



Balancing sulfur accumulation and nitrous oxide emissions during sulfide autotrophic denitrification in moving-bed biofilm reactor-based bioscrubbers

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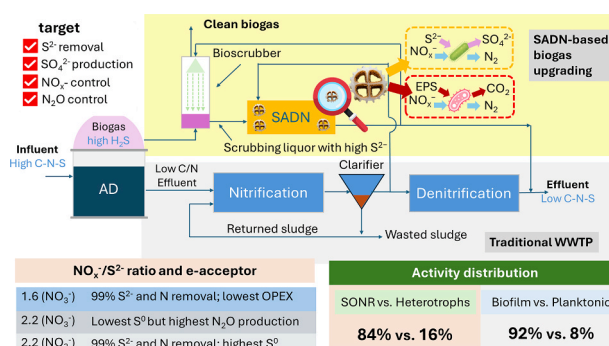
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HIGHLIGHTS

- Excess electron-acceptor enhanced S^0 oxidation to SO_4^{2-} but increased N_2O emissions.
- Mixotrophs shifted electron flow from SADN to HDN and promoted S^0 accumulation.
- NO_2^- substitution and shorter HRT reduced S^{2-} oxidation to SO_4^{2-} .
- Biofilm biomass accounted for 92% of total SADN activity in the MBBR.
- $NO_3^-/S^{2-}=1.6$ achieved a balance among S^0 and N_2O production and cost-effectiveness.

GRAPHICAL ABSTRACT



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ABSTRACT

Sulfide autotrophic denitrification (SADN) is a promising strategy for integrating biogas desulfurization with nitrogen removal in anaerobic digestion-based treatment systems. However, its application in moving bed biofilm reactors (MBBRs) remains limited by biosulfur (S^0) accumulation and nitrous oxide (N_2O) emissions. This study investigated the operational tradeoffs governing SADN performance in an MBBR under different nitrate-to-sulfide (NO_3^-/S^{2-}) ratios, electron acceptors (NO_3^- or NO_2^-), and hydraulic retention times (HRTs). Batch assays, microbial community analysis, and techno-economic evaluation were conducted to elucidate biofilm-planktonic biomass interactions and operational feasibility. Complete sulfide removal (>99%) was achieved under all tested conditions. Increasing the NO_3^-/S^{2-} ratio reduced S^0 accumulation from 16.5% to 5.2%, mitigating carrier clogging, but simultaneously promoted incomplete denitrification and N_2O formation. In contrast, operation at an NO_3^-/S^{2-} ratio of 1.6 achieved complete denitrification without detectable N_2O emissions while maintaining effective sulfur control, representing the optimal techno-economic condition. Using NO_2^- as electron acceptor

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enabled complete denitrification and suppressed N_2O emissions, but increased S^0 accumulation due to limited electron-accepting capacity for complete sulfide oxidation. Microbial analysis identified *Sulfurimonas* as the dominant sulfur-oxidizing genus, while mixotrophic denitrifiers contributed to residual sulfur formation. Activity assays showed that biofilm biomass dominated electron transfer, whereas planktonic biomass enhanced reactor performance through synergistic interactions. Decreasing HRT from 24 to 12 h, with increased S^{2-} and NO_3^- loadings, maintained S^{2-} and NO_3^- removal while reducing reactor footprint. These findings demonstrate that sustainable SADN-MBBR operation requires balancing sulfur control, denitrification completeness, greenhouse-gas mitigation, and reactor stability for scalable low-carbon biogas upgrading applications.

Nomenclature

AD	Anaerobic digestion
Conc.	Concentration
CSTR	Continuously stirred tank reactor
Eff.	Effluent
RE	Removal efficiency
HDN	Heterotrophic denitrification
HRT	Hydraulic retention time
Inf.	Influent
MBBR	Moving bed biofilm reactor
MD	Mixotrophic denitrifier
Nar	Nitrate reductase
Nir	Nitrite reductase
Nor	Nitric oxide reductase
Nos	Nitrous oxide reductase
$\text{NO}_x^-/\text{S}^{2-}$	Nitrogen (nitrate + nitrite)-to-Sulfide molar ratios
$\text{NO}_2^-/\text{S}^{2-}$	Nitrite-to-sulfide molar ratios
$\text{NO}_3^-/\text{S}^{2-}$	Nitrate-to-sulfide molar ratios
SADN	Sulfide autotrophic denitrification
SBR	Sequencing batch reactor
SLR	Sulfide loading rate
SO-NR	Sulfur-oxidizing nitrogen-reducing
Sox	Sulfur-oxidizing enzyme

Sqr	Sulfide:quinone reductase
SRB	Sulfate-reducing bacteria
UBF	Upflow blanket filter
$A_{\text{BF},d}$	Electron-donating activity distribution of biofilm biomass (%)
$A_{\text{PT},d}$	Electron-donating activity distribution of planktonic biomass (%)
$A_{\text{BF},ac}$	Electron-accepting activity distribution of biofilm biomass (%)
$A_{\text{PT},ac}$	Electron-accepting activity distribution of planktonic biomass (%)
$r_{\text{S}^{2-} \rightarrow \text{S}^0}$	Electron-donating rate for oxidizing S^{2-} to S^0 (mol-e•g-VSS ⁻¹ •d ⁻¹)
$r_{\text{S}^0 \rightarrow \text{SO}_4^{2-}}$	Electron-donating rate for oxidizing S^0 to SO_4^{2-} (mol-e•g-VSS ⁻¹ •d ⁻¹)
$r_{\text{NO}_3^- \rightarrow \text{NO}_2^-}$	Electron-accepting rate for reducing NO_3^- to NO_2^- (mol-e•g-VSS ⁻¹ •d ⁻¹)
$r_{\text{NO}_3^- \rightarrow \text{N}_2\text{O}}$	Electron-accepting rate for reducing NO_3^- to N_2O (mol-e•g-VSS ⁻¹ •d ⁻¹)
$r_{\text{NO}_3^- \rightarrow \text{N}_2}$	Electron-accepting rate for reducing NO_3^- to N_2 (mol-e•g-VSS ⁻¹ •d ⁻¹)
$r_{\text{NO}_2^- \rightarrow \text{N}_2}$	Electron-accepting rate for reducing NO_2^- to N_2 (mol-e•g-VSS ⁻¹ •d ⁻¹)

1. Introduction

Energy recovery from wastewater as biogas is a sustainable alternative to fossil fuels and supports circular economy goals through anaerobic digestion (AD) of organic matter. In Thailand, agro-industrial wastewaters, such as those from rubber, pulp and paper, tannery, and food-processing industries, commonly contain high acidity and elevated COD, sulfate (SO_4^{2-}), and total Kjeldahl nitrogen (TKN) concentrations requiring integrated treatment before discharge (Raketh et al., 2022). AD is widely used to treat these high-strength C–N–S wastewaters while simultaneously producing biogas (Raketh et al., 2022; Tilahun and Calli, 2024). However, the generated biogas often contains hydrogen sulfide (H_2S) at levels that limit direct energy recovery because of corrosion problems (Raketh et al., 2022). In addition, most nitrogen remains in the digestate and requires downstream treatment to comply with discharge regulations (Yang et al., 2022). Consequently, AD systems generally require simultaneous sulfur removal from the gas phase and nitrogen removal from the liquid phase.

