BIOPARTICLES FOR PHOSPHORUS REMOVAL FROM WASTEWATER

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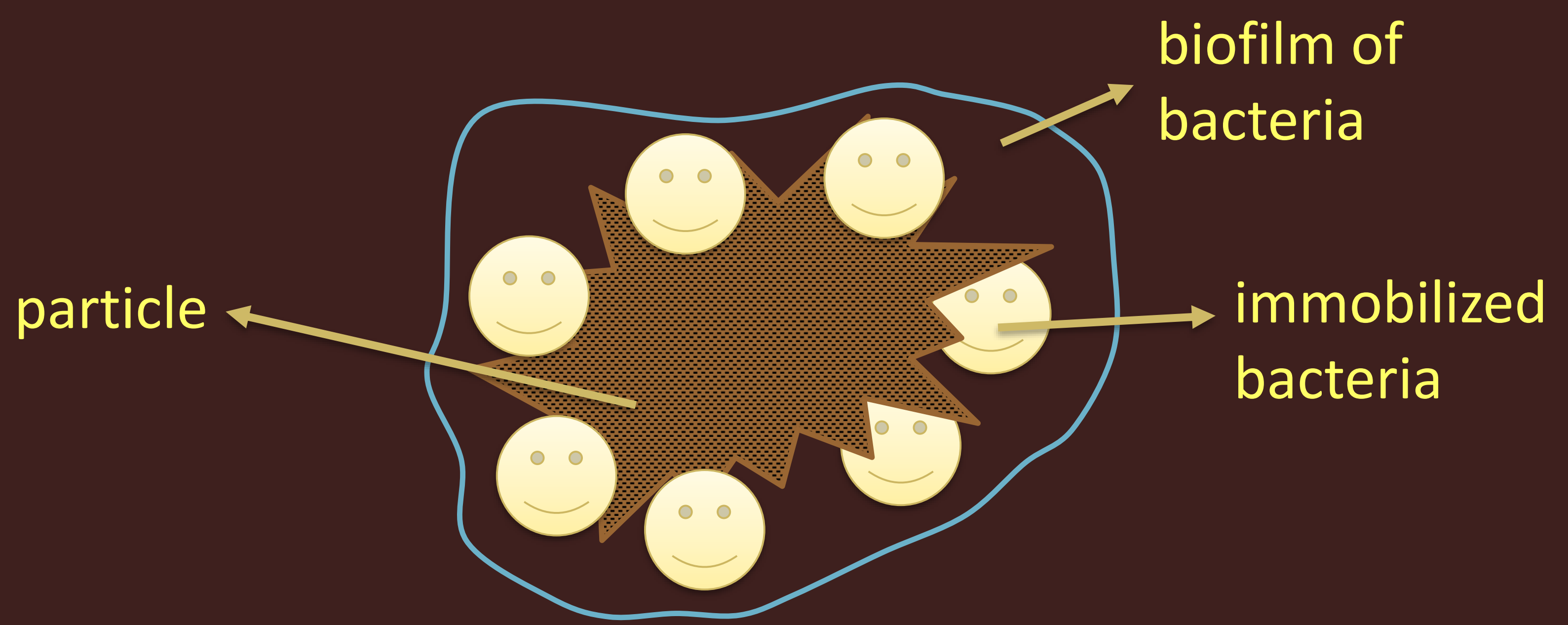
Bioparticles, also known as biosolids for wastewater treatment are consisted of:

Bio = bacteria immobilized onto particle and

Particle = inorganic carrier of bacteria.

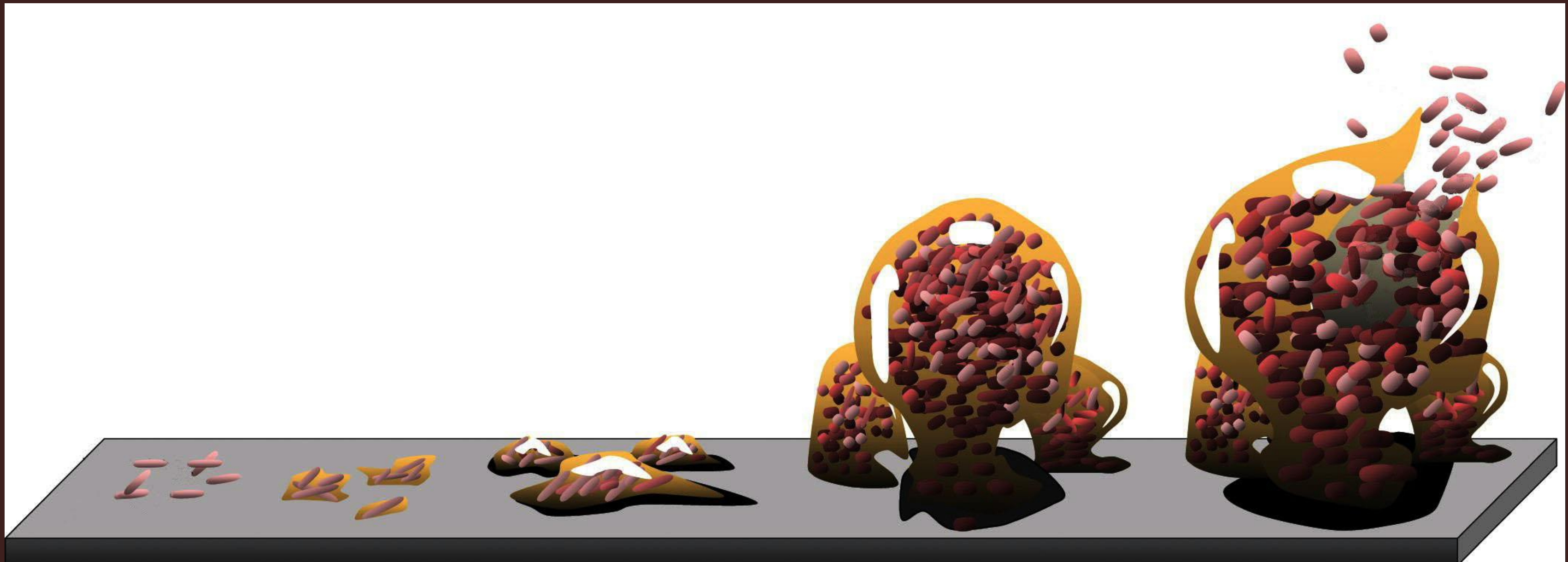
Bioparticles are used as a seed in technology of bioaugmentation, usually in the tertiary stage of wastewater treatment.



The immobilization of desired bacteria onto suitable materials as carriers is currently gaining much attention in biological wastewater treatment.

By the use of immobilized bacteria a higher cell density and metabolic activity in bioreactors can be achieved and based on this,

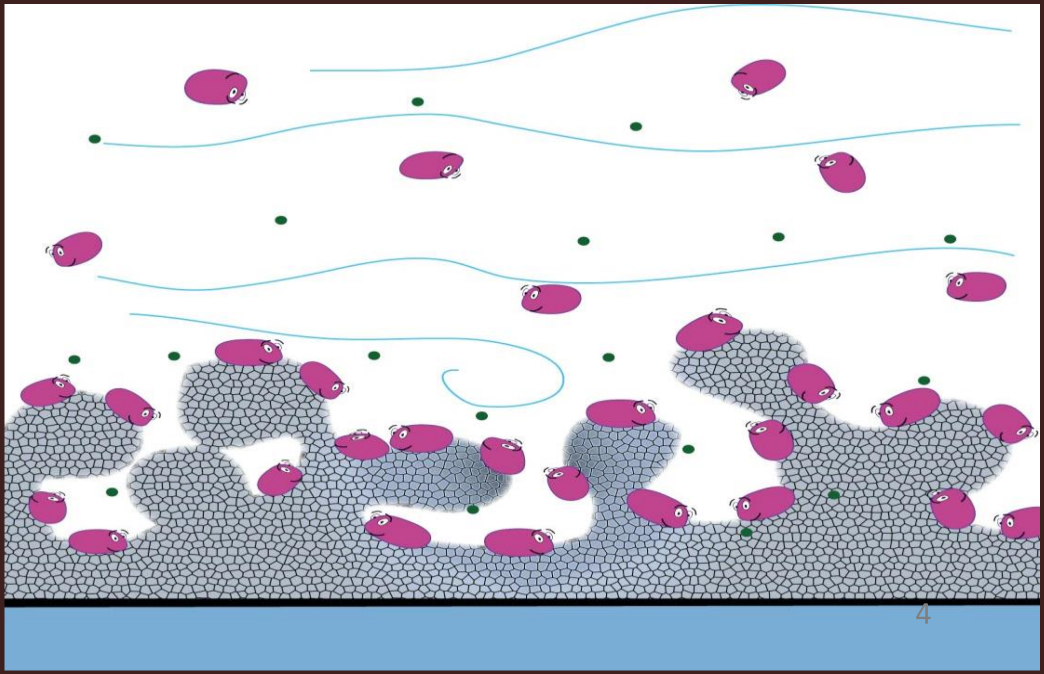
- smaller reactors,
- shorter retention time,
- higher flow rates can be employed or
- better efficiency of the existing system could be achieved.



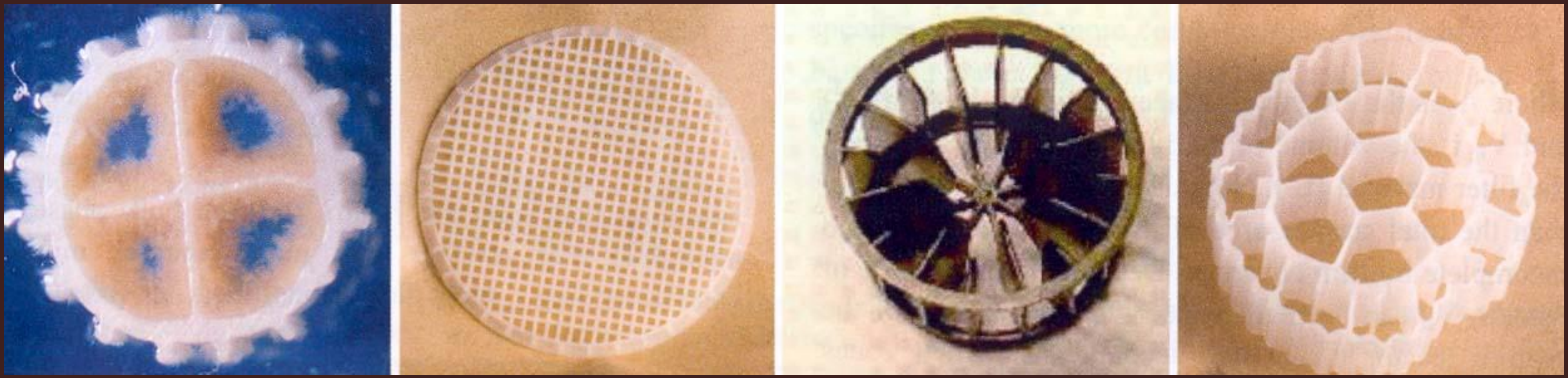
The ideal carrier of bacteria should be:

- inert,
- nontoxic,
- of porous structure,
- relatively cheap,
- easily available,
- environmental friendly and
- should provide a rough, irregular surface available for colonization.

On such carriers the whole external surface, cavities, cervices, irregularities and large pores are accessible for dense bacterial colonization and formation of biofilm.

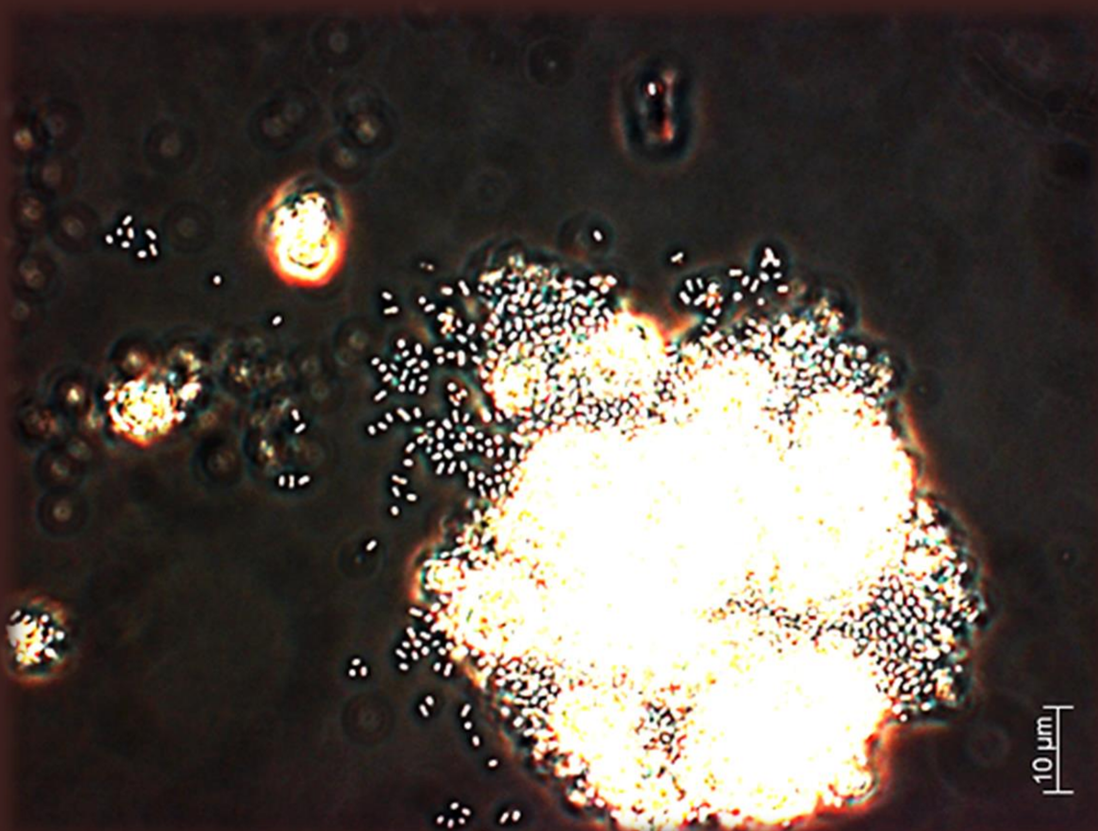


Naturally available materials can replace plastic carriers of bacteria.



