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Synergistic co-digestion of agroindustrial biomass waste for optimized digestion for liquid biofertilizer recovery

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Abstract: The improvement of biomass energy systems through waste valorization strategies represents a promising approach for rural energy infrastructure development. This study evaluated synergistic anaerobic co-digestion approaches to optimize liquid biofertilizer recovery from diversified agroindustrial biomass waste streams in the Ecuadorian highlands. Three formulations were tested: cattle manure, panela-yeast-whey blend, and molasses-milk-ash-Medicago sativa combination using 200 L tubular biodigesters at 37°C under randomized complete block design. Physicochemical characterization followed NTE INEN standards with ANOVA statistical analysis. The optimized co-digestion formulation achieved substantial improvements in nutrient concentration. Organic matter content elevated to 48.8% while bioconversion efficiency maintained 92.4%. Process optimization reduced fermentation time without compromising volumetric yield, demonstrating enhanced biomass throughput for distributed energy applications. Statistical validation confirmed treatment superiority across all macronutrients ($p < 0.001$). This multifunctional biorefinery approach transforms heterogeneous agricultural residues into value-added products, advancing biomass valorization technologies and supporting circular bioeconomy development in smallholder energy systems.

Keywords: anaerobic co-digestion; biorefinery systems; agricultural residues; nutrient recovery; process optimization; waste valorization

1. Introduction

The global imperative for sustainable intensification of agriculture necessitates transformative approaches that simultaneously address food security, climate change mitigation, and circular economy principles [1]. Conventional agricultural systems, heavily dependent on synthetic fertilizers, contribute approximately 1%–2% of global greenhouse gas emissions while depleting soil organic matter (O.M.) and disrupting biogeochemical cycles. In this context, anaerobic digestion technology emerges as a promising biorefinery platform capable of converting organic waste streams into value-added products, including liquid biofertilizers (biol), biogas, and soil conditioners [2,3].

Biol, the nutrient-rich liquid effluent from anaerobic digestion processes, represents a multifunctional agricultural input that delivers essential plant nutrients nitrogen (N), phosphorus (P), and potassium (K) while simultaneously enhancing soil physicochemical and biological properties through O.M. enrichment and microbial diversity promotion[3,4]. Unlike synthetic fertilizers, biol provides a slow-release nutrient profile that aligns with plant uptake patterns[5], reduces nutrient leaching, and supports long-term soil health through carbon sequestration mechanisms.

However, the inherent variability in biol composition stemming from substrate

heterogeneity, process parameters, and operational conditions present significant challenges for standardization and quality assurance in agricultural applications [6]. This compositional variability directly impacts nutrient bioavailability, application rates, and agronomic efficacy, thereby limiting widespread adoption among smallholder farmers who require predictable and reliable fertilizer inputs [7]. Knowledge gaps persist regarding optimal feedstock combinations that can systematically enhance biol nutritional quality while maintaining process stability and economic viability, particularly concerning synergistic effects of co-digesting complementary organic substrates on accelerating microbial metabolism and concentrating macronutrients [8].

The present investigation addresses these gaps through systematic evaluation of synergistic anaerobic co-digestion approaches designed to optimize liquid biofertilizer recovery from diversified agroindustrial biomass waste streams. Building upon established analytical methodologies (NTE INEN 220: 2013, NTE INEN 211: 98) [9,10], this study employs a randomized complete-block experimental design to quantify the effects of strategic feedstock blending on biol nutritional composition, process kinetics, and conversion efficiency. By demonstrating the feasibility of transforming heterogeneous organic waste streams into standardized, high-quality liquid biofertilizers, this research contributes to evidence-based frameworks for integrated waste-to-energy systems and supports the development of multi-functional biomass energy platforms that maximize resource recovery while enhancing agricultural sustainability in resource-constrained environments[11,12].

2. Materials and methods

2.1. Study location and context

This study was conducted at the Tunshi Experimental Station, owned by the Escuela Superior Politécnica de Chimborazo (ESPOCH), located in Licto, Riobamba, -1.750320 , -78.626174 , with an altitude of 2,710 meters above sea level. The site presents a high mountain temperate climate; the mean temperature ranges (≈ 12 – 16 °C) and annual precipitation (≈ 400 – 500 mm) were obtained from the Monthly Meteorological Bulletins and the 2024 Climate Yearbook of the ESPOCH–Tunshi Agrometeorological Station [13]. In comparative terms, the site's altitude is located within the provincial gradient, its location in the Chambo river sub-basin corresponds to the agroecological conditions described for the central Andean Sierra, in accordance with the intervals reported by the Instituto Nacional de Meteorología e Hidrología (INAMHI) for the central Sierra of Ecuador [14].

