Interaction of surfactant-modified zeolites and phosphate accumulating bacteria

J. Hrenovic [a,*], M. Rozic [b], L. Sekovanic [c], A. Anic-Vucinic [d]

[a] *University of Zagreb, Faculty of Science, Rooseveltov trg 6, 10000 Zagreb, Croatia*
[b] *University of Zagreb, Faculty of Graphic Arts, Getaldiceva 2, 10000 Zagreb, Croatia*
[c] *University of Zagreb, Geotechnical Faculty, Hallerova aleja 7, 42000 Varazdin, Croatia*
[d] *University of Zagreb, Faculty of Mechanical Engineering and Naval Architecture,*
Ivana Lucica 5, 10000 Zagreb, Croatia

Abstract

The aim of this study was to determine the interaction of surfactant-modified zeolites (SMZ) and orthophosphate (P)-accumulating bacteria in the process of P removal from wastewater. The SMZ were prepared from the natural zeolite (NZ) of size fractions <0.122 mm and 0.25–0.5 mm. The hexadecyltrimethylammonium (HDTMA) bromide was used to modify the NZ surface from partial monolayer to the bilayer coverage. The surface modification of NZ resulted in the change of zeta potential of particles from negative to positive and great enhancement of the P-adsorption capacity. Only in reactors containing <0.122 mm fraction of partial monolayer coverage of the SMZ, the P was efficiently removed from wastewater by combined adsorption onto the SMZ and bacterial uptake in the biomass. The SMZ with bilayer or patchy bilayer coverage showed the bactericidal effect. To enhance the P removal from wastewater in the aerated biological system, the SMZ can be used, but the special attention should be given to the configuration of sorbed HDTMA molecules and its potential desorption.
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1. Introduction

The activated sludge treatment is the most common way of the purification of wastewaters. The process of enhanced biological phosphorus removal (EBPR) from wastewater is based on the accumulation of orthophosphate (P) in the cells of some bacterial strains present in the activated sludge. According to the recent metagenomic analyses [1], bacteria from the genus *Acinetobacter* are present as a minor component of activated sludge and P removal is carried out primarily by uncultivable species. However, bacteria from the genus *Acinetobacter* have become the model organism for EBPR since it was isolated from the P-removing activated sludge plant [2]. Although *Acinetobacter* spp. were present in extremely low number in the activated sludge plant, their capacity to remove P was the highest among all the P-accumulating isolates [3].

The low cost of natural zeolites (NZ), their superior hydraulic characteristics and high cation exchange capacities makes their use attractive in wastewater treatment. However, the NZ have negligible affinity for anions such as P [4,5]. Treatment of the NZ with cationic surfactants changes their surface chemistry, and the electrical charge reversal induced by the surfactant bilayer allows the retention of anions by ion exchange [6]. Such modified zeolites are commonly known as the surfactant-modified zeolites (SMZ). The SMZ sorb all major classes of water contaminants (anions, cations, organics and pathogens), thus making it amenable to a variety of water treatment applications [7].

There appears to be much opportunity for combining the SMZ adsorption of P with bacterial accumulation of P in order to improve the P removal from wastewater. Up to date, there is no effort to apply the promising SMZ in the EPBR process. The aim of this study was to determine the interaction of the SMZ and P-accumulating bacteria from the standpoint of basic science and possible application.

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* Corresponding author. Tel.: +385 14877700; fax: +385 14826260.
E-mail address: jasnah@zg.biol.pmf.hr (J. Hrenovic).

2. Material and methods

2.1. Bacterium

A P-accumulating bacterium *A. junii* (DSM, 1532) was obtained from the Deutsche Sammlung von Microorganismen und Zellkulturen GmbH [8].

