Iranian Reasearch Organization for Science and Technology (IROST)

Advances in Environmental Technology 12(3) 2026, 274-290.

Advances in Environmental Technology

Journal home page: https://aet.irost.ir

Optimization of fermented rice noodle wastewater treatment using effective microorganisms (EM)

Tiammanee Rattanaweerapan, Karnika Ratanapongleka*, Supatpong Mattaraj, Wipada Dechapanya, Sompop Sanongraj

Department of Chemical and Environmental Engineering, Faculty of Engineering, Ubon Ratchathani University, 34190, Ubonratchathani, Thailand.

ARTICLE INFO

Document Type:
Research Paper

Article history:
Received 19 April 2025
Received in revised form
27 April 2026
Accepted 02 May 2026

Keywords:
Wastewater treatment
Effective Microorganisms (EM)
Fermented rice noodles factory
Response Surface Methodology (RSM)
Chemical Oxygen Demand (COD)

ABSTRACT

The treatment of high-strength wastewater generated from fermented rice noodle production poses significant environmental challenges due to its elevated organic load, acidity, and nitrogen content. This study investigated the optimization of wastewater treatment using Effective Microorganisms (EM), focusing on the effects of initial wastewater pH (6–8) and EM dosage (1–10% v/v) on Chemical Oxygen Demand (COD) and Total Kjeldahl Nitrogen (TKN) removal efficiency. A Central Composite Design (CCD) within the framework of Response Surface Methodology (RSM) was employed to model and analyze the interactive effects of these operating parameters. The results demonstrated that near-neutral pH (6.9) and a low EM dosage (1.2% v/v) yielded the highest COD removal efficiency (80.21%), whereas an alkaline pH (8.0) with a low EM dosage (1% v/v) resulted in the maximum TKN removal efficiency (75.18%). Statistical analysis revealed that EM dosage significantly impacted COD removal ($p < 0.0001$), while initial pH had a more pronounced effect on TKN removal ($p < 0.0001$). The quadratic regression model exhibited strong predictive performance for both COD ($R^2 = 0.9827$) and TKN ($R^2 = 0.9326$) removal. The findings further indicate that COD removal is predominantly governed by biologically regulated microbial metabolism, whereas TKN removal is controlled mainly by pH-driven physicochemical pathways. Overall, the EM application optimized through RSM represents a promising and sustainable strategy for enhancing the simultaneous removal of organic matter and nitrogen from wastewater generated by the fermented rice noodle industry.

---

*Corresponding author Tel.: +81 5447559
E-mail: karnika.r@ubu.ac.th

DOI: 10.22104/aet.2026.7571.2125

1. Introduction

Fermented rice noodles, known as Khanom Jeen in Thailand, are a widely consumed food product produced through a fermentation process. This process generates significant amounts of wastewater characterized by high concentrations of starch residues, suspended solids, organic compounds, and nitrogenous matter [1]. The discharge of untreated or inadequately treated wastewater into water bodies poses a substantial threat to environmental quality. High organic loads in the effluent lead to oxygen depletion, disrupting aquatic life and causing eutrophication, which further degrades water quality and impacts ecosystems [2]. Furthermore, the presence of nitrogen compounds, such as ammonia and organic nitrogen, contributes significantly to nutrient pollution, leading to excessive algal blooms, oxygen depletion, and the creation of dead zones in aquatic ecosystems [3, 4]. Therefore, developing effective wastewater treatment methods is crucial to minimizing environmental harm and supporting sustainable food production practices.

Recent advances in environmental remediation have increasingly emphasized that treatment efficiency is governed not only by the presence of reactive agents or microorganisms, but also by the regulation of their structural, physicochemical, and behavioral characteristics under controlled operational conditions. Studies on engineered polymeric and nanocomposite systems have demonstrated that pollutant removal performance is strongly influenced by regulatory mechanisms such as pH-dependent surface charge, electrochemical conductivity and thermodynamic stability, which collectively dictate adsorption kinetics, and reaction pathways [5-7]. Similar regulatory principles have been applied in the design of electrochemical sensors and controlled-release systems, where precise adjustment of environmental parameters determines functional performance and long-term stability [8, 9]. These findings highlight a broader conceptual framework in which effective environmental treatment systems, whether physicochemical or biological, require fine regulation of operating conditions to optimize system behavior and efficiency. Wastewater treatment methods, such as activated sludge processes, sequencing batch reactors, and membrane-based systems, have been widely applied to various high-strength industrial wastewater [10-12]. However, these approaches often require significant energy inputs, produce substantial sludge volumes, and involve high operational costs [13-15]. In addition, the specific characteristics of fermented rice noodle wastewater (such as low pH, high starch content, and fluctuating organic composition) pose challenges for conventional biological systems, which may exhibit limited biodegradation efficiency and process instability. Previous studies have reported COD removal efficiencies of only 50–70% when treating similar food-processing wastewaters using conventional activated sludge systems [16, 17], underscoring the need for alternative treatment strategies.

In response to these limitations, increasing attention has been directed toward biological treatment methods employing mixed microbial consortia [18-21]. Among these approaches, the application of Effective Microorganisms (EM) has emerged as a promising and sustainable option. EM consists of a complex consortium of beneficial microorganisms, including lactic acid bacteria (*Lactobacillus plantarum, L. casei, Streptococcus lactis*), photosynthetic bacteria (*Rhodopseudomonas palustris, Rhodobacter sphaeroides*), yeasts (*Saccharomyces cerevisiae, Candida utilis*), fermentative fungi (*Aspergillus oryzae, Mucor hiemalis*), and actinomycetes (*Streptomyces albus*) [22]. These microorganisms work synergistically through competitive and cooperative mechanisms to accelerate the biodegradation of organic pollutants, stabilize pH, reduce pathogenic microbial loads, and improve overall water quality parameters [23]. EM-based treatment has been successfully applied to municipal wastewater [24, 25], livestock effluent [26], and various industrial wastewater [27-29], offering advantages such as reduced energy consumption, lower chemical input, and decreased sludge production [30-33].

Importantly, EM does not function as a simple additive but rather as a dynamically regulated biological system. The performance of EM consortia is highly sensitive to environmental regulation, particularly wastewater pH and inoculum dosage, which directly influence microbial growth kinetics, enzyme activity, membrane transport processes, and interspecies interactions [30-33]. Wastewater pH plays a critical regulatory role by controlling enzyme conformation, proton motive force, and substrate transport across microbial membranes; deviations from optimal pH ranges can induce enzymatic deactivation and metabolic stress [34-36]. Similarly, EM dosage governs microbial density and substrate availability. While insufficient inoculum may limit biodegradation rates, excessive EM concentrations can result in metabolic burden, intensified competition for oxygen and nutrients, and inhibitory interactions within the consortium, ultimately reducing treatment efficiency [30, 33, 37].

Despite the extensive application of EM in various wastewater treatment contexts, limited research has systematically investigated the combined regulatory effects of initial wastewater pH and EM dosage on the simultaneous removal of organic matter and nitrogen. This knowledge gap is particularly evident for fermented rice noodle wastewater, which exhibits high organic loading, acidic conditions, and variable nitrogen composition. Moreover, the distinct physiological and metabolic requirements governing carbon degradation and nitrogen transformation within EM consortia remain insufficiently elucidated for this specific wastewater type.

