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Kinetics of Ammonia Biodegradation using EM4 with Palm Sugar as an External Carbon Source

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ABSTRACT

Biological treatment of ammonia requires sufficient carbon availability to maintain stable microbial activity and sustain optimal degradation rates. This study evaluates the kinetics of ammonia biodegradation using an EM4 microbial consortium supplemented with palm sugar as an external carbon source in aerobic batch reactors. Initial ammonia concentrations of 10, 30, and 50 ppm were evaluated over six days of operation. Monitored parameters included ammonia concentration, pH, and biomass (MLSS), while kinetic evaluation applied a pseudo–first-order model via the $\ln(S_t/S_0)$–time relationship. The results showed removal efficiencies of 79.0–83.4%, accompanied by MLSS increases from ~2.000 to ~4.600 mg/L with higher initial concentrations. The pH range of 5.8–8.5 remained conducive to microbial activity. The $\ln(S_t/S_0)$ curves exhibited strong linearity, confirming the suitability of the pseudo–first-order model, and the constants reaction rate increased under higher substrate and biomass conditions. These findings indicate that palm sugar effectively serves as an external carbon source that enhances process stability and ammonia removal. The integration of EM4 with a natural carbon source demonstrates potential as an efficient, economical, and readily implementable biological approach for ammonia treatment in wastewater.

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INTRODUCTION

Based on its mass, ammonia is one of the most widely used chemicals in the world [1]. Ammonia plays a significant role in various industrial sectors, including agriculture, energy, and the development of energy storage systems. The development of ammonia synthesis technology shows that, along with the increasing global energy demand, ammonia production is expected to increase significantly in the coming decades [2]. This increase in production has the potential to lead to the release of larger amounts of ammonia into the environment, including air, water, sediment, and soil. Ammonia is known to have a high level of toxicity to aquatic and terrestrial organisms [3], which can trigger water eutrophication, dissolved oxygen depletion, as well as the formation of hypoxic zones that impact biodiversity decline [4].

The increasing problem of ammonia pollution in water has encouraged the development of sustainable and cost-effective treatment methods. Various methods have been developed to reduce the concentration of ammonia in water, both through physical processes, such as water stripping, ion exchange, and chemical oxidation, as well as through biological processes [5]. The biological method utilizes the natural activity of microorganisms to convert ammonia into a harmless form, significantly reducing operational costs and reducing secondary waste.

Several studies report that the use of Effective Microorganisms 4 (EM4) can significantly reduce ammonia concentrations [6]. EM4 is a consortium of microorganisms that plays a role in accelerating the degradation of organic compounds and improving water quality, as shown by the decrease in Biological Oxygen Demand (BOD) value by up to 72.3% in hospital liquid waste treatment [7]. Nevertheless, the performance of EM4 is strongly influenced by the availability of nutrients, especially carbon sources, which play an important role in microbial growth and metabolic activity [8]. In addition, the addition of external carbon sources is known as an effective strategy to increase microbial activity in the nitrogen biodegradation process in wastewater treatment [9]. Conventional carbon sources, such as methanol and ethanol, are widely used [10], but their utilization is often limited by economic aspects, availability, and operational risks [11]. Palm sugar, as a local natural carbon source, has a high carbon content and has the potential to be utilized in various applications, including water treatment, so it is interesting to study as an alternative source of carbon in ammonia biodegradation systems [12-13]. Furthermore, understanding the biological reaction rate in an ammonia treatment system requires a comprehensive kinetic analysis. Several kinetic models, such as the Monod, first-order, and modified Stover-Kincannon models, have been used to describe the biodegradation process [14]. In addition, supporting parameters such as Mixed Liquor Suspended Solids (MLSS) and pH serve as important indicators that directly affect the effectiveness of the ammonia biodegradation process.

