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Effect of Aeration Rate on the Quality of Bio-Urine Produced from Cattle Urine Fermentation in an Aerobic Bioreactor

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ABSTRACT

The utilization of cattle urine as a raw material for liquid organic fertilizer has strong potential to support sustainable agriculture through the optimization of aerobic fermentation using a bioreactor system. This study aimed to analyze the effect of aeration rate on the quality of bio urine produced. The fermentation process was conducted for 15 days in a 1100 L aerobic bioreactor with three aeration rate treatments of 48, 60, and 90 L/h. The main parameters evaluated included pH, biological oxygen demand (BOD), C/N ratio, microbial population, and macro-nutrient contents (N, P, and K). The results showed that increasing the aeration rate accelerated the reduction of BOD and C/N ratio while maintaining pH stability throughout the fermentation process. The aeration rate of 90 L/h produced bio urine with a C/N ratio below 15, the lowest BOD value, and stable pH, indicating the highest level of fermentation maturity and chemical stability. Under this condition, macro-nutrient contents reached 0.25% N, 0.10% P, and 0.42% K, accompanied by a stable microbial population during the final fermentation phase. Therefore, an aeration rate of 90 L/h was determined as the optimal condition based on fermentation maturity indicators (C/N ratio, BOD, and pH) and improved efficiency of organic matter mineralization.

1. INTRODUCTION

The increasing market demand for agricultural products has encouraged farmers to maximize productivity through the intensive use of chemical (Kurzemann *et al*., 2020; Xing *et al*., 2025). However, excessive application of chemical fertilizers can degrade soil fertility, reduce soil water-holding capacity, and disrupt soil ecosystem balance (Fu *et al*., 2023), ultimately leading to counterproductive outcomes in efforts to increase crop production (Kurzemann *et al*., 2020; Xing *et al*., 2025).

The incorporation of organic matter into cultivated land represents a crucial strategy for mitigating the adverse effects of excessive chemical fertilizer use (Tabbassum *et al*., 2022). Organic materials are commonly derived from agricultural waste, including livestock manure (López-rubio *et al*., 2025). Despite its considerable potential as an organic nutrient source, the utilization of cattle urine remains very limited at the farm level (Ali *et al*., 2020).

Cattle urine contains various essential macronutrients, including nitrogen (N), phosphorus (P), and potassium (K) (Ruiz *et al*., 2025). In addition, it also comprises several micronutrients such as calcium (Ca), magnesium (Mg), aluminum (Al), iron (Fe), sulfur (S), copper (Cu), and molybdenum (Mo), with water accounting for approximately 95% of its composition (Ambigalakshmi *et al*., 2023). This relatively complete nutrient profile highlights the strong potential of cattle urine for use as liquid organic fertilizer (Mudhita & Mulyadi, 2023; Pramudya *et al*., 2024).

Previous studies have successfully processed cattle urine into bio-urine products suitable for agricultural applications (Nuraini & Eka Asgianingrum, 2017; Situmeang *et al*., 2012). Furthermore, field applications have demonstrated positive contributions to soil improvement and crop performance (Oliveira da Silva *et al*., 2023). The production of bio-urine is relatively simple, typically involving urine containers such as jerrycans, buckets, or drums, supplemented with microbial starters, 3% sugar, and 3% urea (Pramana *et al*., 2017; Tabbassum *et al*., 2022). Despite this simplicity, traditional bio-urine production methods exhibit several limitations, including inconsistent product quality, contamination risks, low fermentation efficiency, and relatively long processing times of 10–15 days (Yunus *et al*., 2022; Putra *et al*., 2022). More controlled bio-urine production can be achieved through continuous flow or mechanical mixing, which enhances dissolved oxygen levels in the urine. This approach improves fermentation efficiency when conducted in a bioreactor system, resulting in a more complete process and reduced gas odor formation (Setiyo *et al*., 2023; Kadam *et al*., 2024).

This study aims to investigate the effect of varying airflow rates on bioreactor performance in producing high-quality bio-urine. Bioreactor performance was evaluated using a series of physical, chemical, and biological parameters, including pH, temperature, biological oxygen demand (BOD), total dissolved solids (TDS), electrical conductivity (EC), carbon-to-nitrogen (C/N) ratio, nutrient concentrations, and microbial population dynamics.

2. MATERIALS AND METHODS

2.1. Conceptual Framework

This study was designed to analyze the performance of an aerobic bioreactor in producing bio-urine by regulating three aeration (airflow) rates as the main experimental variable. Aeration rate plays a critical role in determining the availability of dissolved oxygen in the fermentation medium, which subsequently influences the activity and dynamics of aerobic microorganisms during the cattle urine fermentation process.

The experiment employed three bioreactors (Figure 1), each with a total capacity of 1100 L, equipped with an air pump rated at 186.5 W. Air was supplied into the bioreactor through a PVC pipe with a diameter of 25.4 mm and a length of 30 cm, fitted at the end with a fine-porosity ceramic diffuser stone (pore diameter ±1 mm). This configuration generated microbubbles, increasing the air–liquid interfacial area and thereby enhancing oxygen transfer efficiency into the bio-urine. Approximately 3.5% of the oxygen supplied in the airflow was dissolved in the medium, exceeding the minimum Standard Oxygen Transfer Efficiency (SOTE) requirement of 2% for supporting aerobic fermentation (Setiyo *et al*., 2023).

