MICROBIOLOGICAL APPLICATIONS OF NATURAL ZEOLITE

Tomislav Ivankovic[1]*, Jasna Hrenovic*[1]*, Nevenka Rajic*[2]*, Jelena Dikic*[3]*, Sabine Rolland du Roscoat*[4]

[1]Faculty of Science, University of Zagreb, Horvatovac 102A 10000 Zagreb, Croatia
[2]Faculty of Ecology and Environmental Protection, University Union Nikola Tesla, Cara Dusana 62-64, 11158 Belgrade, Serbia.
[3]Innovation centre of the Faculty of Technology and Metallurgy, University of Belgrade, 11000 Belgrade, Serbia
[4] Laboratoire 3SR, Universite Grenoble Alpes, Domaine Universitaire, 38000 Grenoble, France

E-mail: tomislav.ivankovic@biol.pmf.hr

ABSTRACT

Natural zeolites (NZ) have found numerous applications in the life science such as medicine, agriculture, and environmental protection, in practical applications, and in basic research. Various ways in which natural zeolites are used in microbiology, where they are desirable due to their non-toxicity and ion exchange capacity, are presented here. Natural zeolites of the clinoptilolite type are excellent bacterial carriers with potential applications in biotechnology. When modified with heavy metals, the zeolites could fill biofilters and be used in wastewater treatment. Since zeolites facilitate biofilm growth, they can be used as an excellent support and model material that enables biofilm formation in porous media and subsequent visualization of biofilm in 3D by using X-ray microtomography.

Key words: natural zeolite, bacteria, biofilm, carriers, silver, X-Ray tomography.

INTRODUCTION

Natural zeolites have found numerous applications in biotechnology and the life science, both in research and in the industry. Various applications are mainly due to zeolite's unique and beneficial properties: porosity, high adsorption capacity, ion exchange capacity, and, essential in life sciences, zeolites are non-toxic to living organisms and environmentally friendly. Some examples of using zeolites in life science [1,2] are environmental protection (water, air, and soil purification), medicine (drug delivery, surface modifications of bone implants), industry (catalysis, adsorbents, concrete additive), or research (molecular sieves, zeolite coatings, selective membranes, carriers for cell cultures).

In this short overview, the focus will be on research applications of natural zeolites that have been done in the past 15 years at the Faculty of Science of the University of Zagreb, Croatia, mainly with partners from the Faculty of Technology and Metallurgy of the University of Belgrade, Serbia, and Laboratoire 3SR at the Universite-Grenoble Alpes in France. The zeolites have been used as bacterial carriers, antibacterial materials, biofilters, and models for imaging biofilms in 3D using X-ray microtomography.

NATURAL ZEOLITES AS BACTERIAL CARRIERS

Natural zeolites (NZ) show all the necessary properties to be considered as biocarriers in various fields of biotechnology; they are non-toxic towards bacteria and other micro- and aquatic or terrestrial organisms, so they can be safely disposed of in the environment. Compared to synthetic carriers, zeolites are economically feasible and, above all, show a good affinity for adsorption of bacterial cells. In contact with NZ particles, bacteria spontaneously form biofilm on the surface (**Figure 1**), attaching firmly and producing the so-called exopolymeric substances (EPS), essentially a protective polymeric coat.

