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Microwave assisted anaerobic digestion of waste-activated sludge: Integrating multi-scale characterisation and energy assessment for enhanced biogas recovery

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A B S T R A C T

Anaerobic digestion (AD) is a promising technology for energy recovery from waste-activated sludge (WAS), yet its efficiency remains constrained by poor biodegradability and limited substrate accessibility. Pretreatment processes can enhance sludge disintegration and improve methane production, but their energy efficiency and large-scale feasibility remain uncertain. In this study, the impacts of microwave pretreatment (MP) and conventional pretreatment (CP) on sludge characteristics and AD performance were systematically investigated using integrated experimental and analytical approaches. Both pretreatments significantly enhanced methane yields, with moderate microwave intensity (MP600) achieving the highest improvement (69.1 %) compared to CP (57.9 %) and high-intensity microwave treatment (MP1000, 62.8 %). Multi-scale characterisation using fluorescence excitation–emission matrix (EEM) spectroscopy with parallel factor analysis (PARAFAC) and self-organising maps (SOM), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and multi-omics analyses revealed that microwave pretreatment effectively disrupted sludge microstructure, promoted solubilisation of organic compounds, and induced beneficial microbial community shifts that facilitated hydrolysis and methanogenesis. A full-scale energy assessment further confirmed the process feasibility, demonstrating that all pretreatments yielded positive net energy gains. MP600 achieved the highest net balance (13.97 kJ/gVS), surpassing CP (13.22 kJ/gVS) and MP1000 (13.30 kJ/gVS). These results highlight that low-power microwave pretreatment offers the most favourable trade-off between enhanced methane recovery and energy efficiency, representing a technically and economically viable strategy for sustainable sludge management and bioenergy generation.

Abbreviations

AD	Anaerobic digestion	PARAFAC	Parallel factor analysis
AF	After anaerobic digestion	PBS	Phosphate-buffered saline
ARGs	Antibiotic resistance genes	qPCR	Quantitative PCR
BF	Before anaerobic digestion	RG	Reference group
blaTEM	β-lactam resistance	SCOD	Soluble chemical oxygen demand
CP	Conventional (thermal) pretreatment	SEM	Scanning electron microscopy
DOM	Dissolved organic matter	SOM	Self-organising map
EEM	Excitation–emission matrix	Sul1	sulfonamide resistance
EPS	Extracellular polymeric substances	TB-EPS	Tightly bound extracellular polymeric substances
ermB	macrolide resistance	TCOD	Total chemical oxygen demand
FTIR	Fourier-transform infrared	tetA	tetracycline resistance
FDOM	Fluorescent dissolved organic matter	TS	Total solid
LB-EPS	Loosely bound extracellular polymeric substances	VS	Volatile solids
MP	Microwave pretreatment	WAS	Waste activated sludge

(continued on next column)

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1. Introduction

Waste activated sludge (WAS), a primary by-product of wastewater treatment plants (WWTPs), represents a significant economic and environmental burden, with treatment and disposal costs accounting for approximately 50 % of the total operational expenditure of wastewater treatment plants. (Zan et al., 2021). Anaerobic digestion (AD) is commonly employed to treat WAS, owing to its ability to reduce sludge volume and produce renewable energy in the form of biogas (Roopnarain et al., 2021). However, the efficiency of the AD process remains limited, primarily because WAS contains complex organic structures resistant to microbial degradation (Nie et al., 2021). Consequently, enhancing the efficiency of anaerobic digestion remains a critical research priority to achieve sustainable waste management goals.

The hydrolysis stage is widely acknowledged as the rate-limiting step in the AD process, as it involves the breakdown of complex organic materials into simpler, readily biodegradable forms. To address this limitation, numerous pretreatment techniques, including thermal (Scherzinger and Kaltschmitt, 2021), chemical (Cazaudehore et al., 2022), mechanical (Wang et al., 2021), and biological (Basinas et al., 2025) methods, have been explored to accelerate hydrolysis and thereby enhance overall biogas yields. Among these approaches, microwave pretreatment (MP) has recently emerged as an attractive alternative to conventional (thermal) pretreatment (CP), owing to its rapid heating characteristics, targeted energy delivery, and potential to improve the biodegradability of WAS (Li et al., 2024a).

Previous studies have demonstrated that MP can effectively enhance biogas production. Liu et al., (2020) reported that during the co-AD of sewage sludge and food waste, MP increased methane production by approximately 20 % compared to untreated controls. Yue et al. (2021) found that MP promotes lipid mixing, solid dissolution, and macromolecular decomposition primarily through thermal effects, resulting in improved methane yields and production rates. Kor-Bicakci et al. (2019) highlighted that MP enhances the disintegration of complex sludge structures, allowing digesters to operate effectively at higher loading rates and reduced digester volumes without compromising organic removal efficiency. Nevertheless, most reported benefits of MP have been attributed primarily to thermal effects, while the existence and relevance of microwave-specific non-thermal effects such as enhanced molecular rotation, selective energy absorption, or altered cell membrane permeability remain controversial and insufficiently resolved (Bozkurt and Apul, 2020; Liu et al., 2019, 2022; Zhang et al., 2019).

Clarifying whether and how such microwave-specific effects contribute to sludge transformation requires an integrative assessment of physicochemical, structural, and biological responses. Previous work has shown that MP can promote extracellular polymeric substances (EPS) release (Yu et al., 2024), disrupting microbial cell walls (Li et al., 2019), altering spatial conformations of sludge flocs (Vialkova et al., 2021), and modify organic matter composition (Yang et al., 2020). More recent studies further indicate that MP may reshape microbial communities, enriching fermentative and syntrophic populations that support enhanced anaerobic digestion (Fang et al., 2020; Y. Li et al., 2025a; Zhang et al., 2019). However, distinguishing microwave-specific interactions from intensified thermal and heating-rate effects remains a major challenge, particularly when target temperatures are matched.

Against this background, this study systematically compares conventional thermal and microwave pretreatments at different intensities to evaluate their effects on the anaerobic digestion of waste-activated sludge. Sludge samples collected after pretreatment and during subsequent AD were characterised using complementary techniques, including fluorescence excitation–emission matrix (EEM) spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and multi-omics analysis. By integrating these analyses with methane production and energy balance assessments, this work aims to examine how microwave pretreatment intensity governs sludge disintegration, microbial selection, and AD performance, while carefully contextualising potential microwave-specific effects within broader thermal and process-scale interactions. The findings provide mechanistic insight to support optimisation of pretreatment strategies for improved sludge management and bioenergy recovery.

2. Materials and methods

2.1. Experimental setup and sample properties

The raw WAS, used as the substrate, was collected from a municipal wastewater treatment plant in Jinchang, Gansu province, China, which operates using anaerobic/anoxic/oxic processes. The sludge was sampled after sedimentation and centrifugation. The inoculum was sourced from a laboratory-scale mesophilic AD reactor. The primary analytical characteristics of both the substrate and inoculum are presented in Table S1. Sludge samples were transported to the laboratory in a polypropylene plastic container, passed through a 1.0 mm sieve, and stored at 4 °C in a refrigerator until further use.

Microwave pretreatment was performed using a domestic microwave oven (John Lewis, JLSMWO09, UK) at nominal settings of 600 W and 1000 W. Approximately 400g of sludge was placed in sealed borosilicate glass vessels and heated until the centre temperature reached 85 °C, monitored with a fibre-optic probe. Samples were then maintained at 85 °C for 15 min by cycling the microwave power. Conventional pretreatment was carried out in a thermostatic water bath at 85 °C for 15 min. It is noted that while both treatments shared the same terminal temperature and hold duration, the heating ramp profiles differed, with MP achieving 85 °C in approximately 5 min (600 W) or 3 min 30 s (1000 W) compared to around 15 min for CP. Thus, the thermal history (heating rate, transient exposure) was not fully identical. To strengthen attribution of observed effects, future work will incorporate a thermal-mimic control following the exact MP temperature–time trajectory using conventional pretreatment. Accordingly, the present results are interpreted as consistent with potential microwave-specific non-thermal contributions, but do not constitute definitive proof.

In both cases, samples were maintained at 85 °C during the exposure period. The target temperature of 85 °C was selected based on literature reports suggesting that thermal pretreatment within the range of 70–100 °C enhances sludge solubilisation and improves the biodegradability of organic matter, while avoiding the risk of excessive energy use or formation of inhibitory compounds (Ferrer et al., 2008; González-Fernández et al., 2012; Rafique et al., 2010; Skiadas et al., 2005). Following treatment, samples were allowed to cool to ambient temperature before further analysis.

After the pretreatment, the sludge was allowed to cool to room temperature. The pre-treated WAS samples were then mixed with inoculated sludge at a substrate-to-inoculum ratio of 3:1, and the mixture was transferred into a 500 mL laboratory-scale anaerobic digester with a working volume of 400 mL. The experimental setup is shown in Fig. S1. The study was divided into three groups: control, microwave pretreatment, and conventional pretreatment, each conducted in triplicate. The substrate used in the control group was raw WAS without any pretreatment, while pretreated WAS was used in the respective experimental groups.

