

Comparative assessment of magnetic pretreatment across low to moderate intensities for enhancing anaerobic digestion of sewage sludge

Nicola Di Costanzo^{a,b,*}, Alessandra Cesaro^a, Francesco Di Capua^b, Federica Carraturo^c, Michela Salamone^c, Maria Cristina Mascolo^d, Giovanni Esposito^a

^a Department of Civil, Architectural and Environmental Engineering, University of Naples Federico II, Via Claudio 21, Naples, 80125, Italy

^b Department of Engineering, University of Basilicata, Via dell' Ateneo Lucano 10, Potenza, 85100, Italy

^c Hygiene Laboratories, Department of Biology, University of Naples Federico II, Via Cinthia 21, Naples, 80126, Italy

^d Department of Civil and Mechanical Engineering, University of Cassino and Southern Lazio, Via Gaetano di Biasio 43, Cassino, 03043, Italy

ARTICLE INFO

Keywords:

Magnetic pretreatment
Anaerobic digestion
Sewage sludge
Biomethane production
Microbial community shifts

ABSTRACT

This study investigates the effect of static magnetic field (SMF) pretreatment at different field intensities on the anaerobic digestion (AD) of sewage sludge. Three SMF intensities (10, 20, and 50 mT) were applied through tubular magnetic polarizers under varying numbers of magnetization cycles (NMC) to assess their impact on biomethane production. The 20 mT condition with a single NMC yielded the highest improvement, increasing methane production by 21% compared to the untreated sludge. Lower (10 mT) and higher (50 mT) intensities produced modest enhancements, suggesting a nonlinear correlation between SMF strength and methane generation. Volatile fatty acid analysis indicated that pretreatment at 20 mT enhanced hydrolysis and acidogenesis. Microbial investigation further showed that moderate SMF exposure reshaped the sludge community, enriching fermentative and syntrophic taxa that promote organic matter degradation and efficient methanogenesis. Unlike energy-intensive pretreatments, the proposed SMF system operates passively, using permanent magnets to generate magnetic exposure without external power input. Preliminary economic analysis suggests that this energy-neutral approach can lower sludge management costs while improving overall energy recovery.

1. Introduction

The management of sewage sludge, a by-product generated in vast quantities by wastewater treatment plants (WWTPs) worldwide, represents a pressing environmental and economic challenge. This challenge is magnified by increasing urbanization, population growth, and the tightening of environmental regulations aimed at ensuring sustainable waste management [1]. Traditional disposal methods, such as land-filling and incineration, are becoming less viable due to rising costs, public opposition, and stringent legislation regarding greenhouse gas emissions and land use [2]. As a result, innovative strategies to valorize sewage sludge have garnered significant attention in both scientific and industrial fields.

Anaerobic digestion (AD) is recognized as one of the most promising solutions for sludge valorization. This biological process not only reduces the volume of sludge but also produces biogas, a renewable energy source composed primarily of methane, which can be harnessed for electricity, heat, or even upgraded to biomethane for injection into

natural gas grids [3]. However, despite its many advantages, AD is a slow process and often suffers from inefficiencies due to the complex and recalcitrant nature of sewage sludge. The hydrolysis phase represents a critical bottleneck, as the breakdown of particulate organic matter into soluble compounds is inherently slow [4]. To address this issue, pretreatment technologies are increasingly being explored to improve the biodegradability of sludge [5] and boost the overall efficiency of AD [6].

Among the array of pretreatment methods, ranging from thermal and chemical techniques to mechanical and enzymatic approaches, the exposure of sewage sludge to a static magnetic field (SMF) has recently emerged as a novel and underexplored alternative [7]. The application of SMF is based on its potential to influence molecular structures, microbial metabolism, and enzymatic activity, potentially creating conditions conducive to enhanced biodegradability and biogas production [8]. Initial studies (Table 1) have shown that SMF application may alter the physicochemical properties of the sludge, stimulate microbial communities, and increase methane yields during AD.

Notwithstanding these promising findings, the underlying

* Corresponding author. Department of Engineering, University of Basilicata, Via dell' Ateneo Lucano 10, Potenza, 85100, Italy.

E-mail addresses: nicola.dicostanzo@unina.it, nicola.dicostanzo@unibas.it (N. Di Costanzo).

<https://doi.org/10.1016/j.renene.2026.126119>

Received 20 June 2025; Received in revised form 22 June 2026; Accepted 22 June 2026

Available online 23 June 2026

0960-1481/© 2026 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

mechanisms remain poorly understood, and there is a lack of systematic investigations into the influence of different SMF intensities on the digestion process. Zieliński et al. [9] analyzed the effect of SMF on the AD of dairy wastewater, reporting a significant increase in biogas production (373.2 L/kgVS compared to 200.2 L/kgVS in the control reactor) and methane content in the biogas (56.8% compared to 49.1% in the control reactor). In a subsequent study, the same authors investigated the impact of SMF on the AD of sewage sludge [12], demonstrating an improvement in methane production efficiency, although cumulative biogas production did not increase. However, in both studies, the exposure time to the SMF was limited to 16.2 min/day and between 70 and 432 min/day, respectively. Dębowski et al. [11], in their evaluation of SMF effects on the AD of algal biomass, observed that daily exposure to 0.6 T for 216 min resulted in a positive effect on both methane production (exceeding that of the untreated substrate by 11.4%), and methane yield (71% compared to 67% in the untreated condition). Similarly, Haritwal et al. [13] studied the influence of an electrically induced SMF on biogas production from cattle manure. Their results revealed a 19.2% increase in methane production, along with a significant improvement in the methane yield (73.4% compared to 57.8% of the control). Zhao et al. [10] employed a similar approach but used a flat, circular permanent magnet, requiring no external energy source, placed at the base of the digestion reactor. With a SMF of 11.4 mT ($\pm 2\%$) applied to the AD of maize cultivation residues, they observed a 19.5% increase in total biogas volume and a 20% increase in methane production compared to the control reactor. More recently, Hong et al. [14] applied a continuous SMF of 30 mT throughout the AD process of activated sludge, finding a biomethane production increase of up to 25% that was attributed to an enhanced microbial metabolic activity.

While these findings are encouraging, a recurring methodological limitation becomes evident: most studies apply a single SMF intensity and exposure duration, often without justification, to varying substrates and operating conditions. Consequently, reported performance gains, typically within a narrow range, offer limited insight into whether the chosen magnetic parameters were truly optimal.

To address this gap, the present study adopts a controlled experimental framework in which sewage sludge is exposed, under identical operating conditions, to a range of low-to-moderate SMF intensities (10, 20, and 50 mT) generated by a passive tubular magnetic polarizer. The device relies on permanent magnets and, unlike electrically generated magnetic fields or conventional energy-intensive pretreatments, requires no external power supply for magnetic field generation. To the best of our knowledge, this is the first study to systematically evaluate an energy-neutral magnetic pretreatment configuration for sewage sludge anaerobic digestion, directly comparing different magnetic field intensities and exposure cycles on the same substrate. While our recent preliminary work [15] suggested the potential of low-to-moderate intensity static magnetic fields, the present study provides a more structured assessment aimed at identifying the most effective conditions for enhancing biomethane production and reducing sludge volume.

In addition to addressing the lack of systematic comparisons across SMF intensities, this work also fills a second critical gap in the literature,

as no previous studies have evaluated the economic feasibility of magnetic pretreatment. By introducing a preliminary techno-economic assessment, the study links process performance with practical applicability in wastewater treatment. This research evaluates the impact of SMF intensity on key performance metrics, including biogas and biomethane yields, variations in volatile fatty acid (VFA) profiles, and shifts in the microbial composition. By systematically comparing different low-to-moderate SMF intensities under identical operating conditions and complementing the experimental results with a preliminary techno-economic perspective, the study provides a novel contribution to the assessment of SMF pretreatment as a practical strategy for sewage sludge valorization and renewable energy recovery.

2. Materials and methods

2.1. Sludge sampling and chemical profile

The sewage sludge utilized in this study was sourced from a WWTP located in Nola (Campania region, Italy). The plant employs a conventional activated sludge system with a solids retention time exceeding 20 days. The treated wastewater is predominantly of municipal origin, with a minor contribution from industrial discharges in the surrounding industrial zone. The sludge sample was collected from the lower outlet of the pre-thickening unit, stored in a plastic container, and immediately prepared for experimental procedures. The detailed chemical composition of the sewage sludge is provided in Table 2.

