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REGULAR ARTICLE

# RESPONSE SURFACE METHODOLOGY AS OPTIMIZATION TOOL IN STUDY OF COMPETITIVE EFFECT OF $Ca^{2+}$ AND $Mg^{2+}$ IONS IN SORPTION PROCESS OF $Co^{2+}$ BY DRIED ACTIVATED SLUDGE

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## ABSTRACT

The aim of present study was to investigate biosorption of cobalt ions onto dried activated sludge (DAS) from ternary solution Co-Ca-Mg, as a function of pH and concentration of cobalt, calcium and magnesium via Box-Behnken design under Response surface methodology (RSM). The four parameters namely cobalt initial concentration of 2000 μmol/L, calcium initial concentration of 2000 μmol/L, magnesium initial concentration of 2000 μmol/L and pH of 5.5 were chosen as center points from the previous study of metal sorption process. The experimental data on sorption capacity $Q_{\mathrm{exp}}$ of DAS for $Co^{2+}$ ions were obtained by $^{60}Co$ gamaspectrometry and fitted into a quadratic polynomial model using multiple regression analysis. The experimental Box-Behnken design revealed that biosorption of cobalt ions from ternary system increase with increasing pH of the system, initial cobalt concentration and magnesium concentration. Results showed also drastic inhibitory effect of increasing calcium concentration in sorption process of cobalt ions. The present study provides valuable information about combined effect of independent variables on sorption process from multivariable system and enables us to minimize number of needed experiments from 60 to 29.

**Keywords:** sorption, activated sludge, cobalt, co-ions, RSM, $^{60}Co$ gamaspectrometry

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# INTRODUCTION

Under influence of urbanisation in recent decades WWTPs produce large amounts of sewage sludge. Treatment and final disposal are important for reducing overage of sludge that can cause problem in the environment. Traditional sludge treatment methods include landfilling, application to the soil as soil conditioners or incineration. Sludge stored on landfill carries the risk in leaching of accumulated heavy metals to the environment. Incineration plays an important role in recent years. However, concerns over its rising energy cost, ash disposal and gaseous emissions continue to be significant constraints on its more widespread use **(Deng *et al.*, 2009)**. Farmland application is also limited by the uptake capacity of the soil and the potential pollution by the heavy metals. The disposal method appears to be an economical and promising option because sludge can supply crops with large amounts of organic matter and inorganic nutrients and improve soil structure. However, the presence of heavy metals in the sludge significantly restricts their use for agricultural purposes. Unlike organic pollutants, heavy metals are persistent environmental contaminants that cannot be destroyed **(Wade *et al.*, 1993)**. There is a tendency to decrease the amounts of produced sludge or use them as secondary renewable source directly or after appropriate processing. At present, liquid waste waters are mostly treated by the chemical methods, such as chemical precipitation, electrochemical treatment and ion exchange. These conventional methods have several limitations such as inefficient precipitation when metals are present as anions or are complexes, final concentration imposed by the solubility product and formation of unsettleable particles. The alternative use of biosorbents or sorbents prepared from microbial cells for the removal and recovery of heavy metals from industrial effluents can be economical and effective methods for metal removal. The metal removal ability of microorganisms including bacteria (**Erdogan and Ulku, 2012**), moss (**Marešová *et al.*, 2011**), microalgae **(Lourie and Gjengedal, 2008; Kaduková and Virčíková, 2005)** and fungi (**Kumar *et al.*, 2011**) has been studied extensively. Biomaterials, such as waste sludge, digested sludge, and waste biomass, are also suitable for the recovery of heavy metals (**Aksu *et al.*, 2002; Pamukoglu and Kargi, 2006; Wang *et al.*, 2006**). Activated sludge of WWTP contains variable amounts of heavy metals, however, sorption capacity of sludge is generally much higher and it can be consider as cheap sorbent. Waste waters contain not one but many kinds of metal ions. Although much research information is available on the single-metal biosorption and bioaccumulation (**Kaduková *et al.*, 2008)**, relatively less attention has been paid to the biosorption of multi-metal-ion systems (**Aksu and Domnez, 2006**), and no general trend was observed. Examining the effects of divalent cations in various combinations is more representative of the actual environmental problems faced by organisms than the studies of a single metal. Removal of single species of metal ions using microorganisms is affected by several factors. These factors include the specific surface properties of the microorganism and the physico-chemical parameters of the solution such as temperature, pH value, initial metal concentration and amount of biomass. Many other parameters affect the capacity of microorganisms to bind more than one species simultaneously. The combined effects of two or more metal ions to microorganisms also depend on the number of metal ions competing for binding sites, metal ion combination, levels of metal ion concentration, etc. (**Yan *et al.*, 2010**).

