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Review

Digestate Recirculation in Agricultural Biogas Plants: Effects on Process Stability, Methane Production, Water Demand, and Inhibitor Accumulation

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Abstract

Digestate recirculation is increasingly considered a strategy for improving resource efficiency in agricultural biogas plants by reducing process-water demand, retaining active biomass, and enhancing the internal reuse of nutrients and residual organic matter. This review critically examines the effects of digestate recirculation on anaerobic digestion stability, methane production, water balance, inhibitor accumulation, and microbial community dynamics. Previous reviews have largely addressed digestate management, nutrient recovery, fertilizer use, and broader anaerobic digestion optimization, while digestate recirculation itself has received less attention as an operational process-management strategy in agricultural biogas plants. This review addresses this gap by integrating technological, physicochemical, microbiological, and operational evidence and by emphasizing that the effects of recirculation are system-specific rather than governed by a single universal optimum recirculation ratio. Particular attention is given to the influence of recirculation ratio, digestate fraction, solids content, reactor configuration, and feedstock characteristics. Available studies show that recirculation can increase volumetric methane production, support buffering capacity, improve microbial retention, and reduce the need for external dilution water. However, the response is generally non-linear, and excessive recirculation may promote the accumulation of total ammonia nitrogen, volatile fatty acids, soluble salts, sulphides, recalcitrant compounds, and suspended solids. These changes can increase viscosity, impair mass transfer and mixing, and shift methanogenic pathways toward more stress-tolerant microbial consortia. Effective implementation requires integrated control of chemical, physical, and microbiological parameters. Digestate recirculation should therefore be treated as a flexible process-management tool whose benefits depend on system-specific operating conditions and long-term mass-balance control. Future research should define robust monitoring indicators for full-scale operation.

Keywords: anaerobic digestion; digestate recirculation; agricultural biogas plants; methane production; process stability; water reuse; inhibitor accumulation; microbial adaptation; microbial retention; omics

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1. Introduction

Anaerobic digestion (AD) is an established biological process for converting biodegradable organic materials, including agricultural residues and livestock manure, into biogas. In addition to renewable energy recovery, the process generates digestate, which retains water, nutrients, and a fraction of the organic matter originally introduced with the feedstock.

Consequently, the overall performance of an agricultural biogas plant depends not only on methane production but also on how effectively this residual stream is managed and integrated into the plant operation [1].

Agricultural use remains one of the principal pathways for digestate utilization because of its nutrient content and potential fertilizer value. However, the large quantity of water associated with digestate and the need for its storage, transport, treatment, or land application may create additional operational and environmental constraints [1]. Digestate management is therefore increasingly considered as part of the overall resource balance of anaerobic digestion rather than as a separate downstream activity.

Digestate recirculation provides a means of integrating this residual stream more closely with the digestion process by returning water, microorganisms, nutrients, and residual organic matter to the reactor. Its effects, however, depend on the operating conditions and the extent of internal recycling [2–4].

1.1. The Importance of Digestate Management in Agricultural Biogas Plants

The significance of digestate in agricultural biogas plants results primarily from the presence of components that remain available for further use in agricultural production after anaerobic digestion. These include mainly nitrogen, phosphorus, potassium, and residual organic matter [1,5–7]. Their proportions and chemical forms depend on the type and share of the feedstocks used, the course of the digestion process, and the extent of organic matter degradation. Consequently, the fertilizer properties of digestate may differ considerably among biogas plants, which limits its treatment as a material with a constant and predictable composition [5–7].

From the perspective of resource management, digestate represents a potential link for returning nutrients to agricultural systems and partially reducing the demand for mineral fertilizers [5,6]. These benefits, however, depend on whether the quantity and composition of digestate are consistent with the actual nutrient requirements of crops. Where local surpluses of nitrogen or phosphorus occur, direct use of the entire digestate stream may be restricted, and further management may require nutrient recovery, concentration, or selective utilization of individual components [5–7].

Appropriate digestate management is therefore important not only for exploiting its fertilizer value, but also for limiting nutrient losses and reducing potential environmental impacts [1,5,6]. From the perspective of biogas plant operation, increasing attention is being paid to management strategies that maximize the use of resources contained in digestate while reducing the amount of material that must be handled outside the technological system. This provides the basis for considering partial digestate recirculation as an integrated element of biogas-plant resource management.

1.2. Rationale for Digestate Recirculation

The rationale for digestate recirculation is primarily associated with the possibility of modifying the internal operating conditions of anaerobic digestion without introducing an additional external process stream. Depending on the fraction returned to the reactor, recirculation may affect the hydraulic and solids balance, redistribute soluble metabolites and microbial biomass, and modify the interaction between freshly supplied feedstock and material that has already undergone digestion [2–4,8]. Experimental evidence indicates that these effects are strongly dependent on the recirculation ratio and reactor configuration, suggesting that digestate recycling should be regarded as an operational variable rather than as a fixed technological practice [2,8].

An important feature of recirculation is that its function may differ substantially among individual digestion systems. In continuous dry anaerobic digestion, changes in the recir-

culation ratio have been associated with variations in volumetric biogas production and microbial community structure [2]. In systems treating lignocellulosic agricultural feedstocks, recirculation of the liquid digestate fraction has been applied to support long-term operation and improve process stability, although its effect on specific methane yield has not always been pronounced [9,10]. Simulation-based studies on the co-digestion of wheat straw and cattle manure likewise indicate that the benefits of liquid digestate recirculation depend on defining an appropriate operating range rather than simply maximizing the recycled fraction [11].

These observations indicate that the technological rationale for digestate recirculation lies mainly in its capacity to shape internal process conditions while reducing dependence on external auxiliary streams. Increasing internal circulation, however, also decreases the removal of soluble or slowly degradable constituents and may promote their progressive accumulation [3,9]. Practical implementation therefore requires balancing process intensification against long-term control of accumulating compounds [3,9]. The effectiveness of recirculation consequently depends on the properties of the recycled fraction, feedstock characteristics, reactor configuration, solids content, organic loading rate, and the applied recirculation ratio [2,3,8,9].

1.3. Aim and Scope of the Review

The aim of this review is to critically assess digestate recirculation in agricultural biogas plants, with particular emphasis on its effects on anaerobic digestion stability, methane production, process water demand, and the risk of inhibitor accumulation.

Previous review studies have mainly focused on digestate management, nutrient recovery, fertilizer use, downstream processing, and the environmental or techno-economic aspects of digestate valorization [1,5–7]. More recent work has also considered recirculation as part of broader strategies for anaerobic digestion intensification, including both biogas and digestate recirculation [9–11]. However, digestate recirculation itself has received less attention as an operational process-management strategy in agricultural biogas plants, particularly with regard to the balance between process benefits and the long-term consequences of repeated internal recycling.

This review addresses this gap by integrating the technological, physicochemical, microbiological, and operational aspects of digestate recirculation. The analysis covers digestate properties relevant to recirculation, technological configurations, effects on methane production and process stability, accumulation of potentially inhibitory compounds, microbial responses, and the implications of recirculation for water, energy, and resource management.

Rather than seeking a single universal optimum recirculation ratio, this review emphasizes that the effects of digestate recirculation are system-specific and should be interpreted in relation to the characteristics of the recycled stream and the operating conditions of the digestion process. The synthesis therefore focuses on identifying the conditions under which recirculation may improve process performance and resource efficiency, as well as the factors that may limit further increases in the recycled fraction.

1.4. Literature Search and Study Selection

This review was based on a structured literature search focusing on digestate recirculation in anaerobic digestion systems, with particular emphasis on agricultural biogas plants. The literature search was designed to identify studies addressing the effects of digestate recirculation on methane production, process stability, water demand, nutrient retention, and the accumulation of potentially inhibitory compounds. Relevant publications were identified through searches of the Scopus and Web of Science databases, supplemented by Google Scholar and reference screening of selected publications. The search covered stud-

ies published between 2010 and 2026. Combinations of keywords and Boolean operators (AND, OR) were used, including the following search string: ("digestate recirculation" OR "digestate recycling" OR "liquid digestate recirculation" OR "solid digestate recirculation" OR "leachate recirculation") AND ("anaerobic digestion" OR "biogas production" OR "methane production"). Additional searches combined terms such as "process stability", "methane yield", "water consumption", "freshwater demand", "ammonia inhibition", "total ammonia nitrogen", "volatile fatty acids", "salinity", "organic loading rate", and "microbial community" to identify studies addressing specific technological and biochemical aspects of recirculation. The inclusion criteria comprised: (i) peer-reviewed research articles presenting experimental, pilot-scale, or full-scale investigations of digestate recirculation; (ii) studies reporting effects on methane production, organic matter degradation, process stability, water consumption, or inhibitory compound accumulation; and (iii) review articles providing relevant mechanistic or technological background. Particular attention was given to agricultural substrates, including animal manure, crop residues, and energy crops. Studies involving other organic substrates were also considered when their findings contributed to understanding the mechanisms and operational limitations of digestate recirculation. Publications were excluded when they: (i) did not address anaerobic digestion or relevant recirculation processes; (ii) focused exclusively on digestate utilization outside the digestion system without providing information relevant to recirculation; or (iii) lacked sufficient methodological or experimental information to support the interpretation of reported effects.

Study selection involved screening titles and abstracts for relevance, followed by full-text assessment against the inclusion and exclusion criteria. The selected publications were subsequently evaluated and organized according to recirculation strategy, substrate characteristics, operating conditions, and reported process responses.

The final literature search was conducted on 30 September 2026. A total of 81 publications were ultimately included in the review. As a complete screening log was not maintained during the literature-selection process, the exact numbers of records initially identified, duplicates removed, records screened, and full-text publications excluded could not be reliably reconstructed. Therefore, no retrospective estimates of these values were introduced.

2. Digestate Properties Relevant to Recirculation

The effects of digestate recirculation depend to a large extent on the properties of the recycled fraction. Its physicochemical and biological composition is shaped by feedstock characteristics, digestion conditions, the extent of substrate degradation, and the method used for solid–liquid separation [12]. As a result, recirculation may return dissolved compounds, microorganisms, residual organic matter, and inorganic constituents to the reactor.

Repeated recirculation can gradually modify the composition of the digester liquor because some compounds undergo further transformation, whereas others may persist and accumulate over successive recycling cycles. Therefore, the suitability of digestate for recirculation should be assessed primarily on the basis of parameters such as total solids, volatile solids, chemical oxygen demand, alkalinity, ammonia nitrogen, salinity, sulphides, and residual organic matter [13]. The main digestate properties relevant to recirculation and their potential implications for anaerobic digestion are summarized in Table 1.

Table 1. Digestate properties relevant to recirculation and their potential effects on anaerobic digestion.

Digestate Property	Main Origin	Potential Effect of Recirculation	Potential Process Implications	Governing Factors and Key Indicators	Experimental Evidence
TAN/NH_3	Protein degradation	Increased retention of ammoniacal nitrogen may occur, depending on the nitrogen balance and digestate replacement rate.	Ammoniacal nitrogen contributes to nutrient supply and buffering capacity. Elevated free ammonia (NH_3) concentrations may inhibit methanogenic activity under unfavorable conditions.	TAN, pH, T recirculation ratio, microbial adaptation	Increased ammonia concentrations without confirmed inhibition were reported by Li et al. [3]. Temperature-dependent differences in reactor performance were observed by Chen et al. [2]
Soluble COD	Residual organics	Recirculation may return biodegradable and poorly biodegradable dissolved compounds to the reactor.	Additional methane production may be possible if the recycled compounds are biodegradable. An increase in soluble COD alone does not demonstrate inhibition or process deterioration.	Soluble COD, residual BMP, OLR, HRT, VFA concentrations, methane yield	Hu et al. [9] reported increasing COD concentrations during direct digestate recirculation without significant differences in methane yield.
K^+, Na^+, Cl^-	Feedstock/process water	Dissolved ions may be retained or become enriched when recirculation reduces their removal from the system.	Elevated salinity may cause osmotic stress and reduce microbial activity if tolerance limits are exceeded. Effects depend on ionic composition and microbial adaptation	Electrical Conductivity (EC), ion concentration	Potential risk based on known salinity-related inhibition mechanisms; effects require verification under the specific recirculation conditions
Sulphides	Sulphate reduction and degradation of sulphur-containing organic compounds	Dissolved sulphide concentrations may change depending on sulphur input, precipitation, gas transfer and recirculation conditions.	Elevated dissolved H_2S may inhibit microorganisms, whereas sulphide precipitation may reduce the bioavailability of essential trace metals.	Total dissolved sulphide, pH, dissolved H_2S, biogas H_2S, sulphur loading, dissolved metal concentrations	Potential mechanisms; inhibition or trace-metal limitation should be supported by process-specific observations.
TS/VS	Undigested	Recirculation of solids-containing digestate may increase solids retention and alter slurry rheology.	Increased biomass retention may support degradation, whereas excessive solids loading may impair mixing and pumping and adversely affect methane production.	TS, VS, rheology	Li et al. [3] observed solids accumulation and a subsequent decrease in methane yield during prolonged digestate recirculation.
Trace metals	Feedstock, mineral additives and recycled microbial biomass	Recirculation may increase the retention of essential or potentially toxic metals, depending on their solubility and removal pathways.	Improved micronutrient availability may support methanogenesis. Excessive bioavailable concentrations may cause inhibition, while precipitation may lead to micronutrient limitation.	Total and dissolved Fe, Ni, Co, Zn; pH, sulphide concentration, metal speciation	Potential beneficial or inhibitory effects; their occurrence depends on bioavailability and requires system-specific evidence

Table 1. *Cont.*

Digestate Property	Main Origin	Potential Effect of Recirculation	Potential Process Implications	Governing Factors and Key Indicators	Experimental Evidence
Recalcitrant organic matter	Lignocellulosic residues, humic substances and other poorly biodegradable fractions.	Slowly degradable or inert organic fractions may be retained when solids-containing digestate is recycled.	retention may support further degradation of slowly biodegradable material, while excessive retention of inert fractions may increase solids loading without proportional methane recovery.	Residual BMP, VS, lignocellulosic composition, solids retention time, solid–liquid separation efficiency	Potential effects dependent on substrate composition, biodegradability and long-term solids retention
VFAs	Hydrolysis, acidogenesis and incomplete conversion of fermentation intermediates	Recirculation may return VFAs and buffering compounds; net VFA concentrations may increase or decrease depending on their production and consumption rates.	VFA recycling may support further methane formation. Persistent VFA accumulation accompanied by insufficient buffering, declining pH or reduced methane production may indicate process imbalance.	Acetate, propionate, butyrate, total VFA, alkalinity, VFA/alkalinity ratio, pH, OLR, temperature	Chen et al. [2] observed VFA accumulation and reduced methane yield under thermophilic recirculation, whereas Li et al. [10] reported decreased VFAs and improved process stability

2.1. Liquid, Solid, and Whole Digestate: What Is Actually Recirculated?

Digestate can be returned to the process either as whole digestate or, following separation, as liquid and solid fractions. This distinction is fundamental because the resulting streams differ in their contents of solids, dissolved compounds, nutrients, and organic matter. Mechanical separation, for example by screw pressing, concentrates larger particles in the solid fraction, whereas the liquid fraction contains mainly water, dissolved compounds, and finer suspended particles. The efficiency of separation depends, among other factors, on dry matter content, separator design, and operating conditions [14]. Therefore, the term "digestate recirculation" should always be interpreted in relation to the specific fraction that is actually returned to the reactor.