AD process was conducted using the Automatic Methane Potential Test System (AMPTS II; Bioprocess Control, Sweden), which provided real-time measurements of biogas production over a period of 20–40 days (van der Berg et al., 2024). The digester was mixed using a motor-driven stirring rod, operating at a rotation speed of 80 rpm with an agitation frequency of 60s ON and 60s OFF. The produced biogas was directed through flexible tubing to a scrubbing reactor, where acid gases (CO_2, H_2S) were removed using a 4M NaOH alkaline solution. The remaining methane was then passed into a measuring cell equipped with a liquid-displacement gas counter, which recorded gas impulses and converted them into normalised millilitres of CH_4 per gram of volatile solids (NmL CH_4/gVS) using AMPTS® v5 software. The experiments were conducted under mesophilic conditions at 37.5 °C to evaluate the impact of microwave pretreatment on biogas production, with continuous tracking of biogas yield.

2.2. Spectroscopy and microscopy characterisation

2.2.1. Fluorescence excitation–emission matrix analysis

To evaluate the effects of different pretreatment methods on organic matter characteristics in WAS, fluorescence excitation–emission matrix spectroscopy (HACH DR6000, USA) was conducted on sludge samples both before and after AD. EEM spectra were collected for untreated samples and for those subjected to CP and MP to assess transformations in fluorescent organic components throughout the digestion process. In addition, tightly bound extracellular polymeric substances (TB-EPS) and loosely bound EPS (LB-EPS) were separately extracted and analysed to distinguish pretreatment-induced changes in specific sludge fractions.

EEM data were decomposed using parallel factor analysis (PARAFAC) to identify characteristic fluorescent components. The PARAFAC modelling was performed using the DOMFluor toolbox in MATLAB (Stedmon and Bro, 2008). To further visualise sample groupings and highlight differences in fluorescence profiles under various treatment conditions, self-organising map (SOM) analysis was applied using the SOM Toolbox in MATLAB (Cuss and Guéguen, 2016).

2.2.2. Fourier-transform infrared spectroscopy

FTIR spectroscopy (Nicolet iS 50, Thermo Scientific, USA) was employed to investigate the structural and functional group changes in organic matter within WAS samples before and after anaerobic digestion. Freeze-dried sludge samples from all pretreatment conditions were analysed in the mid-infrared region (4000–400 cm^{-1}) to identify key functional groups, such as proteins, polysaccharides, and lipids, and to track their transformation during digestion.

2.2.3. Scanning electron microscopy analysis

SEM was employed to observe morphological changes in sludge flocs before and after pretreatment. Sludge samples were first washed with phosphate-buffered saline (PBS) and fixed in 2.5 % glutaraldehyde (pH 7.2–7.4) at 4 °C overnight. After fixation, samples were washed with PBS for 2 h and post-fixed in 1 % osmium tetroxide for 1.5 h at room temperature. The samples were then rinsed with double-distilled water for 2 h.

To prepare cross-sections, the fixed samples were rapidly frozen in liquid nitrogen and sectioned using a rotary microtome. Dehydration was performed using a graded ethanol series (25 %, 50 %, 75 %, 80 %, 90 %, 95 %, and 100 %). Samples were subsequently dried with a critical point dryer and coated with platinum using an ion coater. SEM imaging was carried out using an XL30 scanning electron microscope (Philips, Holland) to assess structural alterations induced by CP and MP pretreatments.

2.3. Multi-omics analysis

To investigate the effects of pretreatment on the anaerobic digestion of WAS, a multi-omics approach was employed, including metabolomic profiling, microbial community analysis, and quantitative PCR (qPCR) targeting antibiotic resistance genes (ARGs). Metabolomic analysis was conducted on WAS samples collected on day 0 (post-pretreatment) and day 20 of anaerobic digestion to assess changes in metabolic activity induced by different pretreatment methods. Microbial community structure was evaluated via 16S rRNA gene amplification and Illumina MiSeq sequencing, providing insights into taxonomic composition and shifts in microbial populations during digestion. To assess the potential of pretreatment to reduce ARGs, qPCR was performed to quantify the abundance of selected resistance genes (tetA, ermB, bla_TEM, and sul1), with results normalised to 16S rRNA gene copy numbers. All analyses were carried out by Biomarker Technologies Co., Ltd (Beijing, China), and detailed experimental procedures can be found in previously published studies.

2.4. Other analytical methods

Total solids (TS) and volatile solids (VS) were measured according to standard methods (American Public Health Association, 2005). Volatile organic losses during pretreatment were not quantified via TS/VS analysis; however, the observed increases in soluble chemical oxygen demand (SCOD), soluble protein, and methane yield indicate that net solubilisation dominated under the applied pretreatment conditions. The samples were centrifuged at 8000 rpm for 10 min and subsequently filtered through a 0.45 μm cellulose membrane (Huang et al., 2021). The pH of the filtrate was then measured using a pH meter (pHs-3C, Leici Co. Ltd., Shanghai). SCOD and total chemical oxygen demand (TCOD) were quantified using a visible light spectrophotometer (DR3900, HACH, USA). Proteins and polysaccharides concentrations in the supernatant were determined colourimetrically using the Lowry-Folin method (Lowry et al., 1951) and the anthrone-sulfuric acid method (DuBois et al., 1956), with bovine serum albumin and glucose serving as the respective standard reference substances.

3. Results and discussion

This study employs multiple complementary analytical techniques not as independent endpoints but as mechanistic lenses to interrogate how microwave pretreatment alters sludge structure, composition, and microbial ecology, thereby directly influencing AD performance. While thermal effects are shared with conventional pretreatment, microwave pretreatment introduces distinct energy deposition characteristics, rapid volumetric heating, localised thermal gradients, and intensified cell and EPS disruption, thereby modifying substrate availability and microbial selection during AD. Accordingly, the following discussion focuses on how microwave-specific effects propagate across molecular, microbial, and process scales to govern digestion efficiency and energy recovery.

3.1. Impact of pretreatment on methane production

The cumulative methane yield from anaerobic digestion under different pretreatment conditions is shown in Fig. 1. All pretreatment groups significantly enhanced methane production compared to the

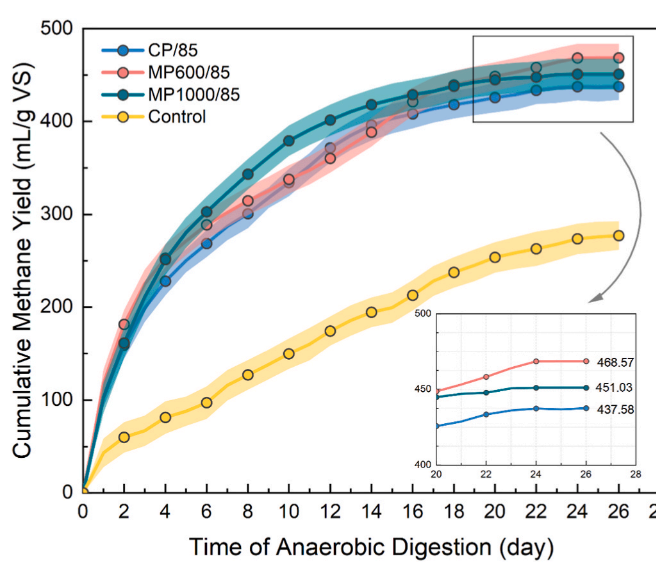

Fig. 1. The effect of different pretreatments on cumulative methane production.

control group, which received no pretreatment. By the end of the 26-day digestion period, the control group without any pretreatments yielded only 277.02 mL/gVS, whereas microwave pretreatment at 600 W (MP600/85) achieved the highest yield of 468.57 mL/gVS, representing a 69.1 % increase. Microwave pretreatment at 1000 W (MP1000/85) and conventional pretreatment (CP/85) achieved 451.03 mL/gVS and 437.58 mL/gVS, corresponding to increases of 62.8 % and 57.9 %, respectively.

In all pretreated groups, methane production accelerated rapidly in the early digestion phase (day 0–10), indicating enhanced hydrolysis and improved biodegradability of the sludge following pretreatment. Among the tested methods, MP600/85 demonstrated the most effective balance between pretreatment intensity and biogas yield. Although MP1000/85 involved a higher energy input, it did not lead to proportionally higher methane production. This trend aligns with previous findings. (Kan et al., 2018; Li et al., 2024b; Tang et al., 2010), which suggests that microwave irradiation has a threshold effect: while moderate energy input disrupts floc structures and microbial cell membranes to enhance biodegradability, excessive irradiation may lead to over-disruption, causing premature release and possible degradation of soluble organics, thereby reducing substrate availability for methanogens (Sapci, 2013). Moreover, energy efficiency tends to decline near this threshold, limiting the effectiveness of further increases in microwave intensity.

3.2. *Impact of pretreatment on organic matter transformation*

3.2.1. *SCOD, protein, and polysaccharide dynamics*

The effects of pretreatment on the solubilisation and transformation of organic matter were evaluated by monitoring SCOD, protein, and polysaccharide concentrations during anaerobic digestion, as illustrated in Fig. 2a and b.

