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Headspace micro-oxygenation as a strategy for efficient biogas desulfurization and biomethane generation in a centralized sewage sludge digestion plant

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ABSTRACT

The biogas produced in a centralized digestion plant treating high-solid sewage sludge under thermophilic conditions was biologically desulfurized via in-situ headspace micro-oxygenation. The removal of hydrogen sulfide (H_2S) from the produced biogas was evaluated for 84 days under decreasing injection flows of oxygen (O_2), resulting in O_2 doses from 0.96(±0.03) to 0.19(±0.01) NL/Nm^3 biogas. A stable H_2S removal efficiency of 98.2(±1.3)% was obtained with an O_2 dose of 0.96(±0.03) NL/Nm^3 biogas, whereas removal efficiencies of 67.4 (±0.7)% were observed at the lowest O_2 dose tested. The response time of the biological desulfurization system to transient oxygen conditions was evaluated through intermittent O_2 injection. Headspace micro-oxygenation did not negatively impact the digestion performance, and the optimization of O_2 dose allowed to reach a biogas quality complying with the specification for biomethane in terms of both O_2 and H_2S contents. Lentimicrobiaceae, Caldicoprobacteraceae, DTU014, Syntrophomonadaceae, and Rhodobacteraceae were the main microbial families responsible for biological H_2S oxidation in digester headspace.

1. Introduction

AD is a biological process by which complex organic substrates, such as sewage sludge, are transformed into biogas, an energy vector, and digestate, a semi-solid residue that is nowadays considered a valuable source of organic matter and nutrients and can be used as a fertilizer [1,2]. The biogas is a well-known energetically valuable product that can be further processed to produce biomethane and/or thermal/electrical energy [3]. It typically contains 55–65% of CH_4, 35–45% of CO_2, and small concentrations of other gases, including H_2S in the range 50–5000 ppm [4]. H_2S in biogas commonly occurs due to the anaerobic fermentation of sulfur-containing organic molecules (i.e., proteins) and the activity of sulfate-reducing bacteria [5]. The presence of H_2S in biogas is often a limitation for downstream processing to generate energy, as H_2S oxidation can release harmful SO_x in flue gases and create corrosive condensates that reduce the operational life of gas pipelines, CHP units, boilers, and biogas upgrading systems [6]. Moreover, dissolved sulfide is toxic to methanogens already at concentrations above 50 mg/L and may cause the inhibition of the AD process [7].

Depending on the use of biogas and required desulfurization performance, several biogas desulfurization technologies have been proposed: biological systems, as biotrickling filters and microaeration [8,9], adsorption media such as granular activated carbon [10,11], hybrid solvent [12] or wet scrubbing systems [13,14]. Physical-chemical processes typically require large amounts of chemicals or water as well as the replacement and disposal of spent media, making operation and maintenance more complex and expensive [6,7]. In contrast, biological methods involve lower operational costs with no need for chemical addition [15]. Among these methods, the in-situ biological desulfurization consists in the injection of a limited flux of air or O_2 into the digester headspace, which triggers the biological oxidation of the H_2S contained in the biogas to SO_4^{2-} and/or S^0 by the SOB growing on the internal surfaces exposed to O_2 [16]. The redox reactions described above are shown by Eqs. (1) and (2) [5,17].

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Abbreviations

AD	Anaerobic Digestion
CH_4	Methane
CHP	Combined Heat and Power
CO_2	Carbon Dioxide
H_2S	Hydrogen Sulfide
N_2	Nitrogen gas
NGS	Next Generation Sequencing
O_2	Oxygen
PSA	Pressure Swing Adsorption
S^0	Elemental Sulfur
SO_4^{2-}	Sulfate
SOB	Sulfur-Oxidizing Bacteria
SO_x	Sulfur Oxides
TS	Total Solids
VFAs	Volatile Fatty Acids
VOCs	Volatile Organic Compounds
VS	Volatile Solids

$$HS^- + 2\,O_2 \rightarrow SO_4{}^{2-} + H^+ \quad \Delta G^0 = -732.6\ kJ/mol \tag{1}$$

$$HS^- + 0.5\,O_2 \rightarrow S^0 + OH^- \quad \Delta G^0 = -169.4\ kJ/mol \tag{2}$$

Despite the use of air can be considered the most convenient option for headspace micro-aeration, it leads to the dilution of the CH_4 produced during the process mainly due to the presence of N_2. This can turn in an important challenge especially if the target is biogas upgrading to biomethane. Indeed, the technical specification UNI/TS 11537:2019 regulating biomethane injection in the natural gas network sets the higher heating value and Wobbe index of biomethane are respectively above 34.95 ÷ 45.28 MJ/Sm^3 and 47.31 ÷ 52.33 MJ/Sm^3. Moreover, standards for CO_2, O_2 and H_2S contents respectively to ≤2.5 %$_{mol}$, ≤0.6 %$_{mol}$, and ≤5 mg/Sm^3 (about 3.5 ppm), are required.

