

Comparative Evaluation of Sodium Alginate and Agar Beads for Ammonia and Phosphate Removal in Algae-Based Phycoremediation Systems

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This study investigates the efficiency of algal beads synthesised with sodium alginate and agar for the removal of ammonia and phosphate in phycoremediation systems, addressing the persistent issue of nutrient pollution in wastewater. The objectives were to optimise bead formulation, compare pollutant removal performance between agar- and alginate-based matrices, and evaluate the statistical significance of the results. Algal biomass was immobilised in 2% sodium alginate and 2% agar to produce uniform beads, and their nutrient removal efficiency was assessed over a three-day period using spectrophotometric analyses; the salicylate method at 660 nm for ammonia and the molybdate method at 880 nm for phosphate. Statistical analyses were conducted using independent t-tests and one-way ANOVA. The alginate-based beads demonstrated significantly higher removal efficiencies for ammonia (up to 85%) and phosphate (up to 80%) compared with agar-based beads, which achieved approximately 65% and 60%, respectively ($p < 0.05$). The superior performance of alginate beads is attributed to their enhanced porosity and nutrient diffusion capacity, while agar beads, despite their mechanical stability, exhibited limited nutrient transport and uneven algal distribution. These findings underscore the importance of bead matrix composition in algae-based phycoremediation and identify sodium alginate as a promising encapsulation material for enhancing nutrient removal efficiency in sustainable wastewater treatment applications.

Keywords: Phycoremediation, sodium alginate, agar, nutrient removal, wastewater treatment

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The increasing concentrations of nitrogen and phosphorus compounds in wastewater pose a major threat to aquatic ecosystems worldwide, primarily through eutrophication and dissolved oxygen depletion [1, 2]. Conventional wastewater treatment methods—biological, chemical, and physical—are widely employed but often face limitations such as high operational costs, energy consumption, and the generation of secondary pollutants [3]. Consequently, growing attention has turned towards phycoremediation, a sustainable, algae-based biotechnology that exploits the nutrient assimilation capacity of microalgae to remove contaminants from wastewater [4, 5].

Microalgae such as *Chlorella*, *Spirulina*, *Oscillatoria*, and *Scenedesmus* are effective phycoremediators due to their non-pathogenic nature, rapid growth, and adaptability to diverse environments [6, 7]. These photosynthetic organisms assimilate nitrogen and phosphorus for cellular growth, converting them into valuable biomass components including

proteins, lipids, and bioactive metabolites [8, 9]. Among these species, *Chlorella sorokiniana* is a robust freshwater chlorophyte that has demonstrated outstanding performance in nutrient removal due to its resilience and high uptake capacity [10, 11].

To improve the practicality and stability of phycoremediation systems, cell immobilisation has emerged as a preferred strategy. Encapsulating algal cells within hydrogel matrices forms *algae beads* that mitigate common challenges of suspended cultures, such as biomass recovery, cell washout, and low mechanical stability [12, 13]. Hydrophilic gels such as sodium alginate and agar enable cell entrapment while maintaining sufficient porosity for nutrient diffusion and light penetration [14, 15]. This microenvironment enhances nutrient availability around immobilised cells, thereby improving assimilation efficiency [16].

Sodium alginate, a biopolymer composed of mannuronic and guluronic acid blocks, is favoured for

its biocompatibility, hydrophilicity, and gel-forming ability in the presence of calcium ions [17, 18]. Its carboxyl functional groups facilitate ionic interactions with ammonium and phosphate ions, providing both adsorption and biological uptake pathways [19, 20]. Conversely, agar, a thermo-reversible gel derived from red algae, offers superior mechanical strength and effective passive adsorption due to its hydroxyl-rich and porous network structure [21, 22]. However, agar beads often exhibit reduced porosity and uneven algal distribution, limiting nutrient diffusion and uptake efficiency [23].

Despite the widespread use of alginate and agar as immobilisation matrices in algae-based wastewater treatment, direct comparative evaluations under identical experimental conditions remain limited. Moreover, previous studies have often employed different microalgal species, wastewater sources or experimental conditions, making direct comparisons between immobilisation matrices difficult. Therefore, this study aims to comparatively evaluate sodium alginate and agar beads for ammonia and phosphate removal using immobilised microalgae in synthetic wastewater of varying nutrient concentrations and real aquaculture wastewater. Using the same *Chlorella sorokiniana* culture, bead loading and treatment conditions for both matrices, this study enables a direct comparison of sodium alginate and agar beads while minimising experimental variability. The findings provide practical guidance for selecting suitable immobilisation matrices for algae-based phycoremediation.

