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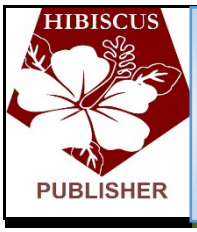

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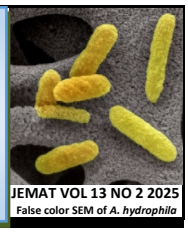

Optimization of Sugarcane Bagasse for Whey Adsorption: A Sustainable Approach to Enhancing Nutrient-Rich Animal Feed

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Abstract

Waste agricultural biomass is often environmentally hazardous when released to the environment, and some residues are toxic to the surrounding ecosystems. These biomasses, when properly valorized, can be a sustainable source of green adsorbents. The ever-increasing need for nutrient-fortified animal feed has become a real challenge for the animal feed industry. In this study we focus on a method of improving the nutrient content of the agricultural waste biomass sugarcane bagasse through the adsorption of whey, a byproduct of the cheese industry. The potential of sugarcane bagasse and spent cheese whey as sustainable adsorbent and adsorbate, respectively, is explored. We first use a one-factor-at-a-time approach to maximize the adsorption capacity of sugarcane bagasse. The factors examined include bed height, pH, temperature, and whey concentration. RSM was successfully used to further improve the optimal conditions. A fixed-bed column packing method was utilized in the adsorption experiments. Parameters optimized include the effects of different bed heights (1 cm to 3 cm), pH levels (4 to 8), temperatures (20 °C to 30 °C), and whey concentrations (0.01% to 0.05%). The optimal conditions include a bed height of 3 cm, pH of 8.0, temperature of 25 °C, and whey concentration of 0.01%. These optimal conditions improve the nutrient content of the agro-industrial waste. The Modified Dose Response (MDR) Model was utilized to analyze the breakthrough curves of the design parameters, which include bed height, flow rate, and initial solute concentration. The results show that a 3 cm bed height yields a q_m value of 79.487 mg/g, pH 8 gives a q_m value of 82.797 mg/g, and a 0.05% whey concentration results in a q_m value of 95.274 mg/g. To the best of our knowledge, this research is the first, and it has the potential to contribute to a sustainable novel approach for whey supplementation in animal feed.

INTRODUCTION

It is expected that in the next few years, the demand for products made from animals will expand exponentially. According to the World Health Organization (WHO) statistics, the average individual will eat roughly 134.8 kg of livestock products per year by 2030. This is an increase of about 18% over the levels seen in the early 2000s [1]. If the efficiency and digestibility of feed roughly remain the same, a growth of over 66% in livestock feed supply will be needed, as the world's population is expected to grow by 2.5 billion people between 1999 and 2030. In addition, at the same time, the agricultural sector is under an intense pressure to meet the rapidly growing needs of the global population, meaning that it needs to produce more food and resources. Based on recent studies, technical advances in farming have increased the amount of food that can be grown in the last 50 years but in the attempt to fight food shortages, this has unwittingly damaged the ecosystem with pesticide and fertilizers runoffs. Agricultural practices are a major source of ecological damage, with waste and hazardous chemicals accumulation presenting considerable threats to the ecosystems and public health.

It is estimated that more than 21% of all greenhouse gas emissions come from farming [2]. Agro-industrial waste production in Asia alone is about 1.3 billion tons per year, which

reflects how enormous this issue is [1–5]. Due to the changing lifestyles and higher demand, the cheese industry in Malaysia has grown a lot in the past decades. However, cheese processing produces waste effluent that is a major byproduct of the dairy industry. This effluent is protein rich and is very bad for the environment. The high COD and BOD are the negative consequences of cheese whey waste, which increase the content of nutrients and organic matter in the environment. D'Alessandro et al. reported that cheese whey effluent exhibited biological oxygen demand (BOD) levels between 27 and 60 kg/m³ and chemical oxygen demand (COD) levels between 50 and 102 kg/m³, which makes it a major pollutant [6]. The biodegradability index of cheese whey effluent is between 0.4 and 0.8, and if not handled correctly, leads to oxygen deprivation, eutrophication, impermeabilization, and environmental pollution [7]. Despite these environmental dangers, cheese waste effluents are still full of nutrients, and roughly 93–94% water and contain soluble proteins and lactose. Sugarcane bagasse is plentifully cultivated by small holders for demand as sugar cane beverages. It is a renewable resource that may be used in many ways.

As the term sustainability has come at the center stage, this has led to a heightened interest in using sugarcane bagasse to solve environmental problems. Sugarcane bagasse is an item that can add value while cutting down on waste. Research has shown that sugarcane bagasse can be reused as reinforcement material in polymer composites, which supports the development of eco-friendly alternatives [8]. Considering Malaysia's agricultural landscape and the increasing focus on sustainable practices, has led to the incorporation of sugarcane bagasse in animal feed. Its use as an adsorbent for cheese waste effluent could provide a double waste to wealth strategy as a substantial advantage for both environmental preservation and agricultural advancement.

The groundbreaking "feedsorption" process we developed earlier [9] is set to revolutionize the valorization of waste effluents originating from industry and agriculture. The enhancement of the nutrient content of Sugarcane Bagasse (SCB) through the adsorption of protein from cheese wastewater (CW) effluent is a novel technique. This adsorption process aims to transform SCB a low-value byproduct of agriculture, into a nutrient-dense and higher-value feed ingredient. A fixed bed column is one of the best configurations for the design of an adsorbent-rich nutrient as it simultaneously traps and adsorbs protein-rich adsorbate. It is anticipated that the use of statistical optimization methods like Response Surface Methodology (RSM) can maximize the effectiveness of the feedsorption process. RSM can provide a valuable insight and enhance the efficiency of the feedsorption system through a systematic analysis of the interactions between critical variables and adsorption as a response. This study aims to advance eco-friendly animal feed solutions to contribute significantly to Malaysia's agricultural sector and lay the groundwork for global adoption of sustainable practices in animal nutrition.

