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The Effect of Static Magnetic Field on Methanogenesis in the Anaerobic Digestion of Municipal Sewage Sludge

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Abstract: The present study aimed to determine the effect of a 17.6 mT static magnetic field (SMF) on the efficiency of anaerobic digestion (AD) of municipal sewage sludge (MSS). The SMF had a significant impact on methane (CH_4) production efficiency, the levels of fermentation rate (η_FMSS) vs. removal rate (ηVS), and the structure of the anaerobic bacteria consortium, but it did not affect cumulative biogas production. The highest CH_4 yield (431 ± 22 dm^3_{CH4}/kgVS) and the highest methane content in the biogas (66.1% ± 1.9%) were found in the variant in which the SMF exposure time was 144 min/day. This variant also produced the highest η_FMSS and ηVS values, reaching 73.8% ± 2.3% and ηVS 36.9% ± 1.6%, respectively. Longer anaerobic sludge retention time in the SMF area significantly decreased AD efficiency and caused a significant reduction in the number of methanogens in the anaerobic bacteria community. The lowest values were observed for SMF exposure time of 432 min/day, which produced only 54.8 ± 1.9% CH_4 in the biogas. A pronounced reduction was recorded in the Archaea (ARC915) and Methanosaeta (MX825) populations in the anaerobic sludge, i.e., to 20% ± 11% and 6% ± 2%, respectively.

Keywords: municipal sewage sludge (MSS); anaerobic sludge (AS); anaerobic digestion (AD); static magnetic field (SMF); biogas; methane (CH_4)

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1. Introduction

The operation of sewage treatment plants directly necessitates optimal sludge management. The municipal sewage sludge (MSS) generated during wastewater treatment must be converted, then properly neutralized and/or managed [1]. Although MSS management techniques and methods are clearly being continuously explored, a universal, economically-viable technology for its effective neutralization has yet to be developed. With increasing MSS quantities and their quality rarely meeting environmental requirements, this ongoing problem is becoming increasingly difficult to solve [2]. The dynamically growing number of new wastewater treatment plants has a direct impact on the increasing amount of sewage sludge, while stringent standards related to sludge management make it necessary to use complex technologies to limit their impact on the environment [3,4].

Natural use is widely held to be the simplest and cheapest form of MSS management. This method is widely promoted both in scientific/technical literature and in national/international legal acts [5]. Unfortunately, in addition to its soil-building and fertilizing properties, MSS is also malodorous and rich in organic compounds susceptible to putrification, which often limits its direct use for agricultural, natural, and reclamation purposes [6,7]. In many cases, MSS poses a sanitary threat due to high concentrations of pathogenic and parasitic organisms [8]. It is often a source of heavy metals and other toxic substances derived from wastewater [9], and its improper management and neutralization frequently lead to the emission of gaseous contaminants and aerosols to the

atmosphere [10]. Thus, there is a pressing need to alleviate the environmentally harmful properties of MSS by improving current technologies and exploring new ones [11].

Anaerobic stabilization through anaerobic digestion (AD) is one technologically-sound and environmentally-friendly method of processing MSS [12]. Well-implemented AD can reduce susceptibility to putrification, improve sanitary conditions, reduce MSS volume, and produce high yields of CH_4-rich biogas [13–15]. These results are often further bolstered through MSS pretreatment and shredding [16,17], i.e., processes that disrupt the sludge's structure by separating flocs, destroying microbial cells, releasing organic substances and extracellular polymers into the dilute phase, etc. [18,19]. There are several pretreatment methods, including for example: mechanical method [20]; high-pressure method [21]; microwave pretreatment [22]; ultrasound energy pretreatment [23]; thermal methods, including heat treatment and freezing/defrosting [24], or low-temperature disintegration [25]; chemical methods, including acidification [26], alkalization [27], ozonation [28], and oxidation technique [29]; as well as biological methods [30]. Coupled disintegration methods, called the hybrid methods, are employed as well [31]. However, these methods drive up investment and operational costs, are technologically complex and often uneconomical for treatment plant operators [32].

As such, there is a legitimate need to seek alternative, prospective, and competitive methods for neutralizing MSS. Few reports can only be found on the use of the static magnetic field (SMF) to improve the anaerobic digestion of MSS. However, there is a basis for research that would verify the impact of the SMF on the anaerobic stabilization processes of this type of waste. In recent studies, it was proved that the magnetic field exerts a positive effect on many properties of fluids, i.e., it changes the polarization, and electric charge decreases surface tension and increases viscosity. In studies conducted so far, the magnetic field has usually been applied to separate solids, like, e.g., activated sludge, from the effluent [33,34]. It was also demonstrated to influence the growth and metabolism of bacteria. For instance, Okuno et al. (1993) showed it did not exert a bactericidal effect but caused changes in the bacteria growth rate [35]. These changes might have been due to magnetic field intensity and frequency, its static or oscillating nature, the waveform, as well as the type and condition of exposed cells [36,37]. Magnetic fields improved phenol biodegradation by immobilized activated sludge [38]. It was observed that by applying a magnetic south pole to the process, biodegradation in the form of biological oxidation was enhanced. A 30% increase in the biodegradation rate was obtained by applying a magnetic south pole of the strength of 0.45 Tesla to the bioreactor with immobilized microbial beads as compared to the control [38].

Several efforts have been made to improve biodegradation effectiveness by using SMF. The influence of SMF depends on its intensity [39]. Generally, using the SMF with magnetic induction higher than 1 T, results in the inhibition of biochemical processes [40]. However, a weak SMF may also increase the efficiency of the biological decomposition of organic compounds [41]. Filipic et al. proved that low intensities of 17 mT SMF could stimulate the activity of enzymes and increase the rate of organic matter biodegradation by the bacterial community [42]. Moreover, the influence of SMF on the metabolism of microorganisms still requires extensive research. Potenza et al. reported that the 300 mT SMF might influence cell multiplication, bacterial biomass growth, and gene expression [43]. In another study, the SMF (200 mT) was applied to remodel the membrane lipid composition of *Salmonella typhimurium* to maintain an optimum level of fluidity [44].

The aim of the present study was to determine the effect of a low range SMF on the course and efficiency of anaerobic digestion (AD) of MSS, as well as on the qualitative composition and yields of the resultant biogas.

