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# Recent Advances and Prospects in Methane Production from Anaerobic Digestion: Process Intensification, Additives, and Biogas Upgrading

**Bonface O. Manono [1,*] and Felix Lamech Mogambi Ming'ate [2]**

[1] Engagement and Extension, Colorado State University, Fort Collins, CO 80253, USA
[2] Department of Environmental Studies and Community Development, Kenyatta University, Nairobi P.O. Box 43844-00100, Kenya; mingate.felix@ku.ac.ke
* Correspondence: bonface.manono@colostate.edu

**Abstract**

Anaerobic digestion (AD) plays an important role in the circular bioeconomy by converting organic waste into renewable methane and nutrient-rich fertilizer. However, consistent, high-quality biomethane production is hindered by four main factors: hydrolysis limitations, fluctuating feedstock quality, microbial instability, and the high cost/energy demand of purification. This review explores three key areas that improve biomethane production: (i) process intensification (pretreatments and advanced reactors), (ii) microbial regulation through additives, and (iii) biogas upgrading for pipeline use. Anaerobic digestion can be greatly improved by combining thermal or hybrid pretreatments, staged digestion, high-solids technology, and electrochemical systems. These methods speed up hydrolysis and help the system handle higher amounts of organic material more effectively. However, actual performance benefits depend on specific substrate characteristics, heat integration, and control complexity. Optimizing the C:N ratio, buffering capacity, and trace-element supplementation, while simultaneously diluting toxic inhibitors, makes co-digestion an effective and adaptable approach to enhancing anaerobic digestion processes. Additives like carbon, iron nanoparticles, enzymes, and buffers can optimize digestion, but their performance is highly dependent on dosage and substrate. Additionally, they lack validation in long-term, industrial-scale applications. Conventional physicochemical techniques continue to be standard for generating high-quality biomethane, but biological methanation and microalgal systems are playing a growing role in integrating Power-to-Gas technology and using $CO_2$ efficiently. Critical research needs to focus on four areas: (1) standardized reporting metrics, (2) AI-enabled monitoring and control, (3) coupled techno-economic and life-cycle analysis (TEA-LCA), and (4) long-term pilot or full-scale validation. Overall, comprehensive optimization of the entire flow is more effective than improving isolated parts.

**Keywords:** anaerobic digestion; methane; biomethane; co-digestion; direct interspecies electron transfer; process intensification; biogas upgrading; techno-economic analysis

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## 1. Introduction

Anaerobic digestion (AD) is a biologically mediated conversion process in which microorganisms decompose organic matter in the absence of oxygen to produce biogas and nutrient-rich digestate [1]. Although widely deployed, AD performance remains strongly shaped by substrate composition, microbial ecology, and reactor design, which sustains active innovation in process engineering and microbial management [2]. The AD pathway

proceeds through four tightly coupled stages, hydrolysis, acidogenesis, acetogenesis, and methanogenesis, whose kinetics and stability determine methane formation [3]. Hydrolysis is frequently the dominant bottleneck for lignocellulosic and some protein-rich substrates because structural recalcitrance restricts enzyme access and slows conversion to soluble intermediates [4]. Because intermediates produced in one phase propagate into the next, phase imbalance can rapidly translate into volatile fatty acid (VFA) accumulation, pH decline, and suppressed methanogenesis under high loading or heterogeneous feedstocks.

AD contributes to climate and circular-economy goals by capturing methane that would otherwise be released during unmanaged decomposition and by generating a renewable-energy carrier [5,6]. Raw biogas typically contains 55–65% methane and 35–45% $CO_2$ with trace contaminants (e.g., $H_2S$ and moisture) that constrain direct use [7,8]. Upgrading to biomethane (>95% methane) broadens end-use options, enabling grid injection and transport fuels with potential emissions benefits relative to diesel [8,9]. In parallel, digestate can support nutrient recovery and reduce reliance on synthetic fertilizers when appropriately managed [10].

While promising, practical methane output is often limited by the slow hydrolysis of complex biomass, system instability due to VFA and ammonia buildup, and the technical demands of refining raw biogas into market-grade biomethane [7,11–13]. These constraints frequently interact, which is why improvements reported for single interventions may not transfer unless reactor conditions, microbial ecology, and gas handling are considered jointly. Accordingly, this review synthesizes recent advances in methane production from AD with a focus on process intensification, additive-enabled microbial/process regulation, and biogas upgrading technologies. It examines how pretreatment, co-digestion, and reactor design improve hydrolysis and process stability. It evaluates additives (e.g., direct interspecies electron transfer (DIET)-promoting materials) for specific control mechanisms and positions upgrade options within the context of operational performance and feasibility constraints.

## 2. Review Methods, Search Strategy and Scope

This structured review examines recent advances that directly improve methane production performance and biomethane quality in AD systems. The scope covers five tightly connected domains: (a) process intensification and pretreatment, (b) co-digestion and reactor design, (c) additives and conductive materials, (d) microbial monitoring and process control, and (e) biogas upgrading and techno-economic feasibility. To strengthen reproducibility, the review was organized as a structured scoping synthesis rather than a PRISMA-style systematic review or meta-analysis. Literature discovery focused primarily on peer-reviewed studies indexed through major scientific databases and citation tracking, with emphasis on work published from 2020 to March 2026 while retaining earlier foundational studies where necessary for mechanistic or technological context.

Search combinations paired core terms such as “anaerobic digestion”, “biogas”, and “biomethane” with topic-specific terms including pretreatment, co-digestion, reactor design, DIET, conductive materials, microbial community, metagenomics, ADM1, machine learning, biogas upgrading, techno-economic analysis, and life-cycle assessment. Studies were prioritized when they reported enough process detail to support interpretation, including feedstock type, reactor configuration, operating conditions, and at least one deployable outcome such as methane yield or rate, methane purity, VFA behavior, pH, ammonia, sulfide, microbial shifts, or techno-economic or environmental indicators. Studies focused only on hydrogen or volatile fatty acid production without a methane endpoint, reports with insufficient operating detail, and duplicate summaries of the same dataset were deprioritized. Review articles were retained when they offered mechanistic synthesis or

comparative framing for fast-moving topics such as DIET-promoting additives, biological methanation, and AI-assisted process optimization [14–16].

Because AD studies differ substantially in substrate type, inoculum, reactor configuration, scale, and reporting metrics, the review uses a comparative synthesis rather than a pooled meta-analysis. Where studies allowed, comparisons were interpreted against decision-relevant variables such as solids content, organic loading rate, hydraulic retention time, methane concentration, methane slip, ammonia or sulfide pressure, and the presence or absence of heat or energy integration. Evidence was weighed qualitatively based on its transferability to real-world applications. High-confidence evidence included continuous, pilot-scale, long-duration, and system-integration studies. Batch and BMP studies were used primarily to clarify mechanisms, while emerging topics lacking long-term validation were classified as promising, lower-TRL (technology readiness level) pathways rather than mature best practices. The review therefore emphasizes patterns, contradictions, semi-quantitative performance ranges, and boundary conditions rather than single best-technology conclusions. Figure 1 provides a conceptual overview of the AD stages and the intervention points synthesized in this review.

