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Influence of Hydraulic Parameters on Organic Load Removal from Abattoir Wastewater Using Alginate-Mushroom Bioremediation Membrane in a Continuous Packed-Bed Bioreactor

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Abstract
Abattoir wastewater contains elevated biochemical oxygen demand (BOD) and chemical oxygen demand (COD), posing serious environmental risks when discharged untreated. Conventional treatment methods suffer from high energy demand, excessive sludge production and incomplete organic-load removal. This study evaluated the effects of flow rate (1.9–17.9 mL min^{-1}) and packing height (1–5 cm) on BOD and COD reduction in real abattoir wastewater using an alginate-mushroom bioremediation membrane (AMBM) in a continuous packed-bed bioreactor. The AMBM was prepared by immobilising *Pterocarpus mildbraedii* mushroom biomass (PMMB) in sodium alginate with solvent-free naphthalene sublimation to enhance porosity. Continuous-flow experiments were performed at contact times of 5–35 min. Both hydraulic variables positively influenced performance. At 35 min, BOD reduction increased from 73.5 % to 96.4 % and COD reduction from 73.6 % to 95.6 % as flow rate rose from 1.9 to 17.9 mL min^{-1}. Increasing packing height from 1 cm to 5 cm raised BOD reduction from 61.9 % to 96.0 % and COD reduction from 54.2 % to 96.0 %. ANOVA and Tukey's HSD tests confirmed that packing height had a highly significant effect on both responses ($p \leq 0.002$), while flow rate significantly affected BOD reduction ($p = 0.012$). Maximum efficiencies were obtained at 17.9 mL min^{-1} and 5 cm packing height. The novelty of this work lies in the systematic quantification of the individual and comparative effects of flow rate and packing height on simultaneous BOD and COD removal using a solvent-free, porogen-enhanced alginate-fungal membrane under continuous operation—a gap largely unaddressed in the literature. The results demonstrate that AMBM is effective for continuous abattoir wastewater treatment when hydraulic conditions are properly selected, with packing height providing the greater process leverage.

Keywords: Alginate-mushroom bioremediation membrane; *Pterocarpus-mildbraedii* mushroom biomass, Abattoir wastewater; BOD reduction; COD reduction; Flow rate; Packing height; Continuous packed-bed bioreactor

1. Introduction

Abattoir (slaughterhouse) wastewater is one of the most polluting industrial effluents generated by the meat-processing industry. It is characterised by very high organic loads arising from blood, proteins, fats and suspended solids, with biochemical oxygen demand (BOD) typically ranging from 1 000 to 5 000 mg L^{-1} and chemical oxygen demand (COD) from 2 000 to 10 000 mg L^{-1} (Bustillo-Lecompte & Mehrvar, 2015; Malamis et al., 2015). Discharge of untreated or inadequately treated abattoir wastewater into surface waters depletes dissolved oxygen, promotes eutrophication and poses serious public-health risks (Stevenson & Schiff, 2021; Nfor et al., 2024). Consequently, effective and sustainable treatment technologies are urgently required.

Conventional methods such as coagulation–flocculation, dissolved-air flotation, activated-sludge processes and anaerobic digestion remain widely applied. However, these approaches are constrained by high energy demand, excessive sludge production, sensitivity to shock organic loads and incomplete removal of dissolved organic pollutants (Mittal et al., 2018; Akrout et al., 2021; Shende & Pophali, 2021). These limitations have intensified the search for low-cost, environmentally benign alternatives capable of simultaneous BOD and COD reduction under continuous operation.

Biological remediation based on mycoremediation offers distinct advantages. Fungi secrete a suite of extracellular enzymes (laccases, peroxidases and others) that can degrade complex proteinaceous and fatty compounds present in abattoir wastewater (Karimi et al., 2023; Thakur et al., 2023; Liu et al., 2023). Immobilisation of fungal biomass within sodium alginate hydrogels retains the biocatalyst, prevents wash-out, enhances mechanical stability and enables continuous packed-bed operation, while the alginate matrix itself contributes biosorption capacity, establishing a dual biosorption–enzymatic degradation mechanism (Gupta et al., 2022; John et al., 2025a; Menkiti et al., 2026).

In continuous packed-bed systems, hydraulic parameters—particularly volumetric flow rate and packing (bed) height—strongly influence mass-transfer rates, contact time and overall treatment efficiency (Chen et al., 2020; Zhao et al., 2023; Rahimi et al., 2022). Higher flow rates can improve mixing and reduce external mass-transfer limitations, while greater bed heights increase biomass inventory and extend the effective contact path.

