New magnetically responsive yeast-based biosorbent for the efficient removal of water-soluble dyes

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Abstract

Fodder yeast (*Kluyveromyces fragilis*) cells were magnetically modified by a contact with the water-based magnetic fluid in order to prepare a new type of magnetically responsive biocomposite material. This procedure enabled a simple separation of modified cells by means of commercially available magnetic separators or strong permanent magnets. It allows using the prepared material as a new inexpensive magnetic affinity adsorbent for the removal of water-soluble dyes.

Magnetically modified cells were characterized by means of magnetic and microscopy methods. Both isolated magnetic nanoparticles and aggregates of particles were present on the cell surface. The prepared material displayed a superparamagnetic behavior at room temperature, with a transition to a blocked state at $T_B \sim 180$ K for the applied magnetic field $H = 50$ Oe.

Seven dyes (crystal violet, amido black 10B, congo red, Saturn blue LBRR, Bismarck brown, acridine orange and safranin O) were used to study the adsorption process. The dyes adsorption could be described with the Langmuir isotherm. The maximum adsorption capacities ranged between 29.9 (amido black 10B) and 138.2 (safranin O) mg of dye per g of dried magnetically modified cells.

Introduction

Enormous amount of dyestuff is produced every year in different branches of industry. There are more than 100,000 commercially available dyes with over 7×10^5 t of dyestuff produced annually [1]. Dyes are widely used in textile, paper, food and cosmetics industries. It is estimated that 2–50% of them are lost into wastewaters, causing environmental contaminations.

Dyes may be toxic and mutagenic, and, if they are discharged directly into the environment, they contaminate not only the environment but also traverse through the entire food chain, leading to biomagnification. For their removal, many physical and chemical methods are available. However, many dyes are difficult to decolorize due to their complex chemical structure and synthetic origin [2,3]. They are resistible on the exposure to light, water and many chemicals. Therefore, there is a considerable interest in development of biological methods (microbial and enzymatic) for the removal of dyes. These procedures are considered to be attractive due to their potential low cost, environmental compatibility and public acceptability [4].

Different types of microorganisms, including bacteria, fungi and algae, are capable of decolorizing a diverse range of dyes. Many bacteria are able to degrade dyes both aerobically and anaerobically. Biodegradation of azo dyes by bacteria is often initiated by azoreductase-driven cleavage of azo bonds, followed by aerobic or anaerobic degradation of resulting amines. On

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the other hand, fungal degradation typically originates from the lignolytic activity to degrade azo dyes aerobically with the aid of lignin peroxidase [4,5].

For many dyes the adsorption on appropriate adsorbents seems to be an efficient procedure for their removal. Bacterial, yeast, fungal and algal cells have been reported for their capability of partial or complete removal of industrial dyes using the adsorption process. Both living and died biomass has been tested with various success.

Although the decolorization of dye wastewater by live or dead microbial biomass has been a subject of various studies, only limited information is available on interactions between the biomass and molecular structure of dyes [6]. Dead cells remove dyes through the mechanism of biosorption, which involves physicochemical interactions such as adsorption, deposition and ion exchange. The extent of dye biosorption depends on the chemical structure of dyes and the functional group of dye molecules.

Recently, magnetic separations have been used in different areas of biosciences, biotechnology and environmental technology [7–10]. These techniques enable a simple magnetic manipulation with the adsorbents using an external magnetic field. Sirofloc process for the waste water treatment uses fine magnetite particles as an adsorbent for the removal of different types of impurities [11]. However, there is only a very limited amount of papers describing magnetic modification of microbial cells and their subsequent use in the course of xenobiotics removal [12]. In this paper we describe a simple preparation of ferrofluid-modified fodder yeast (*Kluyveromyces fragilis*) cells, detailed magnetic characterization of the prepared biocomposite material and its use for the removal of selected dyes.

