BIOLOGICKÉ CENTRUM AV ČR, v. v. i.

Akademie věd České republiky
Strategie AV21
Špičkový výzkum ve veřejném zájmu

MAGNETICKÉ MATERIÁLY PRO BIORAFINACI POTRAVINÁŘSKÝCH ODPADŮ

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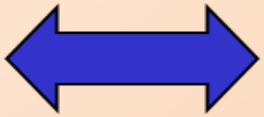
Proč jsou magnetické materiály významné pro biovědy a biotechnologie?

Jedná se o inteligentní materiály!
(smart materials, stimuli responsive materials)

Magnetické materiály vykazují několik typů odezev ke vnějšímu magnetickému poli

Nejdůležitější odezva

Selektivní separace magnetických částic



Magnetické materiály pro biorafinace

- **Magnetické katalyzátory**
  - Magnetické enzymy
  - Magnetické kyselé a basické katalyzátory
  - Magnetické nanočástice s peroxidasovou aktivitou

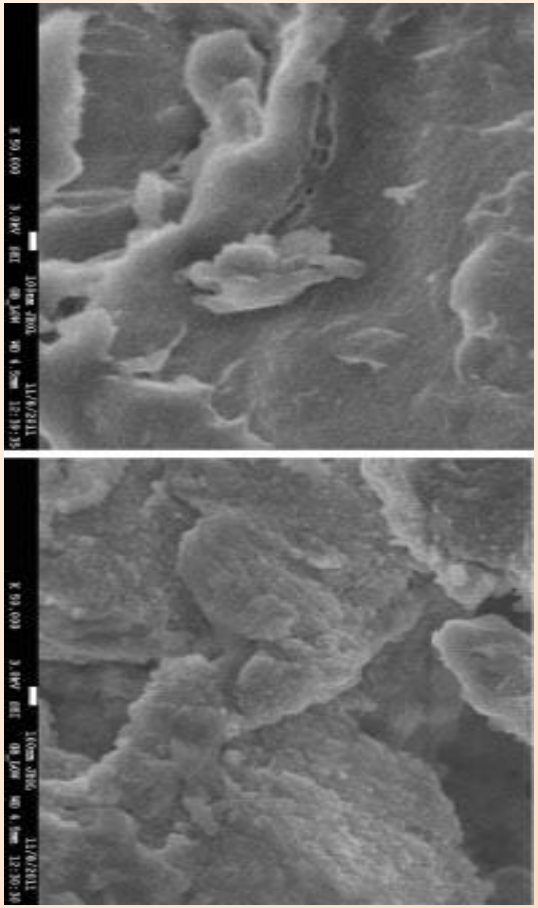
- **Magneticky modifikované buňky**
  - Magnetické celobuněčné biokatalyzátory
  - Magnetické buňky jako adsorbenty

- **Magnetické materiály pro isolaci cílových produktů**
- **Imunomagnetická separace patogenních mikroorganismů**
- **Příprava magnetických biosorbentů pro odstraňování polutantů**

Magnetické katalyzátory

- Jednoduchá separace pevných (imobilizovaných) biokatalyzátorů z reakčního systému
- Zjednodušení biorafinačních procesů
- Nižší cenová náročnost (možnost využití potravinářských odpadů jako nosičů pro imobilizaci enzymů)
- Přeměna biomasy na biopaliva nebo jiné významné produkty
- Biokatalýza (magnetické imobilizované enzymy) nebo magnetické pevné katalyzátory (kyselé, basické)

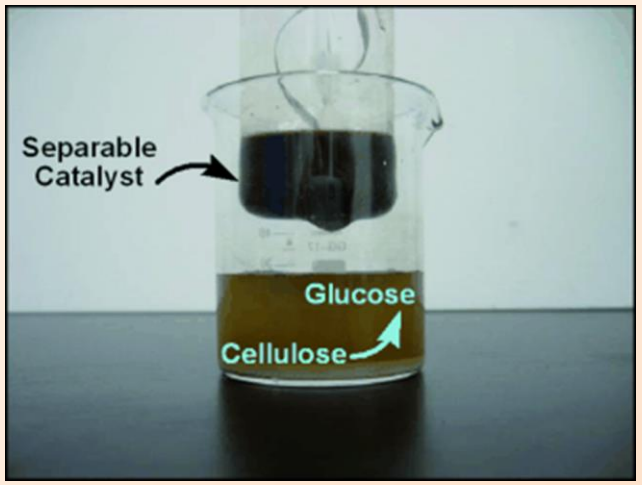
Magnetické imobilizované enzymy využitelné pro biorafinace