Biogas upgrading is therefore necessary before energy utilization. For high- H_2S streams, bioscrubbers are attractive because they prevent methane (CH_4) dilution and associated safety risks by avoiding direct air-gas contact (González-Cortés et al., 2021). In these systems, H_2S is absorbed into a recirculating liquor and biologically oxidized to elemental sulfur (S^0) and/or sulfate (SO_4^{2-}) in a separate reactor (Gabriel et al., 2017). In agro-industrial wastewaters, nitrogen in the AD effluent is usually removed through nitrification–denitrification. However,

heterotrophic denitrification (HDN) requires external organic carbon and may emit nitrous oxide (N_2O), a potent greenhouse gas (Cao et al., 2021). Moreover, operating separate sulfur and nitrogen treatment systems increases energy demand and operational costs (González-Cortés et al., 2021), motivating the development of integrated strategies that couple nitrogen removal with resource recovery while reducing aeration requirements (Cai et al., 2022).

Sulfide autotrophic denitrification (SADN) is a promising strategy for integrating sulfur and nitrogen removal. In SADN, sulfide (S^{2-}) acts as an electron donor for the reduction of oxidized nitrogen species (NO_x^- ; NO_3^- and/or NO_2^-) to N_2 , while being oxidized to S^0 and/or SO_4^{2-} by sulfur-oxidizing, nitrogen-reducing (SO-NR) bacteria (Supplementary Material, Eqs. S1–S9) (Wang et al., 2023). In AD-based systems, SADN enables coupling between sulfide-rich absorbent from biogas scrubbing and NO_x^- generated from nitrification of nitrogen-rich digestate (Supplementary Material, Fig. S1) (Gabriel et al., 2017). This integration can reduce aeration demand and eliminate the need for external organic carbon during denitrification (Cai et al., 2022). In addition, bioscrubber configurations improve operational flexibility because H_2S absorption and biological oxidation of sulfur occur in separate units (Chetawan et al., 2026; Gabriel et al., 2017).

Implementation of SADN in bioscrubbers may provide a cost-effective strategy for biogas upgrading while preserving CH_4 concentration and reducing emissions compared with aerobic sulfide oxidation coupled with HDN (Cao et al., 2021; Wang et al., 2024). However, nitrogenous intermediates such as NO and N_2O can still be formed, making N_2O evaluation essential because of its high global warming potential. Moving bed biofilm reactors (MBBRs) are attractive for SADN

applications due to their high biomass retention, tolerance to fluctuating loadings, and compact design (Kostrytsia et al., 2022). Compared with suspended-growth and granular systems, MBBRs perform well under high sulfur and nitrogen loadings (Cui et al., 2019b). Nevertheless, SADN-based MBBR bioscrubbers for biogas desulfurization remain poorly explored.

SADN performance is strongly influenced by electron-acceptor availability and reactor operation. Depending on the $\text{NO}_x^-/\text{S}^{2-}$ molar ratio, sulfide can be oxidized to S^0 or SO_4^{2-} , while nitrogen may be fully reduced to N_2 or accumulate as NO_2^- and N_2O . Increasing electron-acceptor availability can promote further oxidation of S^0 to SO_4^{2-} and thereby reduce S^0 accumulation. However, excessive NO_x^- supply may increase the risk of N_2O accumulation when denitrification becomes incomplete, particularly at the terminal N_2O -reduction step. Nitrite is particularly relevant because shortcut nitrogen removal intentionally produces NO_2^- , reducing aeration demand compared with full nitrification (Supplementary Material, Eqs. S10–S11) (Zhang et al., 2020) and altering denitrification thermodynamics and kinetics (Oberoi et al., 2021). Moreover, using NO_2^- bypasses the initial NO_3^- -reduction step and reduces the electron requirement for nitrogen reduction, which may help avoid N_2O accumulation under limited electron-donor availability. Hydraulic retention time (HRT) also plays a key role by affecting contact time, S and N loading rates, as well as reactor volume, biomass retention, and capital cost (Tilahun and Calli, 2024).

Despite growing interest in SADN, important gaps remain regarding its application to biogas upgrading. Previous studies have evaluated $\text{NO}_x^-/\text{S}^{2-}$ ratio, HRT, MBBR configuration, and biomass distribution separately, limiting integrated assessment of bioscrubber performance. However, process suitability depends not only on sulfide and nitrogen removal but also on sulfur product distribution, N_2O emissions, maintenance requirements, and operating costs. This is particularly important because conditions that improve sulfur oxidation may increase the risk of incomplete denitrification or utility demand, whereas less oxidative conditions may favor biosulfur accumulation. In addition, biofilm and planktonic biomass may differently affect substrate diffusion, electron transfer, reactor stability, and long-term operation.

While previous studies on SADN have mainly focused on sulfide and nitrogen removal efficiencies, the operational tradeoffs among sulfur accumulation, denitrification completeness, greenhouse gas emissions, microbial ecology, and reactor stability in MBBR systems remain insufficiently understood. In particular, limited information is available regarding the interaction between biofilm and planktonic biomass during SADN operation in MBBRs and how these interactions affect reactor performance and sustainability. Therefore, this study provides an integrated mechanistic and preliminary techno-economic evaluation of SADN-MBBR operation under different electron-acceptor conditions and hydraulic regimes, together with one of the first integrated quantitative assessments of electron-transfer partitioning between biofilm and planktonic biomass during SADN in MBBRs. This work investigated the operational tradeoffs governing SADN performance in an MBBR bioscrubber system, including sulfur oxidation, N_2O emissions, biomass functional partitioning, operational stability, and process cost; moving beyond conventional single-factor optimization and providing a framework for identifying practical operating windows for integrated biogas upgrading and nitrogen removal in AD-based treatment systems. The findings provide practical guidance for the design and operation of integrated sulfur and nitrogen management systems, particularly for agro-industrial processing industries.

2. Methodology

2.1. Biomass and wastewater preparation

The inoculum was planktonic biomass obtained from an existing lab-scale continuously stirred tank reactor (CSTR) performing SADN at an $\text{NO}_3^-/\text{S}^{2-}=0.6$. The MBBR was initiated by seeding the inoculum at an

initial concentration of $1.0 \text{ gVSS}\cdot\text{L}^{-1}$. The MBBR was subsequently fed with synthetic wastewater at a constant sulfide loading rate (SLR), HRT and $\text{NO}_3^-/\text{S}^{2-}$ ratio of $14.9 \pm 0.2 \text{ mmol}\cdot\text{S}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$, 24 h and $1.8 \text{ mol}\cdot\text{N}\cdot\text{mol}\cdot\text{S}^{-1}$, respectively. Biomass acclimation was considered complete once the bioreactor reached pseudo steady-state conditions, defined by variations below $\pm 5\%$ in biofilm and planktonic biomass concentrations, as well as S^{2-} and NO_3^- removal efficiencies. After acclimation, biofilm samples on the carriers were imaged using scanning electron microscopy (Supplementary Material, Fig. S2). Then, the experiment described in Section 2.2.2 was initiated.