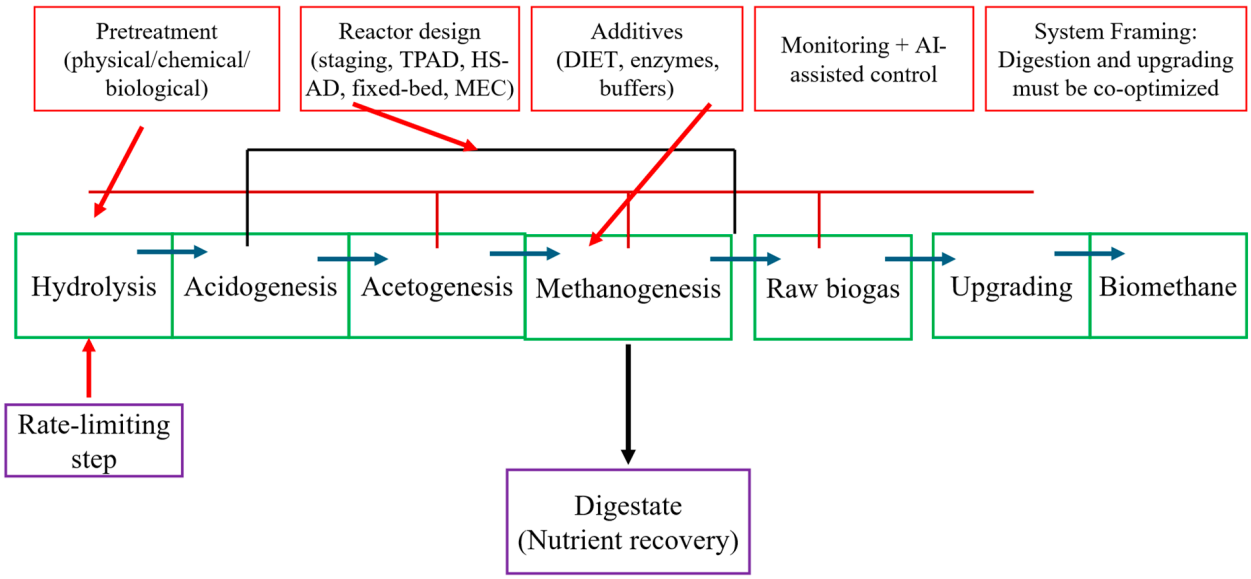



**Figure 1.** Conceptual overview of the AD process showing hydrolysis, acidogenesis, acetogenesis, and methanogenesis as sequential but tightly coupled stages, with hydrolysis highlighted as the dominant kinetic bottleneck for complex feedstocks. The figure identifies the major intervention points synthesized in this review—pretreatment, additives, reactor staging, monitoring, and upgrading—and frames AD as an integrated process train.

## 3. Strategies for Process Intensification and Enhanced Methane Yield

Process intensification in AD has shifted from isolated pretreatment experiments toward integrated strategies that co-optimize substrate accessibility, reactor stability, and methane conversion efficiency [17,18]. Recent studies show that successful interventions do more than increase biodegradability; they stabilize processes by smoothing phase imbalances, enhancing microbial retention, or improving feedstock compatibility. Accordingly, intensification claims must be interpreted within explicit boundary conditions including substrate recalcitrance, solids content, loading rate, retention time, inhibitor burden, heat-integration options, and operator control capacity. Technologies that outperform a baseline in BMP or short-batch tests do not automatically deliver equivalent gains at continuous or industrial scale. This section therefore organizes intensification strategies by mech-

anism, transferability, and operational boundary conditions rather than by technology novelty alone.

### *3.1. Advancements in Physical, Chemical, and Biological Methods for Breaking Down Complex Organic Matter*

Physical pretreatments are essential for overcoming hydrolysis limitations by increasing substrate accessibility through size reduction. However, mechanical methods like milling or grinding offer limited benefits unless paired with thermal or chemical conditioning [19]. Thermal hydrolysis is highly effective for sludge and food waste, as it breaks down cellular structures and increases solubilization. This pretreatment reduces lag phases and boosts methane production [20–22]. However, thermal intensification is only advantageous when heat integration and solids handling are well designed.

Chemical pretreatments provide stronger structural disruption for recalcitrant substrates, particularly lignocellulosic residues. While alkali and acid treatments increase cellulose accessibility by removing partial lignin and hemicellulose, oxidants like ozonation and $KMnO_4$ improve sludge disintegration and speed up hydrolysis [23,24]. The critical limitation is not proof of biochemical benefit, but cost, chemical recovery, and compatibility with downstream biology under continuous operation.

Biological pretreatments and bioaugmentation strategies operate at milder conditions and align more naturally with AD microbiology. Enzyme additives (e.g., cellulase and amylase) often improve methane yield in cellulose-rich feedstocks, but results vary strongly with substrate composition, pH, exposure time, and pretreatment sequence [25,26]. Bioaugmentation can improve resilience or accelerate selected conversion steps, but reproducibility remains context-dependent because introduced strains must compete within established consortia [27]. The literature indicates that hybrid pretreatment design is the most adaptable practice. Combining moderate physical disruption with targeted chemical or enzymatic conditioning often outperforms severe, single-stage pretreatment. This approach boosts hydrolysis efficiency while minimizing chemical consumption and energy use [28]. Table 1 provides a comparative summary of major intensification pathways and their boundary conditions.

**Table 1.** Process intensification methods in anaerobic digestion.

| Method Category | Representative Techniques | Primary Mechanism | Main Performance Effects | Key Limitations/ Implementation Notes | References |
|---|---|---|---|---|---|
| Physical | Milling, grinding; thermal hydrolysis; hydrothermal subcritical water; ultrasound | Increases surface area, cell disruption, solubilization | Faster hydrolysis, improved biodegradability, higher methane yield/rate | Energy demand can offset gains, especially for ultrasound; thermal systems require heat integration and solids management | [20–22,29] |
| Chemical | Alkali/acid pretreatment (e.g., NaOH); ozonation; $KMnO_4$ | Lignocellulose deconstruction, floc disintegration, oxidation of recalcitrant fractions | Improved substrate accessibility and hydrolysis; increased methane production in sludge and lignocellulosic systems | Reagent cost, corrosion, residual chemical handling, and process compatibility at full scale | [23,24] |
| Biological | Enzyme additives (cellulase, amylase); bioaugmentation | Enzymatic depolymerization and targeted microbial function enhancement | Hydrolysis acceleration and improved methane yield, especially for carbohydrate-rich substrates | Feedstock-specific effectiveness; enzyme cost and dosing optimization required | [25,27] |

**Table 1.** *Cont.*

| Method Category | Representative Techniques | Primary Mechanism | Main Performance Effects | Key Limitations/ Implementation Notes | References |
|---|---|---|---|---|---|
| Hybrid | Combined physical–chemical–biological approaches | Multi-mechanism substrate conditioning | Can maximize degradability and yield while improving process robustness | Requires careful techno-economic optimization; operational complexity increases | [28] |

