

CADMIUM BINDING CAPACITY OF DEMINERALIZED WHEY POWDER: A BOX-BOEHKEN DESIGN APPROACH

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Received / Geliş: 15.01.2025; Accepted / Kabul: 10.08.2025; Published online / Online baskı: 06.09.2025

Yıldız Küçük, N. (2025). Cadmium binding capacity of demineralized whey powder: A Box-Boehken design approach. GIDA (2025) 50 (5) 766-779 doi: 10.15237/ gida.GD25019

Yıldız Küçük, N. (2025). Demineralize peyniraltı suyu tozunun kadmiyum bağlama kapasitesi: Bir Box-Boehken tasarım yaklaşımı. GIDA (2025) 50 (5) 766-779 doi: 10.15237/ gida.GD25019

ABSTRACT

This study explores the use of demineralized whey powder (d-WP) as an adsorbent for removing cadmium ions (Cd^{2+}) from wastewater. Characterization of d-WP involved various analytical techniques: FTIR identified surface functional groups involved in adsorption, SEM examined surface morphology, elemental mapping visualized element distribution, and EDX determined elemental ratios. To optimize Cd^{2+} removal, a Box-Behnken Design (BBD) was utilized, evaluating four key parameters: initial Cd^{2+} concentration (10-50 mg/L), adsorbent dosage (0.5-1 g), contact time (10-60 minutes), and temperature (24-50 °C). The optimal conditions for maximum Cd^{2+} removal was found to be an initial concentration of 44 mg/L, a contact time of 26 minutes, an adsorbent dosage of 0.63 g, and a temperature of 45 °C. ANOVA confirmed the significant effects of these parameters on removal efficiency.

Keywords: Response surface methodology, Box-Behnken design, demineralized whey powder, cadmium

DEMİNERALİZE PEYNİRALTI SUYU TOZUNUN KADMIYUM BAĞLAMA KAPASİTESİ: BİR BOX-BEHNKEN TASARIM YAKLAŞIMI

ÖZ

Bu çalışma, demineralize peyniraltı suyu tozunun (d-WP) atık sudan kadmiyum iyonlarını (Cd^{2+}) uzaklaştırmak için bir adsorban olarak kullanımını araştırmaktadır. d-WP'nin karakterizasyonu çeşitli analitik teknikler kullanılarak gerçekleştirilmiştir.

FTIR, adsorpsiyonda yer alan yüzey fonksiyonel gruplarını tanımlamış; SEM, yüzey morfolojisini incelemiş; element haritalama, element dağılımını görselleştirmiş; ve EDX, element oranlarını belirlemiştir. Cd^{2+} uzaklaştırmasını optimize etmek için bir Box-Behnken Tasarımı (BBD) kullanılmış, dört ana parametre değerlendirilmiştir: başlangıç Cd^{2+} konsantrasyonu (10-50 mg/L), adsorban dozu (0.5-1 g), temas süresi (10-60 dakika) ve sıcaklık (24-50 °C). Maksimum Cd^{2+} uzaklaştırma için optimal koşullar, 44 mg/L başlangıç konsantrasyonu, 26 dakika temas süresi, 0.63 g adsorban dozu ve 45 °C sıcaklık olarak bulunmuştur. ANOVA, bu parametrelerin uzaklaştırma verimliliği üzerindeki önemli etkilerini doğrulamıştır.

Anahtar kelimeler: Yanıt yüzeyi yöntemi, Box-Behnken tasarımı, demineralize peyniraltı suyu tozu, kadmiyum

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INTRODUCTION

Whey is a nutrient-rich liquid that is extracted from milk during the process of cheese making. Initially regarded as a mere by-product of the dairy sector, it has now gained recognition as a valuable commodity in its own right. On average, the production of whey amounts to approximately 9 liters for every kilogram of cheese produced (Durmuş et al., 2022). Approximately 120 million tonnes of whey are produced each year. If whey is disposed of directly as wastewater, it can become an environmental pollutant due to its high organic load and elevated chemical oxygen demand (50–80 g L⁻¹) and biological oxygen demand (40–60 g L⁻¹) (Müşerref et al., 2024).

While liquid whey may not be directly applicable in certain contexts, particularly within some food industries, it is essential to transform it into functional and value-added products. This conversion process significantly improves both its utility and nutritional profile, thereby expanding its range of applications. A commonly employed final step in producing whey powder is spray drying, which follows concentration processes such as membrane filtration and evaporation. This technique effectively converts concentrated whey into a powdered form, allowing for a wide array of uses across various industries. Dried whey powder is frequently utilized as a culinary additive, offering benefits including flavor enhancement, texture modification, and nutritional enrichment (Baris et al., 2022; Reza et al., 2022). Whey, a byproduct of cheese production, has the potential to be transformed into various valuable products such as biofuels, biopolymers, electricity, single-cell protein, probiotic dairy products, prebiotics, and ethanol. However, in many developing nations, a significant amount of whey remains unprocessed and is often discarded into the environment. This careless disposal leads to the loss of important nutritional components found in milk, while also causing serious environmental pollution. Thus, there is a need for better whey management practices to utilize its nutritional benefits and minimize environmental damage (Mahdi et al., 2019). Whey is demineralized using electrodialysis

and various membrane techniques (such as ultrafiltration and diafiltration). This process reduces the high mineral content of whey, resulting in demineralized whey (Melnikova et al., 2023).