EXPERIMENTAL

Microalgae Strain

The freshwater green microalga *Chlorella sorokiniana* (NIES-2168) was employed in this study. The strain was obtained from the Marine Biotechnology Laboratory, Faculty of Agriculture, Universiti Putra Malaysia (UPM), and originally sourced from the National Institute of Environmental Studies (NIES), Japan.

Media Preparation and Microalgae Cultivation

Chlorella sorokiniana was cultured in Bold's Basal Medium (BBM), prepared by dissolving 0.705 g/L of BBM powder in 1 L of distilled water. The pH was adjusted to 6.6 using 1 mL of 0.1% (v/v) sulphuric acid solution. The medium was sterilised in an autoclave (Tomy SS 325, Japan) at 121 °C for 15 minutes. Subsequently, 60 mL of algal inoculum was introduced into each of three 500 mL Erlenmeyer flasks containing 240 mL of BBM. Cultures were maintained at room temperature (25 ± 2 °C) under a 12:12 h light–dark photoperiod for 30 days. At the end of the cultivation period, the algal culture reached

an approximate biomass concentration of $3.0\text{--}5.0 \times 10^6$ cells mL⁻¹ and was subsequently harvested for bead preparation.

Sodium Alginate – Algae Beads Preparation

Sodium alginate solutions were prepared at concentrations of 1%, 2%, and 3% (w/v) by dissolving the appropriate amount of sodium alginate in 100 mL of distilled water with continuous stirring for 30 minutes. After degassing overnight, 25 mL of each alginate solution was mixed with 25 mL of concentrated algal sediment and gently agitated to obtain a homogeneous mixture. The mixtures were dispensed dropwise into a 2% (w/v) calcium chloride solution using a 2 mL dropper to form alginate beads. The beads were allowed to harden for 15 minutes, filtered, rinsed with distilled water, and stored in sealed containers at 4 °C until use. Based on bead morphology and handling properties, the 2% (w/v) sodium alginate formulation was selected for subsequent nutrient removal experiments.

Agar-Algae Beads Preparation

Agar solutions were prepared at concentrations of 1%, 2%, and 3% (w/v) by dissolving the appropriate amount of agar in 100 mL of distilled water and heating the mixture in a microwave oven for 2 minutes until fully melted. After partial cooling, 25 mL of the molten agar was mixed with 25 mL of algal sediment. The mixture was dispensed dropwise into 100 mL of cooled vegetable oil using a 2 mL dropper to form agar beads. Solidified beads were filtered, rinsed thoroughly with distilled water to remove oil residues, and stored in sealed tubes for further use.

Synthetic Wastewater Preparation

Synthetic wastewater was prepared using stock solutions of ammonia and phosphate (100 ppm each). Two concentration levels were formulated:

- High concentration: 10 ppm phosphate and 8 ppm ammonia (100 mL phosphate stock + 80 mL ammonia stock diluted to 1 L).
- Low concentration: 5 ppm phosphate and 4 ppm ammonia (50 mL phosphate stock + 40 mL ammonia stock diluted to 1 L).

Real Wastewater Preparation

Real wastewater (2 L) was collected from aquaculture effluent at the Institute of Bioscience, UPM. Baseline ammonia and phosphate concentrations were determined using API colourimetric test kits.

Experimental Setup

For each wastewater type (synthetic and real), identical experimental setups were prepared using agar and alginate algal beads.

- **Agar algae beads treatment:** Triplicate 250 mL conical flasks were prepared for each synthetic wastewater concentration (high and low), each containing 100 mL of wastewater and 2 g of agar algal beads. Triplicate flasks were also prepared for real wastewater treatment under the same conditions.
- **Alginate algae beads treatment:** The same configuration and conditions were applied using alginate algal beads.
- **Control setup:** Control flasks were prepared under identical conditions but without algal beads, serving as baselines for nutrient concentration comparisons.

All flasks were maintained at room temperature under natural light with gentle aeration throughout the 72-hour experimental period.

Ammonia Determination

Ammonia concentrations were quantified using the salicylate method (API Ammonia Test Kit, API, USA). A 5 mL sample was mixed with 8 drops each of Solutions 1 and 2, allowed to react for 5 minutes, and the absorbance was measured at 660 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). Concentrations were determined from a calibration curve generated via linear regression from standard ammonia solutions.

Phosphate Determination

Phosphate concentrations were analysed using the molybdate blue method (API Phosphate Test Kit, API, USA). A 5 mL sample was mixed with 6 drops each of Solutions 1 and 2, incubated for 3 minutes, and the absorbance was measured at 880 nm. Phosphate concentrations were calculated using a standard calibration curve.

Sampling and Data Collection

Nutrient removal was evaluated over a 72-hour period. Samples (5 mL) were collected at 0, 24, 48, and 72 hours for ammonia and phosphate analyses. All measurements were performed in triplicate, and the mean values were used for statistical analysis.

