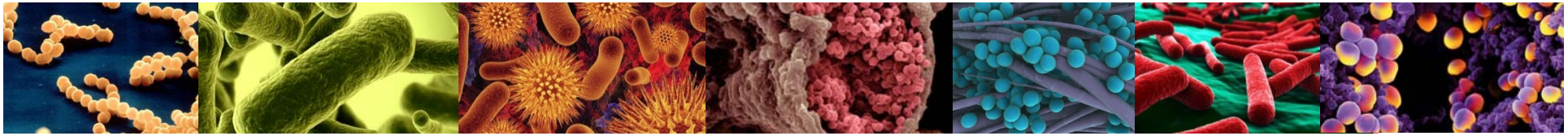
ON THE ANTIBACTERIAL ACTIVITY OF METAL-EXCHANGED ZEOLITES

Jelena Milenković

Innovation Centre of the Faculty of Technology and Metallurgy, University of Belgrade, Serbia
Faculty of Technology and Metallurgy, University of Belgrade, Serbia

7th Slovenian-Serbian-Croatian Symposium on Zeolites, Ljubljana, May 25-27, 2017



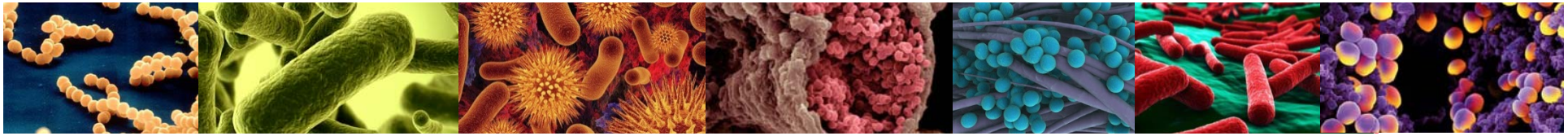
Antibacterial activity

- **Gram-positive**
  - *Staphylococcus aureus* (Deutsche Sammlung von Microorganismen und Zellkulturen GmbH- DSM 799)

- **Gram-negative**
  - *Escherichia coli*

  (DSM 498 and isolates from lake water)
  - *Acinetobacter baumannii*

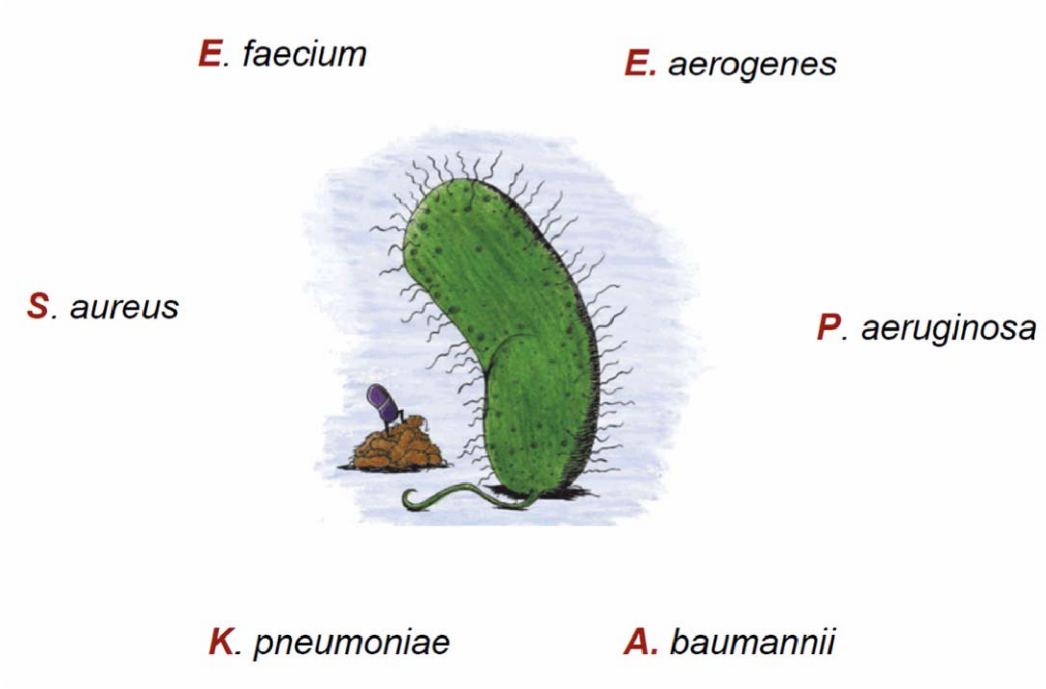
  (clinical isolates belonging to the IC I and II)

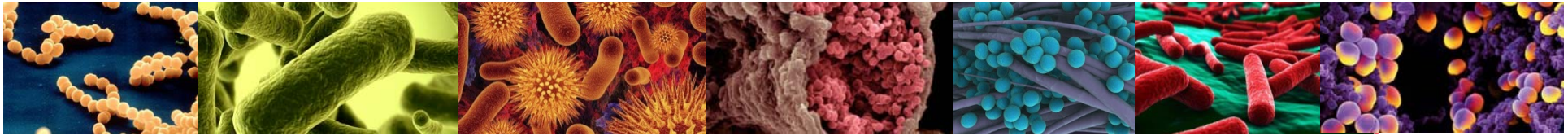


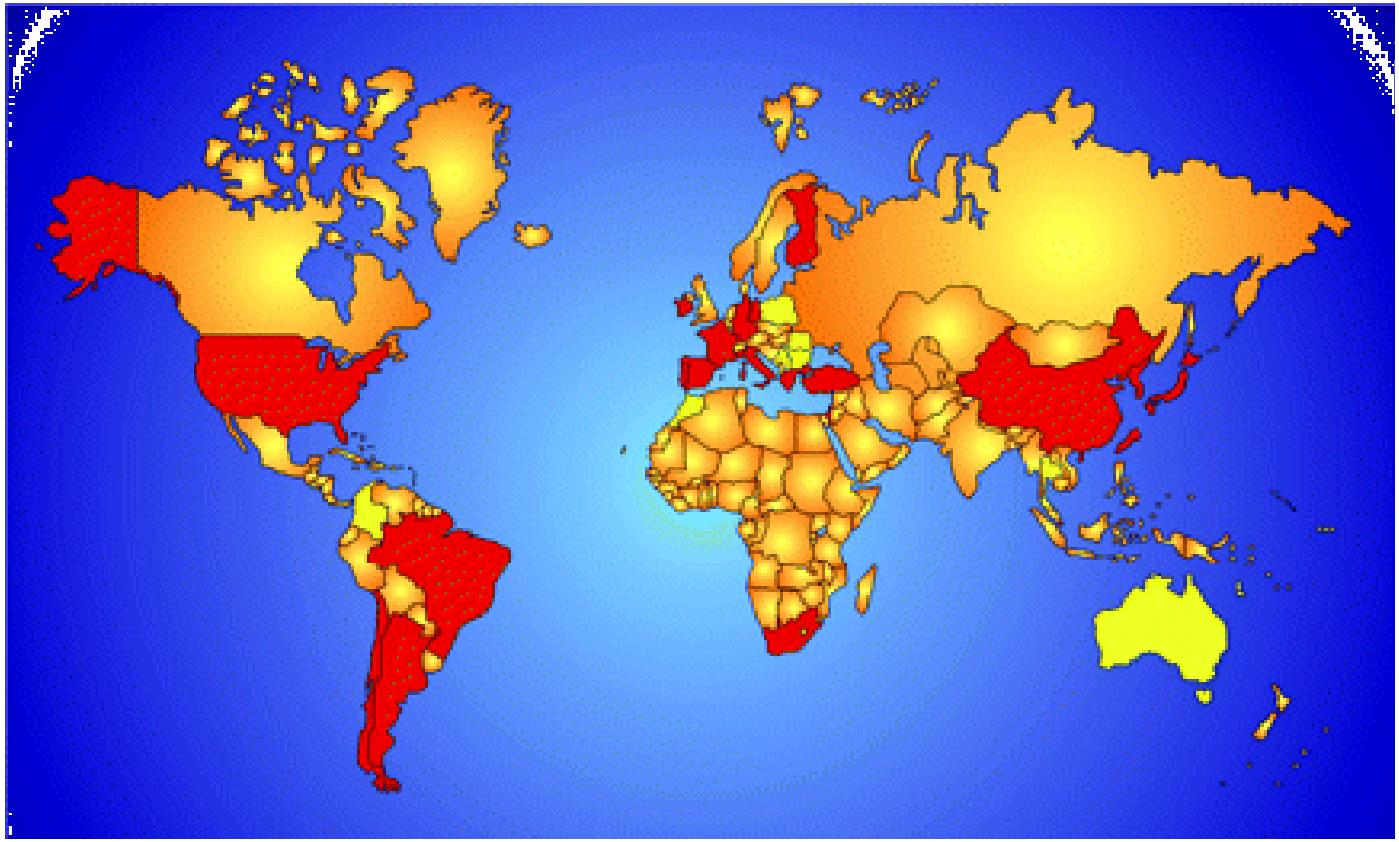
Pathogenic microorganisms

- *E. coli*- indicator of faecal contamination of waters
- *S. aureus* and *A. baumannii*

Infectious Diseases Society of America-IDSA declared „ESKAPE" pathogens:

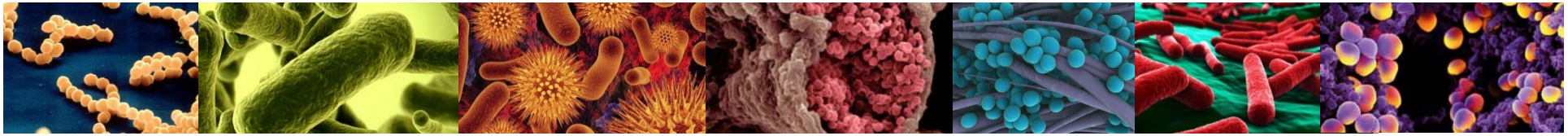




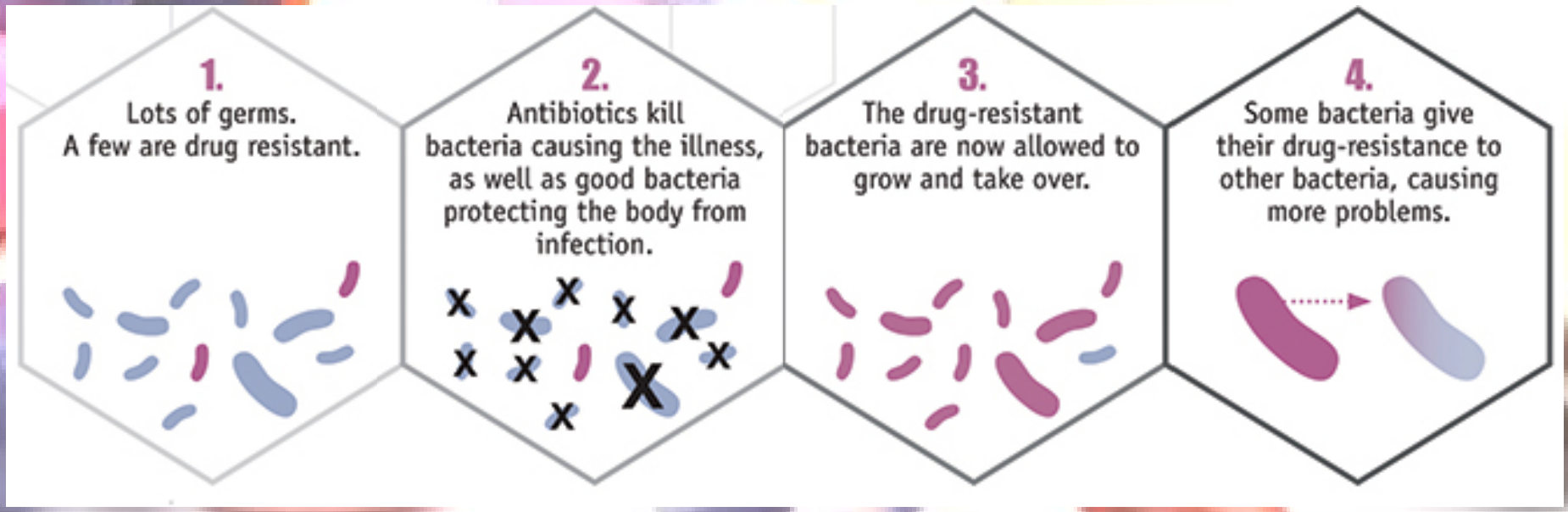


Countries that have reported an outbreak of carbapenem-resistant *Acinetobacter baumannii*. Red - outbreaks before 2006, yellow -outbreaks since 2006.