Cadmium is a toxic heavy metal that can cause serious harm to human health. It accumulates in the kidneys and liver, leading to health issues such as high blood pressure, osteoporosis, anemia, and lung cancer. Additionally, cadmium intake is associated with metabolic disorders and weakened immune system (Öktüren Asri et al., 2007).

The Box-Behnken design (BBD) is an experimental design approach that requires a relatively small number of design points and experiments to produce a comprehensive dataset. This approach is more advantageous in terms of experimental and economic efficiency compared to other designs, as it uses three levels for each factor instead of five (Uçurum et al., 2018; Isra et al., 2021). The aim is to determine the combinations of parameters affecting the adsorption of cadmium heavy metal by demineralized whey powder (d-WP) with predictable properties, using polynomial mathematical equations and response surface graphs. Thus, the BBD will be utilized for this purpose. Calculating the cadmium adsorption capacity of demineralized whey powder using Box-Behnken Design (BBD) contributes significantly to the literature by providing a systematic and reliable approach for optimizing adsorption processes. This method enables precise identification of influential parameters and their interactions, leading to more efficient and sustainable adsorption strategies. Additionally, it reduces the number of experiments required, saving time and costs, and offers a solid foundation for future research in similar areas.

METHODS AND MATERIALS

Materials

d-WP obtained from Enkasut (Konya/Türkiye), Cadmium chloride (CdCl₂, MW 183.31 g mol⁻¹) analytical grade, obtained from Thermo. Stock

solutions of 1000 mg L⁻¹ Cd⁺² were prepared using distilled water.

Characterization

The surface properties of d-WP were investigated using several analytical techniques. To analyze the surface functional groups before and after adsorption, attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was utilized (Agilent Cary 630, USA). Additionally, scanning electron microscopy (SEM) (LEO-EVO 40, Cambridge-UK) combined with energy dispersive X-ray analysis (EDX) (Bruker-125 eV, Berlin-Germany) was employed to assess the surface morphology at various stages of the process.

Design of experiments

In this investigation, the researcher utilized Response Surface Methodology (RSM) with a Box-Behnken Design (BBD) to explore how different independent variables affect the adsorption of dyes onto an adsorbent material. The study focused on four independent variables: initial heavy metal concentration (A), adsorbent dosage (B), contact time (C), and temperature (D). Data gathered from the experiments were analyzed using the Design-Expert software (version 13.0), which facilitated the planning, execution, and statistical analysis of the experiments. The coded values and levels for these independent variables are detailed in Table 1.

Table 1 Codes and ranges of independent variables at their respective levels

Codes	Variables	Level 1 (-1)	Level 2 (0)	Level 3 (1)
A	Initial concentration	10	30	50
B	Adsorbent Dosege	0.5	0.75	1
C	Contact Time	10	35	60
D	Temperature	24	37	50

After conducting the experiments, the resulting data were modeled using a second-order polynomial equation of quadratic form, which is illustrated in Equation (1).

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \sum \beta_{ij} X_i X_j \quad (1)$$

In this equation, Y represents the response, while β_0 , β_{ij} , β_{ii} , and β_i , and refer to the constant

coefficient, the interaction coefficient, the quadratic coefficient, and the linear coefficient, respectively. X_i and X_j denote coded values of the independent variables. A total of 29 experimental runs (Table 2) were performed to optimize levels of independent variables, which include A: Initial Concentration (10-50 mg/L), B: Adsorbent Dosage (0.5-1), C: Contact Time (10-60 min), and D: Temperature (24-50 °C).

Table 2 The BBD matrix and experimental data for Cd removal

Run	A: Initial concentration	B: Adsorbent Dosege	C: Contact Time	D: Temperature	Cd amount
1	50	0.75	10	37	26.102
2	30	0.75	60	24	16.98
3	50	0.75	35	24	21.125
4	50	1	35	37	31.898
5	30	0.5	35	24	10.701
6	30	0.5	60	37	18.156
7	30	0.75	10	24	14.754
8	10	0.75	10	37	5.401
9	50	0.75	35	50	31.1
10	30	1	35	24	16.623
11	30	0.5	10	37	13.137
12	30	0.75	35	37	16.245
13	30	0.75	35	37	16.518
14	10	0.75	60	37	6.346

15	30	0.5	35	50	18.009
16	10	0.75	35	50	6.976
17	10	1	35	37	6.304
18	30	0.75	35	37	17.148
19	10	0.5	35	37	5.128
20	10	0.75	35	24	5.548
21	50	0.5	35	37	24.002
22	30	0.75	10	50	18.555
23	30	1	35	50	20.844
24	30	1	10	37	17.82
25	30	0.75	35	37	16.959
26	30	0.75	60	50	19.542
27	50	0.75	60	37	27.761
28	30	0.75	35	37	16.812
29	30	1	60	37	17.757

