Magnetic Studies of Ferrofluid-Modified Microbial Cells

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Microbial cells (Kluyveromyces fragilis and Chlorella vulgaris) efficiently interacted with maghemite nanoparticles stabilized as low-pH ionic magnetic fluid, leading to the formation of magnetically labeled cells. This simple procedure allows to use the prepared materials as new cheap and easy to get magnetic affinity adsorbents to the removal of water-soluble dyes from polluted water sources using magnetic separation techniques. Magnetically modified cells were investigated by means of electron spin resonance spectroscopy and conventional magnetic methods over the temperature range 4–300 K. The magnetic behavior of these materials was dominated by the superparamagnetic relaxation of isolated single domain maghemite particles although a little amount of agglomerates was also present on the cell surface. However, these agglomerates were sufficiently small to show at static conditions the superparamagnetic behavior at room temperature. Therefore, the ferrofluid-modified microbial cells represent new interesting magnetic affinity adsorbents which could be applied for large-scale magnetic separation processes.

Keywords: Magnetically Labeled Cells, Biocomposite Materials, Magnetic Adsorbents, Maghemite Nanoparticles, Dynamic Susceptibility, Magnetization.

1. INTRODUCTION

Magnetically modified biocompatible materials, containing magnetic nanoparticles as labels, have been successfully applied as magnetic affinity adsorbents for the magnetic separation of various proteins (enzymes, antibodies, antigens, receptors), nucleic acids (DNA, RNA, oligonucleotides), drugs and xenobiotics (carcinogens, water-soluble dyes, heavy metal ions, radionuclides).¹,² Magnetic separation techniques have many interesting applications in different areas of biosciences ranging from cell separation³ to removal of xenobiotics from aqueous wastes.⁴

A large amount of xenobiotics (mainly dyes) is produced every year in different branches of industry. A substantial part of them pollutes many water sources. Many synthetic dyes are difficult to remove by the conventional wastewater systems, due to their complex chemical structure. Therefore, the adsorption on appropriate adsorbents seems to be an efficient procedure for their removal.

There are many adsorbents available, but the main attention is focused on cheap and easy to get materials which could be applied for large-scale processes. Among them, living or dead microorganisms (yeast, bacteria, fungi, algae) are intensively studied (see Ref. [5] and references therein). Each microorganism is able to bind or degrade several types of dyes and on the other side each dye can have an affinity to various microorganisms. In addition, microbial cells efficiently interact with magnetic nanoparticles stabilized as low-pH ionic magnetic fluid, leading to the formation of magnetically labeled cells, which could be easily separated from the system using an appropriate magnetic separator.⁵ Currently, extensive studies are performed in many laboratories to find the optimal microbial biomass enabling to separate contaminating dyes from large volumes of polluted water.

This work reports on new magnetic adsorbents—ferrofluid-modified fodder yeast (Kluyveromyces fragilis) and unicellular algae (Chlorella vulgaris) cells—containing maghemite nanoparticles as magnetic labels. The prepared materials were tested as possible adsorbents for binding of different substances.⁶,⁷ They efficiently adsorbed selected water-soluble organic dyes, namely, crystal violet, amido black, congo red, Saturn blue, acridine orange, aniline blue, Bismarck brown and safranin O.
Table I. Comparison of maximum adsorption capacities of studied microbial cells.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Maximum adsorption capacity of studied <em>Kluyveromyces fragilis</em> cells (mg/g)</th>
<th>Maximum adsorption capacity of studied <em>Chlorella vulgaris</em> cells (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acridine orange</td>
<td>62.2</td>
<td>31.9</td>
</tr>
<tr>
<td>Amido black 10B</td>
<td>29.9</td>
<td>18.5</td>
</tr>
<tr>
<td>Bismarck brown</td>
<td>75.7</td>
<td>201.9</td>
</tr>
<tr>
<td>Congo red</td>
<td>49.7</td>
<td>156.7</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>42.9</td>
<td>42.9</td>
</tr>
<tr>
<td>Safranin O</td>
<td>138.2</td>
<td>115.7</td>
</tr>
<tr>
<td>Saturn blue LBRR</td>
<td>33.0</td>
<td>24.2</td>
</tr>
<tr>
<td>Aniline blue</td>
<td>257.9</td>
<td></td>
</tr>
</tbody>
</table>