### *3.2. Synergistic Benefits of Co-Digestion*

Co-digestion is a powerful way to enhance anaerobic digestion because it can address several constraints simultaneously: it corrects nutrient imbalances, improves alkalinity and pH buffering, provides essential trace elements, and dilutes inhibitory compounds [30,31]. Reported methane gains are often large, but direct comparison across studies is difficult because baseline mono-digestion conditions vary widely. The more transferable insight is mechanistic: co-digestion works when mixing shifts the composite feed toward operating windows that better match microbial demand, for example by moving the C:N ratio toward approximately 20–30:1, improving alkalinity, increasing micronutrient supply, and reducing the effective burden of free ammonia or other inhibitors per unit reactor volume [11,32–34]. Food waste and manure combinations illustrate this clearly. Food waste provides high biodegradability and carbon availability, while manure improves buffering capacity, contributes micronutrients, and often stabilizes microbial activity [32–34]. Similar logic applies to microalgae with carbon-rich co-substrates and to sludge fractions paired with fruit waste, where co-substrates improve pH behavior and VFA conversion [35–37].For optimal anaerobic digestion of lignocellulosic residues, co-digestion with manure or sludge is highly effective because it provides buffering, inoculation, and dilution of inhibitory intermediates [38]. However, a pretreatment step is still required to break down the complex, recalcitrant structure of many crop residues [39]. This reinforces a broader point: co-digestion is not a universal substitute for pretreatment, but a compatibility tool that improves biochemical resilience when blending is matched to substrate chemistry and reactor constraints. A persistent gap is that many co-digestion studies optimize methane yield while underreporting logistical constraints such as seasonal feedstock availability, preprocessing requirements, transport distance, and mixture-specific ammonia or long chain fatty acid (LCFA) risk. These factors often determine whether co-digestion remains advantageous outside laboratory or pilot settings. Representative co-digestion pairings and reported outcomes are synthesized in Table 2.

**Table 2.** Co-digestion feedstock combinations and outcomes.

| Feedstock Combination | Typical Rationale | Reported Outcomes | Boundary Conditions/Notes | References |
|---|---|---|---|---|
| Food waste + animal manure | Balance C:N ratio, improve buffering, dilute inhibitors | Higher methane yield, improved vs. removal, more stable operation | Highly dependent on mixing ratio and loading rate; target C:N is typically 20–30:1 | [32–34,40] |
| Microalgae + organic wastes (e.g., potato waste, glycerol) | Correct unfavorable microalgal C:N and improve biodegradability | Specific methane yield improvements reported (53–128%) and improved economics | Microalgal digestibility and pretreatment requirements remain important | [35] |
| Lignocellulosic residues + manure | Add buffering/nutrients to fibrous substrates; improve inoculation and dilution | Methane yield increases, especially when residues are pretreated | Pretreatment is often necessary for consistent gains | [39] |
| Slaughterhouse/poultry-related streams + sludge | Reduce instability and manage high-strength wastes | Higher methane production and improved stability at optimized ratios | Ammonia risk and protein-derived inhibition require close control | [11] |

**Table 2.** *Cont.*

| Feedstock Combination | Typical Rationale | Reported Outcomes | Boundary Conditions/Notes | References |
|---|---|---|---|---|
| Food waste + crude glycerol | Add concentrated carbon source; improve gas yield | Enhanced biogas production potential | Glycerol dosing must be controlled to avoid acidification | [36] |
| Sewage sludge fractions + fruit waste | Use carbohydrate-rich co-substrate to improve VFA conversion and pH behavior | Large biogas increases reported in optimized systems | pH control and alkalinity management remain critical | [37] |

### *3.3. Novel Reactor Designs and Integrated Systems*

Reactor innovation now focuses less on residence time alone and more on phase management, microbial habitat design, and controllability. Two-stage AD systems decouple acidogenesis from methanogenesis so each phase can operate under more favorable conditions, which often improves stability and methane quality as much as it improves yield [1,24,41]. Temperature-phased anaerobic digestion (TPAD) works well for feedstocks that acidify quickly. It uses two temperature stages to balance the process: a high-heat thermophilic first stage to speed up breakdown, followed by a cooler mesophilic second stage to keep methane production stable [22,42]. TPAD is particularly attractive where pathogen reduction and sludge stabilization are operational priorities, but its benefits depend on thermal integration and operator capability.

Other configurations including fixed-bed systems, high-solid AD (HS-AD), and microbial electrochemical systems reflect a broader, strategic move toward improving microbial retention and enhancing electron transfer [43–45]. While these systems improve methane yield and loading capacity, they also introduce greater operational complexity. In practice, reactor intensification should be selected based on the plant's dominant constraint (hydrolysis, instability, water use, or gas upgrading integration), not on laboratory performance alone. These intensification mechanisms and their interactions are summarized in Figure 2, while key reactor configurations and deployment implications are compared in Table 3.

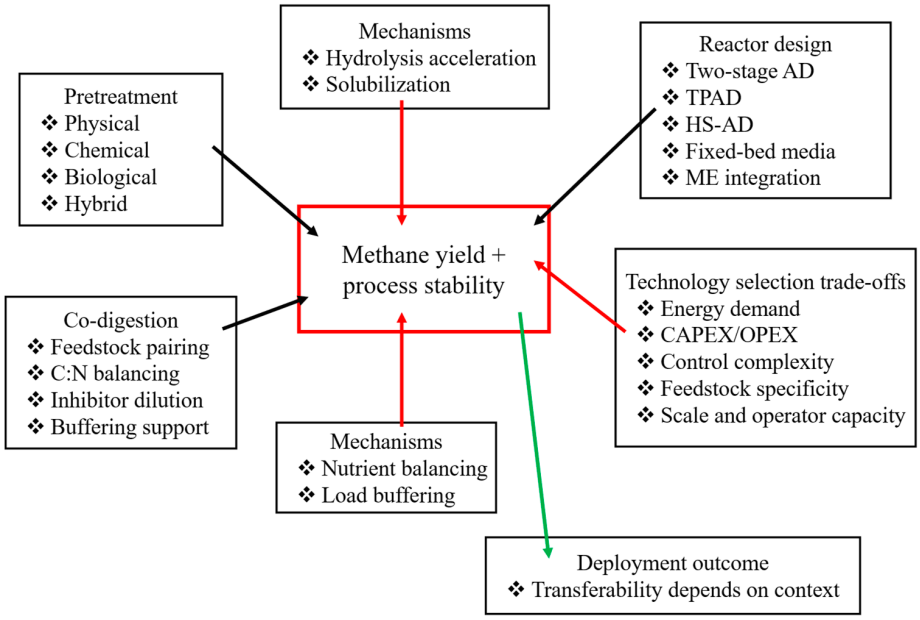



**Figure 2.** Process intensification framework for enhanced methane production. The integrated schematic diagram shows how pretreatment, co-digestion, and advanced reactor configurations act through different mechanisms (hydrolysis acceleration, nutrient balancing, phase separation, biomass retention, electron-transfer enhancement) to improve methane yield and process stability. The figure emphasizes that intensification outcomes are moderated by energy demand, CAPEX/OPEX, and site-specific operating constraints.

