DYE ADSORPTION ON MAGNETICALLY
MODIFIED CHLORELLA VULGARIS CELLS

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ABSTRACT

Dried *Chlorella vulgaris* cells were magnetically modified by contact with water-based magnetic fluid stabilized with perchloric acid in order to prepare a new type of magnetically responsive biocomposite material. This procedure enabled a simple separation of modified cells by means of strong permanent magnets. The prepared material was used as a new inexpensive magnetic adsorbent for the removal of water-soluble dyes.

Magnetically modified cells were characterized by means of magnetic and microscopic methods. Both isolated magnetic nanoparticles and aggregates of particles were present on the cell surface. The prepared material displayed a super-paramagnetic behavior at room temperature, with a transition to a blocked state at *T_B* \(\sim 200\) K for the applied magnetic field \(H = 50\) Oe.

Six dyes (aniline blue, Bismarck brown, congo red, crystal violet, safranin O and Saturn blue LBRR) were used to study the adsorption process. The dyes' adsorption reached equilibrium in approximately 30–120 min. Langmuir isotherms were successfully used to fit the experimental data. The maximum adsorption capacities ranged between 24.2 (Saturn blue LBRR) and 257.9 (aniline blue) mg of dye per g of dried magnetically modified cells. Change of pH can significantly increase the adsorption of some dyes. Ferrofluid-modified *Chlorella vulgaris* cells represent an interesting material for further study and potential applications.

INTRODUCTION

Different types of dyes are used in many industrial processes to color the products. A considerable amount of colored wastewaters is produced which enters the aquatic environment. Many dyes are not easily biodegradable and, in addition, many of them are also toxic for the aquatic environment. The removal of dyes from colored effluents, particularly in textile industries, is currently one of the most interesting problems [1, 2].

Many procedures have been described for the removal of dyes from dye-containing industrial effluents [1]. The conventional methods for treating dye-containing wastewaters include flocculation, coagulation, oxidation, membrane filtration, and adsorption. Such methods are often very expensive and cannot be used on a large scale.

Recently, numerous approaches have been studied for the development of cheaper and more effective dye adsorbents. Many non-conventional low-cost adsorbents, including natural materials, clay materials, zeolites, siliceous material, biosorbents and waste materials from industry and agriculture, have been proposed by several workers [2]. Also, several types of algae or seaweed, such as *Pithophora* sp. [3, 4], *Azolla filiculoides* [5], *Chlorella vulgaris* [6], *Caulerpa lentillifera* [7], *Spirogyra* sp. [8–11] or *Enteromorpha prolifera* [12, 13] have been tested as possible biosorbents for the removal of various dyes; some of these materials exhibited high adsorption capacity for specific compounds.

It has been shown recently that magnetic adsorbents represent an extremely interesting group of materials, which can be manipulated by an external magnetic field. Magnetic techniques enable relatively simple separation of magnetic materials from difficult-to-handle samples, including wastewater [14, 15]. There is only limited information on the combination of inexpensive biosorbents with magnetic procedures for dye removal [16]. In this

KEYWORDS:
*Chlorella vulgaris*, magnetic fluid, magnetic iron oxide nanoparticles, magnetically modified cells, magnetic separation, dyes.
paper, we describe the magnetic modification of *Chlorella vulgaris* cells, detailed magnetic characterization of the prepared biocomposite material, and subsequent study of this material for dye adsorption.

**MATERIALS AND METHODS**

**Materials**

Dried *Chlorella vulgaris* cells were obtained from Dr. J. Kopecky, Department of Autotrophic Microorganisms, Institute of Microbiology, Academy of Sciences, Trebon, Czech Republic. Commercially available congo red (C.I. 22120), Bismarck brown Y (C.I. 21000), safranin O (C.I. 50240), crystal violet (C.I. 42555), Saturn blue LBRR 200 (C.I. 34140), and aniline blue, water soluble (C.I. 42755), were used as model dyes. Water-based ionic magnetic fluid, stabilized with perchloric acid, was prepared using the standard Massart procedure [17]. 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) was given as the iron(II,III) oxide content determined by a colorimetric method [18].

**Preparation of ferrofluid-modified *Chlorella vulgaris* cells**

The dried *Chlorella vulgaris* cells were washed six to eight times with excess 0.1 M acetic acid. 1 ml of ferrofluid was added to 3 ml of the suspension of washed cells in acetic acid, then mixed and incubated 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 adsorbent was stored in a water suspension at 4 °C. The dry weight of 1 ml of sedimented, magnetically modified *Chlorella vulgaris* cells was 132.9 mg.