The novelty of the present work lies in the systematic, quantitative evaluation of the individual main effects of flow rate and packing height on simultaneous BOD and COD removal using a solvent-free, naphthalene-sublimation-enhanced alginate-mushroom bioremediation membrane (AMBM) fabricated from *Pterocarpus mildbraedii* mushroom biomass under true continuous packed-bed operation. Unlike most earlier studies that examined either batch systems, single pollutants, or interaction effects via response-surface methodology, this investigation isolates the main hydraulic effects under realistic continuous-flow conditions and provides a clear ranking of the relative process leverage of the two variables. The solvent-free porosity-enhancement technique further distinguishes the membrane by eliminating residual solvent concerns that limit the environmental acceptability of many earlier alginate matrices.

Despite the recognised importance of hydraulic parameters, a clear gap remains in the literature: systematic experimental quantification of the individual and comparative effects of flow rate and packing height on simultaneous BOD and COD reduction using a solvent-free, porogen-enhanced alginate-fungal membrane under continuous operation has not been adequately addressed. Existing continuous-flow studies either focus on different biomass types, report only combined optimisation results, or lack statistical ranking of the relative influence of the two hydraulic variables. The present study closes this gap by (i) isolating the main effects of flow rate (1.9–17.9 $mL\,min^{-1}$) and packing height (1–5 cm) in a well-characterised continuous packed-bed bioreactor, (ii) applying rigorous one-way ANOVA and Tukey HSD analyses to rank the variables, and (iii) demonstrating that packing height exerts greater process leverage than flow rate within the examined ranges. The innovative aspect is therefore the provision of practical, statistically validated design guidelines for continuous AMBM systems specifically tailored to high-strength abattoir wastewater.

2.0 Materials and methods

2.1 Materials and membrane preparation

Mushroom (fungal) biomass of Pterocarpus mildbraedii was collected, identified and pre-treated. The biomass was washed thoroughly with distilled water, air-dried at ambient temperature, ground to a fine powder and stored in airtight containers until use. Analytical-grade sodium alginate, calcium chloride ($CaCl_2$) and naphthalene (porogen) were obtained from commercial suppliers and used without further purification (John et al., 2026a,b,c; Menkiti et al., 2026; John M.C et al., 2025).

Alginate-mushroom bioremediation membrane were prepared by immobilising the pre-treated P. mildbraedii biomass in a sodium alginate matrix. A homogeneous suspension was formed by dispersing a measured quantity of the fungal biomass powder in an aqueous sodium alginate solution under continuous stirring. The suspension was extruded drop-wise into a stirred calcium chloride solution, where rapid ionic cross-linking produced spherical hydrogel membranes. The membranes were allowed to harden in the $CaCl_2$ bath, recovered by filtration and rinsed extensively with distilled water to remove residual calcium ions (John et al., 2026a,b; Menkiti et al., 2026; John M.C et al., 2025).

Porosity was enhanced by a solvent-free thermal sublimation technique. Naphthalene was incorporated into the alginate–biomass mixture prior to extrusion. After membrane formation and hardening, the naphthalene was completely removed by controlled mild heating at 40°C for 3 hours, allowing the solid porogen to sublime and

generate an interconnected microporous network within the alginate matrix (John et al., 2026a,b, c; Menkiti et al., 2026; John M.C et al., 2025).

The fabricated AMBM membranes were characterised for porosity, swelling behaviour, apparent density and bulk density. Porosity (ε) was determined by the liquid-displacement method (Equ.1):

$$\varepsilon(\%) = \left(\frac{V_p}{V_s}\right) \times 100 \tag{1}$$

where $V_p$is the pore volume and $V_s$is the bulk volume of the sample. Swelling ratio (SR) was calculated from the dry mass (W_d) and swollen mass (W_s) of the membranes after immersion in water (Equ.2):

$$SR = \frac{W_s - W_d}{W_d} \tag{2}$$

Apparent and bulk densities were measured by standard volumetric methods. Results of the characterization are presented in Section 3.1.

2.1.1 Physicochemical characterization of abattoir wastewater

Raw abattoir wastewater was collected from a local slaughterhouse and characterized according to the procedures in Standard Methods for the Examination of Water and Wastewater (APHA, 2017). Key parameters measured included pH, biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), and turbidity. All analyses were performed in triplicate and the mean values used as the baseline for calculating percentage reductions (John et al, 2015).