Nanomaterials are particles that do not exceed 100 nm in at least one dimension. Usual diameter of bacterial cell is 1000 nm. Carrier of bacteria should be larger than bacterium.

Therefore, particles used as carriers usually are not nanomaterials.



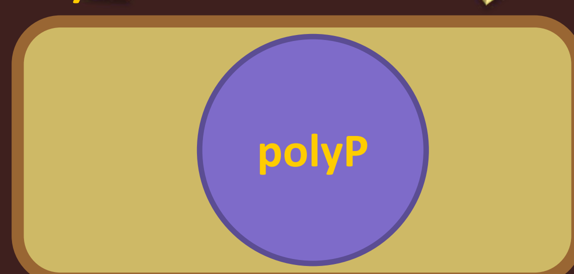
Phosphate (P) is contained in the wastewater and the excess discharge of wastewaters into closed water bodies result in eutrophication of aquatic ecosystems.
Eutrophication leads to significant changes in water quality and lowers the value of surface waters for human uses.

Therefore, there is a need to remove P from municipal and industrial wastewaters before its discharge into natural recipients.

The process of biological P removal from wastewater is based on the accumulation of soluble P present in wastewater in the form of nonsoluble polyP granule inside cells of bacteria - which therefore are called P-accumulating bacteria (PAB).

Phosphate
(polyphosphate kinase)

excess sludge



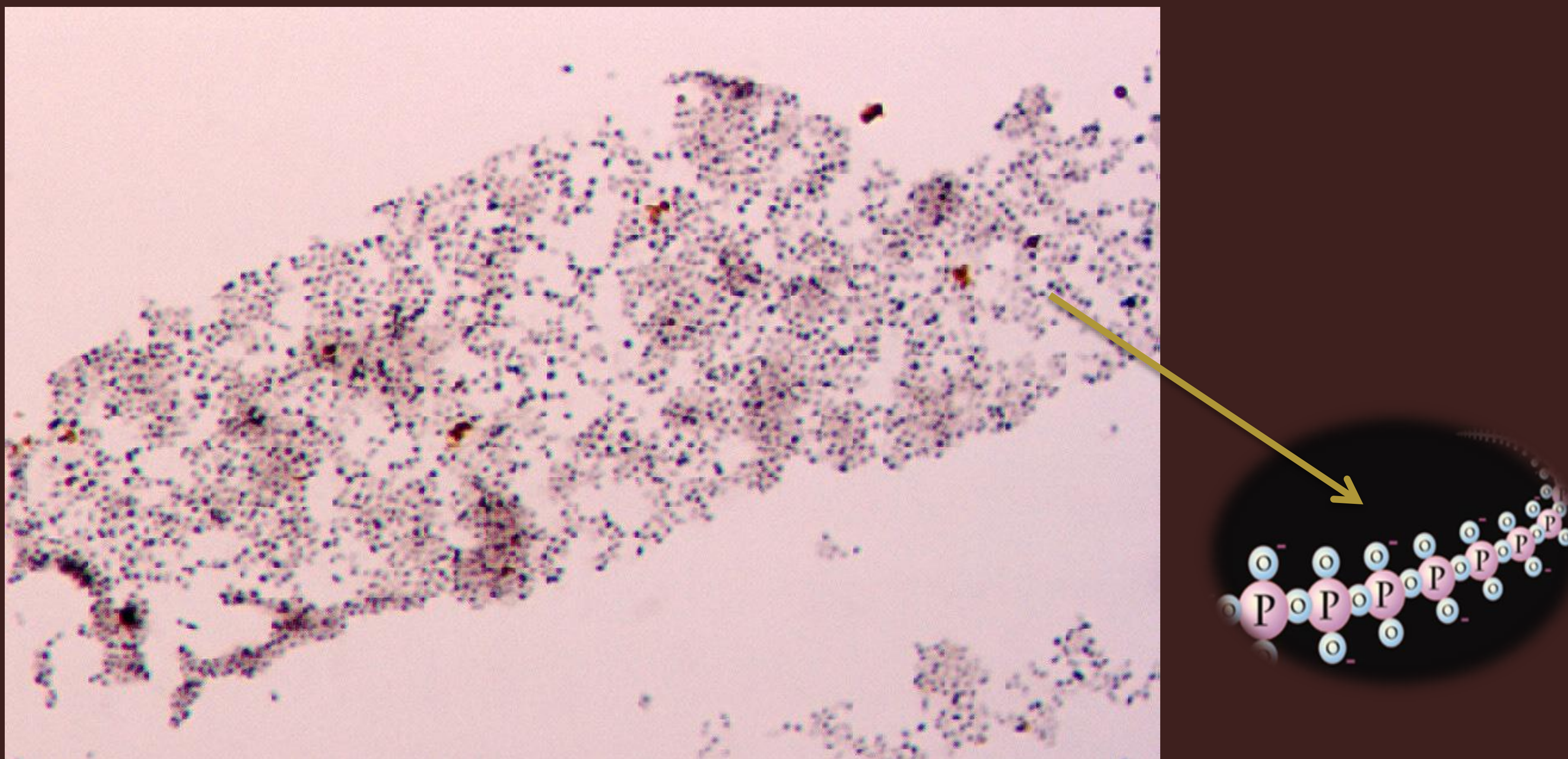
PAB are normally present in activated sludge, but in minority due to its low growth rate.

The use of immobilized PAB in the wastewater treatment process is promising method for the achievement of higher efficiency of the P removal from wastewaters.

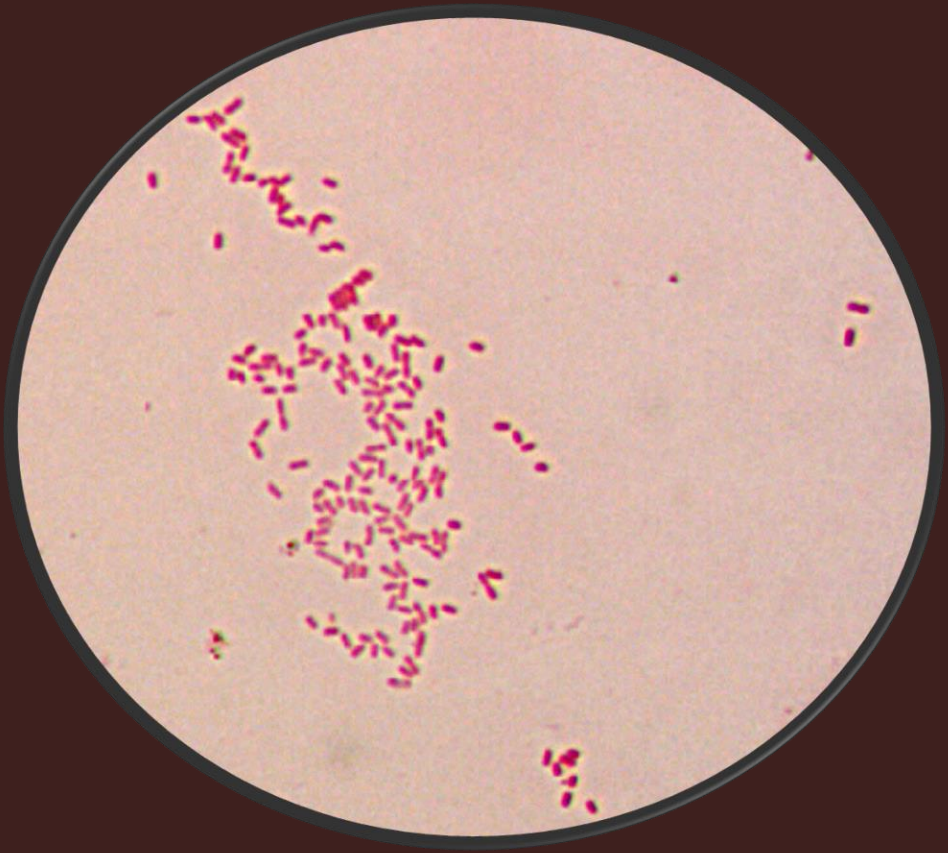
The immobilized PAB will be protected from the grazing of protozoa present in the activated sludge and protected to being washed away by large amounts of wastewater.

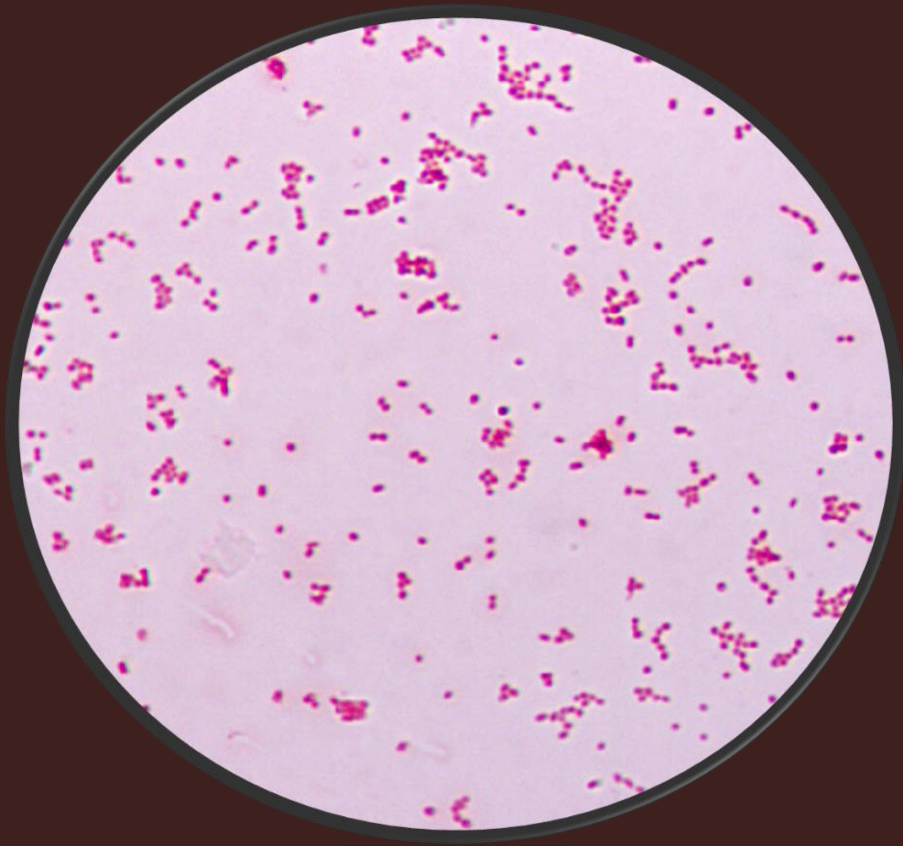
Colonies of PAB *A. junii* grown on nutrient agar

Model bacteria: PAB *Acinetobacter junii* (DSM no. 1532), which was isolated from the activated sludge showing the enhanced biological P removal from wastewater.