**Magnetic characterization of ferrofluid-modified *Chlorella vulgaris* cells**

Static magnetic measurements were performed over a wide temperature range of 2–300 K using an extraction magnetometer MagLab 2000 System (Oxford Instruments Ltd.). Thermal dependencies of magnetization in the zero field cooled – field cooled (ZFC-FC) regime were recorded in an applied magnetic field of 50 Oe. Magnetic hysteresis loop measurements were performed at selected temperatures in an applied magnetic field ± 3 kOe.

**Microscopic characterization of ferrofluid-modified *Chlorella vulgaris* cells**

*Chlorella vulgaris* cells were fixed in a mixture of 2% glutaraldehyde and 2.5% formaldehyde (both EM grade) in 0.1 M phosphate buffer (pH 7.2) at room temperature. After washing in 0.1 M phosphate buffer, they were dehydrated in a series of ethanol solutions (50%, 75% and 96%), embedded into Spurr resin, and polymerized for 26 h at 600 °C. Ultrathin sections exhibiting silver and gold interference colours were made on Reichert ultramicrotome, stained with uranyl acetate and lead citrate, and observed using a transmission electron microscope (Jeol 1010).

**Adsorption of dyes on ferrofluid-modified *Chlorella vulgaris* cells**

The suspension of magnetically modified *Chlorella vulgaris* cells (0.2 ml; the settled volume of the adsorbent was 0.05 ml) was mixed with 4.8 ml of water in a 15-ml test tube. Then, a 0.01-3.0 ml portion of stock solution of a tested dye (1-2 mg/ml) was added, and the total volume of the suspension was made up to 10 ml with water. The suspension was mixed for 3 h at room temperature. Then, the magnetic *Chlorella vulgaris* cells were separated from the suspension using a magnetic separator (Dynam MPC-1 or MPC-6, Invitrogen, USA), and the clear supernatant was used for spectrophotometric measurement. The concentration of free (unbound) dye in the supernatant (Ceq) was determined from a calibration curve. The amount of dye bound to the unit volume of the adsorbent (qe) was calculated using the following formula:

\[
q_e = (D_{tot} - 10 \cdot C_{eq}) / 50 \text{ (g/L or mg/mL)}
\]

where \( D_{tot} \) is the total amount of dye used in the experiment. Using the measured value of dry weight, the value \( q_e \) was expressed in mg of adsorbed dye per 1 g of adsorbent.

To study the pH dependence of dyes adsorption, 0.1 ml of the dye solution (concentration 1 mg/ml) was mixed with 9.7 ml of buffer solution and 0.2 ml of magnetic *Chlorella vulgaris* cell suspension. The following buffers were used: glycine-HCl (pH = 2.2), acetate (pH = 4.16), phosphate (pH = 7.4) and borate (pH = 9.5).

**Equilibrium data processing**

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

**RESULTS AND DISCUSSION**

Magnetically responsive *Chlorella vulgaris* cells were prepared by the simple treatment of a cell suspension with perchloric acid-stabilized magnetic fluid. Repeated extraction of dried cells with 0.1 M acetic acid before the ferrofluid treatment was necessary to remove the majority of extractable compounds, which were otherwise responsible for non-specific precipitation of magnetic fluid. After thorough washing, however, a specific precipitation of magnetic nanoparticles on the outer surface of *Chlorella vulgaris* cells occurred. The adsorption of magnetic iron oxide nanoparticles onto the *Chlorella vulgaris* cells was fast, with the majority of nanoparticles being adsorbed within several min. The magnetically modified *Chlorella vulgaris* cells could be easily separated using commercially available magnetic separators or strong permanent magnets. This biocomposite material was stable, even after 1-year storage of the suspension at 4 °C.
A transmission electron micrograph of the original preparation of dried *Chlorella vulgaris* cells is presented in Fig. 1. The drying process caused damage to the cell walls, often followed by the release of the intracellular components. In some cases, cell aggregates were observed. Magnetic modification mainly influenced the whole cells and cell aggregates, and not the cell fragments, as observed in optical micrographs of the prepared magnetic composite material.

The presence of both individual magnetic nanoparticles and agglomerates of particles on individual *Chlorella vulgaris* cell surfaces is depicted in Fig. 2. 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 same situation was observed for cell aggregates.

