

Article

Biotransformation of Agro-Livestock Residues by *Lactobacillus delbrueckii* subsp. *bulgaricus*: Advancing Circular Bioeconomy in Veracruz, Mexico

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Abstract

Agro-industrial residues from livestock and sugarcane production—particularly abundant in regions such as Veracruz, Mexico, where both agro-industries co-occur in high density—constitute underutilized carbon and nitrogen streams with high potential for biotransformation within circular bioeconomy frameworks. This study aimed to evaluate whether inoculation with *Lactobacillus delbrueckii* subsp. *bulgaricus* SP96 at increasing levels (5, 10, and 15% *v/v*) achieves simultaneous stabilization, carbohydrate-to-lactic-acid conversion, and nutritional enrichment of a thermally pretreated bovine manure (BM)–agro-sugarcane waste (ASCW) mixture, and to describe the underlying growth kinetics using the Gompertz model. Thermal pretreatment reduced *Salmonella* spp. and fecal coliforms to levels compliant with NOM-004-SEMARNAT-2002 Class B biosolid standards, yielding a substrate with suitable fermentability (14.56 gL^{−1} carbohydrates, 0.44% total nitrogen, pH 6.52, and 90.43% volatile solids). Fermentation at 37 °C and 120 rpm for 72 h followed Gompertz kinetics ($R^2 = 0.92$ – 0.97). The 15% inoculum achieved the highest conversion efficiency and product yield (lactic acid and carbohydrate consumption), whereas the 10% inoculum represented the most balanced operating condition (best kinetic fit, $R^2 = 0.97$). However, differences in lactic acid concentration among treatments were not statistically significant ($p > 0.05$). The resulting biomass showed high organic matter (93.45%), increased crude protein (8.5%), a C/N ratio of 39.9, and enrichment in P₂O₅, MgO, Na₂O, and B. These findings validate a low-cost, scalable platform for simultaneous waste stabilization and generation of value-added, protein-enriched biomass with potential application as a feed supplement and soil amendment, pending further safety and functional characterization, from agro-livestock residues.



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1. Introduction

The sustainable management of organic residues represents one of the most pressing challenges of the 21st century, particularly within the framework of a circular economy that aims to convert waste streams into valuable resources [1]. Agro-industrial residues, including those generated by swine, cattle, and other livestock systems, constitute a significant

source of environmental pollution when not properly treated. However, they also represent a substantial opportunity to produce value-added bioproducts through innovative biotechnological processes [2]. Microbial biotransformation has emerged as a promising strategy capable of simultaneously addressing pollution concerns and the growing demand for renewable resources. The scale of this challenge is substantial: livestock manure alone accounts for an estimated 2.5 billion tons of waste generated annually worldwide, alongside comparable volumes of crop and agro-processing residues, positioning agro-livestock waste management as a global sustainability priority rather than a localized concern [3].

Among the available waste valorization technologies, anaerobic digestion for biogas production and aerobic composting remain the most widely implemented because of their technological maturity and their ability to stabilize large volumes of waste [3,4]. However, both approaches target energy recovery or soil amendment primarily, and neither is specifically oriented toward the recovery of high-value, protein-enriched microbial biomass and organic acids through targeted lactic acid bacteria fermentation, representing a comparatively underexplored valorization route for combined livestock–agro-industrial substrates. This distinction has also been explored by the authors' own research group in related biological valorization strategies for other agro-industrial residue streams [5]. At the international level, the stabilization of organic residues is regulated through criteria designed to ensure microbiological safety, pathogen elimination, reduction in vector attraction, and minimization of putrefaction potential [6].

Regulatory frameworks in the United States and the European Union establish physico-chemical and microbiological thresholds that must be achieved for a residue to be classified as stabilized and suitable for agricultural or environmental applications [7]. These standards, originally developed for municipal wastewater sludges, include requirements such as reductions in total and volatile solids, pathogen inactivation, and compliance with limits for heavy metals and toxic compounds. In contrast, Mexico possesses a far more limited regulatory framework, since NOM-004-SEMARNAT-2002 exclusively regulates residual sludges from wastewater treatment plants, establishing criteria such as a minimum reduction of 38% of total solids, vector attraction reduction, and compliance with heavy metal limits. While this regulation allows the classification of treated sludges as biosolids, there is a notable absence of guidelines applicable to agro-industrial waste, especially that of livestock origin, which constitutes a significant regulatory gap [8].

Under this limitation, it becomes relevant to adapt stabilization criteria developed for residual sludges to the context of agro-industrial residues, considering that these materials (especially agricultural and livestock wastes) typically meet the non-hazardous requirements defined by NOM-052-SEMARNAT-2005 (CRETIB criteria) [9] and remain within the permissible heavy-metal limits of NOM-004-SEMARNAT-2002. This adaptation allows emphasis on total solids reduction, directly associated with decreased microbial load and reduced vector attraction. Among the technologies with the greatest potential to achieve these objectives are anaerobic fermentative processes mediated by lactic acid bacteria. *Lactobacillus delbrueckii* subsp. *bulgaricus* is a Gram-positive, thermophilic lactic acid bacterium widely recognized for its ability to convert organic substrates into valuable metabolites such as lactic acid, as its principal metabolite (thereby acidifying the medium and inhibiting pathogenic and undesirable microorganisms) [10,11].

When compared to other lactic acid bacteria frequently used in fermentation processes, this microorganism exhibits high tolerance to acidic environments, rapid carbohydrate conversion capacity, and efficient acidification of the fermentation medium, which contributes to the inhibition of undesirable microbiota during fermentation. Additionally, its Generally Recognized as Safe (GRAS) status and metabolic adaptability to heterogeneous agro-industrial substrates rich in fermentable carbohydrates make it a suitable microor-

ganism for biomass valorization and circular bioeconomy applications [12]. Due to its high nutritional requirements, this microorganism has traditionally been cultivated in synthetic media; however, the growing interest in sustainable bioprocesses has promoted the exploration of agro-industrial residues as low-cost alternative substrates. In this regard, bovine manure and agro-sugarcane waste were selected due to their high regional availability in the state of Veracruz, Mexico, where cattle production and sugarcane agro-industry constitute major economic activities that continuously generate large volumes of organic residues.

Veracruz ranks among the leading Mexican states in both cattle inventory and sugarcane cultivation area, and this geographic overlap between the two agro-industries makes it a representative case study for evaluating the co-valorization of livestock and sugarcane residues within a single fermentation process; a regional assessment of current agro-sugarcane residue management practices among local cattle producers is presented in Section 3.4, providing practical context for the laboratory-scale results reported here. Beyond their regional availability, the selection of BM and ASCW responds to a documented physicochemical complementarity: bovine manure has a low C/N ratio ($\approx 5\text{--}8$) and strong buffering capacity but insufficient carbon for efficient fermentation when used alone, whereas lignocellulosic residues such as sugarcane bagasse and tops provide a high C/N ratio and abundant structural carbohydrates but limited buffering capacity; combining both substrates corrects this mutual imbalance, as widely applied in co-fermentation and co-digestion processes [13,14]. Both raw materials have also been independently validated as fermentable substrates for lactic acid bacteria, including bovine manure under conditions comparable to those used here [15,16] and sugarcane bagasse hydrolysates fermented directly by *Lactobacillus delbrueckii* [5].

This complementary composition creates a suitable substrate matrix for closed, oxygen-limited lactic acid fermentation processes involving heterogeneous agro-industrial residues [17,18]. Bovine manure provides moisture, nitrogen, and mineral content, whereas agro-sugarcane waste contributes structural carbohydrates and lignocellulosic carbon fractions capable of improving the physicochemical stability and C/N balance of fermentation systems. Because this combined matrix retains heterogeneous soluble and structural fractions, valorization requires a versatile microorganism capable of exploiting organic substrates. Accordingly, the metabolic versatility of *Lactobacillus delbrueckii* subsp. *bulgaricus* enables the efficient utilization of these complex substrates, including simple sugars, structural polysaccharides, and partially hydrolyzed lignocellulosic components, making it an ideal candidate for residues of diverse composition, such as livestock wastes [19–21]. Additionally, *L. delbrueckii* subsp. *bulgaricus* has been reported to produce bacteriocins and limited proteolytic activity, primarily through lactocepin (EC 3.4.21.96), a cell-envelope-associated proteinase that hydrolyzes proteins into peptides and free amino acids at the cell surface, together with amylolytic activity mediated by α -amylase (EC 3.2.1.1), which cleaves accessible α -1,4-glycosidic bonds in simple sugars and short oligosaccharides. These enzymatic activities primarily target readily accessible fractions, contributing to nitrogen mobilization and partial depolymerization of soluble carbohydrates, while structural lignocellulosic fractions are expected to remain largely intact, consistent with the species' known enzymatic profile; no assays targeting cellulolytic or hemicellulolytic activity were performed in this study [22].

Through these mechanisms, fermentation not only stabilizes residues but also generates value-added products such as lactic acid (in the liquid phase, serving as a key raw material for the food, pharmaceutical, and bioplastics industries) [23], and microbial biomass (in solid phase), representing a protein-rich resource with potential feed-related applications [24], whose functional and safety characterization (strain viability after pro-

cessing, antibiotic-resistance profile, and efficacy trials) remains to be established. Beyond describing whether fermentation occurs, kinetic modeling provides a quantitative framework to characterize how it occurs over time, allowing direct comparison of treatments through defined parameters such as the lag phase, maximum specific growth rate, and maximum attainable growth, rather than relying solely on single-endpoint measurements. Sigmoidal models such as the modified Gompertz equation are particularly well suited to fermentation processes involving complex, heterogeneous substrates, where microbial growth typically follows a lag–exponential–stationary pattern shaped by substrate accessibility and metabolite accumulation.

Additionally, its descriptive value, kinetic modeling is also a prerequisite for process design and scale-up, since parameters obtained under controlled laboratory conditions can inform reactor sizing, residence time estimation, and future process optimization [25]. Therefore, this study evaluates the anaerobic fermentation of a mixed bovine manure–agro-sugarcane waste (BM–ASCW) system using *Lactobacillus delbrueckii* subsp. *bulgaricus*, aiming to optimize microbial kinetics, maximize carbohydrate-to-lactic acid conversion, and generate stabilized, protein-enriched biomass, while providing laboratory-scale evidence supporting the potential of this approach as a platform for the valorization of agro-livestock residues within a circular bioeconomy framework, supported by kinetic modeling and process performance assessment (carbohydrate consumption, lactic acid yield, and biomass protein content; see Section 2.7). This approach integrates microbiological stabilization with biochemical conversion, establishing a proof-of-concept pathway for transforming heterogeneous organic residues into functional bioinputs with potential agricultural and industrial applications, pending validation at pilot and industrial scale.

2. Materials and Methods

2.1. Obtaining and Conditioning Agro-Industrial Waste

2.1.1. Bovine Manure Sample (Residual Substrate)

Bovine manure (BM) was collected at a semi-technified dairy farm located in Rafael Delgado, Veracruz, Mexico, following USDA-NRCS sampling guidelines [26]. Fresh samples (≤ 2 h post-excretion) were obtained directly from the milking and handling areas, under ambient conditions below 25 °C, to reduce superficial desiccation and limit the proliferation of undesirable saprophytic microorganisms. The selection of fresh samples was prioritized to prevent spontaneous fermentation, oxidation, and decomposition processes that could alter the intrinsic carbon-to-nitrogen (C/N) ratio and substrate composition.

Once collected, samples were transferred into sealed 2 kg high-density polyethylene containers, refrigerated at 4 °C, and processed within 24 h of collection to ensure controlled handling, preserve substrate integrity, and minimize spontaneous fermentation during transport and storage, following standard field sample handling and storage procedures [27]. Following collection, physicochemical and microbiological characterization was carried out by determining pH according to NMX-AA-013-SCFI-2006 [28], total and volatile solids using APHA Standard Method 2540G [29], carbohydrates by the Anthrone–Sulfuric method [30], total nitrogen and proteins by the Kjeldahl method [31], and fecal coliforms, *Salmonella* spp., and helminth eggs following NOM-004-SEMARNAT-2002 [9].