MATERIALS AND METHODS

Preparation of sugarcane bagasse (SCB)

The sugarcane bagasse (SCB) was bought from a vendor at a night market in Serdang in 2024. First, the bagasse was dried in the sun, and then it was dried in an oven at 60 °C for 24 hours. Then SCB was ground and sieved until the particles were 1.25 mm in size. Before use, the bagasse was steeped in distilled water until it was stable.

A simple simulated cheese whey (SCW) effluent was developed by dissolving whey protein (20 grams) from a cheese processing company with 200 mL of distilled water [10].

Standard curve for whey protein based on the Bradford assay

A series of BSA standard solutions as a surrogate for whey protein was set up at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL in distilled water. To 100 µL of the working BSA solution, 1 mL of Bradford reagent was added to a test tube and mixed well. After that, the cuvettes were left at room temperature for 5 minutes. After the incubation time was up, a spectrophotometer (Shimadzu UV-VIS1201) was used to measure the samples' absorbance at 595 nm.

Optimization of whey sorption by sugarcane bagasse using OFAT approach

To get the best adsorption of whey onto sugarcane bagasse, a number of factors were optimized. The parameters were whey content, bed height, pH, and temperature, as each of these could affect the rate of adsorption. The research sought to ascertain the ideal conditions for the enhancement of whey adsorption onto sugarcane bagasse.

Effect of bed height

A glass chromatography column (XK 50/30, GE Helathcare) was utilized to study the adsorption of whey at different bed heights, which was packed with sugarcane bagasse and started with an initial bed height of 1 cm. Whey solutions with a concentration of 0.05% were used. These solutions were passed through the packed column using a peristaltic pump (MasterFlex L/S Model 7518). Eluted samples were collected every minute using a fraction collector (Bio-Rad Model 2110), with approximately 2 mL of solution collected in each test tube. This experiment was repeated for different bed heights of 1.5 cm, 2 cm, and 3 cm [9].

Effect of pH

The influence of pH on whey adsorption was investigated by the initial pH method, where the pH was adjusted between 4 and 8 using 0.1 M hydrochloric acid (HCl) and 0.1 M sodium hydroxide (NaOH). Sugarcane bagasse was packed in a glass column at an optimized bed height of 1 cm. The whey solution, adjusted at various pHs was passed through the packed column, and samples were collected at one-minute intervals via a fraction collector (BioRad fraction collector model 2110) [9].

Effect of temperature

In this experiment, sugarcane bagasse was packed into the column chromatography with a bed height of 1 cm. A 100 mL 0.05% (v/v) whey solution at pH 7 was prepared in a beaker. Sous vide (AUGIENB) was used to set different temperatures (25, 30, 35, and 40 °C) in an ice box for each experiment. The water from the ice box was directed into the water-jacketed glass column via silicon tubes, with water flow pressurized by a peristaltic pump. Then the simulated cheese whey (SCW) ran through the packed sugarcane bagasse in the column, and 2 mLs samples were collected per test tube in a fraction collector. The experiment was repeated at various temperatures of 30 °C, 35 °C, and 40 °C to assess the impact of temperature on whey adsorption [9].

Effect of concentration

A 1 cm bed height of sugarcane bagasse was first packed into a glass column, followed by pumping a 0.05% (v/v) whey solution via a peristaltic pump for a duration of 30 minutes.

Samples were collected at 1-minute intervals and the process was repeated with whey solutions of varying concentrations (0.01%, 0.02%, 0.03%, 0.04%, and 0.05%) [9].

Quantification of Whey Protein Using the Bradford Assay with Bovine Serum Albumin (BSA) Standard

For the determination of residual whey in the collected fractions, 100 µL of each sample collected in the test tube was transferred into a test tube. Then, 1000 µL of Bradford reagent was added to each cuvette, mixed and incubated for 5 min. The absorbance of each sample was measured at 595 nm [9].

Column operation parameters

Creating a graph with the ratio of C_t/C_0 (the concentration of adsorbate in the effluent relative to the influent) against time (t) generates the breakthrough curves, which are defined by the area beneath these curves. The cumulative amount of adsorbate captured by the column is denoted as q_{total} and t_s refers to the stoichiometric time observed in an asymmetrical breakthrough curve [11]:

$$q_{total} = Q \int_{t=0}^{t=t_e} C_r dt$$

$$t_s = \frac{1}{C_0} \int_{t=0}^{t=t_e} C_r dt$$

The data obtained from the experiment can be modeled using various kinetic models to determine the maximum adsorption capacity and to analyze the breakthrough curves. The Thomas and Yoon-Nelson models are commonly used for this purpose, but the Modified Dose Response (MDR) model, proposed by Yan et al. [12], has been shown to minimize errors in the Thomas model, especially at lower or higher times in the breakthrough curve. The Modified Dose Response (MDR) model is described by the following formula:

$$\frac{C_t}{C_0} = 1 - \frac{1}{1 + \left(\frac{V_t}{b}\right)^a}$$

where, a and b are MDR model constants.

C_0 = Amino acid concentration (mg L^{-1}) at time 0.

V = Column flow rate (L h^{-1})

t = Flow time (h)

C_t = Amino acid concentration (mg L^{-1}) at time t.

From the value of b, the value of the maximum adsorption (q_m) of adsorbate (whey) in (mg per g adsorbent) to agrofeed (SCB) was anticipated using the following:

$$q_m = \frac{bC_0}{m}$$

Optimization study using RSM

Response Surface Methodology (RSM) is an effective statistical tool for optimizing processes, and in this study, the Box-Behnken Design (BBD) was employed to optimize the adsorption process of whey onto sugarcane bagasse. The BBD is used for designing experiments, modeling the response surface through regression, and performing optimization. It involves evaluating the relationship between input variables and the response variable (in this case, the maximum adsorption capacity, q_m (mg g^{-1}), using a second-order polynomial equation. The general form of the second-order polynomial equation used in RSM is as follows:

$$y = \beta 0 + \sum \beta i \; k \; i=1 \; xi + \sum \beta iixii \; k \; 2 \; i=1 + \sum \sum \beta ijxixj + error$$

In this study, a three-level, three-factor BBD **(Table 1)** was employed, with three input variables, which could include factors such as bed height, pH, and temperature. The main goal was to maximize the response. q_m (mg g^{-1}), which represents the maximum adsorption capacity (mg/g) of the sugarcane bagasse. The Design Expert software (Trial version) output was a total of 17 randomized experimental runs specified in the design **(Table 2)**, which included 12 factorial points and 5 center points. The center points function to minimize the variability induced by external factors and can offer a significant insight into the effect of curvature. The results from the experimental runs are then analyzed to construct the response surface, which facilitates the identification of optimal conditions for maximum adsorption.