2. Materials and Methods

2.1. Experimental Design

The experiments were divided into seven variants. The only criterion for the division was hydraulic retention time (HRT) of the municipal sewage sludge (MSS) in the SMF

area. Differences in HRT were obtained by the multiple pumping of a mixture of anaerobic sludge and MSS through a magnetic fluid actuator (MFA). The design of completed research works is shown in Table 1.

Table 1. Design of completed research works.

Variant	Number of Pumping Cycles through the SMF Area (times/h)	Performance of Circulating Pump (dm^3/h)	HRT in the SMF Area (min/day)
V1	0	0	0
V2	2	8	72
V3	4	16	144
V4	6	24	216
V5	8	32	288
V6	10	40	360
V7	12	48	432

SMF: static magnetic field; HRT: hydraulic retention time.

2.2. Materials

The MSS and anaerobic sludge (AS), which was the inoculum for anaerobic reactors, originated from the municipal wastewater treatment plant (M-WWTP) in Olsztyn. The M-WWTP uses an activated sludge process with enhanced biogenic compound removal. Daily average flows through the plant are 60,000 m^3/day. The M-WWTP produces approx. 550 tones MSS/day. The MSS is stabilized in two enclosed digesters with a total active volume of 10,000 m^3, operating at an organic load rate (OLR) of approx. 2.4 kg VS/m^3·d, a hydraulic retention time (HRT) of 20 days, and a temperature of 35 °C. The AS inoculum was sourced from the digesters. The characteristics of the MSS and AS used in the study are presented in Table 2.

Table 2. Characteristics of the municipal sewage sludge (MSS) and anaerobic sludge (AS) used in the study.

Indicator	Unit	MSS	AS
pH	-	7.1 ± 0.2	7.3 ± 0.1
Total solids (TS)	(%)	3.5 ± 0.6	1.8 ± 0.2
Volatile solids (VS)	(% TS)	77.4 ± 1.2	67.3 ± 0.7
Mineral solids (MS)	(% TS)	22.6 ± 1.3	31.7 ± 1.1
Total carbon (TC)	(mg/gTS)	460 ± 24	283 ± 17
Total organic carbon (TOC)	(mg/gTS)	371 ± 19	192 ± 11
Total nitrogen (TN)	(mg/gTS)	45 ± 5	24 ± 3
Total phosphorus (TP)	(mg/gTS)	1.7 ± 0.4	1.3 ± 0.2
Carbon to nitrogen ratio (C/N)	-	10 ± 1.2	11 ± 1.4
Protein	(% TS)	19.4 ± 2.1	14.9 ± 2.7
Lipids	(% TS)	12.1 ± 1.6	3.6 ± 0.9
Sugars	(% TS)	17.45 ± 3.3	1.4 ± 0.2

2.3. Experimental Setup

Experiments were carried out in anaerobic reactors with an active volume of 4.0 dm^3 and operating in the complete mixing mode ensured by four-plate vertical stirrers rotating at 45 RPM. The initial concentration of anaerobic sludge was approximately 4.0 gTS/dm^3 (2.69 kgVS/dm^3). AD was conducted at 35 °C at the chamber loading level of 2.0 kgVS/dm^3·d, with an HRT of 20 days. Anaerobic reactors were equipped with a circulating pump, which ensures more intense mixing and fed the AS/MSS mixture into the area exposed to SMF. Depending on the desirable SMF exposure time, the pump's performance ranged from 8.0 to 48.0 dm^3/h . In all variants, concurrent tests were performed for a control rector with similar hydraulic (pumping efficiency) conditions but no magnetic fluid actuators (MFAs). It was found that the operation regime of the circulating pump had no effect on the process or the products of AD. The design of the test site is presented in Figure 1.

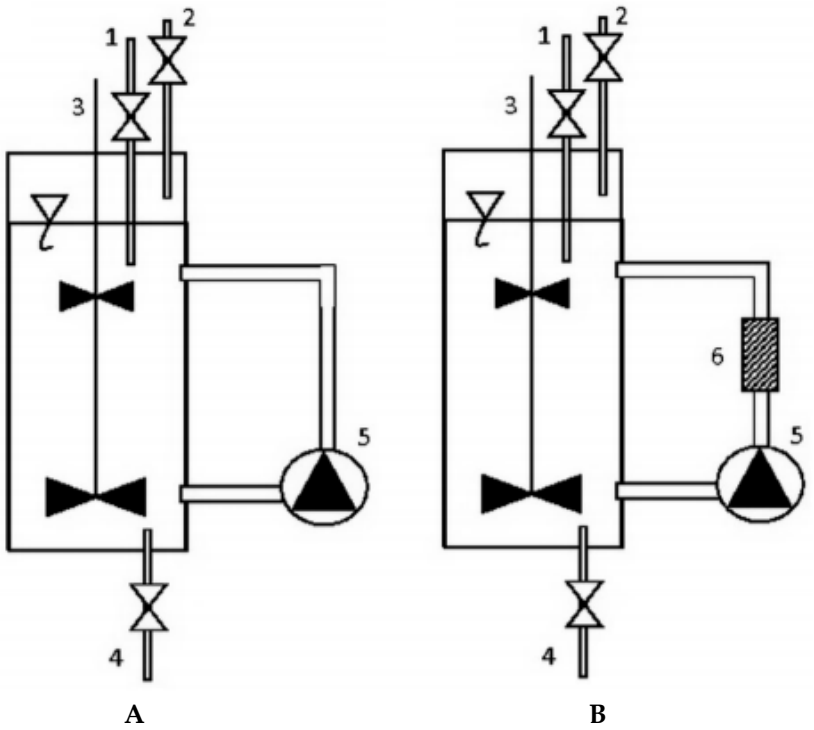

Figure 1. Design of the test site used for the experiments: (A—system without a magnetic fluid actuator (MFA), B—system with MFA, 1—substrate dosing, 2—biogas uptake, 3—vertical axis stirrer, 4—collection of the post-fermentation pulp, 5—circulating pump, 6—MFA).

