Environmental and Climate Technologies
2025, vol. 29, no. 1, pp. 797–808
https://doi.org/10.2478/rtuect-2025-0053
https://content.sciendo.com

Enhancing Methanogenesis in Anaerobic Bioreactors Using *Phragmites Australis* and Additives – A Review

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Received 27.05.2025; accepted 10.10.2025

***Abstract* – Anaerobic digestion of lignocellulosic biomass, particularly *Phragmites australis* (common reed), presents prospects for sustainable biogas production. However, its rigid structure and high lignocellulose content obstruct microbial accessibility and the effectiveness of methanogenesis. The review offers a comparative analysis of recent strategies to enhance methane yield and process stability through iron-based nanomaterials, trace metal additions, enzymatic pre-treatments, and co-substrates. Iron-based nanomaterials reduce process inhibition and improve redox balance by facilitating direct interspecies electron transfer. Supplementation with trace metals such as nickel, cobalt, and selenium enhance methanogenic pathways, thereby promoting microbial enzymatic activity. The optimization of additive requirements, taking into account substrate characteristics, microbial community dynamics, and operational conditions, offers a solid framework for designing and adapting additive strategies to specific AD systems. Enzymatic pre-treatment improves hydrolysis by transforming complex lignocellulosic structures into readily fermentable sugars, thereby accelerating biogas production. Compared to other alternatives, the expenses associated with enzymes and the difficulties in process optimization complicate the large-scale implementation. However, improvements vary widely among studies due to differences in enzyme type, dosage, substrate composition, and operational conditions, leading to the need for standardized evaluation methods. Co-digestion with nitrogen-rich substrates improves the carbon-to-nitrogen ratio, thereby minimizing ammonia inhibition and promoting stable microbial activity. This paper addresses current challenges with feedstock recalcitrance, process inhibition, and biogas quality while also emphasizing the additives' synergistic benefits in maximizing anaerobic digestion performance and offering perspectives for future research and practical implementation of enhanced anaerobic digestion systems.**

***Keywords* – Additives; anaerobic digestion; biogas production; co-digestion; enzymatic pre-treatment; DIET; iron-based nanomaterials; methanogenesis.**

1. INTRODUCTION

Anaerobic digestion is a process in which microorganisms decompose the organic components into methane (CH_4), carbon dioxide (CO_2), hydrogen sulphide (H_2S), and other gases in the absence of free oxygen [1]. Although AD is a highly promising process for producing energy from waste, it has significant limitations, including low conversion efficiency, a lack of process stability, and operational requirements that vary depending on the feedstock composition. The physiological parameters of the biochemical processes

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occurring during anaerobic digestion are influenced by microbial growth at each stage, operational modes, as well as the pH and temperature of the digester [2]. The anaerobic digestion process includes four sequential stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

- Hydrolysis: decomposition of complex, non-soluble biopolymers, including carbohydrates, proteins, and lipids, into soluble monomers such as sugars, amino acids, and fatty acids;
- Acidogenesis: conversion of soluble organic molecules generated during hydrolysis into volatile fatty acids (VFAs), CO_2, and other intermediates;
- Acetogenesis: VFAs are metabolized into acetate, hydrogen (H_2), and supplementary carbon dioxide (CO_2) by acetogenic bacteria;
- Methanogenesis: methanogenic archaea transform acetate, CO_2, and H_2 into CH_4, thereby finalizing the biogas production process [4].

To ensure stable AD performance, digesters with large volumes are usually built, resulting in substantial capital expenditures. Therefore, increasing hydrolysis and methanogenesis rates is critical for increasing biogas output, allowing the organic loading rate (OLR) to be increased while decreasing reactor volume, resulting in significant capital cost savings [4]. Methanogenesis is a key biochemical process of anaerobic digestion during which organic material is transformed into methane by a consortium of organisms, including methanogenic archaea [5]. This process has substantial implications for the production of renewable energy, particularly in biogas plants.

Despite *Phragmites australis* potential as a viable feedstock or renewable energy source, its invasive and fast-growing nature often results in blocking waterways and severely limiting access to recreational areas [6]. Harvesting common reed has the drawback of limited terrain accessibility, causing the need for specific harvesting technologies as well as the necessity of constant mowing, including biomass removal to maintain areas for the development of adaptive biodiversity, which makes it particularly crucial that the proper use of the generated biomass is feasible [7]. *Phragmites australis* biomass can be harvested twice a year, during winter and summer. Biomass harvested during the summer has a high moisture content and is therefore suitable for fermentation and extraction for medical applications, but the dry biomass harvested in the winter period is suitable for the production of construction materials or to produce fuel [8].