Several technologies are today commercially available and implemented for biogas upgrading at commercial biogas plants, including water scrubbing as well as PSA and membrane-based technologies [18,19]. In the PSA process, the raw biogas is compressed at 4–10 bars and introduced into an adsorption column in which CH_4 can be separated from the impurities via selective adsorption on the filter media. However, this approach has some drawbacks, including significant treatment costs due to both a rapid saturation of the adsorbent, high CH_4 losses, and complicated process design [20,21]. Similarly, membrane separation is affected by the rapid contamination of the membranes in the presence of certain VOCs [22], the use of high pressures during the process, and the high maintenance costs, which add to the high price of the membrane [21,23]. Water scrubbing exploits the different solubilities of CH_4, CO_2, and H_2S in water. Specifically, H_2S has the highest solubility in water (3.93 g/L at 20 °C) followed by CO_2 (1.69 g/L at 20 °C) and CH_4 (0.02 g/L at 20 °C). The key parameters that govern the process efficiency are the liquid/gas ratio, pressure, and temperature. In detail, high pressure and low temperature are advantageous to the absorption of gas components into water. Typically, CO_2 is absorbed in the process water and then removed through an air stripper so that the regenerated water can be reused for the absorption process. H_2S in water acts as a weak acid, as it generates an HS^- ion and releases a proton. This last aspect is crucial because in the presence of an acidic environment the absorption of CO_2 into water is disadvantaged and the water solution cannot be easily regenerated [21], causing the production of significant amounts of wastewater and the need for frequent water make-up in the water circuit, which increases water consumption. It also should be noted that N_2 and O_2 cannot be separated by water scrubbing because of their low solubility in water [24]. In view of biogas upgrading to biomethane in AD plants, it is therefore recommended to remove H_2S upstream to optimize CO_2 elimination via water scrubbing and inject small amounts of pure O_2 (micro-oxygenation) instead of air in the digester headspace to prevent N_2 contamination of biogas. Oxygen requirements for desulfurization should take into account the amount incorporated by the process water during the air stripping process for water regeneration, in order to comply with the limits for O_2 content in biomethane.

The present study investigates biological biogas desulfurization via in-situ micro-oxygenation during the centralized digestion of sewage sludge and evaluates the impact of O_2 injection on biogas upgrading to biomethane. The desulfurization performance of three full-scale thermophilic digesters working in series was monitored for 84 days under different O_2 injection regimes with the aim to optimize both H_2S removal and biomethane production. The response times of the biological desulfurization system were verified through intermittent O_2 injection. The impact of O_2 injection on the composition of the microbial communities responsible for H_2S oxidation and methane production was also evaluated.

2. Materials and methods

2.1. Full-scale plant description

The full-scale AD plant is located near Pavia in Northern Italy and consists of three thermophilic reactors working in series and treating a high-solid (about 18.5% dry weight) feedstock mainly composed of sewage sludge. A flow scheme of the full-scale thermophilic digestion plant is reported in Fig. 1. The feedstock is heated by steam injection to 55(±2)°C and mixed with digestate coming from the third digester and water to reach a TS content of 12–14% before being fed to the first digester. Mixing in the reactors is guaranteed by continuous digestate recirculation through external pumps. A side-stream ammonia air stripping unit is coupled to digester to control ammonia build-up in the system during the digestion process, as described by Di Capua et al. [25]. The digestate exiting from the third digester is collected in two 53,000 m^3 storage tanks prior to being used as fertilizer on agricultural fields. The biogas produced during the process is sent primarily to a CHP unit, which provides the electricity needed by the whole plant, and secondarily to a boiler which guarantees the heat to maintain thermophilic temperatures in the digesters. The excess biogas is sent to an upgrading unit to produce biomethane. Once the treatment capacity of the upgrading unit is filled, the possible surplus of biogas is sent to the CHP to generate electricity destined to the national grid.

In-situ micro-oxygenation was performed by dosing O_2 with a 93 (±3)% purity (the rest being mainly N_2) in the headspace of the digesters, thus replacing the previous technique consisting in dosing air. O_2 was produced by feeding air to a PSA unit, ensuring a continuous O_2 supply with a maximum flow rate of 3.5 Nm^3/h. The O_2 flow was controlled by glass tube flowmeters (ASA, Italy). The desulfurized biogas extracted from the digester was dewatered, compressed, and treated in an activated carbon filter prior to feeding the upgrading unit. The latter consisted of a water scrubbing unit with a maximum capacity of 600 Nm^3/h of raw biogas and was formed by three columns: an absorption column operating at 4.5–6.5 bar and liquid-gas ratio of 0.21(±0.04) v/v, a flash column operating at 1.1–1.4 bar, and a stripping column fed with air to regenerate the process water.

2.2. Experimental design

The study covered a period of 84 days divided in four different experimental periods. During the study, the plant treated about 34,500 ton of feedstock composed of 90% of sewage sludge from municipal and industrial (agrifood industry) wastewater treatment plants and 10% of co-products of source segregated domestic food waste. The main operational conditions of the AD plant, including O_2 supply, during the