Magnetic fluid actuator (MFA) used in the experiment was installed on a plastic pipe (2.5 cm in diameter), pumping the contents of the reactor. It consisted of two parts forming a ring. Two SMF-generating rings (i.e., permanently magnetized ceramic frits) were used to increase the area of direct SMF impact. The technical parameters of the MFA were as follows: width of the ring, 65 mm; the height of an individual ceramic magnet, 45 mm; the weight of an individual ring, 0.5 kg; nominal diameter range, 15–30 mm; and nominal intensity of induced SMF, 0.6 T. The induction inside the pipe was measured using the digital Gaussmeter LZ-641H (ENES Magnesy) immersed in the liquid, starting from the edge of the pipe to the opposite side. The distance between measurement points was 0.5 cm. The magnetic induction decreased with the distance from the source of the field from 30 mT directly at the source of the field to 8 mT in the center of the pipe. The cross-sectional area of the conductor, on which the MAP was mounted, was divided into equivalent concentric surfaces. Next, the real magnetic induction was measured at 13 designated measurement points, and its average value was 17.6 mT.

2.4. Analytical Methods

Qualitative analyses of MSS and anaerobic sludge, being an inoculum for anaerobic reactors, were performed in all experiments. The contents of dry matter and volatile and mineral solids were determined with the gravimetric method; whereas these of total carbon (TC), total organic carbon (TOC), and total nitrogen (TN) in the biomass samples dried at 105 °C, using a Flash 2000 elemental analyzer (Thermo Scientific). The total phosphorus (TP) was quantified with the colorimetric method with ammonium metavanadate (V) and ammonium molybdate at a wavelength of 390 mm using a DR 2800 spectrophotometer (Hach Lange), after sample mineralization in a mixture of sulfuric (VI) and chloric (VII) acids. The total protein content was estimated by multiplying the TN value by the conversion factor for proteins, or 6.25. The content of reduced sugars was determined colorimetrically at a wavelength of 600 nm using a DR 2800 spectrophotometer (Hach Lange), whereas that of fat using the Soxhlet method in an extractor (Büchi). The pH

value was measured potentiometrically. The instantaneous and total biogas flow in the reactors was measured using a gas flow meter (Aalborg Instruments and Controls, Inc.). The composition and percentage of individual biogas components were analyzed using a gas chromatograph (7890A Agilent) equipped with a thermoconductometric detector, whereas biogas quality was determined using a GFM 430 analyzer (Gas Data).

2.5. Molecular Analysis

The molecular analysis aimed to determine the percentage of ammonia-oxidizing bacteria AOB in the biofilm using the fluorescent in situ hybridization (FISH) technique. The biomass used for the FISH analysis was fixed immediately after sampling. Having been washed in 1× concentrated sodium phosphate buffer (PBS), the cells were fixed by adding three volumes of a fixation buffer (4% paraformaldehyde in PBS (pH 7.2)) to one volume of the bacterial suspension [45], and afterward gently vortexed and left at 4 °C for 24 h. After centrifugation (3 min at 4000 rpm), the supernatant was removed, and the sample was resuspended in the mixture of 1× concentrated PBS and 96% ethanol (1:1 volume ratio of mixture components) and stored at a temperature of −20 °C for up to a few weeks. A 10 cm^3 sample portion was spread on the well of each glass slide (Marienfeld Laboratory Glassware), dried for about 10 min at 46 °C, and dehydrated by serial immersion of the slide in 50%, 80%, and 96% (v/v) ethanol (3 min each). Afterward, alcohol was evaporated from the samples by air-drying. The hybridization buffer was composed of: 180 cm^3 of 5 mol/dm^3 NaCl, 20 cm^3 of 1 mol/dm^3 TrisHCl (pH 8.0), 550 cm^3 of formamide, 250 cm^3 of ultraclean water, and 2 cm^3 of 10% (w/v) SDS. The washing buffer, composed of100 cm^3 of 5 mol/dm^3 NaCl, 1000 cm^3 of 1 mol/dm^3 TrisHCl (pH 8.0), 500 cm^3 of 0.5 mol/dm^3 EDTA (pH 8.0), 50 cm^3 of 10% (w/v) SDS, and distilled water to complete the buffer to 50 cm^3, was preheated in a water bath to 48 °C.

Four molecular probes were used for hybridization: a Bacteria-universal probe EUB338 [46], an Archaea-universal probe ARC915 [47], a Methanosarcinaceae-targeting probe MSMX860, and a Methanosaetatargeting probe MX825 [48]. Their 10^4pmol/cm^3 portions were used to prepare a mixture with 140 cm^3 of a hybridization buffer. The mixture (12 cm^3) was then transferred into each well, and then the slide was immediately transferred to the hybridization oven and incubated at 46 °C for 3 h. After completed hybridization, the hybridization buffer was quickly washed out from the slide with the washing buffer, and the slide was incubated in the washing buffer for 10 min in a preheated water bath at 48 °C. Afterward, the washing buffer was removed with cold (4 °C) distilled water and dried, whereas the slides were embedded in VectaShieldmedium (Vector Laboratories, Burlingame, CA) to prevent a rapid loss of fluorescence during microscopic examination, and a cover slip was placed on each slide. The slides were then examined under an epi-fluorescence microscope (100x objective, total magnification 1000x; Nikon Eclipse, Nikon, Tokyo, Japan). The abundance of the examined group of microorganisms was estimated in relation to DAPI-stained cells using the Image J software (http://rsbweb.nih.gov/ij/).

2.6. Calculation Methods

The fermentation rate coefficient, i.e., the ratio of the vs. load removed in the reactor to the vs. load fed into the reactor (1) and the vs. removal rate (efficiency) coefficient (2) were determined using the following equations

$$\eta_F MSS = \frac{VS_{in} \times \rho_{in} \times Q_{in} - VS_{out} \times \rho_{out} \times Q_{out}}{VS_{in} \times \rho_{in} \times Q_{in}} \quad (1)$$

$$\eta VS = \frac{VS_{in} \times Q_{in} - VS_{out} \times Q_{out}}{VS_{in} \times Q_{in}} \quad (2)$$

where $\eta_F MSS$—fermentation rate (%), ηVS—vs. removal rate (%), VS_{in}—vs. levels in the influent (g/kg), VS_{out}—vs. levels in the digested sludge (g/kg), ρ_{in}—influent density (kg/dm^3), ρ_{out}—digested sludge density (kg/dm^3), Q_{in}—daily volume of the reactor feedstock (dm^3/d), Q_{out}—daily volume of the discharged sludge (dm^3/d).

