

REMOVAL OF CONTAMINANTS FROM AQUEOUS SOLUTIONS USING HOP (*Humulus lupulus* L.) AGRICULTURAL BY-PRODUCTS

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Abstract: Agricultural wastes can be used as an alternative to the existing sorbents for the removal of metals or synthetic dyes from contaminated liquids. In this work, the fine powdered biomass of the hop (*Humulus lupulus* L.) variety Osvald's clone 72 and variety Bohemie as a sorbent for the removal of Cd from aqueous solutions of CdCl₂ spiked with radionuclide ¹⁰⁹Cd and synthetic dyes thioflavine T (ThT) or methylene blue (MB) from single dye solutions under conditions of batch systems was used. The maximum sorption capacity $Q = 264 \mu\text{mol Cd/g}$ (d.w.) was found in the case of the leaf biomass of hop (*H. lupulus* L.) variety Osvald's clone 72 at the initial concentration of CdCl₂ 10,000 $\mu\text{mol/dm}^3$, whereby the sorption capacity decreased in the order $Q_{\text{leaves}} : Q_{\text{stems}} : Q_{\text{roots}} = 1.0 : 0.8 : 0.7$. The sorbed amount of Cd was removed from the hop biomass with the following increasing desorption efficiency of the extraction reagents: deionised H₂O $\ll 0.1 \text{ mol/dm}^3$ HCl $\leq 0.1 \text{ mol/dm}^3$ EDTA-Na₂. Similarly as in the case of Cd sorption, the kinetics of ThT and MB sorption by the leaf biomass of the hop (*H. lupulus* L.) variety Bohemie were also showed as two-phase processes. The maximum sorption of ThT approx. $Q = 19 \text{ mg/g}$ (d.w.) and MB approx. $Q = 70 \text{ mg/g}$ (d.w.) were found within the range of the initial values of pH 4 – 7. The sorption of both dyes by the leaf biomass from single dye solutions decreased with increasing biomass concentration and on the other hand increased with increasing the initial concentrations of ThT or MB. The process of ThT and MB sorption was better described by the Langmuir model than the Freundlich model of sorption isotherm. From the obtained values of Q_{max} , it was found that in the case of MB the dried leaf biomass showed more than 2-times higher sorption capacity ($Q_{\text{max}} = 184 \text{ mg/g}$; d.w.) in comparison with the value predicted for ThT. Obtained results suggest that dried plant biomass of hop (*H. lupulus* L.) as agricultural by-products can be used as a potential sorbent for both types of studied contaminants.

Key words: cadmium, synthetic dyes, sorption, hop, agricultural by-products, sorbents

1. Introduction

In recent decades, the growth of industrial activities and the increasing water usage worldwide have led to the release of various pollutants into an aquatic environment, such as heavy metals or organic compounds represented by phenols, dyes, pesticides, humic substances, detergents, and other persistent organic pollutants (ABDOLALI *et al.*, 2014).

Heavy metals, such as cadmium, often presented in industrial wastewaters are hazardous to the aquatic ecosystem and pose possible human health risk. Cadmium (Cd) makes its way to water bodies through the wastewaters from metal plating industries, industries of Cd-Ni batteries, phosphate fertilizer, mining, pigments, stabilizers and alloys (LOW and LEE, 1991). Cd is listed as one of the top toxic

metals, since it causes carcinogenic and renal disturbances, lung insufficiency, bone lesions, cancer, anaemia, hypertension, Itai-Itai disease and weight loss (DING *et al.*, 2012). EPA requires water suppliers to limit the concentration in drinking water to $< 5 \mu\text{g}/\text{dm}^3$ for Cd (EPA, 2011). Council Directive 83/513/EEC of 26 September 1983 on limit values and quality objectives for cadmium discharges (CEC, 1983) determines the limit values for industrial discharges to be 0.2 mg of Cd per litre of discharge. According to EPA Wastewater Treatment Manuals, Cd in concentrations of $1.0 - 10.0 \text{ mg}/\text{dm}^3$ can significantly inhibit positive biological activity of activated sludge in the wastewater treatment process.

Many industries, such as textile industry, used dyes to colour their products and thus produce wastewaters containing organic compounds with a strong colour, because within the dyeing processes the percentage of dyes lost in wastewaters is approx. 50 % of the dyes due to the low levels of dye-fibre fixation (MOHAN *et al.*, 2007; SALLEH *et al.*, 2011). Large amounts of dye contaminated wastewaters are being released yearly from the industry of leather, cosmetics, pharmaceutical, and plastics, and results in an impending hazard to human health and the ecosystem (CAO *et al.*, 2014; SEMERARO *et al.*, 2015). Besides dyes, such effluents also contain a number of other contaminants, such as alkalis, acids, electrolytes, heavy metal ions, dissolved and other suspended solids etc. (SAFA and BHATTI, 2011). Today, there are more than 10,000 dyes commercially available. Dyes have a complex aromatic molecular structure and are generally resistant to light, temperature, and oxidizers. These characteristic features make the dyes non-degradable and therefore causes bioaccumulation in living organisms, leading to severe diseases and disorders (BANERJEE and CHATTOPADHYAYA, 2013). Dyes can affect aquatic plants because they reduce the sunlight transmission through the water as well as may impart toxicity to aquatic life and may be mutagenic, carcinogenic and may cause severe damage to human beings, such as dysfunction of the kidneys, reproductive system, liver, brain, and central nervous system (ADEGOKE and BELLO, 2015).

