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REVIEW ARTICLE

The role of sludge microstructure in enhancing anaerobic digestion: a comprehensive review combining bibliometric and experimental insights

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Abstract
The increasing generation of organic waste driven by global population growth and industrialization necessitates sustainable management solutions. Anaerobic digestion (AD) stands out as an effective technology, converting organic waste into biogas and nutrient-rich digestate. This review integrates a bibliometric analysis of 841 peer-reviewed articles with detailed experimental insights from the 50 most-cited studies published between 2020 and 2024, focusing specifically on sludge microstructure's role in enhancing AD performance. Bibliometric results highlight dominant research themes such as microbial community dynamics (32%), operational parameters (24%), biochar integration (22%), and co-digestion strategies (22%). Approximately 65% of experimental studies are predominantly at laboratory scale, with limited pilot-scale validation, underscoring a significant gap in the validation of laboratory findings at pilot and industrial scales. Experimental studies further demonstrate that sludge microstructure significantly influences microbial activity, and biogas yields through targeted pretreatment, biochar supplementation, optimal operational conditions, and strategic co-digestion. The integration of bibliometric and experimental evidence offers a novel dual-perspective approach that not only maps prevailing research trends but also connects them directly to measurable performance outcomes. This comprehensive framework clearly identifies critical knowledge gaps and offers actionable research priorities aimed at improving the scalability, economic viability, and environmental sustainability of AD technologies.

Keywords Sludge microstructure · Microbial activity · Waste management · Bibliometric analysis · Bioenergy

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1 Introduction

The dramatic increase in global waste generation, driven by population growth and industrialization, has created significant environmental and public health challenges. Traditional waste management practices such as landfilling and incineration are increasingly criticized for contributing to greenhouse gas emissions, leachate contamination, and low energy recovery efficiency (Malek et al., 2023; Nanda and Berruti, 2021). These approaches are insufficient to meet the goals of modern sustainable waste policy, underscoring the urgent need for circular, resource-recovering alternatives such as anaerobic digestion (AD). AD has emerged as a transformative technology that simultaneously manages organic waste and produces renewable energy. By converting organic waste into biogas and nutrient-rich digestate (Uddin and Wright, 2023), AD reduces landfill dependency (Shukla et al., 2024), mitigates greenhouse gas emissions (Singh et al., 2023), and generates versatile energy outputs for heating (Khan et al., 2018), electricity (Nunes Ferraz Junior et al., 2022), and transportation (Czekała, 2022). The resulting digestate also serves as a biofertilizer, promoting sustainable agricultural practices (Hidalgo & Martín-Marroquín, 2020). Importantly, anaerobic digestion contributes directly to Sustainable Development Goal (SDG) 7—Affordable and Clean Energy—by transforming organic waste into renewable biogas and expanding access to sustainable energy solutions.

Despite its wide-ranging benefits, the widespread adoption of AD faces significant technical and operational barriers (Ibarra-Esparza et al., 2023). High capital investment costs, coupled with the need for expertise in managing operational parameters, limit scalability (Foster et al., 2021). Furthermore, the heterogeneous nature of organic waste introduces challenges in process stability and biogas yield (Bella & Rao, 2023). For instance, lignocellulosic-rich wastes, such as agricultural residues, are recalcitrant to microbial breakdown due to their complex cellulose–hemicellulose–lignin structure, requiring pretreatment to improve degradability (Anacleto et al., 2024). In contrast, lipid-rich wastes, such as food oils or slaughterhouse waste, can cause digester imbalances through long-chain fatty acid (LCFA) accumulation and microbial inhibition if not carefully co-digested or diluted (Wang et al., 2023). This compositional variability necessitates feedstock-specific strategies to ensure stable process performance. To address these challenges, researchers (Liu et al., 2016; Wang et al., 2018; Wu et al., 2021) have increasingly focused on optimizing sludge microstructure, the primary feedstock in many AD systems. Microstructural properties, such as particle size, surface area, and extracellular polymeric substances, play a pivotal role in influencing microbial activity (Abanades et al., 2022), substrate availability (Xu et al., 2020), and biogas production efficiency (Niu et al., 2020). For instance, smaller particle sizes and larger surface areas promote microbial colonization and enhance substrate degradation, leading to more stable and efficient digestion processes (Halalsheh et al., 2011).

Over the past two decades, significant advancements have been made in understanding sludge microstructure and its influence on AD. Research on sludge pretreatment techniques (Lee and Lee, 2019; Li et al., 2021a; Lu et al., 2020), microbial community dynamic (Lv et al., 2018), and biogas production optimization (Obileke et al., 2024; Srivastava, 2020) has demonstrated the potential of microstructural interventions to improve AD performance. However, despite these contributions, the literature remains fragmented, with studies often focused on isolated parameters or case-specific findings. A comprehensive synthesis that consolidates these findings and reveals overarching patterns has been lacking. This review fills that critical gap by integrating bibliometric mapping with experimental analysis to offer a unified, multi-level perspective on sludge microstructure's role in enhancing AD performance.

This review employs a dual-methodological framework combines bibliometric analysis of 841 peer-reviewed articles with a synthesis of the 50 most-cited experimental studies from 2020 to 2024. The bibliometric component identifies influential publications, collaborative networks (Malacina & Teplov, 2022), thematic clusters, and emerging research areas such as microbial community engineering and biochar-enhanced digestion (dos Santos et al., 2022; Pasalari et al., 2021), while also highlighting gaps in pilot-scale translation.

Complementing this, the experimental synthesis provides detailed insight into the mechanistic and operational effects of sludge microstructure manipulation, including pretreatment techniques, biochar addition, temperature regimes, and co-digestion strategies. Together, these two layers of analysis allow this review to not only contextualize the evolution of the field but also deliver practical, evidence-backed strategies to enhance the scalability, stability, and energy yield of AD systems.

2 Methodology

2.1 Review objectives and research questions

This review aims to systematically investigate how sludge microstructure influences the performance of AD, by combining bibliometric analysis with insights from recent experimental studies. Through this dual approach, the review seeks to provide a comprehensive understanding of current research directions, practical applications, and knowledge gaps in this field. To guide the review, the following research questions were formulated:

1. What are the key trends, influential themes, and knowledge gaps in research linking sludge microstructure and anaerobic digestion, as revealed through bibliometric analysis?
2. How do recent experimental studies address the relationship between sludge microstructure and factors such as microbial activity, biochar integration, operational conditions, and co-digestion strategies?
3. What practical implications can be derived by synthesizing bibliometric and experimental evidence, and how can these inform future research or technological development?

These questions shaped both the bibliometric strategy and the selection of experimental studies, ensuring that the review remains targeted and relevant to advancing anaerobic digestion research.

2.2 Data collection

The data collection procedure is summarized in Table 1. Bibliometric data for this review were collected from two major databases: Web of Science (WoS) and Scopus, both of which offer broad and high-quality coverage of peer-reviewed scientific literature across disciplines. The combined use of these databases ensures a more comprehensive and inclusive

Table 1 Summary of search query iterations and screening criteria

Description	Detail	Results (Number of Articles)
Database Selection	Web of Science (WoS) and Scopus	-
Initial Search Date	May 15, 2025	-
Search Query (Final Iteration)	(((TS= (sludge microstructure) OR TS= (sludge characterization)) AND TS= (anaerobic digestion)) OR TS= (biogas production)) AND TS= (microbial activity) (adapted syntax for both WoS and Scopus)	WoS: 2456, Scopus: 1979; Total: 4435
Time Frame	January 1, 2000, to December 31, 2024	2157
Language Criterion	English-language articles only	1989
Publication Type	Peer-reviewed journal articles (Exclusion of reviews, conference papers, book chapters, editorials)	1632
Deduplication	Removal of overlapping articles indexed in both databases	1460
Manual Relevance Screening	Screening of titles, abstracts, and keywords based on relevance to sludge microstructure, microbial dynamics, anaerobic digestion, and biogas production	841

literature base, capturing a wider range of relevant studies and minimizing publication bias. The initial search was conducted on May 15, 2025, using the following query syntax (adapted for both platforms): (((TS= (sludge microstructure) OR TS= (sludge characterization)) AND TS= (anaerobic digestion)) OR TS= (biogas production)) AND TS= (microbial activity)). All subsequent steps—including deduplication and manual relevance screening—were carried out in the following days to ensure accuracy, completeness, and data integrity.

The publication date range was limited to January 1, 2000, to December 31, 2024, covering two decades of research developments. Only English-language, peer-reviewed primary research articles were included. While this language restriction helps maintain consistency in content quality and interpretability, it may introduce geographic bias by underrepresenting research published in other languages, particularly from non-English-speaking countries. This limitation should be considered when interpreting the global distribution of research trends. Review papers, book chapters, editorials, and conference proceedings were excluded to ensure the dataset consisted of studies with original methodologies and empirical findings. The combined search initially yielded 2,157 articles. A two-stage screening process was applied:

a) Deduplication: Articles with overlapping metadata were removed to ensure data integrity.
b) Relevance screening: Titles, abstracts, and keywords of the remaining articles were manually reviewed to assess relevance to the relationship between sludge microstructure and anaerobic digestion. To reduce subjectivity, the screening was guided by predefined inclusion and exclusion criteria, which required that studies (i) focus on

sludge-based feedstocks, (ii) examine microstructural characteristics or their impact on AD performance, and (iii) provide primary experimental or modelling data. Review papers, theoretical studies without empirical support, and articles unrelated to microstructure (e.g., general policy reviews, purely chemical optimization studies) were excluded. To further reduce subjectivity, a subset of the screened articles was independently cross-checked by a second reviewer, and discrepancies were resolved through discussion to ensure consistency.

