

EFFECT OF NATURAL AND SYNTHETIC ZEOLITES ON BACTERIA

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INTRODUCTION

Zeolites display many useful applications in the field of pollution abatement. Uses of zeolite include horticulture product, soil conditioner, animal feed, odour control and hygiene products, air filtration media, cation exchange products, water and wastewater treatment. Currently attention is being drawn to the implementation of zeolites in the wastewater treatment process in order to achieve a higher efficiency of the water purification.

Naturally occurring zeolites include tuffs rich in more than 48 known zeolite mineral species such as chabazite, clinoptilolite, erionite, heulandite, mordenite, phillipsite. The natural clinoptilolite is usually chosen in studies on wastewater treatment on the base of its widespread occurrence in the nature, price-easily accessibility and feasibility, cost effectiveness, large surface area, rigidity, surface functionality, thermal, mechanical and radiation stability. Clinoptilolite is a high-silica member of heulandite group of natural zeolites and occurs in abundant and easy mined sedimentary deposits in many parts of the world. However, the composition, purity and mineralogical characteristics of clinoptilolite tuff may vary widely from one deposit to another and even within the same deposit.

When putted into contact with microorganisms, natural zeolite tuff influences its metabolic activity positively [1, 2, 3, 4], while synthetic zeolite negatively [5]. This review summarizes these effects.

EXPERIMENTAL

The zeolitised tuff from Donje Jesenje, Croatia contained more than 50% of clinoptilolite, some quartz and plagioclase and accessory minerals from the mica group (illite-celadonite and biotite). Among the exchangeable cations, potassium was the dominant one in the sample. Four different fractions of particle size of zeolite tuff were tested. The commercial synthetic zeolite of lynde type A (LTA) from Silkem d.o.o., Kidričevo, Slovenia was used. The material was sodium aluminosilicate with average particle size 3-5 μm . Zeolite samples were washed with demineralised water and then dried at 105°C/16h before the experiments were to commence.

In experiments the pure culture of phosphate-accumulating bacterium (PAB) *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 simulative wastewater was as follows (in mg/L): Na-propionate 300; peptone 100; MgSO₄ 10; CaCl₂ 6; KCl 30; yeast extract 20; KH₂PO₄ 44 or 88. The pH of the synthetic wastewater was adjusted to 7.0 before autoclaving (121°C/15 min).

The experiments were carried out as batch tests so that the bacterial biomass was suspended in 200 mL of synthetic wastewater. In reactors different concentration of zeolite was added. The reactors left without the addition of zeolite served as control reactors. The

flasks were sealed with a sterile gum cap and thereafter aerated with filtered air (1L/min) and agitated (70 rpm) in a water bath at 30°C during 24h. The phosphate (P-PO₄³⁻) concentration in the synthetic wastewater was measured spectrophotometrically in a DR/2500 Hach spectrophotometer using the molybdovanadate method (Hach method 8114). The number of cells of *A. junii* was determined as colony-forming units (CFU) grown on nutrient agar after 24h of incubation at 30°C [5].

RESULTS AND DISCUSSION

Figure 1 shows the total numbers of cells of *A. junii* when cultivated in reactors containing zeolite tuff originating from Croatia at concentration 1g/L and in control reactors without addition of zeolite tuff. The numbers of PAB *A. junii* were significantly higher when cultivated in reactors with addition of natural clinoptilolite than in control reactors without the clinoptilolite addition, regardless which particle size of material was used. Besides the positive influence on the biomass formation, clinoptilolite addition positively influenced the phosphate removal from wastewater. Natural clinoptilolite tuff has a negligible capacity for adsorption of phosphate anions [5]. Therefore, all phosphate removal from wastewater can be ascribed to the activity of PAB *A. junii*. It is obvious that more metabolically active bacteria can take up more phosphate, which directly influenced the final percent of phosphate removal. The performance of reactors with addition of clinoptilolite was dependable on the performance of control reactors. When analysing the particles of clinoptilolite tuff at the end of experiment, it is observed that most of the total bacterial population was immobilised onto carriers by adsorptive growth while the rest of the bacteria remained in the supernatant as planktonic cells (Figure 2). The number of immobilised cells was the highest on the smallest particle size, and decreased with the increase of particle size, while the planktonic cells showed the opposite trend. It can be summarized that natural clinoptilolite act as a carrier of immobilized cells of *A. junii* and shows the positive influence on the multiplication of bacteria and yield of bacterial biomass. The higher concentration of metabolically active cells resulted in a higher efficiency of phosphate removal from wastewater.