The yields of biogas/CH_4 in relation to the vs. removed (3) and the yields of biogas/CH_4 in relation to the vs. load fed with the influent into the reactor (4) were calculated as:

$$Y_{b/CH_4}^{VS_{removed}} = \frac{V_{b/CH_4}}{(s_{in}^{VS} \times \rho_{in} \times Q_{in} - s_{out}^{VS} \times \rho_{out} \times Q_{out})/1000} \quad (3)$$

$$Y_{b/CH_4}^{VS_{in}} = \frac{V_{b/CH_4}}{(s_{in}^{VS} \times \rho_{in} \times Q_{in})/1000} \quad (4)$$

$Y_{b/CH_4}^{VS_{removed}}$—biogas yield in relation to $VS_{removed}$ ($dm^3/kgVS_{removed}$), $Y_{b/CH_4}^{VS_{in}}$—biogas yield in relation to the vs. in the influent ($dm^3/kgVS_{in}$), V_{b/CH_4}—the volume of biogas/CH_4 produced from the influent load (dm^3), s_{in}^{VS}—vs. levels in the influent (g/kg), s_{out}^{VS}—vs. levels in the digested sludge (g/kg), Q_{in}—the volume of a single dose of influent fed into the reactor (dm^3), Q_{out}—the volume of a single post-AD discharge of digested sludge from the reactor (dm^3).

2.7. Statistical Analysis

The study was conducted over 60 days in each of the process variants, i.e., three times longer than the HRT (20 days). AS and biogas were sampled for analysis every three days. Statistical analysis of the obtained results was performed using the STATISTICA 13.1 PL software package. Verification of the hypothesis concerning the distribution of each tested variable was determined using the Shapiro–Wilk W-test. Univariate analysis of variance was conducted to determine the significance of differences between variables. Verification of the homogeneity of variance in the groups was performed using the Levene test. Tukey's honestly significant difference test was applied to determine the significance of differences between the analyzed variables. In the tests, a significance level of $p = 0.05$ was assumed.

3. Results and Discussion

Many studies to date have suggested that SMF promotes microbial biocenosis formation and biochemical conversion by pollution-degrading microorganisms. It was shown that the SMF-based systems produced nitrification rates several times higher at an induction strength of 7 mT [49,50]. Chacón Alvarez et al. (2006) determined that SMF (5 mT) exerted a positive effect on the production of nisin by *Lactococcuslactis* [51], whereas Yavauz and Celabi (2000) found that the use of SMF in the range from 8.9 to 46.6 mT in an activated sludge process increased the biodegradation of organic compounds in the effluent by 44% [52]. Literature reports and the authors' own research to date inspired the present study on SMF use to promote anaerobic digestion of MSS. The average SMF induction of 17.6 mT was chosen for the present work on the basis of previous scientific reports. Yavauz and Celabi (2000) found that microorganisms exhibited the highest biochemical activity levels at SMF of 17.8 mT. As the SMF induction levels increased, the efficiency of organic compound biodegradation decreased significantly [52]. This was corroborated by Ji et al. (2010), who examined the effect of 0–20 mT SMF on the bio-treatment of wastewater, and determined an optimum SMF induction for stimulating microbial metabolism and improving treatment efficiency, peaking at 17.8 mT [53].

The present study showed no significant ($p = 0.05$) effect of SMF on biogas yields. The $Y_b^{VS_{in}}$ ranged between 621 ± 37 $dm^3/kgVS$ (V1) and 662 ± 42 $dm^3/kgVS$ (V3) (Figure 2). The daily production was close to 5 dm^3/day regardless of SMF exposure time (Figure 3). No correlation was also observed between the process variant used and the biogas yield (Figure 2).

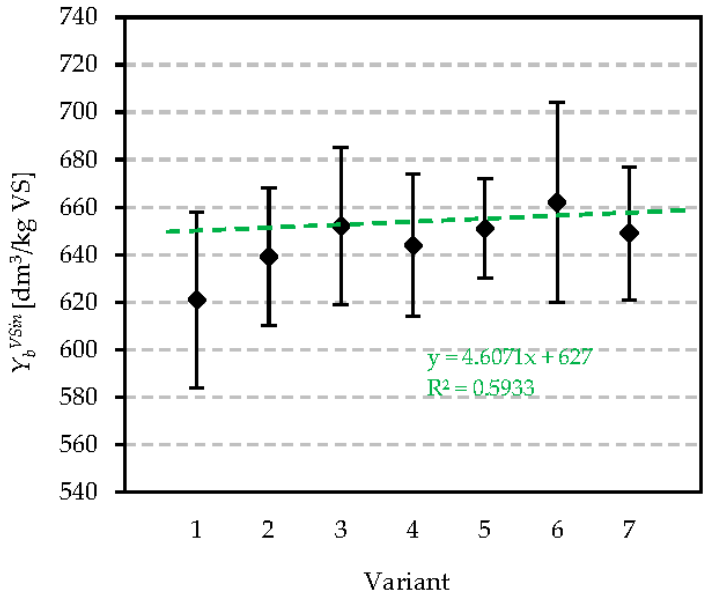

Figure 2. One-time biogas production efficiency.

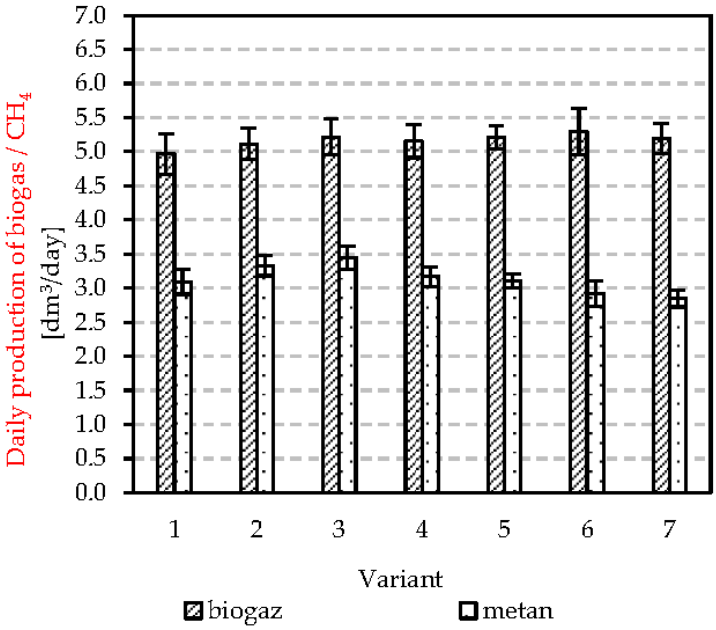

Figure 3. Daily biogas and CH_4 yield.