2.1.2. Agro Sugarcane Waste (Support Substrate)

The agro-sugarcane waste (ASCW) used as the structural support substrate was supplied by a sugar mill located in the central region of Veracruz, Mexico. At the time of collection, care was taken to ensure minimal exposure of the material to intense sunlight and ambient humidity; therefore, sampling was conducted during the early morning hours (06:30–09:00 h). The sample consisted of representative fragments of the various

components of sugarcane field residues, including dry and green stalk pieces, tops, dried grasses, leaves, and cane debris, with approximate lengths of 20–30 cm (inter-node segments). Burned material, soil-contaminated sections, or foreign residues were deliberately excluded. To represent spatial heterogeneity, at least five subsamples were collected across the mill's residue yard and subsequently homogenized to obtain a composite sample.

The collected material was placed in opaque polyethylene bags, sealed to prevent air ingress, and transported to the laboratory under passive cooling conditions (shaded environments, $<25\text{ }^{\circ}\text{C}$) to inhibit microbial proliferation and avoid spontaneous fermentation [27,32,33]. After arrival, the material was oven-dried and then milled using a pulverizing mill (SDV, model SV-MOL-2000; SemillasdeVida, Mexico).

Vida, Mexico) under controlled rotational speed and gradual feed rate to prevent overheating and preserve the structural integrity of the lignocellulosic matrix [34]. The pulverized ASCW was sieved through a No. 50 mesh ($\approx 300\text{ }\mu\text{m}$) to obtain a uniform particle size fraction, removing coarse particles that could impair homogeneous mixing with BM. A 1:10 (w/v) dilution was then prepared (equivalent to 100 g ASCW/L), using sterile distilled water, followed by gentle agitation to ensure complete dispersion of the pulverized particles. This suspension was stored at $4\text{ }^{\circ}\text{C}$ until use to prevent microbiological alteration [35]. Once conditioned, the ASCW sample underwent physicochemical characterization using the same analytical procedures described for BM.

2.2. Thermal Stabilization

This stage is essential to ensure microbiological safety and enable the application of bovine manure in biotechnological processes, as it significantly reduces undesirable microbial loads without compromising the availability of essential nutrients [36,37]. The thermal treatment conditions were selected based on biosolid sanitation and sludge stabilization criteria reported in previous studies and international guidelines [6,38,39], which indicate that temperatures within the range of $70\text{--}90\text{ }^{\circ}\text{C}$ applied for approximately 30 min are effective for substantially reducing pathogenic microorganisms and inactivating highly resistant biological structures such as helminth eggs. The objective of this pretreatment was to significantly decrease the native microbial load and promote the selective establishment of *Lactobacillus delbrueckii* subsp. *bulgaricus* during the subsequent fermentation stage. In the case of BM, a controlled thermal treatment at $90\text{ }^{\circ}\text{C}$ for 30 min was applied, followed by filtration to remove oversized residues or highly recalcitrant fibers that could interfere with mixture homogeneity; the resulting liquid fraction was subsequently used to prepare the substrate blend. On the other hand, ASCW was dried at $110\text{ }^{\circ}\text{C}$ for 8 h, a condition that effectively reduces moisture content to below 10%, thereby inhibiting microbial proliferation and chemically stabilizing the lignocellulosic matrix. This drying step not only ensures biosafety but also enhances milling performance and mixture homogenization, enabling the production of particles with increased surface area and improved enzymatic accessibility during fermentation.

2.3. Preparation of a Mixed Bovine Manure/Agro-Sugarcane Waste Substrate

The BM residue was diluted in distilled water at a 1:5 ratio (w/v) to achieve a total solids content between 1 and 5%, while the ASCW was milled, sieved, and concentration-adjusted to facilitate its dispersion in the liquid medium. Preparation of the mixed substrate (BM/ASCW) was performed through the controlled integration of bovine manure (BM) with agro-sugarcane waste (ASCW) at a 1:1 ratio (v/v), selected as the point at which the system achieves the most suitable balance between fluidity, operational density, and total solids contribution. At this ratio, the mixture exhibits a moderate density ($\sim 1045\text{ kg}\cdot\text{m}^{-3}$),

low enough to allow homogeneous fluid movement under agitation, but high enough to ensure a significant solid load contributed by the ASCW.

It is noteworthy that mixtures with a lower ASCW proportion maintain high fluidity but display reduced total solids content, limiting the availability of structural substrates for fermentation. Conversely, mixtures with a higher ASCW fraction increase system density to the point of hindering uniform dispersion of the solid material, generating regions prone to sedimentation. Once the optimal 1:1 ratio was established, the supporting matrix was prepared by mixing both materials under continuous agitation until obtaining a uniform composition, ensuring proper distribution of the lignocellulosic components from ASCW within the organic matrix of BM [40,41].

2.4. Propagation of *Lactobacillus*

The probiotic bacterium *Lactobacillus delbrueckii* subsp. *bulgaricus* SP96 was obtained from a biological culture bank located in Mexico City. The strain was supplied in lyophilized form at a concentration of 1×10^{11} CFU/g. For reactivation and propagation, 1 g of the lyophilized culture was suspended in 100 mL of sterile MRS broth and incubated at 36 °C for 24 h, following the propagation protocol established in previous studies [42]. This step was carried out not only to reactivate the microorganism but also to promote the propagation of bacterial biomass, allowing the production of metabolically active cultures with an adequate cell concentration for subsequent use as inoculum in the biotransformation assays. A separate propagation batch was prepared for each inoculum treatment (5, 10, and 15% *v/v*); the small differences in initial cell density observed among treatments in Table 3 (4.04, 4.00, and 4.11 log CFU mL⁻¹, respectively; ≤ 0.11 log units) reflect normal batch-to-batch propagation variability and plate-count replicate error, rather than an intended experimental difference, since a single inoculum stock was not divided across treatments.

2.5. Biotransformation Experiments of BM/ASCW Residues

Once the *Lactobacillus* cultures were propagated, growth kinetics assays were performed by inoculating the mixed BM/ASCW substrate with 5, 10, and 15% *v/v* inoculum levels, based on the total working volume of 150 mL in 250 mL Erlenmeyer flasks. Accordingly, inoculum volumes of 7.5, 15 and 22.5 mL were used for each treatment, respectively. The fermentation reactors were equipped with single-bore rubber stoppers that remained sealed throughout the entire process. Sampling was carried out through permanently installed tubing, eliminating the need to repeatedly open the reactors and thereby minimizing oxygen intrusion. Although no external inert gas purging was applied, the combination of a closed reactor configuration, restricted gas exchange through the sealed single-bore stoppers and microbial oxygen consumption during the initial stages of fermentation favored the establishment of anaerobic conditions inside the reactors. To verify the maintenance of these conditions, dissolved oxygen (mgL⁻¹) was measured in situ using a HQ30d portable meter equipped with an Intellical™ LDO101 luminescent/optical dissolved oxygen probe (Hach Company, Loveland, CO, USA). The reactors were incubated at 37 °C and 120 rpm for 72 h under anaerobic conditions and controlled pH (6.0 ± 0.5) [15]. pH was monitored potentiometrically at each 4 h sampling interval and manually adjusted to maintain it within 6.0 ± 0.5 by the dropwise addition of 0.1 N NaOH, counteracting the acidification produced by lactic acid accumulation, following a pH-control approach consistent with recent studies on *Lactobacillus delbrueckii* subsp. *bulgaricus* fermentation of agro-industrial residues [43].

To determine whether the observed acidification could be attributed to the inoculated *Lactobacillus delbrueckii* subsp. *bulgaricus* rather than to spontaneous fermentation by residual native microbiota, a non-inoculated control was included consisting of the ther-

mally treated BM/ASCW substrate [BM/ASCW]_{HT}, processed under identical conditions (37 °C, 120 rpm, 72 h, sealed reactor) but without microbial inoculation, run in triplicate ($n = 3$). This control allowed the effect of thermal pretreatment on the native microbiota to be assessed independently of the inoculation effect. Likewise, reactors containing only bovine manure or only agro-sugarcane waste were not considered, since the study was specifically designed to evaluate the combined valorization of both residues as a single fermentation matrix. Sampling was conducted every 4 h to quantify carbohydrates, lactic acid, and viable cell counts, whereas protein determination was performed at 12 h intervals. Viable cell concentration was determined by plate count on MRS agar and expressed as log(CFU/mL) [44]. Lactic acid (LA) production was quantified by titration following the methodology established in the Mexican Standard NMX-F-716-COFOCALEC-2014 [45]. At the end of fermentation, the reactor contents were centrifuged for 10 min at 10,000 rpm to separate the liquid fraction (supernatant) from the solid fraction (microbial biomass). The recovered biomass was subsequently dried in a forced-air convection oven at 60 °C for 24 h to a constant weight, avoiding thermal degradation of heat-labile organic compounds, and then analyzed to determine its yield and nutritional composition. A schematic overview of the process methodology is presented in Figure 1.

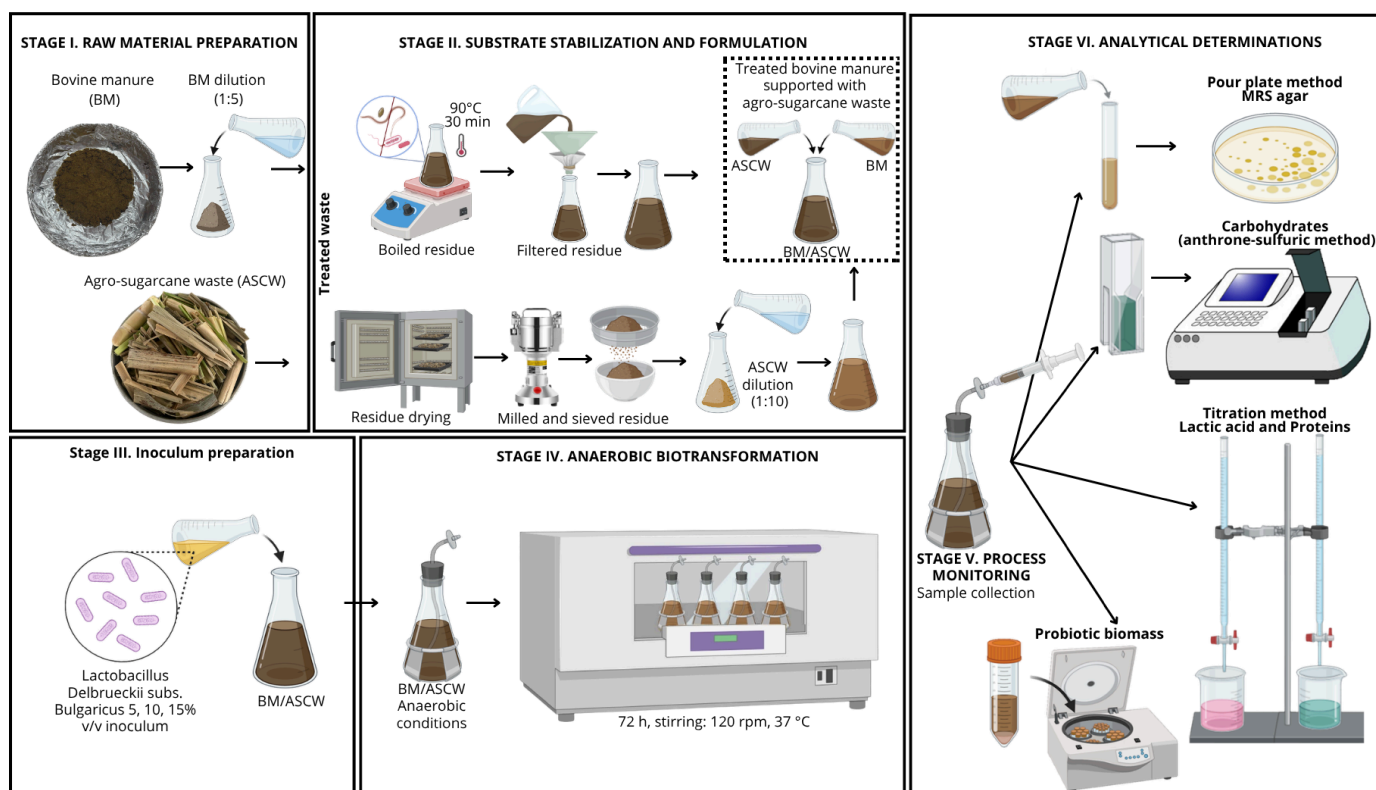


Figure 1. Methodological diagram of the biotransformation process using BM/ASCW and the probiotic species of *Lactobacillus delbrueckii* subsp. *bulgaricus*.

