MAGNETIC NANOBIOCOMPOSITES AND THEIR POSSIBLE APPLICATIONS

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Abstract

Magnetic nano- and microparticles have already found many important applications in various areas of biosciences, medicine, biotechnology, environmental technology etc. These smart materials exhibit different types of response to external magnetic field. In most cases they can be described as composite materials, where the magnetic properties are caused by the presence of iron oxides nano- or microparticles. In this paper attention will be focused on nanobiocomposites where the diamagnetic part of magnetic composites is formed by microbial and algae cells. Such materials can be efficiently separated from difficult-to-handle samples and be used as xenobiotics adsorbents or whole cell biocatalysts.

1. INTRODUCTION

Nanocomposites are materials that are usually created by introducing appropriate nanoparticles (often referred to as filler) into a macroscopic sample material (often referred to as the matrix). The resulting nanocomposites may exhibit drastically changed mechanical, optical or magnetic properties. Such materials are of great importance for new scientific and technological applications [1].

Materials whose physical properties can be varied by application of external magnetic fields belong to a specific class of smart materials. The broad family of magnetic field-controllable materials includes ferrofluids (magnetic fluids), magnetorheological fluids, magnetic polymers, magnetic inorganic materials, magnetically modified biological structures, magnetic particles with bound biomolecules etc. In many cases magnetically responsive composite materials consist of small magnetic particles (most often formed by magnetite, maghemite or various ferrites), usually in the nanometer to micrometer range, dispersed in a polymer, biopolymer or inorganic matrix; alternatively magnetic particles can be adsorbed on the outer surface of diamagnetic particles, such as microbial cells. Different chemical routes for the synthesis of superparamagnetic iron oxide nanoparticles (classic synthesis by precipitation, high-temperature reactions, reactions in steric environments, sol-gel reactions, decomposition of organometallic precursors, polyol methods, etc.) and their use for the preparation of magnetically responsive composite materials have been reviewed recently. In addition to synthetic magnetic nano- and microparticles, biologically produced magnetic particles (e.g., magnetosomes present in various magnetotactic bacteria) have been successfully used for biotechnology applications [1].

Magnetic nano- and microparticles have found many important applications in various areas of biosciences, medicine, biotechnology, environmental technology etc. In most cases magnetic composite particles, prepared from biocompatible synthetic polymers (e.g., polylactic acid, polyvinyl
alcohol, polyethylene glycol, polystyrene), biopolymers (e.g., cellulose, chitosan, dextran, starch, agarose, alginate, albumin, whole egg white), silica or porous glass have been used. These particles can be efficiently separated from difficult-to-handle samples (e.g., suspensions or viscous solutions) and targeted to the desired place, used as contrast agents for magnetic resonance imaging, to generate heat during exposure to alternating magnetic field, to modify biomolecules and biological structures etc. Different areas of biosciences and biotechnology have already benefited from the application of biologically active compounds (antibodies, enzymes, lectins, nucleic acids, oligonucleotides, specific affinity ligands) immobilized to magnetic nano- and microparticles (e.g., immunomagnetic assays of target compounds, affinity separation of cells, cell organelles or molecules, molecular biology applications, magnetofection, etc.). Biocompatible and biodegradable magnetic materials have been used as contrast agents during magnetic resonance imaging, for magnetic drug targeting, magnetic fluid hyperthermia and detoxification of biological fluids. Magnetically responsive microbial cells or lignocellulose, useful for xenobiotics removal, have been prepared by ferrofluid modification of the starting materials [1].

This short paper will show selected examples of new types of magnetically responsive nanobiocomposite materials prepared from microbial and algae cells and their possible applications.

2. MAGNETIC CELL-BASED NANOBIOCOMPOSITES

An absolute majority of prokaryotic and eukaryotic cells is diamagnetic (i.e., without magnetic properties). In order to prepare magnetically responsive cells, the native cells have to be magnetically modified, usually by forming complexes with magnetic nano- and microparticles. Generally the cells can be modified by the non-specific attachment of magnetic nanoparticles (e.g., by the magnetic fluid treatment), by binding of maghemite or magnetite particles on the cell surface, by specific interactions with immunomagnetic nano- and microparticles, by the biologically driven precipitation of paramagnetic compounds on the cell surface, by covalent immobilization on magnetic carriers, by cross-linking of the cells or isolated cell walls with a bifunctional reagent in the presence of magnetic particles or by entrapment (together with magnetic particles) into biocompatible polymers. In most cases the magnetic properties of the modifiers are caused by the presence of nano- or microparticles of magnetite (Fe$_3$O$_4$) or maghemite ($\gamma$-Fe$_2$O$_3$); in some cases also ferrite particles or chromium dioxide particles have been used. Alternatively the modification can be performed by binding paramagnetic cations on acid groups on the cell surface. In many cases the attached magnetic particles or ions do not have a negative effect on the viability and phenotype alternation of modified cells [2].

