

Review

Plant-Derived Bioactive Compounds in Agricultural Waste Anaerobic Digestion: Mechanisms of Inhibition, Process Stability and Methane Production

Anna Rygała-Galewska *  and Kinga Borek 

Institute of Technology and Life Sciences—National Research Institute, Falenty, 3 Hrabaska Avenue, 05-090 Raszyn, Poland; k.borek@itp.edu.pl

* Correspondence: a.rygalo-galewska@itp.edu.pl

Abstract

Anaerobic digestion (AD) plays a key role in the circular bioeconomy by converting organic waste into renewable energy and facilitating the sustainable utilisation of waste materials. Agricultural and agro-industrial by-products are increasingly recognised as valuable AD feedstocks due to their widespread availability and significant bioenergy potential. However, many of these substrates contain plant-derived bioactive compounds, such as polyphenols, tannins, flavonoids and terpenes, which can influence microbial communities and process performance. Depending on their concentration and chemical characteristics, these compounds may inhibit microbial activity, impair process stability, and ultimately decrease methane production. This review critically synthesises current knowledge on the occurrence, bioavailability and biological activity of plant-derived bioactive compounds in agricultural feedstocks used for anaerobic digestion, with particular emphasis on their implications for process performance and reactor stability. The principal mechanisms through which phytochemicals influence anaerobic digestion include enzyme inhibition, membrane disruption, interference with syntrophic interactions and trace metal chelation. The available evidence demonstrates a pronounced dose-dependent response, whereby low concentrations may exert neutral or selective modulatory effects. In contrast, elevated concentrations disrupt microbial activity, leading to volatile fatty acid accumulation, prolonged lag phases and reduced methane production. Current mitigation strategies include substrate pretreatment, co-digestion, microbial adaptation, adsorbent-assisted detoxification and the use of DIET-promoting materials. An integrated evidence matrix is proposed to link phytochemical composition with reactor configuration, operational parameters and mitigation strategies, thereby providing a practical framework for feedstock-specific process optimisation. Overall, the available evidence demonstrates that reliable evaluation of agricultural feedstocks should extend beyond conventional biochemical methane potential assessment to incorporate phytochemical composition, microbial functional responses and key operational parameters. Such an integrated approach can improve the prediction of methane recovery and support evidence-based optimisation of anaerobic digestion within circular bioeconomy systems.

Keywords: anaerobic digestion; agricultural waste; bioactive compounds; methane yield; process inhibition; co-digestion; lignocellulosic biomass; circular bioeconomy



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1. Introduction

Anaerobic digestion (AD) is an important technology that supports the development of the bioeconomy and the circular economy. This process facilitates the concurrent valorisation of diverse organic waste streams and the production of renewable biogas, while generating digestate with considerable fertilising potential [1–3]. In agriculture, AD integrates waste management, renewable energy production, and nutrient recycling within the carbon, nitrogen, and phosphorus cycles [4,5].

The range of substrates investigated for anaerobic digestion has broadened considerably in recent years. Alongside conventional feedstocks such as manure and maize silage, increasing attention has been given to agricultural and agro-industrial residues, many of which contain high concentrations of plant secondary metabolites [6–8]. Examples include fruit-processing residues, pomace, winery by-products, citrus and olive wastes, herbal residues, and leafy biomass. Besides their high organic matter content, these substrates often contain plant-derived bioactive compounds, including polyphenols, flavonoids, terpenes, alkaloids, saponins, and essential oils [7,9].

In plants, bioactive metabolites serve protective and allelopathic functions. Under anaerobic digestion conditions, these compounds can substantially affect microbial communities. High concentrations may reduce methane production, promote volatile fatty acid accumulation, and destabilise digestion. These effects are characterised by a reduction in methane yield, an accumulation of volatile fatty acids (VFAs), and a destabilisation of pH [10–12]. Polyphenol- and terpene-rich residues, such as winery waste, olive-processing by-products, and aromatic plant biomass, often exhibit particularly strong inhibitory effects.

The effects of plant-derived bioactive compounds are not exclusively inhibitory. Several studies have shown that, at low or moderate concentrations, certain compounds can enhance specific stages of anaerobic digestion. These effects include improved hydrolysis, reduced competition for substrates by non-methanogenic microorganisms, and changes in microbial community composition that favour process stability [10,13].

Despite the growing body of literature addressing the influence of inhibitory compounds on anaerobic digestion, the available evidence remains fragmented and difficult to translate into engineering practice. Most experimental studies investigate individual feedstocks, selected classes of phytochemicals, or laboratory-scale reactor systems under highly variable operational conditions. Moreover, substantial differences in analytical methods, inhibitor quantification, concentration units (e.g., $\text{mg}\cdot\text{g}^{-1}$ VS versus $\text{mg}\cdot\text{L}^{-1}$), inoculum characteristics, reactor configurations, and operating conditions considerably hinder direct comparison among studies and limit the establishment of broadly applicable inhibitory thresholds [14–16]. Consequently, quantitative relationships among bioactive compound concentrations, microbial responses, process stability, and methane production remain insufficiently characterised, thereby limiting the development of practical recommendations for agricultural biogas systems.

Several recent reviews have discussed inhibitory compounds and mitigation strategies in anaerobic digestion, including the comprehensive review by Gao et al. [17]. However, these reviews primarily address anaerobic digestion from a broad process perspective and encompass a range of organic substrates. Unlike municipal organic waste or mixed food waste, agricultural and agro-industrial residues are characterised by highly heterogeneous lignocellulosic matrices and naturally occurring plant secondary metabolites whose occurrence, chemical composition, bioavailability, and inhibitory potential vary considerably among feedstocks. These substrate-specific characteristics create unique challenges for microbial adaptation, process stability, and reactor operation that have not yet been comprehensively synthesised within the context of agricultural biomass.

Rather than providing another general overview of anaerobic digestion inhibition, the present review specifically focuses on agricultural and agro-industrial feedstocks. It integrates current knowledge on the occurrence of plant-derived bioactive compounds, their physicochemical characteristics, mechanisms of microbial inhibition across successive stages of anaerobic digestion, microbial community adaptation, and engineering strategies for mitigating phytochemical-induced process instability. Rather than merely summarising the available literature, this review critically evaluates the consistency of published evidence, identifies current knowledge gaps, and discusses the practical implications of existing findings for the operation of agricultural anaerobic digestion systems. Particular emphasis is placed on dose-dependent responses, interactions between feedstock composition and microbial processes, and the implications for maintaining stable methane production under agricultural operating conditions.

By integrating evidence dispersed across studies involving different agricultural residues, this review provides a mechanistic framework linking feedstock composition, plant-derived bioactive compounds, microbial responses, and reactor performance. The resulting synthesis is intended to support substrate selection, process optimisation, and future research aimed at improving the stability, resilience, and methane productivity of anaerobic digestion systems processing agricultural biomass.

Literature Search Strategy

The present review was developed using a structured literature search followed by a critical narrative synthesis of the available evidence. Although this manuscript is presented as a narrative review rather than a formal systematic review, the literature identification, screening and selection process followed key principles of the PRISMA 2020 statement to improve the transparency and reproducibility of the review methodology.

The literature search was performed using four major scientific databases: Scopus, Web of Science Core Collection, PubMed, and Google Scholar. Publications from January 2000 to March 2026 were considered, with particular emphasis on studies from the last five years to capture recent advances in anaerobic digestion, microbial ecology, omics technologies, and process optimisation. The final database search was completed in May 2026.

The search strategy combined controlled vocabulary and free-text keywords related to anaerobic digestion, agricultural and agro-industrial residues, plant-derived bioactive compounds, phytochemicals, phenolic compounds, tannins, flavonoids, terpenes, alkaloids, saponins, methane production, microbial communities, metagenomics, biochar, hydrochar and direct interspecies electron transfer (DIET). Boolean operators (AND, OR) and database-specific search syntax were applied to maximise retrieval efficiency. In addition, the reference lists of relevant review papers and key original publications were manually screened to identify further studies not retrieved during the initial database search.

Eligible publications were limited to peer-reviewed articles published in English that investigated the occurrence, transformation or biological effects of plant-derived bioactive compounds during anaerobic digestion of agricultural or agro-industrial biomass. Both original experimental studies and high-quality review articles were considered when they provided information on inhibitory mechanisms, microbial responses, reactor performance, operational parameters or mitigation strategies. Studies focusing exclusively on municipal wastewater treatment, aerobic biodegradation, composting, rumen fermentation, or other biological systems that are not directly relevant to anaerobic digestion were excluded. Conference abstracts, editorials, patents and publications lacking sufficient methodological information were also excluded.

The relevance of retrieved publications was evaluated through sequential screening of titles, abstracts and full texts. Particular attention was given to studies reporting quantitative information on phytochemical concentrations, inhibitory thresholds, reactor operating conditions, microbial adaptation and process performance. Greater weight was assigned to investigations employing well-characterised substrates, continuous reactor systems, advanced molecular techniques (including metagenomics, metatranscriptomics and metaproteomics) and comprehensive analytical characterisation of plant-derived bioactive compounds.

The final evidence synthesis integrated findings from the most methodologically robust and scientifically relevant studies to provide a comprehensive assessment of the occurrence, mechanisms of action, inhibitory effects and mitigation strategies associated with plant-derived bioactive compounds during anaerobic digestion of agricultural biomass.

2. Agricultural Waste as Feedstock for Anaerobic Fermentation

2.1. Types and Origin of Agricultural Waste

Agricultural waste is among the most widely used substrate groups in anaerobic digestion. These materials include lignocellulosic residues such as cereal straw, maize stalks, rice straw, and post-harvest residues, as well as agro-industrial by-products, including pomace, pulps, and fruit- and vegetable-processing waste [6,18] (Table 1). Agricultural residues are generated in large quantities worldwide, although a substantial proportion remains underutilised [19].

Table 1. Typical characteristics of major agricultural feedstocks relevant to anaerobic digestion.

Feedstock	Predominant Bioactive Compounds	Typical Lignin (% DM)	Typical C/N Ratio	Principal Phytochemical-Related Limitation	References
Olive pomace	Hydroxytyrosol, tyrosol, oleuropein derivatives, phenolic acids, tannins	22–35	30–45	High phenolic load; inhibition of methanogens; VFA accumulation	[20–22]
Grape pomace	Polyphenols, flavonoids, condensed tannins, anthocyanins	20–35	25–40	Phenolic enzyme inhibition; reduced hydrolysis; concentration-dependent methanogenic inhibition	[23–25]
Citrus processing waste	Monoterpenes (mainly <i>d</i> -limonene), flavonoids, essential oils	3–10	25–35	Membrane disruption; methanogenic inhibition; prolonged lag phase; VFA accumulation	[11,26,27]
Apple pomace	Phenolic acids, flavonoids, tannins	15–22	30–45	Rapid acidification combined with phenolic stress	[28–30]
Herbal and aromatic plant residues	Essential oils, terpenoids, phenolic acids	10–18	20–30	Strong antimicrobial activity; membrane disruption; concentration-dependent inhibition of hydrolysis and methanogenesis	[27,31,32]
Wheat straw	Ferulic acid, <i>p</i> -coumaric acid, lignin-bound phenolics	15–22	60–100	Structural recalcitrance and release of lignin-derived phenolics during pretreatment	[33–35]
Rice straw	Ferulic acid, <i>p</i> -coumaric acid, tannins, lignin-derived phenolics	8–15	50–80	High lignin/silica content; phenolic inhibition following pretreatment; reduced methane kinetics	[36–38]
Maize stover (corn stover)	Ferulic acid, vanillic acid, hydroxybenzoic acid, lignin-derived phenolics	12–18	45–70	Limited hydrolysis; release of phenolic acids during pretreatment; inhibition at elevated phenol concentrations	[39–41]
Maize silage	Low concentrations of phenolic acids; lignin-associated phenolics	4–7	25–35	Generally low inhibition risk; recalcitrance mainly associated with lignocellulosic fraction	[42–44]
Sugar beet pulp	Low levels of phenolic acids; pectin-associated phenolics	1–3	20–30	Low lignin content; phenolic compounds may form after severe pretreatment; rapid acidification	[45–47]

Typical lignin contents and C/N ratios represent approximate ranges compiled from published studies and may vary depending on plant cultivar, agronomic conditions, harvesting stage, processing technology, and analytical methodology. Therefore, the reported values should be interpreted as representative rather than universal.