Table 2. Experimental design and results of Box-Behnken for the adsorption of Simulated Cheese Whey (SCW) on SCB.

		Factor 1	Factor 2	Factor 3	Response 1
Std	Run	A: pH	B:Bed height	C:Conc	qm
			cm	%	mg/g
12	1	6.5	1	0.1	56.8112
2	2	8	1	0.075	58.5432
15	3	8	2	0.1	42.8427
5	4	8	2	0.05	78.7278
9	5	5	2	0.1	78.3017
7	6	6.5	2	0.075	64.8886
3	7	8	3	0.075	83.0762
17	8	5	2	0.05	69.0128
11	9	5	1	0.075	97.6495
13	10	6.5	2	0.075	58.3174
16	11	6.5	3	0.05	83.9871
14	12	6.5	3	0.1	69.8471
4	13	6.5	2	0.075	60.2762
10	14	6.5	2	0.075	60.5504
1	15	6.5	2	0.075	58.2452
6	16	6.5	1	0.05	81.4237
8	17	5	3	0.075	91.3546

All experiments were carried out duplicate, and the average values are presented in this study. The data were analyzed using the trial version of **Design Expert 11.0** software from **Stat-Ease, Inc.** (Trial version), which includes **ANOVA** to identify the significant factors among the variables.

Table 1. Coded and uncoded levels of the independent variables

Factor	Name	Units	Type	SubType	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	pH		Numeric	Continuous	5.00	8.00	-1 ↔ 5.00	+1 ↔ 8.00	6.50	1.06
B	Bed height	cm	Numeric	Continuous	1.0000	3.00	-1 ↔ 1.00	+1 ↔ 3.00	2.00	0.7071
C	Conc	%	Numeric	Continuous	0.0500	0.1000	-1 ↔ 0.05	+1 ↔ 0.10	0.0750	0.0177

Statistical Analysis
The values, reported as means ± SD, were obtained in triplicate. Group comparisons were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test or the Student's t-test. A p-value below 0.05 was deemed statistically significant.

RESULTS AND DISCUSSION

BSA Standard Curve

Optimization of whey sorption by SCB using OFAT approach

The classical One-Factor-at-a-Time (OFAT) method functions by analyzing multiple factors but not at the same time but through varying a single factor while maintaining the others at a constant level. The key factors that influences whey adsorption are optimized sequentially, one after the other and these include concentration, bed height, pH, and temperature, which is similar to several other previous studies utilizing agricultural biomasses [13–18]. This study employed a constant particle size of 1.25 mm. The particle size is anticipated to swell as Saw et al. [19] indicate that the water retention capacity of PKC increases as particle size decreases from 4.825 mm to 0.513 mm. This swelling is attributed to an increase in surface area and pore volume [20].

Mathematical modeling encompasses kinetic and isotherm analyses, which provide a significant insight into adsorption characteristics. Isotherm models facilitate the evaluation of the biosorption process by defining the correlation between the equilibrium concentration of whey and the quantity adsorbed by SCB. The adsorption data were analyzed using modified dose-response (MDR) kinetic models to examine the adsorption behavior and performance of the fixed-bed column. These models are crucial for predicting dynamic performance, breakthrough behavior, and comprehending the influence of various factors on the adsorption process [12].

Effect of bed height

The binding capacity of whey to sugarcane bagasse was examined utilizing bed heights of 1, 2, and 3 cm (**Fig. 1**). Initially, sugarcane bagasse with a particle size of 1.25 mm was arranged to a bed height of 1 cm. A 0.05% whey solution was subsequently passed via a peristaltic pump at a constant flow rate through the packed sugarcane bagasse in the column. Effluent samples were systematically collected at regular intervals utilizing a fractionating collector, with approximately 2 mL obtained in each test tube. The eluted samples were quantified with Bradford reagent to identify the presence of whey, through the formation of a blue color. The raw data was normalized to a value of 1 to standardize the readings. The normalized breakthrough curves were modeled employing modified dose-response (MDR) models.

The breakthrough curve was accurately modeled using the modified dose-response (MDR) method. The resultant modeling depicts the influence of different bed heights on the adsorption rate, which shows a strong correlation with the experimental data points. **Fig. 1** illustrates that a bed height of 3 cm produced the longest breakthrough curve, which suggests a minimal presence of whey in the effluent samples. This is followed by the 2 cm bed height, and the worst was 1 cm bed height.

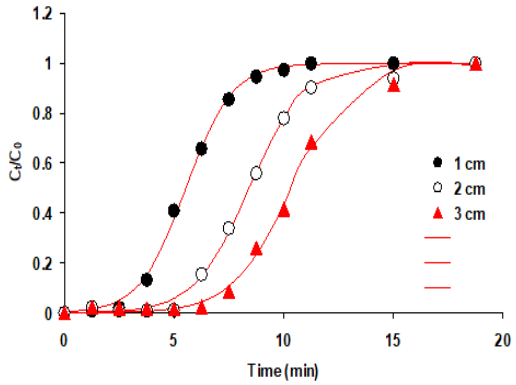


Fig. 1. Column breakthrough curves for SCW sorption by sugarcane bagasse at various bed heights. Red lines indicate model-fitted results.

Longer breakthrough curves indicate an increased adsorption of whey onto SCB, as they signify reduced protein content in the effluent samples. ANOVA analysis indicated a significantly longer breakthrough curve ($p < 0.05$) for the 3 cm bed height. The increased bed size is anticipated to facilitate enhanced whey adsorption onto SCB, as a larger bed size allows a longer contact time between the adsorbate and adsorbent, resulting in a more effective interaction and diffusion of adsorbate molecules into the pores of the adsorbent [21].