Therefore, this study aims to investigate the effects of initial wastewater pH and EM dosage on the removal efficiencies of COD and TKN from fermented rice noodle wastewater. RSM with a CCD is employed to evaluate the individual and interactive effects of these regulatory parameters and determine optimal operating conditions to maximize treatment performance. The findings of this study provide mechanistic insight into the regulation of EM-based wastewater treatment systems and offer a practical foundation for developing cost-effective and sustainable treatment strategies tailored to the fermented food processing industry.

2. Materials and methods

2.1. Wastewater Collection and Characterization

Wastewater samples were collected from the wastewater collection pond of a fermented rice noodle factory in Ubon Ratchathani, Thailand. The samples were analyzed for pH, Suspended Solids (SS), Total Kjeldahl Nitrogen, Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand using standard analytical methods. The characteristics of wastewater are shown in Table 1. The wastewater had a slightly acidic pH of 4.70 ± 0.20, a milky-white appearance, and a strong odor due to the fermentation process. It contained high organic pollutant levels, with a COD of 5600 ± 11.13 mg/L and a BOD of 560 ± 17.33 mg/L. These values indicate that although the organic load is high, only a small fraction is readily biodegradable. This is reflected by the low BOD/COD ratio of approximately 0.1, which is characteristic of fermented starch-based wastewater where complex or partially recalcitrant organics are commonly present. The concentrations of suspended solids (262 ± 2.17 mg/L) and TKN (180 ± 1.44 mg/L) further contributed to the wastewater's turbidity and nitrogen content.

Table 1. Characteristics of wastewater from a fermented rice noodle factory.

Parameter	Analysis method	Average value
pH	pH meter	4.70+0.20
SS (mg/L)	Gravimetric Method	262+2.17
TKN (mg/L)	Kjeldahl Method	180+1.44
BOD (mg/L)	Azide Modification	560+17.33
COD (mg/L)	Closed Reflux Titrimetric Method	5600+11.13

Although the low BOD/COD ratio typically indicates limited immediate biodegradability under conventional biological treatment, fermented rice noodle wastewater can still undergo effective biological transformation when supplemented with a diverse microbial consortium such as Effective Microorganisms (EM). Fermentative, acidogenic, and facultative microbial groups within EM are capable of initiating the breakdown of complex organics that are resistant to rapid degradation. This characteristic supports the suitability of EM-assisted treatment for wastewater of this nature despite its inherently low biodegradability index.

2.2. Wastewater Treatment Using EM

The study was conducted in a 20-liter, opaque white, cylindrical plastic reactor. Wastewater collected from a fermented rice noodle factory was first adjusted to the desired pH levels using NaOH before being transferred into the reactor. Effective Microorganisms purchased from Kasama, Thailand, were then added to the system to ensure a total working volume of 10 liters in the reactor.

Intermittent aeration was supplied through a porous sparger at a rate of 3 L/min to maintain oxygen availability for microbial activity. A controlled aeration cycle of 1 hour aeration followed by 1 hour without aeration was applied continuously throughout the 7-day operational period. This cycle was selected to create frequent alternating aerobic and anoxic phases. Aerated periods promote the aerobic degradation of organic matter, while non-aerated phases potentially enable nitrogen transformation. This intermittent approach also prevents uncontrolled ammonia losses, often caused by excessive continuous aeration. Additionally, continuous mixing was maintained to ensure homogeneous conditions within the reactor.

Samples were periodically collected for pH and COD analysis following the Standard Methods for the Examination of Water and Wastewater (APHA), while TKN measurements were performed using the Kjeldahl method (APHA 4500-Norg B) [38].

The factors investigated in this study included the initial pH of the wastewater (6, 7, and 8) and the EM dosage (1.0%, 5.5%, and 10% v/v). RSM was applied to determine the optimal conditions by analyzing interactions between these parameters. The experimental design followed a CCD approach, as shown in Table 2. Each factor was tested at three levels (low, medium, and high); a total of 13 runs were conducted, with triplicate trials performed to ensure statistical reliability.

Table 2. Factors and levels used in the experiment.

Factors	Levels		
	Low	Medium	High
Initial wastewater pH	6	7	8
EM dosage (%v/v)	1	5.5	10

2.3. Statistical Data Analysis of Experimental Results

The experimental results for both COD and TKN removal were analyzed using RSM with CCD implemented in Design Expert 13 software (Trial Version, Stat-Ease Inc., Minneapolis, USA). The design consisted of 13 experimental runs, including five center points to estimate pure error and assess the reproducibility of the method. COD and TKN removal efficiencies were selected as the response variables to evaluate the treatment performance.

The experimental data for both responses were fitted to a second-order polynomial model to establish the relationship between the response variable (Y) and the independent variables (A: initial wastewater pH and B: EM dosage), as shown in Eq. (1) for COD and a similar equation for TKN.

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A^2 + \beta_{22} B^2 \tag{1}$$

where Y represents COD removal efficiency (%), 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. The statistical significance of the model and individual parameters for both responses was evaluated using Analysis of Variance (ANOVA) at a 95% confidence level ($p < 0.05$). The quality of the fitted model was assessed through the following multiple criteria:

1. Coefficient of determination (R^2) to measure the proportion of variance explained by the model
2. Adjusted R^2 to account for the number of predictors in the model
3. Predicted R^2 to evaluate model predictive capability
4. Coefficient of variation (C.V.%) to express the model precision and reproducibility
5. Adequate precision to measure the signal-to-noise ratio

The adequacy of the model was verified by analyzing the residual plots. The normality of the residuals was examined using normal probability plots, while homogeneity of variance was assessed by plotting residuals against predicted values. These diagnostic plots were used to confirm that the assumptions for regression analysis were met for both COD and TKN removal.

Three-dimensional response surface plots were generated to visualize the interactive effects of the initial wastewater pH and EM dosage on COD and TKN removal efficiency. These plots helped identify the optimal ranges for each factor to maximize treatment performance for both pollutants.

Finally, numerical optimization was performed using the desirability function approach, a multi-criteria decision-making tool used for identifying the optimal combination of factors that maximizes the desired response for both COD and TKN removal efficiencies. The predicted optimal conditions were subsequently validated through confirmatory experiments conducted in triplicate to verify the accuracy and reliability of the model.

3. Results and discussion

3.1. Experimental Data and Model Development

The study employed RSM with a CCD to evaluate the treatment of fermented rice noodle wastewater using EM. The investigation focused on the impact of initial wastewater pH (6, 7, and 8) and EM dosage (1% v/v, 5.5% v/v, and 10% v/v) on COD and TKN removal efficiencies. The CCD design consisted of 13 experimental runs, allowing assessment of both individual and interactive effects of these variables.

3.1.1. COD Removal Efficiency

The COD removal efficiencies obtained from the experimental runs ranged from 68.47% to 80.21%, as shown in Table 3. The highest COD removal (80.21 ± 0.44%) was achieved at an initial wastewater pH of 7 with an EM dosage of 1% v/v. This result indicates that organic matter degradation by the EM consortium is strongly favored under near-neutral pH conditions combined with a relatively low microbial inoculum. Near-neutral pH conditions are widely recognized as optimal for the metabolic activity of fermentative and heterotrophic microorganisms commonly present in EM, particularly lactic acid bacteria and yeasts. Under these conditions, key enzymes involved in carbohydrate hydrolysis and glycolytic pathways exhibit maximum stability and catalytic efficiency while membrane transport processes and proton motive force are maintained at favorable levels [35, 39]. As a result, substrate uptake and organic matter conversion proceed efficiently, leading to enhanced COD removal.

