

Propionate production and degradation in the biological wastewater treatment: A mini review on the role of additives in anaerobic digestion

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ABSTRACT

Propionate production and consumption are influenced by thermodynamic constraints, microbial competitions, and metabolic inhibition. Accumulation of propionate in wastewater can destabilize anaerobic digestion and lead to process failure. Anaerobic digestion is one of the viable biological methods for its recovery and utilization. Additives have been shown to modulate propionate production and consumption, effectively influencing the overall performance of anaerobic digestion. This mini review systematically examines the application of various additives and their effects on: (I) propionate production and recovery (less CH₄ and more propionate) and (II) propionate degradation (less propionate and more CH₄) goals in anaerobic digestion. This review studied and listed recent studies on the most used anaerobic digestion additives and classified them according to their impact on propionate concentration. To our knowledge, limited research has been conducted on the role of additives in propionate recovery. No studies have reviewed the impact of additives on propionate recovery from anaerobic digestion or their potential to mitigate its inhibitory effects. This mini review enables researchers to select the most suitable additive to recover propionate or boost CH₄ production by mitigating its inhibition, as well as discussing the role of modern bioreactors.

1. Introduction

Anaerobic digestion (AD) is a biological process that plays a crucial role in waste management and renewable energy production. AD relies on microorganisms to break down organic matter in the absence of oxygen, transforming it into valuable byproducts. AD generally begins with the hydrolysis stage, during which complex biopolymers are broken down into their monomers. It then progresses to the acidogenesis stage, where the biochemicals are converted into intermediate biochemicals, primarily volatile fatty acids (VFAs). Following acidogenesis, the acetogenesis stage ensues, during which all intermediate biochemicals are further degraded into simpler forms, mainly acetate (Ac). Finally, AD concludes with the methanogenesis stage, where the remaining biochemicals are converted into biogas [1]. These intermediate products are key to the overall efficiency of the AD system [2].

Among these intermediates, propionate (Pr) stands out as a central player with significant implications for the success of the process. Pr (molecular weight = 76 g/mole) is the conjugate base of propionic acid (CH₃CH₂COOH), which is the second VFA, being a colorless, water-soluble, and corrosive carboxylic acid (pK_a = 4.87) with a sharp, somewhat unpleasant odor [3]. The intricate balance of Pr's metabolism depends heavily on the symbiotic relationship between different groups of complex microbes, such as acidogens, sulfate-reducing bacteria (SRB), acetogens, and methanogens, during its production in the hydrolytic acidification phase and subsequent biodegradation [4]. Therefore, understanding Pr in AD such as thermodynamics, microbiology, and inhibition, which can be observed at a low concentration of 10 mM [5], is essential for optimizing and manipulating AD [3,6].

Pr's importance in AD goes beyond its role as a metabolic intermediate. It exerts a profound influence on the thermodynamics of the

Abbreviations: Ac, Acetate/Acetic Acid; AC, Activated Carbon; AD, Anaerobic Digestion; Bu, Butyrate/Butyric Acid; CM, Chicken Manure; DIET, Direct Interspecies Electron Transfer; FW, Food Waste; OLR, Organic Loading Rate; Pr, Propionate/Propionic Acid; SM, Swine Manure; SS, Sewage Sludge; SRB, Sulfate-Reducing Bacteria; VFA, Volatile Fatty Acid; WAS, Waste-Activated Sludge; ZVI, Zero Valent Iron

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process and plays a crucial role in complex microbiological interactions [3]. The delicate balance of Pr within the AD can significantly impact the quality and efficiency of CH₄ production [2,7]. On the other hand, Pr serves as a valuable chemical with diverse applications, including its role as a food additive, preservative, pesticide, industrial precursor, etc. [8,9]. Therefore, recent scholarly attention has been directed toward the recovery of Pr from AD, recognizing it as a valuable end product with potential applications beyond its role as an intermediate [10].

To enhance the efficiency of AD, few researchers have explored the impact of various additives and chemicals on the process, focusing specifically on Pr. These additives play a critical role in directing Pr metabolism toward desired outcomes. The choice of additives becomes crucial, depending on whether the goal is to increase Pr degradation for improved CH₄ production or to accumulate Pr for recovery purposes. This review consolidates insights from significant recent studies providing a comprehensive understanding of how additives can influence Pr in the context of AD [11,12]. While there exist numerous additives employing various mechanisms to alleviate Pr inhibition, including Iron-based and carbon-based materials, the most prevalent ones suitable for the recovery purposes are antimethanogens like phenol, chloroform, and hydrogen peroxide [11–13].

This paper seeks to enhance current understanding by providing a brief mini review of the impact of additives on Pr in AD. By reviewing recent findings, it addresses the existing research gap concerning the lack of publications reviewing the control of Pr metabolic balance in AD by various additives, as well as the oversight in previous studies focusing solely on CH₄ production without considering the role of additives in regulating intermediate biochemicals, particularly Pr. This review summarizes and lists the additives and their mechanism and applications for manipulating Pr in AD. The insights presented in this paper are intended to inform future research directions and practical applications in the fields of biochemicals (Pr) recovery and production as well as renewable energy production from AD.

