P-093 **Assessment of ceramic beads as a carrier for bacteria immobilisation**

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INTRODUCTION AND OBJECTIVES

Ceramic products with controlled porosity can be used for efficient, cost-effective remediation applications. The high surface area ensures efficient contact between the water/air/soil and the beneficial bacteria (Mihailova 2008). The ceramic carriers are mechanically stable and could be chemically and steam cleaned for re-use (Lee 2004; Sheldon 2005). The aim of this study was to evaluate immobilisation of bacterial consortium with nitroaromatics-degrading activity onto ceramic beads.

MATERIALS AND METHODS

Bacterial consortium, including *Pseudomonas fluorescens*, as well as four strains belonging to *Stenotrophomonas maltophilia*, was cultivated at 28 °C, 48 h, with agitation 140 rpm. Medium composition was as follows, g/l: Na_2HPO_4 – 6.0; KH_2PO_4 – 3.0; NaCl – 0.5; molasses – 5.0; yeast extract – 2.0. Cell concentration was expressed as colony-forming units (CFU) per ml and determined by making serial decimal dilutions and plating on TGA (Sifin, Germany). CFU were counted after 72 h plate incubation at 28 °C. Ceramic beads were prepared at 1200 °C (Table 1). Ceramic beads were rinsed with distilled water then heated at 120 °C for 5 h. Two series of experiments were performed. The 1st experiment. Ceramic beads were soaked in culture liquid with bacteria (8.4×10^8 CFU/ml), with and without 1% agar, during 10 min. Afterwards, ceramic beads were dehydrated in Petri dishes at 28 °C overnight and at 37 °C for 3 days. For fluorescein diacetate (FDA) hydrolysis assay one ceramic bead was placed in a tube with 5 ml 0.06 M phosphate buffer pH 7.6, containing 0.4 mg FDA in 0.2 ml acetone. FDA hydrolysis activity was determined after 60 min incubation at 28 °C, in fourplicate. After incubation, beads were rinsed in 5 ml sterile distilled water twice and tested for FDA hydrolysis assay once more. FDA hydrolysis was measured in liquid phase after beads incubation, having added an aliquot of acetone, centrifuged at 4000 rpm and read at 490 nm (Chen 1988). The 2nd experiment. Ceramic beads were incubated in a growing bacteria culture (initial concentration 1.1×10^6 CFU/ml), for 192 h at 28 °C with periodic agitation. Beads were taken for analysis in 18 h; 120; and 192 h incubation, dehydrated and tested for FDA hydrolysis activity, as indicated for the 1st experiment.

Table 1 : Physical characteristics of ceramic beads

Characteristics	Value
Diameter, cm	1.18±0.63
Weight, g	0.63±0.07
Particle density, g/cm^3	0.45
Water absorption (24 h / 28 days), %	28.5 / 52.0
Particle porosity, %	72.0
Pore size on the surface, nm	60 ÷ 10 000
The predominant pore size, μm	0.5 ÷ 10

RESULTS AND DISCUSSION

An efficiency of bacteria immobilisation onto ceramic beads was tested by measurement of microbial enzymatic activity. Hydrolysis of FDA has been suggested as an appropriate method in integrated bioecosystem studies because the ubiquitous lipase, protease, and esterase enzymes are involved in the hydrolysis of FDA (Green 2006).

Effect of bead rinsing to biofilm integrity

One of the most important biofilm characteristics is the maintaining its integrity after rinsing. Bacteria immobilised on the surface of ceramic beads were tested for enzymatic activity to hydrolyse FDA, before and after rinsing. As shown in Fig. 1, rinsing procedure did not affect microbial activity of FDA hydrolysis.

Dependence of biofilm formation on exposition time

Incubation of ceramic beads in liquid medium with inoculum showed a strong dependence of FDA hydrolytic activity onto bead surface on the period of incubation. Thus, FDA activity after 18 h, 120 h, and 192 h was found to be 0.3; 1.8; and 2.2 μM/bead/h, respectively (Fig. 2). Dependence of FDA hydrolysis activity on cell concentration in liquid broth was found to be linear (Fig. 3).

