Bulgarian Chemical Communications, Volume 56, Special Issue C (pp. 72-77) 2024 *DOI: 10.34049/bcc.56.C.SI-9*

Biosorption of Cu (II) ions on a waste material of plant origin using factorial design approach

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Received: July 31, 2024: Revised: August 28, 2024

The present study deals with the application of an experimental design approach in order to clarify the optimal experimental conditions for biosorption output signals (A% and Q_e) depending on three input factors (pH, mass and concentration C_0). The major goal is to create adequate regression models able to correctly describe the area determined by the intervals of variation of the inputs and, to use the modes for predictive and optimization studies. The experimental design procedure offers very reliable regression models (with multiple correlation coefficients r^2 for Qe and A% for calculated vs. experimentally obtained values of 0.923 and 0.921, respectively). Additional contribution of the study is the use of linear regression models without mixed coefficients, which improves the model interpretation.

Keywords: *Melissa officinalis* L., waste material, adsorption, copper ions, experimental design

INTRODUCTION

Melissa officinalis L. (also named: lemon balm, balm, bee balm, sweet balm, common balm, honey balm or balm mint) is a widespread and well-known all over the world aromatic perennial medicinal plant of Lamiaceae (mint family). It is intensively cultivated in Europe [1-3] and particularly in our country. Thanks to its components, the plant is used for treatment of numerous diseases and health problems [4, 5]. Biological activities of *Melissa officinalis* L. include antioxidant, antimicrobial, antiviral, anti-inflammatory, antidepressant, anxiolytic, neurological, sedative, analgesic, spasmolytic and other effects, which are related to the therapeutic properties of its essential oils and its main active constituents as volatile compounds (geranial, neral, citronellal and geraniol), triterpenes (ursolic acid and oleanolic acid), phenolic acids (rosmarinic acid, caffeic acid and chlorogenic acid), tannins and flavonoids (quercetin, rhamnocitrin, and luteolin) [1-3, 5, 6].

Environmental contamination by heavy metals is a serious problem worldwide, due to their toxic effects and persistence. Copper is among the most common pollutants, found in surface water and groundwater, as well as in industrial wastewater [7]. Copper pollution is generated by various industrial processes, mainly from mining, metallurgical and electroplating industries.

Although copper is an essential element for humans, it can be very harmful at high concentrations and has to be removed from contaminated waters. The conventional methods for the removal of metal ions are often restricted because of technical or economic constraints [8, 9]. This has led to the development of new and improved methods for treatment of polluted waters. Biosorption, based on metal binding capacities of various biological materials, is an effective technique for removal of toxic metals.

In recent years, essential oil plants have attracted increasing interest as biosorbents, as they contain numerous active phytocomponents capable of binding metals. Our experience with materials based on several medicinal plants as adsorbents for Cu(II) including *Melissa officinalis* L. [10-15] show that plant materials possess abilities for removal of Cu(II) ions from contaminated waters. The medicinal plant *Melissa officinalis* L. also proved to be a good adsorbent for Cu(II) ions [14]. The production of essential oils from medicinal plants like *Melissa officinalis* L. is well developed worldwide [16]. In addition to the medical uses of *Melissa officinalis* L. essential oils, they are useful for cosmetics, food industry, aromatherapy, etc. As the amount of essential oils in *Melissa officinalis* L. is particularly low - between 0.01 and 0.72% [2], enormous amounts of wastes are generated during the process of extraction of these essential oils.

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It is an important task to find practical application for these wastes. The biosorption of toxic metals from contaminated waters is a suitable possibility for such application.

For several decades Bulgaria is one of the leaders in the herbal export list in Europe – between 13 000 and 17 000 t herbs annually. About 25 % of this export is obtained from the cultivated ethereal oil plants. On the other hand, Bulgaria is a major producer of copper and copper concentrates and is one of the largest in Europe. Among the copper mining and processing companies are Aurubis Bulgaria plant in the Srednogorie region, Ellatzite Med with open-pit mine located near the town of Etropole and the flotation complex in Mirkovo village, Stara planina Mountain. Therefore, the removal of copper ions from contaminated waters is a problem of great significance [13].

Using renewable or waste materials, biosorption can be economically even more attractive than known conventional cleaning methods. In this regard, it was a challenge to study the biosorption capacities of waste plant materials provided to us by "Essential Oils and Herbs" distillery, Zhelyo Voivoda village, Burgas city, Bulgaria. This offered us an opportunity to utilize the wastes after extracting the valuable essential oils from the plants and applying them in adsorption processes related to environmental protection. It is proved by our previous studies, that the initial concentration, the adsorbent amount and the acidity of initial solution are the most important parameters that affect the adsorption process.

