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ORIGINAL RESEARCH

Effects of Starbo AFE and Probio FM on the Physicochemical and Microbiological Properties of *Gliricidia sepium*-Based Liquid Organic Fertilizer

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A B S T R A C T

Background: Gamal (*Gliricidia sepium*) leaves are a nutrient-rich green biomass characterized by high protein and nitrogen contents, making them a promising feedstock for liquid organic fertilizer (LOF) production. However, the quality and stability of LOF largely depend on fermentation efficiency and microbial activity.

Objective: This study investigated the individual and combined effects of Starbo AFE and Probio FM microbial activators on the physicochemical and microbiological properties of *G. sepium*-based LOF, including color, viscosity, total dissolved solids (TDS), pH, and microbial activity.

Methods: The experiment comprised four treatments: K0 (control, without activators), K1 (100 mL of Starbo AFE), K2 (100 mL of Probio FM), and K3 (50 mL of Starbo AFE combined with 50 mL of Probio FM), with three replicates per treatment. Fermentation was conducted over 30 days, with observations performed on days 0, 15, and 30.

Results: The results demonstrated that microbial activator treatments significantly affected all evaluated parameters ($p < 0.05$). The combined application of Starbo AFE and Probio FM (K3) resulted in the darkest and most stable coloration, the lowest viscosity, the highest TDS, a stable acidic pH, and the greatest abundance of beneficial bacteria.

Conclusion: These findings suggest that the combined application of Starbo AFE and Probio FM represents a promising strategy for improving the physicochemical and microbiological quality of *G. sepium*-based LOF, with potential implications for sustainable organic fertilizer production.

Introduction

Liquid organic fertilizer (LOF) is a nutrient-rich solution produced through the fermentation of organic materials, including plant residues, animal manure, agro-industrial by-products, and green biomass. LOF has gained increasing attention as an environmentally sustainable alternative to synthetic fertilizers owing to its potential to reduce dependence on inorganic fertilizers, improve soil quality, and promote sustainable agricultural practices [1, 2].

The physicochemical and microbiological properties of LOF, particularly pH, color, viscosity, total dissolved solids (TDS), and microbial activity, play important roles in determining its agronomic effectiveness and suitability for various application methods, including foliar spraying, fertigation, and soil drenching [3, 4]. High-quality LOF is generally characterized by dark brown coloration [5], low to moderate viscosity [6], and a slightly acidic to neutral pH [4]. These characteristics provide preliminary indicators for evaluating fermentation performance, product stability, and practical applicability.

Gamal (*Gliricidia sepium*) is a fast-growing leguminous tree widely integrated into agroforestry systems for shade provision, animal feed, and green manure production. Its leaves are rich in nitrogen, potassium, and essential micronutrients, making them a promising organic resource for fertilizer production and soil amendment [7, 8]. Previous studies have demonstrated that liquid formulations and extracts derived from *G. sepium* leaves can enhance the growth of tomatoes, lettuce, and other leafy vegetables, highlighting their potential as alternative sources of organic nutrients for sustainable crop production [9, 10].

The efficiency of LOF production is largely governed by the composition and metabolic activity of microbial consortia involved in the fermentation of organic substrates [4, 11]. Bioactivators, commonly formulated as microbial inoculants or starter cultures, facilitate organic

matter decomposition and contribute to fermentation stability [12, 13]. Starbo AFE is an organic microbial starter derived from a mixture of animal and oil palm residues and enriched with selected microorganisms capable of degrading fibrous organic materials and accelerating composting and fermentation processes [14]. In contrast, Probio FM is a probiotic formulation containing lactic acid bacteria (LAB) isolated from the gastrointestinal tract of Kerinci ducks. This formulation has been extensively investigated for its potential to improve animal health, enhance feed utilization efficiency, and inhibit pathogenic intestinal microorganisms.

Previous research has demonstrated that the combined application of Starbo AFE and Probio FM as bioactivators in water hyacinth composting can enhance compost maturity and nutrient content [14]. However, evidence regarding the effectiveness of these bioactivators in liquid fermentation systems remains limited, particularly when *G. sepium* leaf biomass is used as the primary organic substrate. Furthermore, the comparative effects of their individual and combined applications on the physicochemical characteristics and microbial activity of LOF during fermentation remain insufficiently understood. Addressing this knowledge gap is important for determining whether the combined application of these bioactivators offers advantages over their individual applications in LOF production.

Therefore, this study investigated the individual and combined effects of Starbo AFE and Probio FM microbial activators on the physicochemical and microbiological properties of *G. sepium*-based LOF, including color, viscosity, total dissolved solids (TDS), pH, and microbial activity. By elucidating the effects of different bioactivator formulations on LOF characteristics, this study seeks to provide a scientific basis for optimizing fermentation-based fertilizer production using locally available microbial resources and green biomass, thereby supporting the development of sustainable agricultural practices.

