Magnetically modified microbial cells: A new type of magnetic adsorbents

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Abstract

Microbial cells, either in free or immobilized form, can be used for the preconcentration or removal of metal ions, organic and inorganic xenobiotics or biologically active compounds. Magnetic modification of these cells enables to prepare magnetic adsorbents that can be easily manipulated in difficult-to-handle samples, such as suspensions, in the presence of external magnetic field. In this review, typical examples of magnetic modifications of microbial cells are presented, as well as their possible applications for the separation of organic xenobiotics and heavy metal ions.

Keywords: Microbial cells; Magnetic modification; Magnetic iron oxide nanoparticles; Magnetic adsorbents; Magnetic fluids; Xenobiotics

1. Introduction

The use of microorganisms in the preparation of various foods and beverages has been known since ancient times. Production of wine, bread, various oriental fermented dishes and beverages, production of cheese, pickles and sauerkraut, together with the harvest and consumption of protein-rich blue-green algae of the genus Spirulina by the ancient Aztecs may serve as examples. However, in most cases people protected their food against undesirable microorganisms as a cause of food spoilage. In the 20th century microorganisms were even more widely used for the preparation of various products, the production of proteins and other biotechnology applications (Volfova, 1984).

Many types of microbial cells have found numerous new applications in different areas of biosciences, biotechnology, chemistry and environmental technology in recent years. Many important microorganisms can be easily cultivated and biomass can be obtained in large amounts. Large quantities of waste and dead biomass are also available as byproducts of pharmaceutical and enzyme manufacture or the fermentation industry.

Recently several types of microbial cells have been used as efficient adsorbents for the concentration and/or removal of different biologically active compounds and xenobiotics. Such organisms (both free and immobilized) can be used to decrease pollution by using them to treat industrial waste streams before their introduction into the environment. Their ability to remove and to degrade hazardous organic compounds, such as fuels, from water, soils, sludge and residues, making them environmentally safe is widely recognized and utilized in bioremediation processes. Microbes have also been applied to the reclamation of heavy metals and radionuclides from waste streams and sludge, industrial effluents and mine water, as well as for the recovery of precious metals from processing streams in the electroprocessing and jewellery industries. Microorganisms may also be employed to immobilize metals in moderately polluted fields, thus allowing the fields to be used for agriculture, or to leach metal constituents from ores or rocks. Biosoorption is an alternative to conventional or traditional methods of pollution removal, such as incineration, catalytic destruction, and electrolytic recovery, use of adsorbents, ion-exchangers and the physical removal and subsequent destruction of pollutants (Aksu, 2005; Godlewska-Zylkiewicz, 2006; Vieira & Volesky, 2000).

Microorganisms immobilized on various inorganic and polymeric carriers have been used many times for the preconcentration and speciation of trace metal ions (Bag, Lale, & Turker, 1998; Bag, Turker, Lale, & Tunceli, 2000; Baytak & Turker, 2005; Baytak, Turker, & Cevrimli, 2005; Turker & Baytak, 2005).
2. Magnetic modification of microbial cells

Magnetotactic bacteria are exceptional microorganisms having the ability to synthesize intracellular biogenic magnetic nanoparticles (based either on magnetite or greigite), which enable their magnetic separation. On the contrary, an absolute majority of prokaryotic and eukaryotic microbial cells is diamagnetic (i.e., without magnetic properties). In those cases, when a magnetic separation technique is applied, cells have to be first magnetically modified, usually by forming complexes with magnetic particles. Generally microbial cells can be modified by the non-specific attachment of magnetic nanoparticles (e.g., by the magnetic fluid treatment) (Safarikova, Ptackova, Kibrikova, & Safarik, 2005), by binding of maghemite particles (Dauer & Dunlop, 1991) or magnetite particles (Mac Rae & Evans, 1983; Sze, Lu, & Wong, 1996; Wainwright, Singleton, & Edyvean, 1990; Wong & Fung, 1997) on the cell surface, by specific interactions with immuno-magnetic nano- and microparticles (Safarik & Safarikova, 1999; Safarik, Safarikova, & Forsythe, 1995), by the biologically driven precipitation of paramagnetic compounds on the cell surface (Bahaj, Ellwood, & Watson, 1991), by covalent immobilization on magnetic carriers (Safarikova et al., submitted-a), by cross-linking of the cells or isolated cell walls with a bifunctional reagent in the presence of magnetic particles (Al-Hassan et al., 1991; Ivanova, Hristov, Dobrev, Al-Hassan, & Penchev, 1996; Patzak, Dostalek, Fogarty, Safarik, & Tobin, 1997) or by entrapment (together with magnetic particles) into biocompatible polymers (Brady, Nigam, Marchant, McHale, & McHale, 1996). As can be seen, in most cases the magnetic properties of the modifiers are caused by the presence of nano- or microparticles of magnetite (Fe₃O₄) or maghemite (γ-Fe₂O₃); in some cases also ferrite particles (Lee et al., 2004) or chromium dioxide particles can been used (Widjojoatmodjo, Fluit, Torenisma, & Verhoef, 1993). Alternatively the modification can be performed by binding paramagnetic cations on acid groups on the cell surface (Zborowski, Malchesky, Jan, & Hall, 1992). In many cases the attached magnetic particles or ions do not have a negative effect on the viability and phenotype alternation of modified cells.