All pretreatment methods significantly increased SCOD at day 0 compared to the control group (as shown in Fig. 2a), indicating enhanced solubilisation of organic components immediately after pretreatment. The MP600/85 group exhibited the highest initial SCOD value of approximately 4300 mg/L, corresponding to a 106.86 % increase relative to the control. This was followed by MP1000/85 (103.92 %) and CP/85 (92.16 %). These findings suggest that both microwave and conventional pretreatments effectively disrupted sludge flocs and cellular structures, with MP 600 W achieving the most pronounced solubilisation effect.

Additionally, protein and polysaccharide concentrations in sludge supernatants were also significantly influenced by pretreatment, as shown in Fig. 2b. At the initial digestion stage (day 0), MP600/85 yielded the highest protein (620 mg/L) and polysaccharide (210 mg/L) levels, substantially exceeding those of MP1000/85 and CP/85, which confirms its greater efficacy in disrupting microbial cells and EPS. Throughout the digestion period (days 14 and 26), protein and polysaccharide concentrations consistently decreased in all pretreated groups, reflecting effective microbial uptake and conversion of solubilised organic matter into biogas. Notably, the MP600/85 group maintained higher protein and polysaccharide degradation rates over time compared to CP/85 and MP1000/85, suggesting better microbial utilisation of solubilised substrates. These observations reinforce the hypothesis that moderate microwave irradiation not only enhances initial organic solubilisation but also facilitates complete and more sustained biodegradation during anaerobic digestion.

These quantitative solubilisation trends provide bulk-phase confirmation of the molecular-scale transformations identified by fluorescence EEM–PARAFAC and FTIR analyses in the later discussion section, where protein unfolding, EPS disruption, and intracellular material release were shown to govern both the extent and biodegradability of solubilised organic matter.

However, microwave pretreatment released more soluble organic matter than conventional pretreatment under the same temperature conditions (85 °C). This enhancement is due to the unique heating mechanism of microwave irradiation, which induces rapid, volumetric heating through dielectric polarisation and molecular friction. Unlike conventional conduction-based heating, microwave energy penetrates the sludge matrix directly, leading to localised superheating and formation of micro-hotspots, which can intensify cell membrane rupture and EPS disintegration (Kocbek et al., 2022; Tyagi and Lo, 2013).

3.2.2. *PARAFAC analysis of DOM*

To further explore the differences in solubilisation mechanisms between CP and MP, EEM spectroscopy was conducted to characterise the dissolved organic matter (DOM) in the sludge supernatant before and after anaerobic digestion. Fig. S2 presents the EEM spectra of supernatants subjected to different pretreatments, highlighting changes in fluorescent DOM components during digestion. To extract and interpret the underlying fluorophores, PARAFAC modelling was applied to the EEM data, and three distinct components were identified: tyrosine-like (Component 1, Ex/Em ≈ 230/340 nm) (Yu et al., 2015), humic-like (Component 2, Ex/Em ≈ 220/400 nm) (Chen et al., 2003; Li et al., 2014), and tryptophan-like (Component 3, Ex/Em ≈ 220, 280/345 nm) (Yang et al., 2015), as shown in Fig. 3a–c. It should be noted that EEM–PARAFAC selectively characterises fluorescent dissolved organic matter (FDOM) and does not capture non-fluorescent or weakly fluorescent organic fractions, such as certain carbohydrates, lipids, and low-molecular-weight aliphatic compounds. Accordingly, PARAFAC results reflect compositional and structural changes within the fluorescent DOM pool rather than the total dissolved organic matter.

The concurrent reduction of C1 (tyrosine-like) and C2 (humic-like)

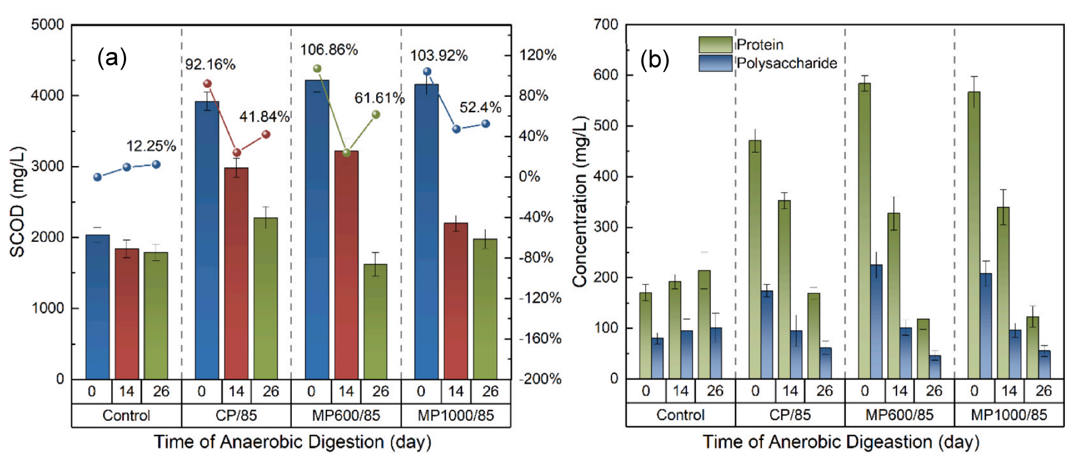

Fig. 2. The effects of pretreatments on (a) SCOD, (b) protein and polysaccharide.

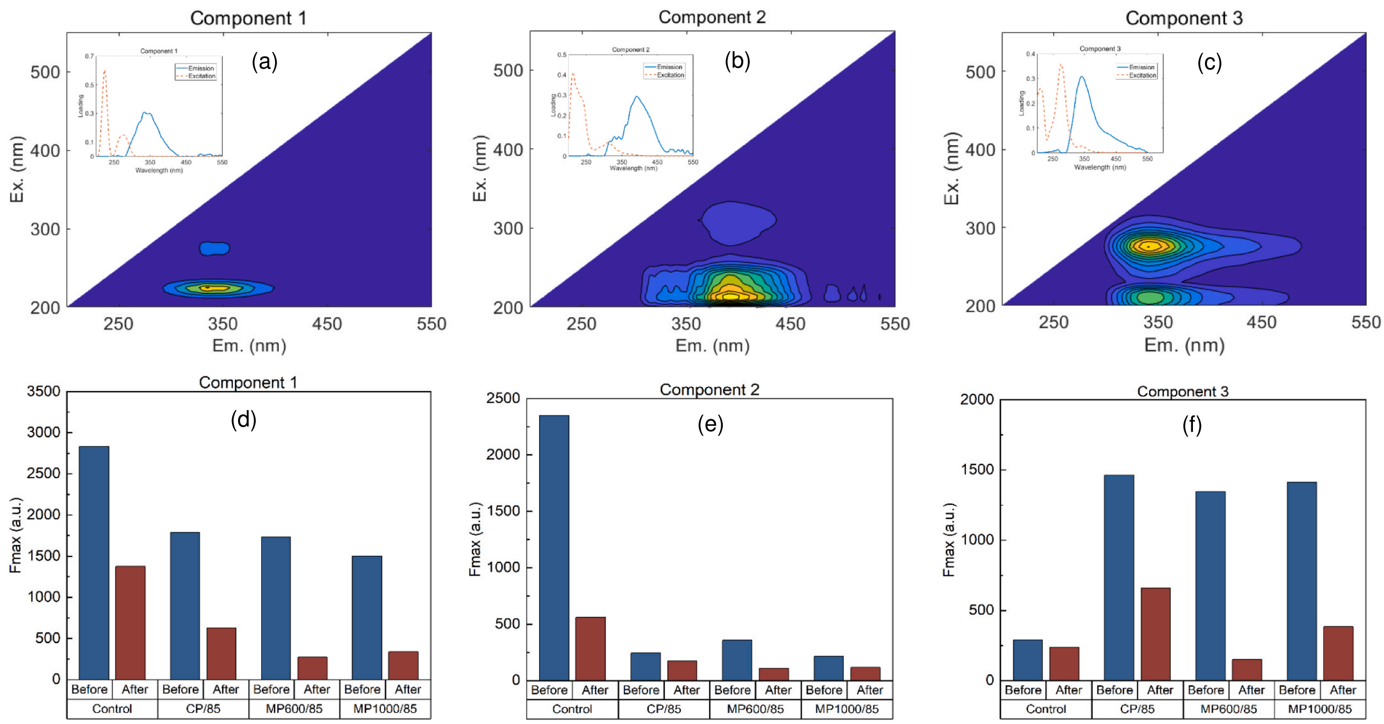

Fig. 3. Three fluorescent components decomposed by the PARAFAC model (a, b, c). F_{max} of fluorescence components before and after anaerobic digestion (d, e, f).

fluorescence after pretreatment reflects the disruption and transformation of loosely bound proteinaceous substances and humic-associated matrices originally present within sludge flocs and EPS (Fig. 3d and e). Microwave and thermal pretreatments promote depolymerisation, unfolding, and partial breakdown of these macromolecular structures, diminishing their characteristic fluorescence signatures while transferring organic carbon from particulate-associated pools into the soluble phase. This transformation is consistent with the pronounced increases in SCOD and soluble polysaccharide concentrations observed immediately after pretreatment, indicating enhanced release of previously immobilised organic matter.