Zhao et al. (2020) reported opposite results, indicating SMF could potentially enhance biogas production by converting agricultural waste into energy. An experimental biogas production system, composed of a flat circular permanent magnet with a central hole to provide an SMF, was designed to boost biogas production during anaerobic fermentation of non-pre-treated corn stover substrate. 11.4 mT (±2%) was found to be the optimal SMF strength, leading to an increase in biogas production of 19.5% against the non-SMF system [54]. Similarly, Dębowski et al. (2014) found that SMF exerted a positive effect on biogas production in an anaerobic reactor used to treat milk-industry sewage. The experiment examined the magnetically-active filling (MAF) equipped with neodymium magnets at the induction of 0.39 T. The MAF components were placed in the zone of hydrolysis. The study showed increased biogas production and CH_4 levels when SMF was used. The efficiency of milk-industry sewage digestion was found to directly correlate with the number of MAF components incorporated into the anaerobic reactor, i.e., rising with

the number of magnetically-active fittings introduced into the reactor [55]. Similar results were obtained in a respirometric assay used to examine the digestion of milk-industry effluent under static conditions [19]. A magnetic intensity of 230–260 mT increased gas production from cow manure by 203% in a medium-temperature anaerobic fermentation environment [56].

The authors' own research showed that MFAs had a significant impact on CH_4 production efficiency (Figure 4), as well as on η_FMSS and ηVS, and the structure of the anaerobic bacteria consortia (Table 3). CH_4 levels in V1 averaged 62.3% ± 3.1% (Figure 5), with $Y_{CH_4}^{VS_{in}}$ at 387 ± 23 $dm^3{}_{CH4}$/kgVS (Figure 6). The η_FMSS reached 68.6% ± 2.1%, with ηVS at 33.8% ± 1.4% (Figure 7). Bacteria (EUB338) and Archaea (ARC915) predominated in the microbial community with proportions of 71% ± 12% and 24% ± 9%, respectively (Table 3).

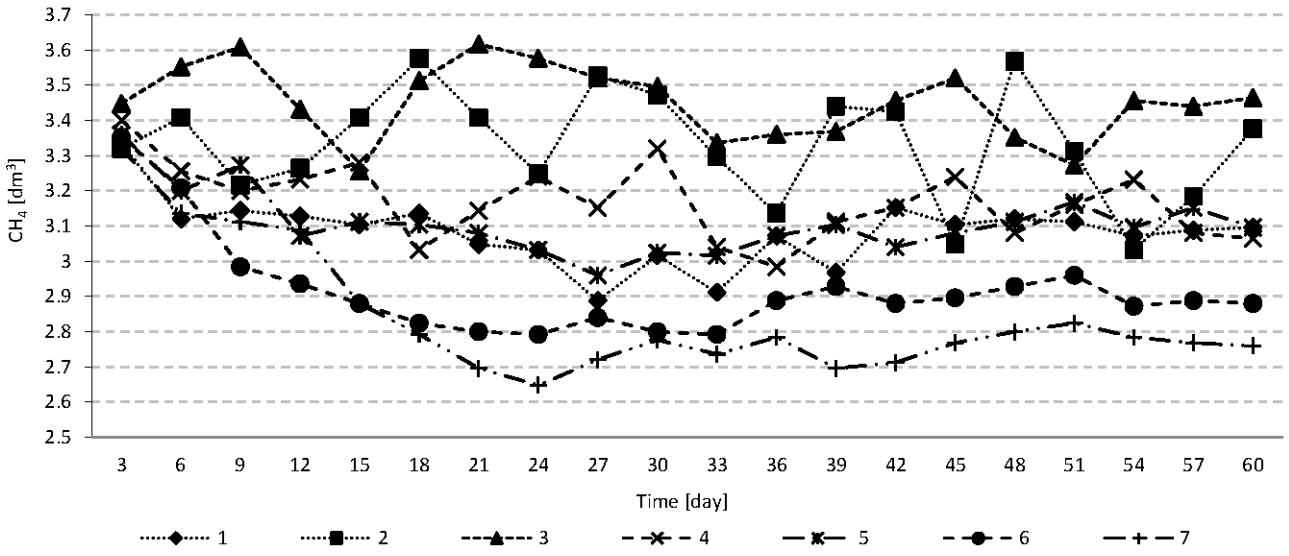

Figure 4. The course of CH_4 production during the experiment.

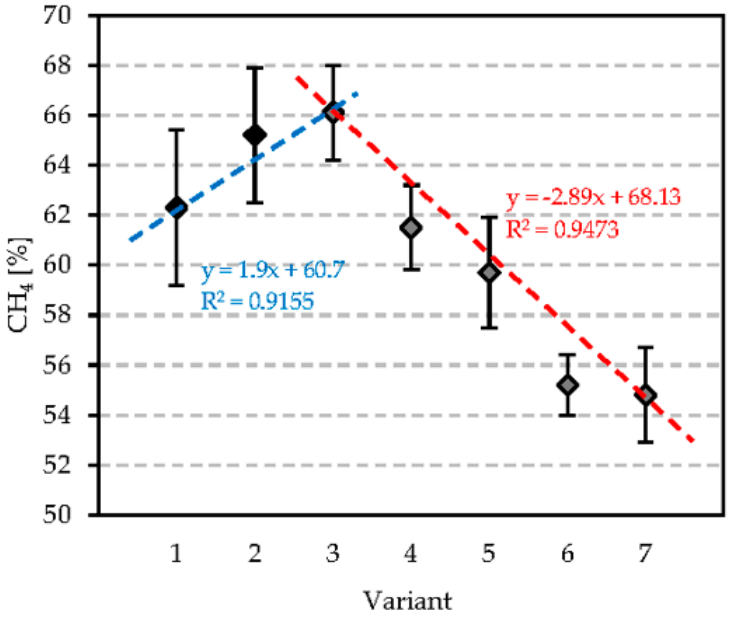

Figure 5. CH_4 in biogas.