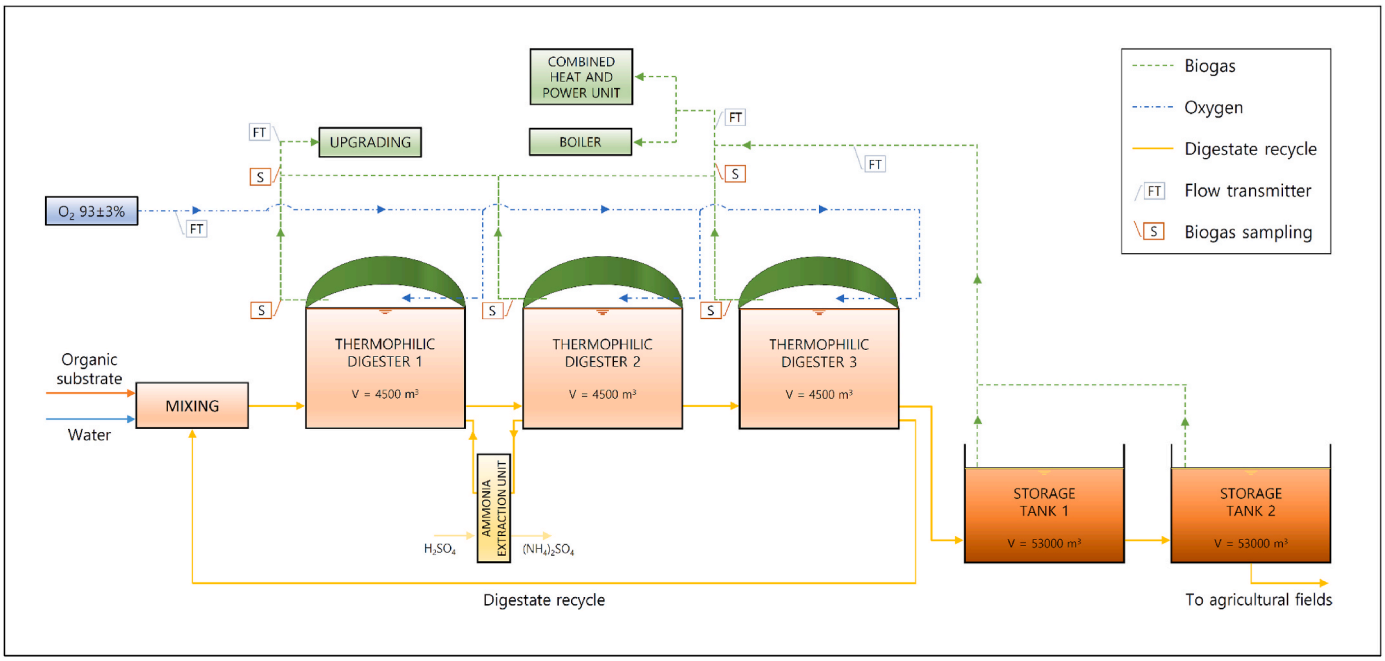

Fig. 1. Flow scheme of the full-scale thermophilic digestion plant. Biogas, oxygen, and digestate flows are indicated with lines of different colors. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

experimental campaign are described in Table 1. A full analytical profile of the sludge feedstock entering the AD plant is reported by Di Capua et al. (2020). After a start-up phase, the O_2 flow rate injected in the digesters was gradually decreased from 94.4(±1.0) to 27.5(±0.6) m^3/week, and the desulfurization and digestion performances of the plant evaluated by daily and weekly measurements. During the study, the HRT and OLR were in the range of 21.5–27.5 d and 3.3–4.4 kg VS/m^3d, respectively (Table 1).

2.3. Monitoring and analytical methods

Biogas composition in the three digesters and upstream the main biogas pipeline was monitored five times/d using an Optima 7 portable biogas analyzer (MRU GmbH, Germany). The O_2 content in the biogas sent to the upgrading unit was continuously measured through an O_2 transmitter Senz-Tx (NTRON, Ireland). The composition of the biomethane produced with the upgrading unit was provided by an assembled skid (Endress + Hauser, Switzerland). This system is composed by two gas analysers with tunable diode laser absorption spectroscopy for monitoring H_2S (Mod. SS2100i-1) and water dew point (Mod. J22), an O_2 analyser (GPR1500 GB, MICHELL, UK), and a gas chromatograph (NGC8206, ABB, Switzerland).

Feedstock and digestate samples were periodically collected for VS and TS analyses, which were carried out according to standard methods [26]. VFAs and carbonate alkalinity (defined as the proton accepting capacity of the carbonate weak acid subsystem and indicated as H_2CO_3*

Table 1
Operational conditions of the full-scale thermophilic digesters during the study.

Parameter	Unit of measure	Start-up phase	Period 1	Period 2	Period 3	Period 4
Operational time	d	0–21	22–42	43–56	57–70	71–84
O_2 flow	m^3/week	86.4 (±8.9)	94.4 (±1.0)	74.1 (±0.9)	53.8 (±4.8)	27.5 (±0.6)
HRT	d	27.5	26.7	22.0	21.5	22.1
OLR	kg VS/ m^3d	3.3	3.4	4.3	4.4	4.4

alkalinity) concentrations in the digestate were measured as described by Lahav et al. [27].

The reported H_2S concentration was monitored upstream the main biogas pipeline and calculated as a weekly average concentration, according to Eq. (3), and reported with the corresponding standard deviation.

$$\overline{H_2S} = \sum_{i=1}^{n} H_2S_i \Big/ n \quad (3)$$

with "n", the number of measurements performed during the observation week.

2.4. Microbial community characterization using next generation sequencing

Samples for the identification of microbial communities were collected at the end of the study from the surface and the wall of the three thermophilic digesters (Fig. 2) and stored at −20 °C. Total DNA extraction was performed to sequence the genome of the whole microbiota, using NGS, targeting bacterial 16S rRNA gene. For samples under analysis, 10 g were aliquoted and centrifuged to extract DNA, transferring 2 mL supernatant in sterile vials containing 0.5 g glass beads. The recovery of total DNA was performed using CTAB extraction protocol [28]; extracted DNA samples were amplified employing PCR, utilizing V3 and V4 primers, complementary to the V3–V4 variable region of 16S rRNA bacterial gene (V3: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWCGAG; V4: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC), and then sequenced with NGS analysis employing MiSeq Illumina platform, with 2 × 300 bp paired end, 600 cycles, according to manufacturer's instructions (Illumina MiSeq, USA). The diversities in the microbial communities data were evaluated, using weighted UniFrac distance and ANOVA (using Bray Curtis distance, Mothur), by anosim [29].