Thus, the removal of heavy metals and dyes as xenobiotics from polluted waters is of great importance from an environmental and industrial point of view. The most widely used methods for removal these contaminants from wastewaters include ion-exchange, chemical precipitation, reverse osmosis, evaporation, membrane filtration, adsorption, biological treatment, coagulation, flocculation, electrochemical treatment, electrodialysis, photo-catalysis, and photo-oxidation (RANGABHASHIYAM *et al.*, 2014; OGUNTWEIN, 2015). But major drawbacks of these technologies are long operation time, low specificity, high cost, and eco-unfriendliness.

Biological systems have the availability to accumulate specific metals and compounds from wastewaters. This process is called bioremediation. One type of bioremediation is phytoremediation, which uses dead tissues from plants to remove contaminants from waters (LÓPEZ *et al.*, 2005). It is based on (bio)sorption processes. The cost of such sorption technology application can be reduced, if the sorbent is inexpensive. So, there is a constant search for alternate low-cost sorbents (GHASEMI *et al.*, 2014). The limitations which considered when choosing suitable sorbents are: the sorption and regeneration abilities, market availability, and kinetic parameters.

Sorption capacity parameter is very important for estimation of process costs. Regeneration of the sorbent is also important in cyclic processes when the used sorbents are expensive (SADEEK *et al.*, 2015).

Natural materials that are available in large quantities, or certain waste products originated from industrial or agricultural operations may have the potential as inexpensive sorbents. Agricultural wastes – plant residues – are renewable and available abundantly at no or low cost (ŠCIBAN *et al.*, 2008). Agricultural wastes can be used as an alternative to the existing conventional technologies as the sorbents for the removal of metals or xenobiotics (synthetic dyes) from aqueous solutions. Several studies characterized the sorption properties of waste materials derived from plants, such as hop (LÓPEZ *et al.*, 2005), rice (WU *et al.*, 2016), maize (GARCÍA-ROSALES and COLÍN-CRUZ, 2010), sunflower (OGUNTIN, 2015), wheat (GORGIEVSKI *et al.*, 2013), sugar beet (AKSU and İŞOĞLU, 2005), and tobacco (QI and ALDRICH, 2008) biomass. Also, some papers suggest on the utilization of physical or chemical approaches for the modification of waste plant biomasses or techniques for production of biochar with the aim to increase their sorption capabilities (PELLERA *et al.*, 2012; ZAFAR *et al.*, 2015; WANG *et al.*, 2016).

Our previous papers deal with the study of sorption processes and characterisation of sorption capabilities of lichen, moss or algae biomass to bind metals or synthetic dyes (PIPIŠKA *et al.*, 2007; MAREŠOVÁ *et al.*, 2011; HORNÍK *et al.*, 2013; ŠUŇOVSKÁ *et al.*, 2015). The aim of this work was to evaluate the capability of fine powdered hop (*Humulus lupulus* L.) biomass as an agricultural by-product to removal of Cd or synthetic dyes thioflavine T (ThT) and methylene blue (MB) from aqueous solutions. Also, the effects of time, metal or dye concentration, amount of hop biomass, and pH on sorption processes were studied. For these purposes, a radionuclide ^{109}Cd with gamma-spectrometry and UV-VIS spectrophotometry as detection methods and approaches were applied.

2. Materials and methods

2.1 Hop biomass

In the experiments, the plants of hop (*Humulus lupulus* L.) variety Oswald's clone 72, genotype K-72/6/13 obtained from Plant Production Research Institute in Piešťany (Slovak Republic) and leaf biomass of hop (*H. lupulus* L.) variety Bohemie obtained from Hop Research Institute Co., Ltd., Žatec (Czech Republic) were used. Leaves, stems, and roots of variety Oswald's clone 72 and leaves in the case of variety Bohemie were dried 3 days at 60 °C, ground and sieved for a particle size < 0.45 mm (variety Oswald's clone 72) or between < 0.63 mm and > 0.31 mm (variety Bohemie).

2.2 Sorption experiments

All solutions of CdCl_2 (analytical grade; CAS 10108-64-2; Sigma-Aldrich, USA) or synthetic dyes thioflavine T (ThT; C.I. 49005; Mr 318.86; CAS 2390-54-7; Fluka, USA) and methylene blue (MB; C.I. 52015; Mr 319.85; CAS 61-73-4; Fluka, USA)

were prepared in deionised water. In the case of CdCl_2 , solutions were spiked with a standardized $^{109}\text{CdCl}_2$ solution. The initial pH of experimental solutions was adjusted with 1 mol/dm^3 HCl or 1 mol/dm^3 NaOH solutions.

