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Advancing Psychrophilic Anaerobic Digestion of Cattle Manure through Microbial Adaptation, Bioaugmentation and Pathways to Reliable Low-Temperature Biogas: A Critical Review

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REVIEW ARTICLES

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Abstract: Low-temperature anaerobic digestion of cattle manure can recover methane, but slow conversion and delayed start-up can limit reliable energy delivery. This critical narrative review examines biochemical constraints, microbial adaptation and bioaugmentation, performance measurement and translation to useful energy. International evidence is synthesized, with the Ngorongoro Conservation Area of northern Tanzania serving as a motivating application context. The synthesis identifies coordination of hydrolysis, syntrophic conversion and methanogenesis as central to methane onset, production rate and stability. Cold-adapted inocula and targeted consortia can improve methane recovery in selected controlled systems, but differences in substrates, inocula, dosing and reactor operation restrict cross-study comparison and field extrapolation. Combined microbial and carbon amendments require controls that distinguish biological effects from additional substrate contributions. Reliable interpretation also requires explicit solids accounting, gas volume reference conditions and separation of measured recovery from modeled asymptotic potential. Higher methane recovery alone does not establish improved pH stability, methane fraction or microbial persistence. Net energy and economic benefits of these interventions remain insufficiently resolved because methane gains must be assessed alongside process and intervention inputs, gas losses and useful conversion to cooking heat, boiler heat or electricity. Priorities are replicated factorial experiments, integrated monitoring, kinetic validation and seasonal pilot trials linking biological enhancement to measured energy services and locally grounded economic assessment.

Keywords Biochemical methane potential, Inoculum selection, Methanogenesis, Process stability, Volatile fatty acids.

1. Introduction

Anaerobic digestion (AD) links cattle-manure management with methane capture and renewable gas production (Song et al., 2023). For household applications, its value depends on sustained fuel availability and usable energy. East African experience shows that installation alone does not ensure sustained cooking use where maintenance, cost and continued use of other fuels remain constraints (Clemens et al., 2018). Cooking-related discussion in this review concerns fuel availability and use; the review does not quantify stove emissions, household exposure or life-cycle benefits.

Low-temperature operation can reduce reactor-heating demand, while lower temperatures constrain microbial growth, enzymatic activity and conversion rates (Akindolire et al., 2022; Si et al., 2026). The resulting challenge concerns both methane availability and the balance between intermediate formation and methanogenesis. Accordingly, this review distinguishes recovery within an operating period from ultimate methane potential. Published temperature classifications vary, including the broader psychrophilic range discussed by Si et al. (2026). As operational eligibility boundaries, the core range here is ≤20 °C, with selected studies above 20 °C and up to 25 °C used as supporting mechanistic or reactor evidence.

Cattle manure adds a substrate-specific constraint because its fibrous fraction can restrict hydrolysis (Song et al., 2023). Enhancement strategies should therefore be assessed against the limiting function they address and the downstream capacity to convert released intermediates. In batch cattle-manure/corn-straw co-digestion at 20 °C, targeted cold-tolerant bioaugmentation improved methane production and reduced volatile fatty acid (VFA) accumulation (Xu et al., 2023). This supports a biological opportunity while leaving transfer to manure monodigestion and unheated field systems to be assessed separately. Biochar may influence microbial retention and electron transfer through complementary mechanisms (Zhao et al., 2024).

Carbon-containing amendments create a further attribution problem. Gosgot Angeles et al. (2024) combined molasses and Caesalpinia spinosa meal in heated cattle-manure experiments. That design cannot isolate the contribution of molasses, the meal or an introduced microbial culture. Consequently, increased gas after a combined amendment should not be attributed to microbial augmentation without controls that separate the added organic matter from the microbial intervention. This distinction is particularly important when assessing commercial mixed cultures activated with fermentable substrates.

This critical narrative review examines microbial adaptation, bioaugmentation and complementary strategies for improving low-temperature cattle-manure digestion. The Ngorongoro Conservation Area (NCA) in northern Tanzania provides the motivating application context for evaluating their relevance to unheated pastoral biogas systems. The scientific synthesis draws on international literature to examine mechanisms, methane performance, methodological limitations and implications for useful energy recovery. NCA-specific implications are presented as research and implementation considerations requiring local validation.

2. Review Scope and Methodology

This study adopts a critical narrative review to integrate evidence on microbial mechanisms, reactor performance, measurement practices and implementation constraints in low-temperature cattle-manure digestion. Its purpose is to explain relationships across these themes, assess the limits of existing evidence and identify research priorities. This integrative purpose supports a narrative design, with the review method aligned to its questions and intended contribution (Snyder, 2019). The search and selection procedures described below support a reasoned synthesis of selected literature; comprehensive systematic coverage is not claimed. No pooled effect estimate was calculated because the assembled studies differ in substrate composition, inoculum, temperature, amendment dose, reactor configuration, operating duration and outcome normalization. These differences limit the interpretation of a single combined estimate. Systematic reviews can also synthesize findings without meta-analysis; therefore, heterogeneity explains the decision not

to pool results, while the explanatory and integrative purpose explains the narrative review design (Campbell et al., 2020).

Literature searches were conducted using PubMed-indexed records and supplemented by searches of relevant publisher platforms, DOI based bibliographic records, and backward and forward citation tracking from recent review articles and highly relevant primary studies. Search terms were combined according to the principal review themes. Representative search combinations included ("psychrophilic anaerobic digestion" OR "low-temperature anaerobic digestion") AND ("cattle manure" OR "cow manure"); ("bioaugmentation" OR "microbial consortium" OR "methanogenic culture" OR "cold-adapted inoculum") AND ("anaerobic digestion" OR "biogas"); ("direct interspecies electron transfer" OR "DIET" OR "biochar") AND "anaerobic digestion"; ("Modified Gompertz" OR "biochemical methane potential" OR "methane yield" OR "lag time"), and ("clean cooking" OR "biogas") AND ("Tanzania" OR "East Africa"). Review articles were used primarily to map the field and identify relevant primary literature, whereas quantitative claims concerning methane enhancement, temperature response, lag reduction, VFA dynamics, or microbial community changes were preferentially supported by primary experimental studies.

Sources were considered eligible when they (i) were published within the defined 2018–2026 period; (ii) addressed at least one of the principal review themes, including low-temperature digestion, cattle-manure conversion, microbial enhancement, process kinetics, gas quality, process-stability monitoring, or low-input biogas implementation; and (iii) provided sufficient information on experimental conditions and outcome measures to allow meaningful interpretation. Policy and adoption studies were retained when they directly informed the East African or Tanzanian clean-cooking context. Publications outside the specified period and studies unrelated to methane producing anaerobic digestion were excluded from the core technical synthesis.

Evidence was appraised qualitatively according to experimental replication, substrate and organic-loading comparability, inoculum-to-substrate ratio, amendment accounting, methane normalization, control procedures, process diagnostics and experimental scale. Gas-volume correction, digestion duration and sampling frequency were considered because they affect the interpretation of reported methane performance. Because the reviewed studies differed considerably in substrate composition, temperature, reactor configuration, inoculum source, dosing strategy, digestion duration, and methane normalization procedures, no pooled effect size was calculated.

The synthesis distinguishes direct evidence from cattle-manure digestion from supporting mechanistic evidence obtained with other substrates and contextual evidence concerning implementation. Quantitative findings are interpreted within their experimental conditions, with biogas production, methane yield, time-bounded recovery and fitted methane potential treated as distinct outcomes. Table 1 summarizes principal studies used in the synthesis and is not an inventory of all cited sources. Evidence-strength descriptions reflect the appraisal dimensions above and the relevance of each study to the claim being assessed.

Comparisons in Table 1 are interpreted within each experiment. Similar operating temperatures do not establish equivalent substrate loading, inoculum activity, amendment dose, control conditions or observation periods. Consequently, the reported fold enhancements cannot rank bioaugmentation against biochar or predict an expected response in cattle-manure monodigestion. Supporting evidence from swine slurry, food-waste mixtures and electrochemically assisted systems informs mechanisms and experimental design, while its transferability requires separate assessment.

Table 1. Principal evidence supporting the review and limits of experimental comparability

A. Primary experimental studies

Study	Substrate and relevance	Operating conditions and comparison	Evidence used in this review	Limits of comparison
Lendormi et al. (2022)	Fresh swine slurry; supporting evidence for inoculum selection	Laboratory reactors progressively filled through weekly feeding at 13 °C; comparison of five manure-derived inocula	Inoculum origin and history influenced start-up and acclimation	Different substrate and feeding regime from cattle-manure batch tests; loading and gas-reference basis
Xu et al. (2022)	Cattle manure and corn straw; substrate ratio	Batch digestion at 20 °C; methanogenic propionate-degrading culture compared with a non-bioaugmented control; dose denominator, initial loading, inoculum ratio and duration	Reported methane enhancement of 2.80–4.20-fold and T_{80} reduction of 11–22 days	Source-specific treatment effects; gas-reference conditions, VS accounting and T_{80} definition
Xu et al. (2023)	Cattle manure and corn straw; substrate ratio	Batch digestion at 20 °C; cold-tolerant methanogenic culture compared with a non-bioaugmented control; reported optimum tested dose of 12%, with percentage basis	Reported methane enhancement of 3.4–4.8-fold and T_{80} reduction of 28–34 days; reported yields of 15.40 and 73.01 mL CH_4 g^{-1} VS for the control and optimum treatment, respectively	Initial loading, inoculum ratio, duration, gas-reference conditions, VS denominator and T_{80} definition
Jaimes-Estévez et al. (2023)	Cheese whey and cattle manure; mixture ratio	Batch biochemical methane potential tests at 15 °C; favorable reported condition used gasified pinewood biochar at 30 g L^{-1} and a particle-size setting of 0.575 mm	Reported methane potential increased from 230 to 340 mL CH_4 g^{-1} VS added after unit-prefix conversion	Co-digestion evidence; inoculum ratio, substrate loading, duration, blank treatment and gas-reference conditions
Zheng et al. (2023)	Electrochemically assisted digestion; substrate identity	Microbial electrolysis cell coupled with AD at 20 °C, using a modified cathode and applied-voltage comparisons; operating mode and loading	Methane performance and microbial/metabolic responses to electrochemical assistance	Reported methane yields use a COD basis; conversion to a VS basis requires corresponding composition data; electrical input must be assessed separately
Gosgot Angeles et al. (2024)	Cattle manure with combined molasses and Caesalpinia spinosa meal	Batch experiments in 3 L digesters over 43 days; controlled-heating and solar-heating systems; combined amendments compared with manure treatments at corresponding dilutions	Biogas volume and methane fraction changed with treatment and heating conditions	Combined-amendment response does not isolate microbial augmentation; temperature profiles and VS-normalized comparison basis
Rama et al. (2024)	Cattle manure and food waste	Cold-adaptation and batch bioaugmentation experiments; psychrophilic augmentation at 15 °C; assessment over 14 weeks	Methane production associated with changes in microbial communities and predicted functional capabilities	Co-digestion and differing adaptation histories constrain transferability; inferred functions require direct validation; loading, inoculum ratio and gas-reference basis