2.5. Statistical analysis

The statistical significance of the data was assessed through a one-

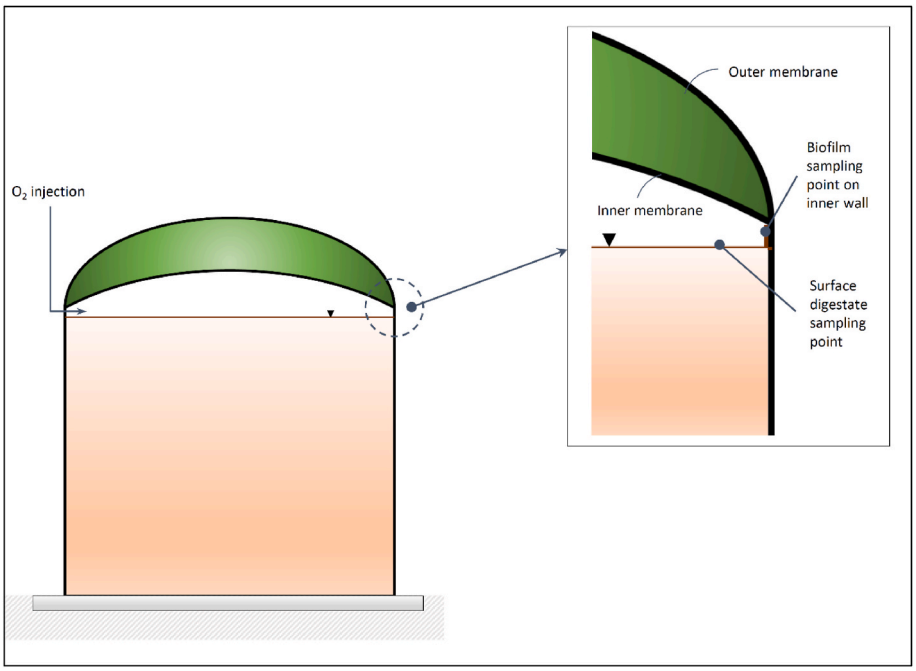

Fig. 2. – Sampling point for the identification of microbial communities in the three thermophilic digesters.

way ANOVA statistical test using the Microsoft Excel (Office 365, Microsoft Corporation, USA) statistical package and considering significant the differences among the different datasets when p-value <0.05 was obtained.

3. Results and discussion

3.1. Desulfurization performance of headspace micro-oxygenation

The temporal profiles of O_2 dosage, H_2S concentration, and biogas produced from the three thermophilic digesters monitored in this study are shown in Fig. 3.

H_2S concentration in the produced biogas was 2.6(±0.3) ppm during the first experimental period, when O_2 was dosed at approximately 1 NL/Nm^3 biogas to the digester headspace. H_2S concentration in the biogas during this period was already compliant with the admissible limit for biomethane according to UNI/TS 11537:2019. Reducing the O_2 flow rate to 0.59(±0.03) NL/Nm^3 biogas in period 2 increased the H_2S levels in the biogas to >10 ppm and up to 32.6(±0.7) ppm ($p < 0.05$) in the last experimental period when O_2 dose was decreased to 0.19 (±0.01) NL/Nm^3 biogas (Fig. 3). Based on the obtained data, a linear relationship ($R^2 = 0.97$) between the logarithmic of the residual H_2S concentration in biogas and the oxygen dose was observed, as outlined in Fig. 4.

It should be pointed out that a specific logarithmic relationship between H_2S concentration and O_2 dosage can be defined for every plant, as it depends on the feedstock, operational conditions, and reactor configuration. This relationship can provide a decision-making tool indicating an optimal O_2 dosing for desulfurization, which takes into account the daily fluctuation of the produced biogas, and can help plant operators to reduce the costs for oxygenation while improving biogas quality.

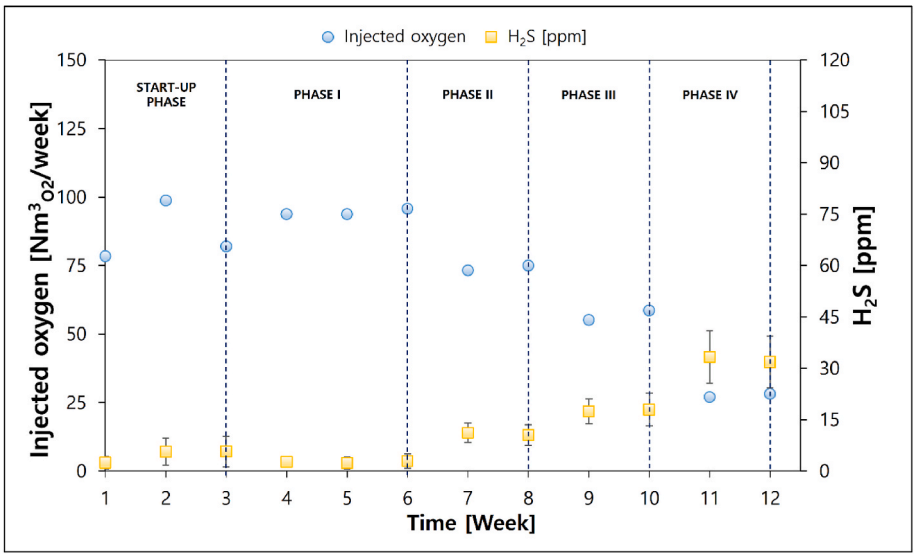

Fig. 3. Temporal profiles of injected O_2, biogas flows, and average H_2S levels in the produced biogas.

