JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2026, 27(2), 110–123
https://doi.org/10.12911/22998993/211059
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Received: 2025.09.09
Accepted: 2025.12.15
Published: 2026.01.15

Optimizing ammonia removal using immobilized microalgal-bacterial beads: A response surface methodology approach

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ABSTRACT

Ammonia contamination from fertilizer production, palm oil processing, livestock, and food industries is a major contributor to eutrophication, aquatic toxicity, and ecosystem degradation. Conventional treatment methods often struggle with high ammonia concentrations, prompting interest in biological approaches. One promising solution is the use of immobilized microalgal–bacterial beads (IMBB), which combine microbial synergy with enhanced stability and reusability. Building on this approach, this study optimized ammonia removal and developed an empirical predictive model using IMBB, while characterizing their physicochemical properties through SEM–EDS, FTIR, and mechanical strength analysis. Three independent variables ammonia concentration (250–500 mg L^{-1}), bead dosage (20 – 40 g L^{-1}), and bead size (3–7 mm) were evaluated via response surface methodology (RSM). Optimal conditions were identified at 422.65 mg L^{-1} ammonia, 37.88 g L^{-1} beads, and 7 mm bead size, achieving a predicted removal efficiency of 93.83%. SEM revealed a dense and uniform bead structure; EDS detected C, O, Na, and Ca as dominant elements; and FTIR confirmed O–H, COO^{-}, and C–O–C functional groups. Mechanical testing showed a strength reduction from 136.30 to 7.62 gForce post-operation. These findings demonstrate the potential of IMBB as a robust, sustainable biotechnology for efficient ammonia removal in industrial wastewater treatment.

Keywords: ammonia, immobilized microalgae–bacteria beads, optimization, response surface methodology.

INTRODUCTION

Ammonia is a major pollutant in domestic, agricultural, and industrial wastewater, with concentrations ranging from 100–280 mg L^{-1} in fertilizer industry effluents and up to 500 ppm in petrochemical discharges (Aprilia et al., 2025; Gova, 2019). Excess ammonia contributes to eutrophication, aquatic toxicity, oxygen depletion, and odor generation through nitrification processes (Badrah et al., 2021). Maintaining ammonia concentrations below 1 mg L^{-1} is essential to safeguard aquatic ecosystems, underscoring the need for innovative and sustainable treatment strategies.

Immobilized microalgal-bacterial beads (IMBB) have emerged as a promising biotechnology for wastewater treatment (Guo et al., 2021; Montgomery, 2017). In this symbiotic system, *Chlorella* sp. secretes extracellular polymeric substances (EPS) to adsorb ammonia, assimilates nitrogen into biomass via photosynthesis, and supplies oxygen to support nitrifying bacteria (Afifah et al., 2021; Alkahf et al., 2021; Aprilia et al., 2025). *Nitrosomonas* sp. oxidizes ammonia to nitrite, which is further converted to nitrate by *Nitrobacter* sp. under aerobic conditions (Gova, 2019; Respati et al., 2017). Studies have reported ammonia removal efficiencies of 40–60% by *Nitrosomonas* sp. alone (Severo et al., 2024; Wang et al., 2021) and 75–80% by *Chlorella* sp. (Afifah et al., 2021; Guo et al., 2021; Khotimah, 2021). Their integration not only enhances removal performance but also generates microalgal biomass that can be valorized as biofertilizer or bioenergy feedstock, offering environmental and economic benefits (Afifah, et al., 2021).

Research on algal-bacterial systems has demonstrated promising ammonia removal under various operational conditions. For example, activated sludge–algae systems achieved 74.9% removal at 430 mg L^{-1} ammonia (Jia and Yuan, 2018), immobilized *Chlorella vulgaris* beads achieved 93% removal at 40 g L^{-1} biomass (Oluwole et al., 2019), and chitosan–alginate beads immobilizing *Bacillus subtilis* achieved 96.5% removal at 20 g L^{-1} in swine wastewater (Guo et al., 2021). Optimization of calcium alginate beads for nitrate removal also demonstrated performance improvements at 10 g L^{-1} biomass (Bahrami et al., 2020). Bead size has been identified as a critical factor, with optimal diameters varying by wastewater type – 5.3 mm for nutrient-rich effluents (Özgür and Göncü, 2023), 3 mm for aquaculture wastewater (Porkka, 2021), and dosage-dependent effects for domestic wastewater (Kardena et al., 2020).

Recent studies have highlighted the advantages of statistical optimization tools such as response surface methodology (RSM) over traditional factorial designs, particularly for identifying parameter interactions and minimizing experimental runs (Ya'acob et al., 2022). However, systematic optimization of IMBB systems combining *Chlorella* sp. and *Nitrosomonas* sp. remains scarce. This study addresses this gap by optimizing ammonia removal through a combined IMBB system, focusing on the effects of initial ammonia concentration, bead dosage, and bead size. RSM was applied to establish predictive models, determine optimal operational conditions, and advance IMBB as a sustainable biotechnology for industrial wastewater treatment.

RESERARCH METHODOLOGY

Cultivation of *Nitrosomonas* sp. and *Chlorella* sp.

The regenerated culture of *Nitrosomonas* sp. was prepared by inoculating a single bacterial colony into 7.5 mL of nutrient broth (NB) supplemented with 2.5 mL of ammonia solution (50 mg L^{-1}) in a test tube, followed by incubation for 24–48 h. The resulting culture was then transferred to a 100 mL Erlenmeyer flask containing 50 mL of NB and 50 mL of ammonia solution (150 mg L^{-1}) and incubated for an additional 24–48 h (Respati et al., 2017) (Figure 1). After regeneration, bacterial density was quantified using the total plate count (TPC) method. The culture was serially diluted to 10^{-6}, and aliquots from the 10^{-5} and 10^{-6} dilutions were spread on plate count agar (PCA) and incubated at 30 °C for 48 h, followed by incubation at 35–37 °C for an additional 24–48 h (Riadi, 2022). This procedure yielded a bacterial concentration of approximately 10^6 CFU mL^{-1} for subsequent immobilization [14]. Colony counts were determined using the standard TPC calculation.

$$N = \frac{1}{dilution\ factor} \times the\ number\ of\ bacteria \quad (1)$$

Chlorella sp. was cultured by combining 100 mL of algal inoculum and 400 mL of Bold's Basal Medium (BBM) with 3.5 L of distilled water, and maintained under natural sunlight at ambient temperature (25–28 °C). The culture was propagated until the exponential growth phase, reaching a density of approximately 1×10^6 cells mL^{-1}. Cell density was quantified using a hemocytometer with a cover glass, and cells were enumerated under a light microscope using a manual hand counter (Figure 2).