After applying these criteria, a final dataset of 841 articles was selected for analysis. These articles were exported in text format for bibliometric processing.

2.3 Data processing

The processing of data involved using CiteSpace, VOSviewer, and Bibliometrix for bibliometric analysis. Prior to analysis, the dataset was cleaned and standardized to ensure accuracy, including normalization of author names, institutions, and keywords.

CiteSpace (Chen, 2006) was employed to detect citation bursts, perform keyword clustering, and identify emerging thematic trends. The analysis used the log-likelihood ratio (LLR) for cluster labelling and applied pathfinder network scaling to reduce noise and enhance clarity in co-citation networks. Time slicing was set to one-year intervals to capture temporal evolution.

VOSviewer (van Eck & Waltman, 2010) was used to construct visual maps of keyword co-occurrence and author collaboration. The minimum occurrence threshold for keywords was set to 5 to filter out infrequent terms, and association strength normalization was applied to both keyword co-occurrence and author collaboration networks to measure link strength. The clustering resolution parameter was set to 1.0, balancing interpretability and granularity in cluster formation. Label size, font, and spacing were adjusted to optimize readability of network layouts.

Bibliometrix (Aria & Cuccurullo, 2017) was used to generate co-citation networks, trend analyses, and descriptive statistics, helping to identify influential works and research themes. These combined tools enabled a robust, multidimensional exploration of the research landscape.

The overall research protocol is summarized in Fig. 1, which outlines the data retrieval, processing, and analytical steps. This systematic approach ensures reproducibility and reliability in deriving insights from the dataset.

2.4 Analytical methods

The study employed a combination of bibliometric techniques to examine research trends and interconnections within the field. Co-occurrence analysis was conducted to investigate the frequency and relationships between keywords in the selected publications. This method, visualized through VOSviewer, generated a co-occurrence map that revealed the major research themes and their interconnections. By identifying frequently co-occurring keywords, the analysis provided insights into the dominant topics and emerging trends shaping the field of sludge microstructure and anaerobic digestion.

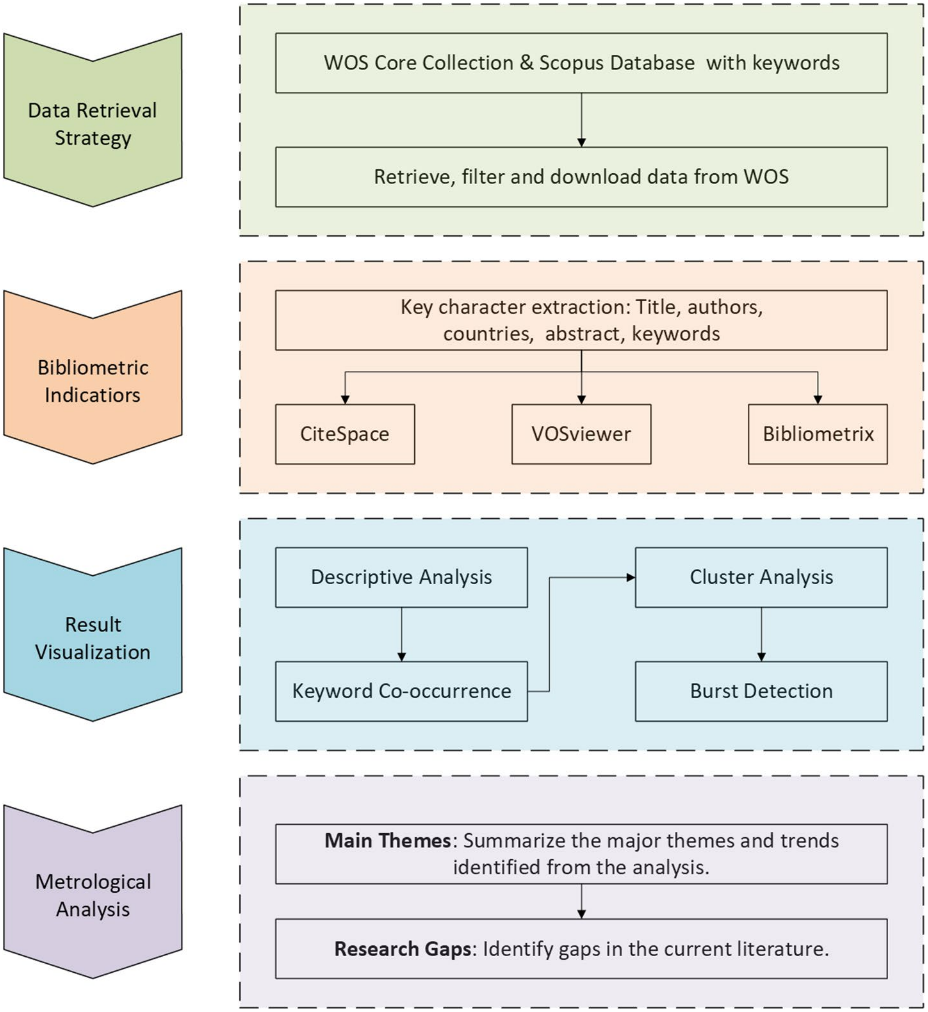

Fig. 1 The research protocols

Co-citation analysis, performed using Bibliometrix, examined how often pairs of publications were cited together, helping to map the intellectual structure of the field. This technique identified influential works and key contributors, highlighting seminal studies that have significantly shaped the understanding of microbial activity, sludge pretreatment, and biogas optimization. The analysis provided a deeper understanding of how foundational studies are interconnected and have influenced the progression of the field.

Cluster analysis, carried out with CiteSpace, grouped related publications into thematic clusters based on citation patterns. Each cluster represented a distinct research focus, such as microbial community dynamics or pretreatment strategies. This clustering approach enabled a detailed exploration of the thematic evolution within the field, identifying both well-established and emerging areas of interest.

To capture dynamic changes in research focus over time, burst detection was employed using CiteSpace. This method identified terms and references that experienced sudden increases in citation frequency, signalling emerging areas of interest or heightened research activity. Burst detection provided valuable insights into the temporal dynamics of research themes, highlighting recent innovations and shifts in the field's priorities.

By integrating these analytical techniques, the study achieved a comprehensive evaluation of the research landscape, combining quantitative metrics such as citation frequency with qualitative insights into thematic evolution. This approach enabled a nuanced understanding of both the breadth and depth of developments in sludge microstructure and anaerobic digestion research.

3 Bibliometric analysis

3.1 Descriptive analysis

As illustrated in Fig. 2(a), the annual number of publications related to sludge microstructure and anaerobic digestion has shown a pronounced upward trajectory over the past two decades, with a particularly sharp acceleration since 2015. This trend underscores the growing recognition of AD as a sustainable solution for integrated waste management, energy recovery, and circular economy goals. The cumulative publication count approaches 900 by 2024, with annual outputs nearing 120 publications—more than doubling the yearly average from a decade ago. The superimposed trend line further confirms the exponential growth pattern, reflecting heightened interdisciplinary interest across environmental engineering, microbiology, and materials science domains (Zhong et al., 2023). This rapid expansion highlights both the urgency and complexity of addressing microstructural factors in AD optimization.

Geographically, China emerges as the dominant contributor to sludge microstructure and anaerobic digestion research, not only in terms of publication volume but also in the breadth of its international collaboration network, as illustrated in Fig. 2(b). China's partnerships with countries such as the USA, Germany, France, Australia, and Korea reflect its strategic investment in global scientific exchange and policy-driven environmental research (Shi et al., 2024). The United States and United Kingdom also maintain extensive co-authorship networks, particularly with European and Asia-Pacific countries, reinforcing their roles in method development and applied bioenergy research (Lu et al., 2024). Notable interregional collaborations—such as between Germany and Japan, France and India, and Australia and Korea—demonstrate the interdisciplinary and cross-continental nature of current AD innovation. Emerging economies like Colombia, Egypt, and Iran are increasingly integrated into the global research landscape, often through bilateral programs with larger contributors. These international collaborations are instrumental in harmonizing methodological standards, facilitating technology transfer, and addressing region-specific challenges in sludge treatment and biogas production (Almansa et al., 2023).