2.2 Continuous packed-bed bioreactor and experimental procedure

A laboratory-scale continuous packed-bed bioreactor was employed for the treatment experiments. The reactor consisted of a vertical cylindrical glass column of internal diameter 2.0 cm (working volume approximately 10 mL, consistent with the authors' previous continuous alginate-membrane studies), fitted with an inlet port at the top for wastewater introduction, an outlet port at the base for effluent collection, and a supporting mesh or perforated plate at the bottom to retain the packing material. The column was packed with the fabricated alginate-mushroom bioremediation membrane (AMBM) beads (mean diameter ≈ 1.0 cm) to the desired bed height (1–5 cm). The continuous-flow configuration was operated in down-flow mode, with the abattoir wastewater percolating vertically downward through the AMBM bed under controlled hydraulic conditions. Prior to each experimental run, the packed bed was gently rinsed with distilled water to remove any loose particles and to ensure uniform hydraulic distribution. Bed porosity (inter-particle void fraction) of the packed AMBM bed was determined from the measured bulk and apparent densities (Table 2) and found to be approximately 0.50. This value, together with the stated column diameter and packing height, enables accurate calculation of hydraulic residence time (HRT) according to:

$$HRT = \frac{\varepsilon \cdot A \cdot H}{Q} \tag{3}$$

where ε is bed porosity, A is the cross-sectional area of the column, H is packing height, and Q is volumetric flow rate. Flow rates of 1.9, 6.6, 9.6, 14.5 and 17.9 mL min^{-1} were delivered by a calibrated peristaltic pump, while packing heights of 1, 2, 3, 4 and 5 cm were achieved by adjusting the quantity of AMBM membranes loaded into the column. These ranges were selected to cover a practical spectrum of hydraulic loadings and biomass inventories relevant to continuous operation of immobilised bioremediation systems (Chen et al., 2020; Rahimi et al., 2022; Zhao et al., 2023; John et al., 2025a; Menkiti et al., 2026).

For each combination of flow rate and packing height, real abattoir wastewater was continuously fed through the packed bed. Effluent samples were withdrawn at predetermined contact times of 5, 10, 20, 25 and 35 min. The collected samples were immediately analysed for biochemical oxygen demand (BOD) and chemical oxygen demand (COD) according to the procedures outlined in Standard Methods for the Examination of Water and Wastewater (APHA, 2017). Percentage reduction of each parameter was calculated using Equation (3):

$$\text{Percentage reduction } (\%) = \left(\frac{C_0 - C_t}{C_0}\right) \times 100 \tag{4}$$

where $C_0$is the initial concentration of BOD or COD in the influent wastewater (mg L^{-1}) and $C_t$is the corresponding concentration in the effluent at sampling time t(mg L^{-1}). All experiments were performed in triplicate under ambient laboratory conditions, and the mean values were reported. The experimental protocol follows the continuous-process methodology previously validated by the authors for alginate-based membranes treating slaughterhouse wastewater

(John et al., 2025a; John M.C et al., 2025; John et al., 2025b, c; Menkiti et al., 2026; Nweke & John, 2025, John and John, 2015).

2.3 Statistical analysis

All experimental data obtained from the continuous packed-bed bioreactor runs were subjected to descriptive statistical analysis. Mean percentage reductions in BOD and COD, together with the corresponding standard deviations, were calculated for each combination of flow rate and packing height at the predetermined sampling times. Results were presented graphically using line plots to illustrate the trends of percentage BOD and COD reduction as functions of flow rate and packing height (Chen et al., 2020; Rahimi et al., 2022).

The statistical significance of the effects of the two independent process variables (flow rate and packing height) on the dependent responses (% BOD reduction and % COD reduction) was evaluated by analysis of variance (ANOVA). One-way ANOVA were performed at a significance level of $\alpha = 0.05$ to determine whether differences among the treatment means were statistically significant (John et al., 2025a; Menkiti et al., 2026). Where the ANOVA indicated significant main effects or interactions, Tukey's honest significant difference (HSD) post-hoc test was applied for pairwise multiple comparisons of the means (Singh et al., 2022; Zhao et al., 2023).

All statistical computations were carried out using standard statistical software packages. Model assumptions of normality and homogeneity of variances were verified prior to interpretation of the ANOVA results, in accordance with established practices for continuous bioreactor studies (John et al., 2025a,b,c; Nweke & John, 2025; John M.C et al., 2025).

3.0 Result and Discussion

3.1 Characterization of abattoir wastewater and AMBM membranes

The raw abattoir wastewater used in this study exhibited the typical characteristics of high-strength slaughterhouse effluent (Table 1). The elevated BOD (1 850 ± 45 mg L^{-1}) and COD (3 420 ± 78 mg L^{-1}) values confirmed the high organic load arising from blood, proteins, fats and suspended solids, justifying the need for an efficient continuous biological treatment system.