2.2. Procedural steps for magnetic pretreatment and anaerobic digestion tests

Three different magnetic polarizers supplied by AMS (Italy) were employed in this study, each providing a nominal SMF intensity of 10, 20, and 50 mT, respectively. Each polarizer consists of magnetic rings made from a mixture of rare-earth metals embedded in a ceramic matrix and sintered, with nominal diameter 50 mm, height 125 mm, and weight 1.5 kg. The magnetic rings are alternately magnetized along the longitudinal axis of the flow path, creating a continuous and uniform radial magnetic field around the treated stream.

For the magnetic pretreatment, 2 L of sludge was placed in a 2 L DURAN® bottle (DWK Life Sciences GmbH, Germany) and positioned on a magnetic stirrer (Argo Lab, Italy) to ensure continuous homogenization. The sludge was pumped through each magnetic polarizer using a 520Du peristaltic pump (Watson-Marlow, UK) operating at a flow rate of

Table 2

Main chemical and physical characteristics of the sewage sludge used in the experiments.

Parameter	Unit	Value
Total Solid	%	3.8 $\pm$ 0.1
Volatile Solid	%	2.3 $\pm$ 0.1
pH	-	6.9
soluble COD	mg/L	498.1 $\pm$ 11.7
Ammonia nitrogen	mg/L	84.4 $\pm$ 5.4

Table 1

Overview of the existing literature on SMF application to enhance the AD.

Substrate	Biomethane production			Methane content		SMF intensity	Exposure time	Reference
	Magnetized substrate	Untreated substrate	Percentage increase	Magnetized substrate	Untreated substrate			
Dairy wastewater	373.2 L/kgVS	200.2 L/kgVS	+86.4%	56.8%	49.1%	20 mT	16.2 min/d	[9]
Corn stover	14.8 L	11.9 L	+19.5%	67.2%	53.8%	11.4 mT	n.a.	[10]
Algal biomass	309.4 L/kgVS	277.7 L/kgVS	+11.4%	70.6%	67.2%	0.6 T	216 min/d	[11]
Sewage sludge	431 $\pm$ 22 L/kgVS	n.a.	n.a.	66.1%	n.a.	17.6 mT	144 min/d	[12]
Cow dug	n.a.	n.a.	+19.2%	n.a.	n.a.	0.42 T	n.d.	[13]
Waste activated sludge	322.5 L/kgVS	257.8 L/kgVS	+25.1%	n.a.	n.a.	30 mT	1440 min/d	[14]

0.1 L/min and recycled through the polarizer for a number of magnetization cycles (NMC) of either 1 or 10. The flow rate and NMC were chosen based on previous studies [16] with the aim to maintain sludge exposure to the SMF within an optimal range, while evaluating the impact of varying exposure on the process. The experimental setup is detailed in Table 3. After completing the pretreatment cycle, the sludge was collected in a second sterile DURAN® bottle.

Following the SMF pretreatment, AD experiments were initiated in triplicate, under mesophilic conditions ($37 \pm 1^\circ\text{C}$) in 250 mL serum glass bottles (OCHS, Germany). Each bioreactor was sealed with a cap consisting of a rubber septum, an aluminium crimp, and a needle equipped with one-way stopcocks (Masterflex, Germany) for gas and liquid sampling. The bioreactors were manually mixed once a day before sampling. Each bottle was filled with sludge, leaving 100 mL of headspace for biogas accumulation. No external inoculum was added to preserve the native anaerobic microbial community in the sludge, ensuring that the impact of SMF exposure on AD performance could be assessed without interference of non-magnetized sludge. To maintain anaerobic conditions, each bottle was flushed with argon gas for 1 min and then vented to atmospheric pressure. Control tests, containing untreated sludge, were conducted simultaneously to evaluate biomethane production in the absence of SMF pretreatment. AD tests were monitored for 30 days, being the maximum retention time typically applied in WWTP digesters.

2.3. Analytical methods

The concentrations of total solids (TS) and volatile solids (VS) were analyzed according to Standard Methods. Prior to the following analyses, all samples were centrifuged at 14,000 rpm for 10 min and the liquid fraction was filtered through 0.45 μm polypropylene-membrane syringe filters (VWR, Italy). The concentration of soluble COD (sCOD) was determined by the closed reflux colorimetric method (APHA, AWWA, WEF, 2023) [17]. The concentration of the ammonium ion was analyzed spectrophotometrically through the indophenol blue method [18]. Anionic concentrations were measured by ion chromatography using a 761 Compact IC (Metrohm, Switzerland). Biogas volume was measured by the water displacement method, while gas composition was quantified five times per week and recorded for 30 days using an HPR-20 RD gas-mass spectrometer (Hiden Analytical, Warrington, UK) equipped with a capillary unit heated to 140°C . VFAs were analyzed by high-performance liquid chromatography using a UVD 340U system (Dionex, USA) equipped with a diode array detector and a Metrosep organic acid column of dimensions 250/7.8 (Metrohm, Switzerland). A water solution with 1% sulfuric acid was used as mobile phase at flow rate of 0.7 mL/min. The concentration of VFAs were converted into COD equivalents in order to express them on a common basis and reported as the sum of acetic, propionic, butyric, caproic and valeric acids expressed in $\text{mg}_{\text{COD}}/\text{L}$, considering the specific COD conversion coefficients as reported by Arhin et al. [19].

2.4. Calculations

A preliminary techno-economic evaluation was carried out to assess

Table 3

Experimental conditions for low-to-moderate magnetic pretreatment of sewage sludge before AD.

Test	SMF (mT)	NMC	Flow rate (L/min)
A	10	1	0.1
B		10	
C		1	
D		10	
E	50	1	
F		10	
Ctrl	-	-	-

the feasibility of the SMF pretreatment in comparison to conventional sludge pretreatment technologies. The analysis was based on a model WWTP processing 10,000 tons of sewage sludge annually. Capital expenditures (CAPEX) were annualized over a 10-year equipment lifespan to reflect typical depreciation practices in WWTP operations. Operational expenditures (OPEX) were estimated by multiplying the energy demand of each technology (kWh/t) by a fixed electricity tariff of 0.10 €/kWh. Maintenance costs were set at 3% of the annualized CAPEX, in accordance with the methodology proposed by Shrestha et al. [20]. Biomethane yield improvements for thermal [21], ultrasound [22], chemical [5], and biological pretreatments [4] were derived from existing literature, while a 21% enhancement was adopted for SMF based on the experimental results of this study, assuming a baseline yield of $180 \text{ Nm}^3 \text{ CH}_4/\text{t}$ of sludge. The resulting methane gain (ΔCH_4 , Nm^3/t) was monetized using a conservative market price of 0.20 €/Nm³. The total additional revenue was then compared to investment costs to compute the simple payback period (SPBP) for each technology, defined as (Eq. (1)):

$$\text{SPBP} = \frac{\text{CAPEX}}{\Delta \text{CH}_4 \cdot \text{price}_{\text{CH}_4}} \quad \text{Eq. 1}$$

Finally, a sensitivity analysis was performed by varying electricity costs and methane prices by $\pm 10\%$ to evaluate the economic resilience of the SMF system under fluctuating market conditions.

2.5. Microbial community characterization

The samples were compared with the control sample, which did not undergo treatment, to evaluate the changes that occurred in the microbial community. The samples were subjected to magnetic treatment at different intensities, as previously reported. To this end, total DNA was extracted in triplicate, following the in-house sample pretreatment protocol previously reported by Di Costanzo et al. [15], to obtain concentrated DNA from 10 g of the initial sample. Next, the V3-V4 hypervariable region was amplified to identify bacteria and archaea, including potential methanogens [23]. After 16S rRNA gene amplification targeting the V3-V4 region, a PCR clean-up was performed to remove free primers and primer dimers. A subsequent limited-cycle PCR was carried out to add multiplexing indices and Illumina sequencing adapters using the Nextera XT Index Kit. A second purification step followed. The resulting libraries were then normalized and pooled. Denoising was performed prior to sequencing on the Illumina MiSeq platform. Taxonomic assignment was carried out using a custom Naive Bayesian Classifier trained on the V3-V4 region of the SILVA 132 database. Microbiome data analysis was performed using the QIIME 2 platform, including steps such as demultiplexing, quality filtering, chimera removal, and taxonomic classification. Sequencing reactions were performed by an external service (Biofab Research, Rome, Italy), according to Illumina MiSeq manufacturer's instructions [24].

2.6. Statistical analysis

The statistical significance of the data was assessed through a one-way analysis of variance (ANOVA) test using Microsoft Excel (Office 365, Microsoft Corporation, USA). The differences among the datasets were considered significant when a p -value < 0.05 was obtained.