Preparation of immobilized microalgal *Chlorella* sp. bacterial *Nitrosomonas* sp. beads

Immobilization was carried out by mixing cultured *Chlorella* sp. and *Nitrosomonas* sp. at a 1:3 (v/v) ratio with 2.5% (w/v) sodium alginate solution. The mixture was dispensed dropwise into 100 mL of 2% (w/v) $CaCl_2$ solution to form alginate–microalgae–bacteria beads. The beads were cured overnight at 4 °C to enhance structural stability and subsequently rinsed with distilled water prior to use (Figure 3).

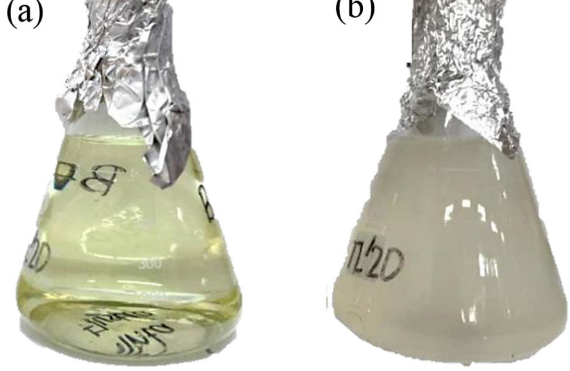


Figure 1. Regeneration of *Nitrosomonas* sp. culture (a) initial regeneration and (b) final regeneration

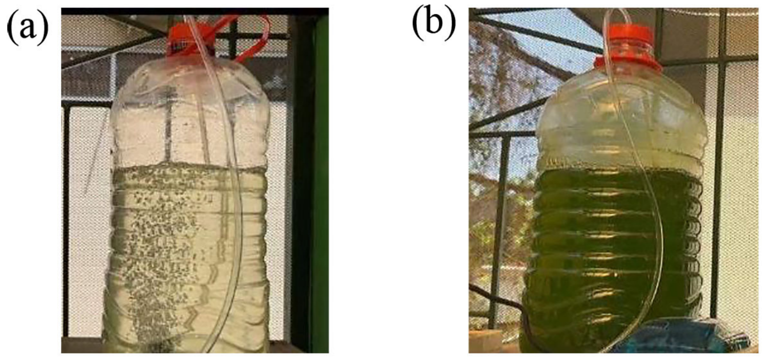


Figure 2. Cultivation stages of *Chlorella* sp. culture (a) initial inoculum and (b) final culture after 8 days under natural sunlight

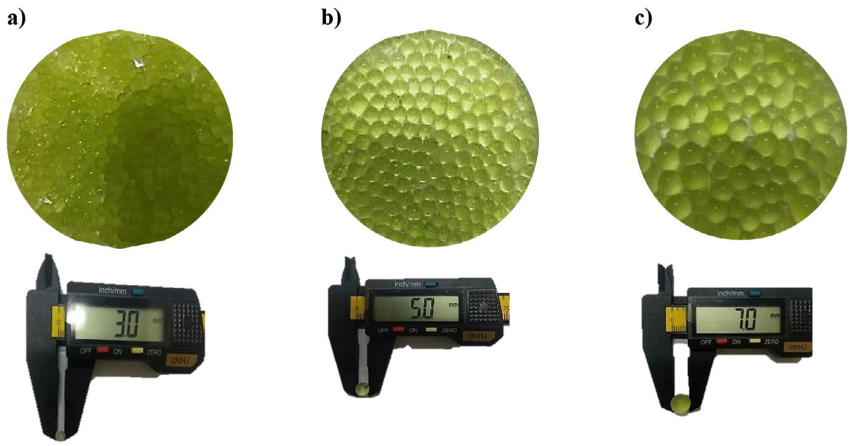


Figure 3. Immobilized microalgal *Chlorella* sp. bacterial *Nitrosomonas* sp. beads with different diameters (a) 3 mm, (b) 5 mm and (c) 7 mm

Design of experiment

Optimization of process parameters was conducted using RSM with a Box-Behnken design (BBD). Three independent variables were considered: ammonia concentration (250–500 mg/L), bead dosage (20–40 g/L), and bead size (3–7 mm), with a treatment duration of 4 days. Each factor was evaluated at three levels, and the BBD matrix generated a total of 17 experimental runs. This design was selected because it provides an efficient approach for estimating both interaction effects and quadratic terms while requiring fewer experiments compared to other quadratic designs. The relationship between the input variables and ammonia removal efficiency was expressed through a second-order polynomial equation, and model adequacy was verified using analysis of variance (ANOVA). The detailed experimental design and corresponding responses are presented in Table 1. Following the 17 experimental runs, the response data were subjected to statistical analyses, including ANOVA, response surface regression, determination of factor significance, residual diagnostics, and visualization through contour and surface plots. Response predictions were further generated to evaluate the effects of factor combinations under different optimization scenarios. Based on the optimal values of each factor obtained from the RSM model, validation experiments were subsequently performed to verify the model predictions. Each validation run was conducted in triplicate to ensure the accuracy and reliability of the results.

Analysis of ammonia parameters

Ammonia concentration in wastewater was quantified spectrophotometrically according to U.S. EPA Method 350.1 (Nitrogen, Ammonia – Colorimetric, Automated Phenate). Ammonia

Table 1. Experimental design and the responses

Run	Factor 1 X_1: ammonia concentration (mg/L)	Factor 2 X_2: beads dose (mg/L)	Factor 3 X_3: beads size (mm)
1	250	20	5
2	500	20	5
3	250	40	5
4	500	40	5
5	250	30	3
6	500	30	3
7	250	30	7
8	500	30	7
9	375	20	3
10	375	40	3
11	375	20	7
12	375	40	7
13	375	30	5
14	375	30	5
15	375	30	5
16	375	30	5
17	375	30	5

removal efficiency (%) was calculated using the following equation

$$Removal\ efficiency\ (\%) = \frac{C_{in} - C_{ef}}{C_{ef}} \times 100\% \quad (2)$$

where: Cin – influent concentration (mg/L), Cef – effluent concentration (mg/L).