2.2. Natural zeolite (NZ) and surfactant-modified zeolites (SMZ)

The NZ tuff from Bigadic, Turkey of two size fractions was used: <0.122 mm and 0.25–0.5 mm. The NZ sample consists of approximately 70% clinoptilolite, subordinate opal-CT and quartz (10–15% of each) and traces of K-feldspar and mica, as estimated by X-ray powder diffraction method by comparison with samples in which clinoptilolite content was determined by internal standard method [9]. No peaks belonging to clay minerals were observed on the recorded diffractogram. The total cation exchange capacity (CEC) of small fraction was determined by measurements of concentrations of exchangeable cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) after saturation of NZ with NH_4^+ ions [10]. The exchangeable cations were determined by atomic apsorption spectrometry (AA-6800, Shimadzu). The results indicated that the main exchangeable cation was Ca^{2+} (77.3 meq 100 g^{-1}). The total CEC of NZ was 134.7 meq 100 g^{-1}. The NZ was washed three times with the 300 mL of demineralised water and then dried at 105 °C in oven for 16 h before the experiments were to commence.

The cationic sufractant – quartery ammonium salt – hexadecyltrimethylammonium bromide, $C_{16}H_{33}N(CH_3)_3Br$ (HDTMA, Merck) was used to modify the NZ surface. The critical micelle concentration (CMC) of HDTMA at 30 °C was 0.94 mmol L^{-1} as estimated by measurements of aqueous HDTMA solutions of different concentrations. The HDTMA solutions were prepared with deionised water. The concentrations of HDTMA were from 0.477 to 9.900 mmol 100 mL^{-1} i.e. less and more than CMC. A 100.0 mL of the HDTMA solution and 2.000 g of the NZ were shaken on a mechanical shaker (Biosan OS-10) at 150 rpm during 48 h in thermostat (Memmert IPP400) at 30.0 ± 0.1 °C (above Kraft point which is 25 °C). Thereafter a 50 mL of each mixture was centrifuged (3000 rpm/5 min). The concentration of residual surfactant in the supernatant was determined as a content of total organic carbon (TOC) on Shimadzu TOC-5050A analyzer. The concentration of sorbed HDTMA (i.e. loading of external mineral surface) was calculated from the mass balance equation as follows:

$$Q_e = (c_0 - c_e)\frac{v}{m}$$

where c_0 and c_e are the initial and equilibrium liquid-phase concentrations of HDTMA solution (mmol L^{-1}), respectively; v the volume of surfactant solution (L), and m the mass of NZ (kg).

The obtained SMZ were filtered through the Büchner funnel and filter paper (blue band). The samples were washed with distilled water until negative reaction to Br^- ions was reached. The SMZ chosen for future experiment were loaded with the HDTMA of starting solution concentrations: 0.477, 1.535, 2.586, 3.665 and 5.187 mmol L^{-1}. The wet particles of the chosen SMZ were again washed 10 times in the membrane filtration system with the 300 mL of deionised water. The wet material was used for experiments. After experiments the particles were dried (105 °C/24 h) and all results were calculated per dry mass of SMZ.

2.3. Phosphate (P) adsorption capacity of NZ and SMZ

The P-adsorption capacity of the NZ and SMZ was determined by equilibrating material within a range (0, 5, 500, 5000 mg L^{-1}) of P solution made from KH_2PO_4, a method adapted from those used previously [8,11,12]. Erlenmeyer flasks containing 1.0 g of material, and a set of flasks with no material (blanks), were set up in triplicate, each with 100.0 mL of P solution. Two drops of chloroform were added in each flask to inhibit microbial growth. Flasks were shaken on a mechanical shaker (Biosan OS-10) at 200 rpm for 5 days in the thermostat (Memmert IPP400) at 25.0 ± 0.1 °C. A 10 mL of sample was taken from each flask in 24 h intervals. Samples were filtered through Whatman filter units of pore diameter 0.2 μm and supernatant was analysed for P. The time of equilibration when the P concentration in the supernatant becomes constant was 72 h. The P that disappeared from the solution was considered to have been adsorbed by the NZ/SMZ. Since the highest percent of the applied P was adsorbed at a lowest initial P concentration (5 mg L^{-1}), these data were used to calculate P-adsorption capacity [8,11,12]. The P retained by the material was taken to calculate the P-adsorption in mg P per kg of dry NZ/SMZ.