3. Results and discussion

3.1. Impact of varying magnetic exposure on biomethane yield and solid reduction

Fig. 1 illustrates the evolution of the specific biomethane production over a 30-day period for the control and the experimental groups exposed to SMF pretreatment at varying intensities (10, 20, and 50 mT)

and NMC (1 or 10).

All SMF-exposed groups demonstrated a consistent enhancement in biomethane production compared to the control, with differences becoming more evident as AD progressed. Among the treatments, the group subjected to 20 mT with a single NMC (group C) achieved the highest specific biomethane production by the end of the experiment ($p < 0.05$). These results highlight that the low/moderate SMF intensity range promotes biomethane production, which can be maximized by modulating SMF intensity and NMC, thereby optimizing the sludge exposure. To provide a quantitative perspective, Fig. 2 summarizes the percentage increase in final biomethane production relative to the control for each pretreatment condition.

Condition C (20 mT, NMC = 1) achieved the highest enhancement in biomethane production, resulting in a 21% increase compared to the control. Group D (20 mT, NMC = 10) exhibited a slightly lower improvement of 15%, suggesting that additional NMC does not provide benefits compared to a single exposure. At the minimum intensity of 10 mT, groups A and B showed modest increases of 6% and 8%, respectively; even when the NMC was increased, the overall effect remained limited. This indicates that too low intensities only limitedly promote biomethane production. Similarly, sludge exposure to the highest intensity tested of 50 mT (Groups E and F) resulted in 9% and 12% increases, respectively. These improvements, although slightly higher than those observed at 10 mT, are still lower than those achieved at 20 mT. These results suggest that an optimal SMF intensity exists for sludge exposure to the SMF, which in the proposed configuration was identified as 20 mT. Lower intensities may not exert sufficient influence on AD, likely due to inadequate stimulation to overcome barriers in substrate degradation. Conversely, higher intensities may lead to over-exposure, potentially causing metabolic stress or disrupting the balance of microbial consortia essential for effective digestion, thereby counteracting potential benefits. Indeed, as previously reported, increasing the field strength up to 1.5 T proves to be highly detrimental [16]. Consequently, the relationship between SMF intensity and AD performance appears nonlinear, with an optimal window that avoids both insufficient stimulation and excessive perturbation. Further examination of the interplay between field intensity and exposure cycles reveals that, while increasing the NMC to 10 can enhance biomethane production at 10 mT and 50 mT, the highest yield at 20 mT is obtained with a single cycle. Mechanistically, the superior performance of Group C suggests that a 20 mT SMF with limited exposure effectively enhances microbial activity and substrate bioavailability, facilitating hydrolysis

and acidogenesis processes, as supported by the subsequent analysis of VFAs evolution and microbial composition. This may be attributed to improvements in microbial cell membrane permeability, favourable shifts in enzymatic kinetics [7], or more efficient electron transport within the microbial community [14]. Beyond the increase in biomethane production itself, this result is relevant because the best-performing condition was obtained using a passive magnetic device that does not require external energy input for magnetic field generation. Therefore, the observed improvement cannot be interpreted only as a biological response to SMF exposure, but also as evidence that a properly tuned, energy-neutral magnetic pretreatment can enhance AD performance without introducing the high energy demand typically associated with conventional sludge pretreatments. This aspect differentiates the proposed approach from most previously investigated pretreatment strategies and reinforces the importance of identifying an optimal SMF intensity rather than simply increasing the magnetic exposure.

Fig. 3 shows that, in all samples, there was an evident reduction in TS and VS by the end of AD, confirming that the digestion process was effective overall.

Across the various magnetic pretreatment conditions that were tested, the differences in TS and VS between the stages are relatively modest. This observation suggests that the improved biomethane production observed is not primarily a result of a higher degree of solid degradation. It is more likely that the enhanced AD performance was due to a more efficient conversion of the available organic substrate into biomethane. This implies that magnetic pretreatment may influence microbial activity at a biochemical level, optimizing metabolic pathways, leading to greater biomethane yields without a substantial increase in solid degradation.

The next sections will delve into the shifts of VFAs, N-NH_4^+ and microbiological composition to further elucidate these mechanisms and provide deeper insights into the enhanced biomethane production.

3.2. Effect of SMF intensity and NMCs on the production of volatile fatty acids and ammonia

Fig. 4 presents the evolution of both total and specific VFAs during the AD of both pretreated and untreated sludge.

Although the initial values at day 0 are comparable across treatments, notable differences emerge early on. For instance, while the control starts with a baseline dominated by propionic acid, its total VFAs

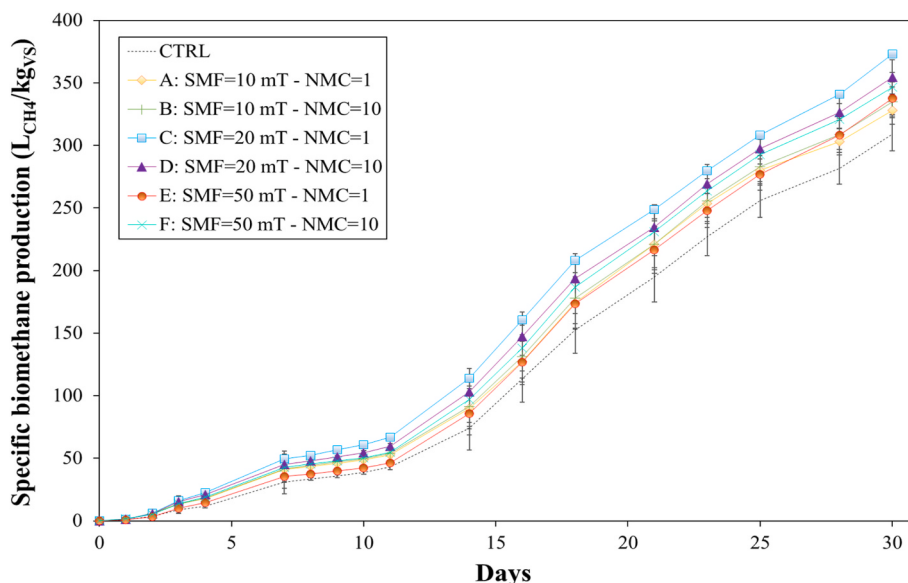


Fig. 1. Evolution of specific biomethane production over a 30-day period for the control and the experimental groups exposed to SMF.

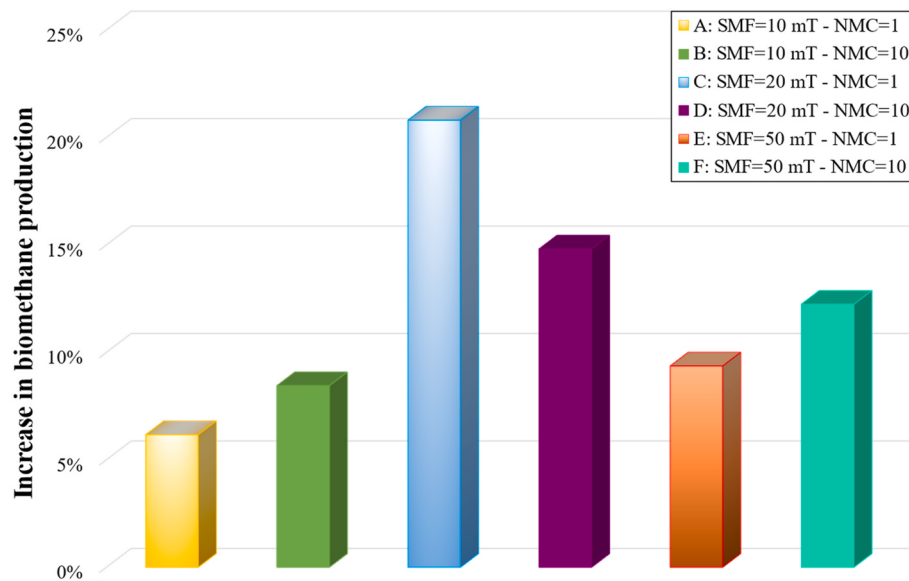


Fig. 2. Percentage increase in final biomethane production relative to the control for each pretreatment condition.

