ZEOLITIZED TUFF AS A CARRIER OF BACTERIA

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INTRODUCTION

There is a growing interest to immobilize desired bacteria onto inexpensive materials as bacterial carriers. Bacterial carriers can find wide application, such as additive in order to improve the efficiency of wastewater treatment process. Usage of bacterial carriers in bioreactors can result in a higher cell density and based on this, smaller reactors, shorter retention time or higher flow rates can be employed. Immobilisation of microorganisms has been investigated using different synthetic and natural materials, such as alginate, ceramics and zeolitized tuff [1-7]. Natural zeolite has been shown as a promising material for the immobilisation of microorganisms due to its roughness, large surface area and high porosity.

The extent of bacterial immobilization depends on the particle size of zeolite tuff [5-7], but it also varies when using different zeolite tuffs of the same particle size. The aim of this work was to elucidate the crucial factors which determine the extent of bacterial immobilization onto different zeolite tuffs.

EXPERIMENTAL

Three types of natural zeolitized tuffs of particle size <0.125 mm, originating from Croatia, Turkey and Serbia were examined. The quantitative chemical composition of zeolitized tuffs was determined by classical chemical analysis. The mineralogical composition was estimated by X-ray powder diffraction method by comparison with samples in which clinoptilolite content was determined by internal standard method. Zeolite samples were washed with demineralised water and then sterilized by drying at 105°C/16h before the experiments were to commence.

The cation exchange capacity (CEC) of zeolitized tuffs was determined by measurement of equilibrium concentrations of exchangeable cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) in the supernatant by atomic absorption spectrometry (AA-6800, Shimadzu) after saturation of the samples with $[NH_4]^+$ ions [8]. The zeta potential of particles was measured using the Zetasizer 3000-Malvern Instruments, which automatically calculates the electrophoretic mobility of the particles and converts it to the zeta potential using the Smoluchowski equation. The phosphate adsorption capacity of each carrier was determined by equilibrating a material within a range (0, 5, 50, 100, and 500 mg/L) of phosphate solutions made from KH_2PO_4 [7].

In experiments the pure culture of phosphate-accumulating bacterium *Acinetobacter junii* (DSM 1532), which was isolated from the activated sludge showing the enhanced biological phosphate removal from wastewater, was used. The composition of the synthetic wastewater was as follows (in mg/L): Na-propionate 300; peptone 100; $MgSO_4$ 10; $CaCl_2$ 6; KCl 30; yeast extract 20; KH_2PO_4 88. The pH of the synthetic wastewater was adjusted to 7.0 before autoclaving (121°C/15 min).

The bacterial biomass was suspended in 100 mL of synthetic wastewater and in reactors 1.0 g of zeolitized tuff was added. The reactors were sealed with a sterile gum cap and thereafter aerated with filtered air (1 L/min) and agitated (70 rpm) in a water bath at 30°C during 24h. The phosphate ($P\text{-}[PO_4]^{3-}$) concentration in the synthetic wastewater was measured spectrophotometrically in a DR/2500 Hach spectrophotometer by molybdovanadate method (Hach method 8114). The number of cells of *A. junii* was determined as colony-forming units (CFU) grown on nutrient agar after 24 h of incubation at 30°C [7].

RESULTS AND DISCUSSION

Results of the quantitative chemical analysis of the studied zeolitized tuffs are given in Table 1. The mineralogical composition (Figure 1, Table 2) showed that all three samples are clinoptilolite rich tuffs.

Table 1. Chemical analysis of zeolitized tuffs (weight %). *n.m. - not measured.

Zeolitized tuff	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	Loss by ignition at 1000°C
Croatia	68.51	0.13	11.17	0.16	0.41	1.04	3.48	2.31	11.27
Turkey	56.60	0.65	16.97	4.38	2.05	2.30	1.16	1.63	13.34
Serbia	57.68	n.m.	13.86	2.05	n.m.	6.46	0.66	0.85	14.61