Lignocellulosic residues are characterised by high organic carbon content and considerable energy potential but often exhibit low biodegradability due to their complex

structure [48,49]. In contrast, fruit- and vegetable-processing residues contain readily fermentable compounds, such as soluble sugars and pectins, but are often rich in plant-derived bioactive compounds [50,51].

Pomace, pulps and associated agro-industrial residues represent a particularly intriguing group of substrates from the standpoint of integrated bioenergy systems. Certain agro-industrial residues, including olive pomace and citrus-processing waste, contain high concentrations of polyphenols and essential oils that may influence anaerobic digestion performance [11,50]. The chemical heterogeneity of agricultural waste indicates that it cannot be regarded as a uniform substrate category; rather, it requires individual characterisation of its suitability for AD applications.

Despite their considerable bioenergy potential, agricultural residues cannot be evaluated solely based on conventional compositional parameters such as lignocellulose content or C/N ratio. Growing evidence indicates that naturally occurring phytochemicals are an additional substrate property that can substantially modify microbial activity and reactor performance. Consequently, agricultural feedstocks should be characterised not only by their biochemical methane potential but also by their bioactive compound profile.

2.2. Chemical and Structural Composition of Substrates

Most agricultural residues consist primarily of cellulose, hemicellulose and lignin, which together form a structurally complex matrix with limited enzymatic accessibility [49,52]. The interactions between these fractions, facilitated by covalent and hydrogen bonds, result in a compact, highly organised matrix with limited enzymatic accessibility.

The recalcitrant structure of lignocellulosic biomass limits hydrolysis, which is often the rate-limiting step of anaerobic digestion. Lignin restricts enzyme access to structural carbohydrates, while cellulose crystallinity further reduces degradation efficiency [53,54]. Furthermore, the high crystallinity of cellulose has been shown to reduce its degradation rate, resulting in lower actual methane yields relative to the substrate's theoretical biochemical methane potential [55].

Pretreatment is often required to improve substrate accessibility and enhance methane production from lignocellulosic residues [56,57]. In the absence of appropriate pretreatment, the anaerobic digestion of recalcitrant lignocellulosic feedstocks frequently results in reduced methane yields and increased process instability, particularly in continuous reactor systems, where prolonged exposure to inhibitory compounds and limited hydrolysis may progressively impair microbial activity [11,58,59].

In addition to structural constraints, many agricultural residues contain bioactive plant metabolites such as polyphenols, tannins and essential oils. These compounds may interact with proteins and carbohydrates, reduce substrate bioavailability, and alter microbial activity during anaerobic digestion [10,27]. Consequently, the structural and chemical composition of agricultural residues determines not only their bioenergy potential, but also the risk of microbial inhibition, with direct implications for the design and operation of stable AD systems. Importantly, structural recalcitrance and phytochemical-mediated inhibition should not be regarded as independent constraints. During the hydrolysis of lignocellulosic biomass, lignin-associated phenolic compounds may be progressively released into the liquid phase, resulting in delayed inhibitory effects that become particularly relevant during prolonged operation of continuous anaerobic digesters [10,60,61].

2.3. Fermentative Value and Technological Limitations

The fermentative value of agricultural residues depends primarily on the C/N ratio, the availability of soluble carbohydrates, and the proportion of readily biodegradable organic matter [6,48] (see Figure 1).

The C/N ratio is a fundamental indicator of biomass suitability for anaerobic digestion, as it governs the metabolic balance between microbial biomass synthesis and methane production. Lignocellulosic agricultural residues, such as straw and post-harvest remains, are characterised by high C/N ratios (often exceeding 40), which may result in nitrogen deficiency, restricted microbial growth and reduced fermentation efficiency [49]. Conversely, fruit- and vegetable-processing wastes typically exhibit lower C/N ratios; however, in many cases, they still fall outside the optimal range for AD, which is generally considered to be 20–30 [48].

Fruit-processing residues often contain large amounts of soluble sugars and pectins, which are rapidly converted into VFAs during hydrolysis and acidogenesis. Although this can accelerate biogas production, it may also increase the risk of process instability [17,50,51].

The proportion of soluble organic matter is another important factor affecting the fermentative value of agricultural residues. Fruit- and vegetable-processing wastes are often characterised by high concentrations of readily available soluble compounds, which facilitate rapid organic matter conversion. However, the rapid degradation of these substrates may also promote volatile fatty acid accumulation and pH decline under unfavourable operating conditions [11,58]. In contrast, lignocellulosic residues contain relatively low amounts of soluble organic matter, resulting in slower hydrolysis and longer hydraulic retention times (HRT) required for efficient methane production [55].

The presence of plant-derived bioactive compounds further influences the performance of agricultural residues in anaerobic digestion. Polyphenols, tannins, and terpenes can reduce substrate availability by interacting with structural carbohydrates and may also inhibit fermentative microorganisms [62]. As a result, the actual fermentative value of these substrates may be considerably lower than expected based on their chemical composition alone. This effect is particularly evident in olive- and citrus-processing residues, where high concentrations of bioactive compounds can reduce methane productivity despite the substantial energy potential of these waste streams [11,50].

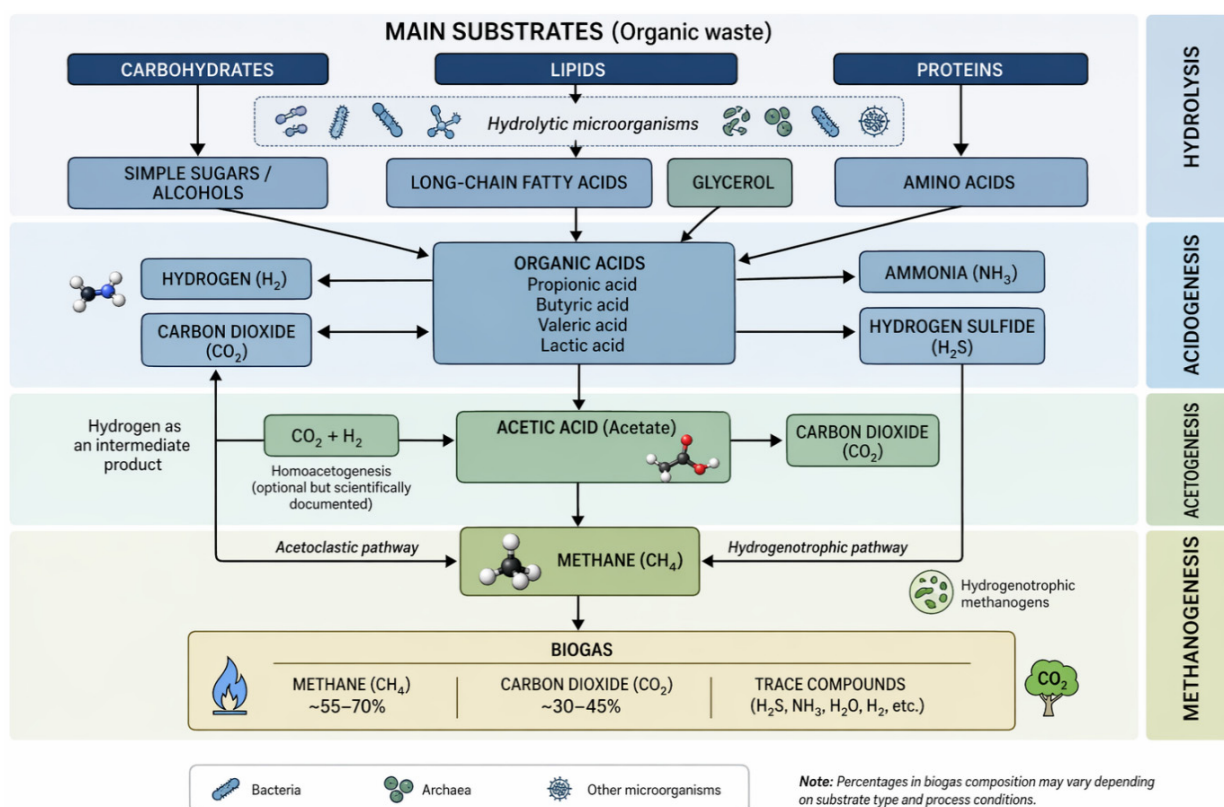


Figure 1. Anaerobic degradation pathways [10,63,64].

To overcome these limitations, co-digestion is commonly applied to improve nutrient balance and process stability. Operational parameters, such as organic loading rate and hydraulic retention time, should also be adjusted to the substrate's characteristics [7,63].

However, the relative contributions of substrate composition, phytochemical content, and reactor operating conditions to overall methane productivity remain difficult to quantify because most available studies differ substantially in feedstock preparation, reactor design, inoculum source, and analytical methodology. The practical significance of these limitations is particularly evident in continuous reactor systems, where prolonged exposure to inhibitory compounds and increasing organic loading rates may amplify phytochemical-induced process instability compared with short-term batch assays.

3. Occurrence, Mechanisms of Action and Microbial Responses to Plant-Derived Bioactive Compounds During Anaerobic Digestion

3.1. Sources of Bioactive Compounds in Agricultural Waste

Agricultural and agro-industrial residues naturally contain a broad spectrum of plant-derived bioactive compounds originating from secondary plant metabolism. Unlike synthetic inhibitors, these substances are intrinsic constituents of plant biomass and are therefore inevitably introduced into anaerobic digestion systems together with the feedstock. In plants, they fulfil diverse ecological and physiological functions, including defence against pathogens and herbivores, protection against oxidative stress, and adaptation to environmental conditions [65–67]. However, when these materials are used as substrates for anaerobic digestion, many of these compounds may unintentionally interfere with microbial metabolism due to their antimicrobial and antioxidant properties [27,68].

The occurrence and composition of phytochemicals vary considerably among agricultural feedstocks. Fruit-processing residues, particularly olive, grape and citrus by-products, are generally rich in polyphenols, flavonoids, tannins and terpenes. In contrast, lignocellulosic residues such as cereal straw and maize stover predominantly contain lignin-associated phenolic acids, including ferulic, p-coumaric and vanillic acids. Herbal and aromatic plant residues constitute another important source of essential oils and terpenoids with well-documented antimicrobial activity. The predominant classes of plant-derived bioactive compounds associated with the major agricultural and agro-industrial feedstocks are summarised in Table 1. At the same time, their concentration-dependent inhibitory effects are presented in Table 2.

Table 2. Concentration-dependent inhibitory effects and principal mechanisms of selected plant-derived bioactive compounds during anaerobic digestion.

Compound/Group	Representative Feedstock	Reported Threshold Concentration *	Primary Inhibitory Mechanism	Observed Effect on AD	Reactor/Assay Type	References
Limonene (monoterpene)	Citrus waste, citrus peel	~400–1000 mg·L ^{−1}	Membrane disruption	Strong inhibition of methanogenesis; reduced CH ₄ yield; prolonged lag phase; inhibition becomes severe near 1000 mg·L ^{−1}	Batch BMP assays	[27,69]
p-Cymene/terpene metabolites	Citrus-derived biomass	~600 mg·L ^{−1}	Membrane disruption	Near-complete inhibition of methane production	Batch anaerobic toxicity assays	[27]
Hydroxytyrosol	Olive mill wastewater (OMWW)	Usually <40–170 mg·L ^{−1} during stable operation; toxicity increases at higher accumulation	Oxidative stress	Partial inhibition of methanogenic activity; reduced biodegradability	Thermophilic UPBR/continuous reactor	[70–72]
Total phenolic compounds	Olive mill waste, distillery stillage	~0.5–2.0 g·L ^{−1}	Enzyme inhibition	Reduced methane yield; inhibition of methanogens; VFA accumulation; concentrations >2 g·L ^{−1} considered strongly inhibitory	Batch BMP and continuous AD systems	[35,73,74]

Table 2. Cont.