**Table 3.** Reactor designs and performance implications in anaerobic digestion.

| Reactor Design | Core Design Principle | Typical Benefits | Main Constraints/ Deployment Considerations | References |
|---|---|---|---|---|
| Two-stage AD | Separates acidogenesis and methanogenesis into distinct reactors | Improved stability, better phase-specific control, higher biogas quality/yield potential, possible $H_2$ + $CH_4$ integration | Higher capital and control complexity; requires stable inter-stage management | [1,24,40,41] |
| TPAD | Thermophilic front-end followed by mesophilic methanogenic stage | Enhanced hydrolysis, pathogen reduction, improved solids destruction, more stable methanogenesis than fully thermophilic systems | Thermal integration and energy demand are critical | [22,42] |
| Fixed-bed/media-supported reactors | Packing media improve biomass retention and local microbial density | Higher COD removal, improved stability, greater loading tolerance | Media fouling/clogging and replacement cost | [43] |
| HS-AD | Operates at elevated solids to reduce water use and reactor volume | Lower water demand, compact footprint, strong performance for dry residues | Mixing and mass-transfer constraints; local inhibition risk | [22,46,47] |
| Microbial electrochemical integration | Electrochemical stimulation of microbial pathways and electron transfer | Faster methane production, improved microbial enrichment, possible $CO_2$-to-$CH_4$ support | Electrical infrastructure and scale-up uncertainty | [44,45,48,49] |

## 4. Role of Additives and Conductive Materials

In AD, additives have shifted from general supplements to targeted process-control tools. Recent research shows that specific additive classes map to distinct functional improvements: conductive materials for DIET and syntrophic efficiency, enzymes for accelerated hydrolysis, and buffering/trace-element systems for microbial stability [50,51]. This mechanistic framing is important because additive performance is rarely universal. Reported gains depend on substrate composition, baseline instability, additive dosage, and operating regime.

### *4.1. Nanoparticles and Nano-Catalysts*

The addition of conductive materials, including biochar, graphene, and $Fe_3O_4$ nanoparticles, significantly enhances anaerobic digestion by promoting direct interspecies electron transfer (DIET) between syntrophic bacteria and methanogenic archaea [14,15,52]. In many systems, DIET reduces reliance on diffusional hydrogen transfer and can accelerate methanogenic conversion, particularly under stressed or high-loading conditions. Biochar is especially versatile because it combines conductivity, pH buffering, and microbial attachment surfaces [53]. However, DIET-promoting materials should currently be classified as emerging, lower-TRL intensification tools rather than as mature plant-wide strategies. Most evidence comes from batch or short-duration reactors, where changes in VFA turnover, lag phase, and methane rate are easier to observe than long-term operational reliability. While research highlights increased methane yields and hydrogenotrophic methanogen enrichment, transitioning these additives to real-world applications is limited by several factors. Specifically, unresolved issues with additive longevity, accumulation in digestate, poor recoverability, material costs, and sensitivity to mixed-feedstock variability impede industrial adoption [31,54–56]. In practice, DIET-oriented additives are best viewed as promising control tools for specific instability or high-loading scenarios pending long-duration pilot and full-scale validation.

### *4.2. Enzyme Additives*

Enzyme additives directly target the hydrolysis bottleneck and are most useful where the dominant substrate polymers are known. Cellulase and amylase additions can improve

hydrolysis and methane yield in cellulose-rich materials such as crop residues [25,26]. Their strongest contribution is often not a dramatic absolute yield increase but a reduction in lag time and improved process predictability. However, enzyme-based intensification is sensitive to operational conditions, especially pH, temperature, retention time, and substrate accessibility [57]. In mixed waste systems, enzyme benefits may be limited unless the substrate has already been physically or chemically preconditioned [58]. This is why enzyme addition is often more effective within a staged intensification strategy than as a standalone intervention [59,60].

### *4.3. pH and Nutrient Regulation*

Buffering systems and trace-element supplementation remain among the most practical additive strategies because they address instability directly. Ash, biochar, and phosphate buffers can stabilize pH in reactors exposed to VFA accumulation, while trace elements such as Fe, Co, and Ni support enzymatic pathways required for methanogenesis [51,61,62]. Biochar and ash are particularly attractive because they can provide simultaneous buffering, adsorption, and sulfur-management effects; some studies also report lower $H_2S$ and a shift toward methanogenic dominance over sulfate-reducing populations [63]. Nonetheless, trace-element dosing windows are narrow and site-specific, and excessive supplementation can create toxicity or precipitation problems. To optimize anaerobic digestion, additives should not be selected arbitrarily, but rather to address the specific bottleneck or failure mode dominating the plant. In anaerobic digestion, conductive materials best address electron-transfer bottlenecks, enzymes combat rate-limiting hydrolysis, and buffers or trace elements resolve pH and micronutrient instability [64]. The dominant additive-mediated mechanisms are illustrated in Figure 3, and representative additive classes, effects, and caveats are summarized in Table 4.

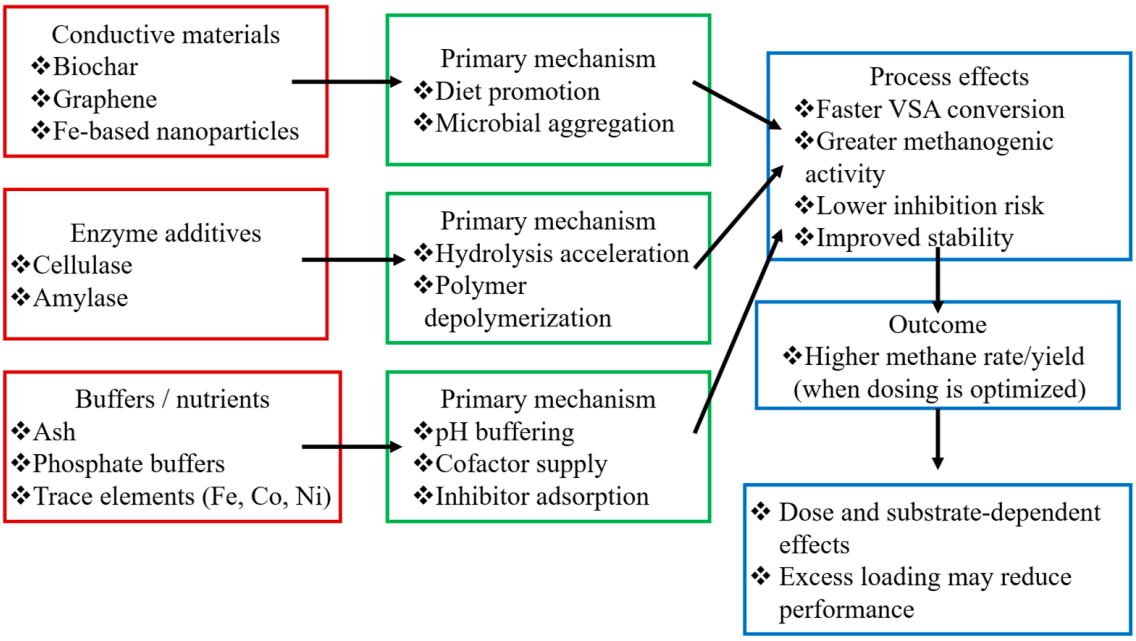



**Figure 3.** Additive-mediated mechanisms enhancing methane production in anaerobic digestion. Mechanistic schematic linking conductive materials, enzyme additives, and buffering/trace-element systems to their primary process effects (DIET promotion, hydrolysis acceleration, and pH/nutrient stabilization, respectively). The figure also highlights that methane gains are conditional on dose, substrate composition, and reactor operating conditions.