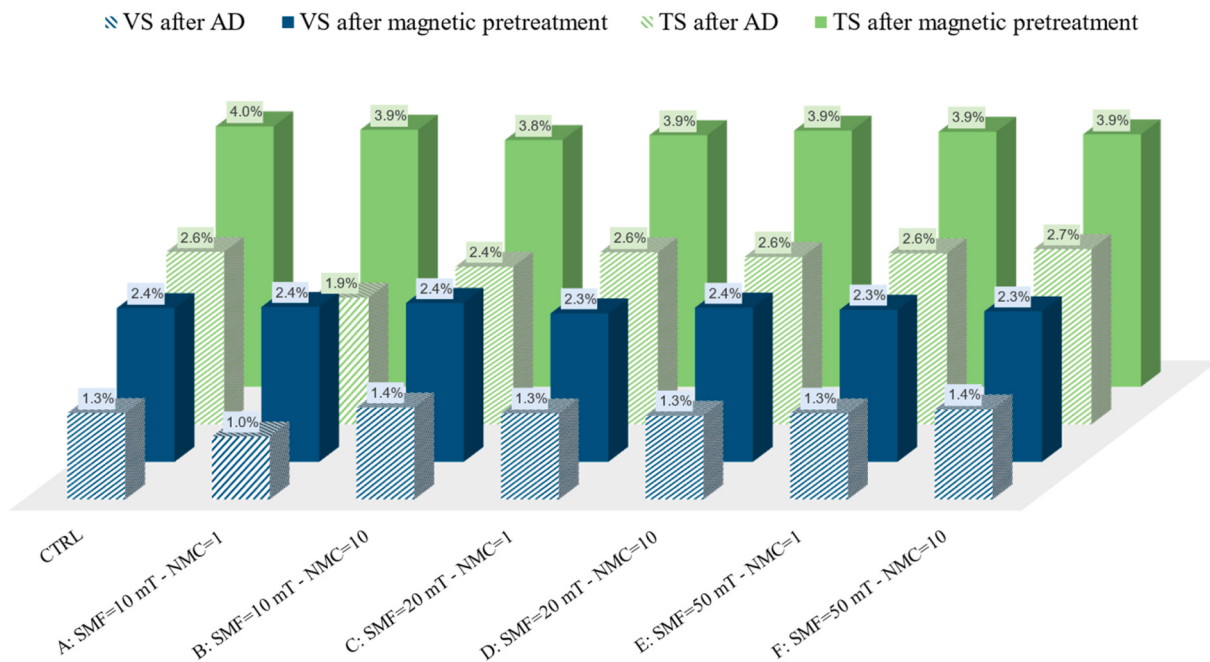


Fig. 3. Solid reduction after magnetic pretreatment and AD process.

do not exceed 1168 mg_{COD}/L throughout the experiment. In contrast, group C shows a pronounced surge by day 5 and reaches its peak at day 10, where total VFAs exceed those of all other conditions ($p < 0.05$) with a particularly high contribution of propionic and caproic acids. In contrast, the sludge pretreated at 50 mT displayed a delayed VFA accumulation, suggesting that the higher field intensity may have altered microbial kinetics and extended the acidogenic phase before full methanogenic activation occurred. Meanwhile, the 10 mT treatments consistently exhibited a lower and more fluctuating VFA profile ($p < 0.05$), regardless of whether 1 or 10 cycles were applied, indicating that this SMF intensity provides insufficient stimulation of the microbial consortia responsible for robust acidogenesis.

Within group C, our results revealed a significant and sustained VFA production characterized by notably high levels of propionic acid and relatively lower concentrations of butyric acid. The prominent propionic

acid is particularly important for methanogenesis as it is syntrophically oxidized to acetic acid [25], a direct substrate for acetoclastic methanogens. Although butyric acid was less abundant, it assumes a significant importance for the AD process as it is typically converted into acetic acid and hydrogen, thereby fueling hydrogenotrophic methanogenesis [26]. Furthermore, even the less prevalent valeric and caproic acids can be degraded into shorter-chain VFAs, thus broadening the substrate pool available for methanogens.

The highest VFAs generation observed in group C indicates an enhancement of the acidogenic activity at an applied SMF of 20 mT, which correlates positively with the higher biomethane production observed at the same magnetic intensity. In contrast, treatments at 10 and 50 mT yielded less favourable VFAs temporal profiles, which corresponded with a lower biomethane production compared to 20 mT. It is plausible that sludge pretreatment at 20 mT optimized the mechanisms

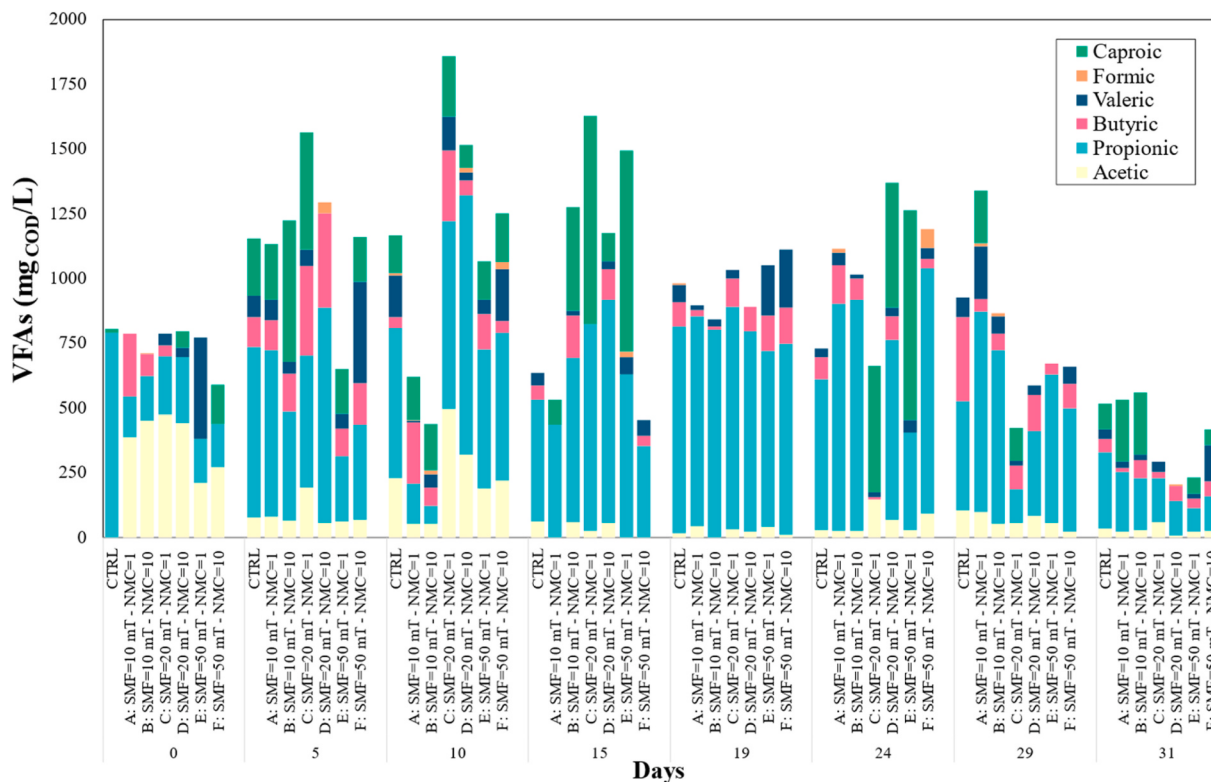


Fig. 4. VFAs production during AD batch tests with sludge exposed at different magnetic intensities and NMCs. Each bar is segmented to represent the distribution of individual VFAs, providing their speciation and relative contributions to the total concentration.

activated through SMF exposure and identified in the literature as beneficial for biomethane production, such as the enhancement of membrane permeability [7], a higher enzyme activity, conformation and orientation, and an optimal redox environment [10]. In contrast, sludge exposure at 10 mT seemed to provide insufficient stimulation of acidogenic consortia, while exposure at 50 mT seems to delay VFAs production, resulting in slower conversion to biomethane and prolonged residence time in the digesters. Collectively, these findings underscore the critical importance of fine-tuning SMF pretreatment parameters. Specifically, the condition observed at 20 mT with a single

magnetization cycle appears to maximize substrate conversion, enhances fermentative and acidogenic activity, thereby setting the stage for enhanced methanogenic activity and higher specific biomethane production.

Fig. 5 illustrates the temporal changes in N-NH_4^+ concentrations during the AD process. Overall, N-NH_4^+ profiles are quite similar, although some differences can be highlighted at the different conditions tested.