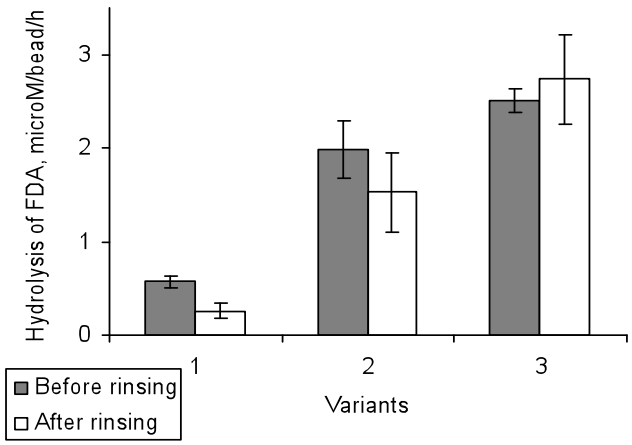

Figure 1 : Hydrolysis of FDA by bacteria cells immobilised on one ceramic bead. 1 – without cell immobilisation procedure (incubation in sterile liquid medium); 2 – cell immobilisation by 10 min contact with culture liquid with bacteria; 3 – as variant No 2, in the presence of 1 % agar. (Description in Materials and methods as the 1st experiment.)

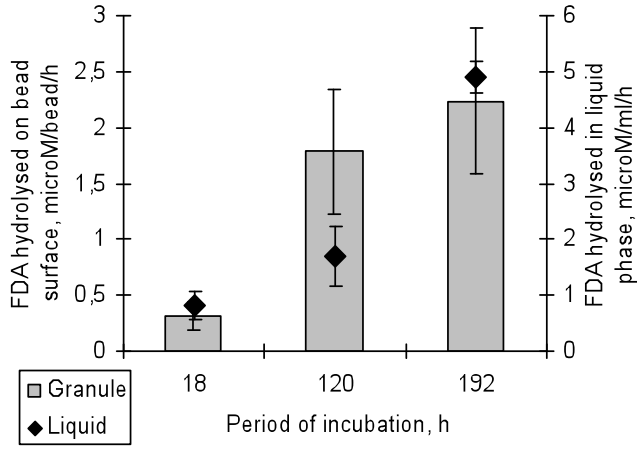

Figure 2 : Microbial activity of FDA hydrolysis on ceramic bead surface and in liquid in dependence on the period of incubation. (Description in Materials and methods as the 2nd experiment.)

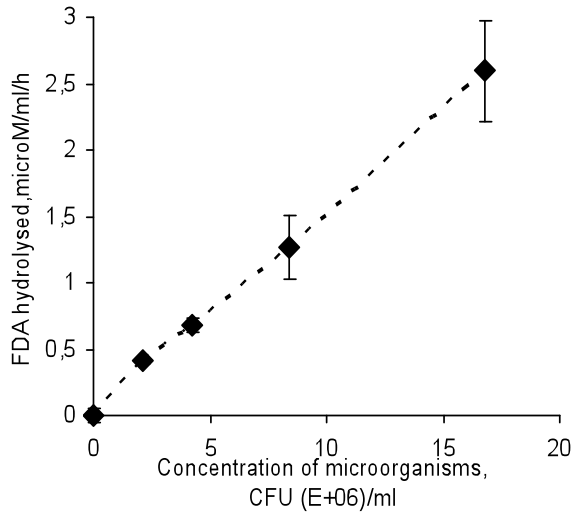

Figure 3 : Dependence of FDA hydrolysis activity on bacteria concentration.

CONCLUSIONS

Summarizing the results obtained in these experiments with nitroaromatics-degrading bacteria consortium, ceramic beads could be evaluated as an appropriate carrier for bacteria immobilisation.

Enzymatic activity of microorganisms depends on many factors, such as physiological state of microorganisms, chemical composition and physical properties of environment. A correlation of enzymatic activity and the number of sessile microorganisms or the overall biomass is not yet possible. It is not known how long hydrolytic enzymes are active after their mother cells may have died (Flemming 2000). At the same time, immobilisation of the both, microorganisms and enzymes on the carrier surface could provide efficient biodegradation. For further experiments, the main goal will be to distinguish immobilisation processes of enzymes and whole bacteria cells, as well as to assess the ceramic beads in biodegradation model experiments.

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