CADMIUM AND ZINC UPTAKE BY DRIED ACTIVATED SLUDGE: EQUILIBRIUM AND EXPERIMENTAL DESIGN STUDY

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Abstract: Removal of Cd^{2+} and Zn^{2+} ions from single and binary solutions by dried activated sludge was studied in batch experiments. It was shown that the metal removal is a rapid process significantly influenced by solution pH. Maximum uptake of both Cd and Zn was reached at pH 6.0 and negligible uptake was observed at pH 2.0. The Langmuir isotherm was found to well represent the measured equilibrium sorption data in single metal systems and the maximum sorption capacities Q_{max} of the activated sludge (d.w.), calculated from Langmuir model were 540 ± 16 μmol/g for Zn^{2+} and 510 ± 17 μmol/g for Cd^{2+} ions. The Response surface methodology (RSM) was used for investigation of interaction and competitive effects in binary metal system. It was found that dried activated sludge in binary system Cd-Zn has slightly higher affinity for Cd^{2+} comparing with Zn^{2+} ions. Competitive effect of Cd on Zn uptake increased with increasing solution pH and Cd initial concentration. Maximum sorption capacities of the activated sludge were 321 μmol Cd^{2+}/g and 312 μmol Zn^{2+}/g. RSM appears to be a better tool for the evaluation of interaction and competitive effects in binary systems than both the simple extrapolation from single-component systems and experimentally difficult study of multi-component systems.

Key words: cadmium, zinc, biosorption, activated sludge, isotherms, RSM

1. Introduction

Industrial effluents contain both organic and inorganic pollutants. These pollutants upset natural balance in water ecosystems, interfere with organisms, accumulate in biota and enter into the food chain with human on the top. Contaminants removal by conventional treatment methods, such as chemical precipitation, membrane separation, evaporation and ion-exchange is often limited due to their low efficiency and economic viability (NAYAK and LAHIRI, 2006). Therefore, there is a need for an effective and economical treatment alternative. Biological processes such as biosorption and bioaccumulation represent possible interactions of toxic pollutants with biological systems in contaminated environment. Current research activity in the field attempts to evaluate whether biosorption may eventually provide such an effective and economical treatment process alternative (NAJA *et al.,* 2010).

Many researchers studied biosorption from single systems (GHODBANE *et al.,* 2008; KANG *et al.,* 2007), although the degree of removal of metal ions from wastewaters by biosorption depends mainly on the competitive interactions of co-ions when present in solution (MA and TOBIN, 2003). Results from single component systems can provide useful data about the uptake capacity of used biosorbent, but they do not exactly reflect the real situation in wastewaters (GÖNEN and AKSU, 2009). Therefore it is necessary to study sorption process as complex process, which consists of many mechanisms and can be affected by many parameters. Effect of various parameters (pH, temperature, sorbent particle size, initial concentration of sorbates) can be studied by the classical approach, when one variable is changed at a time. However this procedure represents a time consuming and less effective method. Multivariate optimization is faster and overcome circumstances not explained by the traditional methods such as interactions between the variables that influence the response e.g. sorption capacity (ZOLGHARNEIN *et al.,* 2010). Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for designing experiments, building models and analyzing the effects of the several independent variables (MUNE *et al.,* 2008). Main advantage of statistical design of experiments is the reduced number of experiments to be performed as well as it considers interactions among the variables and can be used for optimization of the operating parameters in multivariable systems (GÖNEN and AKSU, 2009). From the literature it was found that RSM has been widely used for optimization of biosorption processes mainly in single systems. Only a few studies utilized RSM methodology for statistical analysis of individual and interaction effects of parameters in binary and ternary sorption systems (FEREIDOUNI *et al.,* 2009; PAKSHIRAJAN and SWAMINATHAN, 2009).

Within this context the objective of present study was firstly to quantify the ability of the biosorbent prepared from dried activated sludge to sorb Cd^{2+} and Zn^{2+} ions. Equilibrium isotherm models according to Langmuir and Freundlich were used for mathematical description of sorption equilibria in single systems. The second objective was to study the competitive and interaction effects of above mentioned ions in binary Cd^{2+}-Zn^{2+} system at various solution pH values using Response surface methodology (RSM).

2. Material and methods

2.1 Biosorbent preparation

Activated sludge was obtained from waste water treatment plant in Enviral a.s. (Leopoldov, Slovak Republic) producing fuel ethanol. The sludge was washed twice in deionised water, oven-dried for 72 h at 90°C. After drying activated sludge was ground to various particle sizes, from which particle size <450 μm was used in biosorption experiments.