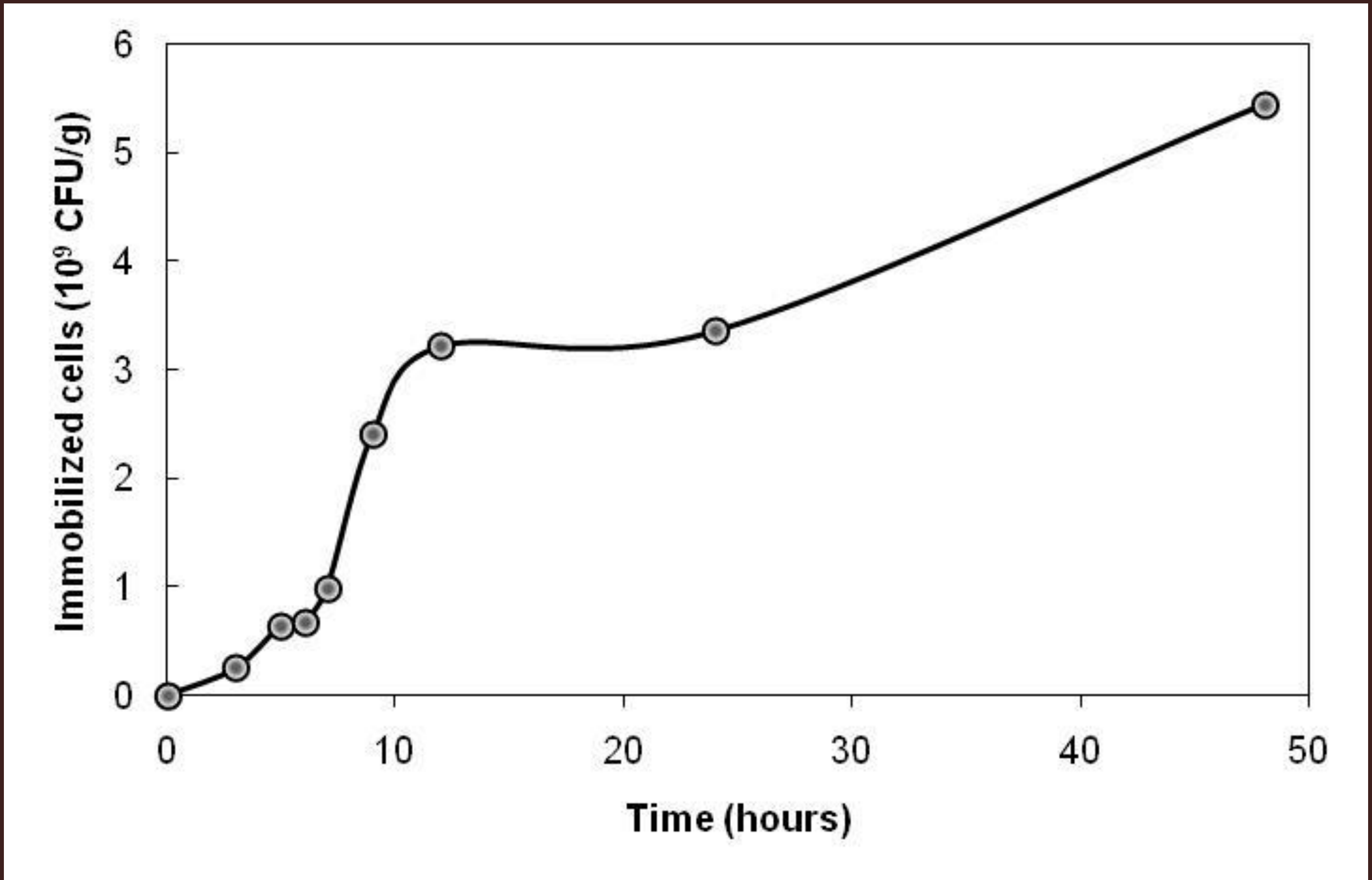


Blue granule of polyP inside cells of *A. junii*

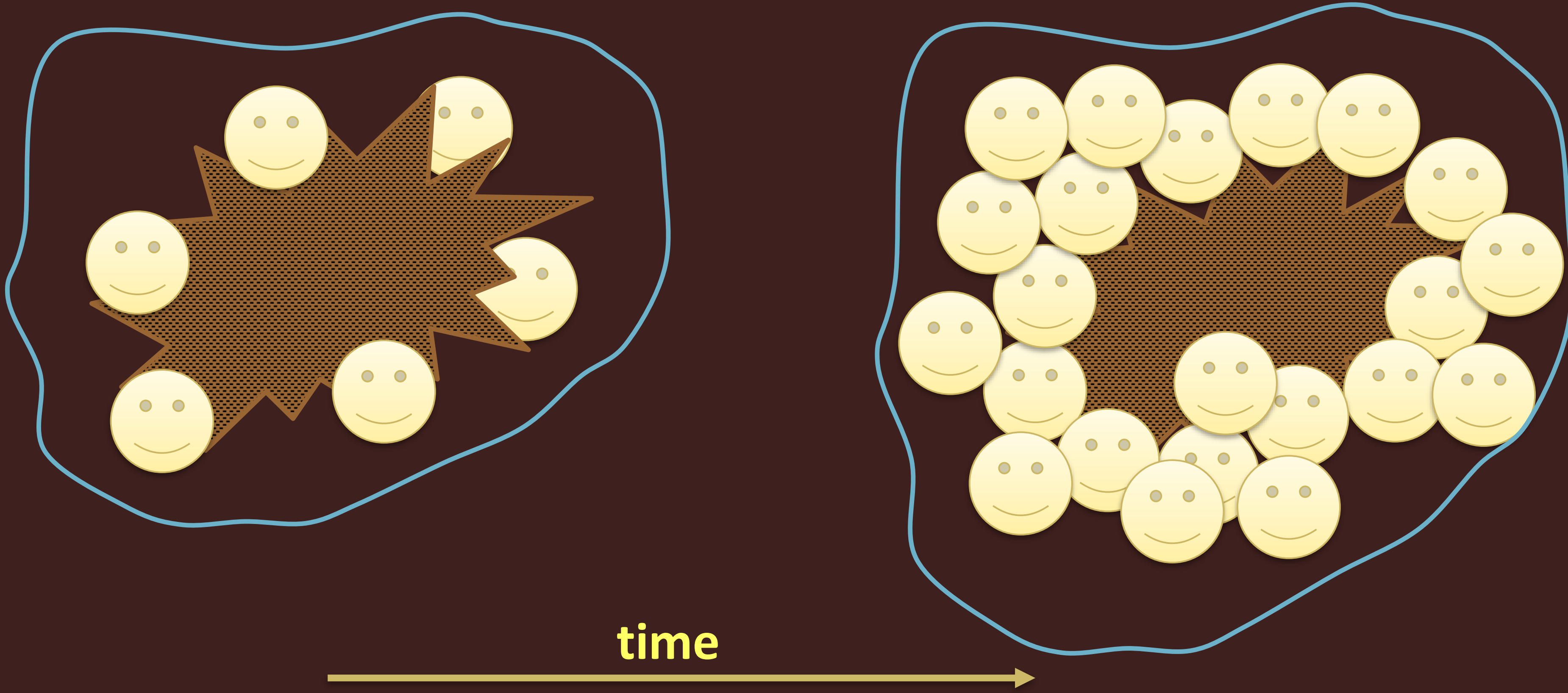




Gram-negative, non-motile, rod cells of *A. junii* become spherical in the stationary phase of growth, when the polyP accumulation occurs.

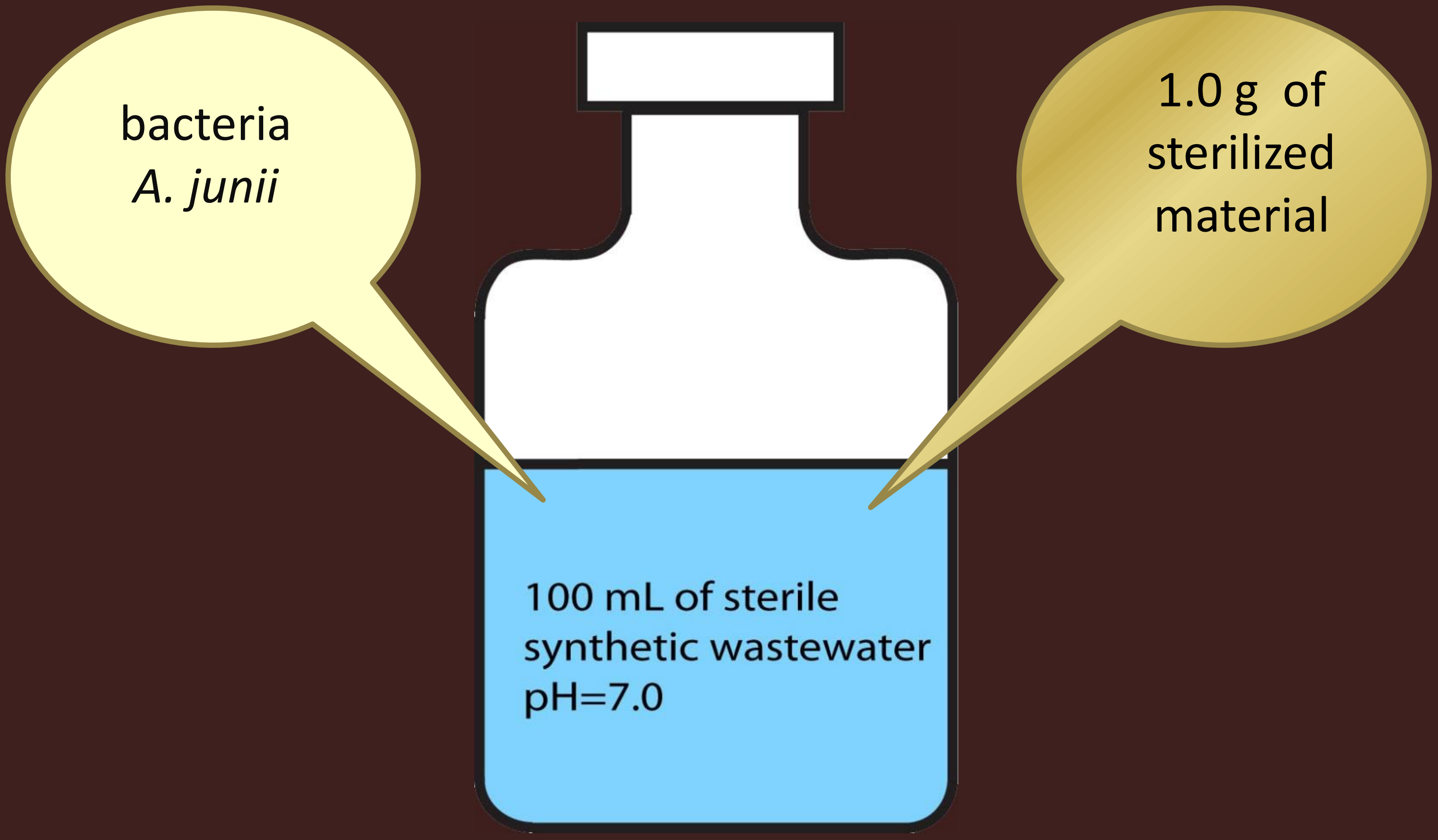


Number of immobilized *A. junii* per one gram of dry carrier (natural zeolite) during 48h of contact.



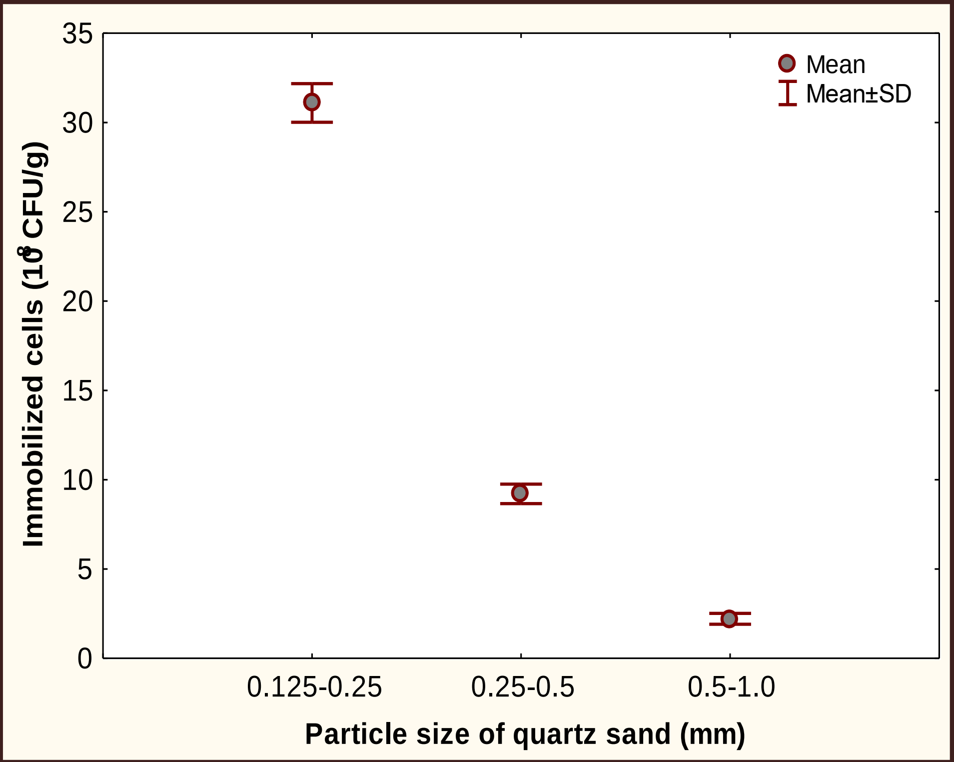
Number of immobilized bacteria will increase during time if there is available nutrients in water – due to the bacterial multiplication inside initially formed biofilm.

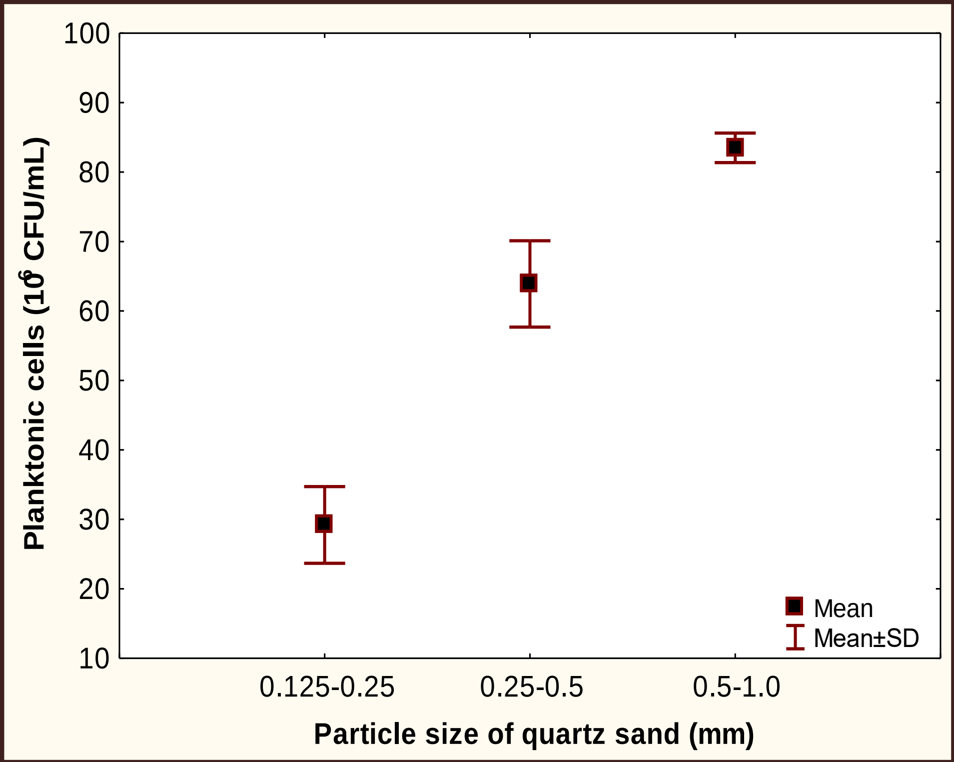
Spontaneous immobilization of *A. junii* during 24 h: 30°C, aeration with filtered air (1L/min) and stirring (70 rpm).