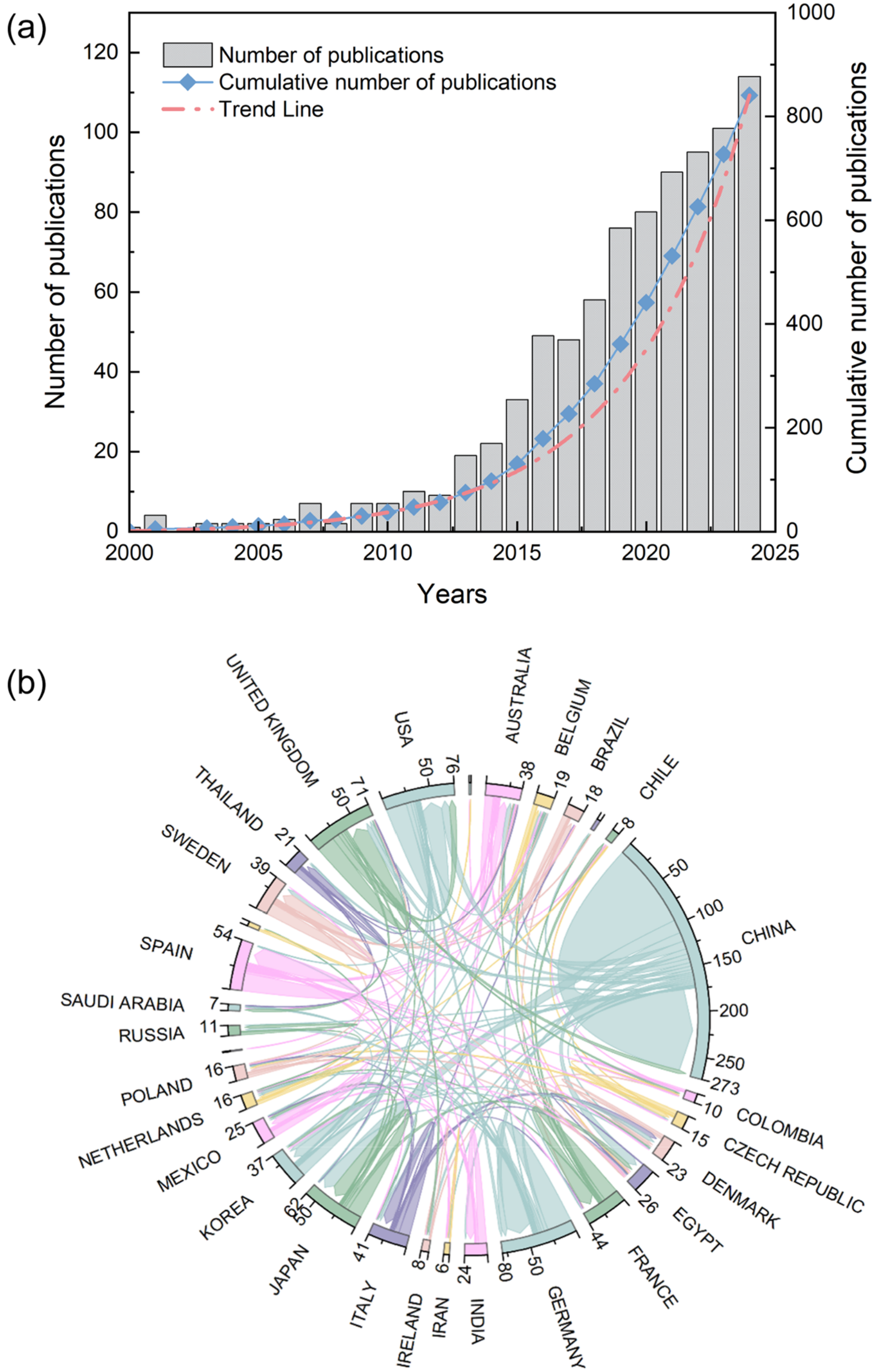

Fig. 2 (**a**) Annual number of published document (2000–2024); (**b**) The most contributing countries/regions in research area

3.2 Keyword Co-occurrence analysis

Keyword co-occurrence analysis, visualized using VOSviewer, highlights the major themes and research trends within sludge microstructure and AD. Figure 3 presents a network map where nodes represent keywords, and links indicate their co-occurrence in publications. Node size reflects keyword frequency, and link thickness represents co-occurrence strength.

Three dominant thematic clusters emerge from the analysis. The first and most central cluster is built around "anaerobic digestion", "biogas production" and "microbial community", underscoring the critical role of microbial structure in determining AD performance. Numerous studies have demonstrated that microbial community composition and dynamics directly influence process stability and methane yield. For example, Lin et al. (2016) and Westerholm et al. (2018) observed that shifts in methanogenic populations under varying operational conditions (e.g., temperature and organic loading rate) significantly affect system efficiency and resilience.

The second major cluster, including keywords such as "pretreatment", "hydrolysis" and "optimization", reflects growing research on enhancing substrate accessibility and hydrolysis efficiency through physical, thermal, or chemical pretreatments. For instance, Jin et al. (2016) demonstrated that thermal pretreatment at 70 °C improved solubilization of organic matter in food waste, leading to a 12.5% increase in methane yield. Similarly, alkaline or microwave pretreatments of lignocellulosic substrates have been shown to disrupt the cellulose–lignin structure, improving enzymatic hydrolysis and microbial degradation (Li et al., 2022; Pellera and Gidarakos, 2017; Shetty et al., 2017).

The third cluster is centered on "co-digestion", "food waste", "manure" and "methane yield", emphasizing strategies to combine substrates for improved nutrient balance and buffering capacity. Co-digestion of food waste with manure or sludge has been widely studied to overcome the limitations of single-substrate digestion, achieving better process stabil-

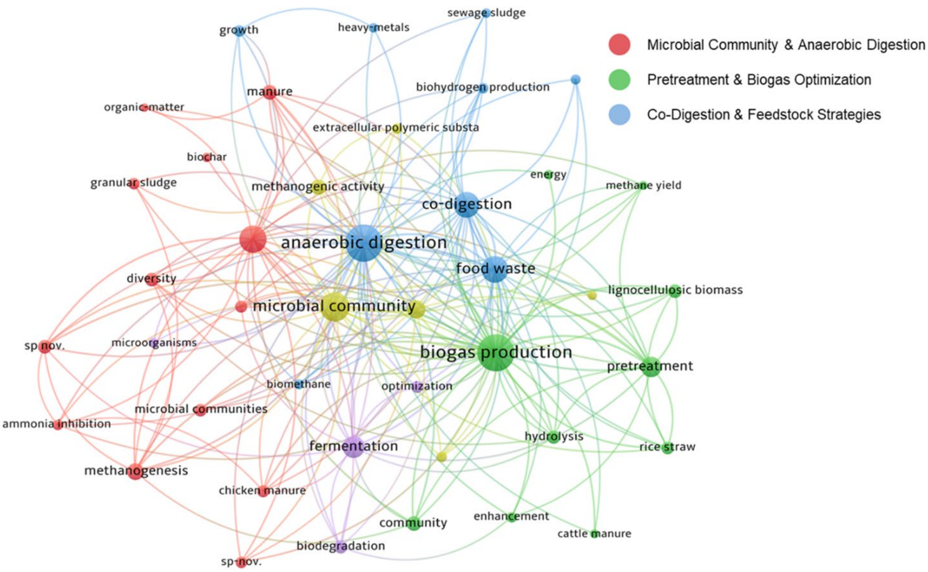

Fig. 3 Visualization of keyword co-occurrence within the field of sludge microstructure and anaerobic digestion

ity and enhanced methane productivity (Chen et al., 2022; Ravindran et al., 2022; Zhang et al., 2022).

Beyond these main clusters, inter-cluster synergies are also evident. For example, "biochar" is closely linked to both microbial and performance-related themes such as "anaerobic digestion", "microbial community" and "granular sludge", highlighting its role as a multifunctional additive. Biochar enhances microbial colonization (Cai et al., 2023), stabilizes pH (Saif et al., 2022), reduces ammonia inhibition (Zhao et al., 2024), and promotes direct interspecies electron transfer (Wang et al., 2021). Similarly, the co-location of "extracellular polymeric substances" with microbial and substrate-related terms suggests increased attention to sludge microstructure–microbe interactions and their impact on biodegradability (Li et al., 2022).

To further elucidate evolving research trends, Fig. 4 presents the top 20 keywords with the strongest citation bursts between 2000 and 2024, capturing periods of intense scholarly focus on specific terms. Earlier bursts, such as "renewable energy" (2010 — 2014) and "microbial activity" (2011 — 2014), reflect foundational concerns with sustainable energy recovery and microbial roles in AD. As the field matured, attention shifted toward microbial structure and analytical tools, evidenced by bursts in terms like "microbial consortium" (2016 — 2019), "high-throughput sequencing" (2016 — 2018), and "community structure" (2015 — 2016). These trends align with the increasing use of omics-based techniques to dissect microbial dynamics in sludge.

More recent bursts (2019 — 2024) suggest an emerging emphasis on system-level performance and optimization. Terms such as "volatile solids", "cow manure", "enzymatic

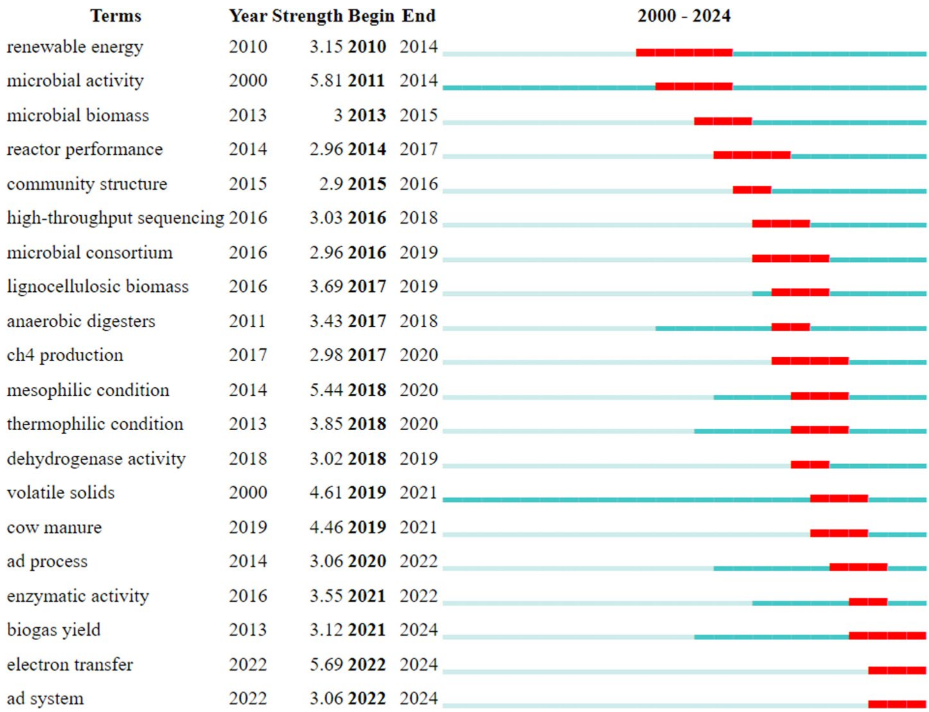

Fig. 4 Top 20 terms with the strongest citation bursts

activity", and "biogas yield" illustrate rising interest in feedstock composition and substrate degradation pathways. The presence of "electron transfer" and "AD system" as 2022–2024 burst terms indicate a growing focus on advanced microbial interactions and integrated process modelling, resonating with broader efforts to bridge micro-scale understanding and macro-scale engineering outcomes.