The selection of this site strategically aligns with the study's objective of developing replicable anaerobic digestion technologies for smallholder farming communities throughout the Andean region. The environmental conditions of the experimental station, including altitude, temperature fluctuations, and precipitation patterns, reflect those faced by typical highland agricultural communities, which allows the findings of this research to be applicable and reproducible in similar contexts of the Andean region, thus facilitating their effective implementation in sustainable agricultural practices. Furthermore, the proximity to diverse agroindustrial activities in the region provides access to representative organic waste streams

commonly available to local farmers, enhancing the transferability of the proposed co-digestion strategies.

2.2. Feedstock selection and community-based rationale

The formulation design prioritized the utilization of locally abundant agroindustrial residues and organic materials readily accessible to smallholder farmers in highland Ecuador. This approach ensures that the developed co-digestion strategies can be realistically implemented at the community level without requiring external inputs or specialized supply chains. The selected substrates represent typical waste streams generated by local agricultural and food processing activities, including cattle farming, sugarcane processing (panela and molasses production), dairy operations, and forage cultivation.

Cattle manure serves as the primary substrate and inoculum source, providing essential methanogenic microorganisms and buffering capacity while representing the most universally available organic resource in highland farming systems [15]. The incorporation of sugar-rich residues (panela and molasses) addresses the common challenge of carbon-to-nitrogen ratio optimization in anaerobic digestion, while these materials are consistently produced by local sugarcane processing facilities throughout the region [16]. Protein-rich components (milk, whey, yeast) enhance microbial nutrition and nitrogen content, utilizing dairy waste streams that are often underutilized or disposed of inappropriately in rural communities [17].

The inclusion of ash from agricultural burning practices transforms a typically problematic waste product into a valuable pH buffer and micronutrient source, while *Medicago sativa* (alfalfa) leaves provide additional nitrogen and trace elements essential for microbial metabolism [18]. This holistic approach to substrate selection not only optimizes biodigestion performance but also addresses local waste management challenges, creating synergistic benefits for community-level implementation.

2.3. Experimental design and process optimization

A randomized complete block design (RCBD) was adopted with three treatments (biol formulations) and three blocks ($n = 9$ experimental units). The blocking was defined by raw material batch/temporal loading window, in each block a single batch of manure and inputs (molasses, milk, *Medicago sativa* and ash) was prepared and three tubular continuous flow biodigesters were operated under the same environmental conditions. This arrangement was employed to absorb predictable sources of variation unrelated to treatment and the intrinsic heterogeneity of batches and increase the precision of contrasts between formulations. Within each block, the three treatments were randomly assigned to the biodigesters. Each unit remained in operation for the time corresponding to its treatment (14–30 days); upon completion, the equipment was washed and conditioned to start the next block. In the statistical analysis, the block factor was incorporated to isolate variation between batches and focus inference on the formulation effect, maintaining operational parameters compatible with community-scale implementation.

The temperature was fixed at 37 °C (mesophilic window 35–40 °C) through

automated heating with calibrated sensors. This set point was selected because consortia derived from bovine manure show stable performance in mesophily [19], the energy cost is lower than in thermophily in community schemes, and the presence of co-substrates rich in sugars and proteins (molasses, milk, *Medicago sativa*) favors hydrolysis/acidogenesis kinetics adequate at 37 °C without compromising methanogenesis [20,21].

The pH was controlled at 6.8–7.2 through sodium bicarbonate dosing with continuous monitoring by electrode. This neutral interval limits the free ammonia fraction associated with protein nitrogen at 37 °C, maintains operational alkalinity to buffer volatile fatty acid pulses linked to molasses input, and benefits from the background alkalinity provided by ash [22]; bicarbonate was used as fine adjustment to avoid deviations from the target interval [23]. The value of 70% moisture refers exclusively to the moisture of the feeding substrate (influent) on wet basis, not to environmental humidity nor to a global reactor parameter [17]. Before each load, composite samples were taken from the prepared batch and moisture/TS/VS were determined by gravimetry (mass loss at 105 °C; total and volatile solids); with that measurement adjustment with process water was made up to 70% $\pm$ 2% to ensure pumpable rheology and appropriate mass transfer [24]. The water content of the reactor was followed by mass balances and flow control; environmental humidity was not part of the process control.

