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Monod Kinetics for EM4-Driven Ammonia Biodegradation Under Molasses Variation and Microbial Acclimatization

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Abstract

Ammonia is a toxic pollutant parameter in petrochemical wastewater and can impair the quality of aquatic environments. This experimental study evaluated the kinetics of ammonia biodegradation by an Effective Microorganisms-4 (EM4) consortium using the Monod model approach, focusing on molasses concentration as an external carbon source and the effect of microbial acclimatization. This experiment was conducted in aerobic batch systems with initial ammonia concentrations of 10, 30, and 50 ppm and the addition of 5% and 10% molasses. Mixed Liquor Suspended Solids (MLSS) and pH were monitored to evaluate environmental dynamics and biomass growth. The results showed that increasing the molasses concentration from 5% to 10% increased the maximum specific growth rate (μ_{max}) from 6.29 to 7.17 day^{-1} and decreased the half-saturation constant (K_s) from 23.22 to 14.01 mg/L; therefore, a lower K_s indicates higher apparent substrate affinity. The acclimatization further improved the kinetic response, yielding higher μ_{max} and lower K_s than the non-acclimated system, alongside higher MLSS and a faster pH decrease consistent with more intensive biodegradation activity. Overall, these results confirm that the combination of microbial acclimatization and sufficient molasses addition can enhance the kinetic performance of ammonia biodegradation by EM4.

1. Introduction

Ammonia (NH_3/NH_4^+) is a critical water quality parameter due to its toxicity to aquatic organisms and its contribution to eutrophication [1]. In the industrial context, ammonia is a major component of petrochemical wastewater generated by processes such as synthetic ammonia production, urea manufacturing, nitration, hydrocarbon cracking, as well as utility streams, including cooling tower blowdown and condensate wastewater [2]. Ammonia concentrations in petrochemical effluents can reach levels that inhibit natural biological processes and may pollute the environment if not properly treated. Therefore, ammonia removal a crucial aspect of modern petrochemical wastewater management [3].

The bioremediation of ammonia using microbial consortia has been widely investigated as an effective approach for wastewater treatment. One microbial product commonly used in Indonesia and reported in applied studies is Effective Microorganisms 4 (EM4). EM4 has demonstrated acceleration of organic transformation and helps reduce nutrient parameters, including ammonia, when implemented in biofloc, biofilm, or controlled-aeration systems. Field and laboratory-scale studies have shown the potential of EM4 to reduce ammonia and other indicators, such as BOD/COD, in various wastewater streams, including hospital wastewater [4], surfactant-containing wastewater [5], tempeh industry wastewater [6], and shrimp pond effluent [7].

The selection of a supplementary organic carbon source (nutrient) plays an important role in the degradation of ammonia by heterotrophic and autotrophic microbial communities. Molasses is often used as a low-cost and readily available carbon source to enhance heterotrophic microbial activity and promote biotransformation processes [6]. Numerous studies have examined the use of molasses as a carbon source in wastewater treatment and fermentation processes, as well as its effects on removal efficiency and microbial community stability [8]. The application of molasses increases the availability of degradable carbon that supports the growth of competent microorganisms involved in ammonia uptake and assimilation [9].

In this context, the commercial EM4 consortium is primarily composed of heterotrophic microorganisms, including lactic acid bacteria, yeasts, and other fermentative microbes [10]. Under conditions with abundant organic carbon supplied by molasses, ammonia removal is expected to occur predominantly through heterotrophic nitrogen assimilation into microbial biomass rather than through autotrophic nitrification [11]. Therefore, the kinetic parameters obtained in this study should be interpreted as indicators of microbial growth and ammonia assimilation performance.

Kinetic modeling can be used to understand the rate and mechanisms of ammonia biodegradation by microbial consortia. The Monod model is widely applied to determine kinetic parameters (μ_{max} and K_s) that help characterize the degradation capability of a given inoculum toward a specific substrate [12]. The applicability of the Monod model in describing microbial growth and substrate utilization makes it a useful tool for evaluating the performance of biological ammonia removal systems under different operational conditions.

An operational factor that determines the success of biodegradation is the acclimatization of microorganisms to new environmental conditions and substrates. Acclimatization (gradual adaptation) can increase microbial tolerance to higher ammonia concentrations, alter microbial community composition, and improve degradation kinetics [13]. Studies on wastewater treatment systems have shown that acclimatized microbial communities are able to tolerate elevated ammonia concentrations and maintain more stable ammonia removal performance than non-acclimated communities [14]. Gradual adaptation promotes physiological adjustment, improves substrate utilization efficiency, and enhances process stability under high ammonia loading conditions [15]. Therefore, evaluating the effect of acclimatization becomes important when applying EM4 under different conditions and in combination with carbon sources such as molasses.

The performance of microorganisms in biodegradation processes, including cultures such as EM4, is strongly influenced by environmental conditions such as pH and biomass concentration, as represented by mixed liquor suspended solids (MLSS) [16]. Several studies have shown that when pH deviates from the optimal range, microbial growth and ammonia assimilation efficiency can decrease significantly due to reduced enzymatic activity and metabolic performance [17, 18]. Meanwhile, high MLSS allows sufficient accumulation of microbial biomass, thereby increasing degradation potential and resilience to environmental fluctuations. Recent studies even indicate that systems with high MLSS can optimize nitrogen and ammonia removal efficiently while maintaining the stability of biological reactors [16]. Therefore, in this study, in addition to variables such as molasses and acclimatization, monitoring pH and MLSS is essential as control indicators of EM4 performance in ammonia degradation.

