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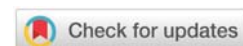
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Review Article

Anaerobic Digestion for Pathogen Reduction in Waste Treatment and Safe Agricultural Use of Digestates

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Abstract

Anaerobic Digestion (AD) is a widely used process for treating organic wastes and producing renewable energy. This review examines the effectiveness of AD in reducing pathogens in various waste streams and evaluates the safety of using digestates as agricultural fertilizers. The mechanisms of pathogen inactivation during AD are explored, including the roles of temperature, pH, ammonia, and microbial competition. Case studies demonstrate pathogen reduction rates ranging from 1-5 log units for different microorganisms. While AD can significantly reduce pathogen loads, challenges remain in achieving consistent and complete sanitization. Factors affecting digestate safety, such as feedstock composition, operating conditions, and post-treatment, are discussed. The review also addresses methodologies for enhancing pathogen reduction in AD systems.

Introduction

Anaerobic Digestion (AD) is a biological process that breaks down organic matter in the absence of oxygen, producing biogas and a nutrient-rich residue called digestate. This process has gained significant attention in recent years as a sustainable waste management strategy, offering multiple benefits such as renewable energy production, greenhouse gas reduction, and nutrient recycling [1]. AD can be applied to a wide range of organic waste streams, including municipal wastewater sludge, animal manures, food waste, and agricultural residues.

The AD process involves a complex microbial ecosystem that

degrades organic compounds through a series of biochemical reactions. These reactions occur in four main stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During these stages, various groups of microorganisms work synergistically to convert complex organic molecules into simpler compounds, ultimately producing biogas (primarily composed of methane and carbon dioxide) and digestate [2].

One of the key advantages of AD is its ability to reduce pathogen loads in organic waste streams. Pathogens, including bacteria, viruses, protozoa, and helminths, are often present in high concentrations in raw organic wastes, particularly in animal manures and sewage sludge. These pathogens pose

potential risks to human and animal health if not properly managed. The AD process can significantly reduce pathogen concentrations through various mechanisms, including thermal inactivation, competition for nutrients, and exposure to inhibitory compounds produced during the digestion process [3]. However, the effectiveness of pathogen reduction in AD systems can vary depending on several factors, such as the type of waste being treated, the operating conditions of the digester (e.g., temperature, retention time), and the specific pathogens present. Understanding these factors is crucial for optimizing AD systems to achieve consistent and reliable pathogen reduction.

The use of digestate as an agricultural fertilizer has gained increasing interest due to its high nutrient content and potential to replace synthetic fertilizers. Digestate contains valuable plant nutrients, including nitrogen, phosphorus, and potassium, in forms that are readily available for plant uptake. Additionally, the organic matter in digestate can improve soil structure and water retention capacity. However, the safe use of digestate in agriculture depends on effective pathogen reduction during the AD process and the prevention of pathogen regrowth in the final product [4].

Conventional techniques for reducing pathogens in waste before agricultural application include several key methods. Physical treatment involves sedimentation, which allows heavier particles, including some pathogens, to settle out of wastewater, and filtration methods such as sand filters and membrane filtration, which can physically remove pathogens [5]. Chemical treatment encompasses disinfection methods like chlorination, ozonation, and UV irradiation, which effectively reduce pathogens [5]. Chlorination adds chlorine to wastewater, reducing pathogens but not eliminating all antibiotic-resistant bacteria [6], while ozone and UV treatments are also effective but less commonly used. Biological treatment includes the activated sludge process, which utilizes microorganisms to degrade organic matter and pathogens, significantly reducing pathogen levels, and stabilization ponds that use natural processes involving bacteria and algae to treat wastewater [7].