2.4. Zeta potential of SMZ and bacteria

The zeta potential of the NZ and SMZ was measured by using a Zetasizer 3000-Malvern Instruments, equipped with microprocessor unit. This unit automatically calculates the electrophoretic mobility of the particles and converts it to the zeta potential using the Smoluchowski equation. A 0.010 g of material of particle size <0.122 mm was added in 50 mL of distilled water. The particles were dispersed on a mechanical shaker (Biosan OS-10) at 200 rpm for 15 min. The samples were allowed to stand for 5 min to let larger particles to settle. An aliquot taken from the supernatant was used to measure the zeta potentials. The zeta potential of bacterium *A. junii* was measured as previously described [13]. The procedure was repeated three times for each measurement.

2.5. Simulative wastewater

The composition of the synthetic medium used to simulate the sewage was (in mg L^{-1} of distilled water): Na-propionate 300; peptone 100; $MgSO_4$ 10; $CaCl_2$ 6; KCl 30; yeast extract 20; KH_2PO_4 88 [14]. The pH of the simulative wastewater was adjusted to 7.00 ± 0.02 with 1 M NaOH or 1 M HCl before autoclaving (121 °C/15 min).

2.6. Experimental design

In order to define which part of P is removed by biological/NZ or SMZ process and which part by sorption onto NZ or SMZ particles, the experiment containing the simulative wastewater and different fractions of NZ or SMZ was firstly set up. In each 300 mL Erlenmeyer flask 1.0 g of the NZ or SMZ was added. The flasks were sealed with a sterile gum cap and thereafter aerobically agitated (70 rpm) in a water bath (Julabo SW23) controlled with a thermostat ($30.0 \pm 0.5\,^{\circ}$C). The aeration rate of $1\,\text{L}\,\text{min}^{-1}$ with filtered air was provided during the 24 h of experiment.

In order to examine the efficiency of biological/NZ or SMZ process, the bacteria were pre-grown on the nutrient agar (Biolife, Italy) for 20 h at $30.0 \pm 0.1\,^{\circ}$C. Thereafter the biomass was placed in sterile 0.3% NaCl and dispersed by shaking (2700 rpm for 2 min using the test tube shaker Kartell TK3S). One milliliter of dispersed biomass was inoculated into 100 mL of simulative wastewater. In each flask 1.0 g of the NZ or SMZ was added. The flasks were sealed with a sterile gum cap and thereafter aerobically agitated (70 rpm) in a water bath (Julabo SW23) at $30.0 \pm 0.5\,^{\circ}$C. The aeration rate of $1\,\text{L}\,\text{min}^{-1}$ with filtered air was provided during the 24 h of experiment. All experiments were carried out as triplicate tests.

2.7. Analytical methods

The pH-value of water was measured with WTW 330 SET. The water samples (75 mL) were filtered before the P and TOC measurements through Whatman filter units of pore diameter $0.2\,\mu\text{m}$. The P ($P\text{-}PO_4^{3-}$) concentration in water was measured spectrophotometrically in a DR/2500 Hach spectrophotometer by the ascorbic acid (Hach method 8048) or molybdovanadate (Hach method 8114) method. The HDTMA concentration was measured as a content of TOC on Shimadzu TOC-5050A analyser. When the HDTMA concentration was measured in simulative wastewater, the final TOC values were corrected for initial TOC values.

The number of viable bacterial cells was determined as colony-forming units (CFUs) grown on the nutrient agar after incubation at $30.0 \pm 0.1\,^{\circ}$C for 72 h. At the end of experiments the particles of NZ and SMZ were washed three times with 300 mL of sterile 0.3% NaCl, and cell counts were performed in order to determine the number of immobilised cells. Each carrier was aseptically placed in a tube containing 9 mL of sterile 0.3% NaCl, crushed with a sterile glass rod and dispersed by shaking (2700 rpm/10 min). This procedure, similar to [15] remove the immobilised cells so that they remained as individual cells on carrier (compared to the previous more than $10\,\mu\text{m}$ tick multi-layer cell coverage of material), as confirmed by microscopy. Suspension of immobilised cells prepared in this way was serially diluted (10^{-1} to 10^{-9}). Volumes of 0.1 mL were plated (spread plate method) onto nutrient agar. After incubation, the bacterial colonies were counted and reported as CFUs per one gram of dry NZ and SMZ. At the same time, the CFUs were performed on the supernatant in order to determine the number of free cells/mL of water. All measurements were done in