Mechanical agitation operated at predetermined intervals to maintain homogeneous substrate distribution and prevent stratification within the biodigesters [25]. The agitation protocol balanced mixing efficiency with energy conservation for practical community applications.

Fermentation duration ranged from 14 to 30 days depending on formulation characteristics. Process completion was determined through organoleptic evaluation including odor assessment, color changes, and texture modifications [26]. This monitoring approach enables farmer implementation without specialized equipment while providing reliable completion indicators.

2.4. Treatment formulations

The treatments were defined to cover a substrate–nutrition complexity gradient with community replication feasibility. The quantities are expressed in wet mass (kg) and volume (L) as they were dosed. To allow valid comparisons of biol yield (also biogas) and substrate efficiency, after mixing each formulation the influent was adjusted with process water until reaching a target moisture of 70% ($\approx$ 30% TS, wet basis) with $\pm$ 2% tolerance, determined by moisture gravimetry in composite samples of the batch. In this way, the total solids load (and its volatile fraction, VS) was kept comparable between treatments as shown in **Figure 1**.

2.5. Sample collection and physicochemical analysis

Biol samples were collected upon completion of each biodigestion cycle using sterilized sampling equipment to prevent cross-contamination between treatments. Multiple subsamples were obtained from different positions within each biodigester and composited to obtain representative samples for analysis. Samples were

immediately refrigerated at 4 °C and analyzed within 24 h for pH and dissolved nutrients, while parameters requiring extended storage followed established preservation protocols.

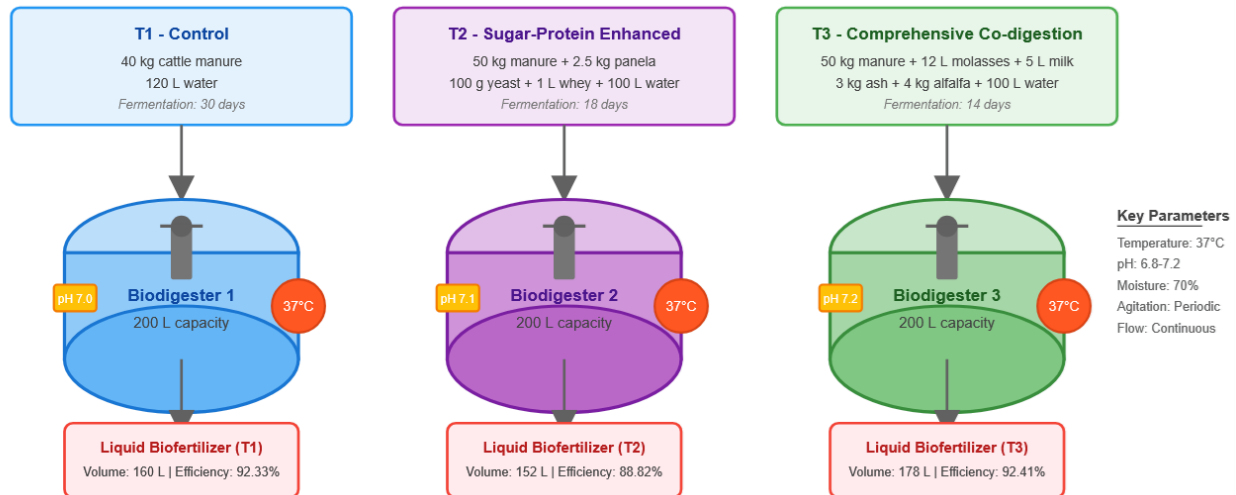


Figure 1. Experimental setup diagram.

Nutrient analysis encompassed both macronutrients and micronutrients essential for agricultural applications. Total nitrogen was quantified using the Kjeldahl digestion method, while phosphorus and potassium were determined through atomic absorption spectrophotometry and flame photometry, respectively. Secondary nutrients (Ca, Mg) and trace elements (Fe, Zn, Mn, Cu) were analyzed using atomic absorption spectrophotometry following acid digestion protocols.