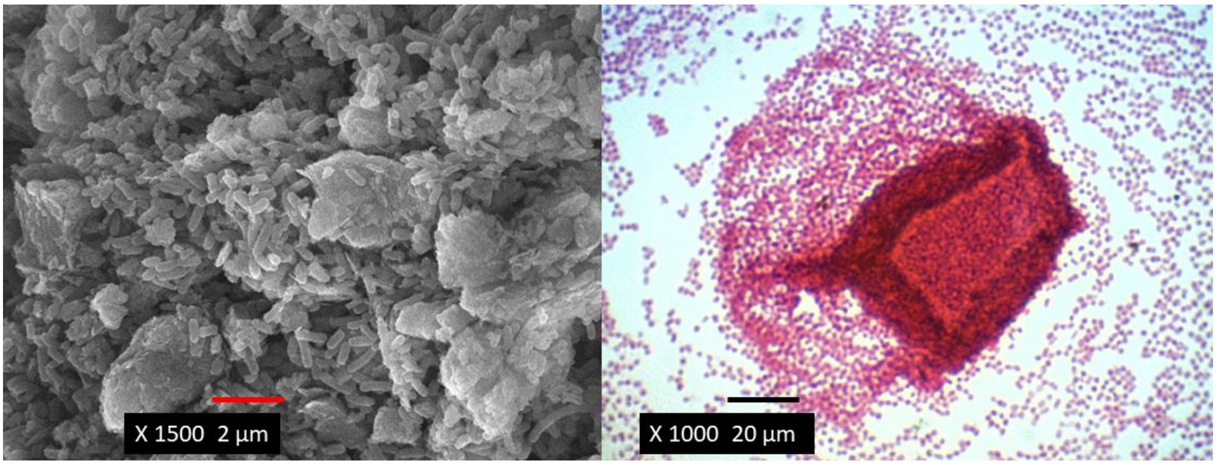

Figure 1: Bacterial cells attached to natural zeolite particles, imaged by scanning electron microscope (left) and light microscope (right).

Multiple experiments showed that NZ containing clinoptilolite shows a high affinity for bacterial attachment with numbers constant at ~10^8 cells per gram of NZ [3]. A significant property affecting the extent of immobilization was the particle size fraction, with the number of attached cells rising with a decrease in particle size. This is expected, as the surface available for immobilization, when considered per mass unit of the carrier, increases when smaller particles are used. In our experiments, the bacterial attachment was not dependent on the zeta potential of NZ particles [3,4,5], but the chemical composition showed significant influence. Namely, when NZ went through ion exchange with Mg^2, bacterial immobilization increased significantly compared to non-exchanged NZ [3,4].

NATURAL ZEOLITES AS ANTIBACTERIAL MATERIALS

Due to its ion-exchange capacity, the NZ has been proposed as an adsorbent of heavy metal pollution from wastewater. As a resulting material, minerals with high concentrations of, i.e., Cu, Ni, or Zn can be obtained. Such materials were tested for antibacterial effect towards standard model bacteria and showed satisfactory antibacterial efficacy, especially the Cu- and Zn-loaded zeolite [6]. It was, therefore, suggested that heavy-metal-loaded zeolites could be used as fillings in biofilters for wastewater disinfection. Indeed, such a biofilter was tested in subsequent experiments by filling the filter with Cu- and Ag-exchanged NZ of clinoptilolite type [7]. The silver has been shown to exhibit supreme antibacterial activity when loaded onto NZ compared to other heavy metals, both towards environmental and clinical bacterial isolates [8,9].

In the experiment described in Ivankovic et al. [7], the small laboratory-sized biofilter was fed with real effluent wastewater for 96 hours, during which time the Ag-exchanged NZ removed 80 – 100 % of various clinically significant bacteria from the wastewater. The activity of modified NZ was not only due to the leaching of Ag ions to the wastewater but also from bactericidal activity of direct contact between bacterial cells and NZ surface loaded with Ag. An exciting feature described in the mentioned research was the use of X-ray microtomography that enabled the 3D reconstruction of the biofilter (**Figure 2**), revealed the creation of preferential pathways after several days of run, and accented the importance of particle size to be used for filling of biofilters.