triplicate with mean values presented. The Neisser stain was performed to confirm poly-P granules in cells of *A. junii* and the Gram stain to confirm immobilization of the cells onto NZ and SMZ. Stained samples were examined using an inverted microscope (Axiovert 200 MAT, Carl Zeiss MicroImaging Inc.) at magnification of $1000\times$.

2.8. Data analysis

The results were statistically analysed using the Statistica program [16]. The results obtained for the reactors containing different materials were compared. Since the data were independent, ordinary Student's *t*-tests was performed. The null hypothesis tested by the analysis was that reactors with different SMZ and reactor with NZ showed no difference in performance. Results were considered significant at the 5% level ($p = 0.05$). The correlation between variables was estimated using the Pearson linear correlation.

3. Results and discussion

3.1. HDTMA sorption on NZ, zeta potential, P-adsorption on NZ and SMZ

The results of the determination of HDTMA sorption onto NZ are presented in Fig. 1. The obtained amount of the HDTMA sorbed on the NZ was depended on the HDTMA available in aqueous solution. The HDTMA sorbed on the NZ was somewhat higher when using the small fraction of the NZ compared to the large fraction. The maximum sorption of HDTMA was achieved in the solution of starting concentration $5.187\,\text{mmol}\,\text{L}^{-1}$. In this solution $204.0\,\text{mmol}\,\text{HDTMA}\,\text{kg}^{-1}$ was sorbed onto small fraction of NZ and $179.0\,\text{mmol}\,\text{HDTMA}\,\text{kg}^{-1}$ onto large fraction. According to the HDTMA sorption isotherms, the SMZ chosen for future experiment were loaded with the HDTMA from
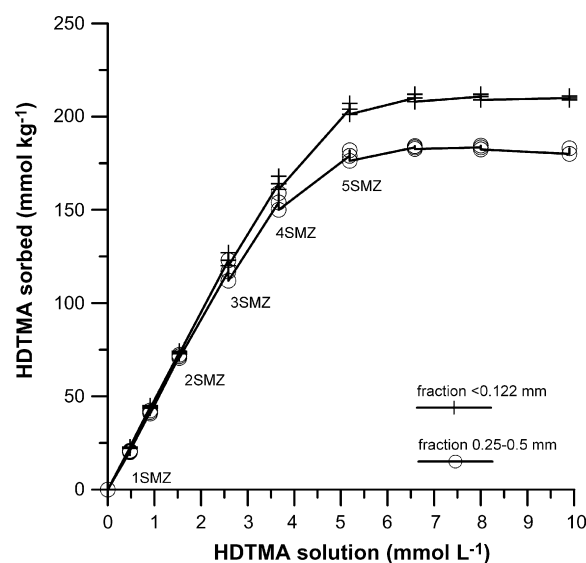

Fig. 1. The HDTMA sorption isotherms after equilibrating the different fractions of natural zeolite (NZ) in different HDTMA solutions.
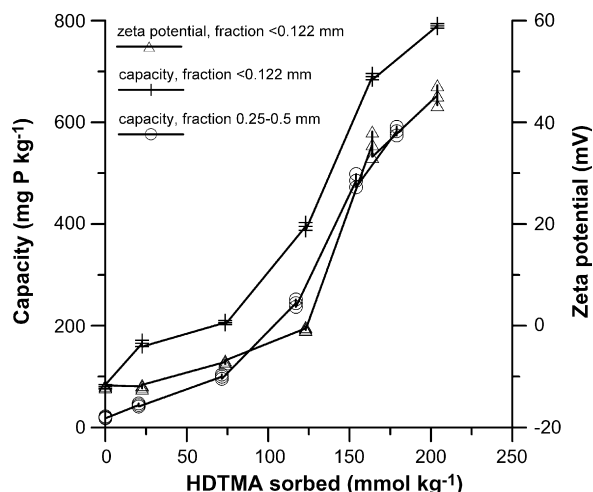
Fig. 2. Zeta potential and phosphate (P) adsorption capacity as a function of the HDTMA sorbed on different fractions of surfactant-modified zeolites (SMZ).