2.6. Kinetic Modeling of *Lactobacillus delbrueckii* Growth

The bacterial growth profile was modeled using the four-parameter Gompertz equation [Equation (1)], a widely applied sigmoidal function for describing microbial evolution under controlled fermentation conditions [25], which allows direct comparison between experimental data and model predictions. This formulation is widely applied in microbial kinetics to describe sigmoidal growth behavior under batch fermentation conditions, par-

ticularly due to its ability to represent asymmetric growth curves more accurately than classical logistic models [46].

$$y_A = b + (A)\exp(-\exp(-K_s(t - t_i))) \quad (1)$$

where $y_A = \log(N_t)$ represents the microbial cell concentration at time t , expressed as the logarithm of colony-forming units per milliliter [$\log(\text{CFU/mL})$]; parameters a and b correspond to the maximum and minimum cell concentrations [$\log(\text{CFU/mL})$] estimated by the model, and their difference $A = (a - b)$, represents the asymptotic extent of microbial growth as $t \rightarrow \infty$ as estimated by nonlinear regression—analogue to the parameter $A = \log(N_{\max}/N_0)$ in the original Zwietering et al. parameterization [46]. The parameter t_i corresponds to the inflection point of the curve, which represents the time at which the maximum growth rate occurs. Meanwhile, the parameter K_s is associated with the slope of the exponential phase and determines the rate at which the system transitions from the lag phase to active growth. Based on these parameters, the maximum specific growth rate ($\mu_{\max} = K_s/e$) and the lag phase duration ($\lambda = t_i - (1/K_s)$) were calculated [46].

The Gompertz model provides a robust framework for interpreting microbial growth dynamics, as it captures the transition between physiological adaptation, exponential proliferation, and growth deceleration due to substrate depletion or metabolite accumulation [47]. In this context, the parameter K_s reflects the rate of transition from the lag phase to active exponential growth, while t_i provides insight into the temporal dynamics of microbial adaptation and growth acceleration. Furthermore, the application of this model enables the quantitative description of the relationship between biomass formation and fermentation progress, facilitating the comparison between different operational conditions, such as inoculum concentration [25]. This is particularly relevant in systems where microbial growth is coupled to metabolite production and substrate conversion [48], as it allows the identification of conditions that maximize growth performance and process efficiency.

The validity of the model was verified through the coefficient of determination (R^2), ensuring a strong correlation between experimental observations and predicted values. The high goodness-of-fit obtained supports that the Gompertz model adequately describes the growth behavior of *Lactobacillus delbrueckii* under the evaluated anaerobic fermentation conditions. Additionally, the consistency between experimental and simulated curves indicates that the model reliably captures the different phases of microbial growth, including lag, exponential, and deceleration stages. This agreement supports the suitability of the selected parameters to represent the biological system and suggests that the model can be used for predictive purposes under similar operational conditions. Moreover, the robustness of the fitting reinforces the applicability of the Gompertz approach for analyzing complex fermentation systems involving heterogeneous substrates [25].

2.7. Statistical Analysis

A completely randomized experimental design was applied, consisting of three replicates per treatment, with a total of 4 treatments (5%, 10%, 15%, and non-inoculated control) and twelve experimental units. Three inoculum levels (5, 10, and 15% v/v) were selected to span the practically relevant range reported for lactic-fermentation inoculation of complex agro-industrial substrates, and were evaluated in triplicate to allow variance estimation while keeping the batch design tractable for detailed sampling every 4 h over 72 h. This replication level is consistent with recent lactic acid bacteria fermentation studies employing comparable batch designs and sampling intensity [49]. The independent variables were the inoculum percentage (5, 10, and 15% v/v) and the presence/absence of inoculation, while the response variables included nutritive biomass, carbohydrate consumption, lactic acid production, protein synthesis, and viable cell count. The collected data were analyzed

using a one-way ANOVA followed by Tukey's post hoc test with a significance level of $p < 0.05$. The relationship between carbohydrate consumption and lactic acid production was assessed using Pearson's correlation analysis, following statistical approaches consistent with recent lactic acid fermentation studies [49]. Additionally, Dunnett's test ($\alpha = 0.05$) was used to compare each inoculated treatment against the non-inoculated control for lactic acid and residual carbohydrate concentration at 72 h. All statistical analyses were performed using Statistica version 7.0.

2.8. Biomass Valorization

The biomass obtained from the biotransformation process of the BM-ASCW substrate treated with *Lactobacillus delbrueckii* subsp. *bulgaricus* was subjected to a comprehensive physicochemical characterization. The determinations included moisture content, ash content, organic matter, pH, electrical conductivity, C/N ratio, and total nitrogen, as well as a detailed mineral profile (P_2O_5 , MgO, K_2O , Na_2O , Fe, and B). These analyses enabled the establishment of the elemental composition and the assessment of parameters associated with the material's stability and nutritional quality. The information generated was used to evaluate the biotechnological potential of the microbial biomass (Table 1).

Table 1. Physicochemical and microbiological characterization methods of BM/ASCW biomasses.

Parameter	Units	Method
Moisture	% w/w	Gravimetric
Ash	% w/w	Gravimetric
Organic matter	% w/w	Gravimetric
pH		Potentiometric
Electrical conductivity	S·dm ⁻¹	Conductimetry
Total carbon	% w/w	Dry combustion
Total nitrogen	% w/w	Kjeldahl method
C/N ratio		Carbon-nitrogen balance
Protein	% w/w	Kjeldahl method
Phosphorous (P_2O_5)		Acid digestion
MgO, Na_2O , K_2O , Fe, B		Acid digestion and spectroscopy

Note: Moisture, ash, organic matter, pH, electrical conductivity, total carbon, and the acid-digestion/spectroscopy determinations of P_2O_5 , MgO, Na_2O , K_2O , Fe, and B were performed following standard procedures for the characterization of composted and organic residual materials [50]. Total nitrogen and protein content were determined by the Kjeldahl method [51].

2.9. Identification of Agro-Sugarcane Residue Utilization Practices in Bovine Production Systems

A complete census of all registered bovine production units (BPUs) across 12 zones—municipalities in the state of Veracruz, Mexico, selected based on their proximity to sugarcane mills. The total study population was $n = 405$ herds, because this represents a complete enumeration of the target population (BPUs within the 12 selected municipalities), rather than a probabilistic sample drawn from it, the reported adoption rate constitutes the actual population parameter for this target group rather than a sample estimate; consequently, no inferential statistical testing (e.g., confidence intervals or hypothesis tests) was applied, consistent with standard census-based reporting practice. Adoption was defined as the reported use of any agro-sugarcane waste (ASCW: sugarcane tops, dry leaves, bagasse, or their combinations) either as part of the feeding ration or as a nutritional supplement during the previous 12 months. The primary variable was the adoption rate (%), calculated following standard adoption-rate methodology used in livestock technology studies [51] as:

$$\% \text{ Adoption} = \frac{\text{BPUs incorporating ASCW into feeding system}}{\text{Surveyed bovine production units (BPUs)}} \times 100. \quad (2)$$

The type of residue and the mode of utilization were also recorded (e.g., energy–protein supplement, total mixed ration, silage).

3. Results

3.1. Physicochemical and Microbiological Characterization of the Residues

Table 2 summarizes the results of the physicochemical characterization. A pH of 6.52 was recorded for BM–ASCW_{HT}, a value consistent with this type of agro-industrial substrate [52]. ASCW_R exhibited a stronger acidic tendency (pH 5.93), thereby lowering the final pH when incorporated into BM. This behavior is advantageous, since the proposed bioprocess requires a moderately acidic environment (pH 6.0 ± 0.5) to promote optimal probiotic growth [53,54]. Regarding total solids (TS), the BM–ASCW_{HT} mixture showed a content of 4.29%, which is appropriate for the process, as TS values below 8% facilitate adequate mixing and operational handling [55]. Carbohydrate concentration increased markedly in BM–ASCW_{HT}, improving 3.29-fold relative to the initial BM substrate and reaching a final concentration of $14.56 \text{ g}\cdot\text{L}^{-1}$. With respect to chemical composition, the incorporation of ASCW into BM yielded a total nitrogen content of 0.44%, which lies within the optimal range required for efficient lactic acid fermentation (0.1–0.5% N) [56].

Table 2. Physicochemical and microbiological characterization of residues.

Parameter	ASCW _R	BM _R	BM _{HT}	[BM/ASCW] _{HT}
pH	5.93	6.80	7.16	6.52
TS [%]	5.71	1.53	1.86	4.29
TVS [%]	97.03	86.96	70.06	90.43
Carbohydrates [$\text{g}\cdot\text{L}^{-1}$]	17.28	1.93	4.43	14.56
Total nitrogen [%]	0.43	0.23	0.15	0.44
Proteins (dry base) [%]	2.68	1.42	0.96	2.75
Fecal coliforms [MPN/g TS]	<1000	≥ 2400	<1000	<1000
<i>Salmonella</i> spp. [MPN/g TS]	<3.00	4.6×10^{10}	<3.00	<3.00
Helminth eggs [eggs/g TS]	N.D.	N.D.	N/A	N/A

Note: R = raw residue, HT = heat-treated residue, N.D. = not detectable, N/A = not applicable.

The higher-than-expected carbohydrate and protein content of the [BM/ASCW]_{HT} mixture, relative to the simple average of its two component streams, is attributed to additional thermal solubilization occurring at the point of mixing, when the freshly heat-treated BM_{HT} stream (90 °C) was combined with the ASCW suspension. This residual thermal effect likely promoted further release of soluble carbohydrates and nitrogenous compounds from the ASCW particles beyond what was captured when ASCW was characterized independently under refrigerated conditions (4 °C), consistent with the disproportionately smaller increase observed in total solids (a bulk gravimetric measure insensitive to the soluble/insoluble distribution) relative to the larger increases observed in carbohydrates and protein (both measured via methods specific to the soluble fraction).

The microbiological parameters highlight the critical importance of assessing livestock-derived residues, as raw samples exhibited values $\geq 2400 \text{ MPN g}^{-1} \text{ TS}$ for fecal coliforms and $4.6 \times 10^{10} \text{ MPN g}^{-1} \text{ TS}$ for *Salmonella* spp. These pathogenic loads were eliminated after thermal treatment, substantially reducing their potentially harmful impact, since they pose a risk to both human and animal health [57,58]. Post-treatment values for fecal coliforms and *Salmonella* spp. were <1000 and <3 MPN g^{−1} TS, respectively, thus complying with the maximum permissible limits for Class B biosolids established under NOM-004-SEMARNAT-2002. Helminth eggs were analyzed exclusively in bovine manure (BM), as this substrate represents the fraction with potential fecal contamination and therefore the most likely source of parasitic ova. No helminth eggs were detected in BM under the

analytical conditions employed, whereas analysis was not applicable to agro-sugarcane residue (ASCW) because it is a plant-derived lignocellulosic material with no expected risk of helminth contamination. The effectiveness of the thermal stabilization step is particularly relevant, as the presence of competing microorganisms can interfere with the optimal growth of probiotic species by initiating substrate competition and metabolic antagonism [59].

3.2. *Lactobacillus* Growth and Metabolite Generation

Dissolved oxygen measurements consistently yielded values close to zero ($<0.2 \text{ mg L}^{-1}$) throughout the 72 h fermentation period, confirming the persistence of anaerobic conditions within the system. The non-inoculated, thermally treated BM/ASCW_{HT} control ($n = 3$) showed no evidence of fermentative activity over the 72 h incubation period. pH remained virtually unchanged in all three replicates (initial: 6.2, 6.4, 6.3; final: 6.2, 6.4, 6.3), and carbohydrate content showed no consumption, with final values (15.4, 14.9, 14.5 g L^{-1} ; mean $14.9 \pm 0.5 \text{ g L}^{-1}$) statistically indistinguishable from the initial substrate concentration (14.56 g L^{-1}). Lactic acid remained below the detection limit in all replicates, and no viable colonies were recovered by the pour-plate method at any of the dilutions tested, confirming the absence of culturable bacterial growth. Dissolved oxygen in the control reactors ($0.2, 0.1, 0.1 \text{ mg L}^{-1}$) remained within the same range measured in the inoculated treatments ($<0.2 \text{ mg L}^{-1}$), confirming that the absence of fermentation was not attributable to insufficient oxygen limitation, but to the lack of a metabolically active inoculum. These results demonstrate that the thermal pretreatment effectively inactivated the native microbiota of the BM–ASCW substrate and that the fermentative acidification observed in the inoculated treatments is directly attributable to the introduced *L. delbrueckii* subsp. *bulgaricus*.