In contrast, C3 (tryptophan-like substances) increased substantially following pretreatment, especially under microwave irradiation. Tryptophan-like fluorescence is widely associated with aromatic amino acids and intracellular protein fragments, which are typically shielded within intact microbial cells and tightly bound EPS structures (Lu et al., 2018; Sun et al., 2016). The sharp increase in C3 therefore indicates extensive cell lysis and EPS disruption, resulting in the liberation of intracellular proteins and protein-rich soluble substrates. This behaviour closely aligns with the elevated soluble protein concentrations quantified after pretreatment, particularly for MP600/85, confirming that microwave irradiation is highly effective in mobilising proteinaceous matter that is otherwise resistant to conventional pretreatment.

Following anaerobic digestion, fluorescence intensities of all three components declined, reflecting microbial consumption and transformation of solubilised substrates. Notably, MP600/85 exhibited the most pronounced post-digestion reduction in C3 fluorescence, reaching levels comparable to the untreated control. This shows that the proteinaceous compounds released under moderate microwave conditions were not only efficiently solubilised but also readily bioavailable and extensively degraded during anaerobic digestion. In contrast, residual C3 fluorescence persisted in CP/85 and MP1000/85, implying incomplete degradation of certain protein-derived fractions. To contextualise the analytical scope of fluorescence results, EEM–PARAFAC interpretations were evaluated alongside bulk solubilisation indicators (SCOD, soluble protein, and polysaccharides) and FTIR-derived structural information, ensuring that conclusions regarding organic matter transformation are supported by complementary, non-fluorescence-based evidence.

The differential behaviour of C1–C3 highlights the importance of pretreatment intensity in controlling both the nature and fate of solubilised organic matter. While higher microwave power enhances cellular disruption, it may also promote the release of partially recalcitrant or inhibitory proteinaceous compounds that resist complete microbial conversion. In contrast, moderate microwave pretreatment (MP600/85) achieves a favourable balance between organic matter release and biodegradability, maximising SCOD and soluble protein generation while minimising the accumulation of residual fluorescent DOM. These fluorescence-based insights therefore provide a mechanistic explanation for the solubilisation and biodegradation trends quantified through SCOD, protein, and polysaccharide measurements in Section 3.2.1.

3.2.3. *PARAFAC – SOM analysis of EPS*

To further investigate how pretreatment influences the structural transformation of EPS, SOM was applied to PARAFAC-modelled fluorescence data from LB-EPS and TB-EPS. Samples were collected from an untreated reference group (RG), a conventionally pretreatment group (CP), and microwave pretreatment groups (MP600 and MP1000), both before (BF) and after (AF) anaerobic digestion. The SOM component planes (Fig. 4a–c) visualise the distribution of three fluorescent components: tyrosine-like (C1), humic-like (C2), and tryptophan-like (C3), previously identified in the DOM analysis (Section 3.2.2).

For LB-EPS, samples before digestion (e.g., RG-BF-LB, CP-BF-LB, MP600-BF-LB, MP1000-BF-LB) were distributed in the upper-right region of the SOM and aligned with strong C1 signals, indicating a dominance of tyrosine-like proteinaceous compounds. This is typical of the loosely bound layer, which is known to be rich in soluble proteins (Xiao et al., 2017; Zhang et al., 2020). Following digestion, samples such as RG-AF-LB, CP-AF-LB, MP600-AF-LB, and MP1000-AF-LB migrated toward the upper-left neurons, where C3 intensities were elevated, reflecting the accumulation of tryptophan-like fluorophores likely released through microbial cell lysis and enzymatic degradation. This transformation from C1 to C3 dominance supports the earlier observation that digestion, particularly with microwave pretreatment, promotes

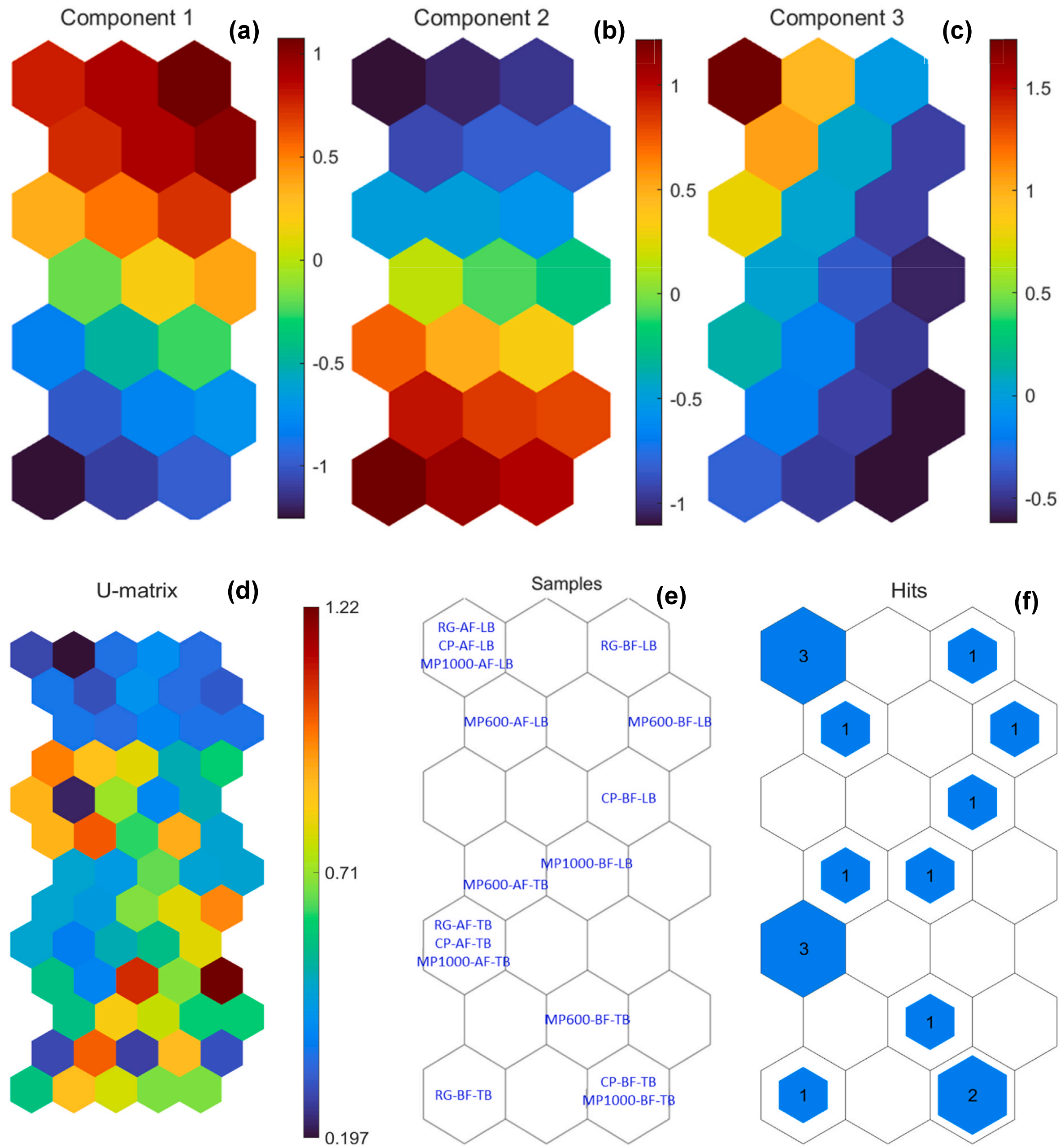

Fig. 4. Self-organising map (SOM) analysis of EPS fluorescence components based on the PARAFAC model. (a–c) Component planes showing the distribution of Component 1 (tyrosine-like), Component 2 (humic-like), and Component 3 (tryptophan-like) across SOM neurons. (d) U-matrix illustrating the inter-neuron distances and cluster boundaries. (e) Sample distribution map indicating the location of each LB- and TB-EPS sample before (BF) and after (AF) anaerobic digestion under different pretreatment conditions (RG, CP, MP600, MP1000). (f) Hit map showing the frequency of samples mapped to each SOM neuron, revealing sample clustering patterns.

solubilisation of intracellular materials and conversion into more aromatic protein forms.

TB-EPS followed a distinct compositional evolution. All pre-digestion TB-EPS samples (RG-BF-TB, CP-BF-TB, MP600-BF-TB, MP1000-BF-TB) were clustered in the bottom region of the SOM, which corresponds to high C2 (humic-like) intensities. This confirms that TB-EPS, as the more tightly structured matrix, harbours a greater proportion of humic material that is more resistant to degradation (Song et al., 2025; Ye et al., 2020). After digestion, TB-EPS samples such as RG-AF-TB, CP-AF-TB, MP600-AF-TB, and MP1000-AF-TB shifted toward upper neurons, where C3 was more prominent, while C2 declined. This suggests partial degradation of humic substances and a concurrent increase in proteinaceous residues, likely due to the breakdown of the recalcitrant matrix and exposure of embedded tryptophan-like components.

