OPTIMIZATION OF MICROBIAL BIOPREPARATIONS FOR SOIL QUALITY IMPROVEMENT: TESTING NEW FORMULATIONS

OLGA MUTER[1], VIZMA NIKOLAJEVA[2], MĀRIS KĻAVIŅŠ[3]

[1]Institute of Microbiology & Biotechnology, University of Latvia; [2]Department of Microbiology & Biotechnology, Faculty of Biology, University of Latvia; [3]Department of Environmental Science, University of Latvia

INTRODUCTION

Microorganisms play a vital role in fixing, solubilizing, mobilizing, and recycling of nutrients in agricultural eco-systems. These microorganisms occur in soils naturally, but their populations are often scanty. In order to increase the quality of soil and crop yield, the microorganisms with target properties are isolated from soil and artificially cultured, often with further incorporation into suitable carriers. These are known as biofertilizers. Another important application of active microbial biomass lies in environmental biotechnologies with emphasis on soil, water and air clean up.

Recently, many remediation technologies are insufficiently understood because of variable and complex environmental conditions, improper evaluation of the level and content of contamination, and poor capabilities of introduced microbial communities in the field.

The problem of maintenance of microbial activity in different types of biotechnologies is considered as one of the most significant worldwide.

The aim of our experiments was to study a feasibility of different organic and inorganic materials served as a carrier for immobilization/incapsulation of beneficial microorganisms.

METHODS, RESULTS & DISCUSSION

Immobilization and enzyme activity of bacterial consortium on ceramic beads

Seven types of ceramic beads fabricated from two types of Devonian clay, were compared in terms of their appropriateness for bacteria cell attachment and further use for soil/air cleaning technologies (Table 1, Fig.1). The effect of different ceramic beads to the microbial growth and biofilm formation was studied for pure culture *Pseudomonas putida* MSCL 650 and for bacteria consortium MDK.EKO-7. The highest CFU number recovered from the bead surfaces after 72h cultivation, was in the sets No. 4, 6, and 7, corresponding to one Liepa red and two Planci clay samples, respectively.
Besides, a fluoresceine diacetate (FDA) hydrolysis activity of the attached bacteria served as a criterion of biofilm formation.

Sample No.	Composition	Tempe-rature, °C	Apparent density (ρ), g/cm³	Water uptake, %	pH (H_2O)
1	Liepa red clay + 3% sawdust, extrused (whole spheres)	1175	2.09	n.d.	5.7
2	Liepa red clay + 3% sawdust, extrused, crushed (half-spheres)	1175	1.56	n.d.	5.9
3	Liepa red clay + 3% sawdust	1150	1.90	10.89	6.0
4	Liepa red clay + 3% sawdust, reduced	1150	1.95	7.07	6.0
5	Liepa red clay + 3% sawdust + clay shamot	1150	2.15	12.90	6.1
6	Planci clay + 3% sawdust	1200	n.d.	n.d.	6.1
7	Planci clay + 3% sawdust	1100	1.95	n.d.	6.1

Table 1. Physicochemcial properties of ceramic beads.

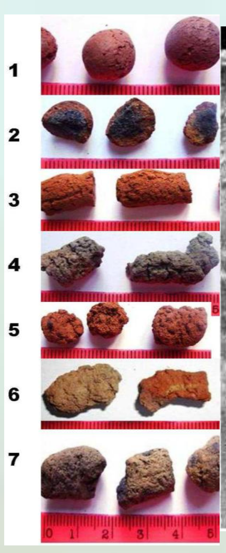

Fig. 1. Ceramic beads.