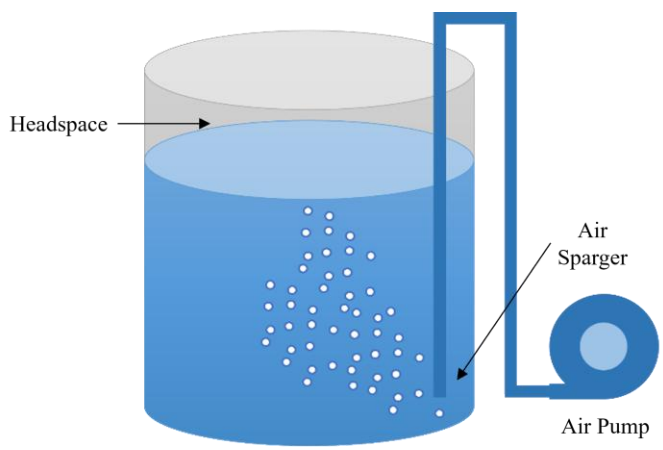

Figure 1. Bioreactor schematic

Variations in airflow rates resulted in differences in oxygen supply and biomass mixing intensity within the bioreactor. These conditions directly affected the growth and metabolic activity of aerobic microorganisms, particularly organic matter–degrading microbes and nitrifying bacteria. Optimal microbial activity accelerates the degradation of complex organic compounds into simpler and more stable forms.

Sequentially, changes in microbial activity induced by different aeration rates were reflected in variations of physical, chemical, and biological parameters, including temperature, pH, biological oxygen demand (BOD), total dissolved solids (TDS), electrical conductivity (EC), C/N ratio, and microbial population dynamics. These parameters served as indicators of fermentation maturity and stability of the produced bio-urine.

2.2. Materials

Materials used in this study were classified into two categories: materials for bio-urine production and materials supporting sample analysis. The primary raw material for bio-urine production was fresh cattle urine, which exhibited natural variation in initial microbial populations, measured at 3.2 and 3.6 log CFU. This variation was not defined as an experimental treatment but was recorded as an inherent characteristic of the raw material, commonly arising from differences in collection time and initial urine conditions. The initial microbial population was not artificially standardized to better represent field application conditions. All fermentation treatments were applied identically, ensuring that variations in bio-urine quality were primarily attributable to differences in aeration rates. Supporting chemicals used for sample analysis included distilled water, Fe_2SO_4, H_2SO_4, $K_2Cr_2O_7$, Na_2SO_4, $CuSO_4$, NaOH, NH_4OH, HCl, Na_2SO_5, $BaCl_2$, NH_4-acetate, and 80% alcohol.

2.3. Experimental Procedure

The experiment was conducted using three replications, where each treatment was applied to three separate fermentation reactors (n = 3) for each initial microbial population level of 3.2 log CFU and 3.6 log CFU. The experimental procedure consisted of the following steps:

a) The primary material used was cattle urine obtained as livestock waste, which was filtered to remove solid impurities or foreign materials. Preliminary analysis indicated initial microbial populations of 3.2 log CFU and 3.6 log CFU.

b) Cattle urine was loaded into three bioreactors at a volume of 1000 L each, leaving headspace filled with air. Subsequently, 5% nitrifying bacteria, 2% urea, and 2% molasses were added to each reactor.

c) During bio-urine production, the biomass was aerated using an air pump with three different aeration rate treatments applied to separate bioreactors, namely V1 = 48 L/h, V2 = 60 L/h, and V3 = 90 L/h. Each treatment was observed over a 15-day fermentation period.

d) Carbon content was analyzed using the Walkley–Black method, and microbial population dynamics were assessed every three days by collecting 1000 mL samples from a total urine volume of 1000 L. Microbial population analysis was conducted using the Total Plate Count (TPC) method. Analyses of P_2O_5 (Bray II method), K_2O, and other relevant nutrients were performed on day 15.

2.4. Data Analysis

Data obtained during the fermentation process were analyzed using statistical and descriptive approaches. The main parameters observed included temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), biological oxygen demand (BOD), C/N ratio, and microbial population dynamics. In addition, the quality of bio-urine at the end of fermentation (day 15) was evaluated based on macro- and micronutrient content, cation exchange capacity (CEC), and other chemical properties.

All quantitative data were subjected to analysis of variance (ANOVA) at confidence levels of 95% ($\alpha = 0.05$) and 99% ($\alpha = 0.01$) to identify significant differences among aeration rate treatments. When ANOVA results indicated significant differences, Duncan's Multiple Range Test (DMRT) was applied to determine the minimum significant differences among treatments.

In addition to statistical testing, data were presented in tables and graphs using Microsoft Excel to facilitate interpretation. Descriptive analysis was employed to explain temporal patterns of each parameter throughout the fermentation process, including trends in temperature, pH, EC, TDS, BOD, and microbial population dynamics.

3. RESULTS AND DISCUSSION

3.1. Temperature Changes during the Fermentation Process

During the fermentation process, biomass temperature exhibited dynamic fluctuations influenced by several factors, including urine volume, microbial activity, aeration rate variation within the bioreactor, initial C/N ratio of the urine, and bioreactor dimensions (Figure 2). The presented data represent the average values of three replications for each treatment. Overall, temperature increased from day 0 to day 5 across all treatments, indicating that heat generated by fermentation reactions exceeded heat losses to the surrounding environment.