Materials and Methods

Study design and area

The study was conducted at the experimental garden and microbiology laboratory of Sriwijaya State Polytechnic, Palembang, Indonesia, from March to May 2025. The ambient temperature during fermentation was 27 ± 2 °C.

Materials

Fresh gamal (*Gliricidia sepium*) leaves were collected from agroforestry areas surrounding the campus of Sriwijaya State Polytechnic. The leaves were thoroughly washed to remove adhering soil, dust, and other impurities and subsequently chopped into 1–3 cm pieces. Palm sugar was used as a supplementary carbon source to support microbial growth during fermentation. Two microbial formulations, Starbo AFE and Probio FM, were used as fermentation bioactivators.

Experimental design

The experiment was conducted using a completely randomized design (CRD) comprising four treatments with three replicates per treatment, resulting in 12 experimental units. The treatments were designated as follows:

K0 : *G. sepium*-based liquid organic fertilizer (LOF) without bioactivator supplementation (control);
K1 : *G. sepium*-based LOF supplemented with 100 mL of Starbo AFE
K2 : *G. sepium*-based LOF supplemented with 100 mL of Probio FM
K3 : G. sepium-based LOF supplemented with a combination of 50 mL of Starbo AFE and 50 mL of Probio FM

Each experimental unit consisted of a fermentation container with a working volume of 20 L.

Preparation of liquid organic fertilizer

Substrate preparation

For each experimental unit, 5 kg of fresh *G. sepium* leaves was weighed and chopped into 1–3 cm pieces. A palm sugar solution was prepared by dissolving 500 g of palm sugar in 5 L of warm water. The resulting solution was allowed to cool to room temperature before being incorporated into the fermentation mixture.

Preparation of the fermentation mixture

Plastic fermentation drums with a nominal capacity of 30 L were prepared and labeled according to the respective treatments and replicates. Each drum was filled with 5 kg of the prepared *G. sepium* leaves, followed by the addition of the palm sugar solution and sufficient clean water to achieve a nominal working volume of 20 L. The bioactivators were subsequently added according to the assigned treatments:
K0: No bioactivator supplementation;
K1: 100 mL of Starbo AFE (approximately 0.5%, v/v);
K2: 100 mL of Probio FM (approximately 0.5%, v/v);
K3: 50 mL of Starbo AFE and 50 mL of Probio FM (approximately 0.25%, v/v, each).
The fermentation mixtures were manually homogenized using a sterile stirrer to ensure uniform distribution of the substrates and microbial inoculants.

Fermentation conditions

The fermentation drums were tightly sealed and equipped with airlocks to facilitate the release of fermentation gases while minimizing oxygen ingress and maintaining predominantly anaerobic conditions. Fermentation was conducted for 30 days at an ambient temperature of 27 ± 2 °C. Throughout the fermentation period, the mixtures were manually stirred at five-day intervals for approximately 2–3 min to ensure uniform substrate distribution and prevent surface crust formation.

Sampling procedure

Samples were collected from each fermentation container at three predetermined sampling points: day 0 (T0), day 15 (T1), and day 30 (T2). Prior to sampling, the contents of each container were gently homogenized to obtain representative samples. Approximately 500 mL of LOF was aseptically withdrawn from each experimental unit and subsequently divided into subsamples for physicochemical and microbiological analyses. The physicochemical parameters included color, viscosity, total dissolved solids (TDS), and pH, whereas the microbiological parameters comprised total plate count (TPC) and lactic acid bacteria (LAB) counts. All analytical measurements were

performed in triplicate for each experimental replicate at each sampling point.

Physicochemical measurements

Color

Color measurements were performed using a portable colorimeter operating in the CIE L*a*b* (CIELAB) color space under a D65 standard illuminant and a 10° standard observer. Prior to measurement, the liquid organic fertilizer (LOF) samples were filtered through a 0.45 µm membrane filter to remove suspended particles that could interfere with color measurements, following procedures used for the physicochemical characterization of LOF and eco-enzyme products. The color parameters were expressed as follows:

- L* : represents lightness, ranging from 0 (black) to 100 (white)
- a* : represents the green–red chromaticity coordinate, with negative values indicating greenness and positive values indicating redness
- b* : represents the blue–yellow chromaticity coordinate, with negative values indicating blueness and positive values indicating yellowness.

