Chapter 9

MAGNETICALLY MODIFIED BIOLOGICAL MATERIALS AS PERSPECTIVE ADSORBENTS FOR LARGE-SCALE MAGNETIC SEPARATION PROCESSES

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ABSTRACT

Novel magnetically modified biological materials, containing magnetic iron oxides nanoparticles as labels, have been successfully developed and applied as magnetic affinity adsorbents for the magnetic separation of various biologically active compounds and xenobiotics.

The main attention was focused on cheap and easy to get magnetic adsorbents which could be applied for large-scale processes. Among them magnetically modified plant-based materials (sawdust) and microbial cells (yeast and algae) were taken into consideration. An inexpensive, extremely simple procedure was proposed for the preparation of such magnetic adsorbents using standard water-based ferrofluids containing maghemite nanoparticles with the diameter of about 12 nm. Such ferrofluids can be prepared in a simple way (almost in any lab) and such nanoparticles can be used to prepare biocomposite materials enabling their simple magnetic separation with standard permanent magnets. Both of these properties are important for possible large-scale applications.

The structural, adsorption and magnetic properties of the developed materials were studied in detail by means of scanning electron microscopy, transmission electron microscopy, spectrophotometric measurements, ESR spectroscopy and conventional magnetic methods (DC magnetization and AC susceptibility measurements). The prepared materials efficiently adsorbed selected biologically active compounds and
xenobiotics (mainly different enzymes, water-soluble organic dyes and heavy metal ions). Their magnetic behavior was dominated by the superparamagnetic relaxation of isolated single domain maghemite nanoparticles, although a little amount of agglomerates was also present. However, these agglomerates were sufficiently small to show at static conditions the superparamagnetic behavior at room temperature which allows to use the developed materials as magnetic adsorbents in the magnetic separation techniques. Moreover, the prepared materials exhibit the peculiar features enabling their rapid and efficient removal not only from solutions, but also from suspensions. Such materials could be efficiently used to isolate rare biologically active compounds from difficult-to-handle materials including raw extracts, blood and other body fluids, cultivation media, environmental samples, etc.

Inexpensive raw materials, extremely simple preparation method, affinity to various biologically active compounds and both organic and inorganic xenobiotics, and distinctive magnetic properties make the developed materials greatly suitable as magnetic adsorbents for large-scale magnetic separation processes.

1. INTRODUCTION

Magnetically modified biocompatible materials, containing magnetic nanoparticles as labels, have attracted much attention because of their great potential as magnetic affinity adsorbents for various biologically active compounds. They have been successfully applied for the magnetic separation of various proteins (enzymes, antibodies, antigens, receptors, lectins), nucleic acids (DNA, RNA, oligonucleotides), low-molecular weight biologically active compounds (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 cells separation [3] to removal of xenobiotics from aqueous wastes [4]. They are alternative methods to gravitational, centrifugal or filtration separation techniques and enable a simple magnetic manipulation with the adsorbents using an external magnetic field.

Magnetic affinity adsorbents can be efficiently used for work in difficult-to-handle materials including raw extracts, blood and other body fluids, cultivation media, environmental samples, etc. However, for large-scale applications (e.g., in biotechnology or environmental technology), the finding of relatively cheap and readily available magnetic adsorbents is necessary. That is the reason why an extremely simple procedure was here proposed for the preparation of such magnetic adsorbents using standard water-based ferrofluids (which can be prepared in any lab) and inexpensive raw materials (sawdust and microbial cells).

The sawdust and microbial cells have been chosen for magnetic modification because of their well known affinity for various xenobiotics (mainly water-soluble organic dyes and heavy metal ions [5-8]) which allows to use these materials for the large-scale removal of xenobiotics from polluted water sources.

A large amount of environmental contaminants (among them dyes) is produced every year in different branches of industry. A substantial part of them pollutes many water sources. Even a very small amount of dye in the water \((10 – 50 \text{ mg/dm}^3)\) affects the water transparency and aesthetic values [9, 10]. There are more than 100,000 commercially
available dyes and over $7 \times 10^5$ t of them are annually used in textile, paper, leather, plastics, food and cosmetics industries [11]. It is estimated that 20-50% of these dyes are lost into wastewaters, causing environmental contaminations.

