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Enhancing Agricultural Biogas Desulfurization: Improving Cost-Efficiency and Robustness Through Micro-Aeration with Psychrophilic Anaerobic Liquid/Solid Media

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Abstract: This study endeavors to develop an economical and user-friendly biological sulfide oxidation system and explore its mechanism for generating biological elemental sulfur under micro-aerobic conditions using psychrophilic anaerobically digested media (liquid/solid inoculums obtained from agricultural livestock wastes) for sulfide-free biogas production. With an initial hydrogen sulfide concentration of 5000 ppm, a biogas flow rate ranging from 0.9 to 1.8 L/h-$L_{inoculum\text{-}mix}$, and an air injection rate of 0.6–1% (oxygen concentration in biogas), a remarkable biodesulfurization efficiency of 99–100% was attained using solid inoculum as the biodesulfurization medium. This efficiency was achieved without compromising the methane quality in the treated biogas. Compared to liquid inoculum, solid inoculum requires less than half the volume and no mixing equipment, such as bubble column reactors. The biodesulfurization reactor requires only 1 m^3, which is approximately 1.5% of the volume of a wet anaerobic digester and 3% of a dry anaerobic digester, while processing cow manure (Total Solids: 20%) at 1.03 m^3 of manure per day. Moreover, it can be operated at (19–20 °C), leading to substantial reductions in cost and footprint.

Keywords: anaerobic medium; biogas; desulfurization; hydrogen sulfide; methane quality; psychrophilic

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1. Introduction

Transitioning to renewable energy sources such as manure biomethanization offers a promising way to reduce greenhouse gas (GHG) emissions in agriculture [1,2]. Anaerobic digestion (AD) is a key carbon-neutral technology with environmental and health benefits, including GHG reduction, waste disposal and pathogen destruction. The methane-rich biogas produced can be utilized for heating, electricity or as a supplement to natural gas [3,4]. In the United States, 263 agricultural anaerobic digesters produced 1.4 million MWh in 2020, supplying 115,039 homes [5]. In Europe, Germany leads the way, with 8780 biogas installations producing 31.5 TWh/year of electricity and 16.5 TWh/year of heat in 2018, followed by the UK, with nearly 1000 installations [6]. Meanwhile, in Asia, China has 40 million anaerobic digesters, while Nepal has 50,000 [7,8].

Biogas, an essential renewable energy source, is produced by the anaerobic fermentation of organic matter, consisting mainly of methane (CH_4: 55–70%), carbon dioxide (CO_2: 30–45%), hydrogen sulfide (H_2S: 0.1–5%), as well as trace elements such as water vapor, siloxanes and ammonia, where composition depends on the feedstock [3,9,10]. However, the widespread industrial application of biogas encounters numerous challenges, primarily stemming from the presence of H_2S, a toxic and corrosive gas which requires removal to ensure its safe and efficient utilization for biogas valorization [11]. Additionally, elevated H_2S levels, a high CO_2 content, and the presence of water vapor can slow hydrolysis rates under anaerobic conditions, while also rendering anaerobic bacteria sensitive to changes in wastewater composition [12,13]. The treatment processes required for biogas purification

vary according to its intended use. H_2S remains a significant issue, particularly for downstream applications such as heating boilers, electricity generation using internal combustion engines, and catalytic processes for methanol and biodiesel production [14]. For biogas to be suitable for combined heat and power (CHP) technology, the H_2S concentration must be below 1000 ppmV [15], while engine manufacturers generally set a stringent limit, below 100 ppmV [16].

The AD of sulfate or protein-rich waste streams, such as animal waste, can result in the formation of various sulfur-containing compounds, including H_2S and mercaptans. These compounds are problematic due to their foul odor, toxicity and corrosive properties, hence the necessity to remove sulfide from biogas. Several methods have been employed for this purpose, such as chemical precipitation, water/gas scrubbing, membrane separation, adsorption on activated carbon or metal oxides, and absorption in organic solvents [17]. However, these elimination techniques present their own set of difficulties. They often require specialized equipment, resulting in high operating costs and frequent maintenance [11,16]. In addition, these processes can consume a significant amount of energy, potentially negating the energy benefits derived from biogas production [16,17]. The disposal of spent materials or waste streams generated during the disposal process also presents difficulties [12,13]. Combined, these factors make sulfide disposal technologies less economically viable and less scalable, particularly for small and medium-sized operations [14,16].

Furthermore, research by Haghighatafshar et al. [14] highlighted the differences in sulfide inhibition between thermophilic and mesophilic AD processes. Methanogenesis in thermophilic reactors was inhibited at a sulfide concentration of around 22 mg/L (equivalent to 10,000 ppm in biogas). In contrast, mesophilic reactors showed inhibition at sulfide levels up to 50 mg/L (equivalent to 17,000 ppm in biogas). This suggests that thermophilic AD is more sensitive to sulfide inhibition during methanogenesis than mesophilic AD. However, there is limited research on low-temperature AD processes, such as psychrophilic conditions (19–20 °C), for the biogas desulfurization of livestock wastes, indicating the need for further research in this area.

In situ H_2S removal is possible in the existing AD system using micro-aeration or micro-oxygenation. The limited air/oxygen supply converts H_2S to elemental sulfur, which settles in the headspace and on the digester walls, potentially leading to clogging and requiring frequent cleaning [18,19]. In addition, there is a risk of explosion due to the presence of O_2 and methane in the biogas [20]; therefore, the oxygen concentration supplied for removal as well as the residual oxygen concentration must be carefully monitored. Nonetheless, the inclusion of iron salts or oxides in anaerobic digesters can reduce H_2S concentrations, but does not remove H_2S to the degree of purity required by engine manufacturers [20].

Shifting the focus toward the agricultural sector, livestock manure (poultry, swine and dairy) exhibits significantly higher H_2S concentrations during anaerobic digestion (AD) compared to other feedstocks like agricultural, food, and municipal wastes, with levels reaching up to 6000 ppm [21,22]. These elevated H_2S concentrations are toxic to methanogens, inhibiting anaerobic processes and lowering biogas quality [23]. There is a scarcity of studies on optimizing low-temperature biogas desulfurization specifically for livestock waste and high H_2S concentrations (5000 ppm). Innovative methods are needed to effectively address in situ removal challenges and enhance the removal efficiency with minimal energy consumption and cost.