The kinetic behavior of *Lactobacillus delbrueckii* subsp. *bulgaricus* showed a consistent overall pattern across inoculum levels, with the 5% and 10% treatments displaying broadly similar growth and conversion trajectories, while the 15% treatment showed a more distinct profile (Figure 2). At the lowest inoculum level (5% *v/v*), the process reached 5.8 log CFU/mL, with a lag time of 0.24 h as estimated by the Gompertz model (Table 4), and a delayed accumulation of lactic acid ($\approx 10.23 \text{ g L}^{-1}$) relative to the higher inoculum treatments. Increasing the inoculum to 10% (Condition B) resulted in a comparable final conversion efficiency (87.01% substrate conversion, 11.15 g L^{-1} LA) to that of the 5% treatment, achieving 6.4 log CFU/mL within 24 h, although with a longer estimated lag time ($\lambda = 1.32 \text{ h}$) than the 5% treatment. Notably, the 15% inoculum exhibited the longest lag time of the three treatments ($\lambda = 6.04 \text{ h}$; Table 4), despite reaching the highest final cell density and conversion efficiency, suggesting that the larger initial cell load delayed the onset of active growth—plausibly due to intracellular substrate competition or early metabolite accumulation—without ultimately compromising overall process performance. Despite this extended lag phase, the 15% inoculum ultimately achieved the highest lactic acid concentration (13.4 g L^{-1}) and carbohydrate consumption (92.9%), with a maximum specific growth rate (μ_{max}) of 0.15 h^{-1} —the highest among the three treatments. Once the initial adaptation period was overcome, the rapid establishment of acid-dominant, oxygen-limited conditions likely favored continuous acidogenesis until nutrient limitation occurred, consistent with the higher final biomass and conversion efficiency observed at this inoculum level. The correlation between increased inoculum density and process efficiency confirms that higher initial cell counts, despite an extended adaptation period, ultimately facilitate greater substrate utilization and enhanced tolerance to end-product inhibition.

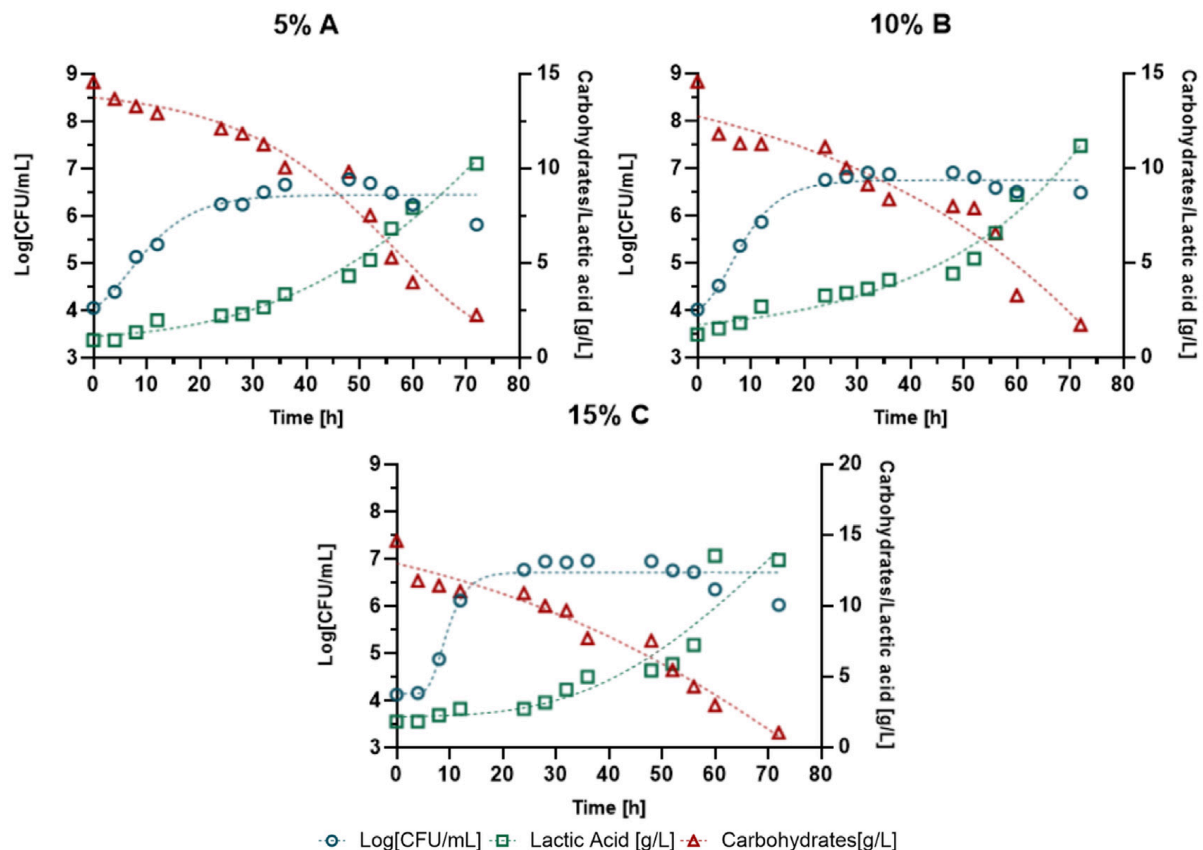


Figure 2. Growth kinetics of *Lactobacillus delbrueckii* subsp. *bulgaricus* in BM/ ASCW_{HT} at different inoculum concentrations: (A) 5%, (B) 10% and (C) 15% v/v.

Comparatively, the kinetic profiles reveal that fermentation efficiency improved consistently with inoculum concentration across the tested range (5–15%); whether this trend would continue at higher inoculum levels, where diminishing returns due to nutrient depletion or crowding effects might be expected, was not evaluated in this study and is recommended for future work. From a statistical perspective, data were evaluated by one-way ANOVA followed by Tukey's post hoc test ($\alpha = 0.05$) to identify significant differences among inoculum levels, and by Pearson's correlation analysis to quantify the relationship between carbohydrate consumption and lactic acid production. Significant differences ($p < 0.05$) were observed in residual carbohydrate content at 72 h as a function of inoculum percentage, with the treatments at 10% and 15% showing statistically higher degradation than 5%. Degradation levels were 83.37%, 87.01% and 92.9% for 5%, 10% and 15% inoculum, respectively (Figure 2). A strong negative Pearson correlation ($r = -0.91$) between carbohydrate concentration and fermentative acidification indicated that substrate depletion was closely associated with organic acid production during the process. This behavior is consistent with the metabolic characteristics commonly reported for *Lactobacillus delbrueckii* subsp. *bulgaricus* and suggests a predominantly acidogenic fermentation behavior under the evaluated conditions [60,61].

The increase in carbohydrate content from BM~R~(1.93 g L⁻¹) to BM~HT~(4.43 g L⁻¹) reflects thermally induced solubilization of soluble/extractable carbohydrates rather than structural (lignocellulosic) hydrolysis. Accordingly, the 92.9% carbohydrate consumption reported for the 15% inoculum refers to depletion of the soluble carbohydrate fraction quantified by the anthrone–sulfuric acid method, not to total structural carbohydrates in the substrate. Direct quantification of structural fiber content before and after fermentation (e.g., Van Soest fiber analysis) was not performed in this study and is recommended in

future work to establish the extent, if any, of structural carbohydrate degradation during the process. These findings align with previous reports that demonstrated that optimized inoculum levels are critical to maintaining the metabolic equilibrium between growth, substrate utilization, and product formation. In summary, the comparative analysis establishes that the 15% inoculum achieved the highest measured conversion efficiency and lactic acid yield among the tested conditions, whereas the 10% inoculum represented the most balanced operating condition in terms of kinetic robustness and process stability; differences in lactic acid concentration among treatments were not statistically significant ($p > 0.05$). These findings provide laboratory-scale evidence supporting the technical feasibility of the process for the biotechnological valorization of agro-industrial residues under circular bioeconomy frameworks.

In Table 3, the values derived from the growth kinetics of *Lactobacillus delbrueckii* subsp. *bulgaricus* are summarized, complementing the graphical information to facilitate a more robust interpretation of the results. Regarding carbohydrate consumption and LA production, both exhibited directly proportional relationships between the quantified variable and the inoculum percentage, indicating efficient substrate utilization accompanied by parallel acid synthesis. Likewise, the protein content increased from 2.75% (initial protein for all inoculum levels) to 6.88, 7.70, and 8.50% for the 5, 10, and 15% inoculum treatments, respectively, confirming the generation of biomass enriched in nitrogenous compounds.

Table 3. Initial and results of the growth kinetics of *Lactobacillus delbrueckii* subsp. *bulgaricus* during the anaerobic bioconversion of the BM/ASCW substrate.

Inoculum Concentration (% v/v)	Cell Density (Log CFU/mL)		Carbohydrates (g·L ⁻¹)		Lactic Acid (g·L ⁻¹)		Proteins (%)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
5	4.04	5.80	14.56	2.23	0.90	10.23	2.75	6.87
10	4.00	6.47	14.56	1.71	1.20	11.15	2.75	7.70
15	4.11	6.01	14.56	1.03	1.50	13.40	2.75	8.50

The fermentation performance was primarily evaluated through pH reduction and titratable acidity as indirect indicators of organic acid production during the process [60]. Although the observed acidification behavior is consistent with lactic acid fermentation by *Lactobacillus delbrueckii* subsp. *bulgaricus*, the analytical approach employed in this study was not intended to specifically identify or quantify individual metabolites present in the complex agro-industrial substrate mixture. Therefore, the results were interpreted in terms of overall fermentative acidification behavior associated with microbial metabolic activity under the evaluated conditions [61]. To provide a conceptual, literature-based interpretation of the experimental behavior observed during fermentation, a coupled metabolic pathway is proposed, as shown in Figure 3, based on the well-established homofermentative metabolism of lactic acid bacteria [62]. In this scheme, fermentable carbohydrates derived from the BM–ASCW matrix are assumed to be metabolized through the Embden–Meyerhof pathway, generating pyruvate as the central metabolic intermediate, which is subsequently reduced to lactic acid by lactate dehydrogenase, allowing continuous NAD⁺ regeneration and sustained glycolytic activity.