Although studies have examined the use of EM4 and conventional carbon sources in ammonia processing, studies using local natural carbon sources such as palm sugar are still very limited, particularly those that integrate pseudo-first-order kinetic analysis with the dynamics of microorganism biomass and changes in the pH of the system. The relationship between increased initial ammonia concentration, biomass growth, and constant reaction rate in EM4-based biological systems with palm sugar supplementation has also not been comprehensively studied. Therefore, this study was conducted to evaluate the biodegradation of ammonia using EM4 and palm sugar as natural carbon sources through reaction kinetics, biomass growth (MLSS), and pH stability approaches, as an effort to develop an effective, economical, and local resource-based ammonia treatment system.

RESEARCH METHODS

Research Design

This study was a laboratory-scale experimental investigation aimed at evaluating the biodegradation of ammonia using a consortium of effective microorganisms (EM4) in an aerobic batch reactor system. The aerobic system was selected to support microbial activity in utilizing ammonia as a nitrogen source, facilitated by continuous aeration throughout the treatment process. The independent variable in this study was the initial ammonia concentration, which was varied at 10, 30, and 50 ppm. The dependent variable was the change in ammonia concentration during the treatment period. Supporting parameters observed included pH and Mixed Liquor Suspended Solids (MLSS), which served as indicators of microbial biomass activity and growth.

Materials and Equipment

The materials used in this study included synthetic ammonia solution (NH_4OH) as the nitrogen substrate source, EM4 as the microbial inoculum, and palm sugar as a natural carbon source. The main equipment used consisted of a closed batch reactor equipped with an aeration system, a calibrated pH meter, and a UV–Vis spectrophotometer for ammonia concentration analysis. Supporting equipment included an analytical balance, an oven, and MLSS analysis apparatus. The research methodology describes the overall research design, scope or object of the study, primary materials and equipment, research location, data collection techniques, data analysis methods, and operational definitions of the research variables.

Figure 1. Schematic diagram of the aerobic reactor system

Experimental Procedure

Ammonia biodegradation was conducted in a batch reactor operated under aerobic conditions with continuous aeration. Ammonia solutions with predetermined initial concentrations according to the experimental variations were introduced into the reactor. Subsequently, EM4 was added at a sludge-to-ammonia solution volume ratio of 1:10. Palm sugar was supplied as an external carbon source to support the metabolic activity of microorganisms. The reactor was operated for 144 hours (6 days) at room temperature. Throughout the process, aeration was maintained to ensure homogeneous mixing and to provide favorable environmental conditions for microbial activity. Samples were collected every 24 hours to analyze ammonia concentration and pH. At the end of the treatment period, the Mixed Liquor Suspended Solids (MLSS) value was measured to evaluate the growth of microbial biomass.

Ammonia Analysis (Nessler Method)

The concentration of ammonia was determined using a UV–Vis spectrophotometer at a wavelength of 425 nm with a cuvette optical path length of 1 cm. The ammonia concentration was quantified based on a calibration curve prepared from standard solutions with concentrations of 0, 2, 3, 5, 7, and 10 ppm, which exhibited a linear relationship between absorbance values and ammonia concentration. Liquid samples were periodically collected from each reactor with a volume of 10 mL. When necessary, the samples were diluted with deionized water to ensure that the concentration fell within the linear measurement range. Subsequently, 0.5 mL of Nessler reagent was added to the sample solution. The mixture was then homogenized and allowed to stand at room temperature for approximately 10 minutes until a stable yellowish-brown color developed. The resulting absorbance values were applied to the linear regression equation of the calibration curve to determine the residual ammonia concentration in the reactor throughout the six-day observation period.