Despite the extensive application of EM4 as a microbial consortium in biological wastewater treatment, quantitative information regarding its kinetic behavior during ammonia removal remains limited, particularly under conditions representative of petrochemical wastewater. Previous studies have primarily focused on the general effectiveness of EM4 in reducing pollutant concentrations, while relatively little attention has been given to the determination of kinetic parameters governing microbial growth and ammonia utilization. Furthermore, the combined influence of external carbon supplementation and microbial acclimatization on the kinetic performance of EM4 consortia has not been comprehensively investigated. This limitation restricts the understanding of how operational conditions affect ammonia removal efficiency and microbial growth dynamics, thereby hindering the optimization and scale-up of biological treatment processes. To address these gaps, the present study evaluates the Monod kinetic characteristics of ammonia removal by an EM4 consortium under different operational conditions.

The novelty of this work lies in the integrated assessment of molasses supplementation and microbial acclimatization as factors influencing ammonia biodegradation kinetics. In addition, the study emphasizes heterotrophic ammonia assimilation as the dominant nitrogen removal pathway under high-carbon conditions, providing a more mechanistic interpretation of the role of EM4 in ammonia removal. Experimental variables include molasses concentrations of 5% and 10%, acclimatized and non-acclimatized microbial cultures, and the monitoring of ammonia concentration, pH, and MLSS throughout the treatment process. The resulting kinetic parameters (μ_{max} and K_s) are expected to provide valuable information to improve process design, operational control, and the future development of biological ammonia treatment systems.

Based on the above considerations, this study investigates the Monod kinetics of ammonia removal by an EM4 consortium under varying molasses concentrations and acclimatization conditions. Given the heterotrophic nature of the EM4 microbial community and the high availability of organic carbon from molasses, ammonia removal is interpreted primarily as heterotrophic nitrogen assimilation into microbial biomass. Furthermore, pH and MLSS dynamics were evaluated to provide additional insight into biomass development and process performance during ammonia biodegradation.

2. Experimental

2.1. Materials

The materials used in this study included synthetic ammonia solution (NH_4OH) as the substrate, EM4 as the microbial inoculum, molasses as the carbon source, and buffer solutions for pH control. The main equipment consisted of covered glass batch reactors, an aerator, a pH meter, a UV–Vis spectrophotometer for ammonia analysis, an analytical balance, an oven, and supporting equipment for MLSS analysis.

2.2. Procedures

2.2.1. Microorganism Acclimatization

The acclimatization process was carried out in an aerobic glass batch reactor with a working volume of 1,500 mL. EM4 was inoculated into the reactor at a volume ratio of 10% (v/v) relative to the medium. The acclimatization medium consisted of deionized water supplemented with molasses at an initial concentration of 500 mg/L, which was then gradually increased to 1,000 and 1,500 mg/L every 48 h.

The system was operated under aerobic conditions using an aquarium aerator at an airflow rate of approximately 2–3 L/min, maintaining dissolved oxygen above 3 mg/L. Homogenization was achieved using a magnetic stirrer at 100 rpm. The operating temperature was maintained at 27 ± 2°C. The pH of the solution was monitored and maintained within the range of 6.8–7.5 using a digital pH meter and, when necessary, by adding phosphate buffer solution. The acclimatization process lasted 6–8 days until a stable condition was achieved, as indicated by a relatively constant pH. Beyond pH stabilization, the acclimatization process was quantitatively defined as successful and 'stable' when the biomass concentration, measured as MLSS, reached a steady state with a variance of less than 5% over three consecutive days. Additionally, a consistent daily reduction in ammonia during this period was used as a supplementary criterion to ensure that the microbial consortium had fully adapted to the toxic ammonia levels.

2.2.2. Ammonia Degradation

Ammonia removal tests were performed using synthetic ammonia solutions (NH_4OH) at initial concentrations of 10, 30, and 50 ppm. Experiments were conducted in aerobic glass batch reactors with a working volume of 1,000 mL for each concentration. The NH_4OH solutions were prepared by diluting concentrated NH_4OH in deionized water to reach the target concentrations.

EM4 was then added to each reactor at a volume ratio of 1:10 (v/v) relative to the NH_4OH solution, resulting in 100 mL of EM4 and 900 mL of ammonia solution in each reactor. The reactors were operated aerobically using aquarium aerators at an airflow rate of approximately 2– 3 L/min, maintaining dissolved oxygen above 3 mg/L. Mixing was provided by a magnetic stirrer at 100 rpm to ensure homogeneity. All experiments were carried out at room temperature (27 ± 2°C) and lasted for 6 days (144 h).

During the experimental period, pH was measured every 24 h using a calibrated digital pH meter to monitor environmental conditions affecting microbial activity. In addition, MLSS were measured every 24 h using the gravimetric method. A 5 mL sample was taken from each reactor and placed in a crucible dish. The sample was then dried in an oven at 105°C to constant weight, cooled in a desiccator, and weighed again. MLSS values were calculated as the weight difference between before and after drying, divided by the sample volume.

Sampling for ammonia concentration analysis was also performed every 24 h throughout the experiment. The temporal changes in ammonia concentration were subsequently used to evaluate ammonia removal efficiency and biodegradation kinetics.