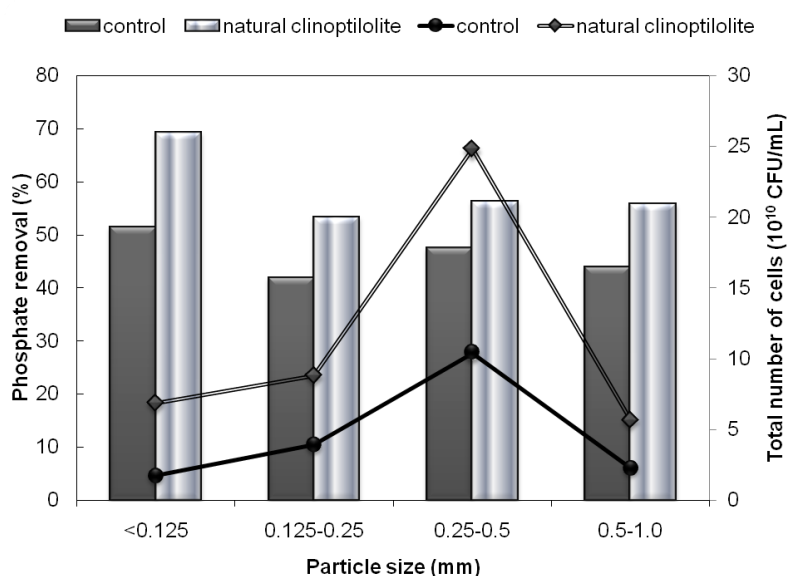


Figure 1. Total number of *Acinetobacter junii* (CFU) and percent of phosphate removal in reactors with and without (control) addition of clinoptilolite tuff. [t_0 CFU (10^9 CFU/L)] = 8.36; [t_0 P-PO₄ (mg/L)] = 13.62.

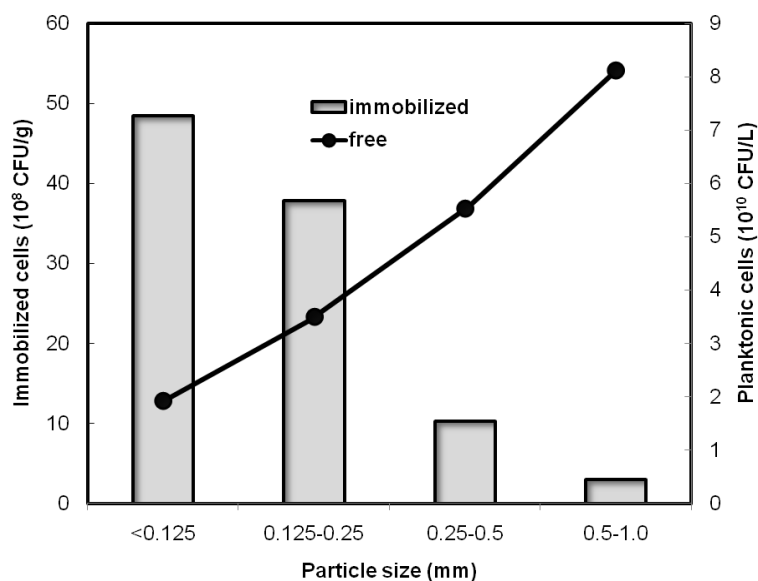


Figure 2. Number of planktonic and immobilized cells of *Acinetobacter junii* (CFU) per dry mass of clinoptilolite tuff.

In spite of natural zeolites, the synthetic zeolites of lynde type A (LTA), which has no natural analogue, display the toxic effect against PAB *A. junii*. The inhibition effect of zeolite LTA against the pure culture of *A. junii* is shown in Table 1. The obtained EC_{50} value of 0.16 g/L indicate the antibacterial property of examined zeolite LTA and eliminate it as a possible carrier of the PAB *A. junii* or material which stimulates its growth. The observed antibacterial property of zeolite LTA is consistent with the material safety data sheet where aquatic toxicity against alga, *Daphnia* and fish is reported. Warne & Schiffko [6] showed that zeolite used in the production of detergent has EC_{50} value against the cladoceran *Ceriodaphnia dubia* of 0.43 g/L.