Synthetic wastewater was prepared by mixing two different solutions before feeding the reactor: (1) a sulfide solution (Influent 1, designated Inf-1) prepared by dissolving $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ (AR grade, Sigma-Aldrich, Germany) at 14.8 mM ($490 \text{ mg}\cdot\text{S}\cdot\text{L}^{-1}$), and (2) a nitrogen solution (Influent 2, designated Inf-2) containing either NO_3^- or NO_2^- and nutrients, the former prepared using NaNO_3 and NaNO_2 (AR grade, Merck, Germany). Nutrients and trace element solutions were prepared according to Cui et al. (2019a). Oxygen in the headspace of the feeding tanks was removed by providing 99.99% N_2 (Linde, Ireland) for 1 min.

2.2. System configuration, operating strategy, and experiments

2.2.1. Moving bed biofilm reactor (MBBR) setup

The MBBR, with a total volume of 3.6 L and an effective volume of 3.1 L (Fig. 1), was used for long-term operation. The MBBR main body was constructed from transparent PVC with a height and an internal diameter of 31.6 cm and 10.0 cm, respectively. The moving carriers were Kaldnes-K1 carriers that filled 60% of MBBR's effective volume (Kostrytsia et al., 2022). The total amount of carriers was approximately 500 (± 5) pieces. Inf-1 and Inf-2 were fed by using a peristaltic pump with a two-channel head (PERIMAX 12, SPETEC, Spain). The mixing system comprised an 8-cm-diameter three-blade propeller (impeller-to-reactor diameter ratio = 0.80) mounted on a stainless-steel shaft and operated at 60 rpm using a mixer (F201A0156, VELP SCIENTIFICA, Italy; nominal motor rating 190 W). At the 60% carrier filling fraction, carriers circulated continuously without persistent settling, agglomeration, or apparent stagnant zones. Actual shaft power was not measured. pH inside the bioreactor was maintained between 7.65 and 7.85 by a pH controller (PH 28, CRISON, Switzerland) connected to an acid feeding pump for 1.0 M HCl addition. Temperature was controlled at 25°C by placing the MBBR in a temperature-controlled room. During the experiment, the reactor was operated under fully anoxic conditions by purging N_2 through a ceramic diffuser at a fixed flow rate of $0.5 \text{ NL}\cdot\text{d}^{-1}$.

2.2.2. Long-term operation of the SADN-MBBR

Three long-term experiments were carried out in the MBBR. Experiment 1 was designed to assess the effects of $\text{NO}_3^-/\text{S}^{2-}$ on the SADN process under S^{2-} -limiting conditions. Experiment 1 was initiated following the acclimation phase. The MBBR was fed at a constant SLR and HRT of $14.9 \pm 0.2 \text{ mmol}\cdot\text{S}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ and 24 h, respectively, while the influent $\text{NO}_3^-/\text{S}^{2-}$ was varied at 1.8, 1.6, 2.0, and 2.2 according to Table 1. Experiment 2 was designed to evaluate the influence of HRT on MBBR performance. In this experiment, inlet S^{2-} and NO_3^- concentrations were maintained at 14.9 mM and 32.8 mM, respectively, corresponding to a constant $\text{NO}_3^-/\text{S}^{2-}=2.2 \text{ mol}\cdot\text{N}\cdot\text{mol}\cdot\text{S}^{-1}$, while HRT was varied at 24, 18, 12, and 6 h (Table 1). Experiment 3 aimed to assess the impact of electron acceptor type on the SADN process. The operating conditions followed a similar approach to Experiment 1, but the $\text{NO}_x^-/\text{S}^{2-}$ ratio was fixed at 2.2, selected based on the highest SO_4^{2-} production observed in Experiment 1, and the electron acceptor was switched from NO_3^- to NO_2^- . As during biomass acclimation, each operating condition was maintained until pseudo steady-state conditions were achieved before transitioning to the next condition.

2.2.3. Biofilm and planktonic biomass activity tests

The total MBBR activity was assessed by monitoring S^{2-} oxidation

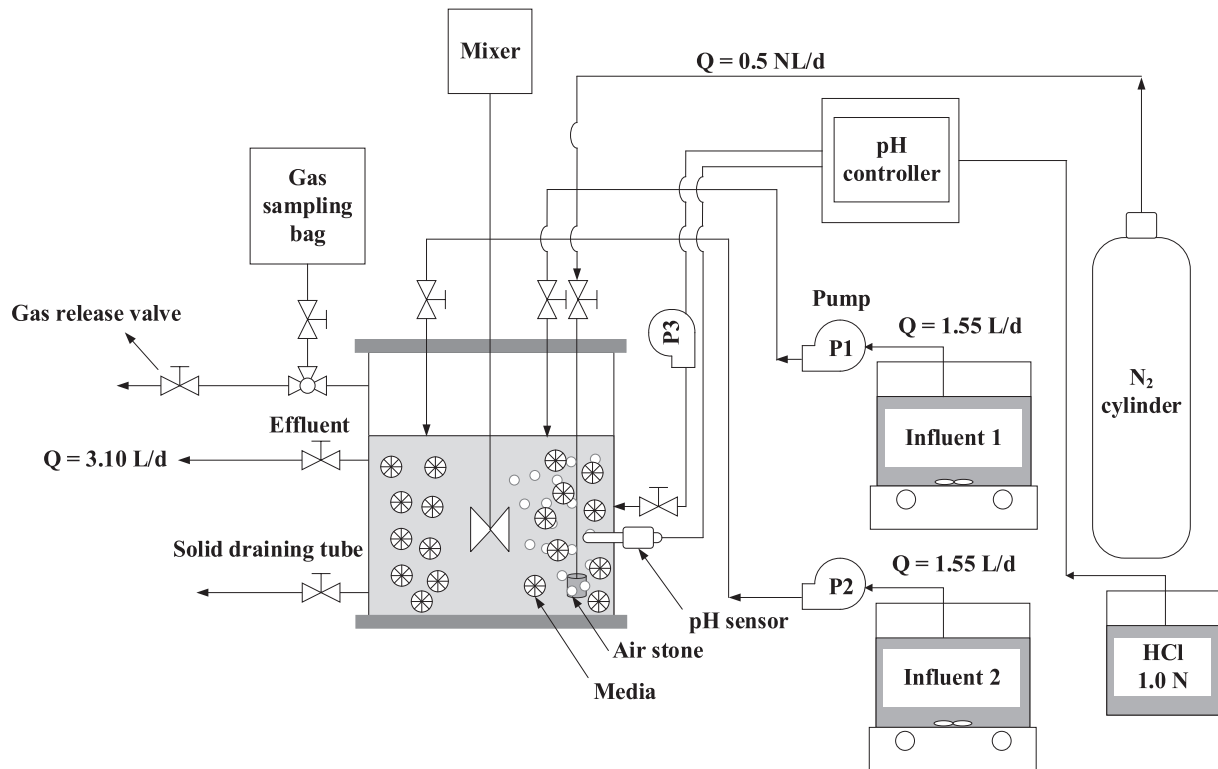


Fig. 1. Schematic of the MBBR. The body hosted five ports for pH sensor, acid inlet for pH control, draining, outlet and gas release port. The top was closed with a PVC lid holding four ports for Inf-1, Inf-2 and N₂ inlet and the mixer.