Notably, these citation bursts also reflect broader external influences such as shifting government priorities, targeted R&D funding, and policy frameworks promoting renewable energy and waste valorisation. For instance, the surge in interest around 'renewable energy' coincides with major international commitments like the Paris Agreement (Alengebawy et al., 2024), while the rise of system-level terms after 2020 may reflect national bioeconomy strategies and increased investment in circular economy innovation.

Collectively, these temporal patterns reveal a clear research trajectory—from basic microbial function to complex system enhancement—underscoring the importance of integrating microbiology, process control, and material science to drive the next generation of AD technologies.

3.3 Co-citation analysis

Co-citation analysis allows for an in-depth examination of how research papers are interconnected through shared references, thus identifying intellectual linkages and the development of key research areas over time. The top 10 cited papers in this review (Table 2) have been instrumental in advancing the understanding of sludge microstructure and anaerobic digestion. By analysing the co-citation patterns, it is evident that several core themes have emerged, reflecting the interdisciplinary nature of anaerobic digestion research.

However, it is important to acknowledge that co-citation patterns tend to favour older publications, as more recent studies have had less time to accumulate citations and become embedded within the scholarly network. This can result in a phenomenon known as "citation inertia," where foundational but possibly outdated research continues to dominate the intellectual landscape, potentially obscuring recent breakthroughs. To address this potential citation bias, we have incorporated a complementary analysis in Sect. 4, which reviews the 50 most-cited original research articles from 2020 to 2024. This more temporally balanced approach helps ensure that emerging innovations and evolving research directions are also captured and contextualized within the broader knowledge structure.

Based on the co-citation results, several core thematic clusters emerge, reflecting the interdisciplinary and evolving nature of AD research. The first major theme identified is the enhancement of biogas production via process optimization. Papers such as Feng et al. (2009) and Huang et al. (2011) highlight the role of sludge pretreatment and the optimization of operational parameters like pH, HRT, and SRT. Both papers have frequently been co-cited together, underscoring their contribution to optimizing the sludge conversion processes. Their study on adjusting pH to improve VFA production and findings on the effect of SRT in submerged anaerobic membrane bioreactors both emphasize how operational conditions can modulate microbial activity and improve digestion efficiency. These co-citations reflect a shared interest in process optimization strategies for increasing methane yield, which has become a cornerstone in anaerobic digestion research.

Another closely related cluster of co-citations is the research on microbial community dynamics and its influence on anaerobic digestion. Westerholm et al. (2016), which focuses

Table 2 Top 10 most cited papers in sludge microstructure and anaerobic digestion research

Rank	Title	Year	Journal	Total Citations	Main findings	Ref.
1	Enhancement of Waste Activated Sludge Protein Conversion and Volatile Fatty Acids Accumulation during Waste Activated Sludge Anaerobic Fermentation by Carbohydrate Substrate Addition: The Effect of pH	2009	Environmental Science & Technology	529	Adjusting pH (optimal at pH 8.0) and adding carbohydrates improved protein conversion and VFA production, especially propionic acid. This finding is crucial for enhancing microbial activity in anaerobic digestion and improving sludge breakdown efficiency.	(Feng et al., 2009)
2	Submerged Anaerobic Membrane Bioreactor for Low-Strength Wastewater Treatment: Effect of HRT and SRT on Treatment Performance and Membrane Fouling	2011	Water Research	525	Longer solids retention time (SRT) improved biogas production and methanogenesis, but increased membrane fouling due to smaller particle size and high soluble microbial product concentration.	(Huang et al., 2011)
3	Biogas production through syntrophic acetate oxidation and deliberate operating strategies for improved digester performance	2016	Applied Energy	348	Syntrophic acetate oxidation (SAO) dominates in high ammonia conditions, improving methane production. Key factors influencing SAO include ammonia concentration, acetate levels, temperature, and methanogenic community structure.	(Westerholm et al., 2016)
4	Effect of organic loading rate on VFA production, organic matter removal and microbial activity of a two-stage thermophilic anaerobic membrane bioreactor	2011	Bioresource Technology	346	Higher organic loading rates increased VFA production and microbial activity in a two-stage thermophilic anaerobic membrane bioreactor, with acetic and butyric acid as the dominant VFAs. However, excessive loading led to decreased removal efficiency.	(Wijekoon et al., 2011)
5	Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops	2007	Journal of Applied Microbiology	318	Microbial community composition differed between sugar beet and grass substrates, with Firmicutes dominating in grass digestion. Hydrolysis rates became the limiting factor after day 10 for both substrates, impacting biogas production.	(Cirne et al., 2007)

Table 2 (continued)

Rank	Title	Year	Journal	Total Citations	Main findings	Ref.
6	Comparative characterization of digestate versus pig slurry and cow manure – Chemical composition and effects on soil microbial activity	2017	Waste Management	311	Digestate had higher ammonium content, but lower volatile fatty acids compared to pig slurry and cow manure, with digestate generally having a stimulating effect on ammonium oxidation and lower carbon utilization, indicating better suitability for fertilizing heavier soils.	(Risberg et al., 2017)
7	Batch anaerobic co-digestion of pig manure with dewatered sewage sludge under mesophilic conditions	2014	Applied Energy	299	Co-digestion of pig manure with dewatered sewage sludge at a 2:1 ratio enhanced methane yield by 82.4% compared to digesting sludge alone, improving hydrolysis and microbial activity. The modified Gompertz model better predicted methane yields.	(Zhang et al., 2014)
8	Effect of cerium dioxide, titanium dioxide, silver, and gold nanoparticles on the activity of microbial communities intended in wastewater treatment	2012	Journal of Hazardous Materials	297	Low concentrations of cerium dioxide and titanium dioxide nanoparticles enhanced methane production by stimulating microbial activity, while silver and gold nanoparticles had inhibitory effects on anaerobic digestion.	(García et al., 2012)
9	Acetoclastic methanogenesis led by Methanosarcina in anaerobic co-digestion of fats, oil and grease for enhanced production of methane	2019	Bioresource Technology	270	Methanosarcina replaced Methanosaeta under high acetate concentrations during co-digestion of fats, oils, and grease (FOG), leading to a 217% increase in methane production. The dominance of acetoclastic methanogenesis highlights the importance of microbial shifts in digestion efficiency.	(Kurade et al., 2019)
10	Profiling of the metabolically active community from a production-scale biogas plant by means of high-throughput metatranscriptome sequencing	2012	Journal of Biotechnology	258	High-throughput metatranscriptome sequencing revealed that Firmicutes and Euryarchaeota dominate biogas-producing communities, with methanogenesis being a highly active pathway. This underscores the importance of microbial dynamics for efficient anaerobic digestion.	(Zakrzewski et al., 2012)

on syntrophic acetate oxidation (SAO) under high ammonia conditions, and Kurade et al. (2019), which explores the dominance of Methanosarcina under high acetate concentrations, are co-cited with papers that study microbial interactions, such as Zakrzewski et al. (2012). These papers highlight the importance of understanding microbial community structures and their shifts under different conditions, a theme that is crucial for optimizing biogas production and enhancing sludge treatment efficiency. The co-citation of these papers reflects the growing recognition of the need for a microbiological perspective in understanding the efficiency and resilience of anaerobic digestion processes under varying environmental stressors.

A third prominent co-citation cluster revolves around the impact of co-digestion on digestion efficiency. Zhang et al. (2014) and Cirne et al. (2007) are often cited together in studies investigating the benefits of co-digestion strategies for enhancing methane yield. Zhang et al.'s research demonstrates the synergy between pig manure and dewatered sewage sludge, while Cirne et al. emphasizes the role of microbial community changes in co-digestion. Their frequent co-citation suggests that co-digestion is a pivotal strategy for overcoming the limitations of single-substrate digestion by improving the hydrolysis rate and enhancing microbial activity. The co-citation of these studies with others focused on microbial activity, indicates an ongoing effort to integrate chemical and microbial optimization strategies to enhance digestion performance.

Lastly, the co-citation analysis highlights the increasing focus on environmental implications and material recovery from anaerobic digestion processes. Risberg et al. (2017) contribute to this theme by comparing the nutrient content and microbial impacts of digestate, pig slurry, and cow manure. Their co-citation with studies on microbial activity shows an emerging interest in understanding the dual role of anaerobic digestion as both a waste management technology and a source of valuable by-products, such as digestate, which can be utilized in agriculture.

In summary, the co-citation analysis underscores four core research areas: process optimization, microbial dynamics, co-digestion benefits, and environmental impacts of digestate. These interconnected themes reflect the evolving landscape of anaerobic digestion research, where the focus is shifting towards a more integrated understanding of sludge treatment, biogas optimization, and sustainable waste management. The co-citation patterns also suggest that future research may continue to explore the synergies between chemical and microbial approaches, aiming for even greater efficiency and sustainability in anaerobic digestion processes.