There are many statistical approaches of assessment of effect these factors. Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing the effects of several independent variables on the response (**Box and Draper, 1987**). RSM has an important application in the process design and optimization as well as the improvement of existing design. This methodology is more practical compared to theoretical models as it arises from experimental methodology which includes interactive effects of the variables and, eventually, it depicts the overall effects of the parameters on the process (**Bas and Boyaci, 2007**).

The aim of our present research was to study the competitive effect of the initial concentration of $Co^{2+}$, $Ca^{2+}$, $Mg^{2+}$, and the pH of the solution on $Co^{2+}$ biosorption using dried activated sludge (DAS) from model aqua system.

## MATERIAL AND METHODS

### Preparation of sorbent and chemicals

Activated sludge was obtained from wastewater treatment plants (WWTP) in Enviral Inc. (Leopoldov, Slovak Republic). After washing twice in deionised water, the sludge was oven dried at 90 °C for 72 h, ground and sieved. Powdered dried activated sludge (DAS) of particle size <450 µm was used for metal sorption studies. Standardized $^{60}CoCl_2$ solution (5.181 MBq/ml; 20 mg/L $CoCl_2$ in 3 g/L HCl) was obtained from the Czech Institute of Metrology. For determining of the competitive effect of $Me^{2+}$ ions, water solutions of $CaCl_2$ and $MgCl_2$ in sorption experiments were used.

## Sorption experiments

Batch sorption experiments were carried out by suspending of DAS biomass (2.5 g/L d.w.) in water solutions of $CaCl_2$, $CoCl_2$ and $MgCl_2$ whose concentrations were set according to experimental design methodology. Solutions were spiked with $^{60}CoCl_2$ and adjusted to desired pH (5.95-6.10) values by chloric acid. Flasks were agitated on a reciprocal shaker (120 rpm) at 22°C, and after experiments, samples were taken for equilibrium study. Biomass was filtered out, washed twice in deionised water and the radioactivity of both activated sludge and liquid phase was measured. The metal uptake was calculated according to:

$$Q_{eq} = (C_0 - C_{eq})\frac{V}{M} \qquad (1)$$

where $Q$ is the uptake (μmol/L), $C_0$ and $C_{eq}$ are the initial and equilibrium liquid-phase concentrations of metal (μmol/L), $V$ is the volume (L), and $m$ is the amount of dried biosorbent (given in grams).

## Analytical equipments

The gamma spectrometric assembly using well type scintillation detector 54BP54/2-X, NaI(Tl) (Scionix, the Netherlands) and the data processing software Scintivision 32 (ORTEC, USA) were used for $^{60}$Co determination in DAS and supernatant fluids at the energy of γ-photons – 1173.24 keV. Determination of the initial cobalt content in sorbent was made by atomic absorption spectrometry (ETAAS) device ShimadzuAA-6300 (USA) with an electrothermal atomization equipment Shimadzu GFA-EX7i using a Shimadzu autosampler ASC6100 and background correction method of Smith-Hieftje, after decomposition of samples for elemental analysis using high-pressure digestion mineralization equipment DAH406Berghof(DE).