Solution properties including pH, electrical conductivity (EC), and total dissolved solids (TDS) were measured using calibrated potentiometric methods. O.M. content was determined through loss-on-ignition via controlled calcination at 550 °C. Physical properties including density and moisture content were characterized using gravimetric and volumetric methods. All analytical procedures followed NTE INEN 211:98 [10] standards and National Institute of Agricultural Research (INIAP) [27] guidelines to maintain consistency with national quality frameworks.

2.6. Data collection and statistical analysis

The quantitative variables (e.g., N, P, K, pH, EC, organic matter) were measured per experimental unit (in triplicate) according to the procedures described in the methodology. In parallel, qualitative observations oriented to process and final product monitoring were recorded (odor, color, presence of foam/supernatant, and appearance of remaining solids).

These categories, of extended use in technical guides for bio-inputs and digestates at small scale reported in the INIAP manual [27,28], were employed as practical stabilization indicators: “organoleptic stabilization” was considered when odor in two consecutive readings separated 24–48 h. This criterion was used only to define the final sampling window within the retention time established by treatment, avoiding sampling during transitory phases.

The qualitative variables were coded as ordinals and summarized descriptively

(median and range); they did not enter the inferential models. Their function was documentary and process control (support to set the sampling moment and to describe the biofertilizer appearance).

For quantitative variables, a model according to the randomized complete block design was applied (treatment as fixed factor; block as control effect), with assumption verification and multiple comparisons when appropriate, maintaining $\alpha = 0.05$. The qualitative results were not used to support hypothesis tests; their contribution was interpretative/descriptive in the discussion of process progression and final product state.

Statistical analysis employed ANOVA appropriate for the randomized complete block design. Prior to analysis, fundamental assumptions were verified: normality through Shapiro-Wilk test, variance homogeneity through Levene's test, and independence through Runs test. Treatment differences were identified using Fisher's LSD test ($p < 0.05$) for pairwise comparisons.

For variables failing parametric assumptions, non-parametric alternatives were applied: the Friedman test as the ANOVA equivalent, followed by pairwise comparisons using the Wilcoxon signed-rank test with Bonferroni correction ($p < 0.05$). All analyses were conducted using *R* version 4.4.0, ensuring reproducibility and transparency of the statistical procedures.

3. Results and discussion

3.1. Biomass valorization through co-digestion: Physicochemical properties

The physicochemical characterization of liquid biofertilizers derived from different co-digestion strategies demonstrates the potential for agroindustrial biomass waste valorization (**Table 1**). Each formulation exhibited distinct nutrient profiles reflecting the synergistic effects of substrate diversification on digestate quality and agricultural value.

Table 1. Physicochemical characteristics of biol formulations.

Parameter	T1 (Control)	T2 (Improved)	T3 (Optimized)
E.C.	7.25 ± 0.12	3.19 ± 0.1	7.07 ± 0.06
Density	0.96 ± 0.01	10.28 ± 0.07	0.96 ± 0.01
Moisture	99.88 ± 1.04	85.27 ± 4.8	100.19 ± 1.5
pH	8.31 ± 0.06	7.2 ± 0.17	7.61 ± 0.2
TDS	4.66 ± 0.09	1.77 ± 0.03	3.5 ± 0.2
Calcium	0.07 ± 0.01	0.09 ± 0.01	3.31 ± 0.57
Chloride	0.0036 ± 0.0002	0.0043 ± 0.0002	0.003 ± 0.0003
Copper	0.0001 ± 0	0.0006 ± 0.0001	0.0682 ± 0.0037
Phosphorus	0.03 ± 0	0.12 ± 0.01	0.3 ± 0.03
Iron	0.52 ± 0.01	0.17 ± 0.01	0 ± 0
O.M	32.43 ± 0.79	50.32 ± 4.35	48.7 ± 0.72
Magnesium	0.56 ± 0.01	0.78 ± 0.01	1.71 ± 0.28

Table 1. (Continued).

Parameter	T1 (Control)	T2 (Improved)	T3 (Optimized)
Manganese	0.0007 ± 0.0001	0.0014 ± 0.0001	0 ± 0
Nitrogen	0.04 ± 0.01	0.11 ± 0.01	0.2 ± 0.02
Potassium	0.02 ± 0	0.2 ± 0.02	0.55 ± 0.02
Sulfur	0 ± 0	0.15 ± 0	0.17 ± 0.02
Zinc	0.03 ± 0	0.02 ± 0	0.0006 ± 0.0002

Note: E.C. = Electrical Conductivity, TDS = Total Dissolved Solids. Values represent mean ± standard deviation ($n = 3$).

