations of the leached metals from Z were measured after 24 h of the experiment. The results (Table 2) showed that the leaching occurred for all studied samples but the leached concentrations differ for different metal cations.

The leaching of Ag and Zn is significantly prominent in comparison with Cu and the leached concentration for Zn is higher than for Ag. For both, Ag and Zn, the leached concentrations are higher than maximum allowable concentration (MAC) for drinking water [17,18]. The leaching of Cu is considerably lower being below MAC [19]. The observed effect could be attributed to different crystallographic sites occupied by these cations in the clinoptilolite lattice. Namely, for Cu and Zn, structural analyses showed that the position of Cu in the clinoptilolite lattice differs from that of Zn [20,21]. Cu forms two coordination geometry inside clinoptilolite

Table 2
Concentrations of the leached metals from M–Z in seawater after 24 h

M–Z	Concentration of leached metals (mg dm^{-3})	
	E. coli	*A. baumannii*
Ag–Z	2.57	2.60
Cu–Z	0.28	0.42
Zn–Z	4.92	5.20

Table 3
Elemental analysis of seawater and spring water

Water medium	Concentration (mg dm^{-3})												
	F^-	Cl^-	Br^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	HCO_3^-	Li^+	Na^+	NH_4^+	Mg^{2+}	K^+	Ca^{2+}
Seawater	2.03	19,576.10	73.50	4.30	0.00	2,892.00	171.65	0.230	12,031.73	19.93	1,597.57	361.90	483.07
Spring	0.05	1.52	0.00	0.89	3.07	6.28	354.77	0.003	2.47	0.00	36.23	1.66	64.48

lattice: a disordered octahedral and square planar ones which could influence its realise from the lattice.

Moreover, the leaching of Ag and Zn is more noticeable in seawater than in spring water [10]. This can be explained by a significant higher concentration of Na^+ ions in seawater (12,031 mg dm^{-3}) than in spring water (2.5 mg dm^{-3}) (Table 3). A high concentration of Na^+ in seawater provokes leaching of the metal cations by ion exchange reaction:

$$Na^+(aq) + M\text{–}Z\ (s) \rightarrow M^{2+}/M^+(aq) + Na\text{–}Z(s)$$

The obtained results indicate that that the leaching of all three metals could be responsible for the antibacterial activity. Although the leached concentration of Cu is lowest, it has been reported that millimolar concentrations of Cu have bactericidal effect for *E. coli* and *A. baumannii* strains [22,23].

4. Conclusions

The Ag–, Cu– and Zn-containing clinoptilolite showed antibacterial activity toward Gram-negative bacteria *E. coli* and *A. baumannii* in natural seawater. Bactericidal activity for Ag– and Cu-containing clinoptilolite was found after 3 h toward *E. coli*, and after 6 h of exposure toward *A. baumannii*. High concentration of Na^+ in seawater was suggested to be responsible for leaching of all three cations. The leaching of Cu occurred in the smallest extent, which was explained by its different crystallographic position in the clinoptilolite lattice. Since the concentration of the leached Cu is lower than MAC for drinking water this material could be recommend for use as a disinfectant in treatment of seawater.

Acknowledgments

This work was financially supported by the Croatian Science Foundation (project no. IP-2014-09-5656) and by the Serbian Ministry of Education, Science and Technological Development (project no. 172018).

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