In contrast, COD removal efficiency declined when the initial pH was increased toward alkaline conditions (pH 8). Alkaline environments can adversely affect enzyme conformation and reduce the stability of pH-sensitive metabolic pathways, thereby impairing microbial activity. Elevated pH may also disrupt proton gradients across microbial membranes, which are essential for energy conservation and substrate transport in heterotrophic microorganisms [36, 40]. These physiological constraints explain the observed decrease in COD removal efficiency at higher pH levels, despite the continued presence of active microbial populations.

A notable finding of this study is that the highest COD removal efficiency occurred at the lowest EM dosage tested (1% v/v), whereas intermediate (5.5% v/v) and high (10% v/v) EM dosages resulted in reduced performance. This behavior reflects a non-linear relationship between microbial inoculum concentration and treatment efficiency. At low EM dosage, microbial density is sufficient to initiate effective biodegradation without imposing excessive metabolic or environmental stress on the system. Under these conditions, substrate availability per cell remains relatively high, supporting efficient microbial growth and organic matter utilization.

Conversely, increasing the EM dosage beyond this optimal level likely introduced several inhibitory effects. High microbial density can intensify competition for readily biodegradable substrates and dissolved oxygen, particularly under the intermittent aeration regime employed in this study. Such competition can reduce the specific substrate utilization rate and shift microbial metabolism toward maintenance energy requirements rather than biomass growth and pollutant degradation [41]. In addition, excessive inoculum levels may exacerbate intra-consortium competition among microbial groups within EM, including lactic acid bacteria, yeasts, and photosynthetic bacteria, leading to metabolic uncoupling and reduced overall system efficiency.

These effects are consistent with microbial kinetic principles, where substrate-limited conditions at high biomass concentrations result in diminishing returns in treatment performance. Similar observations have been reported in bioaugmentation studies where excessive inoculation failed to improve and, in some cases, reduced organic matter removal efficiency due to oxygen limitation and metabolic burden [41]. The non-linear influence of EM dosage on COD removal was effectively captured by the quadratic regression model, as reflected by the statistically significant model terms and high coefficient of determination ($R^2 = 0.9827$).

The quadratic regression equation describing COD removal (Eq. (2)) accurately represented the experimental data, with close agreement between predicted and observed values across all runs (Table 3). The strong predictive capability of the model confirms that COD removal in this system is governed by a balance between microbial activity, enzyme stability, and substrate availability, all of which are regulated by initial pH and EM dosage. These findings highlight the importance of optimizing microbial inoculum levels rather than assuming that higher EM concentrations necessarily lead to improved treatment performance.

$$Y_1 = 19.51252 + 18.25851A - 1.17488B + 0.053333AB - 1.34966A^2 - 0.034304B^2 \quad (2)$$

where Y_1 represents COD removal efficiency, A denotes initial wastewater pH, and B represents EM dosage (%v/v).

3.1.2. TKN Removal Efficiency

The experimental results for TKN removal are summarized in Table 4, with removal efficiencies ranging from 57.78% to 76.11%. The highest TKN removal efficiency (76.11 + 1.22%) was achieved at an initial wastewater pH of 8 with an EM dosage of 1% v/v. In contrast, lower removal efficiencies were observed under acidic conditions (pH 6) and at higher EM dosages, indicating that nitrogen removal in the EM-assisted system is strongly influenced by pH regulation rather than inoculum concentration. Statistical analysis confirmed that initial wastewater pH was the most significant factor affecting TKN removal ($p < 0.0001$), while the effect of EM dosage was not statistically significant within the tested range.

This behavior contrasts with COD removal and suggests that distinct physicochemical and biological mechanisms govern nitrogen transformation in the system. The linear increase in TKN removal with increasing pH indicates that alkaline conditions favor nitrogen removal pathways under the experimental conditions employed in this study. At elevated pH levels, the equilibrium between ammonium ions (NH_4^+) and free ammonia (NH_3) shifts toward the un-ionized ammonia form. At pH values approaching 8, a greater fraction of total ammonia nitrogen exists as NH_3, which is more volatile and readily transferred from the aqueous phase to the gas phase. Consequently, physicochemical ammonia stripping becomes increasingly significant under alkaline conditions.

Table 3. COD removal efficiency of wastewater from a fermented rice noodles factory using EM.

No.	initial wastewater pH	EM dosage (%v/v)	COD removal efficiency (%)	
			Experimental data	Predicted values
1	6	1	79.68+0.39	79.59
2	6	5.5	74.42+0.62	74.74
3	6	10	68.72+0.52	68.50
4	7	1	80.21+0.44	80.35
5	7	5.5	76.66+1.22	75.74
6	7	5.5	76.72+1.28	75.74
7	7	5.5	75.46+0.58	75.74
8	7	5.5	74.83+1.21	75.74
9	7	5.5	75.59+0.45	75.74
10	7	10	69.34+0.71	69.74
11	8	1	78.47+0.35	78.42
12	8	5.5	73.82+0.53	74.05
13	8	10	68.47+0.48	68.29

Table 4. TKN removal efficiency of wastewater from fermented rice noodles factory using EM

No.	initial wastewater pH	EM dosage (%v/v)	TKN removal efficiency (%)	
			Experimental data	Predicted values
1	6	1	58.33+0.67	58.70
2	6	5.5	60.00+2.33	57.87
3	6	10	57.78+1.04	57.04
4	7	1	67.78+1.13	66.94
5	7	5.5	66.67+0.85	66.11
6	7	5.5	62.22+4.19	66.11
7	7	5.5	65.00+1.41	66.11
8	7	5.5	66.12+0.31	66.11
9	7	5.5	66.10+0.30	66.11
10	7	10	63.89+1.69	65.28
11	8	1	76.11+1.22	75.18
12	8	5.5	73.89+0.76	74.35
13	8	10	75.56+2.34	73.52

This mechanism is particularly relevant in systems with active aeration, where air–water mass transfer enhances ammonia volatilization. The intermittent aeration strategy applied in this study further reinforced this pH-dependent nitrogen removal mechanism. During aerated phases, increased turbulence and gas exchange promote the transfer of free ammonia from the liquid phase to the atmosphere, thereby reducing TKN concentrations. During non-aerated phases, ammonia stripping is temporarily reduced. However, these periods may facilitate partial mineralization of organic nitrogen by heterotrophic microorganisms within the EM consortium.

The alternating aerobic and non-aerobic conditions thus create a coupled physicochemical–biological removal pathway, even in the absence of fully established nitrification–denitrification processes. Although biological nitrification typically occurs under aerobic conditions and is favored at pH values between 7.5 and 8.5, the intermittent aeration cycle and relatively short operational time in this study likely limited the development of stable nitrifying bacterial populations.

As a result, complete biological nitrification followed by denitrification was unlikely to be the dominant nitrogen removal pathway [42]. Instead, TKN reduction was primarily attributed to a combination of organic nitrogen mineralization and ammonia volatilization, both of which are enhanced under alkaline conditions.