The liquid fraction is generally easier to pump and recirculate than digestate with a high solids content, which is relevant to pump selection and to the integration of recirculation with reactor mixing systems [15]. By contrast, recirculation of whole digestate returns both dissolved constituents and particulate material, together with microorganisms and residual biodegradable organic matter [16]. Consequently, the choice of recycled fraction affects not only the water balance but also the amount of solids and biologically active components retained within the system.

Solid–liquid separation does not, however, remove all constituents to the same extent. Particulate material and part of the phosphorus may be preferentially retained in the solid fraction, whereas highly soluble compounds, such as ammonium and potassium, remain mainly in the liquid phase. Recirculation of the liquid fraction can therefore reduce the return of larger particles without eliminating the re-entry of dissolved constituents into the reactor. At the same time, the hydraulic effect of recirculation depends not only on flow rate but also on reactor geometry and the applied mixing strategy [17].

The importance of the recycled fraction has been demonstrated in different anaerobic digestion configurations. Liquid digestate recirculation preferentially returns dissolved compounds, fine suspended particles, and microorganisms associated with the liquid phase [18],

whereas recirculation of a solids-retentive fraction increases the return of particulate material and microorganisms associated with that fraction [19]. In solid-state anaerobic digestion systems, recirculated leachate performs a distinct function as a mobile liquid phase that enables the transport of moisture, dissolved compounds, and microorganisms through the heterogeneous substrate matrix [20].

Accordingly, liquid, solid, and whole digestate should not be treated as equivalent recirculation streams. Their different physical and chemical properties determine both the technical implementation of recirculation and the nature of the material reintroduced into the reactor.

2.2. Total Solids, Volatile Solids, Chemical Oxygen Demand, Alkalinity, and Nutrient Profile

Parameters describing solids content and residual organic matter are among the fundamental criteria for assessing digestate intended for recirculation. Total solids (TS) and volatile solids (VS) influence both the physical properties of the recycled stream and the amount of organic material returned to the reactor. Available studies indicate that increasing solids concentration does not automatically improve organic matter conversion, and that the relationship between TS, VS degradation, and overall process performance depends on feedstock characteristics and digestion conditions [21]. From the perspective of recirculation, the key issue is therefore not the TS or VS value alone, but their effect on the internal solids balance and the potential progressive retention of less biodegradable material within the system.

Chemical oxygen demand, particularly its soluble fraction (SCOD), provides complementary information on the residual organic matter remaining in digestate. Studies on digestate streams show that a fraction of organic compounds may remain available for further biological transformation after the main digestion stage [22]. At the same time, SCOD may comprise both readily biodegradable metabolites and compounds with limited susceptibility to further degradation. For this reason, COD/SCOD should not be interpreted as a direct measure of additional methane potential, but rather as part of a broader assessment that also considers VFA concentrations, VS, and the extent of previous substrate conversion.

The role of alkalinity is similarly context-dependent. Recirculated digestate may contribute buffering capacity to the system, but its actual significance depends on the composition of the recycled stream, the rate of acid formation, and the operating conditions of the receiving reactor. Studies conducted under different digestion configurations indicate that recirculation can support pH control, although it does not necessarily eliminate the need for additional alkalinity adjustment or process control [23,24]. The buffering potential of digestate should therefore be evaluated on a case-specific basis rather than treated as an inherent benefit of recirculation.

The nutrient profile is equally important because individual elements differ in their distribution between the liquid and solid phases. Following anaerobic digestion, nitrogen is present to a considerable extent in ammonium form, phosphorus may occur both in dissolved form and associated with particulate matter, whereas potassium remains largely in the liquid phase [13,14]. Consequently, the fraction selected for recirculation may selectively modify the internal nutrient balance of the reactor. This becomes particularly relevant during long-term recirculation, as digestate composition is not static but may gradually change as a result of differences among nutrient input, biological transformation, and removal from the system.

Taken together, the available evidence indicates that TS, VS, COD/SCOD, alkalinity, and nutrient profiles should be evaluated jointly. None of these parameters alone is sufficient to characterize the suitability of digestate for recirculation, whereas their combined

interpretation provides a more informative basis for assessing residual biodegradability, buffering potential, and the likelihood of progressive changes in digester liquor composition.

2.3. Total Ammonia Nitrogen, Free Ammonia, Salinity, Sulphides, and Trace Elements

Ammonia nitrogen is one of the most important digestate constituents from the perspective of recirculation, particularly in plants treating nitrogen-rich feedstocks such as livestock manure and protein-rich substrates. During anaerobic digestion, a substantial fraction of organic nitrogen is mineralized to ammonium forms, which remain mainly in the liquid phase and may therefore be repeatedly returned to the reactor. As a result, long-term recirculation may lead to a gradual increase in total ammonia nitrogen (TAN) concentration [25]. The significance of this accumulation depends not only on TAN itself but also on the fraction present as free ammonia (NH_3), which is strongly influenced by pH and temperature [26]. Total ammonia nitrogen (TAN) comprises ammonium nitrogen (NH_4^+-N) and free ammonia nitrogen (NH_3-N, FAN), whose relative proportions depend primarily on pH and temperature. Increasing pH shifts the NH_4^+/NH_3 equilibrium towards the un-ionized form, while higher temperatures further increase the free ammonia fraction. Consequently, identical TAN concentrations may result in substantially different FAN concentrations and inhibition risks under mesophilic and thermophilic conditions [27,28]. Mlinar et al. [25] demonstrated that methanogenic archaea respond predominantly to NH_3 concentrations, whereas bacterial populations are affected by both ammonia species, highlighting the importance of considering ammonia speciation rather than TAN alone. Experimental investigations confirm that ammonia inhibition is strongly dependent on exposure conditions and microbial adaptation. Gaspari et al. [29] reported that an ammonia shock load of 5 g NH_4^+/L increased FAN to 200 ± 16 mg NH_3-N/L and caused a temporary 41% reduction in methane yield, followed by recovery of reactor performance after approximately 14 days. Conversely, Dai et al. [30] demonstrated that acclimated microbial communities could maintain stable biogas production under considerably elevated TAN and FAN concentrations. Similarly, Bucci et al. [31] reported sustained methane production at ammonium nitrogen concentrations reaching 20 g NH_4^+-N/L following more than 20 months of gradual microbial adaptation.

A similar mechanism applies to highly soluble inorganic compounds. Ions such as K^+, Na^+, and Cl^- may remain in the liquid digestate fraction and progressively increase salinity under intensive recirculation [32]. Therefore, assessment of digestate intended for recycling should consider not only the concentrations of individual ions but also their combined contribution to the osmotic conditions prevailing in the reactor.

Sulfur compounds represent another relevant group. Under anaerobic conditions, sulfur-containing compounds can be converted into sulfide species, the distribution of which depends, among other factors, on pH and temperature [33]. From the perspective of recirculation, particular attention should be given to the repeated return of dissolved sulfur species to the reactor, since limited removal may result in a gradual increase in the internal sulfide load.

Trace elements, including Fe, Co, Ni, Mo, Se, and Zn, constitute a distinct group of compounds relevant to anaerobic digestion. Several of these elements are involved in key enzymatic and metabolic functions of anaerobic microorganisms, but their effects depend not only on total concentration but also on chemical speciation and bioavailability [34]. Recirculation may promote their retention within the system, while their actual biological impact is further modified by precipitation, complexation, and sorption onto solid material.

Taken together, the available evidence indicates that the assessment of digestate for recirculation should place particular emphasis on compounds that are not effectively removed during anaerobic digestion and may therefore become progressively concentrated within a closed-loop system. TAN and NH_3, dissolved salts, sulfides, and trace elements

should consequently be considered as components of the long-term mass balance of the digestion system rather than merely as individual digestate-quality parameters.

From a mass-balance perspective, digestate recirculation involves the continuous return of water, dissolved compounds, suspended solids, and microorganisms to the reactor. During repeated recycling, some constituents are transformed through biological or physicochemical processes, whereas others may accumulate if their input exceeds their removal. Ammonium, soluble salts, and refractory organic matter are of particular interest because their concentrations may increase over successive cycles, while sulphur compounds and trace elements may undergo transformations, precipitation, or retention in the solid fraction. Their net accumulation therefore depends on substrate inputs, internal transformations, recirculation, and removal with biogas, separated digestate, or other output streams. Monitoring these flows and their associated concentrations is essential for assessing long-term process stability.

2.4. Rheology, Viscosity, and Mixing Behaviour

The rheological properties of digestate directly affect its pumpability, mixing behaviour, and suitability for recirculation. Available studies indicate that digestate from agricultural biogas plants commonly exhibits non-Newtonian, shear-thinning behaviour, while its apparent viscosity and yield stress are influenced primarily by solids content, particle size, temperature, and feedstock characteristics [35]. Increasing TS generally increases apparent viscosity and flow resistance, whereas reducing particle size may mitigate these effects [36].

The significance of rheology extends beyond hydraulic transport alone. High viscosity may impair mixing and restrict mass transfer within the reactor, resulting in zones with uneven distribution of substrate and dissolved compounds. Studies on high-solids anaerobic digestion indicate that increasing solids content can reduce effective diffusion and deteriorate mass-transfer conditions [35]. Therefore, assessment of digestate intended for recirculation should consider not only its chemical composition but also its flow properties. Mixing should provide sufficient homogenization of the reactor contents without causing unnecessary energy demand. Available evidence suggests that both insufficient and excessive mixing may reduce process efficiency, and that mixing intensity should therefore be adjusted to the rheological properties of the reactor contents [37]. In systems with high solids concentrations, recirculation of a liquid stream may additionally support local transport of moisture and dissolved compounds, thereby partially alleviating mass-transfer limitations [20]. From the perspective of digestate recirculation, the interaction among solids content, viscosity, mixing conditions, and mass transfer is therefore of particular importance. These parameters should be considered jointly because they determine both the technical feasibility of digestate recycling and the way in which the recycled stream is distributed throughout the reactor volume.

2.5. Microbial Load and Residual Biodegradability

Digestate is not a biologically inert process stream. It contains a complex microbial community originating from anaerobic digestion, including fermentative and syntrophic bacteria as well as methanogenic archaea. Consequently, digestate recirculation returns not only dissolved compounds and organic matter to the reactor, but also microbial cells, although their viability and metabolic activity after recirculation cannot be assumed [38]. The recycled stream may therefore perform an inoculum-like function, provided that the transferred microorganisms remain viable, adapt to the physicochemical conditions of the receiving reactor, and maintain sufficient metabolic activity to contribute to substrate degradation and methane production. The detection of microbial cells or DNA in recirculated digestate does not, by itself, demonstrate that the introduced microorganisms remain viable, become established in the receiving reactor, or contribute measurably to methane

production. Residual biodegradability represents another relevant characteristic of digestate. Experimental studies investigating digestate recycling as inoculum in high-solids anaerobic digestion systems, such as Chakrabarty et al. [39], support the potential relevance of microbial recycling. However, changes in methane production or microbial community composition do not necessarily demonstrate a direct functional contribution of the transferred microorganisms, as recirculation also modifies the physicochemical conditions of the system. After the main digestion stage, a fraction of organic matter may remain susceptible to further degradation, depending on feedstock characteristics, organic loading rate, retention time, and process configuration. Recirculation may therefore provide an opportunity for additional conversion of this residual fraction and increase the overall utilization of organic matter remaining in the digestate [3].

Studies conducted in multi-stage anaerobic digestion systems further indicate that microorganisms adapted to different physicochemical conditions may also be transferred between reactors together with the recycled digestate [40,41]. Such microbial transfer may influence the composition and diversity of the microbial community in the receiving reactor; however, the resulting effect is not necessarily beneficial and depends, among other factors, on recirculation intensity, hydraulic conditions, and the compatibility between the receiving environment and the requirements of the transferred populations. The detailed mechanisms of microbial responses to digestate recirculation are discussed later in this review.

From the perspective of evaluating digestate for recirculation, its process value therefore results from both the presence of active microbial biomass and the remaining biodegradable fraction of organic matter. Both aspects should be considered when interpreting the effects of recirculation, as they may influence further organic matter conversion and the biological continuity of the digestion process.

3. Technological Configurations of Digestate Recirculation

Digestate recirculation can be implemented in different technological configurations depending on the type of stream returned, the way it is conditioned, and the point at which it is reintroduced into the digestion system. Whole digestate, its liquid or solid fraction, or, in multi-stage systems, intermediate streams between individual process stages may be returned to the digester. A distinct configuration is the recirculation of leachate or percolate in dry anaerobic digestion systems.

The selected configuration affects system hydraulics, the solids balance, the transport of dissolved constituents and biomass, as well as pumping, mixing, and separation requirements. Therefore, the effects of recirculation cannot be interpreted solely on the basis of the percentage recycled, without considering the type of recirculated fraction and the technological configuration of the digester. The main technological configurations of digestate recirculation, together with their typical application conditions, reported recirculation ranges, advantages, and limitations, are summarized in Table 2.

Table 2. Comparison of digestate recirculation configurations applicable to agricultural anaerobic digestion systems.

Recirculation Configuration	Application Conditions	Reported Range/Conditions	Main Advantages	Main Limitations	References
Whole digestate	Dry/high-solids AD; manure–crop residue mixtures	50–60%; 60–80% in investigated dry-AD systems	Moisture supply; biomass and alkalinity retention; residual-organic conversion	TAN, VFA and SCOD accumulation; higher TS, viscosity, and pumping/mixing demand	Chen et al. [2]; Li et al. [3]

Table 2. *Cont.*

Recirculation Configuration	Application Conditions	Reported Range/Conditions	Main Advantages	Main Limitations	References
Liquid digestate fraction	CSTR/semi-continuous AD; crop residues and manure-based mixtures requiring dilution	Partial to complete LFD recirculation; study-specific	Freshwater substitution; alkalinity/biomass return; improved stability and OLR tolerance	Ammonia, COD and VFA accumulation during long-term operation	Hu et al. [9]; Li et al. [10]; Shao et al. [18]
Solid digestate fraction	Agricultural CSTR plants; increased solids retention	~4–6% of total feedstock mass in reported plants	Longer SRT; further degradation of residual organics; potential biogas gain	Higher TS; increased mixing, pumping, and material-handling energy demand	Brémond et al. [42]
Interstage recirculation	Serial or multi-stage CSTR systems	Return from downstream to upstream digestion stage	Transfer of alkalinity/biomass; lower VFA; improved stage integration	Soluble-compound and ammonia accumulation; stage-loading dependence	Li et al. [10]
Leachate/percolate	Dry/high-solids AD of lignocellulosic agricultural residues; leach-bed systems	Cyclic/continuous recirculation; study-specific flow rates	Moisture redistribution; improved mass transfer and substrate–microorganism contact	Channeling/uneven flow; possible soluble-inhibitor accumulation	Seboka et al. [20]

3.1. Recirculation of Whole Digestate

Recirculation of whole digestate is one of the simplest technological configurations because it does not require prior separation into liquid and solid fractions. The stream returned to the digester contains water, dissolved organic and inorganic compounds, suspended solids, and active biomass. As a result, this configuration affects both the hydraulic balance and the retention of solids and microorganisms within the system [3,43].