2.2 Batch sorption experiments in single systems

The biosorption kinetics was determined by suspending of activated sludge (2.5 g/L, d.w.) in metal solutions (pH 6.0) containing 1000 μmol/L $CdCl_2$ or $ZnCl_2$ spiked with $^{109}CdCl_2$ or $^{65}ZnCl_2$. The content was agitated on a reciprocal shaker (120 rpm) at 20°C and in time intervals liquid samples were taken and the radioactivity was measured.

The metal sorption capacity of dried activated sludge was determined by suspending of activated sludge (2.5 g/L, d.w.) in metal solutions (pH 6.0) containing $CdCl_2$ or $ZnCl_2$ in concentration range 100-4000 µmol/L spiked with $^{109}CdCl_2$ or $^{65}ZnCl_2$ and exposing for 4h at 20°C on a reciprocal shaker (120 rpm). To analyze the influence of pH, activated sludge was shaken in metal solutions containing 1000 µmol/L $CdCl_2$ or $ZnCl_2$ spiked with $^{109}CdCl_2$ or $^{65}ZnCl_2$ for 4 h on a reciprocal shaker at 120 rpm and 20°C adjusted to different pH values (2.0 – 9.0) by adding 0.5 M HCl or 0.1 M NaOH. At the end biomass was filtered out, washed twice in deionised water and radioactivity of both activated sludge and liquid phase was measured. The metal uptake was calculated as

$$Q = V(C_0 - C_{eq}) / m \tag{1}$$

where Q is the uptake (µmol/g, d.w.), C_0 and C_{eq} is the initial and the final metal concentrations in solution (µmol/L) and m is the amount of dried biosorbent (given in grams). All experiments were performed in duplicate.

2.3 Experimental design of binary Cd-Zn system

The Box-Behnken design under Response surface methodology (RSM) was used to investigate interaction and competitive effects in binary metal system Cd^{2+}-Zn^{2+}. Levels of factors (initial concentrations C_0 of Cd^{2+} and Zn^{2+} ions and initial pH of solution) considered for sorption in binary system are shown in Table 1. The design matrix of 16 experiments is given in Table 3. Based on the matrix, experiments were performed in Erlenmeyer flasks, activated sludge (2.5 g/L, d.w.) was added and the content was agitated on a reciprocal shaker (120 rpm) for 4 h at 20°C.

Table 1. Levels of factors considered for sorption of Cd^{2+} and Zn^{2+} ions from binary system using Box-Behnken design.

Factor	**Unit**	**Coded levels**			
		Factor code	**-1**	**0**	**1**
C_0 Cd^{2+}	µmol/L	A	1000	2000	3000
C_0 Zn^{2+}	µmol/L	B	1000	2000	3000
pH		C	3.0	4.5	6.0

The behavior of the binary sorption system is explained by the following empirical second-order polynomial model

$$Q = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j \tag{2}$$

where Q is the predicted response (specific sorption Q_{eq} of both Cd and Zn), x_i, x_j, ...x_k are the input variables (C_0 Cd^{2+}, C_0 Zn^{2+} and solution pH) which affect the response Q, β_0 is the intercept term, β_i is the linear effect, β_{ii} is the quadratic effect and β_{ij} is the interaction effect (FEREIDOUNI *et al.,* 2009).

2.4 Radiometric analysis

The gamma spectrometric assembly using the well type scintillation detector 54BP54/2-X, NaI(Tl) (Scionix, the Netherlands) and the data processing software Scintivision 32 (ORTEC, USA) were used for ^{109}Cd and ^{65}Zn determination in activated sludge and supernatant fluids at the energy of γ- photons: ^{109}Cd – 88.04 keV and ^{65}Zn – 1115.52 keV. Standardized $^{109}CdCl_2$ solution (3.937 MBq/ml; 50 mg $CdCl_2$/L in 3 g/L HCl) and $^{65}ZnCl_2$ solution (0.8767 MBq/ml; 50 mg $ZnCl_2$/L in 3 g/L HCl) were obtained from the Czech Institute of Metrology, Prague (Czech Republic).

