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Use of Natural Zeolite to Upgrade Activated Sludge Process

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Summary

The objective of this study was to achieve better efficiency of phosphorus removal in an enhanced biological phosphorus removal process by upgrading the system with different amounts of natural zeolite addition. The system performance for synthetic wastewater containing different carbon sources applied at different initial concentrations of phosphorus, as well as for municipal wastewater, was investigated. Natural zeolite addition in the aerobic phase of the anaerobic/aerobic bioaugmented activated sludge system contributed to a significant improvement of phosphorus removal in systems with synthetic wastewater and fresh municipal wastewater. Improvement of phosphorus removal with regard to the control reactors was higher with the addition of 15 than with 5 g/L of natural zeolite. In reactors with natural zeolite addition with regard to the control reactors significantly decreased chemical oxygen demand, ammonium and nitrate, while higher increment and better-activated sludge settling were achieved, without changes in the pH-values of the medium. It was shown that the natural zeolite particles are suitable support material for the phosphate-accumulating bacteria *Acinetobacter calcoaceticus* (DSM 1532), which were adsorbed on the particle surface, resulting in increased biological activity of the system. The process of phosphorus removal in a system with bioaugmented activated sludge and natural zeolite addition consisted of: metabolic activity of activated sludge, phosphorus uptake by phosphate-accumulating bacteria adsorbed on the natural zeolite particles and suspended in solution, and phosphorus adsorption on the natural zeolite particles.

Key words: *Acinetobacter calcoaceticus*, activated sludge, bioaugmentation, natural zeolite, phosphorus removal, wastewater

Introduction

Eutrophication is a worldwide water pollution problem and the control of the access of phosphate (*o*-P) to the aquatic environment is widely used as an eutrophication control strategy, thus requiring its removal from effluents by chemical and/or biological means (*1*). Enhanced biological phosphorus removal (EBPR) from wastewater is based on the enrichment of activated sludge with P-accumulating organisms. These microorganisms (also called P-removing or poly-P bacteria) are able to store intracellular phosphorus (P) as polyphosphate (poly-P). The requirement for the achievement of a P-removing bacterial population in an activated sludge system is the exposure of activated sludge to cyclically anaerobic and aerobic (or anoxic) conditions (*2*).

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Bacteria from the genus *Acinetobacter* have become the model organism for EBPR since they were isolated from a P-removing activated sludge plant (*3*). Although *Acinetobacter* spp. were present in extremely low number in the activated sludge plant, their capacity to accumulate poly-P intracellularly was the highest amongst the all isolates (*4*). These strictly aerobic bacteria (*5*), in the absence of oxygen, transport volatile fatty acids (*e.g.* acetate, propionate) into the cell and subsequently convert and store these as poly-hydroxy-alkanoates (PHA) (*6,7*). The energy for this transport and storage is supplied by hydrolysis of intracellularly stored poly-P to *o*-P, which is released from the cell to the liquid. Under aerobic conditions, stored PHA would be catabolised, using oxygen as electron acceptor to generate energy for cell growth, maintenance, glycogen formation and poly-P synthesis, resulting in the uptake of *o*-P in a quantity greater than the amount previously released (*6,7*).

Natural zeolites (NZ) are the main absorptive, low-cost material used in agriculture and industry (*8*). Although additions of NZ in wastewater treatment evidently reduce the final concentration of P (*8–11*), the mechanism of this reduction is unclear. One of the promising approaches to improve the efficiency and increase the capacity of biological wastewater treatment plants (hydraulically or biologically overloaded plant), without increasing the size, is based upon the application of NZ in the aeration basin.

The objective of this study was to achieve better efficiency of P removal in EBPR system by adding different amounts of NZ in the aerobic phase. The system performance for synthetic wastewater, containing different carbon sources, applied at different initial concentrations of P, as well for municipal wastewater, was investigated.

Material and Methods

Microorganism

Lyophilised culture of *Acinetobacter calcoaceticus* DSM1532, which has been described as a P-accumulating bacterium, was obtained from DSM-Deutsche Sammlung von Microorganismen und Zellkulturen GmbH. A strain of *A. calcoaceticus* DSM1532 was maintained on nutrient agar medium, subcultured monthly and stored at 4 °C.

Natural zeolite

The zeolite tuff from the Aegean Region, Turkey consists of more than 70 % clinoptilolite-heulandite + mordenite, minor quartz and opal-CT, as estimated by X-ray powder diffraction method. The chemical composition (estimated by X-ray fluorescent spectroscopy method) of the NZ (wt %) is: SiO_2 68.5; TiO_2 0.07; Al_2O_3 11.18; Fe_2O_3 1.21; MnO 0.02; MgO 0.71; CaO 1.98; Na_2O 0.33; K_2O 3.88; P_2O_5 0.01; H_2O^- 5.51; H_2O^+ 5.78 *(12)*. Sodium was the dominant cation in the sample. Granulometrical analysis showed that the sample consists of the following size fractions (%): <0.125 mm 4.2; 0.125–0.25 mm 0.2; 0.25–0.5 mm 27.3; 0.5–1 mm 67.5; >1 mm 0.8. The NZ tuff was washed three times with demineralised water and then dried at 105 °C for 16 h before the use in experiments. The estimated equilibrium adsorption capacity for P was P-PO_4 48.5 mg/kg of the sample.