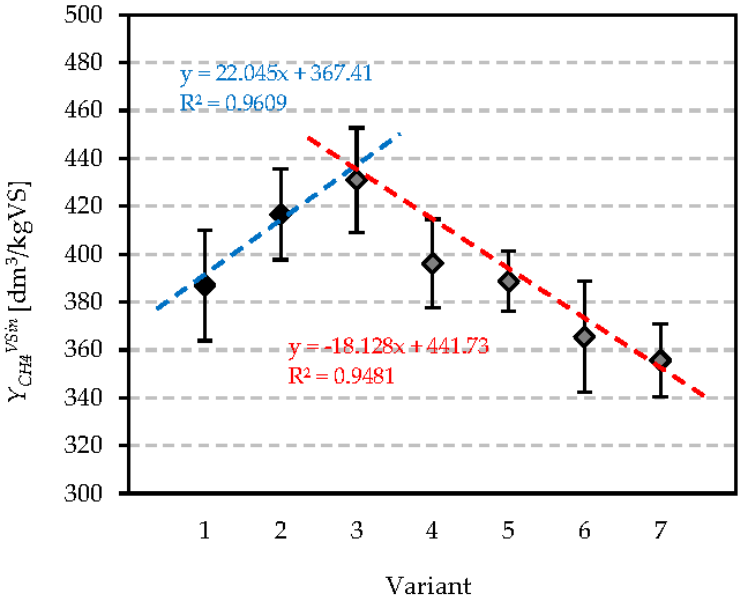

Figure 6. One-time CH_4 production efficiency.

Table 3. Microbial taxonomy in AS depends on the experimental variant.

Consortium	Variant						
	V1	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**
Bacteria (EUB338)	71 ± 12	68 ± 14	68 ± 11	70 ± 18	71 ± 17	73 ± 13	73 ± 14
Archaea (ARC915)	24 ± 9	27 ± 11	29 ± 13	27 ± 7	23 ± 12	21 ± 10	20 ± 11
Methanosarcinaceae (MSMX860)	12 ± 5	13 ± 4	16 ± 7	13 ± 4	11 ± 6	9 ± 3	9 ± 3
Methanosaeta (MX825)	9 ± 4	8 ± 5	8 ± 3	7 ± 3	8 ± 4	7 ± 3	6 ± 2

Higher $Y_{CH_4}^{VS_{in}}$ was noted in V2 and V3, reaching 417 ± 19 dm^3_{CH4}/kgVS and 431 ± 22 dm^3_{CH4}/kgVS, respectively (Figure 6). The CH_4 content in the biogas was at 65.2% ± 2.7 (V2) and 66.1% ± 1.9% (V3) (Figure 5). The average values were higher compared with V1, but the differences observed were not statistically significant (p = 0.05). The η_FMSS reached 70.3% ± 1.7%, with ηVS at 34.9% ± 2.2% (V2), η_FMSS at 73.8% ± 2.3%, and ηVS at 36.9% ± 1.6% (V3). These AD indicators in V3 were significantly (p = 0.05) higher than in V1 (Figure 7). The SMF exposure time was found to have a positive linear relationship with the CH_4 levels in the biogas, $Y_b^{VS_{in}}$, η_FMSS, and ηVS. The coefficient of determination (R^2) for these indicators was >20.5%. Other research has found that CH_4 yield increased by 11.7% in anaerobic fermentation treatment with the addition of magnetic biochar using municipal solid waste as a substrate [57]. SMF has been found to increase methane production using algae as a substrate [58]. High biogas production (449–457 dm^3/kgVS) with large CH_4 content (65.0%) were obtained from algae biomass under a constant magnetic field of 0.6 T [58].

The proportion of Archaea (ARC915) in the anaerobic microbial communities increased to 27% ± 11% (V2) and 29% ± 13% (V3) (Table 3). A very strong correlation was found between the proportion of Archaea (ARC915) and Methanosarcinaceae (MSMX860) in the methanogenic microorganism consortium and $Y_{CH_4}^{VS_{in}}$. The coefficients of determination were R^2 = 0.914 and R^2 = 0.911, respectively (Figure 8). However, the proportion of Bacteria (EUB338) was found to have no effect on the efficiency of methanogenesis, with a slight negative correlation between this group of microorganisms and ηFMSS (R^2 = 0.612) (Figure 9).

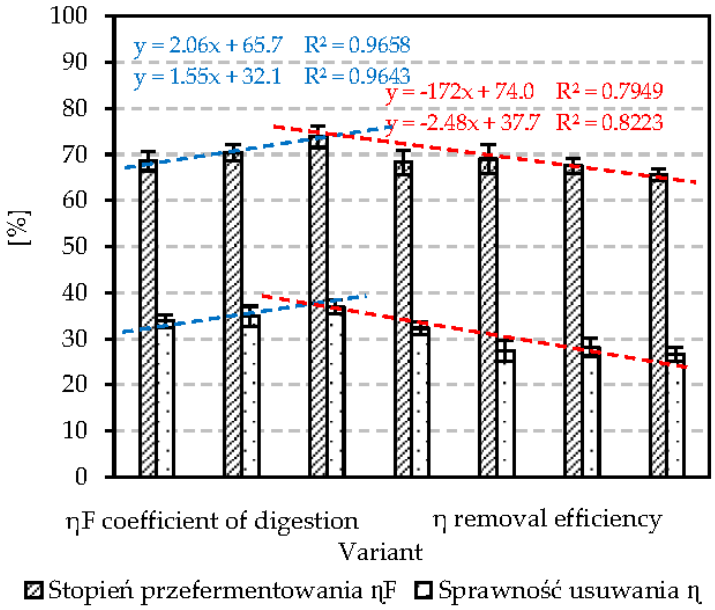

Figure 7. AD performance indicators.