2.2.3. Ammonia Analysis Using the Nessler Method

Ammonia concentration was determined using the Nessler spectrophotometric method. A 10 mL sample was taken from the reactor and, if necessary, diluted with deionized water to fall within the measurement range. Then, 0.5 mL of Nessler reagent was added, the mixture was homogenized, and allowed to react for 10 minutes at room temperature until a yellow–brown color developed. To account for the background color from residual molasses, a sample blank was prepared for each treatment, containing wastewater and molasses without Nessler reagent.

The absorbance of the blank was measured at 425 nm and subtracted from the absorbance of the corresponding Nessler-treated sample prior to ammonia concentration calculation. Residual molasses may contribute to background absorbance because its natural brown coloration can partially absorb light in the visible wavelength range used for the Nessler assay. Since the Nessler reaction produces a yellow–brown complex whose intensity is measured at 425 nm, residual molasses color may artificially increase absorbance values and lead to overestimation of ammonia concentration if not properly corrected. Therefore, treatment-specific sample blanks were used to eliminate the contribution of molasses-derived background color from the ammonia measurements.

The absorbance was measured using a UV–Vis spectrophotometer at a wavelength of 425 nm with a 1- cm glass cuvette. Ammonia concentration was determined using a calibration curve prepared from standard ammonia solutions at 0, 2, 3, 5, 7, and 10 ppm, which showed a linear relationship between absorbance and ammonia concentration. The resulting linear equation was used to calculate ammonia levels in the reactor samples over the 6-day period.

2.2.4. Monod Kinetic Analysis

The Monod kinetic model can be linearized using the Lineweaver–Burk approach, resulting in Equation (1).

$$\frac{1}{\mu} = \frac{K_S}{\mu_{max}} \times \frac{1}{[S]} + \frac{1}{\mu_{max}} \quad (1)$$

Where, μ is the specific growth rate of microorganisms (day^{-1}), $[S]$ is the ammonia substrate concentration (mg/L), μ_{max} is the maximum specific growth rate (day^{-1}), and K_s is the half-saturation constant (mg/L).

The Monod kinetic parameters were determined using a linearization approach based on ammonia concentration depletion data during biodegradation. The specific growth rate (μ) was approximated from the ammonia substrate consumption rate, assuming that the rate of ammonia decrease is proportional to microbial growth activity in the batch system. In this study, the specific growth rate was estimated indirectly from ammonia utilization data rather than from direct measurements of biomass growth. This approach assumes that ammonia removal is predominantly associated with microbial assimilation and growth, with a relatively constant biomass yield throughout the cultivation period. Accordingly, changes in ammonia concentration were used as a proxy for microbial activity to evaluate the kinetic behavior of the EM4 consortium.

A plot of the inverse specific growth rate ($1/\mu$) versus the inverse ammonia substrate concentration ($1/[S]$) was constructed, and the resulting linear relationship was analyzed using linear regression to obtain the line equation. The intercept was used to determine μ_{max}, and the slope to calculate K_s. This approach enables the characterization of ammonia biodegradation kinetics by EM4 based directly on substrate consumption data. All batch experiments, including acclimatized treatments, non-acclimatized treatments, and controls, were performed in triplicate to ensure the reliability and reproducibility of the results.

3. Results and Discussion

3.1. Influence of Molasses Concentration

3.1.1. Monod Kinetics

In the present study, the substrate concentration (S) used in the Monod model corresponds to total ammonia nitrogen (TAN) as determined by the Nessler spectrophotometric method [19]. Although TAN includes unionized ammonia (NH_3) and ammonium ions (NH_4^+), the experimental pH was predominantly neutral to slightly acidic, where ammonium is the dominant species [20]. Consequently, the measured TAN values are considered to primarily reflect the availability of ammonium for heterotrophic microbial assimilation. Therefore, TAN was used as the substrate concentration in the kinetic analysis.

Figure 1 presents the linear relationship between $1/\mu$ and $1/S$ based on the linearized Monod (Lineweaver–Burk) model at molasses concentrations of 0%, 5%, and 10%. All molasses variations produced straightforward correlations, indicating that ammonia biodegradation by EM4 can be adequately fitted using the Monod kinetic model. Differences in the slopes and intercepts of the two regression lines reflect differences in microbial kinetic characteristics resulting from variations in carbon source availability. The Monod kinetic results obtained at molasses concentrations of 0%, 5%, and 10% (Table 1) indicate that increasing molasses concentration as a carbon source significantly affects the kinetic parameters of ammonia biodegradation by the EM4 inoculum. In general, increasing the molasses concentration from 0% to 10% accelerated microbial growth rates and improved substrate utilization efficiency.

The higher μ_{max} value at 10% molasses (7.17 day^{-1}) compared to 5% molasses (6.29 day^{-1}) and 0% molasses (0.89 day^{-1}) indicates that greater carbon availability enhances the maximum microbial growth capacity. This finding is consistent with previous reports showing that increasing the supply of carbon sources can accelerate heterotrophic metabolism and the consumption rate of nutrients such as nitrogen/ammonia in biological systems, as more energy and electrons from carbon substrates are available for microbial growth and degradation processes [21]. In addition, the lower K_s value at 10% molasses (14.01 mg/L) than at 5% molasses (23.22 mg/L) and 0% molasses (24.16 mg/L) suggests greater substrate affinity at higher molasses concentrations.