B. Reviews providing background and appraisal context

Study	Evidence type	Role in the present synthesis
Akindolire et al. (2022)	Critical review	Cold adaptation, microorganisms, enzymes and biological enhancement
Tiwari et al. (2021)	Critical review	Low-temperature process constraints and enhancement strategies
Alipoursarbani et al. (2026)	Systematic review	Functional classification, experimental controls and interpretation of bioaugmentation evidence

Notes: AD, anaerobic digestion; COD, chemical oxygen demand; VS, volatile solids. Experimental values apply to the cited studies and their respective controls. Fold enhancement is retained using the authors' terminology and has not been converted into percentage improvement. The definition and cumulative-production reference used for T_{80} require confirmation before comparing digestion-time effects. The biochar values were reported as 0.23 and 0.34 m^3 CH_4 kg^{-1} VS added; their conversion above changes unit prefixes only and does not establish a common gas temperature or pressure basis.

3. Effects of Low Temperature on Anaerobic Digestion

Anaerobic digestion is a cascade of hydrolysis, acidogenesis, acetogenesis and methanogenesis. Each step depends on different microbial guilds and on the successful transfer of intermediates between them (Harirchi et al., 2022). Under favorable conditions, soluble hydrolysis products are fermented to VFAs, alcohols, hydrogen and carbon dioxide, syntrophic bacteria oxidize reduced intermediates such as propionate and butyrate and methanogens convert acetate or hydrogen/carbon dioxide to methane (Meegoda et al., 2018). The sequential conversion pathway and the principal microbial groups involved in cattle-manure anaerobic digestion are illustrated in Figure 1.

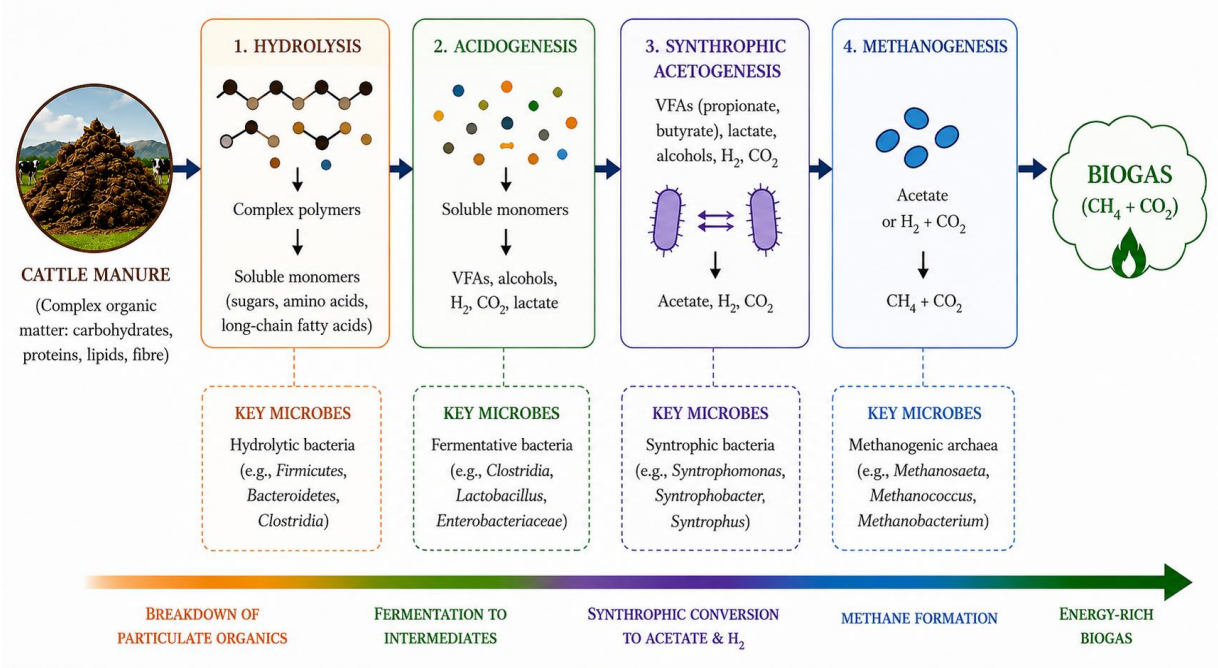


Figure 1. Principal biochemical stages and key microbial groups involved in the anaerobic digestion of cattle manure. Original figure by the authors; conceptual synthesis informed by Akindolire et al. (2022), Kelif Ibro et al. (2024), and Meegoda et al. (2018).

The microbial conversion pathway illustrated in Figure 1 is strongly influenced by temperature at cellular, enzymatic and physical levels. Lower temperatures reduce enzyme turnover, membrane fluidity and microbial growth rates, while simultaneously increasing liquid viscosity and potentially decreasing substrate diffusion and gas transfer (Rodríguez-Jiménez et al., 2026). The effect is especially important for hydrolysis of particulate or fibrous substrates and for syntrophic reactions that operate near thermodynamic limits (Si et al., 2026). As a result, psychrophilic digestion generally requires longer adaptation and retention times than mesophilic digestion (Akindolire et al., 2022; Tiwari et al., 2021).

The low-temperature penalty is often expressed as a widening imbalance between upstream and downstream reactions (Xu et al., 2022). Hydrolysis and acidogenesis can continue sufficiently to release fermentation products, while acetogenic and methanogenic conversion may respond more slowly (Tiwari et al., 2021). Propionate and acetate can therefore accumulate, increasing acid load and suppressing pH if buffering capacity is insufficient (Xu et al., 2023). This explains why VFA/alkalinity and pH are more informative stability indicators than pH alone: a digester can remain near neutral while its buffering reserve is being consumed (Tiwari et al., 2021; Xu et al., 2023).

Psychrophilic operation does not necessarily imply low ultimate biodegradability. Several reviews emphasize that low-temperature primarily changes kinetics, although the final methane recovery can also decline when the experiment is terminated before conversion is complete (Yao et al., 2020). This distinction is central to interpretation. A 60-day or 90-day low-temperature test may show a low endpoint methane yield

because conversion is incomplete, not because the substrate lacks methane potential (Akindolire et al., 2022). Consequently, time-bounded recovery, onset time and modeled lag should be reported alongside endpoint yield (Akindolire et al., 2022; Yao et al., 2020).

Temperature changes also restructure the microbial community (Lendormi et al., 2022). Cold adaptation selects organisms able to maintain membrane function, enzyme flexibility and energy conservation at lower temperature (Rama et al., 2024). Methanogenic pathways can shift and the relative importance of acetoclastic versus hydrogenotrophic routes depends on inoculum history, substrate, ammonia and other stresses. This makes inoculum origin and acclimation history a core experimental variable rather than a minor methodological detail (Lendormi et al., 2022; Rama et al., 2024; Zhu et al., 2025).

Finally, low-temperature interacts with reactor design. Biofilm and granular systems can retain slow growing biomass, staged reactors can separate hydrolysis from methanogenesis, conductive materials can promote syntrophic electron transfer and bioelectrochemical systems can assist thermodynamically difficult reactions (Si et al., 2026; Zheng et al., 2023).

4. Cattle Manure as a Psychrophilic Substrate

Cattle manure is attractive because it is locally available in livestock systems, has substantial moisture content and carries indigenous anaerobic microorganisms (Song et al., 2023). Yet it is also a difficult substrate. A significant fraction of the organic matter is associated with lignocellulosic plant residues that have already passed through the rumen and the residual fiber can be comparatively resistant to further hydrolysis (Li et al., 2021; Song et al., 2023). Reviews focused on cow manure identify this recalcitrant fraction as a major reason why monodigestion often gives lower methane productivity than digestion of readily degradable food residues (Li et al., 2021).

The physicochemical composition of cattle manure varies with diet, bedding, water addition, storage and manure collection method (Abid et al., 2024). Total solids, volatile solids (VS), fiber, nitrogen ash and pH therefore differ markedly among studies (Rodríguez-Jiménez et al., 2026). This variability complicates cross-study comparison because methane yield may be expressed per reactor volume, per kilogram of wet manure, or per gram of VS (Li et al., 2021). Manure dilution may improve mixing but reduces volumetric organic loading; conversely, high solids operation reduces water demand but increases viscosity and mass transfer resistance, which become more pronounced at low-temperature (Abid et al., 2024; Rodríguez-Jiménez et al., 2026).

Nitrogen is another dual-purpose factor. Cattle manure usually provides a relatively balanced nutrient supply, but ammonification can increase total ammonia nitrogen and free ammonia, particularly as temperature, pH and loading change. Although psychrophilic temperatures reduce the free-ammonia fraction compared with warmer systems, chronic ammonia stress can still restructure syntrophic pathways (Bae et al., 2025). Recent continuous cow manure work shows that ammonia tolerance is associated with specific syntrophic microbiomes and methanogenic shifts rather than a simple concentration threshold that applies universally.

