PREPARATION OF BIOPARTICLES FOR PHOSPHORUS REMOVAL FROM WASTEWATER IN REAL SCALE

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ABSTRACT

Phosphorus (P) is a non-renewable and life-sustaining element. The excessive discharge of P to aquatic ecosystems is a worldwide problem causing eutrophication and deterioration of water quality. To protect the environment, P must be removed from wastewater before it is discharged into natural recipient. P-accumulating bacteria such as *Acinetobacter junii* are very efficient in P-removal from wastewater, since they accumulate the soluble P in the form of insoluble intracellular poly-P. By immobilizing *A. junii* onto solid materials, bioparticles are formed. In this form bacteria are protected from different stressors in wastewater. Previous reports on the preparation of bioparticles for P-removal from wastewater have been carried out under sterile laboratory-controlled conditions, that are not suitable for the preparation of larger quantities of bioparticles intended for use in a real scale. This work presents a possible method of bioparticle preparation for P-removal from wastewater, intended for the real environment. Five commercially available solid materials were tested as growth carriers for *A. junii*. Prepared bioparticles were further tested for the capacity of P removal from wastewater in real situation. Bioparticles with immobilized *A. junii* were successfully prepared in non-sterile laboratory conditions at room temperature. The efficiency of P removal from wastewater depended on the type of the growth carrier. Of the tested materials, Aqualite containing 60 wt.% clinoptilolite proved to be the best carrier for the preparation of metabolically active bioparticles for P removal from wastewater in the real world.

Keywords: phosphorus, *Acinetobacter junii*, immobilization, natural zeolites.

INTRODUCTION

Phosphorus (P) in the form of orthophosphate (PO_4^{3-}) is a limiting nutrient for algal growth in surface waters. Discharge of untreated wastewater rich in P removes this limitation, causing the accelerated eutrophication of ecosystems, instead. Therefore, there is a need to remove the excessive amount of P from wastewaters before any discharge into natural recipients.

A physiological group of bacteria capable of accumulating the soluble P in the form of insoluble intracellular poly-P is known as P-accumulating bacteria. Among different bacterial species, *Acinetobacter* spp. has the highest capacity of P accumulation [1]. Bacterium *A. junii* (formerly *A. calcoaceticus*) has become a model P-accumulating bacterium since it was isolated from the wastewater treatment plants with enhanced biological P-removal [2]. *A. junii* can be used in a variety of wastewater treatment systems to enhance the P-removal. However, when planktonic form of *A. junii* is added into wastewater, bacteria can easily be grazed by protozoans or washed away by large amounts of wastewater. Therefore, the implementation of *A. junii* in the form of bioparticles ensures protection of bacteria from different biotic and abiotic stress factors [3].

Bioparticles can be easily prepared by the immobilization of bacteria onto solid material due to the natural ability of bacteria to adhere to the solid surface. Natural zeolitized tuff (NZ) is a promising material for the bacterial immobilization since it is: inert, nontoxic, with porous

structure, relatively cheap, easily available, environmentally friendly and provides a rough, irregular surface available for bacterial colonization [3]. The number of immobilized *A. junii* onto different NZs in range of 10^9 CFU (Colony Forming Units)/g was reported [3]. The number of bacteria that can be immobilized depends on the size of NZ particles. The number of immobilized *A. junii* decreased from 9.7 to 8.5 log CFU/g when NZ with the particle size of <0.125 mm was replaced by 0.5-1.0 mm size fraction [4].

However, these numbers of immobilized bacteria were achieved in sterile laboratory-controlled conditions. Requirement of sterile conditions is not suitable for the preparation of large quantity of bioparticles intended for the field use. This work demonstrates a possible method of bioparticle preparation for P-removal from wastewater in the real environment.

EXPERIMENTAL

Several commercial solid materials were used as substrates for the preparation of bioparticles. Their phase composition was determined by X-ray diffraction (XRD) since their commercial descriptions were vague. The granulometric composition of the material was determined by dry sieving (Table 1).

Table 1. Solid materials used as substrate for bioparticles, their commercial description, semi-quantitative composition determined by XRD, and median grain size.

Solid material	**Commercial description**	**Phase composition [wt.%]**	**Grain size [μm]**
1. Filter-Ag® Plus, Clack Corporation, USA	mixture of natural materials sintered at high temperature	amorphous	590
2. Aqualite™, Josab International AB, Sweden	natural clinoptilolite tuff (K, Ca, Mg)	clinoptilolite 60, sanidine 15, opal-CT 15, quartz 5, smectite 5	595
3. Turbidex™, Hydro Source LLC, USA	natural clinoptilolite tuff (K, Ca, Na)	clinoptilolite 63, smectite 15, sanidine 10, quartz 10, opal-CT 2	610
4. Magno-Dol, RS Minerals Ltd, UK	half burnt dolomite	calcite 70, dolomite 10, periclase 20	1160
5. Crystal-Right™ CR-200, Mineral-Right Inc, USA	synthetic zeolite (Na-aluminosilicate)	amorphous	630

The P-accumulating bacterium *A. junii* was purchased from the German Collection of Microorganisms and Cell Cultures (strain number 1532). The initial bacterial biomass was cultivated in tubes containing 10 mL of sterile Nutrient broth at 22 °C/24 h. The biomass was suspended in a commercially available spring water (10 mL of biomass/1L of water). Spring water is free from nutrients. This prevents P accumulation by *A. junii*. Nylon tea bags were filled with 1 g of various solid materials (in duplicate) and put into bottles containing 100 mL of bacterial suspension. The bottles were left at 22 °C/24 h while *A. junii* immobilized onto particles of material, forming bioparticles. After 24 h one tea bag was taken out for counting the number of immobilized cells (Nutrient agar, 35 °C/24 h). Colonies of *A. junii* are easily recognized, since the spring water contains ≤20 bacterial colonies/mL. Initial number of *A. junii* in the suspension was determined in the previous day.