Materials and methods

Materials

Dried fodder yeast VITEX (*K. fragilis*) was produced by Biocel, Paskov, Czech Republic. Acridine orange (C.I. Basic Orange 14; C.I. 46005) and amido black 10B (C.I. Acid Black 1; C.I. 20470) were obtained from Merck, Germany. Bismarck brown Y (C.I. Basic Brown 1; C.I. 21000) and safranin O (C.I. Basic Red 2; C.I. 50240; a mixture of dimethyl safranin and trimethyl safranin) were provided by Sigma, USA. Crystal violet (C.I. Basic Violet 3; C.I. 42555) was produced by Loba Chemie, Austria. Saturn blue LBRR 200 (C.I. Direct Blue 71; C.I. 34140;) was obtained from Synthesia, Czech Republic. Congo red (C.I. Direct Red 28; C.I. 22120) and common chemicals were obtained from Lachema, Czech Republic. Water-based ionic magnetic fluid stabilized with perchloric acid was prepared using the standard Massart procedure [13]. The ferrofluid was composed of magnetic iron oxides nanoparticles with diameters ranging between 10 and 20 nm (electron microscopy measurements). The relative magnetic fluid concentration (25.8 mg/mL) is given as the iron (II,III) oxide content determined by a colorimetric method [14].

Preparation of ferrofluid modified yeast cells

Dried *K. fragilis* cells were washed six to eight times with an excess of 0.1 M acetic acid. One milliliter 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. 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 adsorbent was stored in a water suspension at 4 °C. The dry weight of 1 mL of sedimented magnetically modified yeast cells was 155.4 mg.

Magneticcharacterizationofferro?uidmodi?edyeastcells

2.3. *Magnetic characterization of ferrofluid modified yeast cells*

Thermal dependencies of magnetization in the zero-field cooled–field cooled (ZFC–FC) regime were recorded using an extraction magnetometer MagLab 2000 System (Oxford Instruments Ltd.) in the applied magnetic field of 50 Oe. The ZFC curve was obtained by first cooling the sample in zero magnetic field from 300 to 2 K. The magnetic field $H = 50$ Oe was then applied and the magnetization was measured with increasing temperature. The FC curve was obtained in a similar manner except that the sample was cooled in the same measuring field $H = 50$ Oe.

The hysteresis loops measurements were performed at selected temperatures in the applied magnetic field ±3 kOe.

Microscopy characterization of ferrofluid modified yeast cells

For transmission electron microscopy, magnetic yeast cells were fixed in 2% glutaraldehyde and 2.5% formaldehyde (EM grade) in 0.1 M phosphate buffer, pH 7.4, for 1 day at room temperature. After washing in pure 0.1 M phosphate buffer, yeast cells were washed in bidistilled water and dehydrated in 50%, 75%, 96% and absolute ethanol. Later, they were embedded into Spurr resin (a standard mixture). Ultrathin sections were made with a Reichert Ultracut ultramicrotome. They were stained with uranyl acetate and lead citrate (a standard recipe). Then they were studied in a Jeol 1010 transmission electron microscope.

For scanning electron microscopy, objects were transferred into 96% ethanol for 1 h and then they were dried on air on aluminium discs. Discs were gold coated by sputtering and observed in Jeol 6300 scanning electron microscope. Images were analyzed using the computer program ACC Structure and Object Analyzer v. 5.0 (an adaptive contrast).

Adsorption of dyes on ferrofluid modified yeast cells

0.200 mL of the suspension of magnetically modified yeast cells (the volume of the settled adsorbent was 0.050 mL) was mixed with 4.8 mL of water in 15 mL test tube. Then 0.01–3.0 mL portion of stock water solution (1–2 mg/mL) of a tested dye was added and the total volume of the suspension was made up to 10.0 mL with water. The suspension was mixed for 3 h at room temperature. Then the magnetic yeast cells were separated from the suspension using a magnetic separator (MPC-1 or MPC-6, Dynal, Norway) 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 [12]:

$$q_{eq} = \frac{D_{tot} - 10C_{eq}}{50} \ (\mu g/\mu L \text{ or } mg/mL) \quad (1)$$

where D_{tot} is the total amount of dye used in the experiment. Using the measured value of dry weight (see Section 2.2) the value q_{eq} was expressed in mg of adsorbed dye per 1 g of adsorbent.