Cattle manure is also microbiologically distinct because it carries rumen derived and fecal communities. These indigenous populations can contribute hydrolytic and fermentative functions, but they are not automatically optimized for methanogenesis in a sealed digester at low-temperature (Günel et al., 2025). Studies using rumen derived inoculation and manure acclimated inocula demonstrate that inoculum origin can change methane kinetics and microbial succession. Therefore, the idea of a universal inoculum dose is unlikely to be robust across manure sources (Günel et al., 2025; Lendormi et al., 2022).

For low-input systems, cattle manure nevertheless has a major advantage because it does not require a separate waste supply chain. In pastoral settings, the feasibility question is therefore often not feedstock availability, but whether sufficient manure can be collected consistently, mixed with available water, and converted into useful gas under temperatures that follow the local environment (Gill-Wiehl et al., 2023). This

shifts the optimization objective from maximizing laboratory methane potential toward achieving reliable energy service per unit of locally available manure, water, labor, and amendment cost (Aamaas et al., 2024; Gill-Wiehl et al., 2023; Tunguhole et al., 2025). Figure 2 integrates these considerations and illustrates how the physicochemical composition, lignocellulosic recalcitrance, nitrogen dynamics, indigenous microbial communities and operating conditions of cattle manure collectively influence its suitability for psychrophilic anaerobic digestion.

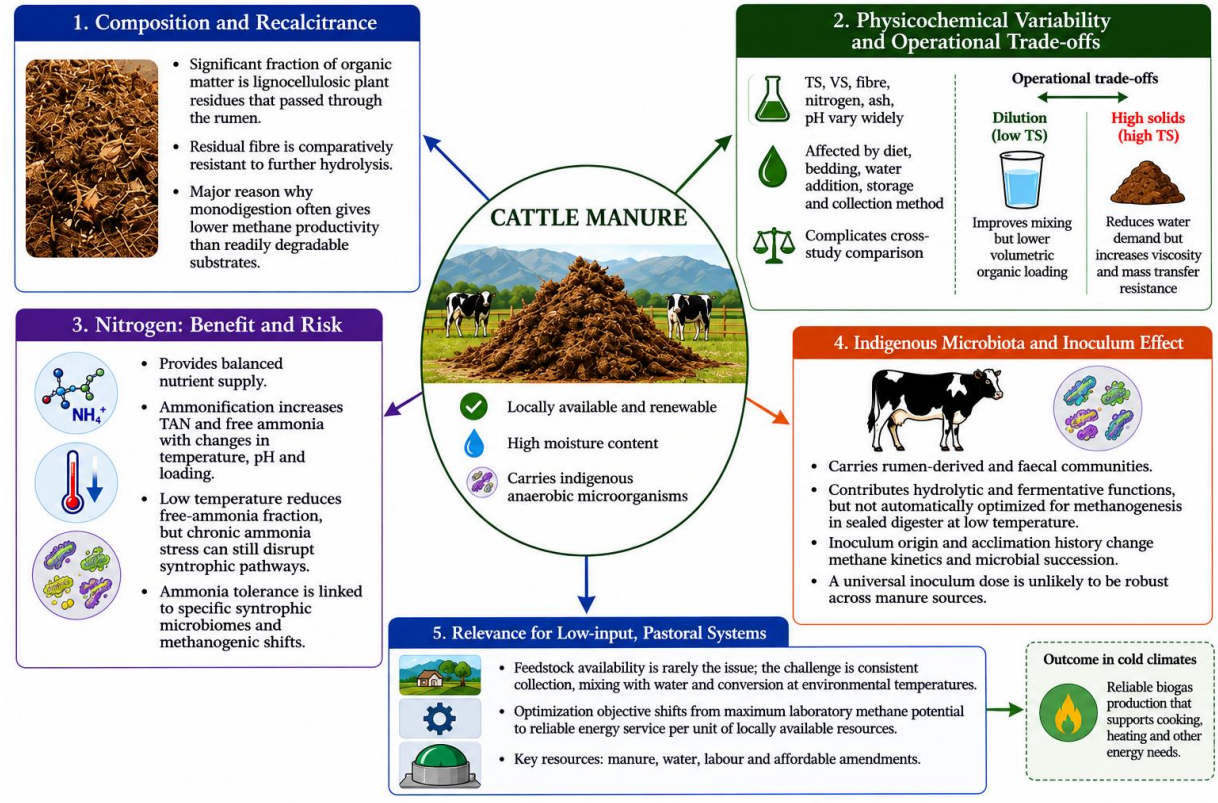


Figure 2. Key characteristics, challenges, and opportunities associated with cattle manure as a substrate for psychrophilic anaerobic digestion. Original figure by the authors; conceptual synthesis informed by Abid et al. (2024), Bae et al. (2025), Günel et al. (2025), Li et al. (2021), Rodríguez-Jiménez et al. (2026), and Song et al. (2023).

5. Microbial Ecology, Syntrophy and Cold Adaptation

5.1. Functional guilds and metabolic coupling

The AD microbiome is a trophic network. Hydrolytic bacteria release sugars, amino acids and fatty acids, acidogens produce short chain organic acids and hydrogen, syntrophic bacteria oxidize reduced VFAs and methanogenic archaea consume acetate or hydrogen/carbon dioxide (Harirchi et al., 2022). The thermodynamic feasibility of several syntrophic reactions depends on rapid removal of hydrogen, formate or electrons by methanogens. This means that methane production is highly sensitive to communication and metabolic balance among community members (Wang et al., 2022).

At low-temperature, the balance becomes more fragile because guilds do not slow at identical rates. If acid formation exceeds downstream conversion, intermediates accumulate (Xu et al., 2022). The bioaugmentation studies are particularly informative because their improved low-temperature methane performance was associated with enrichment of propionate oxidizing bacteria, syntrophic butyrate oxidizers and acetoclastic methanogens rather than with a generic increase in total microbial abundance (Xu et al., 2023). The key implication is that function targeted consortia can be more effective than nonspecific inoculum addition.

Cold adaptation is achieved through multiple physiological strategies, including changes in membrane lipid composition, production of cold active enzymes, altered protein flexibility and stress response regulation (Akindolire et al., 2022). The microbial composition observed after acclimation therefore reflects both selection and functional reorganization (Rama et al., 2024). Recent metagenomic work on cattle manure and food waste under psychrophilic conditions highlights the importance of identifying not only which taxa persist but also which metabolic pathways remain active (Akindolire et al., 2022; Zhu et al., 2025).

5.2. Interspecies electron transfer

Traditional syntrophic methanogenesis is often described through mediated interspecies electron transfer, particularly hydrogen and formate transfer (Park et al., 2018). These pathways are diffusion dependent and can become rate-limiting when syntrophic partners are spatially separated or when intermediate partial pressures move outside the narrow thermodynamic window required for oxidation reactions (Wang et al., 2021). Direct interspecies electron transfer (DIET) provides an alternative route in which reducing equivalents are exchanged through conductive cellular structures or external conductive materials (Kang et al., 2021).

DIET is relevant to psychrophilic digestion because faster electron exchange could compensate for otherwise slow syntrophic metabolism (Kang et al., 2021; Park et al., 2018). Conductive materials such as biochar, activated carbon and iron containing materials have repeatedly been associated with shorter lag, improved VFA conversion and greater methane production, although these effects are not uniquely diagnostic of DIET (Pilarska, 2026). Adsorption of inhibitors, microbial immobilization, trace element release and pH buffering can produce similar performance improvements. Consequently, claims of DIET should be supported with electrochemical, molecular or functional evidence rather than inferred from methane improvement alone (Kang et al., 2021; Pilarska, 2026; Zhao et al., 2024).

The biochar study summarized in Table 1 used cheese whey and cattle manure, so its methane response represents co-digestion evidence rather than manure-only conversion (Jaimes-Estévez et al., 2023). The reported enhancement supports further investigation of biochar under cold conditions. However, the methane response alone cannot determine the relative contributions of microbial retention, buffering and electron transfer, or establish the intervention's net energy benefit.

Bioelectrochemical assistance represents a more engineered version of the same principle. A microbial electrolysis cell integrated with psychrophilic digestion can alter metabolic pathways and community structure, providing an external means of facilitating electron transfer (Zheng et al., 2023). Such systems are scientifically valuable but may be less compatible with the simplicity required in household scale pastoral digesters unless low cost, passive designs are developed (Zheng et al., 2023).

6. Bioaugmentation and Microbial Consortia

6.1. What bioaugmentation is intended to accomplish

Bioaugmentation is the deliberate addition of selected microorganisms or microbial consortia to strengthen functions that are insufficient in the resident community (Alipoursarbani et al., 2026; Zhu et al., 2025). In AD, augmentation may target hydrolysis, acidogenesis, syntrophic VFA oxidation, methanogenesis or stress tolerance (Zhu et al., 2025). Recent systematic reviews show that the literature is heavily weighted toward hydrolytic and methanogenic functions, while targeted acetogenic and syntrophic propionate/butyrate oxidation remain less represented (Chen et al., 2025). This distribution matters because the rate-limiting function under cold cattle manure digestion may differ from the rate-limiting function in food waste or high ammonia systems (Alipoursarbani et al., 2026).

Successful augmentation requires more than inoculation. The added organisms must survive, compete or cooperate with the resident microbiome, retain activity at the operating temperature and receive suitable substrate. Dose and inoculum to substrate ratio influence whether a treatment improves methane productivity, ultimate conversion or both (Xu et al., 2022). A high dose may accelerate startup but be

economically unattractive; a low dose may be cost effective but fail to establish. These tradeoffs are clearly visible in psychrophilic dose-response studies (Xu et al., 2023).

A useful conceptual distinction is between functional augmentation and biomass augmentation. Simply adding more active anaerobic sludge can shorten lag by increasing the initial methanogenic population, whereas targeted augmentation introduces a function that the resident community lacks or performs poorly (Li et al., 2021). Strong experimental designs therefore require more than a no addition control; ideally, they should include a biomass equivalent control, an inactive cell control or a functionally different inoculum where feasible (Alipoursarbani et al., 2026; Li et al., 2021).