(see Table I). However, from the point of view of potential applications of investigated materials as the magnetic affinity adsorbents in magnetic separation procedures these materials should have the best both adsorption and magnetic properties. They should be superparamagnetic to that they would exhibit magnetic properties when placed within a magnetic field, but retained no residual magnetism when removed from the field. They should form stable colloidal suspensions and they should not sediment or aggregate in magnetic fields. Therefore, in this work we present detailed magnetic studies of the ferrofluid-modified microbial cells using various complementary magnetic methods (EPR, dc magnetization and ac susceptibility measurements). These studies are of considerable interest for development of new inexpensive magnetic affinity adsorbents, which could exhibit the peculiar features enabling the rapid and efficient removal of dyes from the polluted water sources.

2. EXPERIMENTAL DETAILS

2.1. Preparation of the Samples

Dried algae (*Chlorella vulgaris*) cells were obtained from Dr. Kopecky, Department of Autotrophic Microorganisms, Institute of Microbiology, Academy of Sciences, Trebon, Czech Republic. Dried fodder yeast (*VITEX Kluyveromyces fragilis*) was produced by Biocel, Paskov, Czech Republic. The microbial cells were modified by contact with water-based magnetic fluid containing maghemite nanoparticles.

Water-based ionic magnetic fluid stabilized with perchloric acid was prepared using the standard Massart procedure; the relative magnetic fluid concentration (25.8 mg/ml) was given as the maghemite content determined by a colorimetric method. The presence of maghemite was confirmed by Mössbauer measurements (data not shown).

Dried microbial cells were washed six to eight times with an excess of 0.1 M acetic acid. 1 ml of ferrofluid was added to 3 ml of the suspension of washed cells in acetic acid (1 + 3, v/v) and the suspension was mixed at room temperature for 1 h on a Dynal MX1 sample mixer (Invitrogen, USA). The residual ferrofluid was removed by washing with 0.1 M acetic acid 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 adsorbents were stored in water suspensions at 4 °C.

2.2. Magnetic and Microscopy Characterization

All magnetic measurements were performed in a wide temperature range 4–300 K.

DC magnetization and AC susceptibility data were collected using an extraction magnetometer MagLab 2000 System (Oxford Instruments Ltd.). DC magnetization was measured in the applied magnetic field ± 3 kOe. For AC magnetic susceptibility measurements, we have used an excitation field of 10 Oe and driving frequencies in the range 36–2237 Hz.

Electron spin resonance (ESR) measurements were performed by means of a standard X-band spectrometer (Bruker EMX-10/12) operating at 9.46 GHz with 100 kHz field modulation. Resonance absorption was measured as a first derivative of the absorbed microwave power versus magnetic field.

The magnetically modified microbial cells and the ferrofluid used for magnetic modification were also studied by means of a Jeol 1010 transmission electron microscope (for details see Refs. [7, 8]).

3. RESULTS AND DISCUSSION

3.1. Transmission Electron Microscopy (TEM) Measurements

Analysis of TEM micrographs showed the presence of both isolated magnetic nanoparticles and their aggregates on the cell surface. The nanoparticles are roughly spherical in shape and externally attached to the microbial cells walls. The outer cell surface preferentially accumulated magnetic nanoparticles even in the case of ruptured cells; only negligible binding of magnetic nanoparticles on the inner cell wall surface was observed.

The TEM analysis of the ferrofluid used for magnetic modification allowed the determination of dimensional distribution of nanoparticles. Figure 1 shows the TEM image of the ferrofluid and the histogram of particle size distribution obtained using samples containing at least 500 particles. The size histogram is satisfactorily described by a log-normal distribution of the particle diameters with a mean particle diameter $\langle D \rangle = 12.6$ nm and the standard deviation $\sigma = 0.31$. 
3.2. Dc Magnetization Measurements

In order to test if the obtained ferrofluid-modified microbial cells could be used as the magnetic adsorbents in the magnetic separation procedures, we performed magnetization measurements as a function of temperature and applied magnetic field. Figure 2 shows temperature dependencies of magnetization measured in zero field cooled–field cooled (ZFC–FC) regime at the applied magnetic field of 50 Oe for both magnetically modified Kluyveromyces fragilis and Chlorella vulgaris cells. The ZFC curves were obtained by first cooling the samples in zero magnetic field from 300 to 2 K. Then the magnetic field $H = 50$ Oe was applied and the magnetization was measured with increasing temperature. The FC curves were obtained in a similar manner except that the samples were cooled in the same measuring field $H = 50$ Oe.