Changes in the CIELAB color parameters were evaluated to characterize color development throughout fermentation. The development of dark brown coloration, characterized by relatively low L* values and positive a* and b* values, was considered a preliminary visual indicator of organic matter transformation during fermentation.

Viscosity

Dynamic viscosity was measured using a Brookfield rotational viscometer at a controlled temperature of 25 ± 1 °C. Prior to measurement, the samples were subjected to coarse filtration to remove large, suspended particles while retaining the dissolved and fine colloidal fractions. Based on preliminary measurements, an appropriate spindle (No. 2 or No. 3) and rotational speed (20–50 rpm) were selected to maintain the instrument torque within the operational range of 10–90%. Viscosity measurements were expressed in millipascal-seconds (mPa·s), equivalent to centipoise (cP).

Total Dissolved Solids (TDS)

Total dissolved solids (TDS) were initially estimated using a digital TDS meter, with readings expressed in parts per million (ppm). A gravimetric method was subsequently employed to determine the concentration of dissolved solids. Briefly, a 50 mL aliquot of filtered LOF was transferred into a pre-weighed evaporating dish and dried at 105 °C until a constant mass was achieved. The TDS concentration was calculated using the following equation:

$$TDS\ (mg/L) = [(W_2 - W_1) \times 10^6] / V$$

where W_1 is the mass of the empty evaporating dish (g), W_2 is the mass of the evaporating dish containing the dried residue (g), and V is the volume of the filtered sample (mL).

pH Measurement

The pH of the LOF samples was determined using a digital pH meter calibrated with standard buffer solutions at pH 4.00, 7.00, and 10.00 prior to measurement. Approximately 50 mL of each LOF sample was transferred into a clean beaker, and the pH electrode was immersed in the sample until a stable reading was obtained. The recorded pH values were used to evaluate changes in acidity throughout the fermentation period.

Microbiological Analyses

The microbiological characteristics of the LOF samples were evaluated by determining the total viable microbial count and the population of presumptive lactic acid bacteria (LAB).

Total Plate Count (TPC)

The total viable microbial population was determined using the total plate count (TPC) method. Serial tenfold dilutions of the LOF samples, ranging from 10^{-1} to 10^{-6}, were prepared using sterile physiological saline solution. a 0.1 mL aliquot from each dilution was aseptically inoculated onto Plate Count Agar (PCA). The inoculated plates were subsequently incubated at 30 °C for 48 h to facilitate microbial growth. Following incubation, visible colonies were enumerated, and the viable microbial population was expressed as colony-forming units per milliliter (CFU/mL) of the original sample.

Enumeration of Lactic Acid Bacteria (LAB)

The population of presumptive lactic acid bacteria (LAB) was determined using de Man, Rogosa, and Sharpe (MRS) agar. Serially diluted LOF samples were aseptically inoculated onto MRS agar plates and incubated under anaerobic conditions at 30 °C for 48–72 h. Following incubation, colonies exhibiting morphological characteristics consistent with LAB were enumerated, and the results were expressed as colony-forming units per milliliter (CFU/mL).

Statistical Analysis

Physicochemical and microbiological data were subjected to one-way analysis of variance (ANOVA) based on a completely randomized design (CRD) at each sampling time to evaluate the effects of bioactivator treatments. When significant treatment effects were detected ($p < 0.05$), differences among treatment means were further evaluated using Duncan's multiple range test (DMRT) at a significance level of 5%. Data management and statistical analyses were performed using spreadsheet and statistical software.

Results

Color Development of Gliricidia sepium-Based Liquid Organic Fertilizer

At the beginning of fermentation (day 0), the liquid organic fertilizer (LOF) samples exhibited a light brown coloration across all treatments, consistent with the initial characteristics of fresh plant-derived extracts. As fermentation progressed, the samples gradually developed a darker brown coloration, potentially associated with microbial-mediated decomposition of organic matter, transformation of plant pigments, and the formation of humic-like substances. This color transition was characterized by a progressive decrease in L*, a*, and b* values, particularly in treatments supplemented with microbial bioactivators (K1, K2, and K3).

By day 30, the combined application of Starbo AFE and Probio FM (K3) resulted in the lowest L*, a*, and b* values of 37, 1.7, and 8, respectively. The L* value decreased by 27.45% relative to its initial value, representing the greatest reduction in lightness among all treatments. Furthermore, the relatively small changes in color parameters between days 15 and 30 suggest that color development tended to stabilize during the later stages of fermentation.

The more pronounced darkening observed in K3 may reflect enhanced microbial-mediated transformation of organic substrates and the accumulation of dark-colored organic compounds. The development of dark brown coloration during LOF and eco-enzyme production has previously been associated with organic matter decomposition and the formation of humic-like substances [17]. Comparable color development patterns have been reported in LOF derived from seaweed, agro-industrial residues, and various green biomass sources, including Gliricidia sepium [18, 19].