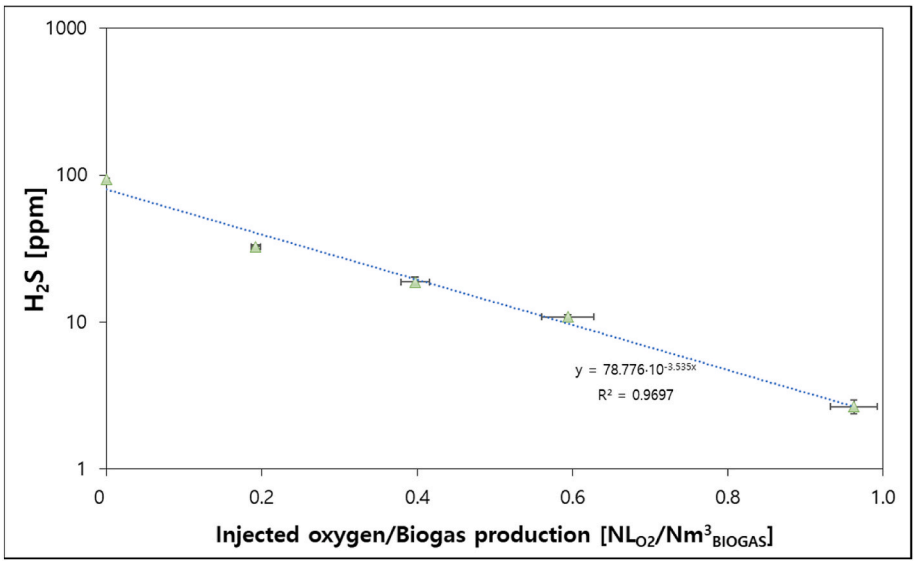

Fig. 4. Relationship between the residual H_2S concentration in biogas (logarithmic axis) and the ratio between injected O_2 and biogas produced from the three thermophilic digesters.

Fig. 4 also shows that the H_2S concentration without O_2 injection in the biogas was estimated to be around 100 ppm. This value is lower than that reported in other studies for AD plants treating sewage sludge [16, 30], which could be related to the presence of the ammonia stripping unit. Specifically, during the AD process the ammonia content in the digestate is controlled via a side-stream ammonia stripping with air as stripping agent. Within this process, part of the H_2S contained in the digestate can be stripped together with ammonia and reduce the H_2S content of the biogas. As a consequence of the low H_2S levels, the specific O_2 dosage was lower compared with previous experiences. For instance, Kraakman et al. [31] removed over 80% of the H_2S from the biogas under microaerobic condition at full scale by providing an amount of O_2 of about 0.26–0.70 L per L of feed sludge (i.e., 0.86–2.04 L of injected O_2 per Nm^3 of biogas produced). In another study, Jeníček et al. [30] reported that a H_2S removal efficiency of about 99% was achieved in 7 full-scale microaerobic digesters when 6.72 L of O_2 were fed per Nm^3 of biogas produced. Similarly, Kobayashi et al. [16] indicated a complete desulfurization at a O_2 dosage of 4.65 L per Nm^3 of biogas produced in a full-scale anaerobic digester. On the other hand, the mass of O_2 required per g of H_2S oxidized observed in this study was higher than that reported in previous experiences.

Based on the data collected during the test, it was possible to estimate an O_2 mass required of 3.99 g per g of H_2S oxidized. This ratio is approximately twice compared to that estimated according to the stoichiometry (**Eq. (1)**) and to those reported by Jeníček et al. [30] for biogas desulfurization during conventional AD of sewage sludge, being respectively of 2 and 2.02 g O_2/g H_2S (Table 2). This difference can be associated to the oxidation of organic compounds (i.e., VOCs including alkanes and aromatic hydrocarbons) in addition to H_2S within the biogas. Indeed, centralized AD can lead to the generation of higher concentrations of VOCs due the high solid content of the sludge entering the process [32]. The higher O_2/H_2S ratio estimated in this study is of fundamental importance for the design of the desulfurization system of full-scale centralized plants performing AD of high-solid (dewatered) sewage sludge, as it indicates that O_2 dosage should be doubled compared to conventional AD to ensure satisfactory biogas desulfurization.

The results of the intermittent O_2 feeding to the digester headspace are illustrated in Fig. 5. After stopping O_2 injection, H_2S concentration in biogas increased gradually from about 13 ppm to a maximum of 95 ppm within 9 h. After reinjecting O_2 at a flow rate of 0.55 NL/Nm^3 biogas, H_2S dropped to a stable value of about 10 ppm in 5 h. The test revealed that H_2S build-up in the digester as well as the response time to the restart of oxygen feeding after a period of interruption occurred within 10 h. This information is useful for plant operators as it indicates a time interval for interventions to prevent reaching high H_2S levels in the biogas which could deteriorate biogas quality in view of its upgrading to biomethane.

Table 2
Comparison of biogas desulfurization performances obtained with in-situ micro-aeration/oxygenation during AD of different feedstocks including sewage sludge.

Digester Scale	Feedstock	Working conditions	H_2S removal	gO_2/gH_2S	Reference
Full-scale	Primary sludge + waste activated sludge	Air dose of 1.2 m^3/h	99.5%	2.02	Jeníček et al. (2017)
Full-scale	Dairy cow manure	Air dose of 20 m^3/d	68.2%	1.75	Kobayashi et al. (2012)
Lab-scale	Cow manure	Air dose with an O_2 load of 4.2 mL/gVS	99.7%	n.a.	Song et al. (2020)
Lab-scale	Primary sludge	Air dose of 20 mL/h	90%	n.a.	Andreides et al. (2021)
Industrial pilot-scale	Sewage sludge	O_2 dose of 12–34 L/m^3/d	99%	n.a.	Ramos et al. (2014)
Pilot-plant scale	Sewage sludge	O_2 dose of 1.8 (±0.1) NmL/min	98%	n.a.	Díaz et al. (2011)
Pilot scale	Dairy manure	Air dose ranging from 5 to 150 mL/min	99%	n.a.	Mulbry et al. (2017)
Full-scale	High-solid sewage sludge	Air dose ranging from 0.09(±0.02) to 0.77 (±0.13)	~100%	n.a.	Giordano et al. (2019)
Full-scale	High-solid sewage sludge	O_2 dose of 0.96(±0.03) NL/Nm^3 biogas	98.2%	3.99	*This work*

[1] n.a.: The data provided by the authors do not permit the computation of the value specified in the table.