Statistical Analysis

Nutrient removal efficiency (%) was calculated using following equation:

$$\text{Removal Efficiency (\%)} = \frac{C_i - C_f}{C_i} \times 100$$

Where C_i and C_f are the initial and final nutrient concentrations (mg/L), respectively. All experiments were performed using three independent biological replicates unless otherwise stated. Data are presented as mean $\pm$ standard deviation. Prior to statistical analysis, the assumptions of normality and homogeneity of variance were assessed. Independent t-tests and one-way ANOVA were subsequently applied to evaluate significant differences among treatments and time points, with statistical significance accepted at $p < 0.05$.

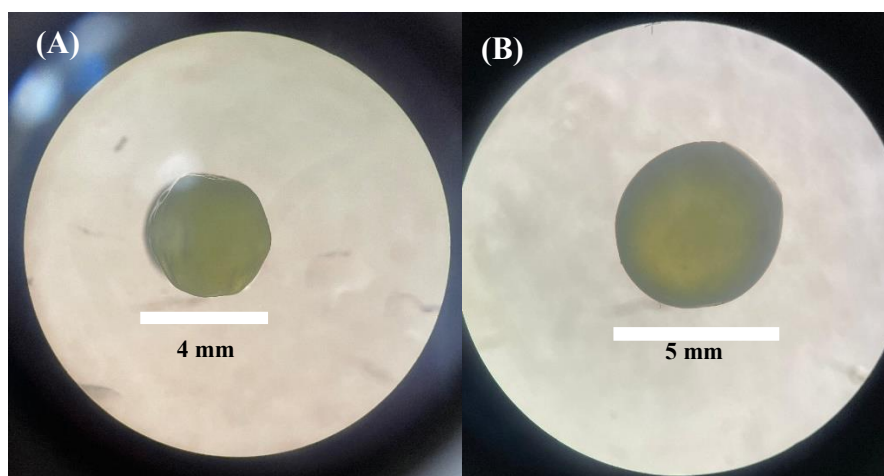


Figure 1. Morphological comparison of alginate (A) and agar (B) algae beads observed under a dissecting microscope at 40 $\times$ magnification. Alginate beads exhibited a smaller diameter (~4 mm) with a softer, more uniform structure, whereas agar beads were larger (~5 mm) and mechanically firmer.

RESULTS AND DISCUSSION

Optimisation of Bead Formation

Optimisation of bead formation revealed that agar and alginate behaved differently according to their concentrations and gelling conditions. Beads prepared with 1% agar were mechanically weak and easily deformed, whereas 3–4% agar solutions were excessively viscous, hindering droplet formation and producing irregular structures. A 2% agar concentration provided the best balance, forming stable and uniform beads suitable for handling. Similarly, sodium alginate trials at 1%, 2%, and 3% showed that only the 2% formulation produced spherical, uniform beads with consistent morphology. Lower concentrations (1%) yielded deformed shapes due to insufficient viscosity, while higher concentrations (3%) limited extrusion, resulting in irregular beads. For alginate, a 2% calcium chloride solution produced optimal crosslinking, forming beads with uniform morphology and minimal shrinkage. Lower CaCl_2 concentrations caused incomplete gelation, whereas higher concentrations led to over-crosslinking and excessive shrinkage.

Under these optimised conditions, alginate beads measured approximately 4 mm in diameter and exhibited a soft, gel-like consistency, while agar beads were firmer, averaging 5 mm (Figure 1). These differences in morphology are attributable to the distinct gelation mechanisms of the two polymers: alginate undergoes ionic crosslinking with calcium ions, whereas agar solidifies through thermo-reversible gelation [26, 27].

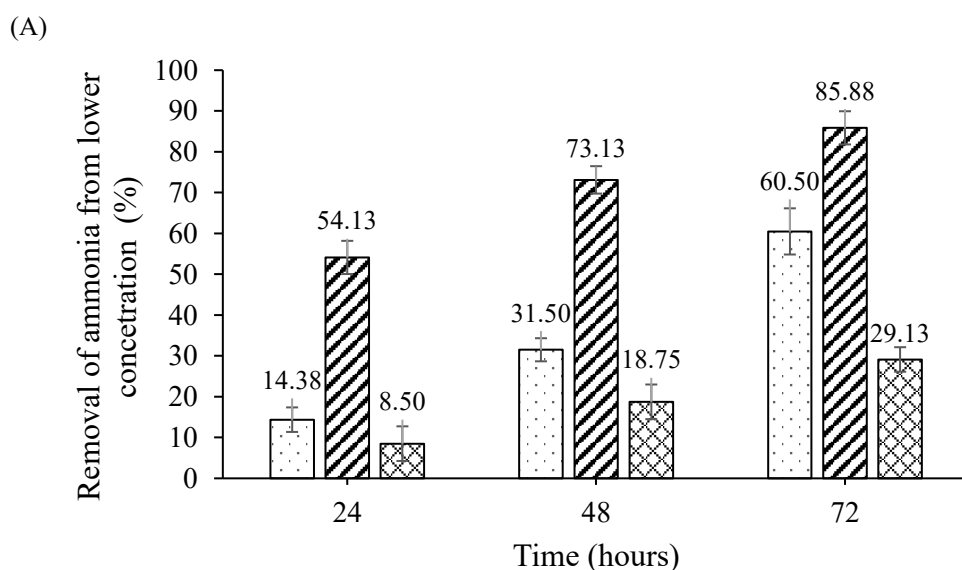
These morphological differences are functionally important, as bead porosity and flexibility influence nutrient diffusion, light availability, and algal