Effect of pH

The binding efficiency of protein to the adsorbent surface is influenced by multiple factors, with pH being a major determinant. This study investigates the impact of pH variations to the sorption capacity of SCB at various pH levels between 4 and 8. **Fig. 2** illustrates that a bed height of 3 cm was selected as a constant variable for the subsequent parameter optimization.

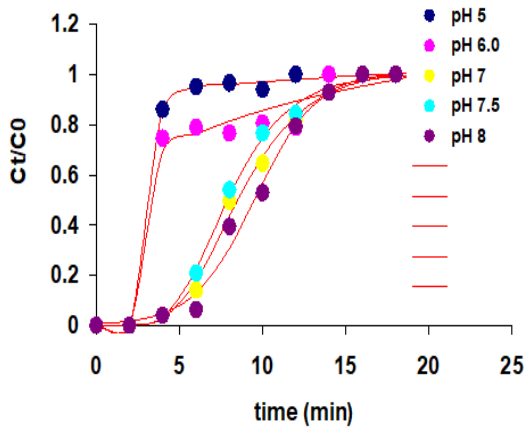


Fig. 2. Column breakthrough curves for SCW sorption by SCB at various pH. Red lines indicate model-fitted results.

The absorbance readings were normalized, and the breakthrough curve was evaluated through the modified dose-response model. **Fig. 2** illustrates the adsorption efficiency of SCB at various pH levels. The longest breakthrough time were observed at pH 8, with pH 7 exhibiting comparable adsorption behaviors. ANOVA analysis indicated no significant differences ($p > 0.05$) in absorbance across pH values of 5, 6, 7, 7.5, and 8, implying that whey adsorption by SCB was optimal within this pH range.

Data at pH 4 were excluded as outliers, likely due to the elevated concentration of H^+ ions in acidic conditions, which compete with adsorbate molecules for active sites on the adsorbent, potentially reducing the adsorption capacity [22]. Bernal et al. indicated that low pH may alter the chemical state of adsorbate molecules. This alteration can impede their interaction with the adsorbent surface and reduce adsorption efficiency [23]. Most proteins in cheese whey possess an isoelectric point (pI) below 5. This results in negative charges on whey proteins at elevated pH levels. The repulsion among negatively charged proteins exposes their functional groups, thereby enhancing their binding capacity with SCB. The selection of pH 8 as the optimal condition for subsequent parameter studies was based on its association with the longest breakthrough time observed.

Effect of temperature

Temperature is a costly factor that governs the economics of an adsorption process. However, it is essential for establishing optimal conditions that can enhance the adsorption efficiency of the adsorbate to the adsorbent. In this study, a temperature range between 20 and 30 °C was chosen, which reflects the Malaysia's average annual temperature. A 0.05% (v/v) SCW solution at pH 8 was introduced into the SCB column. The absorbance of the effluent samples was measured at 595 nm as before. Curve fitting exercise to fit the model to raw data was carried out using the modified dose-response model to determine the optimal temperature for SCW adsorption, which was identified as 25 °C.

Fig. 3 illustrates that the temperature of 25 °C displayed the longest breakthrough curve compared to other temperatures, which indicates that this is the best temperature. Conversely, elevated temperatures of 30, 35, and 40 °C led to a shorter breakthrough curve. This suggests an increased whey content in the effluent samples and indicates a decrease in adsorption efficiency. ANOVA statistical analysis indicated a significant difference ($p < 0.05$) at 25 °C relative to other temperature conditions. This observation correlates with a decrease in thermal agitation at lower temperatures, which facilitates a more stable and effective binding of BSA to the adsorbent surface. A similar temperature-dependent adsorption is observed in the work of Budnyak et al. [24], who reported that lower temperatures favor adsorbate–adsorbent interactions through the minimization of the molecular motion and lead to an enhanced equilibrium binding.

The results in this study conform to established adsorption theory, in which the temperature shows an important role in the determination of adsorption equilibrium, kinetics, including molecular interaction energy. In addition, Santos [25] similarly observed that variations in temperature was found to significantly influence the adsorption of whey protein under dynamic conditions. This finding reinforces the note that excessive thermal energy can lead to a disruption of molecular bonding and a reduction in adsorption efficiency. To conclude, this study supports prior studies demonstrating that moderate thermal conditions (around 25 °C) is the optimal conditions for the interaction between whey and sugarcane bagasse.

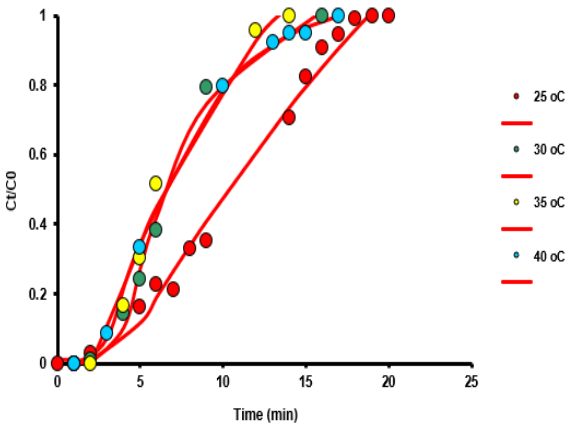


Fig. 3. Column breakthrough curves for whey sorption by SCB at different temperatures. Red lines indicate model-fitted results.

The modified dose-response (MDR) model was employed to identify the optimal temperature for SCW adsorption, which was found to be 25 °C. As shown in **Fig. 3,** this temperature exhibited the longest breakthrough curve when compared to the other temperatures. On the other hand, temperatures of 30, 35, and 40 °C led to shorter breakthrough curves, indicating an increase in whey content in the effluent samples. ANOVA analysis revealed a statistically significant difference ($p < 0.05$) at 25 °C when compared to the other temperatures. This can be attributed to lower thermal agitation at 25 °C, which, as observed by Budnyak et al. [24], promotes better binding of adsorbate molecules to the adsorbent surface. As a result, 25 °C was chosen as the optimal temperature for subsequent experiments.

