P-053 **Entrapment of yeasts cells in sol-gel derived organic-inorganic hybrid materials**

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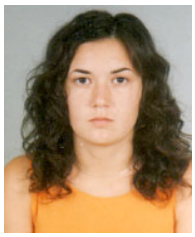

INTRODUCTION AND OBJECTIVES

The sol-gel process, taking place at a molecular and atomic level, gives the possibility of synthesizing new kind of materials with different properties, high level of purity and homogeneity at low temperatures (Brinker 1990).
Environmental pollution with metals and xenobiotics is a global problem, and the development of new cost effective technologies for metal removal from waste waters is therefore of significant interest. The accumulation of metal ions from waste waters in the microbial cells and use of biotechnological methods may help to obtain alternative methods for detoxification and removal of these industrial pollutants. Of crucial importance for the application of the biotechnological methods during the extraction of heavy metals from polluted waters is the multiple use of the bisorbent with the possibility of extraction of a desired metal by an electrolite pathway. The development of a bioremediation process needs further investigations in the field of modeling regeneration of the biosorbent material and of testing of immobilized microorganisms on industrial pollutants (Espinoza-Quiñones 2009, Godjevargova 2006).
The yeasts belonging to the *Trichosporon cutaneum* and *Candida intermedia* are successfully applied for phenol and its derivatives transformation and biosorption of heavy metal ions (Espinoza-Quiñones 2009, Godjevargova 2006).The ability of the yeasts stains to grow in the media supplemented with high content of phenols revealed that the capacity of stains to sustain toxic concentrations of heavy metals in the medium often refers to its ability to accumulate harmful ions in the cells. The stains are isolated from soils, industrial waste waters and wood wastes. The investigations during the recent years showed that by using microbial technologies for detoxification of contaminated waters from ions of heavy metals the technologies are more economical compared to the already existing technologies.
The aim of the present work is to study the possibilities for entrapment and vitality of two types of yeast cells in hybrid organic-inorganic matrices.

MATERIALS AND METHODS

The yeast strain *Candida intermedia PL 50*, maintained in the culture collection of Bulgarian National Bank of Industrial Microorganisms and Cell Cultures under N198 and the filamentous yeast strain *Trichosporon cutaneum R57* maintained under N 2414 were used in the investigations. The cultivation was carried out in a medium according (Georgieva 2008). The sterile glucose solution (20 g/l) was sterilized separately and was added to the growth medium. Starter cultures were prepared by loop-inoculating 100 ml liquid medium and incubating for 18 hours on a rotary shaker (180 rpm at 30^0C). For experimental cultures, 100 ml of medium was inoculated with 5 cm^3 of the starter culture (to an initial biomass concentration of approximately 0,1 mg dry wt/ml) and incubated on the shaker for 24 hours at 30^0C. Cells were harvested in the late exponential phase of growth.
A prehydrolyzed solution of tetraethylsiloxane (TEOS, H_2O, HCl) is first prepared, then dissolved organic parts is added and its pH is increased up to about 7 by adding the phosphate buffer before entrapment. It is then mixed rapidly with the cell suspension (10 ml, approximately 0.22 g-dcw). Gelation occurs within few minutes and a porous silica network is formed around cells. Gels are then aged for several days at room temperature.
Structural details were obtained using a JEOL JSM- 5510 Scanning Electron Microscope (SEM).
N_2 adsorption at 77 K was utilized to determine the specific surface areas and porosities of the prepared hybrids. A gas adsorption manometry apparatus was used for the N_2 adsorption experiments. The BET equation was used for the calculation of the specific surface area.

RESULTS AND DISCUSSION

Synthesized hybrid materials were applied as carriers for entrapment of yeast cells *Trichosporon cutaneum* starin R 57 and *Candida intermedia* starin PL 50.

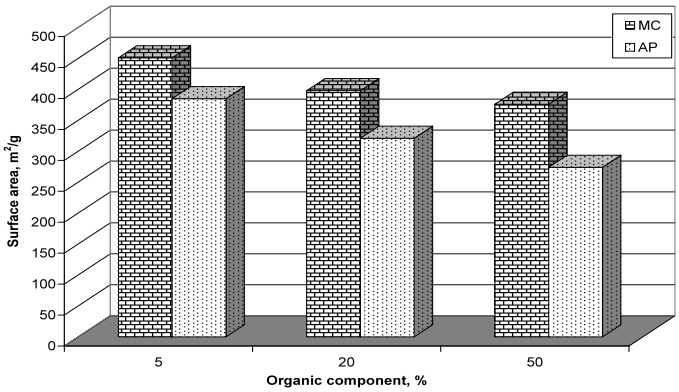

Figure 1 : BET analysis of the obtained hybrid materials with methylcellulose (MC) and pectin (AP)

The results of BET analysis proved that the pore diameter size is about 1.5 nm, and the specific surface area is in the range from 156 to 500 m^2/g depending on the hybrid chemical composition (Figure 1).

