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Magnetically modified sheaths of *Leptothrix* sp. as an adsorbent for Amido black 10B removal

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ABSTRACT

The goal of this study was to assess the biosorption of Amido black 10B dye from aqueous solutions on magnetically modified sheaths of *Leptothrix* sp. in a batch system. The magnetic modification of the sheaths was performed using both microwave synthesized iron oxide nano- and microparticles and perchloric acid stabilized ferrofluid. The native and both magnetically modified sheaths were characterized by SEM. Various parameters significantly affecting the adsorption process, such as pH, contact time, temperature and initial concentration, were studied in detail using the adsorbent magnetized by both methods.

The highest adsorption efficiency was achieved at pH 2. The maximum adsorption capacities of both types of magnetized material at room temperature were found to be 339.2 and 286.1 mg of dye per 1 g of ferrofluid modified and microwave synthesized particles modified adsorbent, respectively. Thermodynamic study of dye adsorption revealed a spontaneous and endothermic process in the temperature range between 279.15 and 313.15 K. The data were fitted to various equilibrium and kinetic models. Experimental data matched well with the pseudo-second-order kinetics and Freundlich isotherm model.

The *Leptothrix* sheaths have excellent efficacy for dye adsorption. This material can be used as an effective, low-cost adsorbent.

1. Introduction

Various chemical industries including textile, plastic, paint, paper, leather and rubber ones generate large volumes of toxic dye wastes. Many dyes are difficult to decolorize due to their complex structure and synthetic origin [1]. Even a very low dye concentration is highly visible and can significantly affect aquatic life as well as food web.

Application of biosorption to remove toxic pollutants from wastewaters is one of the progressive environmental technologies. Adsorption is a very simple method compared to other procedures, and has often been used in the treatment of wastewater containing colored impurities and heavy metal ions [2]. Dye removal by adsorption is often based on the use of natural adsorbents so that the process becomes economically feasible. In addition, natural biosorbents are renewable, available in large amounts and less expensive compared to other materials that are used as adsorbents, such as activated carbon [3], zeolite [4], carbon nanotubes [5], noble metals like Pb and Ag loaded on activated carbon [6] or ZnO nanoparticles on activated carbon [7]. The use of microorganisms as biosorbents for removal of synthetic dyes from textile wastewater is promising due to their good performance and low cost [8]. However, no studies have been reported on the removal of Amido black 10B dye using *Leptothrix* sheaths as adsorbent. The synthetic dye chosen (Amido black 10B, also known as Naphthol blue black or Acid black 1) is usually used for staining of proteins in biochemical research, nevertheless, it can be applied to all kind of both natural (cotton, wool, silk) or synthetic (acrylic, polyester, and rayon) fibers or can also be employed in paints, inks, plastics and leather industries [9]. This dye can cause damages of the human respiratory system and skin and eye irritations. Hence, it is considered worthwhile to develop a low-cost adsorbent for the effective removal of

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Amido Black 10B dye from its aqueous solutions [4].

The sheathed bacteria from the genus *Leptothrix* belong to the group of neutrophilic iron bacteria. They can be found in different aquatic habitats as lakes, streams, springs, swamps, iron seeps, and springs rich in iron and manganese, as well as in wastewater treatment systems [10]. Their metal-oxidizing ability is important in biogeochemical cycles of these metals.

Leptothrix sp. is able to accumulate Fe and Mn ions into their sheaths. This ability is employed for removal of iron and manganese from groundwater to clean water for drinking purposes. This technology is used in many water-treatment plants all over the world. Filamentous-looking sheaths of *Leptothrix* sp. as well as the ferric oxides formed are of great interest for different nanotechnology and bioengineering applications as pigments, adsorbents, carriers and others [11–15].

