Optimization of Swine Farm Wastewater Treatment Using Mixed Microalgae: Statistical Modeling and Performance Evaluation via Response Surface Methodology

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Received: 6 November 2025, Revised: 12 December 2025, Accepted: 9 January 2026

Abstract
Swine farm wastewater contains high concentrations of organic matter and nutrients, requiring treatment approaches that are both effective and economically feasible. This study evaluated the performance of mixed indigenous microalgae for the removal of chemical oxygen demand (COD) and total phosphorus (TP) from swine farm wastewater and optimized the effects of initial pH and algal concentration using Response Surface Methodology (RSM) with a Central Composite Design (CCD). Thirteen experimental runs were conducted under outdoor conditions to reflect field applicability. COD and TP removal efficiencies ranged from 74.30–83.85% and 75.17–83.58%, respectively. Statistical analysis showed that pH significantly influenced both COD and TP removal, whereas algal concentration exerted a stronger effect on COD removal but a comparatively weak influence on TP, in agreement with ANOVA results. The quadratic model demonstrated strong predictive performance for COD (R^2 = 0.9879; predicted R^2 = 0.9245), while the TP model displayed limited predictive capability, suggesting that additional unmeasured factors may govern phosphorus reduction. Numerical optimization identified pH 7.69 and algal concentration (A_{600}) 2.072 as the optimal conditions, yielding predicted removals of 78.21% COD and 82.00% TP. Although TP levels approached regulatory thresholds, COD remained above discharge limits, highlighting the need for a polishing step prior to release. Overall, the results demonstrate that mixed indigenous microalgae offer a robust, low-cost treatment strategy for swine wastewater and provide optimized operational conditions to support practical implementation.
Keywords: Mixed microalgae, Swine farm wastewater, COD removal, Phosphorus removal, Response Surface Methodology (RSM)

1. Introduction

Swine farming is a vital agricultural sector but generates substantial volumes of wastewater laden with high concentrations of organic matter, nitrogen, and phosphorus. While anaerobic digestion is commonly employed for primary treatment, the discharge of nutrient-rich effluents remains a critical environmental challenge, driving eutrophication and ecological imbalance in receiving water bodies [1,2]. Consequently, developing cost-effective and sustainable post-treatment technologies to mitigate these pollutant loads is an urgent priority.

Microalgae-based phycoremediation has emerged as a promising approach due to its ability to assimilate nitrogen and phosphorus while supporting aerobic degradation through oxygen release during photosynthesis [3–5]. In addition to nutrient removal, microalgal biomass produced during treatment offers opportunities for resource recovery, contributing to circular bioeconomy strategies [6,7]. Mixed indigenous microalgal consortia, in particular, have gained attention for their higher robustness in real wastewater, stemming from functional diversity and synergistic interactions with bacteria [8–10]. These consortia also exhibit greater tolerance to fluctuating wastewater characteristics compared with monocultures, making them attractive for low-cost outdoor systems.

Despite these advantages, studies focusing on optimization of mixed microalgal consortia, especially under high-strength swine wastewater, remain limited. Most previous investigations have emphasized monoculture systems or examined single operational variables in isolation, which restricts understanding of how key factors interact to influence treatment outcomes. Environmental and operational factors such as pH, light intensity, temperature and initial inoculum concentration strongly affect microalgal activity but few studies have systematically evaluated the combined effects of pH and initial biomass density, two parameters that directly govern nutrient speciation, carbon availability, photosynthetic efficiency and algal–bacterial interactions [11]. Furthermore, most Response Surface Methodology (RSM) studies predominantly focus on monocultures, leaving mixed indigenous consortia underexplored in terms of predictive modeling and optimization [12]. This gap limits the ability to design field-deployable systems that rely on naturally occurring microalgal communities.

To address this gap, this study investigates the interactive effects of initial pH and mixed-microalgae concentration on the treatment performance of swine farm wastewater using a Central Composite Design (CCD) under RSM. The objectives were to (i) quantify how these two key operational parameters influence COD and total phosphorus (TP) removal, and (ii) determine the optimal conditions for maximizing removal efficiencies. By explicitly examining mixed indigenous consortia rather than laboratory monocultures, this study provides new insight into the operational tuning of robust, low-cost and field-relevant phycoremediation systems.