Dried biomass of hop was transferred into a 100 cm^3 Erlenmeyer flasks with 20 cm^3 of mentioned experimental solutions containing defined initial concentrations of CdCl_2 , ThT or MB. The exposure was carried out on a rotary incubation shaker (250 rpm) at $25 \text{ }^\circ\text{C}$. In defined time intervals mixture was centrifuged (10 min at 5000 min^{-1}), supernatant was removed and analysed from the point of view of remaining concentration of CdCl_2 through the scintillation γ -spectrometry or remaining concentration of ThT or MB dyes by UV-VIS spectrophotometry. From the obtained primary data, the specific adsorption Q (mg of metal/dye sorbed per g of dried hop biomass) was calculated using the following equation (Eq. 1):

$$Q = (C_0 - C_t) \frac{V}{M} \quad (1)$$

where Q is the amount of metal/dye sorbed onto hop biomass (mg/g; d.w.), C_0 and C_t represent the initial concentration of metal/dye in solution and the concentration of metal/dye at the end of the experiment (mg/dm^3), respectively, and V and M are the volume of solution (dm^3) and weight of hop biomass (g; d.w.) in the experiments.

In desorption experiments, the sediment of hop biomass after Cd sorption was resuspended by vortexing in deionised water or 0.1 mol/dm^3 EDTA- Na_2 or HCl solutions. The ^{109}Cd radioactivity leached from the hop biomass into the supernatant was determined by similar way as in the sorption experiments and the desorption efficiency D_{eff} was calculated using the following equation (Eq. 2):

$$D_{\text{eff}} = \frac{A_D}{A_B} * 100 \quad (2)$$

where D_{eff} is the desorption efficiency (in %), A_D is the ^{109}Cd radioactivity released from the hop biomass into the supernatant (in Bq) and A_B is the ^{109}Cd radioactivity sorbed onto the hop biomass before carrying out the desorption experiment.

The sorption equilibrium data were described by sorption isotherms according to the Langmuir (Eq. 3; LANGMUIR, 1918) and Freundlich (Eq. 4; FREUNDLICH, 1906). The non-linear forms of these mathematical models are as follows:

$$Q_{\text{eq}} = \frac{b * Q_{\text{max}} * C_{\text{eq}}}{1 + b * C_{\text{eq}}} \quad (3)$$

$$Q_{\text{eq}} = K * C_{\text{eq}}^{(1/n)} \quad (4)$$

where C_{eq} is the equilibrium concentration of the dye in the solution (mg/dm^3), Q_{eq} is the equilibrium specific sorption of dye onto the dried hop biomass (mg/g; d.w.), Q_{max} is the maximum sorption capacity of the dried hop biomass (mg/g; d.w.), b is the Langmuir equilibrium constant characterizing the affinity between the dye and the biomass of the hop (dm^3/mg), K is the Freundlich equilibrium constant related to sorption capacity of the hop biomass (dm^3/g of biomass), n is the Freundlich equilibrium constant related to intensity of the sorption (non-dimensional).

2.3 Scintillation γ -spectrometry

Standardized solution of ^{109}Cd in the form of $^{109}\text{CdCl}_2$ (3.94 MBq/cm^3 , 50 mg/dm^3 CdCl_2 in 3 g/dm^3 HCl) was obtained from The Czech Institute of Metrology (Prague, Czech Republic).

A gamma-spectrometric assembly using the well type scintillation detector NaI(Tl) , 54BP54/2-X (Scionix, Netherlands) and the data processing software ScintiVision-32 (ORTEC, USA) were used for ^{109}Cd determination in the supernatants and sediment of hop biomass. A library of radionuclides was built by selecting characteristic γ -ray peaks (88.04 keV for ^{109}Cd ; 661.66 keV for ^{137}Cs ; $1,115.52 \text{ keV}$ for ^{65}Zn and $1,173.24 \text{ keV}$ for ^{60}Co) for the energy and efficiency calibration. Counting time 600 s allowed obtaining data with measurement error $< 2 \%$, which do not reflect other source of errors.

2.4 UV-VIS spectrophotometry

A UV-VIS spectrophotometer Cary 50 (Varian, Australia) was used for establishing calibration curves for ThT and MB dyes at maximum absorbances $\lambda_{\text{ThT}} = 412 \text{ nm}$ and $\lambda_{\text{MB}} = 650 \text{ nm}$ and for determination of remaining concentrations of ThT or MB dyes in solutions. The influence of pH value on the analysed absorbances was taken into account.

2.5 Speciation modelling

The prediction of Cd speciation for the synthetic wastewater as a function of pH, temperature, ionic strength, and concentrations of cations and anions was calculated using the modelling software MINEQL+ ver. 4.62 (Environmental Research Software, USA). The composition of synthetic wastewater according to IAQUINTA *et al.* (2006) (ppm): $455 - \text{Cl}^-$; $87 - \text{SO}_4^{2-}$; $418 - \text{Na}^+$; $78 - \text{K}^+$; $45 - \text{Mg}^{2+}$; $138 - \text{Ca}^{2+}$.

2.6 Statistical analysis

All analytical determinations were performed in triplicate. Statistical analysis, non-linear regression and graphical interpretation of the obtained data were carried out using programs OriginPro ver. 8.5 (OriginLab Corp., USA), SYSTAT ver. 13 (Systat Software Inc., USA), and SigmaPlot ver. 12 (Systat Software Inc., USA).