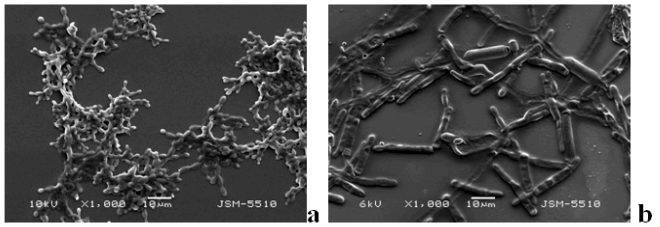

Figure 2 : SEM images of free *Candida intermedia* PL 50 (a) and *Trichosporon cutaneum* R 57 (b) cells

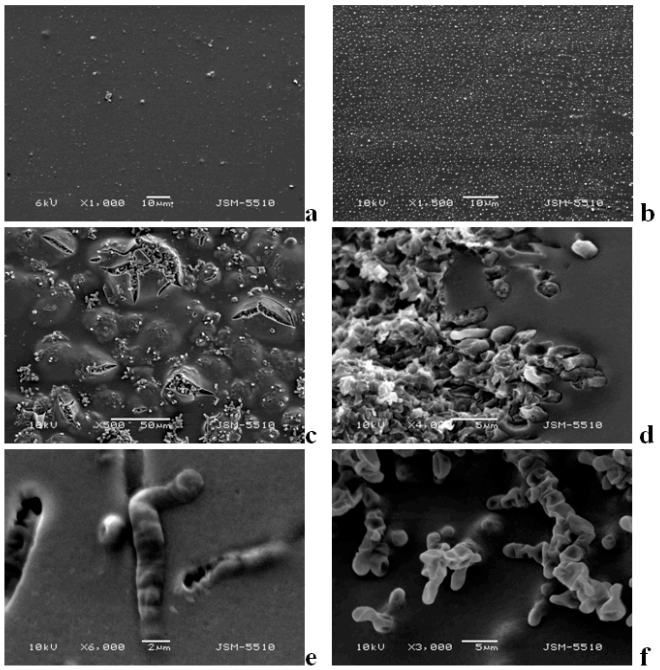

Figure 3 : SEM images of pure matrices containing AP 5 (a) and 50 (b) wt % and entrapped *Candida intermedia* PL 50 (c, d) or *Trichosporon cutaneum* R 57 (e, f) cells

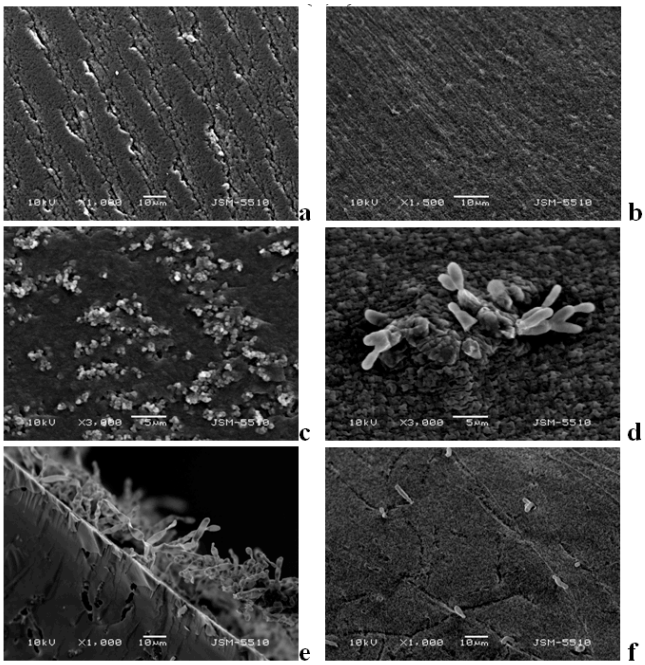

Figure 4 : SEM images of pure matrices containing MC 5 (a) and 50 (b) wt% and entrapped *Candida intermedia* PL 50 (c, d) or *Trichosporon cutaneum* R 57 (e, f) cells

Surface area in obtained samples containing AP (385 m^2/g) is lower than this one containing MC reach to 451 m^2/g. The pore size in the hybrids containing AP lead to 1.54 nm in comparison with this one containing MC (1.24 nm).

The large pore size can lead to penetration of substrate in matrix and increasing of cell activity. The obtained results from SEM and BET analysis are in good correlation. SEM observation of the immobilised by entrapment cells in hybrid matrices showed that yeast cells are uniformly distributed into carriers (Figures 3 and 4). The micrograph on Figure 3 c, d shows more entrapped cells of the strain *PL50* (6.10^6 CFU/mg carrier) into organic-inorganic hybrid containing AP in comparison with strain *R57* (3.10^6 CFU/mg carrier – Figure 3 e, f). The micrographs on Figure 4 e, f show entrapped cells of strain *R 57* into hybrids containing MC and they are 5.10^6 CFU/mg carrier compared with the strain *PL50* ($2.5.10^6$ CFU/mg carrier). Consistent with BET analysis, the large pore size by hybrids containing AP can lead to increasing of entrapped cells by strain *PL 50* in contrast to strain *R 57*, by which is observed decrease in entrapped cells due to filamentous nature of the strain.

CONCLUSIONS

Yeast cells are successfully entrapped in organic inorganic hybrid materials synthesized by sol-gel method. The obtained biocatalysts will be cultivated under conditions of inhibitory concentrations of copper and cadmium ions. The dynamics of the accumulation of biomass in the presence of the corresponding ions, the velocity of depletion of nutrient substrate, the dynamics of extraction of the Cu and Cd ions by the microbial cells will be followed.

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