**Table 4.** Additives and their effects in anaerobic digestion.

| Additive Class | Examples | Dominant Mechanism | Typical Benefits | Key Caveats | References |
|---|---|---|---|---|---|
| Conductive nanoparticles/carbonaceous materials | Biochar, graphene, $Fe_3O_4$ nanoparticles, nano-magnetite, graphite powder | DIET promotion, microbial aggregation, electron-transfer facilitation | Higher methane rate/yield, improved VFA conversion, enhanced microbial/enzyme activity | Dose-sensitive; excessive loading can inhibit activity or reduce diversity | [14,15,31,52,54,56] |
| Enzyme additives | Cellulase, amylase | Hydrolysis acceleration via polymer depolymerization | Improved biodegradability and methane yield in carbohydrate-rich substrates | Performance depends on substrate composition, pretreatment status, and operating conditions | [25,26] |
| pH and nutrient regulators | Ash, biochar, $K_2HPO_4/KH_2PO_4$, trace elements (Fe, Co, Ni) | Buffering, micronutrient supply, inhibitory compound adsorption | Stabilized pH, lower VFA stress, improved methanogenic activity; possible $H_2S$ reduction | Requires site-specific dosing; excess trace metals may cause toxicity or precipitation | [24,61–63] |

## 5. Innovations in Biogas Upgrading to Biomethane

Biogas upgrading to biomethane is now a primary design factor for anaerobic digestion systems rather than an afterthought. Because methane purity, methane recovery, slip, impurity tolerance, and energy demand jointly shape both profitability and environmental performance, upgrading must be integrated into core process strategy rather than optimized as a separate end step [7,8]. Recent innovation is best interpreted as a shift from single-unit upgrading toward integrated gas-management strategies that account for impurities, $CO_2$ valorization, and energy coupling. Accordingly, technology selection should be based on a multi-criteria screening framework that considers methane purity, recovery/slip, specific energy burden, pretreatment requirements, scale fit, and $CO_2$ valorization potential rather than purity alone.

### *5.1. Physicochemical Upgrading Methods*

Physicochemical upgrading remains the most mature route for achieving pipeline-grade biomethane. Due to its modular and scalable nature, membrane separation is a preferred technology. Recent advancements are maximizing efficiency through multi-stage trains and enhanced materials, which minimize methane leakage and improve separation selectivity [65–67]. The primary operational challenge in biogas upgrading is its sensitivity to impurities, specifically $H_2S$ and siloxanes, which necessitate extensive, costly pretreatment [68,69]. Cryogenic separation delivers very high methane purity and a concentrated $CO_2$ stream, making it attractive where $CO_2$ utilization is part of the business model [70,71]. However, its energy intensity and capital cost usually limit application to larger plants or specialized contexts.

Water scrubbing remains relevant because it is robust and comparatively simple, especially for small- to medium-scale plants [72–74]. Chemical absorption systems (e.g., amines) can achieve even higher methane purity, but solvent regeneration energy and chemical handling increase complexity [75,76]. Across these options, methane purity alone is an incomplete comparison metric; methane recovery, slip, impurity tolerance, and specific energy demand are more useful for technology selection.

### *5.2. Biological Biogas Upgrading*

Biological upgrading is important because it converts $CO_2$ to methane instead of only separating it. In in situ biological methanation, hydrogen is injected directly into the digester and hydrogenotrophic methanogens convert $CO_2$ to $CH_4$ [77,78]. This can raise methane concentration without a separate upgrading reactor, but gas–liquid mass transfer,

pH shifts, and $H_2$ control remain difficult under continuously changing digester conditions. Ex situ biological methanation addresses these constraints by separating methanation into a dedicated reactor supplied with $H_2$ and biogas or $CO_2$ [79,80]. This improves control and often stabilizes conversion efficiency, but it adds reactor infrastructure and depends on reliable $H_2$ supply. Microalgae-based upgrading takes a different pathway by using photosynthesis for $CO_2$ removal while generating biomass, but performance is highly sensitive to light, temperature, and harvesting logistics [8,81]. Biological upgrading is therefore best viewed as a carbon-management strategy linked to renewable hydrogen and co-product pathways, not simply as an alternative separator.

### *5.3. Hybrid and Integrated Systems*

Hybrid upgrading systems combine multiple technologies to maximize methane recovery and overcome the limitations of individual methods [82,83]. Examples include membrane pre-concentration followed by scrubbing or biological polishing, and integrated trains that coordinate upgrading with renewable heat and electricity supply [84–86]. Upgrading to hybrid anaerobic digestion can enhance environmental performance, but it also requires sophisticated, integrated control systems and greater initial capital investment [87]. Modern hybrid anaerobic digestion focuses on optimizing the entire plant by linking biomethane production, $CO_2$ valorization, and digestate management into a cohesive, circular value chain [88]. This approach is expected to lead future commercial adoption, as it connects upgrade decisions directly with local energy markets, hydrogen availability, and opportunities for carbon utilization. A comparative map of upgrading pathways is provided in Figure 4, and key performance characteristics across technologies are summarized in Table 5.