3. Results and discussion

3.1 Sorption of Cd by hop biomass

Kinetics of Cd sorption by dried and homogenized biomass of leaves, stems, and roots (particle size $< 0.45 \text{ mm}$) of hop (*H. lupulus* L.) variety Oswald's clone 72 under conditions of batch systems was showed as a two-phase process. The first, fast

phase can be explained as the sorption of Cd^{2+} cations on the hop biomass surface. The second, more slowly phase lasting approx. 30 min can be attributed to the swelling of dried biomass and relating diffusion processes from the outside into the inside of sorbent particles (data not shown). The maximum sorption capacity $Q = 264 \mu\text{mol Cd/g}$ (d.w.) was found in the case of the leaf biomass of hop at the initial concentration of CdCl_2 $10,000 \mu\text{mol/dm}^3$ (Table 1). Generally, cadmium sorption by hop biomass increased with increasing initial Cd concentration in the solution from 10 to $10,000 \mu\text{mol/dm}^3$ of CdCl_2 (Fig. 1). In the case of the leaf biomass, the linear dependence ($R^2 = 0.999$) between the sorption Q and the initial concentration of CdCl_2 was observed. It means that neither at the highest applied initial Cd concentration $C_0 = 10,000 \mu\text{mol/dm}^3$ CdCl_2 the leaf biomass of hop was not saturated by the studied sorbate (Cd^{2+} ions). Mentioned linear dependence was not found for the data describing the sorption of Cd by stem and root biomass of hop under identical conditions (Fig. 1). Thus, the biomass of stem and root of hop showed a partial saturation with Cd^{2+} cations at the highest applied initial Cd concentrations.

Table 1. Desorption of Cd (%) from leaf, stem, and root biomass of hop (*H. lupulus* L.) variety Oswald's clone 72 after 30 min by resuspending of biomass sediment originated from sorption experiments with deionised water ($5.0 \text{ cm}^3/0.05 \text{ g}$; d.w.), 0.1 mol/dm^3 EDTA- Na_2 ($1.0 \text{ cm}^3/0.05$; g d.w.) or 0.1 mol/dm^3 HCl ($1.0 \text{ cm}^3/0.05$; g d.w.) at 25°C .

Biomass	Sorption experiment*		Desorption D_{eff} [%]		
	C_0 CdCl_2 [$\mu\text{mol/dm}^3$]	Sorption Q [$\mu\text{mol/g}$; d.w.]	Deion. H_2O	0.1 mol/dm^3 EDTA- Na_2	0.1 mol/dm^3 HCl
Leaves	10	$0.30 \pm 0.01^{**}$	7.4	70.1	–
	100	3.40 ± 0.10	8.4	72.6	–
	1 000	36.0 ± 0.20	7.8	69.9	–
	10 000	264 ± 3.00	9.1	71.0	71.5
Stems	10	0.40 ± 0.01	6.6	70.5	–
	100	3.40 ± 0.10	7.8	74.3	–
	1 000	32.2 ± 0.40	9.5	74.0	–
	10 000	198 ± 1.00	19.0	73.1	71.2
Roots	10	0.30 ± 0.01	9.4	78.0	–
	100	0.60 ± 0.01	13.4	79.5	–
	1 000	34.3 ± 0.20	16.4	82.5	–
	10 000	190 ± 0.01	18.7	79.2	70.0

* sorption experiments are described in Fig. 1;

** \pm value represents the standard deviation of the mean ($n = 3$).

It can be expected, that the structure and material composition of particular plant organs are different. Therefore, the sorption capabilities of leaves, stems, and roots biomass of hop for Cd were compared. It was found that sorption capacity Q for Cd expressed per gram of dried biomass of hop decreased in the order $Q_{leaves} : Q_{stems} : Q_{roots} = 1.0 : 0.8 : 0.7$ (Table 1). Explanation of the differences in the sorption capabilities of particular plant organs for Cd will require primarily the comparison of differences in the structures of plant tissues of *Humulus* genera in more details. The mean value of sorption capacity for the whole hop biomass at $C_0 = 10,000 \mu\text{mol/dm}^3$ CdCl_2 in deionised water was $Q = 220 \mu\text{mol/g}$ (d.w.).

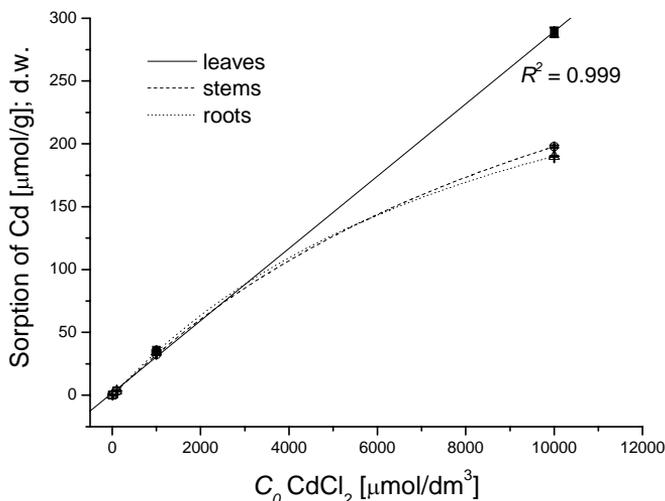


Fig. 1. Sorption of Cd ($\mu\text{mol/g; d.w.}$) by leaf \blacksquare –, stem \circ – and root \triangle – biomass of the hop (*H. lupulus* L.) variety Osvald's clone 72 in dependence on initial CdCl_2 concentration in deionised water, pH 5.0 after 10 min of biomass exposure at 25 °C. Biomass concentration 25 g/dm^3 (d.w.), fraction < 450 μm . Error bars represent standard deviation of the mean ($n = 3$).