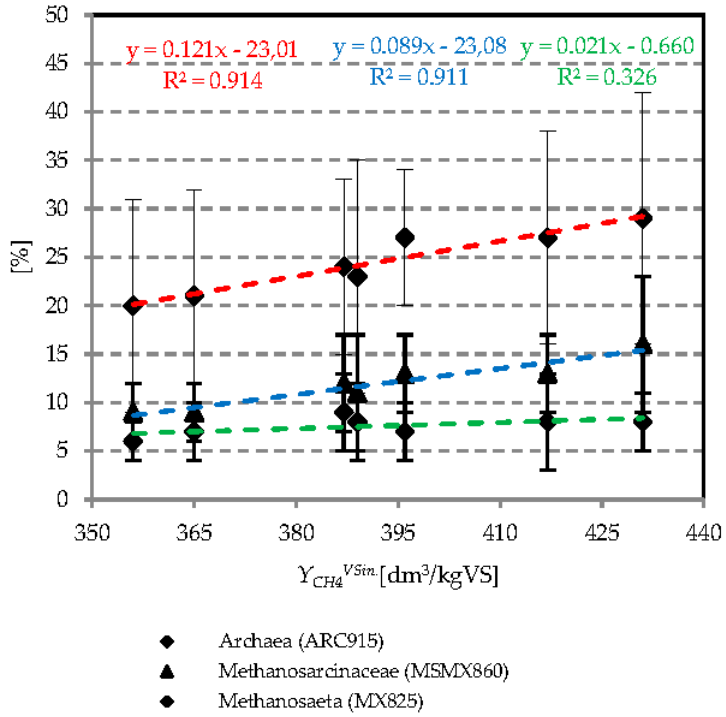

Figure 8. Relationship between the proportions of bacteria and biogas yield in relation to the vs. in the influent ($Y_{CH_4}^{VS_{in}}$).

Increasing the quantity and activity of methanogens is also an effective method to increase gas production [59]. Zhao et al. (2020) showed that 11.4 mT (±2%) SMF led to increases in microbial counts by 85.5%and CH_4 yields by 20.0% against the non-magnetic field (NMF) control. The SMF also changed the structure of bacterial communities by significantly increasing the proportion of methanogenic archaea. The Methanocellales order archaea were the major methanogenic archaea found in the decay stage [54]. The SMF-assisted bioaugmentation during anaerobic fermentation serves to enhance the metabolic activity, enable the selection of a specific bacterial community, and promote cell growth. By acting on metal ions, the SMF stimulates the activity of biological enzymes and the

subsequent metabolic enzymatic reactions [60]. The length of the bacterium Eikelboom Type 0092 increased in the SMF-exposed activated sludge in contrast with the control [61]. Furthermore, the SMF increased the capability of the Defluviimonas genus bacteria to detoxify intermediates in azo dye degradation [62]. Enhanced cell growth and anaerobic metabolism of yeast have been observed under low frequency (10–50 Hz) magnetic fields [63].

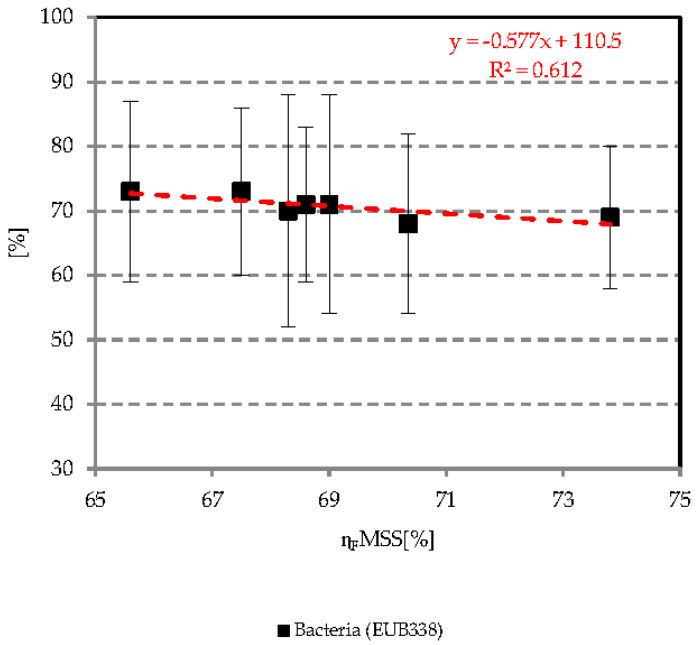

Figure 9. Relationship between the proportion of Bacteria (EUB338) and municipal sewage sludge fermentation rate (η_FMSS).

Longer SMF exposure times significantly (p = 0.05) decreased CH_4 yields in V4 and V5. The CH_4 content in the biogas was 61.5% ± 1.7% (V4) and 59.7% ± 2.2% (V5) (Figure 5), whereas $Y_{CH_4}^{VS_{in}}$ decreased to 396 ± 18 dm^3_{CH4}/kgVS (V4) and 389 ± 18 dm^3_{CH4}/kgVS (V5) (Figure 6). Bacteria (EUB338) prevailed in the anaerobic bacteria consortium, accounting for over 70%. In contrast, a significant decrease was observed in the proportion of Archaea (ARC915) and Methanosarcinaceae (MSMX860), i.e., to 23% ± 12% and 11% ± 6%, respectively (V5) (Table 3). The lowest CH_4 production levels through AD were noted in V6 and V7. The CH_4 content in the biogas fell within the narrow range of 54.8% ± 1.9% (V7) to 55.2% ± 1.2% (V6) (Figure 5). The $Y_{CH_4}^{VS_{in}}$ values were also similar, approximating 360 dm^3_{CH4}/kgVS in both variants (Figure 6). The proportions of Archaea (ARC915) in the anaerobic microbial communities decreased to the lowest of the recorded values, i.e., 20% ± 11%, whereas that of Methanosaeta (MX825) fell to 6% ± 2% (V7) (Table 3). Variants V4 to V7 produced similar levels of η_FMSS—from 65.6% ± 1.3% (V7) to 69.0% ± 3.1% (V5), and ηVS—from 26.6% ± 1.5% (V7) to 32.3% ± 1.3% (V4). The observed efficiencies were significantly (p = 0.05) lower than in variants V2 and V3. No significant variations in $Y_{b/CH_4}^{VS_{removed}}$ were noted in any of the variants. Its values ranged from 1764 ± 180 dm^3/$kgVS_{removed}$ (V3) to 2138 ± 105 dm^3/$kgVS_{removed}$ (V7) in the case of biogas and from 1131 ± 82 dm^3/$kgVS_{removed}$ (V6) to 1194 ± 83 dm^3/$kgVS_{removed}$ in the case of CH_4 (Figure 10).