2.5 Speciation modeling

Prediction of the speciation of Cd and Zn in the aqueous systems as a function of total salt concentration and solution pH was performed using the Visual MINTEQ (version 2.52) program. Visual MINTEQ 2.52 is a chemical equilibrium computer program that has an extensive thermodynamic database for the calculation of metal speciation, solubility and equilibria (GUSTAFSON, 2004).

2.6 Data analysis

To calculate the Q_{max} values and the corresponding parameters of adsorption isotherms non-linear regression analysis was performed by ORIGIN 7.0 Professional (OriginLab Corporation, Northampton, USA) and GraphPad Prism 5.0 (GraphPad Software, USA). Response surface graphs and regression analysis of the obtained data were performed by Statgraphics Centurion XV (StatPoint Inc., USA) and the Design Expert 7.0 (Stat-Ease, Inc., USA).

3. Results and discussion

3.1 Cd^{2+} and Zn^{2+} uptake by activated sludge

In order to determine the minimum necessary time to reach the sorption equilibrium, the time-course studies on the biosorption of cadmium and zinc ions from single metal systems by biosorbent prepared from dried activated sludge were performed. Fig. 1 shows that biosorption of Cd^{2+} and Zn^{2+} ions is a rapid process where equilibrium is reached within several tens minutes. At initial phase driving force is higher and binding sites on activated sludge with higher affinity are occupied. After that time concentration of Cd^{2+} and Zn^{2+} ions in solution decreases and the residual binding sites with lower affinity toward metal ions are occupied slowly. The final equilibrium was reached within 4 hours. Such two-phase sorption has been also reported by YANG *et al.* (2010) in the case of Zn sorption by activated sludge. They found that zinc adsorption capacity increased obviously during the first 60 min and the final equilibrium was reached within 180 min.

From Fig. 2 it can be seen that maximum biosorption of both Cd^{2+} and Zn^{2+} occurred at pH 6.0. This curve is characteristic also for Cd and Zn biosorption by the moss *Rhytidiadelphus squarrosus* (PIPÍŠKA *et al.,* 2010) and biosorption of other metal ions on activated sludge, algae and other biosorbents (WANG *et al.,* 2010; LIU *et al.,* 2009; GUNDOGDU *et al.,* 2009). Observed slightly lower biosorption at pH 4.0 and negligible at pH 2.0 is closely related to protonation of binding sites, resulting in competition between H^+ and Cd^{2+} or Zn^{2+} ions for occupancy of the active sites. In the case of Zn^{2+} at pH 8.0 sharp decrease of biosorption was observed.

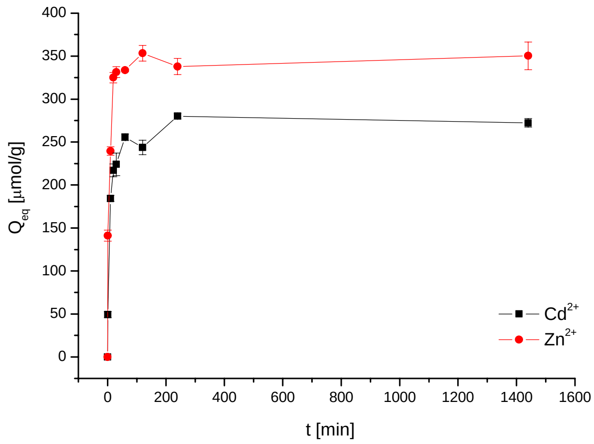


Fig. 1. Effect of contact time on Cd^{2+} (C_0 = 1000 µmol/L, 100 kBq $^{109}CdCl_2$) and Zn^{2+} (C_0 = 1000 µmol/L, 65 kBq $^{65}ZnCl_2$) ions biosorption by dried activated sludge (2.5 g/L d.w.) at 20°C and pH 6.0.

It must be pointed out, that the pH influenced also the level of ionization and speciation of metals in aqueous solution. As can be calculated by Visual MINTEQ speciation program (data not shown) at pH 8.0 besides the divalent cation Zn^{2+}, zinc occurs in solution also in insoluble forms such as $[Zn(OH)]^+$. Therefore the decrease of Zn^{2+} sorption at higher pH values (> pH 8.0) can be caused also by precipitation. According to calculations insoluble cadmium species occurred at pH > 9.0. It is reasonable to suppose that the dependence of metal uptake on pH is related to both the surface functional groups on the biosorbent and the metal speciation in solution.