## Experimental design and optimization

In multivariable systems, the classical approach of changing one variable at a time to study the effects on other variables for a particular response is time consuming. Therefore, an alternative strategy involving statistical approaches, e.g., response surface methodology (RSM), was applied to solve for multiple variables in this complex system. This method of parametric optimization can also be combined very well to monitor the impact of factors affecting the sorption process and to quantify their impact. The advantage of the RSM method is the minimization of the number of experiments and time needed. In the optimization procedure we studied the response of the statistically designed combinations, estimated the coefficients by fitting the experimental data to the response functions, predicted the response of the fitted model and checked the adequacy of the model. We used the Box–Behnken design under response surface methodology (RSM) to investigate competitive effects in the ternary system of $Co^{2+}$-$Ca^{2+}$-$Mg^{2+}$. A total of 29 runs (Tab. 2) were used to optimize the process parameters – the initial pH of the solution, the initial concentration of $Co^{2+}$, $Ca^{2+}$ and $Mg^{2+}$. Each of experiment was performed in triplicate. The statistical software Statgraphics Centurion XV (StatPoint Inc., USA) was used for the experimental design, data analysis, quadratic model building, graph (three-dimensional response surface and contour) plotting and to estimate the responses of pH value, initial concentrations of $Co^{2+}$, $Ca^{2+}$ and $Mg^{2+}$. Levels of factors are shown in Table 1.

**Table 1** Experimental range and levels of independent variables for $Co^{2+}$ sorption using DAS

| **Independent variables** | **Symbols** | **Unit** | **Coded levels** | | |
|---|---|---|---|---|---|
| | | | -1 | 0 | +1 |
| $C_0$ $Co^{2+}$ | A | µmol/L | 1000 | 2000 | 3000 |
| pH | B | - | 3.0 | 5.5 | 8.0 |
| $C_0$ $Ca^{2+}$ | C | µmol/L | 1000 | 2000 | 3000 |
| $C_0$ $Mg^{2+}$ | D | µmol/L | 1000 | 2000 | 3000 |

Regression analysis was performed to determine the surface response as function of second order polynomial equation:

$$Q = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ij} x_i^2 + \sum_{i \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \qquad (2)$$

where $Q$ is predicted response (specific sorption of $Co^{2+}$ ions), $\beta_i$, $\beta_{ii}$, $\beta_{ij}$ represent linear, quadratic and interaction effects. $\beta_0$ is the intercept term and $x_i$, $x_j$, …$x_k$ are the input variables ($C_0$ $Ca^{2+}$, $C_0$ $Co^{2+}$, $C_0$ $Mg^{2+}$ and the pH of the solution) which affect the $Q$ value.

**Table 2** Box-Behnken design matrix, the experimental and predicted values of specific sorption capacity $Q_{eq}$ of DAS for $Co^{2+}$ ions. A. the initial concentration of $Co^{2+}$ (µmol/L); B. the pH of the solution; C. the initial concentration of $Ca^{2+}$ (µmol/L); D. the initial concentration of $Mg^{2+}$ (µmol/L)

| Run | Coded levels | | | | Y ($Q_{eqCo}$) | |
|---|---|---|---|---|---|---|
| | A | B | C | D | $Q_{eq}$ (exp.) (µmol/g) | $Q_{eq}$ (pred.) (µmol/g) |
| 1 | -1 | -1 | 0 | 0 | 78.00 | 87.79 |
| 2 | +1 | -1 | 0 | 0 | 147.00 | 145.29 |
| 3 | -1 | +1 | 0 | 0 | 114.00 | 113.46 |
| 4 | +1 | +1 | 0 | 0 | 192.00 | 179.96 |
| 5 | 0 | 0 | -1 | -1 | 139.00 | 141.29 |
| 6 | 0 | +1 | +1 | 0 | 126.00 | 119.96 |
| 7 | 0 | 0 | -1 | +1 | 141.00 | 144.79 |
| 8 | 0 | 0 | +1 | +1 | 124.00 | 119.46 |
| 9 | -1 | 0 | 0 | -1 | 108.00 | 112.13 |
| 10 | +1 | 0 | 0 | -1 | 162.00 | 164.13 |
| 11 | -1 | 0 | 0 | +1 | 106.00 | 103.63 |
| 12 | +1 | 0 | 0 | +1 | 180.00 | 175.63 |
| 13 | 0 | -1 | -1 | 0 | 123.00 | 120.96 |
| 14 | 0 | +1 | -1 | 0 | 154.00 | 150.63 |
| 15 | 0 | -1 | +1 | 0 | 94.00 | 97.13 |
| 16 | 0 | 0 | +1 | -1 | 126.00 | 127.79 |
| 17 | -1 | 0 | -1 | 0 | 120.00 | 112.92 |
| 18 | +1 | 0 | -1 | 0 | 186.00 | 192.42 |
| 19 | -1 | 0 | +1 | 0 | 111.00 | 107.08 |
| 20 | +1 | 0 | +1 | 0 | 142.00 | 151.58 |
| 21 | 0 | -1 | 0 | -1 | 121.00 | 113.92 |
| 22 | 0 | +1 | 0 | -1 | 124.00 | 128.58 |
| 23 | 0 | -1 | 0 | +1 | 102.00 | 99.92 |
| 24 | 0 | +1 | 0 | +1 | 136.00 | 145.58 |
| 25 | 0 | 0 | 0 | 0 | 137.00 | 137.00 |
| 26 | 0 | 0 | 0 | 0 | 137.00 | 137.00 |
| 27 | 0 | 0 | 0 | 0 | 137.00 | 137.00 |
| 28 | 0 | 0 | 0 | 0 | 137.00 | 137.00 |
| 29 | 0 | 0 | 0 | 0 | 137.00 | 137.00 |

