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Magnetically responsive biological materials and their applications

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ABSTRACT

Diamagnetic biological materials of various origins (e.g., prokaryotic and eukaryotic microbial cells, lignocellulosic materials, food wastes, soluble and insoluble biopolymers etc.) can be magnetically modified in order to obtain smart biomaterials exhibiting an appropriate response to external magnetic field. Magnetic modification of originally nonmagnetic biological materials is usually based on the attachment of magnetic iron oxides nano- and microparticles on the surface or within the pores of the treated material, or by their entrapment in the gel structure. Magnetic modification can be performed using different procedures, e.g., by magnetic fluid treatment, mechanochemical synthesis and by direct or indirect microwave assisted synthesis. This short review will summarize magnetic modification procedures developed by the authors and applications of advanced magnetically modified biomaterials as adsorbents of both organic and inorganic xenobiotics and radionuclides, affinity adsorbents for isolation of target biomolecules, carriers for various affinity ligands, biologically active compounds and cells or whole-cell biocatalysts. The potential of magnetically responsive biomaterials will increase in the near future.

Keywords: Biological materials; magnetic modification; adsorbents; carriers; whole-cell biocatalysts.

Introduction

Magnetically responsive nano- and microparticles have been efficiently used in many areas of biosciences, biotechnology, medicine and environmental technology. Such materials can be described as smart materials, exhibiting several types of responses to external magnetic field. That's why magnetically responsive materials can be utilized for various applications. Such materials can be selectively separated from difficult-to-handle environments by means of a magnetic separator. Alternatively, they can be targeted and localized in a specific place using an appropriate magnetic system. Magnetic particles subjected to high frequency alternating magnetic field generate heat, which can be used for hyperthermia therapy of cancer diseases. Magnetic iron oxide nanoparticles increase a negative T_2 contrast during magnetic resonance imaging. Magnetorheological fluids increase their apparent viscosity when subjected to a magnetic field [**1**]. Recently, it was observed that both naked magnetic nanoparticles and magnetoferritin exhibit peroxidase-like activity [**2, 3**].

Magnetic nano- and microparticles can be successfully used for magnetic modification of diamagnetic biological materials (e.g. prokaryotic and eukaryotic cells or plant-derived materials), biopolymers, organic polymers and inorganic materials, and for magnetic labeling of biologically active compounds and affinity ligands (e.g., antibodies, enzymes, aptamers etc.).

The group of naturally magnetic materials is usually represented by magnetic iron oxides magnetite and maghemite, various types of ferrites or metallic iron, cobalt and nickel. On the contrary, majority of particulate materials exhibit diamagnetic (non-magnetic) behavior. In addition to inorganic particulate materials (e.g., different types of clays, sand, silica, aluminium oxide, titanium dioxide) and organic particulate materials (e.g., polystyrene-based ion exchangers), diverse biological materials are of great interest. Various types of non-magnetic particulate materials can be efficiently used as adsorbents, catalysts, chromatography materials, carriers or whole-cell catalysts. In many cases the application potential of these materials could be improved by their modification leading to the formation of magnetically responsive materials. Such a modification can substantially simplify separation of magnetic materials from complex systems, such as suspensions, culture media etc [**1**].

Magnetic modification procedures

Many procedures for the conversion of non-magnetic biological materials into their magnetic derivatives have been already described [**4**]. Magnetic modification is usually caused by the presence of magnetic labels within

the treated biomaterials pores, on the biomaterials surface or within the biopolymer gels. In general, magnetic properties of the modifiers (labels) are caused by the presence of nano- or microparticles of magnetic iron oxides, namely magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) or their mixtures; in some cases also ferrite particles [**5**], chromium dioxide particles [**6**], nickel [**7**] or metallic cobalt [**8**] have been employed for specific magnetization purposes. Alternatively, magnetic labels are formed by paramagnetic cations [**9**] or by (magneto) ferritin [**10**].

The simplest and most often used approach for magnetic modification of non-magnetic materials is based on the alkali precipitation of ferrous and ferric salts in the presence of the treated material, followed by heating of the aqueous suspension. Magnetic iron oxides (magnetite, maghemite or their mixtures) are usually formed [**4**]. However, specific biomaterials may require more appropriate treatment. Selected, efficiently used magnetic modification procedures are described below.