The individual modification procedures will be described in more details, although not all of them were employed to prepare magnetic microbial adsorbents.

2.1. Binding of magnetic nano- and microparticles on the cell surface

Magnetic modification of microbial cells can be performed using appropriate magnetic fluid. In the simplest way, perchloric acid stabilized by magnetic fluid was mixed with baker’s or brewer’s yeast cells washed with and suspended in acetate buffer, pH 4.6. After a short period of time magnetic particles precipitated on the cell surface. After washing the magnetically modified cells were heated in boiling water bath to kill the cells resulting in the formation of a stable adsorbent (Safarikova et al., 2005).

A different procedure has to be used when working with dried Kluyveromyces fragilis (fodder yeast) and Chlorella vulgaris cells. The cells were thoroughly washed several times with 0.1 M acetic acid to remove substantial portion of soluble macromolecules which otherwise caused spontaneous precipitation of magnetic fluid. After washing and suspending the cells in acetic acid solution the addition of perchloric acid stabilized magnetic fluid resulted in the formation of magnetically modified microbial cells (see Fig. 1) (Safarik et al., 2007).

Another procedure was based on the attachment of submicron, acicular maghemite particles on the yeast cells; the binding occurred irrespective of the solution pH and surface charge and was essentially irreversible (Dauer & Dunlop, 1991). Also magnetite microparticles were used to capture bacterial cells; cells adsorption was best in the pH range 3–6 in the absence of calcium and magnesium, but the pH range was extended up to pH 10 in the presence of these two cations (Mac Rae & Evans, 1983).

2.2. Covalent immobilization of microbial cells on magnetic carriers

Covalent binding is an extensively used technique for the immobilization of biopolymers, but this technique is not used so often for the immobilization of living cells. Covalent bind-
Fig. 1. Transmission electron microscopy of dried Kluyveromyces fragilis cells magnetically modified by perchloric acid stabilized magnetic fluid (Safarik et al., 2007). The bar line corresponds to 200 nm.

ing of microbial cells on a magnetic carrier is usually possible via reactive groups on the matrix or through the aid of a reactive binding which links the cells to the carrier. Various coupling agents (e.g., aminosilane, carbodiimide, glutaraldehyde) may be employed to introduce a specific group on the carrier surface, which subsequently can interact with reactive groups on the cell surface. Immobilization of whole cells or cell walls could produce a system not limited by diffusional limitations (Safarikova et al., submitted-a).

2.3. Entrapment of cells into biocompatible polymers

Microbial cells can be entrapped in natural or synthetic carriers. The carriers can be grouped according to the mechanism with which the gel is obtained. Gels can be formed by polymerization (e.g., polyacrylamide, polymethacrylate), cross-linking (e.g., proteins), polycondensation (polyurethane, epoxy resins), thermal gelation (e.g., gelatin, agar, agarose), ionotropic gelation (e.g., alginate, chitosan) and precipitation (cellulose, cellulose triacetate). The gel is formed in the presence of the cells and appropriate magnetic materials. There are various methods available to obtain particles (beads) containing entrapped cells and magnetic particles (Brodelius & Vandamme, 1987):

- Block polymerization with subsequent mechanical disintegration into particles. This is a simple method but it results in irregular particles of a wide size distribution.
- Molding of particles (beads) in a template form. This method results in a uniform preparation of immobilized cells but it is less suitable for the preparation of large quantities of immobilized cells.
- Bead formation in a two-phase system. Spherical beads can be prepared in large quantities by suspending an aqueous mixture of cells, magnetic particles and polymer in a hydrophobic phase under stirring and subsequently inducing gel formation. Bead formation of ionotropic polymers after dripping the mixture of cells, magnetic particles and polymer into a medium containing appropriate hardening ion.

One of the major drawbacks of entrapment technique is the possible diffusional limitation as well as the steric hindrance, especially when the macromolecular compounds are adsorbed.