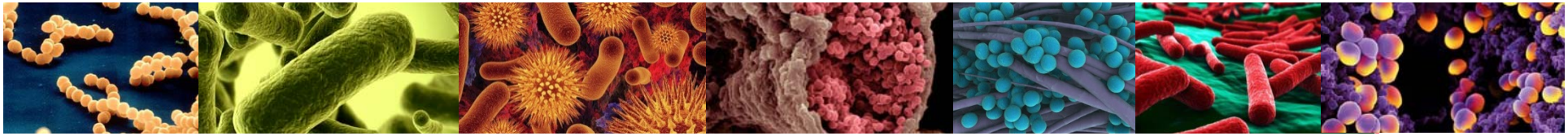
* http://bioquelljp.studiorepublic.com/technology/microbiology/acinetobacter-baumannii/



Multidrug resistance

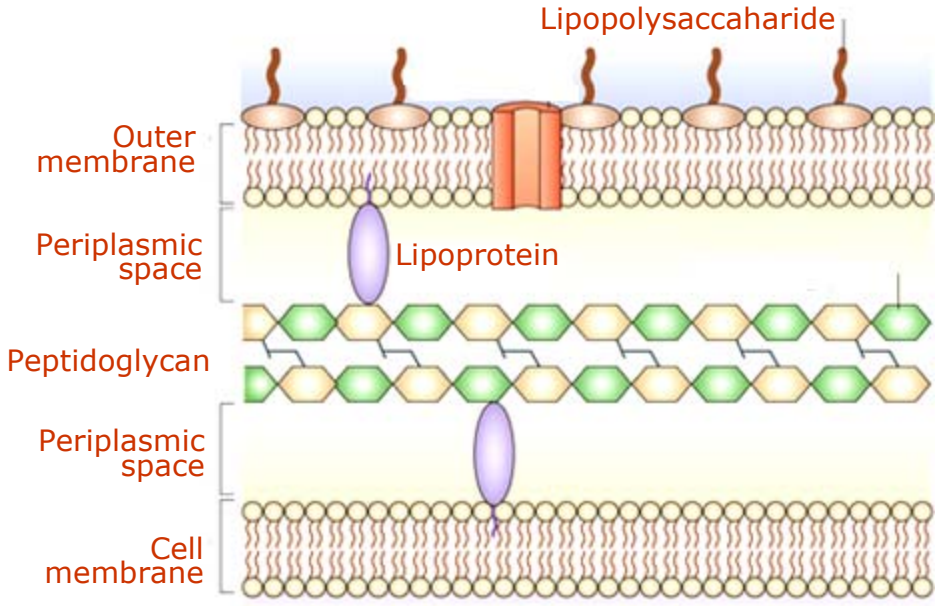


http://www.co-add.org/content/superbug-crisis

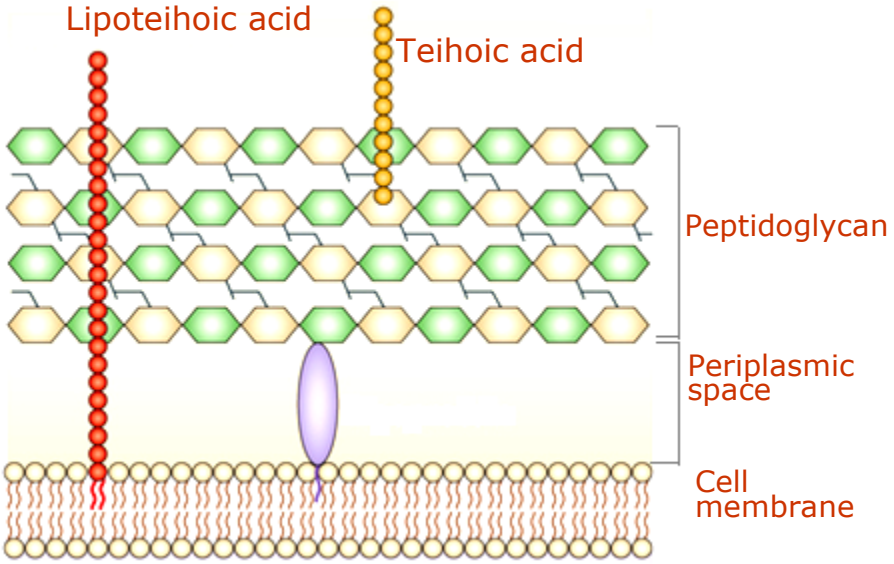


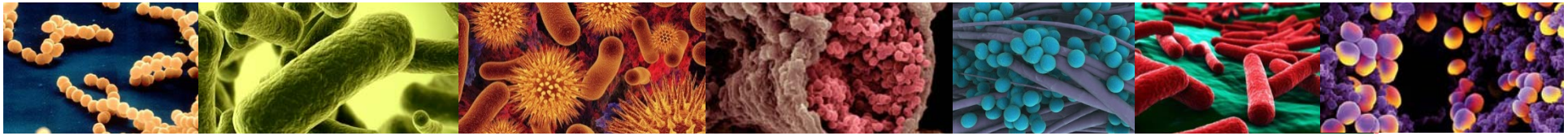
Bacterial cell wall

Gram negative bacteria



Gram positive bacteria





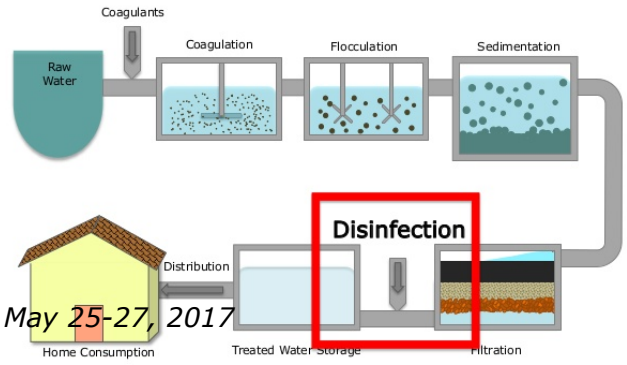
Disinfection of waters

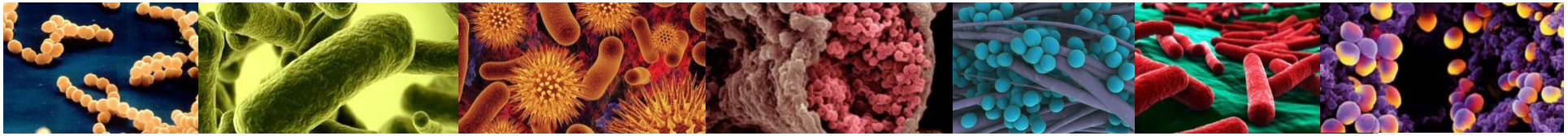
- Physical
  - Heating
  - UV
  - Membrane proceses (ultrafiltration, reverse osmosis)

  } Expensive

- Chemical
  - Chlorination
  - O_3

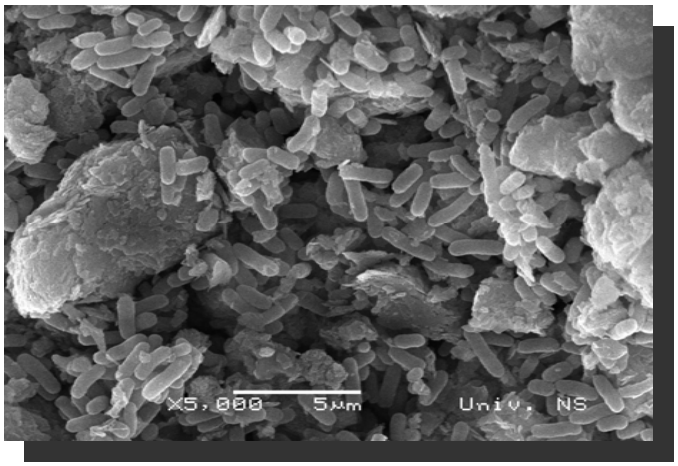
  } Expensive, Formation of cancerogenic by-products

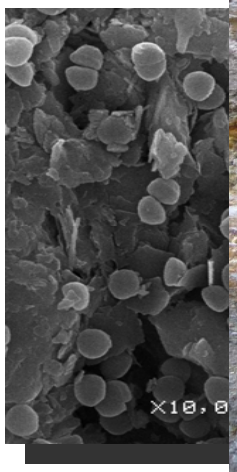




Novel types of disinfectants

- Nano-particles (TiO_2, MgO, Ag, ZnO)
- Carbon nanotubes
- Chitozan
- Clays and clay minerals
- **Zeolites**