It is generally known that the pH optimum for bivalent metal cations sorption by biomass is in the range 4 – 6, thus in the moderate acid conditions. In the strong acid conditions, the efficiency of metal cation sorption is minimal and controlled by competitive effect of H^+ ions (SALIM *et al.*, 2008). On the other hand, at higher pH values > 8 (CORDERO *et al.*, 2004) there exists a formation of sparingly soluble hydroxides $\text{Cd}(\text{OH})_2$ or $\text{Cd}(\text{OH})_3$. The pH value of sorbate solution significantly contributes to ionic equilibria determined by all components presented in the solution. In the Fig. 2, the relative proportion of particular ion forms of Cd in dependence on pH value of solution imitating the wastewaters predicted by the speciation modelling program MINEQL+ is described. In the range of pH 3 – 8, the forms of monovalent CdCl^+ (52 %) and bivalent Cd^{2+} cations dominate at minority participation of other ionic forms. Over the pH 9, the proportion of CdOH^+ ions increases. However, over the pH 10 dominates the proportion of $\text{Cd}(\text{OH})_2$, which already at pH 11 represents 78 % of all Cd forms existed in the model system. In the interpretation of Cd sorption onto living or dead biomass as a potential sorbent, it is necessary to consider the high proportion of monovalent cation CdCl^+ at physiological values of pH 6 – 8. Based on this consideration, it can be concluded that an increasing Cl^- ions concentration in the environment will increase the proportion of CdCl^+ complexes.

In practice, where inorganic sorbents are used for the removal of metals from wastewaters, the regeneration of the sorbent represents an important step in such decontamination technologies. In this step, the application of chelating agents or acids as desorption solutions is generally accepted (ŠUŇOVSKÁ *et al.*, 2015). The efficiency of Cd desorption (D_{eff}) from the hop biomass expressed in %

from the total amount of sorbed Cd in the biomass increased in the order of the following extraction reagents: deionised water \ll 0.1 mol/dm³ HCl \leq 0.1 mol/dm³ EDTA-Na₂ (Table 1). Desorption of Cd with deionised water is more significant in the case of hop biomass originated from the sorption experiments carried out at higher initial CdCl₂ concentration in the solution. This fact point out that in the hop biomass exist binding sites, which are characterized by different stability of the bond Cd–biomass. It can be expected that in the case of Cd sorption from diluted CdCl₂ solutions (10 μmol/dm³), Cd²⁺ cations are binding primarily to the sites with higher stability of the bond and in the case of higher CdCl₂ concentrations (10,000 μmol/dm³) to the sites with lower stability of the bond.

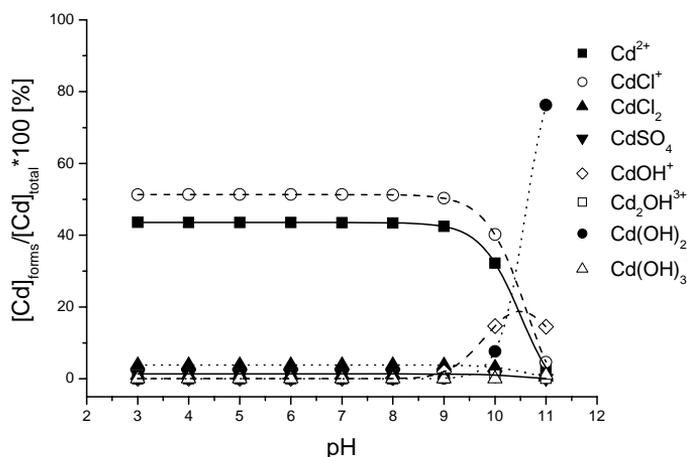


Fig. 2. Speciation forms of Cd in dependence on pH value at the initial concentration of CdCl₂ 10,000 μmol/dm³ and 25 °C in the solution of inorganic salts simulating wastewaters composition according to IAQUINTA *et al.* (2006). Calculated by a speciation program MINEQL+ ver. 4.62.

3.2 Sorption of dyes by hop biomass

Previous experiments with the biomass of leaves, stems, and roots of hop (*H. lupulus* L.) variety Osvald's clone 72 showed that the leaf biomass has the highest affinity and sorption capacity to Cd²⁺ cations. Therefore, in the case of the biomass of hop (*H. lupulus* L.) variety Bohemie only the leaf biomass was studied. Preliminary experiments revealed that the dried and homogenized leaf biomass (particle size < 0.63 mm and > 0.31 mm) of hop (*H. lupulus* L.) variety Bohemie showed a higher affinity to thiazine dye – methylene blue (MB) than to benzothiazole dye – thioflavine T (ThT). Regarding this fact, the next experiments were carried out at different concentrations of these studied dyes. Fig. 3A depicts the kinetics of ThT and MB sorption Q (in mg/g; d.w.) by the leaf biomass of the hop under conditions of batch, single dye systems at the initial ThT concentration $C_0 = 40$ mg/dm³ or MB concentration $C_0 = 80$ mg/dm³, and at the leaf biomass concentration $C_B = 0.5$ g/dm³ (d.w.) and pH value 6.0. Similarly, as in the case of Cd sorption by dried biomass