The purpose of this work was to investigate the adsorption potential of magnetically responsive *Leptothrix* sp. sheaths (MLS) prepared by modification with both microwave synthesized iron oxide nano- and microparticles, and perchloric acid stabilized magnetic fluid. Adsorption of a model dye, namely Amido black 10B was performed in a batch system where MLS with adsorbed dye were easily removed using an appropriate magnetic separator.

2. Materials and methods

2.1. Materials

Natural ocherous sediment containing *Leptothrix* sp. sheaths was collected using glass vessels from an unnamed stream in Ceske Budejovice, Czech Republic (48°58′22.57′′N, 14°27′39.93′′E) (Fig. 1) at the end of February 2016. Sample was concentrated during collection by letting the biomaterial settle down and decanting overlying water. Subsequently, the sediment was promptly transported to the laboratory, where it was repeatedly washed with water. Ferrous sulfate heptahydrate, sodium hydroxide and other common chemicals were obtained from Lach-Ner, Czech Republic. Magnetic fluid stabilized with perchloric acid was prepared using a standard procedure [16]; the relative magnetic fluid concentration (39.5 mg/mL) is given as the maghemite content determined by a colorimetric method [17].

2.2. Modification of Leptothrix sheaths with microwave synthesized magnetic iron oxide nano- and microparticles

The magnetic iron oxide nano- and microparticles were synthesized using a microwave (MW) assisted procedure described previously [18]. 400 mL of completely sedimented (earth gravity, 24 h) *Leptothrix* sheaths (either native or sterilized at 121 °C for 20 min, pH around 7.0) was mixed with 120 mL of iron oxide suspension in water (1 part of completely sedimented (earth gravity, 24 h) microwave synthesized iron oxide nano- and microparticles and 4 parts of water, pH 7.0). The mixture was incubated on a sample mixer (Dynal Biotech Inc., NY, USA; 27 rpm) for 3 h at room temperature, and then, magnetically modified sheaths were repeatedly washed with water (using a magnetic separator) until nonmagnetic material was washed out. Next, magnetized *Leptothrix* sheaths were used to prepare a suspension (1 part of completely sedimented (earth gravity, 24 h) sheaths and 4 parts of distilled water) used in the adsorption experiments. The prepared magnetically responsive sheaths were stored in water at 4 °C.

Fig. 1. The sampling place of ocherous *Leptothrix* sp. sheaths.

2.3. Modification of Leptothrix sheaths with magnetic fluid

400 mL of completely sedimented *Leptothrix* sheaths (the same as above) was mixed with 20 mL of perchloric acid stabilized magnetic fluid (FF). After mixing on a sample mixer (27 rpm) for 3 h at room temperature, the modified sheaths were washed with water. Next, the same suspension as above was prepared for adsorption experiments. The prepared magnetically responsive material was stored in water at 4 °C.

2.4. Characterization of magnetically modified Leptothrix sheaths

The morphology and structure of both native and magnetically modified *Leptothrix* sheaths were studied by optical microscopy and scanning electron microscopy (SEM). The samples were analyzed using a Hitachi SU6600 scanning electron microscope (Hitachi, Japan) with accelerating voltage 1, 3 or 5 kV. Energy dispersive X-ray spectra (EDS) were acquired in SEM using Thermo Noran System 7 (Thermo Scientific, MA, USA) with Si(Li) detector (accelerating voltage of 10 kV, acquisition time 300 s).

2.5. Dye

Amido black 10B is a synthetic amino acid staining diazo dye (CI 20470, molecular formula: $C_{22}H_{14}N_6Na_2O_9S_2$, molar mass: 616.5 g/mol). It was obtained from Merck, Germany (purity: 50%) and was used without further purification. Amido black 10B is a dark red to black powder soluble in water and used as a stain for protein materials. The chemical structure of Amido Black 10B dye is given in Fig. 2. The wavelength corresponding to maximum absorbance for the dye was found to be 618 nm.