Equilibrium data processing

Equilibrium adsorption data were fit to Langmuir, Freundlich and Langmuir–Freundlich adsorption isotherms using SigmaPlot software.

Results and discussion

A simple procedure for the preparation of yeast-based magnetic biocomposite material by modification of dried *K. fragilis* cells with perchloric acid stabilized magnetic fluid was developed. Repeated washing of cells with 0.1 M acetic acid was necessary to remove majority of extractable compounds, which were responsible for non-specific precipitation of magnetic fluid.

After thorough washing, however, a specific precipitation of magnetic nanoparticles on the outer surface of *Kluyveromyces* cells occurred. The adsorption of magnetic iron oxide nanoparticles onto the *Kluyveromyces* cells was fast; majority of nanoparticles was adsorbed within several minutes. The magnetically modified *Kluyveromyces* cells could be easily separated using commercially available magnetic separators or strong permanent magnets.

A scanning electron microscopy picture of the original fodder yeast is presented in Fig. 1. The individual *K. fragilis* cells obtained after cultivation form spherical particles (with a diameter of 5–50 μm) during the drying process. These particles are quite stable; they can withstand both the acetic acid treatment and subsequent magnetic modification. In the same figure a detailed structure of the particles formed by the closely packed dried yeast cells, before and after magnetic modification, is shown. It can be clearly seen that the magnetic modification significantly altered the yeast cells morphology.

The presence of both individual magnetic nanoparticles and agglomerates of particles on the yeast cell surface is documented by a transmission electron microscopy picture (see Fig. 2). The magnetic modification of spherical particles led to the preferential accumulation of magnetic nanoparticles on the exposed parts of the individual yeast cells forming the particles.

In order to test if the obtained material could be used as the magnetic adsorbent in the magnetic separation procedures, we performed magnetization measurements. The ZFC–FC magnetization curves show a typical superparamagnetic behavior (see Fig. 3). They are coincided with each other above the blocking temperature, $T_B \sim 180$ K, and separated below this temperature. The ZFC curve shows a maximum associated to the transition between superparamagnetic and blocked state. Moreover, this maximum is very broad indicating the existence of dipole–dipole interactions between the iron oxides particles and thus a wide distribution in particle size ranging from ultrafine isolated particles up to particle aggregates. Below $T_B = 180$ K, the FC curve decreases very slowly with decreasing temperature. This also confirms the presence of non-negligible dipole–dipole interactions between the particles.

The field dependent hysteresis loops were measured at selected temperatures both below and above the blocking temperature. Fig. 4 shows the exemplary hysteresis curves at 4.2 and 300 K. The magnetization at 4.2 K displays some hysteresis and confirms that the iron oxides nanoparticles are ferrimagnetic below the blocking temperature. The room temperature magnetization curve shows the superparamagnetic behavior indicated by the absence of hysteresis. At $T = 4.2$ K, the remanence-to-saturation ratio, $R = M_R/M_S = 0.37$ is smaller than the expected $R = 0.5$ value for non-interacting, randomly oriented particles with uniaxial symmetry [15]. It is an additional confirmation for the existence of inter-particle dipole–dipole interactions.

The obtained results are very promising from the point of view of using the magnetically modified fodder yeast cells as the magnetic affinity adsorbent in the magnetic separation techniques. Their magnetic behavior is dominated by the superparamagnetic relaxation of isolated single domain iron oxides nanoparticles, although a certain amount of agglomerates of particles coupled by magnetic dipolar interactions is also present. However, these agglomerates are sufficiently small to show at static conditions the superparamagnetic behavior at room temperature.