## RESULTS AND DISCUSSION

Real wastewater contains a wide range of materials (compounds) of organic and inorganic origin. Calcium and magnesium get into the water by leaching of calcite and dolomites eventually also from wastewater coming from industry working with the lime. Calcium and magnesium are conducive to water hardness, which has effect on diseases formation as well as water treatment. For that reason, a Box-Behnken design under RSM was used to analyse interactive effect of the initial concentration of cobalt, calcium and magnesium ions, and the pH value on specific sorption capacity of DAS.

Using multiple regression analysis, the second order polynomial model (Eq. 3), which characterizes the relationship between equlibrium uptake $Q_{eq}$ of cobalt using DAS and studied variables, was obtained.

$$\begin{aligned}Q_{eq} &= +36{,}405 + 0{,}01055 \times C_0Co + 17{,}41333 \times pH + 0{,}014283 \times C_0Ca - 8{,}8.10^{-3} \times C_0Mg \\ &+ 5{,}75.10^{-6} \times C_0{}^2Co - 1{,}78 \times pH^2 - 1{,}75.10^{-6} \times C_0{}^2Ca - 3{,}875.10^{-6} \times C_0{}^2Mg + 9.10^{-4} \times C_0Co \\ &\times pH - 8{,}75.10^{-6} \times C_0Co \times C_0Ca + 5.10^{-6} \times C_0Co \times C_0Mg + 1.10^{-4} \times pH \times C_0Ca + 3{,}1.10^{-3} \\ &\times pH \times C_0Mg - 1.10^{-6} \times C_0Ca \times C_0Mg\end{aligned} \quad (3)$$

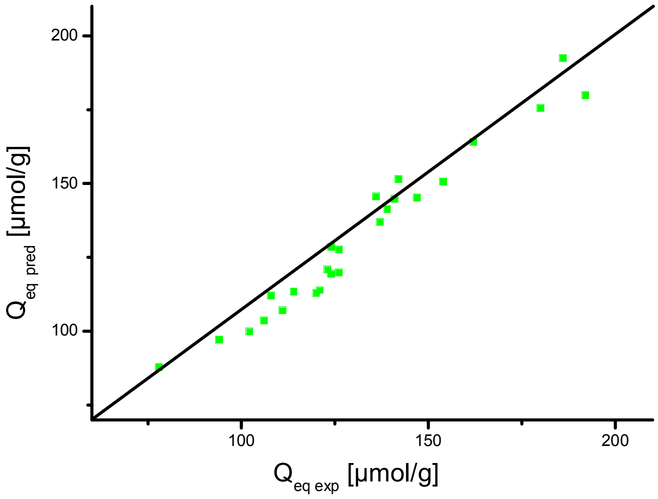


**Figure 1** Correlation between predicted $Q_{eq\ pred}$ and experimental $Q_{eq\ exp}$ values of cobalt sorption by DAS

The statistical significance of the model equation and the goodness of fit were evaluated by $R^2$ and by the $F$-test analysis of variance (ANOVA), which is a statistical technique that subdivides the total variation in a set of data into components associated with specific sources of variation to test hypotheses on the parameters of the model. A large *F*-value indicates that most of the variation can be explained by a regression equation whereas a low *p*-value (<0.05) indicates that the model is considered to be statistically significant (**Myers and Montgomery, 2002**).