Magnetic fluid modification

Diverse types of ionically and sterically stabilized magnetic fluids nanoparticles (magnetic fluids, ferrofluids, FF) can be used for magnetic modification. In the simplest way, perchloric acid stabilized magnetic fluid was mixed with methanol suspension of the powder material to be modified (e.g., sawdust). During mixing magnetic iron oxide nanoparticles from magnetic fluid firmly precipitated on the particles surface [**1, 11**] (**Fig. 1**).

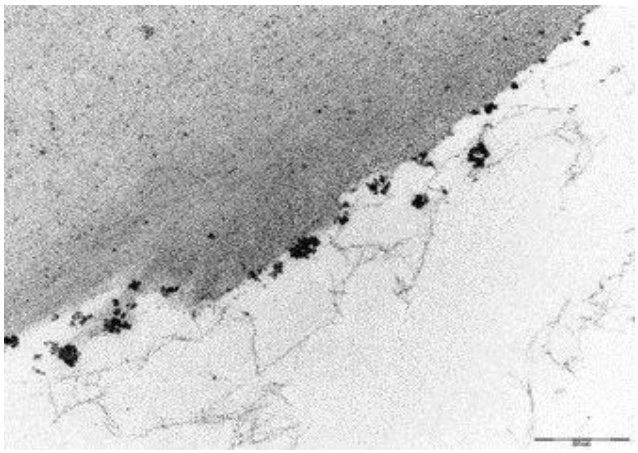

Fig. 1. TEM of ferrofluid modified sawdust particle; the bar corresponds to 200 nm. Reproduced, with permission, from [**1**].

Living baker's or brewer's yeast cells were washed with and suspended in acetate buffer, pH 4.6 or in glycine–HCl buffer, pH 2.2 for magnetic modification with perchloric acid stabilized ferrofluid; alternatively, tetramethyl-ammonium hydroxide stabilized magnetic fluid was utilized for baker's yeast cells modification in 0.1 M glycin–NaOH buffer, pH 10.6. After a short time period, magnetic nanoparticles precipitated on the cell surface (**Fig. 2**). The different physiological state of yeast cells can lead to various magnetic modification; using dormant yeast cells only surface modified cells were obtained, while magnetic modification of actively growing cells led to the accumulation of magnetic modifier in the periplasmic space [**12**].

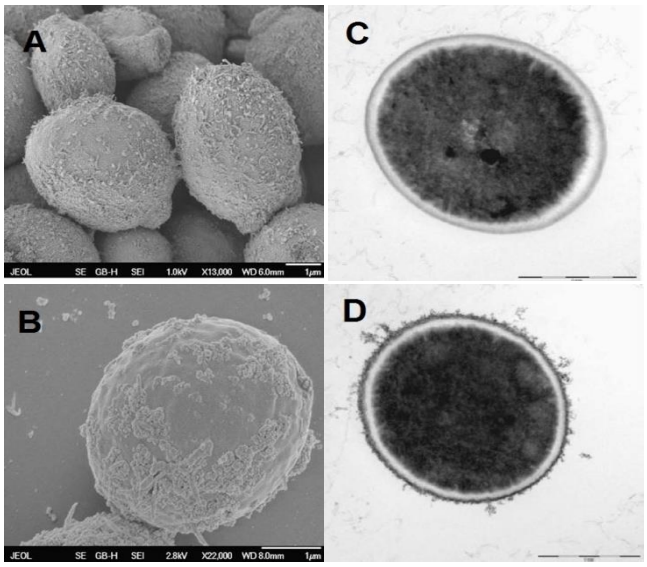

Fig. 2. (A, B) SEM images of ferrofluid-modified *Saccharomyces cerevisiae* cells, showing attached magnetic nanoparticles and their aggregates on the cell surface; bars =1 μm. (C) TEM image of a native *Saccharomyces cerevisiae* cell; bar =1 μm. (D) TEM image of a ferrofluid modified cell with attached magnetic iron oxide nanoparticles on the cell wall; bar =1 μm. Reproduced with permission from [**13**].

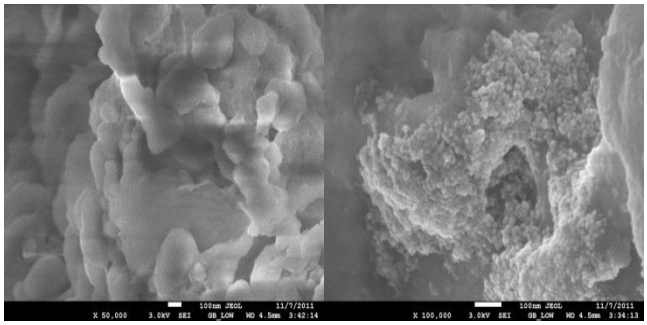

Fig. 3. SEM of native tea leaves (left) and tea leaves modified by direct ferrofluid treatment (right). Reproduced, with permission, from [**14**].