Pseudo First-Order Kinetics Analysis

The pseudo-first-order kinetic approach was applied to describe the rate of ammonia concentration reduction in a simplified manner and to evaluate the performance of the biodegradation process based on the trend of the experimental data obtained during the observation period [15]. This model assumes that the reaction rate is proportional to the concentration of ammonia as the primary substrate, while other factors are considered constant throughout the process [16]. Mathematically, the pseudo-first-order kinetic model can be expressed as follows:

$$\ln\left(\frac{S_t}{S_0}\right) = -k.t \quad (1)$$

Where k is the pseudo-first-order constant reaction rate (day^{-1}), t represents the reaction time (day), S_t denotes the ammonia concentration at time t (day), and S_0 is the initial ammonia concentration. The determination of the constant reaction rate was carried out by analyzing the changes in ammonia concentration over time. The experimental data were plotted as a linear relationship between $\ln(S_t/S_0)$ and time. The value of the rate constant k was obtained from the slope of the straight line generated by linear regression analysis.

RESULTS AND DISCUSSION

Variation of Ammonia Concentration

The change in ammonia concentration over six days showed a consistent decrease across all initial concentration variations, as presented in Table 1. At an initial concentration of 10 ppm, the final ammonia concentration was recorded at 2.10 ppm, corresponding to a removal of 7.90 ppm and a removal efficiency of 79.0%. At an initial concentration of 30 ppm, the final ammonia concentration was 5.50 ppm, with a removal of 24.50 ppm and an efficiency of 81.7%. Meanwhile, an initial concentration of 50 ppm resulted in a final concentration of 8.30 ppm, corresponding to a removal of 41.70 ppm and an efficiency of 83.4%. These results indicate that increasing the initial concentration not only increases the absolute amount of ammonia removed but also leads to a slight improvement in removal efficiency. This suggests that within the concentration range of 10–50 ppm, ammonia continues to function as a substrate that supports microbial activity without causing inhibitory effects [17].

Table 1. Dynamics of Ammonia Concentration and Final Removal Efficiency

Ca* NH_3 (ppm)	Cb* NH_3 (ppm)	Time (days)	Final Removal Efficiency* (%)
10	2.10	6	79.0
30	5.50	6	81.7
50	8.30	6	83.4

*C_a : initial ammonia concentration; C_b : final ammonia concentration.

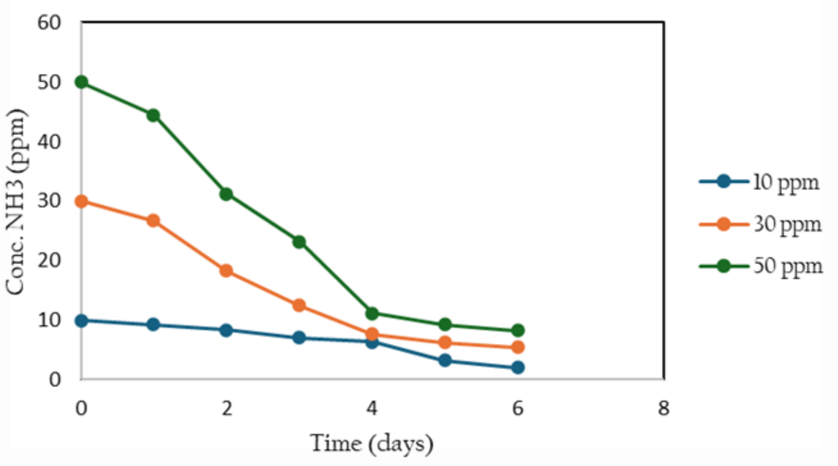

Figure 2. Profile of Ammonia Concentration Reduction

As illustrated in Figure 2, the profile indicates a more rapid decrease in ammonia concentration during the initial phase, followed by a slower decline toward the end of the observation period. This trend reinforces that the high availability of substrate at the beginning of the process enables microorganisms to perform optimally, whereas the reduction in degradation rate at the later stage is attributed to the decreasing ammonia concentration within the system. In addition, the greater absolute removal observed at higher initial concentrations indicates that the EM4-based biological system remains responsive to higher ammonia loads, particularly with the supplementation of palm sugar as an external carbon source that maintains sufficient energy availability for microbial metabolism [18]. Overall, the data presented in Table 1 confirm that the biological system employed demonstrates stable, adaptive, and effective performance across various initial ammonia concentrations.