Effect of concentration

In fixed-bed column studies of a comparable experiment involving the adsorption of BSA to palm kernel cake (PKC), the concentration of BSA was found to be critical in identifying the optimal concentration for BSA to PKC, with higher protein concentrations proving to be suboptimal [9]. To determine the optimal concentration for maximum SCW absorption by SCB, the effects of SCW concentrations of 0.01% (v/v), 0.02% (v/v), 0.03% (v/v), 0.04% (v/v), and 0.05% (v/v) were examined. **Fig. 4** illustrates the effect of these concentrations on the SCW absorption rate by SCB. The results demonstrate that a concentration of 0.01% (v/v) SCW is optimal for maximizing absorption, as evidenced by the longer breakthrough curve observed. The findings indicate that a higher concentration of SCW improves the whey absorption rate by SCB, presumably because a greater number of SCW molecules are present to interact with SCB at elevated concentrations. ANOVA analysis confirmed that 0.01% (v/v) is the optimal concentration, with statistically significant differences noted ($p < 0.05$).

Optimization of whey sorption by SCB using RSM

This research utilized the One-Factor-at-a-Time (OFAT) approach to determine that room temperature (25 °C) is the optimal condition for whey adsorption.

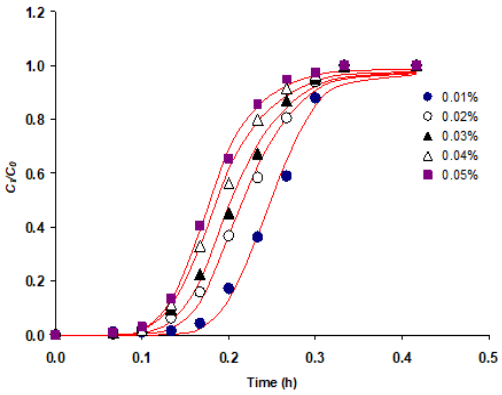


Fig. 4. Column breakthrough curves for SCW sorption by SCB at various concentrations. Red lines indicate model-fitted results.

The selected temperature served as a constant parameter for subsequent optimization employing Response Surface Methodology (RSM) and the Box-Behnken Design (BBD). In contrast to another design, the Central Composite Design (CCD), the Box-Behnken Design (BBD) utilizes three levels for each factor (+1, 0, -1), resulting in a reduction of the number of experimental runs while adequately capturing all of the possible linear, quadratic, and interaction effects. An important benefit of BBD is its capacity to circumvent experimental conditions in which all variables are at extreme levels, thus minimizing the probability of adverse results. It necessitates a minimum of three factors and is particularly appropriate for second-order polynomial modeling [26].

Moderate temperatures are essential for optimizing adsorption efficiency, as they increase molecular kinetic energy while maintaining adsorption stability. It was observed that temperatures greater than 35 °C can lead to protein desorption, particularly when adsorption is primarily influenced by weak noncovalent interactions, including hydrogen bonding and van der Waals forces. Elevated temperatures can induce structural alterations in proteins, which result in a fouling on equipment surfaces, a prevalent concern in industries such as dairy processing [27]. Maintaining a temperature of 25 °C ensures stable adsorption conditions and avoids such challenges. The BBD was applied to three independent variables—pH, SCW concentration (% v/v), and bed height (cm)—each tested at three levels (low, medium, high). Experimental runs were conducted to maximize the primary response variable (q_m). The data were analyzed using Design-Expert software to evaluate linear, two-factor interaction, and quadratic models. A quadratic model, which includes linear terms, interaction effects, squared terms, and an intercept, was found to best describe the data. **Table 3** provides a comprehensive overview of the variable design scheme, along with experimental results, predicted responses, and residuals.

The F-test evaluated the statistical significance of the quadratic model, as shown in **Table 4**. The ANOVA results shows that the model is statistically significant, as evidenced by an F-value of 23.858 and a low P-value of 0.0001. The lack of fit was not significant (P-value > 0.05), which is it should be, indicating the model's suitability and reliability for predicting the response variable. **Table 4** demonstrates that all variables incorporated in the model are statistically significant. The correlation coefficient ($R^2 = 0.968$) and adjusted correlation coefficient (Adj $R^2 = 0.928$) demonstrate the model's robustness, which indicates that 96.8% of the variation in the response data can be explained by the model. The difference in value of between the Predicted R^2 and Adj R^2 was slightly greater than 0.04, indicating a reasonable agreement and further validating the model's predictive accuracy. Adeq Precision, which quantifies the signal-to-noise ratio in the experiment, produced a value of 18.89. The model exhibited a sufficient signal, which facilitates an effective exploration of the design space, as values exceeding 4 are deemed desirable.

Table 4. ANOVA analysis of the fitted Box-Behnken design.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3283.18	9	364.80	23.86	0.0002	significant
A-pH	668.48	1	668.48	43.72	0.0003	
B-Bed height	143.12	1	143.12	9.36	0.0183	
C-Conc	533.81	1	533.81	34.91	0.0006	
AB	237.59	1	237.59	15.54	0.0056	
AC	510.17	1	510.17	33.37	0.0007	
BC	27.42	1	27.42	1.79	0.2224	
A^2	283.26	1	283.26	18.53	0.0035	
B^2	825.05	1	825.05	53.96	0.0002	
C^2	8.69	1	8.69	0.5682	0.4756	
Residual	107.03	7	15.29			
Lack of Fit	77.88	3	25.96	3.56	0.1257	not significant
Pure Error	29.15	4	7.29			
Cor Total	3390.21	16				

Table 3. Design scheme of variables with experimental, predicted values of response and the residuals.