Compound/Group	Representative Feedstock	Reported Threshold Concentration *	Primary Inhibitory Mechanism	Observed Effect on AD	Reactor/Assay Type	References
Phenolic acids (vanillic, ferulic, syringic, p-coumaric acids)	Distillery stillage	0.5–1.0 g·L ⁻¹ inhibition onset; ~2.0 g·L ⁻¹ severe inhibition	Electron transfer disruption	Significant decrease in methane production rate and archaeal abundance; complete inhibition at ~4 g·L ⁻¹	Batch biomethanization assays	[35]
p-Hydroxybenzoic acid	Olive mill effluents	~0.3 g·L ⁻¹ negligible effect; ~6 g·L ⁻¹ strong inhibition	Methanogenic toxicity	Methanogenesis inhibition mainly at elevated concentrations	Anaerobic digestion of OME	[75]
Tannins (condensed/hydrolysable)	Grape pomace, olive residues, woody biomass	Typically, inhibitory above ~1–2 g·L ⁻¹ (total phenolics equivalent)	Protein complexation	Enzyme complexation; reduced hydrolysis; inhibition of methanogenic activity	Batch digestion assays	[73,74]
Flavonoids/polyphenolic mixtures	Pepper-processing waste, plant residues	Variable; inhibitory effects generally reported above ~1 g·L ⁻¹ total polyphenols	Membrane perturbation	Reduced methanogenic activity; lower methane productivity	Batch anaerobic digestion	[76]
Substituted phenolic compounds (general group)	Agricultural lignocellulosic residues	800–1600 mg organic C·L ⁻¹	Microbial metabolic disruption	Partial to severe inhibition of methanization and biodegradability	Anaerobic sludge assays	[73]

* Reported values depend strongly on inoculum adaptation, reactor configuration, hydraulic retention time (HRT), substrate composition and operational conditions; therefore, threshold concentrations should be interpreted as approximate inhibitory ranges rather than universal limits.

Beyond botanical origin, the abundance and composition of plant-derived bioactive compounds are strongly influenced by cultivar, climatic conditions, harvest maturity, storage conditions and industrial processing. Furthermore, mechanical disruption, grinding, drying, thermal treatment and chemical pretreatment may substantially modify phytochemical bioavailability by releasing compounds previously bound within the lignocellulosic matrix or generating transformation products with altered biological activity [27,41,52].

Equally important is the distribution of bioactive compounds within the biomass matrix, which largely determines their accessibility to anaerobic microorganisms. Phenolic compounds bound to lignin, structural polysaccharides or proteins are often released gradually during biomass hydrolysis, potentially leading to delayed inhibitory effects, particularly in long-term continuous reactors. In contrast, volatile constituents of essential oils readily partition into the liquid phase and may rapidly affect microbial activity during the initial stages of anaerobic digestion [11,27,60].

Consequently, the inhibitory potential of agricultural biomass cannot be inferred solely from feedstock identity or total phytochemical concentration. Rather, it reflects the combined influence of phytochemical composition, chemical structure, bioavailability, release kinetics, interactions with the surrounding biomass matrix, and reactor operating conditions. This complexity explains much of the variability reported in the literature and highlights why inhibitory thresholds should be interpreted as context-dependent rather than universal values. These factors also provide the basis for understanding the concentration-dependent responses of anaerobic microbial communities discussed in the following sections [11,17].

3.2. Variability of Chemical Composition and Bioavailability

The composition of bioactive agricultural residues varies considerably among feedstocks and directly influences the bioavailability of inhibitory compounds and their effects on anaerobic digestion [15,77].

Plant species largely determine the type and concentration of bioactive metabolites present in agricultural residues. For instance, grape-processing residues are characterised by elevated concentrations of flavonoids and stilbenes, while citrus-processing waste is

notable for its high levels of terpenes and essential oils. Conversely, leaves of forage plants have been found to contain elevated levels of saponins and glycosides [78,79].

The distribution of bioactive compounds also varies among plant tissues. It has been established that skins, seeds, bark and leaves generally contain substantially higher concentrations of phenolics and tannins than pulp tissues. Essential oils, meanwhile, are localised in specialised secretory structures of leaves and flowers [65,80]. Consequently, residues characterised by an abundance of outer plant tissues often exhibit greater inhibitory capacity against AD microbiota.

Processing methods can substantially alter both the concentration and bioavailability of bioactive compounds. It has been demonstrated that mechanical disruption, drying, milling and thermal treatment can lead to an enhancement in the release of phenolic compounds from lignocellulosic matrices. This, in turn, has been shown to increase the accessibility of these compounds to microorganisms whilst simultaneously increasing inhibitory pressure [60,81]. Conversely, intensive extraction procedures have been shown to selectively remove certain metabolites while leaving structurally bound phenolics or poorly extractable terpenoids in the residual biomass. This phenomenon gives rise to highly dynamic, challenging-to-anticipate substrate behaviour during anaerobic digestion.

Soluble and volatile fractions, such as essential oils, are rapidly released into the liquid phase and may cause acute inhibition during the early stages of fermentation [11]. In contrast, it has been demonstrated that compounds associated with lignin, proteins, or cell wall structures may be gradually released during hydrolysis. This has been shown to cause delayed or chronic inhibitory effects in long-term reactor operation [35,38]. This kinetic heterogeneity is a primary factor contributing to the frequent underestimation of inhibition risks observed in continuous systems by short-term BMP (Biochemical Methane Potential) tests.

Agricultural residues should not be regarded as chemically uniform substrates. Nevertheless, the suitability of the substances for AD is contingent on the quantitative characterisation of both the total bioactive content and the compound bioavailability. This is to be combined with an evaluation of the dose–response relationships under realistic operational conditions. Taken together, these findings demonstrate that phytochemical concentration alone is insufficient to predict inhibitory potential without considering compound identity, bioavailability and release kinetics.

3.3. Classes of Plant-Derived Bioactive Compounds and Their Mechanisms of Action

Plant-derived bioactive compounds comprise several chemically distinct groups that differ in their occurrence, biological activity and inhibitory mechanisms during anaerobic digestion (Table 3). Although these compounds differ substantially in chemical structure, many ultimately affect a limited number of conserved microbial targets, including extracellular enzymes, cell membranes and syntrophic electron transfer [9,77].

Polyphenols are among the most abundant plant-derived bioactive compounds occurring in agricultural residues used for anaerobic digestion. Their principal inhibitory mechanisms include extracellular enzyme inhibition, metal chelation and disruption of microbial metabolism (Table 3). Because this group encompasses chemically diverse molecules, their biological activity varies considerably. It is strongly concentration-dependent, ranging from transient microbial adaptation at low concentrations to inhibition of methanogenesis and accumulation of volatile fatty acids at higher exposure levels [14,48].

Tannins represent a particularly important subgroup due to their strong affinity for proteins and enzymes. By forming stable complexes with extracellular proteins and structural carbohydrates, they can reduce hydrolysis efficiency and substrate availability [74,82]. Condensed tannins are generally more resistant to biodegradation than hydrolysable tan-

nins, which may prolong their inhibitory effects during anaerobic digestion as summarised in Table 3.

Terpenes and essential oils are commonly found in residues derived from aromatic plants, spices, and citrus processing. These compounds consist mainly of mono- and sesquiterpenes and related derivatives such as alcohols, aldehydes, and esters. Owing to their pronounced lipophilicity, they readily interact with microbial membranes, disrupting membrane integrity and cellular functions [12,83]. Monoterpenes such as limonene, thymol, and carvacrol are among the most extensively studied compounds in anaerobic digestion systems. Their antimicrobial activity is primarily associated with increased membrane permeability and disruption of membrane-associated energy metabolism, leading to prolonged lag phases and reduced methane production. Methanogenic microorganisms are particularly sensitive to terpene-rich substrates, making citrus-processing residues one of the most challenging agricultural wastes for stable anaerobic digestion [27,84].

Flavonoids, although classified as polyphenols, are discussed separately because their biological activity differs from that of other phenolic compounds. Rather than causing pronounced membrane disruption, flavonoids primarily interfere with microbial enzymatic activity and cellular redox homeostasis. At subinhibitory concentrations, they may also contribute to shifts in microbial community composition, although the underlying mechanisms remain insufficiently understood [17,68].

Alkaloids and saponins have received considerably less attention in anaerobic digestion research. Available evidence indicates that alkaloids may interfere with enzymatic pathways and cellular metabolism, whereas saponins interact with membrane lipids and may destabilise sensitive microbial populations [49,85].

Table 3. Major classes of plant-derived bioactive compounds occurring in agricultural biomass and their principal mechanisms affecting anaerobic digestion.

Class of Compounds	Representative Compounds/Subgroups	Main Sources in Agricultural Waste	Primary Inhibitory Mechanism	Principal Effect on Anaerobic Digestion	References
Phenolic acids	Ferulic, <i>p</i> -coumaric, vanillic, syringic, hydroxybenzoic acids	Straw, maize stover, lignocellulosic residues, distillery stillage	Extracellular enzyme inhibition; interference with syntrophic metabolism and electron transfer	Reduced hydrolysis and methane production; inhibition of methanogens; VFA accumulation at elevated concentrations	[35,41,61]
Polyphenols	Mixed phenolic compounds	Olive pomace, grape pomace, berry waste, fruit-processing residues	Protein denaturation, enzyme inhibition, metal chelation, membrane interactions	Dose-dependent response: stimulation or microbiome modulation at low concentrations; inhibition of methanogenesis and VFA accumulation at high concentrations	[13,17,73]
Flavonoids	Quercetin, catechins, rutin	Fruit peels, citrus residues, berry pomace	Inhibition of microbial enzymatic activity; membrane interactions; antioxidant activity	Reduced microbial activity and methane productivity; concentration-dependent effects	[17,68]
Tannins	Condensed and hydrolysable tannins	Grape pomace, woody biomass, olive residues	Protein precipitation; extracellular enzyme inhibition; reduced substrate bioavailability	Limited hydrolysis and fermentation; inhibition of methanogenic activity at elevated concentrations	[73,74,82]
Terpenes/essential oils	Limonene, thymol, carvacrol, <i>p</i> -cymene	Citrus peels, herb and spice residues, aromatic plant waste	Disruption of cytoplasmic membrane integrity and proton motive force	Strong inhibition of methanogens; decreased methane production; prolonged lag phase; possible microbiome modulation at low concentrations	[11,27,83]
Alkaloids	Nicotine, quinolizidine alkaloids	Tobacco waste, medicinal plants, some legumes	Interference with microbial metabolic and enzymatic pathways	Potential inhibition of anaerobic microorganisms; evidence remains limited	[85]
Saponins (glycosides)	Triterpenoid and steroid saponins	Alfalfa, legumes, oilseed residues	Interaction with membrane sterols; detergent-like membrane disruption	Cell destabilisation, foaming and possible inhibition of methanogenesis	[49,78]

The reported mechanisms represent the predominant modes of action identified across the available literature. Many phytochemicals exert multiple simultaneous effects on anaerobic microbial communities, depending on substrate composition and concentration, reactor configuration, and operational conditions.