Table 1
Experimental conditions in SADN operation.

Experiment	Phase	Day	Electron acceptor	NO _x ⁻ /S ²⁻ (mol-N•mol-S ⁻¹)	HRT (h)	SLR (mmol-S•L ⁻¹ •d ⁻¹)	NLR (mmol-N•L ⁻¹ •d ⁻¹)
Exp. 1	I	0–35	NO ₃ ⁻	1.8	24	14.9 ± 0.2	26.8 ± 0.2
	II	36–48	NO ₃ ⁻	1.6	24	14.9 ± 0.2	23.8 ± 0.2
	III	49–56	NO ₃ ⁻	2.0	24	14.9 ± 0.2	29.8 ± 0.2
	IV	57–76	NO ₃ ⁻	2.2	24	14.9 ± 0.2	32.8 ± 0.2
Exp. 2	V	77–85	NO ₃ ⁻	2.2	18	19.9 ± 0.2	43.7 ± 0.2
	VI	86–92	NO ₃ ⁻	2.2	12	29.8 ± 0.2	65.6 ± 0.2
	VII	93–102	NO ₃ ⁻	2.2	6	59.6 ± 0.2	131.2 ± 0.2
Exp. 3	VIII	103–108	NO ₂ ⁻	2.2	24	14.9 ± 0.2	32.8 ± 0.2

and denitrification under regular operation. In addition, 24 h-tests were performed to evaluate the individual contributions of biofilm and planktonic biomass to S²⁻ oxidation and denitrification. Sole biofilm activity was determined in the MBBR setup (Fig. 1) by draining the planktonic biomass, whereas sole planktonic activity was determined in a separate CSTR with the drained biomass (Supplementary Material, Fig. S3). The detailed procedure followed for the batch tests is described

in Supplementary Material, Section S2.1, while conditions tested are shown in Table 2. All batch experiments were performed in duplicate to ensure reproducibility. Tests were performed on days 35, 48, 56, 77, and 108 of the MBBR operation to assess biomass activity before transitioning to new experimental conditions.

2.3. Analytical methods

Influent and effluent samples were collected and measured for dissolved S²⁻, which was analyzed with a Silver/Sulfide ion-selective electrode (VWR International EuroLab, S.L.). Samples were previously diluted with a S²⁻ antioxidant buffer (SAOB) to minimize oxidation and S²⁻ stripping (Polizzi et al., 2022). Soluble samples were filtered through a 0.22 µm disposable Millipore syringe filter for their measurement. Soluble NO₃⁻, NO₂⁻, SO₄²⁻, and S₂O₃²⁻ concentrations were measured by ion chromatography (Dionex ICS-2000, Thermo Scientific), while S⁰ was estimated via sulfur mass balance as described by Cai et al. (2022).

Mixed liquor suspended solids (SS) and volatile suspended solids (VSS) were measured according to Standard Methods for the Examination of Water and Wastewater (APHA, 2023). Biomass concentration

Table 2
Experimental conditions in batch assays.

NO _x ⁻ /S ²⁻ (mol-N•mol-S ⁻¹)	Electron acceptor	Duration (h)	Initial S and N concentration (mM)		Biomass mass (gVSS)*	
			S ²⁻	NO _x ⁻	Biofilm	Planktonic
1.6	NO ₃ ⁻	24	14.9	23.8	25.8	3.3
1.8	NO ₃ ⁻	24	14.9	26.8	26.1	3.5
2.0	NO ₃ ⁻	24	14.9	29.8	26.0	4.4
2.2	NO ₃ ⁻	24	14.9	32.8	25.8	4.1
2.2	NO ₂ ⁻	24	14.9	32.8	26.3	3.6

*Estimated from organic nitrogen measurements.

was quantified based on organic nitrogen using the LCK338 Hach test kit (Section S2.2, [Supplementary Material](#)) to avoid overestimation of VSS caused by S^0 accumulation in the biomass (Bosch et al., 2007).

Gas samples were collected in Tedlar bags (SKC, United Kingdom). H_2S was analyzed using a portable H_2S sensor (MX6 iBrid, Industrial Scientific) with a detection range of 0–500 ppm. Gaseous N_2O was quantified in the off-gas using an Agilent 8860 gas chromatograph equipped with an electron capture detector and an HP-PLOT Q column ($30\text{ m} \times 0.53\text{ mm} \times 40.0\text{ }\mu\text{m}$), with a detection range of 0–20 ppm_v. Additional operating details are provided in Section S2.3 of the [Supplementary Material](#).

Microbial communities in biofilm and planktonic biomass were analyzed by next-generation sequencing following Illumina protocols. Samples comprised the original inoculum and biomass collected at the steady state of the $NO_3^-/S^{2-} = 1.6$ (day 48), $NO_3^-/S^{2-} = 2.2$ (day 77), and $NO_2^-/S^{2-} = 2.2$ (day 108) phases. Samples were stored at $-20\text{ }^\circ\text{C}$ until DNA extraction. Genomic DNA was extracted following Polizzi et al. (2022), and sequencing was performed using the MiSeq platform and Greengenes database (Genomics and Bioinformatics Service, Universitat Autònoma de Barcelona). Demultiplexed reads were classified to taxonomic levels.