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	56.81	58.00	-1.19	0.750	-0.608	-0.579	0.111	-1.003	12
2	58.54	61.58	-3.03	0.750	-1.552	-1.775	0.723	-3.074[1]	2
3	42.84	38.62	4.22	0.750	2.161	3.467	1.401[1]	6.004[1]	15
4	78.73	77.54	1.19	0.750	0.606	0.577	0.110	0.999	5
5	78.30	79.49	-1.19	0.750	-0.606	-0.577	0.110	-0.999	9
6	64.89	60.46	4.43	0.200	1.267	1.337	0.040	0.668	7
7	83.08	85.45	-2.38	0.750	-1.215	-1.266	0.443	-2.193	3
8	69.01	73.24	-4.22	0.750	-2.161	-3.467	1.401[1]	-6.004[1]	17
9	97.65	95.27	2.38	0.750	1.215	1.266	0.443	2.193	11
10	58.32	60.46	-2.14	0.200	-0.611	-0.582	0.009	-0.291	13
11	83.99	82.80	1.19	0.750	0.608	0.579	0.111	1.003	16
12	69.85	71.70	-1.85	0.750	-0.946	-0.938	0.268	-1.624	14
13	60.28	60.46	-0.1794	0.200	-0.051	-0.047	0.000	-0.024	4
14	60.55	60.46	0.0948	0.200	0.027	0.025	0.000	0.013	10
15	58.25	60.46	-2.21	0.200	-0.632	-0.603	0.010	-0.301	1
16	81.42	79.57	1.85	0.750	0.946	0.938	0.268	1.624	6
17	91.35	88.32	3.03	0.750	1.552	1.775	0.723	3.074[1]	8

Table 5. Final equation in terms of coded and actual factors.

q_m (coded)	=	q_m (actual)	=
+60.46		+248.88257	
-9.14	A	-41.17317	pH
+4.23	B	-93.01423	Bed height
-8.17	C	+1766.10519	Conc
+7.71	AB	+5.13799	pH * Bed height
-11.29	AC	-301.16057	pH * Conc
+2.62	BC	+104.72455	Bed height * Conc
+8.20	A^2	+3.64540	pH^2
+14.00	B^2	+13.99816	Bed $height^2$
-1.44	C^2	-2298.35757	$Conc^2$

The non-significance of the lack of fit in relation to the pure error (P-value > 0.05) is advantageous, suggesting that the model effectively represents the experimental data. The anticipated response is also formed in coded factor levels, as outlined in **Table 5**, in conjunction with an equation that integrates actual factor values to forecast responses across varying conditions. This underscores the model's practical utility in optimizing process parameters. **Table 6** displays the estimated coefficients for the examined factors, including their corresponding standard errors, confidence intervals, and Variance Inflation Factors (VIF), with the latter serving as a diagnostic instrument for evaluating multicollinearity within regression models. This quantifies the extent to which the variance of a regression coefficient is increased because of correlation with other variables in the model. A VIF of 1 is optimal, signifying no correlation between the coefficient and other model terms, which demonstrates orthogonality. VIF values greater than 10 indicate problematic results, indicating significant multicollinearity that may undermine the precision of coefficient estimation. Significant and severe multicollinearity is represented by values that exceed 100 or 1,000 indicate.

These high values indicate undermining the model's reliability. This study found that all variables exhibited a VIF of 1, indicating no significant multicollinearity. This value guarantees the stability and reliability of the regression coefficients. Confidence intervals were employed to assess the significance of each factor's coefficient. A positive estimated coefficients for all components were seen in this study, suggesting a direct relationship with the response variable. The analysis revealed that pH had the highest estimated coefficient value, signifying its significant impact on adsorption efficiency. The incubation period and SCW concentration subsequently indicated that these factors are significant in improving adsorption performance. The lack of multicollinearity and the significant impact of the examined components highlight the strength of the regression analysis, establishing a solid basis for result interpretation and process parameter optimization.

Table 6. Coefficients in terms of coded factors.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	60.46	1	1.75	56.32	64.59	
A-pH	-9.14	1	1.38	-12.41	-5.87	1.0000
B-Bed height	4.23	1	1.38	0.9606	7.50	1.0000
C-Conc	-8.17	1	1.38	-11.44	-4.90	1.0000
AB	7.71	1	1.96	3.08	12.33	1.0000
AC	-11.29	1	1.96	-15.92	-6.67	1.0000
BC	2.62	1	1.96	-2.01	7.24	1.0000
A^2	8.20	1	1.91	3.70	12.71	1.01
B^2	14.00	1	1.91	9.49	18.50	1.01
C^2	-1.44	1	1.91	-5.94	3.07	1.01

To verify the normality assumption, a half-normal probability plot of the residuals **(Fig. 5)** was generated and analyzed. The values of all internally studentized residuals fell between 2 and aligned closely along a straight line, indicating that no transformation of the response is necessary. This conclusion was drawn from our analysis. Additionally, the plot comparing the actual experimental results with the model's predicted values demonstrates a good fit.

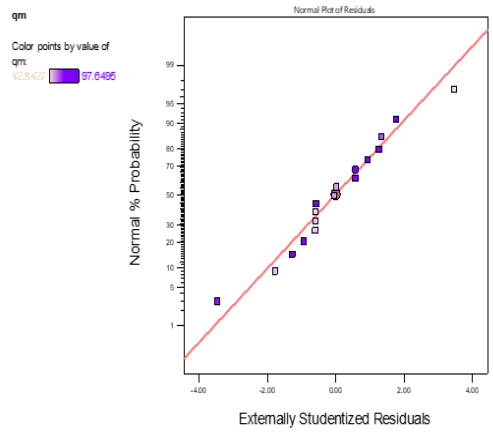


Fig. 5. Half-normal probability plot of the residuals

The plot comparing predicted and actual data for the Box-Behnken design **(Fig. 6)** shows a strong agreement between the predicted and experimental values. The leverage vs. run plot in **Fig. 7** indicates that all the numerical values fall within the standard range of 0-1, suggesting that no design point significantly affects the model fit. If a data point has an unusually high leverage value (greater than 1), it could indicate a problem, such as an unexpected error, because such points can disproportionately influence the model. However, the leverage vs. run plot shows no data points exceeding the average leverage, which would have impacted at least one model parameter.