For long time design of experiment (DoE) proved to be a very useful approach in assessing the impact of many input experimental factors (as independent variables) on one or more selected output analytical signals (as dependent variables). The design is carried out according to a preselected experimental plan allowing simultaneous variation of the inputs used (full factorial design) on two or more levels of variation [17-20].

The major goal of the present study is to use the DoE approach as an appropriate chemometric technique for modeling of the impact of the input experimental factors on predetermined output signals in order to reach an adequate regression model of the system studied and to optimize, if needed, the output signals being registered. In this aspect experimental design of the adsorption process for the plant material *Melissa officinalis* L. after essential oils extraction (denoted as M) was made using the impact of three input factors on two output functions as follows:

- Input factors: X1 – pH; X2 – Co (initial Cu(II) concentration (mg L^{-1}); X3 – mass of adsorbent (g);
- Output functions: Y1 – Qe (amount of adsorbed copper ions per gram sorbent, mg g^{-1}); Y2 – A (% of adsorption).

EXPERIMENTAL

Sample preparation

The plant material M (after essential oils extraction) was washed several times with distilled water to remove surface-adhered and water-soluble particles and dried at 60°C in an electric oven for 48 h. After that it was milled in an electric grinder to a size of particles below 200 μm. No other physical or chemical treatment was performed.

Adsorption experiments

Batch experiments were carried out using stoppered 50-mL Erlenmeyer flasks containing various sample amounts and 20 mL of aqueous solution of Cu(II) ions with different concentrations and different acidity. The mixtures were shaken at room temperature (20°C) on an automatic shaker. After the experiment the biomaterial was removed by filtration through a Millipore filter (0.2 μm).

For the adsorption experiments initial Cu(II) concentrations (C_o) of 100; 200 and 300 mg L^{-1}; pH values of 2.0, 3.5 and 5.0; and sample amounts (mass) of 0.05; 0.20 and 0.35 g were chosen. It is an usual procedure in experimental design to standardize the real factor values with (+1) as the upper level of variation of a certain input factor and (-1) as the low level of variation of the factor. Generally, it helps in calculational work and in rapid check of the output function value according to the model obtained. The different combinations of these input data are given in Table 1.

Table 1. Input factors for full factorial design of the adsorption process of Cu(II) ions – eight basic experiments. Information matrix for DoE (as absolute and as coded values).

Exp. No	pH (X_1)	Initial concentration (X_2) [C_o, mg L^{-1}]	Mass (X3) [g]
1	5.00 (+1)	300 (+1)	0.35 (+1)
2	2.00 (-1)	300 (+1)	0.35 (+1)
3	5.00 (+1)	100 (-1)	0.35 (+1)
4	2.00 (-1)	100 (-1)	0.35 (+1)
5	5.00 (+1)	300 (+1)	0.05 (-1)
6	2.00 (-1)	300 (+1)	0.05 (-1)
7	5.00 (+1)	100 (-1)	0.05 (-1)
8	2.00 (-1)	100 (-1)	0.05 (-1)

The working standard solutions of Cu(II) ions with concentrations of 100, 200 and 300 mg L^{-1} were prepared by stepwise dilution of a stock solution with concentration of 1000 mg Cu L^{-1} ($CuCl_2$ in

H_2O), Titrisol® Merck, (Darmstadt, Germany). All reagents used throughout the experiments were of analytical grade.

The initial and equilibrium copper concentrations were determined by flame AAS model Thermo Elemental SOLAAR - M5 AA spectrometer (Thermo Fisher Scientific, USA) using standard experimental conditions and analytical line of 324.8 nm.

The initial pH of the working standard solutions was adjusted to the required value with 0.1 M HCl and NaOH solutions before mixing the suspension with pH-meter model pH 211 (Hanna instruments, Germany).

The percentage of adsorption (A) and the amount of adsorbed copper ions per gram sorbent Qe were calculated using the following relationships:

$$A(\%) = ((C_0\text{-}Ce)/ Co) \times 100 \quad (1)$$

$$Qe = (C_0 - Ce) \times V/m \quad (2)$$

where Co = initial concentration (mg L^{-1}), Ce = equilibrium concentration (mg L^{-1}); m = mass of adsorbent (g), and V = solution volume (L). All measurements were replicated and the average results were used.