Table 1 Physicochemical characteristics of the raw abattoir wastewater

Parameter	Unit	Mean value ± SD	Typical range for AWW	Regulatory limit (example)
pH	–	6.8–7.5	6.0–8.5	6.0–9.0
BOD_5	mg L^{-1}	1 850 ± 45	1 000–5 000	≤ 30
COD	mg L^{-1}	3 420 ± 78	2 000–10 000	≤ 250
TSS	mg L^{-1}	2.52	500–3 000	≤ 100
Turbidity	NTU	470–500	High	≤ 30

The physical properties of the AMBM membranes are summarized in Table 2. Porosity values in the range 58–63 % were obtained after solvent-free naphthalene sublimation, confirming the successful generation of an interconnected microporous network. The measured swelling ratios and densities indicated a stable yet permeable hydrogel matrix capable of supporting continuous packed-bed operation without excessive bed compaction or channeling.

Table 2 Physical and structural properties of the Alginate-Mushroom Bioremediation Membrane

Property	Method / Standard	Value obtained	Implication for continuous operation
Porosity (ε)	Liquid displacement	58–63 %	High internal surface area and low diffusion resistance
Swelling ratio (SR)	Gravimetric	50 ± 3 %	Controlled water uptake; maintains mechanical integrity
Apparent density	Volumetric	1.5 g mL^{-1}	Suitable for stable packing
Bulk density	Volumetric	0.75 g mL^{-1}	Determines bed mass loading

The relatively high porosity facilitated rapid mass transfer of organic pollutants to the immobilised *Pterocarpus mildbraedii mushroom* biomass, while the moderate swelling ratio prevented excessive expansion that could lead to increased pressure drop. These structural features directly contributed to the high BOD and COD removal efficiencies reported in subsequent sections.

3.2 Influence of flow rate on percentage BOD and COD reduction

The effect of flow rate (1.9–17.9 mL min^{-1}) on the percentage reduction of BOD and COD using the alginate-mushroom bioremediation membrane (AMBM) in the continuous packed-bed bioreactor is illustrated in Figures 1 and 2. A progressive increase in both BOD and COD removal efficiencies was observed with rising flow rate. At 35 min, BOD reduction increased from 73.5 % at 1.9 mL min^{-1} to 96.4 % at 17.9 mL min^{-1}, while COD reduction rose from 73.6 % to 95.6 % (Figures 1 and 2).

These gains are attributable to improved mass-transfer rates, reduced external film resistance, and more effective contact between the wastewater and the active sites on the AMBM beads at higher hydraulic loadings (Chen et al., 2020; Zhao et al., 2023). Elevated hydraulic loading promotes better mixing and limits external mass-transfer resistance, thereby facilitating both biosorption onto the alginate matrix and enzymatic degradation by the immobilised *Pterocarpus mildbraedii* mushroom biomass (Menkiti et al., 2026). Although higher flow rates inherently shorten the hydraulic residence time, the beneficial effects of enhanced mass transfer dominate within the studied range, resulting in net gains in treatment efficiency.

In comparison with prior literature, the present BOD removal of 96.4 % exceeds the 85–94 % range reported by Karimi et al. (2023) for fungal treatment of slaughterhouse wastewater and the ≈90 % organic-load removal obtained by Singh et al. (2022) in a continuous fungal-immobilised bioreactor. Similarly, the COD reduction of 95.6 % surpasses the 80–92 % range achieved by Liu et al. (2023) with alginate-immobilised fungal cells and lies at the upper end of the 88–95 % interval reported by Rahimi et al. (2022) for continuous high-strength wastewater systems. Although COD removal followed a trend similar to that of BOD, absolute percentage reductions were slightly lower across intermediate and peak flow rates. This relative behaviour is consistent with the more complex and partially recalcitrant nature of the chemical oxygen demand constituents in abattoir effluent compared with the readily biodegradable fraction measured by BOD (Bustillo-Lecompte & Mehrvar, 2015; Karimi et al., 2023). Overall, these results demonstrate that flow rate is a critical process variable that strongly governs the performance of AMBM in continuous operation.