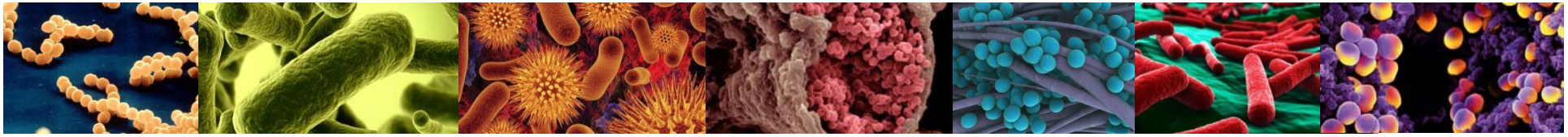




Immobilization of *E. coli* and *S. aureus* on natural zeolite

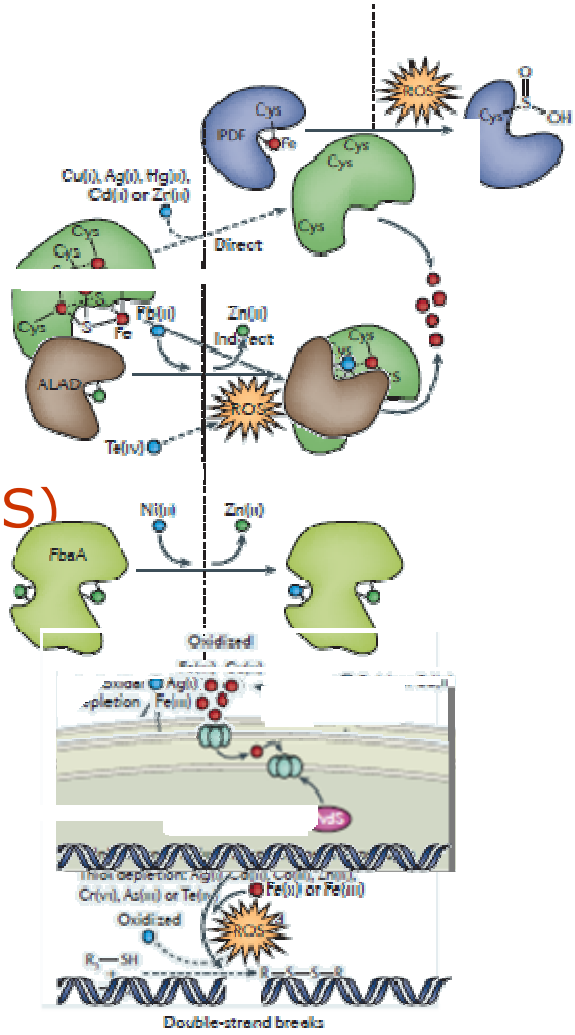
Zlatokop mine, Vranjska banja deposit, Serbia

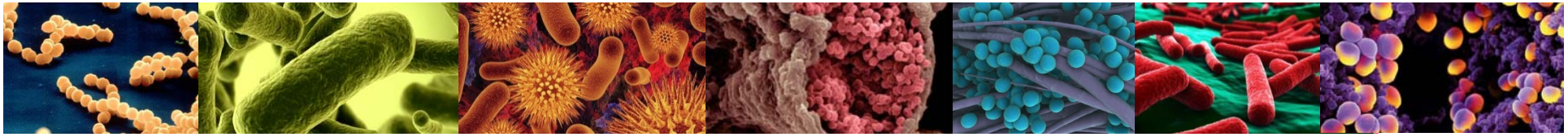
* *Hrenović J, Milenković J, Ivanković T, Rajić N, (2012) J Hazard Mater 201–202:260– 264*



Metals: ancient antibacterial agents

1. Proteine disfunction
   - Oxidation of cysteine in Fe-binding site
   - Destruction of Fe-S cluster
   - Exchange of catalytic metal
   - Exchange of structural metal
2. Production of reactive oxygen species (ROS)
3. Impaired membrane function
4. Interference with nutrient uptake
5. Genotoxicity

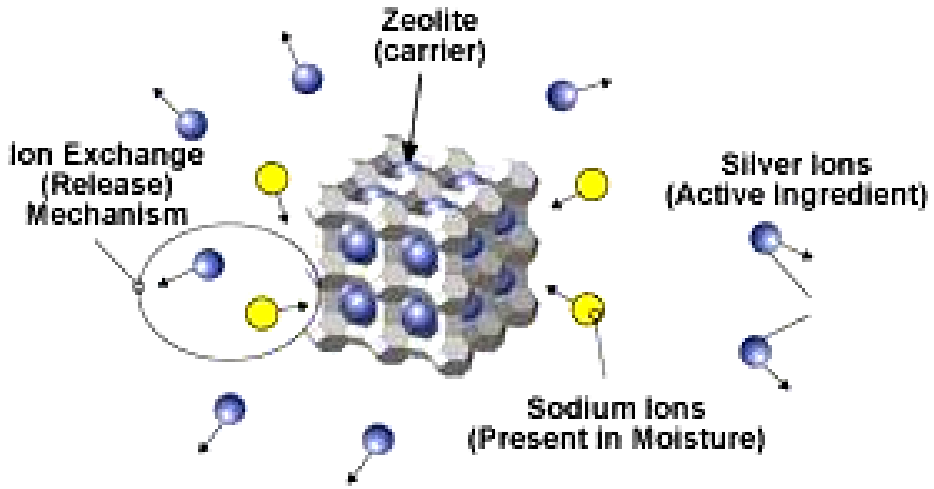


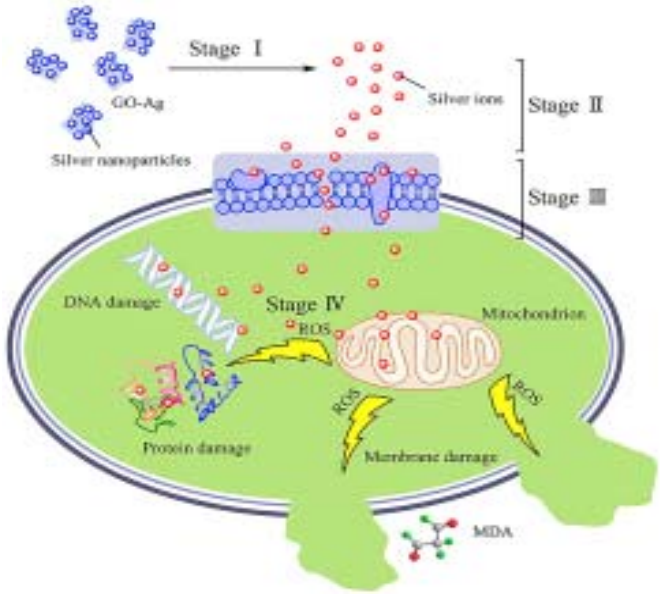


Mechanism of the antibacterial activity of metal-exchanged zeolites

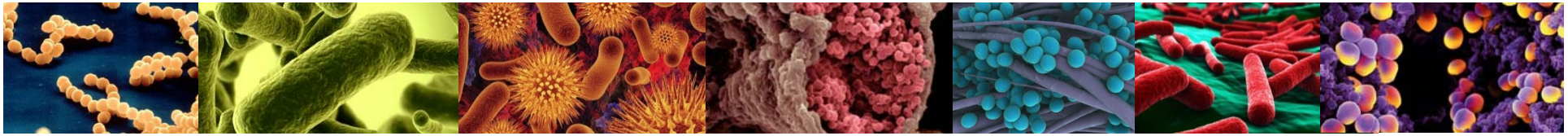
Two assumptions:

- Metal-exchanged zeolites generate the production of ROS;
- Ions leached from the zeolite.



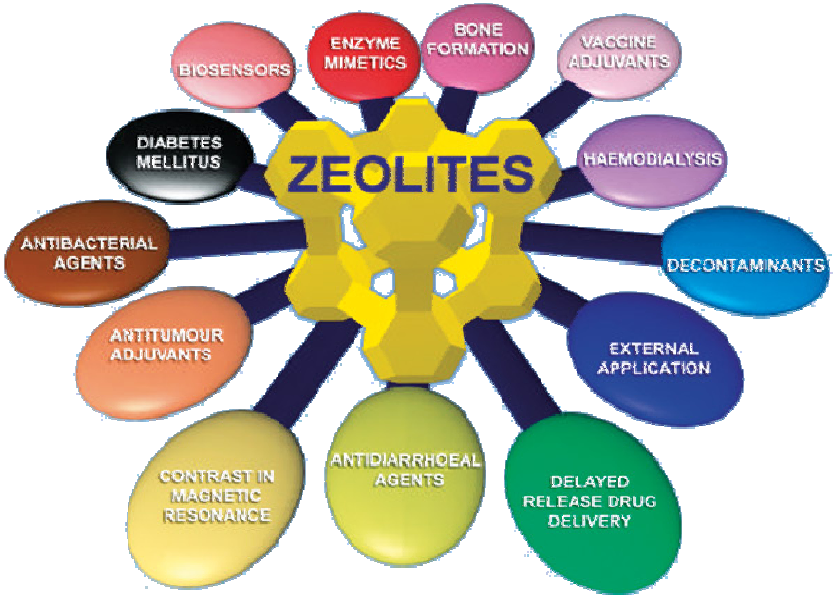


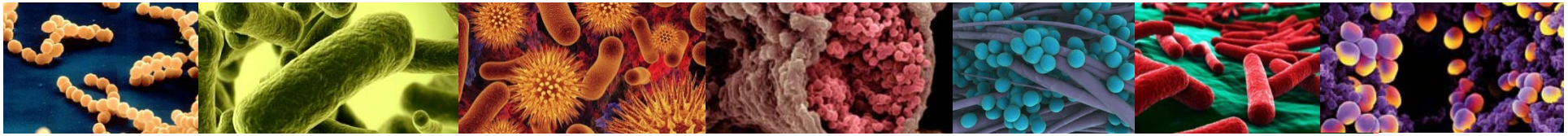
* Matsumura Y, Yoshikata K,Kunisaki S, Tsuchido T, (2003) Appl Environ Microb 69: 4278–4281



Review of research highlights

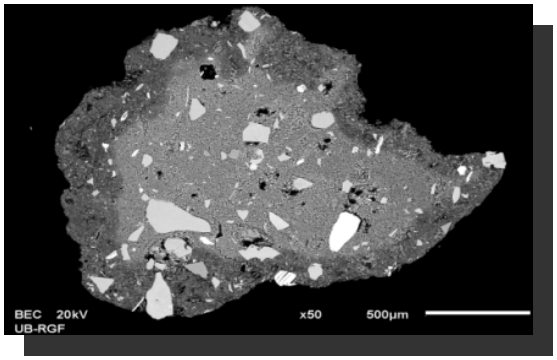
- Study of antibacterial efficiency of two types of zeolites
- Study of antibacterial mechanism
- Application of zeolite-based disinfectants



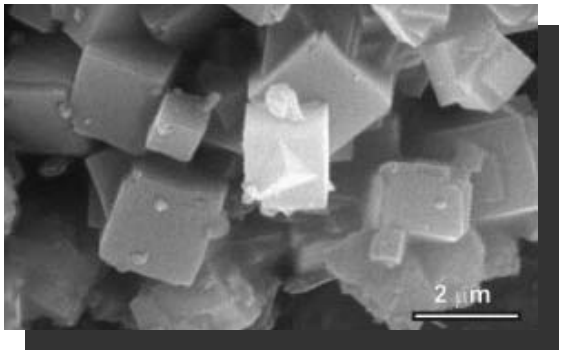


Experimental

Natural zeolite (Z)