2.4. Cross-linking of cells or cell walls

Microbial cell walls contain free amino and/or carboxyl groups, which can easily be cross-linked by bi- or multifunctional reagents such as glutaraldehyde or tolune diisocyanate. The cells are usually cross-linked in the presence of an inert protein like gelatine, albumin, raw hen egg white and collagen. Microbial cells can also be immobilized by ionic cross-linking through a flocculation mechanism by addition of polyelectrolytes. If magnetic particles are used throughout the cross-linking process, magnetic cells or cell walls derivatives can be prepared (Al-Hassan et al., 1991; Ivanova et al., 1996; Patzak et al., 1997).

2.5. Specific interactions with immunomagnetic nano- and microparticles

Immunomagnetic modification of microbial cells implies the use of magnetic microbeads or magnetic nanoparticles—antibody system causing the particles to be selectively attached to target cells when added to a cell suspension. After incubation, cells with attached magnetic particles (and also excess particles) are isolated with the help of an appropriate magnetic separator. Both monoclonal and polyclonal antibodies can be used in the course of magnetic modification. In the direct method the appropriate antibodies are coupled to the magnetic particles, which are then added directly to the sample. Ideally, the antibody should be oriented with its Fc part towards the magnetic particle so that the Fab region is pointing outwards from the particle (Safarik & Safarikova, 1999).

The indirect method can also be used. In the first step, the cell suspension is incubated with primary antibodies, which bind to the target cells. Prior sensitization of the target cells will ensure a proper orientation of the antibodies and an optimal number of interaction possibilities between magnetic particles and cells. Not only purified primary antibodies have to be used; crude antibody preparations or serum can be used, too. After incubation, the unbound antibodies are usually removed by washing. Thereafter, the magnetic particles with immobilized secondary antibodies are added, permitting the beads to bind rapidly and firmly to the primary antibodies on the target cells. Target cells-primary antibody complexes can be also captured by protein A or protein G immobilized on magnetic carriers. Alternatively, primary antibodies can be biotinylated or labelled with fluorescein and magnetic particles with immobilized streptavidin or anti-fluorescein antibodies are used for capturing the target cells (Safarik & Safarikova, 1999).
Table 1
Examples of magnetically modified microbial cells and their applications as magnetic adsorbents for heavy metal ions removal

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Magnetic modification</th>
<th>Adsorbed ion(s)</th>
<th>Matrix</th>
<th>Other details</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterobacter</em> sp.</td>
<td>Adsorption of magnetite microparticles</td>
<td>Ni^{2+}</td>
<td>Aqueous solutions</td>
<td>Batch procedure; diluted citric acid as an eluent</td>
<td>Wong &amp; Fung, 1997</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>Adsorption of magnetite microparticles</td>
<td>Cu^{2+}</td>
<td>Aqueous solutions</td>
<td>Batch procedure; HCl pretreatment of cells</td>
<td>Chua et al., 1998</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>Adsorption of magnetite microparticles</td>
<td>Cu^{2+}</td>
<td>Wastewater</td>
<td>HCl pretreatment of cells</td>
<td>Lei et al., 2000</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>Adsorption of magnetite microparticles</td>
<td>Cu^{2+}</td>
<td>Electroplating effluent</td>
<td>Batch procedure and adsorption in a bioreactor</td>
<td>Sze et al., 1996</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>Adsorption of magnetite microparticles</td>
<td>Cu^{2+}</td>
<td>Industrial water effluent</td>
<td>Semicontinuous biosorption system; HCl pretreatment of cells</td>
<td>Wang et al., 2000</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> cell walls</td>
<td>Cross-linking with magnetic microparticles</td>
<td>Cu^{2+}, Cd^{2+}, Ag^{+}</td>
<td>Aqueous solutions</td>
<td>Batch procedure; epichlorohydrin cross-linking</td>
<td>Patzak et al., 1997</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> subsp. <em>uvarum</em></td>
<td>Magnetic fluid treatment</td>
<td>Hg^{2+}</td>
<td>Water solutions</td>
<td>Batch procedure; regeneration with 0.1 M HNO_{3}</td>
<td>Yavuz et al., 2006</td>
</tr>
</tbody>
</table>

3. Application of magnetically modified microbial cells

As mentioned in the previous section, different procedures are available to convert diamagnetic microbial cells into their magnetic derivatives; only some of them have been really used in magnetic adsorbents preparation. This fact also suggests two possible ways of magnetic separation processes. In the first case, magnetically responsive cells are prepared before the adsorption process is being carried out; it means it is possible to use external magnetic field to manipulate these materials during all stages of the process (cells modified as described in Sections 2.1–2.6). In the second case microbial cells at the beginning of the adsorption process are diamagnetic and they become magnetic in the course of the adsorption process due to the precipitation of paramagnetic compounds (see Section 2.7). Both groups will be described in more details.