**Table 3** Analysis of variance (ANOVA) for $Co^{2+}$ ions sorption by DAS

| **Source** | **Sum of squares** | **DF** | **F value** | **p-value Prob>F** |
|---|---|---|---|---|
| Model | 17855.59 | 14 | 23.45 | <0.0001 |
| $C_{0Co}$ | 11532.00 | 1 | 211.99 | <0.0001 |
| pH | 2730.08 | 1 | 50.19 | <0.0001 |
| $C_{0Ca}$ | 1633.33 | 1 | 30.03 | <0.0001 |
| $C_{0Mg}$ | 6.75 | 1 | 0.12 | 0.7299 |
| $C_{0Co}$×pH | 20.25 | 1 | 0.37 | 0.5516 |
| $C_{0Co}$× $C_{0Ca}$ | 306.25 | 1 | 5.63 | 0.0325 |
| $C_{0Co}$× $C_{0Mg}$ | 100.00 | 1 | 1.84 | 0.1966 |
| pH× $C_{0Ca}$ | 0.25 | 1 | 4.59E-003 | 0.9469 |
| pH× $C_{0Mg}$ | 240.25 | 1 | 4.42 | 0.0542 |
| $C_{0Ca}$× $C_{0Mg}$ | 4.00 | 1 | 0.074 | 0.7902 |
| $C_{0Co}^2$ | 214.46 | 1 | 3.94 | 0.0670 |
| $pH^2$ | 802.80 | 1 | 14.76 | 0.0018 |
| $C_{0Ca}^2$ | 19.86 | 1 | 0.37 | 0.5553 |
| $C_{0Mg}^2$ | 97.40 | 1 | 1.79 | 0.2022 |
| Residuals | 761.58 | 14 | 54.40 | |
| Lack of fit | 761.58 | 10 | 76.16 | |
| Pure Error | 0.00 | 4 | 0.000 | |
| Total | 18617.17 | 28 | | |
| $R^2$ | 0.9591 | $R^2_{adj}$ | 0.9622 | |

The results of ANOVA for sorption efficiency of cobalt ions from ternary system Co-Ca-Mg onto DAS are given in Table 3. The adequacy (statistical significance) of a quadratic model was tested through *F*- and *p*-values. It has long been known that a large *F*-value indicates that most of the variation can be explained by a regression equation whereas a low *p*-value ($<0.05$) indicates that the model is considered to be statistically significant (**Myers and Montgomery, 2002**). The model *F*-value of 23.45 and value of $p$ <0.0001 indicate statistical significance of a quadratic model. On the basis of this investigation, the relationship between the independent variables (the initial concentration of cobalt, calcium and magnesium, and the pH of the solution) and the response (specific sorption capacity of DAS for cobalt) can be explained according to the regression model. The goodness of the model can be confirmed by the coefficient of determination $R^2$ (0.9591) and the adjusted $R^2$. Both values are closed to 1, which are very high and indicate a high correlation between the observed and the predicted values **(Garg *et al.*, 2008).** Value of adjusted $R^2$ (0.9622) suggest that the total variation of 96.22 % $Q_{eq}$ is attributed to the independent variables and only about 3.78 % of the total variation cannot be explained by the model. The adequacy of quadratic model was tested also through the correlation between calculated and experimental values for cobalt sorption by DAS, which is shown in scattered plot (Fig. 3). From correlation between predicted $Q_{eq\ pred}$ and experimental $Q_{eq\ exp}$ values of cobalt sorption by DAS is evident that the regression model can represent the experimental sorption data well. On the basis of the evaluation of ANOVA outputs, the statistical significance of a quadratic model for the response was confirmed and it can be concluded that the model can be used for further analysis of effect of process variables.