The limited influence of EM dosage on TKN removal further supports this interpretation. While EM provides a diverse microbial community capable of degrading organic nitrogen compounds, increasing the inoculum concentration beyond 1% v/v did not significantly improve nitrogen removal. This observation suggests that nitrogen removal was not constrained by microbial abundance but rather by physicochemical conditions governing ammonia speciation and transfer. Similar findings have been reported in wastewater treatment systems where nitrogen removal is dominated by ammonia stripping rather than biological oxidation, particularly under alkaline pH and aerated conditions [43, 44].

The linear regression model developed for TKN removal (Eq. (3)) adequately captured this behavior, with a high coefficient of determination (R^2 = 0.9326) and statistically significant pH dependence.

The close agreement between predicted and experimental values (Table 4) indicates that within the studied domain, TKN removal efficiency can be reliably predicted based on initial wastewater pH. These results highlight the critical role of pH regulation in controlling nitrogen removal pathways in EM-assisted wastewater treatment systems.

$$Y_2 = 9.43635 + 8.24167A - 0.184815B \tag{3}$$

where Y_2 represents TKN removal efficiency, A denotes initial wastewater pH, and B represents EM dosage (%v/v).

3.2. Statistical Analysis

ANOVA was used to evaluate the statistical significance and predictive reliability of the regression models describing COD (Table 5) and TKN removal (Table 7).

This analysis enabled the identification of significant model terms, assessment of model adequacy, and verification of the suitability of the selected regression forms for process optimization.

3.2.1. Statistical Analysis of COD Removal Efficiency

The ANOVA results for COD removal (Table 5) indicated that the quadratic regression model was statistically significant ($p < 0.05$), confirming its suitability for predicting COD removal efficiency within the tested experimental domain. The high F-value (79.50) and the corresponding p-value (< 0.0001) demonstrate a strong model capability in explaining the observed variability in COD removal.

Among the independent variables, EM dosage (B) exerted the most significant influence on COD removal ($p < 0.0001$), suggesting that the amount of microbial inoculum plays a crucial role in the degradation of organic matter [45].

The quadratic term for initial pH (A^2) was also statistically significant ($p = 0.0125$), indicating a non-linear relationship between pH and COD removal efficiency and suggesting the presence of an optimal pH range within the tested conditions. In contrast, the linear effect of initial pH (A) and the interaction term (AB) were not statistically significant ($p > 0.05$), implying that their individual linear and combined effects were relatively limited.

The lack-of-fit was not significant ($p = 0.8621$), indicating that the quadratic model adequately represented the experimental data without systematic deviations. The statistical validity of the model was further supported by the fit statistics summarized in Table 6.

The model demonstrated strong predictive capability, as reflected by the high coefficient of determination ($R^2 = 0.9827$).

The close agreement between the adjusted R^2 (0.9703) and predicted R^2 (0.9594) indicates minimal model overfitting. In addition, the low coefficient of variation (C.V. = 0.8985%) reflects high experimental precision, while the adequate precision value (26.4191) confirms a satisfactory signal-to-noise ratio for model-based prediction and optimization.

Diagnostic plots further verified the adequacy of the model. The normal probability plot of the externally studentized residuals (Figure 1) indicates that the residuals followed an approximately normal distribution.

The residuals versus predicted values plot (Figure 2) shows a random scatter around zero, confirming the absence of systematic bias and supporting the reliability of the regression model for COD removal prediction.

3.2.2. Statistical Analysis of TKN Removal Efficiency

The ANOVA results for TKN removal (Table 7) show that the linear regression model was statistically significant ($p < 0.0001$), indicating its suitability for predicting TKN removal efficiency within the studied experimental range. The F-value of 69.13 further confirms the robustness of the model.

Table 5. ANOVA results for the quadratic regression model of COD removal.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	179.55	5	35.91	79.50	< 0.0001	significant
A-initial pH	0.7073	1	0.7073	1.57	0.2510	
B-EM dosage	168.86	1	168.86	373.82	< 0.0001	
AB	0.2304	1	0.2304	0.5101	0.4982	
A^2	5.03	1	5.03	11.14	0.0125	
B^2	1.33	1	1.33	2.95	0.1296	
Residual	3.16	7	0.4517			
Lack of Fit	0.4889	3	0.1630	0.2439	0.8621	not significant
Pure Error	2.67	4	0.6683			
Cor Total	182.71	12				

Table 6. Fit statistical results of COD removal.

Std. Dev.	0.6721	R^2	0.9827
Mean	74.80	Adjusted R^2	0.9703
C.V. %	0.8985	Predicted R^2	0.9594
		Adeq Precision	26.4191

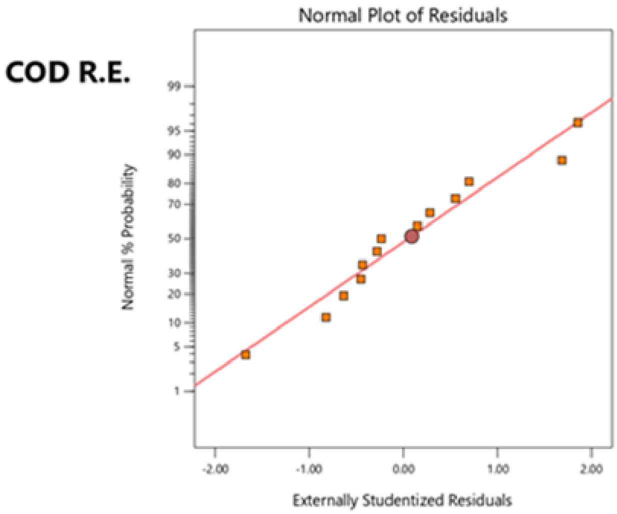

Fig. 1. Externally studentized residuals versus residual normal percent probability of COD removal efficiency.

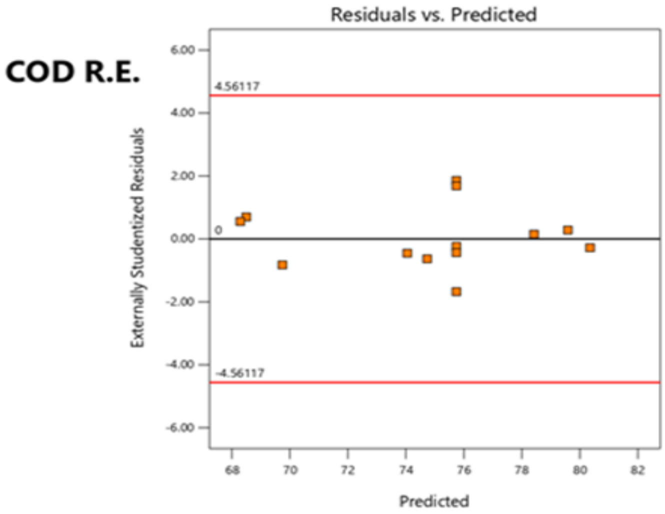

Fig. 2. Residuals vs. predicted plot for COD removal efficiency using EM.

Among the independent variables, the linear effect of initial pH (A) was highly significant ($p < 0.0001$), indicating a strong positive relationship between pH and TKN removal efficiency.