Direct recirculation can increase solids retention time and allow further degradation of organic matter that was not completely converted during its first passage through the digester. At the same time, it reintroduces dissolved and poorly biodegradable constituents into the system, making control of the internal material balance increasingly important as the recirculation ratio rises.

In dry anaerobic digestion systems, high proportions of recycled digestate can be used to regulate substrate moisture content and maintain suitable mass-transfer conditions. Li et al. showed, however, that the relationship between recirculation intensity and process performance is non-linear, and that excessive digestate return may promote the accumulation of VFAs, TAN, and SCOD and reduce biogas production [3].

Overall, the choice between whole-digestate recirculation and the recycling of separated fractions should be based on process configuration and recycled-stream characteristics rather than on recirculation ratio alone [43].

3.2. Recirculation of Liquid and Solid Fractions

Applying separation prior to recirculation allows the properties of the recycled stream to be controlled more selectively. Depending on the separation technology used, the amount of solids returned to the digester can either be reduced or, conversely, their retention can be increased. Separator selection therefore becomes an integral part of the recirculation

configuration because it affects both stream composition and its hydraulic and transport properties [44].

Recirculation of the liquid fraction is used mainly to reduce process-water demand and to return alkalinity, dissolved constituents, and part of the microbial biomass to the digestion system. In CSTR configurations, it can be implemented between successive digestion stages or from the final stage back to the reactor receiving fresh feedstock [10]. Semi-continuous studies also indicate that this approach can improve process stability under increasing organic loading, although its effectiveness depends on feedstock composition and the applied recirculation ratio [18].

Recirculation of the solid fraction serves a different function because it increases the retention of solids and slowly biodegradable organic matter. It can therefore extend the effective solids retention time, but at the same time may increase solids concentration in the digester and the requirements associated with material transport and mixing [45].

Separation therefore enables partial decoupling of hydraulic recirculation from solids retention, and the choice of the recycled fraction should be adapted to digestate properties and plant configuration [44,46].

3.3. Percolate Recirculation and Interstage Recirculation

In dry and high-solids anaerobic digestion systems, percolate recirculation provides a means of internally transporting the liquid phase through the substrate bed without the need for intensive mechanical mixing of the entire mass. The leachate collected at the bottom of the reactor is redistributed over the material, facilitating the transport of moisture, dissolved transformation products, and microorganisms between different zones of the substrate bed [20,47].

The relevance of this configuration has been confirmed by recent studies on high-solids systems. Chakrabarty et al. [47] applied percolate recirculation in a multi-cycle HSAD system in which digestate from previous cycles was also used as inoculum. Seboka et al. [20] demonstrated that stable process conditions could be maintained in a solid-state digestion system with leachate recirculation, highlighting its role in maintaining adequate moisture and supporting mass transfer.

In multi-stage systems, recirculation can also be implemented between individual reactors. Returning a stream from a later stage to an earlier one enables the transfer of alkalinity, biomass, and dissolved constituents between zones operated under different process conditions. Wu et al. [4] showed that such a configuration can influence the interconnection of transformations occurring in successive digestion stages, although its operation requires control of dissolved-compound accumulation, particularly ammonium.

3.4. Recirculation in Wet and Dry Anaerobic Digestion Systems

The technological role of recirculation depends strongly on solids content and the mode of anaerobic digestion. In wet digestion systems, most commonly based on CSTRs, the recycled stream can be relatively easily integrated into the hydraulic circulation of the plant. Recirculation primarily affects the liquid balance, retention of biomass and dissolved constituents, and the amount of material leaving the system [48].

In dry anaerobic digestion systems, recirculation is more closely associated with maintaining adequate moisture and mass transfer under conditions with a limited free liquid phase. Recycled digestate or percolate can improve contact between the substrate, microorganisms, and dissolved transformation products, thereby partly compensating for the limitations associated with high solids content [49,50].

These differences also affect the technical limits of recirculation. In wet systems, constraints are more commonly associated with the increasing internal concentration of

dissolved compounds, whereas in dry digestion, solid accumulation, rheological properties, and mass-transfer efficiency become additionally important. Studies on dry anaerobic digestion of cow manure and corn straw confirm that high digestate recirculation ratios can be technically feasible, although process response remains strongly dependent on both TS content and recirculation intensity [3].

Recirculation in wet and dry anaerobic digestion systems should therefore not be regarded as an identical technological operation. Its configuration and intensity should be adjusted to solids content, mixing strategy, availability of the liquid phase, and the material-transport characteristics of the particular system [48–50].

4. Process Limitations and Inhibitor Accumulation

The properties of digestate relevant to recirculation were discussed in Section 2. Under long-term operating conditions, however, the key issue is not only the presence of individual constituents, but primarily the way in which their retention and accumulation affect the course of anaerobic digestion.

Potential limitations arise when increasing concentrations of dissolved compounds, suspended solids, or metabolic intermediates begin to exceed the system's capacity for further transformation, removal, or biological adaptation. Under such conditions, the benefits of recirculation may gradually be offset by inhibitory effects, deterioration of the physical properties of the digesting mixture, and disturbances in process stability.

4.1. Accumulation of Ammonia and Nitrogen Compounds

Ammonia nitrogen accumulation is one of the most important potential limitations associated with intensive digestate recirculation. As discussed in Section 2.3, during anaerobic digestion a fraction of organic nitrogen is mineralized to ammonium forms, which remain predominantly in the liquid phase and may therefore be repeatedly returned to the reactor. Under conditions of limited nitrogen removal, this can lead to a gradual increase in total ammonia nitrogen (TAN). The significance of this phenomenon, however, depends not only on the total ammonia concentration but also on the proportion present as free ammonia, NH_3, which increases with increasing pH and temperature. For this reason, inhibition risk should not be assessed solely on the basis of a single TAN threshold, but rather in combination with the prevailing process conditions.

The importance of recirculation intensity is well illustrated by the study of Li et al. [3], who investigated semi-continuous dry co-digestion of cow manure and corn straw at digestate recirculation ratios of 60, 70, and 80% and total solids contents of 20 and 25%. Under the investigated conditions (60–80% digestate recirculation and 20–25% TS), increasing recirculation promoted the accumulation of TAN, VFAs, and soluble organic compounds. At 70% recirculation and 20% TS, the highest volumetric methane production rate of 1.17 L L^{-1} d^{-1} was achieved, whereas increasing the recirculation ratio to 80% reduced biogas production by 58.97% [3]. This indicates that the inhibitory effects of accumulated intermediates and nitrogen compounds were associated with the combined influence of recirculation intensity and solids content rather than with a single inhibitor concentration [3]. These results indicate that the relationship between recirculation and process performance is non-linear. Increasing recirculation may improve substrate utilization up to the point at which the accumulation of dissolved intermediates and nitrogen compounds begins to exceed the adaptive capacity of the system. It should be emphasized, however, that the observed optimum applied to a specific feedstock mixture and dry anaerobic digestion configuration and should not be directly extrapolated to other systems.

The effect of increased nitrogen loading is not necessarily uniformly negative, because it may be partly compensated by biomass retention and microbial adaptation. In the semi-

continuous digestion of wheat straw and swine manure, liquid digestate recirculation increased the tolerated OLR from 5.0 g VS L^{-1} d^{-1} in the control system to more than 6.0 g VS L^{-1} d^{-1} [18]. This demonstrates that the effect of accumulated nitrogen compounds cannot be separated from the organic loading conditions and the ability of the microbial community to maintain stable methanogenic activity.

The same study also showed that combining LDR with biochar addition affected enzymatic activity and microbial community structure [18]. These findings suggest that the consequences of increasing nitrogen concentrations should be considered in the context of the overall biological response of the system rather than solely as a function of TAN. The detailed mechanisms of microbial adaptation are discussed in Section 6.

Recirculation may also form part of an active nitrogen-management strategy when combined with ammonia-removal technologies. During long-term high-solids anaerobic digestion of chicken manure at approximately 20% TS, Song et al. [51] reported that TAN accumulation to approximately 8.2 g L^{-1} was associated with severe inhibition, whereas biogas-recirculation-assisted ammonia stripping reduced TAN to approximately 3.0 g L^{-1}. Under the improved operating conditions, VFA concentration decreased to approximately 0.6 g L^{-1}, methanogenic activity increased by a factor of 1.3–2.9, and methane yield reached 0.3 L g^{-1} VS. These results indicate that the inhibitory effect of ammonia should be evaluated together with substrate nitrogen content, solids concentration, and the effectiveness of ammonia-removal mechanisms.

From an operational perspective, ammonia accumulation should therefore not be regarded as an unavoidable consequence of digestate recirculation, but rather as the result of interactions among nitrogen loading, recirculation intensity, pH and temperature conditions, microbial adaptive capacity, and the presence of nitrogen-removal mechanisms. Determining an appropriate recirculation range consequently requires these factors to be evaluated jointly rather than relying on a single universal TAN threshold or recirculation ratio.

4.2. Salinity, Sulphides, Trace Elements, and Recalcitrant Compounds

Recirculation of the liquid digestate fraction may lead to the gradual retention of highly soluble ions such as Na^+, K^+, and Cl^-, and consequently to an increase in the salinity of the digesting liquor. The significance of this phenomenon depends on feedstock composition, the proportion of process water, and the degree of loop closure. Moderate concentrations of certain ions may support microbial metabolism, whereas excessive salinity can induce osmotic stress, disturb enzymatic activity, and alter microbial community structure [32]. Therefore, in systems operated at high recirculation rates, inhibition risk should be assessed not only on the basis of individual ion concentrations but also in terms of the overall salt load. The effect of sulphur compounds was investigated by Chakrabarty et al. [40] in a multi-cycle high-solids anaerobic digestion system treating source-separated organic waste, with digestate recycling ratios of 40 and 50% and percolate recirculation. The 50% digestate recycling condition resulted in a faster decrease in H_2S concentration in biogas and improved process stability compared with 40% recycling. This suggests that sulphide-related effects should be interpreted in relation to substrate composition, digestate recycling strategy, and microbial activity rather than attributed solely to the degree of recirculation. Interactions between sulphides and trace elements are also particularly important. Under anaerobic conditions, metals such as Fe, Ni, Co, and Zn may occur in poorly soluble forms, thereby reducing their immediate bioavailability. Studies on trace-element speciation in anaerobic digestion have shown that a substantial fraction of these elements may be present in non-dissolved forms and that their actual biological availability depends on chemical speciation, pH, and reactor conditions [52]. Total metal concentration should therefore not be interpreted as a direct indicator of availability to microorganisms.

Trace elements also play a dual role in anaerobic digestion. They are essential micronutrients for several metabolic pathways, whereas both deficiency and excessive concentrations may adversely affect process performance [53]. In long-term food waste digestion, Zhang et al. [54] demonstrated that the gradual loss of trace elements with discharged digestate was associated with deterioration in process stability, whereas supplementation helped maintain methanogenic activity [55]. Digestate recirculation may therefore reduce the loss of certain micronutrients, but under highly closed-loop conditions it may also promote their retention and accumulation. The resulting effect depends on speciation, precipitation, sorption, and biological availability rather than on total concentration alone. The importance of operating conditions was also demonstrated [56] during continuous anaerobic digestion of pig manure with liquid digestate recirculation at OLRs ranging from 1.5 to 6.0 g VS L^{-1} d^{-1}. Although recirculation promoted the accumulation of Pb, Mn, Cu, and Zn in the liquid and solid digestate fractions, the study did not establish universal inhibitory concentration thresholds for these elements. Therefore, the potential effects of metal accumulation should be evaluated in relation to organic loading, recirculation intensity, chemical speciation, and metal bioavailability. The accumulation of recalcitrant organic matter represents a different type of limitation. Lignocellulosic fractions, humic substances, and other poorly biodegradable components may remain in the digestate after the main digestion stage and be reintroduced into the reactor during recirculation. Their repeated return does not necessarily result in a proportional increase in methane production, but may increase the proportion of inert or slowly biodegradable solids, contribute to higher viscosity, and impair mass-transfer conditions.

The effects of these factors are interdependent. Increased salinity, sulphide formation, changes in trace-element bioavailability, and retention of recalcitrant organic matter may simultaneously modify the environmental conditions within the reactor. Microbial adaptation may partly mitigate the consequences of these changes, but it does not eliminate the risk of inhibition as internal loading continues to increase. Studies on adaptation to elevated ammonia concentrations have shown that methanogenic communities may alter their structure and functioning in response to prolonged stress [55]; however, the detailed mechanisms of microbial adaptation are discussed in Section 6.

4.3. Increased Viscosity, Foaming, and Mixing Limitations

An increase in solids content represents one of the important physical limitations associated with digestate recirculation, particularly when fractions containing suspended or particulate material are returned to the reactor. Repeated recycling of such streams may increase the TS content of the digesting mixture, extend solids retention time, and gradually increase viscosity. Brémond et al. [42] showed that direct recirculation of solid digestate in agricultural CSTR biogas plants can increase both solids content and solids retention time, thereby affecting the hydraulic and energetic conditions of process operation. Higher viscosity increases flow resistance and may consequently raise the energy demand associated with mixing and circulation.

Ni et al. [52] investigated liquid digestate recirculation during continuous anaerobic digestion of pig manure over 230 days at OLRs ranging from 1.5 to 6.0 g VS L^{-1} d^{-1}. Digestate recirculation improved methane production and process stability at OLRs below 5.0 g VS L^{-1} d^{-1}. However, at an OLR of 6.0 g VS L^{-1} d^{-1}, sludge viscosity increased from approximately 30 to 1000 mPa·s, resulting in impaired mass transfer and reduced methane production. These findings demonstrate that the limiting factor under high organic loading was primarily associated with changes in rheological properties rather than with directly observed ammonia or VFA inhibition. Foaming may represent an additional operational problem in recirculated systems. Its occurrence depends on several interacting factors, including feedstock composition, solids content, rheological properties, gas production

intensity, and mixing conditions. In the study by Ni et al. [52], increasing viscosity and impaired mass transfer at high OLR coincided with deterioration in process stability. Foaming should therefore not be considered a direct and universal consequence of digestate recirculation, but rather as one of the possible outcomes of physical and biological changes occurring in highly loaded systems.

High solids concentrations may also promote heterogeneity within the digesting material and the development of local gradients in substrate and intermediate-product concentrations. This problem is particularly relevant in dry anaerobic digestion, where limited mobility of the liquid phase restricts homogenization and mass transfer [56]. Under such conditions, appropriate mixing intensity becomes essential for maintaining contact between the substrate and active biomass.

Increasing mixing intensity, however, does not automatically improve process conditions. Available studies indicate that both insufficient and excessive mixing may reduce anaerobic digestion efficiency, with the resulting effect depending on feedstock characteristics, slurry rheology, and reactor configuration [57]. Excessive agitation may increase energy consumption, disturb local microbial conditions, and, in some systems, promote VFA accumulation or a decrease in pH. Mixing intensity should therefore be adjusted to the physical properties of the digesting material and the degree of recirculation rather than increased proportionally with rising viscosity.