The anaerobic digestion process is subject to many limitations. Hydrolysis and methanogenesis are often seen as rate-limiting processes, especially because high ammonia levels can slow them down. In the preliminary phase, increased ammonia concentrations obstruct the hydrolysis of biomass, but in the final phase, they hinder methane production. To address these problems, extensive research has focused on increasing methane generation, augmenting biomass conversion efficiency, and refining co-digestion techniques to promote more effective interspecies microbial interactions. The incorporation of metallic and metal oxide nanoparticles, including copper, nickel oxide, hematite, palladium, and silver, has demonstrated potential in enhancing the yield and rate of biohydrogen and methane production by promoting microbial activity and optimizing electron transfer dynamics [9].

Because the common reed's lignocellulosic structure is rigid, it often needs to be pre-treated first to make it more biodegradable before or during anaerobic digestion. Pre-treatment is a widely used method to accelerate anaerobic digestion and increase biomethane production by making previously inaccessible substrates accessible to microorganisms and accelerating substrate conversion [5]. Besides pre-treatment, the incorporation of various additives into the anaerobic digester can significantly enhance the methanogenesis process by increasing biogas yield and overall efficiency and economic viability [10].

2. Enhancement Strategies and Mechanisms of Additives' Action in Anaerobic Digestion

To enhance the anaerobic digestion capabilities of *Phragmites australis,* various additives and process strategies have been investigated. Chemical additives, biological additives, and physical stimulants are the three primary categories into which additives employed to improve methanogenesis can be classified. Each category encompasses different substances or methods that are designed to enhance the activity of methanogenic archaea or the overall efficacy of the anaerobic digestion process [11]. Fig. 1 illustrates the integrative role of various additives and waste sources in enhancing the performance of anaerobic digestion systems. The AD reactor is located at the centre of the diagram, highlighting its role as the biological core where microbial activity converts organic matter into biogas.

This chapter addresses four significant categories: trace metal supplementation, iron-based nanomaterials, co-digestion, and enzymatic pre-treatment. Each method has the potential to enhance methane yield and stability by targeting distinct limiting factors in reed digestion.

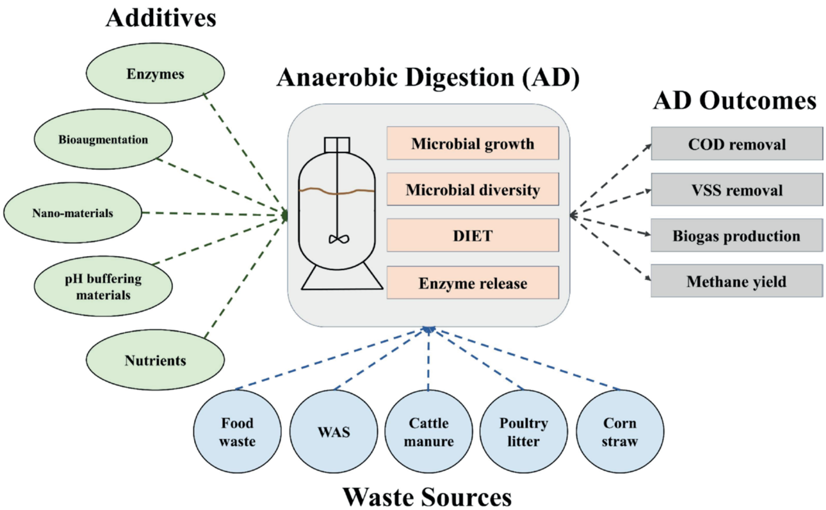

Fig. 1. A schematic diagram of the additives, their impact on AD, and the primary enhancing pathways [11].

Various enhancements are examined to optimize chemical oxygen demand and volatile suspended solids removal, methane output, and biogas production rates. These additions include enzymes, nanomaterials, pH buffers, nutrients, and bioaugmentation agents. Additives can promote microbial growth and diversity, direct interspecies electron transfer (DIET), and enzyme release, which are the main mechanisms and pathways for improved AD process, collectively improving efficiency and stability [11].