When comparing the impact of microwave pretreatment intensity, subtle differences emerged. MP600-AF-LB and MP600-AF-TB samples were mapped closer to the high-C3 regions than those from MP1000, indicating that moderate microwave power led to a more uniform and progressive breakdown of both LB and TB-EPS structures. In contrast, MP1000-treated samples remained more scattered, suggesting uneven solubilisation or possible recondensation of complex organics, which may hinder further microbial conversion. These changes are consistent with the fluorescence shifts observed in DOM, where protein-like components increased while humic signals declined after digestion.

The U-matrix (Fig. 4d) further visualises the divergence between sample types. Regions of higher colour intensity, particularly between lower and upper-SOM zones, indicate significant differences in fluorescence composition before and after digestion. Pre-digestion TB-EPS samples formed a tight bottom cluster, whereas post-digestion samples spread toward more heterogeneous upper regions, indicating substantial transformation under thermal or microwave treatment.

In the sample distribution map (Fig. 4e), LB-EPS and TB-EPS samples were spatially segregated, reflecting their inherent compositional divergence. TB-EPS samples before digestion were in the lower neurons aligned with C2, while post-digestion TB-EPS samples, especially those from microwave treatment, shifted upward, reflecting humic breakdown and protein exposure. The hit map (Fig. 4f) confirmed that most neurons were associated with one or two samples, underscoring the SOM's ability to capture subtle pretreatment-induced changes.

This analysis reveals distinct transformation pathways for LB-EPS and TB-EPS during anaerobic digestion. LB-EPS showed a clearer shift from tyrosine-like to tryptophan-like composition, while TB-EPS evolved from humic-rich toward a more protein-associated profile. Microwave pretreatment, particularly at moderate intensity (MP600), facilitated a more consistent and effective transition in both EPS layers, resulting in greater biodegradability and improved digestion potential. These patterns reinforce the utility of fluorescence-based SOM analysis for elucidating structural EPS changes and guiding pretreatment optimisation to enhance anaerobic processing.

3.3. *Impact of pretreatment on composition*

To comprehensively evaluate the compositional and structural alterations induced by sludge pretreatment, both FTIR spectroscopy and SEM were employed. FTIR analysis was conducted to elucidate changes in the chemical structure and functional groups of organic matter, providing molecular-level insights into how thermal and microwave treatments influence sludge composition. In parallel, SEM was used to observe the surface morphology of sludge flocs, revealing the extent of physical disintegration and cellular damage under different pretreatment conditions. Together, these techniques offer a complementary understanding of how pretreatment facilitates the breakdown of complex sludge matrices, thereby enhancing solubilisation and anaerobic biodegradability.

3.3.1. *FTIR spectroscopy analysis*

To explore how pretreatment influences the biochemical composition of sludge, FTIR analysis was conducted on samples subjected to different thermal conditions: untreated control, conventional pretreatment at 85 °C (CP/85), and microwave pretreatment at 600 W and 1000 W, both reaching 85 °C (MP600/85 and MP1000/85). Fig. 5a displays the FTIR spectra across the 4000–400 cm^{-1} range, with peak assignments summarised in Table S2.

The presence and intensity of these peaks confirm that sludge organic matter consists of proteins, lipids, polysaccharides, and cellulose-like substances (Fig. 5a). Among these, the amide I and II regions (1633 and 1540 cm^{-1}) are particularly valuable for analysing protein secondary structures (Zhang et al., 2023), as amide I band arises primarily from C=O stretching vibrations and is highly sensitive to protein backbone conformation. Deconvolution of this region enables semi-quantitative differentiation of α-helix, β-sheet, aggregated strand, and turn structures, which are further resolved and quantified in Fig. 5b.

As shown in Fig. 5b, thermal pretreatments induced substantial alterations in protein secondary structure, indicative of denaturation and conformational rearrangement. In the untreated control, aggregated strands dominated the protein structure (45 %), followed by 3-turn helices (27 %) and β-sheet structures (12 %), while α-helix content was relatively low. This distribution reveals that sludge-derived proteins are already partially unfolded and heterogeneous, reflecting their origin within complex microbial cells and EPS matrices. Following pretreatment, all thermal conditions resulted in a marked decrease in α-helix content, accompanied by increases in β-sheet, 3-turn helix, and antiparallel β-sheet contents. These shifts are characteristic of protein unfolding, hydrogen bond disruption, and partial refolding into more extended or aggregated conformations (Xiao et al., 2019). Importantly, comparison of protein secondary-structure changes across different pretreatments is based on the relative distribution of deconvoluted amide I components rather than absolute peak intensities. By evaluating changes in the fractional contributions of α-helix, β-sheet, aggregated strand, and turn structures under identical terminal temperature conditions, FTIR enables a semi-quantitative assessment of how different pretreatment strategies alter protein conformation and structural stability.

Such structural transformations are directly relevant to solubilisation behaviour. The loss of ordered α-helical structures and the emergence of less compact or aggregated configurations weaken intramolecular and intermolecular bonding within proteins and EPS frameworks, facilitating their release from sludge flocs into the aqueous phase. This mechanism is consistent with the elevated soluble protein and SCOD concentrations quantified immediately after pretreatment, indicating that protein denaturation observed by FTIR underpins the transfer of organic matter from the particulate to the soluble fraction (Yan et al., 2023).

Microwave-pretreated samples (MP600/85 and MP1000/85) exhibited more pronounced structural changes than conventional pretreatment. Both MP treatments showed higher proportions of aggregated strands and lower α-helix content than CP/85, indicating deeper protein unfolding and matrix disruption. Although β-sheet content in

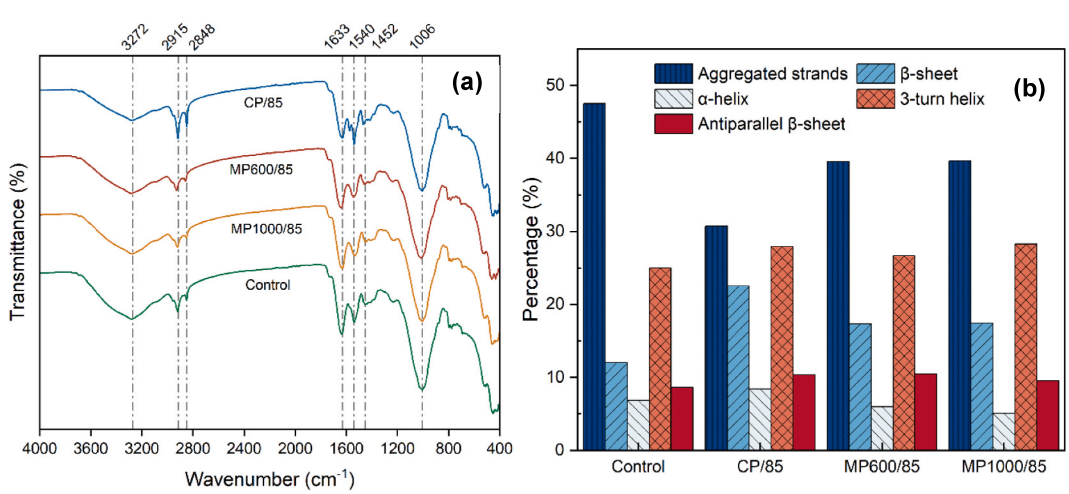

Fig. 5. (a) FTIR spectra of WAS after different pretreatments, (b) secondary structure of proteins in WAS.

MP-treated samples was slightly lower than in CP, the overall shift toward less ordered structures suggests enhanced denaturation and exposure of peptide backbones. These effects are consistent with the rapid and volumetric heating associated with microwave irradiation, in which dipole rotation and ionic conduction generate localised thermal stress that can disrupt hydrogen-bonding networks more effectively than conductive heating (Mishra and Sharma, 2016).

These FTIR-derived structural trends closely align with fluorescence observations. Specifically, the stronger persistence of tryptophan-like (C3) fluorescence after anaerobic digestion under MP1000/85 conditions corresponds to more extensive protein unfolding coupled with incomplete biodegradation. In contrast, MP600/85 exhibited substantial protein denaturation while still enabling efficient downstream degradation, as evidenced by the greater post-digestion reduction in C3 fluorescence and faster depletion of soluble protein concentrations.

Between the two microwave power levels, MP1000/85 induced a slightly more advanced denaturation profile than MP600. While both treatments maintained similarly high levels of aggregated strands (40 %), MP1000/85 produced a modestly higher proportion of 3-turn helix and slightly lower α-helix content. β-sheet content remained relatively stable across the two microwave conditions, indicating that the incremental increase in power did not significantly affect this structural motif. These results suggest that MP1000/85 may push protein unfolding further toward more disordered or flexible configurations than MP600/85, potentially promoting aggregation or reorganisation into less readily biodegradable protein structures, whereas MP600/85 achieves sufficient unfolding to enhance solubilisation without excessive structural condensation.

Overall, FTIR analysis demonstrates that pretreatment intensity governs not only the extent of protein unfolding but also the biodegradability of released proteinaceous matter. While both microwave pretreatments enhance solubilisation relative to conventional pretreatment, moderate microwave power (MP600/85) achieves a more favourable balance between structural disruption and microbial accessibility. This balance explains the superior solubilisation efficiency and digestion performance observed in bulk-phase measurements and highlights the importance of optimising pretreatment conditions to maximise biodegradation while avoiding the formation of resistant residues.