Zeolite A



Ion-exchange procedure

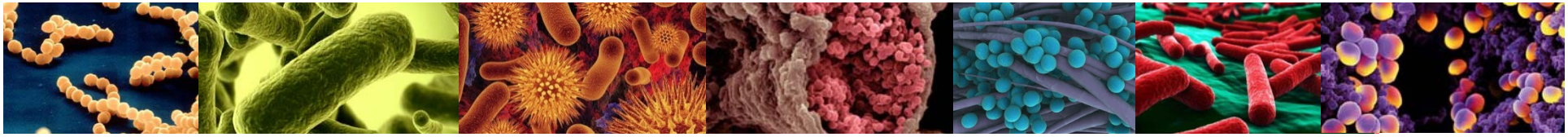
$CuSO_4$ → Cu-Z, Cu-A

$ZnSO_4$ → Zn-Z, Zn-A

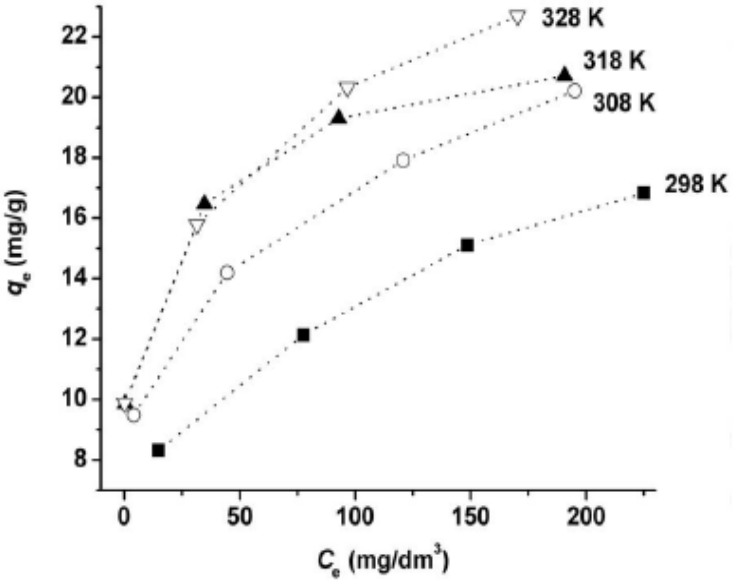
$NiSO_4$ → Ni-Z, Ni-A

$AgNO_3$ → Ag-Z, Ag-A

Ag-Z, Ag-A → **0.25 mmol M/g zeolite**

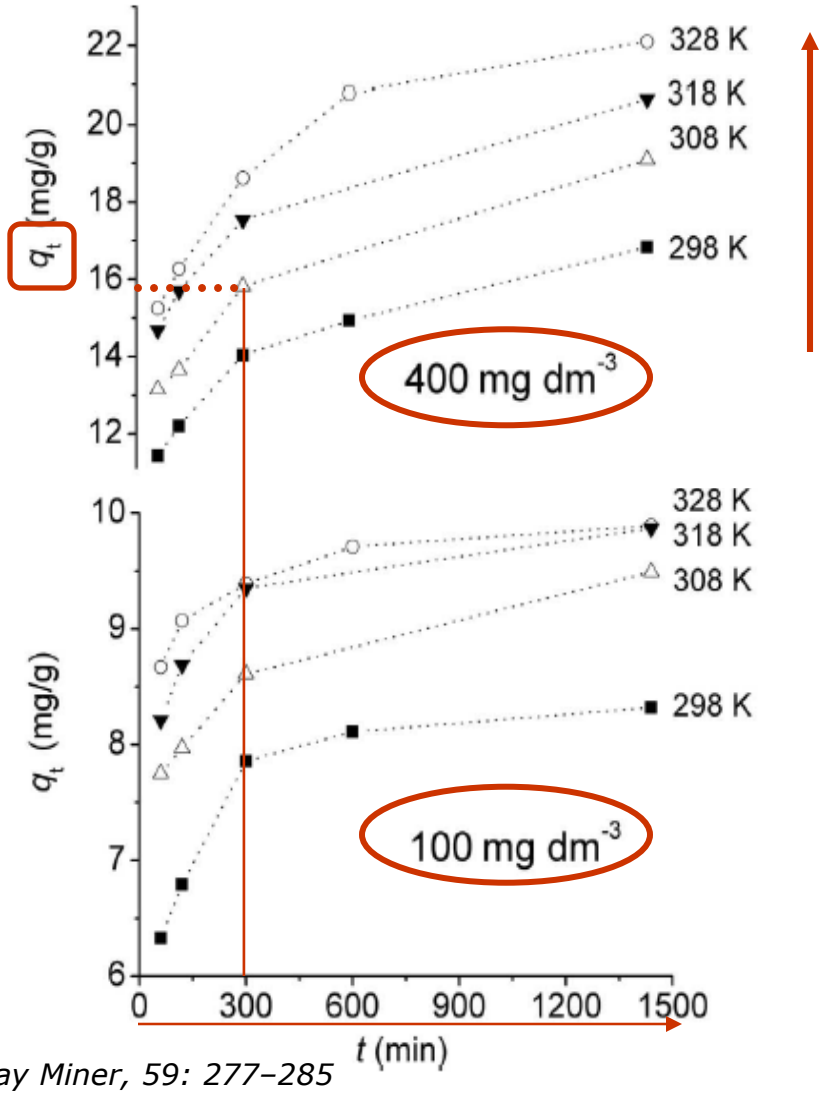


Adsorption isotherms and kinetics study of Cu^{2+}

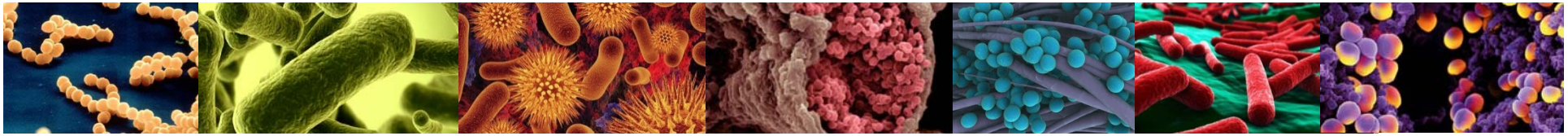


Pseudo-second order model:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^{\,2}} + \frac{1}{q_e} t$$

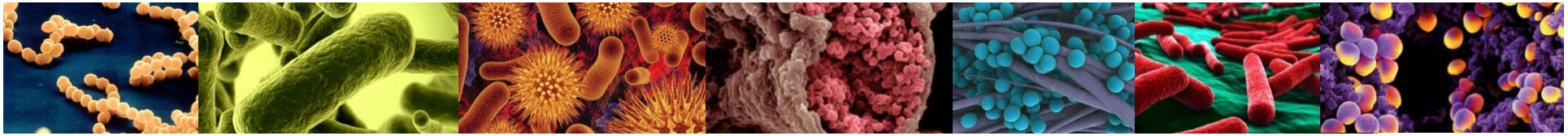


*Stojaković Đ, Milenković J, Daneu N, Rajić N, (2011) Clay Clay Miner, 59: 277–285



Antibacterial tests

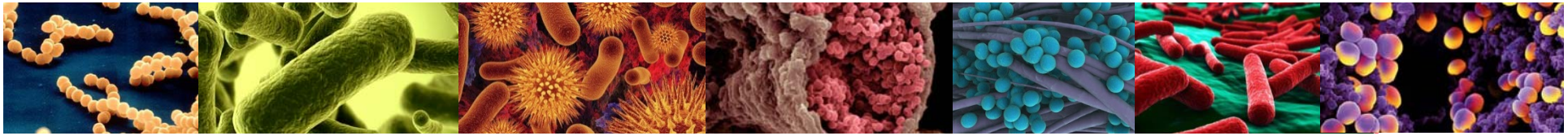
- Treated urban wastewater of the city of Zagreb, Croatia
- Water from the Sava lake in Belgrade, Serbia
- Synthetic wastewater
- Commercial spring water
- Phosphate buffer solution (PBS)



Reduction in the numbers of *E. coli* and *S. aureus*

Bacteria	Medium	Cu-Z		Zn-Z		Ni-Z	
		Reduction (%) for different time (1-24 h)					
		1	*24*	*1*	*24*	*1*	*24*
E. coli	Real effluent	40.1	93.5	8.09	95.1	1.74	18.5
	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
	Synthetic water	42.8	87.3	3.31	82.3	1.28	10.13

t_o (*E. coli*)= 1.1×10^7 CFU cm^{-3}, t_o (*S. aureus*)= 9.9×10^6 CFU cm^{-3}

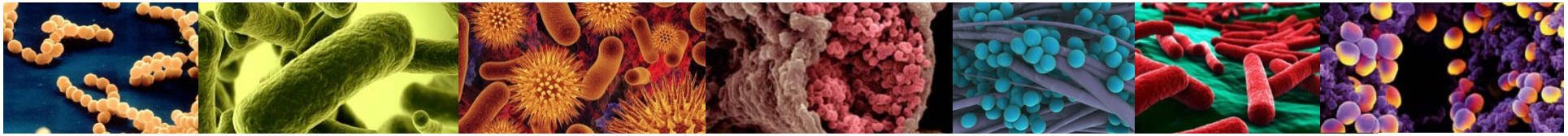


Metal leaching from zeolite lattices

Sample	Bacteria			
	E. coli		*S. aureus*	
	Leached metal (mg dm^{-3})	Final pH	Leached metal (mg dm^{-3})	Final pH
Cu-Z	0.0	7.14	0.0	6.85
Zn-Z	2.36	7.18	1.58	7.13
Ni-Z	1.79	7.97	4.75	7.90

Maximum allowable concentration in drinking water:

- Cu - 2 mg dm^{-3}
- Zn - 3 mg dm^{-3}
- Ni - 0.02 mg dm^{-3}



Reduction in the numbers of *E. coli*

E. coli	Medium	Cu-Z		Zn-Z		Cu-A		Zn-A	
		Reduction (%) for different times (1-24 h)							
		1	*24*	*1*	*24*	*1*	*24*	*1*	*24*
Isolate 1	Real effluent	73.6	**100**	40.3	**100**	60.7	**100**	1.37	1.68
	Commercial water	84.5		82.1		83.6		0.31	26.4
Isolate 2	Real effluent	60.2		10.0		51.1		3.09	6.68
	Commercial water	64.0		26.7		62.0		1.95	14.0

t_o(*Isolate 1*) =2.0x10^7 CFU cm^{-3}, t_o (*Isolate 2*)= 2.2x10^7 CFU cm^{-3}

* *Milenkovic J, Hrenovic J, Matijasevic D, Niksic M, Rajic N, (2017) Environ Sci Pollut R, accepted for publication*

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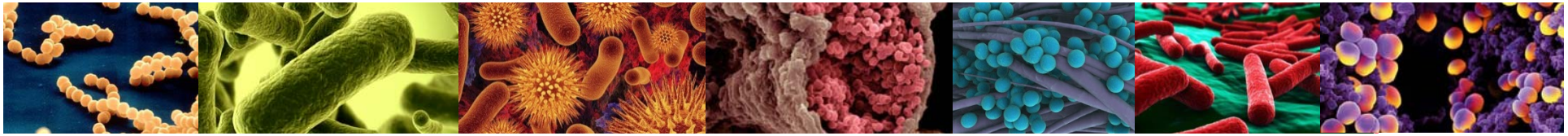
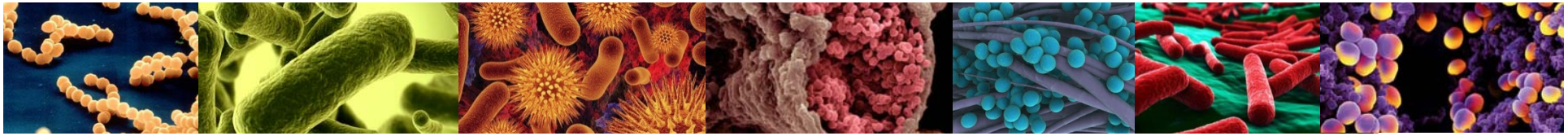