2. Common AD additives affecting propionate

In AD, there is a consistent connection between the pathways that produce and break down Pr. Various additives can be employed to raise Pr levels to increase Pr production, resulting in more Pr being generated than consumed. On the other hand, additives can also be utilized to lower Pr levels to enhance Pr breakdown, leading to less Pr being generated than consumed. This may entail using additives for the purpose of recovering Pr or for improving Pr degradation.

These additives have various effects on pH, conductivity, direct interspecies electron transfer (DIET), changing available surface and active area, inhibiting special microbial activity, and enzymic activity [11,12].

DIET involves syntrophic bacteria exchanging electrons directly, bypassing the need for intermediate compounds. Certain bacteria form electrically conductive connections, such as pili or nanowires, enabling the transfer of electrons between bacteria, and enhancing the efficiency of AD by facilitating direct electron exchange in the degradation of complex organic matter. DIET significantly impacts Pr generation, a bottleneck in AD, by promoting the efficient breakdown of substrates into Pr. This improved Pr generation is vital for maintaining a balanced microbial community and preventing intermediate accumulation. DIET also contributes to effective Pr consumption, preventing its inhibitory effects and ensuring a stable microbial community in the face of substrate fluctuations, ultimately improving the overall performance and resilience of AD systems [14–17].

Iron-based additives are commonly used additives in anaerobic digester reactors, particularly in the context of Pr metabolism. The addition of iron compounds, such as ferrous sulfate or magnetite, has been found to enhance the performance of the anaerobic digester reactors by promoting syntrophic interactions among microorganisms. In Pr-rich environments, iron serves as a crucial mediator in electron transfer reactions, facilitating the conversion of Pr to Ac through the reduction of electron carriers. This mechanism is attributed to the role of iron as a redox catalyst, aiding in the transfer of electrons within microbial consortia. Iron supplementation is recognized for reducing the inhibitory effects of high Pr concentrations on methanogenic archaea, leading to a more stable and efficient anaerobic digestion process. The use of Iron-based additives is crucial in enhancing Pr degradation and increasing biogas production in anaerobic digestion systems [18].

Zero valent iron (ZVI) is a promising additive in anaerobic digester reactors, particularly in addressing the accumulation of Pr. ZVI functions as a catalyst in AD systems, promoting the reduction of organic compounds and facilitating microbial metabolism. In the context of Pr, ZVI's mechanism involves its ability to serve as an electron donor, promoting the conversion of Pr to less inhibitory substances like Ac. The electron transfer reactions mediated by ZVI help mitigate Pr buildup, enhancing the overall efficiency of AD. Additionally, ZVI can aid in the precipitation of metals and improve the settling characteristics of biomass, contributing to the stability and effectiveness of AD systems. Overall, the incorporation of ZVI holds promise for optimizing the anaerobic digester reactors, particularly in managing Pr concentrations and improving biogas production [19–22].

Zeolite and magnetite, both with unique properties, have emerged as promising additives in AD, particularly in addressing the accumulation of Pr. Zeolite's porous structure and ion-exchange capabilities provide a favorable habitat for syntrophic microorganisms and adsorb inhibitory substances, promoting Pr reduction and overall AD stability. Meanwhile, magnetite acts as an electron transfer mediator, facilitating syntrophic interactions and enhancing Pr reduction. Its magnetic properties also facilitate separation and reuse. These innovative approaches hold the potential for optimizing AD systems and advancing sustainable waste treatment [23–25].

Carbon-based additives, including activated carbon (AC), biochar/hydrochar, and conductive carbon cloth, have emerged as promising additives in AD, particularly in addressing challenges associated with Pr accumulation. These additives with their high surface area and porosity facilitate the adsorption of inhibitory substances like Pr, providing a favorable environment for microbial consortia and promoting syntrophic interactions. Additionally, they serve as electron shuttles, enhancing electron transfer processes and improving microbial activity. The incorporation of these additives represents a promising strategy to optimize AD and address challenges associated with Pr accumulation [26–28].

The inclusion of trace elements and bioavailable metals such as zinc, copper, nickel, and molybdenum as additives in AD holds significant promise for addressing Pr accumulation. These elements serve as vital cofactors for key enzymes involved in microbial metabolic pathways, particularly those responsible for Pr degradation. The presence of bioavailable metals enhances the activity of syntrophic microbial consortia engaged in Pr oxidation, promoting the conversion of Pr to Ac. Additionally, these elements play a crucial role in electron transfer reactions, optimizing the efficiency of Pr degradation. The strategic supplementation of trace elements and bioavailable metals not only fosters Pr metabolism but also contributes to the overall stability and resilience of the anaerobic microbial community. This approach proves