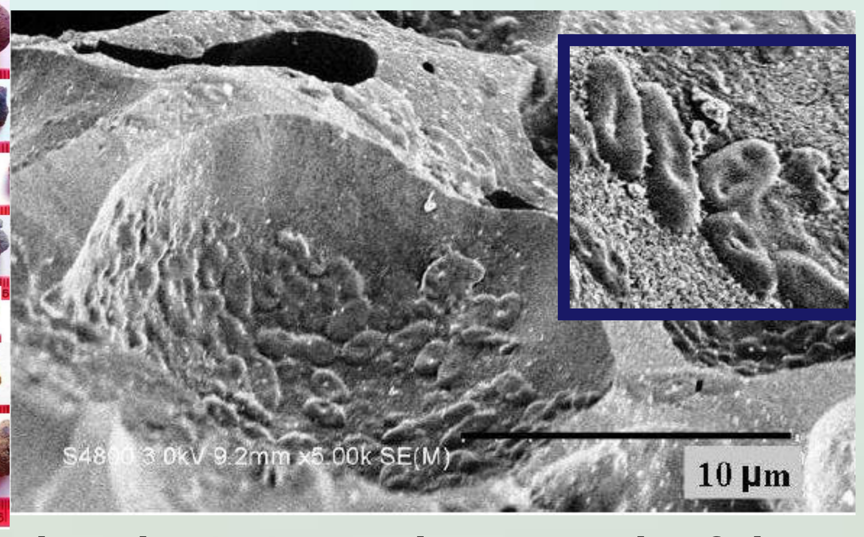

Fig. 2. SEM micropgraph of the bead surface with bacterial cells after immobilization.

Among ceramic beads with biomass, FDA hydrolysis activity on the half-sphere beads was significantly ($p<0.05$) higher than that on the surface of the whole sphere beads fabricated from Liepa red clay. SEM micrographs of the bead surface showed an uneven distribution of bacteria on the surface. The craters (pores) of ceramic bead seem to be the most appropriate sites for bacteria attachment (Fig.2). Experiments on dehydration of the attached *P. putida* at 22 °C showed a decrease of cell viability up to zero in 16 days (Muter et al., 2012).

Immobilization of *Rhizobium leguminosarum* on peat, clay and ceramics and bacteria viability during storage

Five sterilized materials were tested for immobilization of root nodule bacterium *Rhizobium leguminosarum*: peat, clay powder, two kinds of oval aggregates of expanded clay and characterized cylindrical ceramic granules made from Planci deposit of Devonian clay. Immobilization was done during 2.5 h at 20 °C. Afterwards peat, powder and granules were scrubbed and ground in a sterile mortar with a pestle in sterile water to recover the adhered bacteria. The number of colony-forming units was determined by plate count technique. Viability was also detected by LIVE/DEAD cell viability assay.

***Rhizobia* were successfully immobilized in all of the tested carrier materials. One gram of the carrier contained from log 9.4 CFU ("Kano-p") to log 9.8 CFU (peat) of *Rhizobia* (Fig. 3). However, these materials had different effects on bacterial viability during storage.**

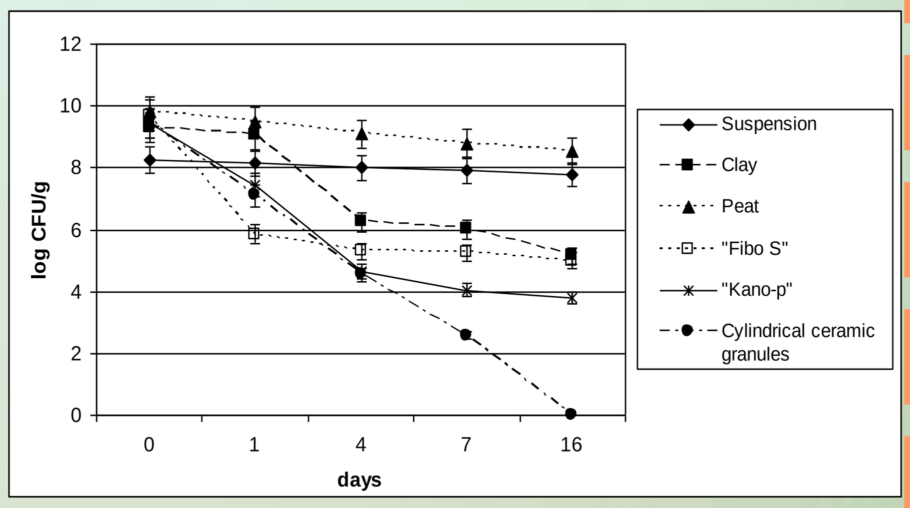

Fig. 3. Survival of *R. leguminosarum* in suspension and five carrier materials stored at 20 °C.