starting solution concentrations of: 0.477, 1.535, 2.586, 3.665 and 5.187 mmol L^{-1}. Samples were named as 1SMZ, 2SMZ, 3SMZ, 4SMZ and 5SMZ, respectively (Fig. 1).

The results of measurements of zeta potential for small fraction of NZ/SMZ are presented in Fig. 2. The zeta potential of 1SMZ stayed negative as that of NZ, probable because of low concentration of formed hemimicelles in relation to the number of active exchangeable positions on the outer surface of NZ [17]. The surface modification of the NZ with higher HDTMA loadings resulted in the change of zeta potential of particles from negative to positive. Isoelectric point or the zero point of charge is characterised by the value zero for electrokinetic potential. Sorption above this level is expected to be in the form of a bilayer or patchy bilayers, while sorption below this point is likely in the form of monomers or hemimicelles [18]. As can be estimated on zeta potential, 1 and 2SMZ have a partial monolayer, 3SMZ a monolayer, 4SMZ a partial bilayer, and 5SMZ a bilayer HDTMA coverage. The zeta potential of large fraction of SMZ was not

measured due to the technical limitation of instrument for measurement of large particles. The zeta potential of the pure culture of *A. junii* after 24 h of incubation (-18.4 ± 1.8 mV) becomes significantly ($p < 0.05$) more negative (-21.3 ± 1.1 mV) during the prolonged 48 h incubation. The cell surface charge of *A. junii* is comparable to subpopulation 1 of *Enterococcus faecalis* [13], but less negative than subpopulation 2 of *E. faecalis* [13] or *Pseudomonas putida* [19].

The P-adsorption capacity of the NZ was greatly enhanced by surface modification with HDTMA (Fig. 2). The higher the HDTMA sorbed, the higher was the effect. The P-adsorption capacity of the small SMZ fraction was higher than that of the large fraction, which is in agreement with the reported negative correlation of the P-adsorption capacity and particle size of materials [14]. The estimated P-adsorption capacity of the small SMZ fraction showed linear correlation ($r = 0.979, p < 0.05$) with the zeta potential of particles.

3.2. Experiment with simulative wastewater and NZ/SMZ

Little is known about how NZ and SMZ would behave in a complex medium. In mixed solutions of sulphate, nitrate and P competitive anion sorption was reported for P sorption onto the SMZ [5]. In the system containing the simulative wastewater, NZ or SMZ and bacteria numbers of competitive interactions are expected. We supposed that the P would be removed from simulative wastewater by combined adsorption onto NZ or SMZ and bacterial uptake in the biomass. In order to define which part of P is taken by bacteria and which part by NZ or SMZ particles, the experiment containing the simulative wastewater and different fractions of NZ and SMZ, but without bacteria was set up. The results (Table 1, column 7) showed that the NZ and SMZ addition resulted in the P removal from wastewater, but in variable extent. The amount of P removal was higher when using small fraction of SMZ and increases with increasing the HDTMA loading on SMZ. The amounts of P removed by NZ and SMZ addition were used for corrections of bacterial uptake

Table 1

Performance of reactors containing different fractions of natural zeolite (NZ) or surfactant-modified zeolites (SMZ) with bacteria (number of immobilised, free and total viable cells (CFU), phosphate (P) uptake rate per total CFU, percent of P removal; [c_0 CFU (10^6 CFU mL^{-1})] = 15.29 ± 5.52; [c_0 P-PO$_4$ (mg L^{-1})] = 21.82 ± 0.62) and without bacteria (percent of P removal; [c_0 P-PO$_4$ (mg L^{-1})] = 23.20 ± 1.03)