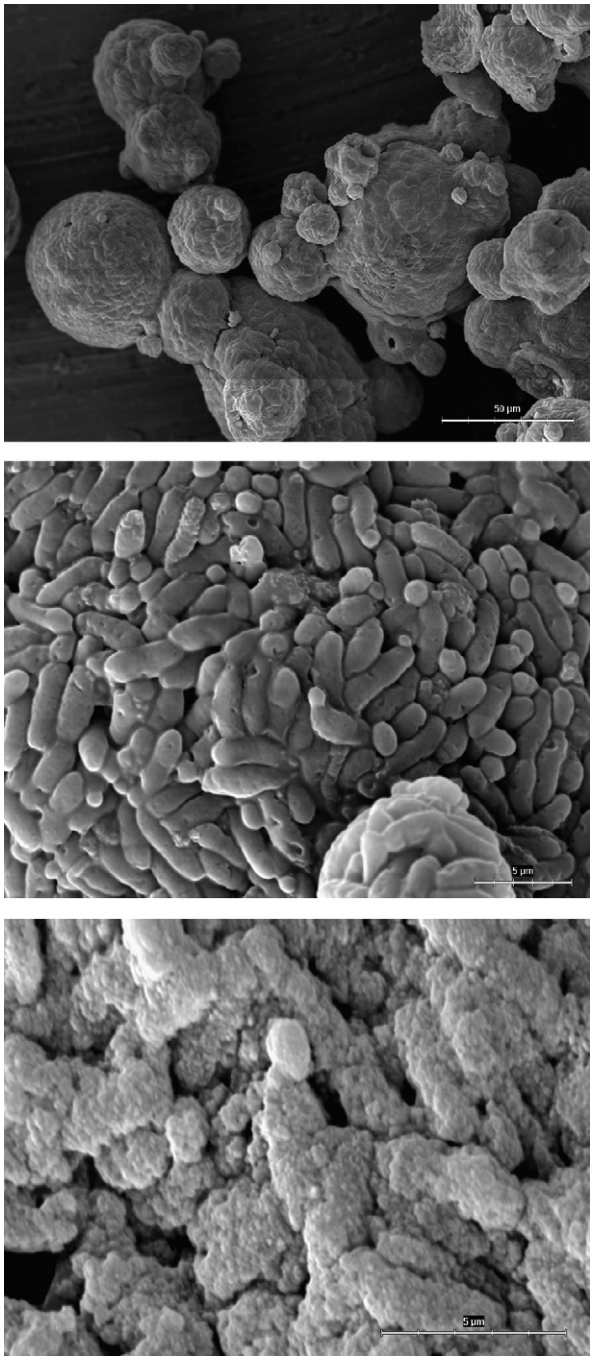

Fig. 1. Scanning electron microscopy pictures of the original fodder yeast (top), detailed structure of particles formed by the closely packed dried yeast cells (middle) and magnetically modified material (bottom). The bar lines correspond to 50 and 5 μm, respectively.

Magnetically modified yeast cells were used as an adsorbent to study the binding of seven water-soluble dyes belonging to different dye classes. These dyes were selected according to their adsorption capacities during the preliminary experiments with more than 20 dyes. The tested group of dyes comprehend crys-

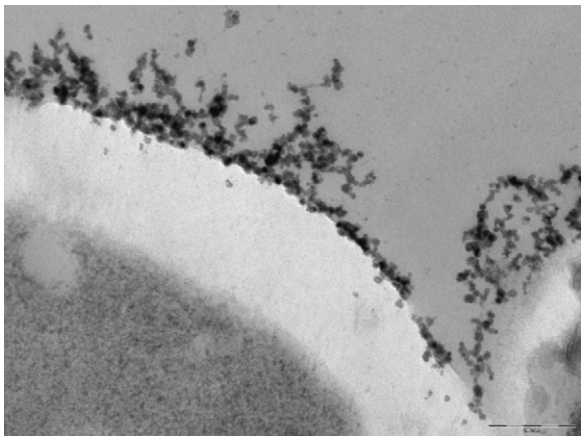

Fig. 2. Transmission electron microscopy picture of magnetically modified yeast cells. The bar line corresponds to 200 nm.