The number of live bacteria decreased by log 1.3, log 4.1, log 4.7, log 5.6 and log 9.5 in the peat, clay, "Fibo-S", "Kano-p" and cylindrical ceramic granules, respectively, after 16 days at the temperature of 20 °C. Studies have shown that carrier material influences the success of immobilization and maintenance temperature influences the survival of *Rhizobium leguminosarum*. The best results were achieved with maintenance of bacteria in the suspension and immobilization on the peat. We recommend keeping *R. leguminosarum* products at a temperature of -18 °C or 4 °C.

Ceramic beads after wastewater treatment process in the model column cascade

Nitrogen and phosphorus removal from wastewater remains one of the serious environmental problems worldwide. The present study was aimed at combining the both nitrification and phosphorus accumulation processes in the laboratory-scale model system with synthetic wastewaters. Blue crystals were found on the bead surface (Fig.4). Accumulation of nitrogen and phosphorus on the beads was also detected. Vegetation experiments have revealed some stimulation effect of the beads applied as an amendment to loamy sand soil, to the growth of rye and cress.

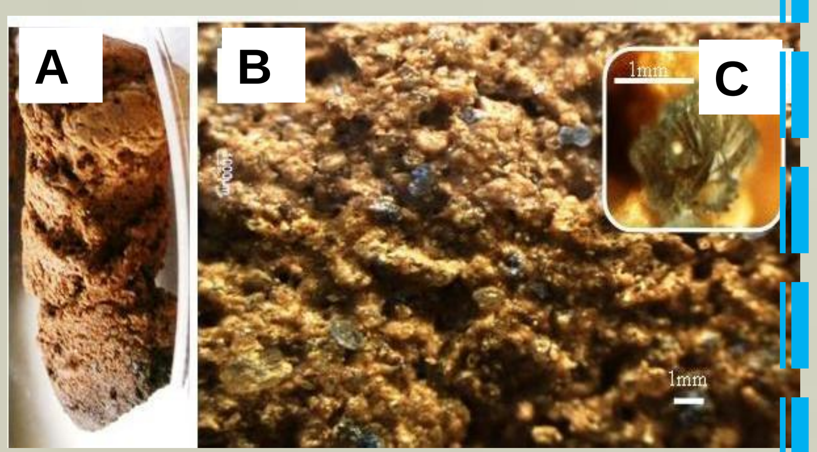

Fig.4. Micrographs of the surface of ceramic beads sampled from the Column III (A, B, C) at the end of the experiment. Blue crystals on the beads in Column III were formed during wastewater treatment.

Latvian peat for the use in biopreparations

Peat can be characterized as organic material which is widely distributed and have high specific surface area. There are differences between peat properties from ombrothropic bogs and fens.