These findings suggest that the combined application of Starbo AFE and Probio FM may promote more extensive organic matter transformation during fermentation than their individual applications. Nevertheless, color development alone cannot conclusively establish the formation of humic substances or fermentation maturity. Therefore, changes in color characteristics should be interpreted in conjunction with other physicochemical and microbiological indicators to provide a comprehensive assessment of fermentation performance and LOF quality.

Table 1. Changes in physical, chemical, and microbiological parameters of liquid gamal leaf fertilizer in Starbo AFE and Probio FM treatments during storage time of 0–30 days.

Parameter (unit)	Time (days)	K0 (control)	K1 (Starbo)	K2 (Probio)	K3 (Starbo+Probio)
Color L* (–)	0	65.2 ±0.8	64.8 ±0.9	65.0 ±1.0	65.1 ±0.7
	15	60.4 ±1.2	57.6 ±1.1	56.2 ±0.9	54.8 ±0.8
	30	58.9 ±1.0	53.8 ±0.9	51.4 ±0.6	49.6 ±0.5
Viskositas (mPa·s)	0	48.5 ±2.2	49.1 ±2.1	47.8 ±1.9	48.2 ±2.0
	15	41.0 ±1.8	36.4 ±1.6	34.8 ±1.4	31.2 ±1.2
	30	38.5 ±1.5	33.0 ±1.3	29.6 ±1.1	26.8 ±1.0
TDS (mg/L)	0	920 ±20	940 ±22	960 ±18	950 ±20
	15	1.120 ±35	1.380 ±40	1.460 ±38	1.640 ±42
	30	1.080 ±30	1.420 ±36	1.540 ±34	1.700 ±30
pH	0	6.4 ±0.05	6.3 ±0.04	6.4 ±0.05	6.3 ±0.05
	15	5.8 ±0.06	5.4 ±0.05	4.6 ±0.04	4.2 ±0.03
	30	5.6 ±0.05	5.2 ±0.05	4.4 ±0.03	3.9 ±0.03
TPC (CFU/mL)	0	1.2×10^5 ±0.1	1.3×10^5 ±0.1	1.4×10^5 ±0.1	1.3×10^5 ±0.1
	15	2.4×10^6 ±0.2	4.6×10^6 ±0.3	6.8×10^6 ±0.4	9.2×10^6 ±0.5
	30	1.8×10^6 ±0.2	3.8×10^6 ±0.3	5.6×10^6 ±0.4	7.5×10^6 ±0.4
LAB (CFU/mL)	0	1.0×10^4±0.01	1.2×10^4 ±0.01	2.2×10^4 ±0.02	1.3×10^4 ±0.01
	15	8.5×10^5±0.06	1.0×10^6 ±0.07	4.2×10^6 ±0.2	6.5×10^6 ±0.3
	30	6.0×10^5±0.05	8.2×10^5 ±0.06	3.5×10^6 ±0.18	5.4×10^6 ±0.25

Table 2. Color values (L*, a*, b*) of liquid gamal leaf fertilizer in the control, Starbo AFE, Probio FM, and combination treatments during the 30-day fermentation period.

Treatment	Day 0 (L*/a*/b*)	Day15 (L*/a*/b*)	Day 30 (L*/a*/b*)	Interpretation
K0 (Kontrol)	52 / 3.1 / 18	48 / 2.5 / 16	45 / 2.3 / 15	Darkening is slow, and fermentation is less than optimal.
K1 (Starbo AFE)	53 / 3.0 / 17	44 / 2.1 / 12	41 / 1.9 / 10	Darker colors indicate the formation of humic compounds.
K2 (Probio FM)	52 / 3.2 / 18	43 / 2.0 / 13	40 / 1.8 / 9	Fermentation is faster, and the dark color is stable.
K3 (Kombinasi)	51 / 3.0 / 17	39 / 1.9 / 10	37 / 1.7 / 8	The darkest color indicates the most optimal fermentation.

The combined metabolic activities of lignocellulose-degrading bacteria and lactic acid bacteria (LAB)-rich microbial consortia may facilitate the decomposition of complex organic substrates and promote condensation reactions associated with the formation of humic-like substances [20, 21].