Biologically, the lower K_s value observed in the 10% molasses treatment indicates a greater apparent capacity of the microbial community to utilize ammonia at relatively low substrate concentrations. However, this result should not be interpreted as evidence of an intrinsic increase in microbial affinity toward ammonia [22, 23]. Rather, the lower K_s likely reflect enhanced metabolic activity and biomass growth promoted by the additional carbon source supplied by molasses [24]. The improved carbon availability supports cellular energy production and microbial proliferation, thereby increasing the overall efficiency of ammonia assimilation under the experimental conditions [24].

The relatively high μ_{max} values observed in this study may be partially attributable to the indirect estimation approach, in which specific growth rates were derived from ammonia consumption rather than from direct biomass growth measurements. Because ammonia removal was used as a proxy for microbial activity, the calculated μ_{max} values should be interpreted as apparent kinetic parameters reflecting the overall ammonia assimilation performance of the microbial consortium [25]. In addition, the presence of readily biodegradable carbon supplied by molasses may have stimulated rapid microbial metabolism and biomass development, contributing to higher apparent growth rates under the experimental conditions [26].

The μ_{max} values obtained in this study were generally higher than those reported for several microbial systems involved in ammonia removal. For example, activated sludge used for NH_4^+-N nitrification has been reported to exhibit a μ_{max} value of approximately 0.057 d^{-1} [27], while Nitrosomonas PY1 reported a μ_{max} of 0.20 d^{-1} [28]. In addition, *Comamonas* sp. GAD4 and *Agrobacterium* sp. LAD9 showed μ_{max} values of 0.433 h^{-1} and 0.286 h^{-1}, respectively [29]. The relatively high μ_{max} values observed in the present study may be attributed to the availability of readily biodegradable carbon supplied by molasses, which can stimulate microbial metabolism and biomass growth.

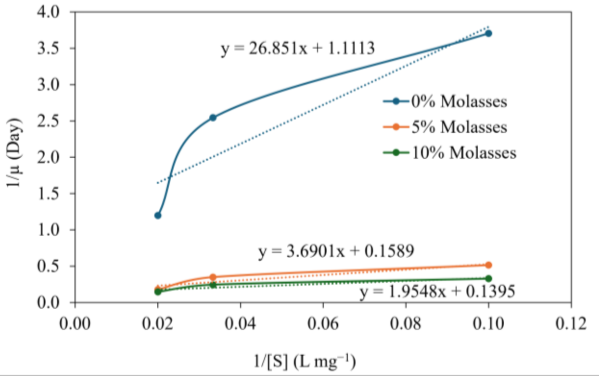

Figure 1. Comparison of Monod kinetic parameters at 0%, 5%, and 10% molasses concentrations

Table 1. Effect of molasses addition on μ_{max} and K_s

Percentage (%)	μ_{max} (day⁻¹)	K_s (mg/L)
0	0.89	24.16
5	6.29	23.22
10	7.17	14.01

Furthermore, differences in microbial consortium composition, substrate characteristics, reactor operation, and kinetic estimation methods may contribute to variations in μ_{max} values reported across studies. Therefore, direct comparison should be made with caution, and the μ_{max} values obtained should be interpreted as apparent kinetic parameters that reflect the overall ammonia-assimilation performance of the EM4 consortium rather than intrinsic microbial growth rates.

While the kinetic parameters obtained provide valuable information on ammonia removal by the EM4 consortium, they should be interpreted as indicators of heterotrophic ammonia assimilation performance rather than as direct measures of microbial growth kinetics. Since the specific growth rate was estimated indirectly from ammonia consumption data, the calculated values represent apparent growth rates associated with substrate utilization under the experimental conditions. In addition, biomass production, microbial community composition, and yield coefficients were not measured directly, preventing a detailed assessment of the intrinsic growth characteristics of the microbial consortium. Therefore, the reported kinetic parameters should be viewed as system-level indicators of ammonia assimilation efficiency rather than absolute descriptors of microbial growth behavior.

3.1.2. pH

Figure 2 illustrates pH variations over six days of observation, showing distinct dynamics among the systems supplemented with 0%, 5%, and 10% molasses. In general, all treatments showed a decreasing pH trend over time, indicating ongoing microbial metabolic activity during biodegradation. The initial pH stability suggests that microbial activity was still balanced, with the production of acidic compounds from organic metabolism being neutralized by the system. The subsequent decrease in pH indicates intensified biodegradation activity, particularly the formation of organic acids and acidic intermediates from nitrogen metabolism.

In contrast, in the 10% molasses treatment, the initial pH was lower (around 5.8–5.9) and showed a faster, more consistent decline than in the 5% molasses treatment. From day 4 to day 6, the pH of the 10% molasses system ranged from approximately 5.4 to 5.5. This sharper decrease in pH indicates that increased carbon availability accelerated heterotrophic microbial metabolism, leading to the production of more by-products, such as organic acids, dissolved CO_2, and other intermediates, thereby lowering the solution pH.

The decrease in pH observed during the experiment can be attributed to the formation of volatile fatty acids (VFAs) during molasses fermentation and the dissolution of CO_2 produced by microbial metabolism [30, 31]. These processes contribute to the accumulation of acidic compounds in the system, resulting in lower pH values. In addition to VFA production and CO_2 dissolution, the relatively acidic initial pH may also have influenced microbial activity during the treatment process [32]. pH is an important environmental factor affecting microbial growth, enzyme activity, and nutrient uptake [33]. Although the microorganisms present in EM4 are generally tolerant of mildly acidic conditions, excessively low pH can reduce metabolic activity and biomass development, thereby limiting ammonia assimilation efficiency [33].