Magnetické mláto

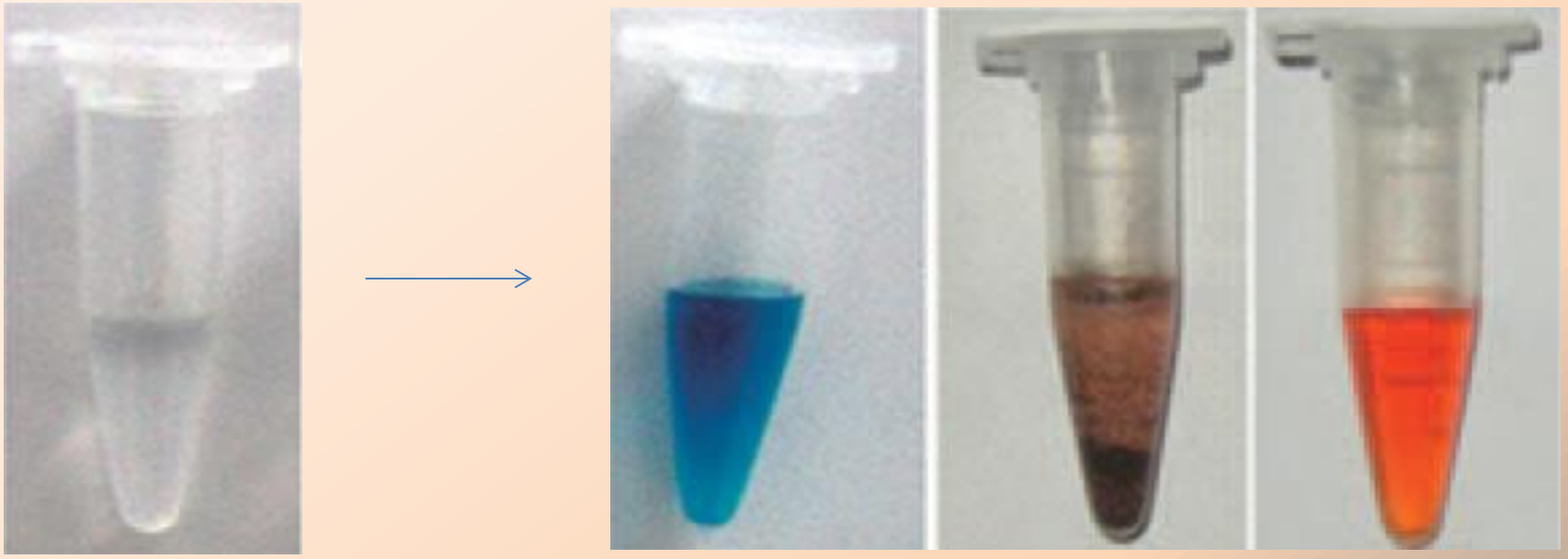
Enzyme	Magnetic carrier	Carrier preparation and activation	Immobilization of enzyme	Application of immobilized enzyme	Additional note
Cellulase mixtures (CellicCTec2)	Non-porous magnetic particles (0.5-1 μm), silica coating	Activated with cyanuric chloride / polyglutaraldehyde	Covalent immobilization + blocking with BSA	Hydrolysis of lignocellulose (wheat straw)	Easy recycling; study of surfactants effects
Cellulose (crude commercial preparation)	Superparamagnetic nanoparticles (12 nm)	Coprecipitation of Fe^{2+} and Fe^{3+} ions, hydrothermal conditions	Covalent binding after glutaraldehyde activation	Hydrolysis of steam-exploded corn stalks	Better pH tolerance, thermal and storage stabilities
Cellulose (*Trichoderma reesei*)	Zn-dopped magnetite nanoparticles	Hydrothermal method (Fe^{2+} + Fe^{3+} ions + $ZnCl_2$)	Covalent binding after glutaraldehyde activation	Saccharification of cellulose and *Cannabis* biomass	Economization of the production process cost of fermentable sugars
β-glucosidase (*Aspergillus niger*)	Magnetic nanoparticles	Coprecipitation of Fe^{2+} and Fe^{3+} ions, hydrothermal conditions	Covalent binding after glutaraldehyde activation	Cellobiose hydrolysis	Improved thermostability after immobilization
Lipase (*Aspergillus niger*)	Magnetic nanoparticles +PEA coverage	Commercial particles	Cross-linked enzyme aggregate on magnetic particles	Synthesis of glycerol carbonate from bio-glycerol	Carbonylation with dimethyl carbonate, solvent free conditions
Lipase B (*Candida antarctica*)	Magnetite nanoparticles with amino groups	Coprecipitation of Fe^{2+} + Fe^{3+} ions, functionalized by APTS	Immobilization on MNPs-NH_2 via GA activation; formation of mCLEAs	Synthesis of biodiesel	Reusability, easily magnetically recovered
Lipase (Bacillus)	Magnetite nanoparticles with amino groups	Coprecipitation of Fe^{2+} + Fe^{3+} ions, functionalized by APTS	Immobilization on MNPs-NH_2 via GA activation	Synthesis of biodiesel	Palm oil, cotton seed oil, waste vegetable oil, *Chlorella* oil used
Lipase (*Candida rugosa*)	Ionic liquid-modified magnetic nanoparticles (10-15 nm)	Covalent binding of ionic liquid on Fe_3O_4 nanoparticles	Covalent binding (ionic liquids – coupling reagent)	Ionic liquids controlled esters hydrolysis at oil-water interface	Nanoparticle-grafted lipase located at the oil–water interface

Magnetické kyselé katalyzátory využitelné pro biorafinace



Type of solid acid catalyst	Magnetic material	Preparation of magnetic solid acid catalyst	Application of magnetic solid acid catalyst	Additional note
Perfluoroalkylsulfonic (PFS) and alkylsulfonic (AS) acid-functionalized magnetic nanoparticles	Cobalt spinel ferrite nanoparticles (10 nm), coated with silica	Magnetic nanoparticles (silanol groups) modified by PFS / AS (sulfonic acid groups)	Pretreatment of wheat straw (hydrolysis of hemicelluloses to soluble oligosaccharides)	Significantly higher efficiency compared with the hydro-thermolysis
Magnetic core–shell Fe_3O_4@C-SO_3H nanoparticle catalyst	Magnetic Fe_3O_4 core encapsulated in a sulfonated carbon shell	Fe_3O_4@C synthetized in autoclave ($FeCl_3$, glucose, urea), sulfonated by H_2SO_4	Hydrolysis of cellulose	Recyclable
Magnetic solid acid with mesoporous structure (Fe_3O_4-SBA-SO_3H)	Magnetic Fe_3O_4 nanoparticles (20 nm) + Pluronic 123 + TEOS (assembling)	Modification by 3-mercaptopro-pyltrimethoxy-silane and oxidation (H_2O_2), compolymer extraction by ethanol	Hydrolysis of biomass	Biomass saccharification and levulinic acid production
Core shell Fe_3O_4@SiO_2–SO_3H acid catalyst	Silica-encapsulated Fe_3O_4 nanoparticles	Sulfonic acid groups modification (chlorosulfonic acid)	Hydrolysis of cellulose in ionic liquids	Reusability for several times without the significant loss of its activity
Magnetic silica nanoparticles with sulfonic acid groups	$CoFe_2O_4$-embedded silica nanoparticles	Sulfonic acid groups modification	Hydrolysis of sucrose, cellobiose, starch, cellulose	Much higher activity than strongly acidic ion-exchange resins
Sulfonic acid-functionalized silica-coated magnetic nanoparticle catalysts	Silica-coated magnetic nanoparticles	Grafting by four different sulfonic acids	Deprotection reaction of benzaldehyde dimethylacetal	Comparable activity to other commercial heterogeneous acidic resins