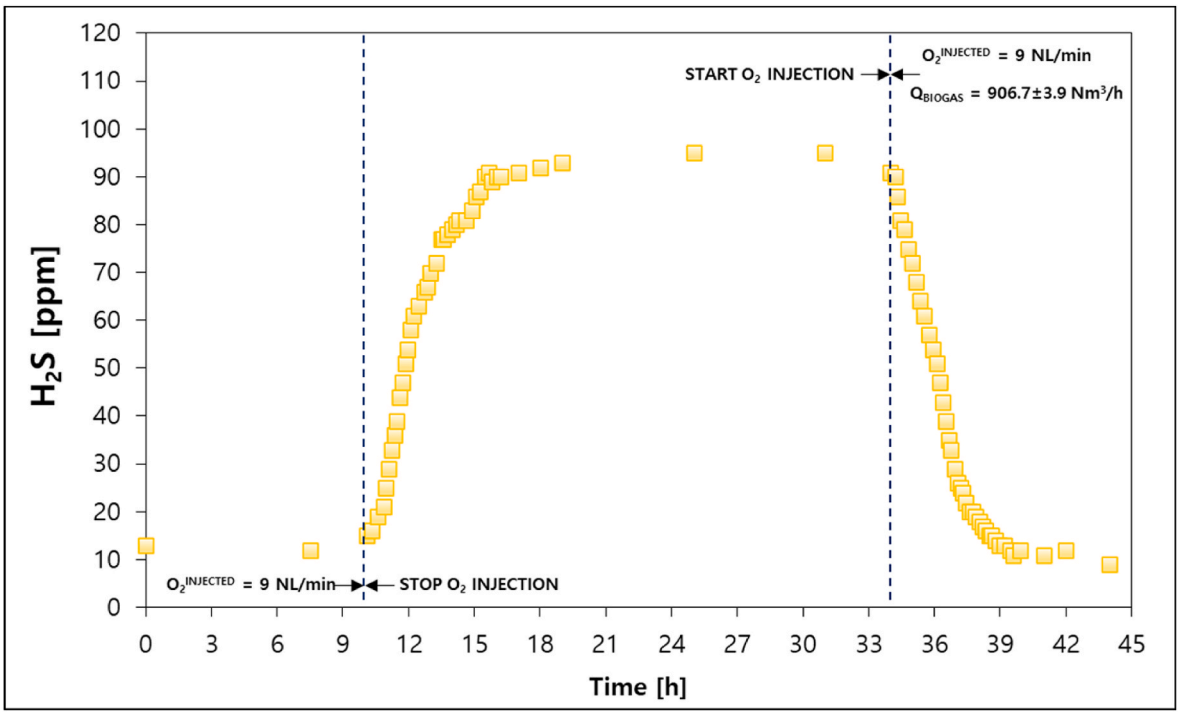

Fig. 5. H_2S build-up and consumption in the biogas collected from the three digesters during the intermittent oxygenation test.

3.2. *Impact of micro-oxygenation on anaerobic digestion and biomethane quality*

During the experimental campaign, no detrimental impact due to headspace micro-oxygenation were observed on the AD process (Table 3). The total VFAs and carbonate alkalinity at the outlet of the third digester remained stable at a daily average of 0.68(±0.14) g HA_c/kg digestate and 10.7(±0.4) g $CaCO_3$/kg digestate ($p > 0.05$). Similar results were reported in previous studies [33,34], indicating that injecting small quantities of oxygen in the digester headspace does not inhibit the anaerobic degradation of the organic matter contained in the sludge. Besides the limited amount of injected oxygen, O_2 diffusion limitation into the digestate and the rapid O_2 consumption by autotrophic SOB and facultative or aerobic microorganisms thriving closer to the surface can also play a role in protecting strictly anaerobic microorganisms. Also, some methanogens (e.g., the genera *Methanosarcina* and *Methanocella*) have shown the ability to survive to the presence of O_2 [35]. Specifically, as reported by Nguyen and Khanal [36] these microorganisms could survive after exposure of up to 500 μM H_2O_2, 1 h of aeration, and O_2 in headspace at 5% v/v, or dissolved oxygen up to 5.6 mg/L.

Biogas upgrading to biomethane started at an O_2 dosage of 0.7 ± 0.1 NL/Nm^3 biogas in the headspace of the three full-scale digesters, and the quality and composition of the produced biomethane was monitored for 30 days. The applied O_2 dosage (0.7 ± 0.1 NL/Nm^3 biogas) ensured the production of biomethane complying with the specifications required for its use (Table 4). Reducing O_2 dosage would increase H_2S concentration above the limits imposed by the technical specification (UNI/TS 11537:2019) for biomethane quality, while increasing O_2 dosage above the tested range (≥1 NL O2/Nm^3 biogas) may result in excess residual O_2. It should be noted that the O_2 content (0.33 ± 0.05%) reported in Table 4 is due to the contribution of the air stripping column for CO_2 removal, since the residual O_2 in the raw biogas fed to the upgrading unit was constantly below the detection limit of 0.1%.

Table 4
Composition and properties of biomethane generated by the upgrading unit compared with UNI/TS 11537:2019 specifications.