In contrast, the effect of EM dosage (B) was not statistically significant ($p = 0.2651$), indicating that within the tested range, variations in inoculum concentration had a comparatively minor influence on TKN removal. The non-significant lack-of-fit ($p = 0.5721$) confirmed that the linear model adequately described the experimental data. Fit statistics (Table 8) indicate good predictive performance of the model, with an R^2 value of 0.9326 and reasonable agreement between the adjusted R^2 (0.9191) and predicted R^2 (0.8893).

The coefficient of variation (2.61%) and adequate precision value (21.8918) further support the reliability of the model for prediction and optimization. Residual diagnostics for the TKN removal model are presented in Figures 3 and 4. The normal probability plot suggests that the residuals were approximately normally distributed, while the residuals versus predicted plot shows a generally random distribution around zero, indicating acceptable variance homogeneity and model adequacy for predicting TKN removal efficiency.

3.3. Optimization of Treatment Conditions Using Response Surface Methodology

RSM was further employed to investigate the interactive effects of initial wastewater pH and EM dosage on the removal efficiencies of COD and TKN and to identify the optimal operating conditions for maximizing these responses.

Table 7. ANOVA results for the quadratic regression model of TKN removal.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	411.70	2	205.85	69.13	< 0.0001	significant
A-initial pH	407.55	1	407.55	136.88	< 0.0001	
B-EM dosage	4.15	1	4.15	1.39	0.2651	
Residual	29.78	10	2.98			
Lack of Fit	17.04	6	2.84	0.8920	0.5721	not significant
Pure Error	12.74	4	3.18			
Cor Total	441.48	12				

Table 8. Fit statistical results of TKN removal.

Std. Dev.	1.73	R^2	0.9326
Mean	66.11	Adjusted R^2	0.9191
C.V. %	2.61	Predicted R^2	0.8893
		Adeq Precision	21.8918

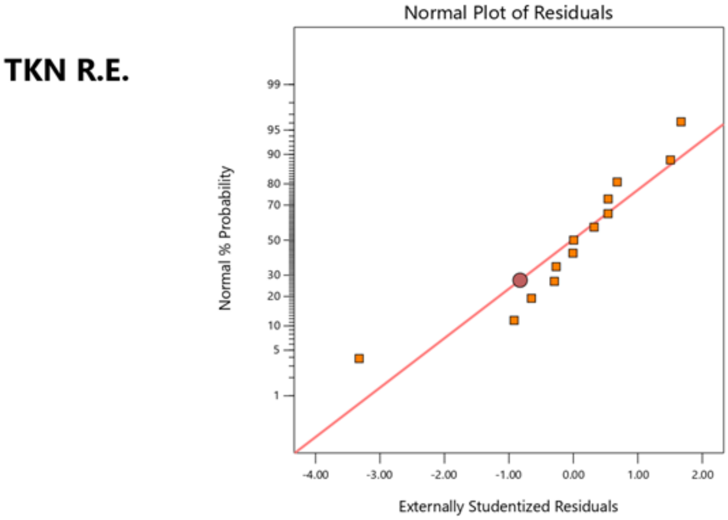

Fig. 3. Externally studentized residuals versus residual normal percent probability of TKN removal efficiency.

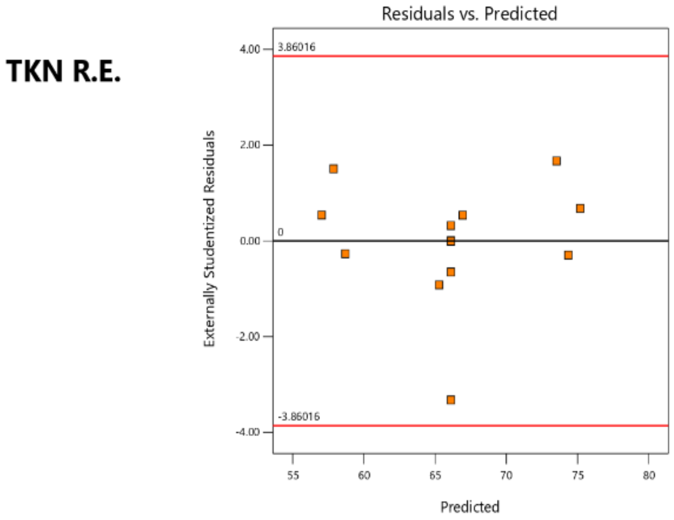

Fig. 4. Residuals vs. predicted plot for TKN removal efficiency using EM.

The three-dimensional response surface plots (Figure 5 for COD and Figure 7 for TKN) provide a graphical representation of the relationship between the independent variables and their corresponding removal efficiencies.

3.3.1. Optimization of COD Removal

The three-dimensional response surface plot for COD removal (Figure 5) reveals a pronounced non-linear relationship between initial wastewater pH and EM dosage, with a distinct optimum observed at near-neutral pH (approximately 6.9) and a low EM dosage (around 1.2% v/v). This well-defined peak confirms that organic matter removal in the EM-assisted system is maximized within a narrow operational window.

The observed non-linear behavior is consistent with the microbial and enzymatic constraints discussed in Section 3.1.1, where both pH-dependent metabolic activity and inoculum-related limitations govern COD degradation efficiency. The quadratic regression model effectively captured this behavior, as evidenced by the statistically significant quadratic pH term and the high coefficient of determination ($R^2 = 0.9827$).

Numerical optimization using the desirability function identified an optimal operating point at pH 6.9 and an EM dosage of 1.2% v/v, yielding a predicted COD removal efficiency exceeding 80% (Figure 6). Experimental validation confirmed close agreement between predicted and observed values, with deviations of less than 0.5%. These results demonstrate that Response Surface Methodology provides a reliable framework for identifying optimal operating conditions for EM-assisted organic matter removal while avoiding excessive microbial dosing or unfavorable pH conditions.

3.3.2. Optimization of TKN Removal

The response surface for TKN removal (Figure 7) showed a strong linear increase with pH, while EM dosage had a comparatively weaker effect, consistent with the statistical model. Numerical optimization identified the highest TKN removal (75.18%) at pH 8 and 1% v/v EM, which aligned closely with experimental values.

Numerical optimization predicted a maximum efficiency of 75.18%, which closely matched experimental results. The desirability score of 0.950 further supported the suitability of the linear model for capturing TKN removal behavior within the studied domain (Figure 8). The progressively increasing trend suggests that within the tested range, pH is the dominant factor influencing nitrogen removal pathways.

3.4. Comparison and Contrast of COD and TKN Removal

The treatment of fermented rice noodle wastewater using EM exhibited fundamentally different response patterns for COD and TKN removal, reflecting distinct controlling mechanisms for organic matter degradation and nitrogen reduction. COD removal was governed primarily by biologically regulated processes, whereas TKN removal was dominated by pH-driven physicochemical pathways. Optimal COD removal occurred under near-neutral conditions with low EM dosage, consistent with the metabolic requirements of heterotrophic microorganisms within the EM consortium. In contrast, TKN removal increased linearly with pH and reached its maximum under alkaline conditions, indicating that nitrogen removal efficiency was largely independent of microbial abundance within the tested range. This divergence highlights an inherent trade-off in single-stage EM-assisted treatment systems. Conditions that favor efficient biodegradation of organic matter do not coincide with those that enhance nitrogen removal, as these processes are controlled by different physiological and physicochemical constraints. Consequently, simultaneous optimization of COD and TKN removal within a single operational setting is limited. From a process design perspective, these findings emphasize the importance of clearly defining treatment objectives or adopting staged operational strategies to address competing removal mechanisms. The mechanistic distinction between carbon and nitrogen removal provides a conceptual basis for future system configurations aimed at achieving balanced treatment performance.

