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Optimisation of casein adsorption onto alkaline-treated rice husk using response surface methodology for sustainable protein recovery

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Abstract. With rising environmental concerns and a need for sustainable resource management, protein recovery from dairy wastewater has gained significant attention. This study investigates the use of alkaline-treated rice husk as an adsorbent for casein recovery, offering a low-cost and eco-friendly alternative for waste valorization. Using response surface methodology (RSM) and a Box-Behnken Design (BBD), the effects of contact time, adsorbent dosage, and pH on adsorption capacity were systematically evaluated. Optimal conditions were determined as 30 minutes of contact time, 4 mg/mL adsorbent dosage, and pH 9.93, achieving a predicted adsorption capacity of 111.547 mg/g. Validation experiments confirmed the model's accuracy, yielding an actual adsorption capacity of 110.0 mg/g, with no significant difference from the predicted values ($p > 0.05$). These findings demonstrate the potential of alkaline-treated rice husk for efficient protein recovery, contributing to sustainable nutrient recycling and environmental protection. This optimized adsorption process holds promise for industrial applications in waste management within the dairy sector, supporting resource conservation and sustainable practices. However, the study is limited by the lack of long-term stability testing of the adsorbent and its performance in real industrial wastewater, which should be addressed in the future.

Keywords: casein adsorption, rice husk, response surface methodology, protein recovery, sustainable waste management

INTRODUCTION

The dairy industry plays a critical role in the global economy, providing essential nutrition to a growing population (Kanire *et al*., 2024; Maree *et al*., 2024). However, it is also one of the largest sources of food processing wastewater, which, if untreated, can lead to significant environmental pollution (Singh *et al*., 2023). Extensive water use in cleaning, sanitizing, heating, and cooling processes during dairy production results in large wastewater volumes rich in organic matter, including valuable proteins like casein (Kowalska, 2024). Typically, this wastewater is treated in sewage plants using the activated sludge process (Joshiba *et al*., 2019). This results in the loss of high-value casein, representing both a waste of resources and a missed opportunity for nutrient recovery. Therefore, efficient casein recovery methods are needed to maximize environmental and economic benefits.

Casein, the primary protein in milk, is well-regarded for its nutritional value, offering essential amino acids, calcium, and phosphorus vital for growth, bone health, and muscle development (Górska-Warsewicz *et al*., 2019). Due to its slow digestion rate, casein provides a sustained release of amino acids, making it particularly valuable in animal feed where continuous nutrient absorption supports optimal growth and health (Kanda *et al*., 2016). In poultry and aquaculture feeds, casein enhances the diet's amino acid profile, improving

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feed conversion efficiency, promoting weight gain, and boosting the nutritional quality of feed (Cho *et al*., 2008; Abbasi *et al*., 2018). By incorporating casein into feed formulations, producers can ensure a balanced and steady protein source, contributing to better animal performance and product quality.

Various protein recovery methods, such as precipitation, membrane filtration, and adsorption, have been explored for their effectiveness in reclaiming proteins from wastewater (Shahid *et al*., 2021). Among these, adsorption is particularly advantageous due to its simplicity, efficiency, and adaptability (Satyam & Patra, 2024). Membrane separations are notable for their efficiency, however, they are challenged by membrane fouling (Tang *et al*., 2021). While for precipitations, the process involves the application of heat or chemicals that may cause protein loss and environmental contamination (Ia Cour *et al*., 2019). Adsorption binds protein molecules to the surface of solid adsorbents, often those with high surface areas and porous structures, like activated carbon, chitosan, or agricultural by-products such as rice husk (Kainth *et al*., 2024). The versatility of adsorption allows for protein recovery with minimal energy and chemical inputs, making it an eco-friendly approach to wastewater treatment. By enabling the recovery of proteins through adsorption, industries can mitigate environmental impacts and recycle valuable nutrients for applications like animal feed and bioproducts.

Rice husk, an abundant agricultural by-product, shows significant promise as an adsorbent in protein recovery due to its unique structural and chemical properties. Composed largely of cellulose, hemicellulose, and silica, rice husk has a naturally high surface area and porous structure that can be enhanced through chemical treatment, improving its adsorption capacity (You *et al*., 2021). Its fibrous nature and silica content provide durability and stability in aqueous solutions, making it suitable for adsorbing various compounds (Kordi *et al*., 2024). Moreover, rice husk's low cost and availability in rice-producing regions make it economically viable, especially in applications focused on sustainable waste valorization. Utilizing rice husk for casein adsorption from dairy wastewater not only reduces effluent pollution but also adds value by creating a protein-enriched animal feed additive. Thus, rice husk serves as a practical and sustainable adsorbent in protein recovery, offering a resource-efficient solution for environmental management and nutrient recycling.