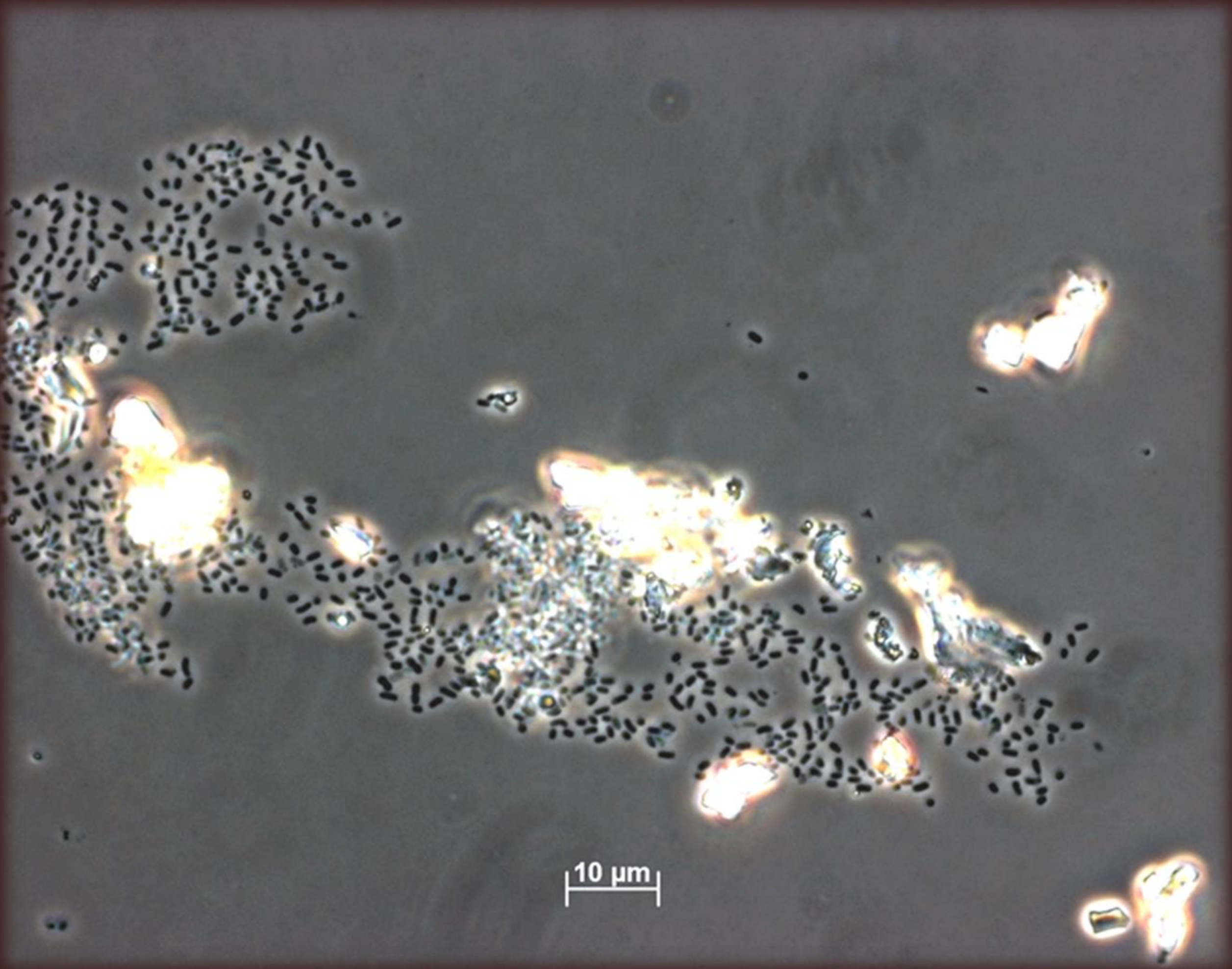
Quartz sand

Bacteria *A. junii* were spontaneously adsorbed on the surface of quartz sand by extracellular substances.





Number of immobilized and planktonic bacteria *A. junii* in reactors with quartz sand of different particle size.
c_0 CFU (10^6/mL) = 18.94±4.10.



Cells of *A. junii* immobilized onto quartz sand originating from Croatia.

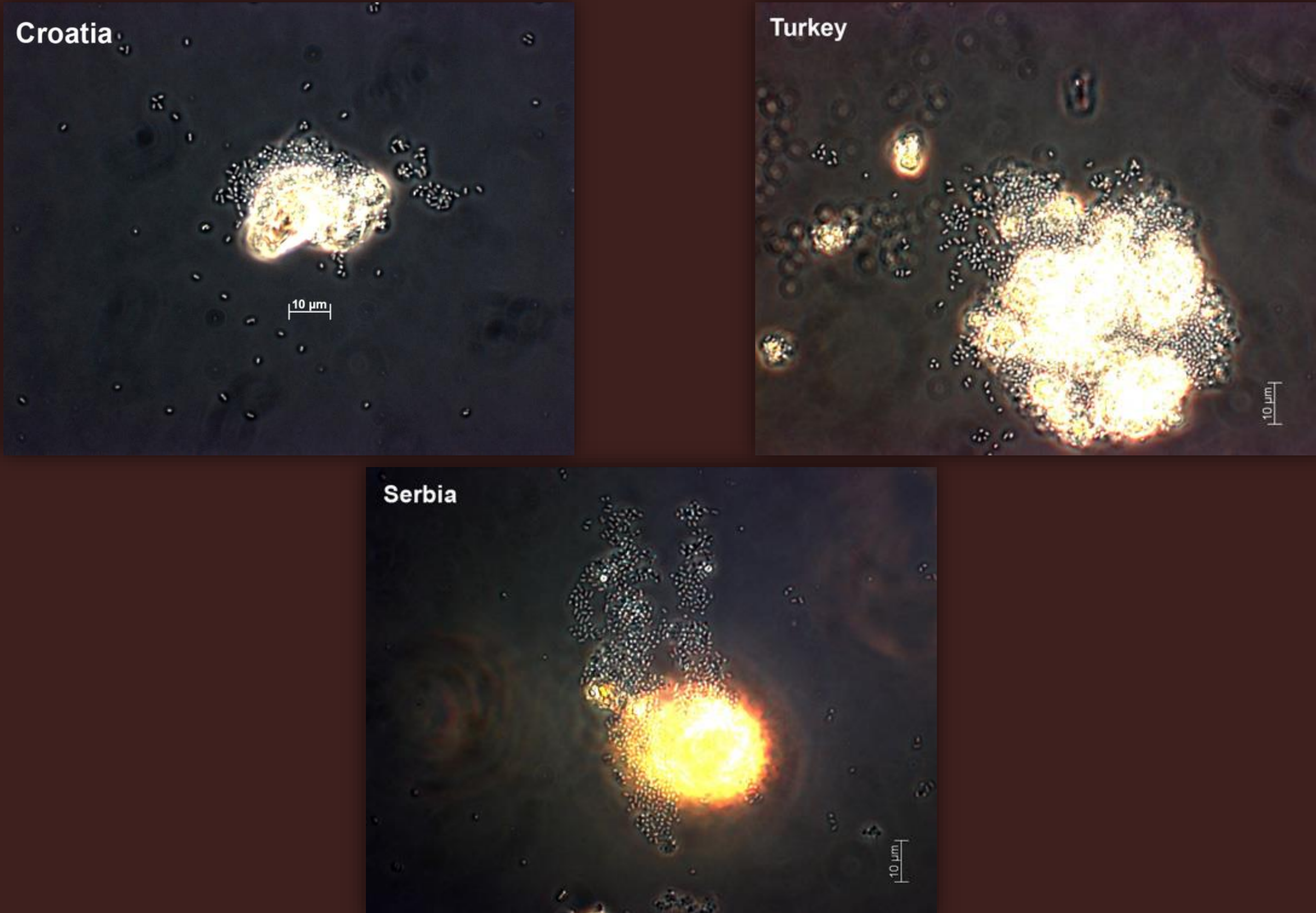
Water SA 2005, 31.

Natural zeolitized tuffs

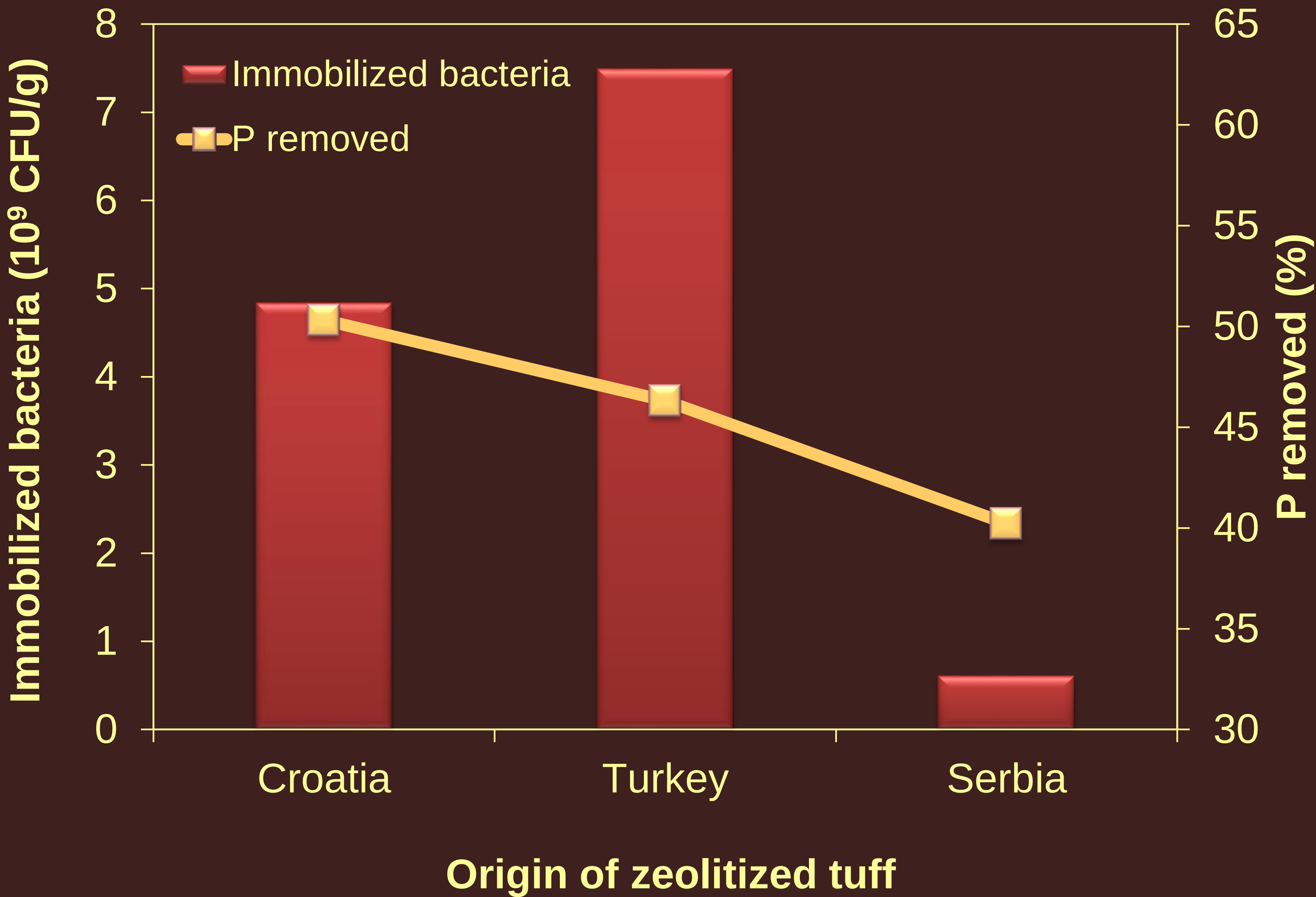
Three types of zeolitized tuffs of particle size <0.125 mm, originating from:

- Croatia (50% of clinoptilolite),
- Turkey (70% of clinoptilolite),
- Serbia (75% of clinoptilolite) were examined.

J Hazard. Mater. 2009, 116.



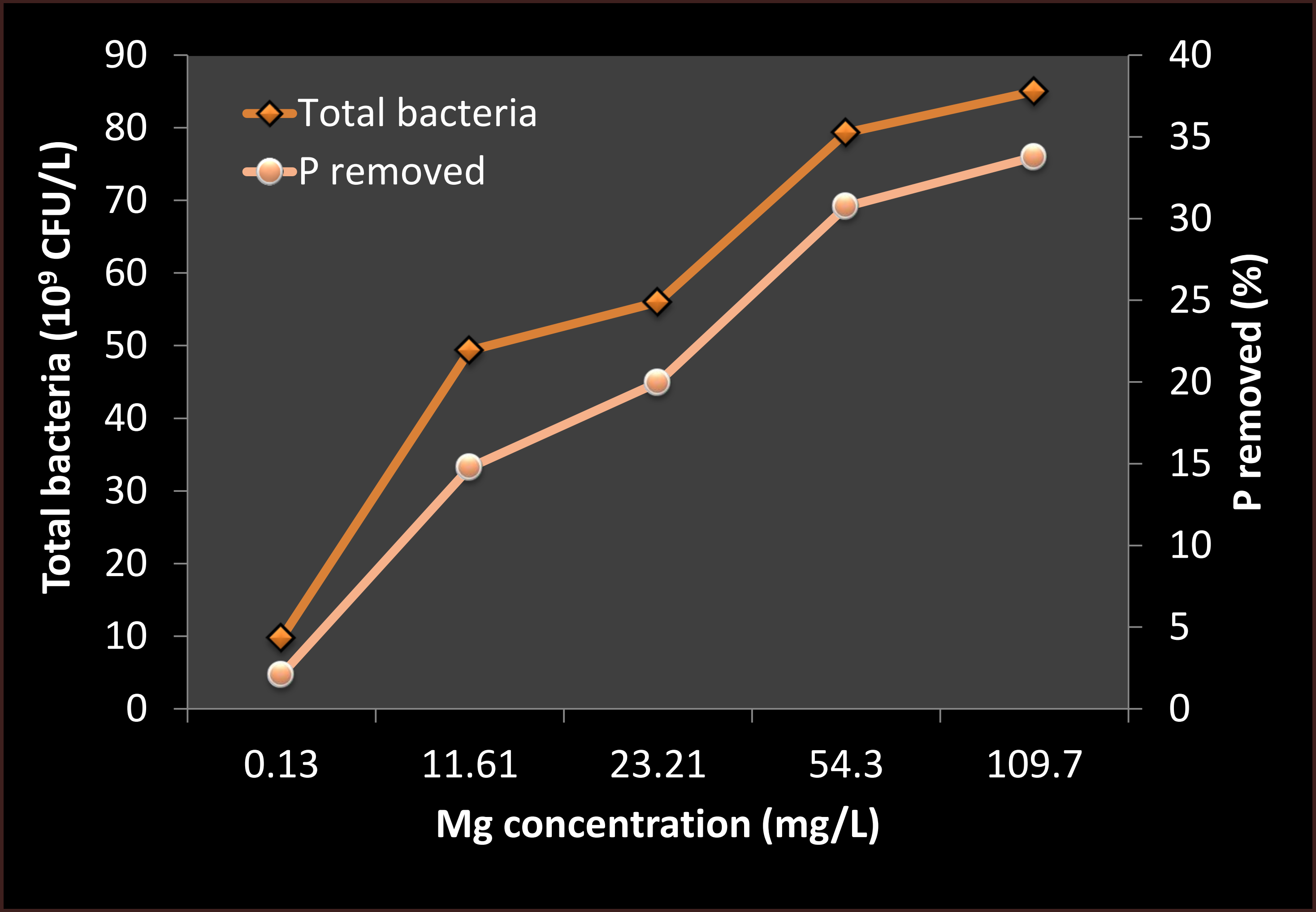
Cells of *A. junii* immobilized onto zeolitized tuffs originating from Croatia, Turkey and Serbia.