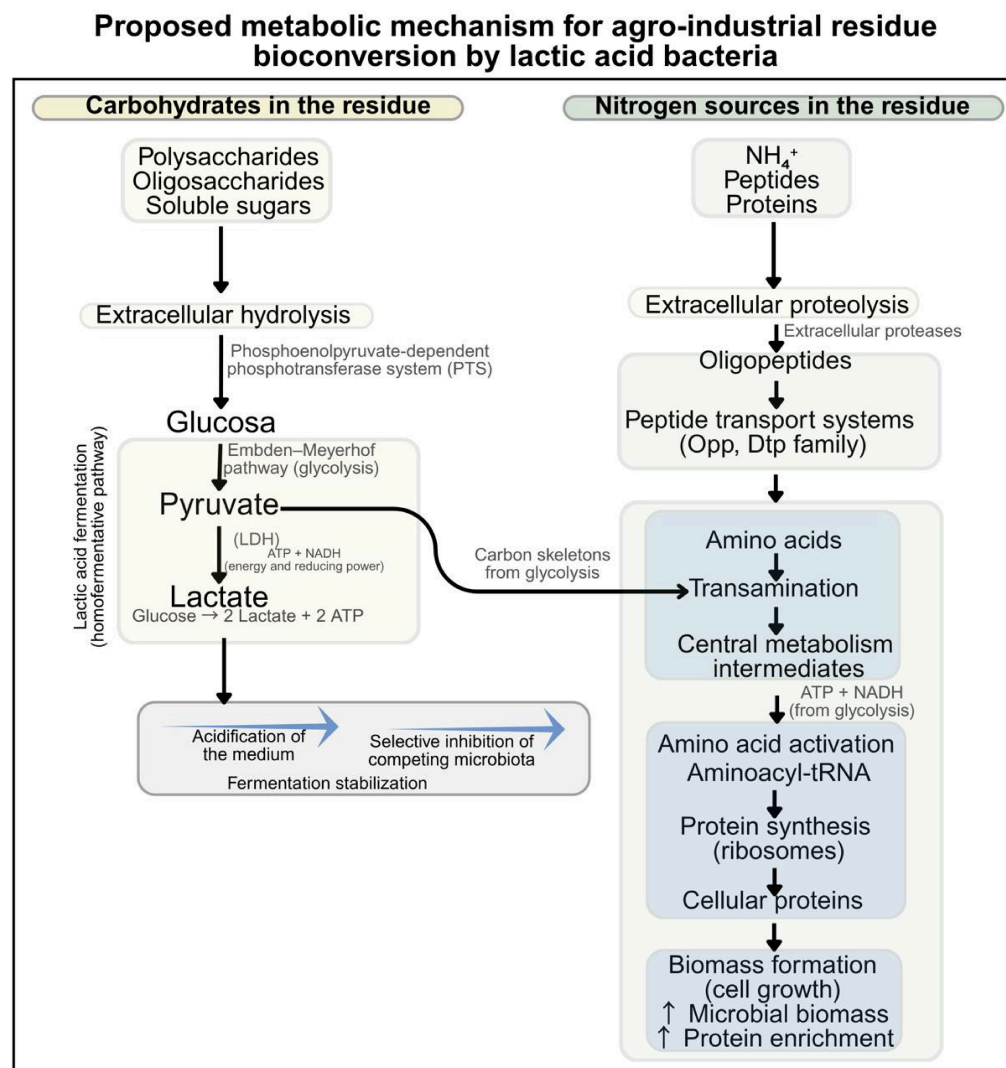


Figure 3. Source: authors' own elaboration, based on the established homofermentative metabolic pathway of lactic acid bacteria [62]; individual steps were not experimentally verified in this study.

Nitrogen assimilation is similarly assumed to proceed through extracellular proteolysis, peptide transport, and amino acid biosynthesis, consistent with the known metabolic capabilities of *L. delbrueckii* subsp. *bulgaricus*. This proposed pathway is consistent with, and offers a plausible explanation for, the simultaneous increase in lactic acid concentration and protein content observed experimentally at the 10% and 15% inoculum levels; however, none of the individual enzymatic steps or metabolic intermediates depicted in the scheme (e.g., pyruvate, NAD^+/NADH ratio, specific peptide/amino acid pools) were directly measured in this study, and the pathway should therefore be interpreted as a conceptual framework rather than as experimentally validated mechanism.

The kinetic parameters obtained for *Lactobacillus delbrueckii* subsp. *bulgaricus* during the anaerobic fermentation of the mixed BM–ASCW substrate are presented in Table 4. The four-parameter Gompertz model exhibited determination coefficients (R^2) ranging from 0.92 to 0.97, indicating a strong correlation between experimental observations and theoretical predictions. This strong fit validates the model's applicability for describing microbial growth dynamics and lactic acid production in complex fermentative matrices [25]. The increase in inoculum percentage was associated with a progressive rise in the maximum specific growth rate (μ_{max}), which increased from 0.06 h^{-1} for the 5% treatment to 0.15 h^{-1} at 15% inoculum. This behavior is consistent with the fact that higher initial inoculum

loads reduce the lag phase and favor the exponential phase, since a greater proportion of physiologically prepared cells are immediately available to initiate active growth [63,64].

Table 4. Kinetic parameters for the biotransformation process of BM/ASCW using *Lactobacillus delbrueckii* subsp. *bulgaricus*.

Inoculum (%)	a (log CFU/mL)	$Y_{P/S}$	μ_{max} [h ⁻¹]	K_s [h ⁻¹]	λ [h]	LA [g L ⁻¹]	R^2
5%	6.43	0.83	0.06	0.15	0.24	10.23 ^a	0.92
10%	6.73	0.87	0.07	0.20	1.32	11.15 ^a	0.97
15%	6.70	0.99	0.15	0.39	6.04	13.40 ^a	0.93

Note: a = maximum CFU concentration; $Y_{P/S}$ = LA production yield; μ_{max} = specific growth rate; K_s = Gompertz rate parameter (associated with the slope of the exponential growth phase); λ = lag or latency duration; LA = lactic acid production; R^2 = correlation coefficient; ^a = are not significantly different (Tukey's HSD, $p > 0.05$).

It is essential to distinguish here between μ , the intrinsic growth rate—defined as the theoretical maximum per capita division rate under unconstrained, optimal resource availability—and μ_{max} , the empirical parameter obtained by fitting a sigmoidal growth model (such as the modified Gompertz equation) to experimental data. This distinction is further compounded by the fact that the original mathematical parameters of the Gompertz equation carry no inherent biological meaning on their own, and only acquire biological interpretability once reparametrized in terms of λ and μ_{max} , as originally proposed by Zwietering [46]—a transformation that, while standard practice, does not eliminate the underlying dependence of the fitted parameters on the specific experimental conditions under which the growth curve was generated [65]. These two quantities are not interchangeable: rigorous analyses of growth-curve inference have demonstrated that fitted μ_{max} values systematically deviate from, and typically overestimate, the true intrinsic growth rate μ , with the magnitude of this deviation depending on experimental factors such as inoculum size, sampling resolution, and the detection method used [66]. Consequently, the μ_{max} values reported in Table 4 should be interpreted strictly as fitted, model-derived parameters characterizing the observed growth curves under each specific inoculum condition, rather than as direct estimates of an inoculum-independent intrinsic biological constant.

The apparent inoculum-dependent increase in μ_{max} observed here is consistent with this known limitation of growth-curve-derived kinetic parameters, rather than implying that the organism's true intrinsic growth capacity changed with inoculum size. Consistently, the values of the Gompertz rate parameter (K_s) increased from 0.15 to 0.39 h⁻¹, indicating a faster transition from the lag phase to active exponential growth at higher inoculum concentrations—a common phenomenon in fermentations involving partially hydrolyzed lignocellulosic residues, where a larger initial cell density accelerates the onset of active substrate utilization [15]. The lactic acid production yield ($Y_{P/S}$) showed a consistent increase (from 0.83 to 0.99 g LA/g carbohydrates), reaching its maximum at a 15% inoculum concentration, indicating a more efficient conversion of the available carbohydrates. This trend aligns with previous studies in which *L. delbrueckii* subsp. *bulgaricus* displays a stable homofermentative metabolism and high carbon-to-lactate conversion efficiency in media enriched with soluble carbon compounds [61]. The final lactic acid (LA) concentration increased from 10.23 to 13.4 g·L⁻¹, reflecting a direct relationship with cellular activity and nutrient availability; although the 15% inoculum treatment exhibited the highest lactic acid concentration, no statistically significant differences were observed among treatments ($p > 0.05$). Regarding the latency parameter (λ), a substantial increase was observed from 0.24 h (5% inoculum) to 6.04 h (15%), suggesting that the microorganism requires a longer physiological adaptation period when the initial cell density is high, likely

due to intracellular competition for substrate and the early accumulation of secondary metabolites [15,61,64]. However, this prolonged lag phase did not negatively affect overall productivity, indicating that the system rapidly reached a functional balance between substrate consumption and acid production. The inoculum-dependent trends observed in this study are consistent with previous reports on *Lactobacillus delbrueckii* fermenting agro-industrial residues, where increasing inoculum size similarly influenced lactic acid production efficiency, with an optimal inoculum level (25% *v/v*) identified beyond the range evaluated here [67]. However, the direction of the inoculum–lag phase relationship observed in the present study contrasts with findings reported for other *Lactobacillus* species modeled using the same Gompertz framework, where increasing initial biomass concentration shortened the lag phase and accelerated the transition to the stationary phase [68].

This divergence may reflect substrate-specific differences: unlike the soluble lactose or lignocellulose-derived sugars used in those studies, the heterogeneous, particulate BM–ASCW matrix used here may impose mass-transfer or nutrient-accessibility constraints that become more pronounced at higher cell densities, potentially explaining the extended lag phase observed at the 15% inoculum level. Overall, the treatment with a 10% inoculum exhibited the most balanced relationship between growth rate, conversion efficiency, and process stability ($R^2 = 0.97$), consistent with the most balanced operating condition already identified above, whereas the highest lactic acid yield remained associated with the 15% inoculum. This behavior is consistent with recent studies emphasizing the importance of optimizing inoculum level to maximize productivity while minimizing constraints associated with substrate depletion or the accumulation of inhibitory metabolites [69]. Accordingly, the results confirm that *Lactobacillus delbrueckii* subsp. *bulgaricus* is an efficient microorganism for the biotransformation of lignocellulosic agro-industrial residues, demonstrating performance comparable to that reported in similar processes employing lignocellulosic substrates such as sugarcane bagasse, corn husk, or tropical fruit residues [11,61,69,70].

Figure 4 illustrates the protein content as a function of fermentation time. The protein dynamics observed during anaerobic fermentation with *Lactobacillus delbrueckii* subsp. *bulgaricus* reveal a markedly inoculum-dependent behavior, reflecting both cellular proliferation and the metabolic synthesis associated with bacterial growth and adaptation on a complex substrate. Across all treatments, protein concentration increased substantially from an initial uniform value of $2.75 \text{ g} \cdot \text{L}^{-1}$ to 6.88, 7.70, and $8.50 \text{ g} \cdot \text{L}^{-1}$ for inoculum levels of 5%, 10%, and 15%, respectively. This ascending trend confirms that under anaerobic conditions, and in the presence of carbon-rich matrices such as bovine manure and ASCW, *Lactobacillus delbrueckii* not only consumes fermentable carbohydrates but also redirects part of the metabolic flux toward the biosynthesis of intracellular and extracellular proteins.

3.3. Analysis of Nutrient Biomasses

The dilution ratios used to reach the working total-solids range in this study (1:5 *w/v* for BM, 1:10 *w/v* for ASCW; Sections 2.1.1 and 2.1.2) are appropriate for laboratory-scale kinetic characterization but imply a substantial freshwater demand that would not be economically or operationally viable if extrapolated directly to commercial scale. As detailed in Section 5 (Future Perspectives), a pilot-scale unit processing 1 metric ton day^{-1} of each residue would require approximately $13 \text{ m}^3 \text{ day}^{-1}$ of water at these ratios, a demand that could be further reduced through strategies such as reusing wash water from technified cattle facilities. This same dilution and centrifugation step also generates a second stream requiring dedicated management: an acidic, lactic-acid-rich supernatant, containing up to 13.4 g L^{-1} of lactic acid at the 15% inoculum level, with a correspondingly low pH. This supernatant was not further characterized or valorized in this study, and its

final destination requires explicit consideration before this process can be implemented beyond laboratory scale, since discharge or land application of an untreated, high-acidity, high-organic-load stream carries a risk of soil or water impact.

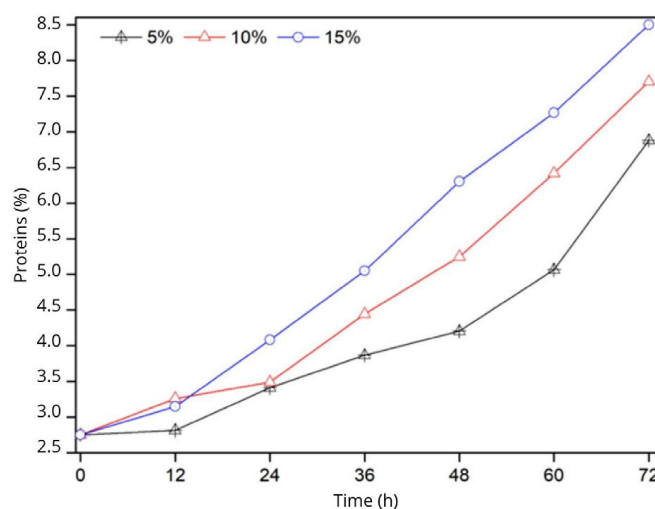


Figure 4. Protein content with different percentages of inoculum (5, 10 and 15%).