distribution within the beads, which collectively affect phycoremediation efficiency. The optimisation confirmed that 2% sodium alginate (with 2% CaCl_2) and 2% agar were the most suitable formulations for bead preparation.

Ammonia Removal Performance in Different Wastewater Systems

Ammonia removal efficiency was evaluated using alginate beads, agar beads, and a control system across synthetic wastewater (low and high ammonia concentrations) and real aquaculture wastewater. In all systems, ammonia removal increased progressively over time (24–72 h), indicating continuous adsorption and biological uptake by immobilised microalgae (Figure 2). Across all wastewater types, alginate beads consistently exhibited superior ammonia removal performance compared with agar beads and the control. At 72 h, alginate beads achieved ammonia removal efficiencies ranging from 85.88% to 90.44% in synthetic wastewater and 89.24% in real wastewater. In contrast, agar beads demonstrated moderate removal efficiencies (52.88–60.50%), while the control exhibited limited natural ammonia reduction (~26%).

When averaged across all wastewater conditions, alginate beads achieved a mean ammonia removal efficiency of 89.12%, which was significantly higher than that of agar beads (57.30%) and the control (26.78%) (Figure 3). Statistical analysis confirmed that bead type had a significant effect on ammonia removal efficiency (one-way ANOVA, $p = 0.001$). Independent t-test analysis further demonstrated that alginate beads removed 31.82% more ammonia than agar beads, confirming the superior performance of alginate-based immobilisation systems.