New and efficient postmagnetization procedure is based on the use of water based magnetic fluid stabilized with perchloric acid, which was directly thoroughly mixed with material to be modified (in a typical procedure, 1 g of nonmagnetic powder and 1 mL of FF is used) and dried completely. This technique is extremely simple and various biological, inorganic and polymer materials have been successfully transferred into their magnetic derivatives [**14**]. Tea leaves modified by this procedure are presented on **Fig. 3**.

Microwave assisted magnetic modification

Microwave assisted synthesis of magnetic iron oxides particles from ferrous sulfate has been described recently [**15**]. In general, microwave irradiation can accelerate many chemical reactions in organic and inorganic syntheses. In comparison with conventional heating methods, reactions under microwave irradiation have usually higher reaction

rates and the product can be obtained in a shorter period of time.

Extremely simple, one-pot, direct magnetic modification procedure employing just iron(II) salt (e.g., very cheap iron(II) sulfate) at high pH in the presence of the treated material has been developed recently. The suspension was treated in the regular kitchen microwave oven (700-750 W, 2450 MHz) for appropriate time. Submicrometer magnetic iron oxides nano- and microparticles formed during the microwave treatment deposited on the surface of the treated materials in the form of individual particles and their aggregates [**16**].

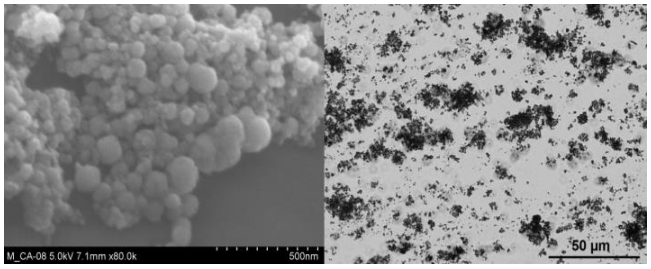

Fig. 4. SEM (left) and optical microscopy (right) images of magnetic iron oxides particles prepared by microwave-assisted synthesis. Reproduced, with permission, from [**18**].

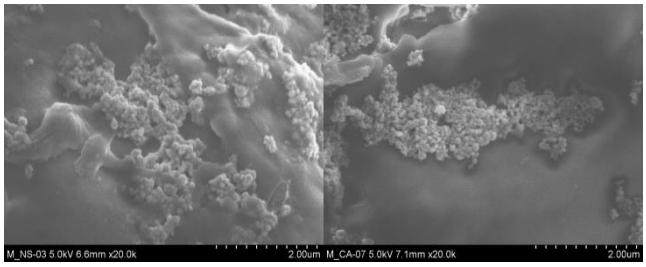

Fig. 5. SEM image of native (left) and chemically treated (right) barley straw magnetically modified with microwave synthesized magnetic iron oxides nano- and microparticles. Reproduced, with permission, from [**18**].

Direct microwave assisted magnetization procedure can be especially used for modification of heat and high pH stable materials. However, in order to enable magnetic modification of also more sensitive materials, an indirect microwave assisted modification has been developed [**17**]. At first, magnetic iron oxides nano- and microparticles (the nanoparticles diameter ranged between ca 25 and 100 nm; during the synthesis, the nanoparticles formed micrometer-sized stable aggregates with the maximum aggregate size ca 20 µm, see **Fig. 4**) have been synthesized from ferrous sulfate at high pH in a microwave oven. After particles washing, materials to be magnetically modified were thoroughly mixed with iron oxides particles suspension and dried completely at slightly increased temperature (e.g., 60º C). Magnetic modification led to the stable deposition of iron oxide nanoparticle aggregates on the surface of the modified material (**Fig. 5**).