Dyes may be toxic and mutagenic. They contaminate not only the environment but also traverse through the entire food chain, leading to the biomagnification. To decrease the dyes concentration, different procedures such as coagulation, flocculation, chemical degradation, oxidation, photodegradation, aerobic and anaerobic biodegradation, etc., are used [10]. However, such methods are often very expensive and cannot be used on a large scale. Moreover, many synthetic dyes are difficult to remove by the conventional wastewater systems, due to their complex chemical structure [10]. 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 [8] 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, the microbial cells efficiently interact with magnetic nanoparticles present both in low-pH and high-pH ionic magnetic fluids, leading to the formation of magnetically labeled cells, which could be easily separated from the system using an appropriate magnetic separator [8].

Another cheap and easy available adsorbents which could be applied for large-scale processes are plant-based materials (e.g. different types of sawdust). It has been known for a long time that these materials have an affinity for different biologically active compounds such as enzymes (e.g. trypsin [12], urokinase [13], elastase [14], cellulases [15]) or polyphenols [16]. Moreover, many organic compounds such as acid and basic dyes and oils [5, 6] and heavy metal ions [7] have been efficiently adsorbed on these materials. Therefore the sawdust seems to be also promising adsorbent for the removal of dyes.

Currently, extensive studies are performed in many laboratories to find the optimal magnetic adsorbents with best magnetic and adsorption properties enabling to separate contaminating xenobiotics from large volumes of polluted water. These materials should be superparamagnetic to that they would exhibit magnetic properties when placed within a magnetic field (thus enabling their simple magnetic separation from the treated systems), but retained no residual magnetism when removed from the magnetic field. They should form stable colloidal suspensions and they should not aggregate in magnetic fields. They should also have an affinity to adequate xenobiotics.

Inspired by these experiments, we directed our attention to the possibility of application of magnetically modified sawdust and microbial cells as new inexpensive and readily available magnetic adsorbents, which could be used for the separation and purification of biologically active compounds and xenobiotics. Therefore new ferrofluid-modified materials – spruce sawdust [17, 18], baker’s yeast (Saccharomyces cerevisiae) cells [19], brewer’s yeast (Saccharomyces cerevisiae subsp. uvarum) cells [20, 21], fodder yeast (Kluyveromyces fragilis) cells [22] and unicellular algae (Chlorella vulgaris) cells [23] – containing maghemite nanoparticles as magnetic labels – were prepared and tested as possible adsorbents for binding of different substances. The structural and magnetic properties of the developed materials were studied in detail by means of scanning electron microscopy, transmission electron microscopy, ESR spectroscopy and conventional magnetic methods (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 their rapid and efficient removal not only from solutions, but also from suspensions. Such materials could be efficiently used to isolate rare biologically active compounds from difficult-to-handle materials including raw extracts, blood and other body fluids, cultivation media, environmental samples, etc. They are also greatly suitable as magnetic adsorbents for large-scale magnetic separation processes.

2. MAGNETIC MODIFICATION OF BIOLOGICAL MATERIALS

An absolute majority of biological materials is diamagnetic (i.e. is not attracted to a magnetic field). Therefore, when a magnetic separation technique is applied, these materials have to be first magnetically modified, usually by forming complexes with magnetic particles.

Different procedures are available to convert diamagnetic biological materials into their magnetic derivatives [8]. However, for large-scale applications (e.g. in biotechnology or environmental technology), relatively cheap and readily available magnetic adsorbents are necessary. That is the reason why the new, extremely simple procedures for magnetic modification of biological materials were developed and presented here.

According to these procedures the biological materials are magnetically modified by a contact with water-based ionic magnetic fluids stabilized with perchloric acid or tetramethylammonium hydroxide. The ferrofluids are prepared using the standard Massart procedure [24, 25] and contain magnetic iron oxide nanoparticles (usually in the form of maghemite) with the diameter of about 12 nm. Figure 1 shows exemplary TEM image of the obtained perchloric acid stabilized magnetic fluid and the corresponding log-normal distribution of particle diameters.