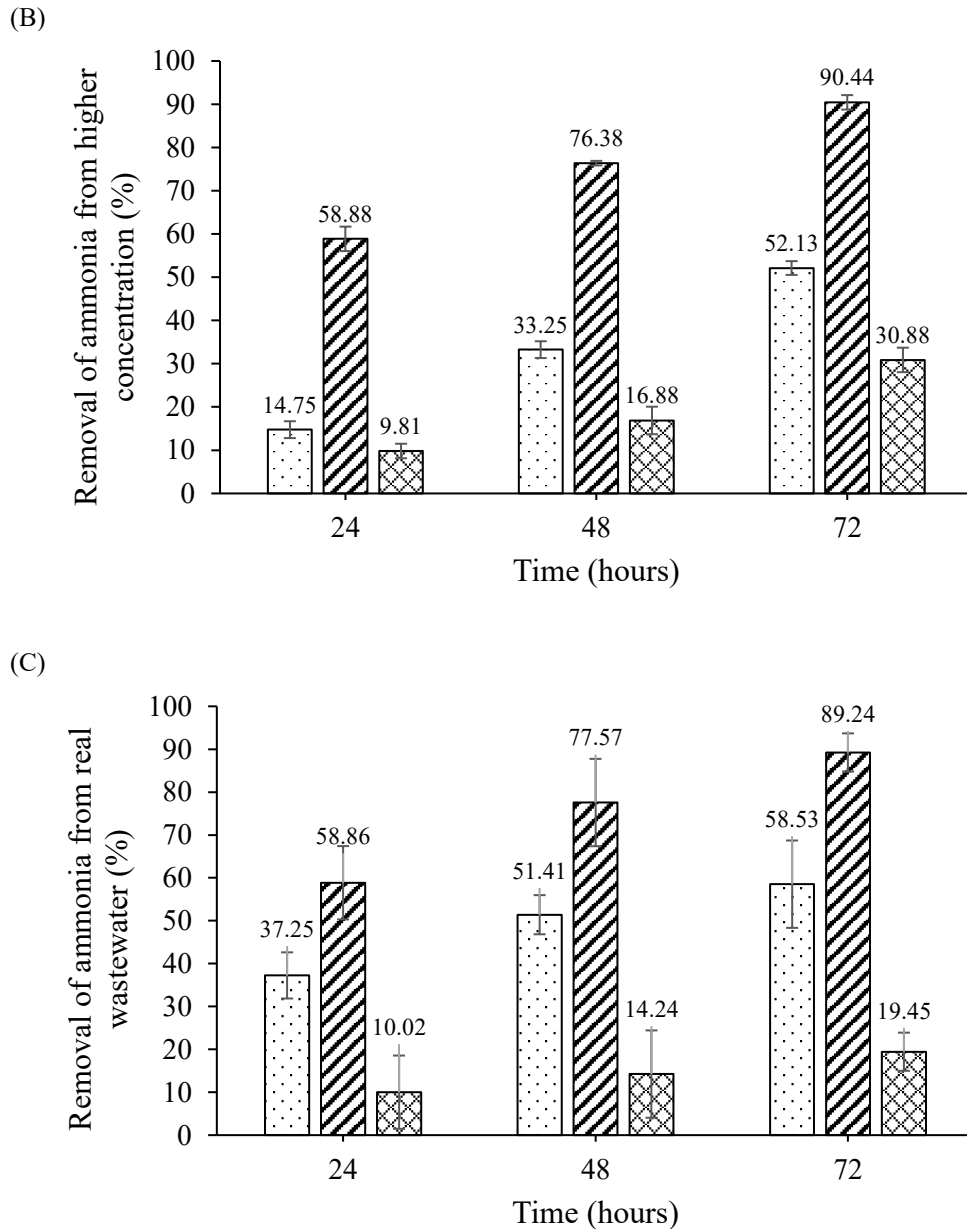


Figure 2. Percentage removal of ammonia (%) over time (hours) in (A) lower ammonia concentration, (B) higher ammonia concentration and (C) real wastewater, for agar beads , alginate beads  and the control . Data are presented as mean $\pm$ standard deviation (SD) ($n = 3$).

The enhanced ammonia removal observed for alginate beads can be attributed to their porous, calcium-crosslinked gel structure, which facilitates efficient nutrient diffusion and promotes sustained algal metabolic activity. The ionic nature of alginate enhances ammonium adsorption, allowing both physical adsorption and biological assimilation to occur simultaneously [26, 28, 35]. In contrast, the denser and less permeable agar matrix restricts nutrient transport, leading to slower ammonia uptake and reduced overall efficiency [29, 34].

It should be noted that the present study did not include blank beads without immobilised microalgae. Therefore, the respective contributions of matrix adsorption and biological uptake could not be distinguished quantitatively. The higher nutrient removal observed in the alginate beads is likely attributable to the combined effects of adsorption by the calcium alginate matrix and assimilation by *Chlorella sorokiniana*. Future studies incorporating blank-matrix controls would enable these mechanisms to be evaluated separately.

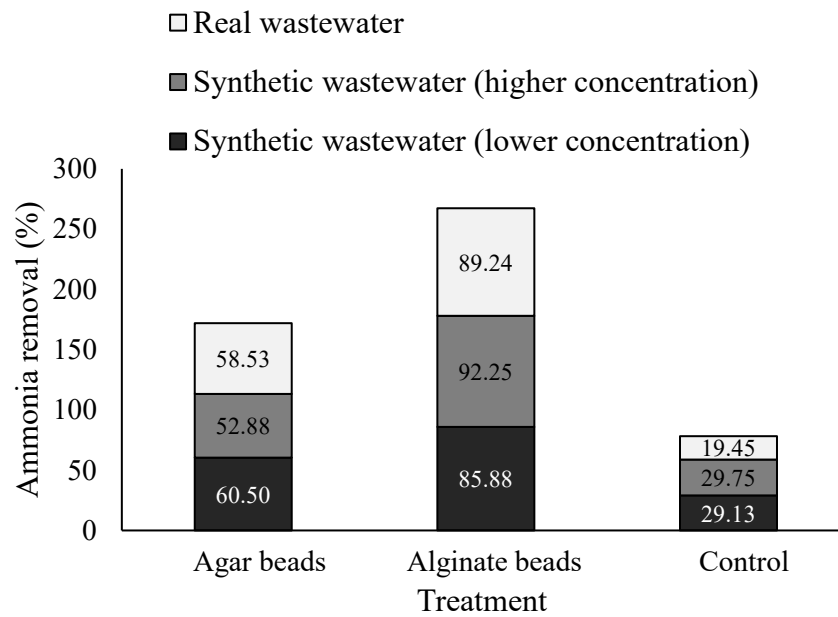


Figure 3. Ammonia removal efficiency after 72 h for different bead types in three ammonia sources: real wastewater, synthetic wastewater with higher ammonia concentration and synthetic wastewater with lower ammonia concentration.

Table 1. Descriptive statistics for ammonia percentage removal between agar beads and alginate beads.