pH

The observational results indicate that the pH values in all variations of ammonia concentration exhibited an increasing trend throughout the treatment period. During the initial phase, the system pH ranged from 5.78 to 5.99, reflecting slightly acidic conditions. This condition was likely caused by the addition of brown sugar as an external carbon source, which, during the early stages of biodegradation, may produce acidic organic compounds due to the fermentative activity of microorganisms. As the process progressed, the pH gradually increased, reaching approximately 7.2–8.56 during the middle to the final stage of reactor operation. This increase in pH is consistent with the metabolic activity of microorganisms present in EM4, which are known to modify the chemical conditions of their environment through substrate consumption and the production of certain metabolites, as reported in previous studies [19].

The observed pH range remained within the optimal range for microbial activity, namely 6.5–8.5 [20], thereby supporting the continuous biodegradation of ammonia. The comparison among different ammonia concentration variations shown in Figure 3 indicates that higher initial concentrations tend to produce a more rapid increase in pH and reach higher final values. This phenomenon can be associated with increased microbial metabolic intensity resulting from greater substrate availability, leading to more significant changes in ionic equilibrium within the system.

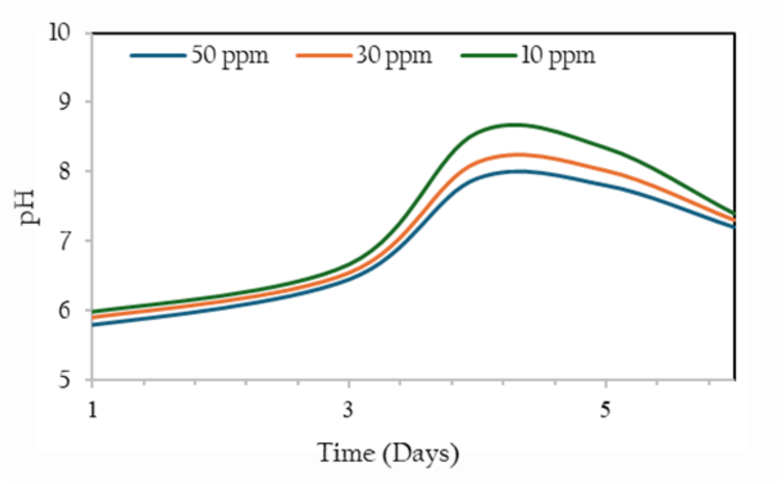

Figure 3. Comparison of pH profiles based on variations in ammonia concentration.

The correlation between the increase in pH and the decrease in ammonia concentration indicates that near-neutral to slightly alkaline pH conditions play an important role in enhancing the efficiency of the biodegradation process. The stability of pH within this range suggests that the microorganisms are able to adapt effectively to environmental changes during the treatment process. Therefore, the progressive pH changes not only reflect the dynamics of microbial activity but also demonstrate the system's ability to maintain reactor conditions that support optimal ammonia oxidation and utilization.

Mixed Liquor Suspended Solids (MLSS)

The Mixed Liquor Suspended Solids (MLSS) values in the biological system showed a clear increase with rising initial ammonia concentrations. Suspended solids measurements conducted through filtration of 5 mL samples resulted in MLSS values of approximately 2.000 mg/L at 10 ppm, 3.200 mg/L at 30 ppm, and 4.600 mg/L at 50 ppm. This gradual increase indicates that higher availability of ammonia substrate can support more intensive microbial growth and activity, considering that ammonia functions as an essential nitrogen source for cellular synthesis.

In addition to nitrogen, the presence of a carbon source also influences biomass dynamics. Microorganisms in EM4 not only utilize ammonia as a nitrogen source but also require sufficient carbon to maintain the C/N balance. The supplementation of palm sugar provides a readily biodegradable carbon source, thereby supporting optimal biomass growth, which is reflected in the increased MLSS values across all concentration variations.