MATERIALS AND METHODS

Material

Rice husks were purchased from supplier in Selangor, Malaysia. They were washed multiple times with distilled water to remove soluble impurities, then dried in an oven at 105°C for 24 hours. All chemicals and reagents used in the experiments were of analytical grade and procured from Sigma Aldrich, Germany.

Rice husk preparation

Rice husk was first subjected to chemical modification to enhance its adsorption capacity. The husk was soaked in 1.25% (w/v) potassium hydroxide (KOH) solution, using a 10% (w/v) ratio. This mixture was incubated at 100°C for 30 minutes. Following this, the rice husk was filtered and washed with hot water until it reached a neutral pH of 7. The residue obtained from this process was dried in an oven at 100°C for 24 hours, ground into a fine powder using a universal grinder, and sieved through a 40-mesh screen to achieve uniform particle size.

Equilibrium batch adsorption study

To assess the adsorption capacity of the treated rice husk, 0.1 g of the ground rice husk was placed in a series of conical flasks. A 0.5 mg/mL casein solution was adjusted to pH 8 and added in 20 mL portions to the flasks. A control flask containing only distilled water was included in each set of experiments. After adding 50 µL of each solution to individual falcon tubes, 1.5 mL of Bradford reagent was introduced, followed by a 10-minute incubation at room temperature. The flasks were incubated in a rotary shaker for 24 hours at 20 rpm. Protein concentration was measured by adding a 50 µL aliquot of a 0.5 mg/mL casein solution at pH 8 to 1.5 mL of Bradford reagent. The mixture was incubated at room temperature for 10 minutes and then measured for absorbance at 595 nm using a Shimadzu™ UVmini-1240 spectrophotometer. The absorbance values obtained were compared to a casein standard curve to quantify protein concentration. The adsorption capacity was measured using Eq. (1).

$$qt = \frac{(Co - Ct)V}{W} \quad (1)$$

where *qt* is the amount of casein adsorbed at time (mg/g), *Co* and *Ct* are the initial and final concentrations of casein (mg/mL), *V* is the volume of the solution (mL), and *W* is the weight of the rice husk used (g).

Optimization of casein adsorption by alkaline-treated rice husk

The Box-Behnken Design (BBD) of response surface methodology (RSM) was used to study the effects of adsorption process conditions (time, adsorbent dosage, and pH) on the adsorption of casein by alkaline-treated rice husk adsorbents. These ranges were selected based on preliminary studies and literature, ensuring sufficient coverage of conditions relevant to casein adsorption and practical application scenarios. Given the three process factors, a total of 17 experimental runs were conducted, including 12 factorial runs and 5 centre runs. Experimental data were analysed using Design Expert software (statistical) version 7.0 (STAT-EASE Inc., Minneapolis, USA). The experimental ranges and the levels of the process input variables for casein adsorption are given in Table 1. To analyse the data, a second order quadratic polynomial expression relate the three independent factors to adsorption capacity as the response (*Y*). The equation for this model is given in Eq. (2).

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ii} x_i x_j \quad (2)$$

Y is the predicted response, β_0 is the intercept, β_i are linear coefficients, β_{ii} are quadratic coefficients, β_{ij} are interaction coefficients, x_i and x_j are the factors.

The experimental data were analysed using analysis of variance (ANOVA). Analysis of variance was chosen for its ability to assess multiple independent variables and their effects on the dependent variable, as well as to identify the optimal levels of each independent variable. To evaluate the quality of fit for the polynomial model, both the adjusted R^2 and the coefficient of determination ($R^{2)}$ were used as evaluation metrics. Additionally, to assess the statistical significance and adequacy of the model, metrics such as the probability value (Prob > F), Fisher's F-ratio (F-value), and adequate precision were considered. Furthermore, a lack-of-fit test was performed to determine whether the selected quadratic polynomial model adequately captured the variability of the experimental data. The lack-of-fit test compares the residual error from the model to the pure error obtained from replicated experimental runs. This comparison assesses whether the model's predictions are consistent with the observed data within the experimental domain.

Validation of model

The mathematical model generated by the RSM approach was validated by conducting experiments under the optimal medium settings. To confirm the model's reliability, a statistical t-test was used to assess the closeness of the experimental response to the model's predictions.

Table 1. Experimental ranges and levels of the independent factors.