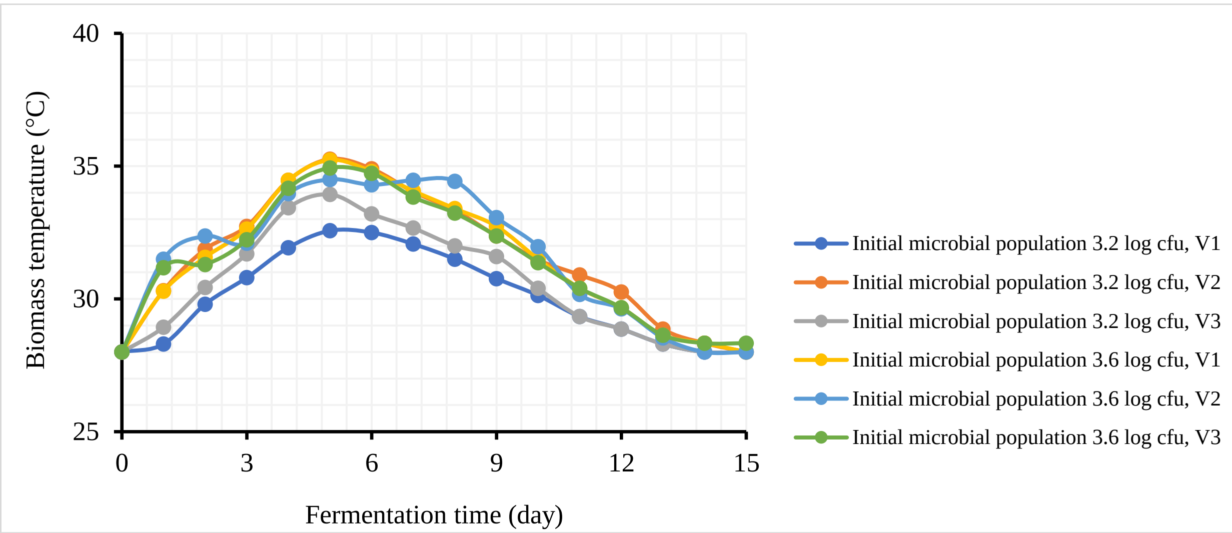

Figure 2. Temperature changes during the fermentation process

This temperature increase is closely associated with intense microbial metabolic activity involved in degrading complex organic compounds into simpler molecules. Such biochemical reactions release energy in the form of heat, gradually elevating the medium temperature (Yang *et al*., 2024). The early temperature rise indicates that microorganisms were in the logarithmic growth phase, during which organic substrates derived from cattle urine and added molasses were optimally utilized as energy and carbon sources (Liu *et al*., 2024).

From day 6 to day 14, the temperature of the fermented urine gradually decreased and stabilized within the range of 29 – 33 °C by day 14. This trend suggests a decline in fermentation reactions, where heat generated by biochemical processes became lower than heat dissipated from the bioreactor to the environment. Furthermore, this decrease reflects a transition from the exponential to the stationary microbial growth phase, as substrate availability diminished and the bioreactor system reached equilibrium between oxygen supply, heat dissipation, and metabolic activity (Ge *et al*., 2020). During the final fermentation phase (days 11–15), temperature remained relatively stable with a slight downward trend, indicating reduced microbial activity due to limited organic availability (Asadu *et al*., 2020).

Variations in aeration rates (V1 = 48 L/h, V2 = 60 L/h, and V3 = 90 L/h) influenced temperature patterns. The mathematical relationships between fermentation time and temperature for an initial microbial population of 3.6 and 3.2 log CFU were presented in Table 1 for three different aeration rates. These equations indicate that higher aeration

Table 1. Mathematical relationships of fermentation time and temperature for an initial microbial population of 3.6 and 3.2 log CFU

V (L/h)	Initial microbial population 3.6 log CFU	V (L/h)	Initial microbial population 3.2 log CFU
V1 = 48	$y = -0.106x^2 + 1.36x + 29.54$	V1 = 48	$y = -0.098x^2 + 1.27x + 29.492$
V2 = 60	$y = -0.087x^2 + 1.003x + 28.276$	V2 = 60	$y = -0.082x^2 + 0.82x + 22.26$
V3 = 90	$y = -0.0741x^2 + 1.003x + 28.276$	V3 = 90	$y = -0.0711x^2 + 1.003x + 21.276$

rates (V3 = 90 L/h) promoted more stable temperature conditions due to enhanced oxygen supply supporting aerobic fermentation. Conversely, lower aeration rates (V1 = 48 L/h) resulted in more pronounced temperature fluctuations due to uneven oxygen and heat distribution. These findings highlight the critical role of aeration control in maintaining temperature stability and optimizing aerobic fermentation conditions (Urra *et al*., 2020).

3.2. pH Changes during the Fermentation

During the fermentation process, biomass pH exhibited a fluctuating pattern closely associated with microbial activity, initial microbial population, and aeration supply regulation within the bioreactor (Figure 3). In the initial phase (days 0–4), pH increased from approximately 7.1 to 7.5. This increase is likely attributed to demineralization processes promoted by oxygen supplied through aeration, which facilitated the decomposition of organic matter into cations and anions. The dominance of cationic species during this process resulted in an overall increase in pH.

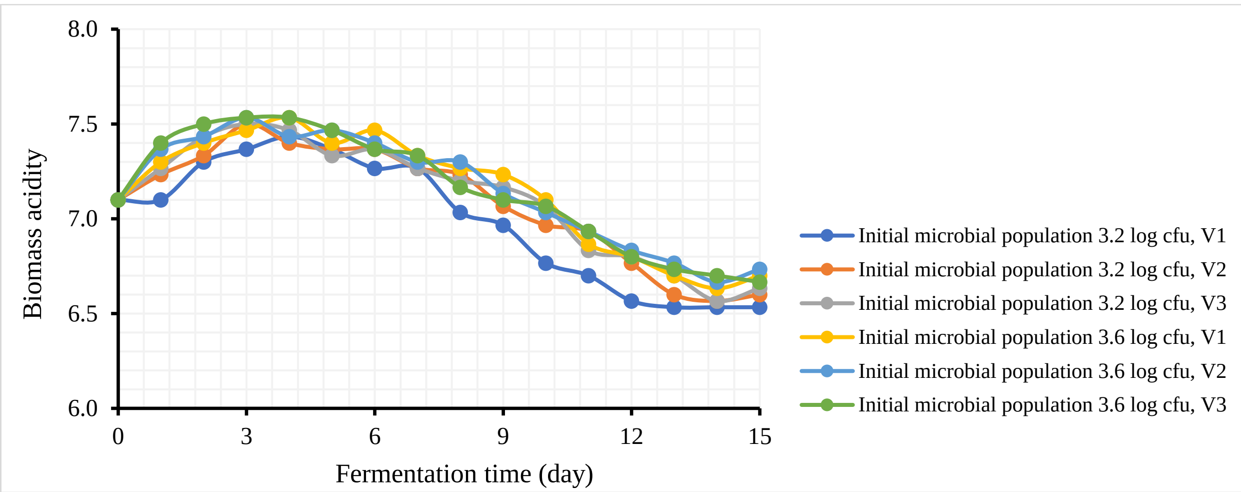

Figure 3. Changes of pH during the fermentation process

From day 5 to day 11, pH decreased rapidly from 7.5 to approximately 6.9. This trend indicates a shift in microbial dominance from fermentative microorganisms to nitrifying bacteria, such as Nitrosomonas and Nitrobacter, which oxidize ammonia into nitrite and nitrate. These oxidation processes alter the ionic balance of the medium and contribute to changes in pH dynamics (Ribera-Guardia & Pijuan, 2017).