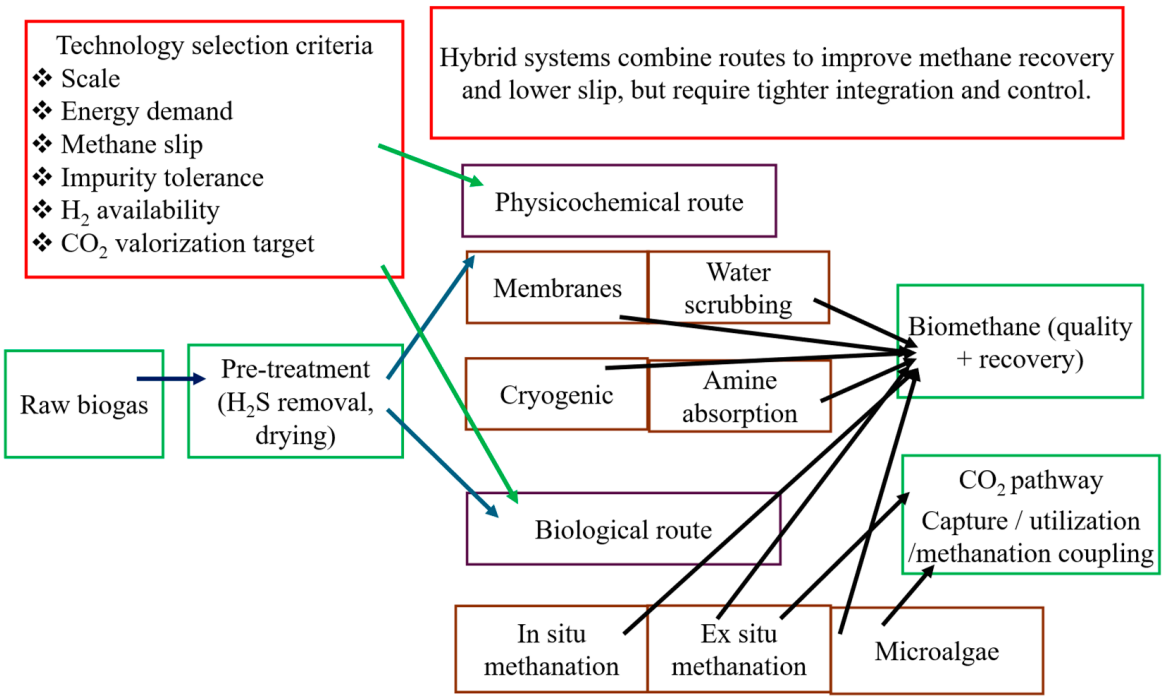



**Figure 4.** Biogas upgrading pathways from raw biogas to biomethane. Comparative process schematic showing pretreatment, physicochemical upgrading routes (membrane, cryogenic, water scrubbing, chemical absorption), and biological routes (in situ/ex situ methanation and microalgae), converging on biomethane production and $CO_2$ handling options. The figure emphasizes selection criteria such as plant scale, energy demand, impurity tolerance, methane slip, and $CO_2$ valorization potential.

**Table 5.** Biogas upgrading technologies summarized as a multi-criteria screening matrix for methane purity, scale fit, impurity tolerance, methane slip or energy burden, and $CO_2$ valorization potential.

| Technology Class | Method | Operating Principle | Typical Methane Purity | Key Strengths | Main Limitations | References |
|---|---|---|---|---|---|---|
| Physicochemical | Membrane separation | Selective permeation of $CO_2$ and other gases through membranes | Up to >95% | Modular, scalable, continuous operation, strong $CO_2$ removal | Sensitive to impurities; fouling- and pressure-related energy demand | [13,65–67] |
| Physicochemical | Cryogenic separation | Cooling to liquefy/solidify $CO_2$ and impurities | Very high purity (>95%) | High biomethane purity, concentrated $CO_2$ stream, low methane slip | High CAPEX/OPEX and process complexity) | [70,71] |
| Physicochemical | Water scrubbing | Dissolution of $CO_2$ and $H_2S$ in water under pressure | ~95% | Robust, relatively simple, suitable for small/medium plants | Water use, regeneration energy, methane dissolution losses | [72–74] |
| Physicochemical | Chemical absorption (amines) | Selective solvent absorption and thermal regeneration | Up to >99% | High purity and effective $CO_2/H_2S$ removal | Solvent regeneration energy, corrosion, solvent management | [75,76] |
| Biological | In situ methanation | $H_2$ injected into digester; methanogens convert $CO_2$ to $CH_4$ | Up to ~90% in some cases | $CO_2$ conversion within digester, potentially lower equipment cost | Gas transfer and pH control challenges; difficult co-optimization | [7,77] |
| Biological | Ex situ methanation | Separate methanation reactor with $H_2$ and biogas/$CO_2$ | ~80–90% | Better control and stable conversion performance | Additional reactor and $H_2$ infrastructure increase complexity/cost | [79,80] |
| Biological | Microalgae photobioreactors | Photosynthetic $CO_2$ uptake with biomass production | Enhanced gas quality | $CO_2$ removal with biomass co-product; potential $H_2S$ co-removal | Land/lighting requirements, climate sensitivity, harvesting cost | [8,81] |
| Hybrid/Integrated | PVT-coupled or multi-method trains | Combines upgrading methods and renewable heat/electricity supply | Often >95% | Improved energy efficiency, reduced carbon footprint, system flexibility | Higher upfront cost and control-system complexity | [84–86] |

## 6. Microbial Community Dynamics and Process Monitoring

### *6.1. Microbial Community Dynamics and Methane Production*

AD performance is fundamentally driven by microbial interactions, which are shaped by engineering conditions [89,90]. Hydrolytic, acidogenic, acetogenic, and methanogenic guilds must remain functionally coupled for methane production to be stable [91,92]. *Methanosarcina* is frequently associated with resilient systems because of metabolic flexibility, while *Methanobacterium* and related *hydrogenotrophs* often dominate when hydrogen transfer or DIET-linked pathways are favored [48,93–95]. Microbial community composition responds strongly to pH, temperature, loading rate, substrate type, trace-element availability, and inhibitor exposure [96–99]. This means microbial analysis is most informative when interpreted together with reactor kinetics and chemistry. Community data alone can suggest mechanisms, but causal inference requires coupling sequence-based diagnostics with methane rates, VFAs, pH, total/free ammonia nitrogen (TAN/FAN), sulfide, and gas composition.

Three classes of inhibitors warrant consideration, as they consistently influence methanogenic failure or alterations in metabolic pathways. First, total ammonia nitrogen and especially free ammonia can suppress acetoclastic methanogens, alter electron flow, and select for more ammonia-tolerant syntrophic or hydrogenotrophic consortia [11,12]. Second, sulfide or $H_2S$ can impair sensitive populations and change competition between methanogens and sulfur-cycling microorganisms, particularly in sulfur-rich feedstocks or sulfate-bearing wastewaters [51,63,91]. Third, substantial lipid loading and the buildup of long-chain fatty acids may adhere to biomass, impede mass-transfer processes, and extend lag phases, even though fats possess considerable theoretical potential for methane production [91]. These inhibitors are therefore not secondary operational details; they help determine whether intensified systems remain stable under higher loading and more heterogeneous feedstocks.

Recent microbial analyses are also moving beyond 16S-only descriptions. Metagenomic, metatranscriptomic, and broader multi-omics approaches increasingly link taxonomic shifts to functional genes, stress responses, and pathway re-routing, especially under ammonia stress or during process perturbation [12,91]. Their current value is strongest in mechanistic diagnosis, inoculum selection, and additive assessment, while routine deployment in full-scale plants remains constrained by sampling cost, turnaround time, and bioinformatic standardization.

### *6.2. Advanced Monitoring, ADM1, and Data-Driven Control*

Monitoring is moving from periodic measurement toward continuous, model-informed control. Routine parameters such as pH, VFAs, alkalinity, gas composition, and temperature remain essential, but their value increases when paired with soft sensors, mechanistic models such as ADM1, and automated anomaly detection [100,101]. In practice, ADM1-type frameworks help reconcile sensor signals with state variables that are difficult to measure directly, including latent overloading, alkalinity stress, and changing substrate-conversion dynamics. This provides a biochemical backbone for control decisions rather than relying only on empirical trend interpretation. 16S rRNA-based microbial profiling has improved mechanistic understanding and can support troubleshooting, especially when evaluating additives, inocula, or process transitions [81,102,103]. However, routine plant use remains limited by cost and turnaround time, which is why soft-sensor and biosensor approaches remain especially promising for near-real-time estimation of hidden variables or toxicity risk [15,104,105].