2.1. Trace Elements Supplementation

Optimization of both the effectiveness and stability of biogas production, trace elements (TEs), also known as micronutrients, are essential additives in the anaerobic digestion process. Numerous studies have been conducted to determine the requirements and ideal concentrations of trace metals, specifically Fe, Co, and Ni in pure cultures of methane fermentation [12]. These elements help to maintain the balance of the microbial community and prevent process upsets. The primary functions of TEs are as follows: (i) promoting the metabolic activity of microorganisms, which results in increased biogas production and a higher methane content; (ii) ensuring the proper functioning of enzymes and metabolic pathways, which helps maintain a stable biodegradation process; (iii) reducing inhibitory compounds, which prevents the accumulation of inhibitors such as VFAs and ammonia; and (iv) preventing toxicity caused by the excessive accumulation of certain metals, such as copper or zinc [13].

Iron (Fe) is the most extensively researched element regarding additive effects, performing numerous roles in AD, primarily due to the exceptionally high reducing potential, the operation of numerous enzymes such as hydrogenases and ferredoxins, which are fundamental to electron transfer and hydrogen metabolism, and the facilitation of methanogenic bacteria in converting CO_2 to CH_4. Therefore, sufficient iron concentrations can enhance the production of biogas and sustain the anaerobic digestion process [4], [13]. Nickel (Ni) is crucial for the activity of many enzymes, including methyl-coenzyme M reductase, which plays an important part in the final stage of methanogenesis and the first step of methane oxidation. Anaerobic bacteria are significantly reliant on Ni, utilizing carbon dioxide and hydrogen as their exclusive energy sources. DIET can be used to accelerate biogas and methane production by allowing for direct cell-to-cell electron transfer between bacteria and methanogens, resulting in metabolically favourable conditions and the rapid conversion and production of organic acids and alcohols into methane and biogas [14].

Cobalt (Co) is a fundamental component of vitamin B12, which activates carboxypeptidase and is necessary for the metabolism of specific methanogens. Corrinoids, including vitamin B12, which contain a cobalt ion, associate with methyl-coenzyme M (CoM) reductase, facilitating methane production in acetoclastic methanogens and hydrogenotrophic bacteria. Cobalt can enhance methane production and augment the metabolic functions of acetoclastic methanogens. In addition to enhancing the methanogenesis process, the incorporation of Co is thought to promote acetogenesis during the early phase of anaerobic digestion. Cobalt is a significant factor in the proliferation of acetogens [13].

Trace metals in nanoparticle form show a unique biostimulating influence on methanogenic activity from the initiation of the anaerobic process to the end of the experiments [15].

2.2. Iron-Based Nanomaterials

Diverse nanoparticles (NPs) have been widely applied across many industrial sectors due to their distinct physicochemical characteristics in the field of technological advancements. Metallic, especially iron-based NPs, are widely recognized as the most effective enhancement additives for anaerobic digestion [2], [16]. The main challenge for implementing NPs in full-scale anaerobic digesters is their cost. Most studies are conducted on laboratory or pilot scales, making them difficult to implement. To overcome this, two strategies can be explored: using low-cost iron-based NPs, which can be scalable and obtained from waste, and reusing NPs in multiple cycles of anaerobic digestion. However, no evidence exists for anaerobic digestion with NPs, except for semicontinuous processes. Another challenge is the characterization and possible uses of digestate, as iron is a nontoxic metal that may accumulate in digestate over time and influence nutrient balance and soil properties, requiring careful assessment [17].

The oxidation state of iron nanoparticles is considered a crucial factor in the enhancement of biogas or methane production. Certain research has thoroughly investigated the oxidation state, emphasizing the role of the iron oxidation state as a catalyst for enhanced methane production in anaerobic digestion. Fig. 2 illustrates a schematic representation of the process of oxidation state and nanostructure of iron-based materials, enhancing biogas mechanisms.