Experimental factorial design

The system subject to experimental design takes into account the impact of three input factors on two output functions as described above.

The values of the output functions Y1 – Qe (amount of adsorbed copper ions per gram sorbent, mg g^{-1}); Y2 – A (% of adsorption) are presented in Table 2.

Full factorial design on two levels of variation of the input factors (2^3 type) was organized for each one of the output functions. Totally 8 experiments were performed for each Yi and carried out in a random way to avoid memory effects of block experiments. In order to estimate the experimental error replicas of each experiment were done. The software package used for calculation of the regression models and graphical presentation of the results was *CHEMOFACE*.

The experimental design used allows to estimate polynomial regression models with 8 regression coefficients assessing the intercept (presented by one coefficient), the single factor impact (Xi – totally 3 coefficients), the two-factor interaction impact (XiXj – totally 3 coefficients) and three-factor interaction impact (XiXjXk – totally 1 coefficient). The models obtained for each one of the output functions are of the type:

$$Y = a_o + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 + a_{123}X_1X_2X_3 \quad (3)$$

The DoE procedure needs to assess the statistical significance of each regression coefficient and to offer a linear or nonlinear regression model. An important task of the experimenter is to make the final decision which model to select based on the own experience with the system in consideration and of the interpretation of the physical meaning of the interaction in the system (the role of the mixed regression coefficients).

RESULTS AND DISCUSSION

Output function Q_e

In Figure 1 the effects of the inputs on Q_e are illustrated (Pareto chart). As seen in the figure, the ranking of the significant ($p>0.05$) impacts is as follows: *Mass, pH,* mixed (nonlinear) interaction *Mass/pH*, *C_o*, mixed (nonlinear) interaction *Mass/C_o*. The other two mixed interactions are statistically insignificant. It could be concluded that *Mass* is the most significant effect for the system in consideration. The effect of mass-input is negative – increasing "mass" would lead to a decrease in the output function Q_e. The acidity has also a significant impact on the output function, and C_0 is with the lowest impact as separate input. Two mixed interactions both of which include the mass input could be considered as contributors to the output function. However, it is our decision to use a linear model to describe the system since it is possible the significant influence of the mass to cause the additional mixed effects. It is a simple way to describe the system studied and if the linear model is valid (adequate) the optimization of the system becomes easier.

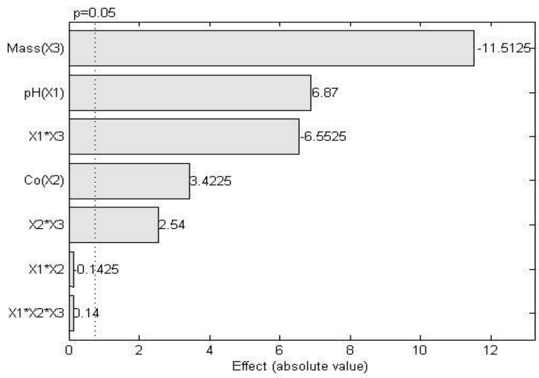

Fig. 1. Pareto chart for DoE (Qe as output function)

It is important to note that the effects found are a qualitative description of the system responses and in order to get a model the regression coefficients should be calculated and statistically assessed. In Table 3 the calculated regression coefficients are presented along with the statistical assessment of their significance (ANOVA).

Table 2. Output parameters for full factorial design of the adsorption process of Cu(II) ions (mean values of two parallel adsorption experiments).

Exp. No	Qe (1) [mg g^{-1}]	Qe (2) [mg g^{-1}]	Qe mean ($\bar{Y}1$) [mg g^{-1}]	A (1) [%]	A (2) [%]	A mean ($\bar{Y}2$) [%]
1	10.03	9.85	9.94	69.64	68.45	69.045
2	9.65	9.60	9.625	60.14	59.79	59.965
3	3.96	4.00	3.98	75.48	76.29	75.885
4	3.64	3.68	3.66	66.93	67.72	67.325
5	24.75	25.90	25.325	24.60	25.79	25.195
6	13.17	11.20	12.185	11.74	9.96	10.85
7	25.15	24.30	24.725	68.66	66.21	67.435
8	11.38	10.66	11.02	29.92	28.08	29.00

Table 3. Statistical (ANOVA) assessment of the results from regression analysis for Q_e

	b	error	t	p	significance
b_0	8.80	0.56	15.81	2.56e-07	significant
X1	2.90	0.11	21.59	2.23e-08	significant
X2	0.02	0.002	19.75	4.92e-06	significant
X3	-38.37	1.06	-36.17	063.74e-10	significant