From an operational perspective, digestate recirculation may therefore create an interrelated set of effects involving solids accumulation, increasing viscosity, mass-transfer limitations, and higher mixing requirements. Once the system can no longer maintain adequate homogenization, these physical constraints may limit further increases in recirculation even when digestate chemistry does not yet indicate a direct risk of inhibition.

4.4. Risk of Volatile Fatty Acid Accumulation and Process Destabilization

Volatile fatty acids (VFAs), primarily acetate, propionate, and butyrate, are key intermediates in anaerobic digestion, and their accumulation generally indicates an imbalance between their production rate and the capacity of methanogenic microorganisms to further convert them [58,59]. Digestate recirculation may affect this balance in two directions. On the one hand, the recycled stream may return alkalinity, active biomass, and partially biodegradable organic matter, thereby supporting process stability. On the other hand, increased retention of dissolved organic compounds may promote VFA accumulation when methanogenic activity is insufficient. The effect of recirculation intensity is strongly dependent on process configuration. In continuous dry anaerobic digestion, Chen et al. [2] showed that increasing the digestate recirculation ratio from 50 to 60% initially resulted in only limited VFA accumulation, after which VFA concentrations decreased and stabilized at a low level. At the higher recirculation ratio, an initial increase in VFA concentration to a maximum of approximately 1.6 g L^{-1} was followed by a gradual decrease and stabilization at approximately 20 mg L^{-1}. The higher recirculation ratio was associated with improved biogas production, reaching a volumetric production rate of 1.6 L L^{-1} d^{-1}, and changes in the microbial community supporting the utilization of metabolic intermediates. The higher recirculation rate was also associated with increased biogas production and changes in the microbial community related to more efficient utilization of metabolic intermediates [2]. These findings indicate that the presence of VFAs in the recycled stream does not necessarily lead to persistent destabilization when the conversion capacity of the system remains sufficient.

A similar relationship was observed in a long-term high-solids anaerobic digestion system with percolate recirculation, where 50% recirculation resulted in a lower VFA/alkalinity ratio and more stable methanogenic conditions than 40% recirculation [47]. This finding suggests that increasing recirculation may improve process stability under specific

conditions when the additional retention of alkalinity and active biomass compensates for the increased organic load. However, this effect should be regarded as system-specific rather than as a general relationship with recirculation ratio.

A contrasting response was observed during whey permeate digestion with solids-retentive digestate recirculation. Recirculation without activated carbon addition resulted in pronounced VFA accumulation and an approximately 20% reduction in methane yield compared with the control reactor [19]. The experiments were conducted in semi-continuous stirred-tank reactors at an OLR of 2.5 g VS L^{-1} d^{-1}. Solids-retentive digestate recirculation without activated carbon addition resulted in approximately 9% higher total VFA concentrations than in the control reactor, accompanied by a decrease in methane yield from 56.77 to 45.02 NL CH_4 kg^{-1} VS, corresponding to a reduction of approximately 20%. However, combining solids-retentive recirculation with a single activated carbon addition of 15 g L^{-1} improved methane production despite continued VFA accumulation. Under these conditions, propionate and acetate concentrations reached 31.82 and 10.67 g COD L^{-1}, respectively, during the final hydraulic retention period, while methane yield increased to 60.53 NL CH_4 kg^{-1} VS [19].

This indicates that in systems treating readily acidifiable substrates, increased retention of intermediate products may exceed the capacity of syntrophic and methanogenic populations for further conversion. The simultaneous application of an appropriately selected process modification, in this case activated carbon addition, restored process stability, highlighting the importance of interactions between recirculation and other technological parameters.

Alkalinity, organic loading, and reactor configuration are also important determinants of the process response. In a temperature-phased anaerobic digestion system with recirculation of mesophilic digestate, greater resistance to shortened retention time, higher methane yield, and an almost 10% increase in net energy output were obtained compared with the system without recirculation [40]. In contrast, in a recirculated two-phase system treating waste activated sludge, the best performance was achieved at an intermediate recirculation ratio, whereas further increasing the ratio did not result in an additional improvement in methane production [41].

The available evidence therefore indicates that the risk of VFA accumulation during digestate recirculation does not depend on recirculation ratio alone. The outcome is determined by the balance among the rate of intermediate-product formation, buffering capacity, methanogenic activity, organic loading, and reactor configuration. VFA concentrations, particularly when interpreted in relation to alkalinity, should therefore be regarded as dynamic indicators of the system's ability to maintain stability as the degree of digestate recirculation increases.

5. Microbial Responses to Digestate Recirculation

5.1. Microbial Retention and Changes in Methanogenic Pathways

In biogas plant processes, digestate recirculation is not merely the return of water, alkalinity, and undegraded organic matter. It also reintroduces active bacterial and archaeal biomass and may therefore increase the effective retention time of microorganisms independently of the hydraulic retention time (HRT).

This is particularly important for slow-growing syntrophic microorganisms and methanogens. In high-solids anaerobic digestion (HSAD) systems, recirculated digestate may even serve as an inoculum for subsequent cycles. Chakrabarty et al. [47] showed that 50% digestate recycling resulted in a stable methanogenic community dominated by *Methanosarcina* and *Methanoculleus* in the digestion chamber, together with improved process stability. *Methanosarcina* archaea are regarded as some of the most metabolically versatile

and robust methane producers, with high tolerance to stress, whereas *Methanoculleus* represents a group of hydrogenotrophic methanogens. In addition, the abundance of the ammonia-tolerant VadinCA11 group in the percolate increased from a few percent to nearly 50%. This group belongs to the family Methanomassiliicoccaceae, performs hydrogen-dependent methylotrophic methanogenesis, and has been shown to tolerate the relatively high ammonia concentrations observed in the percolate.

Recirculation does not cause one universal direction of microbial change. The literature indicates that the direction of the shift depends on the feedstock, TAN/NH_3 concentration, total solids (TS), temperature, organic loading rate (OLR), and recirculation intensity. The difference reported across studies suggest that microbial responses to digestate recirculation should be interpreted in the context of the entire reactor operating regime rather than as a direct consequence of the recirculation ratio alone. The effects of recirculation depend on the balance between microbial retention and the accumulation of potentially inhibitory compounds. Reactor configuration and feedstock characteristics determine the content to which recirculation improves biomass retention and substrate availability, whereas temperature, organic loading rate, and ammonia acuumulation influence the metabolic conditions under which retained microorganisms operate. Consequently, a shift toward *Methanosarcina* or other stress-tolerant methanogens does not necessarily indicate improved process performance. Moderate recirculation may support microbial stability, whereas excessive recirculation may intensify inhibitory stress and reduce methane production. The contrasting responses reported in different studies therefore highlight the importance of considering microbial composition, metabolic function, and process performance together.

In the study by Li et al. [3] on dry anaerobic digestion of cattle manure and corn straw, increasing recirculation resulted in progressively greater dominance of *Methanosarcina*. Increasing the recirculation ratio to 80% produced adverse effects and reduced biogas production. In another study, Wu et al. [40] investigated temperature-phased anaerobic digestion with recirculation (TPAD-R). Recirculation increased archaeal diversity, and part of the previously dominant *Methanothermobacter* population was replaced by Methanosarcina and Methanothrix. The Shannon index increased from 1.41 to 1.84, while the TPAD-R system achieved approximately 10% higher net energy yield than TPAD at the highest loading rate.

Under ammonia stress, which may occur during digestate recirculation, the direct conversion of acetate by acetoclastic methanogens, typically associated with Methanothrix and part of the metabolism of *Methanosarcina*, may become less important. Adams [60] reported that many hydrogenotrophic methanogens show greater resistance to ammonia stress and highlighted the importance of syntrophic interactions under such conditions. Consequently, syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis (SAO-HM) may become increasingly important.

However, the relative abundance of a microorganism alone is insufficient to determine which methanogenic pathway is active, because the metabolic activity of a given methanogenic group may change. Using metagenomics and metatranscriptomics, Gaspari et al. [61] showed that during a strong ammonia shock, *Methanothrix* sp. and Methanosarcina flavescens were able to shift their metabolic activity from the acetoclastic pathway toward CO_2 reduction, probably selecting a thermodynamically more favorable route as a survival mechanism. Transcriptomic analysis also revealed increased expression of numerous genes associated with osmotic balance and genes involved in the synthesis and transport of osmoprotectants specific to hydrogenotrophic archaea (*nhaP2*, *ablB*, and *betT*), which function as microbial cell-survival mechanisms. Increased expression of these osmoprotectant synthesis and transport genes may explain the greater tolerance of hydrogenotrophic archaea to elevated ammonia concentrations compared with acetoclastic species.

5.2. Microbial Adaptation to Ammonia and Other Inhibitors

In anaerobic digestion systems with digestate recirculation, long-term retention of ammonium nitrogen is particularly important. Tiefensee et al. [62] indicated that ammonia inhibition of methanogenic digestion may lead to butyrate accumulation and, consequently, to a decrease in methane production efficiency. Microorganisms that are most vulnerable to ammonia stress, particularly acetoclastic archaea, may alter the way acetate is metabolized under elevated ammonia concentrations, resulting in a shift from acetoclastic methanogenesis toward syntrophic acetate oxidation (SAO).

Unfortunately, these processes, interactions, and microorganisms are still insufficiently understood. Studies indicate that ammonia-tolerant syntrophic butyrate-oxidizing bacteria may play a key role in mitigating ammonia inhibition. One such bacterium was recently isolated and characterized using sequencing and phylogenetic analysis, together with its interactions with other acetate-oxidizing bacteria and hydrogenotrophic methanogens.

In a subsequent study, Bucci et al. [63] adapted microbial consortia under mesophilic conditions for 20 months to gradually increasing concentrations of ammonium chloride in a synthetic medium. Without acidification, ammonium concentrations of up to 20 g NH_4^+-N/L were achieved. Such conditions may promote adaptive mutations, thereby increasing tolerance to the toxic effects of ammonia. Changes in microbial community composition were investigated using strain-resolved metagenomics, allowing individual strains to be tracked through characteristic allele patterns among single nucleotide variants (SNVs) within species. The analysis confirmed the presence of syntrophic acetate-oxidizing bacteria (SAOB), including *Keratinibaculum* sp. *ma44* and *Acetomicrobium* sp. ma133. The methanogenic population, in turn, was dominated by *M. bourgensis*. The study confirmed the occurrence of syntrophic associations and indicated the presence of multiple microorganisms capable of functioning as SAOB under syntrophic conditions.

Digestate recirculation also causes the accumulation of selected ions, including Na^+, K^+, and Cl^-, leading to changes in osmotic pressure and increased electrical conductivity. Qian et al. [64] described changes in microbial community structure caused by salinity and found that archaeal populations underwent the most pronounced restructuring, including a reduction in the dominance of acetoclastic methanogens.

Digestate recirculation may also result in the presence and accumulation of sulphides. These compounds affect the microbiome in several ways. Hydrogen sulphide can directly disturb methanogenic cellular metabolism, while sulfate-reducing bacteria indirectly compete with methanogens for hydrogen and acetate. Sulphide formation can also precipitate metals, thereby reducing their availability as cofactors in enzymatic reactions.

A recent study by Chen et al. [65] used genome-centric metagenomics to investigate anaerobic digestion under sulphide stress and demonstrated the role of quorum-sensing signaling in maintaining syntrophic metabolism and microbial self-adaptation.

Importantly, ammonia inhibition, salinity-related osmotic stress, and sulphide toxicity should not be considered exclusively as independent stressors in digestate recirculation systems. Recirculation may promote the accumulation of ammonium and dissolved ions, while sulphides may also accumulate depending on the feedstock composition and reactor conditions. Their simultaneous occurrence may expose microbial communities to multiple physiological constraints affecting cellular homeostasis, substrate conversion, syntrophic interactions, and methanogenic activity. These effects may be particularly important for microorganisms involved in acetate conversion and hydrogen transfer, because disturbances in one functional group can affect the activity of other members of the syntrophic community. However, the extent to which these stressors act additively, synergistically, or antagonistically remains insufficiently characterized in the context of digestate recirculation. The individual studies discussed above provide evidence of responses to specific inhibitory conditions,

but they do not establish a general quantitative relationship between combined inhibitor exposure, microbial community restructuring, and methane production. Therefore, the combined effects of ammonia, salinity, and sulphides should be treated as an important research gap rather than as a fully established mechanism.

5.3. Emerging Role of Omics-Based Monitoring

An increasing number of molecular tools and techniques are now available for assessing digestate recirculation in anaerobic digestion, enabling monitoring of changes in bacterial and archaeal communities (Table 3). Sequencing of the 16S rRNA gene remains the most accessible tool for tracking bacterial and archaeal community structure. However, the complexity of changes in the pangenome of recirculated microbial communities requires more detailed approaches, such as shotgun and genome-centric metagenomics, including the reconstruction of metagenome-assembled genomes (MAGs), identification of interactions between populations, and analysis of genes associated with hydrolysis, acidogenesis, acetogenesis, syntrophy, and methanogenesis [66]. For practical monitoring, the choice of molecular method should depend on the monitoring objective, required response time, analytical capacity, and cost. Routine assessment should primarily rely on established process parameters, including pH, temperature, total ammonia nitrogen (TAN), the free-ammonia fraction where relevant, electrical conductivity, sulphide concentration, volatile fatty acids (VFAs), and methane production. These measurements provide direct information on reactor conditions and process performance and can be used to identify changes that warrant further investigation. Targeted qPCR or ddPCR may complement process monitoring when rapid assessment of selected microbial groups or functional genes is required. In contrast, 16S rRNA sequencing is more suitable for periodic assessment of community restructuring, whereas shotgun metagenomics, metatranscriptomics, metaproteomics, and metabolomics are primarily valuable for detailed functional investigation and research-oriented diagnosis. However, the evidence supporting the application of metatranscriptomics and metaproteomics specifically to digestate-recirculation systems remains limited. Although these methods may provide insights into transcriptional responses and protein-level functional activity, their relevance to recirculation-associated process monitoring has not yet been sufficiently demonstrated in dedicated studies. Therefore, their proposed applications in this context should be interpreted as research perspectives rather than validated monitoring strategies. These approaches differ in cost, analytical complexity, and interpretation requirements and should not be regarded as interchangeable routine monitoring tools. Combining selected microbial indicators with process parameters may help distinguish changes in microbial composition from changes in metabolic activity and determine whether an observed community shift is associated with declining process performance.

Table 3. Omics-Based Methods for Monitoring Microbial Communities and Functional Responses in Digestate Recirculation Systems.

Method	What It Reveals	Usefulness for Recirculation Monitoring	Main Limitation	Reference Evidence
16S rRNA amplicon sequencing	Which microorganisms are present in the system	Changes in *Methanosarcina*, *Methanoculleus*, *Methanothrix* and SAOB; α/β-diversity	Provides limited information on actual function	Chen et al. [2]: enrichment of *Methanobacterium* and VadinBC27. Li et al. [3]: increasing *Methanosarcina* dominance at higher recirculation ratios. Li et al. [10]: changes in microbial richness and diversity. Wu et al. [40] and Chakrabarty et al. [39]: shifts in archaeal community composition.