For instance, the samples pretreated at 10 mT demonstrated an initial acceleration of N-NH_4^+ release, with condition B showing a

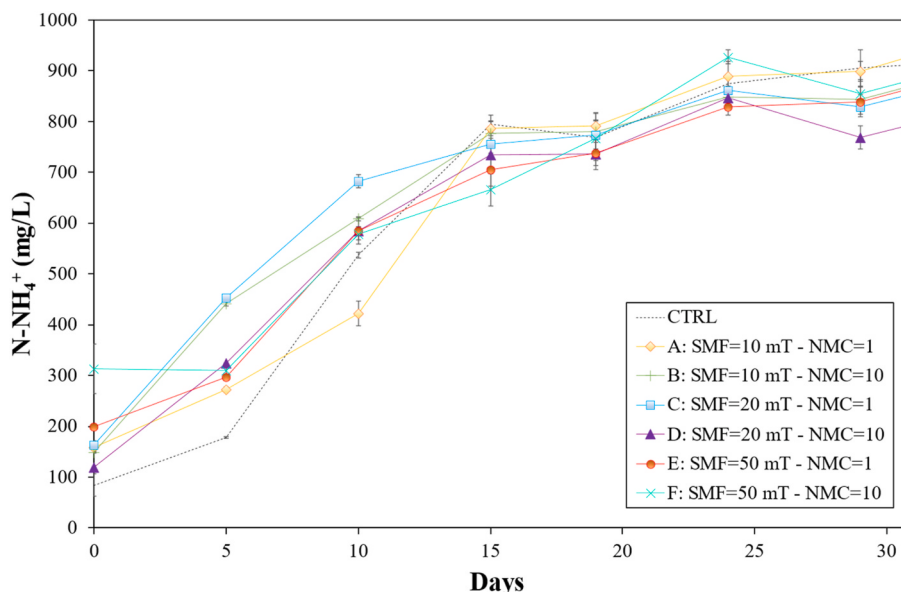


Fig. 5. Temporal profile of N-NH_4^+ during AD tests of sewage sludge exposed at different magnetic intensities and NMCs.

sharper increase at early stages, although both conditions eventually converged towards values comparable to the control. At 20 mT, condition C showed a pronounced ammonium peak ($p < 0.05$) at day 10 (683.5 mg/L), while condition D revealed a more gradual accumulation, suggesting that the NMC can modulate the rate of protein degradation. Sludge pretreated at a moderate SMF intensity (50 mT, conditions E and F) showed similar trend and final N-NH_4^+ concentration.

3.3. Shifts in microbial composition induced by the magnetic pretreatment

The taxonomic composition at genus level of the microbial communities with a relative abundance $>1\%$ in magnetized and untreated sludge is shown in Fig. 6.

As shown, the application of different SMF pretreatments induced marked shifts in the microbial community relative to the untreated condition. Specifically, under condition C, several key taxa implicated in AD exhibited notable percentage changes. For example, *Arcobacter* sp., typically associated with early hydrolysis under microaerophilic conditions [27], decreased modestly from 9.42% to 9.15%, which may reflect a transition toward a community more specialized in strictly anaerobic fermentative processes. In contrast, *Macellibacteroides* sp., known for their role in the anaerobic degradation of proteins [28] and amino acids in sewage sludge [29], contributing to the overall breakdown of organic matter, increased from 6.08% to 6.45%, thereby potentially enhancing the VFAs pool essential for subsequent methanogenesis. *Bacteroides* sp., central to the degradation of polysaccharides and proteins [30], experienced a moderate decline from 6.10% to 5.34%, suggesting that alternative fermentative pathways might be compensating for VFAs production in condition C. Additional shifts were observed in taxa indirectly contributing to the digestion process. For instance, the relative abundance of *Anaerolineaceae*, involved in generating crucial metabolic intermediates and fostering synergistic interactions with other microbes to enhance methane production [31] and overall process stability [32], increased slightly from 3.23% to 3.54%. Notably, condition C also promoted the proliferation of taxa absent or undetectable in the control. Specifically, the family *Leptotrichiaceae*,

undetectable in the control, appeared at 2.37%, indicating the establishment of novel niches that may enhance substrate fermentation. Similarly, *Saprospiraceae* and *Desulfovibrio* sp. increased up to 1.51% and 1.02%, respectively, suggesting that these groups not only support the breakdown of macromolecules [33] but also facilitate interspecies hydrogen transfer, thereby reinforcing the syntrophic interactions [34]. Opportunistic genera such as *Acinetobacter* sp., which produce specific enzymes and biosurfactants that enhance the solubilization and breakdown of recalcitrant organic compounds [35], increased from 1.71% to 2.25%, and members of the *Comamonadaceae* family, known for their ability to metabolize aromatic and otherwise resistant compounds [36], also increased from 1.55% to 2.04%, potentially indicating an enhanced capacity for degrading recalcitrant compounds, thereby contributing to a more efficient overall digestion process. In addition, taxa including *Pseudomonas* sp., *Comamonas* sp., *Aeromonas* sp., and *Caldilineaceae*, which were absent in the control, emerged in condition C at low levels (ranging from about 1.10% to 1.28%), likely contributing to metabolic niches [37] such as the conversion of complex polymers into simpler compounds [38].

In contrast, other SMF pre-treatment conditions, such as those involving a higher NMC or different field intensities, induced a different community shift. Under these alternative conditions, opportunistic taxa, microbes that normally remain at low levels but can quickly proliferate when conditions suddenly favour their growth, like *Acinetobacter* sp. and *Aeromonas* sp., surged markedly, reaching up to 19.63% and 16.50%, respectively. Such pronounced increases suggest that excessive SMF exposure may trigger stress responses that destabilize the microbial consortium and impair the effective conversion of complex substrates. Overall, the balanced and moderately shifted community observed in condition C, characterized by controlled increases in key fermentative, syntrophic, and degradative taxa and the emergence of beneficial niche groups, together with the observed VFAs dynamics, likely optimizes hydrolysis, acidogenesis, and methanogenesis, thereby contributing to the enhanced biomethane production under this treatment regime.

As shown in Fig. 7, applying a SMF pretreatment at 20 mT with a single magnetization cycle resulted in a higher overall relative

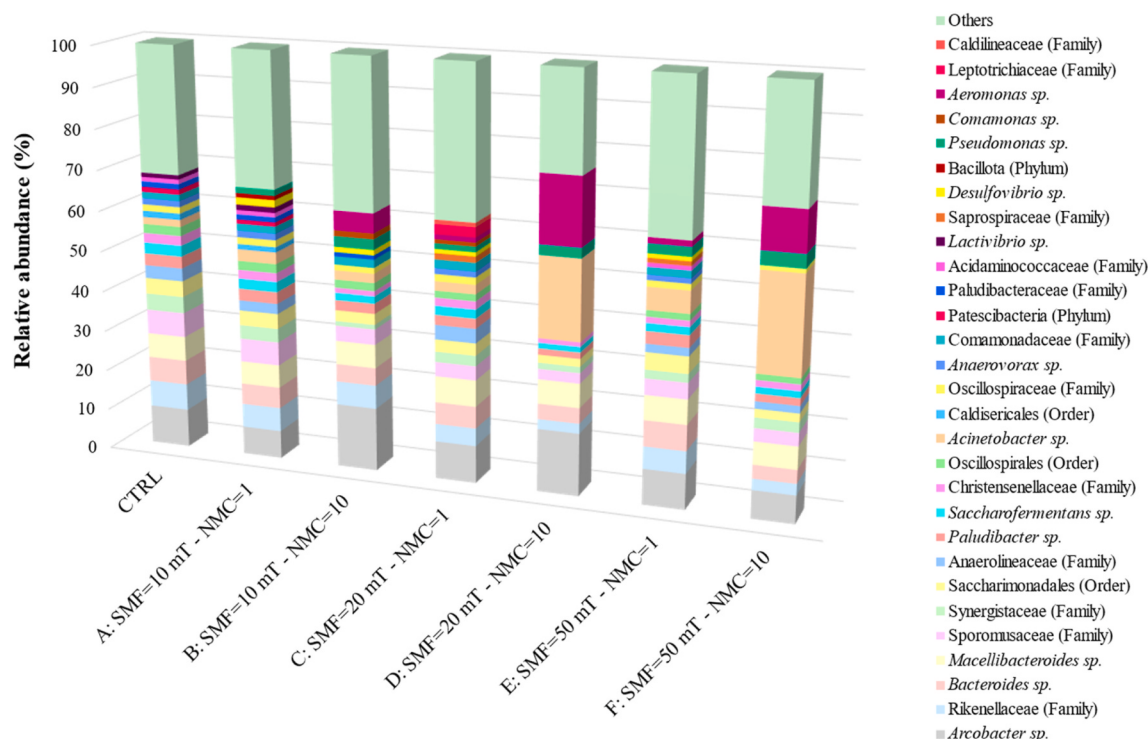


Fig. 6. Microbial community composition at genus level (relative abundances $>1\%$).

abundance of methanogenic taxa compared to the control.