of leaves, stems, and roots of hop (*H. lupulus* L.) variety Oswald's clone 72, the ThT and MB sorption were also showed as a two-phase process. In the first time of analysis ($t = 10$ min) of remaining dye concentration in the solution, the sorption of both dyes is characterized as a rapid process and followed by a gradual increasing amount of sorbed dye within the next 350 min of exposure up to reaching the concentration equilibrium. Similar results also observed HAMEED (2009) in the case of MB sorption by the biomass of the grass waste. Despite of the mentioned reason that the ratio of initial concentrations of dyes C_0 (MB) : C_0 (ThT) was 2 : 1, the sorption of MB by the leaf biomass of the hop was 4-times higher than in the case of ThT. This fact confirms that the dried leaf biomass of the hop showed a higher affinity to the thiazine dye MB than to the benzothiazole dye ThT. In both dye molecules, the positive charge is on the quaternary nitrogen = N^+ , but on the different positions within the molecule. Thus, in this case the steric effects can play an important role.

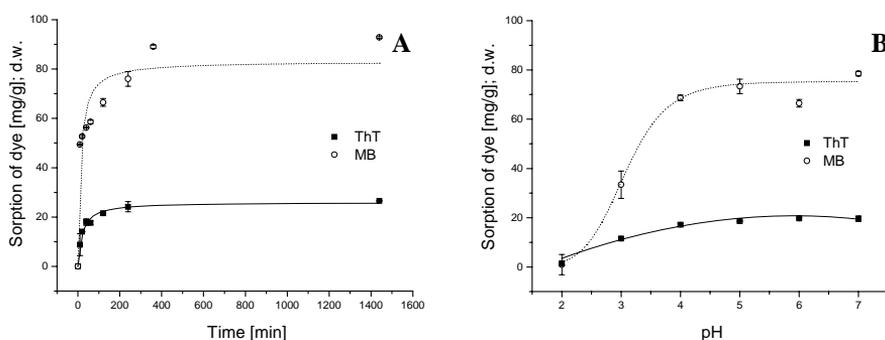


Fig. 3. A. Kinetics of ThT and MB sorption by the leaf biomass of the hop (*H. lupulus* L.) variety Bohemie during 1 440 min of exposure in deionised water containing ThT $C_0 = 40$ mg/dm³ or MB $C_0 = 80$ mg/dm³ at the initial value of pH 6.0 and 25 °C. B. Effect of initial value of the pH on ThT and MB sorption by the leaf biomass of the hop (*H. lupulus* L.) variety Bohemie after 360 min of exposure in deionised water containing ThT $C_0 = 40$ mg/dm³ or MB $C_0 = 80$ mg/dm³ at 25 °C. Error bars represent standard deviation of the mean ($n = 3$).

As it was mentioned, the decisive parameter in metal ions or dyes sorption is the pH value of the solution, which affects the sorption capacity of biomass mainly due to the changes in the dissociation of relevant functional groups responsible for their binding. In addition to this, the pH also affects the chemical speciation of metals as well as the chemical dissociation of dye molecules, and thus the sorption of these contaminants. From the Fig. 3B, it can be seen that the maximum sorption of ThT approx. $Q = 19$ mg/g (d.w.) and MB approx. $Q = 70$ mg/g (d.w.) were found within the range of initial values of pH 4 – 7. Approx. 50 % decrease in both dyes sorption was observed at the pH 3 and the minimal sorption $Q \approx 1$ mg/g (d.w.) for both dyes was calculated at the initial value of pH 2. The similar dependence between the values of specific sorption (in mg/g; d.w.) and the pH value of solutions also described BARKA *et al.* (2011) for the case of MB sorption and the biomass of vascular plant *Scolymus hispanicus*. Moreover, they found that this dependence can be caused by the changes in surface charge of the dye and in functional groups

localized on the surface of biomass, and the value of isoelectric point of the plant biomass ($pI = 5.2$) as well.

In the next experiment, the effect of the leaf biomass concentration on the ThT and MB sorption was studied. The sorption of both dyes by the leaf biomass of the hop from single dye solutions decreased with increasing concentration of the biomass under conditions of batch systems (Fig. 4A). The relationship between the sorption of dyes Q and the concentration of leaf biomass C_B showed a non-linear dependence. KUMAR and PORKODI (2007) explain this dependence on the basis of their results, when in the systems with a higher concentration of biomass a negative aggregation and changes in the specific surface area (m^2/g ; d.w.) of biomass, as well as changes in conditions of effective mixing the biomass within the whole system were described. On the other hand, the efficiency of the removal of both dyes (in %) from the single dye solutions non-linearly increased with increasing concentration of the leaf biomass (data not shown). HAMEED (2009) reported that the increase of the percentage of dyes removal from the solution with increasing concentration of biomass relates with increasing the available surface binding sites responsible for dyes sorption.