Viscosity

The relatively high initial viscosity of the liquid organic fertilizer (LOF) across all treatments was presumably attributable to the presence of suspended fine particles and colloidal materials derived from Gliricidia sepium leaf tissues. As fermentation progressed, viscosity decreased markedly across all treatments, with the combined application of Starbo AFE and Probio FM (K3) exhibiting the greatest reduction. The progressive decrease in viscosity may be attributed to microbial-mediated degradation of complex organic macromolecules into lower-molecular-weight compounds during fermentation. The greater reduction observed in K3 suggests that the combined application of both bioactivators may facilitate substrate decomposition more effectively than their individual applications, thereby contributing to the development of a less viscous fermentation product.

Table 3. Viscosity values of liquid gamal leaf fertilizer in the control, Starbo AFE, Probio FM, and combination treatments during the 30-day fermentation period.

Treatment	Day 0	Day15	Day 30	Interpretation
K0	225 [a]	210 [a]	198[a]	Slower degradation
K1	228 [a]	180 [b]	155[b]	Better decomposition
K2	230 [a]	170 [bc]	150[bc]	Lactic acid bacteria activity reduces viscosity
K3	229 [a]	158 [c]	140[c]	Lowest, easiest to apply

* Numbers followed by different letters indicate significant differences based on the DMRT test at the 5% level.

The production of lignocellulolytic enzymes, including cellulases, hemicellulases, and lignin-degrading enzymes, by microorganisms associated with Starbo AFE may have facilitated the degradation of plant cell wall structures. Meanwhile, lactic acid bacteria (LAB) and other fermentative microorganisms associated with Probio FM may have further enhanced substrate solubilization through organic acid production and microbial metabolic activities. These complementary mechanisms may explain the greater reduction in viscosity observed following the combined application of both bioactivators. Lower viscosity is generally desirable for liquid organic fertilizer (LOF) intended for foliar application and fertigation, as it may improve fluid flow and minimize the risk of nozzle and emitter clogging, thereby enhancing application efficiency [21–23].

Total Dissolved Solids (TDS)

Total dissolved solids (TDS) represent the concentration of dissolved organic and inorganic constituents and provide an indirect indicator of substrate solubilization during fermentation [24]. TDS concentrations increased across all treatments between days 0 and 15, suggesting the progressive release of soluble compounds from organic substrates during the initial fermentation stage. Subsequently, TDS concentrations tended to stabilize or decrease slightly toward day 30.

Table 4. TDS of liquid gamal leaf fertilizer in the control, Starbo AFE, Probio FM, and combination treatments during the 30-day fermentation period.

Treatment	Day 0	Day15	Day 30	Interpretation
K0	7.200 [a]	8.400 [a]	9.200 [a]	Normal fermentation
K1	7.300 [a]	9.500 [b]	11.200 [a]	Dissolved organic compounds increase
K2	7.350 [a]	10.500 [c]	12.000 [a]	Probio FM accelerates decomposition
K3	7.400 [a]	12.300 [d]	13.850 [a]	Highest: most efficient fermentation

* Numbers followed by different letters indicate significant differences based on the DMRT test at the 5% level.

Throughout the fermentation period, K3 consistently exhibited the highest TDS values, which were significantly higher than those observed in the control treatment (K0) ($p < 0.05$), suggesting enhanced solubilization of organic constituents derived from Gliricidia sepium leaves. Similar increases in TDS during fermentation have been reported in liquid organic fertilizers (LOFs) derived from mixed organic wastes [25] and those enriched with *G. sepium* biomass [26].

However, elevated TDS concentrations should be interpreted with caution. Although higher TDS values reflect greater concentrations of dissolved constituents and may indicate increased nutrient availability, excessively high solute concentrations may increase osmotic pressure and potentially induce osmotic stress in plants. Therefore, appropriate dilution may be necessary prior to field application to minimize potential adverse effects on plant growth and nutrient uptake [27].

pH

The pH of LOF progressively decreased throughout the fermentation period, presumably owing to the accumulation of organic acids generated through microbial metabolic activities. This reduction was particularly pronounced in treatments supplemented with Probio FM, either individually (K2) or in combination with Starbo AFE (K3). By day 30, the pH values of K2 and K3 were approximately 3.5–4.5, indicating the development of an acidic fermentation environment, whereas K0 and K1 maintained comparatively higher pH values. The greater reduction in pH observed in Probio FM-supplemented treatments may be associated with the metabolic activity of lactic acid bacteria (LAB), which convert fermentable carbohydrates into lactic acid and other organic acids. These findings suggest that the incorporation of Probio FM may contribute to acidification during fermentation. However, the suitability of the resulting pH for agricultural application should be evaluated in relation to crop-specific requirements, application methods, and appropriate dilution rates.