Magnetické nanočástice s peroxidasovou aktivitou

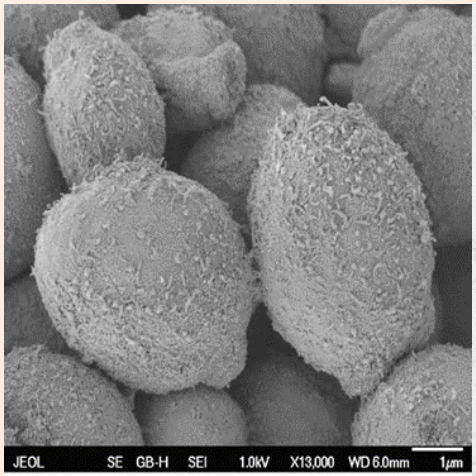


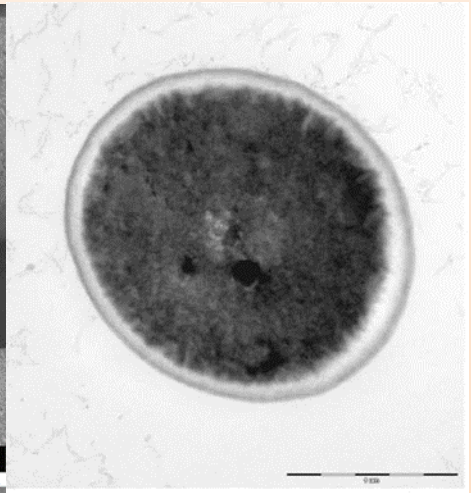
$$2AH + H_2O_2 \longrightarrow 2A + 2H_2O$$

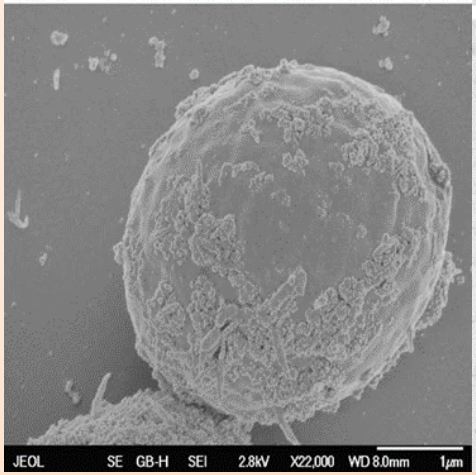
Magneticky modifikované buňky

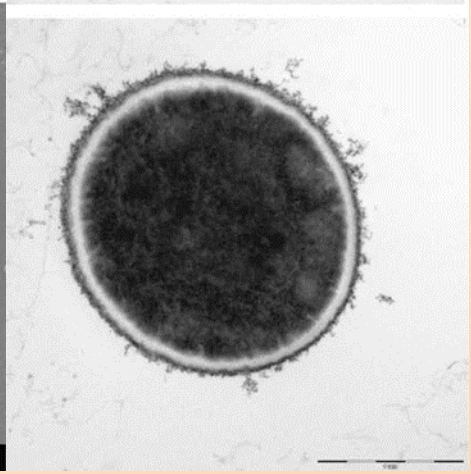
- Možnost jednoduché separace
- Využití jako celobuněčné biokatalyzátory
- Využití jako adsorbenty vybraných biologicky aktivních látek nebo xenobiotik
- Magnetické částice na povrchu buněk nebo v protoplasmě, imobilizace na magnetické nosiče, zabudování buněk do magnetických gelů