Parameter	Unit	This study	UNI/TS 11537:2019
Higher Heating Value	MJ/Sm^3	36.86 ± 0.1	34.95 ÷ 45.28
Wobbe Index	MJ/Sm^3	48.73 ± 0.36	47.31 ÷ 52.33
CH_4	%mol	97.7 ± 0.21	≥96
CO_2	%mol	1.32 ± 0.31	≤2.5
O_2	%mol	0.33 ± 0.05	≤0.6
H_2S	mg/Sm^3	0.03 ± 0.02	≤5

Table 3
Biogas production and composition under the different conditions tested in this study.

Parameter	Unit of measure	Start-up phase	Period 1	Period 2	Period 3	Period 4
Operational time	d	0–21	22–42	43–56	57–70	71–84
Biogas production	Nm^3/week	96,207 ± 6305	98,276 ± 4094	125,278 ± 8611	138,788 ± 2226	143,315 ± 292
Specific O_2 injection	NL O_2/Nm^3 biogas	0.95 ± 0.15	0.96 ± 0.03	0.59 ± 0.03	0.40 ± 0.02	0.19 ± 0.01
CH_4	%	63.9 ± 0.9	63.7 ± 1.1	64.8 ± 0.9	64.2 ± 0.9	63.8 ± 1.5
CO_2	%	36 ± 0.7	36.3 ± 0.8	35.3 ± 0.8	35.6 ± 0.5	36 ± 0.9
H_2S	ppm	5.7 ± 0.1	2.6 ± 0.3	10.8 ± 0.3	18.7 ± 1.5	32.6 ± 0.7
H_2S removal	%	95.4 ± 1.5	98.2 ± 1.3	89.2 ± 0.3	81.3 ± 1.5	67.4 ± 0.7
Residual O_2	%	<0.2	<0.2	<0.2	<0.2	<0.2
VFAs[a]	g HAc/kg digestate	0.49 ± 0.08	0.55 ± 0.05	0.72 ± 0.06	0.78 ± 0.05	0.82 ± 0.04
Alkalinity[a]	g $CaCO_3$/kg digestate	10.4 ± 0.5	10.7 ± 0.2	10.7 ± 0.1	10.7 ± 0.2	11.1 ± 0.3

[a] VFAs and alkalinity concentrations refer to the third digester.

3.3. Sulfur-oxidizing communities in the digester headspace

The taxonomic composition at family level of the microbial communities within the three digesters (Fig. 6, Table S1) included 5 main groups (with relative abundances above 1%) that can be classified as SOB: Lentimicrobiaceae, Caldicoprobacteraceae, DTU014 (Firmicutes), Syntrophomonadaceae, and Rhodobacteraceae. Lentimicrobiaceae, represented by the genus *Lentimicrobium* (Table S2), was the dominant family, among those classified as SOB [37,38], in all collected samples being present at relative abundances between 7.1% and 26.4%. With reference to the Digester 2 and Digester 3, Lentimicrobiaceae showed higher relative abundances in the samples collected from the surface (12.4% and 26.4%, respectively), compared to those sampled from the inner walls (11.6% and 12.7%, respectively). The higher concentration of macro- and micro-nutrients in the digestate likely stimulated the growth of SOB and may explain the higher relative abundance of these bacteria on the digestate surface then on the internal walls. Indeed, the intake of water and nutrients necessary for the optimal growth of SOB is continuously provided by the digested sludge and, therefore, more available on the surface compared to the walls of the digester [16]. Conversely, the relative abundance of SOB on the digestate surface of Digester 1 was half (7.1%) than that on the internal walls (13.9%). Caldicoprobacteraceae, represented by the genus *Caldicoprobacter*, were detected in all samples with similar relative abundances of 1.2–3.6%. Bacteria belonging to Caldicoprobacteraceae are classified as autotrophic and capable of oxidizing sulfide and thiosulfate and of accumulating S^0 extracellularly [39]. The *DTU014* family was present in the three digesters with roughly comparable values ranging from 1.5% to 3.2%. Microorganisms belonging to this family have been previously described as syntrophic acetate oxidizing bacteria [40,41] and typically found in full-scale anaerobic digesters even with high ammonia levels [42].

Two other families of SOB, i.e., Syntrophomonadaceae and Rhodobacteraceae, referred as responsible for H_2S oxidation in biogas [43,44], were also present. Both were found to be predominant in the samples collected from the digestate surface (0.4–2% and 1.1–2.1%, respectively) compared to the samples from the digester wall (0.4–1% and 0.2–1.9%, respectively). Interestingly, none of the main SOB identified by Kobayashi et al. [16] as responsible for H_2S oxidation in anaerobic digesters and populating the digester walls, i.e., *Halothiobacillus neapolitanus* and *Sulfurimonas denitrificans*, were detected in the microbial communities of the three digesters analyzed in this study. It should be noted that the two applications referred to AD performed at different temperatures (thermophilic vs mesophilic) and with a different feedstock (sewage sludge vs cow manure). These differences were most likely responsible for the different composition of the microbial communities in the digesters. As an example, *Caldicoprobacter* is a thermophilic bacterium, while *Sulfurimonas* and *Halothiobacillus* prefer moderate temperatures (28–35 °C) [45].

Besides SOB, other bacteria involved in the sulfur cycle were observed in the digesters, although at relative abundances less than 0.5% (Supplementary material). For instance, sulfur-reducing bacteria including Desulfitobacteriaceae, Desulfuribacillaceae, and Dethiobacteraceae [46] were identified. These bacteria are strictly anaerobes and can reduce oxidized sulfur compounds to H_2S or S^0. The internal walls were also rich in fermentative microorganisms, whose presence should be attributed to digestate spraying. In terms of fermentative microorganisms, the most abundant families found on both surface and internal walls (27.1–39.6%) of the three digesters belong to the class of *Proteinovoracales*, gathering well-known haloalkaliphilic anaerobic bacteria able to ferment proteinaceous substrates to produce VFAs and hydrogen [47].