Characterization of immobilized microalgal *Chlorella* sp. bacterial *Nitrosomonas* sp. beads

The beads were characterized using SEM–EDS, FTIR, and mechanical strength testing to evaluate their physicochemical properties and performance. SEM–EDS was used to examine bead morphology and elemental composition, FTIR to identify functional groups, and mechanical testing to assess structural durability.

RESULTS AND DISCUSSION

Model prediction and statistical analysis

The experimental results obtained from the combinations of the three independent variables are summarized in Table 1. Analysis of variance (ANOVA) confirmed that the quadratic model provided the best fit for predicting ammonia removal efficiency in the immobilized microalgae–bacteria system (Table 2). The model achieved a coefficient of determination (R^2) of 0.8832, indicating that 88.32% of the variation in removal efficiency was explained by the selected variables, while the remaining variation was likely due to uncontrolled environmental or experimental factors. A significance level of $p < 0.05$ further supported the model's robustness and predictive accuracy. Overall, these findings demonstrate that response surface methodology (RSM) effectively captures complex parameter interactions and reliably predicts system performance under varying conditions. The strong predictive power of the model highlights its potential for scaling up ammonia removal processes in real wastewater treatment applications, where optimization of operational parameters is essential for ensuring both efficiency and sustainability.

The analysis further demonstrated that ammonia concentration, bead dosage, and bead size significantly influence the optimization of conditions supporting maximum ammonia removal. The effects of these variables on ammonia removal efficiency under the quadratic model are presented in Table 3, based on the p-values obtained from the ANOVA results.

The ANOVA results for the quadratic model (Table 3) demonstrated statistical significance (F = 14.45, p = 0.0010), confirming its reliability in predicting ammonia removal efficiency. The model achieved a high coefficient

Table 2. Model summary on ammonia removal efficiency response

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.1897	0.0154	0.1361	-0.2240	
2FI	0.1212	0.0227	0.3557	-0.0488	
Quadratic	0.0016	0.3898	0.8832	0.5561	Suggested
Cubic	0.3898		0.8965		Aliased

Table 3. Analysis of variance (ANOVA) of the quadratic model for ammonia removal efficiency

Source	Sum of Squares	F-value	p-value	Description
Model	447,79	14,45	0,0010	Significant
X_1 ammonia concentration	7,93	2,30	0,1731	
X_2 beads dose	129,93	37,72	0,0005	
X_3 beads size	2,79	0,8109	0,3978	
X_1X_2	91,50	26,57	0,0013	
X_1X_3	42,60	12,37	0,0098	
X_2X_3	7,12	2,07	0,1938	
X_1^2	69,29	20,12	0,0028	
X_2^2	13,84	4,02	0,0850	
X_3^2	67,40	19,57	0,0031	
Residual	24,11			
Lack of Fit	11,90	1,30	0,3898	Not Significant
Pure Error	12,21			
Cor Total	471,90			
R^2	0,9489			

of determination (R^2 = 0.9489), indicating that 94.89% of the variability in removal was explained by the fitted equation, with only 5.11% attributed to unexplained factors. Bead dosage (X_2) had the strongest individual effect (p = 0.0005), while significant interactions were observed between ammonia concentration and bead dosage (X_1X_2, p = 0.0013) and between ammonia concentration and bead size (X_1X_3, p = 0.0098). Quadratic effects were also significant for ammonia concentration (X_1^2, p = 0.0028) and bead size (X_3^2, p = 0.0031). By contrast, ammonia concentration (X_1), bead size (X_3), and the interaction of bead dosage with bead size (X_2X_3) were not significant ($p > 0.05$). The non-significant lack-of-fit test (p = 0.3898) further confirmed the adequacy of the model in representing the experimental data. The resulting empirical model is expressed as a quadratic polynomial equation:

This model illustrates the combined linear, interaction, and quadratic effects of the three variables on ammonia removal efficiency, with the intercept (87.73734) representing baseline performance at the central point of the design space. Residual analysis confirmed near-normal and random distribution of errors, suggesting the absence of systematic bias (Miao et al., 2022). Furthermore, the strong agreement between predicted and observed values in the predicted versus actual plot, together with the high R^2 value (0.9489), validated the model's predictive capability and its suitability for optimizing operational conditions in immobilized microalgal–bacterial bead systems (Li et al., 2018) (Figure 4).

The normal probability plot of residuals indicated that the data points closely followed the diagonal line with a symmetrical distribution and no distinct pattern, suggesting that the residuals were approximately normally distributed and the

$$\begin{aligned} \textit{Removal efficiency} &= 87{,}73734 + 0.011274\,(NH_3\ \textit{concentration}) - 1.03182\,(\textit{beads dose}) + \\ &+ 5.64127\,(\textit{beads size}) + 0.003826\,(NH_3\ \textit{concentration} \times \textit{beads dose}) + 0.013054\,(NH_3\ \textit{concentration} \\ &\times \textit{beads size}) - 0.000260\,(NH_3\ \textit{concentration})^2 - 1.02411\,(\textit{beads size})^2 \end{aligned} \quad (3)$$

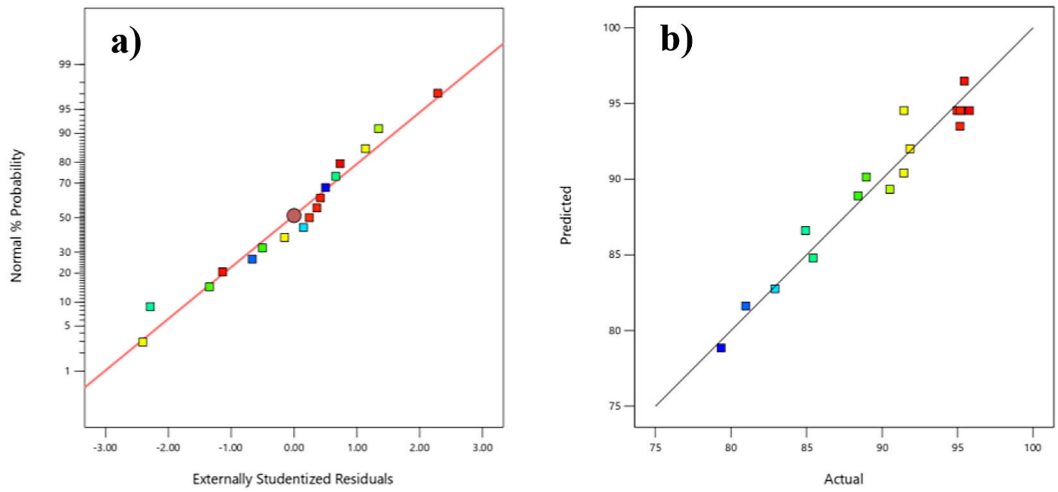


Figure 4. (a) Normal probability plot of residuals, and **(b)** Predicted vs. actual values