Batch adsorption study

In each run, d-WP was placed in a 100 mL Erlenmeyer flask, which was then filled with 50 mL of CdCl_2^{2+} solution. These flasks, containing solution, were agitated at a constant shaking speed of 150 cycles per minute in an isothermal water bath shaker (miprolab, msl 40-40). In analyses performed using an Atomic Absorption Spectrometer (AAS, Agilent 240FS AA), the calibration curve for the element cadmium (Cd) was generated using a 1000 mg/L ICP multi-element standard solution IV stock solution. The wavelength of Cd was 228.8 nm. After establishing the calibration curve, the initial and final concentrations were determined, and the heavy metal removal (q_t) was calculated using Equation (2) (Tang et al., 2024).

$$q_t = \frac{(C_0 - C_t)V}{w} \quad (2)$$

Table 2 presents the experimental design matrix and the corresponding response values.

RESULTS AND DISCUSSION

Characterization of d-WP

FTIR analysis

The infrared (IR) spectroscopy data reveals several significant absorption peaks that provide insights into the molecular structures present in the sample, and this spectrum is shown in Figure 1 (Ming et al., 2021).

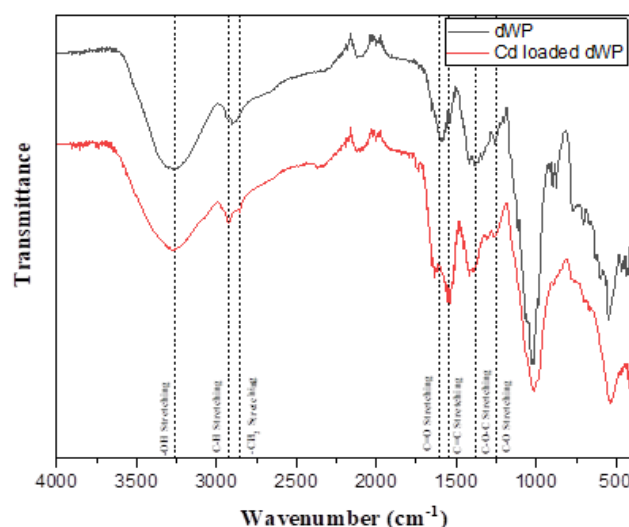


Fig. 1 FTIR spektrum d-WP an Cd load d-WP

One notable absorption peak detected at approximately 3261 cm^{-1} is indicative of the stretching vibrations characteristic of hydroxyl (-OH) groups. This peak suggests the presence of alcohols or phenols within the molecular framework, highlighting their functional significance in the overall composition (Varol et al., 2023). In addition to this, two prominent peaks located in the range of 2935 to 2857 cm^{-1} are associated with the stretching vibration modes of carbon-hydrogen (C-H) and methylene (-CH₂-) groups. These peaks reflect the aliphatic nature of the molecule, pointing towards the presence of long hydrocarbon chains or branched structures, which are common in various organic compounds. Another crucial peak appears at 1605 cm^{-1} , which corresponds to C=O stretching vibrations linked to carboxyl (-COOH) groups. This finding is essential as it indicates the presence of organic acids within the sample, a feature that can enhance the biological activity of the compound (Kalpana et al., 2020). Meanwhile, the band observed at 1549 cm^{-1} is attributed to the stretching vibrations associated with carbonyl (C=O) and alkenyl (C=C) bonds. However, it is more precisely associated with C=C double bonds, which implies that unsaturation is present in the molecular structure, a characteristic that can influence the chemical reactivity and properties of the compound (Asemi et al., 2020). Furthermore, the peaks located at approximately 1379 cm^{-1} are indicative of stretching vibrations of C-O-C bonds, reflecting the presence of ether functionalities or other similar functional groups that contribute to the diversity of the chemical structure. Lastly, the peaks around 1255 cm^{-1} are attributed to C-O stretching vibrations, corroborating the presence of various ether or alcohol functionalities within the molecular framework (Albadarin et al., 2017). Overall, the detailed analysis of these absorption peaks not only elucidates the different functional groups present in the sample but also provides critical information on the molecular interactions and potential applications of the compound in various fields.

SEM-EDX and mapping analysis

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX) techniques were employed to evaluate the surface morphology and chemical composition of demineralized whey powder before and after adsorption, with the results illustrated in Figure 2.

Prior to adsorption, the surface of the demineralized whey powder exhibited a smooth and homogeneous structure. Following adsorption, significant changes in the surface were observed due to the accumulation of Cd ions, resulting in some agglomeration. This indicates the interaction of the powder with metals and provides insights into the chemical reactivity of the surface. EDX was utilized to determine the presence of Cd and the chemical composition of the powder analysis. EDX analysis conducted after adsorption revealed a significant presence of Cd within the powder. Additionally, changes in the concentrations of other elements (such as calcium, phosphorus, etc.) were also recorded. These results indicate that the powder can effectively retain cadmium ions. Mapping maps were used to observe the distribution and localization of the elements present in the powder. The distribution of cadmium was observed to be concentrated on the surface of the demineralized whey powder, while other elements were distributed in a more background manner. These maps visually represent the processes arising from adsorption, clearly illustrating the interactions among different elements and their arrangements on the surface.