Advanced AD techniques enhance sludge treatment by stabilizing waste, reducing volume, and converting organic matter into biogas. Key methods include Thermal Hydrolysis Pretreatment (THP), which uses high temperature and pressure to improve biogas yield by 15% - 20% [8]. Ultrasound and microwave pretreatments disrupt sludge flocs, increasing solubilization and boost biogas production by 10-30% and 10-20%, respectively. Acid/base pretreatment enhances organic matter solubility, improving yields by 20-50% [9]. Two-stage AD separates hydrolysis and methanogenesis for better control, while high-rate reactors achieve higher loading rates [10]. Finally, biogas upgrading removes impurities, producing biomethane suitable for energy applications, and promoting sustainability in wastewater treatment.

This review aims to critically assess the efficacy of AD in reducing pathogen loads in various waste streams and evaluate the safety of using digestates as agricultural fertilizers. We will examine the mechanisms of pathogen inactivation during AD,

analyze case studies demonstrating pathogen reduction rates in different AD systems, and discuss factors affecting digestate safety. Additionally, we will explore regulatory frameworks governing the use of digestate in agriculture, risk assessment methodologies for evaluating potential health risks, and emerging technologies for enhancing pathogen reduction in AD systems. By providing a comprehensive overview of the current state of knowledge on pathogen reduction in AD and the safe use of digestates, this review seeks to inform researchers, engineers, and policymakers working in the fields of waste management, renewable energy, and sustainable agriculture. Understanding the potential and limitations of AD in pathogen reduction is crucial for developing effective strategies to maximize the benefits of this technology while minimizing potential risks to human and environmental health.

Mechanisms of pathogen inactivation in anaerobic digestion

AD works at several stages in the valorization of organic matter harnessing the strength of the microbes in the bioprocess. Figure 1 presents a simple sketch of the AD in waste valorization and the production of digestate for farming. Combining different feedstocks in the synergy and diverse microbial consortia as co-digestion offers higher addition to higher bio-methane yield and diverse quality digestates [11]. Animal manure can harbor a diverse array of pathogenic bacteria, viruses, protozoa, and helminths. These pathogens can pose a significant risk to human health if not properly managed. Some pathogens, such as spore-forming bacteria (e.g., *Clostridium spp.*), viable but non-culturable (VBNC) bacteria, and persistent pathogens (e.g., *Mycobacterium paratuberculosis*), exhibit increased resistance to environmental stresses and treatment processes.

AD has the potential to inactivate pathogens through a combination of factors, including temperature, retention time, reactor configuration, and microbial competition. Thermophilic AD (55-60°C) is generally more effective than mesophilic AD (35-37°C) in terms of pathogen reduction. However, even under mesophilic conditions, AD can achieve significant pathogen reduction (95-98%) for common pathogens. The principal factors controlling pathogen destruction during AD include: Temperature plays a crucial role in pathogen reduction. Mesophilic (35-37°C) and thermophilic (55°C) AD systems exhibit different inactivation rates, with thermophilic conditions generally achieving higher pathogen reduction [12]. Higher temperatures (e.g., thermophilic conditions) generally result in faster pathogen inactivation. Several factors contribute to pathogen inactivation during AD: For example, *Escherichia coli* O157:H7 is reduced by 4 log units in thermophilic AD compared to 2 log units in mesophilic AD [13]. pH: The pH fluctuations during AD (typically 6.5-8.5) can adversely affect certain pathogens. Extreme pH values outside the optimal range for pathogen survival contribute to their inactivation [14]. Ammonia: Free ammonia produced during protein degradation exhibits antimicrobial properties. Studies have shown that ammonia concentrations above 80 mg/L can significantly inhibit pathogen growth [15]. Microbial