Table 3. *Cont.*

Method	What It Reveals	Usefulness for Recirculation Monitoring	Main Limitation	Reference Evidence
qPCR/ddPCR	Abundance of selected genes or taxa	Targeted quantification of selected methanogenes, SAOB, and SRB; complementary monitoring of microbial indicators alongside process parameters, selected methanogens, SRB	Requires prior selection and validation of target genes or taxa; does not directly demonstrate metabolic activity or process performance	Cerrillo et al. [67] applied DNA- and RNA-based qPCR to investigate microbial populations in a thermophilic anaerobic digestion system with a recirculation loop.
Shotgun metagenomics	Metabolic potential of the whole microbiome	Methanogenic pathways, SAO, osmoregulation, resistance traits	Presence of a gene does not equal its activity	Gaspari et al. [61]: microbial responses to ammonia shock, without direct testing of digestate recirculation.
Genome-resolved metagenomics	MAGs and functions of specific populations	Identification of uncultured SAOB, syntrophic interactions, strain-level changes	Cost and bioinformatic complexity	Bucci et al. [63]: microbial adaptation to elevated ammonium levels. Chen et al. [59]: microbial responses to sulphide stress.
Metatranscrip-tomics	Which genes are currently expressed	Active response to NH_3/H_2S and shifts in methanogenic pathways	RNA is more difficult to collect and stabilize	Gaspari et al. [61]: gene-expression responses and methanogenic pathway changes under ammonia stress in anaerobic digestion; indirect evidence, not direct validation in digestate-recirculation systems.
Metaproteomics	Proteins and enzymes actually produced	Validation of metabolic activity	Difficult protein identification in complex samples	Ehiosun et al. [68]: discussion of integrated omics approaches; methodological perspective rather than direct experimental evidence for metaproteomics-based monitoring of digestate recirculation.
Metabolomics	Products of active metabolism	VFAs, osmoprotectants, stress-related metabolites	A metabolite cannot always be assigned to a specific organism	Wu et al. [4]: correlations between metabolites and process performance during two-stage digestion with recirculation. Chen et al. [2] and Li et al. [10]: changes in VFA concentrations.
Integrated multi-omics	Integration of 'who is there', 'what they can do', and 'what they are actually doing'	Potential to integrate microbial and functional indicators with process parameters for process diagnosis and model development	High cost, data integration and standardization requirements, limited comparability across studies, and the need for independent model validation	Ehiosun et al. [68]: methodological basis for integrated diagnosis of anaerobic digestion

Moreover, the latest studies [68] indicate that the current direction is no longer the use of individual omics approaches in isolation, but integrated meta-omics, that is, the mathematical and bioinformatic integration of microbial, functional, and process data into a single diagnostic and predictive model. Such an approach may enable more precise control of anaerobic digestion, particularly in systems operated with digestate recirculation. Despite

this potential, the practical application of integrated multi-omics for predictive process control remains challenging. Differences in feedstock, reactor configuration, operating conditions, and analytical methods may limit model transferability. Reliable prediction requires standardized datasets, consistent metadata, and independent validation under full-scale conditions. Until these requirements are met, integrated multi-omics should be considered a promising research tool rather than an established method for routine process control.

6. Effects on Process Stability and Methane Production

6.1. Specific Methane Yield and Volumetric Methane Production

The effect of digestate recirculation on methane production should be evaluated using both specific methane yield (SMY) and volumetric methane production (VMP), because these parameters describe different aspects of reactor performance [69]. SMY expresses the amount of methane produced per unit of organic matter supplied, whereas VMP relates methane production to reactor volume and time. This distinction is particularly important in recirculated systems because an increase in volumetric productivity does not necessarily correspond to a proportional increase in the conversion efficiency of fresh substrate.

Available studies indicate that the relationship between recirculation ratio and methane production is generally non-linear. Chen et al. [2] demonstrated during continuous dry anaerobic digestion that increasing digestate recirculation from 50 to 60% increased both biogas and methane production. At 60% recirculation, volumetric biogas production reached 1.6 L L^{-1} d^{-1}. The initial increase in VFA concentration was followed by their subsequent conversion and stabilization of the process, indicating that increasing recirculation may improve reactor productivity when the biological capacity of the system to convert recycled intermediates remains sufficient [2].

An even clearer non-linear response was reported by Li et al. [3] during semi-continuous dry co-digestion of cow manure and corn straw. Among recirculation ratios of 60, 70, and 80%, the highest methane production was obtained at 70% recirculation and 20% TS, where VMP reached 1.17 L CH_4 L^{-1} d^{-1}. Further increasing recirculation to 80% did not improve performance and was associated with a substantial decrease in biogas production [3]. These results confirm that maximizing the amount of recycled digestate does not automatically maximize methane production.

Recirculation does not necessarily lead to a significant increase in SMY. Hu et al. [9], investigating long-term digestion of maize stover, found no significant differences in biomethane yield among the system without recirculation and systems with direct and aerated recirculation of the liquid digestate fraction. At the same time, progressive accumulation of COD and ammonia nitrogen was observed in the direct-recirculation system [9]. These findings indicate that the benefit of recirculation may arise from improved long-term process operation, water reuse, or reduced liquid digestate discharge even when SMY does not increase markedly.

A comparable response was observed in a serial CSTR system treating corn stover. Li et al. [10] reported only a modest increase in methane production of approximately 2.3%, whereas total biogas production increased by 10.8%. At the same time, stability-related parameters, including alkalinity and VFA concentrations, improved [10]. This indicates that the effect of digestate recirculation may be more evident in process stability and reactor productivity than in specific methane yield alone.

Reactor configuration is also an important determinant of process response. In multi-stage systems, recirculation may modify the transfer of alkalinity, soluble intermediates, ammonium, and biomass between individual digestion stages [4]. Therefore, results ob-

tained in single-stage, serial, and multi-stage systems should not be compared solely on the basis of recirculation ratio.

Available evidence therefore indicates that digestate recirculation should not be assessed only through changes in SMY. In some systems, its principal benefit may be an increase in VMP, greater tolerance to organic loading, or maintenance of stable operation without a substantial increase in methane yield per unit of fresh substrate. SMY and VMP should therefore be interpreted jointly, together with organic loading rate, solids content, and indicators of metabolite and inhibitor accumulation. Such an approach makes it possible to distinguish a genuine improvement in conversion efficiency from process intensification resulting from more effective use of reactor volume.

6.2. Organic Matter Degradation and Removal Efficiency

The effect of digestate recirculation on organic matter degradation should not be assessed solely on the basis of VS, COD, or SCOD concentrations in the reactor and effluent. In recirculated systems, part of the material leaving the digester is returned to the process and may pass through the reactor multiple times. Therefore, changes in the concentrations of individual organic matter fractions should be interpreted in relation to the internal mass balance, with a distinction between fresh substrate and organic matter reintroduced with the recycled stream [70].

The importance of this approach was demonstrated by Menzel et al. [70] during hydrolysis of maize silage and bedding straw in a plug-flow reactor with partial thin-slurry recirculation. The authors showed that, under continuous operating conditions, assessment of hydrolysis based solely on the relationship between soluble and total COD may lead to misinterpretation because part of the organic matter is repeatedly returned to the system. Application of a COD mass balance that accounted for the organic matter introduced with the recycled stream made it possible to distinguish actual solubilization from internal circulation of previously released organic compounds [70].

In practice, such a mass balance requires quantification of the main inlet, internal-recycle, and outlet streams. At minimum, the flow rate and organic-matter content of the fresh feed and recirculated digestate should be measured, together with COD, SCOD, and VS in the relevant streams. The outgoing balance should include the final digestate or purge stream and, where solid–liquid separation is applied, the separated liquid and solid fractions. Biogas production and methane concentration should also be included to account for the fraction of organic matter converted to gaseous products. Expressing these measurements on a mass-flow basis rather than as concentrations alone allows the organic matter introduced with the recycled stream to be separated from the fresh substrate load and makes it possible to distinguish true conversion from repeated internal circulation.

Recirculation may increase contact between partially degraded organic matter and active biomass and hydrolytic enzymes, thereby promoting further degradation of residual fractions. This effect is particularly relevant for lignocellulosic substrates, for which hydrolysis represents one of the rate-limiting steps. Menzel et al. [70] showed that recirculation may prolong the beneficial effect of bioaugmentation and increase specific hydrolysis rates, although the magnitude of this effect depended on substrate composition and the proportion of less biodegradable lignocellulosic material.

An increase in SCOD should not, however, be automatically interpreted as an improvement in overall organic matter degradation. Li et al. [3] demonstrated during semi-continuous dry co-digestion of cow manure and corn straw that increasing the recirculation ratio promoted acidogenesis and increased the concentration of soluble organic matter. This indicates that enhanced hydrolysis and solubilization may increase the pool of soluble products, but if their subsequent conversion proceeds more slowly, accumulation may occur.

SCOD should therefore be interpreted together with VFA concentrations and methane production in order to distinguish intensified hydrolysis from incomplete conversion of intermediate products [3].

Long-term studies confirm that sustained substrate conversion does not exclude the simultaneous accumulation of residual organic matter. Hu et al. [9] found no significant differences in biomethane production, biomethane yield, substrate reduction, or pH among the system without recirculation and systems with direct and aerated recirculation of the liquid digestate fraction. At the same time, progressive COD accumulation was observed during 360 days of operation in the direct-recirculation system, whereas COD concentrations were more stable in the control and aerated-recirculation systems during the later stages of operation [9]. These results indicate that a similar degree of fresh-substrate conversion may coexist with increasing concentrations of residual organic matter within a closed recirculation loop.

In other configurations, recirculation may instead increase the actual removal efficiency of organic matter. In a temperature-phased anaerobic co-digestion system treating food waste and paper waste, Li et al. [71] obtained higher removal efficiencies of COD, TS, VS, carbohydrates, and proteins following the application of recirculation. Recirculation also supported cellulose degradation and reduced the tendency toward over-acidification in the thermophilic stage [71]. This demonstrates that the benefits of recirculation are strongly dependent on reactor configuration and substrate characteristics.

Further evidence is provided by Wu et al. [40], who investigated continuous temperature-phased anaerobic digestion of high-solid sludge under substantially shortened retention time. Digestate recirculation improved process tolerance and methane production under intensified operating conditions and promoted hydrolysis, protein degradation, and substance transfer between digestion stages [40]. Although this system differs from conventional agricultural digesters, it provides additional evidence that recirculation may enhance overall conversion capacity when shortened retention time or high solids loading increases the demand on hydrolysis and downstream biological conversion.

These findings also emphasize that organic matter retained within a recirculation loop is not uniformly biodegradable. Digestate contains both residual compounds with further conversion potential and poorly degradable fractions that may persist after repeated passage through the reactor [3,9]. As recirculation intensity increases, these refractory fractions may become progressively concentrated. High residual COD or VS concentrations should therefore not automatically be interpreted either as evidence of poor reactor performance or as additional methane potential; the biodegradability of the retained organic matter is more informative than its total concentration alone.

In systems with a high degree of recirculation, a system-level mass balance is therefore particularly important. Such a balance should include fresh organic matter input, the amount of recycled organic matter, conversion to gaseous products, residual digestate, and the fraction ultimately leaving the system [70]. This makes it possible to distinguish actual degradation from internal redistribution and concentration of organic compounds.

COD, SCOD, TS, and VS should therefore be treated as complementary indicators. VS reduction provides information on the conversion of the organic solids fraction, whereas COD and SCOD also include dissolved and suspended organic compounds. SCOD in particular should be interpreted together with VFA concentrations, methane production, and the overall mass balance [3,70]. Such an approach allows assessment of whether digestate recirculation genuinely enhances organic matter degradation, merely redistributes organic matter within the system, or promotes progressive retention of slowly biodegradable and refractory fractions [3,9,40].

6.3. Effects on pH, Alkalinity, and Volatile Fatty Acids

The stability of anaerobic digestion under digestate recirculation depends largely on the balance between acid production and the buffering capacity of the system. Recirculation can modify both of these components because the recycled stream returns not only alkalinity but also volatile fatty acids (VFAs), ammonium compounds, and other soluble metabolites to the reactor. Therefore, pH alone provides limited information on the metabolic state of the system, particularly when high buffering capacity delays measurable acidification. Stability assessment should consequently include the combined interpretation of pH, alkalinity, total and individual VFAs, and indicators describing the relationship between acid accumulation and buffering capacity [2,10].

Experimental studies indicate that digestate recirculation can improve alkalinity and stabilize pH under appropriately controlled operating conditions. Li et al. [10], investigating liquid digestate recirculation in a serial CSTR system treating corn stover, reported increased pH and alkalinity together with decreased VFA concentrations and a lower VFA-to-alkalinity ratio. At the same time, the increase in methane production was relatively modest [10]. This suggests that one of the principal effects of recirculation may be stabilization of the acid–base environment even when its effect on methane yield remains limited.

The buffering role of recycled digestate was also evident in the two-phase system investigated by Ma et al. [72]. Under high organic loading conditions, recirculation increased the average alkalinity of the methanogenic phase by approximately 1.3-fold and enabled stable operation at an OLR of up to 6.0 g VS L^{-1} d^{-1}. The authors identified the total VFA-to-total alkalinity ratio (TVFA/TA) as a sensitive early-warning indicator, with a value of approximately 0.5 corresponding to conditions approaching instability in the investigated system [72]. This value should, however, be regarded as specific to the particular process configuration rather than as a universal threshold.

Increased buffering capacity does not eliminate the risk of transient or persistent VFA accumulation. Chen et al. [2] observed during continuous dry anaerobic digestion at 60% recirculation an initial increase in total VFA concentration to approximately 1.6 g L^{-1}, followed by a decline and stabilization at a substantially lower level, while pH remained close to 7.3. This example shows that a temporary increase in VFAs does not necessarily indicate persistent destabilization when the microbial conversion capacity of the system remains sufficient [2].

Nevertheless, pH should not be treated as a sufficient stand-alone indicator of process stability. Bicarbonate and other buffering species may maintain pH within an apparently acceptable range despite ongoing VFA accumulation, and a pronounced decrease in pH may occur only after a substantial part of the buffering capacity has already been consumed [73]. Therefore, combined indicators such as VFA/TA, FOS/TAC, or the relationship between bicarbonate alkalinity and total alkalinity may provide an earlier indication of developing imbalance [72,73]. Comparative studies of anaerobic digesters subjected to organic overloading have shown that no single parameter is universally suitable for detecting destabilization, whereas the combined interpretation of total VFA, VFA/TA, and bicarbonate alkalinity/total alkalinity provides a more reliable indication of deteriorating process conditions [73].

The interpretation of VFA concentrations should also account for the composition of individual acids. Acetate is a direct substrate for acetoclastic methanogenesis, whereas propionate must first be syntrophically oxidized to acetate, hydrogen, and carbon dioxide before further conversion [74]. This process is thermodynamically less favorable and requires very low hydrogen partial pressure. Therefore, an increasing proportion of propionate may indicate deterioration of syntrophic conversion earlier than changes in total VFA concentration alone [74]. Mu et al. [74] demonstrated that increasing propionate

loading can substantially reduce its conversion rate, confirming the particular value of this parameter in the assessment of process overloading or destabilization.