With this in mind, the present study aimed to (i) develop a low-cost, easy-to-use biological sulfide oxidation system capable of handling high H_2S concentrations (up to 5000 ppm), and (ii) investigate a rapid biological sulfide oxidation mechanism that produces biological elemental sulfur (S^0) under anaerobic conditions. To achieve these goals, the study was conducted in two phases, each utilizing a different anaerobically digested psychrophilic medium (liquid and solid inocula) as biodesulfurization media under low-temperature conditions (19–20 °C), focusing on their distinct advantages for sulfide-free biogas production. Liquid inoculum was selected for its ability to provide effective gas–liquid interaction

and stable H_2S conversion under micro-aerobic conditions, with scalability demonstrated in larger reactor setups. Solid inoculum, on the other hand, was explored for its potential to reduce the reactor size, lower the environmental footprint, and simplify operations by eliminating the need for diffusers. However, initial challenges with airflow resistance in solid inoculum systems were resolved by incorporating wood shavings as a structural medium, which optimized gas flow and enhanced the desulfurization efficiency. Additionally, the solid inoculum offered a larger surface area for microbial growth, fostering the proliferation of sulfur-oxidizing microorganisms. Together, these strategies demonstrate the complementary roles of liquid and solid inocula in achieving efficient and sustainable biogas purification.

2. Materials and Methods

2.1. Experimental Set-Up

2.1.1. Liquid Inoculum as Biodesulfurization Medium (Phase 1)

The experimental setup for Phase 1 aimed to evaluate the use of liquid inoculum/sludge from an anaerobic digestion process for biodesulfurization under micro-aerobic conditions. Figure 1 illustrates the schematic of the experimental setup, illustrating the bioreactor, air injection system, and gas flow monitor. A 40 L reactor was inoculated with 15 L of sludge (representing the active volume), serving as the sole liquid component; this was obtained from a psychrophilic anaerobic digester treating swine manure, with the digestate containing total solids (TS) of 2.5–4% and volatile solids (VS) of 2.1–3.2%. Air was injected into the biogas at a flow rate ratio of 5:100 (i.e., air-to-biogas ratio, % v/v), indicating the volume of air relative to the volume of biogas, which ensured micro-aerobic conditions for the biochemical conversion of H_2S into elemental sulfur (S^0). The liquid inoculum environment maintained approximately 3 $\pm$ 1% total solids (TS), and the airflow rate was adjusted to achieve O_2-to-biogas concentrations of 1% (v/v), serving as the electron acceptor. The system operated with a biogas flow rate of 0.9 L/h per liter of inoculum, and both H_2S and methane (CH_4) concentrations were monitored throughout the 48-day experimental period. Synthetic biogas was utilized in the process, with CH_4 concentrations ranging from 65 to 70% and H_2S levels between 1000 and 4000 ppm, while the remainder consisted primarily of CO_2 for this phase of the study.

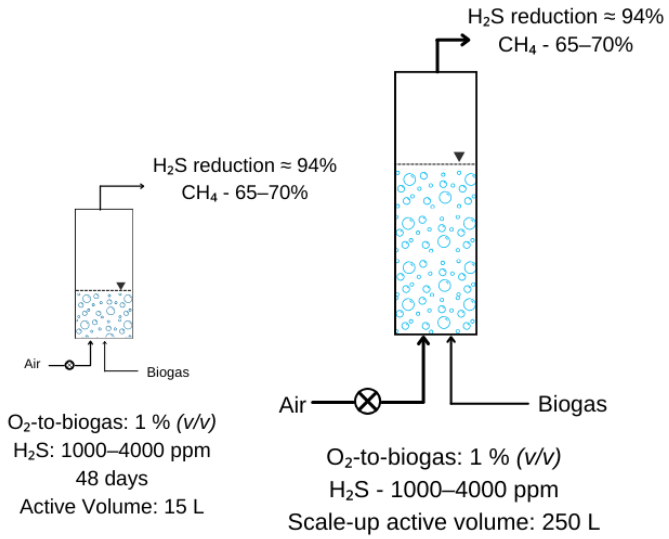

Figure 1. Experimental set-up for Phase 1 of the study using liquid inoculum media.

To validate scalability, the setup was replicated in a larger 250 L (active volume) bubble column reactor under the same conditions. This set-up provided a controlled environment for assessing the biodesulfurization potential of anaerobic sludge in agricultural biogas systems.

2.1.2. Solid-Inoculum as Biodesulfurization Medium (Phase-2)

In Phase 2, solid inoculum was sourced from a pilot-scale psychrophilic dry anaerobic digester (4 m^3 working volume) treating solid-separated cow manure (TS, 20–21%; VS, 18–18.5%). Figure 2 presents the schematic of the experimental setup for this phase. Initially, 100% solid inoculum resulted in high airflow resistance and the short-circuiting of biogas due to its high viscosity at the given flow rate. Therefore, identifying a suitable structural medium was essential for optimizing the biodesulfurization efficiency while preventing gas flow short-circuiting. To address this, wood shavings were chosen as a cost-effective and readily available structural medium to improve gas distribution and prevent short-circuiting. Various volumetric ratios of solid inoculum and wood shavings (%w/w) were tested to optimize the biogas flow rates and enhance the H_2S removal efficiency.

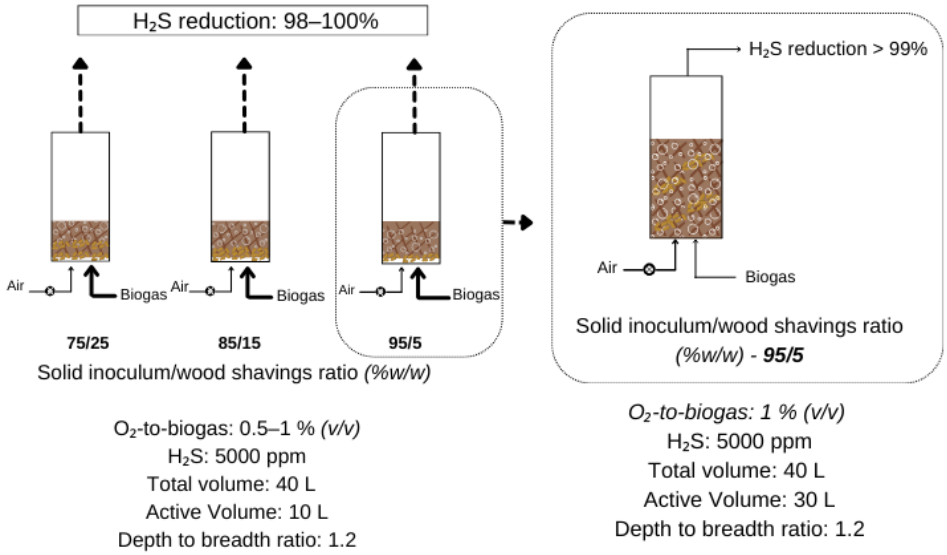

Figure 2. Experimental set-up for Phase 2 of the study using liquid inoculum media.