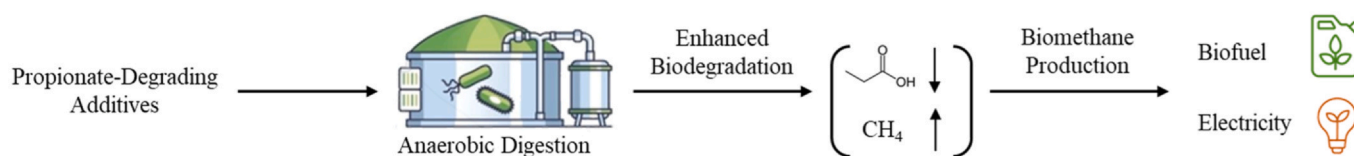


Fig. 1. The schematic effect of Pr-degrading additives in AD (causing Pr reduction and CH₄ production enhancement).

valuable in fine-tuning the anaerobic digester reactors, ultimately leading to enhanced Pr degradation and improved biogas production [29,30].

SO₄²⁻, as an additive in the anaerobic digester reactors, plays a crucial role in shaping microbial communities and influencing metabolic pathways, particularly in the context of Pr degradation. The addition of SO₄²⁻ promotes syntrophic associations between SRBs and methanogenic archaea. In the presence of SO₄²⁻, SRB outcompete methanogens for H₂, forming H₂S as a byproduct. This competition leads to a redirection of metabolic pathways, suppressing the accumulation of Pr, which is often a bottleneck in AD systems. The SO₄²⁻ reduction process acts as an electron sink, enhancing the conversion of Pr to Ac and ultimately improving overall CH₄ production efficiency. Therefore, the inclusion of SO₄²⁻ as an additive in the anaerobic digester reactors provides a strategic means to mitigate Pr accumulation and optimize the performance of the system by fostering syntrophic interactions among key microbial players [31,32].

In addition to the aforementioned additives, researchers employed various methods and supplementary additives. The forthcoming discussion will center on these alternative approaches and additives, emphasizing their impact on Pr, while some additives might cause both Pr better degradation and its generation pathway due to their dual mechanisms. The effectiveness of these additives depends on a variety of factors such as the conditions of anaerobic digestion, the type and composition of feedstock, the amount used, and the method of adding the additive. For instance, although ZVI is commonly known as an iron-based additive for degrading phosphorus, Yu et al. [33] found that nano ZVI initially inhibited methane production and led to phosphorus accumulation in small quantities, but when used with additional material, it resulted in improved Pr degradation and CH₄ production. Additionally, certain additives expedite both Pr and CH₄ production simultaneously, without inhibiting methanogens or accumulating Pr. Thus, these additives are not solely effective for Pr accumulation goals and are best utilized in conjunction with other methods. For instance, activated carbon, as reported by Wang et al. [34], or hydrochar, as reported by Wu et al. [35] and Wang et al. [36], are examples of this. Moreover, the precise mechanisms of certain additives remain unclear, with researchers reporting conflicting results, necessitating further investigation. For example, as already explained, the mechanism for zeolite additive is enhancing microbial syntrophy leading to enhanced P_R degradation, but Wang et al. [37] reported Clinoptilolite, a type of zeolite, to be a Pr-accumulating additive.

3. Propionate degradation and propionate-degrading additives

Pr is a crucial intermediate in AD, but its excessive accumulation can lead to system failure. When Pr levels rise beyond the optimal range, it can cause several detrimental effects. Firstly, it can inhibit the activity of methanogens, the microorganisms responsible for CH₄ production, leading to reduced biogas generation. Secondly, high Pr concentrations can cause acidification, lowering the pH of the digester and disrupting the delicate microbial balance. Additionally, Pr can directly exert toxic effects on certain microbial populations, further hindering

the overall digestion process. These cumulative effects of excessive Pr accumulation can result in a significant decline in digester performance and CH₄ production [7,38].

Additives and the use of different materials, particles, or chemicals in AD have been extensively studied to enhance Pr degradation and performance. Several studies have investigated the effects of various additives on microbial communities, degradation rates, and CH₄ production. The utilization of different materials, particles, or chemicals in AD systems offers promising strategies for enhancing Pr degradation, improving system performance, and mitigating the accumulation of Pr. These studies provide valuable insights into the effects of various additives and their mechanisms on microbial communities, degradation rates, and CH₄ production [13]. Fig. 1 depicts the schematic effect of Pr-degrading additives in AD.

The crucial role of Pr in influencing overall efficiency has led to the investigation of various additives and chemicals aimed at directing Pr metabolism toward desired outcomes. A detailed analysis of these additives is provided in this section, illuminating their mechanisms and effectiveness in promoting Pr degradation. Table 1 provides a concise summary of the various additives that have been studied recently and their effects on Pr, showcasing some of the most important cases. This compilation of key findings aims to offer readers a comprehensive understanding of advancements in Pr degradation enhancement, facilitating informed decision-making for future research efforts and practical applications in the field of AD. The additives include iron-based (e.g., Fe₃O₄, FeCl₂), carbon-based (e.g., activated carbon, biochar, hydrochar, graphene, zero-valent iron), bioavailable metals (e.g., Zn, Cu, Ni, and Mo), and other metallic-based additives (e.g., Se, Co, Ni, and Mo).