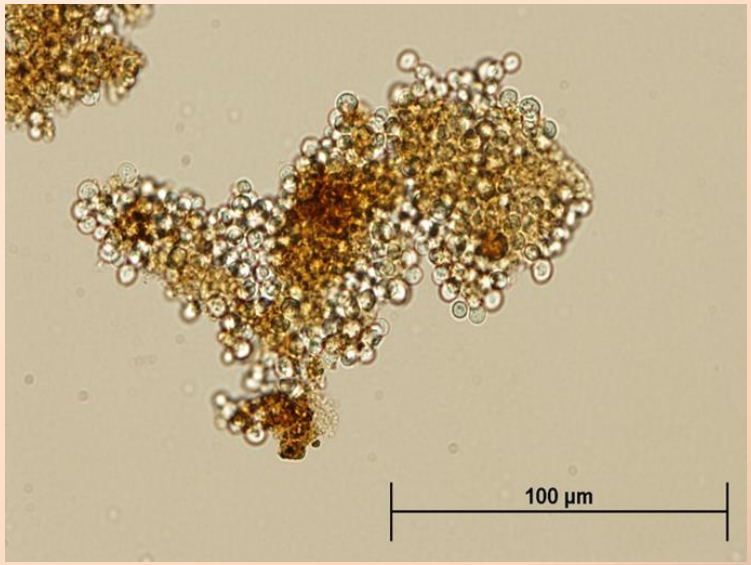
Magneticky modifikované buňky







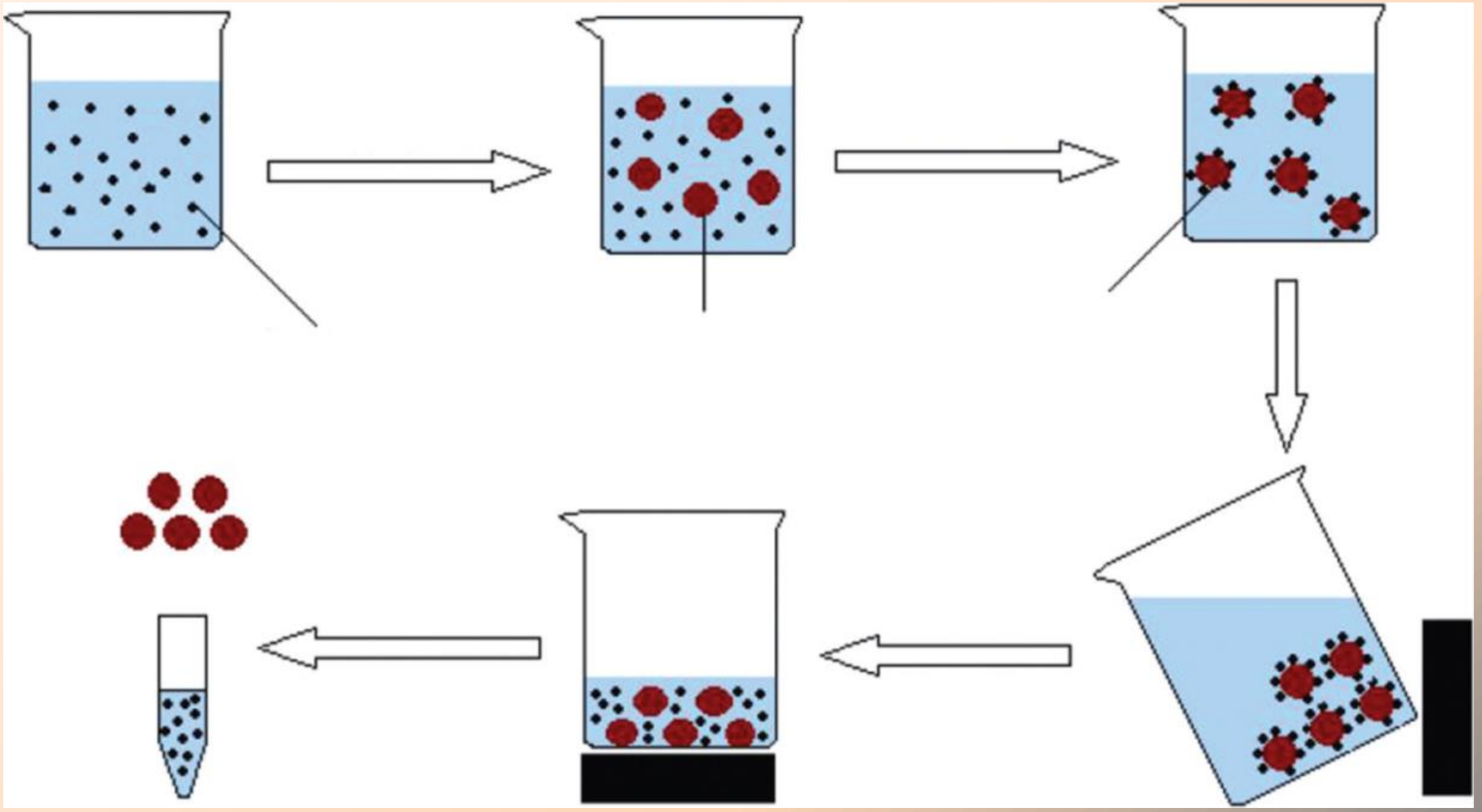




Magneticky modifikované kvasinkové buňky (*Saccharomyces cerevisiae*)

Magnetic particles	Magnetic modification	Application	Other notes
Magnetite	Immobilization in microwave-synthesized magnetic chitosan	Sucrose hydrolysis; H_2O_2 degradation	Also used for organic dye removal
Microwave-synthesized magnetic iron oxides	Incubation of cell suspension with magnetic particles	Sucrose conversion into glucose and fructose	With or without glutaraldehyde as cross-linking agent
Magnetite	Immobilization in alginate/magnetic nanoparticles	Ethanol fermentation	Theoretical yield of ethanol from starch hydrolysates~ 85%
Magnetite	Immobilization in chitosan-magnetite microparticles	Ethanol fermentation	Glutaraldehyde as cross-linking agent; theoretical yield ~ 73%
Magnetite	Immobilization in cellulose-coated magnetic nanoparticles	Ethanol fermentation	Theoretical yield ~ 70%
Magnetite	Encapsulation in magnetic alginate microbeads	Invert sugar formation	Long term stability
Mn-Zn ferrite	Immobilization in magnetic alginate beads	Ethanol fermentation	Continuous ethanol fermentation using magnetically stabilized fluidized bed bioreactor
Magnetite	Encapsulation in magnetic alginate beads	Hydrogen peroxide removal	Long term stability
Magnetite or magnetic fluid	Immobilization in magnetic calcium alginate beads	Ethanol production	No changes in properties after magnetization
Magnetite or magnetic fluid	Immobilization in magnetic calcium alginate beads	Ethanol production	Enzyme activity higher after incubation in nutrient medium

Magnetické materiály pro isolaci cílových produktů



Safarik,I., Safarikova,M.: Magnetic techniques for the isolation and purification of proteins and peptides. BioMagn. Res. Technol. 2 (2004) 7