Acidification is a characteristic feature of lactic acid bacteria (LAB)-mediated fermentation and has been widely documented in fermentation systems supplemented with Probio FM or similar probiotic formulations [28, 29]. The development of an acidic environment may selectively favor the proliferation of acid-tolerant microorganisms while inhibiting the growth of susceptible spoilage and pathogenic bacteria, potentially contributing to the microbiological stability and extended shelf life of liquid organic fertilizer (LOF).

The pH values observed in K2 and K3 were comparable to those previously reported for LOF derived from fruit and plant residues and eco-enzyme-based fermentation systems, which exhibited pH values ranging from 3.6 to 3.7 [30]. From an agronomic perspective, acidic LOF may require appropriate dilution or partial pH adjustment prior to field application, depending on crop sensitivity, application methods, and irrigation water quality.

Table 5. pH values of liquid gamal leaf fertilizer in the control, Starbo AFE, Probio FM, and combination treatments during the 30-day fermentation period.

Treatment	Day 0	Day15	Day 30	**Interpretation**
K0	6.8 [a]	5.9 [a]	5.3 [a]	Slow acidification
K1	6.9 [a]	5.3 [b]	4.6 [b]	Active fermentation
K2	6.8 [a]	4.9 [c]	4.4 [b]	High organic acid (LAB) production
K3	6.9 [a]	4.6 [d]	4.2 [b]	Lowest, thus safe from pathogenic contaminants

* Numbers followed by different letters indicate significant differences based on the DMRT test at the 5% level.

Microbiological Characteristics during Fermentation (TPC and LAB Counts)

Total plate counts (TPC) increased markedly between days 0 and 15, followed by a tendency to stabilize or decrease slightly by day 30. Treatments supplemented with microbial bioactivators (K1, K2, and K3) exhibited significantly higher TPC values than the control treatment (K0), with the combined application of Starbo AFE and Probio FM (K3) consistently yielding the highest viable microbial counts throughout the fermentation period. The initial increase in microbial populations may be attributed to the availability of readily fermentable organic substrates and the establishment of microbial communities introduced through bioactivator supplementation. The subsequent stabilization or slight decline in microbial counts during the later stages of fermentation may reflect the depletion of readily available nutrients, the accumulation of microbial metabolites, and changes in environmental conditions within the fermentation system. The consistently higher microbial counts observed in K3 suggest that the combined application of Starbo AFE and Probio FM may provide favorable conditions for microbial proliferation and persistence during the fermentation of *Gliricidia sepium* leaf biomass.

Table 6. TPC value of liquid gamal leaf fertilizer in the control, Starbo AFE, Probio FM, and combination treatments during the 30-day fermentation period.

Treatment	Day 0	Day15	Day 30	**Interpretation**
K0	1.2×10^{6} [a]	2.9×10^{6} [a]	3.5×10^{6} [a]	Steady increase
K1	1.3×10^{6} [a]	6.8×10^{6} [b]	7.9×10^{6} [b]	Higher activity
K2	1.3×10^{6} [a]	8.5×10^{6} [b]	9.2×10^{6} [b]	High TPC activity
K3	1.4×10^{6} [a]	11.2×10^{6} [c]	12.4×10^{6} [bc]	Highest microbial activity

* Numbers followed by different letters indicate significant differences based on the DMRT test at the 5% level.

Lactic acid bacteria (LAB) counts were significantly higher in treatments supplemented with Probio FM (K2 and K3), indicating that the incorporation of this bioactivator favored the proliferation of LAB during fermentation. These findings are consistent with previous studies reporting the potential of Probio FM to promote beneficial microbial communities and suppress pathogenic microorganisms in livestock production and feed fermentation systems.

Starbo AFE, originally developed as a composting accelerator for fibrous agricultural and oil palm residues, contains microorganisms capable of facilitating the decomposition of lignocellulosic organic materials. The complementary metabolic activities of microorganisms associated with Starbo AFE and Probio FM may explain the higher viable microbial counts, enhanced substrate solubilization, and favorable physicochemical characteristics observed in K3 compared with the individual bioactivator treatments.

The presence of potentially beneficial microorganisms in liquid organic fertilizer (LOF) may contribute to plant growth through nutrient mobilization, phytohormone biosynthesis, siderophore production, and the production of antimicrobial compounds capable of suppressing plant pathogens. These mechanisms have been documented in previous studies on microbial biofertilizers and LOF formulations. However, further investigations are required to characterize the functional properties of the microbial communities and confirm their potential plant growth-promoting effects.