In addition to the aforementioned additives, there are also several unclassified additive methods and hybrid additive methods (using multiple additives or mechanisms) that influence Pr degradation, as shown in Table 2. These methods have not been fully studied or characterized, but they have the potential to significantly impact Pr degradation (e.g., tire particles, Urea, NaOH, Polyacrylamide, ashes, NH₄HCO₃). Further research is needed to better understand these methods and their effects.

Based on Tables 1 and 2, as well as the broader industrial applicability of Pr-degrading materials, sulfate additives (which enhance syntrophy between SRB and methanogens), iron-based materials (particularly ZVI, to control redox conditions and inhibit Pr production), and conductive materials (such as metallic ions or conductive carbon particles, which improve the DIET mechanism) appear to be the most promising for mitigating Pr accumulation and inhibition. Optimal combinations of these materials could be particularly effective in achieving these goals [11,15].

4. Propionate recovery methods and additives

In the second quarter of 2023, the global Pr market exhibited mixed trends. The US market experienced robust growth, driven by steady demand in the food, pharmaceutical, and agriculture sectors. In Asia, the Indian Pr market faced a bearish trend with declining prices, while

Table 1
Recent Cases Investigating Additives for Pr Degradation Enhancement.

Additive Type	Source/Waste	Additive and Method	Remark	Reference
AC	FW	Activated charcoal	Significantly increased the degradation rate of Pr and accelerated the CH ₄ production rate (104 ~ 371 %)	[39]
	FW	Granular AC Powdered AC	Improved H ₂ uptake, which in turn enhanced Pr degradation and CH ₄ production At 2% of the feed total solids, enhanced CH ₄ production contributed to the removal of Pr and reduced the color in digested sludge by about 27%	[40] [41,42]
	FW, Agricultural, and Animal Waste	Biochar	In both powdered (< 5 μm) and granular (0.5–1 mm) forms, increased CH ₄ production by 13.3 %, reduced Pr concentration by 55 %	[27,43–46]
Carbon-based (Others)		Biosolids-derived biochar	Increased CH ₄ production by 430 % and reduced Pr accumulation by 58.3 % at an organic loading rate (OLR) of 3 g COD/L.d	[47]
	Dairy manure	Biochar	Shortened the lag phases at all temperatures and lowered the concentration of total VFAs and Pr, while increasing cumulative CH ₄ and yield by up to 35.71 % and 26.47 %, respectively	[48]
	Corn stover waste FW	Modified hydrochar Sawdust biochar	Increased CH ₄ production by 42.3 % and Pr degradation by 58.3 % at 15 g/L modified hydrochar Sawdust biochar at 15 g/L improved synthetic oxidation of VFAs and enhanced CH ₄ production.	[49] [50,51]
		Carbon nanotubes	Sawdust biochar Increased CH ₄ yield by 24.09 % and decreased Pr levels by 9.96 % Carbon nanotubes stimulate the biodegradation of Pr, leading to a 15–16 % increase in CH ₄ production rate	[52]
		Conductive carbon cloth Conductive carbon-felt particles	Increased cumulative CH ₄ production and Pr degradation by 15.4 % and 19.67 %, respectively. Enhanced Pr degradation (<i>Clostridium</i> , <i>Syntrophobacter</i> , <i>Methanospirillum</i> , <i>Ceobacter</i> , <i>Anaerolineaceae</i> , and <i>Methanoseta</i> were among the identified genera)	[5] [53]
		Conductive carbon fibers	Stimulated the methanogenic conversion of Pr and Bu, resulting in increased specific CH ₄ (more abundant <i>Methanoseta</i> species in the presence of carbon fibers) enabling DIET co-degradation	[54]
		Graphene oxide	CH ₄ production was reduced by up to 17.1 % but Pr degradation enhanced	[55]
	SM	Corn straw and SS	Fe ₃ O ₄ enhanced DIET, increasing Ac and decreasing Pr concentration	[56]
	SM		Improved accumulative CH ₄ production	[57]
		Beer factory wastewater	Iron chloride or iron hydroxide Magnetite	Significantly improved process stability and efficiency, and reduced Pr and Ac accumulation by promoting the growth of acetlastic methanogens of the genus <i>Methanoseta</i> and <i>Methanosarcina</i> spp. Alleviated Pr accumulation and enhanced AD performance enabling DIET between fermentatives and methanogens, and more CH ₄ production (16 % higher)
Metallic Additives		Magnetite and foam carrier	Foam carrier accelerated Pr transformation while magnetite intensified the methanogenesis (60 % increase in CH ₄)	[61]
	FW	Magnetite nanoparticles	Promoting DIET between acetogens and methanogens accelerates both early degradation and methanogenic conversion of Pr	[23,62]
	Saline wastewater Waste-activated sludge (WAS), municipal sludge, and leachate	Magnetite nanoparticles and KCl ZVI	Synergistically promoted the degradation of Pr and Bu, and enhanced CH ₄ production by 124.85 % At 5 g/L, addition significantly reduced the Pr proportion from 49.8 % to 30.9 %, resulting in a 54.2 % higher CH ₄ yield. CH ₄ production increased by 43.5 % in WAS and 30.1 % in municipal sludge	[63] [64–68]
		Bioavailable metals (Zn, Cu, Ni, and Mo) Nano MnO ₂	Increased CH ₄ yield and reduced Pr accumulation at OLR = 4 g/L.d and SRT = 66.7 d	[69]
	WAS	Nickel foam Nickel-containing granular AC	Significantly enhanced the production rate of CH ₄ in Pr and butyrate (Bu) degradation by 25.6 % and 21.7 %, respectively Increased CH ₄ yield by 18.1 % and 15.9 %, respectively, for Pr and Bu	[70] [71]
	Artificial wastewater Industrial waste	Selenium (Se) Se, Co, Ni, Mo, and W SO ₄ ²⁻	Increased maximum CH ₄ production rate, and improved degradation rates of Pr and Bu by 1.14–20.70 times and 1.01–2.16 times, respectively Stimulated CH ₄ production and suppressed Pr accumulation	[72] [73] [74]
	CM	Animal waste and SS	Enhanced Pr degradation in OLR of 15 kg COD/m ³ .d up to 0.15 mM	[31,75]
	Complex organic waste FW and SM	Iron oxide-zeolite Zeolite	Increased specific Pr removal rate of 28 mol/g SS/d, but not Ac oxidation Reduced Pr concentration and increased CH ₄ yield in the acidification phase of two-phase AD Improves bacteria-archaea syntrophy needed for Pr degradation and CH ₄ production. Added at 60 g/L accelerated Pr and Bu consumption, resulting in increased CH ₄ yield (20 %)	[25] [76,77] [39]