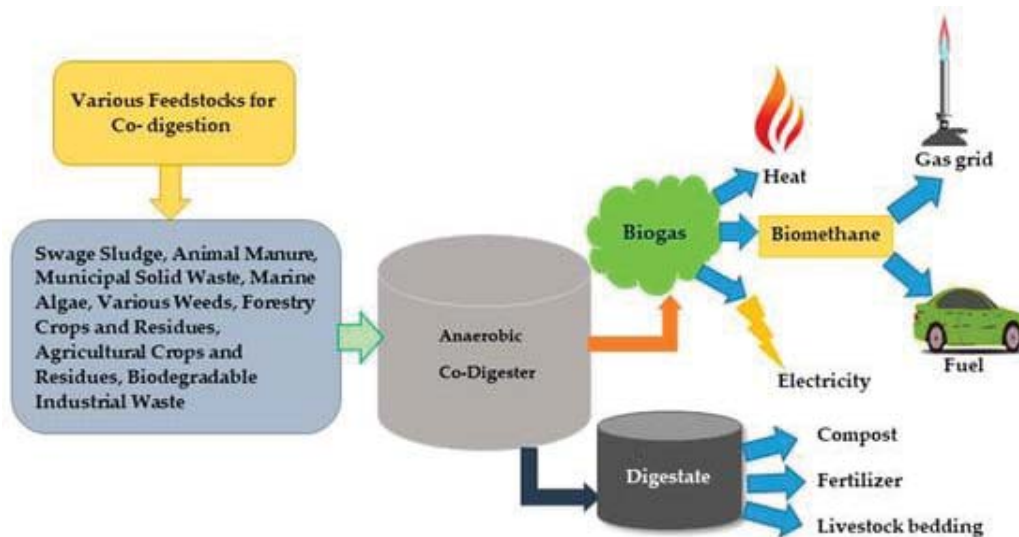


Figure 1: The Co-digestion of multi-feedstocks for waste reduction and digestates for agriculture [11].

Competition: The diverse microbial community in AD systems competes with pathogens for nutrients and space, potentially suppressing their growth and survival [16]. **Retention time:** Longer retention times in the digester allow for more exposure to adverse conditions and increased pathogen die-off. **Reactor configuration:** Well-mixed reactors with minimal dead zones and short-circuiting are more effective in utilizing the entire digester volume for pathogen inactivation. **Microbial competition:** The presence of a diverse microbial community in the digester can outcompete pathogens for resources and produce inhibitory substances, contributing to pathogen reduction [16].

Case studies on pathogen reduction in anaerobic digestion

Highlights on different feedstock's pathogen reduction in anaerobic digestion

AD plays a crucial role in pathogen reduction in municipal wastewater treatment. Studies have shown that various operating conditions impact the efficacy of AD in reducing pathogens. Research has highlighted that two-staged AD systems, particularly the thermophilic-mesophilic configuration, exhibit higher efficiency in volatile solids removal, methane yield, and pathogen reduction compared to single-stage systems [9]. Pre-treatment such as acid, alkaline, heat, and ozonation have been found to enhance pathogen inactivation, ensuring effluent safety levels are met [17]. Additionally, the introduction of hybrid disintegrated Waste-Activated Sludge (WAS) into the fermentation chamber before AD has shown promising results in reducing bacterial pathogens and helminth eggs, further enhancing the overall efficiency of the process [9]. These findings underscore the importance of proper AD configurations and pre-treatments in achieving effective pathogen reduction in municipal wastewater treatment systems.

A study on agricultural waste treatment investigated a thermophilic AD system operating at 55°C with a 15-day

retention time for treating pig manure and crop residues. The research highlighted the efficiency of the thermophilic AD process in terms of methane production potential, with a significant increase observed after heat pre-treatment at 100 °C [18]. Additionally, the study emphasized the importance of optimizing process parameters, such as temperature and C/N ratio, to enhance biogas production in solid-state AD systems, showcasing a maximum biogas yield of 241.4 mL gVS⁻¹ at 47.3°C [19]. Furthermore, the investigation of a two-stage anaerobic process treating pig manure demonstrated higher removal efficiency of volatile solids in the thermophilic-mesophilic system compared to a single-stage mesophilic process [20]. These findings collectively underscore the potential of thermophilic AD systems in effectively treating agricultural waste and maximizing biogas production.

