

ADSORPTION OF DYES ON MAGNETICALLY LABELED BAKER'S YEAST CELLS

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INTRODUCTION: Textile industry (and especially its part focused on the dyeing process) belongs among important sources of contamination responsible for the continuous pollution of the environment. The production of textile industry, as well as the volume of waste water containing processed textile dyes, steadily increases. The release of dyes into the environment constitutes only a small proportion of water pollution, but dyes are visible in small quantities due to their brilliance. Many dyes reaching the water sources are difficult to decompose and may cause problems due to their possible carcinogenicity etc. That's why the control of water pollution has become more important in the last years [1].

Textile dyes differs in their chemical composition and stability and that's why different approaches have to be used to lower their content in water sources. In general, chemical, physical and biological treatment procedures can be used for this purpose. The individual procedures differ in their efficiency to remove or degrade the dyes and also in the cost required for the treatment of the comparable volumes of polluted water.

Various adsorbents have been tested and used for the removal of dyes from polluted water, such as activated carbon, silica gel, natural clay, peat, wood chips, rice husk ash (a waste from rice mills), living or dead microbial biomass etc.

Biologically based procedures use low-cost materials, namely living or died microorganisms. Both adsorption on the surface of the cells (biosorption) and exploitation of cell's enzymes for biodegradation can be used for removal of textile dyes from wastewater. Interactions between microorganisms (yeast, bacteria, fungi, algae) and dyes depend on chemical properties of all the reaction partners. Each dye can have affinity to various microorganisms and on the other side one microorganism is able to bind more types of dyes.

Currently an extensive research is perform in many laboratories to find optimal (and as cheap as possible) microbial biomass and reaction conditions in order to develop an optimal technological

processes enabling to separate contaminating dyes from large volumes of polluted water.

Magnetic adsorbents can be efficiently used for the separation of various types of compounds both from solutions and suspensions. During our experiments we have observed that yeast cells efficiently interact with some types of water based ferrofluids, leading to the formation of magnetically labeled cells which could be easily separated from the system using an appropriate magnetic separator. This material might be a promising adsorbent for various xenobiotics present in water sources. Magnetic properties of the adsorbent could be useful in the course of loaded adsorbent removal.

Adsorption characteristics of this new type of biological adsorbent, based on its interaction with selected model dyes are described in this paper.

METHODS: Baker's yeast (*Saccharomyces cerevisiae*) was obtained locally. Water based ionic ferrofluid (stabilized with perchloric acid) was prepared using the standard Massart procedure [2]. The relative ferrofluid concentration (32.0 mg/mL) is given as the magnetite content determined by a colorimetric method [3]. Acridine orange (C.I. 46005; Loba Chemie, Austria), aniline blue, water soluble (Water Blue; Methyl Blue; C.I. 42755; Lachema, Czech Republic), crystal violet (Basic Violet 3; C.I. 42555; Loba Chemie, Austria), malachite green (C.I. 42000; Roth, Germany) and safranin O (C.I. 50240; Sigma, USA) were used as model dyes.

The following optimized procedure was used for the magnetic modification of yeast cells. The compressed baker's yeast (2 g) was suspended in saline (6 mL), centrifuged and again resuspended in 6 mL 0.1 M acetate buffer (pH 4.6). After next centrifugation the sediment was resuspended in acetate buffer again to obtain ca 33 % yeast suspension (v/v; yeast cells volume determined after sedimentation for 24 h at 1 g). Three ml of the yeast suspension were added to 1 mL of ferrofluid, the suspension was mixed and then incubated at room temperature for one hour without mixing. After this time period the majority of yeast cells was magnetically modified by the added ferrofluid

(the cells responded to external magnetic field). Non-magnetic yeast cells and residual ferrofluid were removed by repeated static magnetic separation using acetate buffer (once) and saline as washing liquids, respectively, until the supernatant was clear. Magnetically modified yeast cells were then heated in a boiling water bath for 15 minutes. Heated magnetically modified yeast cells were washed with saline and stored at 4 °C. This material was used for further experimental work.

The adsorption experiments were performed in the following way. The suspension of magnetically modified yeast cells (200 µL; the settled volume of the adsorbent was 50 µL) in 15-mL test tube was mixed with 4.8 mL of water. Then 0.01 - 5.0 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. In the same manner water solution of the tested dye, used for the construction of a calibration curve, was prepared; instead of 200 µL of magnetic cell suspension 200 µL of water were used. 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, and the amount of dye bound to the unit volume of the adsorbent (q_{eq}) was calculated by difference, using the following formula (Eq. (1)):

$$q_{eq} = (D_{tot} - 10 C_{eq})/50 \text{ (}\mu\text{g mm}^{-3} \text{ or mg cm}^{-3}\text{)} \quad (1)$$

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

RESULTS: Several types of water based ferrofluids were tested for the modification of baker's yeast cells to become magnetic. It was observed that ionic water-based ferrofluid stabilized with perchloric acid enabled rapid magnetic derivatization of the yeast cells. The magnetically modified cells could be easily separated using commonly used permanent magnets or commercially available magnetic separators.