During the final fermentation stage (days 12–15), pH values stabilized within the range of 6.9–7.3. This stability suggests that the bioreactor system had reached microbiological equilibrium, in which organic acid production and nitrification activity occurred in balanced proportions. Neutral pH conditions within this range are considered optimal for sustaining aerobic microbial activity and maintaining the quality of bio-urine as a liquid organic fertilizer (Ge *et al*., 2020).

This pH trend is commonly associated with the formation of organic acids during the early metabolic activity of microorganisms, including lactic acid bacteria and other fermentative microbes that decompose carbohydrates and proteins present in cattle urine and molasses. These processes generate lactic acid, acetate, and other volatile compounds that contribute to pH reduction in the fermentation medium (Yang *et al*., 2024).

Aeration rate variation also significantly influenced pH dynamics. At a high aeration rate (V3 = 90 L/h), abundant oxygen supply enhanced oxidation efficiency, promoted mineral formation, and prevented excessive accumulation of organic acids. Consequently, the final pH values remained at or above 6.7, compared with values below 6.5 observed under lower aeration rates (V1 = 48 L/h). In contrast, limited oxygen supply at low aeration favored facultative anaerobic microorganisms that produce organic acids, resulting in greater pH fluctuations during fermentation (Mal & Chattopadhyay, 2024).

3.3. Biological Oxygen Demand (BOD) Changes during the Fermentation

Biological oxygen demand (BOD) values exhibited a clear decreasing trend throughout the fermentation of cattle urine in the bioreactor (Figure 4). During the initial phase (days 0–7), BOD remained relatively high, ranging from approximately 7.2 to 7.6 mg/L. These values indicate the presence of abundant readily biodegradable organic matter utilized by microorganisms as an energy source. This condition corresponds to the increased heterotrophic microbial activity consuming carbohydrate-rich substrates from molasses and organic nitrogen compounds present in cattle urine (Ahmadi *et al*., 2022). As fermentation progressed into the intermediate phase (days 8–11), BOD values decreased significantly from around 7.6 mg/L to 4.5–5.5 mg/L. This reduction occurred because most easily degradable organic substrates had been consumed, resulting in lower oxygen demand. In addition, the activity of nitrifying bacteria increased, converting ammonia into nitrite and nitrate. This process is more oxidatively efficient and leads to a reduction in total oxygen consumption within the system (Jiang *et al*., 2019). At this stage, fermentation transitioned from heterotrophic microbial dominance toward a more stable system involving autotrophic microorganisms.

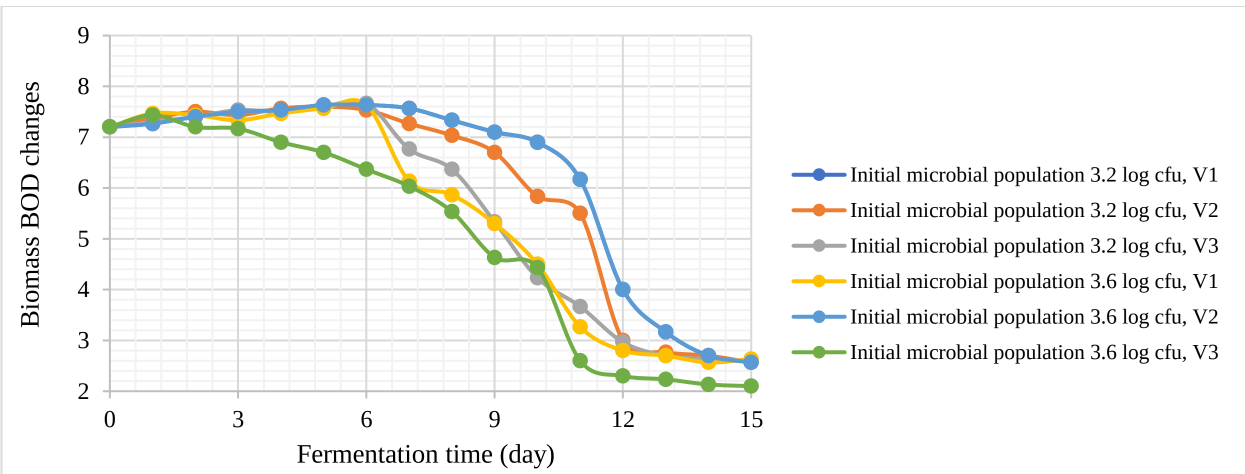

Figure 4. BOD changes during the fermentation process

During the final phase (days 12–15), BOD values reached their lowest levels, ranging from 2.6 to 2.7 mg/L. These low values indicate that most readily degradable organic matter had been depleted and microbial activity declined, marking the stabilization phase of fermentation. Such low BOD values serve as an indicator of good-quality bio-urine with minimal suspended organic matter, making it safer for environmental application and long-term storage (Zhen *et al*., 2025).

Aeration rate variation significantly influenced the rate of BOD reduction. Under high aeration conditions (V3 = 90 L/h) with an initial microbial population of 3.6 log CFU, BOD declined more rapidly due to enhanced dissolved oxygen availability that supported more efficient aerobic degradation. In contrast, limited oxygen supply at lower aeration rates (V1 = 48 L/h) slowed the degradation process, resulting in higher residual BOD values by the end of fermentation (Sun *et al*., 2021).