assumption of normality was satisfied. This implies that the model is free from systematic bias and that the errors are random (Miao et al., 2022). The predicted versus actual plot showed data points near the diagonal line, indicating a high agreement between model predictions and experimental results, with minimal differences between predicted and observed values. This is consistent with the coefficient of determination (R^2) value of 0.9489 for ammonia removal efficiency, demonstrating that the model explains 94.89% of the variability in the experimental data (Kong et al., 2024; Li et al., 2018; Niu et al., 2020; Zhang et al., 2022). These visualizations support the statistical validity of the regression model generated through RSM and confirm that the model meets the fundamental assumptions of regression analysis. Therefore, it can be reliably used as a predictive and optimization tool for the microalgae–bacteria immobilization system. The influence of each factor on ammonia removal efficiency is further illustrated through response surface and contour plots, which display the interaction between two factors while holding the third factor at its midpoint, as shown in Figure 5.

The interactive contour and surface plots (Figure 5A–B) confirm that ammonia concentration and bead dosage synergistically determine system efficacy. Optimal removal (95%) occurs at ammonia loads of 340–500 mg/L and bead dosages of 30–40 g/L. These findings parallel observations by Oluwole et al. (2019), where 97% removal was achieved at 40 g/L, while lower dosages (20 g/L) yielded only ~78%, and higher dosages (80 g/L) did not significantly improve performance. Similarly, work by Tam et al. (2000) showed near-complete NH_4^+ removal at intermediate bead concentrations (12 beads/mL), with performance declining at both extremes. Mechanistically, these studies reflect a classic biomass-to-substrate ratio effect: sufficient biomass ensures high enzymatic capacity and nutrient uptake, while excessive biomass hinders oxygen and substrate diffusion into the beads, diminishing microbial activity. Furthermore, Whitton et al. (2018) research on nutrient loading demonstrated decreasing NH_4^+ uptake beyond 20.8–33.6 g/m³/d, reflecting physical and mass transfer constraints at higher loadings. In the present study, the dosage of 30–40 g/L provides an optimal balance—enough cell density to metabolize high ammonia load without compromising bead integrity or mass transfer. This optimization substantiates the critical role of tailoring both biomass concentration and ammonia levels in RSM-guided design of IMBB systems.

The interaction between ammonia concentration and bead size (Figure 5C–D) revealed a critical operational window, with removal efficiency reaching 94% when bead sizes were between 4–6 mm and ammonia concentrations were within 310–410 mg/L. Bead size significantly influences porosity, diffusion, and oxygen transfer: beads that are too small impair mass transfer, while excessively large beads prevent effective nutrient penetration. This balance supports both adequate biomass retention and microenvironmental stability. This finding aligns with recent literature. For instance, Peng et al. (2025) demonstrated that alginate-immobilized microalgae–bacteria systems benefited from immobilization structure in enhancing nutrient removal pathways and biomass settling, underscoring the structural importance of