Synthetic wastewater

Experiments were carried out for the different types of synthetic wastewaters, presented as wastewater types A, B, C and D. The composition of the synthetic media used to simulate the sewage is reported in Table 1. The concentration of KH_2PO_4, the only sole source of P in the tests, varied from 4 up to 440 mg/L to obtain a concentration of total P in the wastewater ranging from 1 to 100 mg/L. The pH of the synthetic wastewater was adjusted to 7±0.1 with 1 M NaOH or 1 M HCl before autoclaving (121 °C/15 min). Some experiments were carried out with fresh municipal wastewater (E), an influent from the wastewater treatment plant of Ondokuz Mayis University, Samsun, Turkey. The average composition of municipal wastewater was (in mg/L): T-P 5.33; NH_3-N 32.96; NO_3-N 31.04; COD 1430; pH=7.02.

Table 1. Composition of different types of synthetic wastewaters

Component (mg/L)	A	B	C	D
Acetic acid	260	260	500	–
Sodium propionate	40	40	–	–
Glucose	40	40	–	500
Peptone	100	100	100	100
$MgSO_4$	10	10	10	10
$CaCl_2$	6	6	6	6
KCl	30	30	30	30
Yeast extract	20	20	20	20
NH_4Cl	–	38	38	38
KH_2PO_4	4–440	4–440	4–440	4–440

Experimental operation

Laboratory-scale batch experiments were carried out in alternated 24-h anaerobic/24-h aerobic stages in a 500-mL Erlenmeyer flask. The fresh activated sludge was obtained from the aeration tank from a municipal wastewater treatment plant and acclimatised for two weeks in the mineral solution with mixing and aerating at room temperature. The wastewater was inoculated with activated sludge bioaugmented with P-accumulating bacteria *A. calcoaceticus* (DSM1532). The flasks were sealed with a gum cap (*4*) and anaerobically incubated (70 rpm, 20±0.1 °C) in a water bath controlled with thermostat and shaker. After the 24-h anaerobic stage each reactor volume was vigorously shaken and aseptically divided into two reactors. In one reactor, 5 or 15 g/L of NZ was added and the other reactor was left without NZ addition. In the following aerobic phase stirring at 70 rpm, aeration (around 4 L/min) and temperature of 20±0.1 °C was provided.

Analytical methods

All measurements were done according to the Standard Methods for the Examination of Water and Wastewater *(13)*. pH values were measured with a Crison mi-

cro pH 2000 pH-meter. Dissolved oxygen and temperature were controlled with a Jenway 9071 dissolved oxygen meter. Before T-P, ammonia and nitrate measurements, the samples were filtered through Sartorius nitrocellulose filters (pore diameter 0.2 μm). T-P concentration in water was measured after persulfate oxidation by the stannous chloride method in a Cary UV-visible spectrophotometer at 690 nm, while in activated sludge it was determined after perchloric acid digestion. Medium pH of above 7.8 would have shown that P had precipitated as either calcium or magnesium salts, which could erroneously account for the decrease in T-P concentration in the sample. Therefore, the pH of such samples was adjusted between 6.8 and 7.5 before T-P measurement.

Ammonium (NH_3-N) concentration in water was measured by the nesslerization method in a Hitachi 110–40 spectrophotometer at 425 nm. Nitrate (NO_3-N) concentration in water was measured by the ultraviolet spectrophotometric screening method in a Hitachi 110–40 spectrophotometer at 220 nm. Chemical oxygen demand (COD) was determined by the open reflux method. Mixed liquor suspended solids (MLSS) were determined after drying at 105 °C/1 h. Sludge volume index (SVI) was calculated after 30-min sludge settlement. Bacterial number of *A. calcoaceticus* in mixed liquor was determined as colony forming units (CFU) on the nutrient agar. Serial dilutions (10^{-1} to 10^{-8}) of 1 mL sample were prepared. Dilutions (0.1 mL) were plated (spread plate method) onto nutrient agar to obtain a viable cell count. Plates were incubated at 30±0.1 °C for 72 h. After the incubation, the colonies were counted and CFU/L was calculated.

At the end of the experiment NZ particles were washed three times with sterile distilled water and viable cell counts on the nutrient agar were performed in order to determine the immobilised cells. After the isolation of the single colonies, pure bacterial cultures were determined on the basis of Gram staining. Cell shape, size and biochemical characteristics were also specified. Immobilisation of the bacterial cells was determined by direct microscopy in a light microscope (Zeiss, Jenamed 2) under the immersion and magnification of 2000 x. Cell shape was determined by Gram stain. Neisser stain was performed to confirm poly-P granules in cells.

Data analysis

The statistical analyses were done using the program Statistica, Version 6.0 (*14*). The results were set up as control reactors *vs.* reactors with NZ addition. Data of this type are independent, therefore ordinary Student's *t*-test was performed. The null hypotheses tested by the analysis were: reactors with and without NZ additive showed no difference in performance and there was no change in performance between reactors with 5 and 15 g/L NZ additive. Results were taken to be significant at the 5 % level (p=0.05). The correlation between variables was estimated using the Pearson linear correlation.