The experiments were conducted in 40 L reactors, with a working volume of 10 L (referring to the solid structure filling this volume) and a filling height of 16 cm. Initial trials involved three laboratory-scale reactors, using solid inoculum-to-wood shaving ratios of 95/5, 85/15, and 75/25 (%w/w). These ratios were selected based on their ability to balance the H_2S removal efficiency with the minimal use of structural material to reduce costs. This inoculum-to-wood shaving mixture is referred to as the inoculum mix in this study. The airflow rate was adjusted to achieve O_2-to-biogas concentrations between 0.5% and 1.0% (v/v), while maintaining a biogas flow rate of 0.9 L/h per liter of inoculum mix. Synthetic biogas consisting of 69.5% CH_4, 30% CO_2, and 0.5% H_2S (5000 ppm) was used in this phase. Biogas produced from the anaerobic digestion of animal manure typically consists of 50–70% CH_4 and 30–50% CO_2, with other components being negligible.

Once the optimal inoculum structure was determined, further experiments were conducted in a 40 L reactor with a working volume of 30 L (49 cm filling height), using the selected inoculum-to-wood shaving ratio of 95/5 (%w/w), which refers to the solid structure within the 30 L volume. This phase focused on optimizing the O_2 concentrations and biogas flow rates to develop a cost-effective and robust biodesulfurization system. The biogas flow rate was eventually increased from 0.9 to 1.8 L/h per liter of inoculum mix to assess the solid inoculum's capacity to handle larger volumes of biogas without compromising gas quality. Throughout the study, the system demonstrated high H_2S removal efficiency, highlighting the practical applicability of this approach for biogas purification.

2.2. Sampling and Analytical Procedures

The inoculum samples were analyzed for chemical oxygen demand (COD), sulfur, TS and VS using standard methods [24]. The gas samples were taken at regular intervals both

at the inlet and outlet of reactors during the operational period. The biogas composition (methane, carbon dioxide, H_2S and nitrogen) was determined with a HachCarle 400 AGC-gas chromatograph (Hach, Loveland, CO, USA). The column and thermal conductivity detector were operated at 80 °C.

3. Results and Discussion

3.1. Liquid-Inoculum as Biodesulfurization Medium (Phase 1)

Biodesulfurization is essential for improving the quality of biogas for practical use. This Phase 1 study investigated using a liquid inoculum from psychrophilic anaerobic sludge in a bioreactor to convert H_2S into elemental sulfur (S^0) under micro-aerobic conditions. The process demonstrated significant H_2S reduction, with initial concentrations ranging from 1000 to 4000 ppm and stable CH_4 concentrations ranging between 65 and 70% throughout the experimental period. The micro-aerobic conditions facilitated the transformation of H_2S into elemental sulfur, achieving a maximum conversion rate of 2.4 mg $H_2S/L_{inoculum}/h$ and approximately 94% H_2S reduction, particularly at elevated concentrations. The bioreactor demonstrated high H_2S removal efficiency, often reducing H_2S to below detectable limits. Consistent air injection at a controlled ratio ensured sufficient oxygen availability, which is critical for the biochemical conversion process. The optimal air flow and biogas flow rates were established at a 1% O_2-to-biogas concentration (v/v) and 0.9 L/h per liter of inoculum, effectively treating biogas containing 1000–4000 ppm H_2S and 65–70% CH_4. The transformation of H_2S into elemental sulfur aligned with theoretical expectations, validating the role of oxygen as an electron acceptor. Throughout the 48-day operational period, the system maintained stable methane concentrations and effectively managed varying H_2S loads, with CH_4 concentrations remaining unaffected by the biodesulfurization process.

Table 1 summarizes the results from the lab-scale (15 L active volume) and pilot-scale (250 L active volume) bioreactors during Phase 1. Validation using 250 L bubble column reactors confirmed the scalability of the process, matching the efficiencies observed in the initial 15 L bioreactor. Both systems achieved effective H_2S removal (>99%) while maintaining stable CH_4 (65–70%) and CO_2 (25–30%) concentrations. Total solids (TS) and volatile solids (VS) exhibited slight reductions (~3.6% and ~2.9%, respectively). Notably, 1.59 g of sulfur, representing ~15.6% of the total sulfur accumulated in the bioreactor, was recovered as deposits, demonstrating efficient sulfur conversion and recovery.

Table 1. Summary of results for lab-scale and pilot-scale bioreactors (Phase 1).

Parameter	15-L Active Volume Bioreactor	250-L Active Volume Bioreactor
Gas Composition (Inlet)		
CH_4 (Methane)	65–70%	65–70%
CO_2 (Carbon Dioxide)	25–30%	25–30%
N_2 (Nitrogen)	<1%	<1%
O_2 (Oxygen)	1% O_2-to-biogas	1% O_2-to-biogas
Gas Composition (Outlet)		
CH_4 (Methane)	65–70%	65–70%
CO_2 (Carbon Dioxide)	25–30%	25–30%
N_2 (Nitrogen)	<1%	<1%
O_2 (Oxygen)	ND	ND
Other Values (Outlet)		
H_2S Concentration	Reduced from ~1000–4000 ppm (inlet) to <50 ppm (outlet)	Similar rate observed

Table 1. *Cont.*

Parameter	15-L Active Volume Bioreactor	250-L Active Volume Bioreactor
Elemental Sulfur (S^0)	Accumulation rate: ~2.4 mg $S_0/L_{inoculum}/h$	Similar rate observed
COD (Chemical Oxygen Demand)	Initial: ~4000 mg/L, reduced during operation (~15%)	Same as 40 L (~12%, reduction rate)
Total Solids (TS)	~3.5–4.0%, reduced during operation (10–16%)	Similar rate observed (~12%, reduction rate)
Volatile Solids (VS)	~2.8–3.2%, reduced during operation (7–10%)	Similar rate observed (~8%, reduction rate)

This finding suggests that the biodesulfurization technique is viable for practical on-farm applications. The use of liquid inoculum from psychrophilic anaerobic sludge in a controlled micro-aerobic environment effectively reduces H_2S concentrations in biogas, converting it into elemental sulfur efficiently. This study provides a foundation for optimizing and implementing biodesulfurization systems in agricultural settings, contributing to cleaner and more efficient biogas utilization.

3.2. Solid-Inoculum as Biodesulfurization Medium (Phase 2)

3.2.1. Determination of Adequate Structural Mix and O_2 Concentrations

In Phase 2, the use of the solid inoculum demonstrated a biodesulfurization efficiency, representing the rate of H_2S removal, of approximately 99.8%, corresponding to an air injection of 1% O_2 concentration (over biogas) and a biogas flow rate of 0.9 L/h-$L_{incoulum\text{-}mix}$. This efficiency was achieved across all reactors during the initial 14 days of operation, regardless of the ratio of solid inoculum to wood shavings employed, specifically inoculum to structural filling ratios of 75/25 (%*w*/*w*), 85/15 (%*w*/*w*), and 95/5 (%*w*/*w*), respectively. Notably, this efficiency was observed alongside consistent CH_4 and CO_2 concentrations in the treated biogas, as illustrated in Figures 3 and 4.