Magnetically modified yeast cells were then tested as possible dye adsorbents. In order to have stabilized product enabling work for a long period of time, dead yeast cells are preferred. That's why heating of cells in boiling water bath was used in the course of the adsorbent preparation.

The order of heating and magnetic modification of yeast cells is important from the point of view of cells adsorption capacity. It was observed that cells at first magnetically modified with the ferrofluid and then heated (i.e., procedure described in METHODS) exhibit substantially higher dye adsorption capacity than the cells at first heated and subsequently magnetically modified (see Fig. 1). In all experiments the adsorbent prepared by an optimized procedure was used.

The period of heating of magnetically modified cells also influences the adsorption capacity of the adsorbent. As shown in Fig. 2, significantly lower adsorption capacity was observed for preparations heated for 2 minutes, while prolonged cells heating (5 – 15 min) lead to the adsorbents with higher and almost equal adsorption capacity. In all experiments magnetically modified yeast cells heated for 15 min were used.

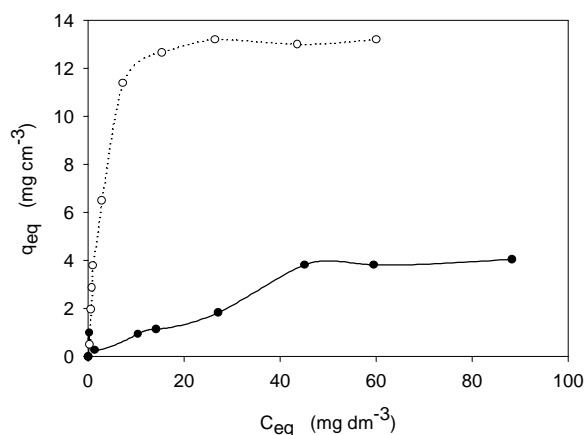


Fig. 1: Equilibrium adsorption isotherms of safranin O using magnetically modified baker's yeast cells as an adsorbent. Bottom curve, the cells were at first heated and then magnetically modified. Top curve, the cells were at first magnetically modified and then heated (15 min in both cases). C_{eq} – equilibrium liquid-phase concentration of the unadsorbed (free) dye (mg dm⁻³); q_{eq} – equilibrium solid-phase concentration of the adsorbed dye (dye uptake) (mg cm⁻³).

Preliminary experiments indicated that the adsorption of the dyes reached equilibrium in approximately 90 minutes (see Fig. 3). In the subsequent experiments, the adsorption of the tested dyes was performed for three hours.

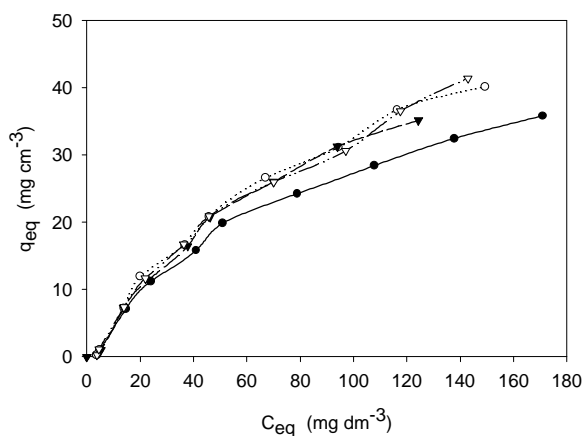


Fig. 2: Equilibrium adsorption isotherms of aniline blue using magnetically modified baker's yeast cells as an adsorbent. Various time of heat treatment of magnetically modified yeast cells was used (●, 2 min; ○, 5 min; ▼, 10 min; ▽, 15 min). The legends are the same as in Fig. 1.

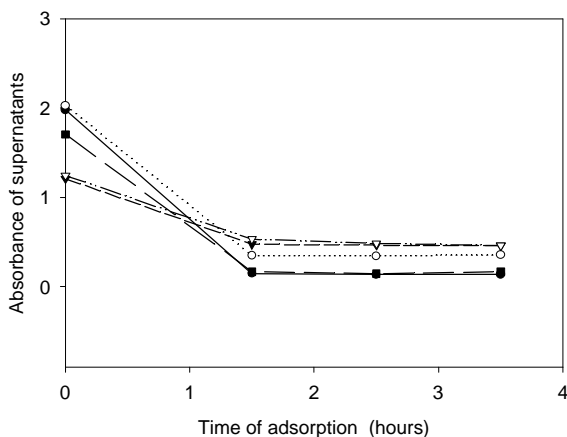


Fig. 3: Time dependence of the adsorption of tested dyes on magnetically modified yeast cells. ●, acridine orange; ■, aniline blue; ○, crystal violet; ▽, malachite green; ▼, safranin O.