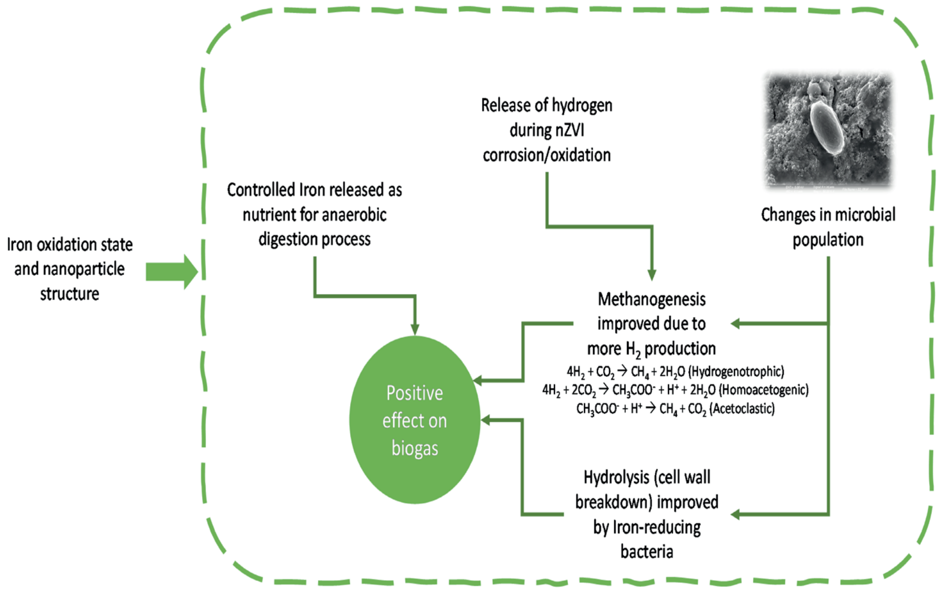

Fig. 2. Mechanisms for enhancing anaerobic digestion using nanomaterials [17].

During AD, iron is gradually released, facilitating hydrogen generation through corrosion reactions, which supports hydrogenotrophic methanogenesis and increases methane yield. Conductive nanomaterials enable DIET between syntrophic bacteria and methanogens, eliminating the necessity for intermediaries. Nanoparticles, such as nZVI or magnetite, facilitate the hydrolysis of lignocellulose by aiding iron-reducing bacteria and offering surfaces for enzymatic degradation. Alterations in microbial communities often enhance the prevalence of electrogenic bacteria and methanogens adapted to DIET [17]. Fe-NPs in the AD process for biogas production ensure the process is economically feasible, as the Fe-NPs recovery from the AD process is costly when compared to the Fe-NPs preparation [18], [19]. Despite high operating costs, nanomaterials are an efficient technology for AD intensification applications due to their excellent performance in improving methane production in comparison to other additives [11].

2.3. Co-Digestion

The simultaneous anaerobic digestion of two or more substrates in the same reactor is referred to as co-digestion. Co-digestion with a co-substrate that is degradable and nutrient-rich can dramatically enhance the performance of *Phragmites australis*. This strategy can introduce positive synergies between different waste streams and address the imbalanced C/N ratio and nutrient deficiencies of reeds. Feedstocks such as livestock manures, food wastes, and sewage sludge are frequently employed as co-substrates in cooperation with lignocellulosic biomass due to their high nitrogen and moisture content, as well as their buffering capacity and active microbial communities. The substrate type has an important impact on how effectively the reactor works, given that different substrates break down at different rates and produce different intermediate chemicals that affect the growth of fermentation, which is particularly relevant for carbohydrates, proteins, cellulose, hemicellulose, lignin, and lipids [20]. Different substrates can complement each other's microbial decomposition. Easily degradable compounds (starches, sugars, fats from food waste or manure) produce VFAs that stimulate the growth of acetogens and methanogens, which in turn may also co-metabolize the tougher components of reed. There is evidence that the presence of a co-substrate can induce a more diverse enzyme production, as microbes adapt to multiple feeds. In essence, co-digestion can create a more robust microbial ecosystem capable of degrading a wider range of materials [21]. The co-digestion of *Phragmites australis* at 25–50 % with a base substrate produced optimal biogas outputs in a study on aquatic plant mixes [7].

The chemical composition of substrates profoundly influences process factors such as OLR, pH, and alkalinity, making an understanding of their composition essential for methane production, biogas prediction, and the optimization of process parameters. Meticulous substrate selection can enhance nutrient equilibrium, biogas production, and overall efficacy in Anaerobic Co-digestion (ACoD) process systems. Another influencing factor, the agitation/mixing rate, refers to the intensity at which organic materials within a digester are mixed, facilitating optimal biogas production. It facilitates interaction between microorganisms and substrates, ensuring equal temperature distribution and minimizing foaming. Effective mixing is essential for solid accumulation, floating layers, and biogas release. Continuously mixed digesters are discouraged due to negative effects on biogas production and higher power consumption. Mixing can be done using hydraulic, mechanical, or pneumatic systems at various frequencies and strengths. The optimal mixing rate should be balanced to ensure enough mixing without high energy consumption [22].