Visual examination revealed the formation of a white crust starting from day 2, indicating the proliferation of sulfur-reducing bacteria within the bioreactors. Consequently, the operation of the reactors employing a 75/25 (%*w*/*w*) ratio of inoculum to structural filling was stopped on day 14, and operations continued with ratios of 85/15 (%*w*/*w*) and 95/5 (%*w*/*w*) to evaluate the potential reduction in the amount of structural medium, considering the cost implications associated with the use of wood shavings. On day 15, the O_2-to-biogas concentration in these two reactors had decreased from 1% to 0.5%, while the continuous monitoring of the biogas composition and H_2S concentrations persisted, with a constant biogas flow rate of 0.9 L/h-$L_{incoulum\text{-}mix}$ maintained throughout the duration of the study. In both reactors, the biodesulfurization efficiency declined from 99.8% to as low as 35% within 4 days, leading to a surge in the effluent H_2S gas concentrations from near-zero levels to a maximum of 3228 ppm. This rapid decrease highlighted the insufficiency of a 0.5% O_2 concentration (over biogas) in effectively mitigating H_2S concentrations in the biogas at the specified flow rate of 0.9 L of biogas/h-$L_{incoulum\text{-}mix}$. Interestingly, while H_2S concentrations began to rise at the reactor outlet, there were no discernible changes in the CH_4 concentrations from days 14 to 18. To determine the optimal conditions for biodesulfurization in biogas, the air injection rate was restored to a 1% O_2 concentration (over biogas) on day 18. Within 3 days, the biodesulfurization efficiency and H_2S concentration reverted to 99–100% and less than 5 ppm, respectively. Notably, the O_2 levels in the biogas remained undetectable, with minimal N levels, as expected due to rapid O_2 consumption by autotrophic sulfur-oxidizing bacteria (SOB).

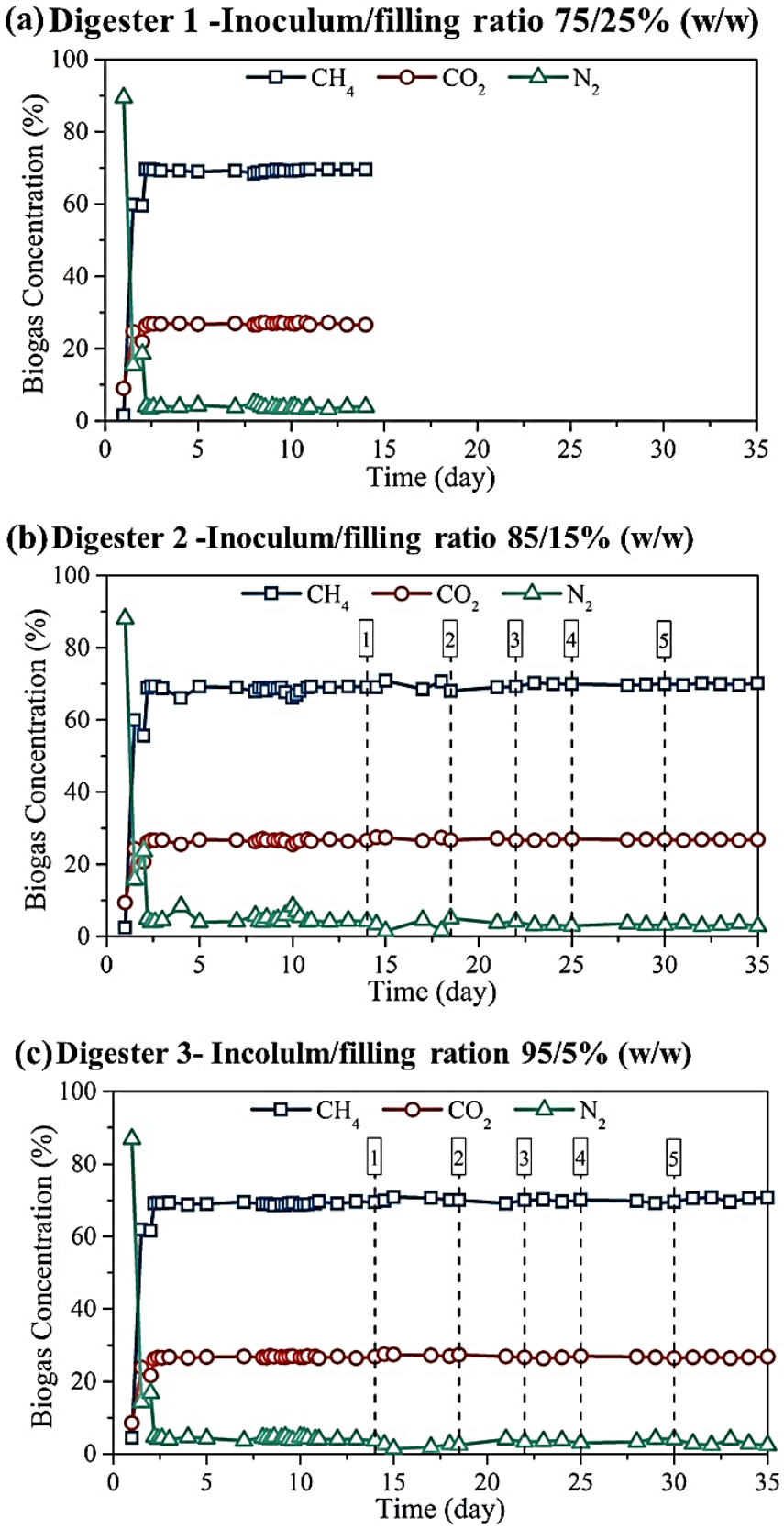

Figure 3. (**a**–**c**). Evolution of CH_4, CO_2 and N_2 concentrations in reactors filled with solid inoculum + wood shavings mix. The O_2 concentrations in the biogas changed to (1) 0.5%; (2) 1%; (3) 0.9%; (4) 0.75%; and (5) 0.6%.