2.1 Preparation of ferrofluid modified microbial cells

Several procedures for magnetic modification of yeast cells (i.e., baker's yeast (Saccharomyces cerevisiae), brewers's yeast (Saccharomyces cerevisiae subsp. uvarum) and fodder yeast (Kluyveromyces fragilis)), algae cells Chlorella vulgaris and fungal mycelium of Aspergillus niger have been developed recently.

Two strategies can be used for magnetic labeling of dead cells used as xenobiotics adsorbents. In the first protocol the cells were washed several times with saline or with 0.1M acetic acid. One milliliter of ferrofluid was added to 3 mL of the suspension of washed cells (1 + 3, v/v) in 0.1 M acetic acid or in
0.1 M acetate buffer, pH 4.6, and the suspension was mixed at room temperature for 1 h. The residual ferrofluid was removed by washing with acetic acid or acetate buffer and then by repeated washing with water, until the supernatant was clear; the magnetized cells were captured using an appropriate magnetic separator. The resultant magnetic adsorbent was stored in a water suspension at 4 °C [3]. The second protocol is derived from the procedure for magnetic modification of sawdust [4]. Dried microbial cells (0.5 – 1 g) were suspended in 7 – 14 ml of methanol and 1 ml of ferrofluid was added. The suspension was mixed on a rotary mixer for 1 h. The magnetically modified cells were then repeatedly washed with methanol and air-dried.

To prepare magnetically responsive whole cell biocatalysts, living baker’s yeast cells were suspended in 0.1 M glycine-NaOH buffer, pH 10.6 and then ferrofluid stabilized with tetramethylammonium hydroxide (pH 13.0) was added [5].

2.2. Adsorption of xenobiotics

Magnetically modified microbial and algae cells were tested as possible adsorbents for binding of different substances. They efficiently adsorbed different organic compounds, such as water-soluble organic dyes, and mercury ions. Eight different dyes belonging to four dye classes were tested, namely, crystal violet and aniline blue (triphenylmethane group), amido black 10B, congo red, Saturn blue LBRR and Bismarck brown Y (azodyes group), acridine orange (acridine group) and safranin O (safranin group). Preliminary experiments also showed that adsorption properties of microbial and algae cells were not significantly influenced by magnetic modification. The adsorption of the tested dyes usually reached equilibrium in approximately 30-120 min. In order to achieve equilibrium during the adsorption process, an incubation time of 3 h was used for all adsorption experiments.

It was shown that Langmuir adsorption isotherm can be generally used to describe the adsorption behaviour. The Langmuir model allows simple calculation of maximum adsorption capacity $Q_{\text{max}}$, which is a very important parameter describing the adsorption process. The results obtained for the magnetically modified microbial and algae cells are presented in Table 1. It can be clearly seen that maximum adsorption capacities differs substantially, depending both on the dye tested and magnetic adsorbent used.

Fig. 1. SEM micrograph of ferrofluid modified *Saccharomyces cerevisiae* cell

2.3 Magnetically responsive whole cell biocatalysts

Living yeast cells magnetically modified under gentle conditions (see Fig. 1) can serve as efficient biocatalysts. Magnetic cells were used to catalyze hydrogen peroxide decomposition and invert sugar formation from sucrose, using the intracellular enzymes catalase (hydrogen-peroxide:hydrogen-
peroxide oxidoreductase, EC 1.11.1.6) and invertase (β-D-fructofuranosidase, E.C. 3.2.1.26). Readily available baker's yeast cells are of special interest, due to their broad application in food industry; they are classified as “Generally recognized as safe (GRAS)“. Also iron oxides and hydroxides are used in food industry as inorganic colorants (E172). Thus, magnetically responsive *Saccharomyces cerevisiae* cells represent a non-toxic, smart whole cells biocatalyst for potential applications in food industry and biotechnology processes [5].

Table 1. Comparison of maximum adsorption capacities ($Q_{\text{max}}$) of magnetically modified microbial and algae cells and mycelium for the tested dyes.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Maximum adsorption capacities of magnetically responsive biomaterials</th>
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<tr>
<td></td>
<td><em>Saccharomyces cerevisiae</em></td>
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<tr>
<td></td>
<td>$Q_{\text{max}}$</td>
</tr>
<tr>
<td>Acridine orange</td>
<td>82.2</td>
</tr>
<tr>
<td>Amido black 10B</td>
<td>11.6</td>
</tr>
<tr>
<td>Aniline blue</td>
<td>430.2</td>
</tr>
<tr>
<td>Bismarck brown</td>
<td></td>
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<tr>
<td>Congo red</td>
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<tr>
<td>Crystal violet</td>
<td>85.9</td>
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<tr>
<td>Safranin O</td>
<td>90.3</td>
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<tr>
<td>Saturn blue LBRR</td>
<td>33.0</td>
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3. CONCLUSION

Due to the low price of biological components (in selected cases waste microbial biomass can be used) and the simplicity of magnetic modification these types of magnetically responsive biocomposites can be considered for possible large scale applications in biotechnology and environmental technology.

4. REFERENCES