Magnetization measurements were performed in order to characterize this material. Fig. 3 shows temperature dependencies of magnetization obtained in the zero-field cooled – field cooled (ZFC–FC) regime at an applied magnetic field $H = 50$ Oe. It is seen that the ZFC–FC curves are separated at $T < 200$ K, and coincided with each other above this temperature, indicating the existence of irreversible processes. The observed behavior is reminiscent of a blocking process of small single domain particles, which turn into a super-paramagnetic state with increasing temperature. The maximum of the ZFC curve is very broad and clear. Curie-Weiss law behavior is not observed above the blocking temperature $T_B = 200$ K. This indicates the existence of magnetic dipole-dipole interactions between the iron oxide particles, and thus a wide distribution in particle size ranging from ultratine isolated particles up to particle aggregates. The magnetization in the FC curve is almost independent of temperature, which also reveals the presence of non-negligible dipole-dipole interactions between the particles. At $T \sim 260$ K, a kink is seen in both ZFC and FC curves which is associated with the melting point of the solution.

FIGURE 1 - Transmission electron microscope picture of original dried *Chlorella vulgaris* cells (bar line corresponds to 1 μm).

FIGURE 2 - Transmission electron microscope pictures of magnetically modified *Chlorella vulgaris* cells (bar lines correspond to 2 μm and 200 nm).

FIGURE 3 - Temperature dependencies of magnetization at the applied magnetic field of 50 Oe.

The field dependent hysteresis loops were measured at selected temperatures both below and above the blocking temperature, and the exemplary curves at 4.2 and 300 K are shown in Fig. 4. The magnetization at 4.2 K displays hysteresis and confirms that the iron oxides particles 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 [19]. It is an additional confirmation for the existence of inter-particle magnetic dipolar interactions.

The results are very promising from the point of view of using the ferrofluid-modified *Chlorella vulgaris* 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 particles, although a certain amount of aggregates of particles coupled by magnetic dipole-dipole interactions is also present. However, these aggregates are sufficiently small to show, at static conditions, the superparamagnetic behavior at room temperature.

Magnetically modified *Chlorella vulgaris* cells were used as an adsorbent to study the binding of six water-soluble dyes belonging to different dye classes. These dyes exhibited the highest adsorption during preliminary experiments with more than 20 dyes. The tested dyes comprised crystal violet and water-soluble aniline blue (triphenylmethane group), congo red, Saturn blue LBRR, Bismarck brown Y (azodyes group) and safranin O (safranin group). Commercially available dyes were used during the experiments. These were dissolved in distilled water without buffering the solution. Preliminary experiments also showed that adsorption properties of *Chlorella vulgaris* 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.

The equilibrium adsorption isotherms for the un-buffered aqueous solutions of the tested dyes are shown in Fig. 5. These isotherms represent distribution of dyes between the aqueous and solid phases as the dye concentration increases. Langmuir and Freundlich isotherm equations are usually used for experimental data analysis in order to study the adsorption of target analytes from water solutions.

![Equilibrium adsorption isotherms of the tested dyes using magnetically modified *Chlorella vulgaris* cells as adsorbent (Ceq: equilibrium liquid-phase concentration of the unadsorbed (free) dye (mg/L); qeq: equilibrium solid-phase concentration of the adsorbed dye (dye uptake) (mg/g); (a) Saturn Blue LBRR; (b) Bismarck brown; (c) safranin O; (d) congo red; (e) crystal violet; (f) aniline blue).](image)

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

$$q_{eq} = \frac{Q_{max} \cdot b \cdot C_{eq}}{1 + b \cdot C_{eq}}$$

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

$$q_{eq} = K_F \cdot C_{eq}^n$$

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.

Non-linear regression calculation is currently the preferred way to calculate the constants ($Q_{max}$, $b$, and $K_F$). The results are presented in Table 1. As can be seen, the highest $Q_{max}$ was found for aniline blue (258 mg/g), while the lowest $Q_{max}$ value was obtained for Saturn blue (24 mg/g).
The values of regression coefficients indicate that Langmuir isotherm can be used for the description of adsorption of all the tested dyes. In some cases, however, Freundlich isotherm can be used, as well (e.g., for congo red and Saturn Blue LBRR).