This increase, although modest in absolute terms, suggests that this kind of pretreatment creates a more conducive environment for methanogenesis. For example, *Methanobrevibacter* sp., organisms critical for hydrogenotrophic methane production via carbon dioxide reduction using hydrogen [39], increased from 0.004% in the control to 0.02% under condition C. Even minor enhancements in these key populations may indicate an optimal activation of metabolic pathways, such as improved enzyme function and electron transfer efficiency, which are essential for the effective reduction of carbon dioxide to methane. Similarly, *Methanothrix* sp., which is pivotal in converting acetate to methane [40], rose from 0.028% to 0.063%, and *Methanospirillum* sp. also showed a positive response, highlighting that different methanogenic groups, each with specialized metabolic roles, can benefit from this pre-treatment.

In contrast, conditions using a higher number of magnetization cycles or different SMF intensities led to a decreased overall relative abundance of methanogens. This decline may be due to stress responses triggered by excessive magnetic exposure, which can compromise cellular membrane integrity or disrupt key enzymatic processes involved in electron transfer. Such stress could impair metabolic activity and selectively disadvantage more sensitive microbial populations, ultimately reducing community diversity and methanogenic potential.

3.4. Economic consideration and future perspectives

The implementation of a magnetic pretreatment for the AD of sewage sludge may result in significant economic advantages. The observed increase in biomethane production in experimental tests implies an improvement in process efficiency, leading to an increase in energy recovery and potential revenues from energy sale. A preliminary economic assessment of this technology must consider several factors. Implementation costs are relatively low, as the proposed system utilizes an SMF device that is fully integrable within the existing technology, consisting in an add-on to a sludge pumping channel. The low influence of NMC compared to SMF intensity further simplifies application at large scale, as no need of multiple recycling though the polarizer would be needed to enhance the AD process. Additionally, the magnetic polarizer itself does not consume energy, since the SMF is passively generated by rings made of a mixture of rare-earth elements. Therefore, the energy demand reported for the proposed pretreatment refers only to sludge pumping through the device, not to magnetic field generation. This

reduces operational costs compared to other pretreatment technologies such as ultrasound, thermal, or chemical treatments. Consequently, only installation, pumping, and maintenance costs must be considered depending on the scale of application. Table 4 provides a brief qualitative comparison of costs and energy requirements between the most reported pretreatment methods, taking into account economic analyses conducted in previous studies.

Building on Table 4, in which the data for the thermal, ultrasound, chemical, and biological pretreatments were drawn from the literature, we conducted an economic evaluation for a reference WWTP treating 10,000 t of sludge per year. CAPEX were annualized over a 10-year service life to reflect typical equipment depreciation. In terms of CAPEX, the proposed SMF pretreatment stands out at just 15–20 €/t, whereas the cost is 300–500 €/t for thermal methods, 150–300 €/t for ultrasound, 100–250 €/t for chemical and 50–150 €/t for biological. Turning to operating costs, an electricity tariff of 0.10 €/kWh was applied to specific energy demand of each technology: SMF application (50–100 kWh/t) costs only 5–10 €/t versus 30–50 €/t of thermal (300–500 kWh/t) and 15–30 €/t of ultrasound (150–300 kWh/t), with chemical and biological processes falling in between. Maintenance expenses were then estimated at 3% of the annualized CAPEX [20], yielding a modest 0.45–0.60 €/t·y for SMF compared to 9–15 €/t·y for thermal, 4.5–9.0 €/t·y for ultrasound, and correspondingly higher

Table 4

Cost, energy requirements and effect on biomethane production of different sludge pretreatments technologies.

Pretreatment technologies	CAPEX (€/t)	Energy requirements (kWh/t)	OPEX (€/t)	Biomethane increase (%)	Reference
Thermal	300–500	300–500	30–50	20–40	[20,41,42]
Ultrasound	150–300	150–300	15–30	20–30	[21,43,44]
Chemical	100–250	100–200	10–20	10–30	[42,44,45]
Biological	50–150	50–100	5–10	5–15	[5,20,43]
SMF application with magnetic tubular polarizer	15–20	50–100	5–10	15–21	This study

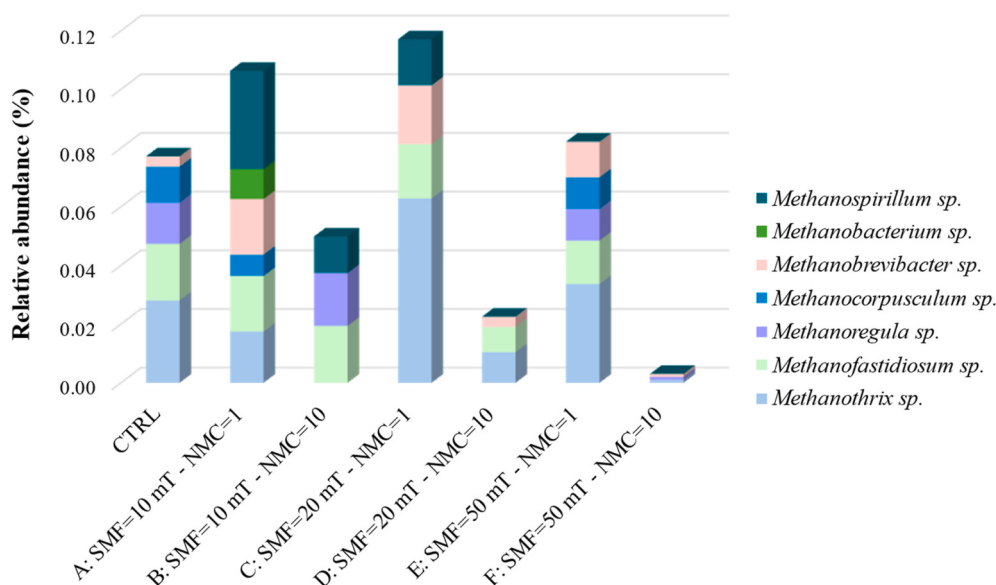


Fig. 7. Variation in the composition of methanogenic archaea across various pretreatment conditions.

values for the other methods. These outcomes highlight that the SMF pretreatment can reduce upfront investment by roughly 90% and cut energy demands by a factor of five to six compared to conventional pretreatments. To translate these cost savings into a project return, a baseline methane yield of 180 Nm³ CH₄/t, typical for sewage sludge anaerobic digestion, was assumed. The 21% improvement measured with SMF corresponds to an extra 37.8 Nm³ CH₄/t, which, at a conservative market price of 0.20 €/Nm³, delivers 7.56 €/t of incremental revenue. Dividing each process CAPEX by its methane-driven uplift gives simple payback times of approximately 2.4 years for SMF, compared to about 37 years for thermal, 25 years for ultrasound, 24 years for chemical, and 28 years for biological pretreatments. Even allowing a ±10% variation in electricity costs or methane prices, SMF remains under three years to pay back. By contrast, all other methods extend well beyond typical WWTP investment horizons, suggesting SMF pretreatment as an economically option for medium-to large-scale facilities.

Despite the promising results obtained in experimental tests, further research is required to optimize the application of SMF treatment at industrial scale. The scalability of the process must be thoroughly studied to optimize the proposed technology in large-scale treatment facilities. For instance, the use of a larger polarizer may vary the distribution of the SMF within the pumping sludge and lead to a different impact on the various AD phases. Therefore, fine-tuning the process at larger scale is an unavoidable step towards the full-scale implementation of the proposed technology. Besides, SMF treatment could be synergistically integrated with other pretreatments, such as thermal or chemical hydrolysis, to further enhance sludge degradability and maximize biogas yield.

4. Conclusions

This study provides new insights into the effects of SMF pretreatment on the AD of sewage sludge, focusing on three different field intensities (10, 20, and 50 mT). The results highlight that SMF application can enhance biomethane production, with the 20 mT treatment yielding the highest improvement (21% increase compared to the control). This suggests that low-intensity SMF can optimize microbial activity and substrate bioavailability without causing metabolic stress. Additionally, SMF pretreatment at 20 mT significantly enhanced VFAs production, particularly propionic and caproic acids, thereby improving the acidogenic phase and supporting subsequent methanogenesis. While TS and VS reductions were comparable across all conditions, the observed improvements in biomethane production suggest a more efficient substrate-to-gas conversion rather than greater overall organic matter degradation. Moreover, N-NH₄⁺ profiles did not exhibit significant deviations from the control, reinforcing that enhanced methane yield is primarily driven by improved metabolic efficiency rather than increased proteolytic activity.