As was already mentioned above, the aim of this study was to analyze the effect of independent variables, namely: the initial concentration of $Co^{2+}$, $Ca^{2+}$ and $Mg^{2+}$ ions as well as the initial pH of the solution, on the biosorption of $Co^{2+}$ ions by DAS. The statistical significance of each coefficient was determined by *p*-values shown in table 3. Lower *p*-value indicates a higher significance of the parameter.

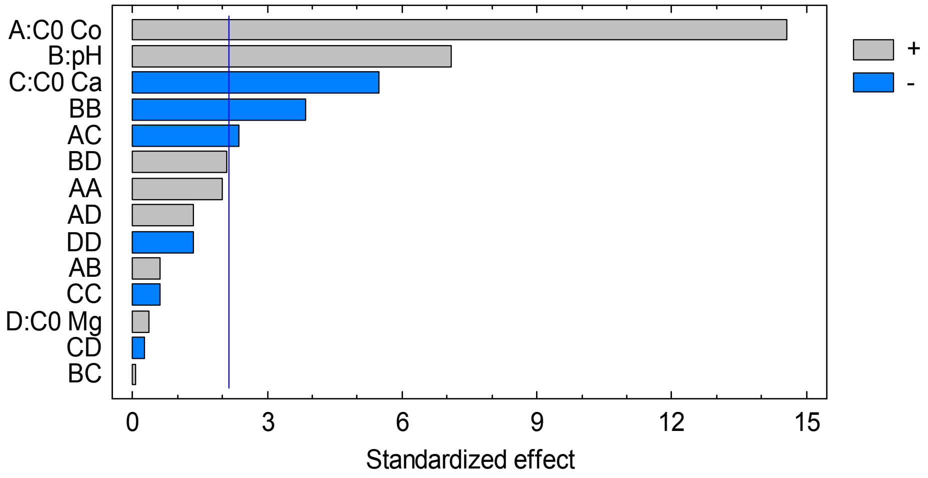


**Figure 2** Pareto chart showing the effects of observed factors and their combined impact on $Co^{2+}$ ions sorption by dry mass (significance level $\alpha = 0.05$)

Obtained results demonstrate the statistical significance of linear quadratic coefficients: A ($F = 211.99$, $p < 0.0001$), B ($F = 50.19$, $p < 0.0001$) and C ($F = 30.03$, $p < 0.0001$), and quadratic coefficient $B^2$ ($F = 14.76$, $p = 0.0018$). Other quadratic forms $A^2$ ($F = 3.94$, $p = 0.0670$), $C^2$ ($F = 0.37$, $p = 0.5553$), $D^2$ ($F = 1.79$, $p = 0.2022$) as well as linear coefficient D ($F = 0.12$, $p = 0.7299$) not confirmed the statistical significance.

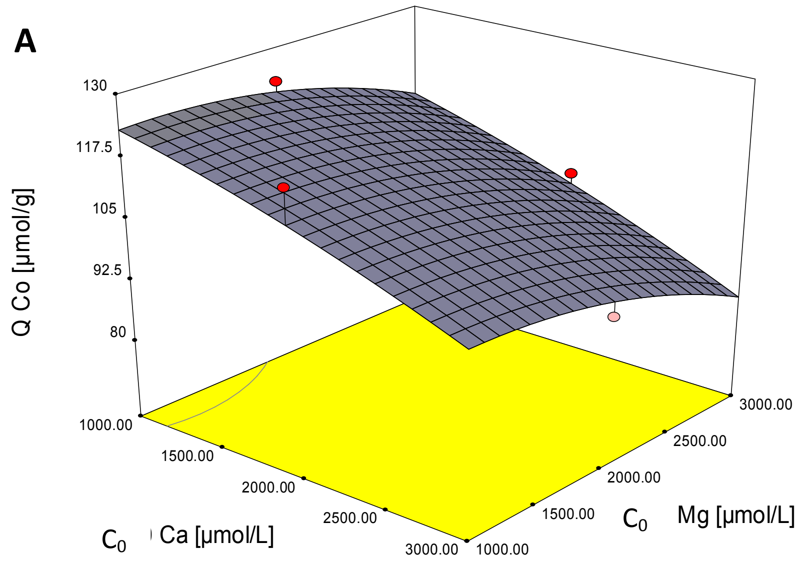
The Pareto chart (Fig. 2) illustrates the standardized effects of the independent variables and their interactions on the dependent variable (sorption of cobalt ions). In the Pareto chart, the effect of each factor as well as the level of its effects on responses is expressed by the length of its bar in the graph.