Number of immobilized bacteria *A. junii* and P removal in reactors containing zeolitized tuffs.

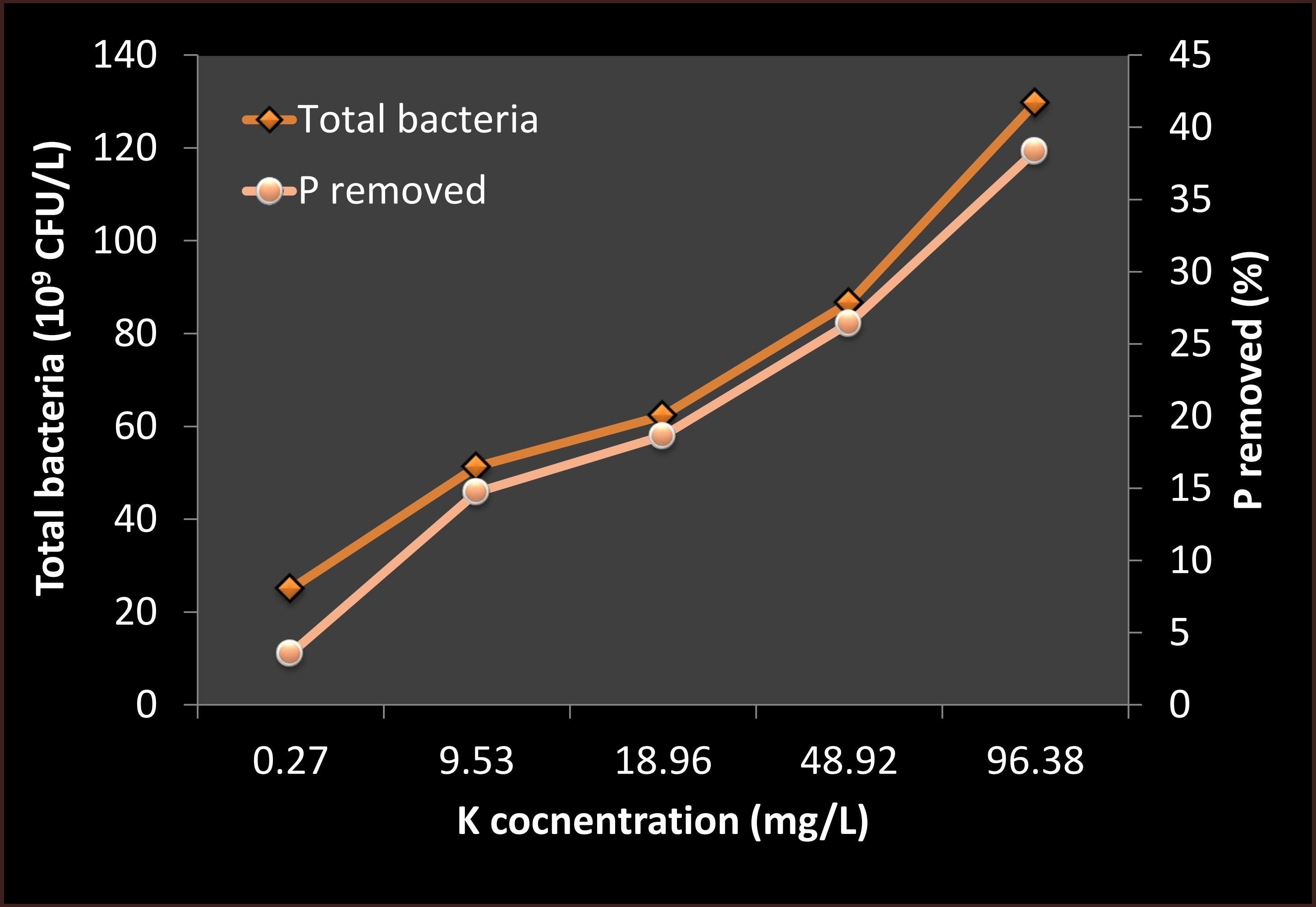
[c_0 CFU (10^6 CFU/mL)] = 12.52±2.03; [c_0 P-PO_4 (mg/L)] = 23.6±0.5.

Zeolitized tuffs modified with magnesium, calcium or potassium



Total number of bacteria *A. junii* and P removal from wastewater containing different concentrations of Mg.

Mg and K ions are important for the formation of biomass and the accumulation of polyP inside cells of PAB *A. junii*.



Total number of bacteria *A. junii* and P removal from wastewater containing different concentrations of K.

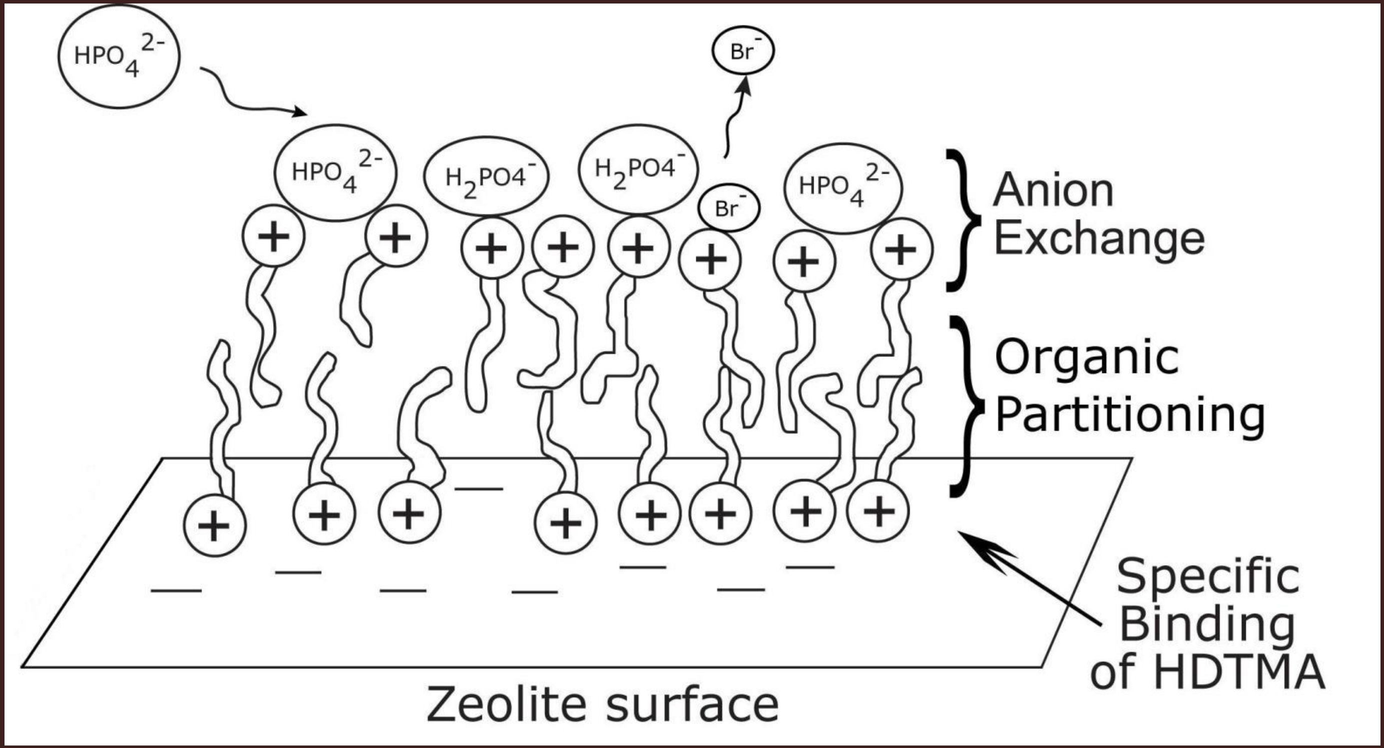
Zeolitized tuffs can serve as an additional source of Mg and K for bacteria.

Number of immobilized and total bacteria *A. junii* and P removal in reactors containing wastewater with shortage Mg, Ca and K (control) and different zeolitized tuffs.

Reactor	Immobilized bacteria (10^9 CFU/g)	Total bacteria (10^6 CFU/mL)	P removed (%)
Control	-	0	0.7
Turkey, Mg exchanged	9.61	139	98.5
Turkey, Ca exchanged	4.83	84	49.1
Turkey, K exchanged	7.67	113	52.7
Serbia, Mg exchanged	1.56	130	74.3
Serbia, Ca exchanged	0.93	66	33.6
Serbia, K exchanged	1.51	96	53.7

J Biosci. Bioeng. 2010, 110.

Zeolitized tuff modified with cationic surfactant hexadecyltrimethylammonium bromide (HDTMA)



J Hazard. Mater. 2008, 156.

Adsorption of HDTMA cations after the equilibration of zeolitized tuff in different HDTMA solutions.

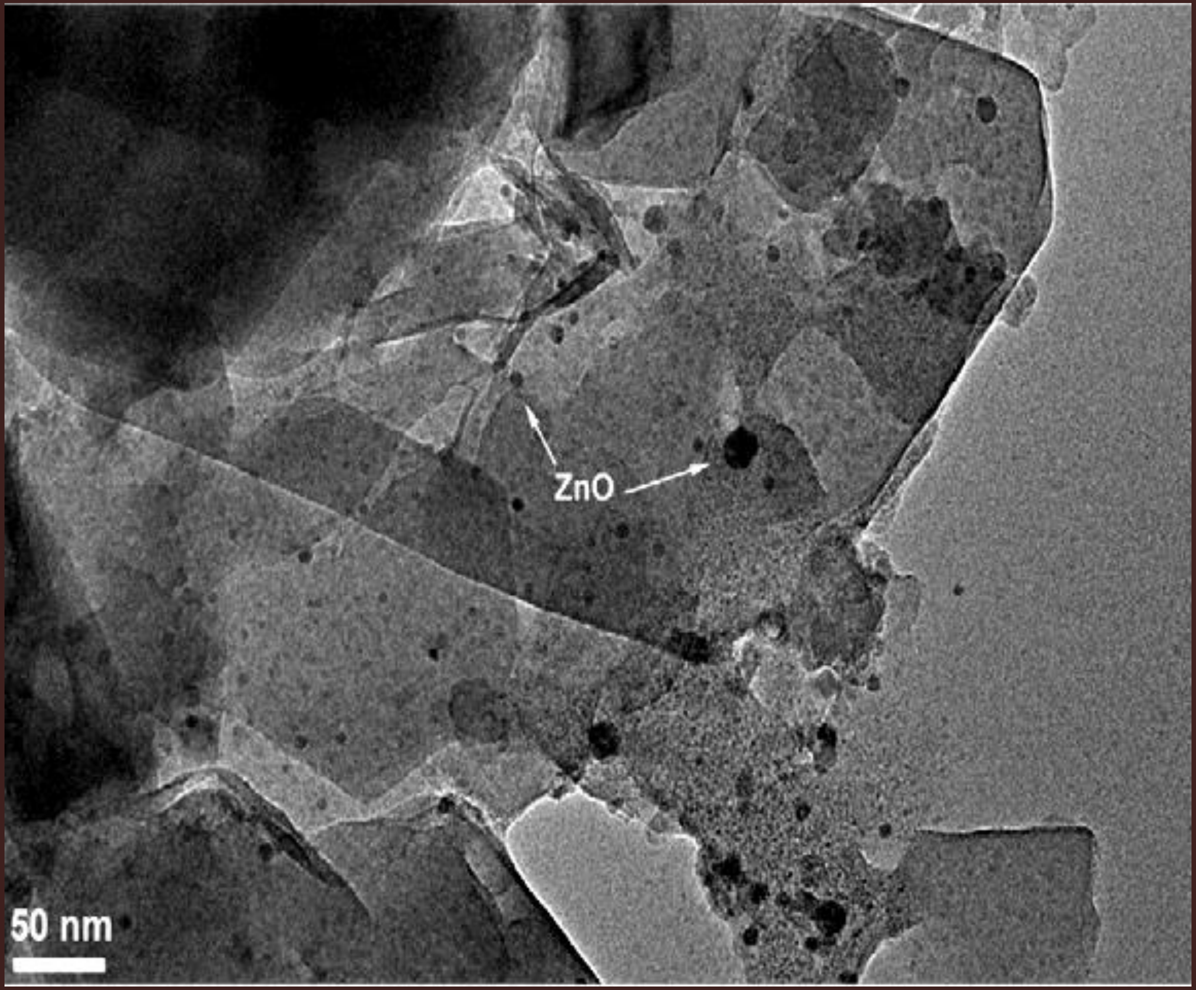
HDTMA adsorbed (mmol/kg)	ECEC occupied (%)	Zeta potential (mV)	Type of HDTMA coverage	Name of material
0.0	0.0	-18.2	none	NZ
22.6	18.8	-18.7	partial monolayer	1 SMZ
73.6	61.3	-15.2	partial monolayer	2 SMZ
123	102.5	3.9	monolayer	3 SMZ
164	136.7	22.4	partial bilayer	4 SMZ
204	170.0	26.1	bilayer	5 SMZ

Performance of reactors containing natural zeolite (NZ) or surfactant-modified zeolites (SMZ) with bacteria ([c_0 CFU (10^6 CFU/mL)] = 17.33; [c_0 P-PO_4 (mg/L)] = 22.2) and without bacteria [c_0 P-PO_4 (mg/L)] = 20.0.