3.4. Total Dissolved Solids (TDS) Changes during the Fermentation

Changes in total dissolved solids (TDS) during the fermentation process reflect the dynamics of microbial activity in degrading dissolved organic matter and the availability of nutrients in the fermentation medium (Figure 5). During the initial phase (days 0–3), TDS values were relatively high, ranging from 900 to 1000 µS. These values indicate the presence of abundant dissolved compounds, such as simple carbohydrates derived from molasses and organic nitrogen compounds from cattle urine, which are readily utilized by microorganisms. According to Zhou *et al*. (2022), elevated TDS levels at the early stage of fermentation are commonly associated with the release of soluble components resulting from the initial enzymatic hydrolysis of organic substrates by microbial enzymes.

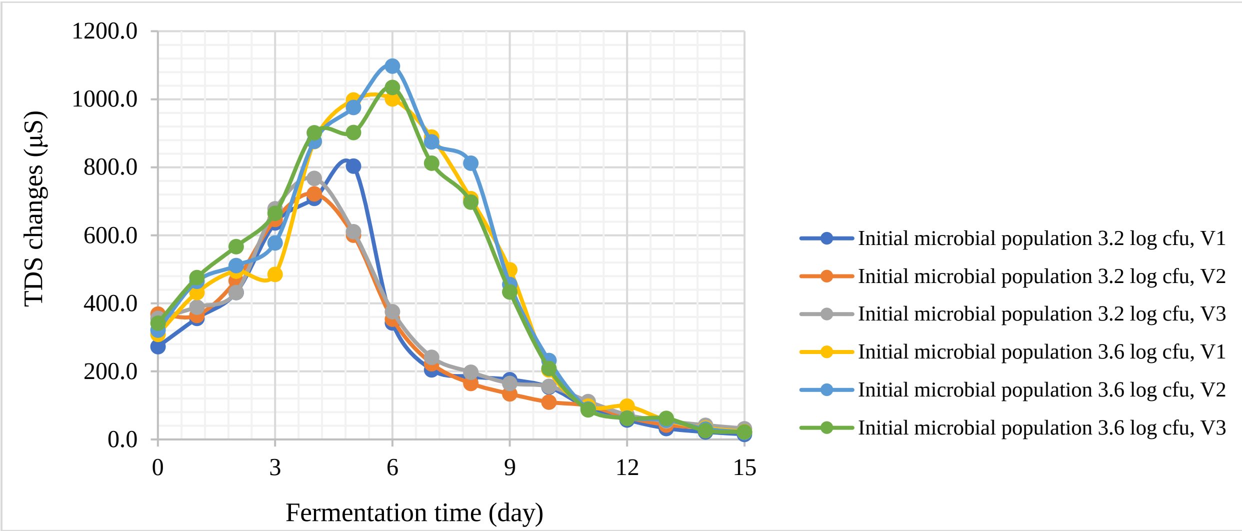

Figure 5. TDS changes during the fermentation process

Between days 6 and 9, TDS values decreased significantly to approximately 500–700 µS. This reduction occurred as complex organic compounds were progressively degraded into simpler inorganic forms, gases, and microbial biomass. This observation is consistent with the findings of Duan *et al.* (2021), who reported that microbial activity during the exponential phase of fermentation leads to intensive consumption of dissolved substances and transformation of nutrients into more stable forms. This phase represents an optimal metabolic condition in which microorganisms utilize most organic substrates as energy and carbon sources for growth.

During the final fermentation phase (days 12–15), TDS values stabilized at relatively low levels, ranging from 200 to 400 µS. This stability indicates that most readily degradable organic matter had been consumed and that the bioreactor system was approaching a dynamic equilibrium. Low TDS values at the end of fermentation suggest improved bio-urine quality, as lower concentrations of dissolved solids reduce the risk of odor generation, microbial contamination, and sediment formation during storage. Similar findings were reported by Kurniawan *et al.* (2024), who identified decreasing TDS values in the final fermentation stage as an indicator of successful substrate conversion into stable metabolites.

Aeration rate variation significantly affected TDS dynamics. At a high aeration rate (V3 = 90 L/h), TDS reduction occurred more rapidly and steadily compared with low aeration conditions (V1 = 48 L/h). This trend reflects adequate oxygen availability, which accelerates organic matter oxidation and facilitates the degradation of dissolved substrates. According to Wang *et al.* (2021), increasing aeration rates enhance aerobic microbial activity, accelerate biochemical reactions, and reduce dissolved solids more efficiently. In contrast, limited oxygen supply under low aeration conditions restricts organic compound oxidation, resulting in persistently higher TDS values until the end of fermentation.

3.5. Electrical Conductivity (EC) Changes during the Fermentation

Electrical conductivity (EC) values exhibited a gradual decreasing trend throughout the fermentation process, reflecting the degradation of organic matter and changes in the concentration of dissolved ions in the fermentation medium (Figure 6). During the initial fermentation phase (days 0–3), EC values were relatively high, ranging from approximately 2.0 to 2.3 mS cm^{-1}. This condition was associated with high concentrations of dissolved ions, such as ammonium (NH_4^+), potassium (K^+), and organic anions derived from cattle urine and molasses that had not yet been degraded (Wang *et al.*, 2024). These ions originate from the early dissociation of simple organic compounds that remain highly soluble in the fermentation medium.