The highest MLSS value observed at an initial concentration of 50 ppm indicates that the system possesses a greater biodegradation capacity when higher substrate levels are available. This relationship is further demonstrated by the tendency for systems with higher MLSS values to exhibit faster ammonia removal rates. Such conditions reflect an increased number of active microbial cells participating in the assimilation and oxidation of ammonia during the treatment period. Consequently, the increase in MLSS not only represents biomass growth but also confirms that a balanced supply of carbon and nitrogen plays a crucial role in maintaining the effectiveness of ammonia biodegradation in the EM4-based biological system.

Comparison of Biological Kinetic Models

The increase in MLSS values observed across the variations in ammonia concentration indicates a close relationship between biomass growth and the rate of substrate degradation. This finding confirms that the selection of an appropriate kinetic model cannot be separated from the biomass dynamics that develop during the treatment process. Therefore, several kinetic modeling approaches were evaluated to determine the method that most appropriately represents the ammonia biodegradation process in this system, as presented in Table 2.

Table 2. Comparative Evaluation of Kinetic Models.

Kinetic Models	Kinetic Parameter	K/Ks value (50, 30, 10)	R^2 (50 ppm)	R^2 (30 ppm)	R^2 (10 ppm)
Zero-order	k_0 (ppm/day)	7.693, 4.463, 1.341	0.942	0.932	0.948
Pseudo-first-order	K_1 (day^{-1})	0.341, 0.316, 0.250	0.958	0.975	0.857
Second-order	K_2 ($ppm^{-1}. day^{-1}$)	0.019, 0.027, 0.056	0.923	0.956	0.737
Monod	r_{max}, Ks	-50.99, -36.65, -1.35	0.783	0.762	0.179

As presented in Table 2, the comparison of four kinetic models—zero-order, pseudo-first-order, second-order, and Monod—reveals distinct differences in their ability to represent the experimental data. The zero-order model produced relatively high rate constants with reasonably good coefficients of determination (R^2 = 0.932–0.948). However, the assumption of a constant degradation rate over time does not adequately represent biological systems, in which the reaction rate generally decreases as the substrate concentration declines.

The second-order model yielded varying rate constants (0.019, 0.027, and 0.056) with relatively high R^2 values at moderate and high concentrations, but a substantial decline at low concentration (R^2 = 0.737). This result indicates that the second-order model tends to overestimate the degradation rate at lower substrate concentrations.

Meanwhile, the Monod model generated non-physical parameters, including negative values for both r_{max} and K_s, along with relatively low R^2 values (0.179–0.783). These results suggest that the Monod model does not adequately fit the experimental data for this simple batch system. In contrast, the pseudo first-order model provided the most consistent results, where the rate constant increased with increasing substrate concentration (0.250, 0.316, and 0.341 day^{-1}) and the R^2 values remained relatively high (0.857–0.975). These findings indicate that the pseudo-first-order model more effectively represents the relationship between substrate concentration, time, and biomass growth (MLSS).

Pseudo First-Order Kinetic Analysis

The evaluation of ammonia removal rates was performed using the pseudo-first-order kinetic model formulated according to Equation (1), where S_0 and S_t represent the ammonia concentration at the initial time and at time t, respectively, while k denotes the constant reaction rate (day^{-1}). This model was selected to describe the degradation dynamics under conditions where other influencing factors—such as carbon availability and aeration—were maintained relatively constant, thereby allowing the reaction rate to depend primarily on the ammonia concentration.