The optimized co-digestion formulation (T3) achieved the highest concentrations of primary macronutrients, with nitrogen, phosphorus, and potassium reaching 0.20%, 0.30%, and 0.55%, respectively. This represents a five-fold increase in nitrogen and ten-fold increase in phosphorus compared to the cattle manure control, demonstrating effective nutrient concentration through strategic biomass blending [29]. The incorporation of molasses provided readily available carbon sources that enhanced microbial metabolism, while milk proteins contributed amino acids for nitrogen mineralization. *Medicago sativa* leaves, recognized for their nitrogen-fixing capacity and high protein content, further elevated the nitrogen profile of the final digestate [30].

The substantial calcium increase in T3 (3.31%) reflects the mineral contribution from ash incorporation, transforming agricultural burning residues into valuable pH buffering agents and secondary nutrient sources. Similarly, magnesium concentrations doubled in T3 compared to the control, indicating improved secondary nutrient availability for plant metabolism and chlorophyll synthesis [31].

O.M. content increased significantly in both enhanced formulations, with T2 and T3 achieving 50.32% and 48.70% respectively, compared to 32.43% in the control. This elevation represents enhanced soil conditioning potential through carbon sequestration and microbial substrate provision [32]. The higher O.M. content in co-digested formulations reflects the incomplete mineralization of complex substrates, preserving carbon-rich compounds beneficial for soil structure and biological activity[33].

The enhanced formulation (T2) demonstrated reduced E.C (3.19 mS/cm) and total dissolved solids (1.77 g/L), indicating lower salinity levels favorable for salt-sensitive crops and prolonged soil application [34]. This reduction suggests that sugar-rich substrates in T2 promoted microbial immobilization of soluble salts, reducing potential phytotoxicity risks.

Iron and zinc concentrations varied inversely with substrate diversification, with the control maintaining higher levels (0.52% Fe, 0.03% Zn) possibly due to concentrated micronutrient presence in undiluted cattle manure [35]. The absence of iron in T3 may reflect precipitation or complexation with organic compounds during extended fermentation periods.

These findings demonstrate that strategic co-digestion of complementary agroindustrial biomass streams can systematically enhance liquid biofertilizer quality while addressing regional waste management challenges. The nutrient concentration achieved through biomass valorization provides smallholder farmers with locally-

produced alternatives to synthetic fertilizers, supporting circular economy principles in agricultural systems [36].

3.2. Bioconversion efficiency and process optimization

Each treatment was operated in tubular biodigesters of 200 L nominal capacity. In each run the working volume was loaded with the previously adjusted influent, leaving a small upper space for gas and foam ($\approx 10\%$ total capacity). Upon concluding the fermentation time established by treatment, the reactor was left at rest to allow gravity separation; then the surface scum/foam was removed, the sediment was maintained at the bottom and the clear liquor was decanted through the lower valve. That decanted volume (**Table 2**) was recorded as “Volume of biol produced” (160 L in T1; 152 L in T2; 178 L in T3).

The reported efficiency (%) corresponds to the proportion of the initial useful volume that remained as stabilized biol after phase separation; meanwhile, it expresses what fraction of the loaded volume was recovered as usable liquid at the end of the process, after discounting losses associated with solids retention, foam and occluded gas.

Table 2. Bioconversion efficiency of co-digestion formulations.

Formulation	Volume of biol produced (L)	Fermentation time (days)	Efficiency (%)
T1	160	30	92.33
T2	152	18	88.82
T3	178	14	92.41

The optimized formulation (T3) achieved the highest volumetric yield (178 L) while requiring the shortest processing time (14 days), representing a 53% reduction in fermentation duration compared to the control treatment. This acceleration reflects enhanced microbial activity resulting from improved carbon-to-nitrogen ratios and substrate bioavailability through strategic biomass blending [37]. The combination of readily fermentable sugars from molasses and protein-rich substrates from milk and *Medicago sativa* created favorable conditions for accelerated hydrolysis and methanogenesis phases.

The enhanced formulation (T2) demonstrated intermediate performance with reduced processing time (18 days) but slightly lower conversion efficiency (88.82%). The incorporation of panela and whey provided easily metabolizable substrates that shortened fermentation time by 40% compared to mono-digestion, though the simpler substrate profile resulted in marginally reduced overall conversion efficiency [38].