2.4. Enzymatic Additives

Enzymes are essential for improving the efficiency of digestion and the rate of biogas production from organic materials by microorganisms, which transform macromolecular solids into soluble and biodegradable substances, improving substrate digestion [4]. Research indicates that enzyme augmentation can enhance the methane potential of agricultural by-products. While enzymatic pre-treatment of *Phragmites australis* is less prevalent in the literature compared to physical and chemical techniques, valuable insights can be gained from analogous substrates. In an experiment with corn straw, the incorporation of a commercial enzyme cocktail into a continuous digester increased the methane yield to approximately 187 mL/g VS, representing a 12 % enhancement compared to the control group devoid of enzymes [23].

Temperature activates and stimulates the enzyme activity, which is an important factor. For instance, enzyme activity is typically minimal in ambient anaerobic digesters (i.e., <25 °C), which can greatly reduce the rates of digestion and biogas production. Enzymes are generally stable and exhibit consistent activity under mesophilic conditions (i.e., 30–40 °C), which enables the production of biogas and the development of microbial organisms in a balanced manner. The activity of enzymes is enhanced in thermophilic AD (i.e., 50–60 °C) compared to mesophilic conditions as a result of the higher reaction rates at high temperatures [11].

Correct use of enzymes can enhance the total cellulolytic activity, thereby eliminating the need for microbial cellulase. This pre-treatment method improves hydrolysis, resulting in higher methane yields and volatile fatty acid production. Additionally, the faster throughput may only slightly enrich acidogens and methanogens [24].

3. COMPARISON OF ENHANCEMENT STRATEGIES FOR ANAEROBIC DIGESTION

The dosage is a critical factor in all environmental uses of nanomaterials. A significant characteristic of these materials is their exceptionally high surface-to-volume ratio compared to non-nanomaterials, which results in improved characteristics such as adsorption and catalytic activity [17]. The benefits of employing conductive materials to address AD process concerns, including a long lag phase, low methane synthesis and yield, and vulnerability to complex conditions.

The improvement of anaerobic digestion efficiency through additives and pre-treatment methods has been thoroughly investigated, although their effectiveness is still dependent on particular conditions. As shown in Table 1, enhancement in methane yield varies among researchers due to differences in substrate composition, digester scale, and operational parameters. These variations demonstrate that process optimization in anaerobic digestion depends on several interrelated factors such as physical structure, nutritional stability, and microbial activity. This section emphasizes the necessity of standardized experimental methodologies to facilitate more dependable comparisons of enhancement strategies and to link laboratory studies with full-scale applications.

TABLE 1. COMPARISON OF ENHANCEMENT STRATEGIES APPLIED TO *PHRAGMITES AUSTRALIS* UNDER BATCH ANAEROBIC DIGESTION CONDITIONS. DATA ARE SUMMARIZED FROM DIVERSE STUDIES, DIFFERING IN SUBSTRATE COMPOSITION AND REACTOR SCALE

Additive type	Dosage Range	Methane Yield	Microbial Community	Ref.
Iron-Based Nanomaterials. ZVI, Magnetite nanoparticles (Nano-Fe_2O_3), Hematite or maghemite (Fe_2O_3)	Optimal concentrations typically range in the low hundreds of mg/L (e.g., 20–100 mg/L); elevated dosages (≥1 % of substrate total solids) provide a risk of inhibition. For instance, research employed 5 g/L Fe° or 5–20 mg/L Fe°.	15–60 % enhancement in methane yield with adequate dosage. For instance, 5–20 mg/L of Fe° elevated CH_4 levels to 1.38–1.59 times that of the control; 15 mg/L of Fe_3O_4 enhanced yield by around 27.5 % with 79 days of reaction time.	Facilitates DIET between syntrophic bacteria and methanogens, hence increasing acetogenesis and methanogenesis. Frequently improves hydrogenotrophic methanogens. The released Fe^{2+} acts as a nutrient and precipitates H_2S into FeS, reducing toxicity.	[25] [26]
Trace Metal Supplementation. Nickel, cobalt, and salts (e.g., $NiCl_2$, $CoCl_2$, $FeCl_3$, Na_2MoO_4 as needed)	Required small amounts of Ni (0.04–0.5 mg per g VS) and Co (~0.01–0.1 mg/g VS) are sufficient, resulting in only a few mg/L. Usually added constantly or intermittently to maintain micronutrient levels.	20–50 % improvements; Supplementing with Ni+Co (with Fe/Mo) increased methane production by 35 % (504 mL CH_4/g vs control).	Cofactors for microbial enzymes may reduce metabolic limitations in methanogens. Ni is needed for the F430 cofactor in methyl-CoM reductase, and Co for vitamin B_{12} analogs; therefore, adding them promotes methanogen development and activity.	[27] [28]