6.2. Direct evidence under psychrophilic conditions

The controlled batch studies of Xu et al. (2023) demonstrate that targeted microbial cultures can improve methane production during cattle-manure/corn-straw co-digestion. Their reported effects and verified operating conditions are presented separately in Table 1. The earlier study's numerical enhancement concerns methane, although some descriptions in the source use the broader term biogas. Both studies support biological enhancement under their tested conditions; their effect ranges should not be transferred directly to cattle-manure monodigestion or compared as evidence of superiority between cultures.

Inoculum-selection evidence also requires attention to substrate and feeding history. Lendormi et al. (2022) compared manure-derived inocula in progressively filled, weekly fed reactors treating fresh swine slurry. This supports the importance of inoculum origin and acclimation, while its operating regime differs from the batch cattle-manure studies. Together, these experiments justify testing functionally suitable inocula, with explicit controls and study-specific interpretation.

6.3. Commercial mixed cultures, EM-type products and molasses

Commercial mixed culture formulations are attractive because they are accessible, easy to dose and do not require local culture development (Gosgot Angeles et al., 2024). However, commercial labels do not provide the same mechanistic information as a defined methanogenic or syntrophic enrichment. Their microbial composition may include facultative fermenters, lactic-acid bacteria, yeasts or other organisms that can influence early fermentation without directly increasing methanogenic capacity (Gosgot Angeles et al., 2024; Harirchi et al., 2022). A higher methane output after adding such a formulation therefore does not by itself demonstrate methanogen bioaugmentation.

The interpretive problem becomes larger when the culture is activated or co-applied with molasses. Molasses is a rapidly fermentable carbon source; it can stimulate microbial activity and act as a co-substrate, but excessive soluble carbon can also increase VFA accumulation (Gosgot Angeles et al., 2024). Recent cattle manure experiments using molasses containing inoculation strategies confirm that gas production and methane fraction can change substantially. Yet without EM only, molasses only and combined treatments, the independent effects cannot be separated (Gosgot Angeles et al., 2024).

For future studies of combined EM type cultures and molasses, a four arm factorial design is therefore the minimum mechanistically informative comparison: untreated control, microbial product only, molasses only and combined microbial product plus molasses (Holliger et al., 2021). Total VS should be equalized or at least fully accounted for across treatments. This design transforms a simple performance comparison into a causal test of microbial, substrate and interaction effects (Gosgot Angeles et al., 2024). It also provides the evidence needed to justify claims of synergy rather than merely co-addition (Xu et al., 2022).

A second priority is microbial verification. Product labels can change, viability can decline during storage and activation conditions can favor some organisms over others (Chen et al., 2025). Culture dependent counts, metagenomics can establish whether the added organisms persist and whether methanogenic or syntrophic pathways actually increase (Rama et al., 2024). This is increasingly feasible and aligns with the direction of modern bioaugmentation research (Alipoursarbani et al., 2026).

Taken together, the available evidence indicates that successful bioaugmentation under psychrophilic conditions depends not simply on adding more microorganisms, but on matching the functional capability of the inoculum to the limiting stage of digestion, while ensuring survival, activity, appropriate dosing, and compatibility with the resident microbial community (Chen et al., 2025; Xu et al., 2022, 2023). For commercial mixed cultures and EM-type products, interpretation becomes particularly important when a readily fermentable carbon source such as molasses is co-applied because microbial and co-substrate effects may occur simultaneously (Alipoursarbani et al., 2026; Gosgot Angeles et al., 2024). Figure 3 integrates these relationships by linking the objectives of bioaugmentation, requirements for successful establishment, appropriate experimental controls, evidence from psychrophilic studies, mechanisms of improvement, and the interpretation of commercial mixed culture and molasses treatments.

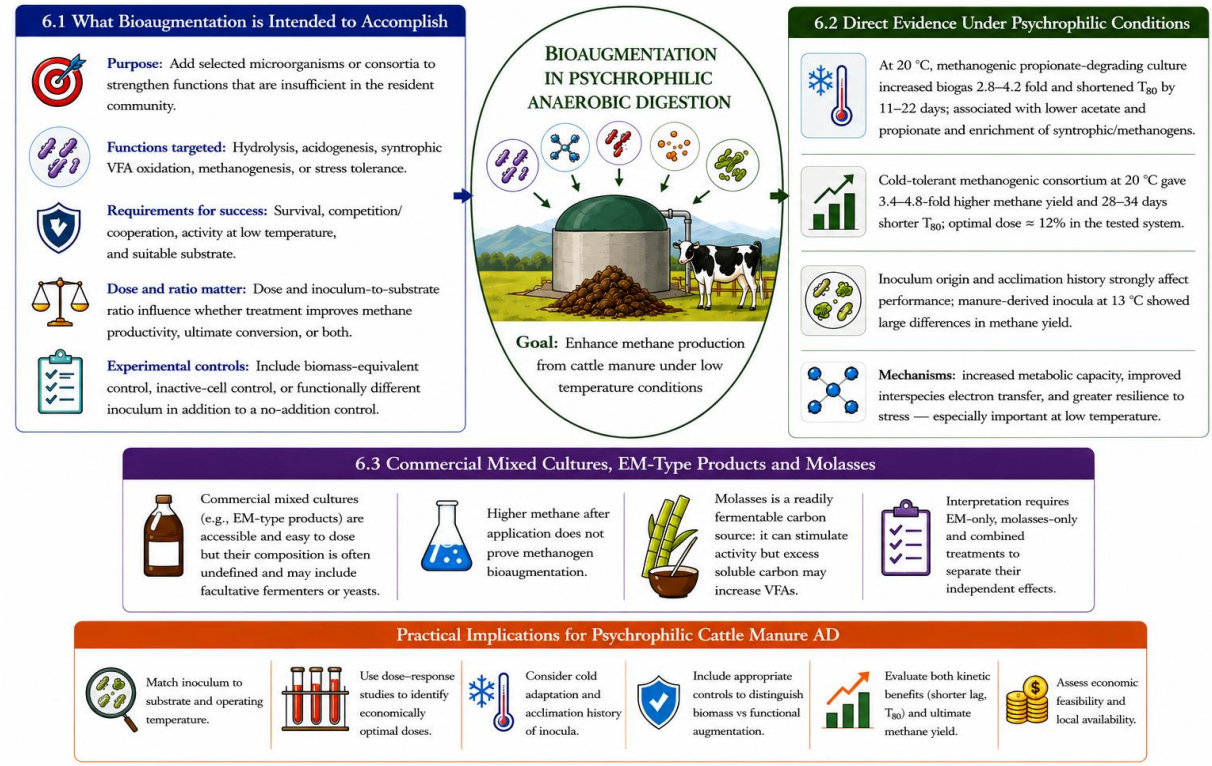


Figure 3. Conceptual framework for bioaugmentation and microbial consortia in psychrophilic anaerobic digestion of cattle manure. Original figure by the authors; conceptual synthesis informed by Lendormi et al. (2022), Zhao et al. (2024), and Alipoursarbani et al. (2026).

6.4. Culture preparation, dosing, immobilization and persistence

The preparation of a bioaugmentation seed determines whether the intervention is reproducible. A culture described only as 'active sludge' or 'microbial consortium' may vary from batch to batch in cell density, functional activity and community composition (Lendormi et al., 2022). For high quality experimentation, the inoculum should be characterized by source, acclimation temperature, enrichment substrate, storage time, total solids or biomass concentration and, where possible, molecular composition (Chen et al., 2025). These details are essential because bioaugmentation performance can be driven as much by inoculum history as by the nominal dose.

Dose-response interpretation depends on the optimization criterion. Xu et al. (2022) distinguished the dose favoring gas production from that favoring microbial bioaugmentation efficiency. Accordingly, a reported optimum should identify the outcome being optimized and the dose denominator. Culture volume, biomass mass and functional activity describe different inputs; percentage doses should not be compared

across studies until their bases are confirmed. A graded response therefore provides more informative evidence than a single dose, particularly when additional inoculum also changes initial biomass.

Immobilization offers a potential solution to culture washout and poor persistence. Cells can be retained on porous carriers, biochar, granular media or other support materials, increasing local cell density and protecting slow growing methanogens from hydraulic loss (Pilarska, 2026). The concept is especially attractive in cold systems because growth is slow and recovery after disturbance can be prolonged (Zhao et al., 2024). However, immobilization changes more than residence time; the support can also alter adsorption, local pH, trace nutrient availability and electron transfer, so biological and physicochemical mechanisms should be separated experimentally (Pilarska, 2026; Zhao et al., 2024).

Persistence should be treated as an outcome rather than assumed from initial dosing. A culture that produces an early methane response but disappears before steady operation may be useful for startup inoculation but not for long term intensification (Rama et al., 2024). Conversely, a consortium that establishes within biofilms or becomes functionally integrated into the resident microbiome may support durable performance (Chen et al., 2025). Metagenomics can distinguish these scenarios and should be paired with methane kinetics whenever possible.

For low-input deployment, the practical question is whether a culture can be maintained locally (Günel et al., 2025). Repeated purchase of proprietary inoculum, refrigerated transport or specialized activation may be acceptable in centralized plants but difficult in pastoral households (Alipoursarbani et al., 2026). Research should therefore compare commercial products with locally acclimated digestate, rumen-derived inocula and simple enrichment approaches, considering not only methane improvement but also shelf life, dosing frequency, biosafety and local capacity for preparation (Günel et al., 2025).

7. Strategies for Improving Psychrophilic Cattle Manure Digestion

7.1. Co-digestion and carbon balance

Co-digestion is one of the most mature strategies for improving cattle manure AD because it can correct nutrient balance, increase readily biodegradable carbon and dilute inhibitory compounds (Bi et al., 2020). Cow manure reviews consistently show that a suitable lignin poor co-substrate can improve methane production and stabilize digestion. Under psychrophilic conditions, however, co-digestion must be controlled carefully because readily fermentable substrates can produce acids faster than methanogens can consume them (Bi et al., 2020; Li et al., 2021).