During sorption experiments pH was not regulated. From Fig. 2 it is evident that the equilibrium pH increased after biosorption experiments what indicates that functional groups on the surface accumulate besides Cd^{2+} and Zn^{2+} ions simultaneously H^+ ions. Similar behaviour observed also CHEN *et al.* (2002) in Cu sorption by activated sludge.

Several mechanisms of heavy metals uptake by activated sludge have been proposed (WANG *et al.,* 2010; LAURENT *et al.,* 2010). Short-term cation uptake is generally regarded as an abiotic process governed by: surface complexation of cations with exposed functional groups (carboxyl-, sulfhydryl-, amine- etc.) on the biosorbent; ion exchange; coordination and chelation of metals; adsorption or by precipitation of solid phases on the cell walls.

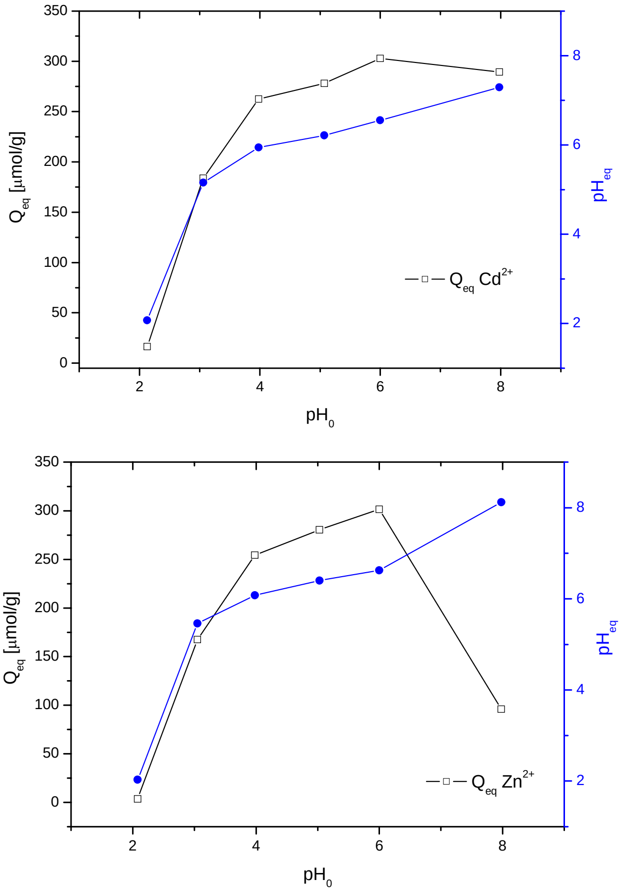


Fig. 2. Effect of initial pH on Cd^{2+} and Zn^{2+} ions (c_0 = 1000 µmol/L) sorption by dried activated sludge (2.5 g/L, d.w.) at 20°C; (-●-) represents shifts in pH after biosorption.

3.2 Sorption equilibrium in single systems

Two well known adsorption isotherm models - Langmuir (Eq. 3) and Freundlich (Eq. 4) were applied for the analysis of the experimental data in single sorption systems.

$$Q_{eq} = \frac{bQ_{\max} C_{eq}}{1 + bC_{eq}} \tag{3}$$

$$Q_{eq} = KC_{eq}^{\;(1/n)} \tag{4}$$

Parameters of these models provide an insight into the sorption process, reflect the nature of the sorbent, surface properties as well as the degree of the affinity of the sorbents and can be used to compare biosorption performance (VOLESKY, 2003). The Langmuir and Freundlich isotherms were fitted to the equilibrium data for Cd^{2+} and Zn^{2+} sorption on dried activated sludge. Isotherm curves and parameters of the models determined from the experimental data using non-linear regression analysis are reported in Fig. 3, 4 and Table 2.