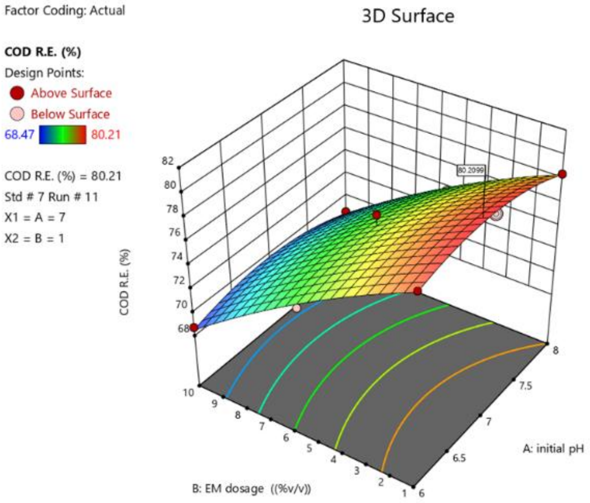

Fig. 5. Three-dimensional response surface plot illustrating the non-linear optimization region for COD removal as a function of initial pH and EM dosage.

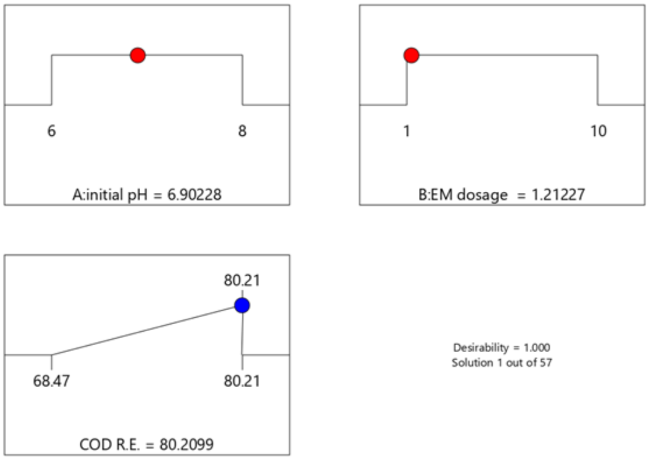

Fig. 6. Desirability ramps of COD removal efficiency.

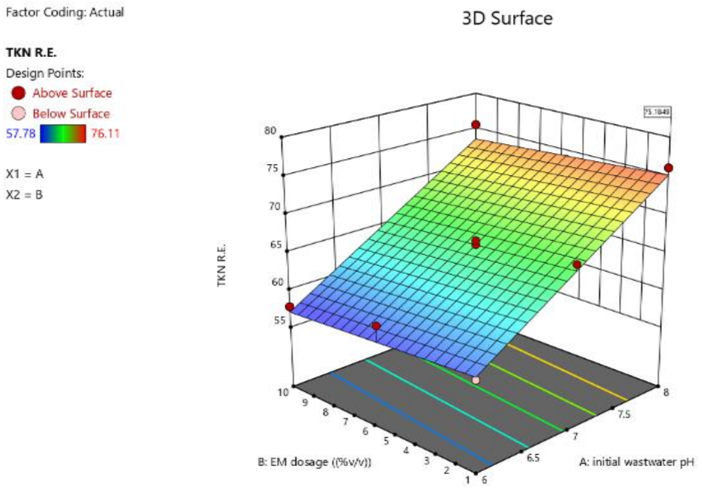

Fig. 7. Three-dimensional response surface plot illustrating the pH-dependent response of TKN removal as a function of initial pH and EM dosage.

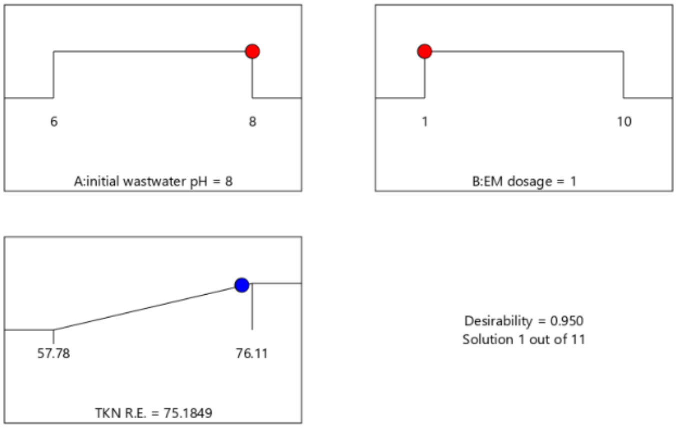

Fig. 8. Desirability ramps of TKN removal efficiency.

4. Conclusion

This study demonstrated that Effective Microorganisms can be effectively applied for the treatment of high-strength wastewater generated from fermented rice noodle production when key operational parameters are appropriately regulated. Using RSM, the influences of initial wastewater pH and EM dosage on COD and TKN removal were evaluated, revealing that organic matter and nitrogen removal are governed by fundamentally different controlling mechanisms.

COD removal was primarily governed by biologically regulated processes and achieved maximum efficiency under near-neutral pH conditions with low EM dosage. In contrast, TKN removal was dominated by pH-regulated pathways and enhanced under alkaline conditions with limited sensitivity to microbial inoculum concentration. This divergence highlights a critical challenge for single-stage EM-assisted reactors where simultaneous optimization of carbon and nitrogen removal is constrained by competing biological and physicochemical requirements.

A notable practical outcome of this study is that consistently high treatment performance was achieved at low EM dosages (1–1.2% v/v) under intermittent aeration. This finding suggests important operational and resource-efficiency advantages compared with conventional biological treatment systems that often rely on high inoculum concentrations or continuous energy-intensive aeration [46].

Although a comprehensive economic assessment was beyond the scope of this study, the combined reduction in inoculum demand and aeration intensity indicates that EM-assisted treatment may offer a cost-effective alternative for managing high-strength wastewater from fermented food industries.

Despite the promising laboratory-scale results, the scalability of EM-based treatment systems warrants careful consideration. Full-scale implementation may encounter operational challenges related to maintaining stable pH conditions, ensuring adequate oxygen transfer under intermittent aeration, and preserving microbial community stability during long-term operation [47]. In addition, hydraulic mixing characteristics and mass transfer limitations in larger reactors may influence overall treatment performance. Consequently, pilot-scale studies are strongly recommended to evaluate system robustness under variable loading conditions and real-world operational fluctuations.

To overcome the inherent limitations of single-stage systems, future research should focus on process configurations that decouple carbon and nitrogen removal requirements, such as sequential pH adjustment, staged aerobic–anoxic operation, or multi-reactor designs. These approaches may enable more effective integration of EM technology into sustainable wastewater treatment strategies for the fermented food industry.