Another tea bag was used for the experiment on the efficiency of P-removal from wastewater. Water from a stream receiving urban wastewater was filtered through 0.45 μm membrane filter to remove suspended particles. Stream water was supplemented with 45 mg

KH_2PO_4/L. Tea bags with bioparticles were immersed into 100 mL of wastewater. Aeration (which is crucial for the accumulation of poly-P in *A. junii* cells) was provided by mixing at 150 rpm. Control bottle contained stream water without bioparticles. Phosphates ($P\text{-}PO_4$) in stream water were measured spectrophotometrically (Hach program 480) after 24 and 48 h of contact. pH value was measured after 48 h of contact.

RESULTS AND DISCUSSION

A. junii was successfully immobilized onto all five tested materials (Table 2). The highest number of *A. junii* was immobilized onto particles of material 2. The number of *A. junii* immobilized onto materials containing clinoptilolite (2 and 3) was comparable to the number (8.5 log CFU/g) obtained by immobilization in sterile conditions on NZ containing 50 % of clinoptilolite of the size fraction 0.5-1.0 mm [4]. The lowest number of immobilized *A. junii* detected on material 4 decreased further to 6.0 log CFU/g after 48 h of contact (data not shown).

Table 2. Number of immobilized *A. junii* onto particles of different materials after 24 h of contact. t_0(log CFU/g) = 7.0±0.1.

Material no.	**Immobilized *A. junii* [log CFU/g]**
1	8.0±0.1
2	8.2±0.0
3	7.8±0.1
4	6.9±0.1
5	7.3±0.0

The prepared bioparticles were tested in a further experiment on the efficiency of P removal from wastewater (Table 3). The P removal in the control bottle was due to the P uptake by the biomass of native bacteria present in stream water and, to a lesser extent, caused by precipitation with Ca cations.

Table 3. Phosphorus removal after 24 and 48 h of contact and final pH values in bottles containing the bioparticles prepared from different materials. t_0(mg $P\text{-}PO_4$/L) = 11.6; t_0(pH) = 7.6.

Material no.	**P removal 24 h [%]**	**P removal 48 h [%]**	**pH 48 h**
Control	29	67	8.0
1	20	81	8.3
2	78	90	8.0
3	22	72	8.4
4	96	96	9.7
5	34	25	8.4

The bioparticles prepared with material 1 did not achieve higher P removal during 24 h of contact in comparison to control, but after 48 h of contact P removal improved. Excellent improvement of P -removal was obtained by bioprticles prepared from material 2 as compared to controls after 24 and 48h of contact. The final pH identical to the control samples suggests that the excessive P-removal was due to the bacterial biomass accumulation. This is in agreement with previous observations that the clinoptilolite rich in Mg is an excellent material for the immobilization of metabolically active *A. junii* [4].

Although *A. junii* was also immobilized onto particles of material 3, the performance of these bioparticles was not higher than that in the control bottle. This phenomenon can be explained by the presence of hydrated clay-type of mineral in sample 3, where the colloidal clay could be adhering onto bacterial cells, limiting bacterial metabolism.

After 24 h, the best performance in P-removal as compared to the control, was obtained in the bottle containing bioparticles prepared with material 4. However, 96% of P-removal did not increase after 48 h, suggesting that the short-term precipitation of Ca-/ and Mg-phosphates was a mechanism of P-removal. Moreover, the final pH value in bottles with material 4 was alkaline (9.7), which explains the above-mentioned antibacterial effect.

The P-removal in bottle containing bioparticles prepared with material 5 was lower than in control. This observation confirms that synthetic zeolites are not suitable for the immobilization of bacteria or as adsorbents of P. This could be due to antibacterial effect of the leached aluminosilicate species [5].

CONCLUSION

Bioparticles composed of P-accumulating bacteria immobilized onto solid materials can be prepared in non-sterile laboratory conditions at room temperature. Packing of bioparticles into the nylon tea bags that are durable in water will ensure easy transport to wastewater systems. Among five tested, commercially available solid materials, Aqualite^{TM} containing 60 wt.% clinoptilolite proved to be the best material for the preparation of metabolically active bioparticles for P-removal from wastewater in the real world.

REFERENCES

1. M. Sidat, F. Bux, H. C. Kasan, Polyphosphate accumulation by bacteria isolated from activated sludge, *Water SA*, 1999, 25, 175-179.
2. G. W. Fuhs, M. Chen, Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater, *Microb. Ecol.*, 1975, 2, 119-138.
3. J. Hrenović, Interaction of zeolites and phosphate accumulating bacteria, Wong, T.W. (ed.): *Handbook of Zeolites: Structure, Properties and Applications*, Nova Science Publishers, Inc, Hauppauge NY, SAD, 2009, 439-467.
4. J. Hrenović, D. Tibljaš, Y. Orhan, H. Büyükgüngör, Immobilisation of *Acinetobacter calcoaceticus* using natural carriers, *Water SA*, 2005, 32, 261-266.
5. J. Hrenović, D. Željezić, N. Kopjar, A. Sarpola, J. Bronić, L. Sekovanić, Antimicrobial activity of commercial zeolite A on *Acinetobacter junii* and *Saccharomyces cerevisiae*, *J. Hazard. Mat.*, 2010, 183, 655-663.