3.1. Adsorption of analytes on magnetically modified microbial cells

Different types of microbial cells (bacteria, yeasts, algae) were magnetically modified to prepare magnetic adsorbents. In most cases adsorption of magnetic particles and magnetic fluid treatment were used. Tables 1 and 2 show the described applications of magnetic microbial adsorbents for heavy metal ions and organic xenobiotics removal, respectively.

Yeast biomass represents an important and promising material for xenobiotics biosorption. The yeast cells of the genus *Saccharomyces* are non-pathogenic, easily available and enable simple manipulation. *Saccharomyces cerevisiae* cells (both baker’s and brewer’s yeasts) were magnetically modified by contact with perchloric acid stabilized magnetic fluid (Azvedo et al., 2003; Safarik, Ptałkowa, & Safarikova, 2002; Safarikova et al., 2005). In order to have stabilized product enabling work for a long period of time, dead yeast cells are preferred. That is why heating of cells in boiling water bath was used in the course of the adsorbent preparation. Essentially there are two methods for the preparation of dead magnetically modified yeast cells (killing the...
Table 2
Examples of magnetically modified microbial cells and their applications as magnetic adsorbents for organic xenobiotics removal from water samples

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Magnetic modification</th>
<th>Adsorbed compound(s)</th>
<th>Other details</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorella vulgaris</td>
<td>Magnetic fluid treatment</td>
<td>Water soluble dyes</td>
<td>Six dyes tested</td>
<td>Safarikova et al., submitted-b</td>
</tr>
<tr>
<td>Kluyveromyces fragilis</td>
<td>Magnetic fluid treatment</td>
<td>Water soluble dyes</td>
<td>Seven dyes tested</td>
<td>Safarik et al., 2007</td>
</tr>
<tr>
<td>Rhodopseudomonas spheroides</td>
<td>Adsorption of magnetite microparticles</td>
<td>Lindane</td>
<td>Optimal pH 4.0–8.0</td>
<td>Mac Rae, 1985</td>
</tr>
<tr>
<td>Rhodopseudomonas spheroides</td>
<td>Adsorption of magnetite microparticles</td>
<td>Chlorinated hydrocarbons</td>
<td>Six compounds tested</td>
<td>Mac Rae, 1986</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>Magnetic fluid treatment</td>
<td>Water soluble dyes</td>
<td>Five dyes tested</td>
<td>Safarik et al., 2002</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae subsp. uvarum</td>
<td>Magnetic fluid treatment</td>
<td>Water soluble dyes</td>
<td>Five dyes tested</td>
<td>Safarikova et al., 2005</td>
</tr>
</tbody>
</table>

cells before or after magnetic modification). Yeast cells which are first magnetically modified and then heated exhibited higher adsorption capacity than yeast cells first heated and subsequently magnetically modified (Safarik et al., 2002; Safarikova et al., 2005).

The fodder yeast cells (Kluyveromyces fragilis) are usually prepared in the dried form, which enables their simple magnetic modification and preparation of an inexpensive adsorbent. Also in this case magnetic fluid treatment was efficiently used (Safarik et al., 2007). The same modification process was used to prepare magnetic Chlorella vulgaris cells (Safarikova, Pona, Mosiniewicz-Szablewska, Weyda, Safarik, submitted-b).

Magnetically modified Saccharomyces and Kluyveromyces cells were used for the adsorption of water soluble dyes from water solutions. The maximum adsorption capacities vary greatly, depending on the dyes structure. Great differences can also be observed even for dyes belonging to the same group (see Table 3). In most cases the adsorption process can be described by the Langmuir adsorption isotherm.

Magnetically modified yeast cells (S. cerevisiae) were also tested as an efficient adsorbent of Hg\(^{2+}\) ions. The adsorption equilibrium data were well fitted to the Langmuir isotherm; the maximum adsorption capacity was ca 115 mg/g. The yeast biomass can be easily regenerated by 0.1 M HNO\(_3\) with high effectiveness (Yavuz, Denizli, Gungunes, Safarikova, & Safarik, 2006). Magnetically modified yeast cell walls were used for the adsorption of Cu\(^{2+}\), Cd\(^{2+}\) and Ag\(^{+}\) ions (Patzak et al., 1997).