2. Materials and Methods

2.1 Sampling of Swine Farm Wastewater

Swine farm wastewater was collected from a small-scale pig farm (80–100 pigs) located in Ubon Ratchathani Province, Thailand. The raw wastewater was screened through nylon mesh to remove large solids and diluted tenfold (1:10) with natural pond water to reduce its organic load before use. This dilution ratio was selected based on preliminary observations and previous studies showing that undiluted swine wastewater may exert strong ammonia toxicity and light attenuation which inhibit microalgal growth; thus, the 1:10 dilution reflects a practical condition commonly used in low-cost open-pond systems. Initial physicochemical characteristics of the wastewater were determined following the Standard Methods for the Examination of Water and Wastewater [13].

2.2. Preparation of Mixed Microalgae Inoculum

A consortium of indigenous mixed microalgae was isolated from a natural pond within the Ubon Ratchathani University campus. The culture was acclimated and pre-cultivated in diluted swine wastewater (1:10) under natural sunlight and ambient temperature (28–32°C). This study employed a naturally occurring consortium without targeted species selection, consistent with field-oriented phycoremediation approaches where functional performance is prioritized.

Continuous aeration was supplied at 2 L/min to maintain homogeneous mixing and prevent sedimentation while optical density at 600 nm (A_{600}) was used to estimate algal concentration. Prior to experimentation, inoculum suspensions were adjusted to the designated A_{600} levels corresponding to the experimental CCD design.

Light intensity was measured using a digital lux meter, yielding daytime values of approximately 8,000–10,000 lux under natural 12 h light: 12 h dark conditions. Measurements were conducted at the reactor surface to reflect the actual irradiance received by the culture.

2.3 Experimental Design using Response Surface Methodology

A Central Composite Design (CCD) under Response Surface Methodology (RSM) was applied to evaluate the effects of two independent variables: initial pH of the wastewater (6, 7 and 8) and mixed microalgae concentration (A_{600} = 1.000, 2.000 and 3.000). A total of 13 experimental runs, including five center points, were performed to estimate experimental variability.

Reactors (10.5 L, diameter 30.5 cm, height 28 cm) were operated under natural sunlight and room temperature. The experiments were run for a fixed retention time of 10 days which corresponded to the period where stable nutrient removal was observed under preliminary trials. Reactors were loosely covered to minimize contamination while allowing gas exchange. Daily evaporation loss (typically <3%) was corrected using distilled water to maintain constant working volume. Continuous aeration ensured mixing and adequate oxygen transfer.

COD and TP were selected as the main response variables for assessing treatment efficiency. Removal efficiency (%R.E.) for each parameter was calculated using Eq. (1):

$$\%R.E.=\frac{(C_0-C)}{C_0}X100 \quad (1)$$

where C_0 and C represent the initial and final concentrations (mg/L) of the parameter after 10 days of treatment, respectively.

2.4 Analytical Methods

Samples collected on Day 0 and Day 10 were analyzed for COD and TP following Standard Methods [13]. COD was measured using the closed reflux titrimetric method and TP was analyzed using the ascorbic acid method after persulfate digestion. Algal growth (A_{600}) and pH were monitored using a spectrophotometer (Shimadzu Corporation, Japan) and calibrated pH meter. All analytical measurements were performed in triplicate to ensure reproducibility.

2.5 Statistical Analysis

Experimental data were analyzed using Design-Expert software (Version 13, Stat-Ease Inc., USA) to fit a quadratic model. Model adequacy was assessed using R^2, adjusted R^2, predicted R^2, lack-of-fit and ANOVA ($p < 0.05$). Residual plots (normal probability and residuals vs. predicted) were examined to verify regression assumptions.

The experimental data were fitted to a quadratic equation (Eqs. (2)) of the form:

$$Y=\beta_0+\beta_1A+\beta_2B+\beta_{12}AB+\beta_{11}A^2+\beta_{22}B^2 \quad (2)$$

where Y represents removal efficiency (%), A is the actual initial pH and B is the actual initial algal concentration. β_0 is the intercept coefficient, β_1 and β_2 are the linear coefficients, β_{12} is the interaction coefficient, and β_{11} and β_{22} are the quadratic coefficients. All regression coefficients therefore correspond to actual experimental values rather than coded factors. Model performance was further evaluated using adequate precision and the coefficient of variation (CV%).

Three-dimensional response surface plots were prepared to visualize interaction effects and numerical optimization was performed using the desirability function.

3. Results and Discussion

3.1 Characteristics of swine farm wastewater

The raw swine farm wastewater exhibited an average pH of 7.79 ± 0.00, COD of 6600 ± 35.5 mg/L, total suspended solids (TSS) of 226.67 ± 16.49 mg/L and total phosphorus (TP) of 78.83 ± 0.09 mg/L. These values exceeded the discharge limits stipulated by Thailand’s effluent standards for livestock farms, indicating the high organic and nutrient strength typical of swine wastewater. The high COD and nutrient content make this wastewater suitable for algal cultivation, as it provides sufficient carbon, nitrogen and phosphorus for photosynthetic growth and nutrient assimilation [14].