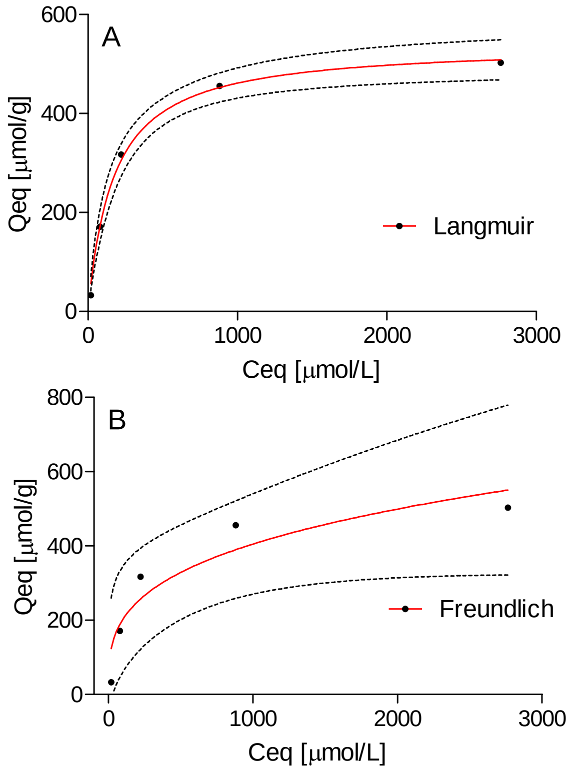


Fig. 3. Isotherms for the sorption of Zn^{2+} ions by dried activated sludge at 20°C and pH 6.0 according to Langmuir (A) and Freundlich (B). Data points represent experimental results, curves represent the calculated values from isotherm models, dotted lines represent the 95 % confidence interval.

The Langmuir isotherm fits the data of both Cd^{2+} and Zn^{2+} ions sorption by dried activated sludge better than the Freundlich isotherm, as is demonstrated by higher values of coefficient of determination (R^2), the more homogeneous standard deviation of each observed parameter and by the lower the sum of squares (*RSS*) values obtained as well as standard deviation of the residuals ($S_{y,x}$) (Table 2). Also HAMMAINI *et al.* (2007) found that the sorption of Cd^{2+} and Zn^{2+} ions by activated sludge was well described using the Langmuir isotherm.

The maximum sorption capacity Q_{max} obtained from Langmuir isotherm for Cd^{2+} was 510 ± 17 µmol/g at pH 6.0. Slightly higher value of Q_{max} was observed in the case of Zn^{2+} sorption, 540 ± 16 µmol/g at pH 6.0. The affinity constant *b* of the isotherms corresponds to the initial gradient, which indicates the biosorbent affinity at low

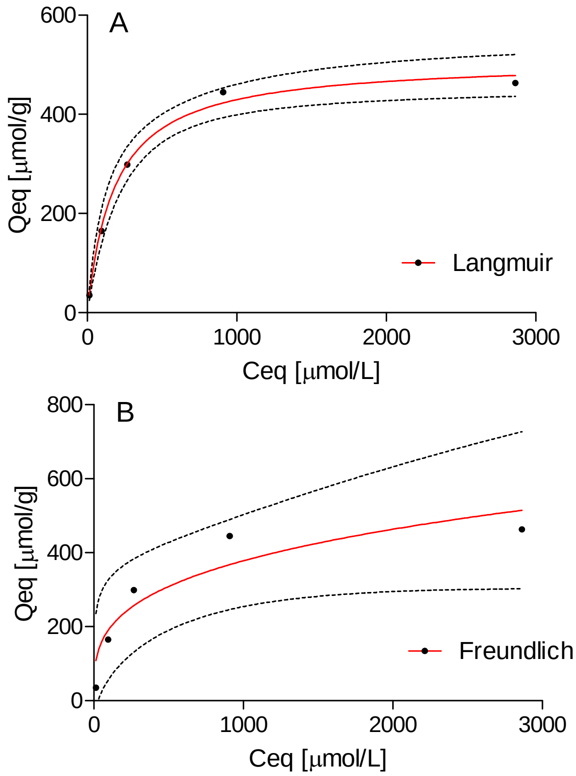


Fig. 4. Isotherms for the sorption of Cd^{2+} ions by dried activated sludge at 20°C and pH 6.0 according to Langmuir (A) and Freundlich (B). Data points represent experimental results, curves represent the calculated values from isotherm models, dotted lines represent the 95 % confidence interval.

Table 2. Langmuir and Freundlich parameters for the sorption of Cd^{2+} and Zn^{2+} ions by dried activated sludge obtained by non-linear regression analysis.