Results

With the NZ addition in the aerobic phase of the anaerobic/aerobic bioaugmented activated sludge system, a significantly ($p<0.05$) higher amount of T-P from synthetic wastewater, as well as from municipal wastewater, was removed at the end of the aerobic phase (Fig. 1). The poorest performance was shown by the system with glucose as the carbon source (D). The amount of T-P removed increased with an increase in the initial P load in the control reactors and in reactors with NZ. T-P removal in reactors with NZ was in correlation with T-P removal in the control reactors, which was shown by the significant positive correlation ($r=0.868$, $p<0.05$) between reactors with and without NZ addition. Higher the amount of NZ addition for each range of starting P load in wastewater, higher was the improvement of T-P removal.

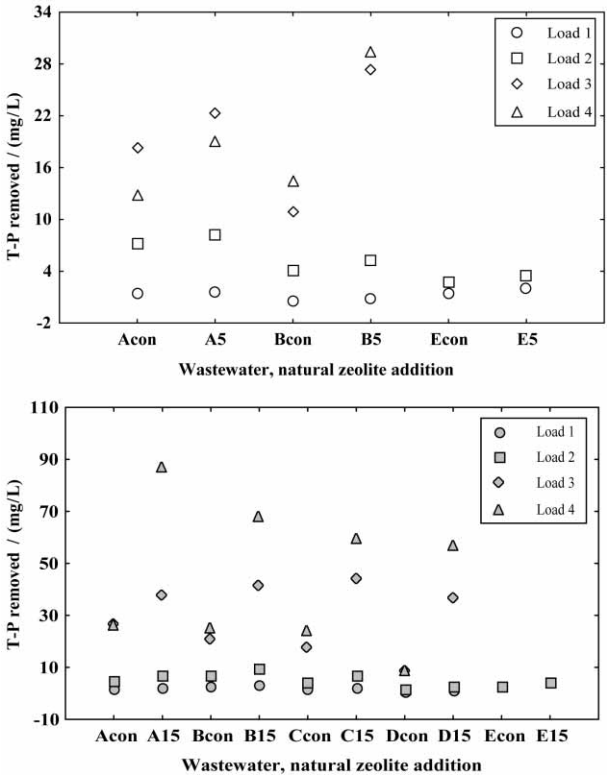

Fig. 1. The amount of total phosphorus (T-P) removed from water at the end of the aerobic phase in reactors without (con) and with 5 g/L (5, empty symbols) and 15 g/L (15, filled symbols) of natural zeolite addition with different wastewaters and initial phosphorus loads. [t_0 T-P (mg/L)] = Load 1 2.35±0.81, Load 2 9.41±2.41, Load 3 58.88±5.19, Load 4 126.23±23.66

In most cases, the amount of T-P accumulated in activated sludge was higher in reactors with NZ, indicating the increased biological activity of the activated sludge. The difference between T-P removed from water and T-P accumulated in activated sludge was in all cases higher in reactors with NZ than in control reactors (Table 2). The difference was on average 2.7 times higher in reactors with 15 g/L than with 5 g/L of NZ addition. These higher amounts of T-P removed from water with lower increase of T-P concentration in activated sludge indicate that P was stored in the media out of the activated sludge, possibly by adsorption on the NZ par-

Table 2. Difference between total phosphorus (T-P) removed from water and total phosphorus accumulated in activated sludge in control reactors and in reactors with natural zeolite at the end of aerobic phase by different wastewaters and initial phosphorus loads

Wastewater	Difference (mg/L) = T-P removed from water – T-P accumulated in sludge							
Natural zeolite	Reactors with natural zeolite				Control reactors			
addition (g/L)	Load 1	Load 2	Load 3	Load 4	Load 1	Load 2	Load 3	Load 4
A) 5	0.39	1.27	8.99	10.84	0.11	0.33	1.16	2.22
A) 15	0.80	3.35	13.50	55.50	0.04	0.56	2.04	1.15
B) 5	0.46	1.24	15.13	14.73	0.15	0.22	1.22	1.37
B) 15	1.19	3.47	22.72	42.67	0.33	0.90	3.52	1.75
C) 15	0.81	2.76	25.84	33.57	0.19	0.54	0.73	0.85
D) 15	0.52	1.65	22.24	46.06	0.10	0.62	1.28	0.20
E) 5	0.76	1.29			0.21	0.56		
E) 15		2.22				0.55		

ticles and in the bacteria biomass adsorbed on the NZ particles.

The number of *A. calcoaceticus* in mixed liquor increased for two orders of magnitude at the end of the aerobic phase with regard to initial values. Final viable cell counts were lower ($p<0.05$) in reactors with NZ than in control reactors (Fig. 2). Microbiological examination of NZ tuff at the end of the aerobic phase showed NZ particles surrounded with biosorbed bacteria. The presence of bacteria from the *Acinetobacter* genus in adsorbed bacterial population was around 80 %. Higher NZ additive provided a larger surface area of substrate particles available to be biosorbed with these P-accumulating bacteria, resulting in lower numbers of suspended cells in mixed liquor. P-accumulating bacteria *A. calcoaceticus*, either attached to the NZ or suspended in solution, participated in the process of P removal, since they contained distinct poly-P granules.