3.4 Cluster analysis

The cluster analysis, conducted using Bibliometrix, reveals key thematic areas in anaerobic digestion and sludge microstructure research over two distinct periods: 2000 — 2019 and 2020 — 2024. The themes identified in these analyses are mapped according to two key metrics: relevance degree (centrality), which indicates how central a theme is to the broader research landscape, and development degree (density), which measures how well-developed a theme is. These metrics allow the themes to be categorized into four distinct types:

- Motor Themes: These themes are both highly relevant and well-developed, representing the driving forces of the research field. They are dynamic areas that influence ongoing research and future directions (Rodríguez-López et al., 2020).
- Basic Themes: These themes are highly relevant but less developed. They form the foundational backbone of the research field, providing essential knowledge without being at the forefront of innovation (Trinidad et al., 2021).

- Niche Themes: These are well-developed but less central themes. Niche themes often represent specialized areas of research, relevant to specific subfields but not widely applicable to the broader research scope (Muñoz-Leiva et al., 2012).
- Emerging or Declining Themes: These themes have both low relevance and low development. They may represent new areas gaining attention or, conversely, areas where research interest is decreasing (Mühl and de Oliveira, 2022).

With this framework, it is possible to observe how the research landscape of anaerobic digestion has shifted between the two periods, revealing both persistent themes and emerging trends.

3.4.1 Thematic evaluation (2000 — 2019)

The thematic analysis for the period 2000–2019 (Fig. 5) highlights methane yield as a dominant motor theme, reflecting its critical role in anaerobic digestion research. Methane yield is a key indicator of process efficiency, and research in this area has focused on enhancing methane production through optimization of process conditions, such as substrate pretreatment (Ventorino et al., 2018) and co-digestion (Amha et al., 2017) strategies. Its positioning as a motor theme indicates its relevance and the extensive research dedicated to improving biogas yields over the years. Simultaneously, anaerobic digestion and biogas production appear as basic themes, indicating their foundational importance. These themes, while highly central to the field, are less developed, suggesting that they represent well-established

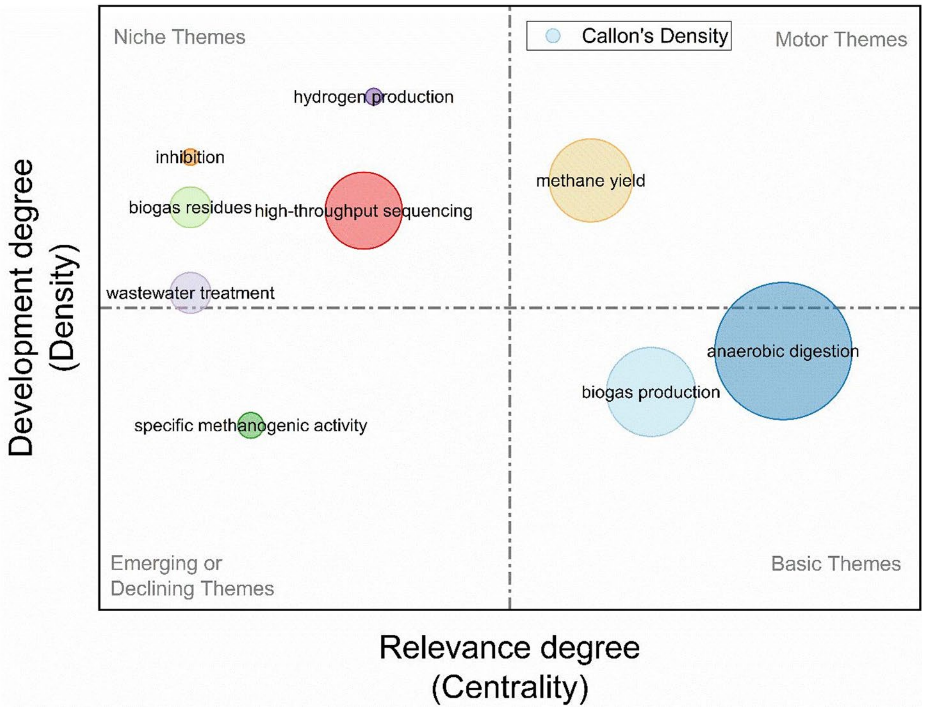

Fig. 5 Thematic evaluation for the period 2000–2019

processes where research has focused on gradual improvements (Jin et al., 2018; Mehryar et al., 2017) rather than significant innovations.

Several niche themes also emerged during this period, including hydrogen production, biogas residues, and high-throughput sequencing. While these themes are less central to the broader field, they are highly developed, representing specialized areas of research. Hydrogen production offers an alternative to traditional biogas production. For example, Hernández and Rodríguez (2013) utilized pig manure for hydrogen production through anaerobic digestion and found an inverse linear relationship between methane and hydrogen concentrations, illustrating the trade-offs in optimizing different bioenergy outputs. Similarly, high-throughput sequencing has become an essential tool for analysing microbial communities and their role in anaerobic digestion. Lim et al. (2018), targeting the 16 S rRNA gene, characterized the microbial consortia of anaerobic digestion sludge samples and emphasized that bacterial community structures are closely related to the sources of biogas plants, suggesting a significant impact of these bacteria on digester performance.

The attention to biogas residues reflects the growing recognition of the need to manage digestate not just as a by-product but as a valuable resource for agricultural use, aligning with circular economy principles (Chen et al., 2012). Meanwhile, emerging or declining themes, such as specific methanogenic activity and wastewater treatment, suggest that traditional areas of focus may be yielding to broader, integrative approaches. For example, specific studies on methanogenic activity (Fu et al., 2016; Gu et al., 2014; Zhang et al., 2007) are now often encompassed within more comprehensive microbial studies that investigate a wider range of microbial interactions. Similarly, research on wastewater treatment has expanded to include a broader variety of substrates, such as food and agricultural waste, reflecting the evolving priorities in optimizing anaerobic digestion for diverse waste streams.

3.4.2 Thematic evaluation (2020 — 2024)

In the second period, 2020 — 2024 (Fig. 6), a clear evolution in research focus is evident. Anaerobic digestion remains a basic theme, but its increased centrality suggests renewed interest in optimizing the process, likely driven by advances in microbial activity (de Jonge et al., 2020; Ottoni et al., 2022) and process integration (González-Arias et al., 2020; Yellezuome et al., 2023). Methane production continues to function as a motor theme, reflecting sustained interest in maximizing energy recovery from waste streams. Most significantly, microbial activity has emerged as a new motor theme, highlighting a critical shift in research priorities toward the biological underpinnings of AD performance.

This shift is closely driven by the rapid advancement of meta-omics technologies, including metagenomics, meta transcriptomics, metaproteomic, and metabolomics. These high-throughput tools have enabled researchers to move beyond traditional microbial enumeration, offering comprehensive insight into microbial structure, functional gene expression, enzyme activity, and metabolic fluxes in real time (Li et al., 2021b; Shi et al., 2021; Sun et al., 2022). This molecular-level understanding has been pivotal in identifying keystone microbial taxa, syntrophic interactions, and stress-response mechanisms—factors directly linked to system stability, biogas yield, and resilience under varying environmental conditions.

For example, Ao et al. (2021) demonstrated that mesophilic digestion supports greater microbial diversity than thermophilic systems, with downstream impacts on methane pro-

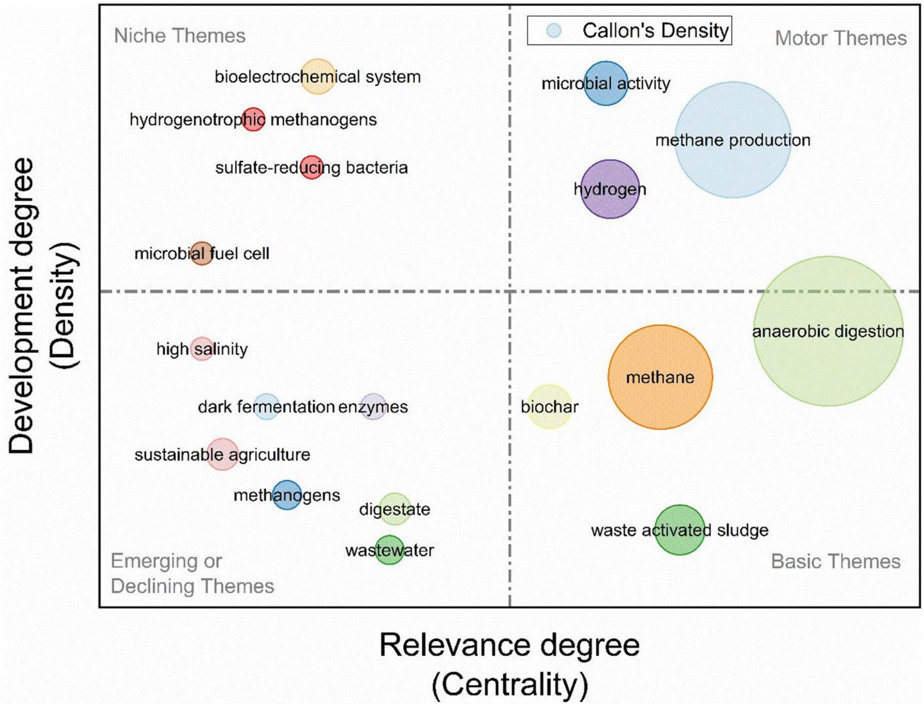

Fig. 6 Thematic evaluation for the period 2020–2024

ductivity. Similarly, Jiang et al. (2020) used microbial profiling to show how temperature variations influence the succession of methanogenic communities during food waste digestion. These findings underscore the growing consensus that microbial ecology, informed by meta-omics, is central to optimizing full-scale AD systems. As such, the prominence of microbial activity in the thematic map is not only timely but also reflects the research community's embrace of biologically driven engineering strategies in AD.