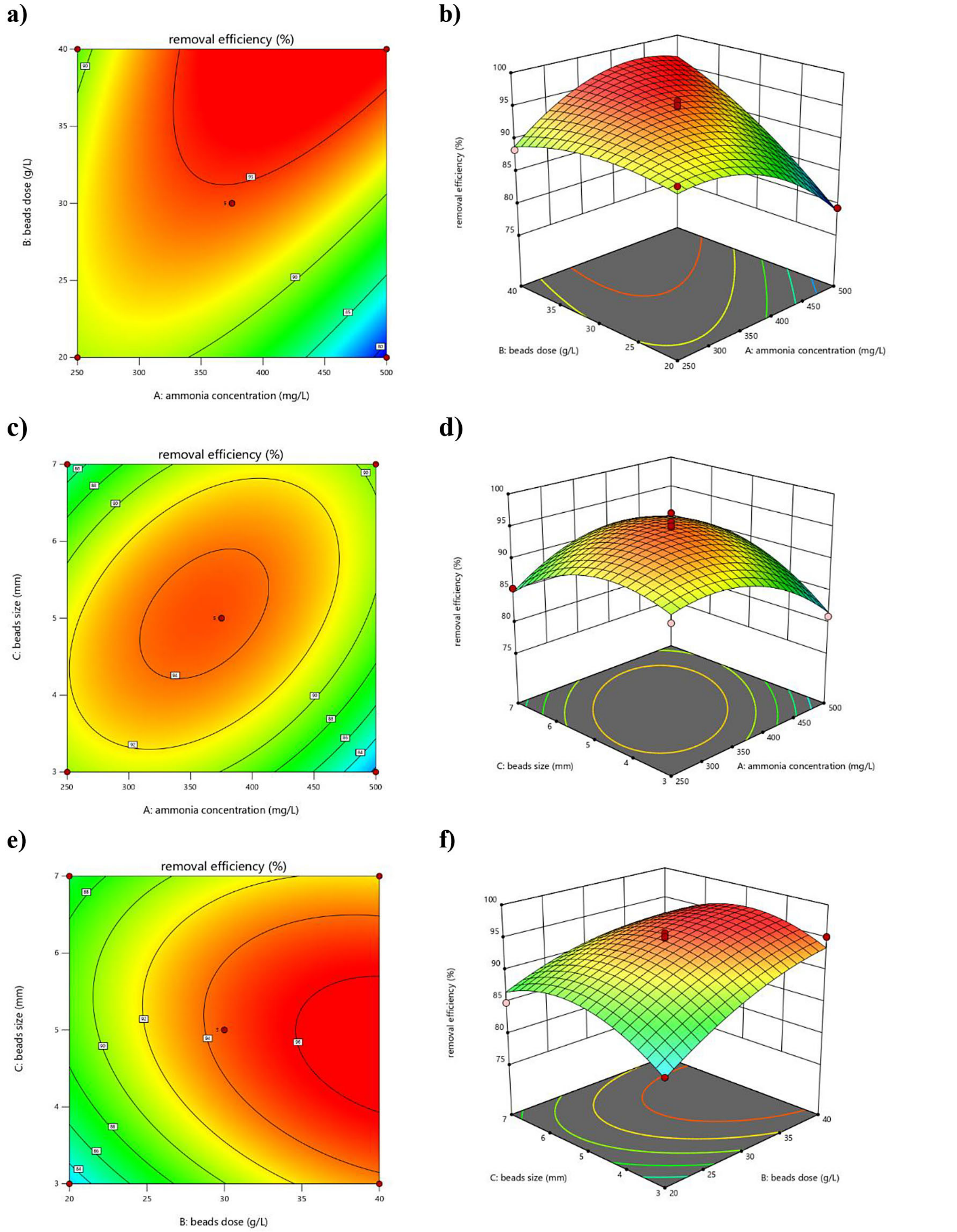


Figure 5. Contour plot & surface plot. (a) Contour plot of X_1, X_2, (b) Surface plot of X_1X_2, (c) Contour plot of X_1, X_3, (d) Surface plot of X_1, X_3, (e) Contour plot of X_2X_3, (f) Surface plot of X_2, X_3

bead composition. Moreover, Zhao et al. (2024) reported high nitrogen (up to 90%) and phosphorus (up to 95%) removal in black-odor water using PVA-alginate gel beads reinforced with inorganic fillers such as zeolite and iron powder, highlighting that bead matrix optimization – including size and material composition – enhances treatment resilience and performance. These studies reinforce the notion that bead design is not merely a physical consideration but a strategic determinant of system efficiency. Mechanistically, beads sized 4–6 mm optimize nutrient and oxygen diffusion to embedded microorganisms while maintaining mechanical stability during agitation. Beads smaller than 4 mm may be more prone to structural damage due to increased fragility and higher surface-to-volume ratios, potentially limiting durability. Conversely, beads larger than 6 mm may suffer from poor penetration of nutrients and slower diffusion to internal microbial communities, reducing metabolic efficiency and reaction rates.

The combined effects of bead dosage and bead size (Figure 5E–F) produced a peak removal efficiency of 96% when bead dosage was maintained at 35–40 g/L and bead size at 4–5.5 mm, highlighting a synergistic interplay between the availability of biomass and the structural stability of the beads. This result is consistent with findings by Oluwole et al. (2019), who reported that *Chlorella vulgaris* immobilized at 40 g/L in calcium alginate beads achieved the highest removal of NH_4^+ compared to lower (20 g/L) or higher (80 g/L) concentrations, emphasizing the existence of an optimal biomass-to-substrate ratio (Oluwole et al., 2019). Additionally, Lee et al. (2020) demonstrated that bead sizes around 3.5 mm provided optimal nutrient removal and microalgal retention in immobilized systems, while larger sizes led to decreased metabolic efficiency due to limited substrate diffusion (Lee et al., 2020). This finding supports the notion that moderate bead size enables effective mass transfer while maintaining bead integrity. Collectively, these studies reinforce the importance of selecting bead dosage and size within the 35–40 g/L and 4–5.5 mm ranges, respectively, to ensure maximal microbial activity, efficient nutrient diffusion, and durable structural performance – essential factors for high-efficiency ammonia removal in immobilized microalgal–bacterial systems.

Overall, ammonia removal efficiency was not dictated by a single factor but by the interplay of concentration, dosage, and bead size, with response surface analysis providing a clear identification of optimal operational regions (Jia and Yuan, 2018). These results emphasize the importance of balancing mass transfer, biomass availability, and structural integrity, thereby confirming the potential of immobilized *Chlorella* sp. – *Nitrosomonas* sp. beads as an efficient and sustainable strategy for wastewater treatment.

Optimum condition for ammonia removal using immobilized microalgal- bacterial beads

Variable limits for ammonia concentration, bead dosage, and bead size were defined within specified ranges, with ammonia removal efficiency set as the response to be maximized. Importance values were assigned using the desirability function, where all independent variables received medium weight (3) and removal efficiency the highest weight (5) (Nandi et al., 2015).

Multi-response optimization identified the optimum operational conditions at an ammonia concentration of 368.829 mg/L, a bead dosage of 38.145 g/L, and a bead size of 4.562 mm, with an overall desirability value of 1. Under these conditions, the ammonia removal efficiency reached 96.409%. The RAMPS graph and desirability bar further confirmed that all three variables were positioned at their optimal levels, with individual desirability values equal to 1 (Figure 6).

The perturbation graph further demonstrated that all three factors exerted a strong and

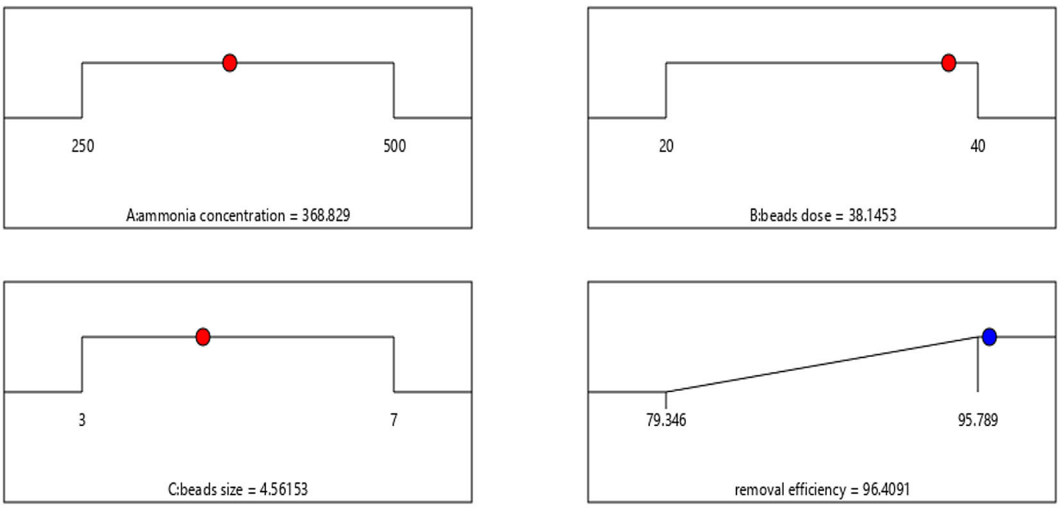


Figure 6. Ramps of optimal condition

predominantly linear influence on desirability and efficiency. Variations in these factors produced significant fluctuations in the system response, thereby confirming the interactive effects among parameters governing overall performance, consistent with previous findings (Zhang et al., 2022).