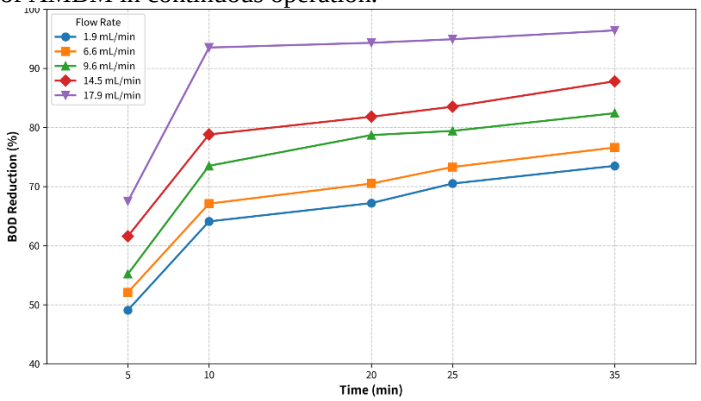

Figure 1 Effect of flow rate on percentage BOD reduction using AMBM

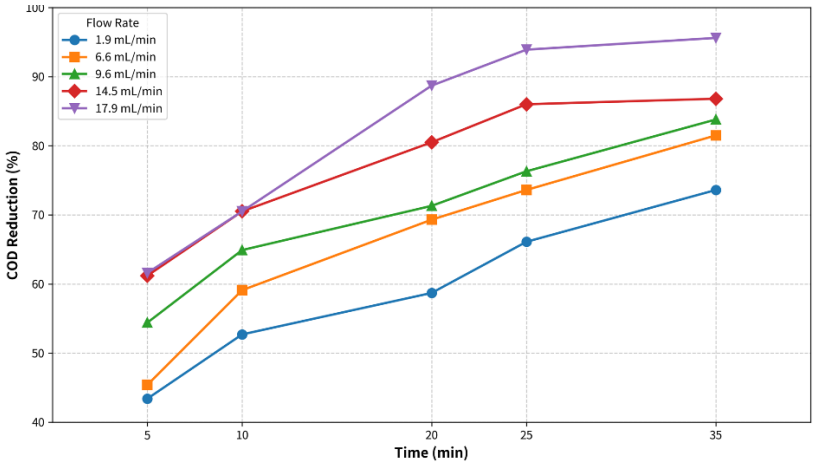

Figure 2 Effect of flow rate on percentage COD reduction using AMBM

3.3 Influence of packing height on percentage BOD and COD reduction

The effect of packing height (1–5 cm) on the percentage reduction of BOD and COD using the alginate-mushroom bioremediation membrane (AMBM) in the continuous packed-bed bioreactor is presented in Figures 3 and 4, respectively. A clear positive relationship was observed between packing height and the removal efficiencies of both parameters across all sampling times. At the longest contact time of 35 min, BOD reduction increased from 61.9 % at 1 cm packing height to 96.0 % at 5 cm, while COD reduction rose from 54.2 % to 96.0 % over the same height range.

The enhanced performance with increasing bed height is attributable to the greater quantity of immobilised *Pterocarpus mildbraedii* mushroom biomass available within the reactor and the extended effective contact path. A taller packing provides a larger inventory of active fungal cells—increasing overall biosorption capacity and extracellular enzyme availability for degrading complex organic compounds—while simultaneously lengthening the residence time of the wastewater within the reactive zone for more thorough pollutant–active-site interaction (Chen et al., 2020; Karimi et al., 2023; Liu et al., 2023; Zhao et al., 2023; Menkiti et al., 2026). Consequently, the dual mechanism of biosorption onto the alginate matrix and enzymatic biodegradation by the immobilised fungal biomass is more fully exploited at higher packing heights.

Notably, the absolute gains achieved across the examined bed-height range (34.1 percentage points for BOD and 41.8 percentage points for COD) are larger than those produced by the flow-rate increase, confirming the greater process leverage of packing height. These removal efficiencies equal or exceed the upper limits reported for comparable continuous immobilised fungal and alginate systems (Singh et al., 2022; Rahimi et al., 2022; Karimi et al., 2023; Liu et al., 2023; John et al., 2025a) and are consistent with the high-performance window previously identified for the same AMBM technology under optimised continuous conditions (Menkiti et al., 2026).