Metal leaching from zeolite lattices

Sample	Isolates of *E. coli*			
	Isolate 1		Isolate 2	
	Leached metal (mg dm^{-3})	Final pH	Leached metal (mg dm^{-3})	Final pH
Cu-Z	0.30	6.80	0.08	6.78
Zn-Z	0.36	6.85	0.47	6.84
Cu-A	0.06	9.08	0.33	8.82
Zn-A	0.01	9.80	0.01	8.81

* *Milenkovic J, Hrenovic J, Matijasevic D, Niksic M, Rajic N, (2017) submitted manuscript*

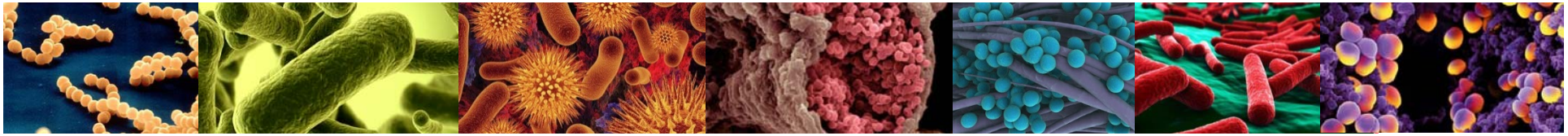


Reduction in the numbers of *A. baumannii*

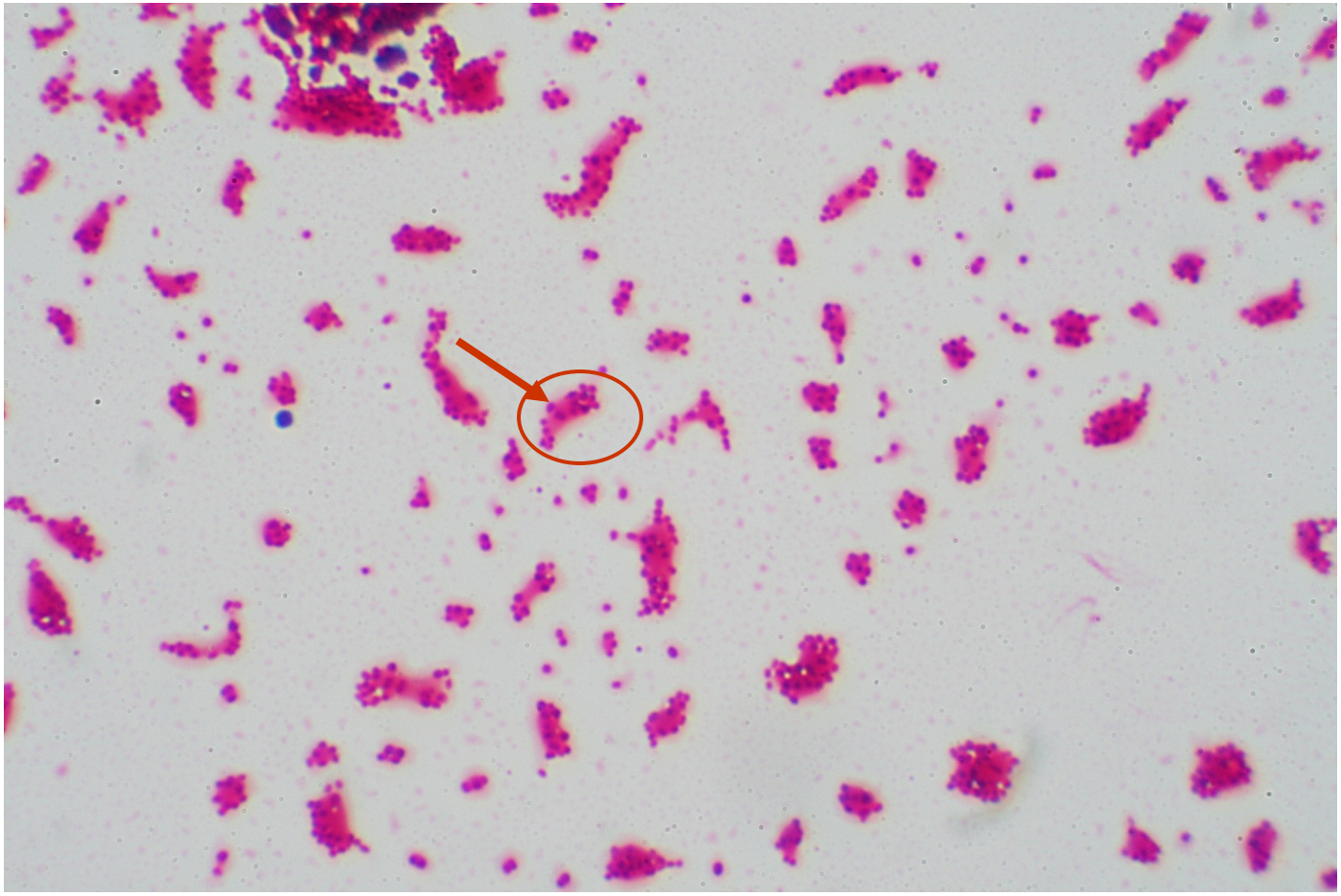
Types	Sample	Reduction (%) for different time (h)		MBC (mg dm^{-3})	Leached metal (mg dm^{-3})
		1	*24*		
IC I	Cu-Z	100	100	250	0.403
	Zn-Z	13.0	28.5	>1000	
	Ag-Z	100	100	500	0.090
IC II	Cu-Z	100	100	125	0.103
	Zn-Z	10.5	22.0	>1000	
	Ag-Z	100	100	62.5	0.130

t_o (IC I)=8.8x10^6 CFU cm^{-3}, t_o (IC II)=1.4x10^7 CFU cm^{-3}

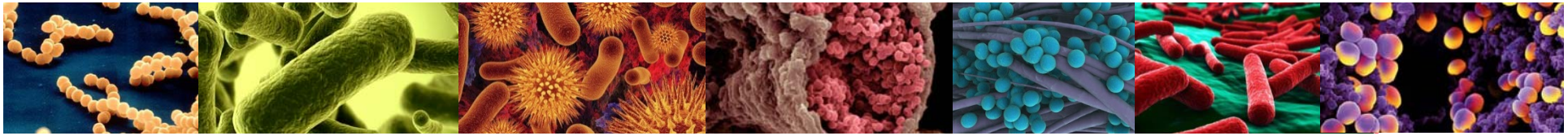
* *Hrenović J, Milenković J, Goić-Barišić I, Rajić N, (2013) Micropor Mesopor Mat 169:148-152*