Independent Variable	Code	Unit	-1	0	+1
Time	A	min	30	135	240
Adsorbent Dosage	B	mg/mL	4	8	12
pH	C	-	7	9.5	12

RESULTS AND DISCUSSION

Response surface methodology for casein adsorption by alkaline-treated rice husk

To investigate the optimum conditions for the adsorption capacity of casein, the parameter with the greatest effect on the observation was identified. Box-Behnken Design was employed to examine the adsorption capacity of casein using rice husk as an adsorbent. Three independent parameters—time, adsorbent dose, and pH, denoted as A, B, and C, respectively—were selected for their influence on adsorption capacity. The Design generated 17 experimental runs for parameter optimization to determine the adsorption capacity of casein. The adsorption capacity response was measured in mg/g. Table 2 summarizes the BBD design, showing the adsorption capacity response, q_t. Based on the completed experimental runs, the polynomial equation for the response, illustrating the interaction between independent and dependent variables, is presented below in Equation 3.

$$Y = 25.40 - 12.96A - 29.99B + 4.45C + 6.82AB - 0.1500AC - 2.85BC + 12.04A^2 + 23.29B^2 - 8.19C^2 \quad (3)$$

In this model, the response Y represents the maximum adsorption capacity of casein, while the factors coded as A, B, and C correspond to time, adsorbent dose, and pH, respectively. Each sign indicates the nature of the effect: negative values represent an antagonistic effect, while positive values indicate a synergistic effect (Stat-Ease Inc., 2019).

The model's adequacy was evaluated using ANOVA and the regression coefficient (R^2) as presented in Table 3. The model's F-value of 7.62 indicates a good fit to the data, and a p-value below 0.050 confirms the model's significance, suggesting that it effectively captures the relationship between the predictors and the response variable, with observed effects unlikely to be due to random variation. In this study, factors A, B, and B^2 were identified as significant. The significance of factors A, B, and B^2 indicates that both the main effects of A and B, as well as the quadratic effect of B, play crucial roles in influencing the response variable. The presence of a significant quadratic term for B suggests a non-linear relationship, where changes in B affect the response in a more complex manner than a simple linear trend. These findings highlight the importance of incorporating both linear and quadratic terms in the model to achieve accurate predictions and effective optimisation of the response.

Table 2. Box-Behnken experiment design layout of the adsorption capacity on Rice Husk.

Run	**Factor 1** A: Time (min)	**Factor 2** B: Dose (mg/mL)	**Factor 3** C: pH	**Response 1** Adsorption Capacity (mg/g)
1	30	4	9.5	110.6
2	135	8	9.5	41.1
3	135	12	7	17.5
4	240	12	9.5	24.5
5	30	8	7	41.3
6	135	8	9.5	23.9
7	135	8	9.5	30.4
8	240	8	7	3.5
9	135	4	7	59.4
10	135	8	9.5	7.8
11	135	8	9.5	23.8
12	135	12	12	15.9
13	135	4	12	69.2
14	30	8	12	55.3
15	30	12	9.5	24.6
16	240	4	9.5	83.2
17	240	8	12	16.9

Table 3. ANOVA and Regression Coefficient for Box-Behnken Design on the Adsorption Capacity of Casein by Rice Husk.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	12105.79	9	1345.09	7.62	0.0069	significant
A Time	1344.21	1	1344.21	7.62	0.0281	
B-Dose	7194.00	1	7194.00	40.78	0.0004	
C-pH	158.42	1	158.42	0.8980	0.3749	
AB	186.32	1	186.32	1.06	0.3383	
AC	0.0900	1	0.0900	0.0005	0.9826	
BC	32.49	1	32.49	0.1842	0.6807	
A^2	610.11	1	610.11	3.46	0.1053	
B^2	2283.40	1	2283.40	12.94	0.0088	
C^2	282.25	1	282.25	1.60	0.2464	
Residual	1234.88	7	176.41			
Lack of Fit	648.82	3	216.27	1.48	0.3480	not significant
Pure Error	586.06	4	146.52			
R^2						0.9074
Adjusted R^2						0.7884
Predicted R^2						0.1532
Cor Total	13340.68	16				

The regression coefficient R^2 of 0.9074 demonstrates the model is highly appropriate for explaining the variability in the response variable within the experimental conditions. An adjusted R^2 value of 0.7884 indicates that the model accounts for approximately 78.84% of the variability in the data, with adjustment for the number of predictors. This value is reasonably good and indicates that the model captures most of the variance in the response variable. Generally, an adj. R^2 above 0.7 is considered acceptable in RSM studies (Leyva-Jiménez *et al.*, 2022). However, A predicted R^2 of 0.1532 indicates that the model has poor predictive power. Ideally, predicted R^2 should be close to adjusted R^2(within 0.2) to show that the model reliably predicts new data. A low predicted R^2 compared to adjusted R^2 suggests overfitting, where the model fits the experimental data well but performs poorly on unseen data. To improve predictive power, future model refinements could include additional replicates at critical points or exploring alternative model structures, such as transforming variables to better align with the observed data trends. Additionally, the model exhibited a non-significant lack of fit, with a p-value above 0.05, indicating that the model fits the data well and is likely sufficient for accurate predictions and optimization within the tested variable range (Stat-Ease Inc., 2019).