Two non-mutually exclusive pathways are proposed for future work: (i) recovery of the supernatant as a separate lactic-acid-enriched co-product; or (ii) neutralization and reincorporation as process water in subsequent fermentation batches, partially offsetting the freshwater demand discussed above. At the end of the fermentation process, the resulting material was centrifuged and dried, yielding a homogeneous solid with an earthy brown coloration that was visually observed to darken progressively as the inoculum proportion increased (Figure 5). This observation was qualitative and descriptive; no colorimetric or spectrophotometric quantification was performed in this study. The visually observed darkening is tentatively attributed to the heightened metabolic activity of the microorganisms during fermentation, which promotes the formation of nitrogenous compounds, pigments, and amines derived from the transformation of organic matter [71]; previous reports indicate that comparable color variations are linked to changes in matrices enriched in amino acids and to Maillard-type browning reactions occurring during microbial metabolic activity [72]. Quantitative colorimetric analysis is recommended in future studies to confirm and characterize this observation objectively.

In Figure 6, the images corresponding to the biomasses obtained under the different experimental conditions: 5% (A), 10% (B), and 15% (C) inoculation with *delbrueckii* subsp. *bulgaricus*; are shown. A progressive increase in the amount of biomass generated is observed, reaching 2.40, 2.64, and 2.67 g, respectively. This increment confirms a greater conversion of the substrate into microbial biomass as the initial microbial load increases, reflecting a higher overall efficiency of the fermentative process.

The results presented in Table 5 describe the physicochemical and nutritional composition of the biomasses obtained under different inoculum levels (5, 10, and 15%). The values reported reflect a material with a high organic matter content ($\approx 93\%$) and low ash fraction ($\approx 6.6\%$), indicating that the mineral portion is minimal relative to the organic matrix, a typical characteristic of residual biomasses with high biochemical valorization potential. The low residual moisture (4.68–5.72%) confirms that the material was effectively dried, ensuring microbiological stability and improved handling properties, which is advantageous for storage or transportation before downstream valorization. The pH decreased from 6.26 to 5.61 as the inoculum proportion increased, a trend associated with enhanced microbial fermentative activity and the concomitant production of organic acids (lactic,

acetic, and other secondary metabolites). This effect aligns with biological stabilization patterns reported in the recent literature [73], demonstrating that inoculation not only alters chemical composition but also activates microbial bioconversion pathways, contributing to the biological stabilization of the substrate.

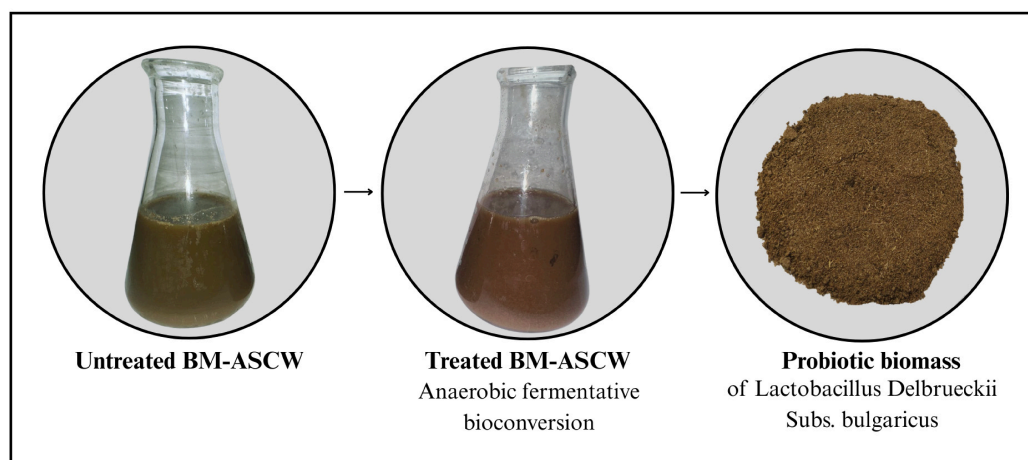


Figure 5. Representative photographs illustrating the visually observed color change in the dried fermented biomass at increasing inoculum levels (5, 10, and 15%) at the end of the *L. delbrueckii* subsp. *bulgaricus* fermentation process. Color assessment was qualitative; no colorimetric quantification was performed.

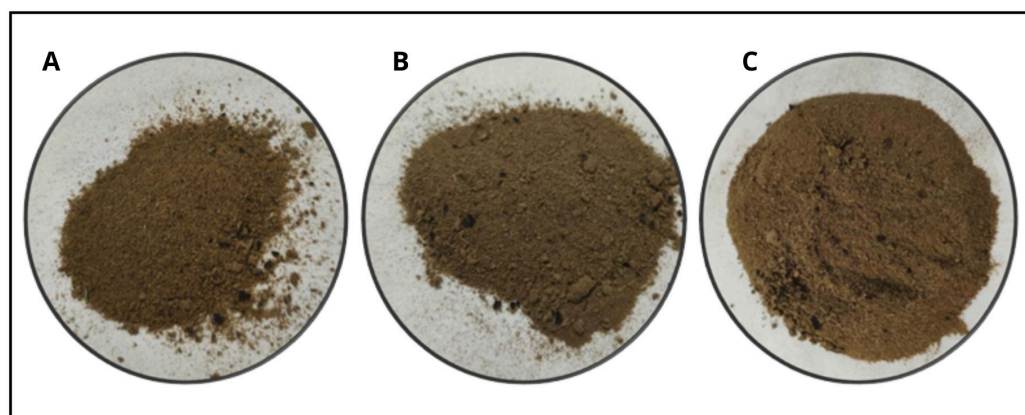


Figure 6. Biomass obtained at the end of the fermentative process with *L. delbrueckii* subsp. *bulgaricus* at 5% (A), 10% (B) and 15% (C) inoculation levels.

Electrical conductivity increased slightly with inoculum level (1.13–1.216 S·dm^{−1}), suggesting a greater release of soluble mineral ions (Na⁺, K⁺, Ca²⁺, Mg²⁺) resulting from partial degradation of organic matter and mineralization of microbial cellular components. This increase correlates directly with greater nutrient availability and elevated microbial metabolic activity under higher inoculation treatments. Total carbon values (~52–54%) and total nitrogen (1.10–1.36%) yielded a decreasing C/N ratio (49.2 to 39.86) as inoculum dosage increased. This shift is highly significant, indicating that the biomass progresses toward a more mature and biologically stable state, as a substantial fraction of carbon is mineralized to CO₂, while the remaining portion is incorporated into microbial biomass (nitrogen-containing compounds such as amino acids, proteins, enzymes), thereby reducing the C/N ratio [74].

Table 5. Physicochemical and Nutrient Composition of Organic Material Samples.

Parameters	Unit	Inoculum 5%	Inoculum 10%	Inoculum 15%
Moisture	%	5.720	5.120	4.680
pH		6.260	5.910	5.610
Electrical Conductivity	(S·dm ^{−1})	1.131	1.180	1.216
Ashes	%	6.690	6.660	6.550
Organic Matter	%	93.310	93.400	93.450
Total Carbon	%	54.124	52.164	54.205
Total Nitrogen	%	1.100	1.250	1.360
C/N Ratio		49.200	44.590	39.860
Calcium (CaO)	%	0.552	0.522	0.490
Magnesium (MgO)	%	0.076	0.0962	0.115
Sodium (Na ₂ O)	%	0.126	0.185	0.249
Potassium (K ₂ O)	%	0.144	0.109	0.089
Phosphorus (P ₂ O ₅)	%	0.182	0.261	0.337
Iron (Fe)	%	0.041	0.039	0.037
Copper (Cu)	%	0.001	0.001	0.001
Zinc (Zn)	%	0.002	0.002	0.002
Manganese (Mn)	%	0.039	0.031	0.023
Boron (B)	%	3 × 10 ^{−4}	5 × 10 ^{−4}	6 × 10 ^{−4}
Proteins (Dry Basis)	%	6.870	7.700	8.500

An optimal C/N ratio between 25 and 30 is ideal for materials intended for controlled fermentation or composting processes, as it prevents carbon-induced inhibition and supports balanced microbial growth [74]. In this context, the treatment with 15% inoculum demonstrates superior utilization of the organic substrate and greater metabolic efficiency, reflected in the generation of nitrogen-rich biomass. The increase in protein content, ranging from 6.87 to 8.50% with higher inoculum levels, confirms the effective bioconversion of organic matter into microbial biomass enriched in proteins—an outcome characteristic of solid-state fermentation or nutritional-enhancement processes. This implies that the introduced microorganisms act as biosynthesizers, transforming simple carbon fractions into high-value biomolecules (proteins, enzymes, structural polysaccharides).

From a biotechnological valorization perspective, this result is highly relevant, as it demonstrates that the treatment of agro-industrial residues with appropriate microorganisms can enhance their nutritional quality, enabling applications such as N- and P-enriched biofertilizers, additives in animal feed, or precursors to produce bioproducts such as amino acids, organic acids, and pigments. These findings align with recent efforts aimed at converting lignocellulosic residues into microbial protein sources or functional biomass [75]. Regarding the mineral profile, selective trends were observed depending on inoculation level: phosphorus (P₂O₅) increased significantly (0.182 to 0.337%), reflecting the release or solubilization of phosphate compounds, an effect typical of phosphate-solubilizing microorganisms. Magnesium (MgO) also increased (0.076 to 0.115%), indicating higher ionic availability relevant for enzymatic biosynthesis [76].

In contrast, potassium (K₂O) and iron (Fe) showed slight decreases, possibly due to their incorporation into biomolecules or organic complexes during microbial metabolism. Sodium (Na₂O) and Boron (B) increased slightly, which may result from partial mineralization of the organic matrix or from the composition of the inoculation medium. Collectively, these changes suggest that the process not only preserves but redistributes essential elements, transforming the material into a chemically balanced biomass with enhanced mineral bioavailability. The overall parameter set indicates that applying inoculant at different proportions enhances the valorization of agro-industrial residues, simultaneously improving their nutritional value and chemical stability. The increase in proteins and mineral nutrients, coupled with the reduction in C/N ratio, evidences controlled biolog-

ical maturation—an essential condition for circular bio-valorization processes. From a circular-economy perspective, these results reaffirm that organic residues can be converted into high-value functional biomass through microbial bioconversion, contributing to waste reduction, bio input generation, and the replacement of conventional protein sources with more sustainable alternatives [76].

3.4. Regional Adoption of ASCW Use in Bovine Production Systems in the State of Veracruz, México

While Sections 3.1–3.3 established the technical feasibility of the BM–ASCW biotransformation process at laboratory scale, its practical relevance ultimately depends on whether the raw materials and resulting practices are already recognized and utilized within the regional production context. To provide this complementary evidence, a regional census of ASCW adoption among bovine production units in Veracruz was conducted; these results do not derive from the laboratory fermentation experiments described above, but rather characterize the socioeconomic and logistical context in which the proposed technology could be implemented.

The field analysis conducted across 12 cattle-producing zones associated with sugar mills in the state of Veracruz enabled the quantification of the adoption level of agro-sugarcane waste (ASCW); primarily sugarcane tops, dry leaves, and bagasse, as feed inputs in bovine diets (Table 6). Of the 405 surveyed cattle operations, 237 (58.5%, 95% CI: 53.7–63.2%, Wilson score interval) reported the use of at least one type of ASCW, whereas 168 (41.5%, 95% CI: 36.8–46.3%) did not incorporate these residues in their feeding practices. This overall adoption rate demonstrates a substantial integration of agro-industrial sugarcane residues into regional livestock nutrition, driven mainly by the local availability of by-products generated by nearby sugar mills. The highest adoption rates were observed in Paso del Macho (65.0%), Carlos A. Carrillo/Tlacotalpan (64.4%), and Atoyac–Potrero Nuevo (63.2%), areas located in direct proximity to the Central Progreso, San Cristóbal, and El Potrero mills, respectively. These regions exhibit a more consolidated utilization of sugarcane residues, reflecting established logistical infrastructure and extensive experience in their handling. Intermediate levels of adoption were recorded in Cosamaloapan/Tres Valles (62.0%), Ángel R. Cabada/Lerdo de Tejada (61.8%), and Cuitláhuac–Carrillo Puerto (59.4%), where ASCW is mainly used for silage or Total Mixed Ration (TMR) formulations incorporating wet bagasse, chopped tops, and dry leaves. These practices correspond to supplementation strategies implemented during the dry season, when the availability of fresh forage declines markedly.

