ENHANCED ADHESION OF MICROORGANISMS TO POLYMER USING SURFACE MOLECULAR IMPRINTING TECHNOLOGY

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Introduction

The technology of molecular imprinting of polymers (MIPs) is very powerful in producing of selectively working sensing systems. The polymeric sensors obtained with this technology are actually used for separation of small molecules and ions [1–4], peptides, cells, microorganisms [5] and some others biological systems like viruses [6]. The MIPs for small molecules separation are usually polymerised as bulk porous materials, which can be used as solid phase for example in chromatography, but for peptides and bigger biological objects a surface molecular imprinting of polymers (SMIPs) methodology has to be used. This methodology combines molecular imprinting technology and stamping technique. This technology leads to obtaining of surface imprinted cavities with molecular memory of particular molecular system-template. The template by means of biological object forms complex supramolecular structure with the functional monomers during the polymerisation and processing [7].

The SMIPs technology was used for obtaining of the polymeric surface imprinting against various microorganisms: yeast *Saccharomyces cerevisiae* and bacteria.

Experimental

Materials

Polymeric matrices were prepared using methacrylate monomers: HEMA, TRIM, an initiator-benzoinethyl ether and solvent-THF.

Processing

The thin-layer polymer film was obtained by purring of the mixture on the microscopy glass using rotating base (*spin-coating*). We applied the stamp method, as is shown in Figure 1, for surface imprinting of the pre-polymerised thin film against big variability of microorganisms: yeast *Saccharomyces cerevisiae* and bacteria. After imprinting the films were washed for removing of the biological materials. After washing the molecularly imprinted films were ready for readsorption

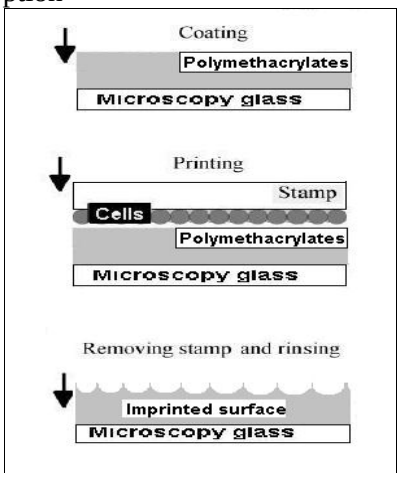


Figure 1 Procedure of surface imprinting. The yeast or bacteria stamping was after preliminarily polymerisation

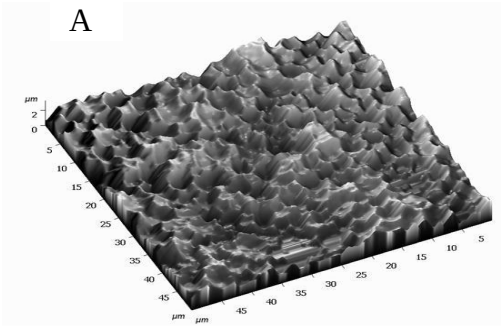
We studied the selectivity of re-adsorption of the cells by the film incubating them in aqueous suspension of particular cells. The Atomic Force Microscopy technique in two modes were used: semi-contact AFM mode with NOVA software package, produced by Solver pro, NT-MDT Co., Moscow, Russia, and adhesion force spectroscopy measurement with a cell-probe immobilised at the end of a standard cantilever.

Results and discussion

Conception of SMIPs is based on hydrogen bonding and van der Waals forces [7,8] that are formed at the interface between monomers and the template-biological material. After removing of the biological material, the modified by imprinting polymeric surface presents functionality leading to selective adsorption of the cells that have been used as template in the processing. Figure 2A and 2B showing the AFM images of polymer surfaces: imprinted A) and for comparison nonimprinted B).

Among others, the experiment with two top-fermenting brewing yeast strains of *Saccharomyces cerevisiae* K2 cat. No. C223 and

LVB Gaffel cat. No. 226 have been used as template and the polymer surface were imprinted.



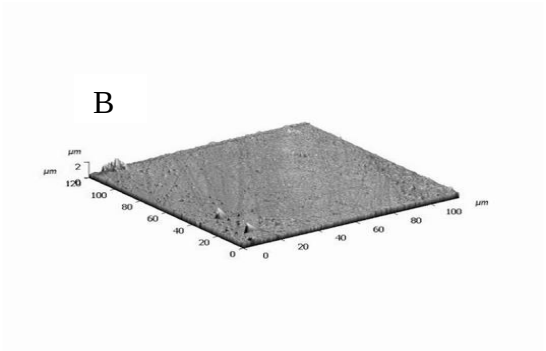


Figure 2. 3D AFM images of imprinted A) and nonimprinted polymer B).

Strong re-adsorption effect of the cells into the cavities, which were empty after the processing and washing was observed when the polymer imprinted against cells 226 were incubated in aqueous suspension of the same strain cells 226. Figure 3 shows AFM image of polymer matrix imprinted with cells 226 after incubation in an aqueous suspension of the same strain cells - 226.

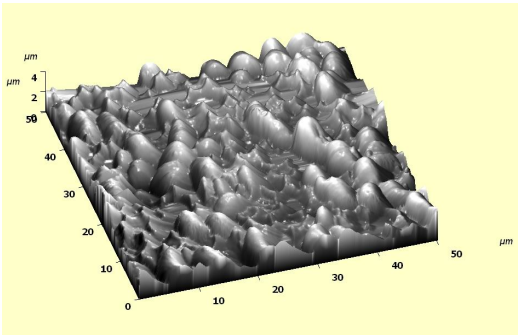


Figure 3. AFM image of polymer imprinted with cells 226 and then incubated for 30 min. with the same strain cells 226.

No adsorption effect of the cells at polymeric surface was observed after incubation of the polymer matrix (imprinted with cells 226) in aqueous suspension of the cells 223 and/or other biological material. The selectivity that we observed results from the specific interactions between polymeric functional groups and the "saccharic-peptides code" at the cell wall, which is characteristic for each type of cells. The specific interactions lead to increase of adhesion forces between of the cell wall and the polymer surface. The Figure 4 shows two distributions of the measured adhesion forces between the probe cell, in this case C226, and the polymer imprinted with the same strain cells (M226) and for comparison imprinted with the other cells (M223). The distribution due to the compatible system cell probe-imprint showing higher adhesion force then the other case.

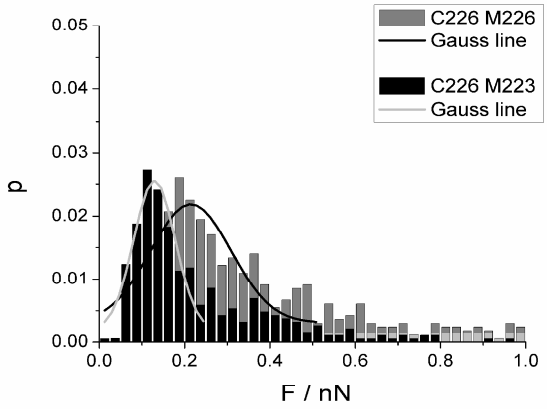


Figure 4. Adhesion force between the probe cell and the polymer, measured by force spectroscopy technique.

Conclusions

The sensing elements obtained by the technology of surface molecular imprinting of polymers (SMIPs) could be applied for identification and quantification of microorganisms in food industry and environmental studies.

References:

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