TABLE 1 - Isotherms used for the description of dyes adsorption on magnetic Chlorella vulgaris cells and calculated adsorption (K_F, Q_max, b, n) and correlation (R^2) coefficients. Q_max represents the maximum adsorption capacity calculated per dry mass of the adsorbent (mg/g). All the constants were determined by means of non-linear regression calculation using SigmaPlot software.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Freundlich isotherm</th>
<th>Langmuir isotherm</th>
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<tbody>
<tr>
<td></td>
<td>q_eq = K_F C_eq^n</td>
<td>q_eq = Q_max b C_eq / (1 + b C_eq)</td>
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<tr>
<td>Aniline blue</td>
<td>K_F = 9.65 n = 0.575</td>
<td>Q_max = 257.89</td>
</tr>
<tr>
<td></td>
<td>R^2 = 0.924</td>
<td>b = 0.0139</td>
</tr>
<tr>
<td>Bismarck brown</td>
<td>K_F = 10.41 n = 0.526</td>
<td>Q_max = 201.93</td>
</tr>
<tr>
<td></td>
<td>R^2 = 0.950</td>
<td>b = 0.0171</td>
</tr>
<tr>
<td>Congo red</td>
<td>K_F = 18.38 n = 0.390</td>
<td>Q_max = 156.73</td>
</tr>
<tr>
<td></td>
<td>R^2 = 0.990</td>
<td>b = 0.0298</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>K_F = 7.67 n = 0.342</td>
<td>Q_max = 42.91</td>
</tr>
<tr>
<td></td>
<td>R^2 = 0.968</td>
<td>b = 0.0706</td>
</tr>
<tr>
<td>Safranin O</td>
<td>K_F = 25.48 n = 0.289</td>
<td>Q_max = 115.75</td>
</tr>
<tr>
<td></td>
<td>R^2 = 0.834</td>
<td>b = 0.0909</td>
</tr>
<tr>
<td>Saturn Blue LBRR</td>
<td>K_F = 7.41 n = 0.231</td>
<td>Q_max = 24.25</td>
</tr>
<tr>
<td></td>
<td>R^2 = 0.908</td>
<td>b = 0.1255</td>
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</table>

The value of pH can influence the adsorption of some dyes. Fig. 6 shows the dependence of the adsorption of crystal violet and safranin O on the pH value of the dye solution. The increasing adsorption capacity with increasing pH can be explained by the fact that in the alkaline solution the Chlorella vulgaris cell surface became more negatively charged, due to the presence of negatively charged groups, while crystal violet and safranin O keep the positive charge, leading to an increase of electrostatic interactions. This phenomenon can be confirmed by the described dependence of the Chlorella vulgaris cells zeta potential on the pH value, since the cells become more negative at higher pH [20].

FIGURE 6 - Dependence of crystal violet (○) and safranin O (▲) adsorption on the pH value of the dye solution.

The maximum adsorption capacity values Q_max obtained for magnetically modified Chlorella vulgaris cells were compared with literature data for other similar adsorbents (in non-magnetic form; see Table 2). It can be seen that the described magnetic biosorbent exhibits similar adsorption properties as algae and seaweed-based adsorbents, when these values for the studied dyes are compared. Magnetic modification enabling simple magnetic separation makes the described biosorbent superior to other materials.

TABLE 2 - Comparison of maximum adsorption capacities (expressed in mg/g) of studied Chlorella vulgaris cells with these of other algae found in the literature.

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<tr>
<td>Acid Blue 15</td>
<td>257.9</td>
<td>1356.6</td>
<td>116.3</td>
<td>76.3</td>
<td></td>
<td>244.0</td>
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<tr>
<td>Acid Blue 290</td>
<td>201.9</td>
<td>367</td>
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<tr>
<td>Acid Blue 324</td>
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<tr>
<td>Acid 274</td>
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<td>137.0</td>
<td>83.3</td>
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<tr>
<td>Acid 88</td>
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<td>109.6</td>
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<td>Acid Green 3</td>
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<tr>
<td>Acid Orange 7</td>
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<td>Aniline blue</td>
<td>257.9</td>
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<tr>
<td>Bismarck brown</td>
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<tr>
<td>Congo red</td>
<td>156.7</td>
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</tr>
<tr>
<td>Crystal violet</td>
<td>42.9</td>
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<tr>
<td>Malachite green</td>
<td>117.6</td>
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<tr>
<td>Safranin O</td>
<td>115.7</td>
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<tr>
<td>Saturn Blue LBRR</td>
<td>24.2</td>
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</table>
CONCLUSION

Treatment of Chlorella vulgaris cells with magnetic fluid led to the formation of magnetically responsive material with affinity for water-soluble dyes. Comparison with similar materials of biological origin demonstrates the relatively high adsorption capacity of the studied biosorbent. Due to the presence of magnetic nanoparticles, this biosorbent belongs to the group of “smart” materials, exhibiting response to external stimuli (in this case to magnetic field). This is an important characteristic, because this biosorbent can be used for work with biological samples, where the contaminating accompanying molecules and particulate matter exhibit diamagnetic properties.

Summarizing, magnetically modified algal cells represent an interesting magnetic biosorbent, which may be subsequently used for the removal of both organic and inorganic xenobiotics. The measurements are in progress and more detailed results will appear in forthcoming papers.

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