2.4. Assessment of S and N conversion

Produced S^0 and N_2 were estimated using a mass balance as shown in Eqs. (1) and (2) (Chetawan et al., 2026). S and N products distributions were calculated according Eq. (3)

$$S^0_{\text{produced}} = S_{\text{Inf.}}^{2-} - SO_{4\text{Eff.}}^{2-} - S_{\text{Eff.}}^{2-} - H_2S_g - 2S_{2O_{3\text{Eff.}}}^{2-} \quad (1)$$

$$N_{2\text{produced}} = NO_{x\text{Inf.}}^- - NO_{3\text{Eff.}}^- - NO_{2\text{Eff.}}^- - N_{2O_g} - \text{Org} - N_{\text{Eff.}} \quad (2)$$

$$S \text{ and } N \text{ distributions} = \frac{S_{\text{Eff.}}^{2-} \text{ or } H_2S_g \text{ or } S^0_{\text{Eff.}} \text{ or } SO_{4\text{Eff.}}^{2-}}{S_{\text{Inf.}}^{2-}} \times 100\%$$

$$\frac{NO_{3\text{Eff.}}^- \text{ or } NO_{2\text{Eff.}}^- \text{ or } N_{2O_{\text{Eff.}}} \text{ or } N_{2\text{Eff.}} \text{ or } \text{Org} - N_{\text{Eff.}}}{NO_{x\text{Inf.}}^-} \times 100\% \quad (3)$$

$$A_{\text{BF,ac}} \text{ or } A_{\text{PT,ac}} (\%) = \frac{((r_{NO_3^- \rightarrow NO_2^-}) + (r_{NO_3^- \rightarrow N_2}) + (r_{NO_3^- \rightarrow N_2O})_{\text{BF or PT}})}{((r_{NO_3^- \rightarrow NO_2^-}) + (r_{NO_3^- \rightarrow N_2}) + (r_{NO_3^- \rightarrow N_2O})_{\text{BF}} + ((r_{NO_3^- \rightarrow NO_2^-}) + (r_{NO_3^- \rightarrow N_2}) + (r_{NO_3^- \rightarrow N_2O})_{\text{PT}})} \times 100 \quad (11)$$

where sub-indexes Inf., Eff., and g. represent the influent (inlet), effluent (outlet) and off-gas (outlet) of the bioreactor, respectively. The empirical formula $C_5H_7NO_2$ was used to represent cellular biomass and EPS for mass-balance estimation. Only Org-N was considered for estimating assimilatory biomass synthesis, while sulfur and nitrogen product distributions were calculated based on the S and N mass balances.

2.5. Specific activity of biofilm and planktonic biomass

The specific activity of the biomass was quantified from electron transport between sulfide oxidation (electron donation) and denitrification (electron acceptance) mediated by the biomass (Oberoi et al., 2021). Electron transport rates (r , mol-e•g-VSS⁻¹•d⁻¹) were derived from measured influent–effluent concentration differences and normalized by biomass. Electron donating rates (r_s) were the sum of electrons released from S^{2-} oxidation based on S^0 and SO_4^{2-} concentrations in the effluent where S^0 was obtained by a sulfur balance (Eqs. (4) and (5)). Electron accepting rates (r_N) were the sum of the electrons consumed in denitrification based on concentrations of NO_2^- and N_2O in effluent and off-gas (Eqs. (6) and (7); N_2 was obtained by nitrogen

balance Eq. (8) and (9), when using NO_3^- and NO_2^- , respectively.

$$r_{S^{2-} \rightarrow S^0} = \frac{(S_{\text{Inf.}}^{2-} - S_{\text{Eff.}}^{2-} - 2 S_{2O_{3\text{Eff.}}}^{2-} - SO_{4\text{Eff.}}^{2-}) \times Q_{\text{Eff.}}}{32 \text{ g/mol} \times \text{conc.}_{\text{biomass}}} \times 2 \quad (4)$$

$$r_{S^0 \rightarrow SO_4^{2-}} = \frac{(SO_{4\text{Eff.}}^{2-} - SO_{4\text{Inf.}}^{2-}) \times Q_{\text{Eff.}}}{32 \text{ g/mol} \times \text{conc.}_{\text{biomass}}} \times 6 \quad (5)$$

$$r_{NO_3^- \rightarrow NO_2^-} = \frac{(NO_{2\text{Eff.}}^- - NO_{2\text{Inf.}}^-) \times Q_{\text{Eff.}}}{14 \text{ g/mol} \times \text{conc.}_{\text{biomass}}} \times 2 \quad (6)$$

$$r_{NO_3^- \rightarrow N_2O} = \frac{(N_{2O_g} \times Q_g)}{14 \text{ g/mol} \times \text{conc.}_{\text{biomass}}} \times 4 \quad (7)$$

$$r_{NO_3^- \rightarrow N_2} = \frac{(NO_{3\text{Inf.}}^- - NO_{3\text{Eff.}}^- - NO_{2\text{Eff.}}^-) \times Q_{\text{Eff.}} - (N_{2O_g} \times Q_g)}{14 \text{ g/mol} \times \text{conc.}_{\text{biomass}}} \times 5 \quad (8)$$

$$r_{NO_2^- \rightarrow N_2} = \frac{(NO_{2\text{Inf.}}^- - NO_{2\text{Eff.}}^-) \times Q_{\text{Eff.}} - (N_{2O_g} \times Q_g)}{14 \text{ g/mol} \times \text{conc.}_{\text{biomass}}} \times 3 \quad (9)$$

The activity of SO-NR bacteria in the MBBR was estimated from the total electron-donating rate associated with sulfur oxidation ($r_{S^{2-} \rightarrow S^0} + r_{S^0 \rightarrow SO_4^{2-}}$). However, the contribution of mixotrophic activity was estimated from the difference between the total electron-accepting rate for nitrogen reduction ($r_{NO_3^- \rightarrow NO_2^-} + r_{NO_3^- \rightarrow N_2O} + r_{NO_3^- \rightarrow N_2}$) and the total electron-donating rate. Activity distribution between the biofilm (BF) and the planktonic biomass (PT) was calculated based on electron-donating ($A_{\text{BF,d}}$ and $A_{\text{PT,d}}$) (Eq. (10)) or electron-accepting ($A_{\text{BF,ac}}$ and $A_{\text{PT,ac}}$) (Eq. (11)) rates during S^{2-} oxidation and nitrogen reduction.

$$A_{\text{BF,d}} \text{ or } A_{\text{PT,d}} (\%) = \frac{((r_{S^{2-} \rightarrow S^0}) + (r_{S^0 \rightarrow SO_4^{2-}}))_{\text{BF or PT}}}{((r_{S^{2-} \rightarrow S^0}) + (r_{S^0 \rightarrow SO_4^{2-}}))_{\text{BF}} + ((r_{S^{2-} \rightarrow S^0}) + (r_{S^0 \rightarrow SO_4^{2-}}))_{\text{PT}}} \times 100 \quad (10)$$

2.6. NO_x^-/S^{2-} influence on selected operating costs in SADN-MBBR

Laboratory-scale experimental data were used for an initial estimate of selected operating costs (OPEX) of an industrial-scale SADN-MBBR design of a latex processing factory in Songkhla Province, Thailand. As a basis for calculations, a biogas flowrate of $250\text{ m}^3\text{h}^{-1}$ containing 55% CH_4 and 20,450 ppm_v of H_2S at $37\text{ }^\circ\text{C}$ and 1 atm was considered. Based on sulfur and nitrogen rates obtained in this study, an MBBR with an effective reactor volume of 149.4 m^3 was considered operating with an influent flow rate and HRT of $149.4\text{ m}^3\text{d}^{-1}$ and 1 d, respectively, that corresponded to a SLR of $1.034\text{ kg}\cdot\text{S}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ ([Supplementary Material, Fig. S1](#)).

