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Ferrofluid-modified plant-based materials as adsorbents for batch separation of selected biologically active compounds and xenobiotics

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Abstract

Spruce sawdust was magnetically modified after contact with water-based magnetic fluid. Magnetic and microscopy characterization of the prepared material was performed. Magnetic sawdust was efficiently used for the adsorption of water-soluble organic dyes (maximum adsorption capacity reached $50\,mg\,g^{-1}$) and purification of hen egg white lysozyme (96% purity achieved in a single step).

Keywords: Magnetic fluid; Sawdust; Lignocellulose; Dye adsorption; Lysozyme purification; Magnetic purification

1. Introduction

Currently, chromatography procedures represent the most often used techniques for the separation and purification of biologically active compounds. Standard affinity adsorbents are usually prepared by immobilization of appropriate affinity ligands on inert carriers. In some cases, however, the carrier exhibiting affinity against the target molecules can be used as affinity adsorbent without the use of any immobilization step.

Sawdust (lignocellulose material) is a cheap material, which has a potential as an (affinity) adsorbent. From the separation science point of view sawdust can be considered as a chromatography material or adsorbent where lignin is immobilized on the polysaccharide matrix. It has been shown recently that sawdust has affinity for

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different biologically active compounds such as enzymes (e.g., trypsin [1], urokinase [2], elastase [3], cellulases [4]) or polyphenols [5]. Also different organic compounds such as acid and basic dyes and oils [6,7] and heavy metal ions [8] were efficiently adsorbed on different types of sawdust.

Magnetic separations have currently found many applications in different areas of biosciences, especially in the laboratory scale. Magnetic affinity, ion exchange or non-specific adsorbents can be efficiently used for work in difficult-to-handle materials including raw extracts, blood, other body fluids, cultivation media, environmental samples, etc. Magnetic affinity adsorbents have been successfully used for the separation of various proteins (enzymes, antibodies, antigens, receptors, histidine-tagged proteins), nucleic acids (DNA, RNA, oligonucleotides), low molecular weight biologically active compounds (drugs) and xenobiotics (carcinogens, water-soluble dyes, heavy metals ions, radionuclides) [9–12].

For large-scale applications (e.g., in biotechnology or environmental technology), however, relatively cheap and readily available magnetic adsorbents are necessary. That is why magnetically modified sawdust was prepared and tested. Up to now, there is no information available about the use of this material for magnetic separation procedures. In this paper, magnetic and microscopic characterization of the prepared material is described. It was observed that selected dyes, as well as hen egg white lysozyme, were efficiently adsorbed on this adsorbent.

2. Materials and methods

2.1. Materials

Spruce sawdust was obtained locally; before use it was sieved to obtain particles with the diameter less than 0.5 mm. Water-based ionic magnetic fluid stabilized with perchloric acid was prepared using the standard Massart procedure [13]; the relative magnetic fluid concentration ($25.8\,mg\,ml^{-1}$) is given as the magnetite content determined by a colorimetric method [14]. Technical preparation of hen egg white lysozyme was from Drubezarsky prumysl, Prague, Czech Republic. Acridine orange (C.I. 46005; Loba Chemie, Austria), Bismarck brown Y (C.I. 21000; Sigma, USA), crystal violet (Basic Violet 3; C.I. 42555; Loba Chemie, Austria) and safranine O (C.I. 50240; Sigma, USA) were used as model dyes.

2.2. Preparation of ferrofluid-modified sawdust

In a test tube 500 mg of sawdust was suspended in 7 ml of methanol and then 1 ml of FF was added. The suspension was mixed on a rotary mixer (Dynal) for 1 h. During this time almost complete adsorption of ferrofluid on sawdust occurred. The magnetic sawdust was then repeatedly washed with water and stored at 4 °C.