The laboratory to farm study of cheese whey and cattle manure illustrates both the opportunity and the risk. Psychrophilic co-digestion was feasible and supported practical energy substitution, but the authors emphasized the importance of organic loading and VFA management. For low-input pastoral systems, co-digestion is attractive only when the co-substrate is locally available, does not create a competing use and can be supplied consistently (Jaimes-Estévez et al., 2022).

7.2. Pretreatment and solids management

Pretreatment can improve hydrolysis of cattle manure fiber through mechanical, thermal, chemical or biological means. Yet the best laboratory pretreatment is not automatically the best field option (Abid et al., 2024). Energy intensive thermal or high-pressure approaches can eliminate the energy advantage of unheated digestion. Low-cost biological pretreatment, size reduction and controlled freeze thaw effects may be more compatible with cold region systems (Abid et al., 2024; Li et al., 2021).

Solids concentration must also be optimized rather than maximized. High solids digestion reduces water demand, a potential benefit in pastoral settings, but increases viscosity and limits mass transfer (Rodríguez-Jiménez et al., 2026). Low-temperature intensifies this rheological penalty. Reactor mixing, slurry dilution and feedstock particle size therefore interact with microbial strategies and should be reported as part of the treatment design (Rodríguez-Jiménez et al., 2026).

7.3. Biochar and other conductive materials

Conductive materials offer a hybrid biological physical enhancement route. Biochar can provide surface area for microbial retention, adsorb inhibitory compounds, buffer local chemistry and promote electron transfer (Zhao et al., 2024). These multiple mechanisms are useful but complicate attribution. Reviews at the community, cellular and genetic levels show that biochar can increase microbial diversity, enzyme activity, electron transfer and functional gene expression (Zhao et al., 2024).

For psychrophilic systems, the strongest practical appeal of biochar is that it may improve kinetics without continuous energy input. The improved methane recovery and increased methanogen abundance observed under an optimized biochar dose demonstrate the potential of this approach (Jaimes-Estévez et al., 2023). Future work should determine whether locally produced biochars from agricultural residues can deliver similar benefits without introducing contaminants or excessive material costs.

7.4. Reactor design, biomass retention and bioelectrochemical assistance

Slow growing psychrophilic biomass can be protected by reactor designs that decouple hydraulic retention from solids retention. Biofilm, granular sludge, membrane and hybrid configurations can retain methanogens and syntrophs even when liquid throughput changes (Si et al., 2026). The newest psychrophilic anaerobic digestion (PAD) reactor design synthesis emphasizes the interaction between mass transfer, microbial retention, enzyme activity, thermodynamics and reactor architecture.

Bioelectrochemical systems are promising where propionate or other intermediates accumulate, because external or internally generated electrochemical gradients can facilitate electron transfer (Zheng et al., 2023). Mechanistic work under psychrophilic conditions shows that microbial electrolysis can reshape microbial communities and metabolic pathways. For household scale application, however, added electrical components increase complexity and simpler passive conductive materials may be more realistic in the near term (Zheng et al., 2023).

7.5. Enzyme assisted and biologically mediated pretreatment

Psychrophilic digestion can also be enhanced by increasing the activity of extracellular enzymes responsible for hydrolysis. Cold active cellulases, proteases and lipases are attractive because they can increase substrate solubilization without heating the entire reactor (Akindolire et al., 2022). Reviews of psychrophilic microorganisms emphasize that enzyme flexibility and cold adaptation are key biological traits that could be exploited as additives or through enrichment of enzyme-producing organisms.

Enzyme supplementation and microbial augmentation should not be treated as equivalent. Enzymes can produce an immediate hydrolytic effect but may be consumed or deactivated, whereas living consortia can reproduce and potentially integrate into the reactor community (Pilarska, 2026). A hybrid strategy may be useful when hydrolysis is the dominant bottleneck: a hydrolytic inoculum or enzyme treatment can release soluble substrate, while a methanogenic consortium prevents the resulting VFAs from accumulating (Harirchi et al., 2022).

The main risk is that accelerating hydrolysis without simultaneously increasing downstream conversion can worsen acidification. This is why a treatment that increases soluble VFA formation is not necessarily an improvement unless methane production and VFA consumption also increase (Tiwari et al., 2021). Future studies should therefore evaluate stage specific rates rather than assuming that faster substrate solubilization automatically translates into higher methane recovery.

For pastoral applications, enzyme based strategies will be competitive only if enzymes can be produced or formulated at low cost and remain active under storage conditions (Pilarska, 2026). Locally produced fungal or bacterial enzyme preparations may eventually be attractive, but their reproducibility, biosafety and shelf stability require considerably more evidence before field recommendation (Akindolire et al., 2022).

The principal strategies for improving psychrophilic cattle manure digestion are synthesized in Figure 4, which shows how substrate management, conductive materials, reactor engineering and biologically assisted

approaches target different limitations imposed by low-temperature operation (Bi et al., 2020; Si et al., 2026; Zhao et al., 2024).

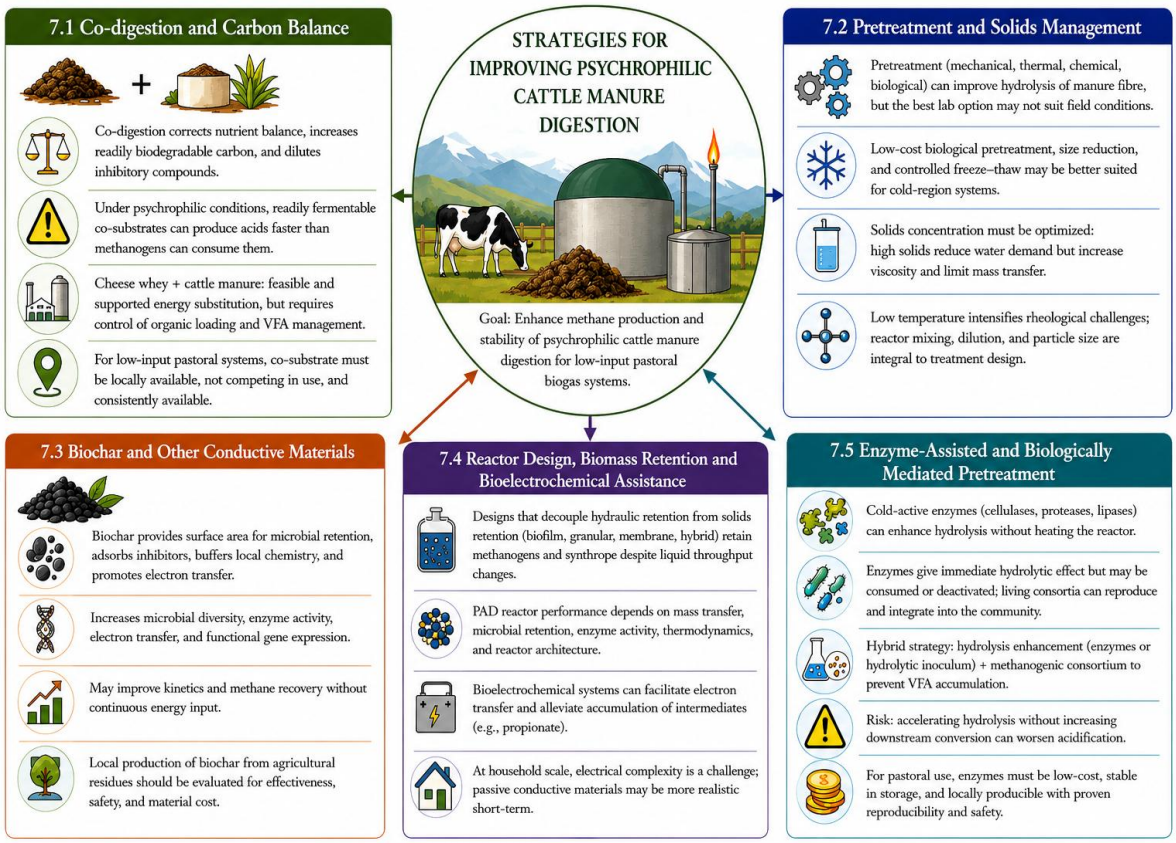


Figure 4. Integrated strategies for improving psychrophilic anaerobic digestion of cattle manure. Original figure by the authors; conceptual synthesis informed by Bi et al. (2020), Si et al. (2026), and Zhao et al. (2024).

8. Process Stability and Biogas Quality

8.1. pH, VFA/alkalinity and ammonia

pH is widely monitored because methanogens generally perform best near neutral conditions, but pH alone is a lagging indicator. Bicarbonate buffering can mask VFA accumulation until the system approaches failure (Meegoda et al., 2018). A stronger monitoring framework combines pH with total VFAs, alkalinity or VFA/alkalinity ratio, ammonia and where possible, soluble chemical oxygen demand (COD) (Tiwari et al., 2021). This is particularly important in PAD because acid consumption is often slower than acid formation (Egwu et al., 2022).

A treatment that increases methane production while maintaining stable VFA and alkalinity is more convincing than a treatment that produces a transient gas pulse accompanied by acid accumulation (Xu et al., 2023). The Xu bioaugmentation studies are notable because improved methane production was accompanied by lower VFA concentrations, directly supporting a mechanism of improved intermediate conversion (Xu et al., 2022).

Ammonia should be measured even in cattle manure systems that are not expected to be strongly inhibited. Recent continuous cow manure research shows that community structure and pathway selection change as total ammonia nitrogen increases (Bae et al., 2025). These shifts can alter the apparent effectiveness of an inoculum or conductive additive and may explain why the same intervention performs differently across manure sources.

Figure 5 summarizes the complementary roles of process-stability indicators. pH should be interpreted alongside VFA accumulation and alkalinity because buffering can mask developing imbalance. Total ammonia nitrogen and free ammonia should be assessed with pH and temperature, while soluble COD provides context on solubilization and subsequent conversion (Bae et al., 2025; Meegoda et al., 2018; Tiwari et al., 2021).