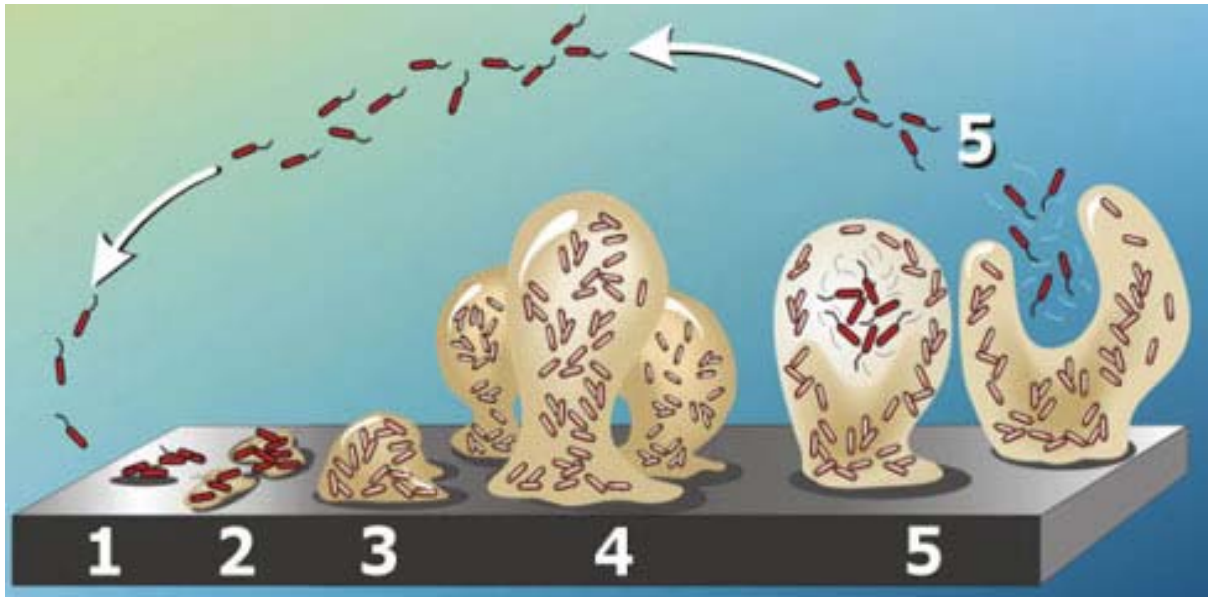
Biofilm



Biofilm of the *A. baumannii*

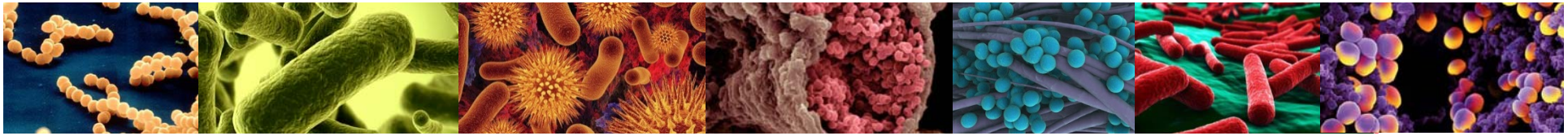


Formation of biofilm

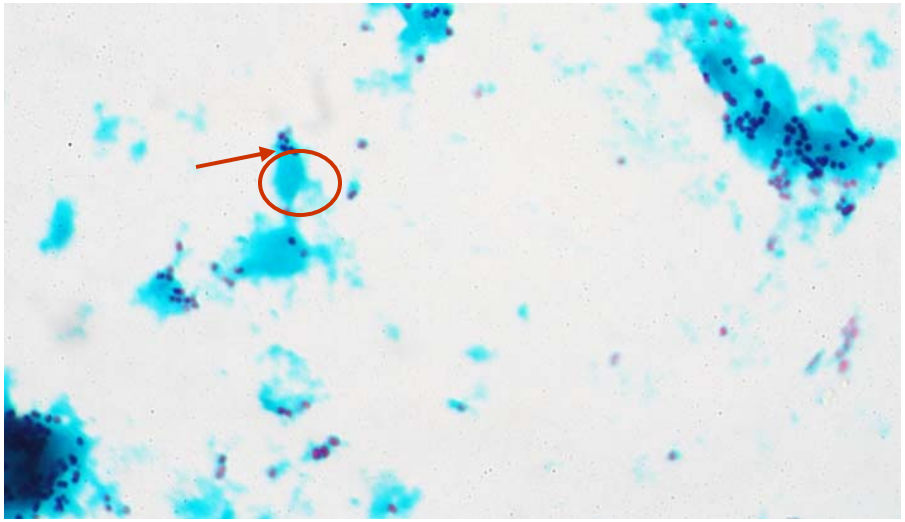


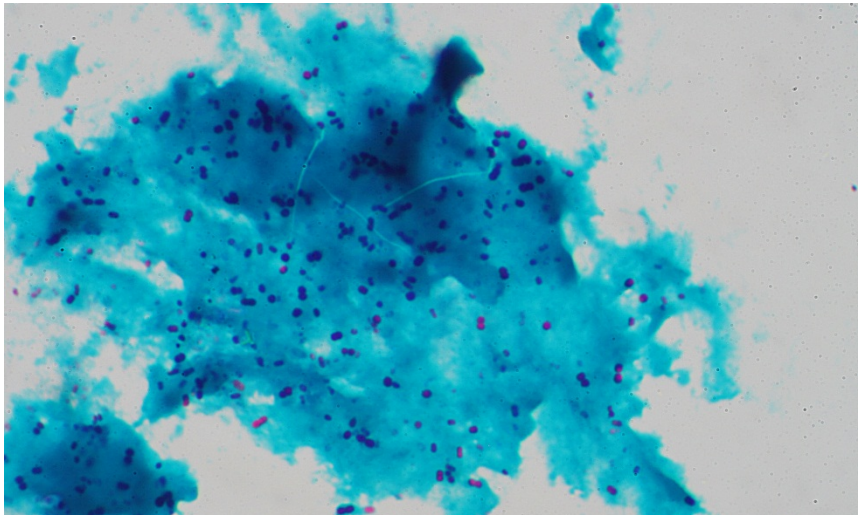
1. Bonding of bacteria cells onto the surface
2. Production of extracellular polymer substance
3. Multiplication of bacteria cell inside the biofilm
4. Biofilm "maturation"
5. Rupturing of the biofilm and releasing of bacteria.

** Stoodley P, Sauer K, Davies D G, Costerton J W, (2002) Annu Rev Microbiol 56:187–209*

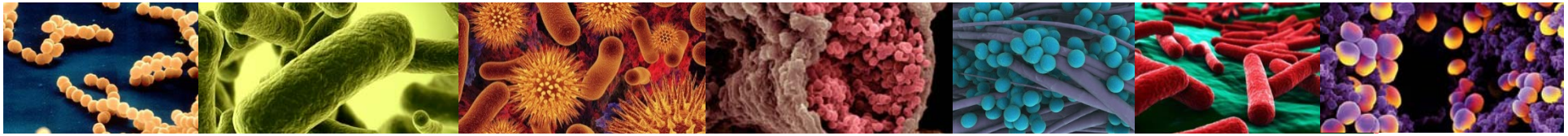


Formation of biofilm of *A. baumannii*



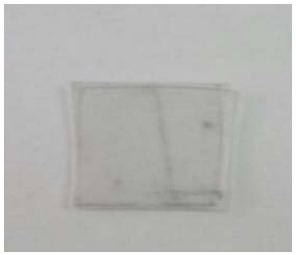


Thick layer of extracellular substances with embedded cells of *A. baumannii* isolates (IC I and II)



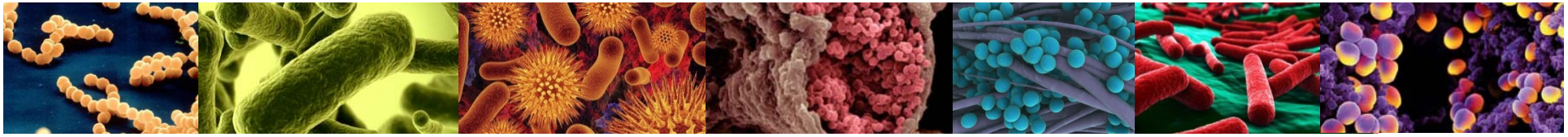
Novel antibiofouling composite

Two series of composites:
PVC/Ag-Z
PVC/Ag-Z + D-Tyrosine

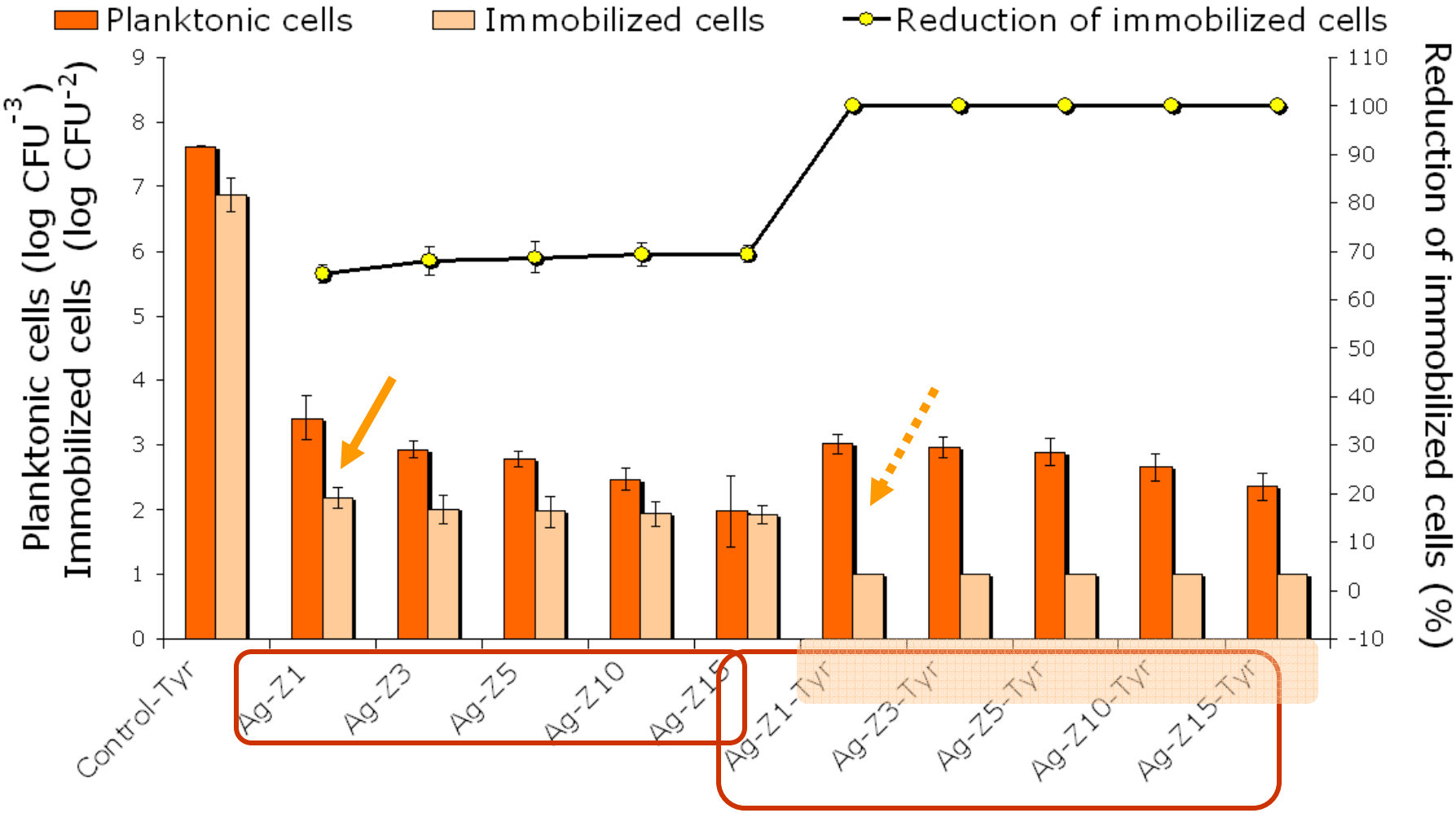


Control sample
pure PVC

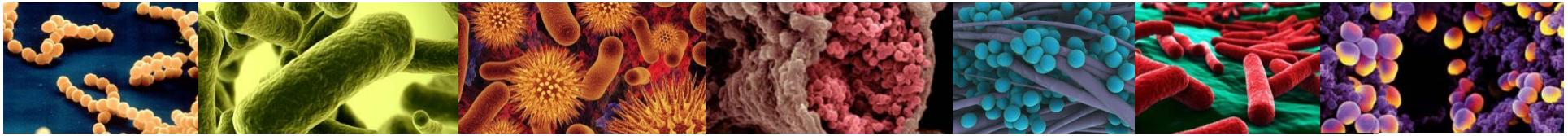
Composite
containing max.
15 wt.% of Ag-Z



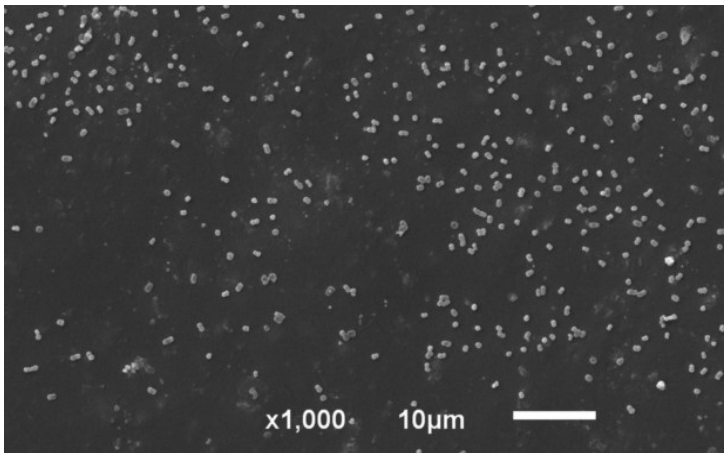
Reduction of *A. baumannii*



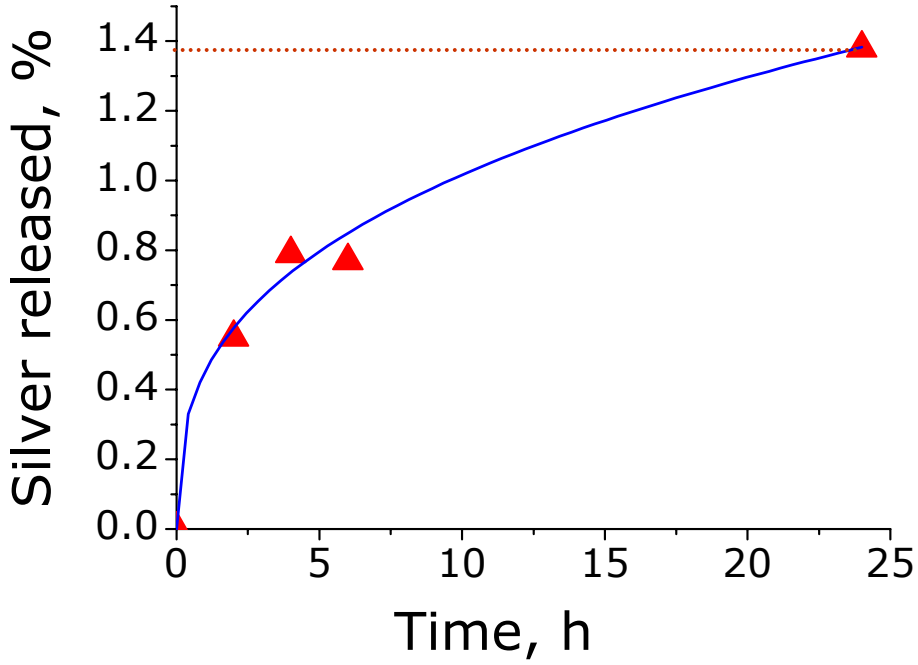
* *Milenković J, Hrenović J, Goić-Barišić I, Tomić M, Đonlagić J, Rajić N, (2014) Biofouling 30:965-973*