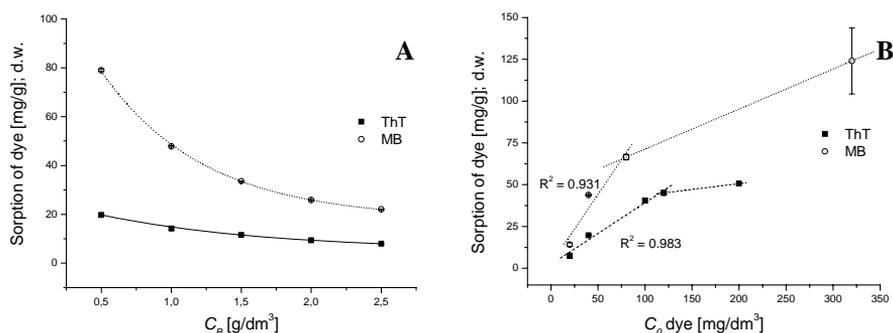


Fig. 4. A. Effect of biomass amount C_B (in g/dm^3 ; d.w.) on ThT and MB sorption by the leaf biomass of the hop (*H. lupulus* L.) variety Bohemie after 360 min of exposure in deionised water containing ThT $C_0 = 40$ mg/dm^3 or MB $C_0 = 80$ mg/dm^3 at the initial value of pH 6.0 and 25 °C. B. Effect of initial concentration of dye C_0 (in mg/dm^3) on ThT and MB sorption by the leaf biomass of the hop (*H. lupulus* L.) variety Bohemie after 360 min of exposure in deionised water containing ThT $C_0 = 20, 40, 100, 120,$ and 200 mg/dm^3 or MB $C_0 = 20, 40, 80,$ and 320 mg/dm^3 at the initial value of pH 6.0 and 25 °C. Error bars represent standard deviation of the mean ($n = 3$).

The efficiency of dyes removal as well as the sorption capacity are also strongly dependent on the concentration of sorbate – dyes in the solution. The Fig. 4B depicts the dependence between the sorption of ThT or MB by the leaf biomass of the hop (mg/g; d.w.) and the initial concentrations of the ThT in the solution within the range 20 – 200 mg/dm^3 or MB within the range 20 – 320 mg/dm^3 . This dependence shows a non-linear trend, whereby the sorption capacity of dried leaf biomass for both studied dyes increased with increasing the initial concentrations of ThT or MB due to the higher probability of collisions between dye and biomass. However, the linear dependences within the concentration range 20 – 120 mg/dm^3 for ThT ($R^2 = 0.983$) and in the case of MB within the concentration range 20 – 80 mg/dm^3 ($R^2 = 0.931$) were observed. Thus, above the concentrations of ThT > 120 mg/dm^3

and the concentrations of MB $> 80 \text{ mg/dm}^3$ the leaf biomass showed the certain degree of the saturation to sorption of both dyes. On the contrary, the efficiency of the removal of both dyes (in %) from the single dye solutions non-linearly decreased with increasing concentration of the dye in the solution (data not shown). As TURABIK (2008) mentioned in his work, at the lower concentrations of dyes practically all present dye molecules can interact with sufficient amount of binding sites localized on the biomass. However, in the case of higher concentrations of dyes the lower efficiency of the removal of dyes from single dye solutions is observed due to the saturation of sorption sites characterized by relevant functional groups.

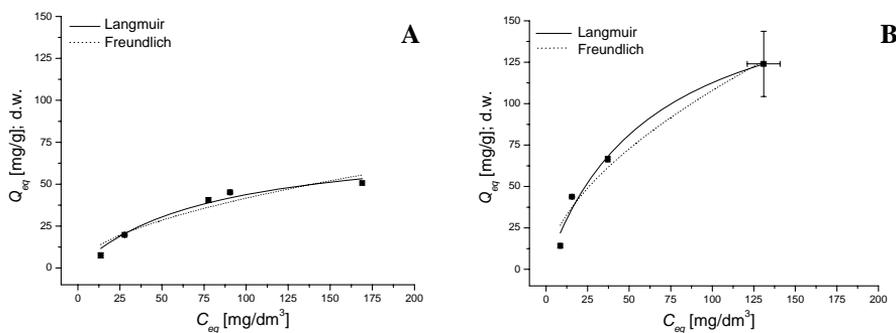


Fig. 5. Description of ThT (A) and MB (B) sorption by the leaf biomass of the hop (*H. lupulus* L.) variety Bohemie according to Langmuir and Freundlich isotherms. Conditions of experiments are described in Fig. 4B. Error bars represent standard deviation of the mean ($n = 3$).

The fact that the experiments concerning the effect of initial concentration of dyes on the sorption of dyes by the leaf biomass of the hop were carried out at constant temperature and within the time, when the concentration equilibrium was reached, allows to evaluate the obtained data by sorption isotherms. Sorption isotherms according to the Langmuir (Eq. 3) and Freundlich (Eq. 4) are often used to describe the sorption data. These mathematical models provide important parameters to characterize the sorption processes of dyes or metals binding by a wide range of sorbents derived from the plant waste biomass from the point of view of the sorption mechanism, characterization of sorption surface or affinity of sorbent to sorbate, and the practical utilization of studied sorbents as well (BANERJEE and CHATTOPADHYAYA, 2013; OGUNTMEIN, 2015). For description of the obtained data, the linear and non-linear forms of these models can be used. However, as noted by several authors (see e.g. NCIBI *et al.*, 2009; ALBADARIN and MANGWANDI, 2015), the application of mentioned models in non-linear forms is preferable than the utilization of their simplified linearized forms, mainly due to the potential changes in the distribution of errors after transforming the data to a linear form.