Other notable trends in the 2020 — 2024 period include the emergence of hydrogen and biochar as niche themes, reflecting the ongoing exploration of alternative bioenergy products and integrated technologies aimed at enhancing environmental sustainability. Biochar has gained significant attention for its potential to improve nutrient recovery and promote carbon sequestration when combined with anaerobic digestion processes. For example, Zhou et al. (2020) used biochar as an additive in the AD of municipal sludge, finding that the effects of biochar on AD largely depend on the specific properties of the biochar. The study emphasized that the selection of appropriate biochar is crucial for achieving higher biogas production and maintaining process stability. Similarly, Sun et al. (2022) highlighted that the electrochemical properties of biochar may play a key role in promoting anaerobic digestion, further underscoring the importance of biochar characteristics in optimizing AD performance.

In addition, the theme of waste activated sludge has gained prominence, indicating continued efforts to optimize sludge management practices, particularly for large-scale applications in municipal wastewater treatment (Dolu & Nas, 2023; Kong et al., 2021; Nguyen et al., 2021). This growing focus reflects the need to improve the efficiency of waste treatment processes while maximizing resource recovery. Meanwhile, themes such as digestate and wastewater appear in the emerging or declining quadrant, suggesting that while these areas remain relevant, research may be shifting towards more innovative and integrated approaches to resource recovery.

The cluster analysis reveals both continuity and evolution in the research landscape of anaerobic digestion. While methane production and anaerobic digestion remain central to the field, the increasing focus on microbial activity reflects a deeper understanding of the biological processes driving anaerobic digestion. The rise of niche areas such as hydrogen production and biochar points to a broader integration of anaerobic digestion with other technologies aimed at improving sustainability. These trends indicate that the field is expanding beyond traditional energy recovery models to address wider environmental and resource management challenges, particularly in the context of the circular economy.

4 Experimental insights into sludge microstructure

The bibliometric analysis of anaerobic digestion research provides valuable insights into how the field has evolved, particularly concerning the role of sludge microstructure in influencing digestion efficiency. The central themes identified—microbial community, biochar, operational conditions, and co-digestion strategies—highlight emerging trends and key areas driving innovation in anaerobic digestion. For a deeper understanding of the relationship between these aspects and anaerobic digestion, as well as the trends in this area, the 50 most-cited papers from the past five years were selected and analysed to provide experimental insights. As shown in Fig. 7, these top 50 papers are categorized into four classifications based on their themes and publication years, offering a clear perspective on the development and focus areas of the field over time.

4.1 Microbial activity and community dynamic

From the top 50 most cited papers, there are in total 16 papers are talking about the relationship between microbial activity and anaerobic digestion. The most published year is 2020 and 2021 (Fig. 7). Microbial communities play a central role in anaerobic digestion by facilitating the sequential breakdown of complex organic matter into biogas. The efficiency and stability of the digestion process largely depend on the composition, structure, and functional activity of microbial consortia. Recent studies have explored how different factors—including pretreatment methods, emerging contaminants and feedstock variability—affect microbial community dynamics and overall digestion performance.

Pretreatment strategies have been extensively studied for their ability to enhance sludge biodegradability by altering substrate structure and chemistry while also shaping microbial consortia. Physical methods such as ultrasound (Mortezaei et al., 2023), microwave irradiation (Yue et al., 2021), and thermal treatment (Gahlot et al., 2022) are widely used to disrupt sludge floc structure and microbial cell walls, facilitating enzymatic hydrolysis. For example, Szaja and Bartkowska (2024) demonstrated that microwave pretreatment at a specific energy (SE) input of 336 kJ/kg TS for 3.5 min resulted in a 50% increase in biogas production compared to the untreated control. Likewise, Çelebi et al. (2021) observed a

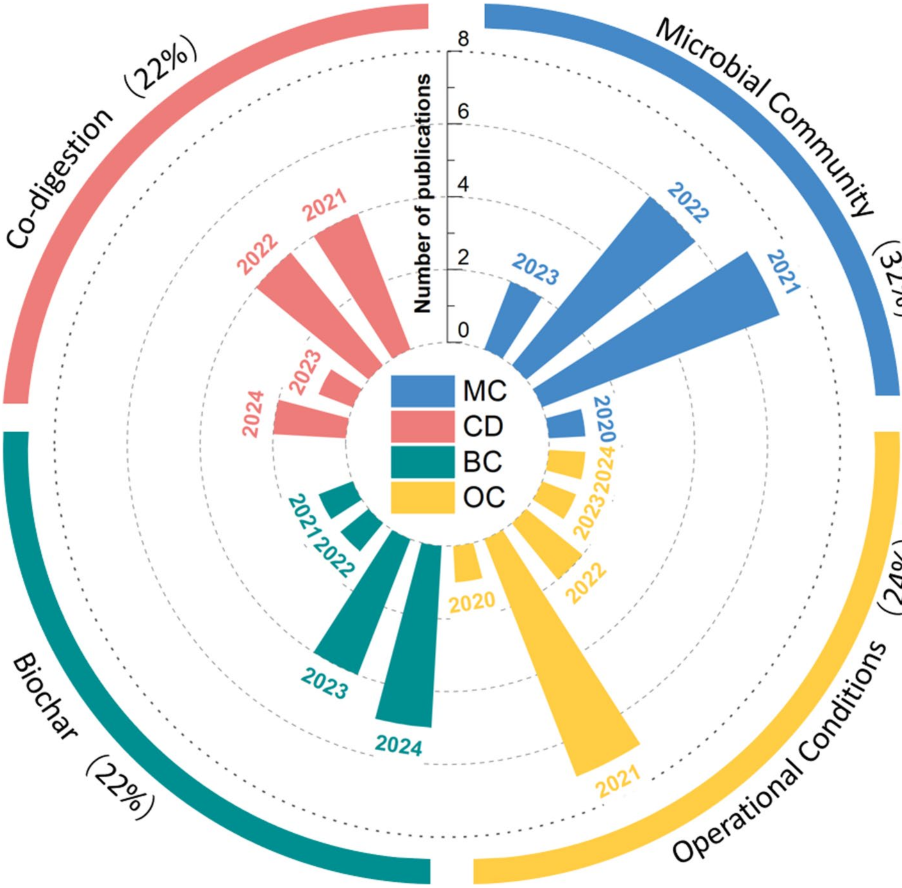

Fig. 7 Overview of the top 50 most-cited papers in anaerobic digestion research over the past five years, categorized into four central themes: microbial community (MC), biochar (BC), operational conditions (OC), and co-digestion strategies (CD)

3.4-fold increase in methane production following ultrasonic treatment with an SE of 12.93 kJ/g TS, corresponding to a 15% increase in total biogas. However, both studies noted that despite enhanced methane yields, the energy recovered from the additional biogas failed to offset the pretreatment energy input, highlighting the need for improved energy recovery strategies and optimization of pretreatment parameters.

Chemical pretreatments also show promise for improving digestion performance, often with lower energy requirements. For example, cation exchange resins (CER) were shown by Geng et al.(2022) to increase methane content from 62.75 to 73.96% by removing organically bound metals and improving sludge solubilization. Similarly, Liang et al. (2020) found that attapulgite-assisted conditioning enhanced the release of soluble organics, while zero-valent iron (He et al., 2020) accelerated hydrolysis through Fenton-like reactions. These chemical approaches tend to offer a more favourable energy return on investment (EROI), making them attractive for full-scale deployment.

However, the effectiveness of chemical pretreatments can vary with substrate type, microbial resilience, and operating conditions. In such cases, integrating them with physical methods—such as microwave or ultrasonic pretreatment—may yield synergistic effects that further enhance microbial activity and biogas recovery. These hybrid strategies, when carefully optimized, offer a pathway to achieving positive net energy balances while maintaining process stability and scalability.

Beyond pretreatment effects, the presence of emerging contaminants such as microplastics and pharmaceutical residues has raised concerns about microbial community stability in anaerobic digestion systems (Wang et al., 2020). Recent research investigating the influence of polyether sulfone microplastics and bisphenol A (Lin et al., 2020) on anaerobic sludge granules revealed that these contaminants can alter microbial diversity, hinder methanogenic activity, and reduce biogas yield. While some microbial populations exhibit resilience and adaptability, long-term exposure to microplastics disrupts microbial interactions, potentially compromising digestion efficiency. Similarly, pharmaceuticals can adversely impact anaerobic digestion processes. Alenzi et al. (2021) found that long-term exposure to pharmaceutical residues induces process instability, resulting in the accumulation of volatile fatty acids (VFAs). These findings underscore the need for further studies on mitigation strategies to counteract the inhibitory effects of environmental pollutants on anaerobic microbial ecosystems.

Feedstock composition also plays a crucial role in shaping microbial communities and affecting digestion performance. Different substrates promote the selection of distinct microbial consortia, leading to variations in system efficiency and biogas output. Research on anaerobic sludge acclimatization with cow manure and food waste digestion demonstrated that microbial communities undergo adaptive changes over extended retention periods, ultimately leading to enhanced biogas yield and system robustness (Xing et al., 2020). Similarly, a long-term study on chicken manure digestion under ammonia stress provided valuable insights into microbial adaptation mechanisms. Over ten years of continuous operation, the system exhibited a gradual shift toward ammonia-tolerant methanogens, ensuring resilience against high ammonia concentrations (Bi et al., 2021). These findings suggest that prolonged exposure to extreme conditions drives microbial evolution, reinforcing the importance of continuous monitoring and adaptive process control in full-scale anaerobic digestion plants.