Microbial community analysis further revealed that SMF pretreatment influences microbial composition, favouring key functional groups involved in hydrolysis and methanogenesis. Notably, the 20 mT pretreatment promoted the enrichment of methanogenic archaea and syntrophic bacteria, potentially explaining the observed increase in methane yields.

From an operational perspective, the application of SMF pretreatment represents an energy-efficient, non-invasive strategy to improve sludge biodegradability and enhance energy recovery in WWTPs. The absence of additional energy consumption and its ease of implementation make this technology a promising alternative to conventional sludge pretreatment methods. However, further research is needed for upscaling and to fully elucidate the underlying mechanisms, optimize treatment parameters, and assess long-term effects on microbial communities and digestion performance. Overall, these findings contribute to the growing body of knowledge on SMF applications in bioprocesses, introducing for the first time a preliminary techno-economic

perspective, and suggest that targeted SMF pretreatment could play a significant role in advancing sustainable sludge management and bio-energy production.

CRediT authorship contribution statement

Nicola Di Costanzo: Conceptualization, Data curation, Formal analysis, Investigation, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Alessandra Cesaro:** Conceptualization, Funding acquisition, Supervision, Visualization, Writing – review & editing. **Francesco Di Capua:** Conceptualization, Funding acquisition, Supervision, Visualization, Writing – review & editing. **Federica Carraturo:** Formal analysis, Writing – review & editing. **Michela Salamone:** Formal analysis. **Maria Cristina Mascolo:** Resources, Visualization. **Giovanni Esposito:** Conceptualization, Funding acquisition, Project administration, Supervision, Visualization, Writing – review & editing.

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.

References

- [1] E. Buonocore, S. Mellino, G. De Angelis, G. Liu, S. Ulgiati, Life cycle assessment indicators of urban wastewater and sewage sludge treatment, *Ecol. Indic.* 94 (2018) 13–23, <https://doi.org/10.1016/j.ecolind.2016.04.047>.
- [2] A. Kelessidis, A.S. Stasinakis, Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries, *Waste Manag.* 32 (2012) 1186–1195, <https://doi.org/10.1016/j.wasman.2012.01.012>.
- [3] N. Di Costanzo, F. Di Capua, A. Cesaro, F. Carraturo, M. Salamone, M. Guida, G. Esposito, A. Giordano, Headspace micro-oxygenation as a strategy for efficient biogas desulfurization and biomethane generation in a centralized sewage sludge digestion plant, *Biomass Bioenergy* 183 (2024) 107151, <https://doi.org/10.1016/j.biombioe.2024.107151>.
- [4] H. Carrère, C. Dumas, A. Battimelli, D.J. Batstone, J.P. Delgenès, J.P. Steyer, I. Ferrer, Pretreatment methods to improve sludge anaerobic degradability: a review, *J. Hazard. Mater.* 183 (2010) 1–15, <https://doi.org/10.1016/j.jhazmat.2010.06.129>.
- [5] U. Brémond, R. de Buyer, J.P. Steyer, N. Bernet, H. Carrère, Biological pretreatments of biomass for improving biogas production: an overview from lab scale to full-scale, *Renew. Sustain. Energy Rev.* 90 (2018) 583–604, <https://doi.org/10.1016/j.rser.2018.03.103>.
- [6] V. Khanh Nguyen, D. Kumar Chaudhary, R. Hari Dahal, N. Hoang Trinh, J. Kim, S. W. Chang, Y. Hong, D. Duc La, X.C. Nguyen, H. Hao Ngo, W.J. Chung, D. Nguyen, Review on pretreatment techniques to improve anaerobic digestion of sewage sludge, *Fuel* 285 (2021) 119105, <https://doi.org/10.1016/j.fuel.2020.119105>.
- [7] R.A. Dar, T.H. Tsui, L. Zhang, A. Smoliński, Y.W. Tong, A.H. Mohamed Rasmeey, R. Liu, Recent achievements in magnetic-field-assisted anaerobic digestion for bioenergy production, *Renew. Sustain. Energy Rev.* 207 (2025), <https://doi.org/10.1016/j.rser.2024.114902>.
- [8] X. Zhang, K. Yarema, A. Xu, Biological Effects of Static Magnetic Fields, 2017, <https://doi.org/10.1007/978-981-10-3579-1>.
- [9] M. Zieliński, M. Zielińska, A. Cydzik-Kwiatkowska, P. Rusanowska, M. Dębowski, Effect of static magnetic field on microbial community during anaerobic digestion, *Bioresour. Technol.* 323 (2021), <https://doi.org/10.1016/j.biortech.2020.124600>.
- [10] B. Zhao, H. Sha, J. Li, S. Cao, G. Wang, Y. Yang, Static magnetic field enhanced methane production via stimulating the growth and composition of microbial community, *J. Clean. Prod.* 271 (2020) 122664, <https://doi.org/10.1016/j.jclepro.2020.122664>.
- [11] M. Dębowski, M. Zieliński, M. Kisielska, A. Hajduk, Effect of constant magnetic field on anaerobic digestion of algal biomass, *Environ. Technol.* 37 (2016) 1656–1663, <https://doi.org/10.1080/09593330.2015.1126362>.
- [12] M. Zieliński, M. Dębowski, J. Kazimierowicz, The effect of static magnetic field on methanogenesis in the anaerobic digestion of municipal sewage sludge, *Energies* 14 (2021), <https://doi.org/10.3390/en14030590>.
- [13] A. Haritwal, D. Mohan, M. Chaudhary, P.P. Asopa, G. Modi, Study on the improved biogas generation through magnetic field modified anaerobic digestion, *Int. J. Eng. Res. Technol.* 4 (2015), <https://doi.org/10.17577/ijertv4is051234>.
- [14] Q. Hong, K. Wang, Y. Huang, Z. Zhang, Y. Jiang, S. Wang, H. Wang, Enhanced methane production from anaerobic digestion of waste activated sludge with weak magnetic field: insights into performances and mechanisms, *Bioresour. Technol.* 408 (2024), <https://doi.org/10.1016/j.biortech.2024.131174>.
- [15] N. Di Costanzo, F. Di Capua, A. Cesaro, F. Carraturo, M. Salamone, M.C. Mascolo, A. Carpentieri, G. Esposito, Low-intensity magnetization pretreatment to enhance