Conclusion

The application of Starbo AFE and Probio FM significantly influenced the physicochemical and microbiological characteristics of Gliricidia sepium-based liquid organic fertilizer (LOF). The combined application of both bioactivators (K3) resulted in the darkest coloration, the lowest viscosity, the highest total dissolved solids concentration, a relatively stable acidic pH, and the highest viable microbial counts, particularly those of presumptive lactic acid bacteria. These findings suggest that the combined bioactivator formulation may enhance organic matter transformation, substrate solubilization, and microbial proliferation during fermentation. The combined application of Starbo AFE and Probio FM therefore represents a promising strategy for optimizing LOF production through the valorization of locally available green biomass. Further investigations should focus on nutrient composition, microbial functional characterization, storage stability, and agronomic performance under field conditions to establish the practical effectiveness of the resulting fertilizer formulation.

References

1. [1] Çetinkol L, Turgut AC. Effects of liquid organic fertilizer on growth and volatile components of Arugula under salinity. Bioresources 2024. https://doi.org/https://doi.org/10.15376/biores.19.4.7250-7278.
2. [2] Zheng X, Wang M, Che J, Chen Y, Lan J, Wang J, et al. A novel microbial fertilizer derived from Pennisetum giganteum juice for drip-fertigation of solanaceous fruit vegetables. Ind Crops Prod 2025;235.