It is seen that the ZFC–FC curves split at $T < T_B$ ($T_B = 190$ K and 210 K for Kluyveromyces fragilis and Chlorella vulgaris cells, respectively), indicating the existence of the irreversible processes. The observed behavior is reminiscent of a blocking process of small single domain particles, which turn to a superparamagnetic state with increasing temperature.

The ZFC curves for both investigated materials show a maximum associated to the transition between the superparamagnetic and blocked state. Moreover these maxima are very broad and a clear Curie-Weiss law behavior is not observed above the blocking temperature $T_B$. This indicates the existence of dipole–dipole interactions between the particles, which may lead to the formation of small agglomerates and thus a wide distribution in particle size ranging from ultrafine isolated particles up to particle aggregates. The magnetization in the FC curves for both materials is almost independent on temperature, which also reveals the presence of non-negligible dipole–dipole interactions between the particles. Such behavior has been observed in several iron oxide particle systems.\cite{11-13}

The obtained results are in good agreement with the TEM measurements,\cite{7,8} where the presence of both isolated nanoparticles and agglomerates was also documented.

At $T \sim 250$ K a kink is seen in both ZFC and FC curves for both materials which is associated with the melting point of the solution.

Above the blocking temperature, the field dependent magnetization curves of both prepared materials show the superparamagnetic behavior indicated by the absence of hysteresis (see Fig. 3). At lower temperatures the magnetization curves display the hysteresis, which confirms that the maghemite nanoparticles are in the ferrimagnetic state.

At $T = 4.2$ K, the remanence-to-saturation ratio for both prepared materials, $R = M_R/M_S = 0.37$, is smaller than the expected $R = 0.5$ value for non-interacting, randomly oriented particles with uniaxial symmetry.\cite{14} It is an additional confirmation for the existence of interparticle magnetic dipole–dipole interactions.

3.3. Ac Susceptibility Measurements

In order to study the effects of interparticle interactions on the dynamics of the blocking process, we have measured...
the temperature dependence of the in-phase (real) component $\chi'(T)$ and the out-of-phase (imaginary) component $\chi''(T)$ of the AC magnetic susceptibility for different frequencies $f$ of the excitation field (see Figs. 4 and 5). The experimental data for both prepared materials exhibit the expected behavior of a superparamagnetic system, i.e., the occurrence of a maximum in both components $\chi'(T)$ and $\chi''(T)$ at different temperatures $T_m$ and $T''_m$ which shift towards higher temperatures with increasing frequency $f$. At about 260 K a kink is seen in both $\chi'(T)$ and $\chi''(T)$ curves for both materials, which is associated with the melting point of the solution. At low temperatures the $\chi''(T)$ curves show oscillations, which are due to the imperfection of the experimental system.

The real component of the AC susceptibility of both materials shows a value not equal to zero for $T$ approaching zero (see Fig. 4). It may be due to the presence of small agglomerates of particles coupled by magnetic dipolar interactions. These interactions modify the magnetic behavior of the system, introducing a collective component which has the influence on the low temperature magnetic relaxation. It has been shown$^{15}$ that the magnetic relaxation of an interacting nanosized magnetic particle system at low temperatures is extended towards longer time scales as compared to the relaxation of a non-interacting particle system. Another indication of the influence of magnetic dipole–dipole interactions on the dynamics of the samples comes from the increasing with increasing frequency of the height of the peak in $\chi''(T)$ for both investigated materials (see Fig. 5), whereas it is almost constant with frequency for a non-interacting system.$^{15}$

The empirical parameter $\Phi$, which represents the relative shift of the temperature $T_m$ per interval of frequency, $\Phi = \Delta T_m/\Delta \log_{10}(f)$, calculated from the frequency dependence of the maximum in the imaginary $\chi''(T)$ part of the AC susceptibility is equal to 0.09 and 0.08 for Kluyveromyces fragilis and Chlorella vulgaris cells, respectively. These values are close to the 0.1–0.13 values found for superparamagnetic systems.$^{16}$ The slightly lower values originate from interparticle magnetic dipole–dipole interactions.