3.3.2. SEM analysis

SEM was employed to visualise the morphological changes in sludge flocs induced by thermal pretreatments (Fig. S3). The microstructure of the untreated sludge (Control) exhibited a compact and dense floc network with relatively smooth surfaces and well-aggregated particles. This structural integrity is typical of raw activated sludge, where EPS and microbial cells form tight aggregates that limit solubilisation and enzymatic accessibility.

After conventional thermal treatment at 85 °C (CP/85), partial surface disruption was observed. The sludge flocs appeared rougher and more porous, with evident fissures and cracks forming between layers. These features indicate the onset of protein denaturation and polysaccharide breakdown, which weaken EPS cohesion and promote matrix loosening. However, large portions of the matrix remained intact, suggesting that CP treatment only partially altered the structural architecture.

Microwave pretreatments (MP600/85 and MP1000/85) induced more pronounced structural disintegration. In the MP600/85 sample, the sludge matrix appeared visibly loosened, with distinct fragmentation and peeling of the surface layers. This suggests effective breakdown of EPS and cell wall components, facilitating solubilisation. The MP1000/85 sample displayed even more extensive damage: irregular cavities, ruptured fragments, and highly disordered surfaces were observed throughout the image field. These features point to advanced cell lysis and matrix collapse, likely driven by rapid internal heating and localised steam formation characteristic of high-intensity microwave exposure.

These morphological observations corroborate the chemical and structural findings in FTIR and fluorescence analyses. In particular, the enhanced fragmentation seen in MP-treated samples is consistent with the disruption of protein secondary structures (Section 3.3.1) and the release of tryptophan- and humic-like compounds identified in EEM–PARAFAC (Section 3.2.2). Furthermore, the greater disintegration observed under MP1000/85 supports earlier conclusions that excessive microwave power accelerates matrix decomposition, though this may also risk generating resistant residues.

Taken together, the SEM analysis highlights the critical role of pretreatment intensity in modulating sludge microstructure. While both CP and MP treatments enhance accessibility by loosening the floc network, microwave pretreatment, particularly at higher power, produces more substantial structural breakdown, potentially improving anaerobic digestibility and downstream dewatering. However, the extent of disruption must be balanced against the risk of forming recalcitrant particulates, as seen in other parts of this study.

4. Multi-omics analysis reveals the impact of pretreatment on metabolites, microorganisms and functional proteins

4.1. Microbial community dynamics

The microbial community structure of sludge was investigated at the phylum level to evaluate the impact of CP and MP pretreatments before and after anaerobic digestion (As shown in Fig. 6a). In the original sludge, the community was dominated by Proteobacteria (33.2 %), Bacteroidota (16.5 %), and Acidobacteriota (12.4 %), which are characteristic of activated sludge systems operating under aerobic or facultative conditions. These phyla are primarily associated with heterotrophic degradation of complex organic matter, polysaccharides, and soluble substrates during wastewater treatment rather than strict anaerobic metabolism (Sun et al., 2017; Xu et al., 2020).

Following conventional pretreatment at 85 °C (CP/85/BF), the phylum-level composition remained largely unchanged, indicating that thermal exposure alone caused limited restructuring of the microbial community prior to digestion. However, after anaerobic digestion (CP/85/AF), a clear ecological transition was observed, marked by a substantial increase in Synergistota (20.4 %) and Firmicutes (6.9 %), alongside pronounced declines in Proteobacteria and Acidobacteriota. This shift reflects the establishment of a strictly anaerobic consortium dominated by fermentative and syntrophic microorganisms, which are essential for protein and amino acid degradation, volatile fatty acid (VFA) conversion, and syntrophic cooperation with methanogens (Mohan et al., 2025).

Microwave pretreatment induced more pronounced and selective microbial restructuring. In the MP600 system, Proteobacteria decreased sharply from 23.8 % (BF) to 8.7 % (AF), while Synergistota increased from near absence to 24.2 % after digestion. A similar but less extreme trend was observed for MP1000, where Proteobacteria declined from 26.5 % to 14.7 % and Synergistota increased to 21.8 %. Firmicutes also increased under both MP conditions, consistent with enhanced proteolytic and fermentative activity following pretreatment. These shifts indicate that microwave pretreatment promotes stronger selection pressure toward obligate anaerobes capable of utilising solubilised proteins and peptides generated during pretreatment (Zhang et al., 2024).

Notably, MP600 produced a more favourable microbial configuration than MP1000, as evidenced by higher Synergistota enrichment and lower residual Proteobacteria after digestion. This suggests that moderate microwave intensity enhances substrate availability while maintaining ecological conditions conducive to efficient syntrophic metabolism. In contrast, excessive pretreatment intensity (MP1000) may disrupt microbial balance, leading to less efficient functional selection despite similar overall solubilisation.

These compositional changes are supported by the heatmap

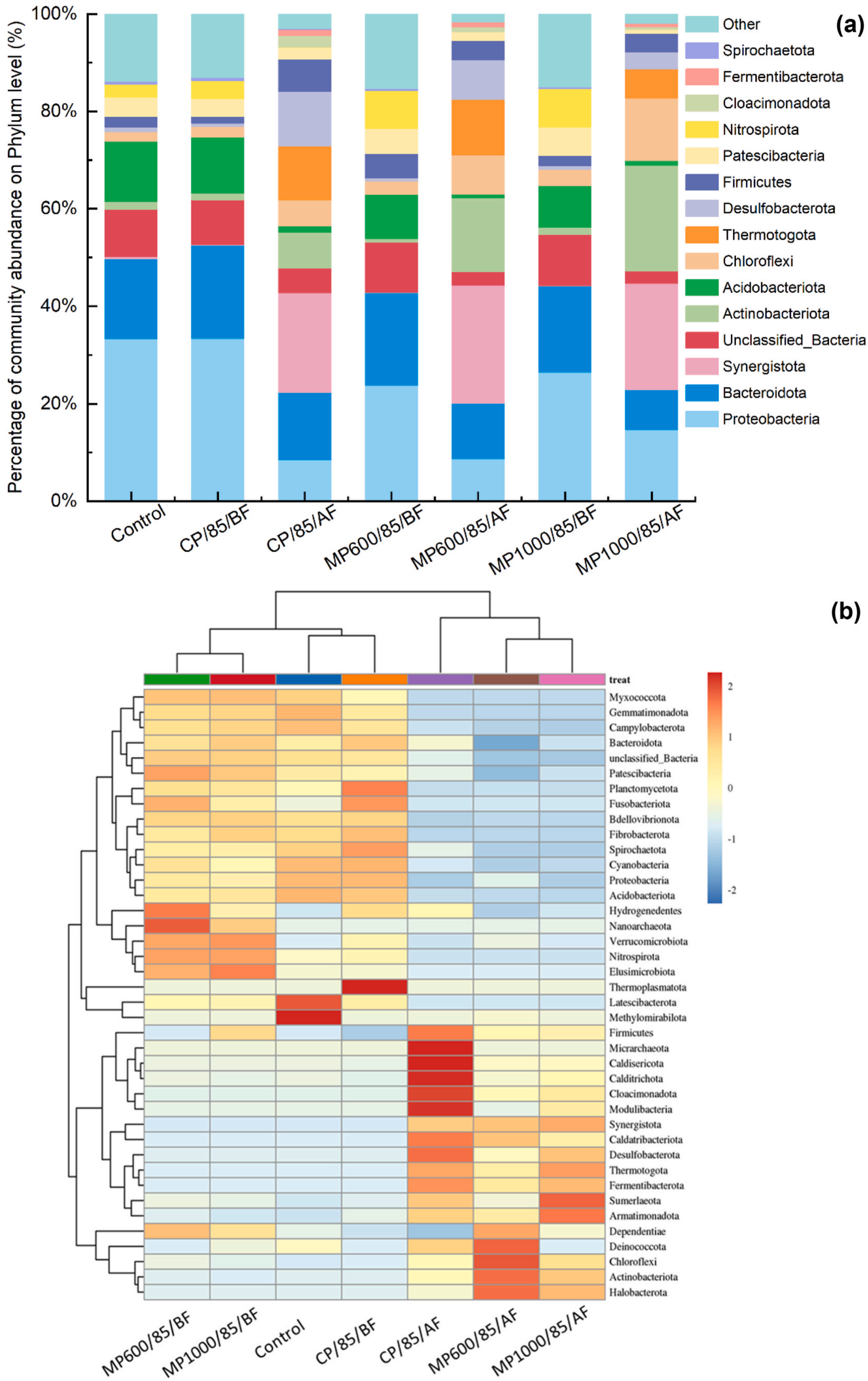

Fig. 6. The relative abundance of microbial community at the phylum level (a) and the corresponding generic level heat map (b).

clustering (Fig. 6b). A clear separation is observed between pre-digestion samples (Control, CP/85/BF, MP600/BF, MP1000/BF) and post-digestion samples (CP/85/AF, MP600/AF, MP1000/AF), indicating that anaerobic digestion is the dominant driver of microbial community restructuring. After digestion, each pretreatment condition exhibits a distinct microbial signature rather than converging toward a common endpoint.