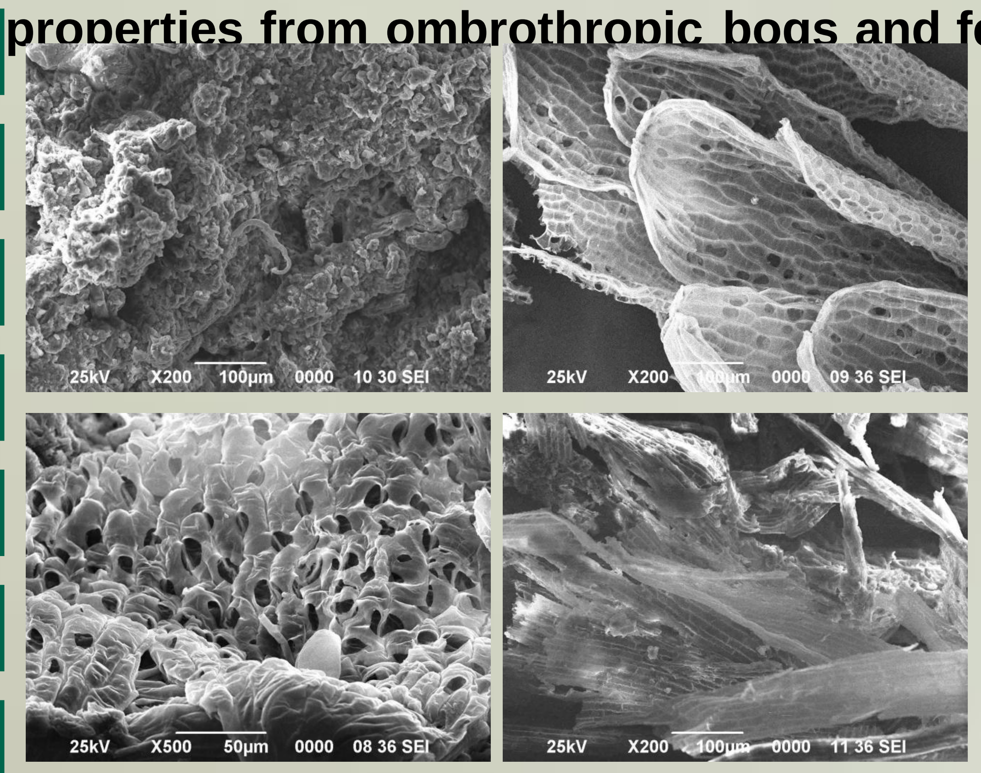

Fig.5. SEM micrographs of the omrothropic bog and fens.

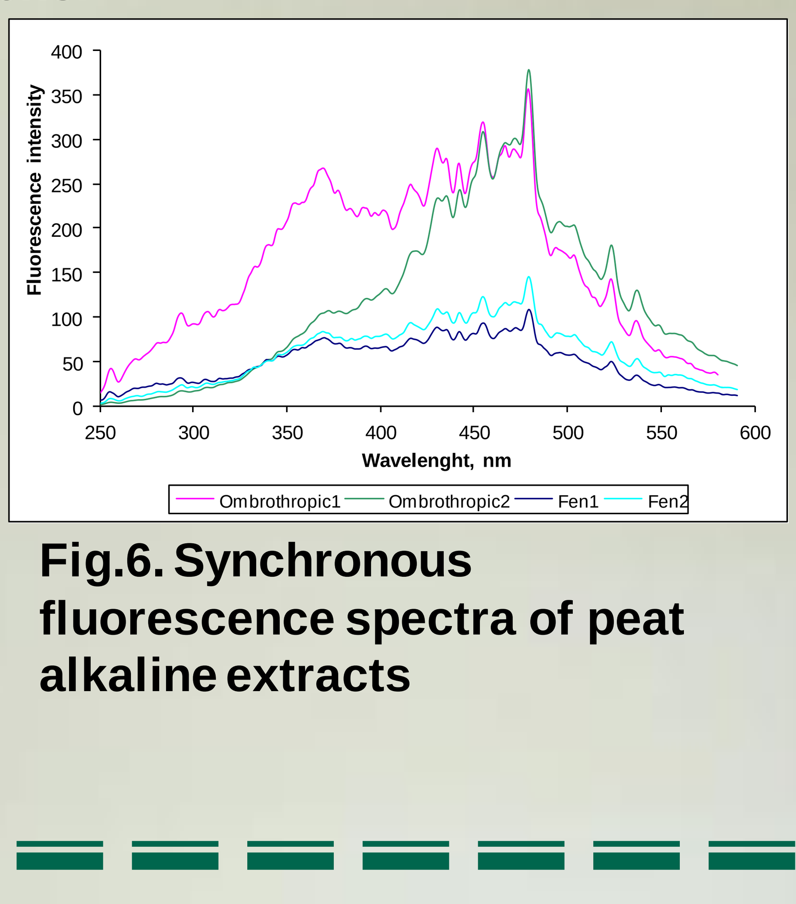

Fig.6. Synchronous fluorescence spectra of peat alkaline extracts

ACKNOWLEDGEMENTS This study was financially supported by Latvia National Research program ResProd.