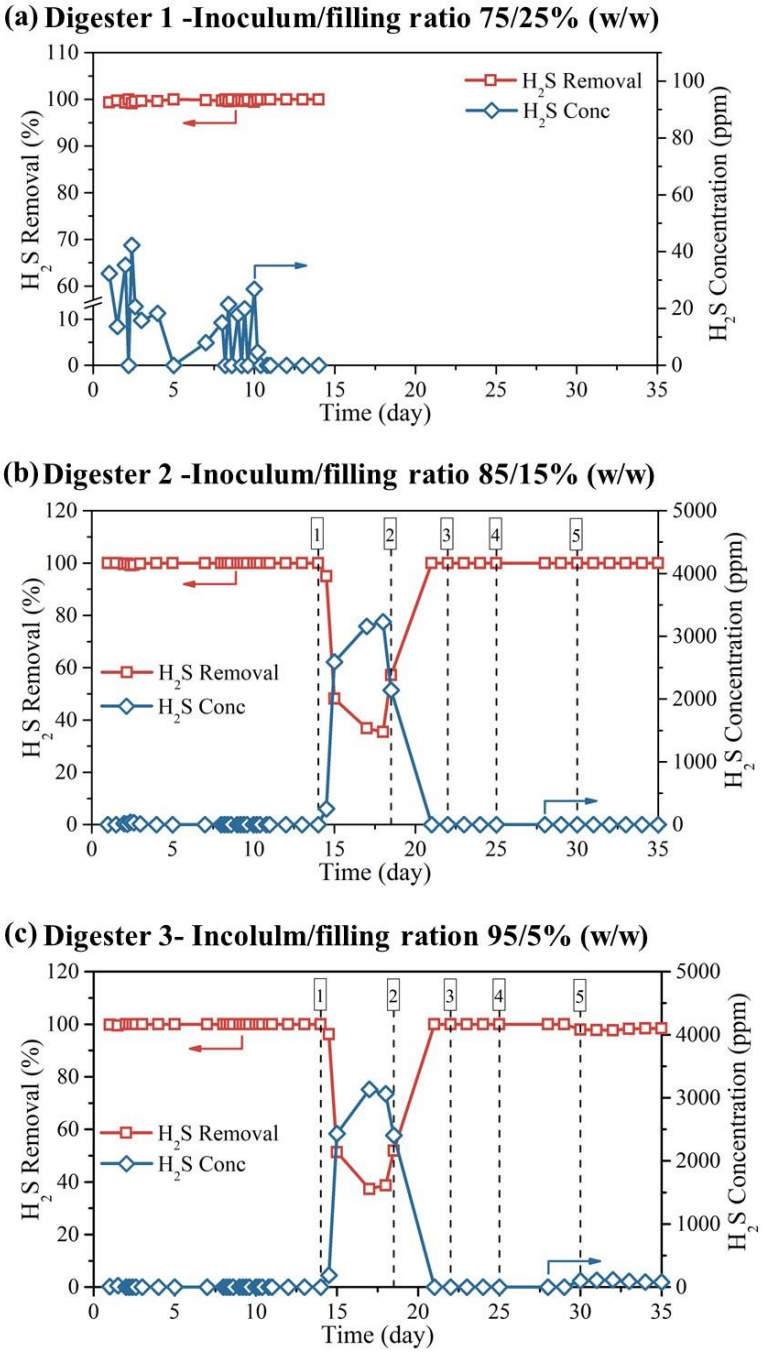

Figure 4. (**a**–**c**). Evolution of H_2S concentrations in reactors filled with solid inoculum + wood shavings mix. The O_2 concentrations in the biogas changed to (1) 0.5%; (2) 1%; (3) 0.9%; (4) 0.75%; and (5) 0.6%.

The resurgence in the biodesulfurization rate also indicates the rapid recovery of the adapted microorganisms present in the reactors from the O_2 concentration shock. Subsequently, the airflow rate was gradually reduced from 1% to 0.90%, 0.75%, and 0.60% (over biogas) on days 22, 25, and 30, respectively. However, biodesulfurization consistently remained within the range of 98–100%, and the concentrations of CH_4 and CO_2 were unaffected throughout this operational study, as depicted in Figures 3 and 4. This phase of the study concluded on day 35, with further experiments thereafter conducted solely using an inoculum-to-structural filling ratio of 95/5 (%*w*/*w*) to minimize the excessive use of structural material and additional costs.

3.2.2. Validation of Experimental Results and Determination of H_2S Removal Saturation Limit

To validate the experimental findings and establish the saturation threshold for H_2S removal, a reactor with a solid inoculum-to-structural filling mix ratio of 95/5 (%*w*/*w*) was selected. Additionally, the depth-to-breadth ratio of the solid medium mix within the reactor was increased from 0.5 to 1.2 to minimize the process footprint, particularly for full-scale applications. During the initial 7 days of operation, the air injection and biogas flow rates were maintained at a 1% O_2 concentration and 0.9 L/h-$L_{incoulum\text{-}mix}$, respectively. Simultaneously, a consistent composition of CH_4, CO_2, and H_2S was ensured at 69.5%, 30%, and 0.5% (5000 ppm), respectively. The performance of the reactors, as indicated by the evolution of the CH_4, CO_2, N_2, and H_2S concentrations, is illustrated in Figure 5.

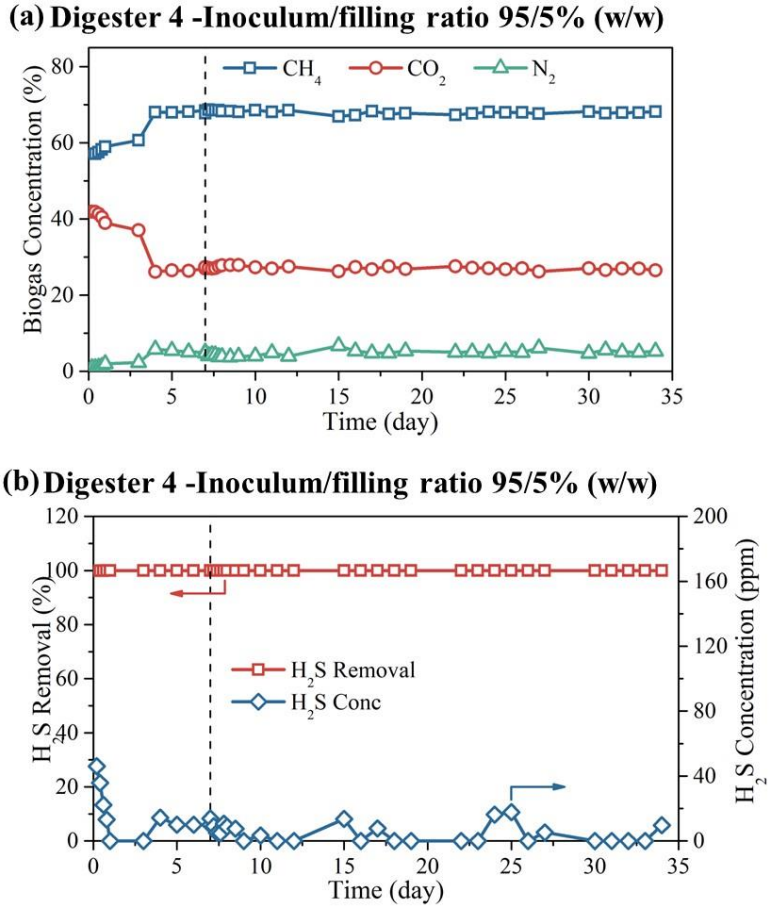

Figure 5. (**a**) Evolution of CH_4, CO_2 and N_2 concentrations; (**b**) evolution of H_2S concentrations, in the reactors filled with a solid inoculum-to-wood shavings ratio of 95/5 (%*w*/*w*).