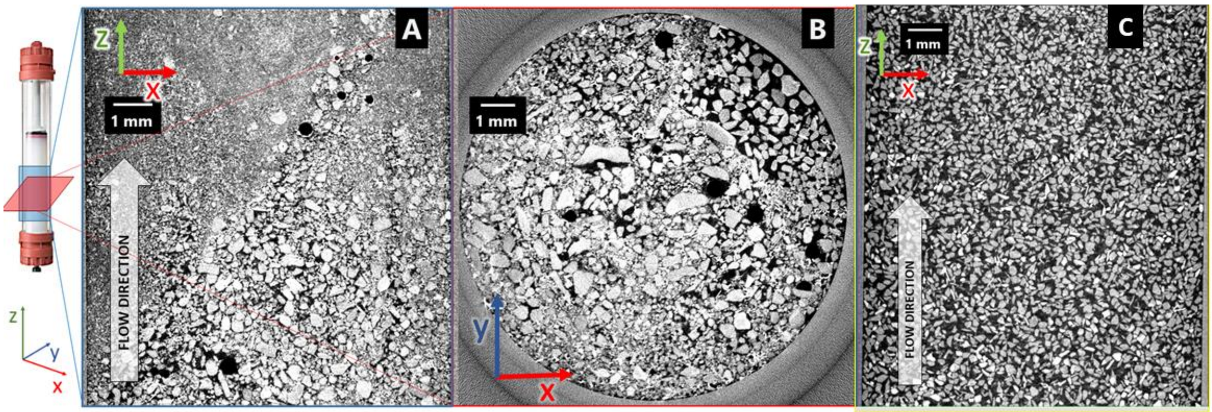

Figure 2: The creation of preferential pathways for wastewater running through the biofilter filled with AgNZ of <0.125 mm particle size for 6 days, viewed from the side (A) and from above (B). The preferential pathways reduced the bactericidal efficacy as the contact with AgNZ was limited, and the antibacterial effect diminished. At this point of the experiment, the column filling was stirred with a sterilized metal rod, after which antibacterial efficacy was regenerated. No preferential pathways were formed when the biofilter was filled with the same material but with particle sizes of 0.5 – 1.0 mm.

NATURAL ZEOLITE AS A MODEL FOR BIOFILM GROWTH AND VISUALIZATION

If used for wastewater purification, the biofilters such as those mentioned above eventually become clogged with the bacterial biofilm, a process known as biofouling. The fouled biofilter is ineffective in purification until regenerated or washed. From a technological point of view, it is highly desirable to understand the process of biofouling and its effect on porous media hydrodynamics, for which a reliable model of biofilm growth in porous media is needed [10]. Since NZ has proven to be an excellent support for bacterial immobilization, it was used as a model porous media for the growth of bacterial biofilm, which was subsequently imaged and visualized in 3D using X-ray microtomography (**Figure 3**). The biofilm volume was directly quantified, and biofilm thickness could have been directly measured with satisfactory reproducibility and accuracy. In the case of natural zeolite, mineral grains should mimic real-life environments as closely as possible, such as those found in aquifer material, soil, sediments, slow sand filters, or percolator filters.