During the treatment period, the mixed microalgae developed into a visibly dense green suspension. Within ten days, the algal population increased approximately 6.7–8.6 times compared to the initial concentration. This rapid proliferation coincided with a marked decline in both COD and TP, suggesting that the microalgae played an active role in nutrient uptake and organic matter removal. The observed reductions reflect complementary activities between microalgae and native bacteria, in which photosynthetically supplied oxygen enhances aerobic oxidation while bacterial CO_2 supports algal carbon fixation [10]. This balanced exchange promoted stable and effective pollutant removal.

3.2 COD and TP Removal by Mixed Microalgae

Across all 13 CCD experimental runs, both COD and TP removal efficiencies exhibited consistent trends with increasing treatment time and were significantly influenced by initial pH, while algal concentration exerted a strong effect on COD but a weaker influence on TP, in accordance with the ANOVA results. COD removal ranged from 74.30 ± 0.13% to 83.85 ± 0.08%, while TP removal ranged from 75.17 ± 0.10% to 83.58 ± 1.18%. Standard errors were used to express variability, reflecting analytical replicates within each biological run. The increase in TSS observed was attributed to algal proliferation rather than incomplete sedimentation.

The removal patterns indicated that a moderately alkaline pH and sufficient algal concentration enhanced nutrient assimilation and organic matter degradation. pH 7-8 provided favorable nutrient speciation and enzymatic activity while lower pH suppressed microalgal metabolism, reducing overall removal efficiency. [15,16] These findings align with earlier reports showing improved nutrient removal at slightly alkaline pH in livestock wastewater [17,18].

TP removal was governed primarily by biological uptake, consistent with the growth trends observed. Under moderately alkaline conditions, pH-induced phosphate precipitation may also occur, although this mechanism could not be confirmed without direct measurement of precipitated species. Similar coupled assimilation-precipitation behavior has been reported in nutrient-rich wastewater systems [19-21].

3.3 Statistical Model Fitting and Validation

The experimental data obtained from the 13 CCD runs were analyzed using Design-Expert to evaluate the effects of initial pH (factor A) and mixed microalgae concentration (factor B) on COD and TP removal. Four model types (linear, two-factor interaction (2FI), quadratic and cubic) were compared. The cubic model produced the highest R^2 value but showed an “Aliased” message, indicating insufficient experimental runs. Accordingly, the quadratic model was selected as the most appropriate compromise between accuracy and complexity.

For COD removal (Table 1), the quadratic model was highly significant ($p < 0.0114$) with $R^2 = 0.9879$, adjusted $R^2 = 0.9506$ and predicted $R^2 = 0.9245$, indicating an excellent agreement between predicted and experimental data. For TP removal (Table 2), the same model provided a reasonable fit ($R^2 = 0.7722$) with a non-significant lack-of-fit ($p = 0.1085$). However, the predicted R^2 for TP (0.0380) was markedly lower than the adjusted R^2, demonstrating limited predictive capacity and suggesting that TP removal was affected by unmeasured factors such as cation composition or pH drift during cultivation.

Table 1 Summary of statistical parameters for model fitting of COD removal efficiency under different model types.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001	0.1201	0.8203	0.6958	
2FI	0.0761	0.1785	0.8619	0.7167	
Quadratic	0.0114	0.8310	0.9506	0.9245	Suggested
Cubic	0.8584	0.4876	0.9349	0.5645	Aliased

Table 2 Summary of statistical parameters for model fitting of TP removal efficiency under different model types.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0361	0.0435	0.3825	0.0961	Suggested
2FI	0.3139	0.0410	0.3909	-0.2405	
Quadratic	0.0257	0.1419	0.7249	0.0380	Suggested
Cubic	0.5408	0.0599	0.6988	-8.2356	Aliased

Analysis of variance (ANOVA), as shown in Tables 3 and 4, indicated that both initial pH and algal concentration significantly influenced COD removal ($p < 0.05$). The interaction term (AB) and quadratic effects (A^2, B^2) were also statistically significant. In contrast, TP removal was driven primarily by pH and its quadratic term, while algal concentration and the AB interaction were not significant, confirming weaker or negligible effects of biomass density on phosphorus reduction. The regression models derived from the RSM analysis are presented as Eqs. (3) and (4).

$$\%R.E.\ COD = 158.156-21.395A+3.615B-1.03AB+1.574A^2+0.069B^2 \quad (3)$$

$$\%R.E.\ TP = -47.605+32.548A+7.363B-0.925AB-2.05A^2 \quad (4)$$

Model diagnostics (results not shown here) confirmed that the residuals followed a normal distribution and exhibited no systematic trends, supporting model adequacy. Residual versus predicted plots showed homogenous variance, supporting the validity of the quadratic model for both pollutants despite the lower predictive performance of the TP model.