![TEM image of perchloric acid stabilized magnetic fluid](image)

**Figure 1.** TEM image of perchloric acid stabilized magnetic fluid obtained according to Massart procedure (the bar corresponds to 200 nm) and the corresponding log-normal distribution of particle diameters. The vertical bars are the experimental data, whereas the solid line represents the fit to the log-normal distribution function.

The above-mentioned ionic ferrofluids can be prepared in a simple way (almost in any lab) and such nanoparticles can be used to prepare biocomposite materials enabling their
simple magnetic separation with standard permanent magnets. Both of these properties are important for large-scale magnetic separation techniques.

Having in mind possible large-scale applications, the inexpensive raw materials should be also used. Therefore spruce sawdust (waste material) and microbial cells (baker’s, brewer’s and fodder yeast and unicellular algae, all of them produced in large quantities and available at low price) were taken into consideration.

In order to produce magnetically modified spruce sawdust the raw material was sieved to obtain particles with the diameter less than 0.5 mm. Then 500 mg of sawdust was suspended in 7 ml of methanol in a test tube and 1 ml of perchloric acid stabilized ferrofluid was added. The suspension was mixed on a rotary mixer (Dynal) for 1 hour. During this time almost complete adsorption of ferrofluid nanoparticles on the sawdust occurred. Then the magnetic sawdust was repeatedly washed with water and the suspension was stored at 4°C [18]. Alternatively, the modified sawdust was washed with methanol and air-dried.

Figure 2. Raw (SEM image) and magnetically modified (optical micrograph) spruce sawdust particles. The scale bars correspond to 50 μm.

Figure 2 shows microscope images of spruce sawdust particle before and after magnetic modification. It is seen that maghemite nanoparticles are attached to the sawdust particle surface as the individual magnetic particles or agglomerates of these particles. The prepared magnetic sawdust can be easily manipulated by means of magnetic field and therefore it can be used as the magnetic adsorbent in magnetic separation techniques.

A different procedure was used when working with dried fodder yeast (*Kluyveromyces fragilis*) and unicellular algae (*Chlorella vulgaris*) cells. The microbial cells were first washed six to eight times with an excess of 0.1 M acetic acid in order to remove substantial portion of soluble macromolecules which otherwise caused spontaneous precipitation of magnetic fluid. Then 1 ml of perchloric acid stabilized 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 adsorption of maghemite nanoparticles onto the microbial cells was fast; majority of nanoparticles was adsorbed within several minutes. 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 [19, 20].
Figure 3. Cross-sections of fodder yeast cells (before and after magnetic modification) observed by TEM. The scale bars correspond to 200 nm.

Figure 4. TEM picture of original dried *Chlorella vulgaris* cell (left) and magnetically modified cell (right). The bar lines correspond to 1 μm.

Figures 3 and 4 show TEM images of original dried and magnetically modified microbial cells. The drying process caused damage to the cell walls, often followed by the release of the intracellular components. Magnetic modification influenced the whole cells not the cell fragments. Analysis of TEM micrographs showed the presence of both isolated magnetic nanoparticles and their agglomerates on the cell surface. The nanoparticles were 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. Obtained this way magnetically modified microbial cells could be easily separated using commercially available magnetic separators or strong permanent magnets and therefore they can be used as cheap magnetic adsorbents. These biocomposite materials were stable even after one year storage of the suspension at 4 °C.

It was found recently that the procedure used for sawdust modification (methanol / acid ferrofluid) can be also successfully used for dried microbial cells modification (unpublished results). To prepare magnetically responsive baker’s yeast cells with highly active intracellular enzymes, tetramethylammonium hydroxide stabilized ferrofluid in glycin-NaOH buffer was used; such cells have been used as non-toxic and efficient magnetically responsive whole cell biocatalysts for hydrogen peroxide decomposition and invert sugar formation [26].