Response surface plots for casein adsorption onto alkaline-treated rice husk

The response surface plots presented in Figure 1 illustrate the interactive effects of contact time, adsorbent dosage, and pH on the adsorption capacity of casein onto alkaline-treated rice husk. These 3D plots provide a visual representation of how each factor and their interactions influence adsorption efficiency, facilitating a deeper understanding of the optimal conditions for maximizing casein uptake.

In examining the interaction between contact time and adsorbent dosage, a clear positive effect on adsorption capacity is observed. As both contact time and adsorbent dosage increase, the adsorption capacity of casein improves significantly, reaching its maximum at the highest levels of both factors. This trend suggests that longer contact times allow for more extensive interaction between the casein molecules and the active binding sites on the rice husk surface (Sazali *et al.*, 2020). Concurrently, higher adsorbent dosages increase the availability of binding sites, further enhancing the adsorption process (Sheraz *et al.*, 2024). These results indicate that both sufficient interaction time and a higher dosage of adsorbent are essential to achieving optimal adsorption efficiency, as they together maximize the binding opportunities for casein molecules.

The interaction between pH and contact time also significantly affects adsorption capacity, though in a more complex manner. The adsorption capacity is relatively stable at lower contact times across various pH levels, indicating that pH alone does not have a strong influence in the absence of adequate contact time. However, as contact time increases, a marked increase in adsorption capacity is observed around moderate pH levels. Extreme pH values, both acidic and basic, appear to reduce adsorption efficiency (Mogashane *et al*., 2024). This effect may be attributed to the ionization state of the rice husk adsorbent or potential denaturation of casein under unfavourable pH conditions, which can disrupt the protein structure and reduce binding affinity. These findings suggest that maintaining a moderate pH, in combination with extended contact times, creates favourable conditions for the interaction between casein and the rice husk, thereby optimizing adsorption.

The interaction between pH and adsorbent dosage further reveals insights into the adsorption process. Higher adsorbent dosages consistently enhance adsorption capacity across all pH levels, with particularly high efficiency observed under slightly acidic conditions. This trend suggests that acidic environments may facilitate better binding of casein molecules to the rice husk surface, potentially due to increased surface charge interactions or enhanced protein stability under these conditions (Kolar & Jin, 2019; Schulte *et al*., 2020). In contrast, at lower adsorbent dosages, adsorption capacity is constrained across all pH levels, emphasizing that the availability of sufficient adsorbent material is critical for maximizing protein uptake. These results underscore the importance of adsorbent dosage as a key factor in achieving high adsorption efficiency, particularly when combined with an optimal pH range.

