P-085

Polyacrylamide carriers for immobilizing chromatographic separation of bacterial cells

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INTRODUCTION AND OBJECTIVES

Necessity in effective chromatographic method of separation of mixed microbic cells objectively exists in biotechnological practice (Szumski 2005). But the decision of this problem is interfaced in presence of serious requirements to applied carriers. The further development of the chromatographic method demands carriers with satisfied characteristics: nontoxicity, chemical stability of material, mechanical strength, long enough durability. Also the carrier should possess high sorbtive capacity and sufficient elasticity.

In this work differently modified polyacrylamide cryogel (cryoPAAG) was used as a carrier for immobilizing separation of mixed bacterial cells. In particular, dodecylaldehyde was applied to modify cryoPAAG. As control for reference the cryoPAAG without modification also was used in experiments. Macroporosity is the specific feature of the cryogel structure (Lozinsky 1998).

It is necessary to note that introduction of specific chemical group in to structure of polymer carrier leads to obtaining of immobilizing chromatographic carriers enabling simple separation of biological particles and cells. So, the main aim of the investigation was the use of carrier with modified present in the mixed cultures and possessing different hydrophobicity of their cell walls.

MATERIALS AND METHODS

Bacterial cells *Pseudomonas sp.* and *Rhodococcus erhytropolis* being soil bacteria were used in the research. The cells are contaminated with oil in a consortium in soils (Chugunov 2000).

Suspension of cells taken in concentration 0.5 g/l were applied in experiments as main modeling objects of investigation. Physiological solution with addition Tween 80 was used as eluent. The carrier was washed with physiological solution pumped at rate 1 ml/min. The loading of immobilizing carrier by cells and their elution from cryoPAAG was done with pumping velocity 0.3ml/min.

Determination of intracellular ATP concentration was realized using luciferine-luciferase method (Efremenko 2003).

Determination of optical density of analyzed solutions was conducted spectrofotometrically at 540 nm. To reveal the cell concentrations in a eluated solution, the calibrating graph was used.

To evaluate the standard microbiological methods were applied, allowing obtain of colony-forming units.

RESULTS AND DISCUSSION

At the first stage of work the immobilizing capacities of investigated carriers in relation of each tested culture was established. During elution of cells from carriers it was revealed that amount of *Pseudomons* sp. cells, removed from cryoPAAG was higher as compared to *R. erhytropolis* (Table 1). Probably, that was due to lager size of *Pseudomonas* cells than *Rhodococcus* cells. Also shape of the cells could influence their retardation in the frame of carrier pores (Schlegel 2006).

Table 1: Immobilizing capacity of used carriers (mg cells/mg carrier) in relation to tested cultures.

Cells carrier	*Pseudomonas sp.*	*R. erhytropolis*
Cryo-PAAG	0.372±0.015	0.299±0.012
C12-cryo-PAAG	0.373±0.017	0.273±0.010

For the further separation of cells from mixed suspensions the carrier C12-cryo-PAAG was used since at insignificant difference in values of immobilizing capacities, this carrier provided more effective elimination of cells from this carrier with washing solution containing detergent.

Probably, it was caused by the additional hydrophobic interactions appeared between cell surface and the carrier. Also it is necessary to note that use of carrier C12-cryo-PAAG guaranteed the peak on chromatogram connected with the beginning of cell eluation. This peak was bigger than peak, obtained during at use of non-modified carrier cryo-PAAG. Also less amount of cells was detected in break through when C12-cryo-PAAG was applied for immobilizing separation.

At separation of a mixture of bacteria as the model sample with suspensions of *Pseudomonas sp.* and *R. erhytropolis* cells take in ratio 1:1 (the general cell concentration was 0.5 g/l) were used (Fig. 1).

It was established that most part of cells *R. erhytropolis* is immobilized on carriers during loading of mixed bacterial

cells. Cells *Pseudomonas sp.,* possessing significantly lower water hydrophobicity of their cellular wall, were removed from the carrier during their washing by physiological solution.

The ratio between cells established in eluate with detergent was 1:4 (*Pseudomonas sp.*: *R. erhytropolis*), whereas their initial ratio in mixed culture was 1:1.

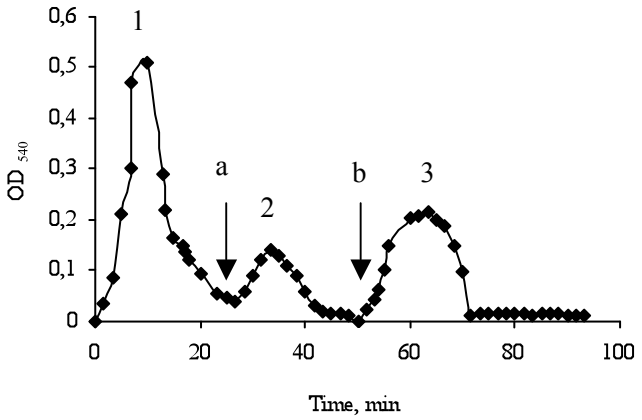

Figure 1: Immobilization and elution of mixed cells *Pseudomonas sp.* and *R. erhytropolis* by use the carrier C12-cryo-PAAG as carrier. Arrows specify the beginning of washing of the carrier by physiological solution (a) and eluation of cells by physiological solution containing detergent (b), correspondently.

The microscopic analysis of probes corresponding to peaks received during separation of mixed bacterial culture (Fig.1), it has been established that at different stages of chromatographyc separation of cells by the help of immobilized chromatography and use of hydrophobic carrier the ratio between cells in eluate changed (Fig. 2).

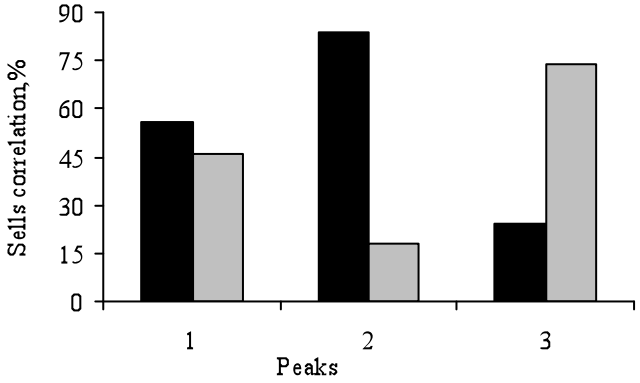

Figure 2: Ratios of cells in probes, collected during immobilizing chromatography separation of bacteria initially introduced to the system as mixed cultures: black columns - *Pseudomonas sp.*, grey columns - *R. erhytropolis.* Number of peaks are presented at Fig. 1.

CONCLUSIONS

Thus, approbation of the offered carriers allowed to establish C12-cryo-PAAG is appropriate carrier for separation of cells *Pseudomonas sp.* and *R. erhytropolis*, present in mixed culture. Conditions of cell separation by means of the chosen carrier can be optimized further to increase efficiency of process.

It should be noted that special scientific interest represents research of possible use of chosen supermacroporous carriers for hydrophobic immobilizing chromatographic separation of the bacterial cells possessing various structures of a cellular walls. Tested mixed culture present in various natural consortia. So, chromatographic isolation of cells from such complex biosystems and definition of concentration of isolated cells constitute new approaches to soil bioremediation presuming allocation of certain type of cells.

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