The results with regard to MLSS show better ($p<0.05$) metabolic and growth conditions of activated sludge in the presence of NZ, especially when larger ammount of NZ was added (Fig. 3). SVI (Fig. 4) was significantly

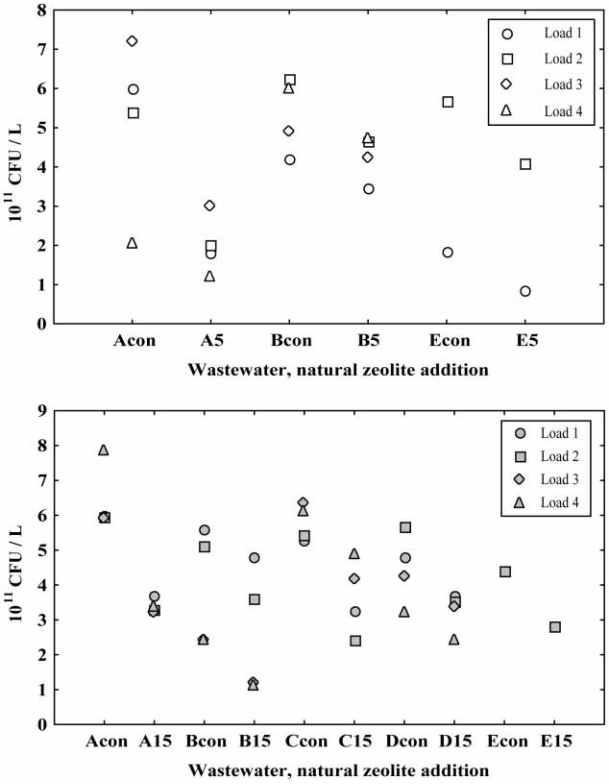

Fig. 2. Colony forming units (CFU) of *Acinetobacter calcoaceticus* at the end of the aerobic phase in reactors without (con) and with 5 g/L (5, empty symbols) and 15 g/L (15, filled symbols) of natural zeolite addition with different wastewaters and initial phosphorus loads. [t_0 CFU (CFU/L)] = $6.00\pm2.60 \cdot 10^9$

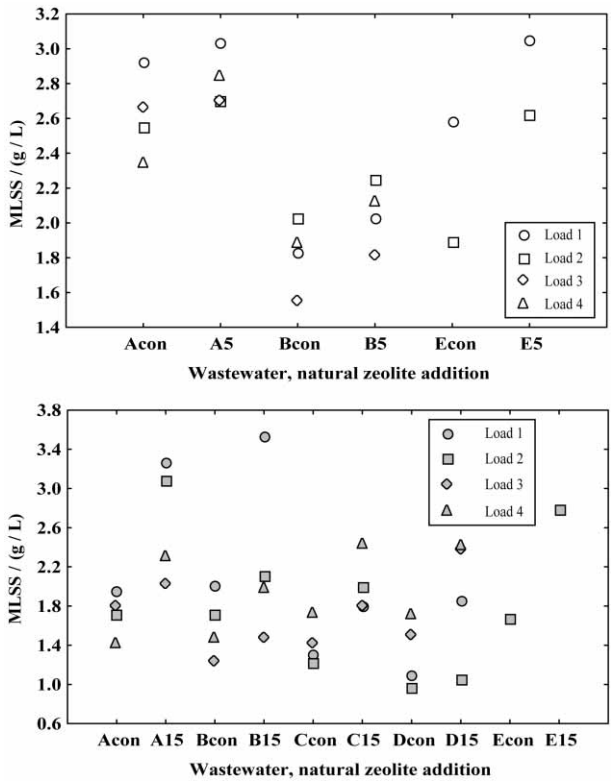

Fig. 3. Mixed liquor suspended solids (MLSS) at the end of the aerobic phase in reactors without (con) and with 5 g/L (5, empty symbols) and 15 g/L (15, filled symbols) of natural zeolite addition with different wastewaters and initial phosphorus loads. [t_0 MLSS (g/L)] = 1.17 ± 0.36