Nevertheless, the pH values observed in this study remained within a range conducive to heterotrophic microbial activity, enabling effective ammonia removal throughout the experimental period. These pH conditions have functional implications because they affect enzymatic activity and the balance of the microbial community. It is important to note that the EM4 consortium consists primarily of heterotrophic microorganisms, such as lactic acid bacteria and yeasts [34]. Therefore, the observed ammonia removal in this system is primarily driven by heterotrophic assimilation (in which ammonia is directly incorporated into newly synthesized microbial biomass as a nitrogen source) rather than by autotrophic nitrification.

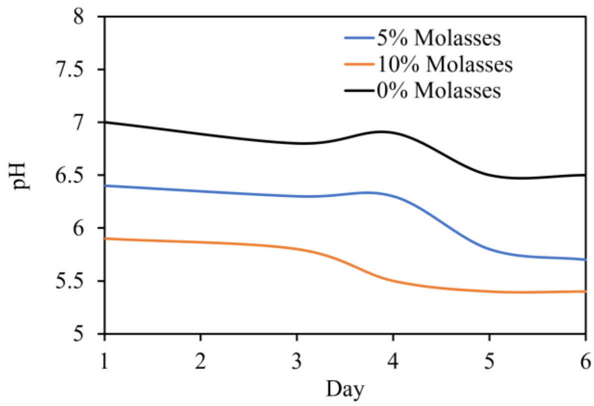

Figure 2. Effect of 0%, 5%, and 10% molasses concentration addition on pH

Consequently, the significant pH decrease, particularly in the 10% molasses system, is driven by robust heterotrophic fermentation of molasses, which generates VFAs and dissolved CO_2, rather than by the release of H^+ ions typically associated with ammonia oxidation [31, 34, 35]. Therefore, these results indicate a trade-off: higher carbon supply enhances degradation rates (higher μ_{max}) but also creates a more acidic environment that, if not controlled, may suppress the activity of pH-sensitive microbial groups [36]. Overall, the pH measurement results confirm that the addition of molasses influences the chemical and biological balance of the system, with higher molasses concentrations accelerating biodegradation activity and leading to a more pronounced pH decrease.

3.1.3. MLSS

The MLSS observed on day 6 shows that increasing the ammonia concentration from 10 to 50 ppm was accompanied by an increase in MLSS (Figure 3). This indicates that ammonia serves as a nitrogen source that supports microbial growth, provided its concentration remains within the microbial tolerance range. The consistently higher MLSS values observed at 10% molasses compared to 0% and 5% molasses confirm that greater carbon availability promotes more intensive microbial biomass growth [37]. Molasses serves as a readily biodegradable carbon source that provides energy and electrons for cell synthesis and metabolic activity [38].

A more balanced combination of nitrogen (ammonia) and carbon (molasses) enhances the formation of active biomass, as reflected by the increase in MLSS. This increase in MLSS is mechanistically linked to the Monod kinetic parameters evaluated in this study. The higher μ_{max} observed in the 10% molasses treatment directly drives more rapid cellular division and active biomass accumulation, explaining the physically higher MLSS values [39]. Furthermore, the lower K_s indicates that this expanded biomass maintains a high affinity for the substrate [40]. This allows the growing microbial population (reflected in the high MLSS) to effectively assimilate ammonia for cell synthesis even as the ammonia concentration gradually declines in the batch reactor.

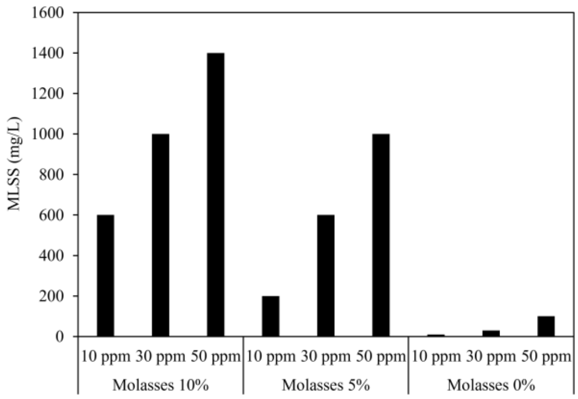

Figure 3. MLSS values on day 6

3.1.4. Ammonia Removal Efficiency

Figure 4 shows that ammonia removal efficiency increased over time across all molasses concentrations, although the trends in both rate and final performance differed markedly. During the initial phase (day 0–6), both systems exhibited very low, nearly stagnant removal efficiencies. This behavior indicates that the microbial community was still in the lag phase, adapting to the new environmental conditions and ammonia substrate [41]. At this stage, microbial metabolic activity remained limited due to incomplete enzymatic adjustment and acclimatization processes [42].

A significant increase in ammonia removal efficiency was observed between day 7 and day 10, particularly in the system supplemented with 10% molasses. This sharp rise suggests that the microorganisms had entered the exponential growth phase, where biodegradation activity and biomass production were substantially enhanced [43]. The faster response observed at 10% molasses indicates that a higher availability of organic carbon effectively stimulates heterotrophic metabolism, thereby accelerating ammonia assimilation [44].