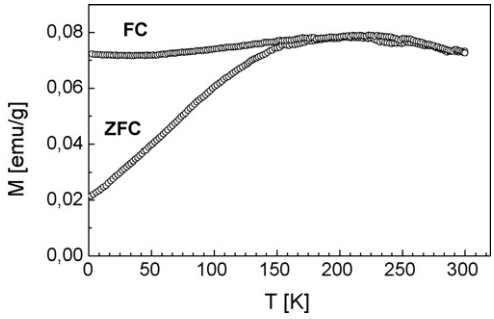

Fig. 3. Zero-field cooled (ZFC) and field cooled (FC) magnetization as a function of temperature in an applied magnetic field of 50 Oe.

tal violet (triphenylmethane group), amido black 10B, congo red, Saturn blue and Bismarck brown (azodyes group), acridine orange (acridine group) and safranin O (safranin group). Pure dyes were used during the experiments in order to study the correlation between the dyes structures and adsorption characteristics. Preliminary experiments also showed that adsorption properties of yeast cells were not significantly influenced by magnetic modification.

The dyes were used for adsorption experiments. They were dissolved in distilled water without buffering the solution. The adsorption of the tested dyes reached equilibrium in approximately 60–90 min. Incubation time of 3 h was used for adsorption experiments. The initial dyes concentration provides an important driving force to overcome all mass transfer resistances of the dye between the aqueous and solid phases. Hence, a higher initial concentration of dye will enhance the adsorption process. The equilibrium adsorption isotherms for the tested dyes are shown in Fig. 5.

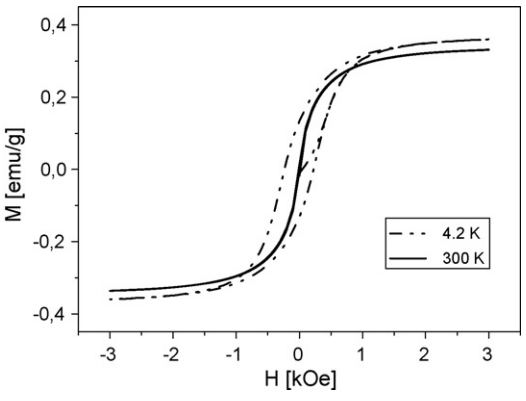

Fig. 4. Field dependent hysteresis loops measured at 4.2 and 300 K.

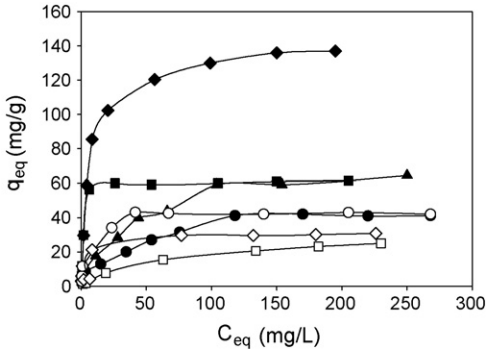

Fig. 5. Equilibrium adsorption isotherms of the tested dyes using magnetically modified fodder yeast cells as adsorbent. C_{eq}: equilibrium liquid-phase concentration of the unadsorbed (free) dye (mg/L); q_{eq}: equilibrium solid-phase concentration of the adsorbed dye (dye uptake) (mg/g). (■) Acridine orange; (□) amido black 10B; (▲) Bismarck brown; (●) congo red; (◯) crystal violet; (♦) safranin O; (◊) Saturn blue LBRR.

In order to study the adsorption process, Langmuir and Freundlich isotherm equations are usually used for experimental data analysis. Several other models have been described, among them the Langmuir–Freundlich isotherm.

The Langmuir model is valid for monolayer adsorption onto a surface with a finite number of identical sites. The well known expression for the Langmuir model is given by

$$q_{eq} = \frac{Q_{max} b C_{eq}}{1 + bC_{eq}} \tag{2}$$

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) [16,17].

The empirical Freundlich equation based on the sorption onto a heterogeneous surface is given by

$$q_{eq} = K_F C_{eq}^{n} \tag{3}$$

where K_F and n are the Freundlich constants characteristic of the system. K_F and n are indicators of adsorption capacity and adsorption intensity, respectively [16,17].