Modified indirect magnetization procedure employing microwave synthesized magnetic particles can also be used for magnetic treatment of thermally sensitive materials, e.g., powdered enzymes. Enzyme powders were suspended in liquid media not allowing their solubilization (e.g., saturated ammonium sulfate and highly concentrated polyethylene glycol solutions, ethanol, methanol) and subsequently cross-linked with glutaraldehyde. Magnetic modification using magnetic iron oxides nano- and microparticles prepared by microwave-assisted synthesis was successfully performed at low temperature in a freezer (-20 °C). It can take several days or even weeks to dry magnetic enzyme powder completely [**19**]. Low temperature drying procedure has also been employed for magnetic modification of enzymes immobilized on non-magnetic carriers and for other sensitive biomaterials such as starch grains [**20**].

Mechanochemical magnetic modification

Recently, mechanochemical procedures have been used to synthesize magnetic iron oxides and ferrites nanoparticles [**21**]. Mechanochemistry represents one of several ways of chemical activation. In solid-state mechanochemistry, nonthermal chemical reactions occur because of the deformation and fracture of solids, which are technically induced by milling or grinding of the materials. During this process the mechanical energy induces chemical reactions and phase transformations [**22**].

In the standard mechanochemical synthesis of magnetic composite materials, hydrated ferrous and ferric chlorides were grounded in a mortar at room temperature for 10 min; to avoid agglomeration, the excess of sodium chloride was added to the precursors before grinding. Then, appropriate amount of target nonmagnetic powdered material was added and after thorough mixing the process continued for another 10 min. As the last step, powdered alkaline hydroxide was added and after mixing the grinding continued for 10 min. After finishing the mechanochemical process, the magnetically modified material was thoroughly washed with water. Variety of inorganic and biological materials, including e.g., potato and maize starch grains (**Fig. 6**), pollen grains, powdered lignocellulosic materials and many others has been successfully modified using this procedure [**23**].

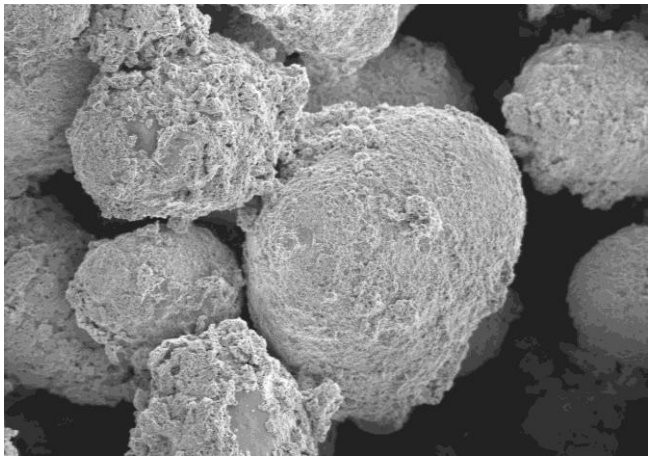

Fig. 6. SEM image of native potato starch magnetically modified using mechanochemical procedure. Reproduced, with permission, from [23].

Magnetically modified biomaterials and their applications

Different types of biological materials have been converted into their magnetic derivatives and subsequently used as adsorbents, carriers, biocatalysts etc. Especially the following materials are of our interest:

1) Individual biopolymers of various origin, such as polysaccharides (e.g., cellulose, chitin, chitosan, alginate, agar, agarose, plant gums), proteins (e.g., casein, keratin, gelatin, ovalbumin, hen egg white) or polyhydroxyalcanoates (e.g., polyhydroxybutyrate)
2) Complex biopolymers, especially lignocellulosic materials of plant origin (e.g., sawdust, straw, spent barley grain, spent coffee grounds)
3) Microbial and microalgae cells, such as baker's yeast (*Saccharomyces cerevisiae*), fodder yeast (*Kluyveromyces marxianus*) or unicellular alga *Chlorella vulgaris*
4) Marine seagrass and marine macroalgae, such as *Posidonia oceanica* (forming so called Neptune balls) or various species of *Sargassum*
5) Inorganic biomaterials, such as egg shells, sheaths of *Leptothrix ochracea* or various diatoms.