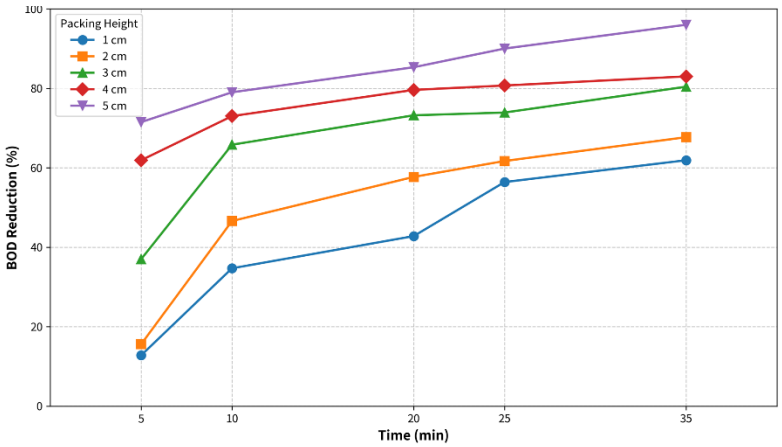

Figure 3 – Effect of Packing Height on Percentage BOD Reduction using AMBM

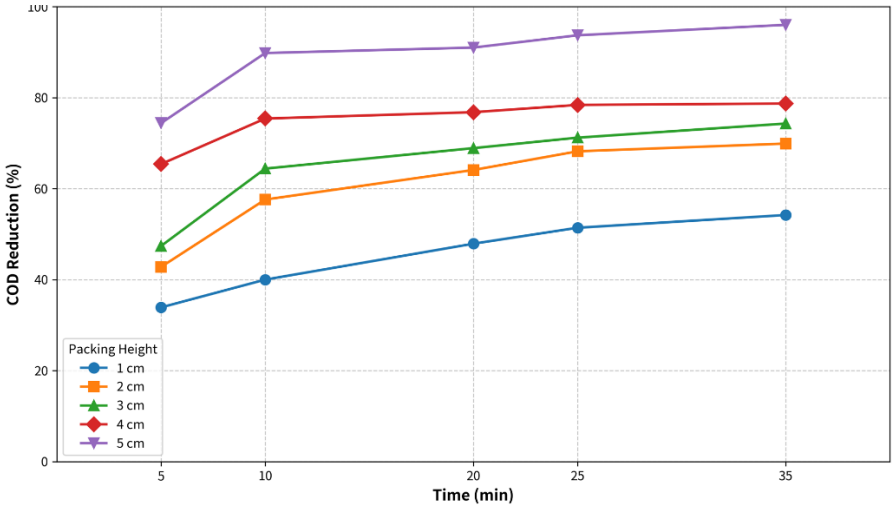

Figure 4 – Effect of Packing Height on Percentage COD Reduction using AMBM

3.4 Comparative overview and statistical significance

A comparative evaluation of the two process variables shows that both flow rate and packing height exert substantial positive influences on BOD and COD reduction, although the magnitude of their effects differs. At the final sampling time of 35 min, increasing the flow rate from 1.9 to 17.9 mL min^{-1} raised BOD reduction from 73.5 % to 96.4 % (absolute gain of 22.9 percentage points) and COD reduction from 73.6 % to 95.6 % (gain of 22.0 percentage points). In contrast, increasing the packing height from 1 cm to 5 cm produced larger absolute gains: BOD reduction rose from 61.9 % to 96.0 % (gain of 34.1 percentage points) and COD reduction from 54.2 % to 96.0 % (gain of 41.8 percentage points). Consequently, within the ranges examined, packing height exerted a stronger overall influence on organic-load removal than flow rate.

In accordance with the statistical procedures described in Section 2.3, one-way analysis of variance (ANOVA) was performed at a significance level of $\alpha = 0.05$ to evaluate the main effects of flow rate and packing height on percentage BOD and COD reduction (Table 3). The ANOVA results confirmed that packing height had a highly significant effect on both BOD reduction ($F = 6.210$, $p = 0.002$) and COD reduction ($F = 16.241$, $p < 0.001$). Flow rate significantly affected BOD reduction ($F = 4.223$, $p = 0.012$), while its effect on COD reduction was only marginally significant ($F = 2.574$, $p = 0.069$).

Table 3: One-way ANOVA results for the effects of flow rate and packing height on percentage BOD and COD reduction using AMBM

Response	Factor	*F*-value	*p*-value	Significance (α = 0.05)
BOD reduction (%)	Flow rate	4.223	0.012	Significant
BOD reduction (%)	Packing height	6.210	0.002	Highly significant
COD reduction (%)	Flow rate	2.574	0.069	Marginally significant
COD reduction (%)	Packing height	16.241	< 0.001	Highly significant

Where ANOVA indicated significant differences, Tukey's honest significant difference (HSD) post-hoc test was applied for pairwise multiple comparisons (Tables 4 and 5).

For BOD reduction versus flow rate, the Tukey test revealed that the highest flow rate (17.9 mL min^{-1}) differed significantly from both the lowest flow rate (1.9 mL min^{-1}; $p = 0.012$) and the 6.6 mL min^{-1} level ($p = 0.031$). Other pairwise comparisons among intermediate flow rates were not statistically significant.

For packing height, the Tukey HSD test showed that the 5 cm bed differed significantly from the 1 cm bed ($p = 0.003$) and the 2 cm bed ($p = 0.019$) for BOD reduction. The 4 cm bed also differed significantly from the 1 cm bed ($p = 0.022$).

Similar patterns were observed for COD reduction. Packing height produced multiple significant pairwise differences (1 cm vs 3 cm, 1 cm vs 4 cm, 1 cm vs 5 cm, 2 cm vs 5 cm, and 3 cm vs 5 cm; all $p < 0.05$), confirming

the strong influence of bed height. In contrast, no pairwise differences among flow rates reached statistical significance for COD at $\alpha = 0.05$, consistent with the marginally significant overall ANOVA result.