Both co-digestion strategies maintain conversion efficiencies above 88%, indicating effective biomass transformation into liquid biofertilizer while substantially reducing processing time requirements. This efficiency demonstrates the technical and economic viability of enhanced formulations for community-scale implementation, where reduced fermentation time translates to increased biodigester throughput and improved resource utilization [39].

The volumetric yield improvements in T3 reflect not only enhanced substrate conversion but also the water-retaining properties of organic amendments, resulting in

increased liquid fraction recovery. This characteristic provides additional value for regions with water scarcity, where maximizing liquid biofertilizer production supports both nutrient management and irrigation efficiency.

3.3. Statistical validation of synergistic co-digestion effects

Statistical analysis confirmed significant differences in macronutrient concentrations among co-digestion strategies, validating the effectiveness of agroindustrial biomass diversification for liquid biofertilizer enhancement (**Table 3**).

Table 3. Parametric analysis (ANOVA–RCBD) of macronutrient concentrations in co-digested formulations.

Parameter	<i>F</i> -value	<i>p</i> -value	Significance	Post-hoc Test (LSD)
Nitrogen (N)	23.41	<0.001	***	T3 > T2 > T1
Phosphorus (P)	19.05	<0.001	***	T3 > T2 > T1
Potassium (K)	17.89	<0.001	***	T3 > T2 > T1
Calcium (Ca)	21.67	<0.001	***	T3 > (T2 = T1)
Magnesium (Mg)	20.34	<0.001	***	T3 > (T2 = T1)

Note: *** indicates statistical significance at $p < 0.001$. Rankings based on LSD post-hoc comparisons.

The ANOVA under randomized complete block design indicated a main treatment effect for all parameters (N, P, K, Ca, Mg), with $F = 17.89$ – 23.41 values and $p < 0.001$. Post hoc comparisons (LSD, $\alpha = 0.05$) showed the following patterns for N, P and K where $T3 > T2 > T1$ was observed, while for Ca and Mg $T3 > (T2 = T1)$ was obtained. Thus the integral formulation T3 statistically exceeded the others in all cases, but T2 and T1 did not differ from each other for Ca and Mg.

The escalation $T3 > T2 > T1$ in N, P and K is coherent with greater ionic availability associated with integral co-digestion. In contrast, the pattern $T3 > (T2 = T1)$ in Ca and Mg suggests that the increment of these cations is mainly linked to the mineral fraction included in T3 (ash), rather than to the organic co-substrates present in T2. In this way the results indicate that T3 maximizes the concentration of macroelements in the liquid digestate, although the magnitude of the effect and its determinant differ according to the evaluated ion.

The enhanced formulation (T2) demonstrated intermediate performance, confirming that even simplified co-digestion approaches using locally available substrates (panela, whey, yeast) can significantly improve liquid biofertilizer quality compared to mono-digestion systems. This finding supports the scalability of co-digestion technologies for smallholder applications where substrate availability may limit formulation complexity.

Statistical assumption verification through Shapiro-Wilk normality tests (**Table 4**), Levene's homogeneity assessments, and residual independence analysis confirmed the validity of parametric testing procedures, ensuring robust statistical inference for treatment comparisons. This methodological validation supports the reliability of observed differences and their practical significance for biomass valorization strategies.

Table 4. Statistical assumptions verification for macronutrient analysis.

Parameter	Shapiro-wilk	Levene	Runs test	Observations
Nitrogen (N)	0.87	0.70	0.12	Assumptions met
Phosphorus (P)	0.41	0.71	1.00	Assumptions met
Potassium (K)	0.48	0.38	0.12	Assumptions met
Calcium (Ca)	0.90	0.70	1.00	Assumptions met
Magnesium (Mg)	0.82	0.71	1.00	Assumptions met

Note: p -values > 0.05 indicate satisfied assumptions for parametric analysis.

3.4. Mechanistic insights into synergistic biomass co-digestion

Various studies on anaerobic digestion and co-digestion of agro-industrial waste show that the increase of N , P and K in the liquid phase does not only obey an “additive” effect of the substrates, but to coupled mechanisms of hydrolysis, mineralization and ionic desorption that accelerate the transfer from the solid/colloidal matrix towards the digestion liquor [40]. This pattern is consistent with the substrate-specific design proposed in T3 and reinforces the mechanistic interpretation described [41].