2.3. Magnetic and microscopy characterization of ferrofluid-modified sawdust

All magnetic measurements were performed in a temperature range 4–300 K. The thermal dependencies of magnetization and hysteresis loops measurements were performed with a vibrating sample magnetometer (Oxford Instruments Ltd.) in the applied field ± 1.1 T. Electron spin resonance (ESR) spectra were recorded in a wide temperature range 4–300 K by means of a standard X-band spectrometer (Bruker ESP 300 E) operating at 9.46 GHz.

Optical microscopy (total preparations) was performed with sawdust particles put on a glass plate in a drop of water; it was covered with cover glass and photographed in an Olympus optical microscope (with Olympus DP-50 CCD camera).

For transmission electron microscopy, sawdust particles were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 1 day at room temperature. After washing in pure 0.1 M phosphate buffer, sawdust particles were washed in bidistilled water and dehydrated in 50%, 75% and absolute ethanol. Later, they were embedded into Spurr resin (standard mixture). Ultrathin sections were made with a Reichert Ultracut ultramicrotome. They were stained with uranyl acetate and lead citrate (standard recipe). Then they were studied in a Jeol 1010 transmission electron microscope.

2.4. Magnetic separation of dyes and lysozyme

The adsorption of the tested dyes to the ferrofluid-modified sawdust was performed essentially in the same way as described previously [15]. Lysozyme was adsorbed to magnetic sawdust during the 1 h incubation period. After washing out the non-adsorbed proteins with water the adsorbed lysozyme was eluted with 0.5 M sodium chloride solution.

2.5. Other procedures

Lysozyme purity was checked using FPLC. The chromatography was carried out on a Mono S HR 5/5 column using 0.05 M acetate buffer, pH 4.4, as a mobile phase A, and the same buffer containing 1 M sodium sulfate as a mobile phase B. The flow rate was $1\,\mathrm{ml\,min^{-1}}$. The elution profile was monitored with diode array detector (Agilent 1100 Series, Agilent, USA).

3. Results

Lignocellulose material (sawdust) was magnetically modified by a simple contact with water-based magnetic fluid. The adsorption of iron oxide nanoparticles onto the sawdust surface proceed in a fast speed (within several minutes). The magnetically modified sawdust could be easily separated using commonly used rare earth permanent magnets or commercially available magnetic separators.

The presence of individual magnetic nanoparticles on the ultrathin sections of sawdust particles can be seen in Fig. 1. However, using optical microscopy aggregates of magnetic nanoparticles can be clearly seen (Fig. 2). The dimensions of these aggregates reach ca 50 μm. The importance of individual states of magnetic nanoparticles on the sawdust surface was resolved using magnetic measurements.

Ferrofluid-modified sawdust was magnetically characterized. Temperature dependencies of magnetization were measured in the zero-field-cooled (ZFC)–field-cooled (FC) regime in the magnetic field of 20 Oe. The ZFC magnetization curve was obtained after cooling the demagnetized sample in zero field and then by measuring the sample magnetization with the increasing temperature in a small applied field (20 Oe). The FC magnetization was also measured on warming but after cooling the sample in the same small applied field. The results obtained, presented in Fig. 3, show the irreversible magnetic behavior (the split between M_{ZFC} and M_{FC} branches) up to around 280 K. The observed behavior is typical of a blocking process of small single domain particles, which

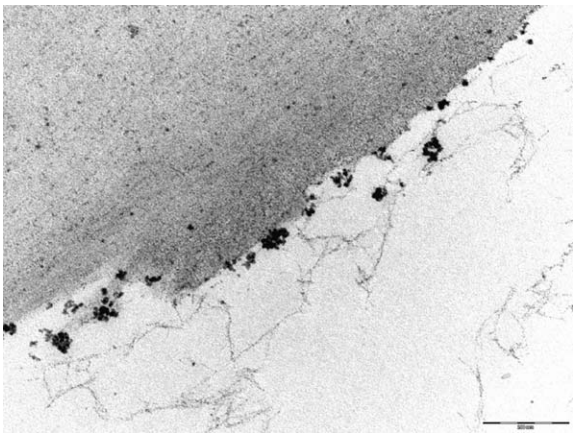

Fig. 1. Ultrathin sections of magnetic sawdust particles observed in transmission electron microscopy. The bar corresponds to 200 nm.