In the later stage (day 10–15), the difference between the two treatments became more pronounced. The system with 10% molasses achieved removal efficiencies approaching 95–100%, whereas the 5% and 0% molasses systems reached only approximately 75–80% and 10- 15%. This result confirms that increasing molasses concentration significantly enhances ammonia biodegradation performance. Mechanistically, molasses serves as a readily biodegradable carbon source that provides energy and electron donors required for heterotrophic microbial growth. A higher carbon availability improves the C/N ratio, facilitating more efficient ammonia assimilation into microbial biomass [45]. In contrast, the lower carbon supply in the 5% and 0% molasses systems limits microbial activity and slows biodegradation. Furthermore, the superior performance of the 10% molasses system is consistent with the kinetic parameters obtained in this study, namely, higher μ_{max} and lower K_s. These parameters indicate enhanced microbial growth capacity and stronger substrate affinity, which contribute to faster and more efficient ammonia removal.

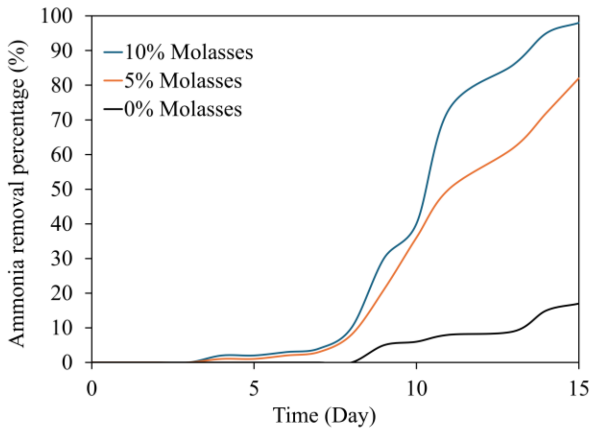

Figure 4. Ammonia removal efficiency at various molasses concentrations

However, it is important to note that increasing molasses concentration may also lead to a more significant decrease in pH due to the formation of organic acids during microbial metabolism [46]. This suggests the presence of a trade-off between enhanced biodegradation kinetics and system stability, particularly in terms of pH control. Overall, these findings demonstrate that the addition of molasses as an external carbon source plays a critical role in improving ammonia removal efficiency, with the 10% concentration providing the most optimal performance under the conditions studied.

3.2. Influence of Microorganism Acclimatization

3.2.1. Monod Kinetics

Figure 5 presents a comparison of the linear relationships between $1/\mu$ and $1/[S]$ based on the linearized Monod model for two conditions: 1) with acclimatization and 2) without acclimatization prior to the ammonia biodegradation process. Both conditions exhibit good linear relationships, indicating that the ammonia biodegradation kinetics in this system can be adequately described using the Monod model. However, differences in the slopes and intercepts of the regression lines indicate that the acclimatization process significantly influences microbial kinetic parameters. The non-acclimatized system shows a steeper slope and higher $1/\mu$ values over the same range of $1/[S]$ compared to the acclimatized system. This indicates a lower specific growth rate and a more limited affinity toward the ammonia substrate. This condition can be attributed to the limited preparedness of the microbial community to cope with the initial ammonia concentrations, such that part of the metabolic energy is diverted to physiological adaptation to environmental stress rather than to growth and substrate degradation.

In contrast, in the acclimatized system, $1/\mu$ values are consistently lower, reflecting higher microbial growth rates at the same substrate concentrations. The acclimatization process allows microorganisms to adjust their enzymatic systems and community composition to the presence of ammonia, thereby improving substrate utilization efficiency [47]. The calculated Monod kinetic parameters presented in Table 2 indicate that microbial acclimatization prior to ammonia biodegradation significantly affects both the μ_{max} and the K_s. The acclimatized system yielded a μ_{max} value of 7.17 day^{-1}, which is higher than that of the non-acclimatized system (6.34 day^{-1}). This increase in μ_{max} indicates that acclimatized microorganisms have a greater maximum growth capacity, reflecting enhanced physiological and metabolic readiness to utilize ammonia as a substrate.

Furthermore, the K_s value in the acclimatized system (14.01 mg/L) is much lower than that in the non-acclimatized system (35.45 mg/L). A lower K_s value indicates a higher substrate affinity, meaning that microorganisms can maintain relatively high growth rates even at lower ammonia concentrations. This demonstrates that acclimatization not only increases growth rate but also improves substrate utilization efficiency within the microbial community. Biologically, these differences in μ_{max} and K_s can be explained by the gradual adaptation of the microbial community during the acclimatization process, which enables the selection of ammonia-tolerant microorganisms and the optimization of metabolic pathways involved in ammonia assimilation and cellular growth [48].

Microorganisms that have undergone acclimatization tend to exhibit more stable enzyme expression and more effective metabolic pathways for ammonia utilization, resulting in better kinetic performance than microorganisms without prior adaptation [47]. Overall, these results indicate that implementing an acclimatization stage prior to the ammonia biodegradation process is highly recommended to enhance microbial kinetic performance, both in terms of growth rate and substrate utilization efficiency. Therefore, acclimatization can be considered an effective operational strategy for treating ammonia-containing wastewater.

The improved kinetic performance observed after acclimatization is likely attributable to a combination of increased biomass concentration [49] and the physiological adaptation of the microbial community [50]. The higher MLSS values obtained in the acclimatized systems indicate greater biomass availability, which contributes directly to enhanced substrate utilization capacity [49]. In addition, gradual exposure to ammonia during the acclimatization period may have promoted the selection of ammonia-tolerant microorganisms and stimulated metabolic adaptation to elevated ammonia conditions [14]. However, because microbial community composition was not characterized in the present study, the relative contributions of biomass increase and community-level adaptation cannot be quantitatively distinguished. Therefore, the observed improvement in kinetic performance is attributed to the combined effects of biomass enrichment and microbial adaptation.