A four-day period was required to stabilize the reactor, primarily to remove residual oxygen during the startup phase. With the H_2S removal concentration reaching nearly 100% for the given O_2 concentration (over biogas) and biogas flow rates, starting from day 8, the biogas flow rate was raised from 0.9 to 1.8 L/h-$L_{incoulum\text{-}mix}$ to assess the solid inoculum's ability to handle a greater volume of biogas without compromising the gas quality. However, the O_2 concentration and biogas compositions stayed consistent. Figure 5 indicates that the quality of CH_4 remained unaffected even with a 200% increase in biogas flow, and that the biodesulfurization efficiency persisted at nearly 99–100% for the first 35 days of operation. The negligible air-flow resistance at these flow rates suggests that increasing the depth-to-breadth ratio of the solid medium mix to 1.2 did not impede gas flow.

Accordingly, the suggested biodesulfurization technology holds significant promise in advancing the use of biogas as a renewable fuel source using feedstocks of high H_2S concentrations. Its implementation can result in reduced maintenance needs for biogas handling equipment, lower CH_4 purification expenses, and decreased emissions of SO_x. Notably, this technology eliminates the necessity for special bacterial inoculation, nutrient addition, trace elements, or pH-controlling chemicals, rendering it a cost-effective and eco-friendly solution for biogas purification. Further research is needed to fully understand the saturation limit of biodesulfurization and refine the operational parameters to ensure maximum efficiency and scalability.

4. Comparative Assessment and Recommendations

4.1. A Summary of Desulfurization Studies

Table 2 summarizes the operating conditions and biodesulfurization efficiencies reported in various studies over the years, focusing on different waste types and highlighting efforts involving micro-aeration. While most studies have been conducted under mesophilic conditions, low-temperature studies remain relatively unexplored. Micro-aeration for sulfur removal has primarily been investigated in municipal wastewater treatment plants (WWTPs), with limited comprehensive studies targeting agricultural and animal waste treatment, presenting an avenue for further research. Overall, the proposed method demonstrates biodesulfurization efficiencies ranging from 68% to over 99%.

Desulfurizing bacterial communities (primarily sulfate-reducing bacteria) are highly sensitive to temperature variations [25]. For instance, the use of a bioscrubber at temperatures below 20 °C has been shown to reduce degradation capacity, as reported in [25]. Similarly, experiments utilizing a fixed-bed trickling bioreactor demonstrated low efficiency in sub-20 °C conditions, attributed to alterations in bacterial community structures. In cold regions, such as North America, the low-temperature biodesulfurization technology presented in this study effectively removes H_2S without the need for additional energy to achieve mesophilic or thermophilic conditions. Furthermore, while several studies have examined sulfate removal using synthetic or municipal wastewater, investigations involving agricultural or animal waste remain limited [21,25].

In situ biodesulfurization (i.e., within AD) using micro-aeration has advantages such as easy H_2S removal without the need for a complex control system, but requires the digester walls to be cleaned at regular intervals due to the deposition of elemental sulfur [20,25], affecting the economic value of the process. The elemental sulfur formed after micro-aeration can be converted back to H_2S when the accumulated products involuntarily return to the fermentation phase [18]. Moreover, while some studies report same removal efficiencies regardless of the air dosing point [26,27], dosing in the liquid phase as opposed to the head space requires more oxygen/air due to the oxidation of the biodegradable organic matter present in the liquid phase [20,28]. As aforementioned, there is also a risk of explosion, which demands optimal process control. To overcome these problems, external or ex situ desulfurization units can be used and are gaining popularity [18] due to their easy integration into existing AD systems and simple operation. Our study explores external biodesulfurization using the AD digestate (i.e., solid/liquid inoculum from digester) as the biodesulfurization medium. Biogas derived from manure waste typically contains 500–6000 ppm [21]. To increase the H_2S content for experimental purposes, chemicals like sodium sulfate or magnesium sulfate were added to the substrates, as can be observed from Table 2. Our preliminary study was close to the realistic scenario, while in the latter part of the study, synthetic biogas was used to obtain a higher concentration of H_2S up to 5000 ppm.

The current study demonstrated recovery from variations in the airflow rate within 3 days when the O_2 concentration was reduced from 1% to 0.5%. Studies have indicated that short-term variations in micro-aeration do not negatively affect process performance [20,26]. In this study, neither the methane quality nor biodesulfurization efficiency was compromised, even with a 200% increase in the biogas flow rate. Increasing the biogas flow rate

enables the same system to process more biogas daily without altering the O_2 concentration. To prevent the dilution of biogas by nitrogen, pure O_2 was used for micro-aeration.

Previous research [18] demonstrated that air can be used as a substitute for pure oxygen, as evidenced by a long-term study (over 12 years) conducted on seven digesters in Central Europe. Similarly, the present study utilized air and achieved a biodesulfurization efficiency exceeding 99%.

Single-pulse and continuous air injection at different air dosages (0.12–0.36 L/d) were used by [21] for the desulfurization of chicken manure at 37 °C and the biodesulfurization efficiency was found to increase with air dose. The O_2 supply and pH of the washing water combined affected the removal efficiency when using a fixed-bed trickling bioreactor. At pH 7, the removal efficiency was directly proportional to the oxygen supply, but at pH 2, it was indirectly proportional [25]. In the present study, 0.5% oxygen was found to be insufficient at a flow rate of 0.9 L of biogas/h-$L_{inoculum\text{-}mix}$; however, 1% was sufficient to obtain a removal efficiency over 99% for the same biogas flow rate.

Table 2. Operating conditions and biodesulfurization efficiency for various wastes.