Machine learning, particularly Artificial Neural Networks (ANNs), Long Short-Term Memory (LSTM) models, and hybrid ML–mechanistic approaches, can improve yield prediction, fault detection, virtual sensing, and set-point optimization when sufficiently large, high-frequency, quality-controlled datasets are available [16,106–108]. However, plant-level usefulness depends on more than predictive accuracy. Robust deployment requires attention to sensor drift, missing data, narrow training domains, overfitting, interpretability, and the need to update models as feedstocks and operating regimes change [106–109]. The most credible near-term pathway is therefore not black-box replacement of process understanding, but hybrid control in which ML complements ADM1 or related biochemical models for forecasting, early warning, and operator decision support. Figure 5 summarizes the microbial interaction network and the main monitoring/control levers discussed.

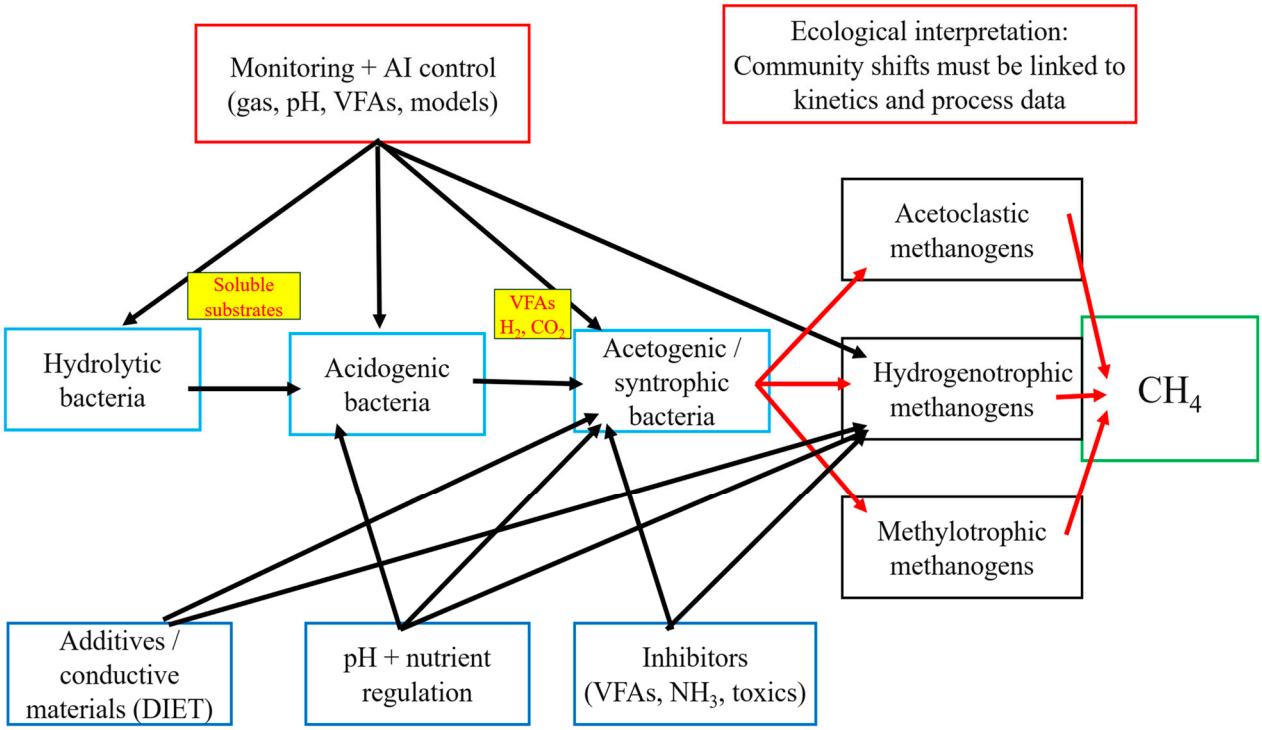



**Figure 5.** Microbial community interactions and process control in anaerobic digestion. Ecological-process schematic showing coupling among hydrolytic, acidogenic, acetogenic, and methanogenic guilds, with methanogenesis differentiated into acetoclastic, hydrogenotrophic, and methylotrophic pathways. The figure also situates additives, inhibitors, and monitoring/AI control as regulators of community function and methane kinetics.

## 7. Challenges, Prospects, and Economic Feasibility

### *7.1. Current Limitations to Large-Scale Implementation*

The primary challenge for AD is not lab-scale efficiency, but ensuring consistent, economic performance in real-world, variable-feedstock applications [110–112]. Capital Expenditure (CAPEX) remains a major barrier when pretreatment, advanced reactors, and upgrading are combined, particularly in farm-scale and decentralized systems [111,113]. Operational complexity is equally important. Intensified systems increase the number of interacting control variables, which narrows the margin for error and raises demands on operator expertise [2,110,114]. As a result, technologies that perform well in pilot studies may underperform when maintenance, staffing, or feedstock quality management is inconsistent [115]. Process instability remains a persistent limitation, especially under variable loading, ammonia stress, or poor feedstock conditioning [12,116,117]. This underscores a core point of this review: future performance gains will depend as much on control architecture and monitoring as on any single pretreatment, additive, or reactor innovation.

### *7.2. Future Research Directions*

Future AD research is moving toward integrated, digitally enabled process optimization, but the immediate goal should be validated hybrid control rather than algorithm proliferation. AI and machine learning will be most useful where they are linked to interpretable features, mechanistic models, and plant-relevant datasets rather than deployed as isolated black boxes [106,107,118,119]. A second priority is truly coupled techno-economic analysis (TEA) with life-cycle assessment (LCA). Performance gains from severe thermal or oxidative pretreatment, specialized additives, or $H_2$-dependent upgrading are not automatically beneficial once parasitic energy demand, material-input burdens, methane slip,

and capital intensity are counted [29,85,118,120]. For example, pretreatments that improve methane yield can still worsen net energy or climate performance when waste-heat recovery is absent, and biological methanation can become environmentally or economically unattractive when $H_2$ is not both low-carbon and reliably available. A third priority is biorefinery integration, where biomethane is co-optimized with digestate valorization, $CO_2$ conversion, and other product streams [121,122]. In this framework, profitability depends on value stacking rather than methane yield alone, and future claims of superiority should increasingly be supported by long-duration pilot or full-scale validation.

### *7.3. Economic Analysis and Feasibility Pathways*

Economic viability in AD increasingly depends on portfolio economics. Biomethane revenue remains central, but digestate valorization, $CO_2$ utilization, tipping fees, and policy incentives are often decisive for project bankability [10,123,124]. Digestate is particularly important because it links AD to agricultural nutrient management and can create stable local value if product quality is standardized [125]. Policy and market design strongly influence outcomes. Feed-in tariffs, renewable-gas standards, carbon pricing, and permitting frameworks frequently determine whether technically viable projects are financially attractive [121,126]. This means TEA results are not universally transferable without policy context.