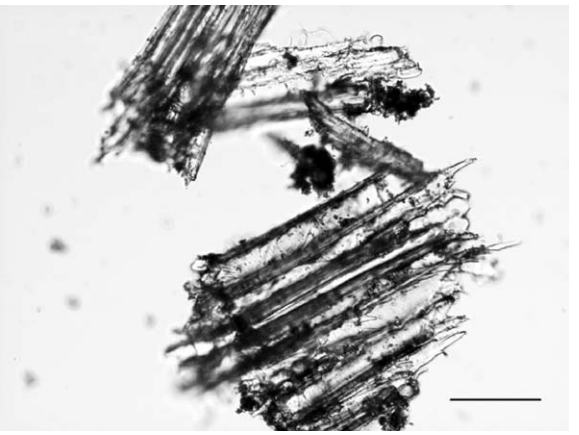

Fig. 2. Optical microscopy of magnetic sawdust particles. The bar corresponds to 100 μm.

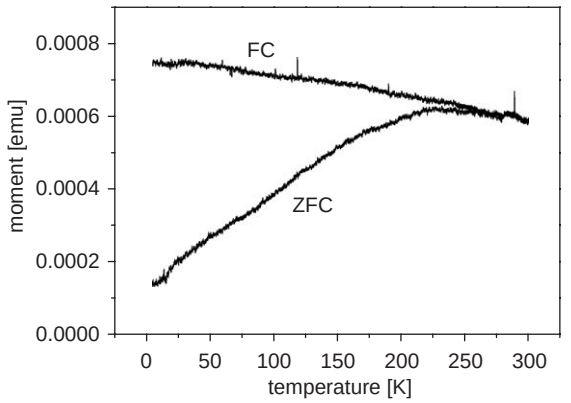

Fig. 3. Temperature dependencies of magnetization at the applied magnetic field of 20 Oe.

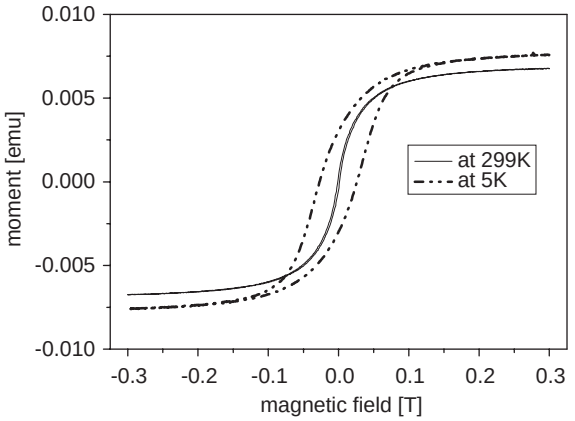

Fig. 4. Hysteresis loops at 5 and 299 K.

turn to a superparamagnetic state with increasing temperature. The maximum in the $M_{ZFC}(T)$ curve, observed at ~225 K, is associated with the mean blocking temperature of the particles. However, the peak in ZFC curve is very broad indicating either a certain coupling between particles by dipolar interactions or a broad distribution of particle sizes.

The hysteresis loops were measured at selected temperatures in the whole temperature range and the exemplary curves recorded at 5 and 299 K are shown in Fig. 4. A continuous reduction of the coercivity and remanence is observed with increasing temperature (Fig. 5) but both parameters do not vanish to zero even at room temperature. It means that at 300 K the pure superparamagnetic state of all the particles is not achieved. The results obtained (see Figs. 3–5) show that the magnetic behavior of the sample is dominated by the superparamagnetic relaxation of isolated particles. However, a certain admixture of a magnetic phase that is stable against the thermal fluctuation is also seen, which indicates that agglomerates of small particles are also present in the sample studied. The presence of two distinct magnetite-based structures in the sample, namely, the isolated nanoparticles and agglomerates of nanoparticles, was also confirmed by ESR (data not shown). These conclusions are supported by the microscopy analysis of the sample (see Figs. 1 and 2).