As mentioned above the new developed procedures of obtaining of magnetically modified biological materials are cheap and extremely simple and may be performed almost in any lab. Therefore they allow to produce also other new magnetic adsorbents which can be used for large-scale magnetic separation.
3. MAGNETIC CHARACTERIZATION OF MAGNETICALLY MODIFIED BIOLOGICAL MATERIALS

Magnetically modified biological materials obtained according to above-mentioned preparation procedure were characterized by means of EPR spectroscopy and conventional magnetic methods in order to test if they can be used as magnetic affinity adsorbents in magnetic separation procedures. As mentioned above, such magnetic adsorbents should be superparamagnetic so that they would exhibit magnetic properties when placed within a magnetic field, but retained no residual magnetism when removed from the field. They should also form stable colloidal suspensions and they should not aggregate in magnetic fields.

3.1. DC Magnetization Measurements

DC magnetization was measured by means of an extraction magnetometer MagLab 2000 System (Oxford Instruments Ltd.) in the applied magnetic field ± 3 kOe and wide temperature range 4 – 300 K. Figure 5 shows temperature dependencies of magnetization measured in zero field cooled – field cooled (ZFC-FC) regime at the applied magnetic field of 50 Oe for magnetically modified spruce sawdust and two kind of magnetically modified microbial cells (Kluyveromyces fragilis and Chlorella vulgaris). The ZFC curves were obtained by first cooling the samples in zero magnetic field from 300 to 4 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 $T < T_B$ ($T_B = 190 K, 210 K$ and $250 K$ for fodder yeast cells, algae cells and spruce sawdust, 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 all 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 all materials does not fulfill the Curie-Weiss law, 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 [27 - 29].

At about $250 K$ and $280 K$ a kink is seen in both ZFC and FC curves for microbial materials and spruce sawdust, respectively, which is associated with the melting point of the solution.

Above the blocking temperature, the field dependent magnetization curves of all prepared materials show the superparamagnetic behavior indicated by the absence of hysteresis (see Figure 6). At lower temperatures the magnetization curves display the hysteresis, which confirms that the maghemite nanoparticles are in the ferrimagnetic state.
Figure 5. Temperature dependencies of magnetization for magnetically modified fodder yeast and algae cells and spruce sawdust recorded at the applied magnetic field of 50 Oe.

Figure 6. Field dependent hysteresis loops for magnetically modified fodder yeast and algae cells and spruce sawdust recorded at 4.2 and 300 K.
At $T = 4.2$ K, the remanence-to-saturation ratio for all 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 [30]. It is an additional confirmation for the existence of interparticle magnetic dipole-dipole interactions.

### 3.2. AC Susceptibility Measurements

In order to study the effects of interparticle interactions on the dynamics of the blocking process, temperature dependence of the in-phase (real) component $\chi'$ (T) and the out-of-phase (imaginary) component $\chi''$ (T) of the AC magnetic susceptibility were measured for different frequencies $f$ of the excitation field for all magnetically modified materials. Measurements were performed by means of an extraction magnetometer MagLab 2000 System (Oxford Instruments Ltd.) using an excitation field of 10 Oe and driving frequencies in the range 36 – 2237 Hz.

The experimental data (see Figure 7) for all 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 $f$ [31]. At about 260 K and 280 K a kink is seen in both $\chi'$ (T) and $\chi''$ (T) curves for microbial materials and spruce sawdust, respectively, which is associated with the melting point of the solution. At low temperatures all $\chi''$ (T) curves show oscillations, which are due to the imperfection of the experimental system.

The real component of the AC susceptibility of all materials shows a value not equal to zero for $T$ approaching zero (see Figure 7). 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 [31] 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 all investigated materials (see Figure 7), whereas it is almost constant with frequency for a non-interacting system [31].