Specifically, CP/85/AF shows enrichment of genera associated with general fermentation and residual facultative metabolism, suggesting a slower or less specialised adaptation to anaerobic conditions. In contrast, MP600F displays the strongest enrichment of syntrophic and thermophilic taxa, including Thermotogota, Cloacimonadota, and Calditrichota, which are widely reported to participate in amino acid fermentation, hydrogen production, and syntrophic interactions with hydrogenotrophic methanogens (Sudiartha et al., 2024; Wu et al., 2025). This enrichment pattern aligns with the superior digestion efficiency and biogas performance observed under MP600 conditions.

The MP1000/AF community also exhibits enrichment of syntrophic taxa, but with a broader, less concentrated distribution, suggesting that higher microwave power induces stronger stress selection while simultaneously promoting microbial redundancy or metabolic inefficiency. This divergence between MP600/AF and MP1000/AF highlights that microbial optimisation in anaerobic digestion is not governed solely by solubilisation intensity but by the balance between substrate release and ecological stability.

Overall, these results demonstrate that microwave pretreatment, particularly at moderate intensity, exerts a dual effect on anaerobic digestion by enhancing organic solubilisation and steering microbial community succession toward functionally specialised, syntrophic consortia. The superior performance of MP600 can therefore be attributed to its ability to couple structural disintegration with targeted microbial selection, reinforcing the importance of pretreatment optimisation from both biochemical and ecological perspectives.

4.2. ARG dynamics following pretreatment and digestion

ARGs are increasingly recognised as emerging environmental contaminants due to their potential role in horizontal gene transfer and in the propagation of microbial resistance. In this study, four representative ARGs were selected to assess the impact of thermal and microwave pretreatments on sludge resistive profiles: tetA (tetracycline resistance), ermB (macrolide resistance), Sul1 (sulfonamide resistance), and blaTEM (β-lactam resistance). These genes are commonly found in activated sludge and serve as indicators for antibiotic resistance proliferation risks during anaerobic digestion (Ni et al., 2020).

As shown in Fig. 7, both conventional pretreatment and microwave pretreatments, followed by anaerobic digestion, altered ARG abundances to varying extents, reflecting the combined effects of microbial community restructuring, DNA degradation, and selective pressure under anaerobic conditions. Among the four ARGs, sul1 exhibited the highest initial abundance in raw sludge (5.69×10^6 copies/g dry sludge), consistent with its frequent association with class 1 integrons and its well-documented environmental persistence. In contrast, ermB showed the lowest initial abundance (1.15×10^4 copies/g), indicating comparatively lower baseline prevalence of macrolide resistance prior to digestion.

Following anaerobic digestion, tetA, sul1, and blaTEM declined across all pretreatment conditions, indicating partial removal or reduced proliferation of bacteria carrying tetracycline-, sulfonamide-, and β-lactam-resistance determinants. Notably, MP600/85/AF achieved the lowest residual abundances for these genes, suggesting that moderate microwave pretreatment enhances microbial cell disruption and DNA

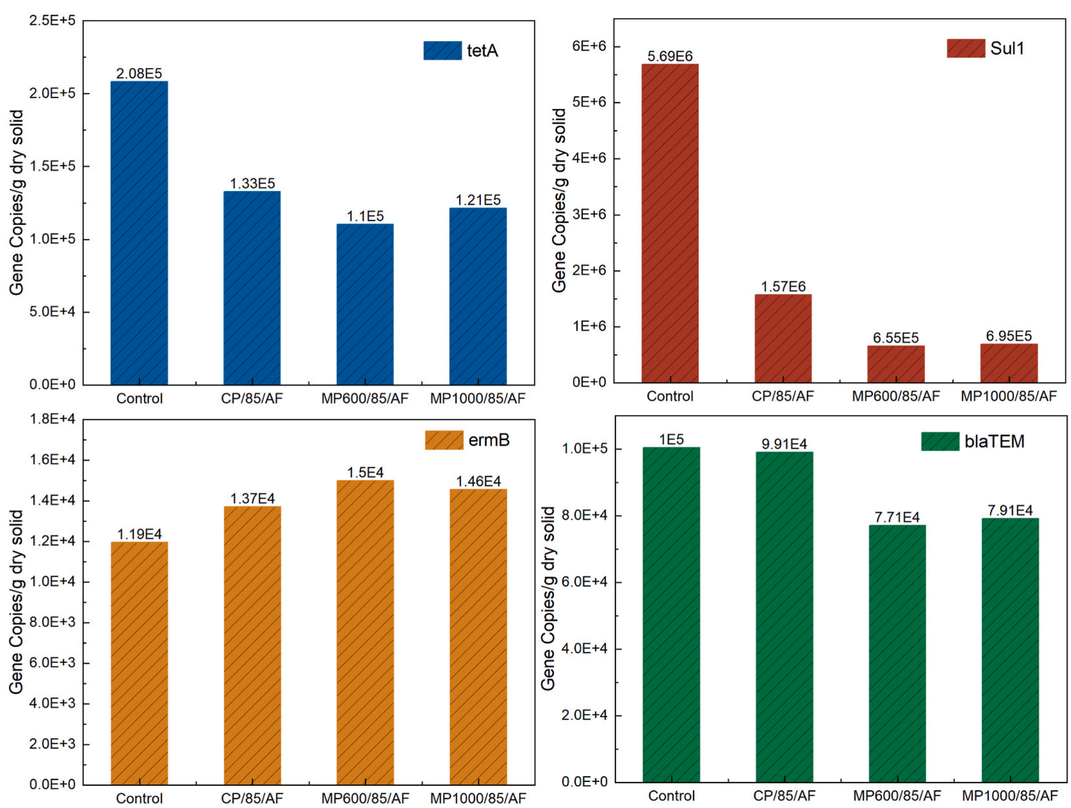

Fig. 7. Abundance of selected antibiotic resistance genes (ARGs) in sludge samples before and after anaerobic digestion under different pretreatment conditions. The ARGs include tetA (tetracycline resistance), ermB (macrolide resistance), Sul1 (sulfonamide resistance), and blaTEM (β-lactam resistance). Pretreatment conditions include no pretreatment (Control), conventional pretreatment at 85 °C (CP/85/AF), and microwave pretreatments at 600 W and 1000 W (MP600/85/AF and MP1000/85/AF). Values are expressed as gene copies per gram of dry sludge.

fragmentation while promoting microbial populations less associated with these ARGs. In contrast, MP1000/85/AF did not yield proportional additional reductions, with slightly higher residual levels of tetA and blaTEM than MP600/85F. This pattern indicates diminishing returns at higher microwave intensity, potentially due to excessive cell lysis, which increases the persistence of extracellular DNA or protects ARG fragments within organic matrices (Z. Li et al., 2025).

In contrast to the declining trends observed for tetA, sul1, and blaTEM, ermB displayed a consistent increase in absolute abundance after anaerobic digestion under all pretreatment regimes, reaching 1.37×10^4 (CP/85/AF), 1.50×10^4 (M600/85/AF), and 1.46×10^4 copies/g dry sludge (MP1000/85/AF). This contrasting behaviour highlights the gene-specific nature of ARG fate during anaerobic digestion and cannot be explained solely by overall biomass reduction or pretreatment severity.

The enrichment of ermB can be mechanistically linked to the microbial community restructuring described in Section 4.1. Macrolide resistance genes such as ermB are commonly hosted by Firmicutes, Synergistota, and other fermentative anaerobes, where they are often located on conjugative plasmids or transposons rather than chromosomal loci or integrons (Guo et al., 2018). These taxa were selectively enriched following anaerobic digestion, particularly under microwave pretreatment, due to their roles in protein and amino acid fermentation and syntrophic metabolism. As a result, ermB abundance appears to track host enrichment rather than pretreatment efficacy, explaining its increase even when other ARGs declined.

In addition, anaerobic digestion imposes sustained physiological stress and nutrient limitation, which may favour resistance mechanisms based on ribosomal methylation (encoded by erm genes) over efflux or integron-associated resistance pathways. Pretreatment-induced cell lysis may further contribute to increased detection of ermB by releasing plasmid-associated extracellular DNA, which is known to persist in anaerobic environments and remain quantifiable by qPCR even when donor cells are no longer viable (Zeng et al., 2021).

Comparing microwave intensities, MP600/85/AF exhibited a more coherent alignment between microbial optimisation and ARG mitigation, reducing most ARGs while avoiding excessive enrichment of ermB relative to MP1000/85/AF. Although both MP conditions promoted fermentative and syntrophic taxa, the broader and less focused community restructuring under MP1000 suggests that excessive pretreatment intensity may enhance ARG persistence through ecological redundancy or increased DNA release without delivering additional functional benefits.