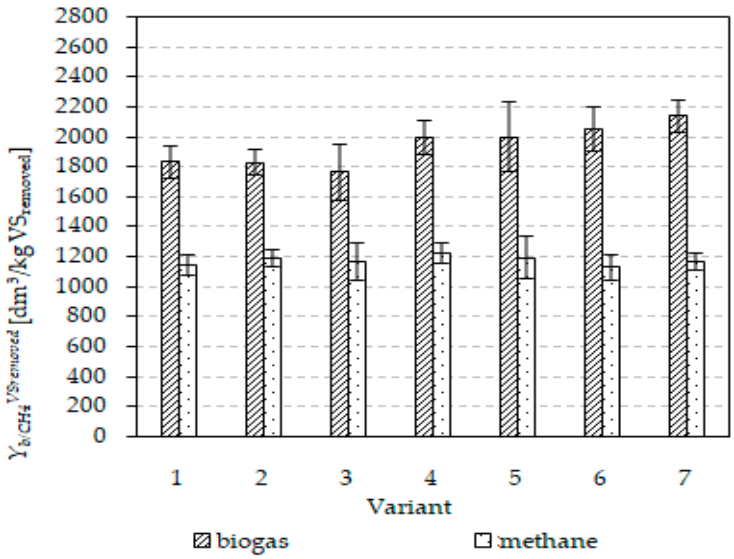

Figure 10. Biogas and CH_4 production in relation to VS removed.

Other studies have also shown a link between SMF exposure time and process performance [64]. The decreased efficiency of AD in variants V6 and V7 may have resulted from the production of free radicals within anaerobic bacteria cells. It was shown that SMF may inhibit the intersystem crossing, leading to decreased levels of free radicals in the biological system through the formation of bonds between them. For this reason, magnetic fields are thought to be drivers of free radical generation [65,66]. The negative impact of magnetic induction increase on the biochemical activity of microorganisms was also reported by Chen and Li (2008), who demonstrated that the SMF induction strength affected the production of polyhydroxyalkanes from short-chain fatty acids in SBR reactors. They showed poly (3-hydroxybutyrate) (PHB) synthesis peaking at the magnetic induction level of 7 mT, with the lowest productivity recorded at 42 mT [67].

The studies to date do not clearly indicate the placement of SMF in the chain of microorganism-based processing. The MFAs were fitted in SBR reactors on activated sludge recirculation hoses, ensuring that activated sludge microorganisms were periodically exposed [50]. MAPs were similarly distributed in the authors' own research examining the potential of using SMF 17.6 mT to improve the efficiency of anaerobic digestion of MSS. Other studies indicated that the use of SMF promoted the formation of free hydroxyl radicals ($OH^{\cdot}$), whose oxidizing potential was used in the process of wastewater treatment. As in the previous example, the wastewater was repeatedly pumped through the SMF area after Fenton's reagent was introduced [68].

In the presented research, a low C/N ratio was observed both in MSS (10 $\pm$ 1.2) and in AS (11 $\pm$ 1.2) (Table 2), which differs from the value considered optimal for AD, ranging from 20 to 30 [69]. However, it should be noted that in many cases, the methane fermentation runs efficiently in a much wider C/N ratio ranging from 9 to 50 [70]. In the present study, the AS (inoculum) and MSS (substrate) were derived from an anaerobic chamber operating for about 20 years on an industrial scale when a C/N ratio of about 10 is used. The anaerobic sludge is adapted to this C/N value. This technological parameter had no negative impact on the course of the AD. Other researchers have also pointed to the possibility of successful adaptation of anaerobic sludge to the low C/N ratio and higher ammonia concentrations [71].

The present study suggests that SMF may be considered a promising and long-term viable method for the anaerobic digestion of MSS. The economics of the process is a very strong argument for MFA use to support MSS processing systems. MFAs are characterized by a simple design and do not require any form of energy supply. When properly operated, their strength is fairly stable in the long-term. In terms of the implementation life-cycle,

they can be used in installations already in-use, without the need for adaptation and complicated upgrade works. They are easy and quick to fit onto (or remove from) any part of an active installation without the use of complex tools.

4. Conclusions

The experiment examined the effect of 17.6 mT SMF on MSS anaerobic stabilization efficiency during AD. It was found that SMF had a significant impact on CH_4 production efficiency, as well as on η_FMSS and ηVS, and the structure of the anaerobic bacteria consortium. However, the use of SMF did not affect cumulative biogas production.

The highest $Y_{CH_4}^{VS_{in}}$ 431 $\pm$ 22 dm^3_{CH4}/kgVS) and the highest methane content in the biogas (66.1% $\pm$ 1.9%) was found in the variant with an SMF exposure time of 144 min/day. The highest values were also found in this variant with regard to η_FMSS 73.8% $\pm$ 2.3% and ηVS 36.9% $\pm$ 1.6%. The proportion of Archaea (ARC915) in the anaerobic microbial communities rose to 29% $\pm$ 13%.

Extended AS retention times in the SMF area significantly decreased CH_4 yields, η_FMSS and ηVS, and methanogenic bacteria population. The lowest process performance was noted for SMF exposure time of 432 min/day. This variant produced only 54.8% $\pm$ 1.9% CH_4 in the biogas, due to a significant reduction in Archaea (ARC915) and Methanosaeta (MX825) biomass in the anaerobic microbial communities—to 20% $\pm$ 11% and 6% $\pm$ 2%, respectively, i.e., to the lowest levels produced throughout the experiment.

The experiment showed a strong correlation between the SMF exposure time and most of the AD performance indicators.

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Abbreviations

SMF	static magnetic field
AD	anaerobic digestion
AS	anaerobic sludge
MSS	municipal sewage sludge
CH_4	methane
M-WWTP	municipal wastewater treatment plant
TS	total solids
VS	volatile solids
MS	mineral solids

TC	total carbon
TOC	total organic carbon
TN	total nitrogen
TP	total phosphorus
$\eta_F MSS$	fermentation rate
ηVS	VS removal rate
$Y_{b/CH_4}^{VS_{removed}}$	biogas yield in relation to $VS_{removed}$
$Y_{b/CH_4}^{VS_{in}}$	biogas yield in relation to the vs. in the influent

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