Two types of dyes were used for adsorption experiments. Triphenylmethane dyes are represented by aniline blue, crystal violet and malachite green, while heteropolyaromatic dyes are represented by acridine orange and safranin O. Fig. 4 shows the equilibrium adsorption isotherms for unbuffered aqueous solutions of tested dyes. Adsorption isotherms represent the equilibrium distribution of the dyes molecules between the aqueous and solid phases as the dye concentration increases. The isotherms follow the typical Langmuir adsorption pattern as shown by the linear transformation of the experimental data (see Fig. 5).

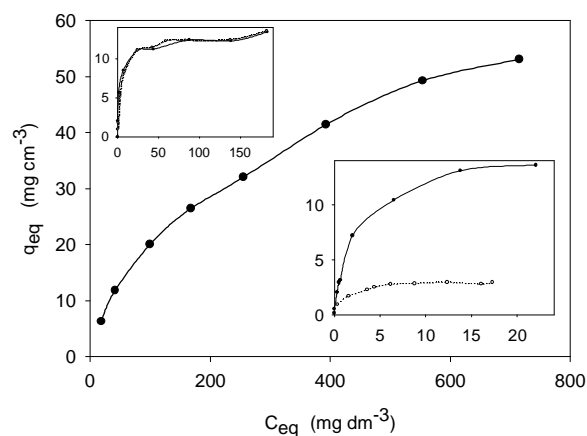


Fig. 4: Equilibrium adsorption isotherms of tested dyes using magnetically modified baker's yeast cells as an adsorbent. Main graph, aniline blue. Top insert, acridine orange and crystal violet. Bottom insert, malachite green (bottom curve) and safranin O (top curve). The legends are the same as in Fig. 1.

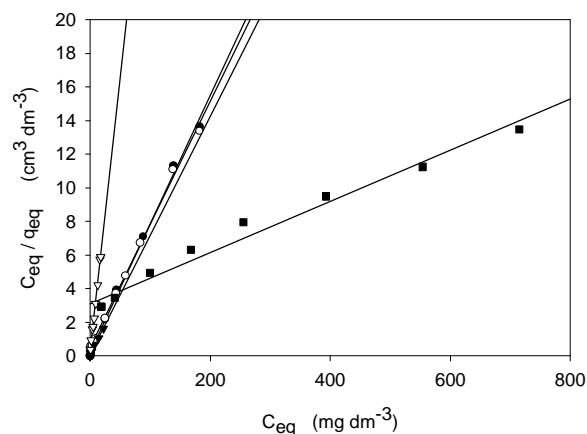


Fig. 5: Langmuir transformation of equilibrium adsorption isotherms of tested dyes. The symbols are the same as in Fig. 3; the legends are the same as in Fig. 1.

The linearized form of the Langmuir isotherm was used to calculate maximum adsorption capacity of the adsorbent (Eq.2):

$$C_{eq} / q_{eq} = 1 / b \cdot Q + C_{eq} / Q \quad (2)$$

where q_{eq} is the amount of dye adsorbed per unit of adsorbent ("adsorbed dye"), C_{eq} is the concentration of dye remaining in solution at equilibrium ("free dye"), Q is maximum adsorption capacity (number of mol or g of the dye adsorbed per unit of adsorbent, forming a continuous monolayer on adsorbent surface) and b is a constant.

The values of magnetic adsorption capacities are shown in Table 1.

Table 1. Maximum adsorption capacities of magnetically modified baker's yeast cells for the tested dyes. Q is calculated using the settled volume of the magnetic adsorbent (mg ml^{-1}) while Q' is calculated using the dry weight of the adsorbent (mg g^{-1}).

Dye	Q	Q'
Acridine orange	13.1	82.8
Aniline blue	68.1	430.2
Crystal violet	13.6	85.9
Malachite green	3.1	19.6
Safranin O	14.3	90.3

DISCUSSION & CONCLUSIONS: Modification of baker's yeast cells with the perchloric acid stabilized ferrofluid lead to the formation of magnetically responsible material which could be used as an efficient adsorbent for the removal of various dyes. The maximum adsorption capacity observed is relatively high, so magnetically modified yeast cells can be a promising dye adsorbent.

It seems that even small changes in the dye structure may significantly influence the adsorption capacity. Very different maximum adsorption capacities were observed for three triphenylmethane dyes (aniline blue, crystal violet and malachite green). The highest adsorption capacity for aniline blue could be caused by the presence of two aromatic rings with sulfonic acid groups present in the molecule while in crystal violet and malachite green four methyl groups are present.

Magnetic modification of other yeast cells and their possible application is currently studied.

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