Name of material	Immobilized bacteria (CFU/g)	Planktonic bacteria (CFU/mL)	Total bacteria (CFU/mL)	P removal with bacteria (%)	P removal without bacteria (%)
NZ	3.36×10^9	8.00×10^7	1.13×10^8	45.5	3.5
1 SMZ	5.28×10^9	1.17×10^8	1.69×10^8	63.4	7.6
2 SMZ	3.18×10^9	1.68×10^8	1.99×10^8	72.5	10.1
3 SMZ	7.40×10^2	1.40×10^2	1.47×10^2	14.8	13.0
4 SMZ	0.00	0.00	0.00	24.1	21.2
5 SMZ	0.00	0.00	0.00	32.8	32.3

Partial bilayer/bilayer HDTMA coverage showed bactericidal activity towards *A. junii*.

Zeolitized tuff modified with zinc



Zn(II) ions are adsorbed from the $ZnCl_2$ solution on the zeolitized tuff. By dehydration at 500°C the formation of nano-sized ZnO particles widespread over the zeolite surface occurs.

Performance of reactors containing *A. junii*, *A. junii* and zeolite modified with Zn^{2+} (NZZn), *A. junii* and zeolite modified with ZnO (NZZnO).
[c_0 CFU (10^{10}/L)] = 5.33±0.52; [c_0 P-PO_4 (mg/L)] = 21.1±0.7.
Bacteria in reactors with NZZn and NZZnO removed less than 0.06% of the initial P.

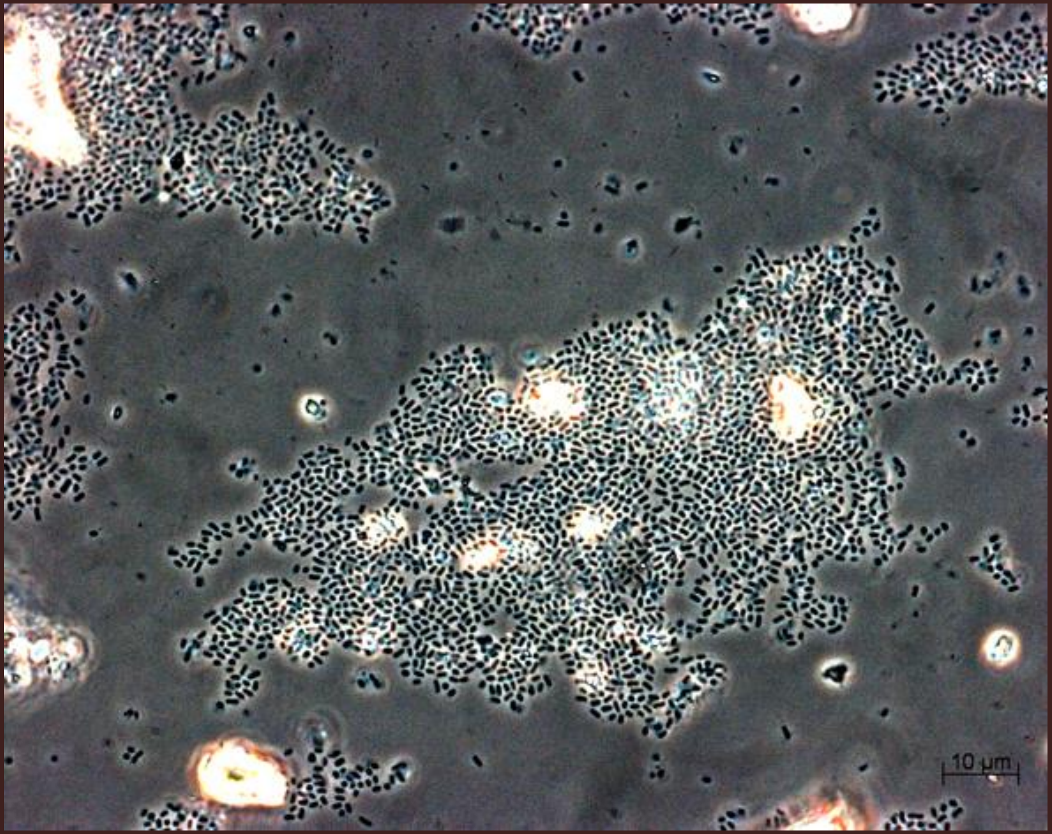
Reactor	Inhibition of bacteria (%)	P removal (%)
A. junii	0.00	38.2
NZZn	99.99	50.1
NZZnO	99.86	80.9

The Zn-modified zeolite can adsorb P from wastewater, but showed antibacterial activity towards *A. junii*.

J Hazard. Mater. 2011, 185.

Bentonite

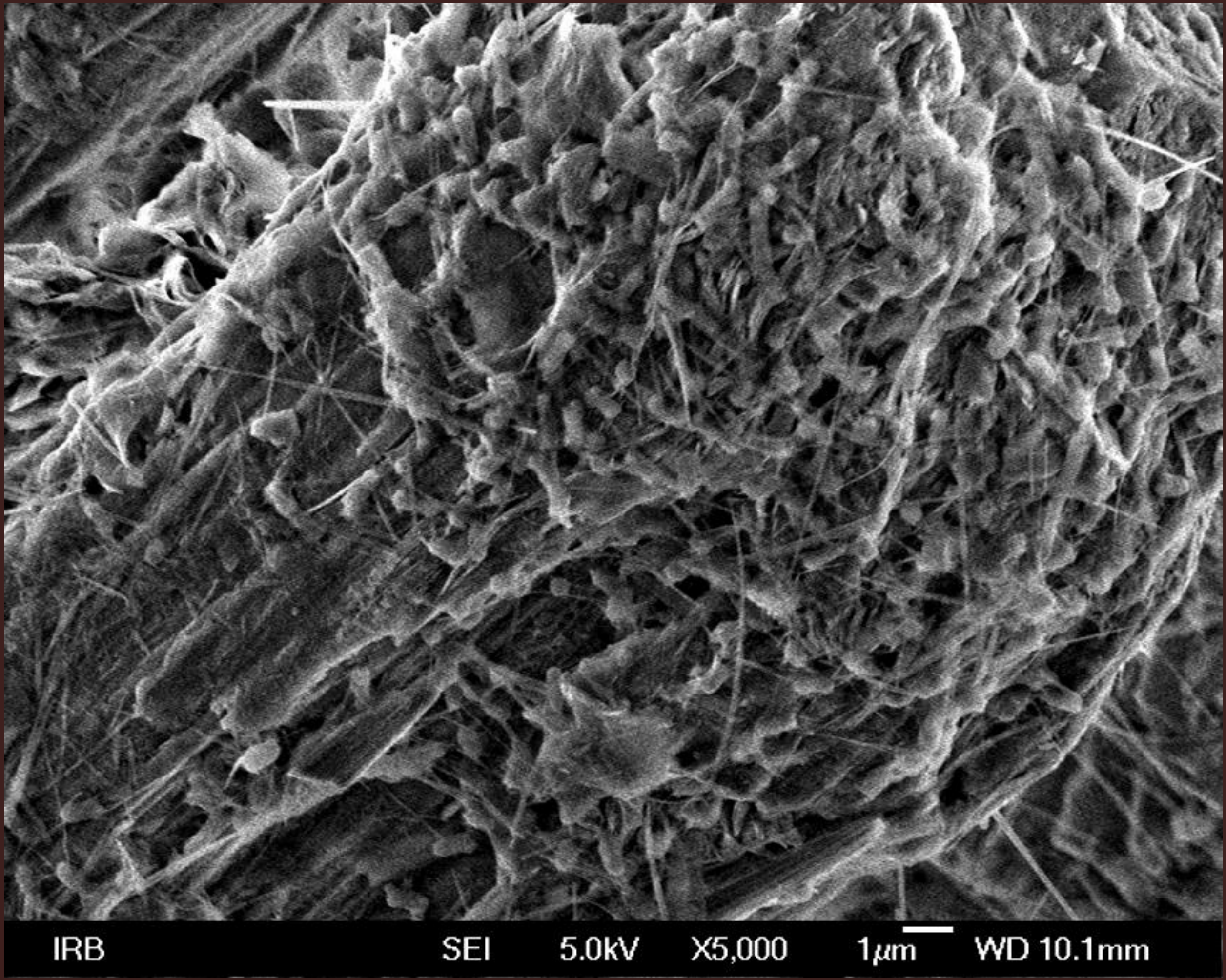
Type of bentonite	Immobilized bacteria (10^9 CFU/g)	Total bacteria (10^8 CFU/mL)	P removed (%)
Bentonite, original	5.82	1.39	46.9
Bentonite, modified with Mg	7.40	1.56	52.5



J Hazard. Mater. 2009, 116.

Sepiolite

Samples originating from China (50% of sepiolite) and Croatia (90% of sepiolite) were examined.



Cells of *A. junii* immobilized onto sepiolite.

Appl. Clay Sci. 2010, 50.

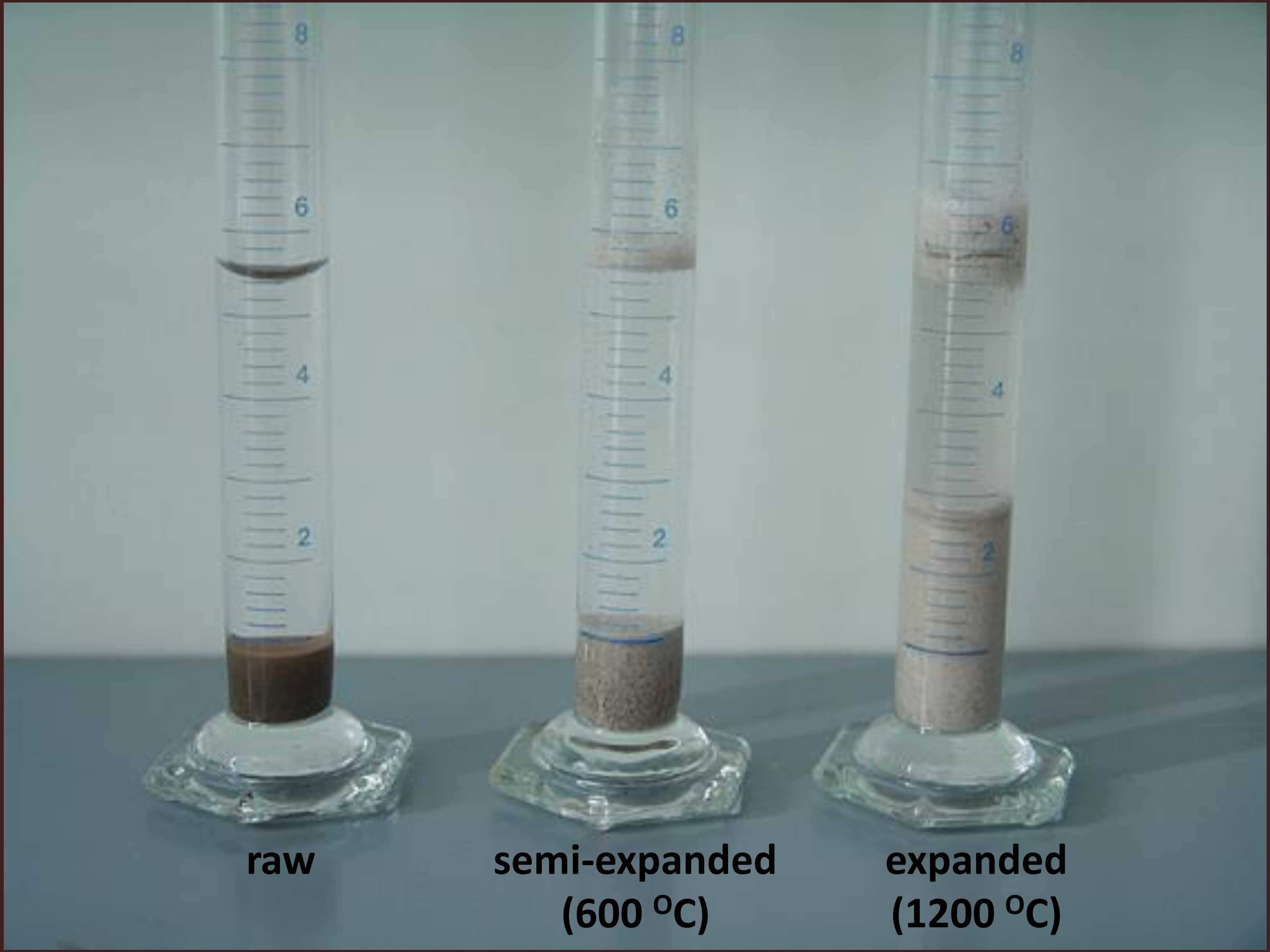
Performance of reactors containing *A. junii* only or *A. junii* and sepiolite from China.
[c_0 CFU (10^7/mL)] = 1.59±0.13; [c_0 P-PO_4 (mg/ L)] = 20.4±0.1.

Reactor	Immobilized bacteria (10^9 CFU/g)	Total bacteria (10^7 CFU/mL)	P removal (%)
A. junii	-	8.41	16.5
Sepiolite, China	5.57	9.93	28.4

Performance of reactors containing *A. junii* only or *A. junii* and sepiolite from Croatia.
[c_0 CFU (10^6/mL)] = 5.78±0.46; [c_0 P-PO_4 (mg/L)] = 18.2±0.3.