A comprehensive study conducted in Wisconsin analyzed the pathogen reduction capabilities of seven full-scale anaerobic digesters treating cattle manure over nine months. The study utilized real-time quantitative Polymerase Chain Reaction (qPCR) to assess the inactivation of various pathogens. The results indicated significant variability in pathogen removal, with log-removal values for *bovine Bacteroides* and *Bacteroidales-like CowM3* averaging 0.78 and 0.70, respectively. These values were lower than expected, highlighting the need for optimization in full-scale AD systems to enhance pathogen inactivation efficiency. The study also revealed that most pathogens ended up in the liquid fraction after manure separation, raising concerns about potential environmental contamination through land application of separated liquids [21].

In Poland, a study focused on thermophilic AD of organic municipal solid waste and food waste assessed the inactivation of pathogens such as *Salmonella Senftenberg* W 775, *Enterococcus* spp., and *Ascaris suum* eggs. Laboratory trials showed that pathogen elimination occurred within 6.06 hours for *Salmonella*, 5.5 hours for *Enterococcus*, and approximately 10 hours for *Ascaris suum*. Full-scale tests using 1500 m³ Kompogas® reactors

confirmed these findings, demonstrating effective sanitization of the digestate. The process maintained stable pH and organic acid concentrations, indicating that thermophilic conditions positively influence pathogen reduction [22]. Further pathogen removal from different feedstocks with the application of AD is presented in Table 1.

Factors affecting digestate safety

The initial pathogen load and diversity in the feedstock significantly influence the final digestate quality. Co-digestion of different waste streams can introduce a wider range of pathogens and affect inactivation rates [16]. Operating Conditions: Digester temperature, retention time, and organic loading rate impact pathogen reduction efficiency. Optimizing these parameters is crucial for maximizing sanitization [25].

Post-treatment: Additional treatments such as composting, heat treatment, or UV irradiation can further reduce pathogen levels in digestates [26]. Regulatory Frameworks and Risk Assessment such as International Regulations: Various countries have established guidelines for the safe use of digestates in agriculture. For example, the EU Animal By-Products Regulation (EC) No 1069/2009 sets specific treatment requirements for AD of animal by-products [27] Risk Assessment Methodologies: Quantitative microbial risk assessment (QMRA) models have been developed to evaluate the potential health risks associated with digestate use. These models consider factors such as pathogen survival, exposure pathways, and dose-response relationships [28].

Emerging Technologies for Enhanced Pathogen Reduction such as Pretreatment Technologies: Advanced pretreatment methods such as ultrasound, microwave, and ozonation can enhance cell lysis and improve pathogen inactivation. Two-stage AD for Separating the hydrolysis/acidogenesis and methanogenesis stages can create more hostile environments for pathogens, potentially improving inactivation rates [29]. Bioaugmentation with the introduction of specific microbial strains or enzymes can enhance the breakdown of complex organic matter and potentially increase pathogen reduction [8].

AD is an effective technology for managing organic waste and reducing pathogens. Several factors significantly influence the efficiency of pathogen removal during the AD process. Understanding these factors can help optimize AD systems for better pathogen control. Table 2 summarizes the main factors affecting AD Each factor is accompanied by brief remarks on its importance or optimal conditions.

pH

The pH level in an anaerobic digester is crucial for microbial activity and pathogen inactivation. The optimum pH range for AD is typically between 6.8 and 7.2, although the process can tolerate values between 6.5 and 8.0.

Impact on Pathogen Removal: A stable pH within this range promotes the growth of methanogenic bacteria, which are essential for effective digestion and pathogen reduction [30]. Extreme pH levels can inhibit microbial activity, leading

Table 1: Presents different feedstocks pathogen removal with AD and its associated limitations and merits.