3.4. Energy demand and operational remarks

In this study, calculations on the energetic need of the descried in-situ desulfurization system were quantified based on an observation period of two months. The specific energy consumption was estimated as 5.9 Wh per Nm^3 of produced biogas when applying an O_2 dose of 0.7 (±0.1) NL/Nm^3 biogas. This value is lower than that of 9.4 Wh per Nm^3 of produced biogas, required for the injection of concentrated O_2 from PSA generators, reported by Díaz et al. [48], who compared this scenario to other microaerobic scenarios (injection of pure O_2 from cryogenic tanks and air injection) in industrial-scale 5000 m^3 anaerobic digesters.

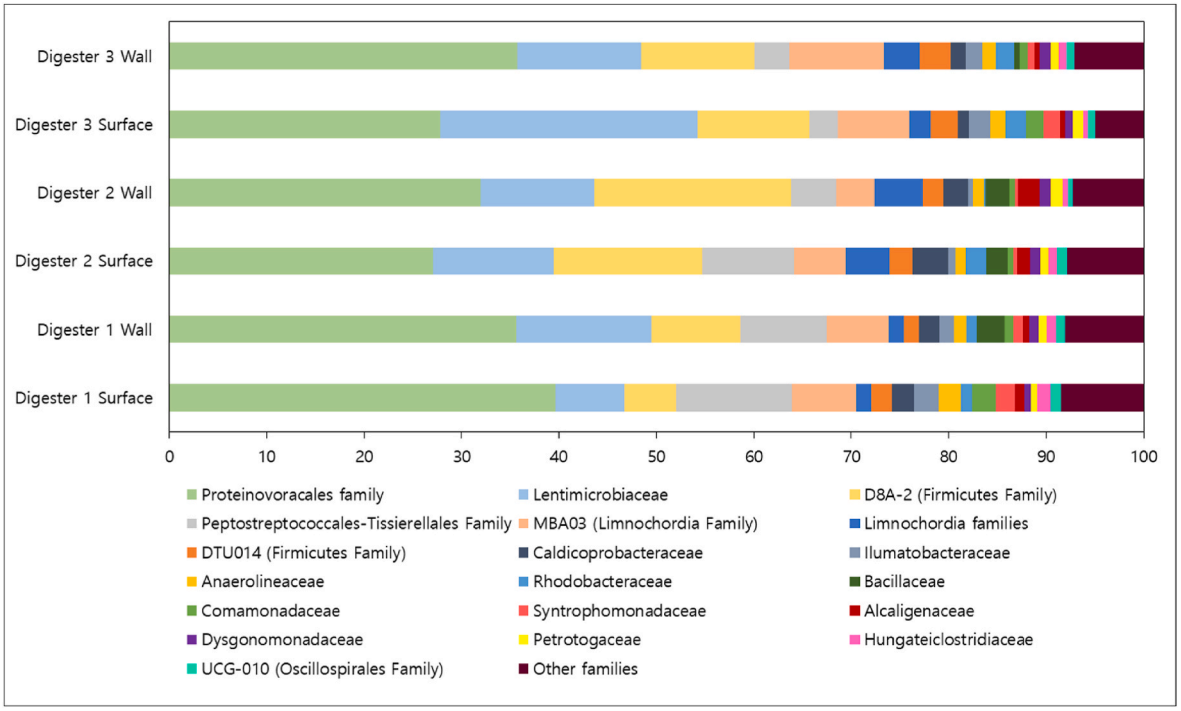

Fig. 6. Microbial community composition characterized in the three digesters at family level (relative abundances >1%).

The energy demand for in-situ biological desulfurization by pure O_2 injection was found to be slightly higher than that established by Giordano et al. [5] with the same AD plant by using air to remove H_2S. Indeed, the authors reported that headspace microaeration performed by side channel blowers required a specific electric consumption of 4.1 Wh per Nm^3 of produced biogas. Despite the slightly higher energy demand of micro-oxygenation compared to microaeration, dosing pure O_2 can be preferred in view of the subsequent biogas upgrading to biomethane, as CH_4 dilution by N_2 is prevented.

4. Conclusions

Micro-oxygenation provided biogas complying with O_2 and H_2S specifications for biomethane in a centralized AD plant treating sewage sludge. An O_2 dose of 0.96(±0.03) NL/Nm^3 biogas was estimated for complete biogas desulfurization. The response to intermittent O_2 injection for full recovery of desulfurization performance was within 10 h. H_2S oxidation was driven by SOB developed both on the digester surface and internal walls. No negative impact on AD was observed at varying O_2 dose. Although energy demand for micro-oxygenation was estimated to be slightly higher than for microaeration, using O_2 is strongly recommended if biogas upgrading to biomethane is targeted. The information provided by this study can be used by plant operators for the design, management, and optimization of micro-oxygenation systems to be implemented in full-scale thermophilic digesters and will help boosting sewage sludge conversion towards biomethane as a sustainable biofuel.

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CRediT authorship contribution statement

Nicola Di Costanzo: Formal analysis, Investigation, Visualization, Writing – original draft, Data curation. **Francesco Di Capua:** Conceptualization, Data curation, Funding acquisition, Investigation, Supervision, Writing – review & editing, Methodology. **Alessandra Cesaro:** Supervision, Writing – review & editing, Investigation. **Federica Carraturo:** Data curation, Investigation, Methodology, Writing – original draft. **Michela Salamone:** Data curation, Formal analysis, Visualization. **Marco Guida:** Resources, Supervision. **Giovanni Esposito:** Supervision, Writing – review & editing. **Andrea Giordano:** Conceptualization, Investigation, Methodology, Resources, Supervision, Writing – review & editing.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biombioe.2024.107151.

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