HIGH EFFECTIVE BIOCATALYTIC SYSTEMS ON THE BASE OF HORSERADISH PEROXIDASE IMMOBILIZED ON MAGNETIC NANOPARTICLES

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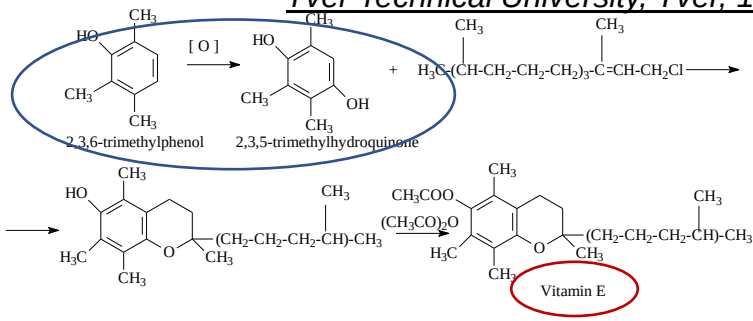
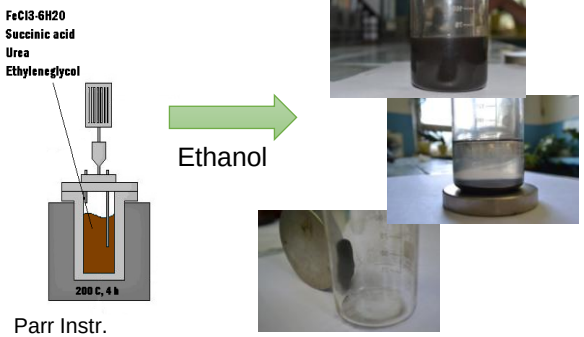


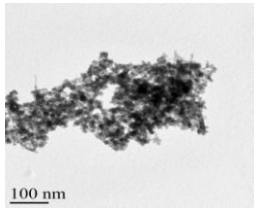
Synthesis of Fe_3O_4

$FeCl_3 \cdot 6H_2O$ (3 mmol), succinic acid (1 mmol) and urea (30 mmol) were completely dissolved in ethyleneglycol (30 mL). The solution obtained was inputted in a teflon beaker (50 mL) in the stainless steel autoclave (Parr Instr.) and was soaked at 200°C for 4 hours. After cooling to the room temperature, the black precipitate was separated with neodymium magnet and washed with ethanol several times until a clear solution.

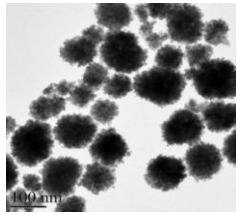


TEM pictures of Magnetic Nanoparticles

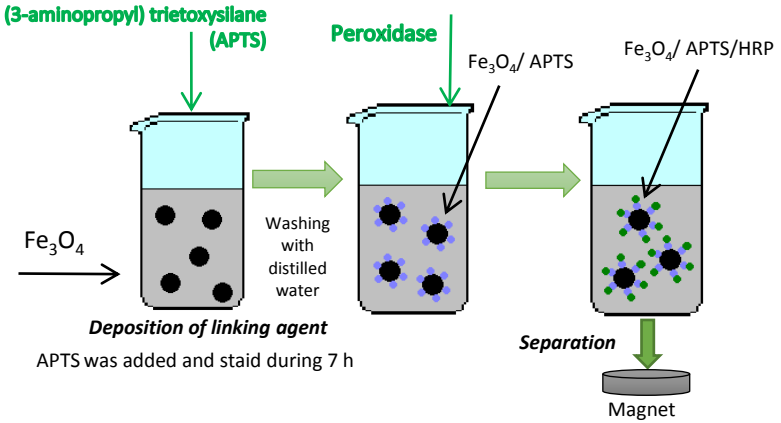
Co-precipitation (Classical Synthesis)



Polyol Method

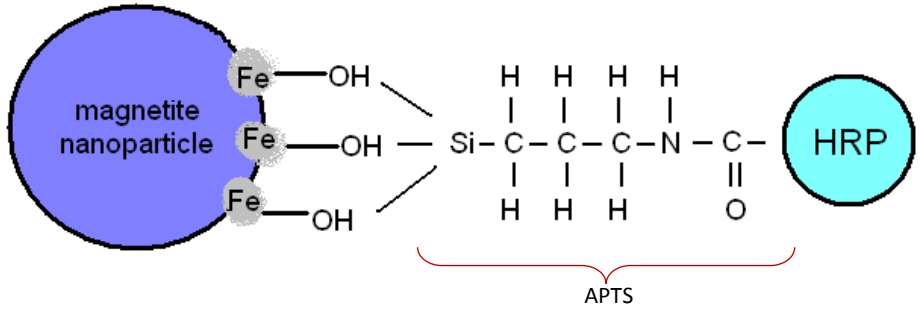


Scheme of Catalyst Synthesis

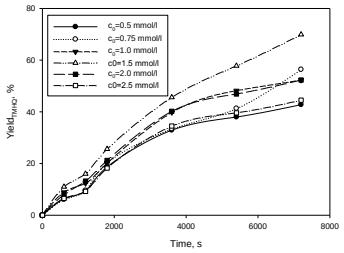


APTS was added and staid during 7 h

Scheme of the structure of the biocatalyst on the basis of HRP immobilized on MNPs



Determination of the optimal concentration of 2,3,6-trimethylphenol

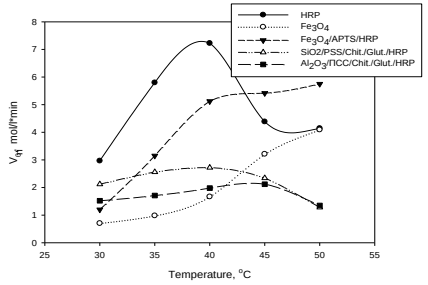


The optimum concentration of TMP was 1.5 mmol / L in both cases

Initial reaction volume 30 mL, biocatalyst concentration 0.2 g/L, pH=6.5, temperature 40 °C, $C(H_2O_2)$=1.5 mol/L

2,3,6-trimethylphenol – TMP
2,3,5-trimethylhydroquinone - TMHQ

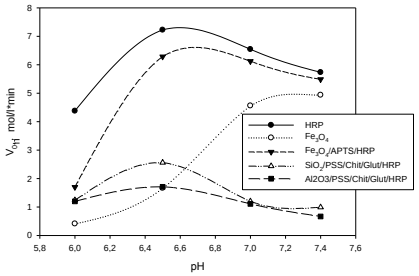
Temperature optimization



The increase of temperature higher than 40°C does not result in the decrease of activity in the case of the HRP immobilized on Fe_3O_4 magnetic nanoparticles in contrast to the native HRP. The enzyme becomes thermostable. This is likely due to the strong binding of the HRP molecule with the support, which results to the stabilization of the enzyme conformation.

Initial reaction volume 30 mL, biocatalyst concentration 0.2 g/L, substrate (TMP) concentration 1.5 mmol/L, pH=6.5, $C(H_2O_2)$=1.5 mol/L

pH optimization



Influence of pH in the range from 6.0 up to 7.4 was carried out

Maximum yield of TMHQ was found at pH 6.5, and this value was used for further investigations

Initial reaction volume 30 ml, biocatalyst concentration 0,2 g/L, substrate (TMP) concentration 1.5 mmol/L, temperature 40 °C, $C(H_2O_2)$=1.5 mol/L

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