Despite their structural diversity, most plant-derived bioactive compounds converge on a limited number of conserved microbial targets (Table 3). These include extracellular hydrolytic enzymes, cytoplasmic membranes, metal-dependent metabolic pathways and syntrophic interactions responsible for interspecies electron transfer. Consequently, different phytochemical classes frequently produce similar process-level responses, including reduced hydrolysis efficiency, volatile fatty acid accumulation, suppression of methanogenic activity and decreased methane production [48,58].

Their transformation within the reactor further modifies the biological activity of phytochemicals during anaerobic digestion. Phenolic compounds may undergo partial degradation during hydrolysis and acidogenesis through dehydroxylation, demethylation, or aromatic ring cleavage, producing metabolites with altered toxicity and biodegradability [10,86,87]. Consequently, the inhibitory effects observed during digestion reflect the combined influence of both parent compounds and their transformation products.

Despite their structural diversity, the phytochemical classes discussed above converge on a limited number of conserved microbial targets, providing a mechanistic framework for understanding process inhibition during anaerobic digestion.

3.4. Effects of Bioactive Compounds on Individual Stages of Anaerobic Digestion

The inhibitory effects of plant-derived bioactive compounds vary markedly across the successive stages of anaerobic digestion because each stage is driven by distinct microbial guilds with differing physiological requirements and stress tolerances. As summarised in Figure 2, phytochemicals target multiple cellular processes, resulting in stage-specific responses that ultimately determine overall process stability. Hydrolysis and acidogenesis initiate anaerobic digestion by converting complex organic matter into soluble intermediates available for further microbial metabolism. The responses of these stages to plant-derived bioactive compounds depend on the type, concentration, and substrate characteristics of the compounds [13,41].

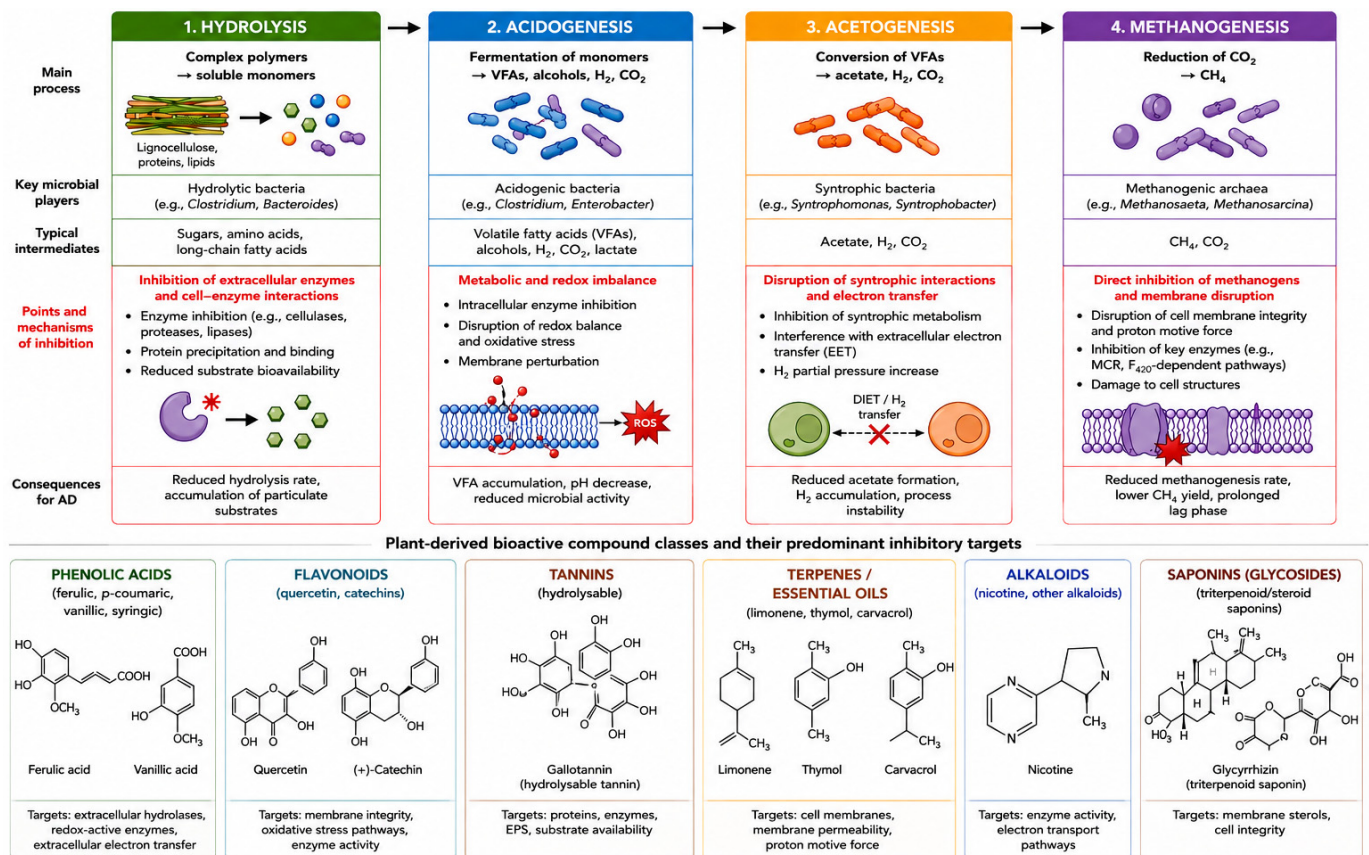
Bioactive compounds influence hydrolysis primarily by inhibiting hydrolytic enzymes such as cellulases, hemicellulases, and proteases (Figure 2 and Table 3). Polyphenols and tannins can form stable complexes with extracellular enzymes, reducing their activity and slowing organic matter degradation [74,82]. Studies on grape and olive pomace have linked elevated tannin concentrations with reduced hydrolysis rates and delayed methane production [60,88].

At low concentrations, some bioactive compounds may selectively suppress competing microbial populations without significantly affecting hydrolytic bacteria. Under such conditions, flavonoids and phenolic acids have been reported to alter microbial community structure, occasionally favouring carbohydrate-degrading microorganisms under subinhibitory conditions [13].

During acidogenesis, bioactive compounds primarily affect the production and conversion of volatile fatty acids. Depending on concentration (Table 2), polyphenols and terpenoids have been associated with increased accumulation of acetate, propionate, and butyrate, indicating disturbances in the balance between acidogenic and acetogenic populations [12]. Moderate VFA accumulation may facilitate microbial adaptation; however, excessive accumulation, particularly of propionate, can reduce pH and inhibit downstream microbial processes [48,89].

Acetogenesis is considered one of the most sensitive stages of anaerobic digestion because syntrophic oxidation of propionate and butyrate operates close to thermodynamic equilibrium [90,91]. Consequently, even relatively small disturbances in hydrogen consumption may rapidly propagate through the entire anaerobic food web. Polyphenols and aromatic metabolites can disrupt syntrophic electron transfer and impair cooperation

between acetogenic bacteria and hydrogenotrophic methanogens, resulting in propionate accumulation and reduced process stability. Experimental studies have confirmed that phenolic metabolites can alter syntrophic VFA-oxidising communities and prolong microbial adaptation periods under both mesophilic and thermophilic conditions [90]. Even small reductions in methanogenic activity may increase hydrogen partial pressure, thereby thermodynamically limiting syntrophic oxidation and promoting VFA accumulation [91]. Phenolic compounds may additionally affect extracellular polymeric substances and sludge granule stability, reducing the activity of syntrophic microorganisms and weakening microbial aggregation [92].



AD – anaerobic digestion; VFA – volatile fatty acids; H₂ – hydrogen; CO₂ – carbon dioxide; CH₄ – methane; DIET – direct interspecies electron transfer; MCR – methyl-coenzyme M reductase; ROS – reactive oxygen species; EPS – extracellular polymeric substances.

Figure 2. Mechanisms of microbial inhibition by plant-derived bioactive compounds during anaerobic digestion.

Methanogenesis is generally regarded as the stage most susceptible to inhibition by plant-derived bioactive compounds because methanogenic archaea exhibit slow growth rates and limited tolerance to environmental stressors. Numerous studies consistently identify methanogenesis as the stage most vulnerable to phytochemical-mediated inhibition, with methanogenic archaea exhibiting slower growth rates and lower physiological resilience than hydrolytic or acidogenic bacteria. Their effects are typically expressed as reduced methane production, accumulation of volatile fatty acids, and suppression of methanogenic activity, with acetoclastic methanogens showing greater sensitivity than hydrogenotrophic methanogens [35,93,94]. Proposed mechanisms include membrane disruption, enzyme inhibition, metal chelation, and interference with electron transfer processes [27,65].

Experimental studies have demonstrated a clear dose-dependent relationship between phenolic compounds and methanogenic inhibition. Significant reductions in methane

production and methanogenic activity have been observed at phenolic acid concentrations of several hundred milligrams per litre [35].

Although individual phytochemicals differ substantially in chemical structure and primary molecular targets, their inhibitory effects frequently converge into similar process-level responses. Regardless of the initial mechanism of action, inhibition of hydrolysis, syntrophic oxidation, or methanogenesis ultimately results in comparable operational consequences, including volatile fatty acid accumulation, reduced methane productivity and prolonged reactor recovery. This functional convergence highlights the importance of evaluating phytochemical mixtures rather than individual compounds when assessing the inhibitory potential of agricultural feedstocks.

Acetoclastic methanogens appear to be more susceptible to plant-derived bioactive compounds than hydrogenotrophic populations, resulting in impaired acetate utilisation and further accumulation of volatile fatty acids [94].

The concentration ranges associated with inhibitory responses of representative phytochemicals are summarised in Table 2. Importantly, the available evidence demonstrates that toxicity is governed not only by compound identity and concentration but also by bioavailability, microbial adaptation and reactor operating conditions. Consequently, inhibitory thresholds vary considerably among phytochemical classes and experimental systems and should therefore be interpreted as context-dependent rather than universal values for agricultural anaerobic digestion.

3.5. Microbial Community Shifts and Adaptation Mechanisms in Response to Plant-Derived Bioactive Compounds

Plant-derived bioactive compounds influence anaerobic digestion not only through direct toxicity but also by reshaping microbial community structure, syntrophic interactions and metabolic functions. Consequently, inhibition should be regarded as a dynamic ecological process rather than a purely toxic response [90,91].

One of the most frequently observed responses to phenolic and terpene stress is a shift from acetoclastic to hydrogenotrophic methanogenesis. Acetoclastic methanogens, particularly members of the genus *Methanothrix* (formerly *Methanosaeta*), are highly sensitive to phenolic inhibitors, volatile fatty acid accumulation, and environmental disturbances. Their inhibition reduces the direct conversion of acetate to methane and disrupts syntrophic acetate oxidation pathways [94,95]. In contrast, *Methanosarcina* species exhibit greater physiological flexibility and often become dominant under inhibitory conditions [95].

Under severe stress, methane production increasingly relies on hydrogenotrophic archaea such as *Methanobacterium* and *Methanoculleus*. This transition is commonly accompanied by altered VFA profiles, increased hydrogen turnover, and reorganisation of syntrophic microbial interactions. Such metabolic restructuring is considered an important adaptation mechanism that helps maintain process stability under adverse conditions [91]. Such community restructuring is frequently accompanied by changes in functional gene abundance and metabolic redundancy, increasing the resilience of anaerobic consortia under prolonged phytochemical stress [90].

Direct interspecies electron transfer has emerged as an important mechanism supporting syntrophic cooperation in anaerobic digestion. Microbial consortia involving genera such as *Geobacter*, *Syntrophomonas* and *Methanothrix* facilitate efficient electron exchange, thereby improving methane production under favourable [96]. Conductive materials, including activated carbon and biochar, further enhance DIET by promoting electrically connected microbial networks [97]. Conversely, membrane-active phytochemicals, particularly phenolics and terpenes, may impair these interactions through membrane destabilisation, disruption of extracellular electron transfer and deterioration of biofilm integrity [90,92].