Overall, these results demonstrate that ARG dynamics during anaerobic digestion are governed by gene-specific carriers and microbial host selection rather than uniform degradation processes. While microwave pretreatment, particularly at moderate intensity, effectively reduces several major ARGs, the enrichment of ermB underscores the importance of interpreting ARG fate within a microbial functional framework. Strategies aimed at mitigating ARG dissemination should therefore consider not only pretreatment severity but also its ecological consequences, including selective enrichment of resistance-associated anaerobic taxa.

5. Energy consumption assessment

An energy consumption assessment was conducted to evaluate the overall feasibility of the pretreatment AD system at an industrial scale. The analysis was based on methane yields obtained from batch experiments, extrapolated to a full-scale digester with a working volume of 1500 m^3, assuming identical reactor performance, constant VS loading, and negligible heat loss through the AD reactor wall. The influent VS concentration was 48.13 kg/m^3 (from the experiment), the operating temperature increased from 25 °C to 85 °C ($\Delta T = 60$ °C), and the heat recovery coefficient was taken as 0.85 (Wang et al., 2024).

The specific energy output of the digester (E_0, $kJ/\ gfed\ VS$) was calculated from the measured methane yield using Eq. (1) (Liu et al., 2020):

$$E_0 = \frac{V \cdot \eta_m \cdot P_{CH_4} \cdot \xi}{Q \cdot VS} \tag{1}$$

Where P_{CH_4} is methane yield (m^3 $CH_4/m^3 \cdot d$); V is working volume of digester (m^3); ξ is the low heat value of methane (35,800 kJ/m^3 CH_4); η_m is the energy conversion factor of methane (0.9), Q is influent flow rate (m^3/d); and VS is the VS concentration of substrate (g Fed VS/m^3).

The specific energy input for conventional pretreatment (E_{CP}, $kJ/gfed\ VS$) required to heat the sludge to 85 °C was estimated as:

$$E_{CP} = \frac{\rho \cdot C_p \cdot \Delta T \cdot (1 - \varnothing)}{\eta_{device} \cdot VS} \tag{2}$$

Where ρ is the sludge density (1000 kg/m^3), C_p is $4.18 kJ/(kg \cdot ^\circ C)$, and η_{device} denotes the efficiency of the heat-delivery system (0.90 for conventional pretreatment).

The specific energy input for microwave pretreatment ($E_{microwave}$, $kJ/gfed\ VS$) required temperature to heat the sludge to 85 °C was estimated as in Eq. (3)

$$E_{microwave} = \frac{P_{MW} \cdot t}{V \cdot VS} \tag{3}$$

Where P_{MW} is the MW irradiation power (W); t is the exposure time(s) of sludge; V is the working volume of the digester (m^3). In the microwave system used, the exposure time *t* corresponds to the effective irradiation (magnetron "on") period rather than total elapsed heating time. Therefore, any cyclic operation of the magnetron is inherently accounted for in the reported irradiation duration. The results of the industrial-scale assessment are summarised in Table 1.

The energy balance presented in Table 1 focuses on the incremental energy inputs associated with sludge pretreatment and the corresponding methane energy recovery, rather than the total operational energy demand of the AD system. Baseline AD energy requirements for mixing, maintenance of mesophilic conditions, and biogas handling were not explicitly included, as these demands are common to all scenarios and largely independent of the pretreatment strategy. Typical full-scale AD systems consume approximately 5–15 % of the recovered biogas energy for internal operation, depending on reactor configuration and heat-integration efficiency.

When such operational energy demands are accounted for, the absolute net energy values would decrease; however, the relative ranking among pretreatment scenarios remains unchanged, as pretreatment does not fundamentally alter the energy requirements of mixing or mesophilic temperature control. Consequently, the comparative advantage of moderate microwave pretreatment (MW 600W) over conventional pretreatment and high-power microwave pretreatment remains robust.

All pretreatment scenarios produced a net positive incremental energy balance under the assumed industrial conditions, indicating the comparative energetic feasibility of thermal and microwave pretreatment prior to AD. Conventional pretreatment required the lowest energy

Table 1
Comparative energy assessment of pretreatment strategies based on incremental pretreatment energy input and methane energy recovery, excluding baseline anaerobic digestion operational energy demands.

Scenario	Energy Input ($kJ/gfed\ VS$)	Energy Output ($kJ/gfed\ VS$)	Energy Balance ($kJ/gfed\ VS$)
No pretreatment	0.00	9.00	9.00
CP 85 °C	0.869	14.09	13.22
MW 600W 85 °C	1.117	15.09	13.97
MW 1000W 85 °C	1.261	14.56	13.30

input due to high heat-exchange efficiency, while microwave pretreatments consumed slightly more electrical energy per unit sludge because of lower cavity and magnetron conversion efficiencies. Nevertheless, microwave pretreatment, particularly at 600 W, yielded the highest methane recovery (15.09 kJ/gVS), resulting in the greatest net energy gain (13.97 kJ/gVS). The superior performance of the low-power microwave (MW 600W) system can be attributed to the more uniform heating pattern and lower non-thermal stress on microbial precursors, which enhances solubilisation and hydrolysis without excessive energy dissipation (Y. Li et al., 2025b). Conversely, the high-power MW 1000 W condition, despite a faster ramp-up, exhibited a slightly reduced net energy efficiency due to higher instantaneous electrical losses and potential overheating of local regions within the sludge matrix.

Beyond cumulative methane yield and energy balance, biogas production rate and the effective digestion time are also critical performance indicators at an industrial scale, as they directly influence digester sizing, throughput, and capital investment. Although the present assessment is based on batch anaerobic digestion experiments and therefore does not allow direct quantification of full-scale digestion time, the enhanced solubilisation, accelerated hydrolysis, and enrichment of fermentative and syntrophic microbial populations observed under moderate microwave pretreatment (MP600/85) strongly suggest improved early-stage digestion kinetics. Such process-level improvements are commonly associated with higher initial methane production rates and reduced effective hydraulic retention times in continuous AD systems.

From a practical perspective, low-power microwave pretreatment offers the most favourable balance between energy input, methane recovery, and potential process intensification, highlighting its suitability for integration into existing AD infrastructure where moderate power densities and effective heat recovery are achievable. Future techno-economic and life-cycle assessments should therefore consider not only the net energy balance but also reductions in digestion time, reactor footprint, and continuous-operation kinetics, together with full AD operational energy requirements and site-specific heat-recovery configurations, to quantify absolute net energy performance at the plant scale.

6. Conclusions

This study systematically demonstrates that microwave pretreatment influences AD performance in an intensity-dependent manner, with a moderate microwave input (600 W) yielding the greatest enhancement in methane production from waste-activated sludge. Compared with untreated sludge, MP600 increased methane yield by approximately 69.1 %, outperforming both conventional thermal pretreatment (57.9 %) and high-intensity microwave irradiation (62.8 %). These results confirm that microwave pretreatment is not simply an alternative heating method, but a controllable process intervention whose intensity governs downstream AD outcomes.

The multi-scale analytical framework employed in this work was used to resolve the mechanisms by which microwave pretreatment interacts with AD, rather than as an end in itself. Fluorescence EEM–PARAFAC, FTIR, SEM, and multi-omics analyses consistently indicate that moderate microwave pretreatment promotes effective sludge disintegration, enhances protein solubilisation and transformation, and selectively enriches fermentative and syntrophic microbial populations that support efficient substrate conversion during AD. In contrast, excessive microwave intensity induces more severe structural disruption without proportional gains in biodegradability, leading to diminished microbial efficiency and persistence of less favourable biochemical traits. While these observations are compatible with microwave-specific effects beyond bulk heating, the present experimental design does not fully decouple such effects from differences in heating rate and thermal gradients. Dedicated thermal-mimic controls will therefore be required to unambiguously isolate non-thermal microwave mechanisms.

From an energy perspective, the industrial-scale assessment indicates that all pretreatment scenarios yield positive incremental net energy balances. Moderate microwave pretreatment (MP600) achieved the highest net energy gain (13.97 kJ/gVS), surpassing both CP (13.22 kJ/gVS) and MP1000 (13.30 kJ/gVS). This advantage arises from the combination of enhanced methane recovery and avoidance of excessive electrical energy input, reinforcing MP600 as the most energetically favourable configuration among the conditions tested.

Despite these promising results, further research is required to support practical implementation. Future studies should evaluate the long-term stability of microwave-shaped microbial consortia under continuous AD operation, assess performance across diverse sludge types and reactor scales, and explore synergistic combinations of microwave pretreatment with chemical or enzymatic approaches. In parallel, a deeper investigation of microwave–cell interactions at molecular and subcellular levels would advance mechanistic understanding. At the industrial scale, optimising microwave reactor design, energy distribution, and integration with heat-recovery systems, together with comprehensive techno-economic and life-cycle assessments, will be essential to ensure that the benefits of microwave-enhanced AD are realised without compromising economic or environmental sustainability.

CRediT authorship contribution statement

Yuxuan Li: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Changjun He:** Writing – review & editing, Resources, Data curation. **Luiza C. Campos:** Writing – review & editing, Supervision, Resources, Project administration. **Shikun Cheng:** Writing – review & editing. **Zifu Li:** Writing – review & editing. **Yukun Hu:** Writing – review & editing, Supervision, Software, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2026.123896.

Data availability

Data will be made available on request.

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