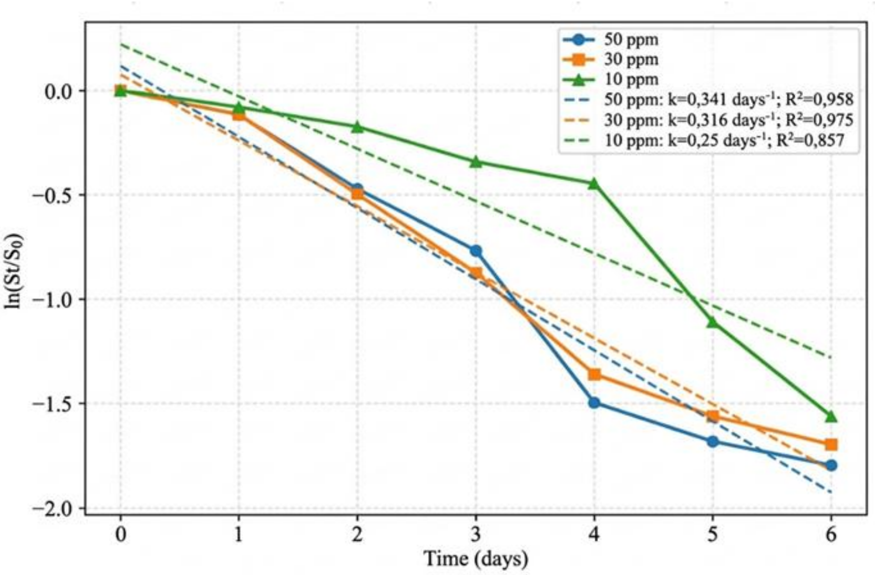

Figure 4. Plot of $ln(S_t/S_0)$ versus time (t), including regression lines and the calculated parameters k and R^2 for initial ammonia concentrations of 10, 30, and 50 ppm.

The plot of $ln(S_t/S_0)$ against time (Figure 4) shows a decreasing linear relationship for all initial concentration variations (10, 30, and 50 ppm), indicating that ammonia removal proceeded continuously and followed pseudo-first-order behavior throughout the operational period. The increasingly negative values of $ln(S_t/S_0)$ with time confirm the progressive depletion of substrate, while the differences in the slopes of the regression lines among treatments reflect variations in the rate constant k resulting from differences in substrate conditions and active biomass. The suitability of the model is further supported by the relatively high coefficients of determination (R^2), demonstrating the reliability of this approach in representing the experimental data.

Table 3. Pseudo First-Order Kinetic Parameters.

Ca NH_3 (ppm)	MLSS (mg/L)	k ($^{day-1}$)	R^2	General Description
10	±2000	0.250	0.857	Relatively low biological activity due to limited biomass [21]
30	±3200	0.316	0.975	Increased biomass drives higher biodegradation rates [22]
50	±4600	0.341	0.958	High biomass increases the frequency of substrate-microorganism contact and reactions [23]

Quantitatively, as presented in Table 3, the system with an initial ammonia concentration of 10 ppm produced a rate constant of k = 0.250 day^{-1} with an MLSS value of approximately ±2.000 mg/L. At 30 ppm, the rate constant increased to k = 0.316 day^{-1} with an MLSS of ±3.200 mg/L, while at 50 ppm, the value of k reached 0.341 day^{-1} with an MLSS of ±4.600 mg/L. The gradual increase in the k value with increasing initial ammonia concentration and MLSS indicates that biomass accumulation enhances the frequency of microbe–substrate interactions, accelerates ammonia transformation, and ultimately increases the effective reaction rate. In other words, the sensitivity of k to biomass levels highlights the significant role of biological factors—namely, the number and activity of microbial cells—in controlling the biodegradation rate in the EM4-based system supported by an external carbon source. Within the concentration range of 10–50 ppm, no evidence of inhibition was observed, as reflected by the increasing rate constant (k) and removal efficiency, indicating that the system operated under conditions favorable for microbial activity. The positive correlation between k and MLSS further suggests that managing the C/N ratio through palm sugar supplementation effectively maintains microbial energy availability, stabilizes the process, and accelerates the degradation kinetics. The strong linearity observed in the $ln(S_t/S_0)$ curve supports the use of k as a simple performance indicator for routine monitoring of the process.

The kinetic analysis using the pseudo-first-order model also demonstrates a consistent relationship between the constant reaction rate (k), biomass growth indicated by MLSS values, and the high linearity of the $ln(S_t/S_0)$ versus time plot. To strengthen the validity of the model, the results of this study were compared with several previous studies that applied similar kinetic models in biological biodegradation systems. The comparison of constants reaction rate and coefficients of determination is presented in Table 4.