Microbial adaptation represents an important determinant of long-term reactor performance. Although short-term batch experiments frequently report strong inhibition, continuous systems often exhibit partial recovery following prolonged exposure to bioactive compounds [90]. Adaptation is typically associated with the enrichment of inhibitor-tolerant microorganisms, the restructuring of syntrophic networks, the activation of stress-response mechanisms, and the gradual development of pathways involved in the degradation of aromatic compounds. Recent metagenomic and metatranscriptomic studies indicate that adaptation is accompanied not only by taxonomic shifts but also by functional reprogramming of microbial communities, including enhanced expression of genes associated with xenobiotic degradation, membrane transport systems and oxidative stress protection [90,92]. Omics studies have further shown increased expression of genes associated with membrane transport, extracellular polymeric substance production, and cellular stress protection [98]. Nevertheless, microbial resilience has practical limits. Excessive concentrations or prolonged exposure to inhibitory compounds may exceed the microbiome's adaptive capacity, leading to persistent instability, reduced methane production, and, in severe cases, reactor failure.

Overall, the response of anaerobic digestion microbiomes to plant-derived bioactive compounds reflects a dynamic balance between inhibition, ecological selection, microbial adaptation and metabolic reorganisation. The final process outcome, therefore, depends not only on the phytochemical concentration but also on the adaptive capacity of the microbial community, the reactor operating conditions, and the duration of exposure.

4. Pretreatment and Process Optimisation Strategies

4.1. Physical and Chemical Pretreatment Methods for Agricultural Substrates

Pretreatment represents one of the principal strategies for mitigating phytochemical inhibition during anaerobic digestion while simultaneously improving substrate biodegradability and methane production (Table 4). As discussed in Section 3, the effectiveness of pretreatment depends on the predominant class of bioactive compounds present in the feedstock. Accordingly, pretreatment should target both structural recalcitrance and phytochemical inhibition rather than lignocellulose degradation alone. Consequently, the choice of pretreatment should be guided by the phytochemical profile of the substrate rather than applying a universal approach to all agricultural residues.

Mechanical pretreatment, involving processes such as shredding, grinding, and homogenisation, has been shown to enhance the specific surface area and partially disrupt lignocellulosic structures. This, in turn, has been shown to accelerate hydrolysis, which is widely acknowledged as the rate-limiting step in AD [99]. Mechanical size reduction improves substrate accessibility but may simultaneously accelerate the release of soluble phenolic compounds from polyphenol-rich residues, thereby increasing short-term inhibitory pressure if applied without complementary mitigation strategies [100,101]. Consequently, authors frequently recommend combining mechanical pre-treatment with complementary strategies to mitigate bioactive stress.

Ultrasonic pretreatment enhances biomass disintegration and increases substrate accessibility. However, excessive sonication may also promote the release of soluble phenolics and terpenes, making optimisation of treatment time and energy input essential, particularly in systems lacking adapted inoculum [102–104].

Alkaline pretreatment using NaOH, Ca(OH)₂ or ammonia is among the most effective approaches for lignocellulosic substrates because it disrupts lignin–carbohydrate complexes and improves substrate accessibility. In addition, chemical modification of phenolic compounds may reduce their inhibitory activity. However, excessive treatment severity may

increase the release of soluble phenolic intermediates, emphasising the need for process optimisation [58,105].

Nonetheless, excessively intensive chemical pretreatment may result in the formation of secondary inhibitors, including low-molecular-weight phenols and nitrogen-containing compounds, which may further burden the fermentative microbiome [100,102].

From the perspective of circular bioeconomy systems, pretreatment should be selected based on the substrate's phytochemical profile rather than on lignocellulosic composition alone. As demonstrated throughout the reviewed studies, integrating pretreatment with appropriate operational strategies generally provides greater process stability than any single intervention applied in isolation.

Table 4. Comparative analysis of pretreatment methods for agricultural waste used in anaerobic digestion.

Pretreatment Method	Methane Yield Improvement (%)	Main Mechanism	Advantages	Limitations	Representative Feedstocks	References
Mechanical milling (roll/hammer milling)	15–25%	Particle size reduction and increased surface area	Simple, scalable, low chemical input	High electricity demand at an industrial scale	Wheat straw, corn stover, grass biomass	[99,105,106]
Alkali pretreatment (NaOH, Ca(OH) ₂)	30–55%	Lignin solubilization and cellulose exposure	Effective delignification	Chemical recovery required	Rice straw, wheat straw	
Ultrasonic pretreatment	40–65%	Cell wall disruption and enhanced solubilization	Strong improvement in hydrolysis	High energy consumption	Lignocellulosic agricultural residues	[102,107]
Steam explosion	35–60%	Hemicellulose disruption and fibre deconstruction	Highly effective for recalcitrant biomass	Formation of inhibitory compounds is possible	Straw, woody residues	[10,106]
Microaerobic pretreatment	20–45%	Partial oxidation and lignin modification	Low operational cost and energy demand	Requires oxygen control	Mixed agricultural waste	[99]
Activated carbon/biochar-assisted detoxification	20–50% recovery under inhibition	Adsorption of phenolics and terpenes	Reduces toxicity and stabilises AD	Additional material cost	Citrus waste, olive pomace	[58,62]
Co-digestion strategy	15–40% increase in methane stability	Dilution of inhibitors and nutrient balancing	Improves C/N ratio and buffering capacity	Feedstock logistics required	Citrus waste + manure; olive waste + sludge	[50,108]

4.2. Strategies for Mitigating Inhibition Caused by Bioactive Compounds

The presence of plant-derived bioactive compounds in agricultural substrates requires implementing mitigation strategies to improve process stability and reduce the risk of microbial inhibition during anaerobic digestion. Current evidence suggests that no single approach is universally effective and that the best results are generally achieved by combining operational, material-based, and biological strategies. The selection of individual mitigation measures should therefore be guided by the dominant phytochemical profile of the feedstock and the prevailing process conditions, as discussed in Section 3. Particular attention has been devoted to limiting the inhibitory effects of phenolic compounds and terpenes, which are among the most frequently reported causes of reduced methane production and process instability [26,105].

One of the simplest operational approaches is the dilution of substrates containing high concentrations of inhibitory compounds. This strategy is particularly relevant for citrus residues rich in limonene and plant materials containing elevated levels of polyphenols. Lowering inhibitor concentrations below critical thresholds can restore microbial activity and improve methane production. Ruiz and Flotats [11] demonstrated that limonene-induced inhibition is partially reversible and that microbial communities can gradually develop increased tolerance following prolonged exposure, highlighting the importance of

inoculum adaptation in addition to simple dilution. Its effectiveness, however, depends on the compatibility of co-substrates, organic loading rate and reactor operating conditions.

Co-digestion is generally considered a more effective long-term solution. Combining bioactive-rich substrates with feedstocks characterised by higher buffering capacity, balanced nutrient composition, and more favourable C/N ratios improves process stability and reduces the impact of local inhibitor accumulation. In addition, co-digestion promotes the development of more diverse microbial consortia that support syntrophic degradation pathways and maintain stable methane production. This strategy has been widely recommended for citrus-processing residues, lignocellulosic waste, and fruit pomace [105,109].

Adsorbent materials such as biochar, hydrochar and activated carbon reduce the bioavailability of inhibitory compounds while simultaneously providing surfaces for microbial colonisation. In addition, conductive carbonaceous materials may promote DIET, thereby enhancing syntrophic cooperation and improving methane production under inhibitory conditions [58,105,110].

Another effective strategy is to remove inhibitors before anaerobic digestion. In citrus-processing residues, limonene extraction by steam distillation or solvent-based methods has been extensively investigated. Partial or complete removal of limonene before anaerobic digestion has consistently been associated with higher methane yields and improved process kinetics. In addition to reducing toxicity, recovery of terpenes may provide an additional value stream within integrated biorefinery systems [101]. This approach is particularly attractive within integrated biorefinery concepts because inhibitor removal and recovery of high-value phytochemicals can be achieved simultaneously.

The underlying mechanisms of microbial adaptation, including community restructuring and metabolic reorganisation, are discussed in Section 3.5.

Overall, effective mitigation of phytochemical-related inhibition requires the integration of pretreatment, operational optimisation, targeted inhibitor removal and microbial adaptation. Rather than relying on a single intervention, the reviewed studies consistently demonstrate that combining complementary strategies provides the greatest improvement in methane production and long-term process stability. The relationships between feedstock characteristics, operational conditions and mitigation approaches are synthesised in Section 5.2.

4.3. Inoculum Management and Operational Parameters

Operational conditions play a key role in determining the resistance of anaerobic digestion systems to plant-derived bioactive compounds. Accordingly, operational optimisation should be considered complementary to pretreatment and inhibitor removal rather than an independent mitigation strategy. In addition to substrate composition, process stability is strongly influenced by parameters such as organic loading rate (OLR), hydraulic retention time (HRT), temperature, and the degree of microbial adaptation to inhibitory compounds [20,59,111].

Organic loading rate is one of the most important factors affecting system resilience to phenolic compounds and other bioactive metabolites. Excessive loading may lead to volatile fatty acid accumulation, reduced methanogenic activity, and overall destabilisation of the process [20,59]. Studies investigating phenolic inhibition have shown that sudden increases in inhibitor concentration often lead to rapid deterioration in methane production. Conversely, gradual increases in loading promote microbial adaptation and improve resilience to phytochemical stress [20,58,112].

Hydraulic retention time is equally important because it determines the retention of slow-growing syntrophic bacteria and methanogenic archaea that degrade aromatic compounds. Short HRT values may result in the washout of key microbial populations,

reducing process stability and limiting the system's capacity to cope with inhibitory substrates. Longer HRTs may therefore partially compensate for phytochemical inhibition by facilitating microbial adaptation and degradation of slowly metabolised aromatic compounds [112,113].

Temperature also influences microbial responses to bioactive compounds. Comparative studies have shown that thermophilic systems are generally more susceptible to volatile fatty acid accumulation and process instability when exposed to elevated concentrations of aromatic inhibitors [114,115]. At the same time, well-adapted thermophilic communities may achieve high degradation efficiencies and methane yields when operating conditions are carefully controlled [104,116].

Inoculum adaptation represents one of the most effective biological strategies for improving long-term process stability. The microbiological mechanisms underlying adaptation, including shifts in syntrophic interactions and methanogenic pathways, are discussed in Section 3.5. Metagenomic studies further indicate that adaptation is accompanied by substantial changes in microbial community structure, with the maintenance of active acetogenic and methanogenic populations being a key determinant of process stability [58,98].

The response of anaerobic digestion systems to bioactive compounds is often threshold-dependent. At low concentrations, many phenolic compounds exert little or no measurable effect on process performance. However, once critical concentrations are exceeded, inhibition frequently manifests as abrupt process destabilisation, characterised by volatile fatty acid accumulation and rapid declines in methane production rather than a gradual reduction in performance [58,59]. The critical operating parameters most frequently associated with successful mitigation of phytochemical inhibition across different agricultural feedstocks are summarised in Section 5.2.

4.4. Influence of Reactor Configuration on Inhibitory Susceptibility

The susceptibility of anaerobic digestion to plant-derived bioactive compounds is highly dependent on the reactor configuration and the operational feeding strategy [117]. While the majority of toxicity thresholds reported in the literature are derived from static batch assays (e.g., Biochemical Methane Potential tests), these conditions do not accurately reflect the dynamics of full-scale continuous systems. In batch reactors, the microbial community is exposed to an acute, single-dose shock of inhibitory compounds. Although this enables rapid identification of baseline toxicity thresholds, it precludes gradual microbial adaptation and does not account for the continuous accumulation of slowly released lignin-bound phenolics.