Material, fraction	Immobilised cells (CFU g^{-1})	Free cells (CFU mL^{-1})	Total cells (CFU mL^{-1})	P-uptake rate (mg P CFU^{-1})	P removal (%) with bacteria	P removal (%) without bacteria
Fraction <0.122 mm						
NZ	3.36 ± 0.27 × 10^9	8.00 ± 1.41 × 10^7	1.13 ± 0.17 × 10^8	1.12 ± 0.02 × 10^{-10}	45.50 ± 8.11	3.50 ± 0.17
1SMZ	5.28 ± 0.16 × 10^9	1.17 ± 0.11 × 10^8	1.66 ± 0.13 × 10^8	1.00 ± 0.06 × 10^{-10}	63.41 ± 8.62	7.61 ± 0.38
2SMZ	3.18 ± 0.15 × 10^9	1.68 ± 0.22 × 10^8	1.96 ± 0.24 × 10^8	8.14 ± 0.15 × 10^{-11}	72.45 ± 9.33	10.07 ± 0.74
3SMZ	7.40 ± 1.15 × 10^2	1.40 ± 0.26 × 10^2	1.47 ± 0.31 × 10^2	0.00 ± 0.00	14.76 ± 0.89	13.02 ± 0.87
4SMZ	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	24.14 ± 1.01	21.19 ± 0.36
5SMZ	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	32.76 ± 1.12	32.33 ± 1.54
Fraction 0.25–0.5 mm						
NZ	2.27 ± 0.14 × 10^9	1.20 ± 0.12 × 10^8	1.44 ± 0.10 × 10^8	1.07 ± 0.03 × 10^{-10}	65.00 ± 4.00	0.58 ± 0.08
1SMZ	9.87 ± 0.19 × 10^8	2.32 ± 0.65 × 10^7	3.49 ± 0.85 × 10^7	1.14 ± 0.07 × 10^{-10}	18.52 ± 7.45	0.96 ± 0.06
2SMZ	1.70 ± 0.55 × 10^3	2.00 ± 1.05 × 10^2	2.20 ± 1.11 × 10^2	0.00 ± 0.00	3.56 ± 1.35	1.71 ± 0.10
3SMZ	0.00 ± 0.00	4.00 ± 2.52 × 10^1	4.00 ± 2.52 × 10^1	0.00 ± 0.00	10.70 ± 2.40	8.06 ± 0.54
4SMZ	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	21.30 ± 3.38	17.66 ± 0.16
5SMZ	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	27.40 ± 3.77	23.50 ± 0.56

of P in the future experiments containing the NZ or SMZ and bacteria.

3.3. Experiment with simulative wastewater, NZ/SMZ and bacteria

By the adsorption of P onto SMZ great amount of P can be removed from wastewater. But, the maximum capacity of SMZ will be reached and there will be no further retention [7]. Therefore, there is a need to transform/degrade the soluble P present in the wastewater by other mechanisms. The P-accumulating bacteria are effective in removal of the P from wastewater by the transport of extracellular present P into the cell and its conversion to the nonsoluble intracellular poly-P [1–3,8]. Currently attention is being drawn to the immobilization of P-accumulating bacteria onto a suitable material in order to achieve a higher cell density in bioreactors, and as a result, a better efficiency of the wastewater treatment process [8,14].