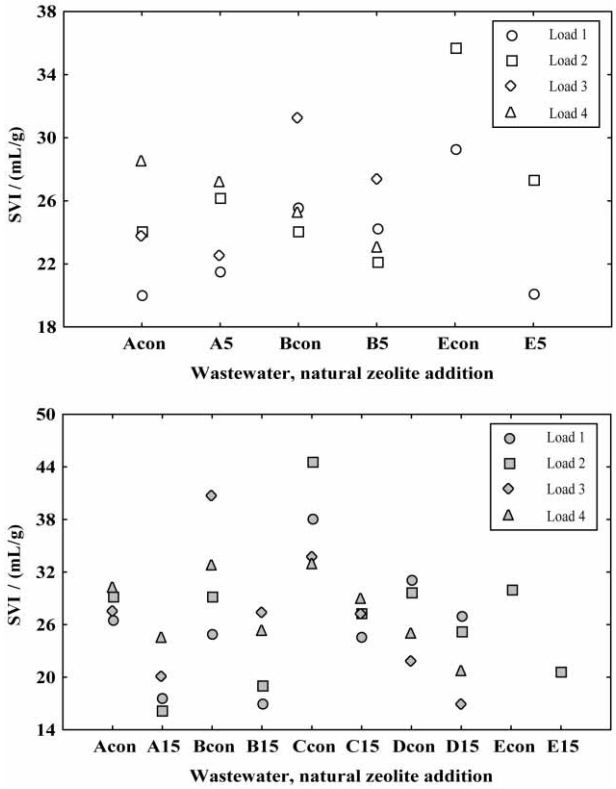

Fig. 4. Sludge volume index (SVI) at the end of the aerobic phase in reactors without (con) and with 5 g/L (5, empty symbols) and 15 g/L (15, filled symbols) of natural zeolite addition with different wastewaters and initial phosphorus loads. [t_0 SVI (mL/g)] = 39.37±10.40

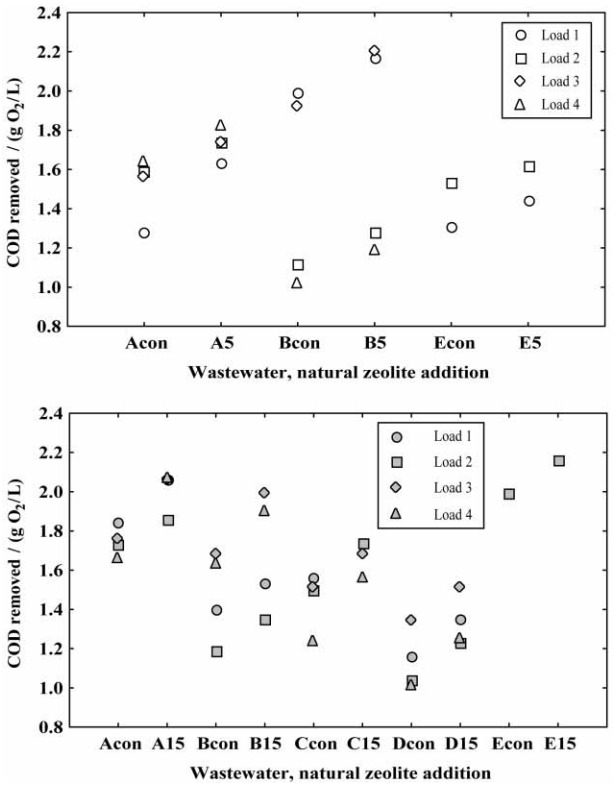

Fig. 5. Decrease in chemical oxygen demand (COD) at the end of the aerobic phase in reactors without (con) and with 5 g/L (5, empty symbols) and 15 g/L (15, filled symbols) of natural zeolite addition with different wastewaters and initial phosphorus loads. [t_0 COD (g O_2/L)] = 1.98±0.30

($p<0.05$) lower in reactors with NZ additive, showing better settling properties of the activated sludge than in reactors without any NZ additive.

P-uptake rates per cell of *A. calcoaceticus* were the highest ($p<0.05$) in reactors with 15 g/L of NZ (11.85±16.24 · 10^{-11} mg T-P/cell), followed by reactors with 5 g/L of NZ (4.54±4.77 · 10^{-11} mg T-P/cell) and control reactors (2.24±2.59 · 10^{-11} mg T-P/cell). P-uptake rates per MLSS were the highest ($p<0.05$) in reactors with 15 g/L of NZ (13.13±13.25 mg T-P/g MLSS), followed by reactors with 5 g/L of NZ (6.01±7.43 mg T-P/g MLSS) and control reactors (5.73±5.82 mg T-P/g MLSS). The highest initial P loads resulted in the highest P-uptake rates.

A significantly ($p<0.05$) higher decrease of COD has been achieved in reactors with NZ, which was higher if more NZ was added (Fig. 5). This can be ascribed to the increased metabolic activity of activated sludge. Decrease of COD in reactors with NZ was correlated with the efficiency of the control reactors. Biomass production of the bioaugmented-activated sludge per mass of COD removed was higher in reactors with NZ (70 %) compared to control reactors (38 %). Carbon incorporation in the biomass in control reactors with wastewater containing glucose as the carbon source was only 6.5 %, which indicated that the activated sludge was in the endogenous phase and that decay was predominant in the biomass synthesis.

With each composition of wastewater, concentrations of ammonia (Fig. 6) and nitrate (Fig. 7) at the end of the aerobic phase were lower ($p<0.05$) in reactors with NZ than in the control reactors. The amount of ammonia and nitrate removed in reactors with NZ addition did not show any correlation with that in the control reactors. The difference in the amount of NZ added (5 or 15 g/L) did not have any significant influence on the amount of ammonia or nitrate removed. Ammonia concentrations at the end of the aerobic phase in the control reactors were higher than the initial concentrations for all synthetic wastewaters but not for municipal wastewater, since in the reactors with NZ they were lower in all cases. Nitrate concentrations at the end of the aerobic phase were lower than the initial concentrations in reactors with and without NZ.

The addition of NZ did not have significant influence on the final pH of the effluent (Fig. 8).