Variance and validation analysis of Box-Behnken design (BBD)

The BBD (Box-Behnken Design) was adopted to design 29 experiments to test four independent variables that could play a significant role in the removal of the heavy metal Cd²⁺ (Table 2). The designed experiments were used to investigate the individual and interactive effects of four independent variables—Initial Concentration (A), Adsorbent Dose (B), Contact Time of the Solution (C), and Temperature (D)—on the removal of Cd²⁺. The experimental data on Cd²⁺

removal was statistically analyzed using Analysis of Variance (ANOVA), and the results are presented in Table 3.

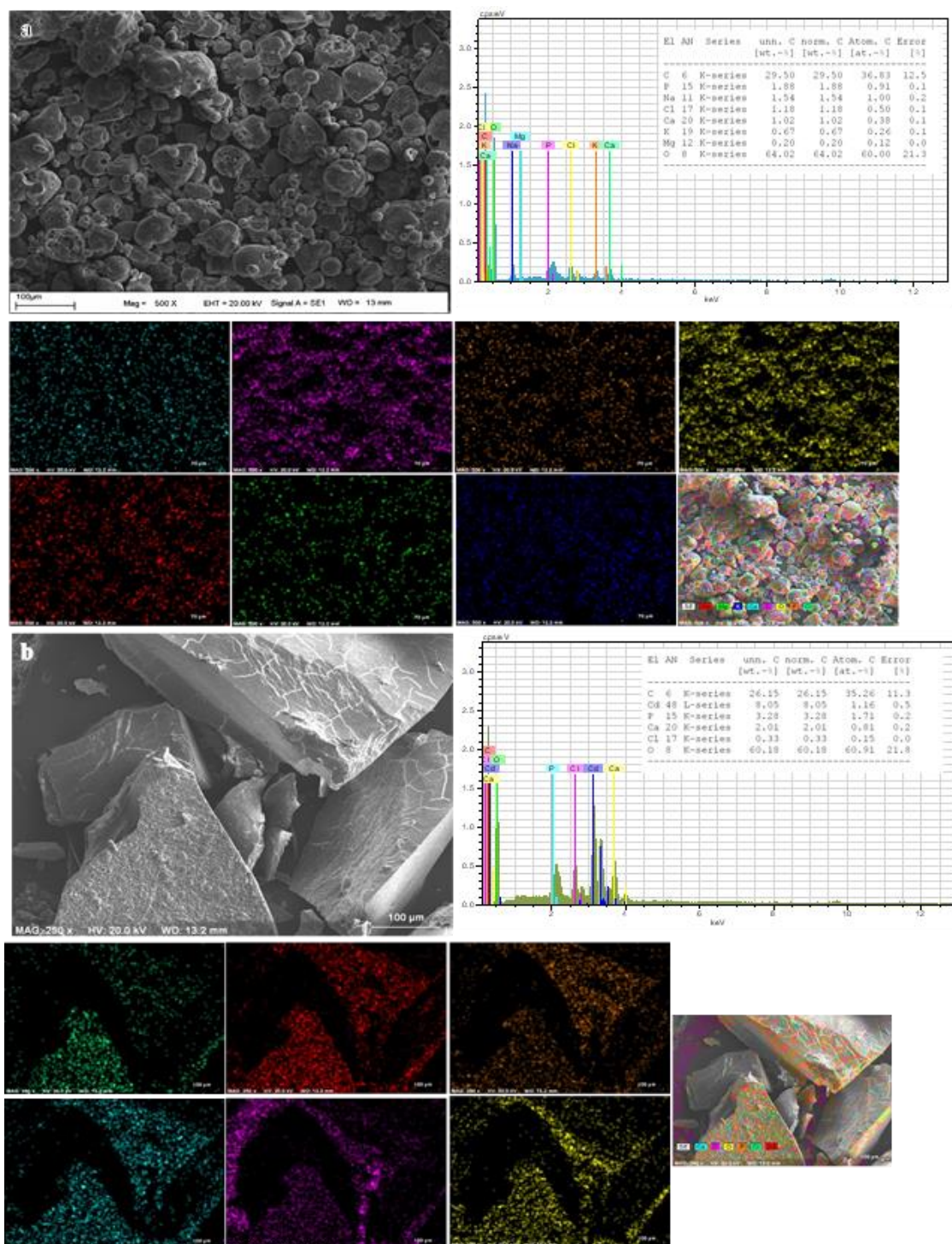


Fig. 2 Sem, EDX and mapping a) d-WP b) Cd loaded d-WP

Table 3 ANOVA for Cd removal

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	1491.22	14	106.52	102.75	< 0.0001
A- Initial concentration	1328.99	1	1328.99	1281.95	< 0.0001
B- Adsorbent Dosege	40.75	1	40.75	39.31	< 0.0001
C- Contact Time	9.67	1	9.67	9.33	0.0086
D- Temperature	71.52	1	71.52	68.98	< 0.0001
AB	11.29	1	11.29	10.89	0.0053
AC	0.1274	1	0.1274	0.1229	0.7311
AD	18.26	1	18.26	17.62	0.0009
BC	6.46	1	6.46	6.23	0.0257
BD	2.38	1	2.38	2.30	0.1518
CD	0.3838	1	0.3838	0.3702	0.5526
A ²	0.7819	1	0.7819	0.7543	0.3998
B ²	0.0008	1	0.0008	0.0008	0.9780
C ²	0.3440	1	0.3440	0.3319	0.5737
D ²	0.0084	1	0.0084	0.0081	0.9294
Residual	14.5137657	14	1.03669755		
Cor Total	1505.7367562069	28			