Table 4 Summary of significant Tukey HSD pairwise comparisons for BOD reduction ($\alpha = 0.05$)

Comparison	Mean difference	*p*-value	Significant
17.9 vs 1.9 mL min^{-1}	+24.44	0.012	Yes
17.9 vs 6.6 mL min^{-1}	+21.40	0.031	Yes
5 cm vs 1 cm	+42.64	0.003	Yes
5 cm vs 2 cm	+34.50	0.019	Yes
4 cm vs 1 cm	+33.92	0.022	Yes

Table 5 Summary of significant Tukey HSD pairwise comparisons for COD reduction ($\alpha = 0.05$)

Comparison	Mean difference	*p*-value	Significant
5 cm vs 1 cm	+43.50	< 0.001	Yes
5 cm vs 2 cm	+28.46	0.001	Yes
5 cm vs 3 cm	+23.74	0.004	Yes
4 cm vs 1 cm	+29.46	< 0.001	Yes
3 cm vs 1 cm	+19.76	0.018	Yes

The stronger statistical and practical impact of packing height aligns with findings by Zhao et al. (2023) and Chen et al. (2020) that bed depth is the dominant geometric variable governing contact time and biomass inventory in packed-bed bioreactors. Taller beds provide a larger inventory of immobilised *Pterocarpus mildbraedii* mushroom biomass and an extended contact path, enhancing both biosorption capacity and enzymatic activity (Liu et al., 2023; Menkiti et al., 2026). Although higher flow rates improve mass transfer, the accompanying reduction in hydraulic residence time partially offsets this benefit, resulting in a comparatively smaller net effect. These observations are consistent with previous continuous packed-bed studies on alginate-immobilised systems (Singh et al., 2022; John et al., 2025a).

Overall, the comparative analysis and post-hoc tests demonstrate that careful selection of both flow rate and packing height is essential for maximising AMBM performance, with packing height offering the greater leverage for process improvement in continuous treatment of abattoir wastewater. Furthermore, the favourable operating window of 17.9 mL min^{-1} and 5 cm packing height identified in the present one-factor-at-a-time (OFAT) study is fully consistent with the response-surface-methodology (RSM)-optimised region previously reported for the identical membrane system (Menkiti et al., 2026).

3.5. Practical implications for continuous reactor design (choice of operating window)

The experimental and statistical findings have direct implications for the design and operation of continuous packed-bed bioreactors employing AMBM. Within the ranges investigated, the highest BOD (96.4 %) and COD (95.6–96.0 %) reductions were consistently achieved at the combination of the highest flow rate (17.9 mL min^{-1}) and the tallest packing height (5 cm). This combination therefore defines a favourable operating window for laboratory-scale continuous treatment of abattoir wastewater.

From a reactor-design perspective, packing height emerges as the more influential variable. Increasing bed height from 1 cm to 5 cm produced larger absolute gains in removal efficiency than the corresponding increase in flow rate. Consequently, designers should prioritise adequate bed depth to maximise biomass inventory and contact path length, while ensuring that pressure drop remains within acceptable limits. Flow rate, although secondary in magnitude of effect, still significantly improved BOD reduction and can be used to enhance mass transfer once a sufficient packing height has been established. An operating strategy that maintains a relatively high packing height

(approximately 5 cm under the present geometry) together with a moderately high hydraulic loading (approaching 17.9 mL min^{-1}) therefore offers the best balance between treatment efficiency and practical throughput.
These guidelines can be scaled by preserving the hydraulic residence time and the ratio of bed volume to volumetric flow rate when moving from laboratory to pilot or industrial systems. Continuous monitoring of effluent BOD and COD, coupled with periodic assessment of pressure drop across the bed, is recommended to maintain performance within the identified operating window.

3.6 Comparison with other continuous immobilised fungal or alginate systems reported in the literature
The removal efficiencies attained with AMBM under optimised hydraulic conditions compare favourably with those reported for other continuous immobilised fungal and alginate-based systems treating high-strength organic wastewaters (Table 6).

Analysis of Table 6 shows that the maximum BOD (96.4 %) and COD (95.6–96.0 %) reductions achieved with AMBM at 17.9 mL min^{-1} and 5 cm packing height equal or exceed the upper limits reported for comparable continuous immobilised fungal and alginate systems. Liu et al. (2023) obtained COD removals of 80–92 % with alginate-immobilised fungal cells, while Karimi et al. (2023) recorded BOD and COD reductions in the range 85–94 % for fungal treatment of slaughterhouse wastewater. Singh et al. (2022) and Rahimi et al. (2022) reported approximately 90 % and 88–95 % organic-load removal, respectively. The present AMBM results therefore lie at the high end of the performance spectrum.