In co-digestions with easily fermentable carbon (e.g., molasses) and proteinaceous co-substrates (e.g., milk, forages), there is faster proteolysis and deamination, predominance of ammonium (NH_4^+) in the dissolved phase under anaerobic conditions (nitrification is insignificant) [42], and retention of N in the liquor due to the buffering effect of alkalinity (pH close to neutral-slightly alkaline), which limits volatilization as NH_3 . Consequently, the N quota in NH_4^+ form increases with respect to organic/colloidal N , and this increment is typically observed when protein-rich sources (milk, *Medicago sativa*) are combined with sugars that “prime” microbial activity [37]. Thus, the N elevation observed in T3 results consistent with a migration towards the dissolved pool promoted by carbon priming and amino acid mineralization [43].

For P , the evidence points to two complementary pathways: one the mineralization of organic P (release of reactive orthophosphate, PO_4^{3-}) favored by hydrolytic activity in the presence of easily available C [44], and the other the solubilization/desorption modulated by pH and alkalinity. The incorporation of agricultural ashes provides buffering agents (CaCO_3 , oxides) and cations ($\text{Ca}^{2+}/\text{Mg}^{2+}$) that reconfigure P speciation; under neutral-slightly alkaline pH, an increase in orthophosphate in liquid phase is usually observed due to (a) displacement of weakly adsorbed P and (b) complexation/organic that prevents readsorption in Fe/Al oxides [45]. Although the presence of Mg and NH_4^+ can lead to struvite in certain pH ranges and molar ratios, several studies show that, in agro-industrial matrices and pH 7–7.5, the precipitation kinetics is limited and P remains mostly as dissolved PO_4^{3-} or in labile fractions, which explains net increments in the liquor under conditions similar to T3 [46]. This behavior agrees with the mineral recovery from ashes and with the reduction of process times by optimizing ionic availability [3].

The K^+ , being monovalent and with low sorption affinity in most organic and mineral matrices, migrates rapidly to the liquid phase during substrate hydrolysis/disintegration [47]. The almost quantitative release of K in the first stages of digestion/co-digestion, with concomitant increments EC and TDS, without this

necessarily implying salinity risks when the process is controlled at adequate pH and load [48]. In systems with ashes and forages (as in T3), this pattern of rapid desorption and stabilization of K in the liquor is recurrent and explains its sustained elevation in the liquid biofertilizer [49].

These synergistic effects extend beyond individual substrate contributions, creating emergent properties through strategic biomass integration [50]. The specific combination of carbon-rich (molasses), protein-rich (milk, *Medicago sativa*), and mineral-rich (ash) components optimizes microbial communities for enhanced nutrient solubilization and O.M. stabilization [3]. This comprehensive approach to substrate selection provides a framework for designing location-specific co-digestion strategies based on locally available agroindustrial residues [51].

Non-parametric analysis of trace elements (pH, copper, manganese) using Friedman tests (**Table 5**) revealed marginal significance ($p < 0.05$), though Wilcoxon post-hoc comparisons with Bonferroni correction showed no pairwise differences between treatments. This conservative statistical approach ensures that only robust treatment effects are identified, supporting the reliability of reported macronutrient improvements while acknowledging the complexity of trace element behavior in co-digestion systems.

Table 5. Non-parametric analysis (Friedman test) of secondary parameters in co-digested formulations.

		<i>p</i> -value	Friedman
Physical	E.C	0.09697	ns
	pH	0.04979	*
Chemical	Copper	0.04979	*
	Manganese	0.04979	*

Note: * indicates $p < 0.05$; ns = not significant.

3.5. Operational implications and design principles for co-digestion

The results allow deriving operation guidelines and substrate selection. The T3 formulation evidenced process intensification with 92.41% conversion efficiency and 53% reduction in fermentation time, which translates into greater productivity and treatment capacity in the same infrastructure [35]. This performance is associated with mixtures that combine rapidly metabolizable carbon and organic nitrogen in proportions that sustain the hydrolysis and acidogenesis stages and stabilize methanogenesis under the evaluated conditions [52].