2.6. Adsorption of dye on magnetically modified Leptothrix sheaths

One mL of settled magnetic *Leptothrix* suspensions (corresponding to 19 mg of dried magnetically modified material) in a series of test tubes was mixed with 1–8 mL portion of stock water solution of tested dye (1 mg/mL; pH 2) and the total volume of the solutions was filled up to 10.0 mL with water (pH 2). The suspensions were incubated on a rotary mixer (Dynal, Norway, 27 rpm) for 2.5 h at room temperature. Various parameters were varied keeping the other parameters constant, to study the influence of pH (2–10), initial dye concentration (0–800 mg/L), contact time (0–240 min), and temperature (6–40 °C). After adsorption, the magnetic adsorbents were separated from the suspensions using a magnetic separator (MPC-1 or MPC-6, Dynal, Norway) and the clear supernatants were used for the spectrophotometric measurements. The concentration of free (unbound) dye in the supernatants (C_e; mg/L) was determined from the calibration curve.

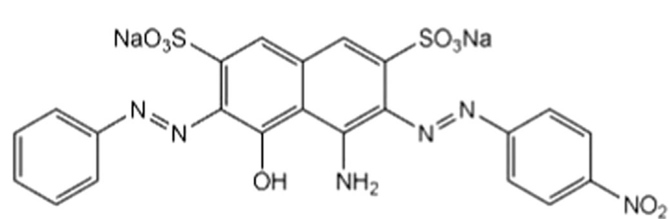

Fig. 2. Structure of Amido black 10B dye.

The amount of dye bound to the unit mass of the adsorbent (q_e; mg/g) was calculated using the following formula:

$$q_e = (C_{tot} - C_e)/1.9 \quad (1)$$

where C_{tot} is the total (initial) concentration of dye (mg/L) used in the experiment. Equilibrium adsorption data were analyzed by the Langmuir and Freundlich adsorption isotherm models using non-linear regression analysis (solver add – in function of the Microsoft Excel), and kinetic data were fitted to the pseudo-first-order and pseudo-second-order kinetic models as described previously [19]. Thermodynamic parameters, namely the standard free energy change ($\Delta G°$; J/mol), enthalpy ($\Delta H°$; J/mol), entropy ($\Delta S°$; J/mol K) and thermodynamic equilibrium constant (K_L) were calculated according to Maderova et al. [20].

3. Results and discussion

3.1. Characterization of the adsorbent

Native *Leptothrix* sheaths were collected from a natural water source. Long sheaths (with the lengths often exceeding 50 µm) are clearly visible using both optical and scanning electron microscopy (Fig. 3). The sheaths were magnetically modified by both simple and quick procedures employing mixing of native *Leptothrix* sheaths with either microwave synthesized magnetic iron oxide particles or water-based magnetic fluid.

The first modification is based on a preparation of nonstoichiometric magnetite particles from an iron(II) salt precursor at high pH. The use of a single salt during the microwave synthesis makes this approach substantially simpler in comparison with analogous techniques where both iron(II) and iron(III) salts have been used simultaneously [18,21].

Water-based magnetic fluid stabilized with perchloric acid was utilized as a second magnetic modifier. Magnetic fluid was composed of maghemite nanoparticles with diameters ranging between 10 and 20 nm (electron microscopy measurements), with a mean particle diameter ca. 12.5–14 nm [22,23].

Thorough mixing of target non-magnetic *Leptothrix* sheaths with both magnetic modifiers led to the deposition of magnetic iron oxide particles onto the surface of the *Leptothrix* sheaths. The presence of iron oxide particles and their aggregates on the surface of the treated sheaths was confirmed by SEM (Fig. 4). The magnetically modified