After 24 h of bacterial cultivation in reactors containing the NZ or SMZ, very different interactions were observed (Table 1, columns 2–6). With the small fraction (Table 1, row 2–8) of the NZ and 1, 2 and 3SMZ one part of the total cell population was immobilised (Table 1, column 2) onto carriers by adsorptive growth while the other part of the bacteria remained as free cells (Table 1, column 3). With the 4SMZ and 5SMZ no bacterial growth was detected. The highest number of immobilised cells obtained with 1SMZ (5.28×10^9 CFU g^{-1}) is somewhat lower than the reported 6.86×10^9 CFU g^{-1} of *A. calcoaceticus* immobilised onto the magnesium-exchanged NZ [14], but higher than 2.9×10^9 CFU g^{-1} of *Acinetobacter* spp. immobilised onto ceramics by the vacuum method [20], 2.5×10^8 CFU g^{-1} of *A. johnsonii* cells immobilised inside alginate beads [21] or 9×10^8 CFU g^{-1} of *P. aeruginosa* immobilised onto a type-Z carrier consisting of silica alumina matrix containing zeolite [15]. The total number of viable cells (Table 1, column 4) was higher in reactors containing 1 and 2SMZ than in the reactor containing NZ. In contrast, decay of bacteria was observed in reactors containing 3 and higher HDTMA loading of SMZ. The P-uptake rates per total CFU of *A. junii* (Table 1, column 5) were on average the highest in reactors containing the NZ, which was insignificantly ($p > 0.05$) higher than 1SMZ and significantly ($p < 0.05$) higher than 2 and higher HDTMA loading of SMZ. The Neisser stain followed by microscopy revealed the presence of poly-P granules in the cells of *A. junii*. It is obvious that more active bacteria can take up more P. Therefore, the significantly higher ($p < 0.05$) percent of P removal was achieved in reactors with 1 and 2SMZ than in the reactors containing NZ (Table 1, column 6). In reactors containing 3 and higher HDTMA loading of the SMZ no enhancement of P removal was achieved when compared to the reactors containing no bacteria (Table 1, column 7).

The large fraction (Table 1, row 9–15) of the SMZ showed in general poorer performance than the small fraction. In the reactors containing the large fraction of NZ more net P was removed than in the reactors containing the small fraction of the NZ due to the higher final number of total cells, but the P-uptake rates per total CFU remained comparable. The addition of even 1SMZ

resulted in the decay of cells, although the remained live bacterial cells showed P-uptake rates comparable to those obtained with NZ. In reactors containing the 2 and higher HDTMA loadings of the SMZ almost complete decay of cells was observed, and no enhancement of P removal was achieved in comparison to the reactors containing no bacteria (Table 1, column 7). Therefore, it can be summarised that only in reactors containing the small fraction of 1 and 2SMZ and large fraction of 1SMZ the P was removed from wastewater by combined adsorption onto the SMZ and bacterial uptake in the biomass.

The final pH-values were the highest in the NZ supplemented reactors (7.54 ± 0.03) and decreased (from 7.44 to 6.70) in the SMZ supplemented reactors without the difference in the size of particles. The pH decrease was a function of the decay of bacteria in reactors containing the SMZ.

3.4. Possible influence of SMZ on bacteria

The microorganisms charged negatively in general, the easiest adhere to the minerals that have a rough and positively charged surface [22]. The amount of *P. putida* adsorbed by colloidal clay minerals was in the order of goethite, kaolinite and montmorillonite, suggesting that the electrostatic properties of mineral surfaces play a vital role in the adsorption of bacteria [19]. The maximum immobilization of cells obtained in this study (5.28×10^9 CFU g^{-1}; Table 1, column 2) is achieved with the small fraction of 1SMS, which had the negative zeta potential as the NZ (Fig. 2). Moreover, the 4SMZ and 5SMZ which had the positive zeta potential did not show immobilization of viable or dead cells, as confirmed by microscopy. Exception of dead cells is explained with the ability of HDTMA to destroy the cell membrane. These suggest that in the case of SMZ mechanisms other than surface charge of the particles plays a role in the process of successful immobilization of cells.

In the preliminary 24 h experiments [23], the estimated EC$_{50}$ values of the aqueous HDTMA for the inhibition of CFUs in the pure culture of *A. junii* was $3.27 \pm 1.12 \times 10^{-7}$ mol L^{-1} and for the inhibition of the P-uptake rates $2.47 \pm 0.51 \times 10^{-6}$ mol L^{-1}. The complete inhibition was observed by the HDTMA concentration of 10^{-5} mol L^{-1} and higher. These results proved a high acute toxicity of the free HDTMA against *A. junii*. In the constructed wetland containing 10 cm radial pacing of the SMZ 100% of *Escherichia coli* was removed from the sewage influent [7].