Model validation

Following the determination of the optimal conditions through RSM namely an ammonia concentration of 368.829 mg/L, a bead dosage of 38.145 g/L, and a bead size of 4.562 mm the model was validated by conducting experiments under these conditions. Validation was deemed successful when the deviation between the experimental results and the model predictions was less than 5%. The validation results show a predicted ammonia removal efficiency of 96.409%. Three replications of the experiment yielded efficiencies of 96.829–96.891%, all with relative errors <5%, indicating the model is accurate. To ensure the model is accurate, the model confirmation is shown in Table 4. The model confirmation results show that the predicted efficiency of 96.3967% is within the specified range of 93.0463–99.7472%. The predicted values, which are all within the confidence range, prove that the RSM model has high accuracy and precision, so it is suitable for use as the basis for setting the operational parameters of the immobilized microalgae-bacteria sewage treatment system.

Mechanism of ammonia removal by immobilized microalgal – bacterial beads

Ammonia removal in immobilized beads occurs through the synergistic interaction of *Chlorella* sp. and nitrifying bacteria. *Nitrosomonas* sp. oxidizes ammonia (NH_3) to nitrite (NO_2^-), followed by conversion to nitrate (NO_3^-) by Nitrobacter sp. via autotrophic nitrification (Bahrami et al., 2020). Concurrently, Chlorella sp. assimilates ammonia as a nutrient and provides oxygen through photosynthesis, which supports bacterial activity, while benefiting from inorganic nitrogen produced by (Li et al., 2018). The immobilization matrix stabilizes the consortium, protects cells

Table 4. Model confirmation

Analysis	Predicted Mean	Predicted Median	Std Dev	N	SE Pred	95% PI low	Data Mean Aktual	95% PI high
Removal Efficiency	96,3967	96,3967	1,85586	3	1,41691	93.0463	96.8603	99.7472

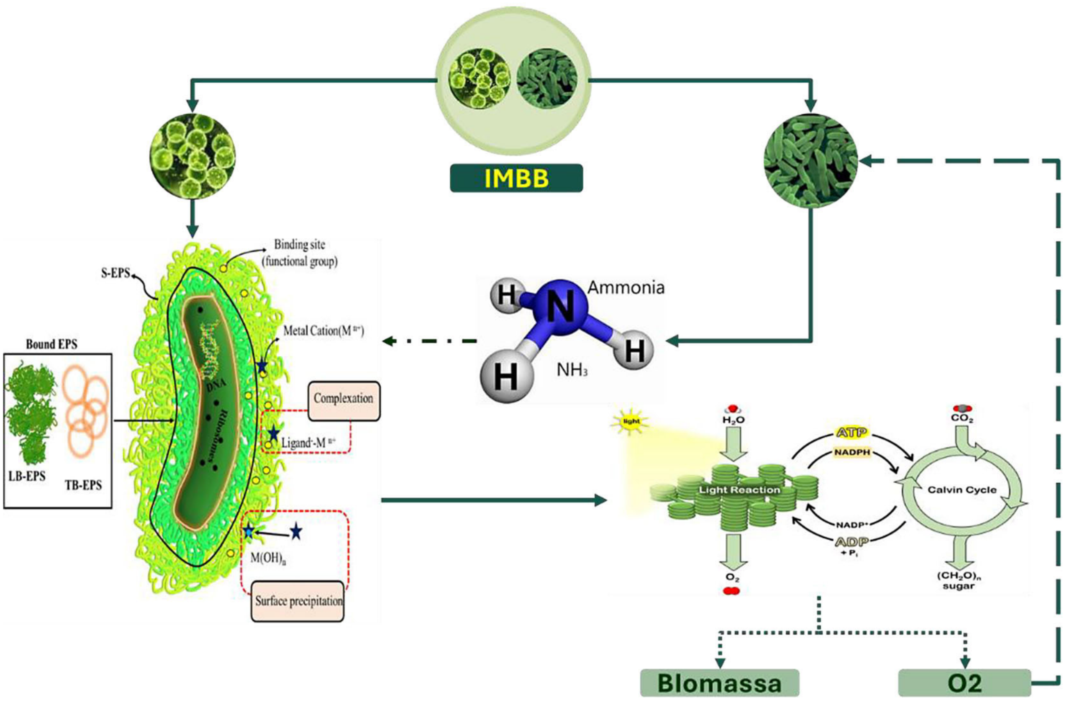


Figure 7. Mechanism of ammonia removal by immobilized microalgal – bacterial beads

from environmental fluctuations, and prevents washout, while the bead porosity allows substrate diffusion and reduces ammonia toxicity at high concentrations (Wang et al., 2024). This synergy enhances efficiency and stability, making the system promising for wastewater treatment with high ammonia loads (Figure 7).

Scanning electron microscopic (SEM) results of immobilized microalgal-bacterial beads

SEM analysis at magnifications of 100× and 5000× revealed that the immobilized *Chlorella* sp.–*Nitrosomonas* sp. beads exhibited a rough, porous surface characterized by cracks and cavities that facilitate mass diffusion and microbial colonization. Rod-shaped *Nitrosomonas* sp. cells were observed to be uniformly attached to the bead surface, whereas *Chlorella* sp. cells were predominantly embedded within the polymeric matrix. The porous microstructure is likely to enhance nutrient transport and contribute to improved ammonia removal efficiency (Figure 8, 9).

Energy-dispersive X-ray spectroscopy (EDS) analysis confirmed that the immobilized beads were predominantly composed of carbon (46.27%), nitrogen (15.50%), and oxygen, reflecting the contribution of microbial biomass and the alginate/PVA–SA matrix. Calcium, which plays a critical role in cross-linking the polymer network, decreased from 17.29% to 1.86% after operation, likely due to ion exchange and leaching during prolonged exposure to wastewater. Trace amounts of sodium, magnesium, aluminum, and chlorine were detected, whereas no toxic metals were observed. These findings indicate that the immobilization matrix maintained its chemical stability, biocompatibility, and capacity to support microbial activity throughout the ammonia removal process(Figure 10, 11).