	N	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation
Agar	3	7.62	52.88	60.50	57.30	2.28	3.95
Alginate	3	6.37	85.88	92.25	89.12	1.84	3.19

Table 2. One-Way ANOVA summary table for ammonia percentage removal between agar beads and alginate beads.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1518.77	1	1518.77	117.74	0.001
Within Groups	51.60	4	12.90		
Total	1570.37	5			

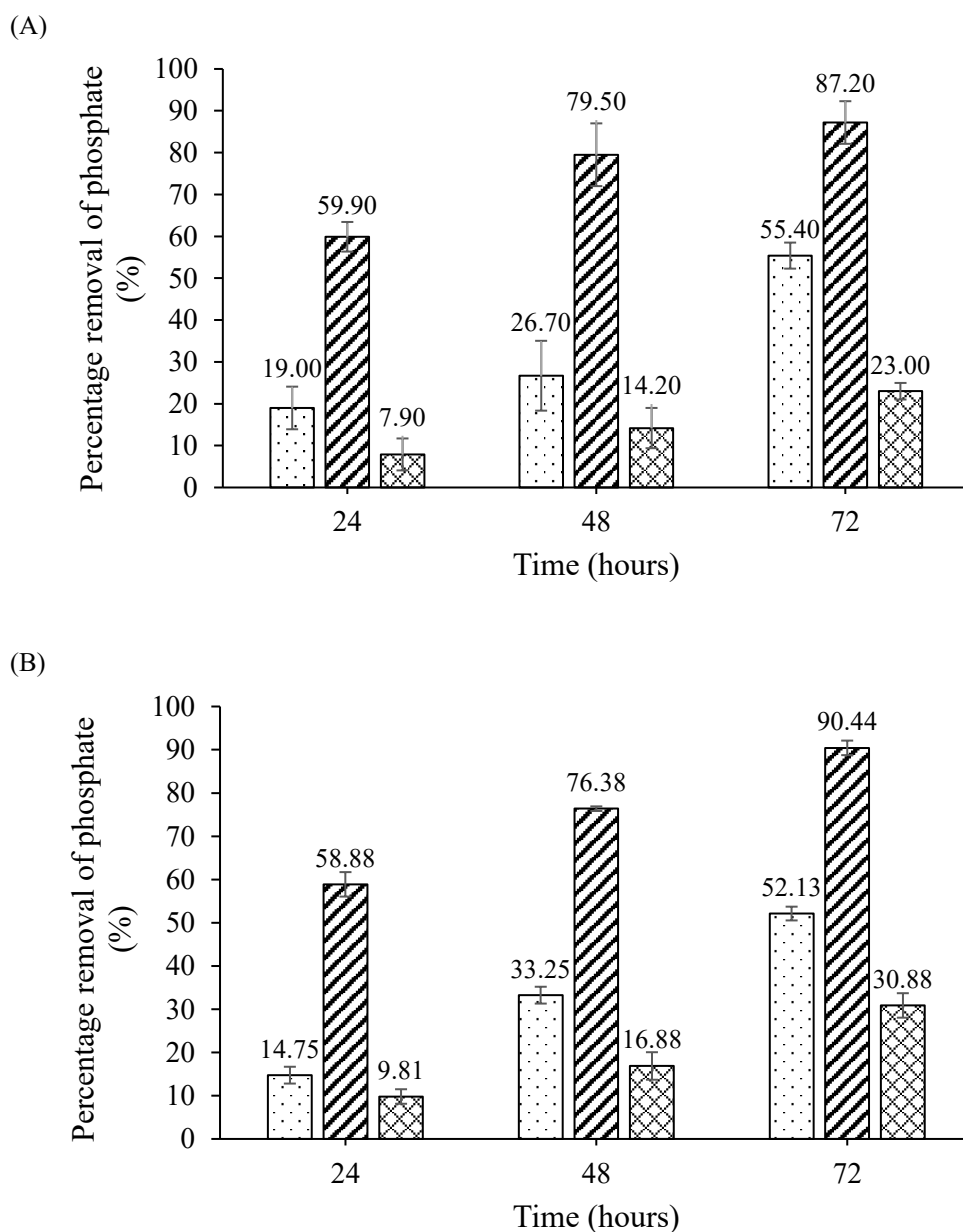
Table 3. Independent T-test for total ammonia percentage removal between agar beads and alginate beads.

Levene's Test for Equality of Variances		t-test for Equality of Means							
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	.323	.600	-10.85	4	.000	-31.82	2.93	-39.96	-23.68
Equal variances not assumed			-10.85	3.83	.001	-31.82	2.93	-40.11	-23.53

Phosphate Removal Performance in Different Wastewater Systems

Phosphate removal followed a similar trend to ammonia removal, with alginate beads consistently outperforming agar beads and the control across all wastewater types. Phosphate removal efficiency increased with exposure time and reached near-maximal values at 72 h, supporting the use of final removal values for comparative analysis (Figure 4).