Enzymatic Pre-treatment. Cellulases, hemicellulases, ligninases	Enzyme loading in tens of milligrams per gram of biomass. Typically, 10–20 mg of cellulase per gram. Pre-treatment time can vary from hours to days. Sequential or single-dose supplementation of enzymes is feasible.	Methane yield increases by 30–60 %, depending on pre-treatment severity. Enzymatic hydrolysis typically increases CH_4 by ~50 % for lignocellulosic. Adding approximately 15 mg/g cellulase (with modest chemical assistance) increased wheat CH_4 output by 64.8 %.	Hydrolysis increases the availability of readily fermentable sugars, thereby shortening the lag phase and promoting earlier volatile fatty acid formation. This enhances substrate flux to downstream microorganisms. Fermentative bacteria can produce biogas more rapidly, and methanogens have a greater supply of acetate/H_2 at an earlier stage.	[29]–[31]
Co-digestion Mixing *Phragmites australis* with complementary substrates	*Phragmites australis* is frequently used as 20–50 % of the feedstock (VS basis). *Phragmites australis* with food waste at a ratio of approximately 1:1. The optimal ratio is contingent upon the co-substrate properties, intending to achieve a C: N ratio of approximately 20–30 and pH 6–8.5.	For instance, the mono-digestion of reed produced approximately 35 mL CH_4/g VS, while co-digestion with food waste resulted in yields of approximately 125–137 mL/g VS (approximately 3–4 times higher). Specific methane yields of around 270 mL/g VS have been attained with the combination of *Phragmites australis* and food waste, surpassing the weighted sum of the individual substrates by approximately 18 %.	Co-digestion promotes a stable consortium with enhanced syntrophy and frequently higher total methanogen counts by providing balanced nutrients and dilution of inhibitors. The co-substrate supplies missing nitrogen, trace elements, or easily digestible organic fractions, resulting in a more diverse and robust microbial community.	[22] [29]

4. DISCUSSION

Co-digestion is the most cost-effective and comprehensive approach to enhancing *Phragmites australis* AD, particularly when complementary waste is available. Trace metal addition is a simple and effective method for promoting the growth of methanogens and can be considered a generally applicable modification for nutrient-limited systems, regardless of whether other methods are used. Iron-based nanomaterials are highly effective in enhancing the kinetics and yields of methane formation, particularly in systems that are subject to higher loads or VFA accumulation. Their effectiveness is maximized when hydrolysis is not the limiting phase, which is why they are well-suited to co-digested or pre-treated feeds [32]. A substantial increase in methane yield was observed when compared to *Phragmites australis* alone. Consequently, co-digestion significantly enhances the biogas potential derived from *Phragmites australis*.

Among the studied approaches, iron-based nanomaterials and enzymatic pre-treatment produce the greatest improvements in methane yield, ranging from 30 % to 60 %. Thus, enzymatic pre-treatment is a promising and preferred approach when the feedstock's recalcitrance is the primary obstacle and the budget allows for a high-impact intervention, as it provides substantial improvements in the biodegradability and methane potential of *Phragmites australis*. Integration of various strategies often yields optimal results, which vary depending on environmental and economic implications. Although the enzymatic pre-treatments are highly effective, their economic feasibility is challenging at large scales due to cost [15]. Conversely, co-digestion and trace metal supplementation are cost-effective and readily implementable, though co-substrate availability may constrain application. Iron-based additives, though moderately priced, require careful dosage to avoid toxicity and optimize economic returns. Trace-metal supplementation resulted in moderate but significant enhancements (20–45 %) by restoring cofactors required for methanogenic enzymes, including coenzyme and hydrogenases. Excessive or poorly controlled dosing can lead to metal precipitation or toxicity, highlighting the necessity for precise dosage optimization in relation to substrate mineral content and digester configuration.