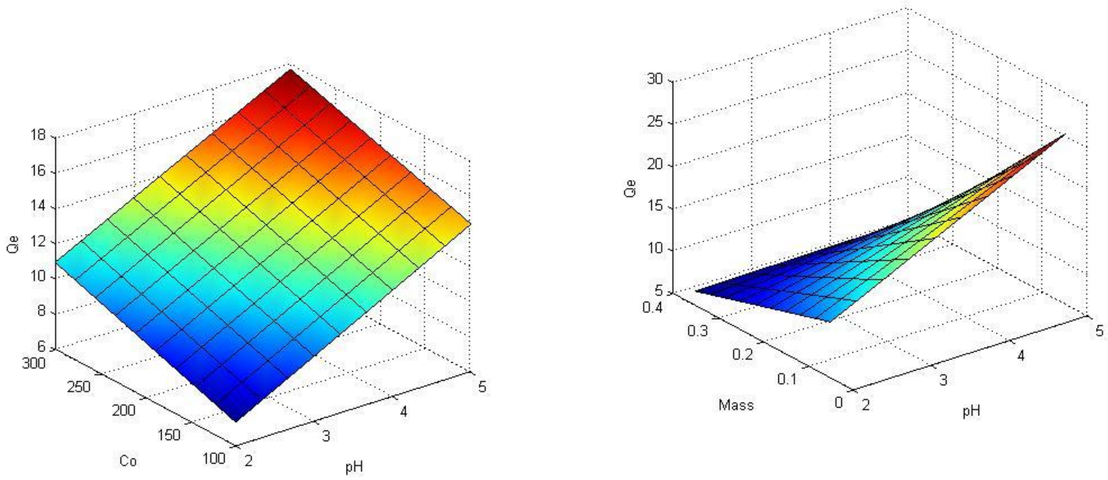

Fig. 2. 3D surface diagrams: (a) Qe = f(pH, Co); (b) Qe = f(pH, mass)

The linear model is as follows:

$$Q_e = 8.80 + 2.9\,X_1 + 0.02\,X_2 - 38.37\,X_3 \quad (4)$$

The regression model was checked for adequateness (by calculating the correlation coefficient for “calculated/measured” values and by least square method). The calculated (adjusted) values of the multiple correlation coefficient prove to be high (r^2 = 0.923). It shows that the model is valid for the area of input factors variation and could be further used for optimization purposes (prediction). Fig. 2a represents a 3D-surface diagram for the relationship between the output function Qe and the input factors X_1 and X_2 (pH and C_o). Since the effect of mass is with highest rank of influence, it is of substantial interest to illustrate the joint effects of the factors with lower rank.

It could be seen that highest responses of Qe are achieved at variation of pH since C_0 values do not affect them significantly. The specific (negatively directed effect of X_3 (mass) on Q_e) is demonstrated on Figure 2b.

Output function A

As seen in Table 4, all effects of the input factors on the output function A are presented and taken into account.

Table 4. Significance of single and mixed effects of inputs on the output function A (statistical significance p = 0.05)

Factors	Effects	Significance
X_1 (pH)	17.61	Yes
X_2 (C_0)	-18.65	Yes
X_3 (mass)	34.94	Yes
X_1X_2	-2.85	No
X_1X_3	-3.96	No
X_2X_3	4.35	No
$X_1X_2X_3$	3.16	No

The highest rank of impact is again for X_3 (mass factor) but for this output function the effect of mass is not only the highest but positive. Factor X_2 is ranked second with relatively high negative impact on A which is another difference as compared to the low positive impact of C_o on the output Qe. X_1 (pH) is with the lowest rank from the single factors but still with very high positive effect. All mixed interactions are not statistically significant which indicated dominantly linear effects for the system in consideration. It could be assumed that the regression model of this system would be rather linear than typical linear although the mixed coefficients could be conditionally used if the linear model proves to be non-adequate. It has to be stressed again that the linear models are preferable for a system of the type considered as mixed coefficient significance is sometime hard to theoretically explain. In Table 5 the calculated regression coefficients are presented along with the statistical (ANOVA) assessment of their significance. The model includes an intercept, which is normal for regression models. The linear model is as follows:

$$A= 25.41 + 5.87\, X_1 - 0.09X_2 +116.45\, X_3 \tag{5}$$

Figs. 3a and 3b illustrate the 2D surface diagrams for the dependence of A on the combination of two input factors. Maximal values for A are achieved for pH 5 and mass above 0.3 (both factors with positive impact). The negative impact of C_0 is well expressed – A shows maximal values (less than that in Fig. 3a) for mass above 0.3 and concentration towards the lower value of 100.