The HDTMA sorbed onto the NZ is subjected to desorption, depending on the initial surfactant loading, a type of mineral substrate and a flow rate [24]. The HDTMA desorption is greater from the SMZ modified to bilayer coverage compared to the monolayer coverage. In the batch experiments a desorption of bilayered HDTMA from the SMZ averages 0.6 and 1.8 mmol L^{-1} [24]. The potential desorption of HDTMA molecules from SMZ, especially those adsorbed by weak forces onto monolayer via hydrophobic alkyl chains interaction, can result in the formation of toxic unbound HDTMA molecules. The experimental data on the HDTMA release form SMZ in the simulative wastewater (Fig. 3) demonstrate that no release occurred in reactors containing NZ and 1SMZ. The HDTMA
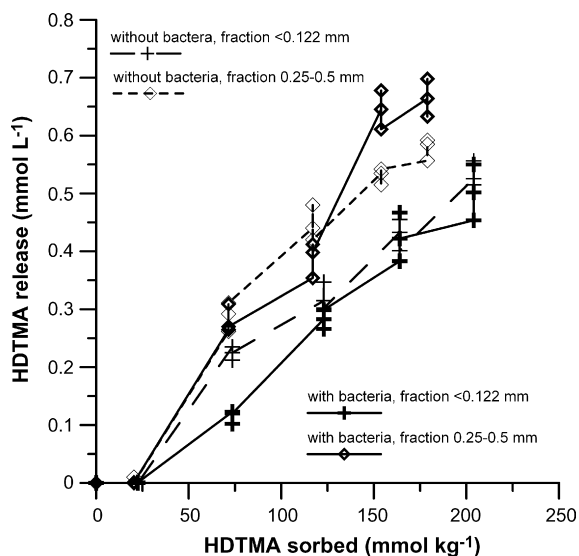
Fig. 3. The HDTMA release from different fractions of surfactant-modified zeolites (SMZ) in experiments without and with bacteria.

release increased by increasing the HDTMA loading on SMZ and was higher when using large fraction of SMZ rather than small fraction of SMZ. The higher HDTMA release from large fraction of SMZ can be explained by lower number of active ion exchangeable positions and as a result lower electrostatic force between the SMZ particles and HDTMA cations in comparison to the small fraction of SMZ. The maximum HDTMA release was observed with the large fraction of 4SMZ and 5SMZ and these values are comparable to Li et al. [24]. These observations can explain the toxicity of the higher HDTMA loadings of SMZ against *A. junii* due to the desorption of HDTMA from SMZ during 24 h in the well aerated and mixed system. A desorption of the HDTMA from the SMZ may be safely ignored for certain applications, such as the permeable barrier for the filtration of groundwater or leachate by low flow-rate conditions [7]. Therefore, a suggested method which will probable protect the HDTMA release from SMZ can be the reduction of turbulence in reactor.

The positively charged polar head of the HDTMA plays a role in the bactericidal action of this compound. It is likely that the SMZ particles with bilayer HDTMA coverage act bactericidal itself.

Although the HDTMA in aqueous solution is bactericidal, if adsorbed onto the SMZ, *A. junii* can remain viable. The toxicity of the HDTMA against bacteria is greatly reduced or eliminated if the surfactant is bound to zeolite [7]. In this case P removal from wastewater can be achieved by the combined P-adsorption onto SMZ particles and bacterial uptake of P in the biomass.

4. Conclusions

The SMZ can be used to enhance the P removal from wastewater in the aerated biological system, but the special attention should be given to the configuration of sorbed HDTMA molecules and its potential desorption. The SMZ with bilayer or patchy bilayer HDTMA coverage showed the bactericidal

effect. The efficient P removal from wastewater can be achieved by the addition of SMZ with partial monolayer HDTMA coverage by the combined mechanisms of P-adsorption onto SMZ and bacterial uptake of P in the biomass.

Acknowledgements

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