In contrast, Continuous Stirred Tank Reactors (CSTRs), which are the most common configuration for agricultural biogas plants, exhibit fundamentally different inhibition dynamics. The continuous feeding and mixing in CSTRs allow for the gradual acclimation of the microbiome to elevated concentrations of polyphenols and terpenes. Furthermore, maintaining a relatively long HRT combined with staged feeding or co-digestion can effectively dilute incoming phytochemicals below their critical toxicity thresholds. However, the planktonic nature of the microbial biomass in CSTRs leaves sensitive methanogenic archaea relatively exposed to sudden organic or inhibitory overloads [118].

High-rate systems, such as Upflow Anaerobic Sludge Blanket (UASB) reactors, rely on the formation of granular sludge, which provides a significant physical barrier against phytochemical stress. The dense matrix of extracellular polymeric substances (EPS) shields the inner core of acetoclastic methanogens from direct contact with membrane-disrupting compounds like limonene. Nevertheless, recent studies indicate that excessive concentrations of specific polyphenols, such as tannic acid, can interact with structural proteins within the EPS, leading to granule disintegration and sudden reactor failure.

Packed-bed reactors and systems utilising conductive support media (e.g., granular activated carbon or biochar) exhibit the highest resilience to bioactive toxicity. As demonstrated by Purwanta et al. [119], fixed-bed reactors processing challenging agricultural residues significantly outperform CSTRs. The formation of robust biofilms on these carriers not only introduces substantial mass-transfer resistance that protects the cells but also promotes DIET. Ultimately, the choice of reactor configuration determines whether the microbial community relies on dilution, spatial shielding, or enhanced syntrophic interactions to survive phytochemical inhibition.

5. Effects of Bioactive Compounds on Anaerobic Digestion Performance

5.1. Process-Level Manifestations of Phytochemical Inhibition

The impact of plant-derived bioactive compounds on AD performance is multifaceted, involving simultaneous physicochemical, enzymatic and microbiological effects occurring across all major stages of anaerobic digestion. The molecular mechanisms underlying these interactions are discussed in Section 3 (Figure 2), whereas this section focuses on their consequences for overall process performance. In contrast to classical inorganic inhibitors, plant secondary metabolites often exhibit selective, dose-dependent effects on microbial consortia, leading to progressive destabilisation rather than immediate process failure (see Table 3 for further details) [11].

Among plant-derived bioactive compounds, terpene-rich substrates consistently exhibit some of the strongest inhibitory effects on AD performance, primarily through reduced methane production and prolonged lag phases. These process-level responses are largely attributed to membrane disruption mechanisms discussed in Section 3 [11,62]. Similarly, phenolic compounds and tannins reduce AD performance primarily by inhibiting enzymes involved in hydrolysis and methanogenesis and by reducing substrate bioavailability (Section 3). At the process level, these effects are reflected in lower methane yields and slower substrate conversion, particularly in phenolic-rich feedstocks [17].

At the level of microbial communities, bioactive metabolites significantly influence syntrophic interactions, which are considered essential for the stability of AD performance. Disruption of syntrophic interactions promotes volatile fatty acid accumulation and reduces methane productivity, particularly under conditions where methanogenic activity is already compromised [90,91]. Redox-active phenolics and flavonoids can act as alternative electron acceptors or disrupt electron transport pathways, reducing methane productivity and altering metabolic fluxes within anaerobic communities [58].

Another significant mechanism involves the chelation of trace metals required by methanogenic enzymes. Polyphenols have been observed to demonstrate a notable binding affinity for iron (Fe), nickel (Ni), and cobalt (Co) ions. These ions are recognised as essential cofactors for hydrogenases and methyl-coenzyme M reductase, which are pivotal in methanogenesis [17,48]. Consequently, trace element limitation may exacerbate phytochemical inhibition even in systems supplied with apparently sufficient micronutrient concentrations.

The inhibitory effects of bioactive compounds are also influenced by their distribution within the substrate matrix. It has been established that soluble and volatile compounds, such as essential oils, can rapidly inhibit during the initial stages of fermentation. Conversely, phenolics bound to lignocellulosic fractions may be released gradually during hydrolysis, thereby causing delayed or chronic inhibition in continuous systems [11]. This kinetic heterogeneity complicates process control and may explain why short-term BMP assays often underestimate the inhibitory effects observed in long-term continuous reactors.

Inhibition should not be viewed solely as a direct toxic effect. The response of anaerobic systems depends strongly on substrate characteristics, buffering capacity, nutrient

availability, organic loading rate, and co-substrate composition. As a result, compounds that are inhibitory in batch experiments may become partially tolerated in adapted continuous systems operated under optimised conditions [35,71]. These interactions explain why inhibition thresholds reported in the literature should be interpreted in the context of reactor configuration, operational parameters and microbial adaptation rather than as fixed toxicity values. The combined influence of these factors across different agricultural feedstocks is synthesised in Table 5.

5.2. Toxicity Thresholds and Dose–Response Effects

The effects of plant-derived bioactive compounds on anaerobic digestion are strongly dose-dependent, though inhibitory thresholds vary widely across substrates and reactor systems. Factors such as reactor configuration, microbial adaptation, exposure time, and substrate composition can substantially modify microbial responses to inhibitory compounds. Representative concentration ranges reported for the major phytochemical groups are summarised in Table 2. Consequently, universal toxicity thresholds cannot be defined for most bioactive metabolites because their effects depend heavily on process conditions and microbial resilience [17]. Much of the variability reported in the literature arises from differences in reactor configuration (BMP versus continuous systems), inoculum adaptation, exposure time and substrate composition rather than from differences in the intrinsic toxicity of individual phytochemicals.

Polyphenols are among the most extensively studied groups of bioactive compounds in AD systems. At low concentrations, they may exert neutral or even mildly beneficial effects by influencing microbial redox balance and selectively suppressing competing microorganisms [1,60]. Although inhibitory concentrations differ considerably among studies, total polyphenol concentrations exceeding approximately $2 \text{ g} \cdot \text{L}^{-1}$ are consistently associated with reduced methane production and process instability (Table 2) [58,62].

Olive mill residues and grape pomace well illustrate the practical implications of dose-dependent inhibition. Olive by-products containing hydroxytyrosol, tyrosol, and oleuropein often exhibit substantially lower methane yields than predicted from their biochemical methane potential because of methanogenic inhibition. Similarly, grape pomace rich in tannins and flavonoids has been reported to impair methane production at concentrations of $20\text{--}60 \text{ mg} \cdot \text{g}^{-1} \text{ VS}$, whereas lower concentrations are often tolerated following microbial adaptation [120,121].

Acetoclastic methanogens are generally more sensitive to phenolic stress than hydrogenotrophic archaea, frequently resulting in a shift towards hydrogenotrophic methanogenesis under inhibitory conditions [94]. Among the phytochemicals reviewed, limonene remains one of the strongest inhibitors, as concentrations naturally occurring in citrus-processing residues may exceed reported inhibitory thresholds, thereby explaining the poor performance frequently observed during the digestion of untreated citrus waste [11,27].

Effective toxicity thresholds are strongly influenced by exposure kinetics and microbial adaptation. Gradual feeding strategies and co-digestion can increase microbial tolerance to phenolic compounds and limonene by preventing sudden inhibitory shocks and improving buffering conditions [11,58]. Long-term exposure may further enrich microbial populations capable of tolerating or partially degrading aromatic compounds [58]. Nevertheless, microbial resilience has practical limits and may be compromised by abrupt increases in organic loading rate, pH fluctuations or sudden increases in inhibitor concentration.

Table 5. Integrated comparative matrix of agricultural feedstocks containing plant-derived bioactive compounds, summarising phytochemical composition, inhibitory responses, process performance and mitigation strategies during anaerobic digestion.

Agricultural Feedstock	Predominant Phytochemicals	Typical Bioactive Load	Most Sensitive AD Stage	Principal Inhibitory Mechanism	Typical Reactor Configuration	Key Operational Parameters Influencing Inhibition	Typical Methane Yield Reduction (%)	Reported Lag-Phase Extension	Recommended Mitigation Strategy	Major Research Gaps	References
Olive pomace	Hydroxytyrosol, tyrosol, oleuropein derivatives, phenolic acids	Very high (total phenolics commonly 2–10% DM)	Acetogenesis; methanogenesis	Enzyme inhibition, membrane stress, disruption of syntrophic metabolism	Mesophilic CSTR; Batch BMP	High OLR increases inhibition; long HRT and adapted inoculum improve resilience	10–50	1–7 d	Polyphenol extraction before AD, co-digestion, activated carbon/biochar, gradual microbial adaptation	Industrial-scale validation; omics-based confirmation of adaptation mechanisms	[20,71,122]
Olive mill wastewater (OMWW)	Soluble phenolics, hydroxytyrosol, tyrosol	Very high (typically 1–8 g L ⁻¹ phenolics)	Methanogenesis	Phenolic toxicity and inhibition of methanogenic archaea	UASB; CSTR; Batch BMP	High phenolic loading; mesophilic operation preferred; low OLR improves stability	20–70	2–10 d	Phenolic recovery, dilution, co-digestion, adsorption	Long-term reactor stability; standardised inhibitory thresholds	[70–72]
Grape pomace	Polyphenols, flavonoids, condensed tannins	High (typically 20–70 mg GAE g ⁻¹ DM)	Hydrolysis; methanogenesis	Protein complexation and extracellular enzyme inhibition	Batch BMP; CSTR	Long HRT; gradual OLR increase; manure co-digestion improves stability	10–40	1–5 d	Co-digestion, microbial adaptation, controlled substrate loading	Harmonised dose-response thresholds; continuous-reactor studies	[23,24,74]
Citrus-processing residues	Limonene, <i>p</i> -cymene, essential oils	Very high (limonene typically 0.5–2% of peel dry matter)	Methanogenesis	Membrane disruption and collapse of proton motive force	Batch BMP; semi-continuous/CSTR	Thermophilic AD increases toxicity; gradual OLR increase; adapted inoculum recommended	20–90	3–15 d	Essential oil removal, co-digestion, activated carbon/biochar, inoculum adaptation	Long-term CSTR validation; chronic terpene exposure	[27,69,123]
Apple pomace	Phenolic acids, flavonoids, tannins	Moderate (typically 5–20 mg GAE g ⁻¹ DM)	Acidogenesis; methanogenesis	Combined phenolic stress and rapid acidification	Batch BMP; CSTR	Buffering capacity more important than temperature; moderate OLR	5–30	0–3 d	pH control, co-digestion, gradual loading	Limited mechanistic and continuous-reactor studies	[28,30]
Tomato-processing residues	Phenolics, carotenoids	Low–moderate	Not clearly identified	Limited phytochemical inhibition	Batch BMP	Moderate substrate loading; extraction does not adversely affect BMP	<10	Not consistently reported	Recovery of bioactive compounds followed by anaerobic digestion (biorefinery approach)	Full-scale validation and continuous-reactor studies	[124–126]
Avocado residues	Polyphenols, saponins	Moderate–high	Methanogenesis	Microbial community restructuring and inhibition of methanogenic archaea	Batch BMP	Limited operational evidence; adaptation appears beneficial	10–35	Limited evidence	Co-digestion, adapted inoculum	Lack of comparative continuous-reactor studies and long-term validation	[127,128]

Table 5. Cont.