The importance of VFA composition is also confirmed by results obtained in closed-loop systems. Azkarahman et al. [19] showed during whey permeate digestion that solids-retentive digestate recirculation without activated carbon resulted in pronounced VFA accumulation and an approximately 20% reduction in methane yield compared with the control system. Process stability was improved following activated carbon addition [19]. This example demonstrates that buffering capacity alone may be insufficient to maintain stability, particularly when highly acidifiable substrates are treated.

From an operational perspective, pH, alkalinity, and VFAs should therefore be interpreted as an integrated stability profile. Stable pH together with adequate alkalinity and low or decreasing VFA concentrations indicates that buffering and methanogenic capacities remain sufficient. In contrast, increasing VFA concentrations, particularly propionate, together with a rising VFA-to-alkalinity ratio may indicate developing imbalance before a clear decline in pH becomes apparent [72–74].

Available evidence therefore indicates that digestate recirculation may stabilize the acid–base balance by increasing alkalinity and supporting the conversion of intermediate products, but this effect depends on recirculation intensity, organic loading, and substrate characteristics [2,10,19,72]. In practice, trends in VFA concentrations, alkalinity, and their mutual relationships provide greater diagnostic value than an isolated pH measurement and should be interpreted in relation to the characteristics of the specific digestion system.

6.4. Process Stability Under Increased Organic Loading

Increasing the organic loading rate (OLR) is one of the main approaches used to intensify anaerobic digestion and increase volumetric reactor productivity. At the same time, a higher organic input increases the amount of hydrolysis and fermentation products that must be processed by downstream microbial populations. Digestate recirculation may extend the range of OLR over which the process remains stable by returning active biomass, alkalinity, nutrients, and intermediate products to the reactor [4,18,52,72]. This effect does not, however, imply an unlimited increase in reactor loading capacity. Available evidence rather indicates the existence of an operational window within which the benefits of recirculation outweigh the effects of progressive accumulation of metabolites, ammonia nitrogen, and solids.

A clear example of this limitation was provided by Ni et al. [52], who operated two CSTRs treating pig manure for 230 days while gradually increasing the OLR from 1.5 to 6.0 g VS L^{-1} d^{-1}. At loadings below 5.0 g VS L^{-1} d^{-1}, liquid digestate recirculation improved bioenergy production and contributed to stable process performance. At an OLR of 6.0 g VS L^{-1} d^{-1}, however, methane production in the recirculated reactor became inhibited [52]. Importantly, the main cause was not ammonia or VFA accumulation but a sharp increase in sludge viscosity, from approximately 30 to approximately 1000 mPa·s, which impaired mass transfer. This demonstrates that the upper loading limit may be determined by the physical properties of the system even when conventional chemical indicators do not yet indicate severe inhibition.

The ability of recirculation to increase OLR tolerance was also demonstrated by Shao et al. [18] during semi-continuous co-digestion of wheat straw and swine manure. The control reactor tolerated an OLR of approximately 5.0 g VS L^{-1} d^{-1}, whereas liquid digestate recirculation enabled operation at loadings above 6.0 g VS L^{-1} d^{-1} [18]. Enzyme-activity and microbial-community analyses indicated enhanced hydrolytic and methanogenic processes. Additional biochar application further increased resistance to loading changes, suggesting that recirculation may improve the biological conversion capacity of the system and that appropriately selected supporting measures may further extend the stable operating range [18].

In multi-stage systems, loading tolerance also depends on maintaining the balance between individual stages of the process. Wu et al. [4] showed that recirculation in two-stage anaerobic digestion positively affected methane production and enabled operation at higher OLR. At the same time, increasing loading and ammonium accumulation progressively disturbed the balance between hydrolytic microorganisms and methanogens [4]. This indicates that the acceptable loading of a multi-stage system depends not only on the amount of substrate supplied but also on the ability of successive stages to maintain coordinated conversion of the resulting intermediates.

A similar role of recirculation was demonstrated by Ma et al. [72]. Recycling the liquid digestate fraction increased alkalinity in the methanogenic phase and allowed stable operation at an OLR of up to 6.0 g VS L^{-1} d^{-1}. At the same time, the TVFA/TA ratio proved to be a sensitive indicator of approaching instability [72]. These results indicate that increased buffering capacity can delay the effects of rising organic loading, but only as long as the methanogenic conversion rate remains sufficient to prevent persistent accumulation of fermentation products.

Organic loading and recirculation intensity should not, however, be treated as independent variables. Li et al. [3] showed during dry co-digestion of cow manure and corn straw that increasing recirculation promoted acidogenic activity, but the best process performance was achieved at an intermediate rather than the maximum recirculation ratio. Excessive recirculation resulted in accumulation of TAN, VFAs, and SCOD and deterioration in gas production [3]. Simultaneously increasing OLR and recirculation may therefore shift the process more rapidly from beneficial intensification toward metabolic overloading or physical limitations.

The importance of recirculation under intensified operating conditions is also supported by the results of Wu et al. [40], obtained during continuous temperature-phased digestion of high-solid sludge under substantially shortened retention time. Recirculation improved process tolerance under more demanding operating conditions, supported hydrolysis and protein degradation, and enhanced material exchange between individual digestion stages [40]. Although this system differs from a typical agricultural biogas plant, the results indicate that the benefits of recirculation may become particularly apparent when process intensification reduces the conventional operational safety margin.

The available studies show that recirculation may increase process resistance to higher OLR through several interacting mechanisms. Retention of active biomass reduces the risk of washout of slow-growing methanogens, recycled alkalinity increases resistance to acidification, and repeated contact of organic matter with active biomass may support further conversion [4,18,40,72]. At the same time, loop closure promotes retention of constituents that would otherwise leave the reactor. As process intensity increases, the benefits of biomass and alkalinity retention may therefore be offset by VFA and ammonia accumulation, increasing solids concentration, and deterioration of rheological properties [3,4,56].

A single critical OLR cannot therefore be defined for all digestate-recirculating systems. The acceptable loading range depends on feedstock type, solids content, reactor configuration, temperature, retention time, recirculation ratio, and the properties of the recycled fraction [3,4,18,56,72]. An OLR of approximately 6 g VS L^{-1} d^{-1} was maintained successfully in some systems [18,72], whereas the same nominal loading caused inhibition in the system investigated by Ni et al. [52] because of severe viscosity increase. OLR values should therefore be interpreted as system-specific operating conditions rather than as universal stability thresholds.

From an operational perspective, increasing OLR in a recirculated system should be implemented gradually and assessed using several groups of indicators. An increase in volumetric methane production accompanied by maintained SMY, stable VFA-to-alkalinity

relationships, acceptable TAN concentrations, and suitable rheological properties indicates that the additional loading remains within the conversion capacity of the reactor. In contrast, persistent accumulation of VFAs or SCOD, increasing VFA-to-alkalinity ratios, declining SMY, or a rapid increase in viscosity indicate that the system is approaching the limit of stable operation [3,56,72].

Digestate recirculation may therefore extend the stable operating range of anaerobic digestion and enable higher OLRs under appropriate conditions [4,18,40,72]. The relationship remains non-linear, however. The operational objective should not be to maximize either OLR or recirculation ratio independently, but to identify an operating window in which increased volumetric productivity is achieved without progressive deterioration of substrate conversion or process stability. The studies summarized in Table 4 should therefore be interpreted as a structured qualitative and semi-quantitative synthesis rather than as a direct ranking of recirculation performance. The reported effects cannot be attributed to recirculation ratio alone because the investigated systems differ substantially in feedstock composition, digestion mode, reactor configuration, solids content, OLR, recycled fraction, and performance metrics. Across the available evidence, beneficial effects are generally observed when recirculation improves moisture distribution, alkalinity, biomass retention, or utilization of residual biodegradable matter without exceeding the biological and physical conversion capacity of the system. By contrast, detrimental effects emerge when increased recycling is accompanied by excessive OLR, accumulation of TAN, VFAs or SCOD, increasing solids concentration, or deterioration of rheological and mass-transfer conditions.

The available evidence nevertheless allows several system-specific operating patterns to be identified. In continuous and semi-continuous dry digestion of manure–straw mixtures, recirculation ratios in the range of approximately 50–70% were associated with improved volumetric gas production or process stability in the investigated systems, whereas further increases to 80% caused accumulation of TAN, VFAs and SCOD and reduced biogas production [2,3]. In liquid-digestate recirculation systems, the response appears to depend more strongly on OLR and reactor configuration than on the recycle percentage itself. For example, liquid digestate recirculation enabled operation above approximately 6 g VS L^{-1} d^{-1} in one straw–manure co-digestion system [18], whereas inhibition occurred at 6 g VS L^{-1} d^{-1} in another system because of severe viscosity increase [56]. Full-scale and modelling studies further indicate that solid or liquid digestate recirculation can improve resource efficiency and methane recovery, but these findings should be interpreted within the specific plant configuration and system boundaries used in each study [11,42].

6.5. Key Monitoring Indicators and Strategies for Gradual Implementation of Digestate Recirculation

Successful implementation of digestate recirculation requires monitoring capable of distinguishing beneficial process intensification from the early stages of destabilization. This is particularly important because VFA accumulation, increasing ammonia concentrations, rising viscosity, or deterioration in substrate conversion may develop gradually before a clear decline in methane production occurs. Monitoring should therefore rely on several groups of parameters and on analysis of their trends relative to the stable baseline of a given reactor rather than on a single threshold value [73,75,76]. It should be noted, however, that some of the monitoring concepts and threshold values discussed in this section are derived from the broader anaerobic digestion literature rather than from studies specifically addressing digestate recirculation. These values are therefore used as supportive reference points and should not be interpreted as recirculation-specific operational limits.

Table 4. Selected studies evaluating the effects of digestate recirculation on methane production, organic loading capacity, and process stability.

Feedstock	Reactor/ AD Mode	Recycled Stream	Recirculation Conditions	OLR/TS	Main Stability/Process Response	Methane/Biogas Response	Reference
Agricultural feedstocks—dry/high-solids AD							
Cow dung + corn straw	Continuous dry AD; horizontal plug-flow reactor	Whole digestate	RR 50 and 60% (TS-based mixing of recycled digestate with fresh feedstock; no explicit RR equation reported)	OLR 4 g VS L^{-1} d^{-1}	Temporary VFA accumulation up to ~1.6 g L^{-1} followed by decline to ~20 mg L^{-1}; pH stabilized around 7.3; 60% recirculation was associated with increased *Methanobacterium* abundance and improved gas-production efficiency	Highest volumetric biogas production at 60% RR: 1.6 L L^{-1} d^{-1}	Chen et al. [2]
Cow manure + corn straw	Semi-continuous dry AD	Whole digestate	RR 60, 70 and 80% (mass-based daily feed; total daily feed 200 g including the recirculated portion)	OLR NR; TS 20 and 25%	Increasing recirculation to 80% caused TAN, VFA and SCOD accumulation and reduced process performance, indicating an intermediate operating optimum	Best performance at 70% RR and 20% TS; VMP 1.17 L CH_4 L^{-1} d^{-1}; peak biogas production 2.71 L L^{-1} d^{-1}; biogas production decreased by ~59% at 80% RR	Li et al. [3]
Agricultural feedstocks—wet, liquid-digestate and multi-reactor systems							
Maize stover	Semi-continuous CSTR AD	Liquid digestate fraction	No recirculation vs direct LFD recirculation vs aerated LFD recirculation; RR = 70% (volumetric fraction of recirculated LFD relative to total separated LFD)	OLR 1.68 g VS L^{-1} d^{-1}; HRT 45 d	Direct recirculation caused progressive COD and NH_3–N accumulation during 360 d; aerated recirculation reduced COD and NH_3–N accumulation and improved long-term stability	Biomethane yields corresponded to 73.8%, 68.2% and 71.8% of theoretical methane potential for control, direct and aerated recirculation, respectively; differences were not significant	Hu et al. [9]

Table 4. *Cont.*

Feedstock	Reactor/ AD Mode	Recycled Stream	Recirculation Conditions	OLR/TS	Main Stability/Process Response	Methane/Biogas Response	Reference
Corn stover	Serial CSTRs	Liquid digestate fraction	LFD recirculation from the second to the first stage; 60–75% of recovered LFD recirculated	TS = 90 g L^{-1}	Increased pH and alkalinity; decreased VFA concentration and VFA/alkalinity ratio; NH_3 increased but remained below inhibitory levels	Methane production increased by 2.3%; total biogas production increased by 10.8%	Li et al. [10]
Pig manure	Mesophilic CSTR	Liquid digestate	LDR; RR = 60% (basis not explicitly defined)	OLR 1.5–6.0 g VS L^{-1} d^{-1}; HRT ≈ 15 d	Viscosity increased from ~30 to ~1000 mPa·s at the highest OLR, causing severe mass-transfer limitation despite TAN and VFA remaining below inhibitory levels	Improved methane production below 5.0 g VS L^{-1} d^{-1}; methane production inhibited at 6.0 g VS L^{-1} d^{-1}	Ni et al. [52]
Wheat straw + swine manure	Semi-continuous CSTR AD	Liquid digestate	Full LDR with and without biochar; all separated liquid digestate (~150–220 mL) returned with fresh feed	OLR increased stepwise from 3.0 to 7.0 g VS L^{-1} d^{-1}; endurable OLR: 5.0 (control) and 6.0 g VS L^{-1} d^{-1} (LDR/LDR + biochar)	LDR improved buffering capacity, hydrolytic and methanogenic activity, and tolerance to increased OLR; biochar further improved loading-shock resistance	At OLR 5.0 g VS L^{-1} d^{-1}, LDR + biochar gave 0.234 L CH_4 g^{-1} VS, ~5.4% above LDR alone	Shao et al. [18]
Supporting evidence from non-agricultural AD systems							
Food waste	Two-stage semi-continuous AD (acidogenic + methanogenic reactors)	Methanogenic effluent/digestate	Effluent recycle ratio = 0.3 (volumetric ratio of returned digestate to base inlet substrate); methanogenic effluent returned to the acidogenic stage	OLR 2–6 g VS L^{-1} d^{-1}; HRT 4 d (acidogenic) + 25 d (methanogenic)	Recirculation increased alkalinity and hydrolysis and improved tolerance to higher OLR; NH_4^+ accumulation disturbed the balance between hydrolytic bacteria and methanogens near the critical loading	At OLR 5 g VS L^{-1} d^{-1}, methane yield reached 398.0 mL CH_4 g^{-1} VS, 26.4% and 18.2% higher than the two-stage and single-stage systems without recirculation, respectively	Wu et al. [4]

Table 4. *Cont.*

Feedstock	Reactor/ AD Mode	Recycled Stream	Recirculation Conditions	OLR/TS	Main Stability/Process Response	Methane/Biogas Response	Reference
Food waste	Ethanol-type two-phase semi-continuous AD	Centrifuged liquid digestate from the methanogenic phase	LDR; RR = 0.4–0.6 (volumetric ratio of recirculated digestate to total influent)	Up to 6.0 g VS L^{-1} d^{-1}	Methanogenic-phase alkalinity increased ~1.3-fold under high loading; TVFA/TA ≈ 0.5 indicated approaching instability	Stable methane production maintained at OLR 6.0 g VS L^{-1} d^{-1}; inhibition occurred after increase to 6.5 g VS L^{-1} d^{-1}	Ma et al. [72]
Food waste + paper waste	Temperature-phased AD	Digestate returned between stages	Interstage digestate recirculation; RR = 0.4 (basis not explicitly defined); digestate returned from second to first reactor	OLR ≈ 4.4–5.3 g COD L^{-1} d^{-1}	Higher removal of COD, TS, VS, carbohydrates and proteins; improved cellulose degradation and reduced over-acidification	Improved biogas production and organic-matter conversion	Li et al. [71]
High-solid sludge	Continuous temperature-phased AD	Digestate	Recirculated digestate/feedstock flow ratio = 1:1; 50% of stage II digestate returned to stage I	OLR 3.96–8.05 g COD L^{-1} d^{-1}; HRT shortened from 20 to 10 d	Improved hydrolysis, protein degradation and interstage substance transfer; recirculation increased tolerance to intensified operation	Methane yield increased by >10% under demanding conditions; net energy output increased by ~10%	Wu et al. [40]

RR—recirculation ratio; LDR—liquid digestate recirculation; LFD—liquid fraction of digestate; OLR—organic loading rate; TS—total solids; VMP—volumetric methane production; NR—not reported or not sufficiently specified in the original study. Recirculation ratios and operational recycle conditions are reported according to the definitions provided in the original publications. Where available, the calculation basis or operational definition has been specified directly in the table. Because these definitions are not standardized across studies and may refer to volumetric ratios, mass-based fractions, flow-rate ratios, or other study-specific bases, numerical recirculation values should not be directly compared unless their underlying definitions are equivalent. Differences in methane production and process stability should likewise be interpreted in the context of feedstock composition, digestion mode, reactor configuration, solids content, OLR, recycled stream, and study scale. Accordingly, Table 4 should be interpreted as a structured qualitative and semi-quantitative synthesis rather than as a direct ranking of recirculation performance across studies.