The ANOVA results are based on F values, sum of squares, and p values to determine significant factors. The significance of the correlation coefficient is illustrated with the F-value and p-value; higher F-values and lower p-values suggest a more significant correlation (Jianhua et al., 2017). In the ANOVA analysis (Table 3), the model exhibited an F-value of 102.75 and a p-value less than 0.0001, indicating that the model is statistically significant. Furthermore, the model's R² value was determined to be 0.9904, while the adjusted R² value was 0.9807. The difference between the R² values is less than 0.20, indicating that the model has good reliability (Wu et al., 2024). Overall, under the selected conditions, model terms with a p-value less than 0.05 (Prob > F < 0.0500) have been considered significant for Cd²⁺ removal. qhip between the test factors and Cd²⁺ removal (response) can be expressed as follows equation 3.

$$\text{Cd}^{2+} \text{ removal} = +16.74 + 10.52 A + 1.84 B + 0.8978 C + 2.44 D + 1.68 AB + 0.1785 AC + 2.14 AD - 1.27 BC - 0.7717 BD - 0.3098 CD - 0.3472 A^2 - 0.0112 B^2 + 0.2303 C^2 + 0.0360 D^2 \quad (\text{Eq. 3})$$

Model validation can be performed by examining the relationship between the predicted Cd²⁺ removal values and the actual Cd²⁺ removal values, as well as by assessing the nature of the

residual distribution (Safo et al., 2022). In Figure 3a, the normal probability plot of the model residuals is presented. The residuals exhibit an almost perfect normal distribution, with the points closely aligning along a straight line. This observation confirms the accuracy of the assumptions and the independence of the residuals (Kamel et al., 2023). Figure 3b shows the relationship between the predicted and actual Cd²⁺ removal, indicating a close proximity between the predicted and actual values. This alignment validates the statistical validity of the model.

The numerical optimization of the BBD model indicated that the optimal removal of Cd²⁺ heavy metal occurred at an initial concentration of 44 mg L⁻¹, a contact time of 26 minutes, an adsorbent dosage of 0.63 g, and a temperature of 45°C. Under these operating conditions, the Cd²⁺ removal was found to be 24.9 mg g⁻¹, which corresponds to a desirability value of one, as illustrated in Figure 4.

The accuracy of this prediction was confirmed through double validation tests using the optimized parameters. Essentially, the findings obtained from experimental observations align well with the data derived from numerical

optimization using desirability functions. These results demonstrate that the BBD model, when integrated with desirability functions, can be effectively employed to optimize the performance

characteristics of Cd^{2+} adsorption by d-WP. Consequently, the ideal input parameters for Cd^{2+} adsorption have been utilized in subsequent studies.

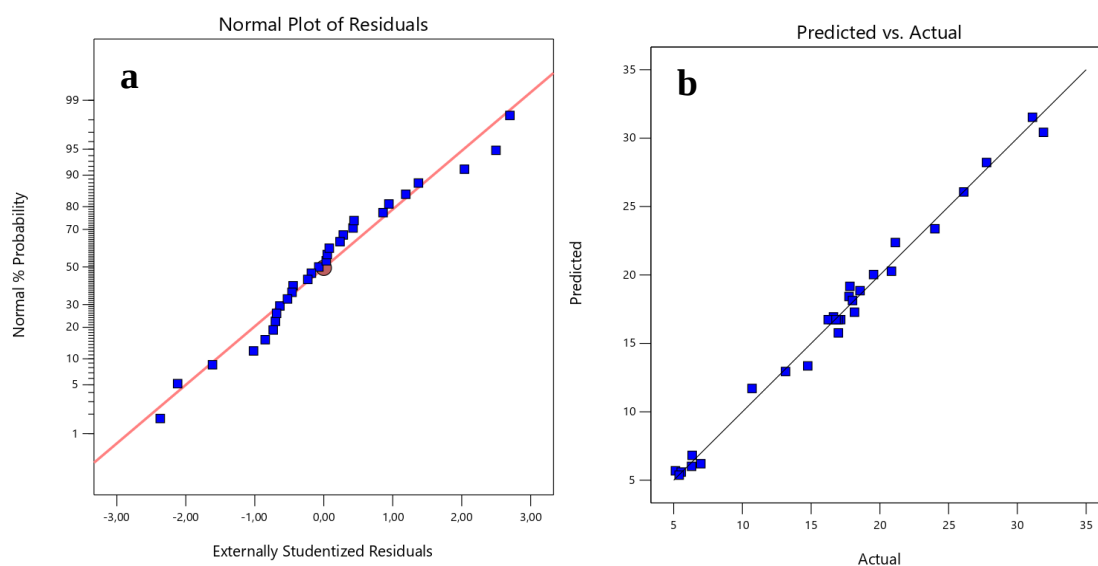


Fig. 3 BBD plots of a normal plot of residuals b predicted vs. actual, for Cd^{2+} removal