A strong synthesis across economic studies is that AD should be evaluated as an integrated waste–energy–nutrient–carbon system. Projects that combine reliable methane recovery with digestate and $CO_2$ valorization often outperform simpler waste-treatment alternatives, but only when feedstock logistics, uptime, and local market conditions are explicitly considered. Integrated biogas projects which combine reliable methane recovery with digestate and $CO_2$ valorization often outperform simpler waste-treatment methods. However, their success hinges on careful planning regarding feedstock logistics, system uptime, and local market conditions [127,128]. Figure 6 summarizes major cost and value pathways, and Table 6 synthesizes economic findings, risks, and improvement strategies for intensified AD systems.

**Table 6.** Economic analysis of anaerobic digestion innovations.

| Economic Domain | Key Findings from Literature | Main Risk Factors | Improvement Strategies | References |
|---|---|---|---|---|
| Capital costs (CAPEX) | Advanced pretreatment, upgrading, and staged/specialized reactors increase upfront cost; farm-scale plants can require high initial investment | Long payback periods, financing barriers, small-scale economics | Modular designs, phased deployment, policy support (subsidies/grants), and design simplification | [65,110,111,113,121,126] |
| Operating costs (OPEX) | Energy-intensive pretreatments and upgrading increase OPEX; skilled labor and maintenance are major contributors | Energy price volatility, operator skill gaps, downtime | AI-assisted optimization and predictive maintenance; renewable-energy integration | [16,29,85,110] |
| Biomethane/biogas revenue | Revenue depends on gas yield, methane purity, and market route | Methane slip, gas quality compliance, market access | Co-optimize intensification and upgrading; route-specific gas polishing and methane-recovery monitoring | [123,129] |
| Digestate valorization | Digestate can provide substantial fertilizer value and additional income | Variable product quality, market acceptance, logistics | Post-treatment, nutrient recovery, quality standardization, and local market development | [10,124] |
| $CO_2$ conversion/utilization | Biological methanation and $CO_2$ use can increase carbon efficiency and value | $H_2$ availability/cost and process integration complexity | Integrate renewable-$H_2$ pathways and target local $CO_2$ use cases | [7] |

**Table 6.** *Cont.*

| Economic Domain | Key Findings from Literature | Main Risk Factors | Improvement Strategies | References |
|---|---|---|---|---|
| Policy and overall feasibility | Incentives and regulation often determine bankability | Policy volatility, weak carbon pricing, permitting barriers | TEA-LCA-informed policy design and bundled value models | [110,118,121,126] |

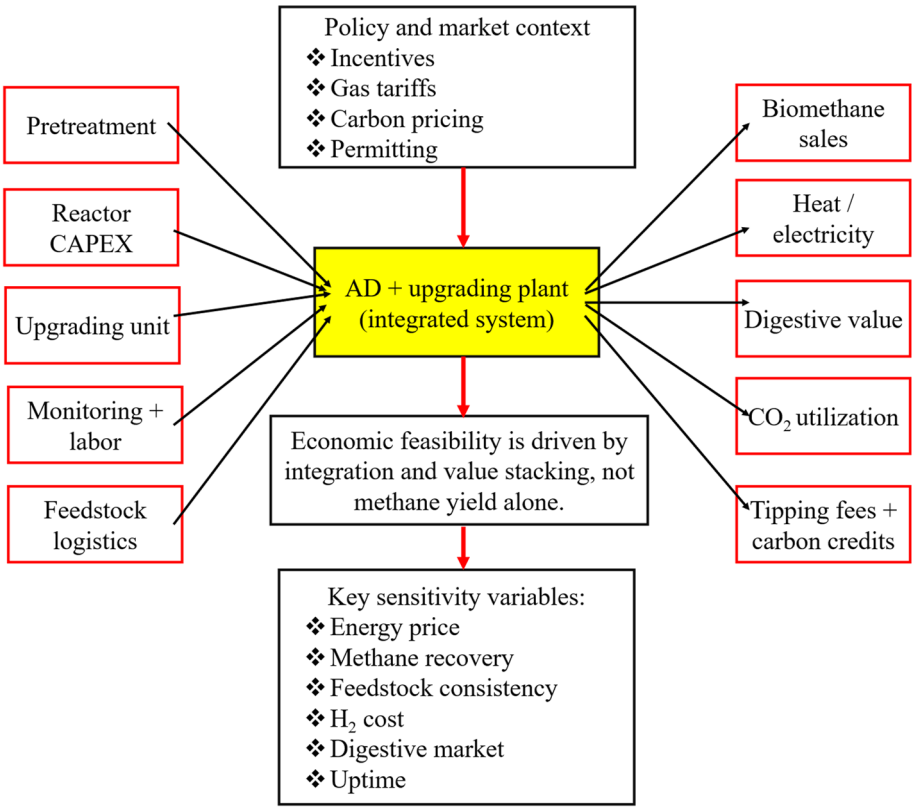



**Figure 6.** Economic value pathways in anaerobic digestion and biomethane systems. Systems-level diagram linking major cost centers (pretreatment, reactor infrastructure, upgrading, monitoring, logistics) with revenue and value pathways (biomethane, heat/electricity, digestate, $CO_2$ utilization, tipping fees, carbon credits). The figure emphasizes value stacking and policy context as primary determinants of project feasibility.

## 8. Conclusions

Recent advances show that methane production from AD is improved most reliably when process intensification, additive-enabled control, and biomethane upgrading are evaluated as an integrated design problem rather than as isolated unit operations. Pretreatment and reactor innovations can reduce hydrolysis and phase-instability constraints, co-digestion can improve biochemical compatibility, and additives can provide targeted support for electron transfer, hydrolysis, or methanogenic stability. However, the strength of evidence differs across interventions: co-digestion, staged reactors, and established physicochemical upgrading routes are comparatively mature, whereas DIET-promoting materials, AI-assisted control, and some biological or hybrid upgrading pathways remain promising but still under-validated at long duration and full scale. Future progress will depend on reproducible comparative reporting, hybrid ADM1–ML monitoring, and TEA-LCA-linked demonstration studies that determine when higher methane yield translates into better net energy, climate, and economic performance.

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## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| AD | Anaerobic Digestion |
| AI | Artificial Intelligence |
| ANN | Artificial Neural Network |
| CAPEX | Capital Costs |
| OPEX | Operating Costs |
| DIET | Direct Interspecies Electron Transfer |
| HS-AD | High-Solid Anaerobic Digestion |
| LCA | Life-Cycle Analysis |
| LCFA | Long Chain Fatty Acid |
| LSTM | Long Short-Term Memory |
| TAN/FAN | Total/Free Ammonia Nitrogen |
| TEA | Techno-Economic Analysis |
| TPAD | Temperature-Phased Anaerobic Digestion |
| TRL | Technology Readiness Level |
| VFA | Volatile Fatty Acid |

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