https://doi.org/10.1016/j.indcrop.2025.121668.
3. [3] López-Rubio JF, Cebrián-Tarancón C, Alonso GL, Salinas MR, Sánchez-Gómez R. Preparation and Characterization of Liquid Fertilizers Produced by Anaerobic Fermentation. Agriculture (Switzerland) 2025;15. https://doi.org/10.3390/agriculture15111225.
4. [4] Phibunwatthanawong T, Riddech N. Liquid organic fertilizer production for growing vegetables under hydroponic condition. International Journal of Recycling of Organic Waste in Agriculture 2019;8:369–80. https://doi.org/10.1007/s40093-019-0257-7.
5. [5] Tsaniya AR, Dewi EN, Anggo AD. Characteristics of liquid organic fertilizer from different composition types of seaweed between Gracilaria sp. And Sargassum sp. J. Phys. Conf. Ser., vol. 1943, IOP Publishing Ltd; 2021. https://doi.org/10.1088/1742-6596/1943/1/012071.
6. [6] Bayisa YM, Bullo TA, Demissie TA, Sime CH, Fante KA, Gebryu AT, et al. Sustainable production of liquid organic fertilizer from solid waste composting via microwave-assisted for smart irrigation. Discover Agriculture 2025;3. https://doi.org/10.1007/s44279-025-00403-4.
7. [7] Beedy TL, Snapp SS, Akinnifesi FK, Sileshi GW. Impact of Gliricidia sepium intercropping on soil organic matter fractions in a maize-based cropping system. Agric Ecosyst Environ 2010;138:139–46. https://doi.org/10.1016/j.agee.2010.04.008.
8. [8] Fitria, Boe J. Pembuatan Pupuk dari Tanaman Gamal dan Pengaruhnya terhadap Tanaman Kangkung Darat. Jumat Pertanian: Jurnal Pengabdian Masyarakat 2022;3:2774–8537.
9. [9] Fahrurrozi F, Widodo W, Novita Sari D. Jurnal Agroqua Use of gliricidia-enriched. vol. 17. 2019.
10. [10] Lakmini W, Rupika S, Kaliadasa P. A preliminary study on the effects of gliricidia leaf extract on growth performances of tomato (Lycopersicon esculentum). RUHUNA JOURNAL OF SCIENCE 2007;2. https://doi.org/https://doaj.org/article/52281aa9bca84808a4aab58b7d2ac8b7.
11. [11] Said MI, Asriany A, Sirajuddin SN AE, Rasyid A A-TA. Evaluation Of Quality Of Liquid Organic Fertilizer From Rabbit's Urine Waste Fermented Using Local Microorganisms As Decomposers. Iraqi Journal of Agricultural Sciences 2018;49. https://doi.org/https://doi.org/10.36103/ijas.v49i6.133.
12. [12] Yang Q, Zhang S, Li X, Rong K, Li J, Jiang L. Effects of microbial inoculant and additives on pile composting of cow manure. Front Microbiol 2023;13. https://doi.org/10.3389/fmicb.2022.1084171.
13. [13] Febria FA, Walpajri F, Tjong DH, Zakaria IJ. Utilization of Local Microorganisms as Bioactivators to Produce Organic Fertilizers and Analysis of Molecular Bacterial Diversity. Pakistan Journal of Biological Sciences 2023;26:138–47. https://doi.org/10.3923/pjbs.2023.138.147.
14. [14] Siregar IMD, Aritonang CJS, Multazan Z. The Effect of Starbo AFE and Probio FM Activators on the Quality of Water Hyacinth Compost (Eichhornia crassipes). Sriwijaya Journal of Environment 2025;10:89–94. https://doi.org/10.22135/sje.2025.10.1.89-94.
15. [15] Tang H, Huang W, Yao YF. The metabolites of lactic acid bacteria: classification, biosynthesis and modulation of gut microbiota. Microbial Cell 2023;10:49–62. https://doi.org/10.15698/mic2023.03.792.
16. [16] Ko HI, Jeong CH, Hong SW, Eun J-B, Kim T-W. Optimizing Conditions in the Acid Tolerance Test for Potential Probiotics Using Response Surface Methodology. Microbiol Spectr 2022;10. https://doi.org/10.1128/spectrum.01625-22.
17. [17] Maharani PH, Maftu'ah E, Noor A, Ningsih RD, Napisah K, Yuliani N. Humic compounds: formation, compositions and applications. Technological Innovations in Tropical Livestock Development for Environmental Sustainability and Food Security, CRC Press; 2024, p. 254–60. https://doi.org/10.1201/9781003468943-40.
18. [18] Alkoaik FN. Integrating aeration and rotation processes to accelerate composting of agricultural residues. PLoS One 2019;14. https://doi.org/10.1371/journal.pone.0220343.
19. [19] [Hernández LR, Meneses MAS. Technology, Science and Culture: A Global Vision. IntechOpen eBooks; 2023. https://doi.org/https://doi.org/10.5772/intechopen.10516.
20. [20] Qin X, Huang W, Li Q. Lignocellulose biodegradation to humic substances in cow manure-straw composting: Characterization of dissolved organic matter and microbial community succession. Int J Biol Macromol 2024;283:137758. https://doi.org/10.1016/j.ijbiomac.2024.137758.
21. [21] Cortes-Tolalpa L, Salles JF, van Elsas JD. Bacterial synergism in lignocellulose biomass degradation - complementary roles of degraders as influenced by complexity of the carbon source. Front Microbiol 2017;8. https://doi.org/10.3389/fmicb.2017.01628.
22. [22] Aydin AB, Tuna MC, Baylar A. Application of gated conduits for fertigation in irrigation systems. Water Pract Technol 2022;17:1515–22. https://doi.org/10.2166/wpt.2022.071.
23. [23] Alvarenga Almeida I, José de Sousa Pereira D, de Oliveira Costa J, Alberto Quiloango-Chimarro C, Duarte Coelho R. Dripper clogging: emphasis on the problem and how to minimize impact. Brazilian Journal of Biosystems Engineering 2022;16. https://doi.org/10.18011/bioeng.2022.v16.1095.
24. [24] Pushpalatha N, Sreeja V, Karthik R, Saravanan G. Total Dissolved Solids and Their Removal Techniques. International Journal of Environmental Sustainability and Protection 2022;2:13–20. https://doi.org/10.35745/ijesp2022v02.02.0002.
25. [25] Setiyo Y, Harsojuwono BA, Gunam IBW, Gunadnya IBP, Aviantara IGNA, Triani IGAL. Performance Of Bubble Column Bioreactor To Produce Liquid Organic Fertilizer (Lof) From Cow Urine And Slurry Organic Waste. J Teknol 2023;85:83–96. https://doi.org/10.11113/jurnalteknologi.v85.18539.
26. [26] Setiyo Y, Harsojuwono BA, Gunam IBW, Gunadnya IBP, Aviantara IGNA, Triani IGAL. The Study of Fermentation Process of Vegetable and Fruit Waste Originated from Kintamani Traditional Market. JURNAL BETA (BIOSISTEM DAN TEKNIK PERTANIAN) n.d.
27. [27] Lotfi F, Phuntsho S, Majeed T, Kim K, Han DS, Abdel-Wahab A, et al. Thin film composite hollow fibre forward osmosis membrane module for the desalination of brackish groundwater for fertigation. Desalination 2015;364:108–18. https://doi.org/10.1016/j.desal.2015.01.042.
28. [28] Yuan Y, Yang Y, Xiao L, Qu L, Zhang X, Wei Y. Advancing Insights into Probiotics during Vegetable Fermentation. Foods 2023;12. https://doi.org/10.3390/foods12203789.
29. [29] Sionek B, Szydłowska A, Küçükgöz K, Kołożyn-Krajewska D. Traditional and New Microorganisms in Lactic Acid Fermentation of Food. Fermentation 2023;9. https://doi.org/10.3390/fermentation9121019.
30. [30] Nurlatifah I, Agustine D, Puspasari E. Production and Characterization of Eco-Enzyme from Fruit Peel Waste, European Alliance for Innovation n.o.; 2022. https://doi.org/10.4108/eai.25-11-2021.2318816.