Agricultural Feedstock	Predominant Phytochemicals	Typical Bioactive Load	Most Sensitive AD Stage	Principal Inhibitory Mechanism	Typical Reactor Configuration	Key Operational Parameters Influencing Inhibition	Typical Methane Yield Reduction (%)	Reported Lag-Phase Extension	Recommended Mitigation Strategy	Major Research Gaps	References
Herbal and aromatic plant residues	Essential oils, terpenoids, tannins	Very high (essential oils frequently 1–5% DM)	Methanogenesis	Membrane disruption and antimicrobial activity	Batch BMP; CSTR (limited)	Gradual substrate loading; dilution of essential-oil-rich biomass	20–60	2–10 d	Dilution, co-digestion, microbial adaptation	Lack of long-term continuous-reactor and industrial-scale studies	[27,127,129]
Wheat straw /Rice straw	Ferulic acid, <i>p</i> -coumaric acid, lignin-derived phenolics	Low (mostly lignin-bound phenolics)	Hydrolysis	Structural recalcitrance and lignin-associated enzyme inhibition	Batch BMP; CSTR (after pretreatment)	Pretreatment severity; long HRT; mesophilic and thermophilic systems reported	5–25	Minor or not consistently reported	Alkali pretreatment, steam explosion, co-digestion	Phenolic release kinetics after pretreatment	[34,37,130,131]
Maize stover/lignocellulosic crop residues	Ferulic acid, vanillic acid, hydroxybenzoic acid	Low–moderate	Hydrolysis	Reduced enzymatic accessibility and release of phenolic acids during pretreatment	Batch BMP; CSTR	Pretreatment severity; long HRT; adapted inoculum improves stability	5–30	Minor; mainly associated with slow hydrolysis	Optimised pretreatment, inoculum adaptation, co-digestion	Continuous-reactor validation; phytochemical dynamics	[39,41,132–134]
Maize silage	Low concentrations of lignin-associated phenolics	Low	Hydrolysis	Structural limitations dominate over phytochemical inhibition	CSTR	Stable mesophilic operation; moderate OLR; standard HRT sufficient	<10	Negligible	Process optimisation rather than detoxification	Interaction with phytochemical-rich co-substrates	[42–44,135]
Sugar beet pulp	Low levels of phenolic acids	Very low	Acidogenesis	Rapid fermentation and acidification rather than direct phytochemical inhibition	Batch BMP; CSTR	OLR and buffering capacity are critical; stable operation depends on appropriate temperature adaptation.	<10	Negligible	Co-digestion, pH control	Phytochemical contribution to inhibition remains poorly characterised	[45–47,136]

Values for methane yield reduction and lag-phase extension represent typical ranges reported across batch BMP and continuous reactor studies. Their magnitude depends on phytochemical concentration, reactor configuration, inoculum adaptation, operating temperature, organic loading rate (OLR), hydraulic retention time (HRT), and co-digestion strategy. The reported ranges are intended for comparative interpretation rather than as universal inhibitory thresholds.

Overall, toxicity thresholds for plant-derived bioactive compounds should be regarded as dynamic rather than fixed values. The actual inhibitory response depends on the interaction between compound concentration, microbial adaptation, reactor operation, and substrate characteristics. Consequently, toxicity thresholds should be regarded as operational rather than absolute values, reflecting the combined influence of reactor configuration, microbial adaptation and substrate characteristics.

5.3. Effects on Process Stability and Methane Yield

Plant-derived bioactive compounds can disrupt metabolic balance in anaerobic digestion, leading to the accumulation of volatile fatty acids (VFAs). This process-level response primarily results from the selective inhibition of acetoclastic bacteria and methanogenic archaea described in Section 3, whereas hydrolytic and fermentative microorganisms often remain comparatively active. Consequently, VFA production may exceed their subsequent conversion to methane, resulting in the accumulation of acetate, propionate, and butyrate [17,58,62].

Among these intermediates, propionate is considered particularly important because its degradation depends on efficient syntrophic cooperation between acetogenic bacteria and hydrogen-consuming methanogens. Inhibition of either group may impair propionate oxidation, leading to VFA accumulation, pH decline, and further suppression of methanogenesis [90]. Such responses are particularly evident in feedstocks combining high concentrations of readily fermentable carbohydrates with phytochemical inhibitors, including fruit pomace and citrus-processing residues [11,50].

Beyond affecting process stability, bioactive compounds also influence methane production kinetics and overall methane yield. Numerous studies have reported substantial discrepancies between theoretical methane potential and actual methane production from substrates rich in phenolics or terpenes [11,62]. These discrepancies are commonly reflected by lower methane yields, prolonged lag phases and reduced maximum methane production rates (R_{max}), indicating impaired microbial activity and delayed adaptation [17,58].

The kinetics of inhibition vary among different groups of bioactive compounds. Lipophilic terpenes such as limonene typically exert rapid inhibitory effects during the initial stages of digestion, whereas phenolic compounds associated with lignocellulosic fractions may be released gradually during biomass degradation, resulting in delayed or chronic inhibition [11]. Consequently, phytochemical concentration alone is a poor predictor of inhibition severity, as release kinetics and substrate structure strongly influence process performance.

The influence of operational strategies, including co-digestion and microbial adaptation, is discussed in Section 4. Operational conditions substantially modify the severity of phytochemical inhibition, explaining why similar substrates may perform differently across reactor systems.

From an operational perspective, long-term exposure to bioactive compounds may reduce reactor robustness, even without a complete process failure. Adapted systems frequently maintain methane production but remain more sensitive to fluctuations in organic loading rate and substrate composition [20,58]. As a result, the anaerobic digestion of bioactive-rich agricultural residues often requires longer hydraulic retention times, lower organic loading rates, and more carefully controlled feeding regimes, all of which have important implications for reactor design, operational costs and the economic feasibility of agricultural biogas systems [48].

Overall, the effects of plant-derived bioactive compounds on anaerobic digestion arise from complex interactions among microbial ecology, substrate composition and operating conditions. Consequently, their impact extends beyond direct toxicity and should be

considered throughout the design, optimisation and evaluation of anaerobic digestion systems treating agricultural residues. These findings further demonstrate that biochemical methane potential alone is insufficient for assessing phytochemical-rich feedstocks. Reliable prediction of methane yield and energy recovery requires the simultaneous consideration of phytochemical composition, microbial adaptation and key operational parameters. The integrated relationships among feedstock characteristics, operating conditions and mitigation strategies are synthesised in Table 5.

6. Microbial Community Responses and Adaptation Mechanisms

6.1. Integrated Framework for Managing Phytochemical Inhibition

The studies reviewed in the preceding sections demonstrate that the effects of plant-derived bioactive compounds on anaerobic digestion cannot be predicted solely from phytochemical composition. Instead, inhibition results from interactions among substrate characteristics, phytochemical profile, microbial adaptation, reactor configuration, and operating conditions. Considering these factors separately provides only limited insight into process performance, highlighting the need for an integrated framework.

To facilitate comparison of the available evidence and support practical interpretation of the literature, Table 5 integrates the principal findings reported for the major agricultural feedstocks. The matrix links predominant phytochemicals, the most susceptible stages of anaerobic digestion, key operational factors, recommended mitigation strategies and the major knowledge gaps identified across the reviewed studies. Unlike conventional summary tables, the integrated matrix highlights that similar phytochemicals may require different management strategies depending on reactor configuration, operating conditions and microbial adaptation. Conversely, different feedstocks may be managed using similar operational approaches when common inhibitory mechanisms are involved.

6.2. Changes in Microbial Consortium Structure: Metagenomic Analyses and MAGs

Recent advances in metagenomics and the reconstruction of metagenome-assembled genomes (MAGs) have considerably improved our understanding of how plant-derived bioactive compounds affect the microbiomes of anaerobic digestion. Unlike conventional 16S rRNA profiling, these approaches provide information not only on microbial community composition but also on the metabolic functions, stress-response pathways and adaptive capabilities of individual populations, thereby enabling functional rather than purely taxonomic interpretation of microbial responses [58,100].

Metagenomic analyses demonstrate that plant-derived bioactive compounds induce selective rather than non-specific shifts in microbial communities. Under terpene stress, populations involved in acetoclastic methanogenesis and syntrophic volatile fatty acid oxidation typically decline, whereas fermentative microorganisms, particularly members of the orders *Clostridiales* and *Bacteroidales*, frequently remain stable or increase in relative abundance [58]. These observations indicate that inhibition results primarily from functional reorganisation of the microbiome rather than from a uniform loss of microbial diversity.

Functional metagenomic analyses further demonstrate that phytochemical inhibition extends beyond taxonomic shifts and directly affects metabolic pathways associated with anaerobic digestion. Phenolic stress has been linked to reduced abundance of genes encoding hydrogenases and enzymes involved in the Wood–Ljungdahl pathway, indicating impaired electron transfer and acetate utilisation [58,105]. In contrast, terpenes such as limonene primarily affect membrane integrity and microbial diversity, illustrating that different phytochemical classes target distinct functional components of the anaerobic microbiome [11,100].

MAG-based studies have also identified microbial populations associated with adaptation to inhibitory compounds. Under prolonged exposure, specialised aromatic-compound degraders may become enriched, enabling partial detoxification of phenolic substrates and improving process resilience. These findings indicate that adaptation involves ecological restructuring of microbial communities by enriching specialised aromatic-compound degraders that sustain process performance under inhibitory conditions. Moreover, shifts towards increased abundance of *Clostridiales* populations have been proposed as early biomarkers of progressing inhibition in anaerobic digesters [17,120].

From a practical perspective, metagenomic and MAG analyses provide powerful tools for monitoring reactor stability and predicting responses to phytochemical-rich substrates before conventional process indicators reveal instability. By integrating microbial community composition with functional gene profiles, these approaches enable earlier detection of inhibitory stress and support evidence-based optimisation of operational strategies, including feedstock selection, co-digestion, inoculum adaptation and the application of conductive materials [17,48,137]. Consequently, integrating metagenomic information with routine process monitoring may facilitate the transition from reactive process control to predictive management of agricultural anaerobic digestion systems [138].

6.3. Microbial Adaptation to Bioactive Stress

Microbial adaptation is one of the main factors determining the stability of anaerobic digestion systems processing substrates rich in plant-derived bioactive compounds. Available studies indicate that the response of anaerobic microbiomes to phenols, polyphenols, and terpenes largely depends on inoculum history, exposure kinetics, and the operational conditions under which inhibitory compounds are introduced into the system [58,100].

In non-adapted inocula, even relatively low concentrations of bioactive compounds may rapidly disrupt microbial activity. Studies involving citrus residues, phenolic-rich wastes, and aromatic plant biomass reported reduced methanogenic activity, accumulation of volatile fatty acids, and a decline in methane production shortly after inhibitor exposure [58]. These observations indicate that the initial response of non-adapted communities is characterised primarily by functional instability rather than irreversible process failure.

In contrast, gradual and prolonged exposure to inhibitory compounds can promote microbial acclimation. Several studies reported partial or complete recovery of methane production after successive adaptation periods, despite the continued presence of phenol or limonene [20,58,62]. The time required for adaptation varies among feedstocks and reactor configurations, but is generally shorter when inhibitor concentrations are increased gradually under stable operating conditions. [58,139]. Although adaptation improves process resilience, it may also be accompanied by reduced metabolic flexibility, making adapted communities more vulnerable to abrupt changes in substrate composition or operating conditions.

Adaptation involves both ecological and physiological changes. Metagenomic studies showed enrichment of microbial populations capable of transforming aromatic compounds into less toxic intermediates, together with a shift towards hydrogenotrophic methanogenesis [100,105]. At the cellular level, adaptation is associated with coordinated physiological responses that improve tolerance to phytochemical stress, although the underlying mechanisms remain only partially understood.

Overall, comparisons between adapted and non-adapted inocula demonstrate that microbial acclimation can substantially improve process resilience under inhibitory conditions. These observations indicate that inoculum adaptation should be regarded as a planned operational strategy rather than a passive consequence of long-term reactor operation. Nevertheless, adaptation cannot fully compensate for excessive phytochemical

loading and should therefore be combined with appropriate substrate selection and process optimisation [19,48].

6.4. Functional Microbial Groups Responsible for Phytochemical Transformation

The transformation of phytochemical inhibitors during anaerobic digestion depends on the coordinated activity of specialised functional microbial guilds. Although phenolic compounds have been studied most extensively, similar syntrophic interactions are required for the detoxification of many plant-derived bioactive metabolites [58,62].