Table 3: Examples of polysaccharide and disaccharide hydrolases purified by magnetic techniques

Purified enzyme	Source	Magnetic carrier	Affinity ligand	Further details	Reference
α-Amylases	Porcine pancreas, *Bacillus subtilis*, wheat germ	Magnetic alginate beads		Elution with 1 M maltose	[4]
	Bacillus amyloliquefaciens, porcine pancreas	Magnetic alginate microbeads		Elution with 1 M maltose	[10]
β-Amylase	Sweet potato	Magnetic alginate beads		Elution with 1 M maltose	[55]
β-Galactosidase	*Escherichia coli* homogenate	Silanized magnetite	p-Aminophenyl-β-D-thiogalactopyranoside	Elution with borate buffer, pH 10	[58]
β-Galactosidase (fusion protein comprising the DNA-binding lac repressor)	Bacterial lysate	Magnetic beads	DNA containing *Escherichia coli* lac operator	Elution with lactose analogue	[64]
Glucoamylase	*Aspergillus niger*	Magnetic alginate beads		Elution with 1 M maltose	[55]
Pectinase	Commercial preparation	Magnetic alginate beads			[82]
Pullulanase	*Bacillus acidopullulyticus*	Magnetic alginate beads		Elution with 1 M maltose	[55]

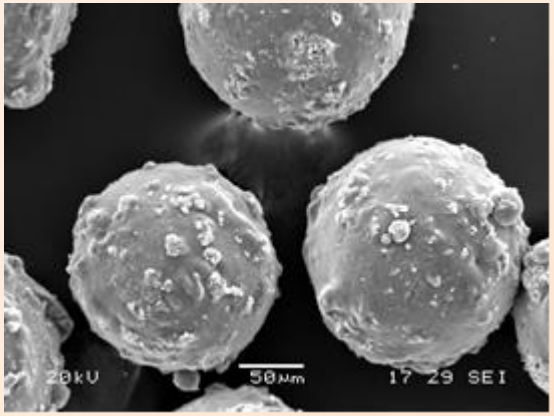
Isolace vaječných proteinů

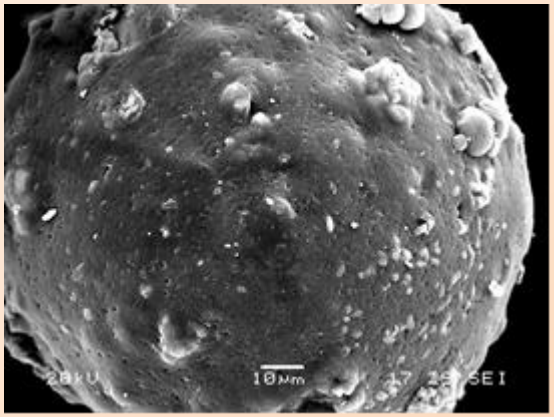
Table 3. Examples of egg white proteins isolated by magnetic separation techniques. Both diluted egg white and commercially available preparations were used as starting materials

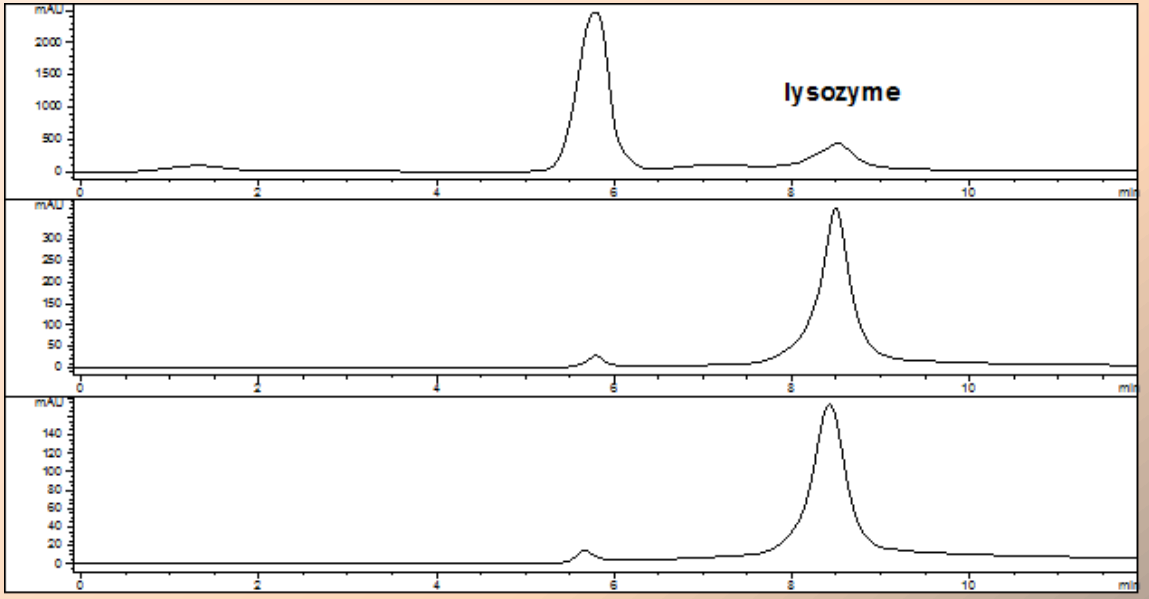
Purified protein	Magnetic carrier	Affinity ligand	Further details	Reference
Avidin	Maghemite nanoparticles	Biotin	Particles diameter 13 nm	(Fan et al. 2003)
Lysozyme	Magnetic chitin, magnetic acetylated chitosan	–	Elution with 0.01 M HCl	(Safarik 1991; Safarik and Safarikova 1993)
Lysozyme	Magnetic poly (2-hydroxyethyl methacrylate)	Cibacron Blue F3GA[a]	Elution with 1 M KSCN	(Odabasi and Denizli 2004)
Lysozyme	Magnetic chitosan beads	–	Magnetically stabilized fluidized bed	(Goto et al. 1995)
Lysozyme	Magnetic cross-linked polyvinylalcohol	Cibacron Blue 3GA[a]	Elution with high salt buffer	(Tong et al. 2001)
Lysozyme	Magnetite – polyacrylic acid nanoparticles	–	Ion-exchange separation	(Liao and Chen 2002)
Lysozyme	Magnetic cross-linked polyvinylalcohol beads	–	Adsorption study	(Xue and Sun 2001)
Lysozyme	Magnetic agarose beads	Cibacron Blue 3GA[a]	Magnetically stabilized fluidized bed	(Tong and Sun 2003)
Lysozyme	Magnetic chitosan	Cibacron Blue 3GA[a]	Study of adsorption properties	(Yu et al. 2000)
Lysozyme	Ferrofluid modified sawdust	–	Elution with 0.5 M NaCl	(Safarik et al. 2005)
Lysozyme	Nano-sized magnetic particles	–	Elution with NaH2PO4 and NaSCN	(Peng et al. 2004)