Note: FW, Food Waste; WAS, Waste-Activated Sludge; SM, Swine Manure; CM, Chicken Manure; ZVI, Zero Valent Iron; AC, Activated Carbon; Bu, Butyrate; OLR, Organic Loading Rate; VFA, Volatile Fatty Acid; Pr, Propionate; AD, Anaerobic Digestion; Direct Interspecies Electron Transfer

Table 2
Hybrid and Unclassified Methods of Additives for Pr-Degradation Enhancement.

Method	Source/Waste	Additive	Remark	Reference
Hybrids	FW	AC and trace elements	Favored Pr consumption after its accumulation	[78]
	FW	Biochar and trace elements in the form of an industrial FeCl ₃ solution	Reduced Pr and increased CH ₄ production	[79]
Unclassified	Rice straw and bran	Cupric sulfate and cupric glycinate	CH ₄ production increased by 28.78%, while Pr concentration was decreased by 32.84%	[80]
	FW	Fe ₂ O ₃ supported on conductive carbon cloth	Increased CH ₄ production and Pr degradation by 15.4% and 19.67%, respectively	[81]
		Granular AC and trace elements	Trace elements allowed faster Pr consumption, and granular AC favored the growth of archaea and syntrophic bacteria, enhanced CH ₄ production	[82]
	Agricultural wastes	Pristine and re-used wood-pellet biochar	At the highest dosage increased Pr-degradation and reduced CH ₄ lag time	[83]
FW	ZVI and AC	Decreased Pr concentration and increased CH ₄ production	[84]	
FW	2-bromoethane sulfonic	Suppressed Pr and CH ₄ production	[85]	
WAS	Alkyl polyglucose	Improve Pr degradation and overall AD performance	[86]	
Coffee grounds and excess sludge	Ammonium bicarbonate (NH ₄ HCO ₃)	reduced Pr content, increased CH ₄ production	[87]	
Municipal biowaste	Aso volcanic limonite	Production of Pr and Bu was reduced and CH ₄ inhibited	[88]	
Berry and plant waste	Bottom ash	Increased CH ₄ production by 57.7% and Pr-degradation by 58.3%, but high levels of ash inhibited CH ₄ production	[89]	
FW	Calcium	Alleviated fat inhibition leads to higher specific Pr and Bu oxidative activities and higher specific acetoclastic methanogenic activities	[90]	
Crop straw and coal chemical industrial cinder	Cinder	This byproduct of the coal industry increased CH ₄ production by 54.61% and Pr degradation by 58.3% at 2.5% cinder	[91]	
Winery wastewater	Fly ash	25 mg/L addition of fly ash increased CH ₄ production by 75% and decreased Pr concentration	[92]	
FW	Glutathione	Enhanced CH ₄ (41.7%) and Pr and Bu bioconversion to Ac	[93]	
Agricultural wastes	High-density polyethylene plastic carrier	Increased CH ₄ production by 17.14-31.61% and reduced Pr concentration by 2-3 g/L.	[94]	
FW	Lipase addition	Significantly increased CH ₄ production (1.773 L CH ₄ /g VS) and decreased Pr production	[95]	
Organic fraction of municipal solid waste	Polyacrylamide	At 20 mg/g total solids, restored methanogenesis, but inhibited Pr-oxidizing bacteria at higher concentrations	[96]	
WAS	Giant reed biomass	Sodium hydroxide (NaOH)	Improved CH ₄ production rate and tolerance for Ac concentration, reduced the lag phase of Pr degradation under thermophilic condition	[97]
		Tire particles	Improve Pr-degradation and overall AD performance	[98]
	Tonalide	Enhanced VFA utility, especially Ac and Pr, and increased CH ₄ production by up to 30%	[99]	
	Urea	2% urea reduced Pr (2-8 times) and increased CH ₄ (18%)	[100]	