Figure 2 shows a significant positive effect of linear factors A (the initial concentration of $Co^{2+}$ ions) and B (the pH of the solution) on a cobalt sorption, indicating the increase of cobalt removal with increasing value of both factors. On the other side, factor C (the initial concentration of $Ca^{2+}$ ions) shows a significant negative linear effect on sorption efficiency. As can be seen from figure 2, it is evident that factor BB and interaction effect AC have a significant negative quadratic effect on the response. However, the effect of the initial concentration of $Mg^{2+}$ ions on the sorption has no significant effect on sorption process.

The interpretation of the interactions simplifies using of three-dimensional plots for the regression model. Such three-dimensional surfaces can provide useful information about the behaviour of the system within the experimental design, facilitate an examination of the effects of the experimental factors on the responses and contour plots between the factors **(Panesar, 2008; Ahmad and Hameed, 2010).**

From figure 3 it can be found out that the sorption of cobalt ions is strongly influenced by the pH of the solution. The initial pH is known as the most critical parameter affecting metal-containing wastewater treatment. The pH value of a solution strongly influences not only the site dissociation of the biomass surface, but also the solution chemistry of the heavy metals: hydrolysis, complexation by organic and/or inorganic ligands, redox reactions, precipitation and the biosorption availability of the heavy metals **(Esposito *et al.*, 2002).** On the basis of the fact, that the pH of real wastewater can vary always at the moment when the number of species rise or when the concentration is changed, the pH range study was between 3.0 and 8.0.

From figure 3 it is evident that the sorption of $Co^{2+}$ ions increased with increasing pH of the solution. It can be seen that maximum biosorption of Co occurred at pH between 6.0 and 7.0. Clearly lower biosorption was observed at pH 3.0. Low sorption efficiency of cobalt at lower pH value can be explained by the increasing concentration of hydrogen ($H^+$) and hydronium ($H_3O^+$) ions competing for binding sites on the DAS surface. As pH levels increase, more ligands with negative charge would be exposed with the subsequent increase in attraction sites to positively charged metal ions **(Setatnia *et al.*, 2004).**