[a] Polysciences Inc., Warrington, PA, USA

Šafařík,I., Šabatková,Z., Tokar,O., Šafaříková,M.: Magnetic cation exchange isolation of lysozyme from native hen egg white. Food Technol. Biotechnol. 45 (2007) 355-359

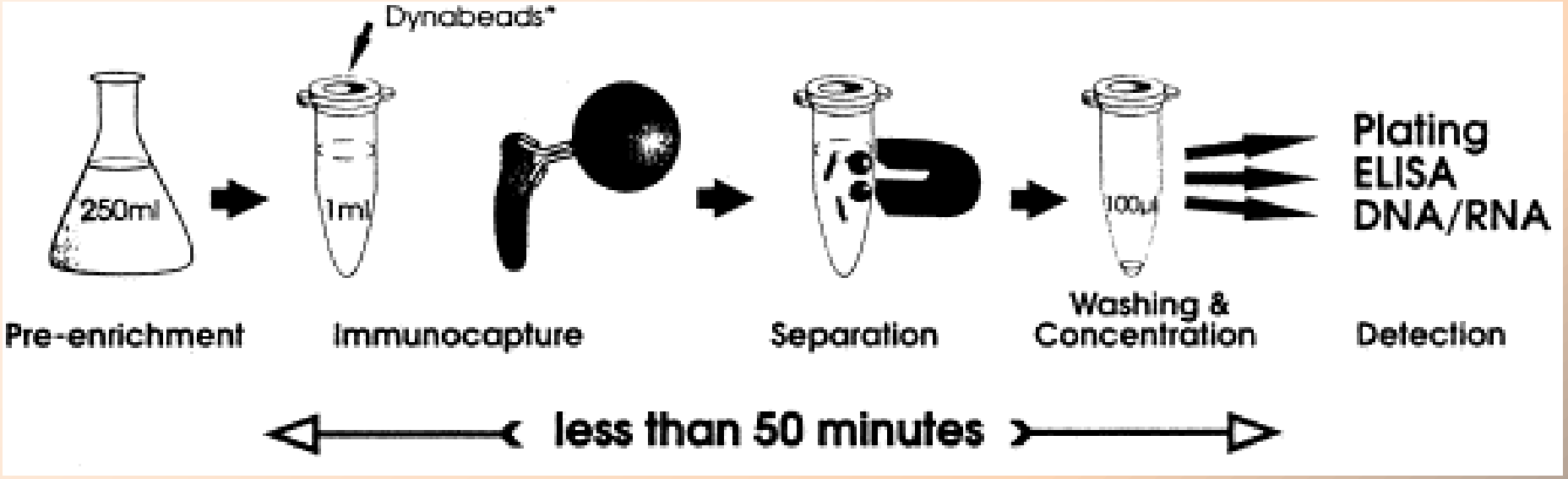






Safarik,I., Horska,K., Martinez,L.M., Safarikova,M.: Large scale magnetic separation of *Solanum tuberosum* tuber lectin from potato starch waste water. AIP Conf. Proc. 1311 (2010) 146 - 151