The Langmuir–Freundlich isotherm (Eq. (4)) describes the adsorption process using three parameters. At low concentrations, the model reduces to the Freundlich model and, in the case of a homogeneous surface, it reduces to the Langmuir model:

$$q_{eq} = \frac{Q_{max} (bC_{eq})^{n}}{1 + (bC_{eq})^{n}} \tag{4}$$

Table 1
Isotherms used for the description of dyes adsorption on magnetic *Kluyveromyces fragilis* cells and calculated adsorption (K_F, Q_{max}, b, n) and correlation (R^2) coefficients

Dyes	Isotherms		
	Freundlich, $q_{eq} = K_F C_{eq}^n$	Langmuir, $q_{eq} = (Q_{max} b C_{eq})/(1 + b C_{eq})$	Langmuir–Freundlich, $q_{eq} = (Q_{max}(bC_{eq})^n)/(1 + (bC_{eq})^n)$
Acridine orange	$K_F = 21.38$, $n = 0.252$, $R^2 = 0.838$	$Q_{max} = 62.22$, $b = 0.443$, $R^2 = 0.986$	$Q_{max} = 59.07$, $b = 0.535$, $n = 1.446$, $R^2 = 0.997$
Amido black 10B	$K_F = 1.69$, $n = 0.509$, $R^2 = 0.988$	$Q_{max} = 29.94$, $b = 0.017$, $R^2 = 0.998$	$Q_{max} = 34.13$, $b = 0.013$, $n = 0.872$, $R^2 = 0.999$
Bismarck brown	$K_F = 8.10$, $n = 0.394$, $R^2 = 0.948$	$Q_{max} = 75.71$, $b = 0.025$, $R^2 = 0.989$	$Q_{max} = 79.40$, $b = 0.022$, $n = 0.916$, $R^2 = 0.990$
Congo red	$K_F = 6.29$, $n = 0.355$, $R^2 = 0.931$	$Q_{max} = 49.71$, $b = 0.024$, $R^2 = 0.945$	$Q_{max} = 62.42$, $b = 0.013$, $n = 0.690$, $R^2 = 0.954$
Crystal violet	$K_F = 13.69$, $n = 0.235$, $R^2 = 0.840$	$Q_{max} = 42.88$, $b = 0.293$, $R^2 = 0.988$	$Q_{max} = 44.05$, $b = 0.250$, $n = 0.908$, $R^2 = 0.989$
Safranin O	$K_F = 32.31$, $n = 0.308$, $R^2 = 0.905$	$Q_{max} = 138.20$, $b = 0.159$, $R^2 = 0.995$	$Q_{max} = 135.21$, $b = 0.170$, $n = 1.087$, $R^2 = 0.996$
Saturn blue LBRR	$K_F = 6.71$, $n = 0.301$, $R^2 = 0.773$	$Q_{max} = 33.04$, $b = 0.084$, $R^2 = 0.856$	$Q_{max} = 30.03$, $b = 0.127$, $n = 11.280$, $R^2 = 0.980$

Q_{max} represents the maximum adsorption capacity calculated per mass of the adsorbent (expressed in mg/g). All the constants were determined by means of the non-linear regression calculation using SigmaPlot software

Table 2
Comparison of maximum adsorption capacities (expressed in mg/g) of studied yeast cells with these found in the literature

Dyes	C.I. number	Magnetic *K. fragilis* [this paper]	Magnetic *Saccharomyces cerevisiae* subsp. uvarum [12]	*Candida utilis* [20]	*Saccharomyces cerevisiae* [19]	Immobilized *Saccharomyces cerevisiae* [22]
Acridine orange	46005	62.2				
Amido black 10B	20470	29.9	11.6			
Aniline blue	42755		228.0			
Bismarck brown	21000	75.7				
Congo red	22120	49.7	93.1			
Crystal violet	42555	42.9	41.7			
Malachite green	42000					17.0
Remazol black B	[a]				88.5	
Remazol blue	[a]			114	84.6	
Remazol red RB	[a]				48.8	
Safranin O	50240	138.2	46.6			
Saturn blue LBRR	34140	33.0				

[a] Information not available in the cited paper.