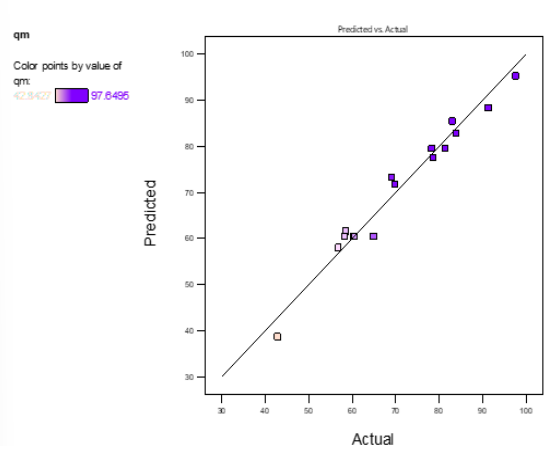


Fig. 6. Diagnostic's plot in the form of the expected/predicted vs real/actual data the Box-Behnken optimization studies.

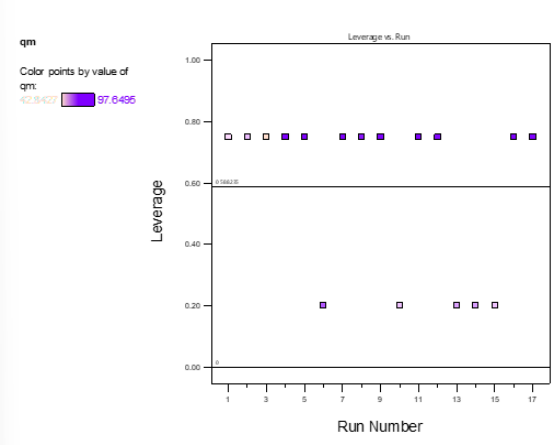
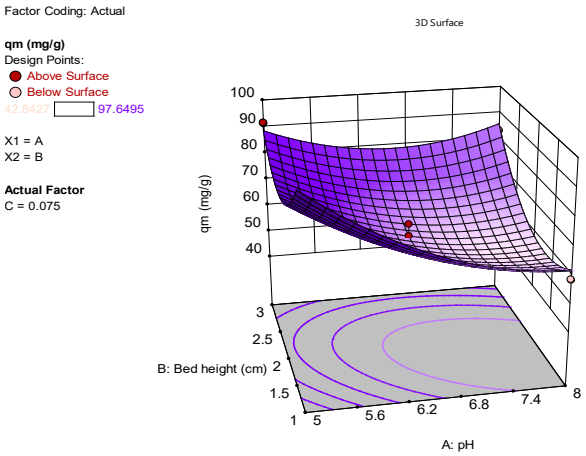


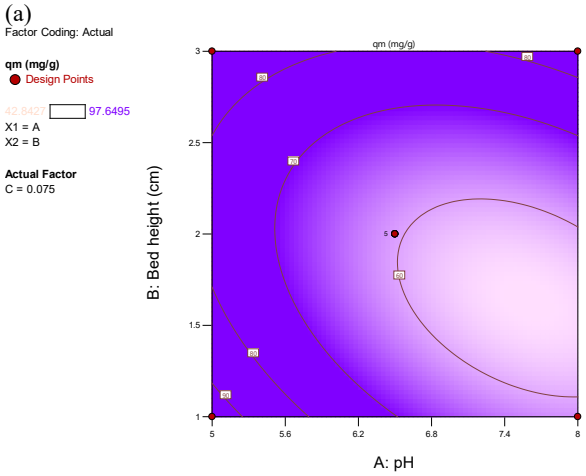
Fig. 7. Diagnostic's plot in the form of leverage vs runs for the Box-Behnken optimization studies.

The 3D plots were developed using the model equation generated by the Design Expert program, providing a visual representation of the interactions between independent factors. These plots were constructed by plotting the response (Z-axis) against any two independent variables, while the third variable was held constant at its midpoint value. This approach allows for the examination of the reciprocal interaction effects between two significant factors within the experimental range. Each graph illustrates how the interaction between the two independent variables affects the response, which is SCW sorption to SCB while the constant factor remains unchanged. The shapes of the plots, whether saddle-shaped, elliptical, or otherwise, reflect the combined influence of the variables on the response and their interaction dynamics.

In the 3D surface plot, where the SCW concentration was fixed at 0.075% (v/v), an elliptical profile is observed (**Fig. 8a**). The plot illustrates the interaction between bed height (B) and pH (A) on the adsorption capacity (q_m). The highest adsorption capacity, 95.274 mg/g, occurs at a bed height of approximately 1 cm and a pH close to 5.0, as indicated by the peak in the plot. The contour plot below the 3D surface further emphasizes this interaction, with elliptical lines suggesting the significant combined effect of the two factors on the response. **Fig. 8b** further highlights this interaction, showcasing the elliptical shape of the contour plot. The elliptical nature suggests a significant interaction between the factors, as the response surface deviates from linearity. This demonstrates that the optimal response is achieved through a balance of bed height and pH rather than the isolated influence of each variable. The combination of the elliptical 3D wireframe and elliptical contour plots emphasizes the importance of considering factor interactions in adsorption studies. It also validates the response surface model as a reliable tool for predicting optimal process conditions.



(a)



(b)

Fig. 8. The 3D response surface plots of between the factor pH and bed height (a) and 3D- (b) contour plots.

When the bed height was held at the midpoint (2 cm), varying the SCW concentration and pH shows a mild elliptical profile indicating a weak relationship of synergistic interaction (**Fig. 9a**). with the highest response (q_m) of 79.487 mg/g (95% confidence interval from 71.479 to 87.495) occurring at the predicted pH of about 5.0 and the highest SCW concentration of 0.1% (v/v) (**Fig. 9b**). When the pH was held at the midpoint (pH 6.5), varying the pH and SCW concentration factors show an elliptical profile indicating interaction (**Fig. 10a**) with the highest response of 82.797 (q_m) (95% confidence interval 74.789 to 90.805) occurring at the predicted SCW concentrations of 0.05% (v/v) and bed height of 3 cm (**Fig. 10b**).