Despite the acceptable performance of the TP model in terms of adjusted R^2 and lack-of-fit, its markedly low predicted R^2 indicates that phosphorus removal was influenced by additional mechanisms not fully captured by the two-factor CCD design. TP dynamics in wastewater-microalgae systems are strongly affected by physicochemical conditions, especially the availability of multivalent cations such as Ca^{2+}, Mg^{2+} and Fe^{3+}, which promote the formation of metal–phosphate precipitates and reduce soluble TP independently of algal uptake [21,22]. Moreover, pH drift during outdoor cultivation alters phosphate speciation and shifts equilibrium toward less soluble forms under alkaline conditions, thereby introducing variability across runs [21]. Microbial interactions may also contribute, as polyphosphate-accumulating bacteria can intermittently store or release phosphorus depending on redox status and carbon availability [22,23]. These unmeasured and dynamic processes likely produced heterogeneity in TP removal that reduced the predictive capability of the quadratic model, suggesting that future optimization studies should incorporate chemical speciation, cation concentrations and pH profiles to improve the accuracy of TP modeling.

Table 3 Analysis of variance (ANOVA) results for the quadratic model of COD removal

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	89.22	5	17.84	47.18	< 0.0001	significant
A-pH	12.04	1	12.04	31.83	0.0008	
B-Algae conc.	66.07	1	66.07	174.67	< 0.0001	
AB	4.24	1	4.24	11.22	0.0123	
A^2	5.97	1	5.97	15.79	0.0054	
B^2	0.0033	1	0.0033	0.0086	0.9287	
Residual	2.65	7	0.3783			
Lack of Fit	0.4739	3	0.1580	0.2907	0.8310	not significant
Pure Error	2.17	4	0.5435			
Cor Total	91.87	12				

Table 4 Analysis of variance (ANOVA) results for the quadratic model of TP removal

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	45.77	4	11.44	6.78	0.0110	significant
A-pH	24.04	1	24.04	14.24	0.0054	
B-Algae conc.	4.73	1	4.73	2.81	0.1325	
AB	3.42	1	3.42	2.03	0.1923	
A^2	13.57	1	13.57	8.04	0.0220	
Residual	13.50	8	1.69			
Lack of Fit	10.74	4	2.68	3.89	0.1085	not significant
Pure Error	2.76	4	0.6909			
Cor Total	59.27	12				

3.4 Response Surface Analysis and Optimization

Three-dimensional response surface plots illustrated the interactive effects of pH and algal concentration on pollutant removal (Figure 1 for COD and Figure 2 for TP). For COD, removal efficiency increased toward slightly alkaline conditions (pH 7.5-8.0) and peaked at moderate algal densities. Beyond this level, efficiency slightly decreased, likely due to self-shading and reduced light penetration, which limit photosynthetic oxygen production. In contrast, TP removal peaked at mid-range pH (7.0-7.5) and moderate algal concentrations (A_{600} = 2.000), where nutrient availability and algal uptake were well balanced.

Numerical optimization using the desirability function identified the optimal operating conditions at pH 7.69 and algal concentration (A_{600}) 2.072, giving predicted removal efficiencies of 78.21 % for COD and 82.00 % for TP. These predictions fall within the observed experimental ranges but do not represent the maximum achieved values. This discrepancy occurs because desirability-based optimization identifies a balanced multi-response solution rather than maximizing a single pollutant removal.

Although no experimental run was conducted exactly at the optimized point, the close agreement between predicted values and nearby experimental data supports the reliability of the model for practical guidance.

To assess practical relevance, final effluent concentrations under optimal conditions were compared with Thai livestock effluent standards. While TP approached permissible limits, COD remained above national discharge thresholds, indicating that microalgal treatment alone is insufficient for full compliance and requires a secondary polishing step.