As fermentation progressed into the intermediate phase (days 6–9), EC values declined to approximately 1.2–1.6 mS/cm. This decrease indicates that a substantial proportion of dissolved ions had been assimilated by microorganisms

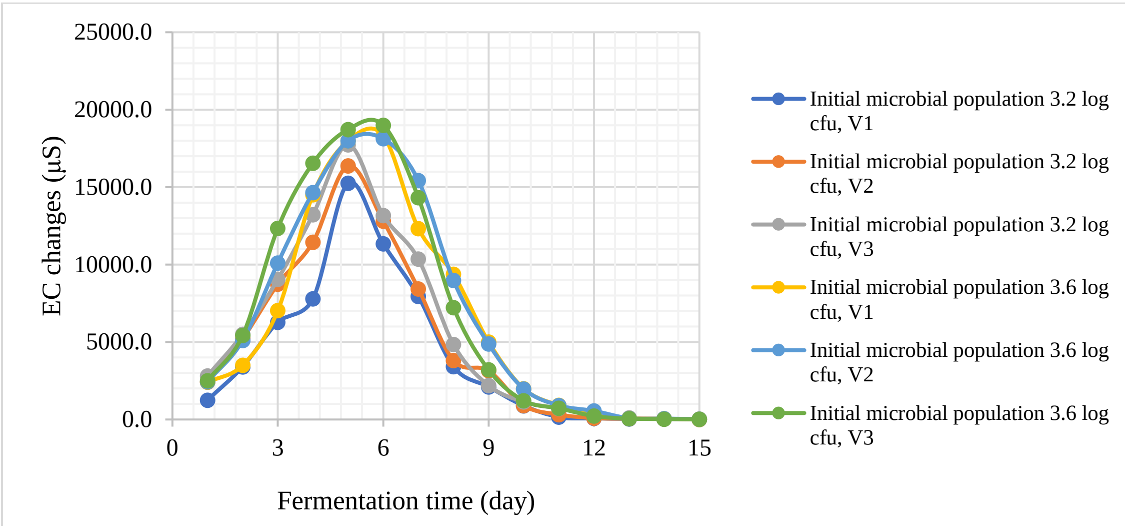

Figure 6. EC changes during the fermentation process

for metabolic activity and biomass formation. In addition, nitrification processes carried out by Nitrosomonas and Nitrobacter converted ammonia into nitrite and nitrate, thereby altering the ionic balance of the medium (Sun *et al*., 2025). Such transformations reduce the number of free ions contributing to the electrical conductivity of the solution.

During the final fermentation stage (days 12–15), EC values stabilized at relatively low levels, ranging from 0.6 to 0.8 mS/cm. This stabilization suggests that the fermentation system had reached equilibrium, with most organic matters decomposed and microbial activity gradually declining. Low EC values at this stage indicate that the produced bio-urine contains low levels of dissolved salts and is therefore safer for plant application, as excessive ion concentrations may induce osmotic stress and interfere with water uptake by plant roots.

Aeration rate variation significantly influenced EC dynamics. Treatments with high aeration rates (V3 = 90 L/h) resulted in faster and more stable EC reductions due to adequate oxygen supply, which accelerated organic matter oxidation and promoted homogenization of dissolved ions within the medium (Perwez & Al Asheh, 2025). In contrast, lower aeration rates (V1 = 48 L/h) limited oxygen availability, thereby restricting aerobic microbial activity and slowing the degradation of organic compounds, resulting in a more gradual decline in EC.

3.6. C/N Ratio Changes during the Fermentation

The carbon-to-nitrogen (C/N) ratio is a critical indicator in the fermentation process, as it reflects the balance between energy sources (carbon) and essential nutrients for microbial growth (nitrogen). During fermentation, the C/N ratio typically decreases over time until reaching a stable condition (Figure 7), indicating the conversion of organic matter into simpler and more bioavailable compounds.

In the early fermentation phase (days 0–3), C/N values remained relatively high due to the dominance of organic carbon derived from molasses and cattle urine. This carbon served as the primary energy source for heterotrophic microorganisms, initiating the early degradation of organic compounds. According to Sun *et al*. (2025), the initial stage of fermentation is characterized by rapid microbial activity that consumes simple sugars, resulting in increased respiration and a reduction in dissolved carbon.

As fermentation progressed into the intermediate phase (days 6–9), the C/N ratio decreased significantly. This trend indicates intensive degradation of organic carbon into CO_2 and microbial biomass, while nitrogen remained available mainly in the form of ammonia and nitrate produced through microbial nitrification. Zhao *et al*. (2025) reported that during this stage, nitrifying and denitrifying microorganisms play a crucial role in converting organic nitrogen into inorganic forms, thereby maintaining nitrogen balance within the fermentation medium.

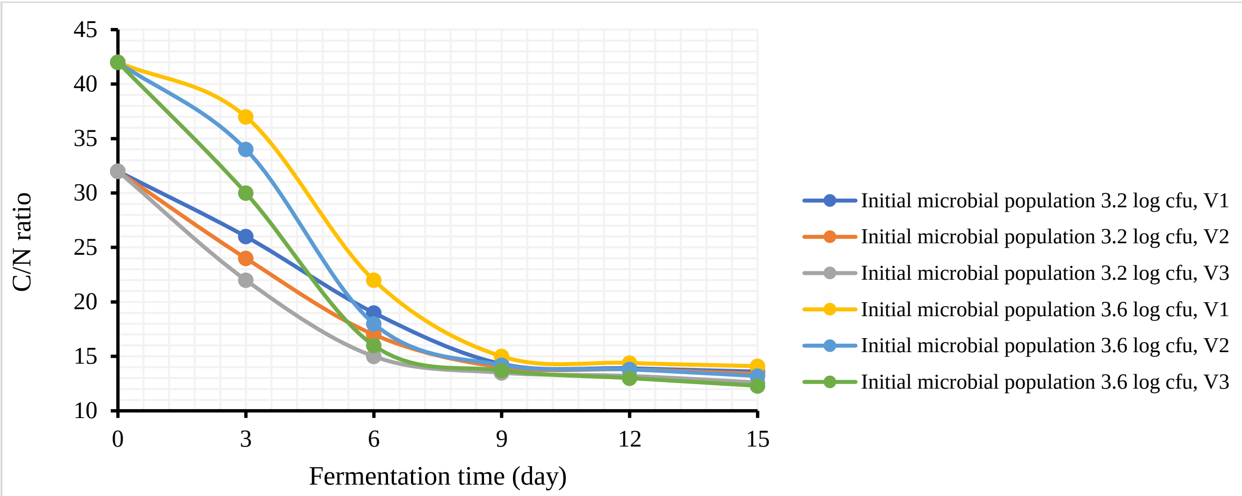

Figure 7. C/N ratio changes during the fermentation process

During the final fermentation phase (days 12–15), the C/N ratio reached low and stable values. Most carbon compounds had been mineralized, and the remaining nitrogen existed in forms readily available for plant uptake. A low final C/N ratio indicates a high degree of fermentation maturity, ensuring that the final product, such as bio-urine, is easily assimilated by plants and does not cause nitrogen immobilization in the soil. Tang *et al*. (2024) reported that organic fertilizers with low C/N ratios enhance mineral nitrogen availability in the root zone and promote plant growth.