In terms of biofertilizer quality, the improved formulations presented higher O.M. content (T2: 50.32%; T3: 48.77%) compared to mono-digestion (T1: 32.43%), which indicates carbon preservation potentially beneficial for soil condition, in parallel to the concentration of nutrients of agronomic interest [34]. This dual-objective available nutrients and O.M. contribution guides design that integrate immediate fertility with soil improvement in the medium term [53].

From the implementation perspective, the process acceleration observed in T3 increases the throughput without equipment expansion, favoring the economic viability of decentralized biofertilizer production schemes and the valorization of local agro-industrial waste within circular economy frameworks [1]. Overall, biomass

diversification and its functional balance allow addressing, in an integrated manner, nutrient management, O.M. supplementation and waste reduction in small-scale agricultural systems [54].

4. Discussion

The synergistic co-digestion approach demonstrated in this study represents a significant advancement in biomass energy systems through enhanced resource recovery and process optimization. The fifteen-fold nitrogen increase and ten-fold phosphorus enhancement achieved through strategic substrate blending illustrates the potential for biomass valorization technologies to generate multiple high-value outputs beyond conventional biogas production [55]. This multi-product approach fundamentally transforms the economic proposition of anaerobic digestion systems, creating integrated biorefineries that maximize energy and material recovery from agricultural residues [56]. The shortened fermentation time from 30 to 14 days while maintaining 92.4% conversion efficiency demonstrates improved process kinetics that enhance biomass throughput and energy system productivity, supporting the development of more efficient distributed energy infrastructure in rural communities [57].

The integration of complementary agroindustrial biomass streams addresses critical challenges in biomass energy development, particularly substrate availability and quality variability. By combining sugar-rich substrates with protein sources and mineral amendments, the optimized formulation creates balanced feedstock compositions that stabilize anaerobic digestion processes while maximizing nutrient recovery [58,59]. This approach mirrors principles employed in advanced biomass energy systems where feedstock diversification enhances process reliability and output quality. The successful transformation of agricultural burning residues into valuable pH buffering agents exemplifies circular economy principles in biomass management, converting problematic waste streams into beneficial system inputs that improve overall energy conversion efficiency and environmental performance [60,61].

From a renewable energy perspective, the enhanced liquid biofertilizer production complements biogas generation by creating additional revenue streams that improve project economics and rural energy access [62]. The concentrated nutrient profiles achieved enable reduced transportation costs and storage requirements, enhancing the practical implementation of decentralized biomass energy systems. The O.M. preservation observed in co-digested formulations also supports soil carbon sequestration strategies that complement renewable energy development goals through integrated carbon management approaches [63]. These findings align with global trends toward multi-functional biomass energy systems that optimize both energy production and agricultural sustainability outcomes [64].

The transferability of the co-digestion approach across diverse agroecological contexts supports widespread deployment of enhanced biomass energy technologies throughout tropical highland regions. The emphasis on locally available substrates ensures that biomass energy systems can be adapted to regional resource availability patterns while maintaining performance standards. This adaptability addresses scalability challenges in biomass energy development where substrate logistics often

limit system viability [65,66]. Future research should focus on integrating advanced process monitoring technologies with community-scale biomass energy systems to optimize real-time performance while maintaining operational simplicity. Investigation of seasonal biomass availability patterns and development of substrate storage protocols would further enhance system reliability and year-round operation capabilities.

While this study demonstrates significant improvements in liquid biofertilizer quality through synergistic co-digestion, several limitations warrant acknowledgment. The research was conducted under controlled that may not fully represent field-scale variability and operational challenges encountered in community implementations [67]. The specific agroindustrial substrates evaluated reflect regional availability in highland Ecuador, potentially limiting direct transferability to other biomass energy contexts without adaptation. Additionally, the study focused on liquid phase outputs without comprehensive evaluation of biogas production characteristics or integrated energy-nutrient recovery optimization. Long-term stability assessment and agronomic field performance validation were beyond the scope of this investigation [68]. Despite these limitations, the substantial nutrient concentration improvements and process optimization achieved provide compelling evidence for the potential of synergistic co-digestion to enhance biomass energy system performance while supporting circular bioeconomy development in agricultural communities.

5. Conclusion

After the evaluation of the synergistic anaerobic co-digestion approaches. The results support the viability of decentralized biomass valorization schemes in high Andean and similar contexts. At the same time, it is suggested to evaluate the integration with biogas systems and validate in the field the agronomic effects of the biofertilizer at pilot scale.

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