Nanomaterials have been studied for biogas production enhancement in the last decade. However, their effectiveness depends on factors like size, concentration, and substrate type. Mixing nanomaterials and studying their interactions could be a leading research area [33]. Studies indicate that iron has demonstrated promising outcomes, which have been mostly connected to its role as an electron donor/acceptor and cofactor of important enzyme activities [34]. The primary reason for the reduced production of biogas in the absence of NPs is a cellular wall that blocks the interaction of catalysts with the substrate. Comprehension of biogas production by examining the impact of various NPs from the perspective of their positive and negative characteristics [33]. Although iron-based nanomaterials have shown promising effects on methane yield and process stability, their environmental implications require careful evaluation. The potential accumulation of nanoparticles in digestate applied to agricultural soils raises concerns about long-term ecotoxicity and metal mobility. Recent research indicates that nanoparticle behaviour and toxicity are strongly influenced by soil characteristics such as pH, organic carbon content, cation-exchange capacity, and redox conditions. However, the specific influence of these parameters on nanoparticle bioavailability and persistence remains poorly acknowledged. It should also be taken into account that ecological concerns are crucial for rationalized usage of nanotechnology [35]. Therefore, a comprehensive assessment of soil-nanoparticle interactions is essential for accurate environmental risk evaluation and for ensuring the safe integration of nanomaterial-assisted anaerobic digestion technologies.

The technological potential of additives and co-digestion for improving reed methanogenesis has been demonstrated. However, translating those findings into an established system will need to overcome these challenges. Future research should focus on real-world tests where harvested *Phragmites australis* is broken down using different methods, while demonstrating not just the amount of gas produced but also the costs, how well it works over time, and its impact on the environment. Also, focusing on integrated additive strategies, including the combination of enzymatic pre-treatment with conductive nanomaterials or trace-metal supplementation, to benefit from potential synergistic effects while reducing environmental and economic disadvantages. Establishing optimized, context-specific enhancement frameworks is essential for ensuring stable, efficient, and sustainable large-scale anaerobic digestion of *Phragmites australis*.

5. CONCLUSION

The utilization of *Phragmites australis* as a methane generation source has limitations due to its lignocellulosic nature, requiring an efficient pre-treatment technique to modify the substrate structure and mitigate lignin's impact on cellulose bioavailability. The process stability and reactor effectiveness of an anaerobic digester may be improved using additives that show specific biochemical activity. Enhancing biogas output is a crucial step for increasing the economic feasibility and sustainability of biogas generation from lignocellulosic biomass. This review illustrates that the strategic application of additives, specifically trace element supplementation and iron-based nanomaterials, may significantly enhance methanogenesis by mitigating microbial constraints, augmenting enzymatic activity, and facilitating complex electron transfer processes, including DIET. Iron has been reported as the cheapest trace metal for improving methane yield and process stability. Apart from trace elements, metal oxide nanoparticles could also improve the catalytic process in AD and offer trace metals for metabolic activity simultaneously. The successful use of enzymatic pre-treatment in augmenting biomethane recovery from lignocellulosic biomass requires further research to clarify the interconnections between biomass composition, enzyme selection, and appropriate pre-treatment parameters. Enzymatic pre-treatments, such as cellulases, hemicellulases, and ligninases, can increase methane yield by 30–60 %, depending on the severity of the pre-treatment. Enzyme production costs as the primary obstacle to full-scale industrial application of enzymatic pre-treatment for biomethane production, despite a lack of comprehensive financial feasibility information. A future study should concentrate on exploring novel approaches to minimize the cost of enzymatic pre-treatment and enhance its economic viability for industrial applications. Co-digestion, which mixes *Phragmites australis* with complementary substrates, can optimize degradability and balance C/N. The performance of AD is affected by microbial communities, bacterial species, the chemical form of CO_2, and the characteristics of nanoparticles. Methanogens demonstrate heightened susceptibility to inhibitors, and the methanogenesis process hinges on their concentration and the presence of Fe-NPs. Additional research is required to clarify the positive impacts of Fe-NPs on methanogenesis and gene transfer. The concentration of Fe-NPs in anaerobic digestion across various substrates and inoculum, where their impact on anaerobic digestion parameters requires investigation.

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