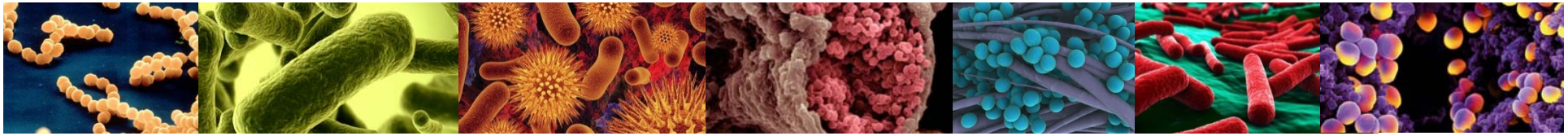
Metal leaching from the composites



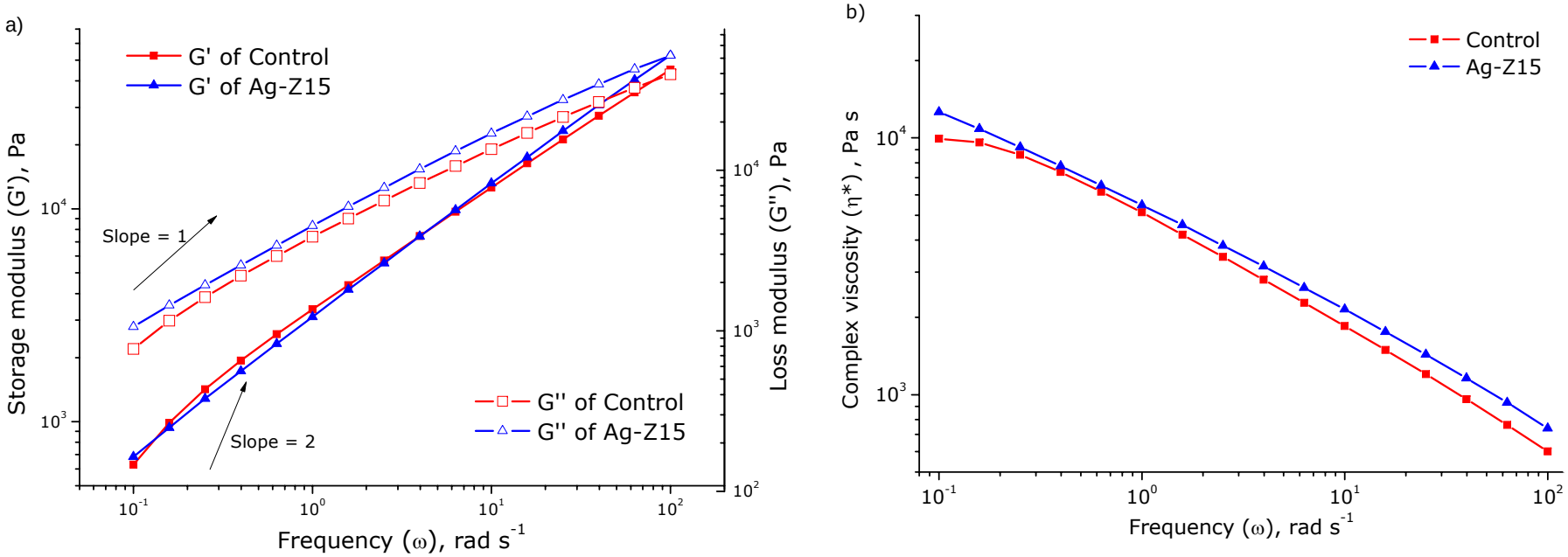
SEM image of immobilized *A. baumannii* on the control surface and the absence of the bacteria on the Ag-Z1/PVC/D-Tyr



Percentage of the Ag released from the composite containing max. amount of Ag-Z over time



Rheological studies of composites



Frequency dependences of (a) the storage and loss modulus, and (b) the complex viscosity of the control material and Ag-Z15.

*Milenković J, Hrenović J, Goić-Barišić I, Tomić M, Đonlagić J, Rajić N, (2014) Biofouling 30:965-973

*Milenković J, Hrenović J, Goić-Barišić I, Tomić M, Rajić N, (2015) J Serb Chem Soc(2015) 80: 819–826

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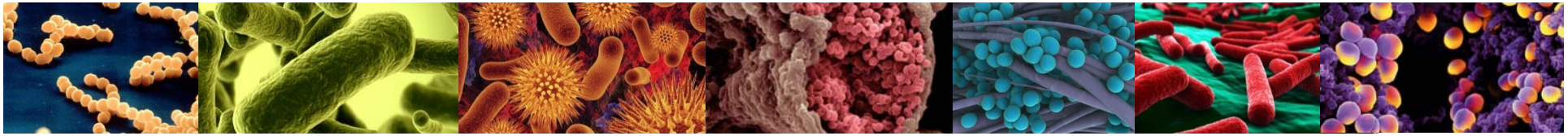
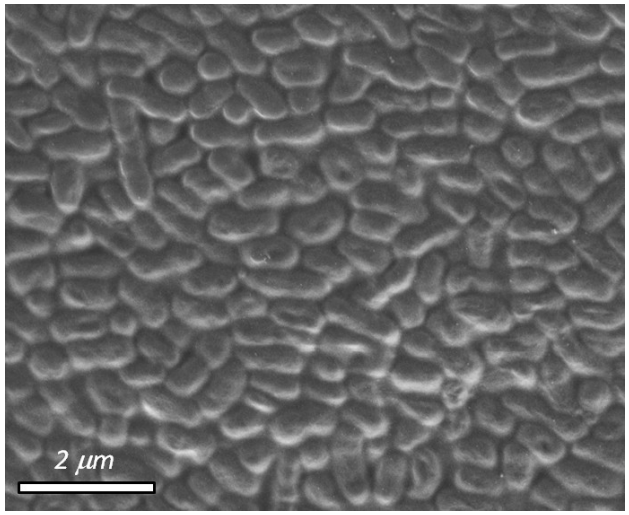
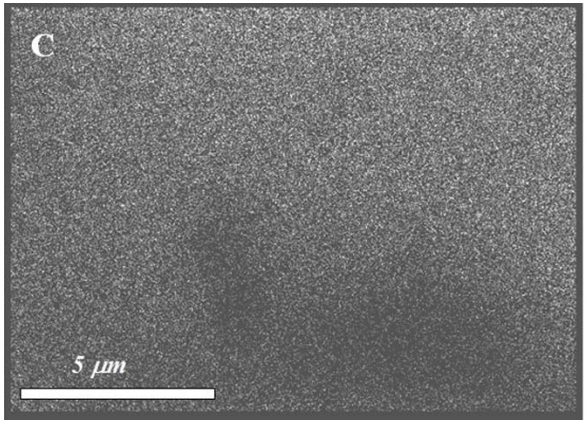
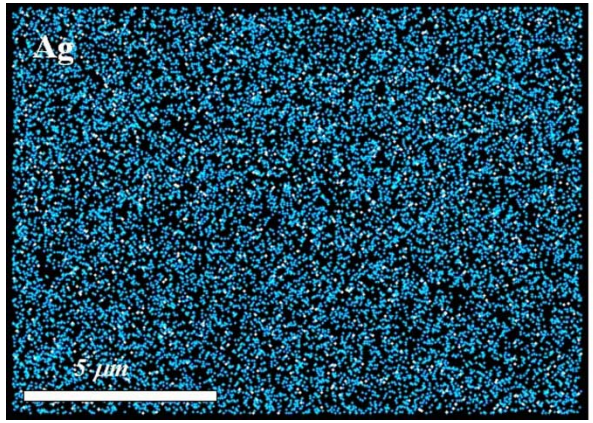


Mechanism of bactericidal activity of Ag^+ toward *A. baumannii*

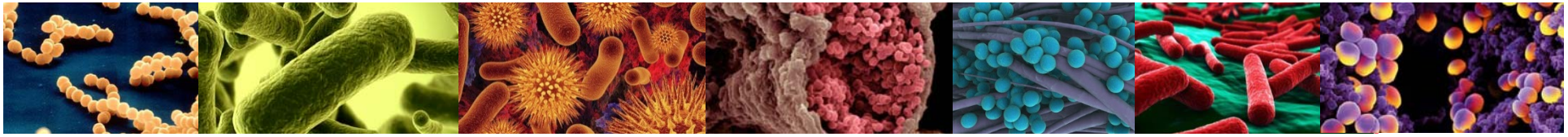
Typical SEM micrograph of the bacterial sample