Aeration rate significantly influenced C/N dynamics. High aeration conditions (V3 = 90 L/h) accelerated C/N reduction by improving oxygen availability and stimulating aerobic microbial activity involved in organic carbon oxidation. In contrast, low aeration conditions (V1 = 48 L/h) slowed C/N reduction due to oxygen limitations that inhibited oxidative processes. These findings are consistent with the results reported by Xiong *et al*. (2017) who demonstrated that increased aeration during liquid fertilizer fermentation accelerates carbon mineralization and improves nitrogen conversion efficiency.

3.7. Microbial Population Changes during Fermentation

During the fermentation process, microbial populations exhibited dynamic changes closely associated with nutrient availability, oxygen supply, and aeration rate regulation within the bioreactor (Figure 8). These changes reflect a complex balance among microbial growth phases, substrate consumption, and metabolite formation throughout the fermentation process. In the initial phase (days 0–3), microbial populations increased significantly from 3.2–3.6 log CFU to approximately 4.5–5.0 log CFU. This increase was driven by the abundance of nutrients, particularly carbon sources from molasses and nitrogen from cattle urine, which supported rapid growth of heterotrophic microorganisms. Early fermentation stages are often characterized by accelerated microbial growth due to high C/N ratios and the availability of easily degradable organic substrates (Gao *et al*. (2024).

During the intermediate phase (days 6–9), microbial populations reached their peak, ranging from 5.5 to 6.0 log CFU. This phase represents the period of highest metabolic activity, during which intensive decomposition of organic matter occurs. Oxygen supply from the air pump played a critical role in maintaining the dominance of aerobic microorganisms, including nitrifying bacteria such as Nitrosomonas and Nitrobacter, which convert ammonia into nitrite and nitrate (Sun *et al*., 2002). Puspitaloka *et al*. (2022) reported that optimal aeration enhances ammonia oxidation efficiency and prolongs the exponential growth phase of microorganisms in aerobic bioreactor systems.

As fermentation entered the final phase (days 12–15), microbial populations declined to approximately 4.0–4.5 log CFU. This decrease was attributed to reduced substrate availability and the accumulation of byproducts such as organic acids and potentially inhibitory compounds that suppress microbial activity (Chidi *et al*., 2018). The relatively stable conditions observed during this phase indicate that the bioreactor system had reached a dynamic equilibrium between microbial growth and decay, marking the end of the active fermentation cycle.

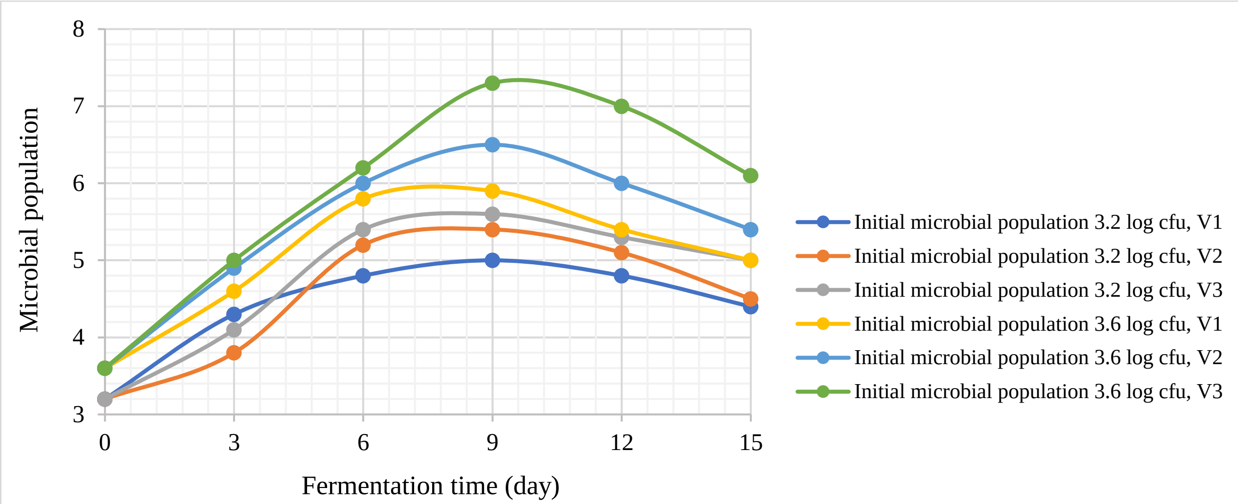

Figure 8. Microbial population changes during the fermentation process

Aeration rate variation significantly influenced microbial population dynamics. Under high aeration conditions (V3 = 90 L/h), microbial populations remained more stable and sustained higher levels until the end of fermentation. More uniform oxygen distribution supported efficient aerobic respiration and prevented the formation of anaerobic zones within the bioreactor (Li *et al*., 2022). In contrast, low aeration conditions (V1 = 48 L/h) limited oxygen availability, resulting in a more rapid decline in microbial activity, as many microorganisms were unable to maintain efficient oxidative metabolism.

3.8. Nutrient Content of Fermented Bio-Urine

Analysis of the nutrient composition of fermented bio-urine under different aeration rates revealed notable variations in the concentrations of macro- and micronutrients (Table 2). The evaluated parameters included nutrient elements, organic matter content, and chemical properties commonly used to characterize soil fertility within the root zone. This approach was employed to assess the extent to which the chemical characteristics of fermented bio-urine correspond to soil fertility parameters required for optimal plant growth.