In the present study, AMBM reached 96.4 % BOD and 95.6–96.0 % COD reduction at 17.9 mL min^{-1} and 5 cm packing height—values that equal or exceed the upper end of the ranges cited above. Similar high efficiencies have been reported by the authors' previous continuous alginate-membrane investigations (John et al., 2025a; Menkiti et al., 2026; John M.C et al., 2025), confirming the robustness of the dual-mechanism (biosorption + enzymatic degradation) approach. The solvent-free porosity enhancement employed in AMBM fabrication further distinguishes the present system by eliminating residual solvent concerns that occasionally limit the environmental acceptability of earlier alginate matrices (Fernando et al., 2020; Gupta et al., 2022).
Collectively, the performance data and statistical evidence position AMBM as a competitive and scalable option for continuous treatment of abattoir wastewater, particularly when operated within the high-flow, high-packing-height window identified in this work. The competitive advantage of AMBM can be attributed to the dual mechanism of biosorption onto the porous alginate matrix and enzymatic degradation by the immobilised *Pterocarpus mildbraedii* mushroom biomass, combined with the solvent-free porosity-enhancement technique that maximises active surface area without residual solvent contamination (Fernando et al., 2020; Gupta et al., 2022). These findings are consistent with the authors' earlier continuous alginate-membrane studies, which also achieved > 95 % BOD and COD reductions under optimised hydraulic conditions (John et al., 2025a; Menkiti et al., 2026).

Overall, the comparative data in Table 6 reinforce the practical conclusion that AMBM, when operated within the high-flow, high-packing-height window identified in this work, constitutes a competitive and scalable technology for continuous treatment of abattoir wastewater.

Table 6. Comparison of BOD and COD removal efficiencies of AMBM with other continuous immobilised fungal or alginate systems

System / Biomass	Wastewater type	Operating mode	BOD reduction (%)	COD reduction (%)	Reference
AMBM (*Pterocarpus mildbraedii*)	Abattoir	Continuous packed-bed	96.4	95.6–96.0	Present study
Alginate-immobilised fungal cells	Industrial effluent	Continuous packed-bed	–	80–92	Liu et al. (2023)
Fungal bioremediation	Slaughterhouse	Continuous	85–94	85–94	Karimi et al. (2023)
Fungal-immobilised bioreactor	High-strength industrial	Continuous	–	≈ 90	Singh et al. (2022)
Continuous high-strength system	High organic-load wastewater	Continuous	88–95	88–95	Rahimi et al. (2022)
Alginate-based membrane (previous work)	Slaughterhouse	Continuous packed-bed	> 95	> 95	John et al. (2025a); Menkiti et al. (2026)

4. Conclusion

This study investigated the influence of two key hydraulic parameters—flow rate and packing height—on the simultaneous removal of organic load (BOD and COD) from real abattoir wastewater using an alginate-mushroom bioremediation membrane (AMBM) in a continuous packed-bed bioreactor.

The central thesis was that both parameters positively affect treatment performance, but that packing height exerts greater process leverage than flow rate within the ranges examined, and that an optimised combination of high flow rate and high packing height can deliver removal efficiencies exceeding 95 %.

The principal findings confirm this thesis. At 35 min contact time, BOD reduction increased from 73.5 % to 96.4 % and COD reduction from 73.6 % to 95.6 % as flow rate rose from 1.9 to 17.9 $\mathrm{mL\,min^{-1}}$. Increasing packing height from 1 cm to 5 cm produced even larger gains (BOD from 61.9 % to 96.0 %; COD from 54.2 % to 96.0 %). One-way ANOVA and Tukey HSD tests established that packing height was highly significant for both responses ($p \leq 0.002$), while flow rate significantly affected BOD reduction ($p = 0.012$). Maximum efficiencies were therefore obtained at the combination of 17.9 $\mathrm{mL\,min^{-1}}$ and 5 cm packing height.

These results demonstrate that the solvent-free, porogen-enhanced AMBM is an effective and competitive technology for continuous treatment of high-strength abattoir wastewater when hydraulic conditions are properly selected. The work provides clear, statistically validated design guidelines—prioritise adequate bed depth while maintaining moderately high hydraulic loading—and positions AMBM at the high end of performance reported for continuous immobilised fungal and alginate systems in the recent literature.

Future research should evaluate the long-term operational stability of AMBM under sustained continuous loading, assess performance at pilot and industrial scales, and explore hybrid alginate matrices that combine fungal and algal biomass to further broaden the spectrum of removable pollutants.

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