The empirical parameter $\Phi$ [32], which represents the relative shift of the temperature $T_m$ per interval of frequency,

$$\Phi = \frac{\Delta T_m}{T_m \Delta \log_{10}(f)},$$

calculated from the frequency dependence of the maximum in the imaginary $\chi''$ (T) part of the AC susceptibility is equal to 0.10, 0.09 and 0.08 for spruce sawdust, yeast cells and algae cells, respectively. These values are close to the 0.1 – 0.13 values found for superparamagnetic systems [32]. The slightly lower values originate from interparticle magnetic dipole-dipole interactions.
Figure 7. Temperature dependencies of the real ($\chi'$) and imaginary ($\chi''$) components of the magnetic susceptibility for magnetically modified fodder yeast and algae cells and spruce sawdust recorded at different excitation frequencies. Arrows indicate increasing frequencies. The data were taken with an external magnetic field $H = 10$ Oe.

3.3. Electron Spin Resonance (ESR) Measurements

In order to give a deeper insight into the nature of the magnetic structures described above, ESR measurements were performed. ESR spectra were recorded 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.

Figure 8 shows the ESR spectra of all magnetically modified materials 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 materials is about 196, 319 and 362 Oe for spruce sawdust, fodder yeast cells and algae cells, respectively. This remarkable resonance field shift is the signature of a strong interaction among the maghemite nanoparticles when attached to the biological materials. The ESR data confirm that maghemite nanoparticles do attach to the microbial cell wall and spruce sawdust surface after incubation of these materials with the biocompatible magnetic fluid sample as revealed by the microscope micrographs (see Figures 2, 3 and 4).

The exemplary ESR spectra of magnetically modified biological materials recorded at various temperatures are presented in Figure 9. The room temperature spectra for all materials show well-defined single broad signals with an effective $g$ value of about 2.1 and the peak-to-peak line width $\Delta H_{pp} = 1040, 990$ and 880 Oe for spruce sawdust, fodder yeast cells and algae cells, respectively. 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 [33]. It suggests the existence of non-negligible dipole-dipole interactions between nanoparticles.

![Figure 8. Room temperature ESR spectra of maghemite nanoparticles in labeled fodder yeast and algae cells and spruce sawdust and maghemite nanoparticles suspended as a stable magnetic fluid samples at different concentrations.](image-url)
Figure 9. ESR spectra of magnetically modified fodder yeast and algae cells and spruce sawdust recorded at various temperatures.
On the spruce sawdust resonance line two more ESR signals are superimposed (see Figure 9), which are clearly seen only at low temperatures due to their paramagnetic behavior:

- A sharp signal, at $g = 2.00$, which seems to be due to free radicals, usually found in biological materials [34].
- A narrow ($\Delta B_{pp} = 19$ mT) resonance at $g = 2.27$, which seems to be attributed to the existence of paramagnetic $\text{Cu}^{2+}$ complexes, sometimes found in wood [34].

Upon decreasing the temperature the main resonance signals for all materials shift to lower fields and gradually broaden, closely following the predictions for the ESR of superparamagnetic nanoparticles systems [35]. Therefore, it may be expected that the broadening and shift of these signals is associated with a blocking of the magnetization in maghemite nanoparticles.

In general, for a superparamagnetic system of particles having a statistical distribution of shapes and sizes, the simple power relation between the resonance field shift $\delta H_{res}$ and the peak-to-peak line width $\Delta H_{pp}$ can be expressed as [35]:

$$\delta H_{res} \sim (\Delta H_{pp})^n$$

(3.2)

where $n = 2$ (3) is predicted for partially (randomly) oriented particles.

In order to test this power relation we have plotted the data of figure 9 on a double logarithmic scale (see Figure 10). It is seen that a straight line with a slope close to 3 (3.08 for spruce sawdust and 3.32 for microbial cells) can well approximate the data for all prepared materials. Thus, the broad ESR signal is shown to be due to the superparamagnetic behavior of small isolated randomly oriented maghemite particles.

Figure 10. Relation between the resonance field shift ($\delta H_{res}$) and peak-to-peak line width ($\Delta H_{pp}$) of the ESR signal for magnetically modified spruce sawdust and fodder yeast and algae cells. Points correspond to the experimental data, the solid line is the best fit according to Equation 3.2.
The presented ESR results for all prepared materials are in good agreement with the magnetization, susceptibility and TEM measurements and confirm that the magnetic behavior of these materials is dominated by the superparamagnetic relaxation of isolated single domain maghemite particles, although a certain amount of agglomerates of these particles coupled by magnetic dipole-dipole interactions is also present. However, these agglomerates are sufficiently small to show at static conditions the superparamagnetic behavior at room temperature. Therefore, the obtained results are very promising from the point of view of using the prepared materials as the magnetic affinity adsorbent in the magnetic separation techniques.