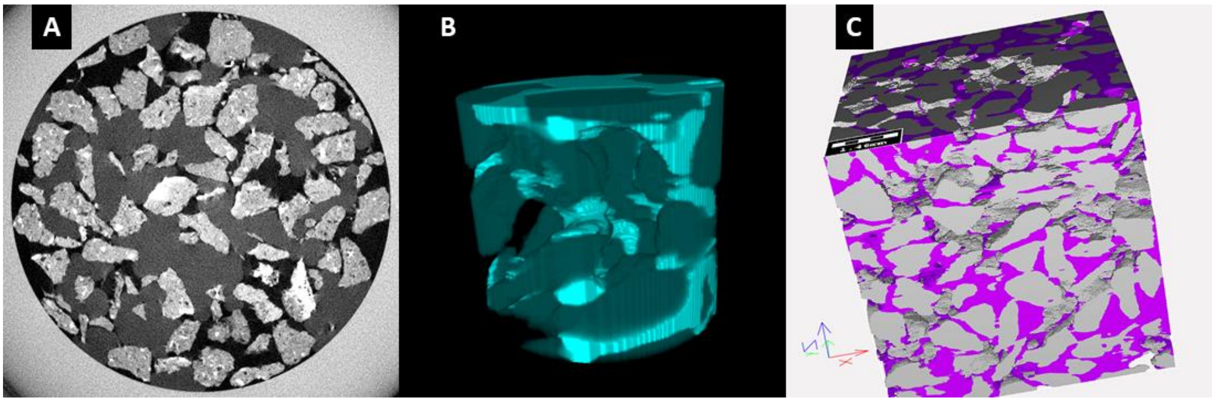

Figure 3: Bacterial biofilm grown on zeolite particles and imaged using X-ray microtomography; in the raw scan (A), the biofilm was black, as opposed to gray grains and the contrast media. The tomography allows for 3D visualization (**B**) and subsequent image segmentation (**C**), where each phase (biofilm, zeolite) is allotted a single color. The Geodict® software can then measure the biofilm volume by measuring the amount of the color, in this case purple.

CONCLUSION

The natural zeolites and bacteria are a good match, especially when zeolite particles are used as bacterial carriers. They not only allow but promote bacterial growth and the formation of a strong and well-established biofilm. Due to easy accessibility and economic feasibility, no doubt the cell carriers made from zeolite will find usage in various branches of biotechnology and as an excellent material for basic biological research. On the other hand, if properly modified, for example with heavy-metals, the NZ can be also an excellent antibacterial material, used for wastewater disinfection.

REFERENCES

[1] L. Bacakova, M. Vandrovcova, I. Kopova and I. Jirka, Applications of zeolites in biotechnology and medicine – a review, *Biomaterials Science*, 2018, 6, 974-989.

[2] S. Montalvo, C. Huilinir, R. Borja, E. Sanchez and C. Hermann, Application of zeolites for biological treatment processes of solid wastes and wastewaters – A review, *Bioresource Technology*, 2020, 301, 122808.

[3] J. Hrenovic, T. Ivankovic and D. Tibljas, The effect of mineral carrier composition on phosphate-accumulating bacteria immobilization, *Journal of Hazardous Materials* 2009, 166, 1377 – 1382.

[4] J. Hrenovic, T. Ivankovic and M. Rozic, Requirement of *Acinetobacter junii* for magnesium, calcium and potassium ions, *Journal of Bioscience and Bioengineering* 2010, 110, 180-186.

[5] J. Hrenovic, D. Kovacevic, T. Ivankovic and D. Tibljas, Selective immobilization of Acinetobacter junii on the natural zeolitized tuff in municipal wastewater, *Colloids and Surfaces B: Biointerfaces* 2011, 88, 208– 214.

[6] J. Hrenovic, J. Milenkovic, T. Ivankovic and N. Rajic, Antibacterial activity of heavy metal-loaded natural zeolite, Journal of Hazardous Materials 2012, 201– 202, 260– 264.

[7] T. Ivankovic, J. Dikic, S. Rolland du Roscoat, S. Dekic, J. Hrenovic, M. Ganjto. Removal of emerging pathogenic bacteria using metale xchanged natural zeolite bead filter. *Water Science & Technology*, 2019, 80, 1085-1098.

[8] J. Milenkovic, J. Hrenovic, D. Matijasevic, M. Niksic and N. Rajic, Bactericidal activity of Cu-, Zn-, and Ag-containing zeolites toward *Escherichia coli* isolates, *Evironmental Science and Pollution Research* DOI 10.1007/s11356-017-9643-8

[9] J. Hrenovic, J. Milenkovic, I. Goic-Barisic, N. Rajic, Antibacterial activity of modified natural clinoptilolite against clinical isolates of *Acinetobacter baumannii*, *Microporous and Mesoporous Materials* 2013, 169, 148-152.

[10] T. Ivankovic, S. Rolland du Roscoat, C. Geindreau, P. Sechet, Z. Huang and J.M.F. Martins, Development and evaluation of an experimental protocol for 3-D visualization and characterization of the structure of bacterial biofilms in porous media using laboratory X-ray tomography, *Biofouling*, 2016, 32, 1235-1244.