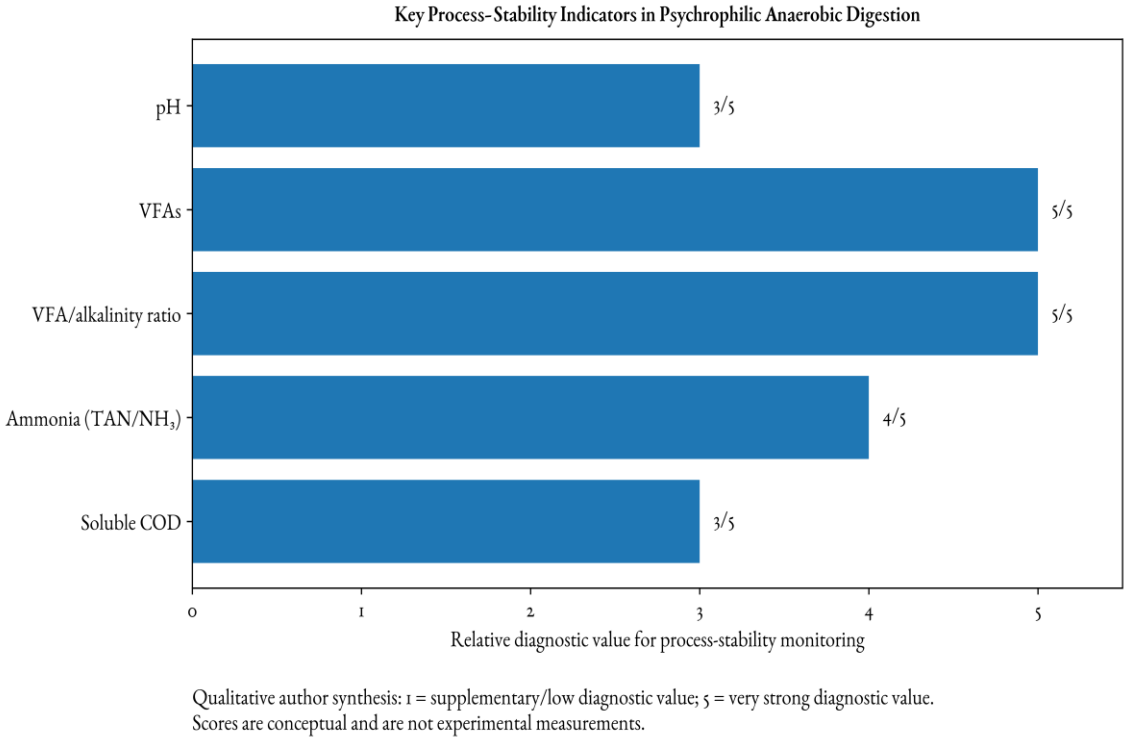


Figure 5. Relative diagnostic value of key process-stability indicators for psychrophilic anaerobic digestion. Original figure by the authors; conceptual synthesis informed by Meegoda et al. (2018), Tiwari et al. (2021), and Zheng et al. (2023).

8.2. Methane fraction, carbon dioxide and trace gases

Biogas quantity and biogas quality should be treated as separate outcomes. Methane fraction determines the energy bearing share of the gas, while carbon dioxide dilutes heating value (Aikeremu & Rajagopal, 2026). A treatment that raises total gas but lowers methane percentage may not improve useful energy to the same extent as the gas volume increase suggests. For household applications, the methane fraction is therefore a direct usability indicator alongside total methane volume (Meegoda et al., 2018).

Trace gases are also important: hydrogen sulfide can corrode fittings and reduce appliance life and oxygen or nitrogen can indicate leakage or air ingress. Many laboratory studies report only CH_4 and CO_2. A field-ready evidence package should include at least CH_4, CO_2 and H_2S, plus leak checks and gas storage performance (Aikeremu & Rajagopal, 2026; Meegoda et al., 2018). This is especially important when transferring biological enhancement strategies to decentralized systems where maintenance resources are limited.

9. Measuring Methane Performance

9.1. Endpoint yield against time-bounded recovery

Methane yield is often presented as if it were a single universal quantity, yet the meaning depends on the experimental endpoint. A standardized biochemical methane potential (BMP) test aims to estimate ultimate biodegradable methane under controlled conditions, whereas a 60-day/90-day field inspired batch test measures methane recovered within a defined operational window (Jaimes-Estévez et al., 2022). The

distinction is especially important at low-temperature because slow conversion can leave substantial biodegradable substrate at the end of a fixed duration test (Holliger et al., 2021).

For this reason, psychrophilic studies should report both the experimental duration and an objective stopping criterion. If cumulative production has not plateaued, the endpoint should be described as time-bounded methane recovery rather than ultimate methane potential (Morais et al., 2021).

9.2. Volatile solids normalization and amendment accounting

Specific methane yield should identify both the numerator and denominator: blank-corrected methane volume divided by added substrate VS. When molasses or another organic co-substrate is supplied, its VS contribution belongs in the substrate denominator used to evaluate the combined feed. Inoculum and augmentation-seed biomass should be characterized separately and addressed through appropriate matched blanks and controls (Alipoursarbani et al., 2026; Holliger et al., 2021).

Normalizing a combined treatment only to manure VS does not measure conversion efficiency of the total added substrate. Where amendment contributions cannot be separated, the result should be described as a whole-treatment response. Wet mass and solids content should be measured; conversion from manure volume to mass requires a documented density rather than an assumed universal value.

9.3. Gas correction and analytical uncertainty

The preferred comparison basis is dry methane expressed as mL CH_4 g^{-1} substrate VS added, with gas volume referenced to 0 °C and 101.325 kPa (Filer et al., 2019). These are gas-volume reference conditions, distinct from reactor temperature. Physical conversion requires the original measurement temperature, absolute pressure and water-vapor basis, together with methane measurements corresponding to the gas-sampling interval.

Where these details are unconfirmed, results retain their reported gas basis and are not labelled as standardized volumes. Unit-prefix conversion alone does not establish physical comparability. COD-based or wet-mass yields cannot be converted to a VS basis without corresponding composition measurements. Measurement methods, calibration and uncertainty should accompany the reported outcome.

9.4. Measurement uncertainty and data quality assurance

Uncertainty should be propagated through derived methane indicators. Gas meter resolution, methane analyzer accuracy, temperature and pressure correction, solids analysis and reactor loading all contribute to uncertainty in specific methane yield (Morais et al., 2021). When treatment differences are small, these measurement components may be comparable to the reported biological effect. Even when differences are large, transparent uncertainty reporting increases confidence in the result and facilitates comparison across laboratories (Holliger et al., 2021).

Independent reactor replication is distinct from repeated measurement of the same reactor. Multiple gas readings improve measurement precision but do not estimate biological variability (Alipoursarbani et al., 2026). High impact experimental studies should therefore use independent reactors as the unit of replication and report means, standard deviations or confidence intervals at the reactor level. This is particularly important for bioaugmentation because microbial establishment can vary stochastically among reactors (Alipoursarbani et al., 2026).

Quality assurance should include routine leak testing, mass-balance checks and consistency checks between total gas volume and gas composition. Apparent decreases in cumulative gas, impossible composition sums or discontinuities in flow can reveal instrumentation or data entry problems (Holliger et al., 2021; Meegoda et al., 2018). Data cleaning rules should be prespecified and raw measurements retained so that derived cumulative curves can be audited.

The same principle applies to microbial data. Relative abundance sequencing can show community shifts but does not measure absolute biomass or activity (Rama et al., 2024). Combining relative sequencing with functional genes, enzyme assays or stable isotope approaches provides a stronger mechanistic basis. The

most informative studies increasingly integrate gas kinetics, process chemistry and microbial measurements rather than treating them as separate datasets (Chen et al., 2025; Zhu et al., 2025).

10. Kinetic Modeling for Psychrophilic Methane Production

10.1. Why kinetics matter more at low-temperature

Kinetic models compress a time series into parameters that can describe the methane production potential, maximum rate and start-up delay. This is especially useful in PAD because two reactors can reach similar endpoints while differing greatly in time to useful gas (Kelif Ibro et al., 2024). For household systems, a shorter lag or earlier onset may have more practical value than a small increase in ultimate yield (Morais et al., 2021).

The modified Gompertz model is widely used for sigmoidal batch methane curves because its parameters are intuitively interpretable: P represents the asymptotic production implied by the model, Rm the maximum fitted production rate and λ the lag parameter (Wang & Guo, 2024). Recent methodological reviews continue to support its usefulness while emphasizing that it is empirical rather than mechanistic.

10.2. Modified Gompertz, logistic, first-order and cone models

First-order models are useful when hydrolysis or overall biodegradable substrate depletion behaves approximately exponentially, but they do not naturally represent a long lag phase (Yahya et al., 2022; Zhang et al., 2021). Logistic and modified Gompertz models provide sigmoidal behavior and are therefore more suitable when start-up is delayed. Cone type or other flexible empirical models can fit irregular curves but their parameters may be less directly connected to biological interpretation (Rubio et al., 2020; Yahya et al., 2022; Zhang et al., 2021).

Model choice should be based on the structure of the data rather than on the highest R^2 alone. A high R^2 can be achieved by an over parameterized or extrapolative model even when the experiment does not approach a plateau (Wang & Guo, 2024). Parameter confidence intervals, residual structure and sensitivity to late time points should therefore be reported. The asymptotic P from a 90-day curve that is still increasing should be described as an extrapolated model parameter, not a measured methane potential.

Observed interval average production rate and fitted Rm should also be distinguished. The former is a finite difference between measured cumulative volumes over a sampling interval; the latter is the maximum instantaneous slope of a fitted continuous curve (Morais et al., 2021; Wang et al., 2018). They can rank treatments differently without being contradictory. This distinction is particularly important when sampling is sparse around the peak production period.

10.3. Emerging data driven modeling

The next modeling frontier is not a replacement of kinetic equations but integration of kinetics with process data. Machine learning frameworks can use temperature, loading, ammonia, VFAs, retention time and other variables to predict methane yield or stability (Aikeremu & Rajagopal, 2026). Recent work in psychrophilic systems shows that interpretable models can identify operational drivers while retaining predictive power (Aikeremu & Rajagopal, 2026) For small experimental datasets, however, mechanistic or low parameter kinetic models remain safer than complex machine learning approaches.