Fourier transform infrared (FTIR) results of immobilized microalgal – bacterial beads

The FTIR spectra provide strong evidence of successful microbial immobilization within the alginate/PVA–SA polymer network, with distinct peaks confirming both the structural integrity of the beads and the metabolic potential of the entrapped microorganisms. The broad absorption at ~3300 cm^{-1}, corresponding to O–H and N–H stretching, reflects the abundance of hydroxyl and amine groups in both the alginate backbone and microbial cell walls, suggesting strong hydrogen bonding interactions that enhance matrix stability. The prominent bands near 1650 cm^{-1} (Amide I) and 1540 cm^{-1} (Amide II) are characteristic of peptide linkages in microbial proteins, verifying the encapsulation of metabolically active biomass.

The carboxylate ($–COO^{-}$) stretching observed between 1400–1600 cm^{-1} further indicates the presence of alginate cross-linked with

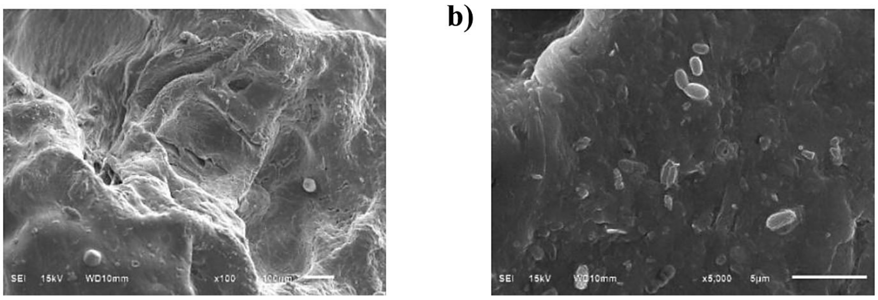
a) b)


Figure 8. SEM observation before treatment (a) 100x magnification and (b) 5.000x magnification

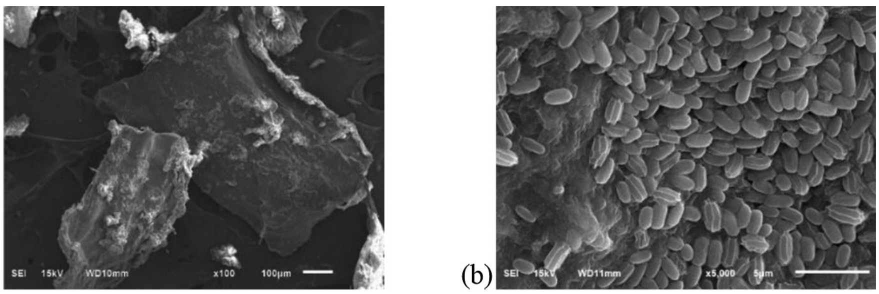
(a) (b)


Figure 9. SEM observation after treatment (a) 100x magnification and (b) 5.000x magnification

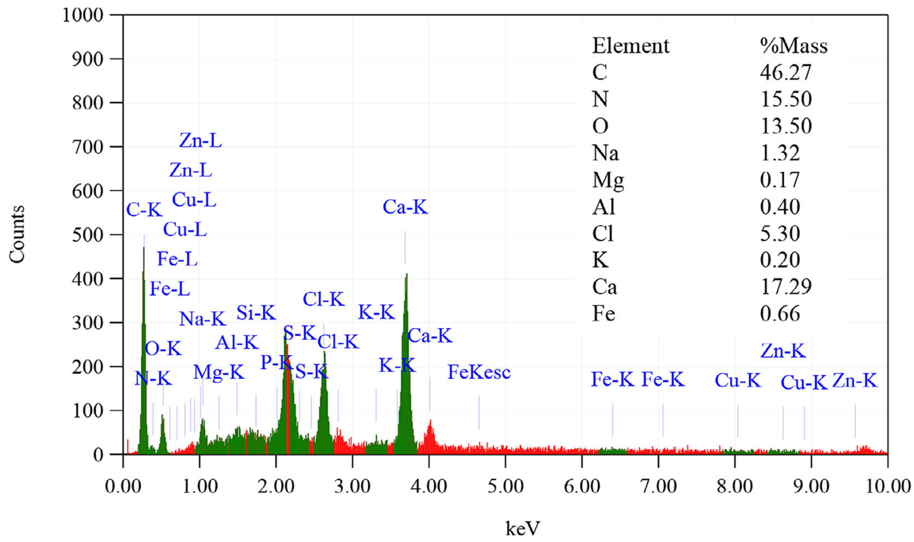


Figure 10. EDS spectrum of IMBB before treatment

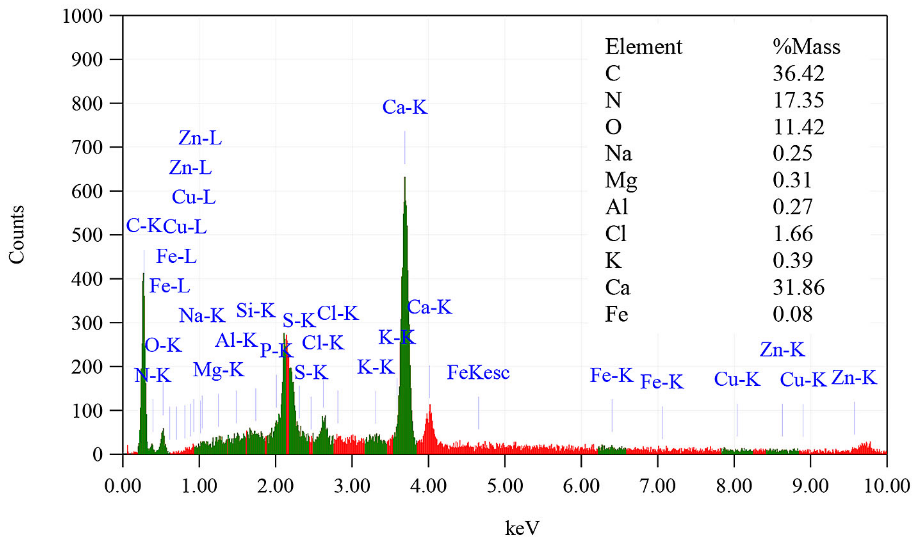


Figure 11. EDS spectrum of IMBB after treatment

Ca^{2+}, while peaks at 1030–1100 cm^{-1}, assigned to polysaccharide (C–O–C) linkages, highlight the structural role of alginate in forming a stable three-dimensional gel matrix. The retention of these functional groups after reactor operation suggests that the immobilization beads maintain their chemical integrity under wastewater conditions, providing a robust microenvironment that protects cells from shear stress and toxic fluctuations. Overall, these FTIR findings confirm that the alginate/PVA–SA beads not only act as a passive support matrix but also create a chemically compatible microhabitat that facilitates nutrient diffusion and ammonia oxidation, thereby validating their application in bioremediation of nutrient-rich wastewater (Figure 12).