Model	**Metal ion**	Q_{max} [µmol/g]	b [L/µmol]	K [L/g]	$1/n$	R^2	*RSS*	$S_{y,x}$
Langmuir	Zn^{2+}	540 ± 16	0.006 ± 0.001	-	-	0.995	723	15.5
	Cd^{2+}	510 ± 17	0.005 ± 0.001	-	-	0.994	769	16.0
Freundlich	Zn^{2+}	-	-	50.3 ± 29.4	0.30 ± 0.08	0.877	18817	79.2
	Cd^{2+}	-	-	49.9 ± 28.9	0.29 ± 0.08	0.878	16347	73.8

concentrations of metal ions. A greater initial gradient corresponds to a higher affinity constant (SHENG *et al.,* 2007). From Fig. 3 and 4 it is evident that the cadmium and zinc isotherms have similar behavior at lower equilibrium concentrations. In the case of *b*, cadmium recorded 0.005 ± 0.001 L/μmol compared to 0.006 ± 0.001 L/μmol for zinc indicating slightly higher affinity of activated sludge for zinc ions. It should be realized that despite the fact that Langmuir isotherm offers no insights into the mechanism of biosorption (LIU and LIU, 2008) it remains a convenient tool for comparing equilibrium data on a quantitative basis (determination of maximum sorption capacity Q_{max} and affinity parameters *b*) and providing information on biosorption potential.

3.3 Experimental design of binary Cd-Zn system

Box-Behnken design under the Response surface methodology (RSM) was used for investigation of interaction and competitive effects between variables in binary system Cd-Zn. According to preliminary experiments and our previous studies (PIPÍŠKA *et al.,* 2010; PIPÍŠKA *et al.,* 2008) the sorption capacity of biosorbent in multi-component systems mainly depends on the initial concentration of primary ion and co-ions in sorption system and on the solution pH. Therefore, initial concentration of Cd and Zn and solution pH were used as process variables in experimental design (Table 3) and two responses – Q_{eq}(Cd) and Q_{eq}(Zn) were studied simultaneously.

Table 3. Box-Behnken experimental design matrix and experimental and predicted values of sorption capacity (Q_{eq}) of Cd^{2+} and Zn^{2+} ions by dried activated sludge from binary system Cd-Zn. A – C_0 Cd (μmol/L), B – C_0 Zn (μmol/L), C – pH.

Run order	Factor			Q_{eq} Cd		Q_{eq} Zn	
	A	B	C	Q_{eq} (**experimental**) (μmol/g)	Q_{eq} (**predicted**) (μmol/g)	Q_{eq} (**experimental**) (μmol/g)	Q_{eq} (**predicted**) (μmol/g)
1	1000	1000	4.5	205.8	206.5	178.0	179.2
2	3000	1000	4.5	321.0	321.7	113.2	122.8
3	1000	3000	4.5	130.7	130.0	312.0	302.4
4	3000	3000	4.5	261.4	260.7	226.2	226.9
5	1000	2000	3	93.77	90.64	138.1	145.5
6	3000	2000	3	211.2	208.0	118.3	115.3
7	1000	2000	6	170.8	173.9	284.9	287.8
8	3000	2000	6	299.3	302.4	193.3	185.9
9	2000	1000	3	180.9	183.4	75.86	69.18
10	2000	3000	3	132.7	136.5	154.0	156.1
11	2000	1000	6	297.8	294.0	151.1	148.9
12	2000	3000	6	206.0	203.5	282.6	289.3
13	2000	2000	4.5	242.5	245.0	213.3	214.6
14	2000	2000	4.5	242.5	245.0	213.3	214.6
15	2000	2000	4.5	254.5	245.0	227.8	214.6
16	2000	2000	4.5	241.1	245.0	198.4	214.6

The behavior of the binary sorption system Cd-Zn is explained by the following quadratic models determined by multiple regression analysis:

$$Q_{eq}(Cd) = -382 + 0.097 \times c0Cd - 2.1 \cdot 10^{-5} \times c0Zn + 194 \times pH - 1.3 \cdot 10^{-5} \times c0^2 Cd - 2.3 \cdot 10^{-6} \times c0^2 Zn - 17.03 \times pH^2 + 3.9 \cdot 10^{-6} \times c0Cd \times c0Zn + 0.0019 \times c0 \times pH - 0.007 \times c0Zn \times pH \quad (5)$$

$$Q_{eq}(Zn) = -394 + 0.008 \times c0Cd + 0.075 \times c0Zn + 187 \times pH + 5.5 \cdot 10^{-6} \times c0^2 Cd - 1.2 \cdot 10^{-5} \times c0^2 Zn - 16.2 \times pH^2 - 4.8 \cdot 10^{-6} \times c0Cd \times c0Zn - 0.012 \times c0 \times pH + 0.009 \times c0Zn \times pH \quad (6)$$