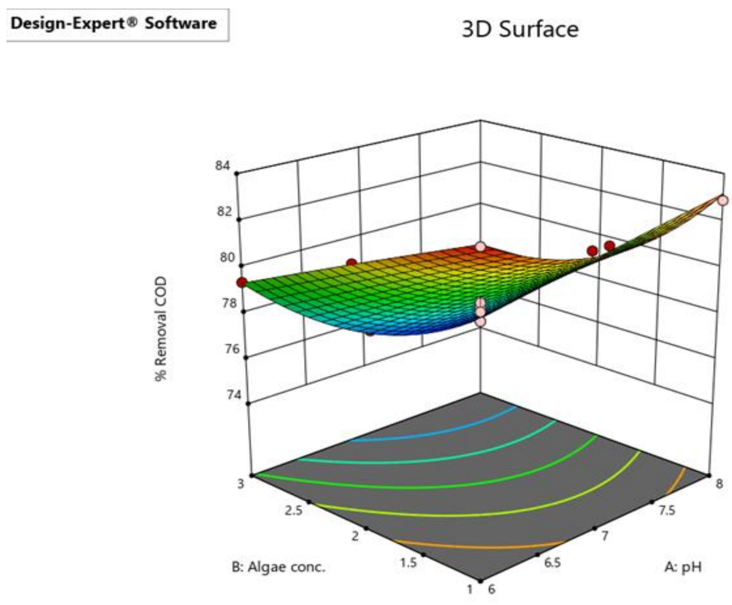

Figure 1 Response surface plots illustrating the interaction effects of initial pH and mixed-microalgae concentration on COD removal efficiency

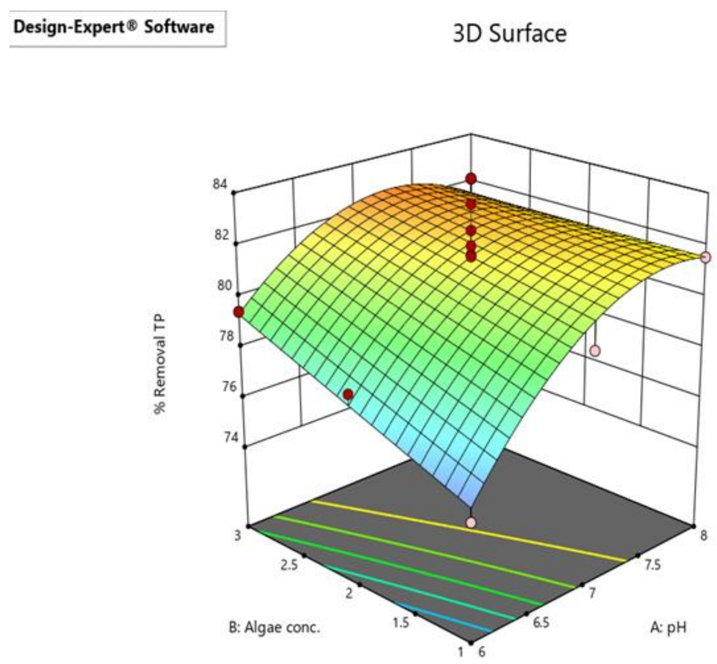

Figure 2 Response surface plots illustrating the interaction effects of initial pH and mixed-microalgae concentration on TP removal efficiency

4. Conclusions

This study demonstrated that mixed indigenous microalgae can effectively reduce COD and TP in swine farm wastewater under outdoor conditions. Initial pH and algal concentration were identified as key variables influencing removal performance and the RSM-CCD model provided reliable predictions, particularly for COD. Optimal conditions (pH 7.69; A_{600} = 2.072) yielded predicted removal efficiencies of 78.21% COD and 82.00% TP.

While the optimized TP removal approached national effluent thresholds, COD remained above regulatory limits, indicating that microalgal treatment alone is insufficient for complete compliance and will require a secondary polishing step.

The findings highlight the practical advantages of using mixed indigenous microalgae, including resilience, adaptability to outdoor conditions and low operational cost. In addition, this work contributes new insight by optimizing a naturally occurring microalgal consortium under outdoor, field-relevant conditions using actual-variable RSM rather than controlled laboratory monocultures. This approach provides an operational window that is directly applicable to decentralized livestock wastewater treatment systems and supports future scale-up.

The limited predictive performance of the TP model indicates the influence of additional chemical and microbial factors that were not included in the current design. Future work should incorporate these factors and evaluate time-resolved nutrient removal behavior and continuous metabolic indicators (e.g., dissolved oxygen profiles, pH drift) to strengthen model robustness and deepen mechanistic understanding.

5. Acknowledgment

The authors gratefully acknowledge the Department of Chemical and Environmental Engineering, Faculty of Engineering, Ubon Ratchathani University, for providing the necessary chemicals, laboratory space, and analytical instruments used for this research work.

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