A promising research design is therefore hierarchical: use a simple kinetic model to summarize each reactor trajectory, then model the fitted parameters or residual deviations as functions of temperature, loading, microbial composition and amendment dose (Ali et al., 2020; Kelif Ibro et al., 2024). This preserves biological interpretability while taking advantage of richer datasets when they become available.

10.4. Sampling design, parameter identifiability and model validation

Kinetic parameters are only as reliable as the sampling schedule. Sparse observations before methane onset can shift the fitted lag parameter by many days, while sparse sampling around the inflection point can distort the fitted maximum rate (Morais et al., 2021; Wang et al., 2018). Psychrophilic studies should therefore use

adaptive sampling, lower frequency during an established zero production period and higher frequency as soon as gas begins to increase. Daily measurements are preferable during the transition from lag to active methanogenesis.

Model identifiability should be assessed explicitly when a curve does not contain all phases of the sigmoidal trajectory. If the experiment ends before a plateau is visible, P and Rm can be strongly correlated, allowing several parameter combinations to fit the observed data similarly. Confidence intervals, parameter-correlation matrices or bootstrap analysis are therefore more informative than R^2 alone (Morais et al., 2021; Wang & Guo, 2024). A nearly perfect R^2 does not prove that the asymptotic methane potential has been estimated accurately.

External validation is rarely possible in a single batch experiment, but simple internal checks can improve credibility. Researchers can fit the model to an early portion of the data and test prediction of later observations, compare multiple kinetic forms, or use information criteria and residual diagnostics (Kelif Ibro et al., 2024; Yahya et al., 2022; Zhang et al., 2021). When several models fit similarly, the model with parameters that best match the research question should be preferred rather than selecting solely by numerical fit.

For future multi reactor datasets, kinetic parameters can also become response variables in statistical models. Lag, Rm and time-bounded recovery can be compared across microbial doses, temperature profiles and loading conditions using mixed effects or hierarchical approaches (Kelif Ibro et al., 2024; Wang & Guo, 2024). This would move psychrophilic bioaugmentation research from descriptive curve fitting toward quantitative inference about the factors controlling methane kinetics.

11. Translating Biological Enhancement to Low-Input Pastoral Systems

11.1. The time to energy problem

The adoption value of a household biodigester is determined by energy service, not methane yield in isolation. East African biogas programs have shown that users value convenience, time savings and reduced fuel expenditure, yet fuel stacking remains common when gas supply is insufficient or unreliable (Clemens et al., 2018). Tanzanian studies similarly show that household fuel choice depends on accessibility, affordability, reliability and user preference rather than on technical availability alone (Choumert-Nkolo et al., 2019; Gill-Wiehl et al., 2023; Tunguhole et al., 2025).

Psychrophilic start-up therefore deserves explicit attention as a user relevant metric. A digester that eventually produces methane but requires many weeks before useful gas becomes available can be technically successful and socially unattractive at the same time. Bioaugmentation evidence is strongest when studies report onset time or lag reduction in addition to endpoint methane yield, because these indicators connect microbiological performance with energy service reliability (Xu et al., 2022; Zheng et al., 2023).

11.2. Ngorongoro and other high-altitude pastoral contexts

High-altitude pastoral environments combine locally available cattle manure with constraints on infrastructure, energy inputs and maintenance. The Ngorongoro Conservation Area is also a multiple use conservation landscape, so technologies must be compatible with pastoral livelihoods and environmental management. Recent literature on Ngorongoro highlights the complexity of balancing livelihoods, infrastructure, tourism and conservation priorities (Koskei & Glyptou, 2025). A low-input digester must therefore be judged not only by methane yield but also by footprint, water demand, ease of relocation or repair, safety and the reliability of locally available inputs.

Tanzania's National Clean Cooking Strategy 2024–2034 provides a policy framework for household cooking-energy transitions (United Republic of Tanzania, Ministry of Energy, 2024). For biogas, sustained cooking use requires continued digester operation and attention to affordability, maintenance and the use of other household fuels, as demonstrated by East African program experience (Clemens et al., 2018).

For this context, an augmentation strategy is attractive only if the additional methane or reduced start-up time compensates for the cost and logistical burden of the amendment. Commercial cultures that require repeated purchase, cold storage or long-distance transport may be less suitable than locally maintainable inocula (Gill-Wiehl et al., 2023). Conversely, a small dose that reliably shortens start-up after seasonal shutdown could have high value even if the final methane yield improvement is modest (Aamaas et al., 2024). Economic assessment should therefore monetize time to energy and reliability, not only methane volume (Aamaas et al., 2024; Gill-Wiehl et al., 2023; Tunguhole et al., 2025).

11.3. Climate and circular economy implications

Capturing manure methane for energy can contribute to climate mitigation, but the net benefit depends on actual gas capture, leakage, displaced fuels and digestate management. Methane mitigation has high near-term climate value (Balcombe et al., 2018; Saunois et al., 2025; Stoerk et al., 2025). At the same time, a poorly sealed digester can lose part of the climate benefit through fugitive methane. Field validation should therefore include leak detection and gas-balance measurements where possible (Olczak et al., 2023).

Digestate is an additional co-product. If safely managed, it can recycle nutrients and reduce waste management burdens. However, digestate value depends on nutrient content, pathogen risk, storage and farmer acceptance (Kimutai et al., 2025; Nleya et al., 2025). A high impact assessment of low-input PAD should therefore move toward a system boundary that includes energy service, manure management, nutrient recovery, leakage and user behavior rather than treating methane yield as the sole outcome (Kimutai et al., 2025).

11.4. Energy balance and techno-economic considerations of low-temperature AD

Low-temperature operation can reduce reactor-heating demand, but its energy advantage depends on methane recovery and the resources required to sustain operation. For heated digesters, thermal demand depends on feed and ambient temperatures, feed throughput, insulation and reactor heat losses. Unheated systems avoid controlled reactor heating, while any pumping, mixing, gas conditioning and storage requirements remain part of the assessment. Biological retention requirements and reactor design must therefore be evaluated alongside heating demand (Si et al., 2026).

The balance should distinguish methane energy from useful energy delivered. Report gas losses, gross conversion output, internal consumption, external energy imports and delivered electricity and heat separately. Specify the heating-value convention and gas-reference conditions, and count internally recycled energy once. The conceptual dairy-manure plant assessed byGarkoti et al. (2024) which allocates recovered heat to digester heating, illustrates why gross thermal output cannot be treated as heat available to users. Report energy in kWh using a common operating period and substrate basis.

End-use selection should follow actual demand. Direct combustion can supply cooking heat or boiler heat, while combined heat and power (CHP) produces electricity and recoverable heat (International Energy Agency, 2025). Credit CHP heat only where it is used, and evaluate conversion at the relevant operating load. Cooking assessments should measure delivered gas, appliance performance and displaced fuel. Jaimes-Estévez et al. (2022) reported cooking use and fuel substitution from psychrophilic cheese-whey/cattle-manure co-digestion at a Colombian rural school. This demonstrates a use pathway, while transfer to pastoral manure-only systems requires separate validation.

Adaptation and bioaugmentation may improve energy recovery by bringing methane production forward or increasing recovery within the operating period. However, the methane responses demonstrated in laboratory co-digestion do not establish a net energy gain (Xu et al., 2023). Compare treated and control systems over the same period, accounting for culture preparation, activation, transport, dosing and any additional process energy. Include molasses and other carbon amendments in substrate and cost accounts; distinguish their energy contribution from improved manure conversion. Culture persistence and repeated

dosing are consequently energy and economic variables as well as microbial outcomes (Alipoursarbani et al., 2026).

Techno-economic assessment should connect capital and operating costs to useful energy delivered, using a stated currency, price year, lifetime and system scale. Relevant costs include amendment supply, water, labor, maintenance, replacement and downtime. Evaluate avoided fuel purchases or usable energy sales against a defined baseline, and test sensitivity to seasonal methane supply, amendment frequency, equipment performance and local prices. Integrated plant assessment provides a useful framework, but its costs and conversion assumptions require local validation before application to pastoral systems (Jalali et al., 2025).

The evidence assembled here does not provide a harmonized inventory linking microbial enhancement to net delivered energy and local costs in unheated cattle-manure systems. Consequently, no general net-energy benefit, conversion efficiency or cost reduction can be assigned to bioaugmentation from this synthesis. Matched seasonal trials combining methane accounting, process-energy measurements and actual heat or electricity use are therefore a priority for pastoral applications, including NCA. The following critical synthesis considers biological enhancement alongside these requirements for demonstrating an energy-service benefit.

12. Critical Synthesis

First, the literature establishes with high confidence that psychrophilic AD is feasible but kinetically constrained. Reviews across manure, wastewater and organic waste systems consistently identify slower hydrolysis, enzyme activity, microbial growth and syntrophic conversion at low-temperature (Tiwari et al., 2021; Yao et al., 2020). The practical implication is that low-temperature systems should be designed around retention, biomass preservation and start-up rather than judged only against mesophilic peak productivity.

Second, targeted bioaugmentation can produce large improvements under controlled psychrophilic conditions. The most compelling evidence involves cold-adapted methanogenic or propionate-degrading cultures that reduce VFA accumulation and shorten digestion time (Xu et al., 2022). However, evidence is strongest for co-digestion and controlled laboratory systems; direct evidence for cattle manure monodigestion in simple household type reactors remains thinner (Xu et al., 2023).

Third, microbial function matters more than the generic label ‘consortium’. Modern sequencing and metabolic analyses show that propionate oxidation, acetate conversion, methanogenic pathway selection and syntrophic electron transfer are central mechanisms (Bae et al., 2025). A publishable augmentation study should therefore define what function the added community is expected to improve and provide evidence that the function changed (Bae et al., 2025; Rama et al., 2024; Zhu et al., 2025).

Fourth, conductive materials and biochar are credible complementary tools, particularly when they support biomass retention and electron transfer without continuous energy input (Park et al., 2018). Yet methane improvement alone is not proof of DIET. Conductive-material studies should separate electrical, adsorption, buffering and trace-nutrient mechanisms wherever possible (Wang et al., 2021; Zhao et al., 2024).