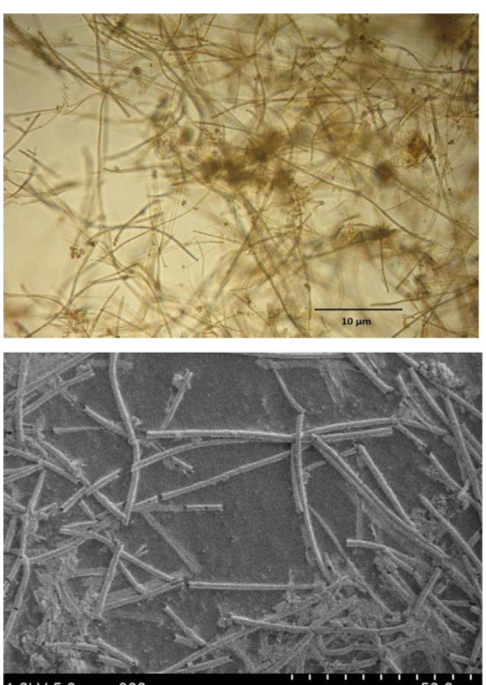

Fig. 3. Images of native *Leptothrix* sp. sheaths: optical microscopy (top); scanning electron microscopy (bottom).

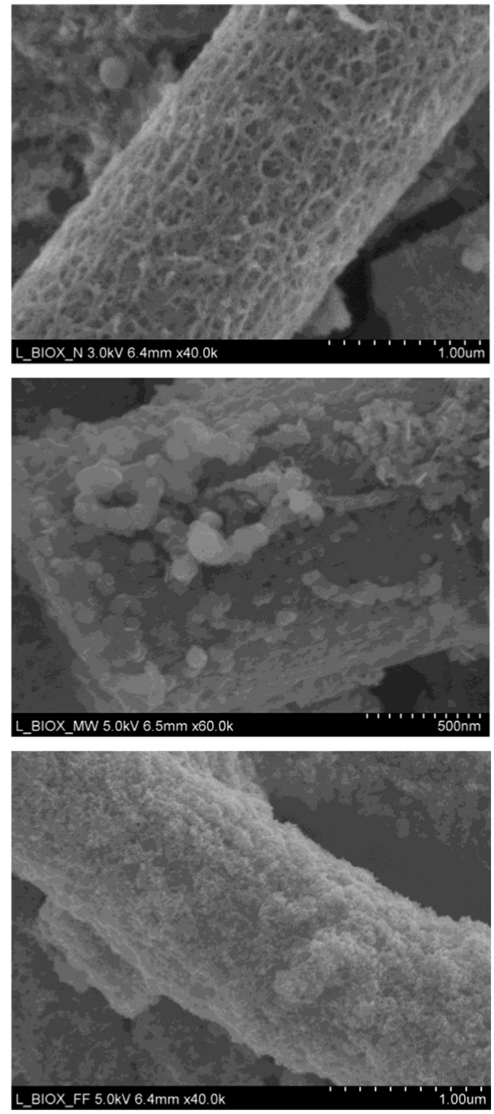

Fig. 4. SEM images of native *Leptothrix* sheaths (top); sheaths modified by microwave synthesized magnetite particles (middle); and sheaths modified by magnetic fluid (bottom).

Fig. 5. Appearance of native *Leptothrix* sheaths suspension (left), suspension of sheaths after magnetic modification (middle) and demonstration of magnetic separation of magnetically modified sheaths (right). Almost the same appearance was observed after magnetic modification with microwave synthesized magnetite particles and with magnetic fluid.

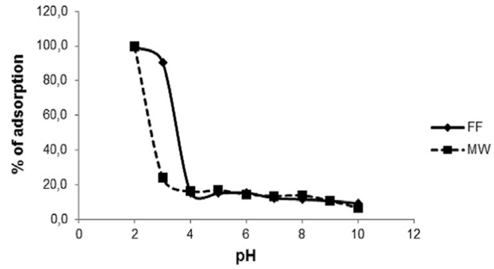

Fig. 6. Dependence of dye removal efficiency on solution pH using MW-MLS and FF-MLS.