Reactor	Immobilized bacteria (10^9 CFU/g)	Total bacteria (10^7 CFU/mL)	P removal (%)
A. junii	-	12.4	25.4
Sepiolite, Croatia	5.60	41.2	55.4

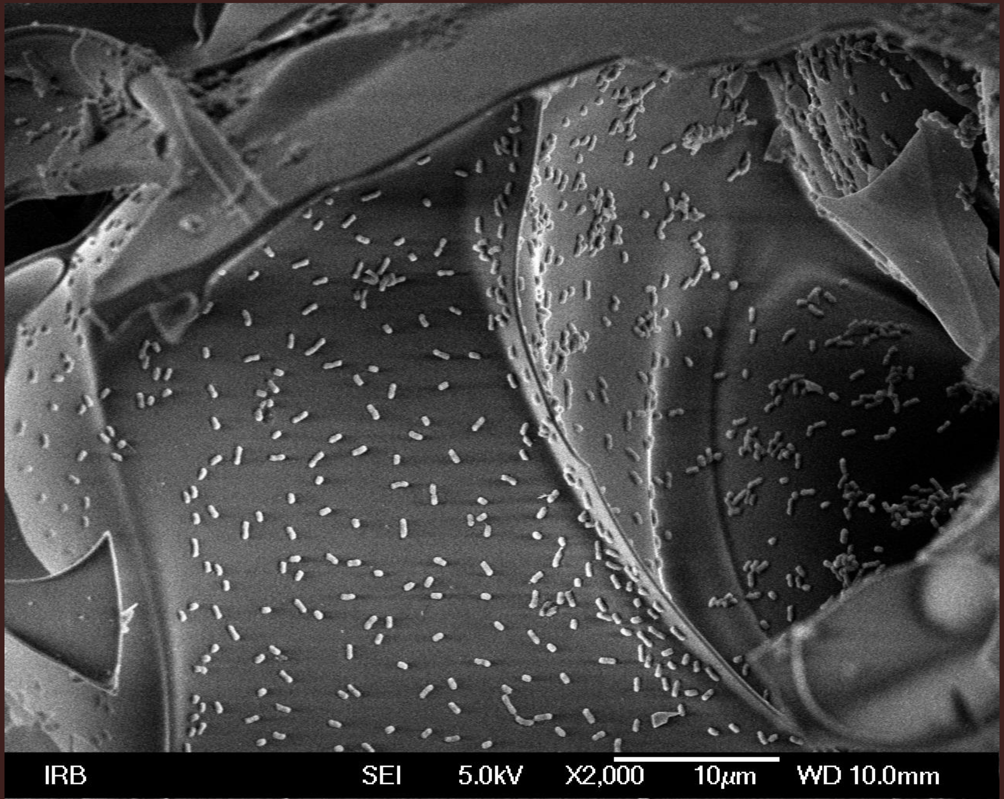
Volcanic glass



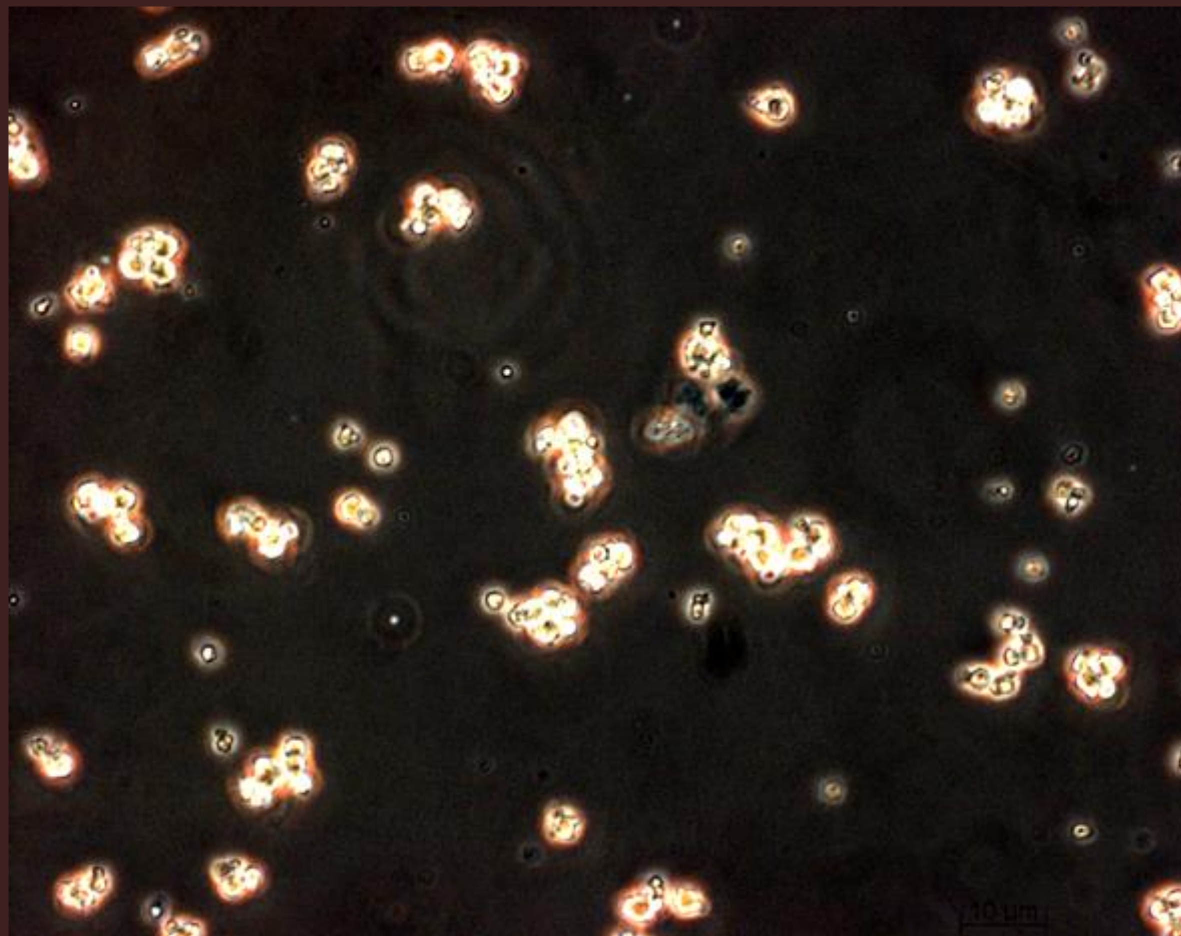
Biochem Eng J. 2010, 51.

Performance of reactors containing *A. junii* and three types of volcanic glass.
[c_0 CFU (10^7/mL)] = 5.27±0.57; [c_0 P-PO_4 (mg/ L)] = 23.9±0.1.

Type of volcanic glass	Immobilized bacteria (10^9 CFU/g)	Total bacteria (10^7 CFU/mL)	P removal (%)
Raw	1.68	10.9	24.79
Semi-expanded	5.57	13.2	32.85
Expanded (perlite)	12.65	15.3	40.97

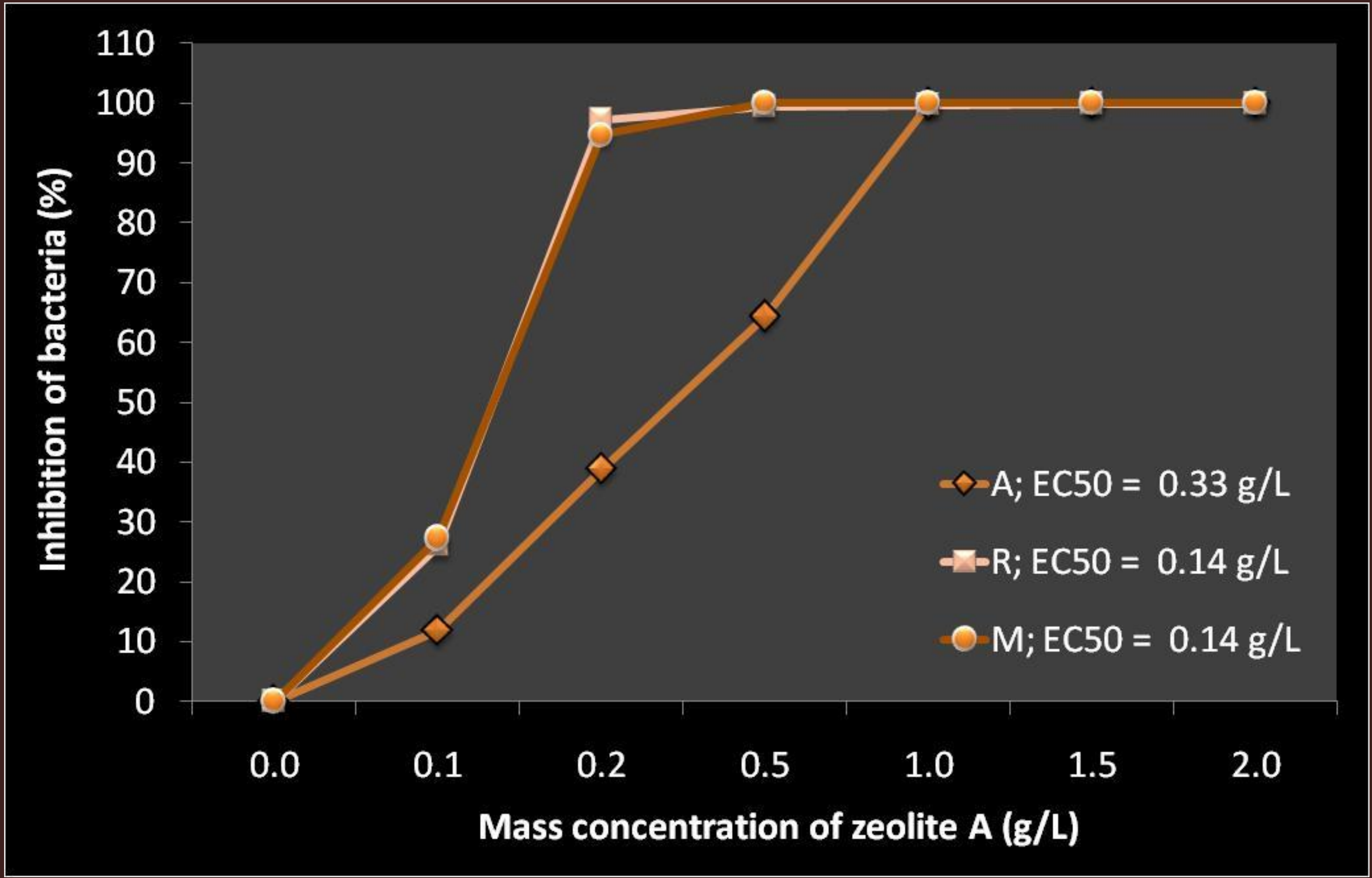


Commercial synthetic zeolite A



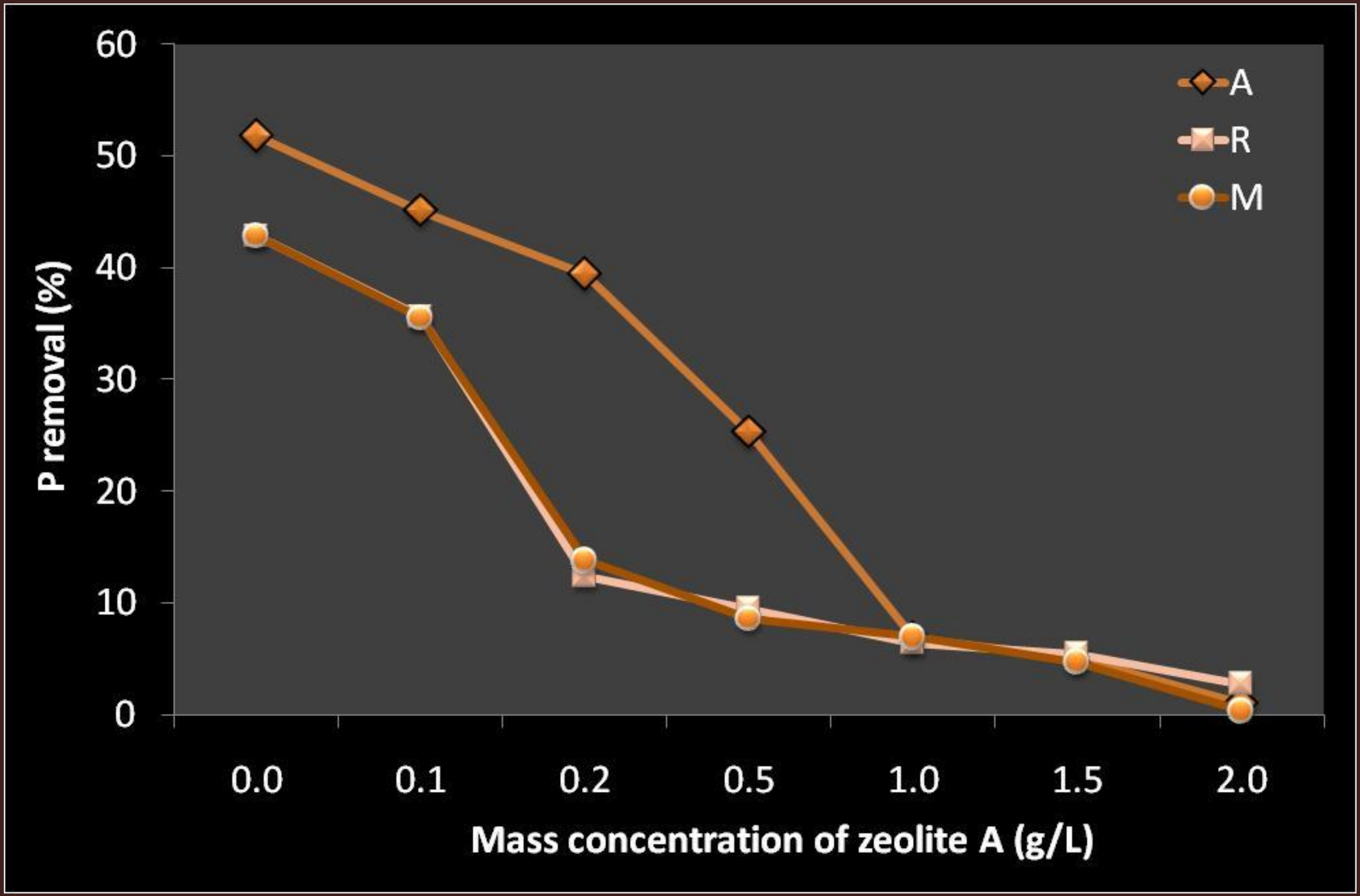
J Hazard. Mater. 2010, 183.