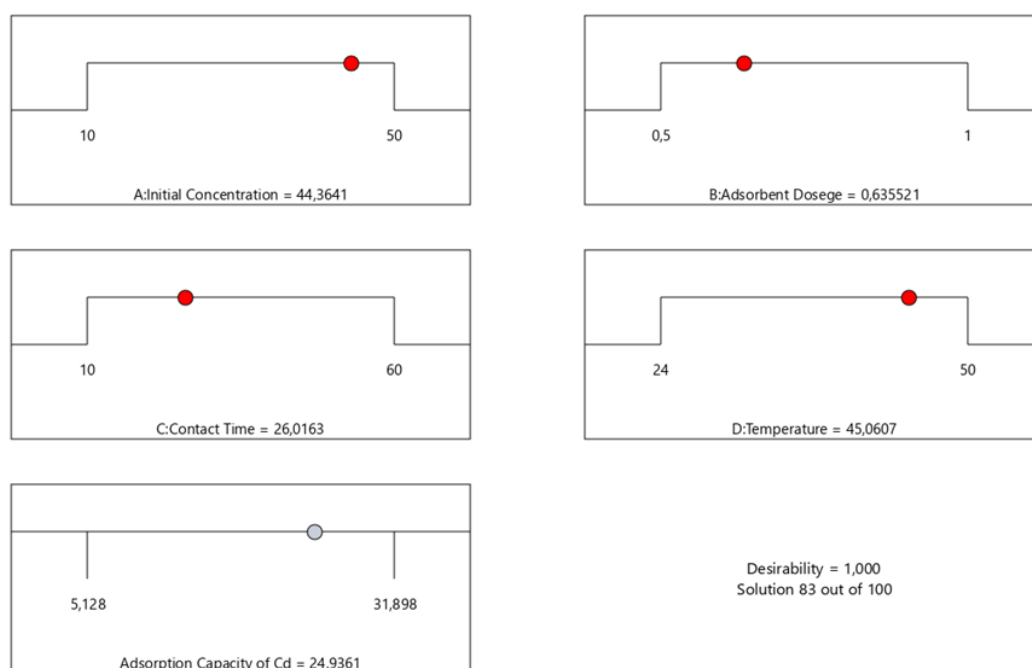


Fig. 4 Desirability ramps for the optimization of important adsorption input factors for Cd^{2+}

Effect of parameters on Cd²⁺ removal

Effect of adsorbent dosages

To investigate the maximum effect of d-WP adsorbent, dosages of 0.5 g, 0.75 g, and 1 g were used for a 50 mL Cd²⁺ solution. The other two parameters, temperature and contact time, were kept at their minimum values, which were 24°C and 10 minutes, respectively. It was observed that the Cd²⁺ removal efficiency at a 0.5 g dosage was lower compared to that at a 1 g dosage under constant initial concentration. Similarly, at the maximum temperature and contact time conditions, the same trend was observed, and adsorption capacity was higher at these values. This situation is illustrated in the 3D graphs in Figure 5(a),(b). The increase in adsorption capacity can be attributed to the greater active sites and larger pores provided by the increased amount of adsorbent for the pollutants. Higher adsorbent dosages enhance the surface area and pore structure, which aids in the greater involvement of pollutants in the adsorption process (Afroze et al., 2018; Islam et al., 2022).

Effect of initial concentration

To investigate the effect of initial concentration on Cd²⁺ removal, 3D graphs were used while keeping other parameters constant, as shown in Figure 5(c), (d). To examine the maximum effect of Cd²⁺ concentration, the other parameters were held constant at both their lowest and highest values. Initial concentrations of 10, 30, and 50 ppm were studied. When the constant values were at their lowest, the adsorption capacity was lower compared to higher constant values. In summary, it was found that the increase in initial concentration led to an increase in adsorption capacity. The analysis of this data indicates that higher initial concentrations help pollutants adsorb onto the adsorbent surface with a stronger driving force. In other words, the increase in initial concentration strengthens the interactions between the pollutant and the adsorbent. Increasing concentrations allow for more effective utilization of the active sites on the adsorbent, thereby enabling all these areas to contribute to the adsorption process (Zaki et al., 2021; Tee et al., 2022). Therefore, the increase in initial concentration not only enhances the

adsorption of pollutants but also elevates the overall capacity of the adsorbent.

Effect of contact time

The effect of contact time on Cd²⁺ removal was evaluated by varying the contact time at three different levels: 10, 35, and 60 minutes. Other parameters were kept at both their lowest and highest levels, as shown in Figures 5(c), (d). With increasing contact time, Cd adsorption increased. When the fixed parameters were at their lowest levels, the Cd²⁺ adsorption capacity was lower compared to the highest fixed parameters. In summary, as time increased, adsorption also increased. This is because the active sites on the adsorbent will interact with more Cd²⁺ over time, allowing more Cd²⁺ molecules to bind (Turp et al., 2020; Gemici et al., 2021; Çiftçi et al., 2022).