Selected OPEX estimates were conducted to compare five experimentally tested conditions and one literature-derived benchmark condition for H_2S removal from biogas. The experimentally tested NO_x^-/S^{2-} conditions were 1.6, 1.8, 2.0, and 2.2 with NO_3^- and 2.2 with NO_2^- ; the NO_3^-/S^{2-} ratio of 0.3 was included only as a literature-derived benchmark. Annual OPEX were estimated for each condition and normalized to the lowest-cost scenario to obtain a relative cost index. The analysis considered media cleaning, biogas-to-electricity losses during cleaning

downtime, N₂O emission fees, acid consumption, and electricity demand for nitrification (Supplementary Material, Section S2.5). A deterministic $\pm 30\%$ sensitivity and uncertainty analysis was additionally performed for key cost variables, including electricity, water, acid, and N₂O-related costs. Detailed assumptions and calculation procedures are provided in Section S2.5.

2.7. Statistical analysis

The performance of the MBBR was derived from data collected under stable conditions, specifically four data points per phase. The average values were analyzed to evaluate the effects of independent factors in the experiments, applying a 95% confidence interval ($\alpha = 0.05$). Statistical comparisons were conducted using Duncan's New Multiple Range Test (DMRT) in SPSS software version 11.0.

3. Results and discussion

3.1. Effects of the $\text{NO}_3^-/\text{S}^{2-}$ ratio on SADN performance

3.1.1. Sulfide and S^0 oxidation and SO_4^{2-} production

Fig. 2a shows the average sulfur oxidation rates at pseudo steady-state as a function of the $\text{NO}_3^-/\text{S}^{2-}$ ratio, ranging from 1.6 to 2.2. Under all conditions, effluent S^{2-} concentrations were below 0.1 mM (Supplementary Material, Fig. S4) with no H_2S in the off-gas. Statistical analysis ($\alpha = 0.05$) confirmed that all $\text{NO}_3^-/\text{S}^{2-}$ ratios led to complete S^{2-} removal. All S^{2-} was used as electron donor by SO-NR bacteria and oxidized to S^0 and SO_4^{2-} via partial and complete S^{2-} oxidation, respectively (Supplementary Material, Eq. S1 – S2 and Eq. S3 – S4). No $\text{S}_2\text{O}_3^{2-}$ was found in the effluent, consistent with its high reactivity and preferential oxidation over S^{2-} and S^0 (Callbeck et al., 2021). SO_4^{2-}

concentrations increased from 12.5 to 14.3 mM with increasing $\text{NO}_3^-/\text{S}^{2-}$ ratio, while residual S^0 showed the opposite trend (Fig. 2a). This pattern indicates that higher NO_3^- availability promoted further oxidation of S^{2-} -derived S^0 to SO_4^{2-} .

In SADN, S^{2-} is first oxidized to S^0 via sulfide:quinone oxidoreductase (Sqr), and S^0 can be further oxidized to SO_4^{2-} through the Sox pathway when sufficient electron acceptor is available (Qin et al., 2019). Under NO_3^- limited conditions, sulfur oxidation can stop at S^0 , whereas sufficient NO_3^- availability promotes continued oxidation to SO_4^{2-} (Cui et al., 2019c). Based on the catabolic stoichiometry (Supplementary Material, Eq. S3), influent S^{2-} can theoretically be fully oxidized to SO_4^{2-} at a $\text{NO}_3^-/\text{S}^{2-}=1.6$ provided enough HRT in the system. However, 16.5% of the removed S^{2-} remained as residual S^0 at $\text{NO}_3^-/\text{S}^{2-}=1.6$, while this fraction decreased to 5.2% at a ratio of 2.2 (Fig. 2c).

Competition among microbial guilds contributed to the observed results. Mixotrophic denitrifiers, including *Chiayiivirga*, *Chryseobacterium*, and *Paracoccus*, were detected in the MBBR (Sections 3.3.1–3.3.2). These organisms can utilize organic carbon derived from EPS turnover and biomass decay as electron donor and utilize NO_3^- as electron acceptor to perform heterotrophic denitrification (see Supplementary Material, Eq. S12) while coexisting with sulfur-oxidizing denitrifiers within the same biofilm matrix (Cui et al., 2019a; Du et al., 2023). Heterotrophic denitrification yields approximately 810 kJ-reaction⁻¹ (Supplementary Material, Eq. S12), which is 1.48–1.6 times higher than autotrophic denitrification using S^{2-} or S^0 as electron donors (501.4 and 547.6 kJ-reaction⁻¹; Supplementary Material, Eqs. S4–S5). This thermodynamic advantage supports the utilization of organic carbon coupled with NO_3^- reduction (Wu et al., 2023). Consequently, part of the influent NO_3^- was consumed by heterotrophic/mixotrophic denitrification, reducing the effective $\text{NO}_3^-/\text{S}^{2-}$ ratio available for sulfur oxidation and leading to increased residual S^0 . This