Note: FW, Food Waste; WAS, Waste-Activated Sludge; AC, Bu, Butyrate; Activated Carbon; ZVI, Zero Valent Iron; Pr, Propionate; VFA, Volatile Fatty Acid; AD, Anaerobic Digestion

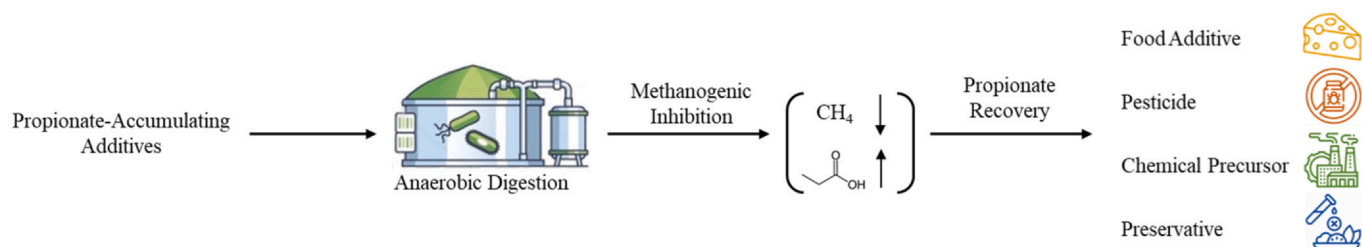


Fig. 2. The schematic effect of Pr-accumulating additives in AD (causing Pr accumulation and less CH_4 production).

in Europe, the market saw steady growth across key industries. Overall, the global Pr market is anticipated to grow at a compound annual growth rate of 3.3%, reaching USD 1801 million by 2028. Factors driving market growth include the increased demand for food preservatives and the use of Pr in the pharmaceutical industry and as an effective pesticide. Europe currently dominates the global market, while Asia-Pacific is expected to experience the highest growth rate [101–103].

Common chemical methods for producing Pr include the hydrocarboxylation of ethylene and the Reppe synthesis. The hydrocarboxylation of ethylene involves the reaction of ethylene, carbon monoxide, and water in the presence of a catalyst, typically involving nickel or cobalt, to produce Pr. This method is energy-intensive and relies on petrochemical feedstocks, contributing to environmental concerns due to the carbon footprint associated with fossil fuel use. The Reppe synthesis is a different approach that includes the combination of ethylene with carbon monoxide and water at elevated pressure and temperature while using a metal catalyst, commonly nickel. Although this method is effective, it also demands a lot of energy and involves the use of dangerous substances and conditions, which can be harmful to both the environment and human health.

Both methods emphasize the need for sustainable alternatives due to their reliance on non-renewable resources and significant environmental impact. However, biochemical and fermentation-based processes, such as AD, have some advantages over those methods [8,104].

AD offers a more sustainable and cost-effective alternative by utilizing organic waste materials, which reduces feedstock costs and aids in waste management. It operates under milder conditions and generates fewer pollutants. The primary goal of AD is to treat organic

waste, with product recovery, such as Pr, being a beneficial side goal. This dual-purpose functionality enhances its appeal, providing multiple benefits simultaneously. Furthermore, AD is generally simpler and less energy-intensive compared to chemical methods [104–107]. Pr production and recovery from AD presents a promising biological and green method for sustainable resource recovery. This approach holds significant potential for advancing sustainable practices and resource utilization [10]. Pr and other VFA production, recovery, and separation from AD have been studied from different sources, such as potato waste [108], organic fraction of municipal solid waste [109], rice straw [110], olive mill wastewater [111], swine manure (SM) [112], tuna waste [113], landfill leachate [114], sewage sludge (SS) and food waste (FW) [115], microalgae biomass [116], fruit waste [117], and animal wastewater [118]. Fig. 2 shows the schematic effect of Pr-accumulating additives in AD.

Pr recovery and separation in AD have been studied using various methods such as membrane-based techniques [119], solvent and in situ extraction [120,121], and Electrodialysis [111]. Different operational manipulations, such as pH control and micro-oxygenation, can enhance its production [122].