Conversely, the lowest adoption levels were reported in Alvarado (45.8%) and La Antigua/Paso de Ovejas (50.0%), where ASCW utilization is limited to seasonal applications or fiber enhancement. In these coastal areas, longer distances to sugar mills and the lack of collection or processing mechanisms restrict the consistent availability of residual sugarcane biomass. Regarding the type of residue used, chopped or ensiled sugarcane tops and dry leaves were predominant, followed by bagasse (fine, wet, or mixed with molasses). The most frequent modes of use included energy–protein supplementation in feedlots (Paso del Macho and Carlos A. Carrillo), blending with fresh forage (Atoyac, Cosamaloapan), incorporation into nutritional blocks or pellets (Tierra Blanca, Omealca), and silage with molasses or urea (San Andrés Tuxtla, Lerdo de Tejada). Overall, the findings confirm that proximity to sugar mills is a determining factor for ASCW adoption, as it ensures continuous residue supply and reduces transportation costs. Likewise, the diversification of usage modalities demonstrates a progressive technical adaptation within the livestock sector aimed at optimizing energy and protein availability in bovine diets throughout the year.

Table 6. Integration of ASCW into Bovine Feeding Practices: Adoption Levels and Utilization Modes.

Zone/Municipality (Region)	Nearest Sugar Mill	Bovine Production Units BPU (n)	BPUs Feeding with ASCW (n)	BPUs not Using ASCW (n)	% Adoption	Type of Waste Used (e.g.)	Reported Mode of Use
Paso del Macho (Altas Montañas)	Central Progreso	40	26	14	65.00	cogollo + dry leaves	energy-protein supplement in feedlot systems
Atoyac—Potrero Nuevo	El Potrero	38	24	14	63.20	chopped cogollo + molasses	mixed with forage (60:40)
Cuitláhuac—Carrillo Puerto	La Providencia	32	19	13	59.40	dry leaves + bagasse	supplementation during the dry season
Amatlán de los Reyes/Córdoba periurbano	El Potrero	28	15	13	53.60	cogollo + crop residues	forage blended with pasture
Omealca	La Providencia	22	12	10	54.50	fine-sieved bagasse	artisanal pelleting
Tierra Blanca	La Victoria	36	20	16	55.60	bagasse + molasses	nutritional blocks
Carlos A. Carrillo/Tlacotalpan	San Cristóbal	45	29	16	64.40	chopped cogollo	milking-time supplement (3–4 kg/day)
Cosamaloapan/Tres Valles	San Cristóbal	50	31	19	62.00	wet yard bagasse	TMR (Total Mixed Ration) base (30%)
Ángel R. Cabada/Lerdo de Tejada	San Pedro	34	21	13	61.80	leaves + ensiled cogollo	urea-treated silage (1%)
San Andrés Tuxtla	San Pedro	30	16	14	53.30	cogollo silage	ND
La Antigua/Paso de Ovejas	La Gloria	26	13	13	50.00	ground dry leaves	ND
Alvarado	El Modelo	24	11	13	45.80	bagasse-cogollo mixture	ND
Totals	—	405	237	168	58.50	—	

In Figure 7, the spatial distribution map of ASCW adoption across cattle-producing regions of Veracruz is presented. In summary, the regional adoption pattern indicates that the use of agro-sugarcane residues constitutes a sustainable production strategy, providing both environmental benefits (reduction in agro-industrial waste) and economic advantages (lower feeding costs and improved herd nutritional balance). This synergistic integration between the sugarcane and livestock sectors emerges as a robust pathway to strengthen rural circular-economy models in the state of Veracruz.

The regional survey results presented above confirm that agro-sugarcane residues are already recognized by local producers as a valuable feeding resource, indicating a favorable socioeconomic context for adopting the BM–ASCW biotransformation process described in this study. Beyond this regional adoption context, however, the practical relevance of the fermented biomass ultimately depends on how its nutritional composition compares with the feed inputs currently used in bovine production systems. To this end, the following section examines the fermented biomass in relation to commercial nutritional supplements, first estimating the national-scale availability of the substrate to contextualize the potential magnitude of this valorization strategy.

3.5. Comparison of the Nutritional Profile of Fermented Biomasses BM/ASCW and Commercial Nuclei: Implications for the Formulation of Bovine Diets

Bovine manure generation is estimated based on the national cattle herd, which comprises approximately 36.6 million head of cattle (SIAP, 2024 [77]). According to Morse et al. (1994) [78], a lactating Holstein cow produces 55–60 kg of fresh manure per day; however, the national herd includes diverse breeds and physiological stages, so the average

production of fresh manure is generally considered to range between 30 and 50 kg day⁻¹. Using an estimated average of 40 kg day⁻¹, daily manure generation at the national scale reaches 1464 million kg, equivalent to 1.46 million metric tons per day. Agro-sugarcane residue (ASCW) comprises the green leaves, dry leaves or straw, and sugarcane tops remaining in the field after harvest, excluding industrial by-products such as bagasse, filter cake, and molasses. Studies in Mexico and Latin America report that sugarcane in its natural state consists of approximately 8–8.5% tops and green leaves, 19–20% dry leaves, and roughly 72% clean stalks, meaning the foliage fraction (leaves + tops) represents 28–30% of the above-ground biomass. According to PRONAC 2021–2024 [79], during the 2023–24 cycle, about 46 million tons of sugarcane were processed, which corresponds to approximately 13.3 million tons of ASCW annually.



Figure 7. Spatial distribution map of ASCW adoption in cattle-producing regions of Veracruz.

The strategy proposed in this study would reduce the environmental impact of bovine and agro-sugarcane residues, as once treated, they have the potential to be reintroduced into the production cycle as nutritional supplements for cattle, thereby promoting circular bioeconomy principles. For this purpose, the following analysis is considered: according to Nasem (2021) [80], dry matter (DM) intake of a lactating dairy cow depends on factors such as body weight, milk production, and diet composition, typically ranging between 12 and 16 kg DM day⁻¹, equivalent to 2.5–3.0% of live weight (LW). For the present analysis, a value of 15 kg DM day⁻¹ is used. Accordingly, national bovine DM intake is approximately 549 million kg day⁻¹, or 549,000 metric tons per day. The daily DM requirement is typically met through a combination of forages and supplements. Cattle are usually fed moist feed components, which contain 25–35% dry matter. Thus, a bovine must consume approximately 43 kg of fresh feed per day to meet its DM requirements. When supplements such as commercial feed nuclei are used, they are typically included at 15% of the diet, and the final mixed feed is recommended at 0.5–1.2% of live weight.

For comparative purposes, a commercial mineral–protein feed nucleus commonly used in bovine production systems in Mexico was selected as a reference (commercial mineral–protein feed nucleus based on reported technical specifications). This product represents a typical formulation designed to supply protein, minerals, and essential nutrients to complement forage-based diets. The compositional values used in this study were obtained from publicly available technical specifications. Table 7 presents the comparative nutrient composition between a commercial feed nucleus and the nutritionally

enriched biomasses obtained through the fermentation process. Although the absolute nutrient concentrations in the fermented biomasses are lower than those of the commercial nucleus, the nutrients in the fermented materials are more bioavailable, as they have been hydrolyzed through the metabolic activity of *Lactobacillus delbrueckii* subsp. *bulgaricus* SP96. This increased bioavailability enhances their accessibility to bovine digestive enzymes. Additionally, probiotic microorganisms help stabilize the intestinal microbiota, compete against pathogens, and are essential for improved gut health, which indirectly enhances the absorption efficiency of all dietary nutrients [81].

Table 7. Comparison of nutrient content between a commercial feed nucleus and the nutritive biomasses obtained from the fermentative process. Based on reported technical specifications [82].

Parameter (Unit)	Commercial Mineral–Protein Nucleus	Nutritive Biomass		
		5%	10%	15%
Protein (%)	15.00 (minimum)	6.870	7.700	8.500
Fat (%)	6.40	ND	ND	ND
Fiber (%)	3.70	ND	ND	ND
Moisture (%)	12.00 (maximum)	5.720	5.120	4.680
Calcium (%)	0.90 (minimum)	0.552	0.522	0.490
Total phosphorous (%)	0.50 minimum	0.182	0.261	0.337

Note: ND, not disponible.

If the nutritive biomasses generated from the fermentative process are considered for application in bovine feeding systems, their low moisture content ($\approx 5\%$), equivalent to approximately 95% dry matter (DM), represents a significant advantage compared to conventional moist feedstuffs (25–35% DM). Under this condition, a greater proportion of nutrients is delivered per unit of fresh weight, improving storage stability, reducing transportation costs, and enhancing handling efficiency. Assuming a feeding strategy in which 70% of the dry matter is supplied by forage or silage and 30% by BM–ASCW-derived fermented biomass, the total fresh feed intake required to meet a daily demand of 15 kg DM would be approximately 35 kg day^{−1}.

This reduction in fresh feed requirement is associated with a higher nutrient density and improved utilization efficiency of the diet. From a nutritional standpoint, the incorporation of fermented biomass does not aim to replace conventional feed nuclei directly, but rather to act as a functional supplement that enhances nutrient bioavailability and supports ruminal microbial activity. The partial substitution of conventional supplements with fermented biomass may contribute to improved feed conversion efficiency, reduced reliance on commercial inputs, and lower overall feeding costs, particularly in systems where feed availability and economic constraints are critical.

The comparison between the nutritional profile of the fermented BM–ASCW biomasses and commercial feed nuclei must be interpreted within a functional rather than compositional framework. In bovine nutrition, feed nuclei are not complete feeds but concentrated supplements designed to provide high levels of specific nutrients, particularly protein, energy, or minerals, to complement forage-based diets. In contrast, the biomasses obtained in this study are not formulated products but biotechnologically transformed materials derived from lactic acid fermentation of agro-livestock residues. Therefore, their nutritional value should not be assessed solely based on absolute nutrient concentration, but rather on their bioavailability and metabolic functionality.

Although the crude protein content of the fermented biomasses (6.8–8.5%) is lower than that of conventional commercial nuclei (typically $\geq 15\%$), this protein fraction is

partially hydrolyzed and associated with microbial biomass, which enhances its digestibility and utilization in the rumen. During lactic acid fermentation, complex organic substrates are converted into simpler compounds, increasing the accessibility of nutrients to ruminal microorganisms. Additionally, the fermentation process promotes the generation of organic acids, microbial metabolites, and enzymatically modified compounds that can improve ruminal fermentation efficiency.

In ruminants, carbohydrates are fermented by rumen microbiota into volatile fatty acids (VFAs), which represent the primary energy source for the animal, while microbial protein synthesized in the rumen constitutes a major source of amino acids. From this perspective, the BM–ASCW biomasses should be considered as functional feed supplements rather than direct substitutes for commercial nuclei. Their value lies in their role as nutrient-enhancing, microbiologically active materials capable of improving feed efficiency and contributing to ruminal metabolic balance.

Furthermore, it is important to emphasize that the biomasses represent a low-cost by-product generated through the valorization of agro-industrial residues. This characteristic provides a significant advantage over conventional feed inputs, as it enables the partial replacement of commercial supplements while simultaneously reducing environmental impact and promoting circular bioeconomy strategies. To strengthen their nutritional applicability, future strategies could include the enrichment of the fermented biomass with nitrogen sources, mineral premixes, or energy-dense substrates, thereby increasing its protein concentration and aligning it more closely with conventional feed nuclei formulations.

3.6. Comparative Evaluation of BM/ASCW Fermented Biomass and Mineral Supplementation Strategies in Bovine Feeding Systems

To further evaluate the applicability of the BM–ASCW fermented biomass within bovine feeding systems, a comparative analysis was conducted against mineral supplementation strategies commonly employed under tropical grazing conditions. In particular, commercial high-concentration inorganic mineral formulations are widely used in Mexico to correct deficiencies in essential macro- and microminerals, including phosphorus, magnesium, sodium, iron, zinc, manganese, copper, and selenium, which are frequently limited in forage-based diets. These mineral supplements are primarily composed of inorganic compounds and are specifically designed to provide highly concentrated and bioavailable mineral elements.