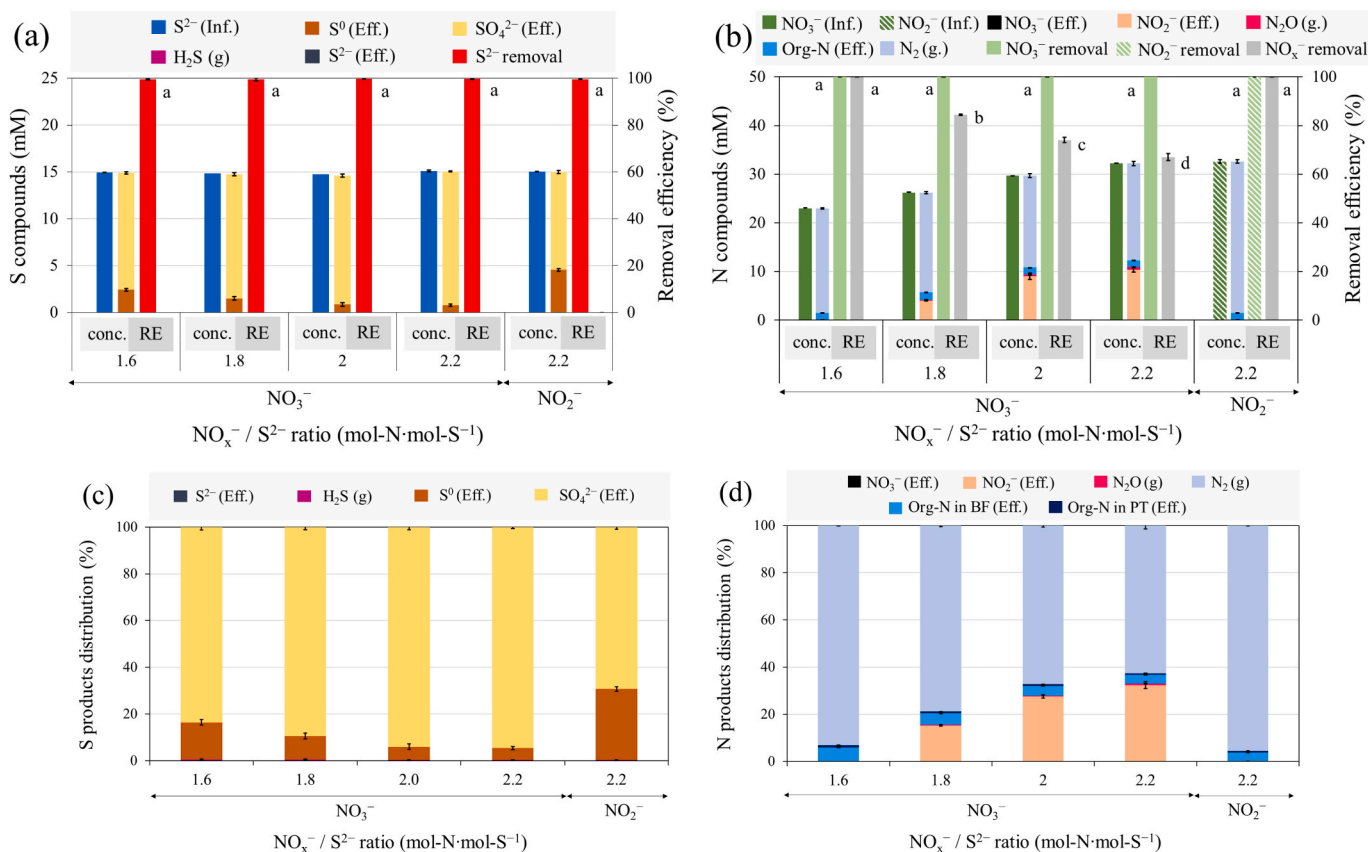


Fig. 2. Averages of the performance at steady state of MBBR at different $\text{NO}_3^-/\text{S}^{2-}$ ratios and electron acceptors: (a) sulfur oxidation, (b) nitrogen reduction, (c) sulfur product distribution, and (d) nitrogen product distribution. Different letters indicate significant difference of the means at $\alpha = 0.05$.

interpretation is supported by the enrichment of mixotrophic genera, the persistence of residual S^0 at $NO_3^-/S^{2-}=1.6$ as well as the batch tests and electron-balance results discussed in Section 3.4.1.

When comparing SO_4^{2-} production, Cui et al. (2019a) reported a SO_4^{2-} production rate of $21.9 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ in an MBBR treating an SLR of $22.8 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ ($NO_3^-/S^{2-}=1.2$, pH 7.2), corresponding to 4.2% ($0.92 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) S^0 accumulation. At the lowest NO_3^-/S^{2-} ratio in the MBBR used in this work ($NO_3^-/S^{2-}=1.6$), a larger accumulation of S^0 was found. Differences in performance with respect to Cui et al. (2019a) could be attributed to a greater abundance of mixotrophs (e.g. *Chiayivirga*, *Chryseobacterium*, and *Paracoccus*) in our MBBR, which likely diverted more NO_3^- to heterotrophic pathways. Meanwhile, Cui et al. (2019a) detected only *Paracoccus* as mixotroph with a relative abundance around half lower than in the MBBR used in this work.

The MBBR performed better compared with similar planktonic and granular bioreactors reported. Yuan et al. (2022) reported a sequencing batch reactor (SBR) operated at a SLR of $13.71 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ and $NO_3^-/S^{2-}=1.1$ (pH 8.0) that produced around 65.3% S^0 . In an upflow blanket filter (UBF) with granular biomass operated at a SLR of $31.25 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ ($NO_3^-/S^{2-}=1.0$, pH 7.8) the SO_4^{2-} production rate was $18.9 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ while the accumulation of S^0 was $10.1 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$. These comparisons indicate that MBBRs are the superior choice when the objective is to maximize SO_4^{2-} production and minimize S^0 accumulation. MBBRs provide high surface area and promote thin active biofilms that shorten diffusion paths, allowing efficient NO_3^- penetration and more complete oxidation of S^{2-} to SO_4^{2-} (Cui et al., 2019c). They also maintain higher biomass concentrations without the washout risk typical of planktonic reactors (Kostretysia et al., 2022). In contrast, granular biomass suffers from strong internal mass-transfer limitations, which promote S^0 entrapment within the bioreactor (Liu et al., 2022). As a result, MBBR biofilms exhibit lower diffusion resistance and better electron-acceptor delivery, yielding higher SO_4^{2-} production with less S^0 . Because the same NO_3^- pool served as the electron acceptor for both sulfide oxidation and denitrification, the observed sulfur-conversion pattern should be interpreted together with nitrogen transformation and gaseous N_2O formation.

3.1.2. Nitrogen transformation and gaseous N_2O emissions

Comparisons of nitrogen conversion and NO_3^- removal efficiency across all conditions tested are shown in Fig. 2b. Complete NO_3^- removal (99.9%) was achieved at NO_3^-/S^{2-} ratios between 1.6 and 2.2, with no significant differences observed ($\alpha = 0.05$). However, complete NO_3^- removal occurred only at $NO_3^-/S^{2-}=1.6$, with N_2 as the dominant nitrogen end product. At higher NO_3^-/S^{2-} ratios of 1.8–2.2, NO_3^- was still fully consumed, but NO_3^- removal significantly decreased from 99.9% to 67.0%, indicating that nitrate reduction was not the limiting step. Instead, downstream denitrification was incomplete, as indicated by NO_2^- and N_2O accumulation from incomplete nitrite and nitrous oxide reduction (Qin et al., 2019; Wu et al., 2022). At $NO_3^-/S^{2-}=2.2$, NO_2^- in the effluent and N_2O in the off-gas increased to 10.3 mM and 0.23 mM, respectively, corresponding to distribution fractions of 32.3% and 0.7%, whereas both intermediates were undetectable at $NO_3^-/S^{2-}=1.6$ (Fig. 2d).