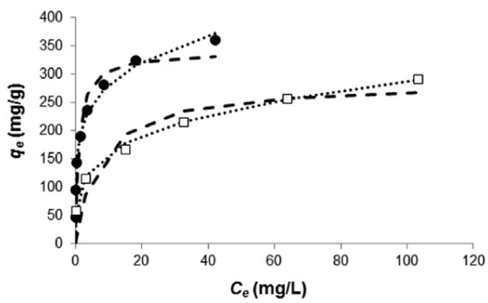

Fig. 7. Langmuir (- - -) and Freundlich (.......) equilibrium adsorption isotherms of Amido black 10B on *Leptothrix* sheaths modified with microwave synthesized magnetite (□) and magnetic fluid (•).

Table 1
Langmuir and Freundlich parameters for Amido black 10B obtained by non-linear regression analysis.

Isotherm model	Parameters	279.15 K		297.15 K		313.15 K	
		FF	MW	FF	MW	FF	MW
Langmuir	q_m (mg/g)	320.8	299.2	339.2	286.1	368.4	382.1
	α_L (L/mg)	0.59	0.83	0.94	0.14	0.95	0.97
	SEE	28.61	44.50	50.27	37.28	75.59	41.73
Freundlich	K_F [(mg/g) (L/mg)$^{1/n}$]	131.8	135.5	178.1	88.9	204.4	190.0
	n	3.44	3.03	5.06	3.94	5.04	2.86
	SEE	23.84	35.31	44.35	7.79	53.42	34.88

materials could be easily separated by rare earth permanent magnets or commercially available magnetic separators (Fig. 5).

3.2. Effect of solution pH on the adsorption process

pH is an important parameter significantly affecting the adsorption processes. To study the effect of initial pH on the adsorption of Amido black 10 B on magnetic *Leptothrix* sheaths, batch adsorption experiments were performed at pH values between 2 and 10 using 100 mg/L dye solution at 27 rpm stirrer speed and 24 °C (297.15 K) for 150 min. Fig. 6 shows the dye removal efficiency (%) profile of MLS over a pH range studied. The percent removal of the dye decreased significantly with the increase in initial pH of the solution as Amido black 10B is anionic in nature [4]. Better adsorption took place at low pH. Hence, all further experiments were conducted at the initial pH of 2.

Table 3
Thermodynamic parameters for both type magnetized *Leptothrix* sheaths.

Adsorbent	ΔH° (kJ/mol)	ΔS° (kJ/mol/K)	ΔG° (kJ/mol)		
			279.15 K	297.15 K	313.15 K
FF	27.161	0.193	−27.285	−28.618	−34.100
MW	8.230	0.128	−28.786	−26.555	−33.685

3.3. Adsorption isotherms, kinetics and thermodynamics

The dye adsorption on both types of the magnetically modified *Leptothrix* sheaths was tested with a water soluble organic dye Amido black 10B at 279.15 K, 297.15 K and 313.15 K. All experiments were carried out at pH 2 where maximum adsorption was achieved. Dye adsorption on both tested magnetic biosorbents was analyzed by two commonly used adsorption isotherm models, namely the Langmuir and Freundlich ones. The fitting of experimental data to each isotherm model was assessed on the basis of standard error of estimate (SEE). 95% of dye content (100 mg/L) was adsorbed within 5 min in both cases of magnetically modified *Leptothrix*. Although the adsorption equilibrium was reached within 15 min, incubation time was set to 150 min. The equilibrium adsorption isotherm models for both tested materials are shown in Fig. 7. The adsorption of Amido black 10B could be well described by the Freundlich model; the values of calculated coefficients are shown in Table 1. The observed maximum adsorption capacity of both types of magnetized materials (MW and FF) with Amido black 10B at room temperature were 286.1 and 339.2 mg of dye per 1 g of adsorbent at pH 2, respectively.

The fitting of experimental kinetic data to the pseudo-first-order and pseudo-second-order kinetic models was evaluated on the basis of obtained correlation coefficients and calculated q_e values; kinetic parameters are summarized in Table 2. The adsorption process followed the pseudo-second-order kinetic model.