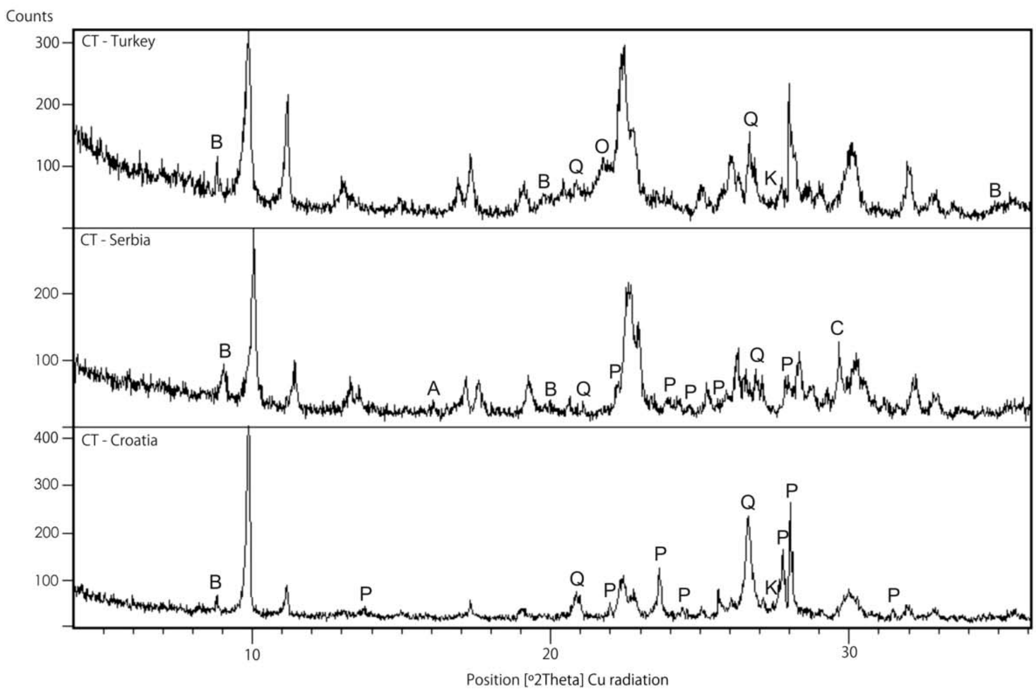

Figure 1. X-ray powder patterns of zeolitized tuffs from Turkey, Serbia, and Croatia. Peaks belonging to "impurities" are marked: (A) analcime, (B) biotite, (C) calcite, (CE) celadonite, (K) potassium feldspars, (O) opal-CT, (P) plagioclase feldspars, and (Q) quartz.

The tuff from Serbia had the largest cation exchange capacity of 1.71 meq/g, followed by tuff from Croatia with 1.60 meq/g and tuff from Turkey with 1.35 meq/g (Table 3). The main exchangeable cation in samples of tuffs from Turkey and Serbia was Ca^{2+}, whereas Na^+ was the main exchangeable cation in the sample of tuff from Croatia.

The particles of the tuff from Croatia had the most negative zeta potential of -25.14 mV, followed by tuff from Turkey (-23.12 mV) and tuff form Serbia (-17.60 mV). The phosphate adsorption capacity was negligible and varied from 22.5 mg P/kg for Serbian tuff, to 30.2 mg P/kg for Croatian tuff and 77.5 mg P/kg for Turkish tuff (Table 3).

Table 2. Mineral composition of zeolitized tuffs (approximately in weight %).

Mineral	Croatia	Turkey	Serbia
Clinoptilolite	50	70	75
Plagioclase feldspars	10-15		5
Quartz	10-15	10-15	5
K-feldspars	5	5	
Celadonite	5		
Opal-CT		10-15	
Biotite		5	5
Anacime			5
Calcite			5

Table 3. Cation exchange capacity (CEC), zeta potential of particles and phosphate-adsorption capacity of zeolitized tuffs.