Acknowledgements

The authors gratefully acknowledge the Department of Chemical and Environmental Engineering, Faculty of Engineering, Ubon Ratchathani University, for providing comprehensive support, including chemicals, experimental equipment, and laboratory facilities necessary for conducting this research. The authors also sincerely thank Tripope Tarasri, Sippawit Leela, and Takdanai Sarakan for their valuable assistance in experimental work and data collection.

Author's contribution

Tiammanee Rattanaweerapan: conceptualization, reviewing and editing. Karnika Ratanapongleka: conceptualization, methodology, visualization, supervision, formal analysis, data curation, writing, reviewing and editing. Supatpong Mattaraj: conceptualization, reviewing and editing. Wipada Dechapanya: conceptualization, reviewing and editing. Sompop Sanongraj: conceptualization, reviewing and editing.

Conflict of interest

No potential conflict of interest was reported by the authors.

Data availability

Not Applicable.

Funding

This research project was financially supported by Faculty of Engineering, Ubon Ratchathani University.

References

[1] Kanchak, A., Kengchuwong, M., Sanghaw, R., & Nuasri, C. (2023). High organic wastewater from fermented rice noodles factories and slaughterhouses treated by anaerobic filter tank system combined with floating plants at different hydraulic retention times. *Burapha Sci. J.*, *28*(3), 1445–1459.

[2] Samorì, G., Samorì, C., & Pistocchi, R. (2014). Nutrient removal efficiency and physiological responses of *Desmodesmus communis* at different HRTs and nutrient stress condition using different sources of urban wastewater effluents. *Appl. Biochem. Biotechnol.*, *173*(1), 74–89. https://doi.org/10.1007/s12010-014-0792-7

[3] Wurtsbaugh, W. A., Paerl, H. W., & Dodds, W. K. (2019). Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. *WIREs Water*, *6*(5), e1373. https://doi.org/10.1002/wat2.1373

[4] de Vries, W. (2021). Impacts of nitrogen emissions on ecosystems and human health: A mini review. *Curr. Opin. Environ. Sci. Health*, *21*, 100249. https://doi.org/10.1016/j.coesh.2021.100249

[5] Obulapuram, P. K., Arfin, T., Mohammad, F., Khiste, S. K., Chavali, M., Albalawi, A. N., et al. (2021). Adsorption, equilibrium isotherm, and

thermodynamic studies towards the removal of reactive orange 16 dye using Cu(I)-polyaninile composite. *Polymers*, *13*(20), 3490. https://doi.org/10.3390/polym13203490

[6] Mohammad, F., Arfin, T., & Al-lohedan, H. A. (2019). Enhanced biosorption and electrochemical performance of sugarcane bagasse derived a polylactic acid-graphene oxide-CeO2 composite. *Mater. Chem. Phys.*, *229*, 117–123. https://doi.org/10.1016/j.matchemphys.2019.02.085

[7] Arfin, T., Bhaisare, D. A., & Waghmare, S. S. (2021). Development of a PANI/Fe(NO3)2 nanomaterial for reactive orange 16 (RO16) dye removal. *Anal. Methods*, *13*(44), 5309–5327. https://doi.org/10.1039/d1ay01402a

[8] Arfin, T., Bushra, R., & Mohammad, F. (2016). Electrochemical sensor for the sensitive detection of o-nitrophenol using graphene oxide-poly(ethyleneimine) dendrimer-modified glassy carbon electrode. *Graphene Technol.*, *1*(1), 1–15. https://doi.org/10.1007/s41127-016-0002-1

[9] Mohammad, F., Arfin, T., & Al-Lohedan, H. A. (2017). Sustained drug release and electrochemical performance of ethyl cellulose-magnesium hydrogen phosphate composite. *Mater. Sci. Eng. C*, *71*(1), 735–743. https://doi.org/10.1016/j.msec.2016.10.062

[10] Gonzales, R. R., Nakagawa, K., Hasegawa, S., Kumagai, K., Matsuoka, A., Okamoto, Y., et al. (2025). Treatment of high-strength wastewater with coupled flocculation and membrane filtration prior to ammoniacal nitrogen enrichment by osmotically assisted reverse osmosis. *Sep. Purif. Technol.*, *352*, 128159. https://doi.org/10.1016/j.seppur.2024.128159

[11] Chan, Y. J., Chong, M. F., Law, C. L., & Hassell, D. (2009). A review on anaerobic–aerobic treatment of industrial and municipal wastewater. *Chem. Eng. J.*, *155*(1-2), 1–18. https://doi.org/10.1016/j.cej.2009.06.041

[12] Mutamim, N. S. A., Noor, Z. Z., Hassan, M. A. A., & Olsson, G. (2012). Application of membrane bioreactor technology in treating high strength industrial wastewater: A performance review. *Desalination*, *305*, 1–11. https://doi.org/10.1016/j.desal.2012.07.033

[13] Yahya, M., Gökçekuş, H., & Ozsahin, D. U. (2020). Comparative analysis of wastewater treatment technologies. *Jurnal Kejuruteraan*, *32*(2), 221–230. https://doi.org/10.17576/jkukm-2020-32(2)-06

[14] Khiewwijit, R., Temmink, H., Rijnaarts, H., & Keesman, K. J. (2015). Energy and nutrient recovery for municipal wastewater treatment: How to design a feasible plant layout? *Environ. Modell. Softw.*, *68*, 156–165. https://doi.org/10.1016/j.envsoft.2015.02.011

[15] Askari, S. S., Giri, B. S., Basheer, F., Izhar, T., Ahmad, S. A., & Mumtaz, N. (2024). Enhancing sequencing batch reactors for efficient wastewater treatment across diverse applications: A comprehensive review. *Environ. Res.*, *260*, 119656. https://doi.org/10.1016/j.envres.2024.119656

[16] Wikaningrum, T., & Putri, A. N. I. (2023). Study on activated sludge composition and concentration setting for increasing COD efficiency in dairy industry wastewater. *IOP Conf. Ser.: Earth Environ. Sci.*, *1268*(1), 012016. https://doi.org/10.1088/1755-1315/1268/1/012016

[17] Canals, J., Cabrera-Codony, A., Carbó, O., Torán, J., Martín, M., Baldi, M., et al. (2023). High-rate activated sludge at very short SRT: Key factors for process stability and performance of COD fractions removal. *Water Res.*, *231*, 119610. https://doi.org/10.1016/j.watres.2023.119610

[18] Aditya, L., Mahlia, T. I., Nguyen, L. N., Vu, H. P., & Nghiem, L. D. (2022). Microalgae-bacteria consortium for wastewater treatment and biomass production. *Sci. Total Environ.*, *838*, 155871. https://doi.org/10.1016/j.scitotenv.2022.155871

[19] Godzieba, M., Zubrowska-Sudol, M., Walczak, J., & Ciesielski, S. (2022). Development of microbial communities in biofilm and activated sludge in a hybrid reactor. *Sci. Rep.*, *12*(1), 12558. https://doi.org/10.21203/rs.3.rs-1592503/v1

[20] Li, L., Chai, W., Sun, C., Huang, L., Sheng, T., Song, Z., et al. (2024). Role of microalgae-bacterial consortium in wastewater treatment: A review. *J. Environ. Manage.*, *360*, 121226. https://doi.org/10.1016/j.jenvman.2024.121226

[21] Cao, Z., Yan, W., Ding, M., & Yuan, Y. (2022). Construction of microbial consortia for microbial degradation of complex compounds. *Front. Bioeng. Biotechnol.*, *10*, 1051233.

https://doi.org/10.3389/fbioe.2022.1051233

[22] Higa, T., & Parr, J. F. (1994). *Beneficial and effective microorganisms for a sustainable agriculture and environment*. International Nature Farming Research Center.