Although the zeolite LTA are evidently toxic against prokaryotic and eukaryotic cells, little is given about the probable mechanism of its toxicity [7, 8]. It can be summarized that leaching of aluminium from zeolite LTA in the water medium is a most probable cause of its toxic effect against the PAB. It is shown that the high-siliceous heulandite-type of zeolites in the same conditions are not toxic against bacteria, which is connected to the higher stability of these types of zeolites, and as a result lower degree of hydrolysis and consequent leaching in the aqueous solution.

Table 1. Inhibition effect of zeolite LTA on the *Acinetobacter junii* [t_0 CFU (10^9 CFU/L)] = 23.27; [t_0 P-PO₄ (mg/L)] = 20.65.

Parameter	Concentration of zeolite LTA (g/L)			
	0	0.1	0.5	1.0
Phosphate removal (%)	62.08	25.26	7.58	0.00
Planktonic cells (10^9 CFU/L)	111.10	63.90	6.91	0.01
Immobilized cells (10^6 CFU/g)	-	4.08	6.27	4.48
Total cells (10^9 CFU/L)	111.10	63.94	6.96	0.06
Inhibition (%)	0.00	42.45	93.73	99.95
EC_{50} (g/L)	0.16			

The zeolites LTA are used in the production of phosphate-free formulations of laundry detergents. Large quantities of detergents enter the sewage treatment facilities. In the process of wastewater treatment the zeolite LTA present in the influent wastewater may disturb the PAB population in the activated sludge and decrease the effectiveness of the phosphate removal from wastewater. This gives enough reason to recommend that interdisciplinary studies on the mechanism of zeolite LTA toxicity are needed, not only with the aim to protect possible failure of the activated sludge wastewater treatment, than to elucidate the general impact on the environment.

CONCLUSION

Depending on the type of zeolite, different interactions between material and examined PAB *A. junii* were observed. Natural clinoptilolite tuff is excellent bacterial carrier and the main mechanism of interaction is immobilization of bacteria onto the surface of material and increase of the total bacterial biomass concentration. In spite, the synthetic zeolites display the toxic effect against the same PAB. Future investigations should be done in order to investigate the influence of different types of zeolite on other bacteria.

REFERENCES

- [1] D.R. Durham, L.C. Marshall, J.G. Miller and A.B. Chmurny, *Appl. Environ. Microbiol.*, 1994, **60**, 3329-3335.
- [2] M. Bauman, M. Mesaric, S. Ribar, V. Maric and M. Tudja, *J. Basic Microbiol.*, 2001, **41**, 7-16.
- [3] S. Shindo, S. Takata, H. Taguchi and N. Yoshimura, *Biotechnol. Lett.*, 2001, **23**, 2001-2004.
- [4] J. Hrenovic, D. Tibljas, Y. Orhan and H. Buyukgungor, *Water SA*, **2005**, 31, 261-266.
- [5] J. Hrenovic, D. Tibljas and L. Sekovanic, *Acta Chim. Slov.*, 2007, **54**, 661-666.
- [6] M.S.J. Warne and A. D. Schifko, *Ecotox. Environ. Safe*, 1999, **44**, 196-206.
- [7] B. Fairman, A. Sanzmedel, M. Gallego, M.J. Quintela, P. Jones and R. Benson, *Analytica Chimica Acta.*, 1994, **286**, 401-409.
- [8] T.P. Flaten, in J. Lag (Ed.), *Geomedical problems related to aluminium, iron and manganese*, Norwegian Academy of Science and Letters, Otta, 1994, 41-50.

UTJECAJ PRIRODNIH I UMJETNIH ZEOLITA NA BAKTERIJE

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SAŽETAK

U kontaktu s mikroorganizmima, prirodni zeolitni tuf pokazuje pozitivan utjecaj na metaboličku aktivnost, dok sintetski zeolite pokazuju negativan utjecaj. U ovom radu prikazani su primjeri ovakvih utjecaja. Ovisno o tipu zeolita, opažena su različita međudjelovanja između materijala i fosfat-akumulirajuće bakterije *Acinetobacter junii*. Prirodni klinoptilolitni tuf je izvrstan nosač bakterija i glavni mehanizam međudjelovanja je imobilizacija bakterija na površinu materijala i porast ukupne bakterijske biomase. Za razliku, sintetski zeolit LTA pokazuje toksični utjecaj na istu bakteriju. Daljnja istraživanja bi trebala biti usmjerena na ispitivanje utjecaja drugih tipova zeolite na druge bakterije.

Ključne riječi: bakterije, klinoptilolit, otpadne vode, zeolit LTA