Two-stage and arrested AD are two modified AD methods that aim to enhance the production and recovery of VFAs, particularly Pr. Two-stage AD separates the acidogenesis and methanogenesis stages into two distinct reactors [25,123], while arrested AD intentionally halts the final methanogenesis step [124–127]. Both methods offer advantages over single-stage AD, including higher VFA production, improved process control, and reduced CH_4 inhibition. However, the common single-staged-4-step AD is still widely used due to its simplicity and cost-effectiveness. The use of additives can help to fill the gap between

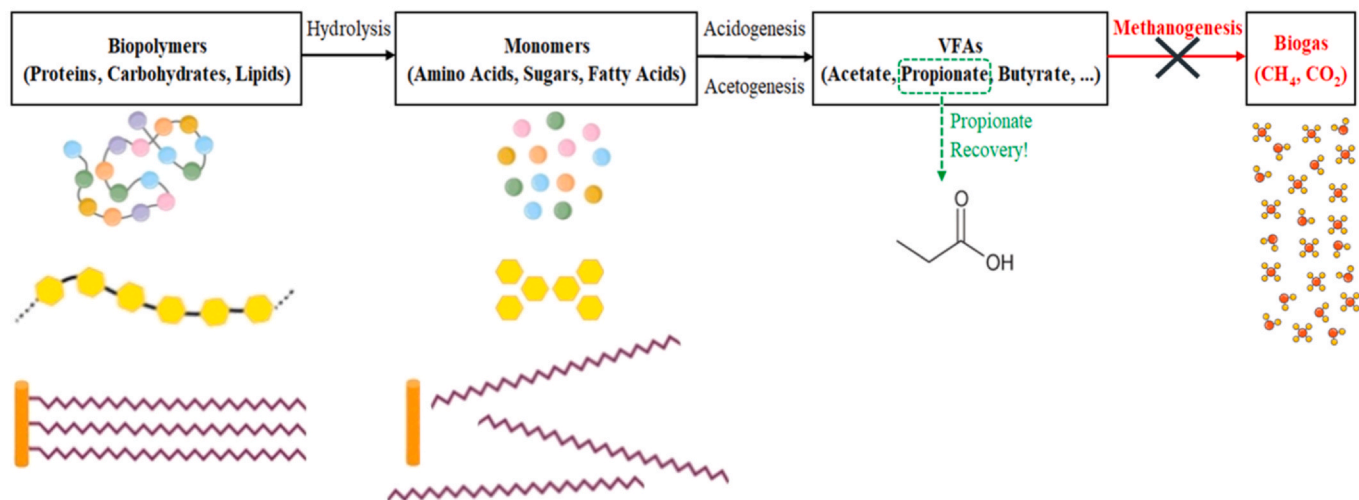


Fig. 3. Mechanism of Pr-accumulating additives in AD (blocking Pr degradation pathways, especially methanogenesis).

Table 3
Overview of Recent Cases Investigating Additives for Enhanced Pr Production Purposes.

Method	Source/Waste	Remark	Reference
Phenol		Pr accumulated to 2750 mg/l and CH ₄ yield was not inhibited	[128]
Biodiesel waste glycerin	Municipal wastewater sludge	1.35 % (v/v) biodiesel waste glycerin increased Pr concentration	[132]
AC	Starch wastewater	AC increased Pr and CH ₄ production	[34]
Cardboard	FW and cardboard	Reduces acid accumulation, slightly increases Pr, maintains CH ₄ yield at increasing substrate loads	[133,134]
Chloroform and acetylene	Solid waste	chloroform enhances the production of Pr and inhibits CH ₄ production	[135]
Clinoptilolite (zeolite)	Kitchen Waste	Increased Pr and CH ₄ production, shorter lag phase, and can both inhibit acidification and improve CH ₄ production	[37]
Glycerol trioleate	Manure	Increased the methanogenesis of Pr in particles > 200 μm and the 50-200 μm fraction	[136]
Hydrochar	WAS	Increased the production of Ac and Pr, which resulted in improved CH ₄ production	[35,36]
Hydrogen peroxide (H ₂ O ₂)		Significantly reduced CH ₄ production and led to higher VFA concentrations, including Pr	[137]
Lincomycin	Alcohol wastewater	Increased CH ₄ production by 20.8 % in anaerobic granular sludge, while impaired Pr and Bu utilization	[138]
Linear alkylbenzene sulfonate		Inhibited the degradation of Pr and CH ₄ , with a 50 % immediate inhibition of Pr degradation at a concentration of 27 mg/L	[139]
Nano ZVI		Inhibited CH ₄ production in the initial stage, but more of it promoted CH ₄ production and Pr degradation	[33]
Pentachlorophenol		Inhibited the activity of methanogenic bacteria significantly reducing CH ₄ production (less Pr degradation)	[140]
Polystyrene microplastics	FW	In different sizes (1 mm, 100 μm, and 1 μm) decreased CH ₄ production (33.08 %) and increased Pr accumulation (58.3 %)	[141]
Sodium dodecyl benzene sulfonate	SS, FW, and green waste	This anionic detergent inhibited methanogenesis and increased Pr accumulation	[142]
Sulfamethoxy pyridazine sodium and zinc	SM and wheat straw	Sulfamethoxy pyridazine increased Pr and CH ₄ production, zinc decreased Pr and CH ₄ production	[143]
Tylosin and chlortetracycline	SM	Chlortetracycline enhanced initial hydrolysis reactions and inhibited CH ₄ , while tylosin did not affect CH ₄	[144]
Persulfate and Biochar	Animal wastewater	Pr and VFA production increased by 12.4 %	[118]
Valine and threonine	CM	At concentrations of 0.2-5.0 %, enhanced Pr and Bu production and decreased the proportion of Ac from 83 % to 47 %	[145]