Despite significant advancements in understanding microbial activity and community dynamics within anaerobic digestion, several critical challenges persist. Figure 8 illustrates that although studies addressing pretreatment strategies, emerging contaminants, and feedstock variability have contributed valuable insights into microbial enhancement of digestion processes, a majority remain confined to short-term laboratory-scale experiments. Particularly, investigations into pretreatment strategies and emerging contaminants are predominantly conducted at laboratory scales (62.5% and 100%, respectively), with limited or no pilot-scale validations and relatively short experimental durations. While pretreatment methods have demonstrated the potential to beneficially manipulate microbial communities and enhance digestion efficiency, the long-term sustainability and resilience of such microbial ecosystems under full-scale operational conditions remain unclear. Additionally, emerging environmental contaminants, such as microplastics and pharmaceutical residues, pose substantial risks to microbial community stability, and their chronic impacts on anaerobic digestion performance require deeper exploration. Therefore, future research

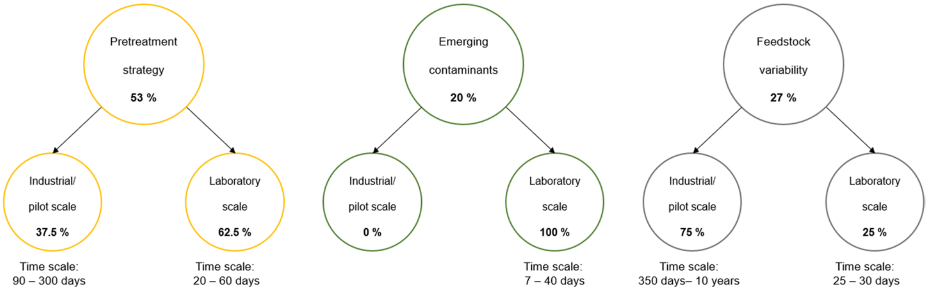

Fig. 8 Overview of the research scale and experimental duration across three key factors influencing microbial activity in anaerobic digestion: pretreatment strategies, emerging contaminants, and feedstock variability

should focus on bridging this gap by conducting prolonged pilot- or industrial-scale experiments, aiming to assess microbial community adaptability, resilience, and ecosystem stability under realistic operational conditions. Addressing these issues effectively will demand an interdisciplinary approach that integrates microbiology, environmental engineering, and computational modelling, thereby advancing our capacity to manipulate microbial consortia purposefully and sustainably in anaerobic digestion systems.

4.2 Biochar integration

Among the top 50 most cited papers, a total of 11 articles focusses specifically on biochar integration into anaerobic digestion processes. Notably, research interest in this area has surged recently, particularly between 2022 and 2023 (Fig. 7). Biochar, a carbon-rich porous material produced via pyrolysis of biomass, has gained attention due to its potential to enhance anaerobic digestion by improving microbial activity, promoting system stability, and mitigating inhibitory conditions.

Recent studies have highlighted biochar's multifaceted benefits within anaerobic digestion systems, attributing improvements primarily to biochar's unique physicochemical characteristics, such as high porosity, abundant functional groups, and exceptional adsorption capacities. These attributes make biochar effective in facilitating microbial colonization, promoting direct interspecies electron transfer (DIET), and alleviating toxic substances (e.g., ammonia and volatile fatty acids), thus enhancing microbial consortium robustness and metabolic efficiency. For instance, Li et al. (2024) demonstrated that Integration of biochar and microbial electrolysis cells was profoundly affected on microbial community, especially the hydrolysis of waste activated sludge and electroactive. Similarly, Wang et al. (2023) reported significant increases in methane yield and digestion stability after integrating biochar into high-ammonia digestion environments, attributing the improvements to biochar's capacity to adsorb ammonia, thereby reducing its inhibitory effects on sensitive microbial groups.

Beyond these physicochemical interactions, biochar amendment has shown the capability to alter microbial community structure beneficially, favouring methanogenic archaea and syntrophic bacteria that are critical for digestion performance. Recent research by Liu et al. (2022) illustrated that biochar integration notably enriched electroactive microbial species, enhancing electron transport efficiency and promoting methanogenesis pathways.

Table 3 Roles of Biochar for improving anaerobic digestion performances

Biochar material	Particle size	Formation conditions	Substrate	Performance	Ref.
Coconut shell	2 µm	Pyrolysis at 800 °C	Cow manure/Aloe peel waste	Cumulative biogas yield and chemical oxygen demand removal rate which are 80.25% and 58.33% higher than those of the blank group	(Xing et al., 2021)
Straw	-	Pyrolysis at 850 °C	Municipal solid waste	After adding magnetic-straw-based biochar (MSBC), the volatile fatty acid production and methane yield were significantly increased by 14.13% and 45.36%, respectively.	(Liu et al., 2022)
Waste apple wood	100 mesh	Pyrolysis at 550 °C	Wheat straw/ swine manure	The biochar application in liquid digestate recirculation (LDR) produced 7.5 and 5.4% more methane than the control and LDR alone, respectively.	(Shao et al., 2022)
Oily sludge	100 mesh	Pyrolysis at 500–700 °C	Municipal solid waste	In AD systems, the addition of 1.2 g biochar prepared at 600 °C obtained the highest accumulative methane production than these with OSBC prepared at 500°Cor 700 °C under the same dosage condition.	(Feng et al., 2023)
Pinecones	100 mesh	Pyrolysis at 500 °C	Liquor wastewater	The utilization of 2.5 g/L pinecone biochar dosage demonstrated a maximum cumulative methane production, indicating a 71% increase in gas production over the control group.	(Song et al., 2024)

Furthermore, a study conducted by Xing et al. (2021) using biochar from cow manure in co-digestion with aloe peel waste found enhanced diversity and metabolic versatility within the microbial communities, ultimately increasing biogas yield by 80% compared to control systems without biochar addition. These studies underline the essential role of biochar not only as a passive carrier but also as an active participant that modulates microbial ecology within anaerobic digestion processes.

Despite promising laboratory-scale results and the growing interest in biochar-enhanced anaerobic digestion, several limitations remain unresolved. As shown in Table 3, biochar characteristics—such as feedstock type, pyrolysis temperature, particle size, surface area, porosity, and functional groups—vary significantly across studies. However, few studies systematically identify or quantify which of these physicochemical properties are most strongly correlated with improved AD performance, microbial colonization, or stability. For instance, high surface area and porosity are frequently associated with enhanced microbial attachment and mass transfer (Ma et al., 2024; Shi et al., 2023), yet inconsistent reporting and lack of standardized characterization methods make direct comparisons across studies difficult. This ambiguity hampers efforts to develop replicable and scalable biochar applications.

To advance the field, future research should prioritize standardized biochar characterization—reporting key metrics such as BET surface area, pore size distribution, pH, electrical conductivity, and elemental composition—to better understand structure–function relationships. Further suggest for establishing a minimum reporting framework for biochar used in AD studies are needed, akin to a "Minimum Information for Biochar Research" (MIBR) dataset. This should include: (i) feedstock source, (ii) pyrolysis conditions, (iii) BET surface area and pore volume, (iv) elemental analysis (C, H, O, N), (v) surface functional groups, and (vi) ash and fixed carbon content.

Such a standardized dataset would facilitate meaningful comparison across studies, promote reproducibility, and support the development of predictive models linking biochar characteristics to AD outcomes. Additionally, studies should explore optimal thresholds for these properties under different digestion conditions. Parallel efforts should focus on validating performance through prolonged pilot- or full-scale trials and developing feasible biochar recovery strategies to offset loss during continuous operations. Without addressing these gaps, biochar integration risks remaining an inconsistent and non-scalable enhancement method for anaerobic digestion.

4.3 Operational conditions

Among the top 50 most cited papers, 12 studies have specifically addressed the impact of operational conditions on AD, highlighting temperature as one of the critical factors influencing microbial activity and digestion performance. Elevated operational temperatures have been widely recognized for their capacity to accelerate hydrolysis—the rate-limiting step of anaerobic digestion—leading to improved overall AD efficiency.

Recent studies indicate that higher temperatures can significantly enhance hydrolysis by promoting microbial metabolism and increasing the solubilization of organic matter, thus facilitating microbial accessibility. For example, Grimalt-Alemany et al. (2020) investigated the effect of incubation temperature on microbial community selection and dominant metabolic pathways, comparing mesophilic (37°C) to thermophilic conditions (60°C). They found that the metabolic network in the thermophilically enriched consortium was notably simpler than that in the mesophilic consortium, leading to significant differences in methane productivity. Similarly, Eng Sánchez et al. (2021) demonstrated that thermophilic conditions substantially increased enzymatic activities involved in hydrolysis, markedly improving the degradation efficiency of complex substrates.

However, maintaining elevated temperatures in anaerobic digestion systems directly relates to the effectiveness and sustainability of pretreatment strategies discussed in previous sections. Pretreatment methods, such as thermal, microwave, or ultrasonic pretreatment, inherently involve energy inputs that lead to elevated temperatures, subsequently promoting hydrolysis. For instance, microwave pretreatment not only enhances substrate solubilization through non-thermal effects but also elevates substrate temperature, thus synergistically accelerating hydrolysis and overall digestion efficiency (Gil et al., 2018). Likewise, thermal pretreatment applied to waste activated sludge significantly facilitates microbial degradation of complex substrates by creating favourable thermophilic conditions, thereby promoting rapid biogas generation (Li et al., 2020; Vandekerckhove et al., 2020). These findings indicate that strategic integration of pretreatment methods with temperature optimization can achieve greater microbial activity and higher anaerobic digestion performance.