At 72 h, alginate beads achieved phosphate removal efficiencies of 87.20% in low-concentration synthetic wastewater, 84.45% in high-concentration synthetic wastewater, and 82.87% in real wastewater. Agar beads exhibited moderate phosphate removal efficiencies (38.95–55.40%), whereas the control showed limited removal (18.70–32.09%). Across all wastewater systems, alginate beads achieved a mean phosphate removal efficiency of 84.84%, which was significantly higher than that of agar beads (47.86%) and the control (approximately 25%).



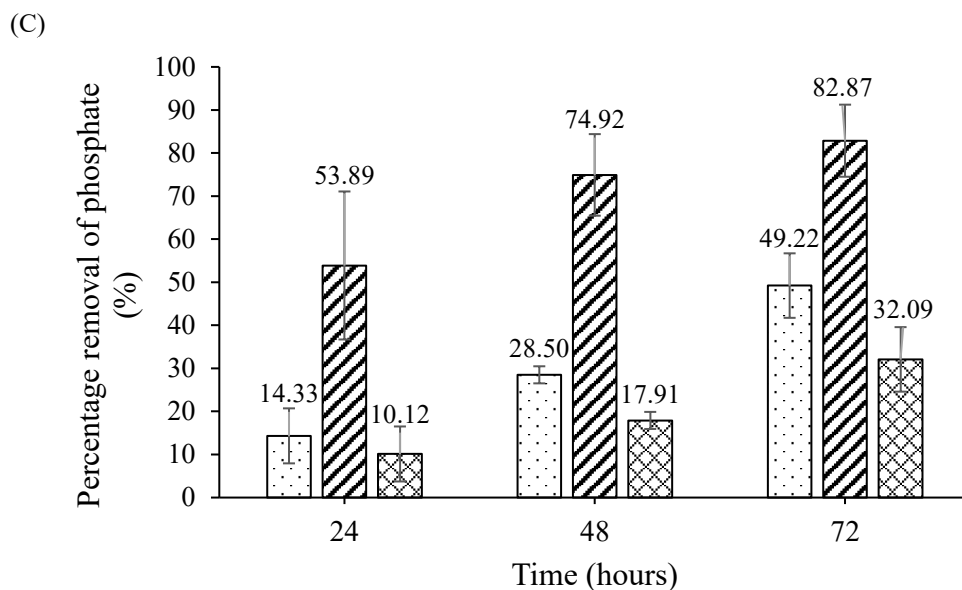


Figure 4. Percentage removal of phosphate (%) over time (hours) in (A) lower phosphate concentration (B) higher phosphate concentration and (C) real wastewater for agar agar beads , alginate beads  and the control . Data are presented as mean $\pm$ standard deviation (SD) ($n = 3$).

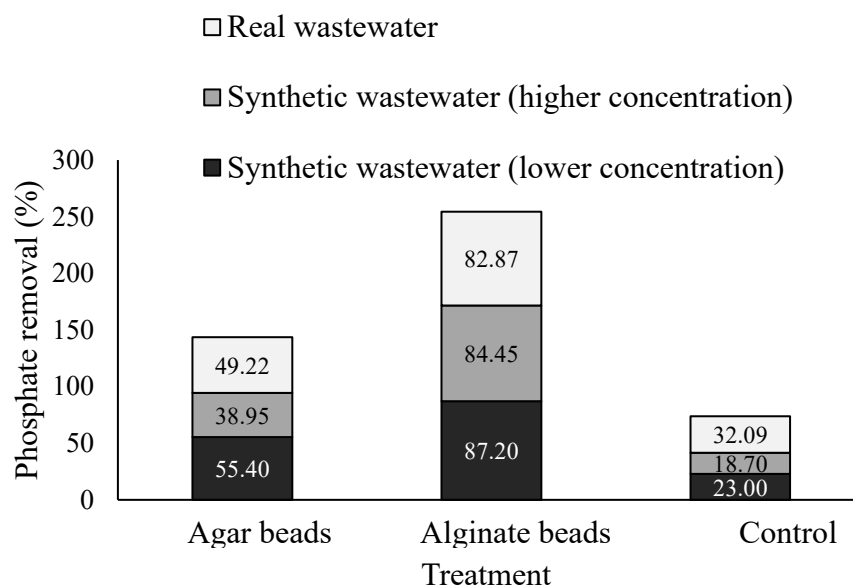


Figure 5. Phosphate removal efficiency after 72 h for different bead types in three phosphate sources: real wastewater, synthetic wastewater with higher phosphate concentration and synthetic wastewater with lower phosphate concentration.