Imunomagnetická separace patogenních mikroorganismů

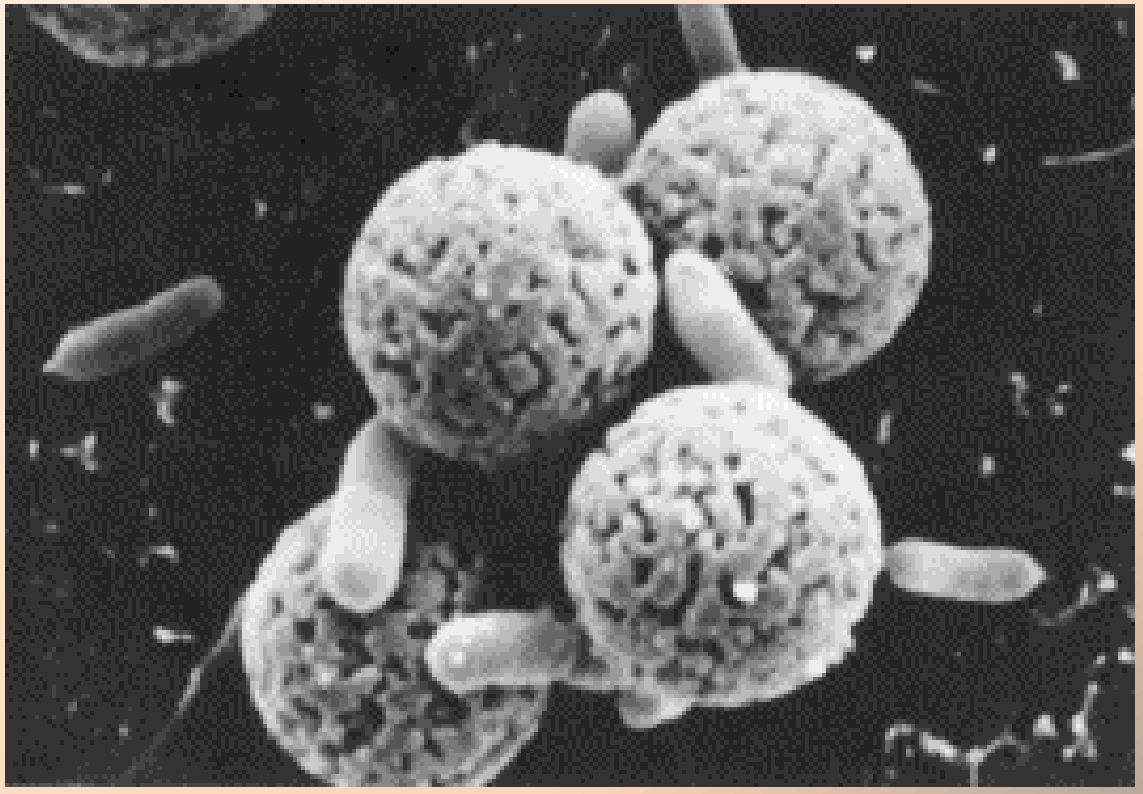


Imunomagnetická separace patogenních mikroorganismů

- *Escherichia coli*
- *Escherichia coli* O157:H7
- EPEC/VTEC O103, O111, O145, O26
- *Helicobacter pylori*
- *Legionella pneumophila*
- *Listeria (monocytogenes* + other species)
- *Mycobacterium avium* subsp. *paratuberculosis*
- *Salmonella* sp.
- *Yersinia enterocolitica*

Buňky *Escherichia coli* O157 vázané na Dynabeads M280

Extrémně nízká infekční dávka (cca 10 buněk; Hemoragická kolitida, hemolyticko uremický syndrom)

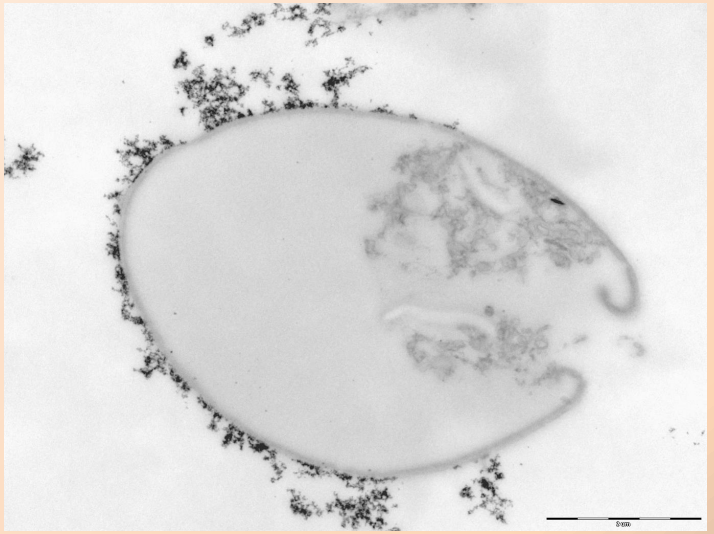


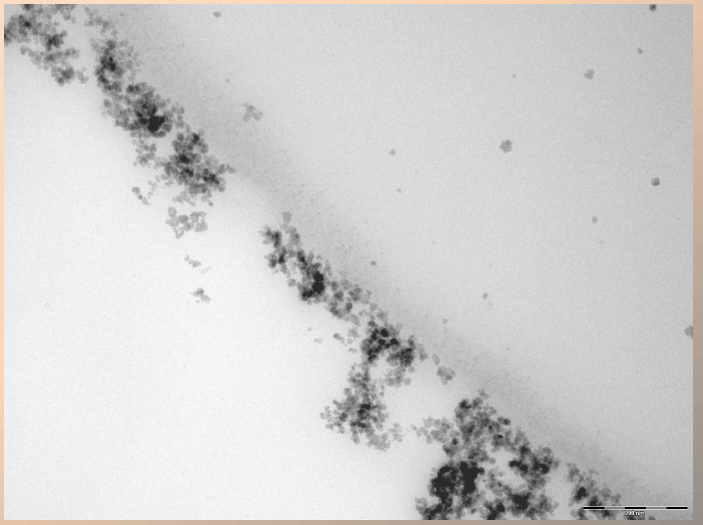
Magnetické biomateriály pro odstraňování xenobiotik

- Intensivní studium biosorbentů pro odstraňování xenobiotik → nízká až nulová cena
- Jednotlivé biopolymery (např. chitosan, chitin, alginát, rostlinné gumy atd.)
- komplexní biopolymery, obvykle rostlinného původu (např. piliny, kávová sedlina, mláto, sláma, čajové lístky, atd...)
- mikrobiální a řasové buňky (biomasa)
- anorganické biomateriály (vaječné skořápky)