The description of obtained data by sorption isotherms according to the Langmuir and Freundlich for both studied dyes in the form of the dependences between the equilibrium specific sorption Q_{eq} (in mg/g; d.w.) of dye on the leaf biomass of the hop and the equilibrium concentration of the dye C_{eq} (in mg/dm^3) in the solution

is depicted in the Fig. 5. In the first step, the suitability of both used mathematical models to description of obtained data was evaluated according to the comparison of determination coefficients R^2 . The obtained values of parameters from mathematical description of the data characterizing ThT and MB sorption by the leaf biomass according to the Langmuir and Freundlich isotherms are mentioned in the Table 2. It is evident that the process of ThT and MB sorption is better described by the Langmuir model ($R^2 = 0.972$ for ThT and $R^2 = 0.982$ for MB) than according to the Freundlich model ($R^2 = 0.913$ for ThT and $R^2 = 0.966$ for MB) of sorption isotherm. Similar results were obtained in our previous work also concerning the sorption of ThT and MB by dried biomass of freshwater moss *Vesicularia dubyana* (PARTELOVÁ, 2012).

Table 2. Obtained values of parameters from mathematical description of the data characterizing ThT and MB sorption by the leaf biomass of the hop (*H. lupulus* L.) variety Bohemie according to Langmuir and Freundlich isotherms.

Model		Q_{max}	b	K	$1/n$	R^2
		[mg/g]; d.w.	[dm ³ /mg]	[dm ³ /g]; d.w.		
Langmuir	ThT	77.6	0.0129	-	-	0.972
	MB	184	0.0159	-	-	0.982
Freundlich	ThT	-	-	3.30	0.548	0.913
	MB	-	-	7.83	0.570	0.966

From the obtained parameters of applied sorption isotherms, the parameter Q_{max} characterizing the maximum sorption capacity (mg/g; d.w.) of the dried leaf biomass of the hop for the given sorbate – dye is the most important parameter. On the basis of this parameter, it is possible to compare the various sorbents derived from the plant waste biomass as well as to study the affinity of different sorbents to the given sorbates (dyes or metals). According to obtained values of Q_{max} for ThT and MB, it can be concluded that in the case of MB the dried leaf biomass of the hop showed more than 2-times higher sorption capacity ($Q_{max} = 184$ mg/g; d.w.) in comparison with the value obtained for ThT ($Q_{max} = 78$ mg/g; d.w.). Thus, the dried leaf biomass has more than 2-times higher affinity to MB than to ThT dye.

4. Conclusions

Experiments focused on the removal of Cd from aqueous solutions of CdCl₂ spiked with radionuclide ¹⁰⁹Cd and synthetic dyes thioflavine T (ThT) or methylene blue (MB) from single dye solutions under conditions of batch systems showed that dried plant biomass of hop (*Humulus lupulus* L.) as agricultural by-products can be used as a sorbent of both types of studied contaminants.

The maximum sorption capacity $Q = 264$ μmol Cd/g (d.w.) was found in the case of the leaf biomass of hop (*H. lupulus* L.) variety Osvald's clone 72 at the initial concentration of CdCl₂ 10,000 μmol/dm³, whereby the sorption capacity decreased in the order $Q_{leaves} : Q_{stems} : Q_{roots} = 1.0 : 0.8 : 0.7$. Individual experiments showed that Cd sorption increased with increasing initial Cd concentration in the solution. For the leaf biomass, but not in the case of stem or root biomass, the linear dependence

between the sorption Q and the initial concentration of CdCl_2 was observed. Thus, the biomass of stem and root of hop showed a partial saturation with Cd^{2+} cations at the highest applied initial Cd concentrations. The sorbed amount of Cd was removed from the hop biomass with the following increasing desorption efficiency (in %) of the extraction reagents: deionised water $\ll 0.1 \text{ mol/dm}^3$ $\text{HCl} \leq 0.1 \text{ mol/dm}^3$ EDTA- Na_2 .

Similarly as in the case of Cd sorption by dried biomass of leaves, stems, and roots of hop (*H. lupulus* L.) variety Osvald's clone 72, the kinetics of ThT and MB sorption by the leaf biomass of the hop (*H. lupulus* L.) variety Bohemie were also showed as a two-phase process. The maximum sorption of ThT approx. $Q = 19 \text{ mg/g}$ (d.w.) and MB approx. $Q = 70 \text{ mg/g}$ (d.w.) were found within the range of the initial values of pH 4 – 7. Approx. 50 % decrease in both dyes sorption was observed at the pH 3 and the minimal sorption $Q \approx 1 \text{ mg/g}$ (d.w.) for both dyes was calculated at the initial value of pH 2. The sorption of both dyes by the leaf biomass of the hop from single dye solutions decreased with increasing concentration of the biomass and on the other hand increased with increasing the initial concentrations of ThT or MB. The process of ThT and MB sorption was better described by the Langmuir model than the Freundlich model of sorption isotherm. From the obtained values of Q_{max} for ThT and MB, it was found that in the case of MB the dried leaf biomass of the hop showed more than 2-times higher sorption capacity ($Q_{\text{max}} = 184 \text{ mg/g}$; d.w.) in comparison with the value obtained for ThT ($Q_{\text{max}} = 78 \text{ mg/g}$; d.w.).

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