Table 2. Effect of acclimatization on μ_{max} and K_s

	μ_{max} (Day^{-1})	K_s (mg/L)
Acclimatized	7.17	14.01
Non-Acclimatized	6.34	35.45

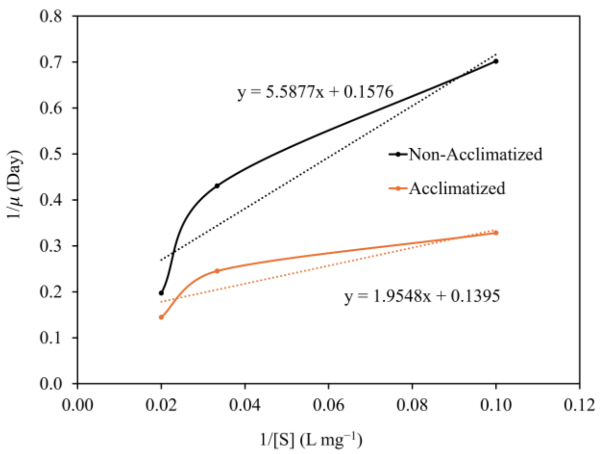

Figure 5. Effect of acclimatization on Monod kinetics

3.2.2. pH

The pH changes during the ammonia biodegradation process in systems with and without acclimatization are shown in the graph above (Figure 6). In both systems, the initial pH was approximately 5.8, then slightly increased on the first day to around 5.9, indicating an initial adaptation phase for microorganisms to the reaction environment and substrate availability. From day 2 to day 4, both systems exhibited a significant decrease in pH; however, the pattern and magnitude of the decrease differed between the acclimatized and non-acclimatized systems. The acclimatized system experienced a faster and greater pH decline, reaching a minimum value of about pH 5.38–5.40 on day 5.

In contrast, the non-acclimatized system showed a slight decrease in pH and tended to stabilize at higher values, around pH 5.48–5.50. Biologically, this decrease in pH is predominantly associated with intensified heterotrophic metabolism of the added carbon source (molasses). As the acclimatized microbial community actively assimilates ammonia for biomass synthesis, it simultaneously ferments molasses at a much higher rate. This rapid metabolic activity leads to a faster accumulation of acidic by-products (such as VFAs) and dissolved CO_2, which is reflected in the sharper pH decrease compared to the non-acclimatized system [51].

In the acclimatized system, the microbial community had already adapted to the presence of ammonia and was therefore able to carry out biodegradation more intensively from the beginning. As a result, the higher biological reaction rates led to a faster accumulation of acidic products, as reflected in the sharper decrease in pH. Conversely, in the non-acclimatized system, microorganisms were still undergoing metabolic adjustment, so ammonia degradation activity proceeded more slowly. This resulted in lower production of acidic compounds, causing the pH to decrease more gradually and remain at relatively higher values than in the acclimatized system [52].

From days 5 to 6, both systems showed a tendency toward pH stabilization, indicating that a balance had been reached between acid production from microbial metabolism and the buffering capacity of the system. Nevertheless, the pH in the acclimatized system remained lower, confirming that biological activity and the rate of ammonia biodegradation were higher than in the non-acclimatized system. Overall, these results demonstrate that microbial acclimatization significantly influences pH dynamics, with the acclimatized system exhibiting faster, larger pH changes due to more intensive metabolic activity. This finding is consistent with the enhanced kinetic parameters (higher μ_{max} and lower K_s) observed in the acclimatized system, which consistently indicate better ammonia biodegradation performance.

3.2.3. MLSS

Figure 7 shows the effect of the acclimatization process on MLSS at ammonia concentrations of 10, 30, and 50 ppm. In general, MLSS values increased with increasing ammonia concentration in both systems, with and without acclimatization. This increase in MLSS indicates that ammonia acts as a nitrogen source that supports microbial biomass growth during the biodegradation process [37].

Nevertheless, across all ammonia concentration levels, the acclimatized system consistently exhibited higher MLSS values than the non-acclimatized system. At an ammonia concentration of 10 ppm, the acclimatized biomass already showed a higher MLSS, indicating that microorganisms that had undergone an adaptation phase were able to utilize ammonia more effectively from the early stages of the biodegradation process [53]. This difference became more pronounced at higher ammonia concentrations, particularly at 30 and 50 ppm, where the acclimatized system maintained more stable and intensive biomass growth.

This difference can be explained by the effects of acclimatization on microbial adaptation. Acclimatization promotes the selection and dominance of microorganisms that are more tolerant to ammonia, as well as the adjustment of enzymatic systems and metabolic pathways involved in nitrogen assimilation. Acclimatized microorganisms tend to exhibit higher substrate utilization efficiency and lower cellular stress levels, allowing more metabolic energy to be allocated to biomass growth, which is reflected in the higher MLSS values [54]. In contrast, in the non-acclimatized system, microorganisms remain in a metabolic adjustment phase in response to ammonia stress, particularly at higher concentrations. This condition can inhibit metabolic activity and cell division, resulting in slower biomass accumulation and consequently lower MLSS values compared to the acclimatized system [54].