The initial stages of phenol transformation are carried out by specialised fermentative and syntrophic bacteria, including members of the genera *Syntrophus*, *Pelotomaculum*, and *Desulfotomaculum*, as well as selected representatives of the *Clostridia*. Metagenomic and MAG-based studies have linked these microorganisms to reductions in phenol concentrations and improved volatile fatty acid conversion in adapted anaerobic systems [58,100]. Efficient degradation of aromatic intermediates depends on close syntrophic cooperation with hydrogenotrophic methanogens, highlighting that detoxification and methane production are tightly interconnected processes [58,105].

Functional metagenomic analyses have identified key pathways involved in anaerobic degradation of aromatic compounds, particularly those associated with benzoyl-CoA metabolism and aromatic ring reduction [58,120]. These transformations ultimately generate simpler intermediates that can be further converted into methane by syntrophic and methanogenic communities [62,105]. Accordingly, the capacity to transform phenolic compounds is primarily determined by the functional potential of the microbial community rather than by the presence of individual taxa.

These observations highlight the importance of maintaining microbial populations involved in aromatic-compound degradation. Operational strategies such as gradual inhibitor loading, appropriate substrate selection, and the use of conductive materials supporting microbial interactions may improve resilience and reduce the risk of phenol-induced inhibition [48,100]. These findings further support the concept that maintaining functional microbial diversity is as important as controlling reactor operating conditions when processing phytochemical-rich agricultural feedstocks.

7. Anaerobic Fermentation Within Circular Bioenergy Systems

Anaerobic digestion (AD) is increasingly recognised as a key technology within circular bioenergy and bioeconomy systems due to its ability to simultaneously valorise agricultural residues, recover renewable energy, and recycle nutrients back into agroecosystems. Beyond waste treatment, AD serves as an integrated bioconversion platform that helps close the carbon, nitrogen, and energy cycles. Agricultural residues, agro-industrial by-products, and food-processing wastes are therefore considered strategic feedstocks for circular bioeconomy development due to their high organic matter content and substantial energy recovery potential. However, as demonstrated throughout this review, their effective utilisation depends not only on biomass availability but also on the phytochemical characteristics that influence anaerobic digestion performance [140,141].

An important advantage of AD is its contribution to greenhouse gas mitigation. The controlled conversion of biodegradable residues into biogas reduces uncontrolled methane emissions associated with conventional waste storage and disposal while partially replacing fossil energy sources. Moreover, digestate recycling supports nutrient recovery and reduces dependence on mineral fertilisers [140–142]. However, these environmental and energetic benefits may not be fully realised when agricultural feedstocks contain high concentrations of plant-derived bioactive compounds that reduce methane production and compromise process stability [11,143,144].

The integration of agricultural biogas plants with local biomass streams is increasingly promoted as a means to improve regional energy security and reduce the environmental burden of managing agricultural waste. This approach is particularly relevant in areas characterised by intensive crop production and food processing, where seasonal surpluses of lignocellulosic residues and fruit-processing by-products are generated [140,141,145]. Effective utilisation of these locally available resources, therefore, requires feedstock-specific management strategies rather than uniform anaerobic digestion approaches.

In this context, effective management of plant-derived bioactive compounds becomes a prerequisite for the successful implementation of AD within circular bioenergy systems. Rather than relying on a single mitigation strategy, successful implementation requires matching feedstock characteristics with appropriate operational conditions and microbial management. The integrated relationships among phytochemical composition, operational parameters and mitigation strategies are summarised in Table 5. Consequently, modern circular bioeconomy concepts increasingly view anaerobic digestion as a biologically complex system whose performance depends on interactions among feedstock characteristics, microbial ecology and process management [141,143,145].

Techno-Economic Implications and Digestate Quality

Implementing mitigation strategies to overcome phytochemical inhibition introduces significant techno-economic challenges that must be evaluated before full-scale application. Advanced pretreatment methods, such as thermal, chemical, or intensive mechanical disruption, successfully reduce the structural recalcitrance of lignocellulosic matrices and degrade complex polyphenols. However, these processes drastically increase both capital expenditures (CAPEX) and operational costs (OPEX) due to high energy requirements and equipment maintenance.

From an economic perspective, co-digestion remains the most cost-effective and operationally viable strategy. It requires minimal infrastructural modifications while significantly improving biogas yields through the synergistic dilution of toxic compounds and the optimisation of the C:N ratio. Furthermore, integrating anaerobic digestion into a broader biorefinery framework offers a promising pathway to improve overall profitability. The sequential extraction and recovery of high-value bioactive compounds (e.g., antioxidant polyphenols from olive or tomato residues) before anaerobic digestion not only prevents microbial inhibition but also generates additional revenue streams without causing a biochemical methane potential penalty [124]. Due to the narrative scope of this review, a full comparative life cycle assessment (LCA) of all detoxification strategies is beyond its scope. However, mitigating phytochemical toxicity must be continually balanced against the net energy and economic gains of the biogas plant.

Beyond energy production, the presence of bioactive compounds in agricultural feedstocks raises important questions regarding the quality and agronomic safety of the resulting digestate. Plant secondary metabolites, particularly recalcitrant condensed tannins and highly concentrated essential oils, may not be fully degraded during the anaerobic process [146]. Consequently, these residual compounds can persist in the digestate, potentially exerting phytotoxic effects or altering the native soil microbiome when applied directly as an agricultural biofertilizer. Future research must urgently evaluate the long-term environmental fate of these residues and establish strict phytotoxicity thresholds for digestates derived from polyphenol- and terpene-rich feedstocks, ensuring that biogas production does not compromise agricultural soil health.

8. Future Perspectives and Research Needs

Although research on the effects of plant-derived bioactive compounds in anaerobic digestion has expanded considerably in recent years, important knowledge gaps remain. Most available data originate from short-term batch experiments conducted under highly variable operational conditions, which limits direct comparisons among studies and complicates the translation of experimental findings into industrial practice. As highlighted by the integrated evidence matrix (Table 5), current knowledge remains uneven across feedstock types, phytochemical classes and reactor configurations, making evidence-based process optimisation difficult.

One of the major challenges is the lack of standardised approaches for quantifying bioactive compounds and reporting inhibitory concentrations. Concentrations are commonly expressed using different units, including $\text{mg}\cdot\text{L}^{-1}$, $\text{mg}\cdot\text{g}^{-1}$ VS, and $\text{mg}\cdot\text{kg}^{-1}$ TS, making cross-study comparisons difficult and preventing the establishment of universal dose–response relationships. Standardisation of analytical methodologies and reporting formats would facilitate cross-study comparisons and enable the development of more robust dose–response models.

Another important research gap concerns chronic inhibition in continuous anaerobic digestion systems. While acute toxicity has been extensively investigated, the long-term effects of phenolics, terpenes, and other secondary metabolites on microbial adaptation, resilience, and recovery remain poorly understood. Future studies should prioritise continuous reactor experiments conducted under realistic loading rates, hydraulic retention times, and substrate fluctuations, using feedstocks representative of those in commercial agricultural biogas plants rather than model substrates.

Future studies should increasingly focus on functional microbiology rather than solely on taxonomic composition, linking metagenomic data with reactor performance and microbial functions.

It is recommended that future research focus additionally on the transformation pathways of bioactive compounds during AD. Current evidence suggests that many phenolics and terpenoids undergo partial transformation during anaerobic digestion, generating intermediate metabolites with potentially different toxicity and biodegradability profiles. Consequently, inhibitory effects should be viewed as dynamic processes involving both parent compounds and transformation products, rather than being interpreted solely from initial concentrations.

Further optimisation of mitigation strategies is also required. Although promising results have been reported for pretreatment, co-digestion, adsorbent supplementation, and microbial acclimation, their combined performance under industrial-scale conditions remains insufficiently understood. Particular attention should be given to conductive materials, such as biochar and hydrochar, due to their dual roles as adsorbents and promoters of DIET-mediated interactions under realistic long-term continuous operation.

Another important priority is the validation of laboratory findings under pilot- and full-scale operating conditions. Most available evidence comes from BMP assays or laboratory reactors, whereas considerably fewer studies have evaluated phytochemical inhibition under long-term industrial conditions. Bridging this scale gap is essential for translating experimental observations into practical recommendations.

A significant future direction involves the development of predictive models and decision-support tools for bioactive substrate management. Machine learning, artificial intelligence, and kinetic modelling approaches have the potential to facilitate the prediction of inhibition risk based on substrate composition, reactor conditions, and microbial community structure. The implementation of such tools has the potential to enhance operational stability and facilitate real-time process control in agricultural biogas plants. Such models

should integrate phytochemical composition, reactor operating conditions and microbial functional data rather than relying solely on conventional process parameters.

Finally, greater attention should be paid to integrated biorefinery concepts that combine the recovery of high-value phytochemicals with the subsequent anaerobic digestion of residual biomass. Selective extraction of polyphenols, terpenes, and flavonoids prior to anaerobic digestion may simultaneously reduce inhibitory pressure and generate additional revenue streams, thereby improving both process sustainability and economic viability.

Future progress will depend on integrating environmental biotechnology, microbial ecology, analytical chemistry and process engineering within unified research frameworks. Such interdisciplinary approaches should support the development of predictive, feedstock-specific management strategies that maximise methane recovery while maintaining process stability. Ultimately, translating these advances into industrial practice will be essential for the efficient utilisation of phytochemical-rich agricultural residues within circular bioeconomy systems.

9. Conclusions

Plant-derived bioactive compounds should be regarded as integral components of agricultural feedstocks rather than secondary constituents of anaerobic digestion systems. The available evidence demonstrates that their effects extend beyond direct microbial toxicity and result from complex interactions among phytochemical composition, microbial adaptation, reactor configuration and operating conditions. Consequently, inhibition cannot be explained solely by the identity or concentration of individual compounds. Still, it should instead be interpreted as a dynamic process shaped by both substrate characteristics and process conditions.

This review demonstrates that effective management of phytochemical-rich agricultural residues requires a feedstock-specific approach integrating phytochemical characterisation with microbial ecology and process engineering. The integrated evidence presented in this review shows that process performance depends not only on the concentration of inhibitory metabolites but also on inoculum adaptation, reactor type, operating conditions and the application of appropriate mitigation strategies. These relationships have been synthesised in the integrated evidence matrix (Table 5), which provides a practical framework for selecting appropriate management strategies based on feedstock characteristics and process conditions.

Future evaluation of agricultural feedstocks should therefore extend beyond conventional BMP assessment to include phytochemical composition, microbial functional responses and key operational parameters. Such an integrated approach will improve the prediction of actual methane recovery, support evidence-based optimisation of anaerobic digestion systems, and facilitate the sustainable utilisation of agricultural residues within circular bioeconomy and integrated biorefinery concepts.

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Abbreviations

The following abbreviations are used in this manuscript:

AD	Anaerobic Digestion
ATP	Adenosine Triphosphate
BMP	Biochemical Methane Potential
CH ₄	Methane
C/N	Carbon-to-Nitrogen Ratio
CSTR	Continuous Stirred-Tank Reactor
DIET	Direct Interspecies Electron Transfer
EPS	Extracellular Polymeric Substances
HRT	Hydraulic Retention Time
MAG	Metagenome-Assembled Genome
OLR	Organic Loading Rate
sCOD	Soluble Chemical Oxygen Demand
TS	Total Solids
UASB	Upflow Anaerobic Sludge Blanket
UPBR	Upflow Packed Bed Reactor
VFA	Volatile Fatty Acid
VS	Volatile Solids
CO ₂	Carbon Dioxide
NaOH	Sodium Hydroxide
Ca(OH) ₂	Calcium Hydroxide
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
LCA	Life Cycle Assessment
CAPEX	Capital Expenditure
OPEX	Operating Expenditure

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