Table 4. Comparison of Pseudo First-Order Kinetic Parameters from This Study and Previous Studies

Researcher & year	System/Substrate	Kinetic Model	K value ($^{day-1}$)	R^2
This study	Batch system, ammonia	Pseudo-first-order	0.25 - 0.34	0.857- 0.975
Gutiérrez-Macías (2022) [24]	Batch system, MBR sludge, pharmaceutical compounds	Pseudo-first-order	0.22 - 0.31	0.88 - 0.95
Pan (2020) [15]	Hydrodynamic system, ammonia	First-order	0.01 – 0.05	0.85 – 0.95
Hooshyari (2008) [25]	General biological systems	First-order	0.06 – 0.16	0.52 – 0.86

Based on Table 4, the comparison between the results of this study and previous studies shows a consistent application of pseudo-first-order and first-order kinetic models to describe the biodegradation kinetics of ammonia and other nitrogen compounds. The constants reaction rate (*k*) obtained in this study fall within a range comparable to those reported by other researchers, while the relatively high coefficients of determination (R^2) confirm the linear relationship between substrate concentration and time. This similarity in trends strengthens the conclusion that the pseudo first-order model represents the most appropriate approach, not only for a simple batch system with carbon supplementation as investigated in this study, but also for various biological reactor configurations reported in the literature. Therefore, the findings of this research are not only internally valid but also demonstrate strong external relevance, suggesting that the pseudo-first-order model can be recommended as a primary method for kinetic analysis of ammonia biodegradation.

Mechanism of Ammonia Biodegradation using EM4 with Palm Sugar Supplementation

Ammonia biodegradation in the EM4-based system supplemented with palm sugar occurs through nitrogen assimilation by microorganisms as well as biological transformation processes under aerobic conditions. Ammonia serves as a nitrogen source for the synthesis of proteins and cellular components, thereby supporting microbial biomass growth during the treatment process. This phenomenon is reflected in the increase in MLSS values from approximately 2.000 mg/L at an initial ammonia concentration of 10 ppm to around 4.600 mg/L at 50 ppm. In addition to assimilation, a portion of the ammonia may undergo biological oxidation by nitrifying microorganisms under continuous aeration conditions. This process results in the gradual transformation of ammonia into more oxidized and relatively stable nitrogen forms. The addition of palm sugar as an external carbon source plays an important role in maintaining the balance of the C/N ratio and providing readily biodegradable organic substrates for heterotrophic microorganisms. The availability of this carbon source can enhance microbial metabolic activity and accelerate the biodegradation process. From a kinetic perspective, this condition is reflected in the increase in the ammonia biodegradation rate constant from 0.250 day^{-1} at an initial concentration of 10 ppm to 0.341 day^{-1} at 50 ppm. The increase in the rate constant indicates that the addition of ammonia substrate within the investigated concentration range still supports microbial activity without causing inhibitory effects. Therefore, ammonia removal in this system is influenced by the combined effects of microbial biomass growth and biological nitrogen transformation processes within an aerobic system enriched with an external carbon source.

CONCLUSION

The EM4-based biodegradation system supplemented with palm sugar effectively reduced ammonia across all tested initial concentrations. The process proceeded in a stable manner, as indicated by the increase in microbial biomass, pH values within the optimal range, and a strong agreement with the pseudo-first-order kinetic model. The increasing constant reaction rate with higher substrate concentrations suggests that system performance is strongly influenced by biomass accumulation and carbon availability. The optimal ammonia concentration variation was 50 ppm, which produced the highest removal efficiency of 83.4%, the greatest MLSS value of approximately ±4.600 mg/L, and the highest constant reaction rate 0.25 t ($k = 0.341$ day^{-1}), thereby reflecting the most optimal biodegradation performance observed in this study.

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