Discussion

Although the predominantly negative surface charge and the lack of sorption sites in NZ would tend to reduce P sorption (*15*), literature data suggest that these materials can adsorb P (*8,10*) and are potentially valuable for use in the removal of P from wastewater. The estimated equilibrium adsorption capacity of the used NZ sample of P-PO_4 48.5 mg/kg of the sample indicates

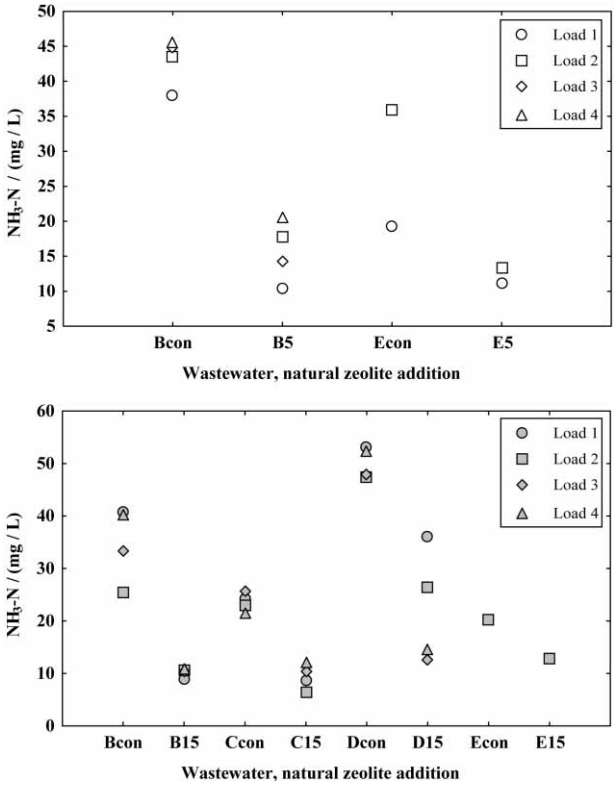

Fig. 6. Ammonia concentration (NH_3-N) at the end of the aerobic phase in reactors without (con) and with 5 g/L (5, empty symbols) and 15 g/L (15, filled symbols) of natural zeolite addition with different wastewaters and initial phosphorus loads. [t_0 NH_3-N (mg/L)] = 21.31±9.22

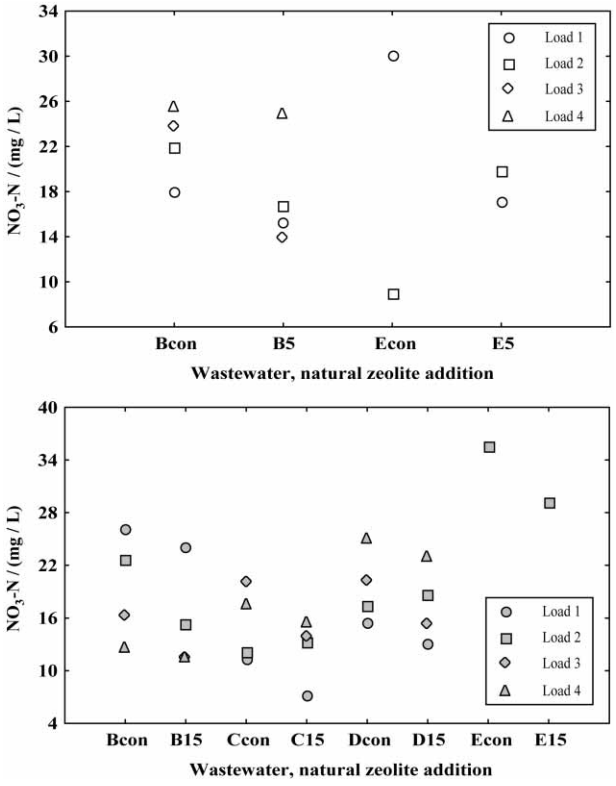

Fig. 7. Nitrate concentration (NO_3-N) at the end of the aerobic phase in reactors without (con) and with 5 g/L (5, empty symbols) and 15 g/L (15, filled symbols) of natural zeolite addition with different wastewaters and initial phosphorus loads. [t_0 NO_3-N (mg/L)] = 44.45±8.05

that NZ addition in a bioaugmented activated sludge system could participate in the process of T-P removal.

The efficiency of a zeolite-containing material in most of the applications, such as ion exchange, selective sorption or catalysis, is in correlation with the amount, structure and composition of the zeolitic component itself. No similar correlation could be observed when clinoptilolite-containing tuffs were used as additive in water treatment or for water filtration (*16*). Higher P removal from pig slurry was achieved with the addition of 2 % of NZ than with the addition of 1 % of NZ (*17*). In this study, a higher amount of NZ added resulted in better T-P removal (Fig. 1) and better system performance, as indicated by other effluent parameters (Figs. 2–8). Better system performance observed with the higher NZ additive (Fig. 1) is explained by the larger surface area of substrate particles available for biosorption with P-accumulating bacteria.