The adequacy of the models was further justified through ANOVA (data not shown). The model *F*-values of 246 (for Cd^{2+} sorption) and 58.3 (for Zn^{2+} sorption) and values of $P<0.0001$ indicate that both models are significant. Good agreement between experimental and predicted values of sorption capacity Q_{eq} (Table 3) confirmed high values of coefficient of determination (R^2), 0.997 (for Cd^{2+}) and 0.987 (for Zn^{2+}). Equations 5 and 6 represent the quantitative effect of the variables (initial concentration of Cd and Zn, solution pH) and their interactive effects on the Q_{eq}(Cd) and Q_{eq}(Zn) in binary system Cd-Zn. A positive sign in the equation implies a synergistic effect of the variables, while a negative sign indicates an antagonistic effect.

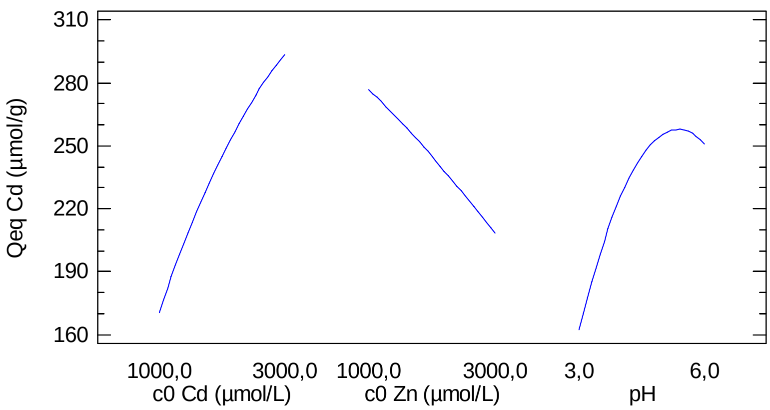


Fig. 5. Main effects plot for Cd^{2+} biosorption by dried activated sludge from binary system Cd-Zn.

Fig. 7A, B, C illustrate the three-dimensional response surface plots of the quadratic model (Eq. 5) for Cd^{2+} sorption from binary system Cd-Zn. The combined effect of initial Cd^{2+} concentration and pH on Cd^{2+} sorption by dried activated sludge at various concentrations of zinc as co-ion is shown. It is evident that Cd^{2+} uptake increased with increasing of initial solution pH as well as with initial Cd^{2+} concentration. On the contrary, increasing Zn^{2+} concentration from 1000 to 3000 µmol/L diminished Cd^{2+} sorption as a result of competition between metal ions (Fig. 5). Increase in solution pH and initial Zn^{2+} sorption caused increase in Zn^{2+} sorption as can be seen from main effects plot (Fig. 6). Similarly, increase in Cd^{2+} concentration decreased Zn^{2+} sorption from binary system Cd-Zn.

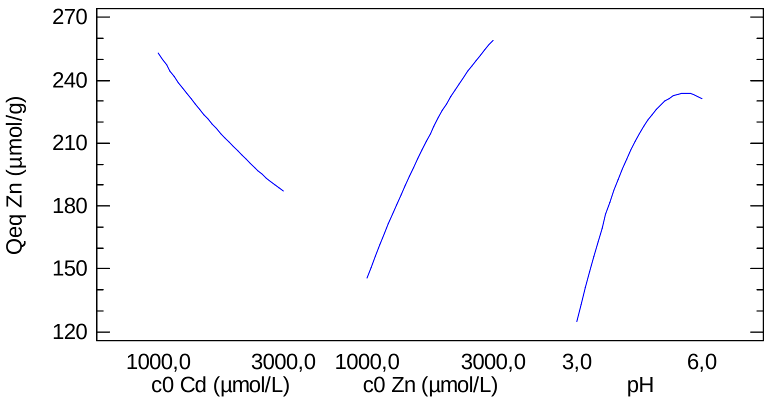
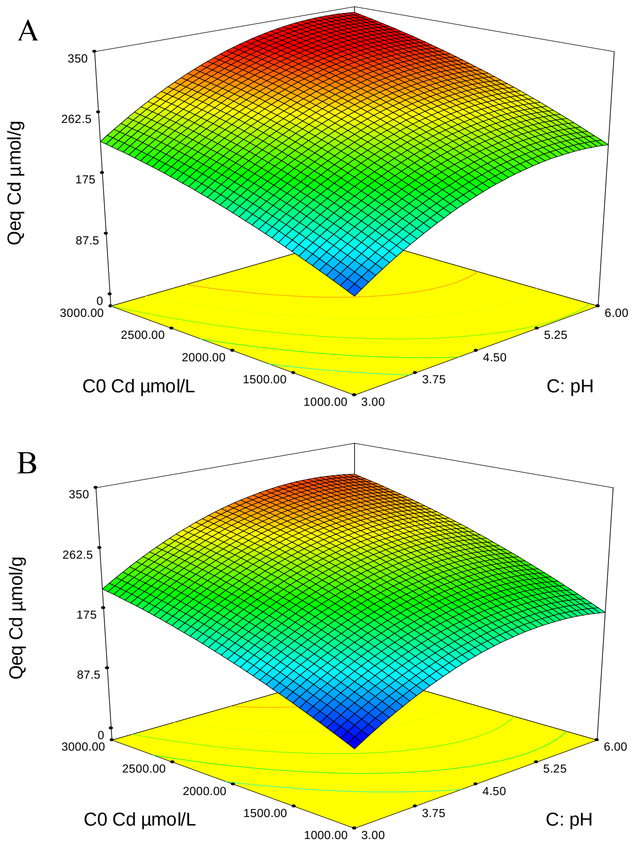