4. ADSORPTION PROPERTIES OF MAGNETICALLY MODIFIED BIOLOGICAL MATERIALS

Magnetically modified spruce sawdust and microbial cells were tested as possible adsorbents for binding of different substances [17-23]. 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). Commercially available dyes were used during the experiments. They were dissolved in distilled water without buffering the solution. Preliminary experiments also showed that adsorption properties of spruce sawdust and microbial cells were not significantly influenced by magnetic modification.

The adsorption of the tested dyes 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.

Measurements were performed in test tubes containing 50 µl of sedimented magnetically modified biological material, appropriate amount of tested dye solution and water to a total volume of 10 ml. The suspension was mixed for 3 h at room temperature. Then the magnetic adsorbent was separated from the suspension using a magnetic separator (Dynal MPC-1 or MPC-6, Invitrogen, USA) and the clear supernatant was used for the spectrophotometric measurement. The concentration of free (unbound) dye in the supernatant (C_{eq}) was determined from the calibration curve. The amount of dye bound to the unit volume of the adsorbent (q_{eq}) was calculated using the following formula [20]:

\[
q_{eq} = \frac{D_{tot} - 10C_{eq}}{50}
\]

where D_{tot} is the total amount of dye used in the experiment.

Examples of equilibrium adsorption isotherms for the unbuffered water solutions of selected tested dyes using magnetically modified fodder yeast and algae cells as adsorbents are presented in Figure 11. These isotherms represent distribution of dyes between the
aqueous and solid phases as the dye concentration increases. They follow the typical Langmuir adsorption pattern given by [22, 23]:

$$q_{eq} = \frac{Q_{max}bC_{eq}}{1 + bC_{eq}}$$

(4.2)

where $q_{eq}$ (expressed in mg/g or mg/ml) is the amount of the adsorbed dye per unit mass or sedimented volume of magnetically modified biomass and $C_{eq}$ (expressed in mg/l) is the unadsorbed dye concentration in solution at equilibrium. $Q_{max}$ is the maximum amount of the dye per unit mass or sedimented volume of biomass to form a complete monolayer on the surface bound at high dye concentration and $b$ is a constant related to the affinity of the binding sites (expressed in l/mg).

The Langmuir model allows simple calculation of maximum adsorption capacity $Q_{max}$, which is a very important parameter describing the adsorption process. The results obtained for the magnetically modified fodder, baker’s and brewer’s yeast and algae cells and spruce sawdust are presented in Table 1.

**Table 1. Comparison of maximum adsorption capacities of magnetically modified microbial cells and spruce sawdust for the tested dyes**

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<tr>
<td>Acridine orange</td>
<td>62.2</td>
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<td>82.8</td>
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<td>24.1</td>
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<tr>
<td>Amido black 10B</td>
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<td>11.6</td>
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<tr>
<td>Aniline blue</td>
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<td>430.2</td>
<td>257.9</td>
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<tr>
<td>Bismarck brown</td>
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<td>201.9</td>
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<td>52.1</td>
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<tr>
<td>Congo red</td>
<td>49.7</td>
<td>93.1</td>
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<td>156.7</td>
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<tr>
<td>Crystal violet</td>
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<td>41.7</td>
<td>85.9</td>
<td>42.9</td>
<td>52.4</td>
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<tr>
<td>Safranin O</td>
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<td>46.6</td>
<td>90.3</td>
<td>115.7</td>
<td>25.0</td>
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<tr>
<td>Saturn blue LBRR</td>
<td>33.0</td>
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The results show that dye sorption by ferrofluid-modified biomass follows a chemical, equilibrated and saturable mechanism. Thus, the adsorption increases when the initial dye concentration rises, as long as binding sites are not saturated. The fact that the Langmuir isotherm fits the experimental data very well may be due to the homogeneous distribution of active sites on the magnetically modified sawdust particles and microbial cells [36].