Routine monitoring should include pH, alkalinity, total and individual VFAs, TAN, free ammonia, TS, VS, COD or SCOD, biogas production, and methane concentration. These parameters provide complementary information on acid–base balance, nitrogen retention, solids accumulation, organic matter conversion, and overall process performance [73,75]. Combined interpretation is particularly useful. Li et al. [73] demonstrated that the combination of total VFA, VFA/total alkalinity (VFA/TA), and bicarbonate alkalinity/total alkalinity (BA/TA) provided more reliable indication of process deterioration than any single parameter.

The usefulness of combined indicators was also confirmed by Ma et al. [72], who identified a TVFA/TA value of approximately 0.5 as a level associated with approaching instability in a system with liquid digestate recirculation. This value should not, however, be regarded as a universal threshold, because recommended limits depend on substrate type, temperature, reactor configuration, and operating conditions [75,76]. In practice, the direction and rate of change of these indicators are often more informative than a single exceedance of a literature-derived value.

Analysis of individual VFAs may further improve monitoring sensitivity. Propionate is particularly important because a persistent increase in its concentration, especially when accompanied by an increasing VFA/TA ratio, may indicate deterioration of syntrophic conversion before a substantial change in pH becomes apparent. Monitoring acetate, propionate, and butyrate separately may therefore help distinguish a transient process response from persistent metabolic imbalance.

Gas-phase monitoring provides an important complement to liquid-phase analyses. Zou et al. [77] showed during full-scale dry anaerobic digestion that combined indicators such as CH_4/CO_2, CH_4/pH, and CH_4/H_2S provided earlier warning of process deterioration than individual parameters, with warning periods of approximately 6, 7, and 10 days, respectively. Neubauer et al. [78] further demonstrated that increased variability in CH_4, CO_2, H_2, and gas volume may precede process disturbances, highlighting the potential of high-frequency gas monitoring and data analysis.

When whole digestate or solids-rich fractions are recirculated, physical properties should also be included in the monitoring strategy. Ni et al. [52] demonstrated that process deterioration may occur even without severe VFA or ammonia accumulation when increasing viscosity limits mixing and mass transfer. Therefore, TS, apparent viscosity, pumping pressure, or mixer power demand may provide important complementary information.

Available evidence supports gradual implementation of digestate recirculation rather than an immediate transition to a high recycle ratio. Experimental studies indicate that intermediate recirculation levels may improve methane production or process stability, whereas excessive recycling may result in accumulation of TAN, VFAs, SCOD, and solids [2,3]. Implementation should therefore begin with characterization of a stable baseline, including at least SMY, VMP, VFA profile, alkalinity, TAN, TS/VS, COD or SCOD, and, where relevant, viscosity.

The recirculation ratio should then be increased stepwise. After each change, the system should be maintained until a new quasi-steady state is reached, and further increases should be introduced only after evaluating the process response [2,3,18,56]. Further intensification may be considered when VMP remains stable or increases without a substantial decline in SMY, VFA concentrations stabilize or decrease, alkalinity remains sufficient, and TAN, SCOD, TS, and viscosity do not show progressive accumulation. Conversely, persistent increases in propionate or total VFA, rising VFA/TA ratios, declining SMY, accumulating TAN or SCOD, or rapidly increasing viscosity should be interpreted as signals that the upper limit of stable operation is being approached [3,56,72]. Operationally, the response to such deviations should follow a stepwise sequence: further increases in recirculation should

first be suspended, the recirculation ratio should then be reduced if adverse trends persist, and the reactor should be maintained under the adjusted conditions until the key stability indicators return toward the previously established baseline. Only after stabilization has been confirmed should another increase in recirculation be considered. The proposed sequence of monitoring, operational response, stabilization, and reassessment is summarized in Figure 1.

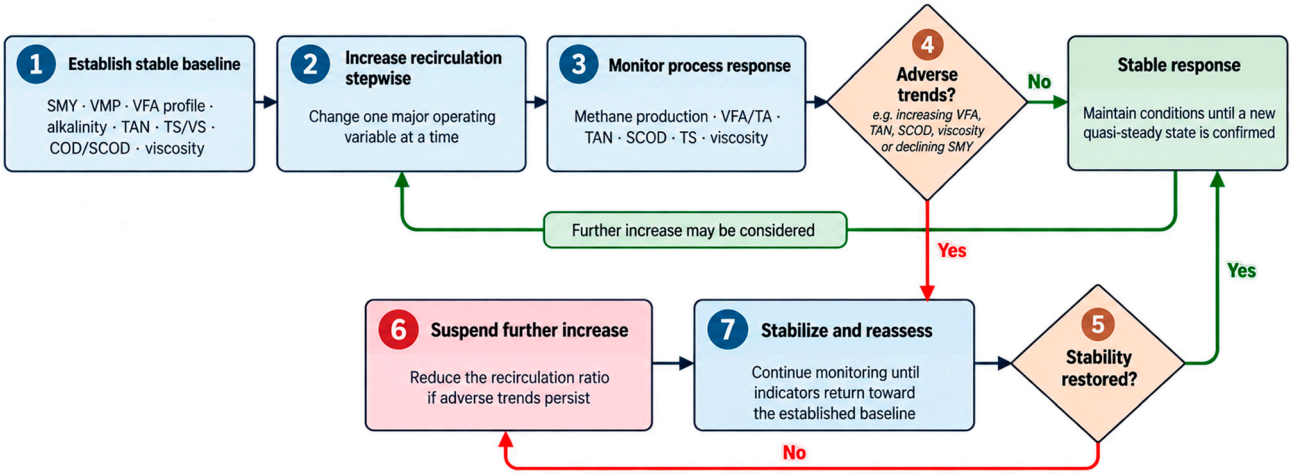


Figure 1. Proposed operational framework for the gradual implementation and monitoring of digestate recirculation. Adverse trends include persistent increases in total or individual VFAs, VFA/TA, TAN, SCOD, TS or viscosity, together with a decline in specific methane yield. The framework emphasizes reactor-specific trends and reassessment of process stability rather than universal threshold values.

Similar caution is required when OLR is increased simultaneously. Changing both OLR and recirculation ratio at the same time makes it more difficult to identify the cause of process deterioration and may accelerate the development of biological or mass-transfer limitations [18,56].

From an operational perspective, changing one major process variable at a time provides a clearer basis for interpretation. The transferability of these recommendations to full-scale agricultural biogas plants should nevertheless be interpreted with caution. Much of the available evidence on digestate recirculation originates from laboratory-scale or semi-continuous systems, which may differ from commercial installations in terms of hydrodynamics, mixing, solids distribution, retention conditions, and process control. Accordingly, the proposed monitoring and stepwise implementation strategy should be regarded as a framework for full-scale adaptation rather than as a set of directly transferable operating limits.

Advanced microbial and molecular analyses may be useful during commissioning, optimization, or unexplained destabilization, but their routine use at full scale is limited by cost and analytical complexity [75,76]. A tiered monitoring strategy is therefore more realistic: frequent or continuous measurement of gas production, gas composition, pH, and temperature; regular analysis of VFAs, alkalinity, TAN, COD, and solids; and targeted advanced analyses when routine monitoring indicates persistent or unusual disturbances.

Successful implementation therefore requires a shift from control based on fixed threshold values toward reactor-specific trend monitoring. No universal recirculation ratio or single stability indicator is suitable for all anaerobic digestion systems [73,75,76]. The most reliable strategy combines biochemical, gas-phase, physical, and performance indicators and increases recirculation only after the process has stabilized at the preceding operating level.

7. Environmental, Energy, and Economic Aspects

Introducing digestate recirculation affects not only the course of anaerobic digestion but also the overall material, water, and energy balances of the biogas plant. Returning whole digestate or its liquid fraction can reduce the amount of material leaving the digestion loop and partially replace external process water, while simultaneously increasing the internal circulation of nutrients, dissolved organic matter, salts, and other constituents. Therefore, the environmental and economic assessment of recirculation should not be based solely on changes in methane production. It should also consider the extent of freshwater substitution, the reduction in liquid digestate requiring further management, additional energy consumption for pumping, mixing, and separation, and the properties of the final digestate stream [1,79].

From a circular-economy perspective, digestate recirculation increases the internal reuse of water and potentially valuable constituents. At the same time, a higher degree of loop closure promotes the retention of compounds that are not completely degraded or removed. Environmental benefits should therefore be assessed together with the operational consequences of their gradual accumulation. Such a plant-wide approach is particularly relevant to agricultural biogas plants, where storage, transport, treatment, and agricultural use of digestate may represent a substantial part of operational requirements [1].

7.1. Water Savings and Reduction in Process Wastewater Generation

Water demand is an important operational consideration in anaerobic digestion, particularly when relatively dry agricultural substrates such as crop residues, straw, or manure-based mixtures require dilution to ensure suitable moisture content, pumpability, and mixing conditions. In such systems, the liquid fraction of digestate can partially replace freshwater used for substrate preparation, thereby converting part of the outgoing stream into an internal process stream.

The magnitude of this effect has been demonstrated experimentally. Zheng et al. [79] investigated liquid digestate recirculation at different straw-to-manure ratios. Under the most favorable conditions, with a straw-to-manure ratio of 3:1, recirculation reduced clean-water consumption by 65.4% and liquid digestate discharge by 81.9% per 1 GJ of energy produced [79]. These benefits were nevertheless dependent on feedstock composition. Recirculation improved process performance in straw-rich systems, whereas manure-rich configurations were more susceptible to ammonia and organic acid accumulation [79]. Water-saving potential should therefore be evaluated in relation to feedstock composition and process stability rather than treated as a universally achievable value.

Similar conclusions arise from long-term studies involving lignocellulosic substrates. Hu et al. [9] showed that direct and aerated recirculation of the liquid digestate fraction during maize stover digestion could reduce the amount of liquid digestate requiring further management and provide moisture to the substrate. At the same time, progressive accumulation of COD and ammonia nitrogen was observed during 360 days of operation in the direct-recirculation system [9]. Reducing external water demand and liquid discharge therefore does not eliminate the need to control the internal accumulation of dissolved constituents.

Plant-scale modelling indicates that even greater water savings may theoretically be achievable. Yuan et al. [11] incorporated liquid digestate recirculation into an ADM1-based model of a 2.5 MW agricultural biogas plant co-digesting wheat straw and cattle manure. At high recirculation levels, the model predicted reductions in fresh process-water demand of up to approximately 95% [11]. These results should, however, be interpreted cautiously because steady-state modelling does not fully reproduce long-term constituent accumulation, microbial adaptation, or changes in the physical properties of the system. Accordingly,

the 65.4% reduction reported by Zheng et al. [79] represents an experimentally observed outcome under defined feedstock and operating conditions, whereas the approximately 95% reduction reported by Yuan et al. [11] represents a model-derived potential rather than a value demonstrated in full-scale operation. These values should therefore not be directly compared or interpreted as generally achievable water-saving efficiencies for agricultural biogas plants.

This limitation is particularly relevant in highly closed-loop systems. Conventional ADM1 formulations do not fully account for the accumulation of conservative and poorly biodegradable compounds, long-term microbial adaptation, or mass-transfer limitations associated with rheological changes [80,81]. Models used to assess intensive digestate recirculation should therefore incorporate these phenomena more explicitly when predicting practical operating limits.

Water savings represent only one component of the environmental benefit. Digestate recirculation also reduces the volume of liquid digestate requiring storage, transport, treatment, or discharge. This may reduce the need for processes such as nutrient recovery, biological treatment, membrane separation, or evaporation, all of which require additional infrastructure and energy [1].

A reduction in liquid digestate volume should not, however, be equated with true zero liquid discharge. As loop closure increases, the removal of salts, nitrogen compounds, refractory COD, and other conservative constituents is reduced. In practice, a controlled purge or additional treatment may therefore remain necessary to prevent their indefinite accumulation. A smaller outgoing stream may simultaneously contain higher concentrations of retained constituents, and both its volume and composition should therefore be considered in the plant water balance [9].

Experimental studies indicate that water-use reductions exceeding 60% can be achieved under favorable agricultural co-digestion conditions [79], whereas plant-scale modelling suggests that even greater reductions may be possible [11]. However, the practical value of increased loop closure depends on maintaining an appropriate internal mass balance and adequate removal of accumulating constituents [1,9,11,79].

The currently available evidence on water savings remains limited and methodologically heterogeneous. Only a small number of studies report directly quantifiable reductions in freshwater demand, while others describe water substitution or reduced liquid-digestate discharge qualitatively. In addition, reported values are based on different system configurations, feedstocks, recirculation strategies, and calculation approaches. Consequently, the available results do not yet support a standardized cross-study comparison of water-saving efficiencies. Future studies should therefore report water balances using consistent definitions and system boundaries to enable more robust comparison between digestate recirculation strategies.

7.2. Effects on Methane Production and Energy Balance

The energetic significance of digestate recirculation should not be assessed solely on the basis of changes in methane yield or volumetric methane production. Although increased methane recovery raises the amount of chemical energy contained in the produced biogas, recirculation also introduces additional energy requirements associated with material transport, pumping, mixing, separation, and, depending on plant configuration, reheating of the recycled stream. Therefore, the appropriate criterion for plant-scale assessment is the net energy balance rather than methane production alone.

Recirculation can increase energy recovery by returning residual biodegradable organic matter to the digester. Brémond et al. [42], who evaluated solid digestate recirculation in five agricultural CSTR biogas plants, estimated that direct recirculation could increase

methane production by approximately 0.6–6.3%, depending on the selected strategy and plant characteristics. In some scenarios, recirculation could also partially substitute fresh feedstock, reducing its requirement by 64–1431 t year^{-1} [42]. The energetic benefit therefore does not arise solely from an increase in specific methane yield, but also from recovering residual methane potential that would otherwise leave the system with the digestate.