Effect of temperature

To investigate the effect of temperature on Cd²⁺ removal, 3D graphs were used while keeping other parameters constant, as shown in Figure 5(e), (f). The fixed parameters were maintained at both minimum and maximum values. It was observed that when held at the maximum level, the adsorption capacity was higher. Additionally, with the increase in temperature, the adsorption capacity also increased. This indicates that the adsorption process is endothermic. As the temperature rises, the number of restricted areas on the adsorbent surface increases. Higher temperatures increase the movement speed of the adsorbate atoms, thereby reducing the density of the solution. Furthermore, the rise in temperature causes adsorbate molecules to become more flexible, leading to a faster formation of monolayers on the surface (Samia et al., 2017; Agarwala et al., 2023). In conclusion, the increase in temperature positively affects the adsorption process.

Cd Adsorption mechanism

FTIR spectra were analyzed to better understand the possible changes before and after the adsorption of Cd²⁺. Theoretically, the adsorption of metal ions onto the adsorbent surface is governed by intermolecular interactions, including electrostatic interactions between the

adsorbent and the metal ions, co-precipitation, surface complexation, cation exchange, and

cation interactions (Wen-Teo et al., 2022). The estimated mechanism is illustrated in Figure 6.

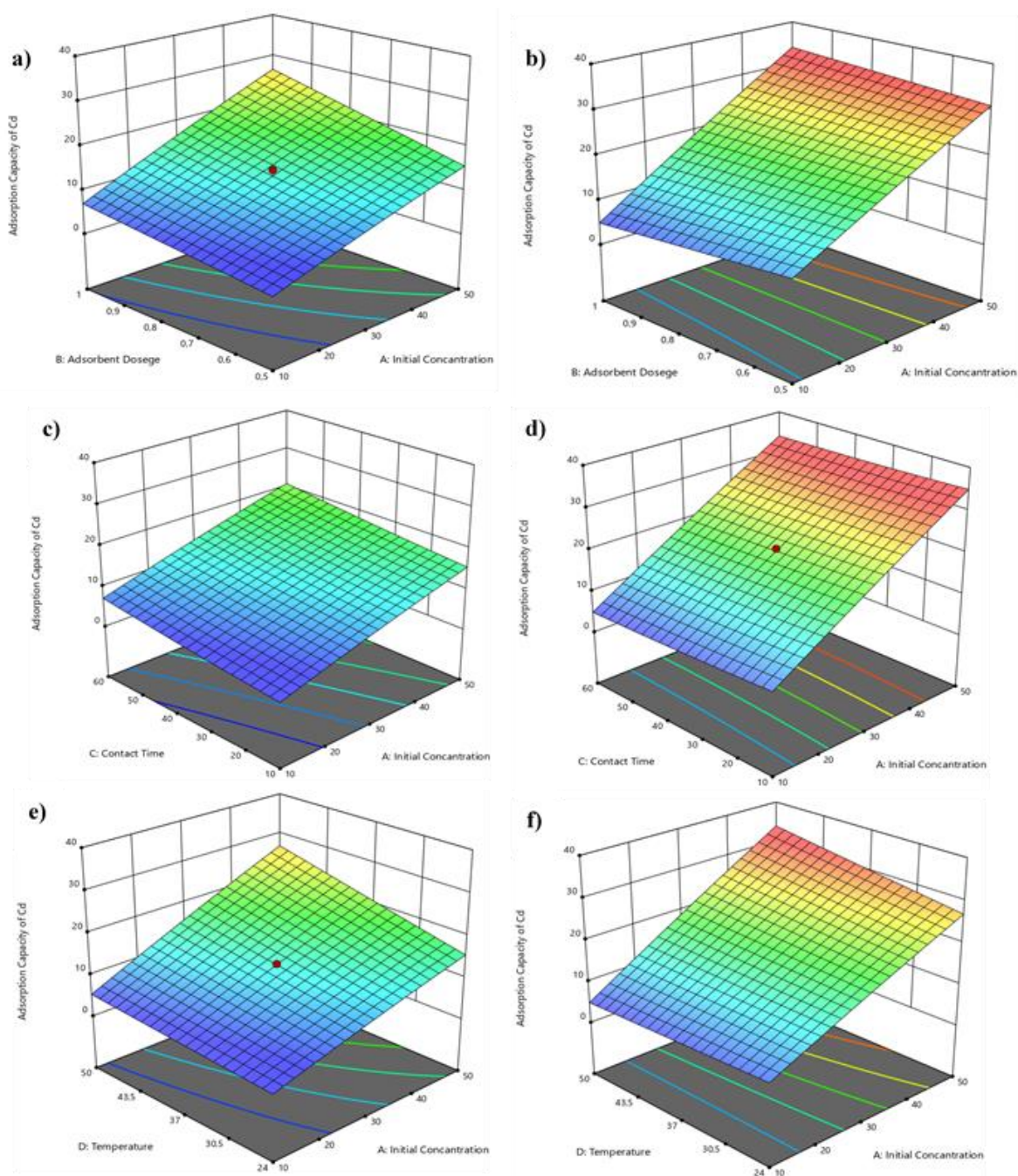


Fig. 5 Plots of 3D response surface for Cd²⁺ removal

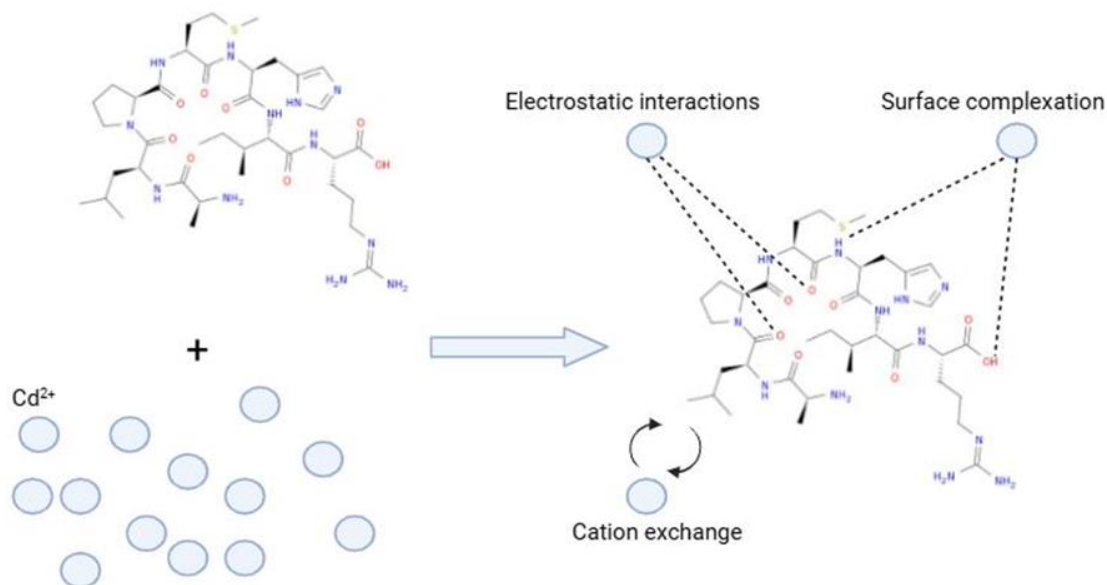


Fig. 6 Estimated adsorption mechanism of Cd

The functional groups on the d-WP surface play a critical role in metal adsorption (Yanfei et al., 2020). Figure 1 shows that the -OH (hydroxyl) stretching vibrations were detected at 3275 cm^{-1} and 3255 cm^{-1} before and after adsorption, respectively. Additionally, the CO (carbonyl, lactones, carboxylic acids, esters, aromatic structures, or benzene rings) was observed at 1595 cm^{-1} and 1639 cm^{-1} (Zulqarnain et al., 2020). After Cd^{2+} adsorption, the peaks at 3275 cm^{-1} significantly weakened and shifted to 3255 cm^{-1} (Jiang et al., 2018). Moreover, the peak at 1595 cm^{-1} both weakened and shifted. This may result from the interaction between Cd^{2+} in the solution and surface hydroxyl groups; this interaction has formed -O-Cd groups on the surface (Zhong et al., 2016).