Type of Waste or Substrate	Reactor Type	Method/Set-Up	Capacity of Reactor, (L, Working Volume)	Operating Conditions								Biodesulfurization Efficiency (%)	Key Remarks	References
				Operating Temperature (°C)	pH	Chemical Added to Raise H_2S Concentration	HRT (Days)	Air Flow Rate (L/d) or O_2 Concentration (%)	Air/O_2 Dose Point	ORP Set Point (mV)	H_2S Concentration (ppm)			
In-situ Desulfurization (within the Anaerobic Digester)														
Synthetic brewery wastewater	UASB and micro aerated UASB	UASB (control) and UMSB (micro aerobic USAB)	2.7	37	7.0–7.6	Na_2SO_4	0.3	1 (air flow)	n.a.	UASB −450 mV UMSB −425 mV	5850	73%	Sulfur removed in the form of inorganic suspended solids and partly accumulated on head space wall and G-L-S separator.	[19]
Municipal WWTP [1]	Anaerobic digesters		Digester P—1600 m^3 [2] Digester M—2600 m^3 [2]	n.a.	n.a.	n.a.	n.a.	P—24,000 (air flow) M—8160 (air flow)	Dosing in sludge recirculation stream (in liquid phase)	n.a.	P—2438 M—507	Dig P—87.9% Dig M—96.0%	Air can be used instead of pure oxygen (no decrease in methane content due to nitrogen dilution when air was used).	[18]
Municipal WWTP	CSTRs	Reactor R1 mixed with Sludge Recirculation (SR) R2 mixed with Biogas Recirculation (BR)	200	35 ± 1	7.1–7.3	Na_2SO_4	20	2.5 [3] (O_2 flow)	R1—SR and head space R2—feed sludge and head space	−510 mV	R1—14,437 R2—12,926	>98%	Similar biodesulfurization efficiencies were achieved regardless of dosing point. BR can be used to remove dissolved sulfide from liquid.	[26]
WWTP	CSTRs	Reactor S1 with Sludge Recirculation (SR) Reactor S2 with Biogas Recirculation (BR)	200	35 ± 1	n.a.	Na_2SO_4	20	S1—4.7 (O_2 flow) S2—4.5 (O_2 flow)	SR, feed sludge	n.a.	S1—9318 S2—10,361	>99%	SR and BR as mixing methods show the same biodesulfurization efficiencies. BR reduced dissolved sulfide concentration by 10 times (compared to SR).	[27]
Animal waste (Chicken manure)	CSTRs	In situ desulfurization	10	37	7.7–8.1	n.a.	40	0.12–0.36 (air flow)	Head space	n.a.	5500	68–99%	Single-pulse air injection gave the lowest removal efficiency (68%) and for continuous air injection, an increase in air dose increased the biodesulfurization efficiency. Micro-aeration enhanced sulfide-oxidising bacteria and increased soluble iron concentration, which had a positive effect on methane yield.	[21]
Municipal WWTP	Anaerobic Digesters	ORP used to regulate oxygen injection	50	35.0 ± 0.2	6.9–7.1	$Mg{\cdot}SO_4{\cdot}7H_2O$	20	12.1 (O_2 flow)	Liquid sludge phase	−320 to −270 mV (baseline −485 mV)	6000	>99%	To obtain a suitable micro-aerobic condition, ORP can be used as a regulating parameter.	[29]
Municipal WWTP	CSTR	Robustness study on a pilot-plant digester (variations in sulfur load and in oxygen rate studied)	200	35 ± 1	7.2–7.4	Na_2SO_4	20	3.6 [4] (O_2 flow)	Head space	n.a.	n.a.	n.a.	The biodesulfurization process showed quick recovery from variations in sulfur load, O_2 supply and from opening the digester for head space cleaning to remove accumulated sulfur.	[30]
Synthetic WW	Continuous-flow anaerobic reactor	Reactor was operated under anaerobic condition until stabilization after which micro-aeration commenced	2.8	n.a.	n.a.	Na_2SO_4	0.5	0.29 (air flow)	Head space	n.a.	0.15 [5]	93	Methane production was reduced due to nitrogen dilution by air. Micro-aeration was technically and economically more feasible than traditional caustic washing for H_2S removal.	[31]

Table 2. *Cont.*

Type of Waste or Substrate	Reactor Type	Method/Set-Up	Capacity of Reactor, (L, Working Volume)	Operating Conditions								Biodesulfurization Efficiency (%)	Key Remarks	References
				Operating Temperature (°C)	pH	Chemical Added to Raise H_2S Concentration	HRT (Days)	Air Flow Rate (L/d) or O_2 Concentration (%)	Air/O_2 Dose Point	ORP Set Point (mV)	H_2S Concentration (ppm)			
WWTP	n.a.	Micro-aeration using air or oxygen	200	35 ± 1	7.2–7.4	Na_2SO_4	20	2.9 (O_2 flow)	Head space	−510 to −480 mV	10,392	>99	Methane in biogas was reduced due to nitrogen dilution when air was used for micro-aeration.	[32]
Ex-situ Desulfurization Unit														
Municipal WWTP	Sulfide Oxidizing Unit (SOU)	1 L (SOU)—connected to output of AD (92 L volume) along with ORP and pH control units	n.a.	25 ± 2	7.1	Na_2SO_4	20	1.4 (air flow)	n.a.	−200 mV	2170	>99%	Constant pH along with ORP controlled aeration can prevent oxygen overdosing.	[16]
Animal manure and energy crops	Fixed-bed trickling bioreactor (FBTB)	External desulfurization unit consisted of FBTB and carbon filter installed between secondary digester and CHP unit	n.a.	35–37	n.a.	No	n.a.	O_2 content 0.5% and 2% in biogas	Biogas supply pipeline	n.a.	500–600	98%	Highest removal efficiency obtained at 30–40 °C. Here, 35 °C was observed as the optimum temperature range for sulfate-reducing bacteria.	[25]
Animal waste (swine, dairy)	Biodesulfurization reactor	Phase 1—liquid inoculum as biodesulfurization medium Phase 2—solid inoculum as biodesulfurization medium	Liquid inoculum digester—15 Solid inoculum digester—10	20 ± 1	n.a.	No	n.a.	O_2 concentration in biogas 0.5% to 1%	n.a.	n.a.	1000–4000	Phase 1—94% Phase 2—99.8%	Volume of solid inoculum (Phase 2) required was at least half of the liquid inoculum needed. Use of diffusers (required in the case of liquid medium) can be neglected.	Present study

HRT—hydraulic retention time, UASB—up-flow anaerobic sludge blanket reactor, UMSB—micro-aerobic UASB reactor, WWTP—wastewater treatment plant, CSTR—continuous stirred-tank reactor, SR—sludge recirculation, BR—biogas recirculation, ORP—oxidation reduction potential, WW—wastewater, SOU—sulfide-oxidizing Unit, FBTB—fixed-bed trickling bioreactor, CHP—combined heat and power. Notes: [1] wastewater treatment plant. [2] data for 2 of 7 digesters; digesters (P,M). [3] represents ~0.25 NL of oxygen per L of feed sludge. [4] represents ~0.33 NL of oxygen per L of feed sludge. [5] mmol/day.