- biomethane generation and the abundance of key microorganisms for anaerobic digestion of sewage sludge, *Bioresour. Technol.* 413 (2024) 131534, <https://doi.org/10.1016/j.biortech.2024.131534>.
- [16] N. Di Costanzo, F. Di Capua, A. Cesaro, M.C. Mascolo, F. Pirozzi, G. Esposito, Impact of high-intensity static magnetic field on chemical properties and anaerobic digestion of sewage sludge, *Waste Biomass Valoriz.* 14 (2023) 2469–2479, <https://doi.org/10.1007/s12649-022-01891-x>.
- [17] A.P.H.A. American Public Health Association, A.W.W.A. American Water Works Association, W.E.F. Water Environment Federation, *Standard Methods for the Examination of Water and Wastewater*, 24th ed., APHA Press, Washington DC, 2023.
- [18] APAT, IRSA-CNR, *Analytical Methods for Water*, 2003.
- [19] S.G. Arhin, A. Cesaro, F. Di Capua, G. Esposito, Recent progress and challenges in biotechnological valorization of lignocellulosic materials: towards sustainable biofuels and platform chemicals synthesis, *Sci. Total Environ.* 857 (2023) 159333, <https://doi.org/10.1016/j.scitotenv.2022.159333>.
- [20] B. Shrestha, R. Hernandez, D.L.B. Fortela, W. Sharp, A. Chistoserdov, D. Gang, E. Revellame, W. Holmes, M.E. Zappi, A review of pretreatment methods to enhance solids reduction during anaerobic digestion of municipal wastewater sludges and the resulting digester performance: implications to future urban biorefineries, *Appl. Sci. (Switzerland)* 10 (2020) 1–28, <https://doi.org/10.3390/app10249141>.
- [21] B. Ruffino, G. Campo, A. Cerutti, M. Zanetti, E. Lorenzi, G. Scibilia, G. Genon, Preliminary technical and economic analysis of alkali and low temperature thermo-alkali pretreatments for the anaerobic digestion of waste activated sludge, *Waste Biomass Valoriz.* 7 (2016) 667–675, <https://doi.org/10.1007/s12649-016-9537-x>.
- [22] S. Pilli, P. Bhunia, S. Yan, R.J. LeBlanc, R.D. Tyagi, R.Y. Surampalli, Ultrasonic pretreatment of sludge: a review, *Ultrason. Sonochem.* 18 (2011) 1–18, <https://doi.org/10.1016/j.ultsonch.2010.02.014>.
- [23] H. Carrère, C. Dumas, A. Battimelli, D.J. Batstone, J.P. Delgenès, J.P. Steyer, I. Ferrer, Pretreatment methods to improve sludge anaerobic degradability: a review, *J. Hazard. Mater.* 183 (2010) 1–15, <https://doi.org/10.1016/j.jhazmat.2010.06.129>.
- [24] N. Yang, C. Tian, Y. Lv, J. Hou, Z. Yang, X. Xiao, Y. Zhang, Novel primers for 16S rRNA gene-based archaeal and bacterial community analysis in Oceanic trench sediments, *Appl. Microbiol. Biotechnol.* 106 (2022) 2795–2809, <https://doi.org/10.1007/s00253-022-11893-3>.
- [25] D.W. Fadrosh, B. Ma, P. Gajer, N. Sengamalai, S. Ott, R.M. Brotman, J. Ravel, An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the illumina MiSeq platform, *Microbiome* 2 (2014) 6, <https://doi.org/10.1186/2049-2618-2-6>.
- [26] J. Zhou, X. You, B. Niu, X. Yang, L. Gong, Y. Zhou, J. Wang, H. Zhang, Enhancement of methanogenic activity in anaerobic digestion of high solids sludge by nano zero-valent iron, *Sci. Total Environ.* 703 (2020) 135532, <https://doi.org/10.1016/j.scitotenv.2019.135532>.
- [27] Y. Wang, Y. Zhang, J. Wang, L. Meng, Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria, *Biomass Bioenergy* 33 (2009) 848–853, <https://doi.org/10.1016/j.biombioe.2009.01.007>.
- [28] S. Raza, K.H. Kang, J. Shin, S.G. Shin, J. Chun, H.U. Cho, J. Shin, Y.M. Kim, Variations in antibiotic resistance genes and microbial community in sludges passing through biological nutrient removal and anaerobic digestion processes in municipal wastewater treatment plants, *Chemosphere* 313 (2023), <https://doi.org/10.1016/j.chemosphere.2022.137362>.
- [29] S.Y. Jeong, T.G. Kim, Determination of methanogenesis by nutrient availability via regulating the relative fitness of methanogens in anaerobic digestion, *Sci. Total Environ.* 838 (2022), <https://doi.org/10.1016/j.scitotenv.2022.156002>.
- [30] L. Yin, A. Zhou, Y. Wei, C. Varrone, D. Li, J. Luo, Z. He, W. Liu, X. Yue, Deep insights into the roles and microbial ecological mechanisms behind waste activated sludge digestion triggered by persulfate oxidation activated through multiple modes, *Environ. Res.* 252 (2024), <https://doi.org/10.1016/j.envres.2024.118905>.
- [31] W. Tukangan, S. Hupfau, M. Gómez-Brandón, H. Insam, W. Salvenmoser, P. Prasertsan, B. Cheirsilp, S. O-Thong, Symbiotic bacteroides and Clostridium-rich methanogenic consortium enhanced biogas production of high-solid anaerobic digestion systems, *Bioresour. Technol. Rep.* 14 (2021), <https://doi.org/10.1016/j.biteb.2021.100685>.
- [32] K. Nakasaki, K.K. Nguyen, F.C. Ballesteros, T. Maekawa, M. Koyama, Characterizing the microbial community involved in anaerobic digestion of lipid-rich wastewater to produce methane gas, *Anaerobe* 61 (2020), <https://doi.org/10.1016/j.anaerobe.2019.102082>.
- [33] S.J. McIlroy, R.H. Kirkegaard, M.S. Dueholm, E. Fernando, S.M. Karst, M. Albertsen, P.H. Nielsen, Culture-independent analyses reveal novel Anaerolineaceae as abundant primary fermenters in anaerobic digesters treating waste activated sludge, *Front. Microbiol.* 8 (2017), <https://doi.org/10.3389/fmicb.2017.01134>.
- [34] N.K. Kim, S. Oh, W.T. Liu, Enrichment and characterization of microbial consortia degrading soluble microbial products discharged from anaerobic methanogenic bioreactors, *Water Res.* 90 (2016) 395–404, <https://doi.org/10.1016/j.watres.2015.12.021>.
- [35] A. El Houari, M. Ranchou-Peyruse, A. Ranchou-Peyruse, R. Bennisse, R. Bouterfas, M.S.G. Urriaza, A.I. Qatibi, R. Guyoneaud, Microbial communities and sulfate-reducing microorganisms abundance and diversity in municipal anaerobic sewage sludge digesters from a wastewater treatment plant (Marrakech, Morocco), *Processes* 8 (2020) 1–22, <https://doi.org/10.3390/pr8101284>.
- [36] D. Pulami, P. Kämpfer, S.P. Glaeser, High diversity of the emerging pathogen *Acinetobacter baumannii* and other *Acinetobacter* spp. in raw manure, biogas plants digestates, and rural and urban wastewater treatment plants with system specific antimicrobial resistance profiles, *Sci. Total Environ.* 859 (2023), <https://doi.org/10.1016/j.scitotenv.2022.160182>.
- [37] L. Hao, A. Bize, D. Conteau, O. Chapleur, S. Courtois, P. Kroff, E. Desmond-Le Quémener, T. Bouchez, L. Mazéas, New insights into the key microbial phylotypes of anaerobic sludge digesters under different operational conditions, *Water Res.* 102 (2016) 158–169, <https://doi.org/10.1016/j.watres.2016.06.014>.
- [38] C. Buettner, M. von Bergen, N. Jehmlich, M. Noll, *Pseudomonas* spp. are key players in agricultural biogas substrate degradation, *Sci. Rep.* 9 (2019), <https://doi.org/10.1038/s41598-019-49313-8>.
- [39] M. Duran, N. Tepe, D. Yurtsever, V.L. Punzi, C. Bruno, R.J. Mehta, Bioaugmenting anaerobic digestion of biosolids with selected strains of bacillus, pseudomonas, and actinomycetes species for increased methanogenesis and odor control, *Appl. Microbiol. Biotechnol.* 73 (2006) 960–966, <https://doi.org/10.1007/s00253-006-0548-6>.
- [40] D. Cam, S. Sayin, O.Z. Sarman, E. Iren, B. Icen, Use of lyophilized and acclimated digestate dominated by methanobrevibacter as a start-up inoculum in anaerobic digester led to higher methane production in biochemical methane potential assays, *Waste Manag. Bull.* 2 (2024) 36–42, <https://doi.org/10.1016/j.wmb.2024.06.004>.
- [41] W. Huang, F. Yang, W. Huang, D. Wang, Z. Lei, Z. Zhang, Weak magnetic field significantly enhances methane production from a digester supplemented with zero valent iron, *Bioresour. Technol.* 282 (2019) 202–210, <https://doi.org/10.1016/j.biortech.2019.03.013>.
- [42] G.C. Mitraka, K.N. Kontogiannopoulos, M. Batsioulas, G.F. Banias, A.I. Zouboulis, P. G. Kougiyas, A comprehensive review on pretreatment methods for enhanced biogas production from sewage sludge, *Energies* 15 (2022), <https://doi.org/10.3390/en15186536>.
- [43] B. Ruffino, G. Campo, G. Genon, E. Lorenzi, D. Novarino, G. Scibilia, M. Zanetti, Improvement of anaerobic digestion of sewage sludge in a wastewater treatment plant by means of mechanical and thermal pre-treatments: performance, energy and economical assessment, *Bioresour. Technol.* 175 (2015) 298–308, <https://doi.org/10.1016/j.biortech.2014.10.071>.
- [44] A. Gonzalez, A.T.W.M. Hendriks, J.B. van Lier, M. de Kreuk, Pre-treatments to enhance the biodegradability of waste activated sludge: elucidating the rate limiting step, *Biotechnol. Adv.* 36 (2018) 1434–1469, <https://doi.org/10.1016/j.biotechadv.2018.06.001>.
- [45] D.O. Tas, C. Yangin-Gomec, T. Olmez-Hanci, O.A. Arikian, D.I. Cifci, E.B. Gencsoy, A. Ekdal, E. Ubay-Cokgor, Comparative assessment of sludge pre-treatment techniques to enhance sludge dewaterability and biogas production, *Clean* 46 (2018), <https://doi.org/10.1002/clen.201700569>.