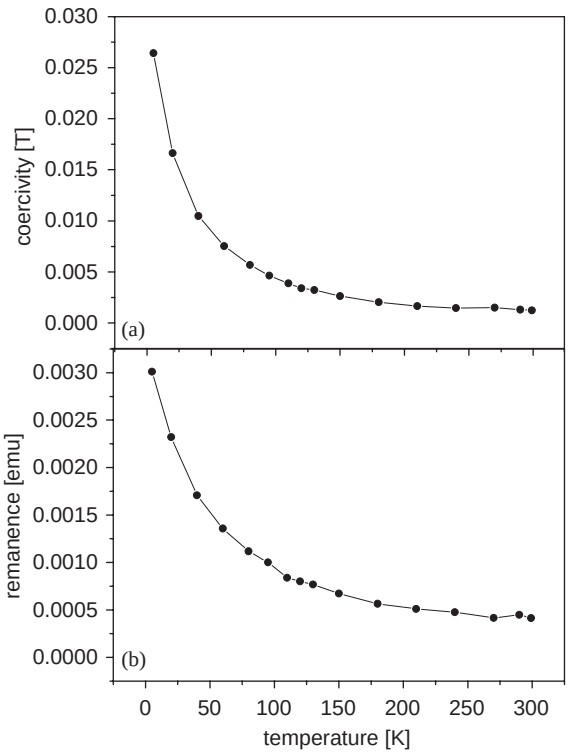

Fig. 5. Temperature dependence of the coercivity (a) and remanence (b).

Ferrofluid-modified sawdust was tested as a possible adsorbent for binding of different substances. Magnetic sawdust efficiently adsorbed

different organic compounds, such as water-soluble organic dyes. Four different types of dyes were tested, namely a triphenylmethane dye (crystal violet), an acridine dye (acridine orange), a disazo dye (Bismarck brown Y) and an azin dye (safranin O). Equilibrium adsorption isotherms for unbuffered water solutions of the tested dyes are presented in the Fig. 6. The isotherms follow the typical Langmuir adsorption pattern, as shown by their linear transformation; this transformation also allows the calculation of maximum adsorption capacities (see Table 1). Both heteropolyaromatic dyes (acridine orange, safranin O) exhibited substantially lower adsorption capacity than the remaining two dyes.

Different enzyme preparations were tested for possible adsorption on the prepared adsorbent. In addition to already described proteolytic enzymes (trypsin, bacterial protease produced by a strain of *Bacillus* sp.), magnetically modified sawdust was successfully used for the batch separation of hen egg white lysozyme. The lysozyme adsorbed to magnetic sawdust from low ionic strength water solutions. Elution of the adsorbed enzyme was performed by increasing the ionic strength of the eluant. The degree of lysozyme purification was checked using FPLC. Fig. 7 documents high affinity of sawdust for lysozyme; the ballast proteins present in the technical lysozyme

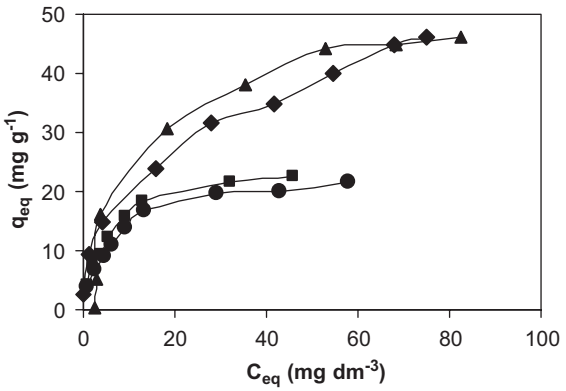

Fig. 6. Equilibrium adsorption isotherms for unbuffered water solutions of acridine orange (●), Bismarck brown (◆), crystal violet (▲) and safranine O (■). C_{eq}—equilibrium liquid-phase concentration of the unbound dye (mg dm^{-3}); q_{eq}—equilibrium solid-phase concentration of the adsorbed dye (mg g^{-1}).