The findings from this study indicate that the methane quality remains unaffected by micro-oxygenation rates, even with O_2 levels of up to 1% following the desulfurization process. These results align with previous research suggesting that small O_2 injections (up to 1%) do not hinder anaerobic degradation [33,34]. The key factors contributing to this resilience include limited O_2 diffusion within the digestate and the rapid consumption of O_2 by autotrophic sulfur-oxidizing bacteria (SOB) and facultative or aerobic microorganisms, effectively shielding strictly anaerobic microbes from harmful O_2 fluctuations. Furthermore, methanogens such as *Methanosarcina* and *Methanocella* have shown the capacity to withstand brief periods of oxygen exposure. Overall, these findings confirm that controlled micro-aeration can be safely implemented without compromising biogas quality, enhancing the feasibility of desulfurization processes.

4.2. Liquid vs. Solid Biodesulfurization Medium

This study explored the mechanisms (physical and biological processes) underlying H_2S removal in liquid and solid inoculum systems, utilizing SOB under micro-aerobic conditions. In the liquid inoculum system, the physical process of gas–liquid mass transfer facilitated the dissolution of H_2S into the aqueous phase, where SOB catalyzed its conversion into elemental sulfur (S^0). This biological process achieved a ~94% H_2S reduction while maintaining stable CH_4 concentrations. Scalability was validated in 250 L (active volume) reactors, but the need for diffusers to ensure proper gas–liquid contact increases the operational complexity, making it less ideal for long-term or large-scale applications. In contrast, the solid inoculum system relied on gas–solid interactions (physical process) and biofilm-mediated sulfur oxidation (biological process). SOB biofilms formed on the solid matrix, enabling the efficient conversion of H_2S into S_0, achieving ~99.8% removal efficiency. The incorporation of wood shavings as a structural medium optimized gas distribution, reduced airflow resistance, and eliminated the need for diffusers. These advantages reduced maintenance costs and the environmental impact while enhancing scalability. The system's ability to handle increased biogas flow rates further emphasizes its potential as a cost-effective and sustainable solution for biogas purification.

When comparing the performance of these systems, the liquid medium demonstrated satisfactory results with a 1% O_2-to-biogas concentration and a biogas flow rate of 0.9 L biogas/h-$L_{inoculum\text{-}mix}$. However, the solid inoculum system excelled with a lower O_2-to-biogas concentration of 0.6% and a higher biogas flow rate of 1.8 L biogas/h-$L_{inoculum\text{-}mix}$, indicating superior efficiency. Additionally, the solid medium required significantly less reactor volume than the liquid medium to achieve comparable or better results. Furthermore, the solid medium demonstrated untapped potential, as its saturation limit was not reached during testing, suggesting room for further optimization. By eliminating the need for diffusers and delivering a superior biodesulfurization performance, the solid inoculum system offers a more practical and cost-effective alternative for biogas treatment, particularly in space-constrained or cost-sensitive applications.

4.3. Solid Medium: Footprint and Cost-Benefit Analysis

For comparison purposes, a farm with 100 cattle was used as an example to estimate biogas production, the size of the anaerobic digestion (AD) system [28], and the capacity required for biogas desulfurization (data from this study). Dairy manure is relatively dense, with each cow producing an average of 10 kg of manure per day. Consequently, for a farm with 100 cattle, the total manure production is approximately 1000 kg per day. Given that the density of cow manure is around 975 kg/m^3, this corresponds to a daily manure production volume of 1.03 m^3 (i.e., 1000 kg ÷ 975 kg/m^3).

On average, cow manure contains 20% total solids (TS) and requires a retention time of 30 days for effective processing. In a wet AD system, 1 m^3 of water is needed to treat waste with a TS content of 10%, resulting in a total volume of aqueous manure of approximately 2.03 m^3 per day (i.e., 1.03 m^3 + 1 m^3). Considering a 1:1 assurance margin, the total capacity required for the AD facility is approximately 67 m^3 (i.e., 2.03 m^3 × 30 days × assurance

factor of 1.1). In contrast, dry AD systems can effectively process waste containing 20% TS for the same 30-day retention period, reducing the required capacity by nearly half compared to a wet AD system, with a total volume of approximately 35 m^3.

Each cow's manure generates approximately 0.33 m^3 of biogas per day, leading to a total biogas production of 33 m^3/day for a farm with 100 cattle. This study evaluated several design parameters to determine the volume of the biodesulfurization reactor filled with solid inoculum and wood shavings as a structural mix. These parameters focused on optimizing the biogas flow rate (1.8 L/h per liter of inoculum-mix) to achieve 99–100% H_2S removal while incorporating an assurance factor to ensure operational robustness. The analysis revealed that the hourly biogas flow rate needed for this removal efficiency was 1.8 L per hour per liter of inoculum mix, translating to a daily rate of 43.2 L per day per liter of inoculum mix, or 43.2 cubic meters of biogas per day per cubic meter of inoculum-mix.

To treat the daily production of 33 cubic meters of biogas, the values from this study indicated a requirement of 0.8 cubic meters of inoculum mix. Incorporating an assurance factor, approximately 1 cubic meter of solid inoculum was determined to be sufficient for handling the daily biogas output. Consequently, the volume of the biodesulfurization unit was set at 1 cubic meter, significantly smaller than that of traditional digestion systems.

In comparison, wet and dry anaerobic digestion systems necessitate capacities of approximately 67 m^3 and 35 m^3, respectively. The biodesulfurization reactor, in contrast, requires only 1 m^3, which is approximately 1.5% of the size of a wet digester and 3% of a dry digester. The system's compact design and ability to operate efficiently at ambient temperatures (20 ± 1 °C) eliminate the need for additional energy inputs such as heating. This underscores its adaptability and potential for cost savings.

The economic benefits of the solid medium system include substantial capital investment reductions due to the smaller reactor size. Additionally, the elimination of diffusers reduces maintenance costs while simplifying operations. The system's scalability for farms of various sizes further enhances its cost-effectiveness. The environmental impacts are equally significant. The reduced reactor size minimizes the land footprint, making the system suitable for space-constrained installations. Furthermore, the elimination of heating requirements significantly reduces energy consumption, contributing to a lower carbon footprint. These advantages highlight that the solid medium biodesulfurization reactor is a sustainable and eco-friendly solution for biogas purification.

5. Conclusions

This study introduces a low-cost technology that can achieve a > 99% biodesulfurization efficiency without the need for additional maintenance, such as pH control, the use of pure oxygen, or the addition of chemicals or catalysts. Operated at low temperatures (19–20 °C), the system demonstrates robustness by effectively recovering from variations in airflow and biogas flow rates. The composition of biogas remains unaffected by microaeration, allowing the use of air instead of pure oxygen. For a farm with 100 cattle, a wet anaerobic digestion (AD) system requires 67 m^3 and a dry AD system 35 m^3, whereas the biodesulfurization reactor requires only 1 m^3 of inoculum-mix—approximately 1.5% of the wet digester's size and 3% of the dry digester's size. This highlights the system's remarkable efficiency, cost-effectiveness, and significantly reduced footprint.

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