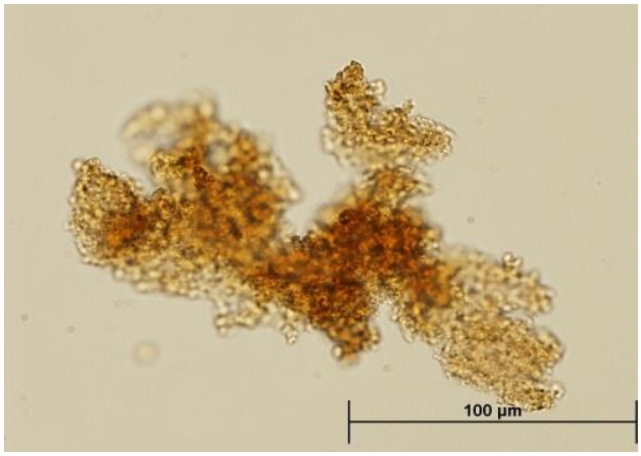

Fig. 7. Optical microscopy image of microwave-synthesized magnetic chitosan microparticles [24]. Reproduced, with permission, from [25].

Applications of magnetically modified biological materials are shortly described in the following section.

1. Chitin and chitosan belong to intensively studied and used polysaccharides. Huge amount of magnetic chitin and chitosan derivatives have been prepared. Recently, an extremely simple, one-pot procedure for magnetic chitosan particles preparation has been developed. In this procedure, chitosan was dissolved in diluted acetic acid solution and then solution of ferrous sulfate was added. Subsequently, solution of sodium hydroxide was added under intensive stirring and the formed brown precipitate was treated in a microwave oven [**24**]. After glutaraldehyde activation, target proteins [**24**] or microbial cells [**25**] can be immobilized. The character of the prepared magnetic chitosan particles is shown in **Fig. 7**.

Magnetic chitin and chitosan derivatives exhibit affinity for specific biologically active compounds, such as hen egg white lysozyme [**26, 27**], lysozyme from the gut of the soft tick *Ornithodoros moubata* [**28**] or plant chitinase from the latex of *Euphorbia characias* [**29**]. Potato (*Solanum tuberosum*) tuber lectin was isolated from tuber extract [**30**] or from potato starch industry waste water; the adsorbed lectin was eluted with glycine/HCl buffer, pH 2.2 and the specific activity of separated lectin increased 27 times during the isolation process [**31**]. Chitin and chitosan binding proteins can be substantially purified in one step using magnetic affinity adsorption [**32**].

Magnetic chitosan can be easily activated by glutaraldehyde and employed as a carrier for enzymes [**24, 33**] and microbial cells [**25**] immobilization. Organic affinity ligands (e.g., reactive textile dyes) have also been successfully immobilized; Ostazin turquoise (a reactive Cu phthalocyanine dye) immobilized on magnetic chitosan or silanized magnetite selectively bound planar organic dyes [**34, 35**] and was used for their magnetic solid phase extraction from water and urine samples [**35-37**].

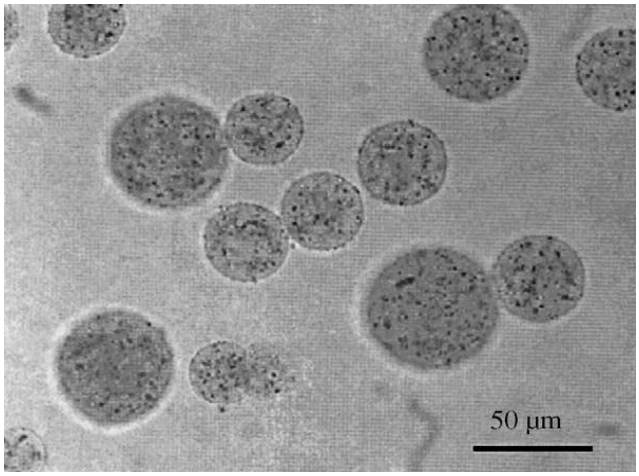

Fig. 8. Optical microscopy image of magnetic alginate microparticles. Reproduced, with permission, from [**40**].

Magnetic cellulose particles in the form of ion exchanger have also been successfully utilized for magnetic ion exchange separation of hen egg white lysozyme directly from native egg white [**38**].

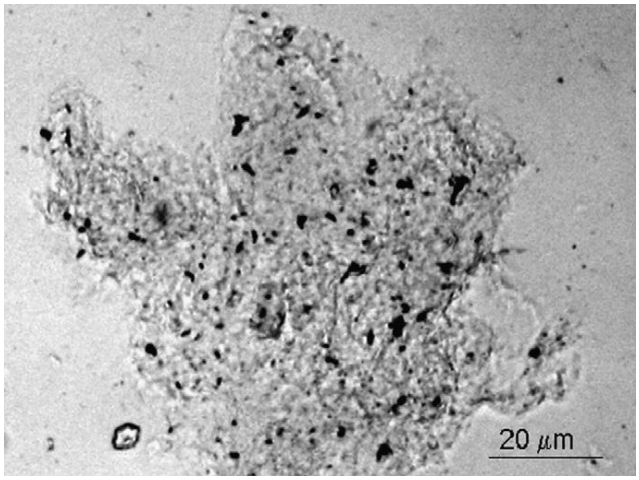

Fig. 9. Optical microscopy image of magnetic ovalbumin microparticles. Reproduced, with permission, from [42].