Feedstock	Pathogen Removal	Advantages	Limitations	Ref.
Cattle Manure	Log removal values for <i>Bacteroides</i> and <i>Bacteroidales</i> were 0.78 and 0.70, respectively.	Reduces zoonotic pathogen transmission, and produces biogas.	Variable inactivation rates; require optimization.	[22]
Swine Manure	95% - 98% reduction of <i>E. coli</i> and <i>Salmonella</i> observed.	High biogas yield; effective pathogen reduction.	Presence of resistant pathogens like <i>Mycobacterium avium</i> .	[21,23]
Food Waste	<i>Salmonella</i> and <i>Enterococcus</i> were eliminated within 6 hours.	Reduces foodborne pathogens; recycles organic waste.	High organic loading can inhibit digestion.	[22]
Municipal Solid Waste	Effective reduction of pathogens under thermophilic conditions.	Diversified feedstock; potential for high energy recovery.	Requires careful management of feedstock composition.	[23]
Paper Sludge	Pathogen reduction is achieved through integrated biorefinery processes.	Reduces landfill waste; high energy yield potential.	Limited research on specific pathogen removal efficiency.	[24]

Table 2: Presents different factors affecting the anaerobic digestion and pathogens associated with the anaerobic digestion process.

Factor	Remarks	Reference
pH	Optimum range: 6.8-7.2; Process can tolerate 6.5-8.0; Crucial for microbial activity	[30]
Temperature	Mesophilic (35 °C - 40 °C) and thermophilic (50 °C - 65 °C) ranges; Affect microbial growth rates and biogas production	[31]
Organic Loading Rate	Measures the amount of volatile solids fed into the system; Overloading can lead to system failure	[32]
Hydraulic Retention Time	Time feedstock remains in the digester; Affects the degree of degradation	[33]
C/N Ratio	Optimal range: 20-30; Affects microbial growth and biogas production	[34]
Feedstock Composition	Affects biogas yield and quality; Lipids, proteins, and carbohydrates have different mechanization potentials	[35]
Presence of Toxins	Can inhibit microbial activity; Examples include ammonia, heavy metals, antibiotics	[30]
Mixing	Ensures uniform distribution of substrates and microorganisms; Improves biogas production	[36]
Particle Size	Smaller particles increase surface area for microbial action and improve degradation rates	[37]
Alkalinity	Provides buffering capacity; Helps maintain stable pH	[23]

to reduced pathogen inactivation. For instance, a study found that maintaining pH levels around 7.0 facilitated the complete eradication of *Salmonella* in thermophilic digesters, while deviations from this range resulted in lower pathogen reduction rates

Temperature

Temperature plays a critical role in the AD process, with mesophilic (35–40°C) and thermophilic (50–65°C) ranges being the most common. Impact on Pathogen Removal [31]: Thermophilic conditions are generally more effective at inactivating pathogens due to higher microbial growth rates and increased metabolic activity. Research indicates that thermophilic digestion can achieve the destruction of pathogens such as *E. coli* and *Salmonella* within 24 hours, while mesophilic conditions may only reduce pathogen levels by 50–70%. The higher temperatures facilitate faster reaction rates and greater pathogen reduction, making thermophilic AD favorable for treating waste with high pathogen loads.

Organic Loading Rate (OLR)

The organic loading rate measures the amount of volatile solids fed into the digester per unit volume.

Impact on Pathogen Removal: Overloading the digester can lead to system failure, resulting in insufficient retention time for pathogen inactivation. High OLRs can also lead to the accumulation of volatile fatty acids (VFAs), which can inhibit microbial activity and reduce the overall efficacy of pathogen removal. Studies have shown that maintaining optimal OLR levels is essential for achieving effective pathogen reduction while maximizing biogas production [32].

Hydraulic Retention Time (HRT)

HRT refers to the average time that feedstock remains in the digester.

Impact on Pathogen Removal: Longer HRTs generally allow for more complete degradation of organic matter and increased pathogen inactivation. Insufficient HRT may not provide adequate time for microbial processes to effectively reduce pathogens. For example, a study demonstrated that extending HRT from 15 to 30 days significantly improved the reduction of pathogens in digestate, highlighting the importance of optimizing retention times [33].