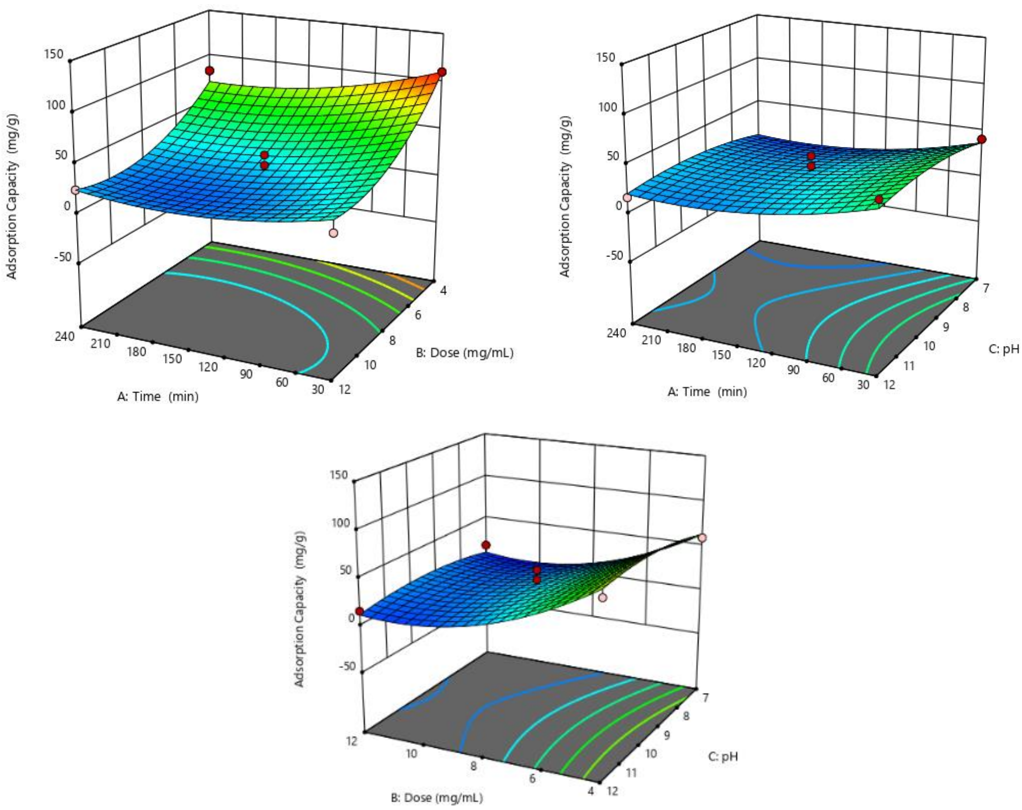

Figure 1. 3D response surface model of the adsorption capacity of casein (Y axis) as a function of contact time (min) and dose (mg/mL) (a); pH and contact time (min) (b); and dose and pH (c), respectively (X-axis).

Optimization of experimental conditions

The optimal conditions for adsorption of casein onto alkaline-treated rice husk were determined using Design Expert software (version 7.0, Stat-Ease Inc., Minneapolis, USA). In the optimization process, contact time and adsorbent dosage were minimized to reduce both time consumption and rice husk usage. pH was set within a broad range to encompass a variety of pH conditions throughout the study. The adsorption capacity was maximized to enhance the efficiency of casein adsorption onto rice husk.

The desirability ramps generated from the optimal points are shown in Figure 2. As indicated in Table 4, the predicted optimal conditions for maximum response were achieved with an incubation time of 30 minutes, an adsorbent dosage of 4 mg/mL, and a pH of 9.93. Under these conditions, the predicted adsorption capacity was 111.547 mg/g. In the actual experiment, the adsorption capacity of casein was measured at 110.0 mg/g, with no significant difference observed between the predicted and actual values ($p > 0.05$).

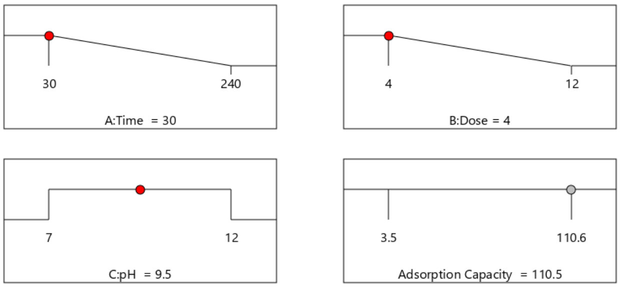

Figure 2. Desirability ramps for optimization of adsorption capacity.

Table 4. Predicted and actual values of response.

	Adsorption Capacity (mg/g)
Predicted	111.547
Actual	110.0
p-value	0.952

CONCLUSION

This study demonstrates the effectiveness of alkaline-treated rice husk as an adsorbent for casein recovery, optimized through response surface methodology (RSM). By systematically adjusting key variables—contact time, adsorbent dosage, and pH—the experimental design achieved a significant adsorption capacity, with optimal conditions determined as 30 minutes of contact time, 4 mg/mL adsorbent dosage, and pH 9.93. The results revealed a close alignment between the predicted and actual adsorption capacities, confirming the reliability of the RSM model in maximizing adsorption efficiency. This optimised process provides an eco-friendly and cost-effective method for protein recovery, with potential applications in nutrient recycling and sustainable waste management. However, scalability challenges remain a critical area for future exploration. For instance, translating laboratory-scale findings to industrial applications may involve addressing issues such as the consistent sourcing and preparation of alkaline-treated rice husk in large quantities, maintaining adsorbent efficiency across varying wastewater compositions, and ensuring process reliability at higher operational scales. Moreover, the economic feasibility of this approach requires detailed cost analyses to balance the expenses of adsorbent production and regeneration with the potential savings from protein recovery and waste valorisation. Identifying strategies to reduce costs, such as reusing treated rice husk or integrating the process into existing industrial workflows, will be essential for broader adoption. Future research could explore the scalability of this approach and evaluate its applicability in industrial wastewater treatment settings, aiming for broader environmental and economic benefits.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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