In addition to temperature, pH is another fundamental parameter that strongly influences microbial dynamics in AD systems. Methanogens are particularly sensitive to pH fluctuations, with optimal methanogenesis typically occurring in the narrow range of pH 6.8–7.2.(Qiu et al., 2023; Ziembińska-Buczyńska et al., 2014) Deviations from this range can cause an accumulation of intermediate products such as VFAs, inhibiting microbial activity and leading to process failure. Cai et al. (2021) demonstrated that maintaining neutral pH not only stabilizes methanogenic pathways but also improves VFA degradation, leading to higher methane yields. pH control strategies often involve the use of buffering agents or co-digestion with alkaline substrates to maintain system stability, especially in high-load or food waste-rich digesters.

Another essential but often overlooked factor is the substrate-to-inoculum (S/I) ratio, which directly impacts the organic loading rate, microbial acclimation, and digestion kinetics. A well-balanced S/I ratio ensures sufficient microbial biomass to degrade the incoming organic matter without overloading the system. A high S/I ratio may lead to rapid acidification and VFA accumulation, while too low a ratio can result in underutilization of microbial capacity and lower biogas yields. For instance, Li et al. (2022) observed that an S/I ratio of 1:3 (on a volatile solid basis) led to optimal methane production and stable pH in co-digestion systems involving kitchen waste and sewage sludge. Adjusting the S/I ratio is particularly crucial when working with substrates that exhibit high biodegradability or C/N imbalance.

While temperature is a central operational parameter that significantly enhances AD efficiency through hydrolysis and microbial activation, pH regulation and substrate-to-inoculum ratio management are equally important for maintaining microbial stability and optimizing biogas production. Future studies should explore synergistic operational strategies that integrate temperature control, pH stabilization, and S/I ratio optimization, especially under dynamic or high-load conditions. These holistic approaches will be essential for scaling AD systems in real-world waste treatment scenarios.

4.4 Co-Digestion strategies

Co-digestion—the simultaneous anaerobic digestion of multiple substrates—has attracted increasing attention due to its potential to balance nutrient ratios, enhance microbial diversity, and positively modify sludge microstructure, ultimately improving overall digestion efficiency. Among the top 50 most cited papers, 11 studies specifically explored co-digestion strategies for enhancing AD performance.

Recent studies have emphasized the benefits of co-digesting municipal sludge with food waste, animal manure, or agricultural residues, highlighting enhanced nutrient availability, improved microbial activity, and beneficial alterations to sludge microstructure. For example, Gulsen Akbay et al. (2021) reported that co-digesting municipal sludge with food waste substantially enhanced biogas production due to the introduction of readily biodegradable organic matter. Similarly, Jiang et al. (2022) employing excitation-emission matrix (EEM) fluorescence spectroscopy and microbial community analysis, confirmed that co-digestion alleviated ammonia nitrogen inhibition, enhanced hydrolytic acidification and methanogenesis, and suppressed excessive humification of organic matter.

Animal manure is widely studied as a co-substrate due to its buffering capacity and microbial richness. However, the extent to which it enhances methane yield is not universal and largely depends on the type of manure. For instance, Li et al. (2020) evaluated the anaerobic co-digestion performance of cow manure (CM) and sheep manure (SM), demonstrating that the synergistic effect of combining CM and SM resulted in enhanced lignocellulose degradation and higher methane yields. These outcomes were attributed to specific microbial consortia naturally present in SM that facilitated the breakdown of complex polymers in CM. Other studies (Liu et al., 2024; Song et al., 2024) have shown that poultry and swine manures may contribute differently due to their higher nitrogen content, which can cause ammonia inhibition if not properly balanced. This variation underscores the need for substrate-specific co-digestion strategies.

Additionally, co-digestion of agricultural residues with animal manure or municipal sludge has provided further insights into the beneficial effects of substrate diversity. Potdukhe et al. (2021), for instance, reported that co-digesting municipal sludge with agricultural waste significantly improved biogas production due to enhanced microbial colonization within sludge flocs, which in turn increased sludge porosity and permeability.

Despite promising laboratory results, co-digestion strategies face several technical and economic challenges at scale. Substrate variability—such as differences in lignocellulosic content, lipids, and nitrogen levels—often lead to operational instabilities like nutrient imbalance, acidification, and unpredictable methane yields in large-scale systems.

Economic barriers are equally important and often underexplored, particularly the costs associated with substrate collection, pre-treatment, transport, and digestion infrastructure. For example, Vinardell et al. (2021) conducted a techno-economic assessment (TEA) of co-digesting sewage sludge and food waste using an anaerobic membrane bioreactor. They found that although co-digestion increased electricity revenue, the additional costs of food-waste acceptance infrastructure and biosolids disposal offset much of the gains, particularly when adding side stream nutrient recovery processes via partial-nitritation/anammox and struvite precipitation.

On the environmental side, Styles et al. (2022) performed a life-cycle assessment (LCA) of integrating anaerobic digestion into decarbonizing energy systems. Their results highlighted that feedstock sourcing and transportation can be primary contributors to greenhouse gas emissions and energy demand in co-digestion scenarios. In addition to technical and economic factors, large-scale co-digestion in municipal settings is often constrained by regulatory hurdles (e.g., cross-sector waste classification and permitting issues) and the lack of flexible infrastructure to handle diverse co-substrates within existing treatment plants.

Going forward, integrating LCA and TEA into co-digestion research is critical. Assessing the environmental and economic feasibility of different animal manures and other substrates—considering logistics, pre-treatment, and digestate management—will help identify scalable, sustainable pathways. Pilot and full-scale trials should evaluate long-term impacts on sludge microstructure, microbial community resilience, and operational costs under diverse co-substrate scenarios.

4.5 Implication for practice

The insights provided in this review have direct implications for enhancing the operational efficiency and sustainability of AD in waste management systems. Optimizing sludge microstructure through targeted pretreatment techniques, biochar integration, co-digestion strategies, and precise control of operational conditions significantly enhances microbial activity and biogas production efficiency. Practically, this translates into greater reliability in renewable energy generation, supporting broader implementation of AD technology at various operational scales.

Policymakers and practitioners are encouraged to explicitly integrate these microstructural interventions within renewable energy and waste-to-energy policy frameworks. For instance, biochar supplementation not only improves digestion efficiency but also aids in carbon sequestration and nutrient recovery, directly contributing to multiple environmental sustainability goals (Afshar and Mofatteh, 2024; Patro et al., 2024). Embedding such guidelines into national and regional policies can effectively accelerate the adoption of enhanced AD technologies, aligning closely with SDG 7—ensuring access to affordable, reliable, sustainable, and modern energy for all.

Municipalities and waste management operators are recommended to systematically integrate microstructure optimization into existing and planned AD facilities. These operational adjustments not only improve overall process efficiency and biogas yield but also significantly decrease the reliance on fossil fuels, thereby contributing to global decarbonization efforts and sustainable energy systems. For example, operational practices involving strategic co-digestion can mitigate feedstock variability challenges and nutrient imbalances, ultimately enhancing the stability and energy output of digestion processes (Chowdhury et al., 2025; Karki et al., 2021).

Ultimately, the practical implementation of these evidence-based strategies provides a clear pathway toward achieving tangible progress in sustainable waste management and renewable energy production, directly aligning with SDG 7 and fostering substantial environmental, economic, and societal benefits.

5 Conclusion

This review systematically evaluated the influence of sludge microstructure on AD performance by integrating comprehensive bibliometric analyses with experimental insights from recent high-impact studies. Bibliometric analysis revealed an evolving research landscape, shifting from traditional process optimization towards microbial-centric approaches, biochar integration, operational parameter refinement, and diversified co-digestion strategies. Recent experimental findings have confirmed these bibliometric trends, clearly illustrating that deliberate modifications to sludge microstructure—achieved via pretreatment methods, biochar amendments, and careful control of operational conditions such as temperature, pH, and substrate-to-inoculum ratio—substantially improve microbial activity, stability, and methane production efficiency.

However, critical research gaps remain, which must be prioritized to transition effectively from laboratory to industrial-scale application. Based on combined bibliometric and experimental analyses, the following areas emerge as high-priority research gaps: (1) standardizing biochar characterization and optimal properties, crucial for predictable enhancement of microbial dynamics; (2) understanding and promoting microbial community resilience, especially under varying operational conditions; and (3) systematically optimizing co-digestion strategies to ensure stable and enhanced methane yields. Addressing these gaps requires extended pilot- and full-scale validation studies, emphasizing rigorous standardization of methodologies and clearly defined operational benchmarks.

Among these, co-digestion strategies and microbial resilience under dynamic conditions align closely with near-term funding priorities in the EU Horizon 2030, UKRI Net Zero programs, and other international bioeconomy initiatives focused on waste-to-energy transitions. These areas are also positioned at higher technology readiness levels (TRL 6–7), making them more amenable to real-world demonstration and commercialization. In contrast, standardized biochar optimization—while promising—remains at a lower TRL and may require targeted material science funding streams and early-stage innovation support.

To tackle scalability challenges concretely, future research must explicitly foster interdisciplinary collaborations: microbiologists and material scientists should jointly optimise biochar characteristics tailored to microbial colonization; environmental engineers and computational modelers should collaborate to develop robust predictive tools for operational management; and economists and sustainability experts must integrate LCA and TEA into early research design. Examples of such integrative efforts can be found in Horizon Europe-funded initiatives like BIOBEST and the IEA Bioenergy Task 37 working group, which facilitate cross-sector collaboration between academia, industry, and policy bodies to scale biogas technologies. These programs demonstrate the practical feasibility and policy relevance of interdisciplinary AD innovation.

These focused interdisciplinary efforts are essential for developing scalable, economically viable, and environmentally sustainable AD solutions, significantly advancing global waste management practices and renewable energy production.

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Declarations

Conflict of 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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