All the constants (Q_{max}, b, n, K_F) were determined by means of non-linear regression calculation using SigmaPlot software. The results are presented in Table 1.

As can be seen, the adsorption can be usually described by the Langmuir isotherm. Such a description allows a simple calculation of the maximum adsorption capacity, which is a very important parameter describing the adsorption process. In the case of seven tested dyes, the highest Q_{max} was found for safranin O (138.2 mg/g), while the lowest Q_{max} value was obtained for amido black 10B (29.9 mg/g). The values of constant b are related to the strength of the bond between the dye and yeast biomass. Highest values of b suggest strongest bonding of acridine orange, as well as crystal violet and safranin O.

In most cases the Langmuir–Freundlich isotherm gave similar results. However, there was a substantial difference in the value of the maximum adsorption capacity in the case of congo red. On the other side, in some cases the non-linear calculation of Langmuir–Freundlich coefficient led to the values without any physical meaning (e.g., the values of the coefficient n higher than 1 in the case of Saturn blue, acridine orange and safranin O). The Freundlich adsorption isotherm is generally not suitable for the study of dyes adsorption on magnetically modified yeast due to the lowest regression coefficients. Also the linear transformation of the experimental data did not lead to the isotherms linearization.

It is difficult to find a straightforward correlation between the dyes structures and maximum adsorption capacities. The dyes belonging to azodyes group exhibit both relatively high adsorption capacity (e.g., Bismarck brown: 75.71 mg/g) and the lowest adsorption capacity from all the dyes tested (e.g., Saturn blue (33.04 mg/mL) and amido black 10B (29.94 mg/g)). The presence of negatively charged sulfo groups in the dyes molecules usually results in low adsorption capacities; this fact corresponds with the negative charge of the yeast cells under physiological pH.

The results obtained for the magnetically modified *K. fragilis* cells were compared with literature data (see Table 2). It can be seen that the described magnetic biosorbent exhibits similar properties as other yeast-based adsorbents when maximum adsorption capacity for the studied dyes is compared. Magnetic modification enabling the simple magnetic separation makes the described biosorbent superior to other materials.

Conclusions

Microbial biomass is a promising adsorbent for the removal of different types of xenobiotics including dyes. In particular,

several types of yeast have been studied for dyes biosorption and biodegradation [18–20]. Magnetic modification of these adsorbents allows using them for the removal of dyes by means of the magnetic separation techniques. These materials should be superparamagnetic so that they 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.

Fodder yeast (*K. fragilis*) cells represent an inexpensive, readily available source of biomass that has a significant potential for dyes bioaccumulation. To improve the manipulation with this biosorbent, the simple magnetic modification was performed. The modification of fodder yeast cells with the perchloric acid stabilized ferrofluid led to the formation of magnetically responsive biocomposite material, which could be used as an efficient adsorbent for the removal of various water-soluble dyes. Magnetic nanoparticles precipitated on the cells surface both in the form of individual particles and agglomerates of particles coupled by magnetic dipolar interactions. However, these agglomerates are sufficiently small to show at static conditions the superparamagnetic behavior at room temperature.

The maximum adsorption capacity of magnetically labeled yeast cells is relatively high, at least for specific dyes (e.g., safranin O), and this value is comparable with those obtained with other types of yeast cells (in non-magnetic state) [20]. The comparison with other inexpensive non-magnetic adsorbents clearly documents the efficiency of this new, magnetically responsive material [21].

Magnetically modified yeast cells can thus be a promising magnetic affinity adsorbent which may be used to the removal of dyes. In addition, other types of xenobiotics (e.g., heavy metal ions), as well as selected biologically active compounds can specifically interact with this new composite material. The measurements are still continuing and more detailed results will appear in a forthcoming paper.

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