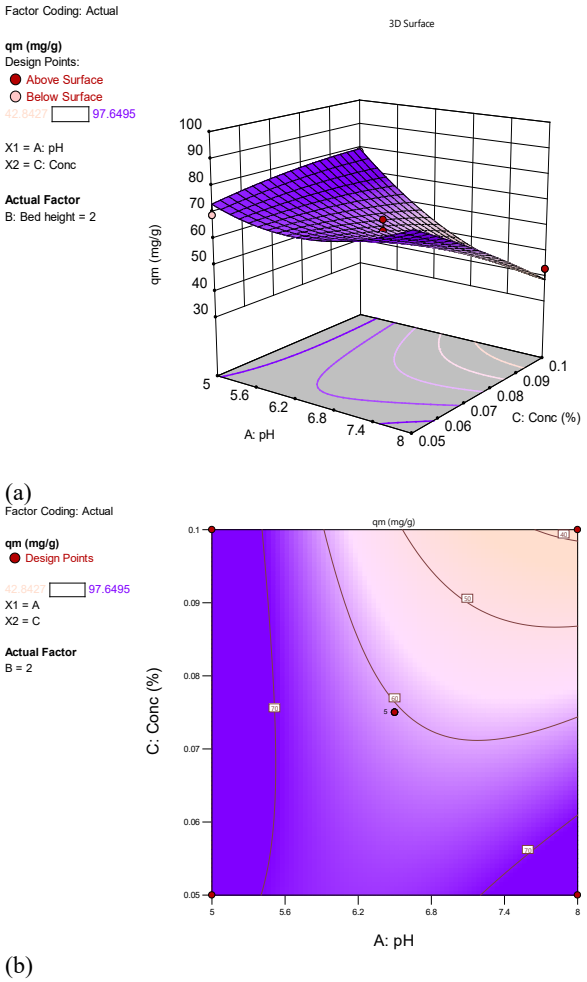


Fig. 9. The 3D response surface plots of between the factor pH and SCW concentration (a) and 3D- (b) contour plots.

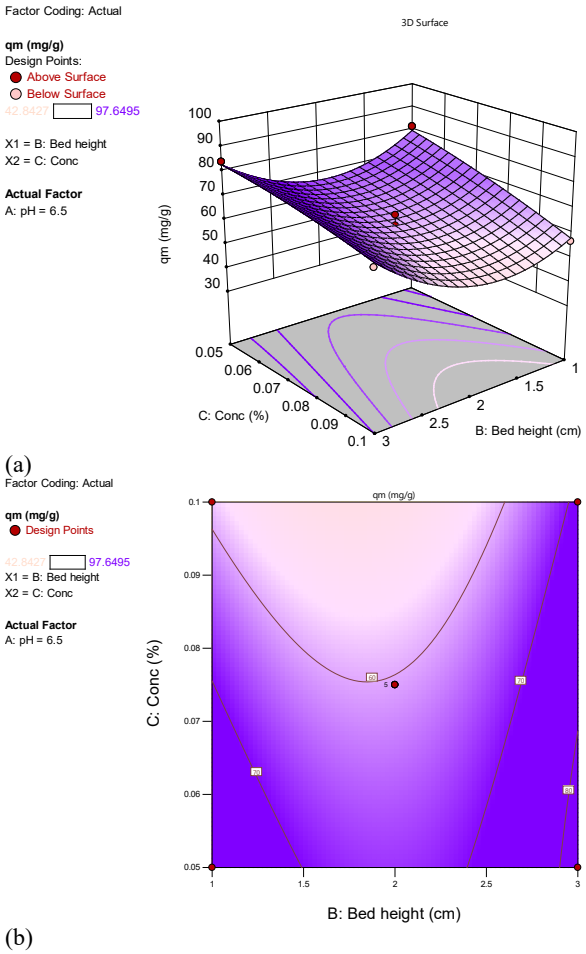


Fig. 10. The 3D response surface plots between the factor SCW and bed height (a) and 2D- (c) contour plots.

Verification of BBD experiment

The predicted ideal conditions were established using the Design Expert software's "Numerical Optimisation" toolset, which was to maximize (q_m) values. **Table 7** shows the solutions for the verification of the first predicted model. The model predicted a maximum (q_m) of 98.026 mg/g (87.901 to 108.151), which was verified through experimental results with an (q_m) of 145.333 mg/g (111.022 to 179.644), with the actual results showing statistically no significant difference (p>0.05) than the predicted values.

Table 7. Suggested parameter for each variable for maximum adsorption of SCW on SCB based on the Box-Behnken design.

RSM target solution	Desira-bility	Predicted (q_m) (95%, C.I.)	Experimental verification (95%, C.I.)	Statistical significant Difference between predicted and experiment
All factors within range, Maximum (q_m)	1.000	98.026 (87.901 to 108.151)	97.81 (79.278 to 116.342)	No significant Difference (p>0.05)

Comparison of optimisation parameters between OFAT and RSM

In comparison, results from OFAT and RSM were gathered and compared to each other (**Table 8**). An improvement of (q_m) was observed for RSM, with the difference was statistically significant (p<0.05).

Table 8. Comparison of optimum conditions and results obtained between OFAT and RSM for the adsorption of SCW on SCB.

	OFAT		RSM		
Factors	Optimum value	(q_m) C.I.)	(95%	Optimum value	(q_m) (95% C.I.)
pH	6.0	72.153		7.96	98.026 (87.901 to 108.151)
Bed height (cm)	1.5	(65.070		to2.95	
SCW (% v/v)	0.03	79.229)		0.05	

CONCLUSION

In summary, this study successfully achieved its objectives, focusing on optimizing the adsorption of whey onto sugarcane bagasse. The research identified that the ideal conditions for maximizing adsorption were a bed height of 3 cm, a pH level of 8, a temperature of 25 °C, and a whey concentration of 0.05% (v/v). Through the integration of mathematical modeling and statistical analysis, the study determined the most suitable fit for each parameter, which significantly contributed to accurately

identifying the maximum adsorption capacity under the specified conditions.

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DECLARATION OF AI AND AI-ASSISTED TECHNOLOGIES

The use of AI-based tools is only at the preliminary stages, and the preparation of this manuscript specifically, ChatGPT, ScholarAI, and Grammarly. These tools acted as supplementary resources. The final interpretations, conclusions, and scholarly work, including the articulation and coherence of the presented arguments, remain the sole responsibility of the authors.

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