Fifth, the field still suffers from methodological fragmentation. Different temperature definitions, substrate ratios, gas corrections, VS denominators, inoculum to substrate ratios and durations create apparent contradictions that are often artifacts of protocol (Alipoursarbani et al., 2026; Holliger et al., 2021). The most important improvement for the next generation of studies may therefore be better standardization rather than a new additive.

Sixth, the practical endpoint for pastoral systems is reliable cooking energy. The strongest research agenda therefore combines biological performance with time to energy, gas quality, safety, maintenance and cost (Aamaas et al., 2024; Tunguhole et al., 2025). This framing connects psychrophilic AD research with Tanzania’s policy objectives for household cooking energy (United Republic of Tanzania, Ministry of Energy, 2024).

12.1. Strength of evidence across the principal claims

The assessments in this section are qualitative judgements based on the appraisal criteria in Section 2. Controlled experiments support improved methane performance under particular substrate, inoculum and operating conditions (Xu et al., 2023). Extrapolation remains limited by experimental heterogeneity and unresolved questions about controls, microbial persistence and implementation requirements (Alipoursarbani et al., 2026). Within the evidence assembled here, seasonal reliability of commercial mixed cultures in unheated cattle-manure systems in NCA has not been demonstrated.

For conductive materials, evidence of improved methane recovery does not by itself identify the mechanism. Electron transfer, adsorption, buffering and microbial retention may contribute concurrently. Mechanistic attribution should therefore be supported by measurements that distinguish these contributions, alongside microbial and process evidence (Zhao et al., 2024).

East African biogas programs report affordability and maintenance constraints alongside continued use of other cooking fuels (Clemens et al., 2018). These findings show that digester installation alone is insufficient evidence of sustained fuel substitution. What remains less certain is the magnitude of the user benefit produced by a specific reduction in digester start-up time (Aamaas et al., 2024; Gill-Wiehl et al., 2023; Tunguhole et al., 2025). Future field trials should therefore connect methane kinetics directly to cooking hours, fuel stacking and user satisfaction.

13. Research Gaps and Future Research Priorities

Despite substantial progress in psychrophilic anaerobic digestion and microbial bioaugmentation, important knowledge gaps remain regarding the mechanisms, reproducibility, long term stability and practical application of enhancement strategies for cattle manure digestion under low-temperature conditions. Addressing these gaps is essential for moving from laboratory scale evidence toward reliable and economically viable biogas systems as shown in Table 2.

14. Implications for Review-Driven Experimental Design in the Ngorongoro Context

The NCA context is used here to develop testable hypotheses from the preceding synthesis. The proposed question is whether a microbial amendment, added fermentable carbon or their interaction can advance methane availability while maintaining stable operation and improving net energy delivery. Local temperature profiles, collectable manure, water availability and energy demand would first need to be measured.

Replicated treatments under matched operating conditions would compare an unamended control, microbial amendment alone, molasses alone and their combined application. Feed and amendment volatile solids would be quantified and loading differences accounted for, with inoculum contributions addressed through appropriate controls and blanks (Alipoursarbani et al., 2026; Holliger et al., 2021).

Measurements would combine observed onset, cumulative methane recovery, production rate, methane fraction, pH, VFAs, alkalinity and ammonia. Fitted lag, maximum rate and asymptotic potential would be reported with uncertainty and distinguished from observed performance (Wang & Guo, 2024).

Subsequent pilot trials would connect methane performance with useful cooking heat, electricity where feasible, seasonal availability, maintenance and cost. Energy accounting would include process and intervention inputs and gas losses, using the existing energy service as the comparison baseline.

Table 2. Evidence, knowledge gaps and research priorities for translating psychrophilic digestion enhancement into useful energy

Evidence from this review (with section reference)	Knowledge gap	Research priority	Energy relevance
Amendment attribution. Combined molasses–tara treatments do not isolate microbial effects (Gosgot Angeles et al., 2024).	Contributions of microorganisms, added carbon and their interaction.	Use replicated factorial controls; quantify amendment volatile solids and distinguish inoculum from substrate contributions.	Prevents additional feed energy being misidentified as improved manure conversion.
Microbial selection and persistence. Bioaugmentation outcomes depend on inoculum and operating context (Alipoursarbani et al., 2026; Xu et al., 2023).	Functional suitability and persistence during sustained operation.	Select inocula for identified functional limitations; track introduced populations, viability and activity throughout operation.	Establishes culture-preparation requirements and the need for repeated dosing.
Monitoring and reporting. Gas correction, solids accounting and endpoint definitions affect interpretation (Holliger et al., 2021).	Comparable evidence of stable methane recovery and measurement uncertainty.	Integrate gas quantity and composition with pH, volatile fatty acids/alkalinity and ammonia; report calibration, blanks, positive controls, reference conditions, replication and endpoints.	Supports defensible methane-energy estimates and detection of unstable operation.
Seasonal resilience. Controlled-temperature co-digestion experiments demonstrate enhancement under specified laboratory conditions (Xu et al., 2023).	Performance under locally observed temperature fluctuations and feeding interruptions.	Test recorded seasonal temperature profiles and feeding disruptions; measure start-up, recovery and sustained production.	Identifies seasonal energy shortfalls and recovery requirements.
Carrier mechanisms. Biochar enhanced whey–manure co-digestion, but several mechanisms may contribute (Jaimes-Estévez et al., 2023; Zhao et al., 2024).	Relative contributions of conductivity, buffering and retention; carrier durability.	Compare appropriate carrier controls; assess mechanisms, reuse, material safety and local supply.	Tests whether production and replacement burdens are justified by useful energy gains.
Kinetic interpretation. Empirical kinetic fits describe production trajectories without establishing biological mechanisms (Wang & Guo, 2024).	Parameter reliability and biological interpretation when observations are incomplete.	Validate predictions against later observations; report uncertainty and connect fitted parameters with process chemistry and microbial evidence.	Improves forecasts of time to usable gas and sustained supply.
Useful energy delivery. Farm-scale co-digestion supplied cooking fuel in a rural school (Jaimes-Estévez et al., 2022).	Transferability to pastoral conditions, including NCA.	Assess cooking, boiler and combined heat and power options against local demand; pilot feasible routes and measure delivered service, storage reliability, maintenance and user experience.	Connects methane production to useful heat, electricity and fuel substitution.
Net energy balance. A conceptual dairy-manure plant allocates recovered heat to internal demands (Garkoti et al., 2024).	Incremental net-energy effects of adaptation and bioaugmentation.	Measure process and intervention inputs, methane losses and useful outputs over matched periods; report heat and electricity separately and avoid double-counting internal heat.	Establishes whether additional methane exceeds process and intervention energy requirements.
Economic and environmental performance. Plant economics depend on scale; East African programs identify cost and maintenance constraints (Clemens et al., 2018; Jalali et al., 2025).	Local cost per useful energy service and environmental outcome.	Use local prices and operating records; assess amendment, labor, water, downtime and replacement costs alongside methane leakage, displaced fuels and digestate management.	Tests affordability and environmental benefit against the existing energy service.

Note: NCA, Ngorongoro Conservation Area. Research priorities are proposed responses to limitations identified in the assembled evidence; they are not findings from an NCA field trial.

15. Conclusion

Cold operation slows and can disrupt the coordination of hydrolysis, syntrophic conversion and methanogenesis, delaying methane onset and increasing the risk of acid accumulation. Methane recovered

within a fixed period therefore reflects both substrate conversion and the time available for microbial adaptation; endpoint yield alone is insufficient to assess practical performance.

Cold-adapted inocula and targeted bioaugmentation can improve methane recovery and production rate under selected controlled conditions. Much of the direct evidence concerns laboratory co-digestion, limiting extrapolation to manure-only systems under seasonal field conditions. Higher methane recovery alone does not establish improved pH stability, methane fraction or persistence of introduced microorganisms.

Combined microbial and carbon amendments leave causal attribution unresolved when their effects are not separated. Replicated factorial comparisons, appropriate inoculum controls and explicit accounting for amendment volatile solids are needed to distinguish microbial effects, additional substrate contributions and interactions.

Reliable comparison requires stated temperature, substrate, inoculum, reactor operation, loading and the basis used to normalize methane yield, with gas volumes expressed at explicit reference conditions. Observed onset, recovery within a defined period and measured production rates should be distinguished from fitted lag, asymptotic potential and maximum rate. Kinetic models require adequate sampling and reported parameter uncertainty; their fitted parameters do not establish biological mechanisms.

The assembled evidence does not establish a net energy or economic advantage for bioaugmented, unheated systems treating cattle manure. Additional methane must be assessed against process and intervention inputs, gas losses and conversion performance. Assessments should quantify useful cooking or boiler heat, or the heat and electricity delivered by combined heat and power, with internal heat use accounted for before crediting energy supplied to users. For NCA, seasonal pilot trials should establish whether earlier methane availability produces sustained, affordable service under local resource and maintenance constraints. These measurements are necessary before laboratory enhancement can support a field recommendation.

Declaration of Ethical Standards

As the authors of this study, we declare that we comply with all ethical standards.

This article is a critical review of previously published literature and did not involve human participants, animals, or the collection of primary experimental data. Therefore, ethical approval and informed consent were not required. The authors declare that the preparation of this manuscript was conducted in accordance with accepted standards of research integrity, responsible authorship, and scholarly publication.

Credit Authorship Contribution Statement

A. Mashaka: Conceptualization, Methodology, Investigation, Literature review, Formal analysis, Data curation, Visualization and Review Writing.

R. Iddphonce: Methodology, Validation, Formal analysis, Writing – Review & Editing, Supervision.

A. Omari: Validation, Critical review, Writing – Review & Editing, Supervision.

All authors reviewed and approved the final version of the manuscript and agreed to be accountable for the integrity and accuracy of the work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests, personal relationships, or other conflicts of interest that could have influenced the work reported in this article.

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Data Availability

No new datasets were generated or analyzed specifically for this review article. All information synthesized in this study was obtained from previously published literature and publicly available sources cited in the manuscript.

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