Increasing the utilization of residual organic matter does not, however, automatically justify additional energy-intensive treatment of the recycled fraction. Brémond et al. [42] showed that thermo-chemical treatment increased the biodegradability of solid digestate by approximately 30–46%, but the associated costs were not compensated by the additional methane recovered. Under the investigated conditions, direct recirculation without intensive additional treatment was more favorable [42]. This result highlights the distinction between maximizing methane recovery and maximizing overall process energy efficiency.

A positive energetic effect has also been demonstrated in multi-stage digestion systems. Wu et al. [40] reported that recirculation in temperature-phased anaerobic digestion increased methane yield by more than 10% under the most demanding operating conditions and simultaneously increased net energy output by approximately 10% [40]. This benefit was associated with improved utilization of organic matter and more effective interaction between individual process stages.

The energetic value of recirculation nevertheless depends on the biodegradability of the recycled material. A stream containing a substantial fraction of residual biodegradable organic matter may provide an additional source of methane, whereas recirculation of a fraction dominated by refractory compounds increases internal material flow without a proportional increase in energy recovery. Potential benefits should therefore be assessed in relation to the residual biomethane potential of the recycled fraction, separation efficiency, and the amount of fresh substrate that recirculation can supplement or partially replace [42]. Thermal requirements are also an important component of the energy balance. If the recycled stream returns to the digester at a temperature below the digestion temperature, reheating is required. The significance of this demand increases with the recirculation ratio and depends, among other factors, on process temperature, heat losses, and the degree of heat-recovery integration within the plant.

Recirculation may also increase solids retention time, allowing further degradation of slowly biodegradable material without increasing reactor volume. Brémond et al. [42] estimated that direct solid digestate recirculation could increase SRT by approximately 11–38%, while simultaneously increasing reactor solids content by approximately 6–20% [42]. This illustrates a clear trade-off between additional methane recovery and increasing operational requirements.

At plant scale, the energetic benefit of recirculation should be evaluated from the net energy balance, including both additional methane recovery and the energy required for recycling, conditioning, and handling the increased internal material flow [40,42].

7.3. Energy Demand for Pumping, Mixing, and Separation

The additional mechanical energy required for digestate circulation, homogenization, and separation represents one of the main auxiliary energy demands associated with recirculation. Its magnitude depends not only on the volume of the recycled stream but also on the physical properties of the digestate and on changes occurring in the reactor contents as a whole. Increasing solids content and viscosity increase flow resistance and consequently raise the energy demand associated with pumping and mixing [31].

The significance of this effect was demonstrated by Ni et al. [52] during pig-manure digestion with liquid digestate recirculation. At the highest applied OLR, sludge viscosity increased from approximately 30 to 1000 mPa·s, accompanied by impaired mass

transfer and a decline in methane production [56]. From an energy-balance perspective, this means that increasing viscosity may require progressively greater energy input to maintain adequate homogenization, without guaranteeing a proportional improvement in biological performance.

Mixing can account for a substantial share of the electricity consumed in biogas plants. Its requirements depend on reactor geometry, solids concentration, mixer design, and operating regime [31,80]. Insufficient mixing may lead to heterogeneity, sedimentation, and zones with limited mass transfer, whereas excessive mixing increases energy consumption and does not necessarily improve digestion efficiency [80]. Increasing viscosity should therefore not automatically be compensated by a proportional increase in mixer power; optimization of operating time, mixing intensity, and mixing strategy may provide a more energy-efficient approach.

Similar relationships apply to pumping. Increasing viscosity and yield stress raise pressure losses in pipelines and may reduce pump efficiency, particularly when the equipment is not designed to handle more concentrated slurries [31]. This effect becomes increasingly relevant in systems where digestate is repeatedly transported between the digester, separator, storage tanks, or conditioning units.

Additional energy demand also arises when digestate is separated into liquid and solid fractions. Carraro et al. [43], based on 175 full-scale observations, showed that centrifuges achieved higher solids and phosphorus separation efficiencies than screw presses but required substantially more energy. Screw presses consumed approximately 4.5 times less energy than centrifuges when processing raw digestate [43]. The higher separation efficiency of centrifuges may additionally be supported by polymer addition, which further increases operating costs and chemical consumption.

The choice of separation technology should therefore be evaluated in the context of the entire recirculation system. A more efficient separator may reduce the amount of suspended solids returned to the digester and thereby limit rheological loading, but at the same time increase direct electricity consumption. Conversely, a lower-energy device may leave a larger proportion of fine solids in the recycled fraction, promoting their gradual accumulation [43].

From a plant-wide perspective, the lowest energy consumption of an individual device does not necessarily correspond to the lowest overall energy demand of the biogas plant. The final balance depends on the recirculated flow rate, rheological properties of the material, mixing regime, pumping configuration, and separation technology. Therefore, the energy required for pumping, mixing, and separation should be explicitly included in the energetic assessment of digestate recirculation strategies rather than treated as a fixed auxiliary load.

At plant scale, selection of a recirculation strategy should therefore be based on a combined assessment of four interacting factors: (i) water savings and reduction in liquid digestate discharge, (ii) additional methane and net energy recovery, (iii) auxiliary energy demand for pumping, mixing, separation, and reheating, and (iv) the risk of solids, salts, nitrogen compounds, and refractory organic matter accumulation. A favorable operating strategy is one in which resource savings and additional energy recovery are achieved without progressive deterioration in process stability, excessive auxiliary energy demand, or loss of digestate quality. This framework shifts the objective from maximizing a single parameter, such as recirculation ratio, methane production, or water substitution, toward identifying a plant-specific operating range that provides the best overall balance among resource efficiency, energy performance, and process stability.

At plant scale, the net energetic effect of digestate recirculation can be conceptualized as the difference between additional methane-derived energy and the additional energy

required for pumping, mixing, separation, reheating, and conditioning of the recycled stream [31,34,42,43].

$$\Delta E_{\text{net}} = \Delta \text{E}_{\text{CH}_4} - \left(E_{\text{pumping}} + E_{\text{mixing}} + E_{\text{separation}} + E_{\text{reheating}} + E_{\text{additional treatment}}\right) \quad (1)$$

where ΔE_{net} represents the change in net energy output associated with digestate recirculation, ΔE_{CH4} represents the additional energy recovered from methane, and the remaining terms represent the additional energy required for pumping, mixing, separation, reheating, and conditioning of the recycled stream. A positive ΔE_{net} therefore indicates that the additional methane-derived energy exceeds the auxiliary energy demand associated with the recirculation strategy.

The available quantitative evidence illustrates the magnitude of these trade-offs but also shows why a single universal economic value cannot be assigned to digestate recirculation. Direct solid-digestate recirculation has been associated with methane-production increases of approximately 0.6–6.3% and potential fresh-feedstock savings of 64–1431 t year^{-1}, whereas temperature-phased digestion with recirculation has been reported to increase net energy output by approximately 10% [34,42]. At the same time, additional treatment may offset these benefits: thermo-chemical conditioning increased digestate biodegradability by approximately 30–46%, but the associated costs were not compensated by the additional methane recovered [42]. Separation technology also strongly affects operating expenditure; screw presses required approximately 4.5 times less energy than centrifuges for raw digestate, although centrifuges achieved higher solids and phosphorus separation efficiencies [43]. Consequently, the economic effect of recirculation depends on the balance between additional methane recovery and avoided water, feedstock, storage, or digestate-management costs on the one hand, and additional energy, chemical, maintenance, and equipment requirements on the other. CAPEX is mainly associated with any additional pumping, separation, heat-recovery, storage, or conditioning infrastructure required, whereas OPEX is primarily influenced by electricity demand, chemical use, maintenance, and handling of the recirculated stream. Because the available studies use different plant configurations, system boundaries, and cost assumptions, these CAPEX and OPEX effects cannot yet be compared on a uniform monetary basis and should therefore be interpreted as system-specific.

7.4. Effects on Digestate Management and Agricultural Use

Digestate recirculation affects not only reactor operation but also the quantity and composition of the material ultimately leaving the biogas plant. Returning part of the liquid or solid fraction can reduce the external digestate stream while increasing the internal retention of nutrients, salts, trace elements, and residual organic matter. This may improve resource utilization, but it can also modify the fertilizer value and environmental safety of the final digestate.

Digestate represents a potential source of nitrogen, phosphorus, potassium, and organic matter, while anaerobic digestion increases the proportion of nitrogen present in ammonium form, which is directly available to plants [1,5]. Its fertilizer value nevertheless depends not only on total nutrient concentrations but also on their chemical forms, plant availability, soil conditions, and crop nutrient requirements.

Recirculation may alter the distribution of nutrients between digestate fractions. Ammonium and potassium remain predominantly in the liquid phase, whereas a larger proportion of phosphorus is associated with suspended and solid material. Therefore, both the separation method and the choice of the recycled fraction influence the composition of the material ultimately removed from the plant [43]. Intensive recirculation of the liquid fraction may increase the internal retention of nitrogen and potassium, whereas solids separation may concentrate phosphorus in the stream intended for further utilization.

Reducing digestate volume can lower storage and transport requirements and may produce a more concentrated fertilizer product. However, it does not reduce the total nutrient load entering the plant. Recirculation primarily redistributes these nutrients and may concentrate them in the final outgoing stream. Under conditions of local nitrogen or phosphorus surplus, this may restrict direct agricultural application and increase the risk of nutrient losses to surface water and groundwater [5,77]. Salinity is another important parameter. Dissolved salts are poorly removed during anaerobic digestion and may accumulate under intensive liquid digestate recirculation. Elevated electrical conductivity may restrict digestate application to soils vulnerable to salinization or to salt-sensitive crops [77]. Agricultural suitability is also influenced by the degree of organic matter stabilization. Recirculation may prolong the contact of residual biodegradable matter with active biomass and thereby support further degradation. However, if the loading exceeds the conversion capacity of the system, VFAs or refractory COD may accumulate. In such cases, the final digestate may require additional stabilization before agricultural application [5]. Quality control should also include heavy metals and other persistent contaminants. Anaerobic digestion does not remove metals, and the reduction in organic matter mass may increase their concentrations on a dry-matter basis. Their environmental significance, however, depends not only on total concentration but also on chemical form, mobility, and bioavailability [78].

When feedstocks may contain pharmaceuticals, veterinary antibiotics, hormones, or other micropollutants, their potential retention within a closed-loop system should also be considered. Removal efficiencies during anaerobic digestion strongly depend on compound properties and process conditions, and some persistent substances may remain in both the liquid and solid fractions [79]. Available evidence is nevertheless insufficient to conclude that digestate recirculation universally increases the concentrations of individual micropollutants. This risk should therefore be assessed primarily in relation to feedstock origin. Recent reviews also identify PFAS and microplastics as emerging concerns in the safe utilization of digestate [80]. From an agricultural perspective, the final digestate should therefore be treated as a fertilizer product of defined quality rather than solely as a residual stream. Its assessment should include nitrogen, phosphorus and potassium availability, organic matter stability, pH, electrical conductivity, VFA concentration, metals, and, where justified by feedstock origin, organic contaminants [5,77–80].

Under intensive recirculation, these parameters should be monitored as trends rather than as isolated measurements. A progressive increase in electrical conductivity, TAN or ammonium concentration, residual VFAs or refractory COD, or concentrations of metals and feedstock-specific organic contaminants may indicate that internal retention is exceeding the removal capacity of the system. In such cases, a controlled purge of the recirculated stream, partial replacement with fresh process water, or additional treatment should be considered to restore the material balance and maintain the agronomic suitability of the final digestate. The choice of intervention should remain system-specific and should account for feedstock composition, the recycled digestate fraction, local fertilizer requirements, and the intended agricultural use of the digestate.

The optimum recirculation strategy from the perspective of reactor operation may not be optimal for final digestate utilization. A high recirculation ratio may reduce the volume requiring storage and transport, but excessive concentration of salts, nitrogen compounds, or contaminants may restrict direct agricultural application. Recirculation strategy should therefore balance reduction in digestate quantity with maintenance of adequate fertilizer quality and environmental safety.

8. Conclusions

- Digestate recirculation should be treated as a system-specific process-management strategy. Its effects depend on the recycled fraction, feedstock characteristics, reactor configuration, solids content, organic loading rate, and operating conditions; therefore, no universal optimum recirculation ratio can be defined.
- The benefits of recirculation are not proportional to recirculation intensity. Under appropriate conditions, recirculation may reduce freshwater demand, improve biomass and residual-organic-matter retention, and increase volumetric methane production. Excessive recirculation may instead promote the accumulation of TAN, VFAs, soluble COD, salts, and solids and impair mixing and mass transfer.
- Recirculation performance should be evaluated using an integrated set of process indicators. Methane production should be interpreted together with organic loading, solids retention, process stability, and the accumulation of potentially inhibitory compounds.
- Further research should prioritize full-scale validation and standardized assessment. Long-term studies, standardized definitions of recirculation ratio, and validated monitoring indicators are needed to establish operating limits for specific digestion systems.

Practical Recommendations

- Increase recirculation gradually. Recirculation should be introduced stepwise under otherwise stable operating conditions to distinguish its effects from changes in OLR or feedstock composition.
- Monitor key process indicators. Routine monitoring should include methane production, VFAs, alkalinity, TAN, COD/SCOD, TS/VS and, where relevant, viscosity and mixing performance. Trends should be assessed relative to the stable baseline of the individual plant rather than to universal threshold values.
- Recognize early warning signals. Persistent increases in VFAs, TAN, SCOD or solids, declining methane production, deteriorating mixing, or loss of buffering stability should be treated as signs that recirculation intensity may be excessive.
- Reduce recirculation and reassess process stability. If adverse trends persist, the recirculation rate should be reduced before further changes in OLR are introduced. Where conservative compounds continue to accumulate, controlled purge or additional treatment of the recycled stream may be required.

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Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Full term
AD	Anaerobic Digestion
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred-Tank Reactor
ddPCR	Droplet Digital Polymerase Chain Reaction
EC	Electrical Conductivity
HSAD	High-Solids Anaerobic Digestion
HRT	Hydraulic Retention Time
LDR	Liquid Digestate Recirculation
LFD	Liquid Fraction of Digestate
MAGs	Metagenome-Assembled Genomes
OLR	Organic Loading Rate
qPCR	Quantitative Polymerase Chain Reaction
SAO	Syntrophic Acetate Oxidation
SAOB	Syntrophic Acetate-Oxidizing Bacteria
SCOD	Soluble Chemical Oxygen Demand
SMY	Specific Methane Yield
SRB	Sulfate-Reducing Bacteria
TAN	Total Ammonia Nitrogen
TPAD	Temperature-Phased Anaerobic Digestion
TPAD-R	Temperature-Phased Anaerobic Digestion with Recirculation
TS	Total Solids
TVFAs	Total Volatile Fatty Acids
TVFA/TA	Total Volatile Fatty Acids-to-Total Alkalinity Ratio
VFA	Volatile Fatty Acid
VFAs	Volatile Fatty Acids
VMP	Volumetric Methane Production
VS	Volatile Solids

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