Thermodynamic parameters were calculated as described recently [20]. As can be seen from the Table 3, the Amido black 10B adsorption on both FF-MLS and MW-MLS adsorbents is a spontaneous (negative value of Gibbs energy) and endothermic (positive value of enthalpy) process. Positive value of entropy indicates an increase in degree of freedom of adsorbed species.

The described procedure for the removal of Amido black 10B employs inexpensive biomaterial produced also during biological water treatment (removal of iron and manganese); the biomass of iron bacteria formed during the process has to be periodically removed. Currently the potential application of this “biological waste” is studied in several countries [13]. The adsorption capacity of magnetic derivative of *Leptothrix* sp. sheaths was quite high, as can be seen from Table 4 where other adsorbents for Amido black 10B are summarized, together with their maximum adsorption capacities.

Table 2
Values of the rate constants, capacities and regression coefficients from pseudo-first-order and pseudo-second-order kinetic models.

Adsorbent	C_{tot} (mg/L)	q_e (mg/g)	Pseudo-first-order model			Pseudo-second-order model		
			q_e (mg/g)	k_1 (1/min)	R^2	q_e (mg/g)	k_2 (g/mg min)	R^2
FF	100	48	1.4	0.0125	0.9431	47.4	0.0742	0.9999
	500	232	73.1	0.0040	0.8523	232.6	0.0023	0.9998
MW	100	58	2.2	0.0170	0.8735	57.8	0.0491	0.9999
	500	276	48.8	0.0156	0.9337	277.8	0.0014	0.9997

Table 4
Examples of adsorbents for Amido black 10B removal.

Adsorbent	q_m (mg/g)	Conditions	Other comments	References
Porous chitosan aerogels doped with graphene oxide	573.5	At 20 °C	P-2-O model followed	[24]
Leptothrix sheaths modified with magnetic fluid	339.2	pH 2 and 24 °C	Endothermic process, Freundlich and P-2-O models followed	Present study
Cross-linked chitosan/bentonite composite	323.6	pH 2 and 20 °C	Endothermic process, Langmuir and P-2-O models followed	[25]
Leptothrix sheaths modified with microwave-synthesized magnetic iron oxides	286.1	pH 2 and 24 °C	Endothermic process, Freundlich and P-2-O models followed	Present study
Mesoporous carbon	270.0	pH 7 and 20 °C	Freundlich model followed	[26]
Polyaniline/iron oxide composite	147.1	At 30 °C	Endothermic process, Freundlich and P-2-O models followed	[27]
Hen feather	88.9	pH 3 and 30 °C	Freundlich model followed	[9]
Kluyveromyces marxianus cells modified with magnetic fluid	29.9	RT	Langmuir model followed	[28]
Fly ash	18.9	At 20 °C	Endothermic process, Freundlich and P-2-O models followed	[29]
Saccharomyces cerevisiae subsp. *uvarum* cells modified with magnetic fluid	11.6	RT	Langmuir model followed	[30]
Palm flower activated carbon	3.8	At 27 °C	Endothermic process, Freundlich and P-2-O models followed	[31]

Notes: RT=room temperature, P-2-O=pseudo-second-order kinetic model.

4. Conclusion

Microwave synthesized iron oxide nano- and microparticles, and water-based magnetic fluid were used for rapid magnetic modification of non-magnetic native *Leptothrix* sheaths. The magnetically responsive materials were used as magnetic adsorbents for the removal of Amido black 10B in a batch system. Maximum adsorption of dye appeared at pH 2. The adsorption kinetics followed the pseudo-second-order kinetic model. It is clearly evident that magnetic *Leptothrix* sheaths have high adsorption capacity towards Amido black 10B dye. The results obtained show that the magnetic *Leptothrix* derivatives are efficient and biocompatible magnetically responsive materials which can substantially improve and simplify biotechnology and environmental technology processes.

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