### 3.4. Electron Spin Resonance (ESR) Measurements

In order to give a deeper insight into the nature of the magnetic structures described above, we performed electron spin resonance (ESR) measurements. Figure 6 shows the ESR spectra of both magnetically modified Kluyveromyces fragilis and Chlorella vulgaris cells plus the spectra of the
concentrated ferrofluid (1:1) used for magnetic modification and diluted ferrofluid (1:100). The huge dilution (from 1:1 to 1:100) of the ferrofluid sample causes a relative shift in the resonance field of about 50 Oe. However, the shift observed in the resonance field between the concentrated ferrofluid sample (1:1) and the ferrofluid-modified microbial cells is about 330 Oe. This remarkable resonance field shift is the signature of a strong interaction among the maghemite nanoparticles when attached to the microbial cell wall. The ESR data confirm that maghemite nanoparticles do attach to the microbial cell wall after incubation of these cells with the biocompatible magnetic fluid sample as revealed by the TEM micrographs.7,8

The exemplary ESR spectra of magnetically modified *Kluyveromyces fragilis* and *Chlorella vulgaris* cells recorded at various temperatures are presented in Figure 7. The room temperature spectra for both materials show well-defined single broad signals with the peak-to-peak line width $\Delta H_{pp} \sim 900$ Oe and an effective $g$ value of about 2.1. The line widths of these signals considerably exceed the magnetocrystalline-anisotropy-determined minimum value, $\Delta H_{pp} = 400$ Oe, for non-interacting single domain maghemite particles.17 It suggests the existence of non-negligible dipole–dipole interactions between nanoparticles.

Upon decreasing the temperature these signals for both materials shift to lower fields and gradually broaden, closely following the predictions for the ESR of superparamagnetic nanoparticles systems.18 Therefore, it may...
be expected that the broadening and shift of the resonance signals is associated with a blocking of the magnetization in maghemite nanoparticles.

The presented ESR results for both prepared materials are in good agreement with the magnetization, susceptibility and TEM measurements and confirm that the magnetic behavior of the samples is determined by two distinct magnetic distributions, namely, the isolated single domain maghemite nanoparticles and agglomerates of these nanoparticles coupled by magnetic dipolar interactions.

4. CONCLUSIONS

Summarizing, the main aim of this work was to find cheap and easy to get magnetic adsorbents enabling to separate contaminating dyes from large volumes of polluted water by means of magnetic separation procedures. Such materials should be superparamagnetic so that it will exhibit magnetic properties when placed within a magnetic field, but retained no residual magnetism when removed from the magnetic field. They should form stable colloidal suspensions and they should not sediment or aggregate in the absence of magnetic fields. They should also have an affinity to corresponding water-soluble organic dyes.

We proposed an inexpensive, extremely simple procedure for the preparation of such magnetic adsorbents using standard water-based ferrofluid containing maghemite nanoparticles with the diameter of about 12 nm. Such ferrofluid can be prepared in a simple way (almost in any lab) and such nanoparticles can be used to prepare biocomposite material enabling its simple magnetic separation with standard permanent magnets. Both of these properties are important for large-scale applications.

The prepared material efficiently adsorbed selected water-soluble organic dyes, namely, crystal violet, amido black 10 B, congo red, Saturn blue LBRR 200, acridine orange, Bismarck brown Y and safranin O.7 8

The magnetic measurements show that the magnetic behavior of the prepared materials is mainly dominated by the superparamagnetic relaxation of isolated single domain maghemite nanoparticles, although a little amount of agglomerates is also present. However, these agglomerates are sufficiently small to show at static conditions the superparamagnetic behavior at room temperature. It means that magnetically modified microbial cells (Kluyveromyces fragilis and Chlorella vulgaris) can thus be new promising magnetic affinity adsorbents which may be used to the removal of dyes.

Acknowledgments: The work was partly supported by Ministry of Education of the Czech Republic under Grant No. OC 108-Action COST 636 and MSM 619859218 and by Ministry of Industry and Trade of the Czech Republic under Grant No. 2A-1TP1/094.

References and Notes


Received: 9 December 2008. Accepted: 20 January 2009.