The dimensions of bacteria are comparable to the sizes of zeolite crystallites and also to the corresponding inter-crystalline pores (*16*); it seems therefore conceivable that P-accumulating bacteria become activated by biosorption on the outer accessible surface of NZ crystallites. The biological activity of the zeolitic tuff is determined by the processes on the outer surface of the embedded NZ crystallites accessible for microorganisms through cavities, macro- and mesopores. Microbiological examination of NZ tuff at the end of the aerobic stage showed NZ particles surrounded with biosorbed bacteria. Washing with sterile distilled water did not mobilise these biosorbed bacteria. The contribution of bacteria from the *Acinetobacter* genus in the adsorbed bacterial population was around 80 %. In the supernatant these P-accumulating bacteria did not show any remarkable differences in the formation of the bacterial population.

NZ particles were good carriers of the P-accumulating bacteria *A. calcoaceticus*, which were adsorbed on the NZ surface, resulting in increased biological activity in the activated sludge. P-accumulating bacteria, either attached to the NZ or suspended in solution, participated in the process of the T-P removal as they contained different intracellular poly-P granules.

In bioaugmented activated sludge systems with NZ, adsorption of *A. calcoaceticus* cells on the NZ particles was the reason for the measured lower viable cell count in the supernatant (Fig. 2) and the higher P-uptake rates at the end of aerobic phase in reactors with NZ.

The bacteria on the NZ surface adhered to each other by extracellular substances. As well as the increased biological activity of the activated sludge, the presence of the microbial extracellular exopolymers may contribute to the increased T-P removal in a bioaugmented activated sludge system. Extracellular exopolymers, which are considered to be responsibile for the incorporation of the microbial cells on the activated sludge flocs, could participate in P removal acting as a P reservoir (*18*).

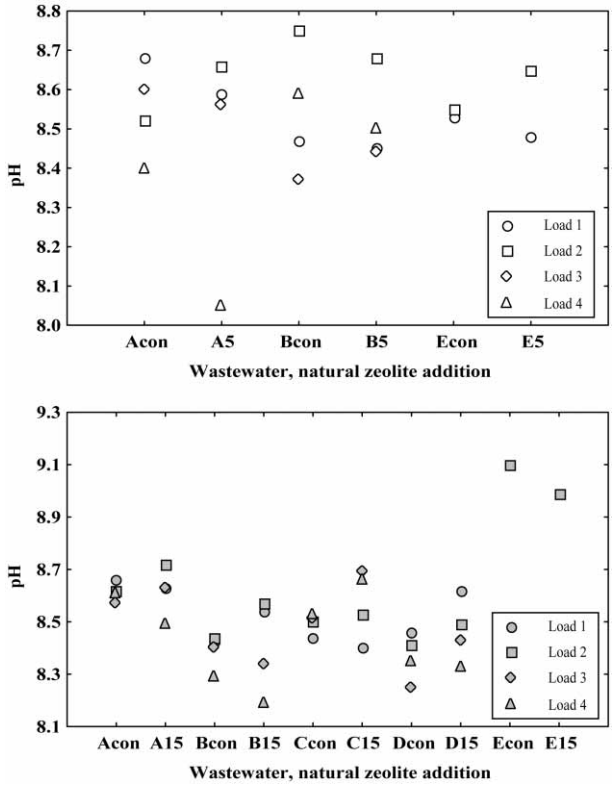

Fig. 8. pH values at the end of the aerobic phase in reactors without (con) and with 5 g/L (5, empty symbols) and 15 g/L (15, filled symbols) of natural zeolite addition with different wastewaters and initial phosphorus loads. [t_0 pH] = 7.02±0.01

In our experiments NZ addition in the aerobic phase of the anaerobic/aerobic bioaugmented activated sludge system contributed to the significant improvement of T-P removal in all types of synthetic wastewater as the substrate. With fresh municipal wastewater as the substrate (wastewater E, Fig. 1) T-P removal was similar to that achieved in synthetic wastewater containing acetate or acetate plus propionate plus glucose, but higher than with glucose as the sole carbon source. In anaerobic/aerobic sequencing batch reactors by the EBPR activated sludge with fermented sewage as the substrate, the achieved *o*-P and organic carbon removal were similar to those achieved with acetate or propionate as the substrate (*19*).

The results with regard to MLSS show better metabolic and growth conditions of activated sludge in the presence of NZ, especially when a larger amount of NZ was used (Fig. 3). SVI was significantly lower in reactors with NZ additive (Fig. 4), showing better settling properties of the activated sludge than in the system without NZ additive. With an upgrade of the anoxic/aerobic activated sludge system by the addition of clay (*20*), an increase of MLSS, better sludge settling and lower SVI were achieved.

Significantly stronger decrease in COD achieved in reactors with NZ as a result of increased addition of NZ (Fig. 5) could be ascribed to the increased metabolic activity of activated sludge. Successful reduction in COD from pig slurry using NZ has been observed (*11*).