The prepared magnetically modified microbial cells were tested as possible adsorbents for binding of heavy metal ions. Heavy metal pollution represents an important environmental problem due to the toxic effects of metals, and their accumulation throughout the food chain leads to serious ecological and health problems. Magnetically modified brewer’s yeast were used to study Hg$^{2+}$ biosorption-desorption process using synthetic solutions in batch system. The biosorption process was fast; 80% of biosorption occurred within 60 min and equilibrium was achieved at around 90 min. The maximum Hg$^{2+}$ biosorption capacity was 114.6 mg/g at 35 °C and this value decreased to 76.2 mg/g at room temperature. The adsorption was well
fitted to the Langmuir isotherm. Results suggest that chemisorption processes could be the rate-limiting step in the biosorption process. The yeast biomass could be easily and efficiently regenerated by 0.1M HNO$_3$. Biosorption of other heavy metal ions from artificial wastewater was also studied; the biosorption capacities were 29.9 mg/g for Cu$^{2+}$, 14.1 mg/g for Ni$^{2+}$ and 11.8 mg/g for Zn$^{2+}$ at room temperature [21].

![Equilibrium adsorption isotherms](image)

Figure 11. Equilibrium adsorption isotherms (calculated from experimental data using Langmuir equation (4.2)) for unbuffered water solutions of tested organic dyes using magnetically modified fodder yeast [22] and algae cells [23]. $C_{eq}$ – equilibrium liquid-phase concentration of the unbound dyes (mg/dm$^3$); $q_{eq}$ – equilibrium solid-phase concentration of the adsorbed dyes (mg/g).

The prepared magnetically modified spruce sawdust was additionally tested as a possible adsorbent for binding of different enzymes [17]. It was successfully used for the batch separation of proteolytic enzymes (trypsin, bacterial protease produced by a strain of Bacillus sp.) and hen egg white lysozyme. The degree of lysozyme purity increased from 65% (a technical preparation) to 96% (after the magnetic sawdust treatment) [17].

### 5. CONCLUSION

The main aim of this work was to find cheap and easy to get magnetic adsorbents which could be used for large-scale magnetic separation procedures. Such materials 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 magnetic field. They should form stable colloidal suspensions and they should not aggregate in magnetic fields. They should also have an affinity to corresponding biologically active compounds and xenobiotics.

We proposed an inexpensive, extremely simple procedure for the preparation of such magnetic adsorbents using standard water-based ferrofluids containing magnetic iron oxides (mainly maghemite) nanoparticles with the diameter of about 12 nm. Such ferrofluids can be prepared in a simple way and such nanoparticles can be used to prepare biocomposite
Magnetically Modified Biological Materials as Perspective Adsorbents...  

materials enabling their simple magnetic separation with standard permanent magnets. Both of these properties are important for large-scale applications.

Inexpensive and easily available raw materials – spruce sawdust, fodder yeast (*Kluyveromyces fragilis*), baker’s yeast (*Saccharomyces cerevisiae*), brewer’s yeast (*Saccharomyces cerevisiae* subsp. uvarum) and unicellular algae (*Chlorella vulgaris*) cells – were used for magnetic modification. It allows to use these materials for large-scale applications.

The prepared magnetic adsorbents efficiently adsorbed several water-soluble organic dyes, belonging to different groups, and also heavy metal ions. It means that they can thus be new promising magnetic affinity adsorbents which may be used to the large-scale removal of environmental contaminants from polluted water sources.

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. The magnetic properties of the prepared magnetic adsorbents enable their rapid and efficient removal not only from solutions, but also from suspensions, so they could be used in the separation process performed directly in unprocessed samples such as waste water, biological fluids, fermentation media, etc.

Summarizing, inexpensive raw materials, extremely simple preparation method, affinity to various biologically active compounds and both organic and inorganic xenobiotics, and distinctive magnetic properties make the developed materials greatly suitable as new magnetic adsorbents for large-scale magnetic separation processes.

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