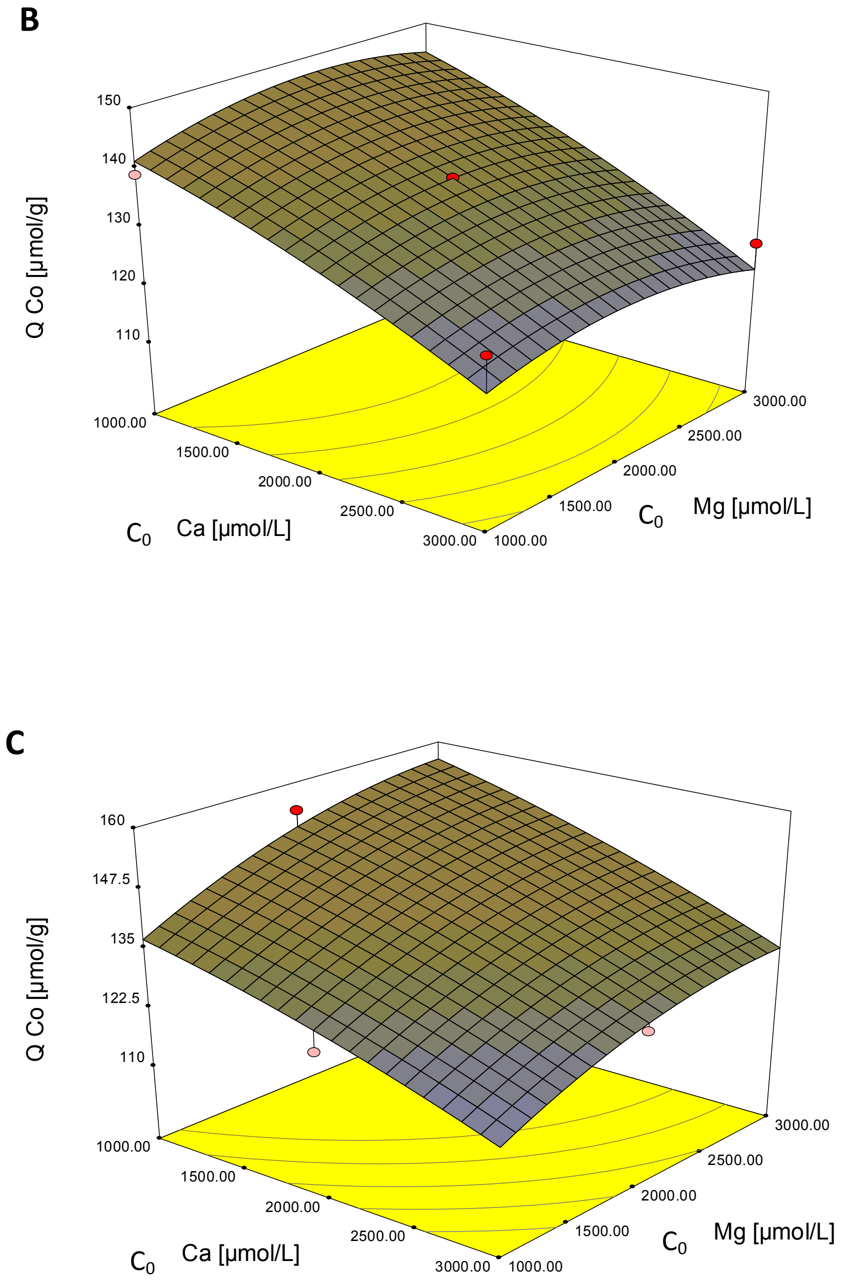


**Figure 3** Three-dimensional surface plots of the combined effect of the initial Ca and Mg concentration on Co sorption by DAS (2.5 g/L) at 22°C, the initial concentration of $Co^{2+}$ 2000 µmol/L and the pH of the solution (A) 3.0, (B) 5.5 and (C) 8.0

Plots of cobalt sorption versus calcium concentration at varying pH revealed that calcium had a drastic inhibitory effect on zinc sorption at all concentrations. Increasing concentration of $Ca^{2+}$ ions caused decrease of $Co^{2+}$ sorption as a result of competition between ions present in ternary solution. However, in case of the presence of magnesium ions, it was observed that cobalt sorption remained unaffected. As can be seen, this trend was independent of the pH of the solution. This fact can be explained by different affinity of studied ions for DAS surface. Using of RSM enables to evaluate plots of cobalt sorption as a function of calcium and magnesium concentration at varying pH values at the same time. Most pronounced inhibition was observed at lower pH (3.0).

## CONCLUSIONS

Sludges produced by all wastewater treatment plants (WWTP) display high fertility properties but the presence of heavy metals in the sludges restricts their use for agricultural purposes. However, considering their surface properties and sorption capacity for toxic pollutants, biomass of activated sludge present an excellent sorbent for toxic pollutants. The present investigation was carried out to study combined effect of the initial concentration of cobalt, calcium and magnesium, and pH value on cobalt sorption from ternary system Co-Ca-Mg by DAS using a Box-Behnken design under the Response surface methodology (RSM). Obtained results demonstrate that $Co^{2+}$ biosorption increased with increasing initial concentration of cobalt and pH value. Sorption process was not sensitive for presence of magnesium. However, Pareto analysis, ANOVA analysis as well as 3D surface plots revealed that calcium had a drastic inhibitory effect on cobalt removal. On the basis of results it can be concluded that RSM present an excellent tool which enables the evaluation of interactions and competitive effects in multicomponent system, considers the role of pH in sorption process and reduces the number of needed experiments in contrast to classical method of changing of one variable at a time.

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