One-way ANOVA confirmed a significant effect of bead type on phosphate removal efficiency ($p = 0.002$). Independent t-test analysis further revealed a mean difference of 36.98% between alginate and agar beads, indicating substantially greater phosphate

removal by alginate-based systems. In addition, alginate beads exhibited lower variability in removal efficiency (standard deviation = 2.19) compared with agar beads (standard deviation = 8.31), suggesting more consistent phosphate removal performance.

Table 4. One-Way ANOVA summary table for phosphate percentage removal between agar beads and alginate beads.

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
Agar	3	16.45	38.95	55.40	47.86	8.31	69.05
Alginate	3	4.33	82.87	87.20	84.84	2.19	4.80

Table 5. One-Way ANOVA summary table for phosphate percentage removal between agar beads and alginate beads.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2051.65	1	2051.65	55.57	.002
Within Groups	147.69	4	36.923		
Total	2199.34	5			

Table 6. Independent T-test for total phosphate percentage removal between agar beads and alginate beads.

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2- tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
Equal variances assumed	3.31	.143	-7.46	4	.002	-36.98	4.96	-50.75	-23.21
Equal variances not assumed			-7.46	2.28	.012	-36.98	4.96	-56.02	-17.94

The superior phosphate removal by alginate beads can be attributed to the presence of negatively charged carboxyl functional groups, which promote electrostatic attraction and complexation with phosphate ions [31, 30]. Furthermore, the flexible gel network of alginate enhances mass transfer and maintains algal viability, enabling sustained phosphate uptake over time [28, 32]. In contrast, the limited porosity and rigidity of agar beads restrict phosphate diffusion and reduce removal efficiency [34].

Comparative Performance of Alginate and Agar Beads

The present findings extend previous reports on immobilised microalgae by demonstrating that the choice of encapsulation matrix has a measurable influence on nutrient removal performance, even when all other experimental conditions are kept constant. Under the same treatment conditions, sodium alginate consistently achieved higher ammonia and phosphate removal than agar. This suggests that differences in matrix structure, particularly porosity and ionic

interactions, play an important role in facilitating nutrient diffusion and maintaining algal activity. While previous studies have reported the effectiveness of either alginate- or agar-based immobilisation systems, direct comparisons between the two matrices remain relatively limited. The present results therefore provide additional evidence to support the selection of sodium alginate as a more suitable immobilisation material for phycoremediation applications.

Overall, the results demonstrate that alginate beads consistently outperform agar beads in both ammonia and phosphate removal across synthetic and real wastewater systems. The superior performance of alginate beads is closely associated with their favourable physicochemical properties, including high porosity, ionic functionality, structural flexibility, and ability to support prolonged algal metabolic activity [25, 33]. While agar beads provide greater mechanical rigidity, their diffusion limitations reduce nutrient uptake efficiency, making them less suitable for high-performance phycoremediation applications [34]. Alginate beads, on the other hand, offer improved nutrient diffusion, enhanced algal retention,

biodegradability, and long-term stability, supporting their application as an effective and sustainable encapsulation material for nutrient removal from wastewater [32, 36].

The enhanced nutrient removal observed for sodium alginate beads can also be explained by the formation of the characteristic "egg-box" structure during ionic crosslinking with calcium ions (Ca^{2+}). This crosslinked network produces a stable hydrogel with interconnected pores that facilitates the diffusion of dissolved nutrients into the bead while maintaining the structural integrity required for microalgal growth. Furthermore, the abundance of negatively charged carboxyl groups in alginate provides additional adsorption sites for ammonium ions before they are assimilated by the immobilised microalgae. The combined effects of initial adsorption by the alginate matrix and subsequent biological uptake by *Chlorella sorokiniana* are likely responsible for the consistently higher ammonia and phosphate removal efficiencies observed in this study. Similar observations have recently been reported for calcium alginate-immobilised microalgae used in wastewater treatment, where improved mass transfer and bead stability contributed to enhanced nutrient removal performance [37].

CONCLUSION

This study demonstrates that the choice of immobilisation matrix plays a critical role in determining the efficiency of algae-based phycoremediation systems. Under identical experimental conditions, sodium alginate beads consistently achieved significantly higher ammonia and phosphate removal efficiencies than agar beads across synthetic wastewater with varying nutrient concentrations and real aquaculture wastewater. The superior performance of alginate beads is attributed to their porous, calcium-crosslinked structure, ionic functionality, and ability to sustain algal metabolic activity, which collectively enhance nutrient diffusion and uptake. Although the present study demonstrated the superior performance of sodium alginate beads over a 72 h treatment period, several limitations should be acknowledged. Long-term stability, bead reusability, adsorption kinetics, and large-scale industrial wastewater applications were beyond the scope of this work. Future studies should investigate these aspects to facilitate practical implementation.

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Further information about the research group is available at albic.my.

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