Magnetic alginate beads have been used for the entrapment of microbial cells [**39**] and as a pseudo affinity adsorbent; spherical magnetic alginate microparticles (25-60 µm in diameter, **Fig. 8**) were prepared using the

microemulsion system, with water-saturated 1-pentanol as the organic phase. The limited solubility of 1-pentanol in water enabled simple removal of the organic solvent from the prepared beads with water solution. The washed alginate microparticles were utilized as magnetic affinity adsorbents for specific purification of alpha-amylases; enzyme activity was eluted by 1.0 M maltose [**40**].

Magnetic porous corn starch was employed as an affinity adsorbent for the efficient and simple scale-up procedure for one-step purification of cyclodextrin glucanotransferase from *Bacillus circulans* culture media; the enzyme purification factor was 19-25 in different batches [**41**].

Simple and low-cost preparation of new magnetic adsorbents from ovalbumin and egg white, based on methanol precipitation and subsequent glutaraldehyde cross-linking has been developed. These adsorbents (**Fig. 9**) were used for preconcentration of two plant lectins from potato (*Solanum tuberosum*) tubers and from wheat (*Triticum* spp.) germs extracts. The adsorbed lectins were eluted with diluted hydrochloric acid. The specific activities of both lectins increased approximately 30–40 times during the preconcentration process [**42**].

Table 1. Comparison of maximum adsorption capacities Q_{max} (mg/g) of magnetically modified plant-based materials for tested dyes.

Dyes	Maximum adsorption capacities of magnetically responsive plant materials (mg/g)					
	Spruce sawdust [45]	Peanut husk [47]	Spent coffee grounds [49]	Spent grain [48]	Spent rooibos [51]	Rye straw [50]
Acridine orange	24.1	71.4	49.3		79.8	42.9
Aniline blue				44.7		
Bismarck brown Y	52.1	95.3	97.8	72.4	119	
Brilliant green					62.4	
Crystal violet	52.4	80.9	36.7	40.2	98.4	
Malachite green			62.9			
Methyl green					117	95.2
Methylene blue					96.5	
Nile blue					142	
Safranin O	25.0	86.1	34.3		128	

A procedure for the determination of proteolytic activity with dyed magnetic gelatine as an insoluble chromolytic substrate has been described; the magnetic nature of the substrate enabled magnetic separation of unhydrolysed substrate from the hydrolysed dyed peptide fragments [**43**]. New magnetic adsorbents for batch isolation and removal of various proteolytic enzymes were prepared by glutaraldehyde cross-linking of bovine, porcine and human erythrocytes in the presence of fine magnetic particles; serine proteinases and proteinases present in various commercial enzyme preparations were efficiently adsorbed on these adsorbents [**44**].

2. Magnetically modified materials of plant origin have been tested as efficient adsorbents of organic and inorganic pollutants. Several magnetic modification procedures have been used, namely ferrofluid treatment for sawdust [**11, 45, 46**], peanut husk [**47**], spent grain [**48**], spent coffee grounds [**49**] or spent tea leaves [**14**], and microwave assisted modification procedure for straw [**18, 50**] or spent rooibos (*Aspalathus linearis*) tea biomass [**51**]. These materials exhibited efficient adsorption of various water soluble dyes (see **Table 1**).

Table 2. Comparison of maximum adsorption capacities Q_{max} (mg/g) of magnetically modified yeast and microalgae cells for tested dyes.

Dyes	Maximum adsorption capacities of magnetically modified yeast and microalgae cells (mg/g)				
	S. cere-visiae [55]	*S. cerevisiae* (*uvarum*) [56]	*K. marxianus [57]*	*S. cerevi-siae* on chitosan [25]	*Ch. vulgaris* [63]
Acridine orange	82.8		62.2		
Amido black 10B		11.6	29.9		
Aniline blue	430	228			258
Bismarck brown Y			75.7		202
Congo red		93.1	49.7		157
Crystal violet	85.9	41.7	42.9	68	42.9
Malachite green	19.6				
Safranin O	90.3	46.6	138.2	111	116
Saturn blue LBRR			33.0		24.2

Wheat grain derived material, namely wheat bran magnetically modified with microwave synthesized iron oxides particles, has been successfully employed for adsorption of uranium [**52**].