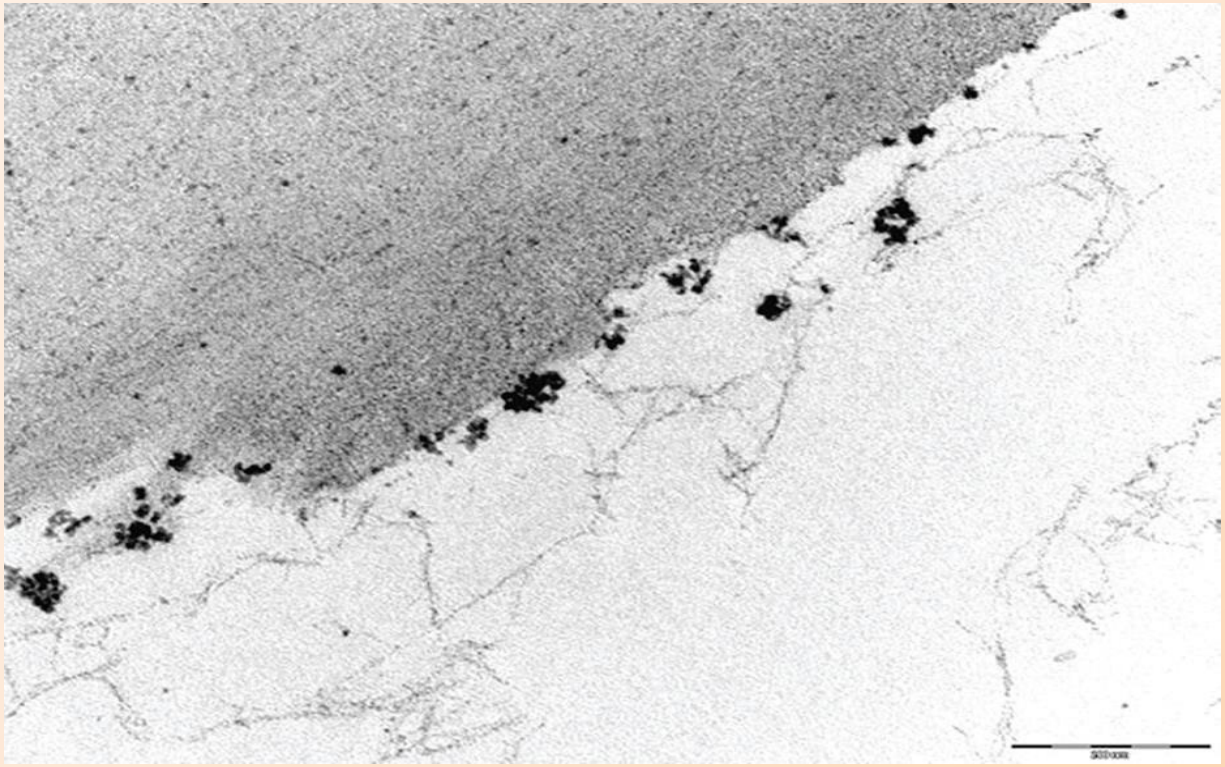
Magnetická modifikace usnadňuje separační proces

Magneticky modifikované buňky *Chlorella vulgaris*





Magneticky modifikované piliny



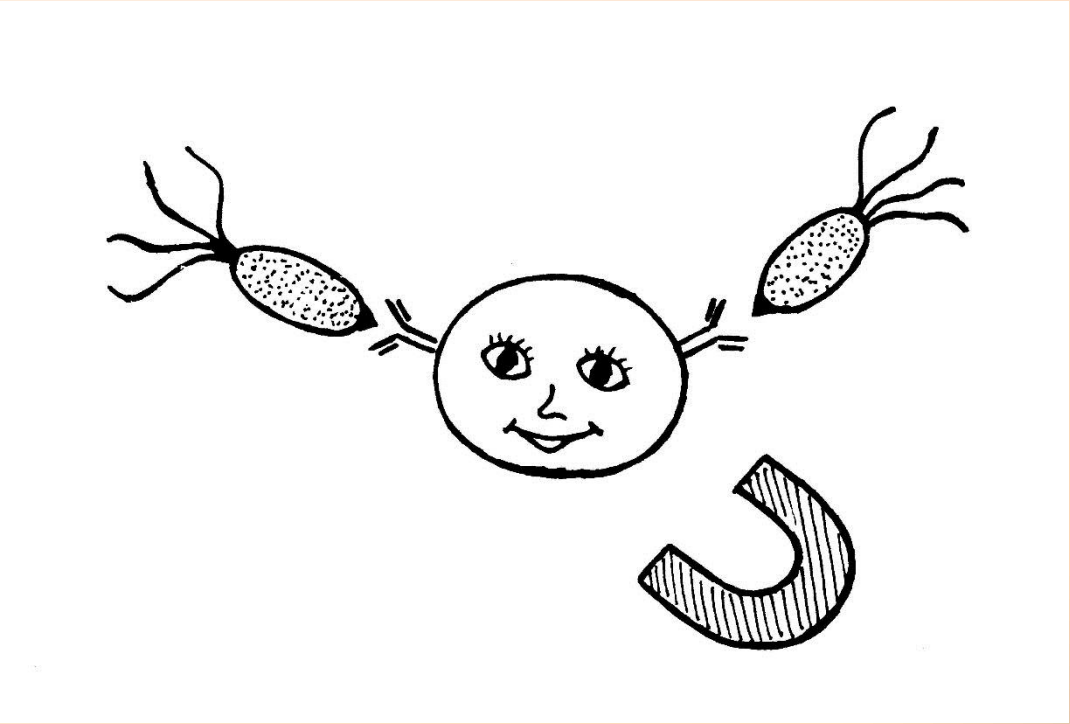
Magnetické materiály mají velkou perspektivu při biorafinačních procesech a při využití odpadních biomateriálů

Safarik,I., Pospiskova,K., Baldikova,E., Safarikova,M.: Development of advanced biorefinery concepts using magnetically responsive materials. Biochem. Eng. J., 2016, v tisku

COST Action TD1203: Food waste valorisation for sustainable chemicals, materials & fuels (EUBis)

Konec akce: 2016

Děkuji za pozornost !



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