Table 1
Maximum adsorption capacities Q of magnetically modified sawdust for the tested dyes

Dye	Q (mg g^{-1})
Acridine orange	24.1
Bismarck brown	52.1
Crystal violet	52.4
Safranine O	25.0

Q is calculated using the dry weight of the adsorbent.

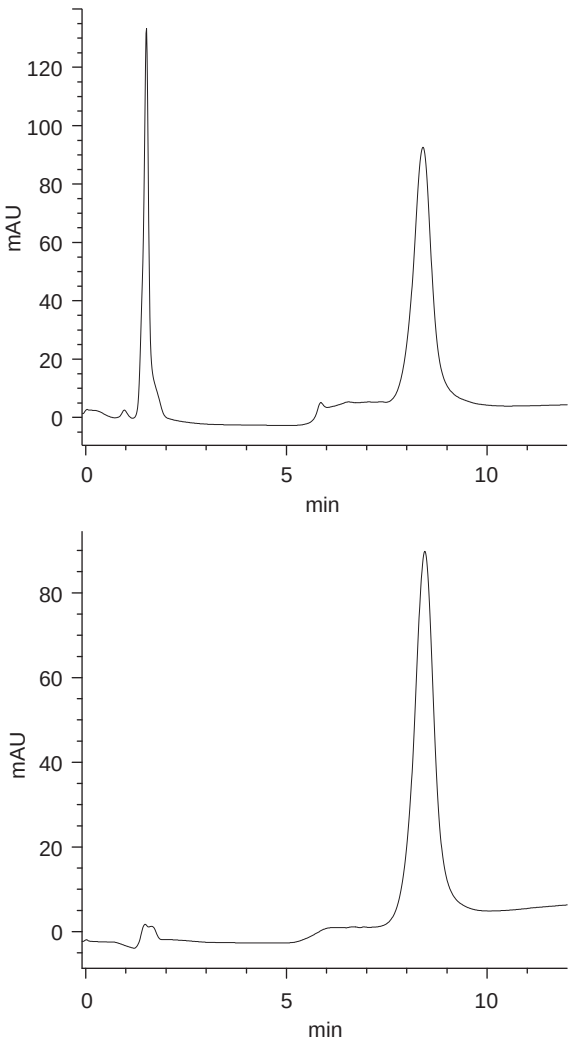

Fig. 7. Fast protein liquid chromatography of technical lysozyme (top) and preparation purified by magnetic sawdust (bottom). The first peak (elution time ca 1.5 min) corresponds to ballast proteins, the second peak (elution time ca 8.4 min) corresponds to lysozyme.

preparation (mainly hen egg white albumin) exhibited almost no affinity to the adsorbent. The degree of lysozyme purity increased from 65% (technical preparation) to 96% (after magnetic sawdust treatment).

4. Discussion

Magnetic sawdust represents an inexpensive adsorbent, which could be used for different separation processes (also at larger scale) in biochemistry, biotechnology and environmental technology. It can be prepared in an extremely simple way. Magnetic behavior of the adsorbent enables its rapid and efficient removal not only from solutions, but also from suspensions. This property is very useful because the separation process can be performed directly in unprocessed samples such as waste water, biological fluids, fermentation media, etc.

There is a complex interaction between the separated biomolecules and sawdust, most probably involving bioaffinity, ion exchange and hydrophobic interactions. Further modification of sawdust prior magnetic modification can lead to the preparation of derivatives with substantially higher adsorption capacities. Also other biologically active compounds could interact with lignocellulose materials; a more detailed search will be performed to find other interesting compounds which could be separated using the described procedure.

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