CONCLUSION

This study investigates the use of demineralized whey powder (d-WP) as an effective adsorbent for the removal of cadmium ions (Cd^{2+}) from wastewater. The results obtained demonstrate that d-WP is an effective adsorbent for Cd^{2+} , showing significant potential in environmental remediation efforts. FTIR analysis confirmed the presence of functional groups on the surface, such as hydroxyl and amino groups,

which are likely responsible for binding cadmium ions. Additionally, the morphological data obtained from SEM revealed that the surface characteristics of the adsorbent are suitable, with a porous structure that enhances contact with contaminants. The multivariate modeling approach conducted using Box-Behnken Design (BBD) allowed for a systematic evaluation of the interactions between key parameters affecting adsorption efficiency and the determination of optimal conditions. Statistically, it was proven that variables such as initial concentration, contact time, adsorbent dosage, and temperature have significant effects on the cadmium removal process, indicating the importance of optimizing these factors for maximum efficiency. The optimal conditions for Cd^{2+} removal obtained in the study are a initial concentration of 50 mg/L , an adsorbent dosage of 1 g , a contact time of 35 minutes , and a temperature of 37°C . These findings highlight that d-WP can serve as a sustainable alternative in wastewater treatment and can contribute to the reduction of environmental pollution caused by heavy metals. Moreover, the use of a renewable and low-cost material like d-WP could offer an eco-friendly and economical solution for large-scale applications. Future studies may explore the

effectiveness of d-WP in removing other heavy metals, potentially broadening the application areas of this material and further emphasizing its usefulness in various environmental remediation contexts

ACKNOWLEDGMENTS

The author gratefully acknowledges İlhan Küçük.

DATA AVAILABILITY

Data analyzed and recorded during the research is included in this article.

FUNDING

This study was supported by Mus Alparslan University Scientific Research Coordination Unit. (Projects Number: BAP-24-SBMYO-4909-01)

CONFLICT OF INTEREST

The author declare that they have no conflict of interest.

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