Table 2. Nutrient composition and chemical properties of fermented bio-urine under different aeration rates

Parameters	Initial microbial population 3.2 log cfu			Initial microbial population 3.6 log cfu		
	V1	V2	V3	V1	V2	V3
Content of carbon, %	3.6	3.81	4.17	4.17	4.32	4.61
Content of nitrogen, %	0.38	0.38	0.4	0.4	0.42	0.48
Content of P2O5, ppm	786	774	792	792	814.4	820
Content of K2O, ppm	500	656	666	666	601.2	621
Content of Na, ppm	79	83	99	99	107	121
Content of Ca mg/kg	1782	1854	1888	1888	2660	2286
Content of Mg, mg/kg	204	208	286	286	234	204
Content of Fe, mg/kg	120	188	1204	1204	1892	1954
Content of Al, mg/kg	5387	5387	12946	7946	8526	9618
Total Organic Matters, %	12.85006	13.35281	22.46924	17.46759	19.61774	20.7766
CEC, mg/100g	25.7	25.9	26.98	25.96	26.23	26.69
EC, µmS	65454.33	99457.67	8231	7798	7875	7910
TDS, ppm	7174.667	6951	7203.667	10025.36	9996	9832.667
BOD	2.6	2.7	2.7	2.6	2.6	1.2
pH	6.5	6.6	6.6	6.8	6.7	6.7
C-N	13.6	13.4	12.6	14.1	13.2	12.3

The results indicated that the bio-urine contained essential macronutrients, including nitrogen (N), phosphorus (P), and potassium (K), at levels sufficient to support plant growth. Nitrogen content ranged from 0.19 to 0.25%, phosphorus from 0.07 to 0.10%, and potassium from 0.35 to 0.42%. This nutrient composition highlights the strong potential of bio-urine as a liquid organic fertilizer, as it provides the primary nutrients required by plants in a sustained manner (Pradhan *et al*., 2018).

In addition to macronutrients, the fermented bio-urine also contained micronutrients such as calcium (Ca), magnesium (Mg), and sodium (Na) at moderate concentrations. These elements play essential roles in enhancing enzymatic activity, chlorophyll synthesis, and maintaining ionic balance within plant tissues. The presence of micronutrients at balanced levels indicates that the bioreactor-based fermentation process not only preserves but may also enhance nutrient availability compared with fresh cattle urine (Oji & Godgift, 2020).

Aeration rate variation was found to significantly influence the resulting nutrient quality. Treatments with high aeration rates (V3 = 90 L/h) tended to produce higher nitrogen and potassium contents compared with low aeration treatments (V1 = 48 L/h). This finding suggests that optimal aeration enhances aerobic microbial activity, thereby accelerating the mineralization of organic compounds into inorganic forms that are more readily available for plant uptake (Perwez & Al Asheh, 2025). In contrast, limited oxygen supply slowed organic matter degradation, resulting in lower nutrient concentrations.

Compared to general standards for liquid organic fertilizers, the nutrient contents of the fermented bio-urine produced in this study fall within acceptable ranges for horticultural applications. The balanced composition of macro- and micronutrients enables bio-urine to function not only as a nutrient source but also as a plant growth stimulant through enhanced soil microbial activity and improved soil chemical properties (Ortiz-Marin *et al*., 2025). Overall, results of our study confirm that the use of a bioreactor with a controlled aeration system can effectively produce high-quality bio-urine. This technology not only improves the efficiency of livestock waste utilization, but also provides an environmentally friendly liquid organic fertilizer alternative that supports sustainable agricultural practices.

The novelty of this study lies in the optimization of aeration rate in a large-scale (1100 L) aerobic bioreactor for cattle urine fermentation and the comprehensive evaluation of its effects on the physical, chemical, and biological characteristics of bio-urine. While previous studies have demonstrated that aeration influences microbial activity during organic waste composting (Ge *et al*., 2020), this study specifically establishes the optimum aeration rate (90 L/h) for cattle urine fermentation by integrating fermentation maturity indicators, including BOD, C/N ratio, pH stability, microbial population dynamics, and nutrient content. Therefore, this study provides a practical engineering reference for improving the efficiency and quality of bio-urine production using a controlled aerobic bioreactor system.

4. CONCLUSIONS AND RECOMMENDATIONS

The findings of this study are highly valuable for advancing the science and technology of liquid organic fertilizer production, which is increasingly needed by local communities. The novelty of this study lies in establishing the optimum aeration rate of 90 L/h in a 1100 L aerobic bioreactor for cattle urine fermentation through the integrated evaluation of physical, chemical, and biological quality parameters. Under this condition, the fermentation process produced bio-urine with a C/N ratio of 12.3–12.6, BOD values of 2.1–2.5 mg/L, EC values of 7,900–8,240 µS/cm, TDS values of 9,200–9,900 ppm, and balanced macro-nutrient contents, providing a scientific basis for improving the efficiency, stability, and quality of bio-urine production using controlled aerobic bioreactor technology. Moreover, the implementation of this simple technology can significantly assist farmers in independently producing fertilizers from available livestock waste, thereby enhancing resource efficiency and supporting sustainable agricultural practices.

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AUTHOR CONTRIBUTION

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
IPSW	✓	✓			✓	✓		✓	✓					
MSM								✓	✓		✓		✓	
YS		✓			✓	✓								

C: Conceptualization	Fo: Formal Analysis	O: Writing - Original Draft	Fu: Funding Acquisition
M: Methodology	I: Investigation	E: Writing - Review & Editing	P: Project Administration
So: Software	D: Data Curation	Vi: Visualization	
Va: Validation	R: Resources	Su: Supervision	

DECLARATION OF THE USE OF GENERATIVE AI AND AI-BASED TECHNOLOGIES IN MANUSCRIPT PREPARATION

During the preparation of this work, the authors used Mendeley as a tool to assist with the management and organization of references in accordance with the writing style of the target journal. All writing, data analysis, and manuscript preparation processes were carried out independently by the authors, and the contents of this publication are entirely the responsibility of the authors.

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