Fig. 6. Main effects plot for Zn^{2+} biosorption by dried activated sludge from binary system Cd-Zn.



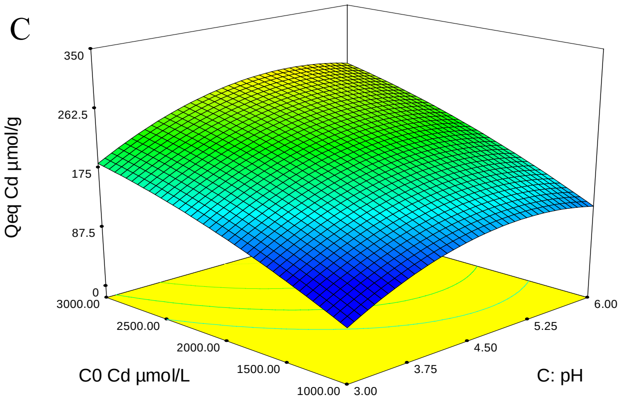


Fig. 7. Response surface plots showing the effect of pH and initial Cd^{2+} concentration on Cd^{2+} biosorption by dried activated sludge (2.5 g/L d.w.) at 20°C and different concentrations of Zn^{2+} as competing ion (A) 1000 µmol/L, (B) 2000 µmol/L, (C) 3000 µmol/L.

Maximum sorption capacities of the activated sludge were 321 µmol Cd^{2+}/g and 312 µmol Zn^{2+}/g. It was found that dried activated sludge in binary system Cd-Zn has higher affinity for Cd^{2+} ions when Cd^{2+} and Zn^{2+} are present in equimolar ratio 1:1 (Table 3) comparing with higher affinity to Zn^{2+} in single systems. This is consistent with the hypothesis that variance in affinity in multi-component systems could be attributed to different ionic characteristics of metal ions (PAKSHIRAJAN and SWAMINATHAN, 2009).

4. Conclusions

Biosorption of Cd^{2+} and Zn^{2+} from aqueous solution by dried activated sludge is a rapid and pH dependent process. Maximum uptake of metals was found to occur at pH 6. The experimental equilibrium data of the single-component systems for Cd^{2+} and Zn^{2+} ions have been well described by the Langmuir isotherm and the maximum sorption capacity Q_{max} were 540 ± 16 µmol/g for Zn^{2+} and 510 ± 17 µmol/g for Cd^{2+} ions. The use of RSM revealed the existence of interaction and competitive effects between variables in binary system Cd-Zn. Cd^{2+} uptake increased with increasing of initial solution pH as well as with initial Cd^{2+} concentration. On the contrary, increasing Zn^{2+} concentration from 1000 to 3000 µmol/L diminished Cd^{2+} sorption as a result of competition between metal ions. Activated sludge in binary system Cd-Zn has higher affinity for Cd^{2+} comparing with Zn^{2+} ions when Cd^{2+} and Zn^{2+} are present in equimolar ratio 1:1. RSM appears to be a useful tool for obtaining interaction and competitive effects in binary systems since it requires less reagents and experimentation time.

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