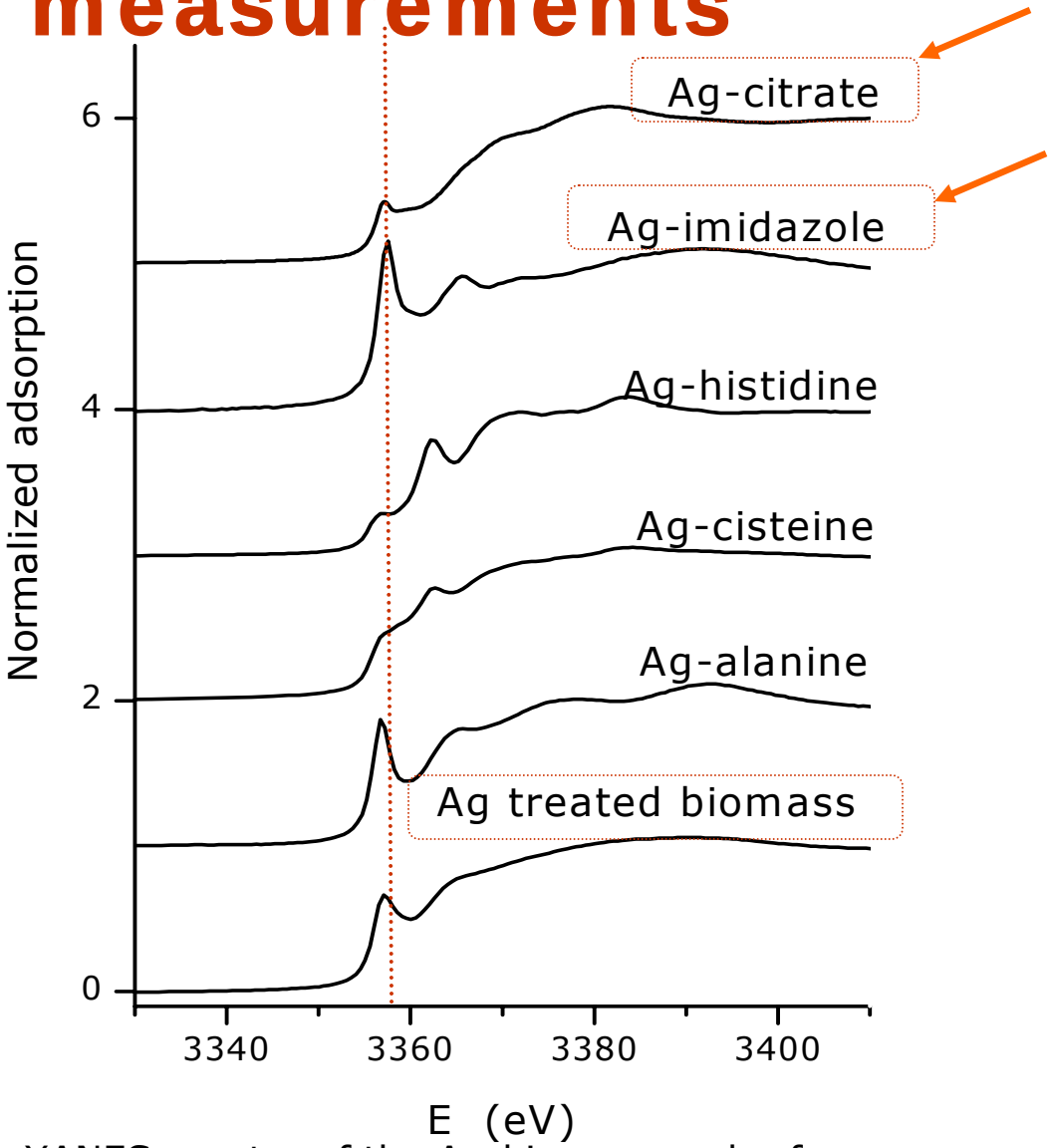




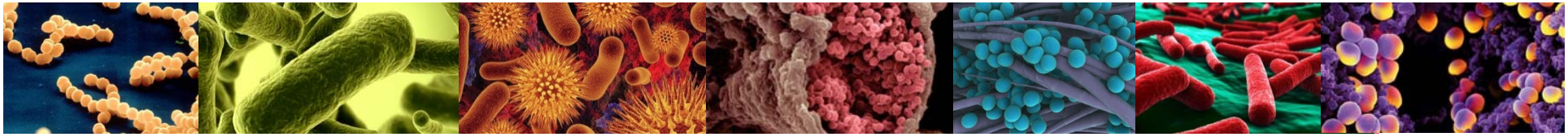
EDAX elemental mapping of the sample showing C (left) and Ag distribution (right).



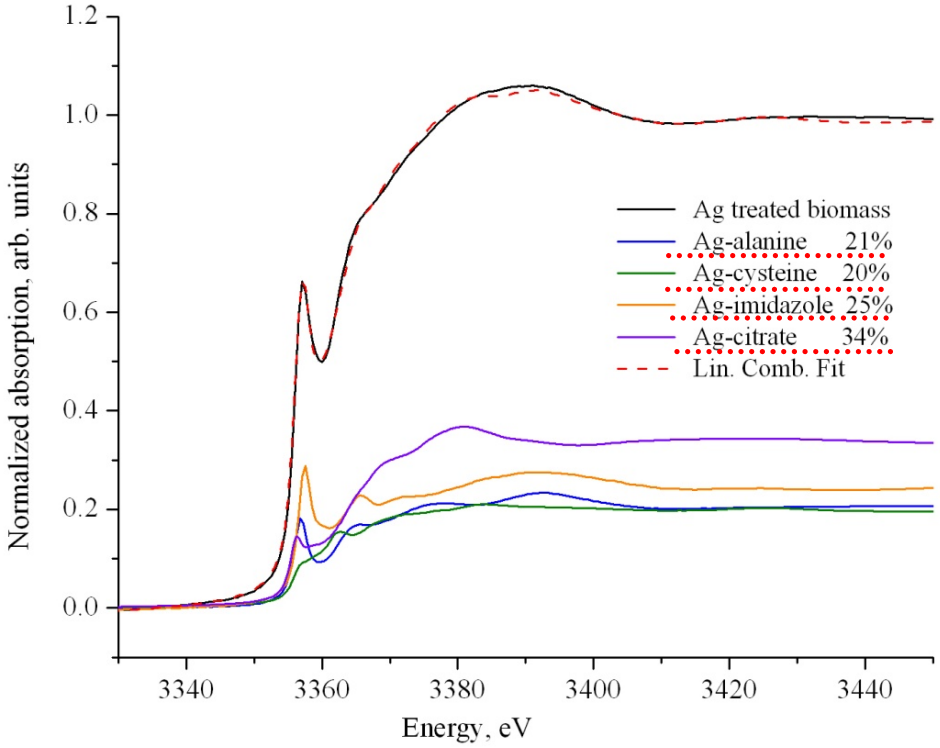
XANES measurements

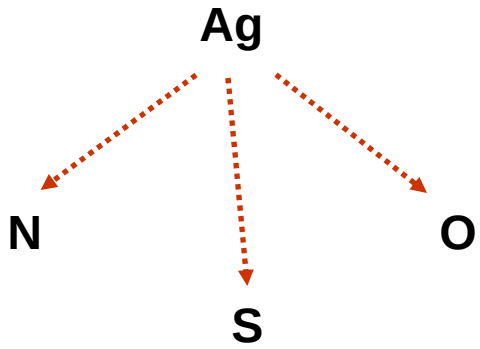


Ag L_3-edge XANES spectra of the Ag-biomass and reference compounds.



XANES measurements





Ag L_3-edge XANES spectrum of Ag-biomass: the best linear combination fit obtained by the reference compounds: Ag-alanine, Ag-cysteine, Ag-imidazole and Ag-citrate

* *Rangus M, Hrenović J, Milenković J, Mazaj M, Sabo T, Rajić N, (2017) J Serb Chem Soc, submitted manuscript*

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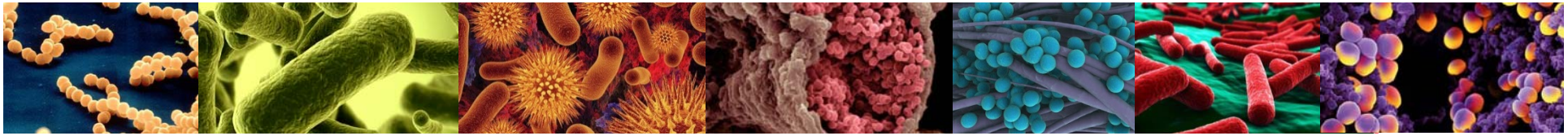
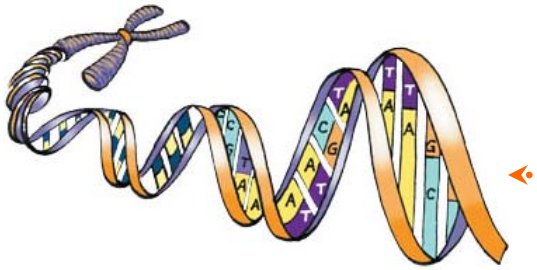
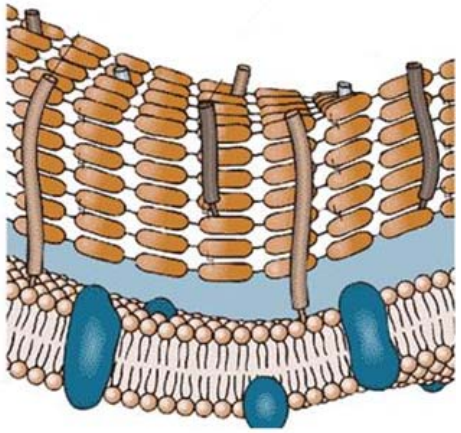


Mechanism of bactericidal activity of Ag^+ toward *A. baumannii*



Intercalates between bases

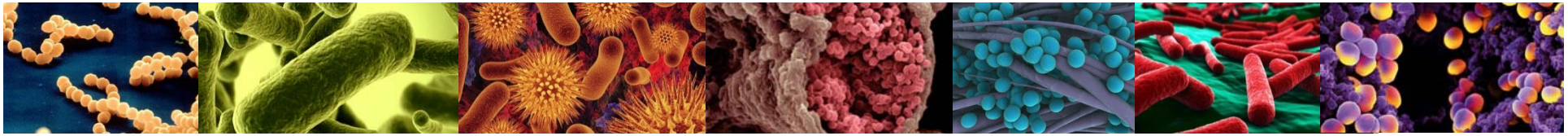
Ag^+



Attaching to the surface of the cell membrane

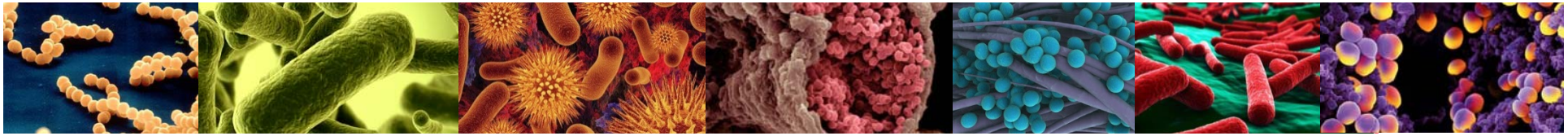
Genotoxicity

Impared membrane function



Conclusions

- Metal-exchanged zeolites show good antibacterial activity towards the studied bacterial species.
- Natural clinoptilolite can be used alone or in composites for removal of pathogenic bacteria.
- Ag-clinoptilolite is perspective antibacterial material for control of multidrug-resistant bacteria.



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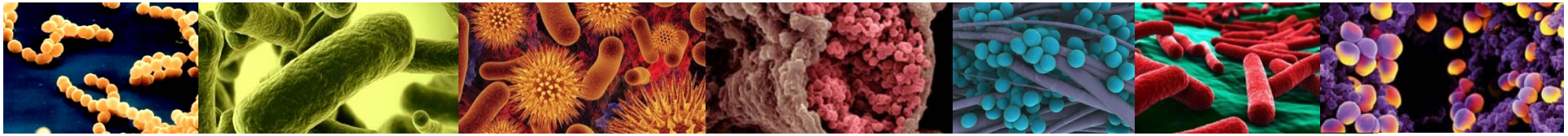

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Jožef Štefan Intitute, Ljubljana