Note: AC, Activated Carbon; ZVI, Zero Valent Iron; FW, Food Waste; WAS, Waste-Activated Sludge; Bu, Butyrate; SS, Sewage Sludge; SM, Swine Manure; CM, Chicken Manure; Pr, Propionate; VFA, Volatile Fatty Acid

single-stage AD and more modern methods, and can also be used in these modern anaerobic digester reactors to further enhance their performance [128].

While the conventional AD goal is CH₄ production, the process can be manipulated to favor VFA production, particularly Pr, through the addition of specific additives and chemical particles. This strategy involves inhibiting methanogenesis, allowing VFAs to accumulate instead of converting to CH₄. This happens because methanogens are more vulnerable than acidogens, and antimicrobial agents have more effect on them [129]. Additives like biochar, persulfate, and iron-based materials can suppress methanogenic activity, while chemical particles like zeolites can adsorb VFAs and prevent their degradation [118]. By selectively controlling the microbial community and manipulating the reaction environment, AD can be transformed into a valuable source of VFAs, especially Pr, with potential applications in biofuel production and chemical synthesis [127,130,131]. Fig. 3 illustrates the general mechanism of Pr-accumulating additives in AD (deviating the common biochemical pathway by inhibiting the Pr degradation pathway which leads to Pr production > Pr degradation).

Recognizing Pr as a valuable end product with applications beyond its role as an intermediate, researchers have investigated

various additives and chemicals to optimize conditions for Pr accumulation. This section provides a comprehensive analysis of these additives, elucidating their mechanisms and effectiveness in promoting Pr recovery. Table 3 is an important resource that provides a summary of significant recent cases. It offers a brief overview of various additives that have been studied and their effects on Pr.

The most common Pr-accumulating additives include agents that alter microbial balance by eliminating methanogens (e.g., Phenol, Chloroform, Hydrogen peroxide, alkylbenzene sulfonate, Lincomycin, Persulfate), along with chemicals (e.g., AC, zeolite, ZVI, Hydrochar). The compilation of key findings in Table 3 provides readers with a nuanced understanding of the advancements in Pr recovery strategies using additives. Based on Table 3 and the previously discussed mechanism of Pr-accumulating additives, antimethanogenic agents such as phenol, chloroform, and hydrogen peroxide are likely the most effective for Pr accumulation. However, due to the limited research on the role and mechanism of these additives, it is recommended to combine them with advanced bioreactor techniques to enhance Pr-accumulating efficiency and improve Pr recovery.

5. Conclusion and future perspectives

Research on additives in Pr recovery from AD is limited while no studies have specifically examined their effects on Pr recovery or their potential to mitigate its inhibitory effects. This mini review explores how additives influence Pr concentrations, impacting AD efficiency and direction. By categorizing various additives, the review provides insights for more efficient Pr recovery and enhanced renewable energy production. Protein plays a crucial role in the management of wastewater and AD, serving as both an inhibitor and a valuable bioproduct.

Some additives enhance Pr degradation to reduce inhibition and boost biogas production, while others aid in Pr recovery and utilization:

- Carbon-based, iron-based, and metallic-based additives show potential for better Pr degradation.
- Antimethanogen agents such as Phenol, Chloroform, Hydrogen peroxide, alkylbenzene sulfonate, Lincomycin, Persulfate, and other chemicals can be used for Pr recovery.
- Coupling modern bioreactor technologies along with proper additive, holds potential for Pr recovery.

Future work is suggested to focus on the simultaneous use of modern digesters, such as two-stage and arrested digesters, with appropriate additive selection to enhance Pr recovery for a more efficient and economic process. Furthermore, it is important to conduct further research on the combination of multiple additives, which will require additional optimization and modeling studies. It is also recommended to examine the effects of these techniques by optimizing process parameters such as temperature and pH for both improved Pr recovery and degradation. It is crucial to explore the impact of these additives and digester techniques in specific wastewater systems with much higher Pr concentrations, like those present in the dairy waste and food industries, as the main goal of AD is biogas production, which can be hindered by Pr accumulation.

CRedit authorship contribution statement

Milad Mehriar: Writing – original draft, Formal analysis, Conceptualization. **Ghazaleh Akhavan:** Writing – original draft, Investigation, Conceptualization. **Milad Mousazadehgavan:** Writing – review & editing, Validation, Investigation, Conceptualization. **Aliyar Javadi:** Writing – review & editing, Supervision, Methodology. **Armin Rahimieh:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis.

Data Availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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