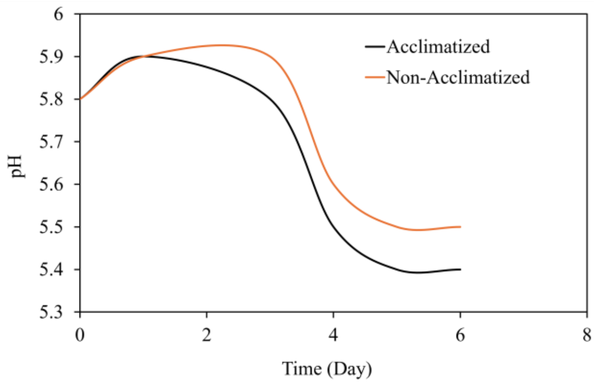

Figure 6. The effect of acclimatization on pH

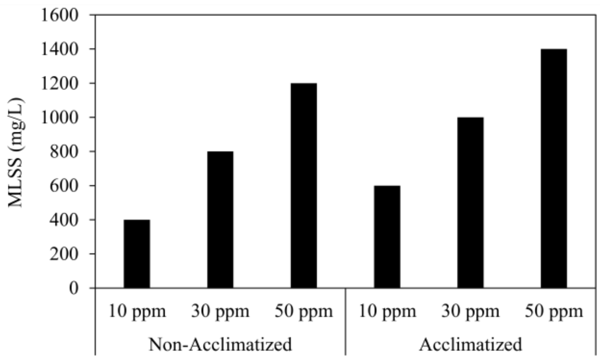

Figure 7. The effect of acclimatization on MLSS (Day 6)

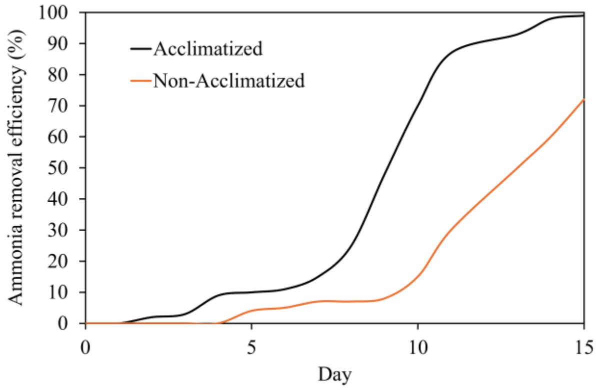

Figure 8. Effect of acclimatization on ammonia removal efficiency

The correlation between the higher MLSS values in the acclimatized system and the previously discussed increase in μ_{max} and decrease in K_s demonstrates strong consistency among the kinetic parameters. A larger and more metabolically active biomass contributes to a higher maximum specific growth rate and improved substrate affinity, leading to overall enhanced ammonia biodegradation performance [55]. Overall, these findings confirm that implementing an acclimatization stage prior to the ammonia biodegradation process provides significant advantages for microbial biomass growth and stability. This is evidenced by increased MLSS across various ammonia concentrations and reinforces the role of acclimatization as a key strategy for improving the efficiency of ammonia-containing wastewater treatment systems.

3.2.4. Ammonia Removal Efficiency

To evaluate the actual biodegradation performance of the system, the temporal changes in ammonia removal efficiency were monitored over a 15-day period (Figure 8). The results clearly demonstrate that microbial acclimatization drastically enhances both the rate and the overall extent of ammonia removal. As depicted in the graph, the acclimatized system exhibited a relatively short lag phase, with the removal efficiency increasing rapidly after day 3. The system entered a robust exponential phase, ultimately achieving near-complete ammonia removal (approximately 98%) by day 15. Conversely, the non-acclimatized system experienced a severely prolonged lag phase, showing minimal ammonia removal for the first 9 days. Consequently, its removal efficiency only reached approximately 70% by the end of the observation period.

These differences can be attributed to the physiological effects of microbial acclimatization. The prolonged lag phase in the non-acclimatized system reflects the physiological stress experienced by the unadapted microbial community. These microorganisms required significantly more time to adapt to ammonia toxicity and to synthesize the necessary enzymes for heterotrophic ammonia assimilation [48, 56]. In contrast, the acclimatized EM4 consortium was already pre-adapted to the environmental stress, allowing it to immediately allocate metabolic energy toward active growth and substrate utilization rather than basic survival. These practical removal efficiency results perfectly corroborate the previously established Monod kinetic findings [57, 58]. The pre-adapted community's ability to achieve 98% removal rapidly is a direct physical manifestation of its higher μ_{max} and superior K_s. This confirms that, beyond theoretical kinetic improvements, microbial acclimatization is a mandatory operational strategy for preventing system failure and ensuring rapid ammonia degradation in industrial applications.

4. Conclusion

This study demonstrates that optimizing external carbon supplementation and implementing microbial pre-adaptation significantly enhance the kinetics and efficiency of ammonia biodegradation by the EM4 consortium. Specifically, increasing the molasses dosage to 10% yielded the most favorable Monod kinetic parameters (maximizing the specific growth rate (μ_{max}) and substrate affinity (K_s)) compared to lower concentrations. Furthermore, microbial acclimatization proved critical; it drastically minimized the lag phase, increased biomass accumulation (MLSS), and achieved an optimal ammonia removal efficiency of approximately 98% by mitigating physiological stress. Collectively, these findings highlight that integrating a dedicated acclimatization stage with adequate external carbon dosing (molasses) is an essential, practical strategy for optimizing biological treatment systems designed for ammonia-rich petrochemical wastewater. Although the results demonstrate the potential of the EM4 consortium for enhancing ammonia removal under controlled laboratory conditions, the present study was conducted in a batch-scale system. Therefore, additional studies using continuous-flow reactors, pilot-scale systems, and real industrial wastewater are required before large-scale implementation can be recommended.

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