Magnetically modified bacterial cells (especially of the genus *Pseudomonas*) were intensively studied as possible adsorbents for heavy metal ions. *Pseudomonas putida* strain isolated from heavy metal ions contaminated samples exhibited high affinity for Cu\(^{2+}\) ions; pretreatment of the cells with diluted hydrochloric acid leads to the increase of the adsorption capacity. EDTA solution (0.1 M) efficiently removed the adsorbed copper ions from the adsorbent (Chua, Wong, Yu, & Li, 1998; Sze et al., 1996).

Another bacterial strain (Rhodopseudomonas spheroides) was used for the adsorption of selected chlorinated hydrocarbons. In a contact time of 20 min, levels of heptachlor, aldrin and *p,p\textprime*-DDT in water were reduced below that of detection of the electron capture detector. *p,p\textprime*-DDT was also completely removed from a spiked wastewater (Mac Rae, 1986).

The same bacterial strain was also used for the adsorption of pesticides. It was observed that sorption of lindane reached equilibrium after 1 min and sorption by live cells was not significantly different from sorption by heat killed cells (Mac Rae, 1985).

3.2. Magnetization of microbial cells by the precipitation of paramagnetic cations

Cultures of Desulfovibrio sp. were grown anaerobically and after centrifugation the cells were suspended in a solution containing iron sulphate together with the ions to be separated. High

Table 3
Maximum adsorption capacities of magnetically modified Saccharomyces cerevisiae (Safarik et al., 2002), Saccharomyces cerevisiae subsp. uvarum (Safarikova et al., 2005) and Kluyveromyces fragilis (Safarik et al., 2007) cells for water soluble organic dyes

<table>
<thead>
<tr>
<th>Dyes</th>
<th>C.I. number</th>
<th>Saccharomyces cerevisiae</th>
<th>Saccharomyces cerevisiae subsp. uvarum</th>
<th>Kluyveromyces fragilis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Maximum adsorption capacities (mg/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acridine orange</td>
<td>46005</td>
<td>82.8</td>
<td></td>
<td>62.2</td>
</tr>
<tr>
<td>Amido black 10B</td>
<td>20470</td>
<td>11.6</td>
<td></td>
<td>29.9</td>
</tr>
<tr>
<td>Aniline blue</td>
<td>42755</td>
<td>430.2</td>
<td></td>
<td>228.0</td>
</tr>
<tr>
<td>Bismarck brown</td>
<td>21000</td>
<td>93.1</td>
<td></td>
<td>75.7</td>
</tr>
<tr>
<td>Congo red</td>
<td>22120</td>
<td>85.9</td>
<td></td>
<td>41.7</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>42555</td>
<td>19.6</td>
<td></td>
<td>42.9</td>
</tr>
<tr>
<td>Malachite green</td>
<td>42000</td>
<td>90.3</td>
<td></td>
<td>46.6</td>
</tr>
<tr>
<td>Safranin O</td>
<td>50240</td>
<td>138.2</td>
<td></td>
<td>33.0</td>
</tr>
<tr>
<td>Saturn blue LBRR</td>
<td>34140</td>
<td></td>
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</table>
concentration of paramagnetic sulphide precipitate was formed near the cell wall of the microorganism, which enabled magnetic separation of the product. The produced sulphides efficiently adsorbed various organic xenobiotics (e.g., pesticides) (Bahaj et al., 1991).

4. Conclusion

As shown by literature search, both free and modified prokaryotic and eukaryotic microorganisms can be used as effective adsorbents for solid phase extraction and removal of different types of organic and inorganic xenobiotics. They offer several advantages compared to adsorbents traditionally used for the separation and preconcentration of trace metals and organic compounds, such as the diversity of active sites on cell walls. Often high selectivity towards certain forms of xenobiotics was observed; it enables the use of these adsorbents for e.g., analysis of metal ions. Biosorption methods are often more effective in removing xenobiotics present at low concentrations from large volumes of solution. The process is usually reproducible.

Magnetic modification of microbial cells enables to prepare smart bioconjugates responsive to external magnetic field. Such materials are useful for work in difficult to handle samples. Standard laboratory equipment, used for routine magnetic separation processes, can be used in analytical procedures implementing magnetically modified microorganisms.

Magnetically modified microbial cells can also find other interesting applications. In some cases, attached magnetic nanoparticles can positively influence the physiological state of the treated cells leading e.g., to better thermal tolerance, increased production of biologically active compounds, increased bioprocess rates, etc. This system can also serve as an efficient model to study biotic–abiotic interactions on the cellular level.

Magnetically modified microbial cells represent new promising tools in biological research, such as magnetically responsive whole-cell biocatalysts, producers of biologically active compounds, parts of advanced sensor systems, sensing and active elements in magnetic microelectromechanical systems (MEMS), specific and non-specific adsorbents, etc. The research in this area is just at the beginning.

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