Plant derived materials can also be used as low cost, biocompatible carriers for enzyme immobilization. Ferrofluid-modified spent grain was utilized as a low-cost, biocompatible and magnetically responsive carrier for the immobilization of *Candida rugosa* lipase. Several immobilization procedures were tested using both native and poly (ethyleneimine)-modified magnetic spent grain. This material can be a promising low-cost magnetic carrier for enzyme immobilization, applicable e.g. in food and feed technology and biotechnology [**53**].

3. Magnetically modified microbial and microalgae cells have found important applications both as adsorbents of important organic and inorganic pollutants [**54**] and whole-cell biocatalysts. Very simple treatment of baker's, brewer's and fodder yeast cells with ionic magnetic fluids led to the formation of magnetic adsorbents of organic water soluble dyes [**55-58**] (see **Table 2**), heavy metal ions [**59,60**] and strontium ions [**61**]. Magnetically modified *S. cerevisiae* cell walls [**62**] and unicellular alga *Chlorella vulgaris* [**63**] can also be used for the same purpose.

Magnetically modified microbial cells can be utilized as whole cell biocatalysts. Several magnetization procedures have been employed, namely application of acid ferrofluid and acetic acid buffer [**13**] (**Fig. 2**), entrapment of cells in magnetically modified millimeter or micron sized alginate particles [**39**] (**Fig. 10**), covalent binding to magnetic chitosan particles [**25**] (**Fig. 11**) or binding of microwave synthesized magnetic iron oxide nano- and microparticles on the cell surface [**64**] (**Fig. 12**).

Magnetic iron oxides particles, especially those prepared by microwave assisted synthesis, can be efficiently used for magnetic flocculation of microalgae cells, e.g., *Chlorella vulgaris* [**65-68**].

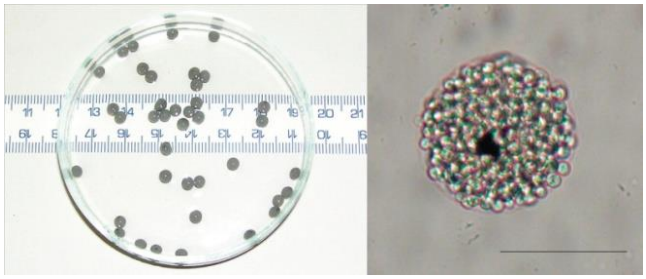

Fig. 10. Magnetically responsive alginate beads containing entrapped *Saccharomyces cerevisiae* cells and magnetite microparticles. Millimeter-sized beads (left) and microbeads (right; the scale bar corresponds to 50 μm). Reproduced, with permission, from [**39**].

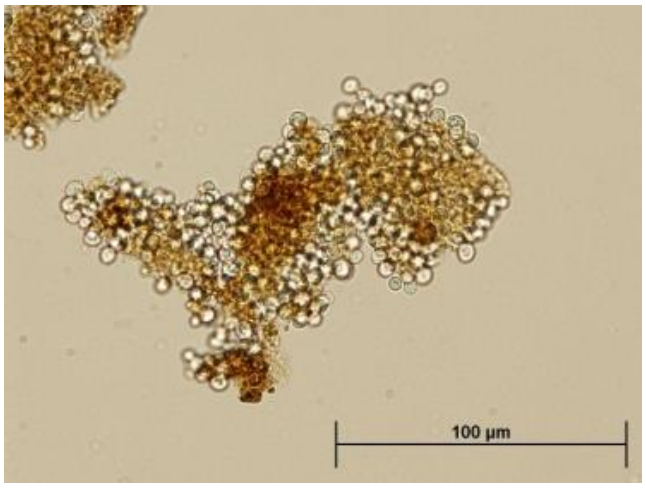

Fig. 11. Optical microscopy image of microwave-synthesized magnetic chitosan microparticles with immobilized *Saccharomyces cerevisiae* cells. Reproduced, with permission, from [**25**].