Commercial zeolite A showed toxic effect against bacteria *A. junii* at mass concentration of 0.1 g/L and higher.

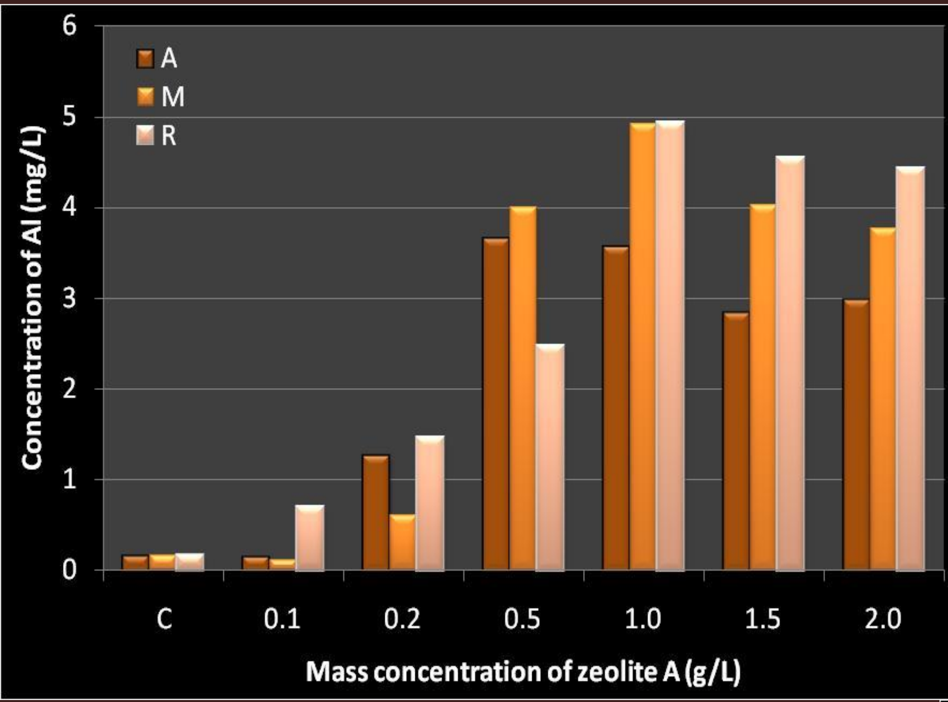


Toxicity of three samples of zeolite A against bacteria *A. junii* in wastewater.

Decay of bacteria *A. junii* caused by zeolite A results in the absence of P removal from synthetic wastewater.

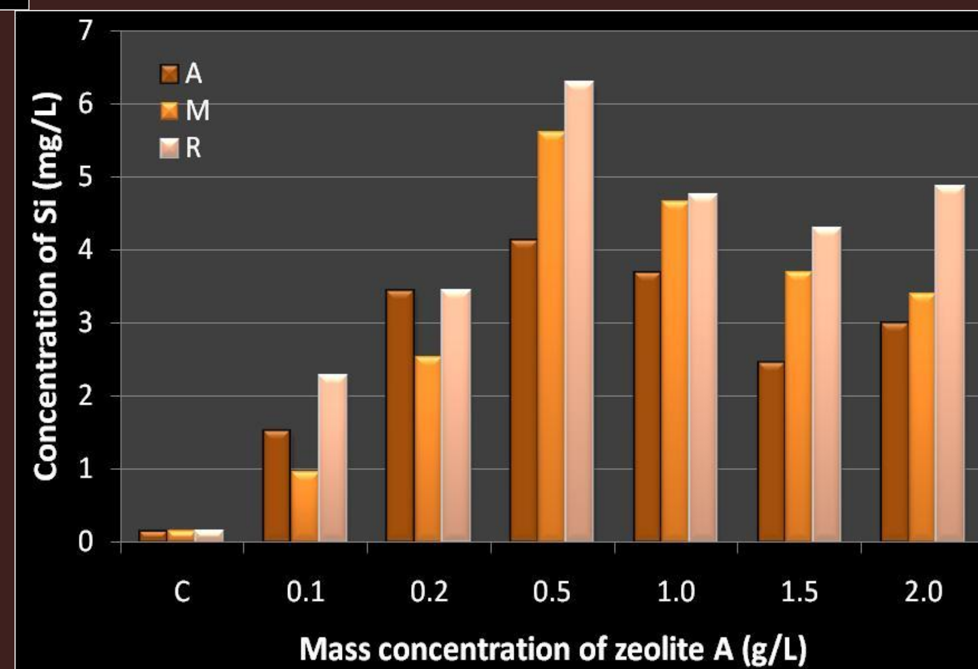


P removal from synthetic wastewater at different mass concentration of zeolite A.

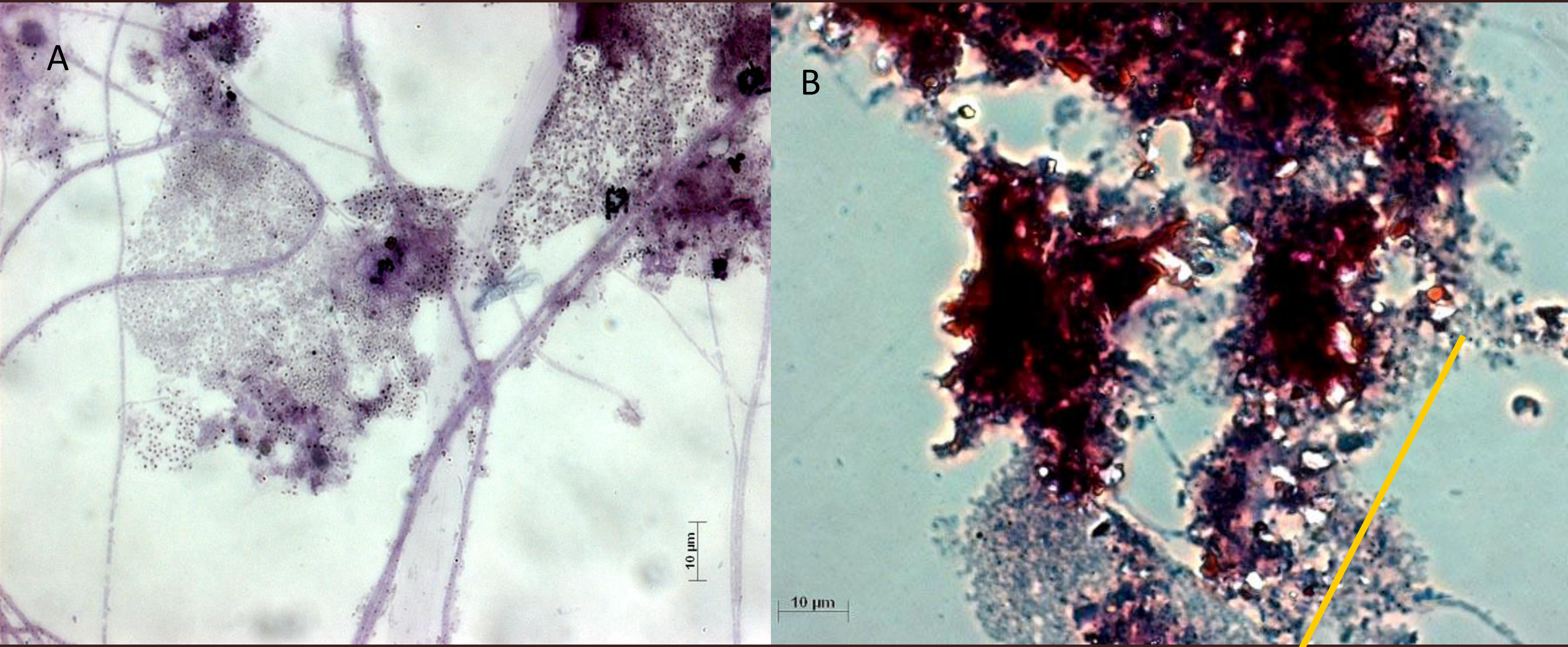


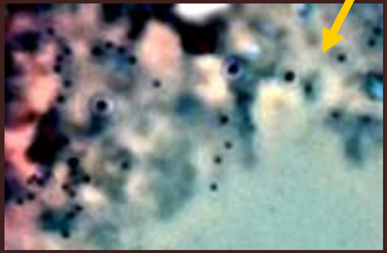
The toxic effect of zeolite A is caused by the hydrolysis of zeolite and consecutive leaching of aluminosilicate molecules $[Al_mH_nNa_pO_qSi_{3\text{-}5}]^{2+}$ and $[(Na(OH))_x(AlO(OH))_y(Si(OH)_4)_{3\text{-}5}]^{2+}$ in wastewater.

Concentrations of Al and Si in water released during the leaching of three samples of zeolite A and corresponding distilled water as negative control (C).



Incorporation of bioparticles (consisted of immobilized bacteria *A. junii* onto materials) in the activated sludge



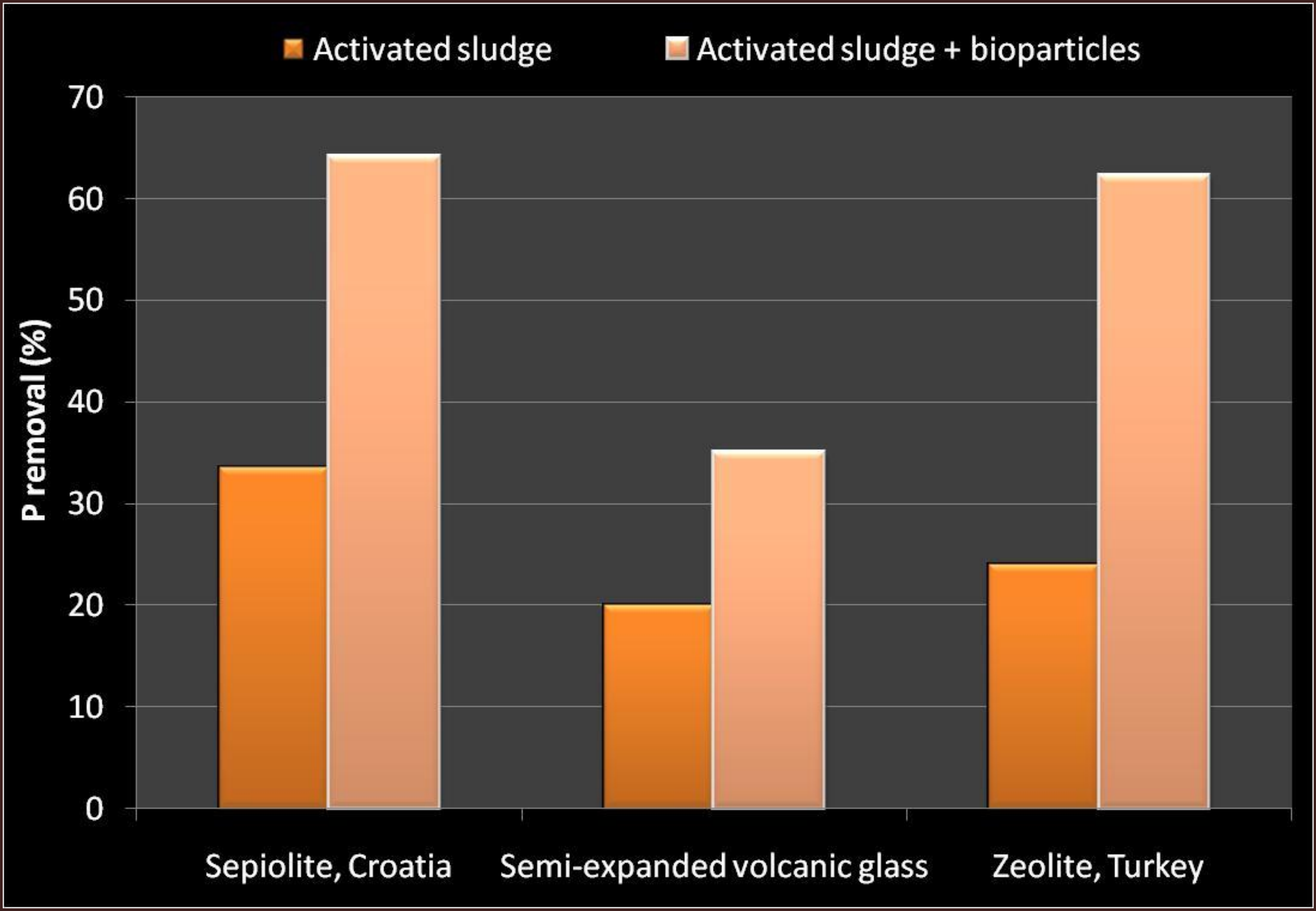


Blue granules of polyP in the activated sludge:

(A) Bioaugmented with *A. junii*

(B) Bioaugmented with *A. junii* immobilized onto natural zeolite tuff.

P removal from synthetic wastewater in reactors containing the conventional activated sludge and activated sludge bioaugmented with 1% of bioparticles (immobilized bacteria *A. junii* onto materials).



Number of immobilized bacteria *A. junii* on the particles of natural materials.

Material	Immobilized bacteria (10^9 CFU/g)
Quartz sand	3.01
Zeolite, Turkey	7.49
Zeolite, Croatia	4.84
Zeolite, Serbia	0.61
Bentonite	5.82
Sepiolite, China	5.57
Sepiolite, Croatia	5.60
Raw volcanic glass	1.68
Semi-expanded volcanic glass	5.57
Expanded volcanic glass (perilte)	12.65

Conclusion:

- Natural materials are good carriers of PAB *A. junii*, which are used for the purification of wastewater.

- Natural materials provide a high density of the metabolically active bacteria in bioreactors. This results in the enhanced P removal from wastewaters, when compared to the rectors without the addition of materials.

- Current research is focused on the implementation of immobilized bacteria in the technological process of wastewater treatment.

A. junii immobilized on perlite found application in constructed wetland ReVive (OneWell AB, Sweden): Installed for treatment of residential sewage and reuse of purified water.

Thank you for attention!