[23] Olle, M., & Williams, I. (2015). The influence of effective microorganisms on the growth and nitrate content of vegetable transplants. *J. Adv. Agric. Technol.*, *2*(1).

[24] Namsivayam, S. K. R., Narendrakumar, G., & Kumar, J. A. (2011). Evaluation of Effective Microorganism (EM) for treatment of domestic sewage. *J. Exp. Sci.*, *2*(7).

[25] Bhandari, B., Babasaheb, B., Pravin, N., Shubham, S., & Nikhil, S. (2021). Review on domestic waste water treatment by using effective microorganisms' technology. *IJSART*, *7*(6), 792–796.

[26] Sulistyaningsih, T., Widiarti, N., Astuti, W., & Harjunowibowo, D. (2019). The proliferation of Effective Microorganism (EM) in vinasse and its application in the manufacture of livestock-waste based fertilisers. *J. Chem. Technol. Metall.*, *54*(4), 727–732.

[27] Ali, M. N., Youssef, T. F., Aly, M. M., & Abuzaid, A. G. (2021). Application of effective microorganisms technology on dairy wastewater treatment for irrigation purposes. *J. Degraded Min. Lands Manage.*, *8*(4), 2917–2923. https://doi.org/10.15243/jdmlm.2021.084.2917

[28] Zhou, G., Li, J., Fan, H., Sun, J., & Zhao, X. (2010). *Starch wastewater treatment with effective microorganisms bacteria*. IEEE.

[29] Anwar, N. Z. R., Hassan, M. A. A., Mahmood, I., & Khamis, A. K. (2013). Treatment of rubber processing wastewater by effective microorganisms using anaerobic sequencing batch reactor. *J. Agrobiotechnol.*, *4*(1), 1–15.

[30] Kaur, B., Choudhary, R., Sharma, G., & Brar, L. K. (2024). Sustainable and effective microorganisms method for wastewater treatment. *Desalin. Water Treat.*, *319*, 100419. https://doi.org/10.1016/j.dwt.2024.100419

[31] Safwat, S. M., & Matta, M. E. (2021). Environmental applications of Effective Microorganisms: A review of current knowledge and recommendations for future directions. *J. Eng. Appl. Sci.*, *68*(1), 1–12. https://doi.org/10.1186/s44147-021-00049-1

[32] Pushpa, T. B., Vijayaraghavan, J., Vijayaraghavan, K., & Jegan, J. (2016). Utilization of Effective Microorganisms based water hyacinth compost as biosorbent for the removal of basic dyes. *Desalin. Water Treat.*, *57*(51), 24368–24377. https://doi.org/10.1080/19443994.2016.1143405

[33] Szymanski, N., & Patterson, R. A. (2003). Effective microorganisms (EM) and wastewater systems. *Future Directions for On-site Systems: Best Management Practice*, 347–355.

[34] Mukred, A. M., Abd Hamid, A., Hamzah, A., & Yusoff, W. M. W. (2008). Growth enhancement of effective microorganisms for bioremediation of. *Pak. J. Biol. Sci.*, *11*(13), 1708–1712. https://doi.org/10.3923/pjbs.2008.1708.1712

[35] Adamberg, K., Kask, S., Laht, T.-M., & Paalme, T. (2003). The effect of temperature and pH on the growth of lactic acid bacteria: A pH-auxostat study. *Int. J. Food Microbiol.*, *85*(1-2), 171–183. https://doi.org/10.1016/S0168-1605(02)00537-8

[36] Kodukula, P. S., Prakasam, T., & Anthonisen, A. C. (2018). *Role of pH in biological wastewater treatment processes*. CRC Press.

[37] Hoo, M. W. S., & Teo, S.-S. (2021). Investigation on the efficiency of effective microorganisms for polluted water treatment. *Appl. Microbiol.: Theory Technol.* https://doi.org/10.37256/amtt.212021683

[38] American Public Health Association, Baird, R., Eaton, A. D., Rice, E. W., Bridgewater, L., American Water Works Association, et al. (2017). *Standard methods for the examination of water and wastewater* (23rd ed.). American Public Health Association.

[39] Yang, E., Fan, L., Yan, J., Jiang, Y., Doucette, C., Fillmore, K., et al. (2018). Influence of culture media, pH and temperature on growth and bacteriocin production of bacteriocinogenic lactic acid bacteria. *AMB Express*, *8*(1), 10. https://doi.org/10.1186/s13568-018-0536-0

[40] van Oort, M. (2009). Enzymes in food technology–introduction. In *Enzymes in Food Technology* (pp. 1–17). https://doi.org/10.1002/9781444309935.ch1

[41] Herrero, M., & Stuckey, D. C. (2015). Bioaugmentation and its application in wastewater treatment: A review. *Chemosphere*, *140*, 119–128. https://doi.org/10.1016/j.chemosphere.2014.10.033

[42] Fan, L., Yao, H., Deng, S., Jia, F., Cai, W., Hu, Z., et al. (2021). Performance and microbial community dynamics relationship within a step-feed anoxic/oxic/anoxic/oxic process (SF-A/O/A/O) for coking wastewater treatment. *Sci. Total Environ.*, *792*, 148263. https://doi.org/10.1016/j.scitotenv.2021.148263

[43] Gupta, V., Sadegh, H., Yari, M., Shahryari, G. R., Maazinejad, B., & Chahardori, M. (2015). Removal of ammonium ions from wastewater a short review in development of efficient methods. *Glob. J. Environ. Sci. Manage.*, *1*(2), 71–94.

[44] Huang, H., Xiao, X., Yan, B., & Yang, L. (2010). Ammonium removal from aqueous solutions by using natural Chinese (Chende) zeolite as adsorbent. *J. Hazard. Mater.*, *175*(1-3), 247–252. https://doi.org/10.1016/j.jhazmat.2009.09.156

[45] Eddy, M. (2014). *Wastewater engineering: Treatment and resource recovery*. McGraw-Hill.

[46] Al-Hazmi, H. E., Lu, X., Majtacz, J., Kowal, P., Xie, L., & Makinia, J. (2020). Optimization of the aeration strategies in a deammonification sequencing batch reactor for efficient nitrogen removal and mitigation of N2O production. *Environ. Sci. Technol.*, *55*(2), 1218–1230. https://doi.org/10.1021/acs.est.0c04229

[47] Garcia-Ochoa, F., & Gomez, E. (2009). Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview. *Biotechnol. Adv.*, *27*(2), 153–176. https://doi.org/10.1016/j.biotechadv.2008.10.006

How to cite this paper:

Rattanaweerapan, T., Ratanapongleka, K., Mattaraj, S., Dechapanya, W. & Sanongraj, S. (2026). Optimization of fermented rice noodle wastewater treatment using effective microorganisms (EM). Advances in Environmental Technology, 12(3), 274-290. DOI: 10.22104/aet.2026.7571.2125