Zeolitized tuff	Ca^{2+} (meq/g)	Mg^{2+} (meq/g)	K^+ (meq/g)	Na^+ (meq/g)	CEC (meq/g)	Zeta potential (mV)	P-adsorption capacity (mg/kg)
Croatia	0.169	0.047	0.215	1.165	1.60	-25.14±0.62	30.2±3.8
Turkey	0.773	0.215	0.133	0.226	1.35	-23.12±0.57	77.5±3.5
Serbia	1.117	0.268	0.073	0.256	1.71	-17.60±2.52	22.5±4.7

The interaction of zeolitized tuffs and phosphate-accumulating bacterium *A. junii* in synthetic wastewater is shown in Table 4. After 24 h of contact with zeolitized tuffs, most of the bacterial population was immobilized onto minerals by spontaneous adsorptive growth, while the rest of the bacteria remained as planktonic cells in supernatant. The highest ratio of immobilized and planktonic cells was obtained in reactors containing the tuff from Croatia (247), followed by tuff form Turkey (151) and tuff from Serbia (10). The highest number of immobilized cells was obtained on the tuff from Turkey ($74.91 x 10^8$ CFU/g), followed by tuff form Croatia ($48.39 x 10^8$ CFU/g), while the tuff from Serbia had the lowest number of immobilized cells ($6.14 x 10^8$ CFU/g). The best bacterial activity measured as phosphate uptake rate per CFU was obtained with tuff from Croatia, followed by tuff from Turkey and tuff from Serbia. As the result of bacterial activity, the final percent of phosphate removal from wastewater decreased in the order: 50.23%, 46.32% and 40.21% for tuff from Croatia, Turkey and Serbia, respectively. The final pH value in all reactors slightly increased form the starting pH value of 7.0, due to the bacterial activity in reactors.

The results showed that three examined zeolitized tuffs are good carriers of metabolically active bacteria. The immobilization rates observed for three examined zeolitized tuffs are comparable to the values reported for other carriers: 68.6×10^8 CFU/g of *A. calcoaceticus* immobilized onto Mg-exchanged zeolitized tuff [5]; 52.8×10^8 CFU/g of *A. junii* immobilized onto surfactant-modified zeolitized tuff [6]; 29×10^8 CFU/g of *Acinetobacter* spp. immobilized onto ceramics [4]; 2.5×10^8 CFU/g of *A. johnsonii* immobilized inside alginate beads [2]; 9×10^8 CFU/g of *Pseudomonas aeruginosa* immobilized onto Type-Z biocarrier [1] and 3.6×10^8 CFU/mL of *Saccharomyces cerevisiae* immobilized onto zeolitized tuff [3].

Table 4. Performance of reactors containing *Acinetobacter junii* and zeolitized tuffs. [c_0 CFU (10^6 CFU/mL)] = 12.52±2.03; [c_0 P-PO_4 (mg/L)] = 23.57±0.51.

Parameter	Croatia	Turkey	Serbia
Total cells (10^8 CFU/mL)	0.67±0.03	1.25±0.02	1.55±0.05
Planktonic cells (10^7 CFU/mL)	1.92±0.29	4.99±0.29	14.85±0.01
Immobilized cells (10^8 CFU/g)	48.39±2.56	74.91±0.01	6.14±0.26
Immobilized / planktonic cells	246.73±48.35	151.08±18.72	10.33±0.33
P removed (%)	50.32±3.54	46.32±0.55	40.21±0.21
P-uptake rate (10^{-11} mg P CFU^{-1})	16.20±0.17	8.45±0.13	6.18±0.03
pH	7.47±0.10	7.89±0.04	7.92±0.04

The extent of immobilization of *A. junii* considerably varies when using zeolitized tuffs of different origin. The zeta potential of *A. junii* was negative (-18.4 mV), as well as the zeta potential of zeolite particles (Table 3) and no correlation between the number of immobilized cells and zeta potential of minerals was obtained. The clinoptilolite content in the zeolitized tuff was not the prevailing factor for the immobilization of bacteria. Bacterial immobilization on the zeolitized tuff was defined by the original structure of material. The factors which determine the bacterial immobilization onto zeolitized tuff still remains to be elucidated.

CONCLUSION

It can be concluded that zeolitized tuff can be a good carrier of phosphate-accumulating bacterium *A. junii*. The extent of bacterial immobilization on single zeolitized tuff cannot be predicted by mineralogical and chemical analysis of mineral, its cation exchange capacity or its zeta potential. The phosphate-accumulating bacteria immobilized onto zeolitized tuff as a biocarrier can find application in the bioaugmentation of activated sludge in order to achieve better phosphate removal from wastewater.

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