In order to decompose hydrogen peroxide (a potential chemical sanitizer for use in the food industry), magnetically responsive alginate beads containing entrapped *Saccharomyces cerevisiae* cells and magnetite microparticles (**Fig. 10**) were utilized. Larger beads (2-3 mm in diameter) were prepared by dropping the mixture into calcium chloride solution, while microbeads (the diameter of majority of particles ranged between 50 and 100 μm) were prepared using the water in oil emulsification process. In general, microbeads enabled more efficient hydrogen peroxide decomposition. The biocatalyst was stable; the same catalytic activity was observed after one month storage at 4 °C, and the microbeads could be used at least five times [**39**]. The same catalyst has also been employed for the hydrolysis of sucrose into glucose and fructose (invert sugar) [**69**].

4. Marine seagrass and marine macroalgae represent interesting and valuable biomaterials, often available in huge quantities. Recently, we have prepared magnetic derivatives of *Posidonia oceanica* (forming so called Neptune balls) and *Sargassum horneri*; both materials exhibited high capacity for dyes adsorption (manuscripts in preparation).

5. Biogenic iron oxides formed mainly by *Leptothrix ochracea*, collected from local water streams and subsequently magnetically modified using water-based magnetic fluid have been characterized in detail and used as an inexpensive magnetically responsive adsorbent for the removal of selected organic dyes from aqueous solutions. The observed maximum adsorption capacities ranged between 34.3 and 97.8 mg of dye per 1 g of absorbent [**70**].

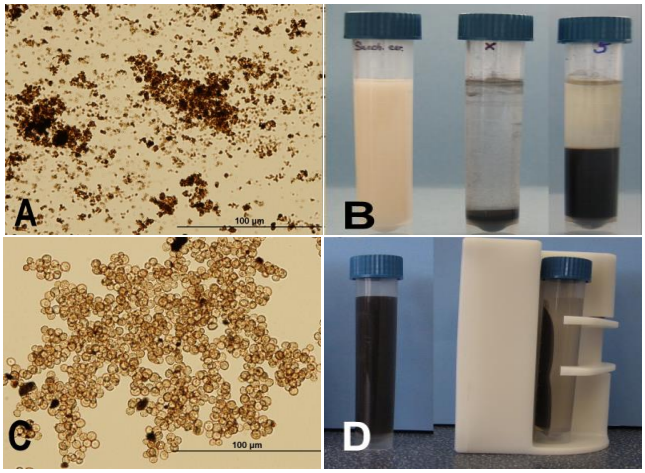

Fig. 12. Optical microscopy of magnetic iron oxide microparticles prepared by microwave-assisted synthesis (A); process of magnetic modification of yeast cells (left tube – *Saccharomyces cerevisiae* cells suspension; middle tube – sedimented iron oxides microparticles for magnetic modification; and right tube – sedimented magnetically modified yeast cells) (B); optical microscopy of *S. cerevisiae* cells modified by iron oxides microparticles (C); magnetic separation of magnetically modified yeast cells (D). Reproduced, with permission, from [**64**].

Conclusion

Different types of progressive materials, among them magnetically responsive nano- and micro(bio)materials, have enormous potential to significantly influence different areas of biosciences, medicine, biotechnology, environmental technology etc., where dozens of extremely important applications, both *in vitro* and *in vivo*, have already been described. It was shown that prepared magnetic (bio)materials can serve not only as adsorbents of diverse inorganic or organic xenobiotics, such as heavy metal ions, radionuclides, water-soluble dyes, endocrine disruptors or drug metabolites highly endangering environment and organisms living in, but also as carriers for immobilization of cells, enzymes and biologically active compounds or as biosensors. Moreover, their application potential is much wider. Due to the presence of magnetic particles, these materials can be successfully employed as contrast agents in MRI or for drug targeting; additional information about their possible utilization can be found in review papers of the authors [**71-86**]. Further progress can be expected particularly in biotechnology and environmental technology, where preparation of cost-effective and biocompatible magnetic particles or (bio) composites is increasingly required. Extremely inexpensive magnetic nanocomposites and microparticles for large scale applications (e.g. separation of biologically active compounds from agricultural wastes and culture media, removal of xenobiotics from wastewater or immobilization

of affinity ligands or biocatalysts) are necessary. Safety and biocompatibility studies of magnetically responsive materials, in particular long-term toxicity studies, have to be carried out. The collaboration of scientists from different fields is necessary. Potential of magnetic nano- and micromaterials will expand in the future.

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