Their function is strictly corrective, aiming to restore mineral balance and improve productive performance without contributing significant amounts of organic matter, protein, or energy to the diet. In contrast, the BM–ASCW fermented biomass exhibits a markedly different compositional profile. As shown in Table 8, the material is characterized by a high organic matter content (~93%), measurable nitrogen levels (~1.36%), and a crude protein content ranging from 6.8 to 8.5%. Additionally, the biomass contains mineral components such as phosphorus (expressed as P_2O_5), magnesium (MgO), sodium (Na_2O), and iron, which overlap partially with the mineral spectrum provided by conventional supplements.

Although the compositional information available for commercial mineral supplements is primarily qualitative, this limitation does not preclude a meaningful comparison with the BM–ASCW fermented biomass. On the contrary, it highlights a fundamental difference in the nature and functional role of both materials within bovine feeding systems. Mineral supplements are formulated as highly concentrated inorganic sources designed to correct specific deficiencies of macro- and microminerals in forage-based diets. Their composition is typically reported in qualitative or targeted quantitative terms, focusing on essential elements such as phosphorus, sodium, magnesium, and trace minerals. However, these formulations lack organic carbon, nitrogen, and bioactive components, and therefore

do not contribute directly to energy supply, microbial growth substrates, or metabolic enhancement beyond mineral correction.

Table 8. Comparative mineral composition of a commercial mineral supplement and BM/ASCW nutritive biomass at different inoculum levels.

Parameter (Unit)	Commercial Mineral Supplement [83]	Nutritive Biomass		
		5%	10%	15%
Moisture (%)	5–10	5.720	5.120	4.680
Ashes (%)	90–98	6.690	6.660	6.550
Calcium (CaO) (%)	Present	0.552	0.522	0.490
Magnesium (MgO) (%)	Present	0.076	0.0962	0.115
Sodium (Na ₂ O) (%)	High	0.126	0.185	0.249
Potassium (K ₂ O) (%)	Present	0.144	0.109	0.089
Phosphorus (P ₂ O ₅) (%)	High	0.182	0.261	0.337
Iron (Fe) (%)	Present	0.041	0.039	0.038
Copper (Cu) (%)	Present	6×10^{-4}	5×10^{-4}	4×10^{-4}
Zinc (Zn) (%)	Present	0.002	0.002	0.002
Manganese (Mn) (%)	Present	0.039	0.031	0.023
Boron (B) (%)	ND	3×10^{-4}	5×10^{-4}	6×10^{-4}

Note: ND, not disponible.

In contrast, the BM–ASCW fermented biomass exhibits a well-defined physicochemical and compositional profile, characterized by high organic matter content (~93%), significant total carbon (~52–54%), measurable nitrogen levels (up to 1.36%), and crude protein values reaching 8.5%. Additionally, it contains relevant mineral fractions, including phosphorus, magnesium, sodium, and iron, which partially overlap with those supplied by mineral supplements. More importantly, the nutrients in the fermented biomass are embedded within an organic matrix that has undergone biological transformation through lactic acid fermentation. This process promotes partial hydrolysis of complex substrates, generation of microbial biomass, and formation of organic acids and bioactive metabolites. As a result, nutrient bioavailability is enhanced, and the material acquires functional properties that can positively influence ruminal microbial activity and digestive efficiency.

From a functional standpoint, this represents a significant advantage. While mineral supplements act exclusively as corrective inputs, the BM–ASCW biomass operates as a multifunctional nutritional matrix, simultaneously providing organic nutrients, fermentative metabolites, and mineral elements. This integrated composition may contribute to improved nutrient utilization, enhanced microbial synthesis, and greater overall efficiency of the digestive process. Furthermore, the biomass is derived from the valorization of agro-industrial residues, making it a low-cost and sustainable alternative. Its use aligns with circular bioeconomy principles by transforming waste streams into value-added products with direct application in animal nutrition.

Therefore, despite the limited quantitative data available for commercial mineral supplements, the BM–ASCW fermented biomass demonstrates clear advantages in terms of compositional complexity, functional properties, and sustainability, supporting its potential as a complementary strategy within bovine feeding systems. However, unlike mineral formulations, the nutrients in the fermented biomass are embedded within an organic matrix that has undergone biotransformation through lactic acid fermentation. This process promotes partial hydrolysis of complex substrates and generates microbial biomass and fermentation-derived metabolites, which may enhance nutrient accessibility and facilitate

their utilization within the ruminal environment. From a functional perspective, this distinction is critical.

While mineral supplements act exclusively as sources of inorganic elements, the BM-ASCW biomass represents a multifunctional nutritional matrix capable of simultaneously supplying organic nutrients, microbial-derived compounds, and selected minerals. This combination may contribute not only to mineral supplementation but also to improved ruminal microbial activity and nutrient utilization efficiency. Therefore, the comparison highlights that the BM-ASCW fermented biomass should not be interpreted as a replacement for mineral supplements, but rather as a complementary strategy within bovine feeding systems. Its integration could enhance the overall nutritional profile of the diet while reducing reliance on conventional inputs and promoting the valorization of agro-industrial residues within a circular bioeconomy framework.

4. Conclusions

The results of this study demonstrate that the anaerobic biotransformation of agro-livestock residues (BM-ASCW) using *Lactobacillus delbrueckii* subsp. *bulgaricus* represents a technically sound, laboratory-scale strategy for the stabilization and valorization of heterogeneous organic matrices, providing proof-of-concept evidence to support future scale-up studies. The applied thermal pretreatment ensured microbiological safety by achieving pathogen reductions compliant with NOM-004-SEMARNAT-2002 biosolid standards, confirming the feasibility of adapting existing regulatory frameworks to agro-industrial residues and highlighting the need to expand current Mexican regulations to formally include agro-livestock waste streams.

From a bioprocess perspective, the system exhibited high metabolic efficiency, achieving lactic acid production of 13.4 g L^{-1} and a maximum conversion yield ($Y_{p/s}$) of 0.99 g LA g^{-1} carbohydrates at a 15% inoculum. The microbial growth dynamics were accurately described by the Gompertz model ($R^2 = 0.92\text{--}0.97$), validating its applicability for complex lignocellulosic substrates. While the 15% inoculum maximized substrate conversion and product yield, the 10% condition provided the most balanced operational performance, reinforcing the importance of inoculum optimization for process stability and scalability. The fermented biomasses obtained displayed a high organic matter content ($\sim 93\%$), low moisture ($\sim 5\%$), progressive enrichment in crude protein (up to 8.5%), and a decreasing C/N ratio (~ 40), indicating effective biological stabilization and efficient nitrogen incorporation into microbial biomass.

Additionally, the presence and redistribution of essential mineral elements (P_2O_5 , MgO , Na_2O , Fe, and B) confirm the transformation of the original residues into a nutritionally enriched material with potential dual application as a functional feed additive and biofertilizer. When compared with commercial mineral supplementation strategies, a fundamental compositional distinction becomes evident. Mineral supplements are composed primarily of inorganic compounds intended to correct specific mineral deficiencies, whereas the BM-ASCW fermented biomass constitutes a complex organic matrix enriched in carbon, nitrogen, microbial protein, and fermentation-derived metabolites. The comparison demonstrates that the biomass provides overlapping mineral elements (P, Mg, Na, and Fe) while simultaneously contributing organic matter and biologically transformed nutrients not present in conventional mineral supplements.

Similarly, when compared with commercial mineral–protein nuclei, the fermented biomasses exhibited lower absolute concentrations of protein and selected nutrients; however, their composition reflects a biologically transformed matrix generated through microbial fermentation. Collectively, these characteristics indicate that the fermentation process successfully converted agro-livestock residues into a stabilized and nutritionally enriched

biomass with multifunctional properties. From a systemic perspective, this study establishes a functional circular bioeconomy model in which agricultural and livestock residues are reintegrated into the productive cycle through microbial biotransformation. This approach contributes to waste reduction, resource recovery, and the generation of value-added bioinputs, strengthening the agriculture–livestock nexus in sugarcane-producing regions.

In the specific context of Veracruz, these findings acquire particular practical relevance. The regional census conducted across 12 municipalities in the state (Section 3.4) revealed that 58.5% of the 405 surveyed bovine production units already incorporate agro-sugarcane residues into their feeding systems, indicating an existing logistical and cultural familiarity with ASCW valorization among local producers. Against this backdrop, the biotransformation process demonstrated here offers a technically grounded pathway to extend current practices beyond direct feeding, enabling producers in sugarcane-cattle overlap zones to convert BM–ASCW residues into a stabilized, nutritionally enriched biomass rather than relying solely on untreated residue incorporation. Given that Veracruz concentrates a substantial share of Mexico’s cattle inventory and sugarcane production within the same geographic areas, this approach represents a realistic, regionally grounded entry point for decentralized agro-livestock waste valorization, complementing broader national efforts to close nutrient and residue cycles in agro-industrial systems.

5. Future Perspectives

Future research should evaluate the nutritional performance of BM/ASCW fermented biomasses under practical livestock production conditions, including digestibility, nutrient bioavailability, ruminal fermentation dynamics, animal productivity, and feed conversion efficiency. Such studies will help determine the extent to which these biomasses can complement conventional mineral and protein supplementation programs. Before any protein-enriched biomass or biofertilizer claim can be substantiated, strain-specific safety and efficacy data (viability after thermal/mechanical processing, antibiotic-resistance profiling, target-species feeding trials) and biofertilizer safety data (phytotoxicity assays, heavy-metal content, and pathogen-regrowth testing) are required and are proposed as priorities for future work. We did not generate these data for the present study and have not implied otherwise. Additionally, the documented regional adoption of agro-sugarcane residues in bovine feeding systems across Veracruz provides a favorable framework for the implementation of decentralized bioprocesses capable of transforming locally available residues into value-added nutritional inputs. From a technical standpoint, the dilution ratios used to reach the working total-solids range (1:5 *w/v* for BM, 1:10 *w/v* for ASCW) imply a water demand that must be addressed progressively as the process advances from the laboratory scale reported here to pilot and, eventually, industrial implementation. Extrapolating these ratios to the national residue generation estimated in Section 3.5 (1.46 million metric tons day^{−1} of BM and approximately 36,400 metric tons day^{−1} of ASCW) illustrates the magnitude of the challenge at full scale, theoretically requiring approximately 5.84 million m³ day^{−1} of water for BM alone and 328,000 m³ day^{−1} for ASCW—volumes that would be environmentally and economically unfeasible under any realistic implementation scenario. At a more actionable pilot scale, however, this demand becomes considerably more manageable: a decentralized unit processing 1 metric ton day^{−1} of BM together with 1 metric ton day^{−1} of ASCW—a throughput comparable to the medium-sized dairy operation sampled in this study—would require approximately 13 m³ day^{−1} of water at the dilution ratios used here (4 m³ day^{−1} for BM, 9 m³ day^{−1} for ASCW). This requirement could be further reduced by reusing wash water from milking parlors and holding pens of technified cattle facilities, which already carries residual organic matter and would therefore contribute additional substrate rather than acting as a purely diluting

stream, partially or fully replacing fresh water in substrate preparation. Systematically evaluating the physicochemical composition of this wash water and its effect on both water demand and fermentation performance is proposed as a specific engineering objective for the pilot-scale phase, together with validating process performance under continuous or fed-batch operation and expanding the experimental design (additional inoculum levels and/or replicates, informed by *a priori* statistical power analysis) to generate confirmatory, scale-relevant data. From an economic standpoint, a preliminary techno-economic evaluation, including pretreatment, fermentation operating costs, water demand at the dilution ratios discussed above, and potential revenue from the fermented biomass and the recovered lactic-acid-rich supernatant, is required before scalability can be claimed, and is proposed as a priority for future work. No cost figures are presented in this study, since a techno-economic analysis was not performed for the present work; such an analysis would need to compare the process against conventional composting or direct land application of untreated residues to establish its economic competitiveness.

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Abbreviations

The following abbreviations are used in this manuscript:

BM	Bovine manure
ASCW	Agro-sugarcane waste
C/N	Carbon to nitrogen ratio
TS	Total solids
TVS	Volatile total solids
HT	Heat-treated residue
R	Raw residue
CFU	Colony Forming Units

MPN	Most Probable Number
LA	Lactic acid
LAB	Lactic acid bacteria
NAD	Nicotinamide Adenine Dinucleotide
ATP	Adenosine triphosphate
tRNA	Transfer RNA
MRS	Man, Rogosa and Sharpe agar
BPU	Bovine production units
DM	Dry matter
LW	Live weight

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