Mechanical strength results of immobilized microalgal – bacterial beads

The significant reduction in bead hardness from 136.30 to 7.62 gforce after prolonged exposure to wastewater highlights the susceptibility of alginate/PVA–SA matrices to polymer degradation and water-induced swelling. This mechanical softening is commonly reported in immobilization systems utilizing calcium-cross-linked alginate, as divalent cation exchange (e.g., Ca^{2+} replaced by Na^{+} or Mg^{2+}) and prolonged hydration disrupt ionic cross-links, resulting in a more elastic structure (Malekjab et al., 2023; Jeoh et al., 2021). Despite this reduction in hardness, the absence of bead fracturability suggests that

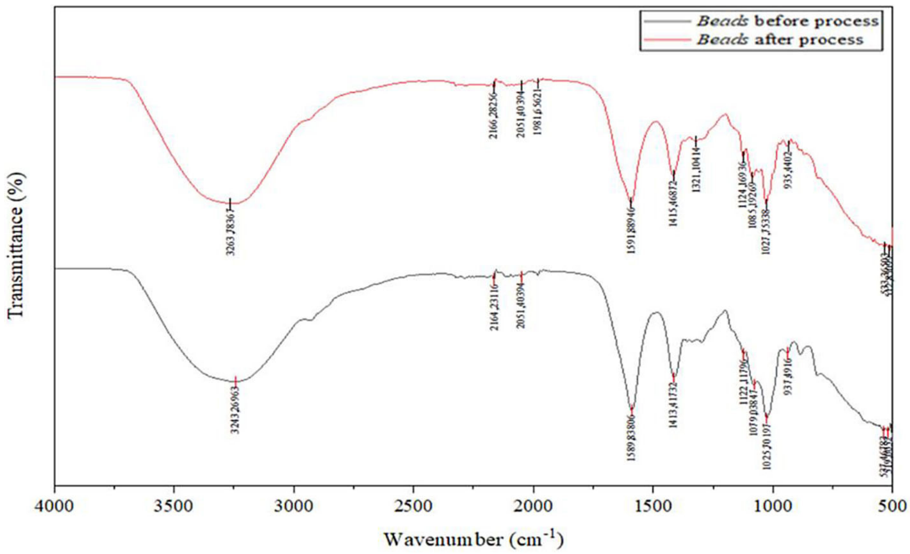


Figure 12. FTIR beads analysis

the beads exhibit elastic–plastic deformation rather than brittle failure, which is advantageous in maintaining bead integrity under continuous mixing and aeration. Similar trends have been observed in alginate-based immobilization studies, where a decrease in compressive strength was noted during long-term bioreactor operation, yet the beads retained structural integrity sufficient for sustained microbial activity. For instance, Zhang et al. (2023) reported a 60–80% reduction in hardness of PVA-alginate beads over 30 days of operation, but the beads maintained high ammonia oxidation rates due to improved nutrient diffusion facilitated by swelling. Likewise, Gao et al. (2022) demonstrated that moderate bead swelling enhances mass transfer, allowing higher substrate accessibility while maintaining microbial viability.

Several studies have demonstrated that immobilized microalgal–bacterial beads not only retain structural stability but can also be reused in real wastewater environments without significant loss of performance. Oluwole et al. (2019) reported that alginate-entrapped microalgal–bacterial consortia maintained over 80% nitrogen removal efficiency after four reuse cycles in palm oil mill effluent. Similarly, Özgür et al. (2023) showed that *Chlorella*-based bacterial beads sustained nutrient removal activity for up to 15 days under semi-continuous operation, even when subjected to fluctuating nutrient concentrations. These findings suggest that moderate bead softening, rather than compromising performance, may actually enhance substrate diffusion, thereby supporting long-term functional stability. More recent investigations in complex wastewater systems have further reinforced the potential for bead reuse. For instance, Severo et al. (2024) demonstrated that sodium alginate beads containing *Tetradesmus obliquus* could be repeatedly applied to swine wastewater while maintaining consistent COD reduction efficiency and biomass productivity over multiple cycles. In another study, Dong et al. (2017) observed that although PVA–alginate beads experienced a 60–80% reduction in mechanical strength over 30 days, ammonia oxidation activity remained stable due to enhanced mass transfer associated with bead swelling. Together, these results confirm that bead reusability under real wastewater conditions is achievable and further validate the robustness of our system.

These findings suggest that, although immobilized microalgal–bacterial beads have shown promising reusability in laboratory and pilot-scale studies, further validation under real wastewater conditions is essential to confirm their long-term stability and practical applicability.

CONCLUSIONS

This study demonstrated the effective application of immobilized *Chlorella* sp. – *Nitrosomonas* sp. beads for sustainable ammonia removal. RSM optimization determined the optimal conditions at 368.829 mg/L ammonia, 38.145 g/L bead dosage, and 4.562 mm bead size, achieving a predicted removal efficiency of 96.41%. Morphological

(SEM), physicochemical (EDS, FTIR), and mechanical analyses confirmed the structural stability, functional integrity, and adaptability of the immobilization matrix under operational conditions. Collectively, these findings highlight the robustness and long-term applicability of the immobilized algal–bacterial system as an environmentally benign biotechnology for wastewater treatment.

Acknowledgments

The authors express their sincere gratitude to the Ministry of Higher Education, Science, and Technology for funding this research under the 2025 Regular Fundamental Research Scheme (Contract 102/C3/DT.05.00/PL/2025 and No. 19591/UN19.5.1.3/AL.04/2025), which was instrumental in conducting the study and achieving the results presented in this report.

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