Carbon to Nitrogen (C/N) ratio

The C/N ratio is a critical parameter that influences microbial growth and biogas production.

Impact on Pathogen Removal: An optimal C/N ratio of 20–30 promotes balanced microbial activity, enhancing both biogas production and pathogen reduction. A low C/N ratio can lead to ammonia accumulation, which may inhibit microbial activity and reduce pathogen inactivation. Conversely, a high C/N ratio may result in insufficient nitrogen for microbial growth, limiting the efficiency of the digestion process [34].

Feedstock composition

The composition of the feedstock significantly affects biogas yield and quality, as well as pathogen removal efficiency.

Impact on Pathogen Removal: Different feedstocks contain varying concentrations of lipids, proteins, and carbohydrates, which have different mechanization potentials. For instance, high lipid content can lead to the formation of long-chain fatty acids that inhibit microbial activity, thereby reducing pathogen removal efficiency. Understanding the composition of feedstock can help in designing digesters that optimize pathogen reduction [35].

Presence of toxins

Toxins such as ammonia, heavy metals, and antibiotics can inhibit microbial activity in anaerobic digesters.

Impact on Pathogen Removal: High concentrations of ammonia can be detrimental to methanogenic bacteria, leading to decreased biogas production and reduced pathogen inactivation. Heavy metals can also have toxic effects on microbial communities [30]. The presence of these toxins can compromise the overall effectiveness of the AD process in reducing pathogens.

Mixing

Mixing within the digester ensures a uniform distribution of substrates and microorganisms.

Impact on Pathogen Removal: Effective mixing enhances contact between microorganisms and pathogens, improving degradation rates and pathogen inactivation. Poor mixing can lead to the formation of dead zones, where microbial activity is insufficient to achieve effective pathogen reduction. Studies have shown that continuous mixing can enhance biogas production and pathogen removal efficiency [36].

Particle size

The size of feedstock particles affects the surface area available for microbial action.

Impact on Pathogen Removal: Smaller particle sizes increase the surface area for microbial colonization, leading to improved degradation rates and enhanced pathogen removal. Larger particles may hinder microbial access and slow down the digestion process, resulting in lower pathogen inactivation rates [37].

Alkalinity

Alkalinity provides buffering capacity, helping to maintain stable pH levels within the digester.

Impact on Pathogen Removal: Adequate alkalinity is essential for neutralizing acids produced during digestion, which can otherwise lead to pH fluctuations detrimental to microbial activity. Maintaining stable alkalinity levels supports optimal conditions for pathogen inactivation and overall digester performance [23]. The effectiveness of AD in pathogen



removal is influenced by a complex interplay of factors, including pH, temperature, organic loading rate, hydraulic retention time, C/N ratio, feedstock composition, presence of toxins, mixing, particle size, and alkalinity. Understanding and optimizing these parameters are crucial for enhancing pathogen reduction in anaerobic digesters, thereby improving the safety of digestate for agricultural applications and contributing to public health protection.

Conclusion and future perspectives

AD has demonstrated significant potential for reducing pathogen loads in various waste streams, with reduction rates ranging from 1–5 log units for different microorganisms. However, challenges remain in achieving consistent and complete sanitization across all pathogen types. The safety of digestate use in agriculture depends on multiple factors, including feedstock composition, operating conditions, and post-treatment processes. Future research should focus on optimizing AD systems for simultaneous energy recovery and pathogen reduction. Developing robust risk assessment tools for digestate application in different agricultural scenarios. Exploring novel technologies and microbial ecology approaches to enhance pathogen inactivation. Establishing standardized methods for pathogen detection and quantification in digestates. By addressing these challenges, the agricultural use of digestates can be further optimized, contributing to sustainable waste management and nutrient recycling while minimizing potential health risks.

Data availability statement: The data will be made available at reasonable request.

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