Table 5. Statistical (ANOVA) assessment of the results from regression analysis for A.

	b	Error	t	p	Significance
b_0	25.41	8.25	2.98	0.011	Yes
X1	5.87	1.62	3.61	0.004	Yes
X2	-0.09	0.02	-3.83	0.0024	Yes
X3	116.45	16.25	7.17	1.14e-05	Yes

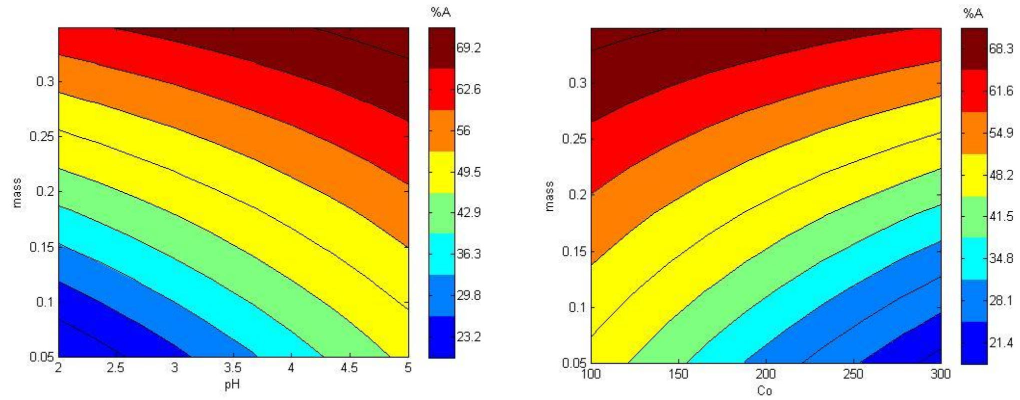

Fig. 3. 2D surface diagrams for: **(a)** A = f(pH, mass); **(b)** A = f(C_0, mass).

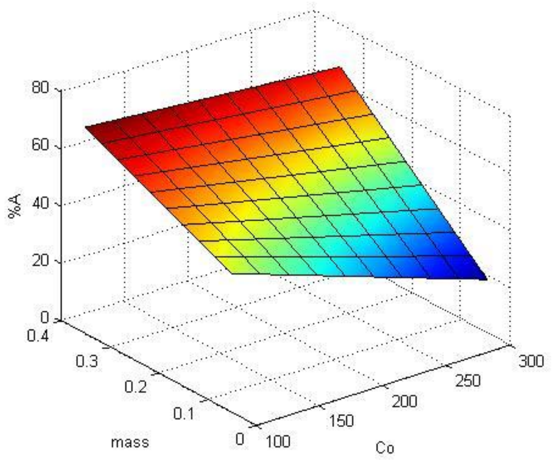

Fig. 4. 3D surface diagram for A = f(C_0, mass)

The same interpretation holds true if a 3D surface diagram (Fig. 4) is constructed for the relationship A = f(Co, mass).

The model for assessing A response function depending on the three input factors mentioned above was checked for adequateness by comparing "calculated to experimentally registered" responses (by calculating the multiple (adjusted) correlation coefficient r^2) and by the least square method. Both approaches proved the model validity ($r^2 = 0.921$) and its ability to correctly describe the area covered by the input factor levels and, additionally, to be used for prediction purposes (optimization).

CONCLUSIONS

The experimental design carried out made it possible to correctly rank the input factors responsible for the performance of the biosorption procedure using plant as a biosorbent. The usage of two output functions indicated that the biosorption yield for each output could differ with respect to the mechanism of registration. Therefore, the experimenter should take into account the way of signal reading when ordering the experimental conditions for the biosorption procedure. The applicability of the simple linear regression models as proven by the experimental design is an easy way to find optimal conditions for its performing.

Acknowledgement: *The authors acknowledge the support from the Operational Programme "Science and Education for Smart Growth" 2018-2023, grant number No BG05M2OP001-1.001-0008-C02 (National Centre of Excellence in Mechatronics and Clean Technologies); Distributed Research Infrastructure INFRAMAT, part of Bulgarian National Roadmap for Research Infrastructures (project Д01-306/20.12.2021) and ESHER, grant agreement D01-161/28.07.22.*

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