Nitrogen compounds can be removed from wastewater during treatment via several mechanisms: volatilisation, adsorption, ion exchange, biological ammonification, nitrification and denitrification. Denitrification by the physiological group of microorganisms, which are facultative anaerobes, require a dissolved oxygen concentration near zero and an available carbon source. At the beginning of our experiments, all types of wastewaters contained nitrogen in the form of ammonia and nitrate. In the anaerobic phase, high increases of ammonia concentrations, with nitrate concentrations near zero, suggest that all nitrates were converted to ammonia by the process of backward ammonification. Ammonia volatilisation is the major removal mechanism in ammonia-dominated wastewater where the pH of water is higher than 8 (*21*). Nitrification by the physiological group of microorganisms, which are obligate aerobes, takes place in aerobic conditions. Denitrification can be performed in aerobic conditions due to the heterogeneity of a mixed culture, where the formation of lumps can form local anaerobic microniches that enable denitrification (*22*). Knowledge to date shows that denitrification can be performed in completely aerobic conditions by some bacterial species (*23*). Cations of Na, K, Ca and Mg, which compensate for the negative charge of the alumosilicate structure of NZ, can be exchanged with other cations from the solution via ion exchange process and therefore NZ can remove cations such as ammonium ions from the solution. Total cation exchange capacity of NZ for the ammonium ion estimated was $N\text{-}NH_4^+$ 23.2 mg/g of the sample (*24*).

In an anoxic/aerobic activated sludge system with NZ addition, ammonia from wastewater was removed by adsorption on the NZ in the anoxic phase, and in the aerobic phase, nitrifying bacteria either attached to the NZ particles or suspended in the media, converted ammonia to nitrite and nitrate (*25*). Addition of NZ-clinoptilolite increased biological nitrification/denitrification activity in the activated sludge and the increased nitrification induced by NZ suggested that NZ was a selective biosorbent for the nitrificants (*16*).

In our experiments, the activated sludge was bioaugmented with P-accumulating bacteria *A. calcoaceticus*, which do not have any nitrification or denitrification capacity. Therefore, it can be concluded that during the aerobic phase in the control reactors ammonia was removed by the process of nitrification, which was reflected in the decrease of ammonia and increase of nitrate concentration in relation to the concentrations at the end of the anaerobic phase. In the reactors with NZ, most of the ammonia was bound to the NZ particles by adsorption. Part of this immobilised ammonia was most probably converted into nitrate, which resulted in lower concentrations of ammonia and nitrate at the end of the aerobic phase than at the beginning of the process (Figs. 6 and 7).

Zeolite has been suggested to act as a pH regulator in the process of glucose fermentation by the yeast *Saccharomyces bayanus* due to its ion exchange capacity that results from the microporous alumosilicate structure (*26*). Our results showed that the NZ addition did not have significant influence on the final pH of the effluent

(Fig. 8), which suggested that NZ did not act as a pH modulator.

It can be concluded that the addition of NZ in the activated sludge system contributed to the significant improvement of T-P, COD, ammonium and nitrate removal, higher increment and better-activated sludge settling, without changes of pH-values in the medium. P was removed from the system due to the metabolic activity of activated sludge, P uptake by P-accumulating bacteria adsorbed on the NZ particles and suspended in solution, and P adsorption on the NZ particles.

Practical aspect is potentially interesting, since by the addition of NZ an improvement of the elimination of P for more than 50 % from water containing more than 100 mg/L T-P can be achieved. The application of NZ in wastewater treatment can be used as a low-cost, efficient and energy-saving technique. The sludge and NZ produced through such biological treatment of P removal from wastewater can be used as a good slow release fertiliser, thus making this process an excellent means of solving environmental problems while, at the same time, recycling huge amounts of energetic substances from waters.

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Korištenje prirodnog zeolita za poboljšanje procesa s aktivnim muljem

Sažetak

Svrha je ovog istraživanja bila postići bolju učinkovitost u procesu biološkog uklanjanja fosfora poboljšavajući sustav s različitim količinama prirodnog zeolita. Ispitan je rad sustava sa sintetskom otpadnom vodom, koja je sadržavala različite izvore ugljika pri različitim početnim koncentracijama fosfora, kao i s komunalnom otpadnom vodom. Dodatak prirodnog zeolita u aerobnoj fazi anaerobno/aerobno poboljšanog aktivnog mulja omogućio je znatno uklanjanje fosfora u sustavima sa sintetskom i svježom komunalnom otpadnom vodom. U usporedbi s kontrolnim reaktorima postignuto je bolje uklanjanje fosfora s 15 g/L prirodnog zeolita nego s dodatkom od 5 g/L. U reaktorima s dodatkom prirodnog zeolita smanjena je kemijska potrošnja kisika, te udjel amonijaka i nitrata, a omo-

gućen je bolji prirast i bolje taloženje aktivnog mulja, bez promjene pH-vrijednosti medija. Pokazano je da su čestice prirodnog zeolita pogodan nosač fosfat-akumulirajućih bakterija *Acinetobacter calcoaceticus* (DSM 1532), koje su se adsorbirale na površini čestica, zbog čega je povećana biološka akivnost sustava. Mehanizam uklanjanja fosfora u sustavu s bioaugmentiranim aktivnim muljem i dodatkom prirodnog zeolita očitovao se u metaboličkoj aktivnosti samog aktivnog mulja, vezanju fosfora na fosfat-akumulirajuće bakterije, adsorbirane na česticama prirodnoga zeolita i raspršene u mediju, te na adsorpciju fosfora na čestice prirodnog zeolita.