Although NO_3^- , NO_2^- and N_2O can serve as electron acceptors in denitrification, the bacterial preference for electron acceptors in S^{2-} oxidation can be explained by their standard redox potentials (E^0), which are $+0.43$ ($NO_3^- \rightarrow NO_2^-$), $+0.35$ ($NO_2^- \rightarrow NO$) and $+1.36$ V ($N_2O \rightarrow N_2$). A higher E^0 indicates a greater electron affinity, also thermodynamically, NO_3^- is more favorable than NO_2^- (Thauer et al., 1977). Although N_2O has higher E^0 than NO_3^- , residual N_2O was still detected. This is because electrons released from S^{2-} oxidation enter the ubiquinone/ubiquinol (UQ/UQH₂) and are transferred through the denitrification chain, in which Nar catalyzes NO_3^- reduction to NO_2^- , Nir catalyzes NO_2^- reduction to NO, Nor catalyzes NO reduction to N_2O , and Nos catalyzes N_2O reduction to N_2 (Oberoi et al., 2021). At higher NO_3^-/S^{2-} ratios, the increased nitrate loading imposed greater electron

demand on this sequence. Consequently, the available reducing power was sufficient to complete NO_3^- reduction through Nar but insufficient to fully sustain NO_2^- reduction via Nir and nitrous oxide reduction via Nos. This incomplete NO_2^- reduction led to NO_2^- accumulation, while incomplete N_2O reduction led to a residual N_2O emission. Conversely, the absence of detectable NO_2^- and N_2O at $NO_3^-/S^{2-}=1.6$ indicates that the available electron supply was sufficient to complete downstream denitrification to N_2 (Oberoi et al., 2021). Importantly, the results indicate that maximizing sulfide oxidation through elevated nitrate availability does not necessarily correspond to the most environmentally sustainable operating condition due to increased N_2O formation. Despite favorable thermodynamics, enzymatic and electron-transfer limitations controlled N_2O reduction.

Cui et al. (2017) demonstrated complete reduction of NO_3^- to N_2 when NO_3^- was fed at $4.3 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ in the MBBR at NO_3^-/S^{2-} ratio and HRT of 1.6 and 4 h (pH 7.5). This finding aligns with our results and further supports 1.6 as alternative operating strategy for nitrogen control. Although incomplete denitrification was observed above an $NO_3^-/S^{2-}=1.6$, operating at ratios of 1.1–1.2 also lead to incomplete denitrification according to Eq. S2 (Supplementary Material). Polizzi et al. (2022) found NO_2^- accumulation of $4.9 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ (8.9 mM) and N_2O production of $0.12 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ (2.1 μM) in a CSTR with planktonic biomass when feeding NO_3^- at $7.2 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$, $NO_3^-/S^{2-}=1.2$ and HRT of 20 h (pH 7.2). Similarly, in a UFB with granular biomass, Yang et al. (2016) reported NO_2^- accumulation of $0.32 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ (0.3 mM) and N_2O production of $0.13 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ (15.5 ppm) at NO_3^- loading rate and HRT of 6.43 mM and 20 h ($NO_3^-/S^{2-}=1.1$; pH 7.5).

However, Cui et al. (2019a) reported complete denitrification in an MBBR operated at a NO_3^- loading rate of $7.2 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ and $NO_3^-/S^{2-}=1.2$ (pH 7.8), despite using an HRT of only 4 h. The higher denitrification is linked to biomass morphology, where planktonic biomass, which lacks a protective EPS barrier, exposes cells to direct contact with inhibitory compounds (Huang et al., 2017). Granules concentrate activity in a thin outer layer at the liquid interface and inhibition of this shell rapidly depresses performance (Yang et al., 2016). By contrast, biofilm exhibit greater tolerance to NO_2^- and N_2O , which arises from its dense, stratified EPS matrix, thus restricting the diffusion of these inhibitors, enabling outer cells to intercept and lower their concentrations before they reach inner cells, thereby maintaining reactor performance even under high nitrogen levels (Cui et al., 2019c). These findings suggest that the MBBR represents a promising alternative also for nitrogen-laden wastewater treatment and that sulfur control and denitrification completeness are strongly interconnected operational objectives in SADN-MBBRs. These results also indicate that the balance between sulfate-oriented sulfide oxidation and complete denitrification depends not only on the NO_3^-/S^{2-} ratio, but also on the type of electron acceptor supplied to the MBBR.

3.2. Effects of electron-acceptor type on sulfur transformation, denitrification, and gaseous N_2O emissions

After the MBBR was fed with NO_2^- at $NO_2^-/S^{2-}=2.2$, the S^{2-} removal efficiency achieved 99% (Fig. 2a). However, the SO_4^{2-} concentration was 1.36-fold lower when NO_2^- was used, reducing the SO_4^{2-} fraction in the effluent from 94.7% to 73.5% (Fig. 2c). This can be attributed to the lower electron-accepting capacity of NO_2^- . One mole of NO_2^- can accept only three electrons per mole, while NO_3^- can accept five, thereby limiting the total electron transfer and causing partial oxidation of S^{2-} to S^0 (Cai et al., 2022). In practical terms, when operating with NO_2^- , achieving the same SO_4^{2-} yield as with NO_3^- typically requires higher NO_2^-/S^{2-} ratio or staged dosing to supply sufficient electron-acceptor capacity for both $S^{2-} \rightarrow S^0$ and $S^0 \rightarrow SO_4^{2-}$.

In addition, the NO_3^- removal efficiency under this condition reached 99.9%, which was 32.9% points higher than that achieved with NO_3^- at the same NO_3^-/S^{2-} ratio, with no detectable N_2O in the off-gas (Fig. 2b). Compared with NO_3^- feeding at the same ratio, switching to NO_2^-

increased residual S^0 from 5.2% to 26.5% while maintaining complete denitrification, providing an alternative strategy for controlling off-gas N_2O . Denitrification from NO_2^- bypasses the initial NO_3^- -reduction step and proceeds through $NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$, requiring three donated electrons per mole of N, compared with five for NO_3^- reduction to N_2 (Wang et al., 2021). At an NO_x^-/S^{2-} ratio of 2.2, the corresponding electron requirement decreased from $164.0 \text{ mmol-e} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$ with NO_3^- feeding to $98.4 \text{ mmol-e} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$ with NO_2^- feeding. This lower electron demand reduced the extent of sulfide oxidation to SO_4^{2-} , thereby increasing residual S^0 , while likely allowing sufficient electron flow through the downstream denitrification steps for complete reduction of N_2O to N_2 .

Although NO_2^- has a lower electron accepting capacity per mole than NO_3^- , it can still drive complete oxidation of S^{2-} to SO_4^{2-} when the NO_2^- loading is increased to meet the total electron acceptor demand, including further oxidation of accumulated S^0 to SO_4^{2-} . These findings highlight the distinct electron-transfer constraints associated with nitrate- and nitrite-based SADN pathways and indicate that further work is still needed to define the full NO_x^-/S^{2-} range that maximizes sulfate production and minimizes residual S^0 in MBBRs. The distinct process responses observed under different NO_x^-/S^{2-} ratios and electron acceptors were further examined in relation to microbial community shifts in the biofilm and planktonic biomass.

3.3. Microbial community responses to the NO_x^-/S^{2-} ratio and electron-acceptor type

3.3.1. Community shifts under different NO_x^-/S^{2-} ratios and electron acceptors

Fig. 3 shows the microbial community composition in the biofilm and planktonic biomass in the MBBR. The initial inoculum was dominated by *Sulfurimonas*, *Enterococcus*, *Megasphaera*, and *Bifidobacterium*. *Enterococcus*, *Megasphaera*, and *Bifidobacterium*, which are anaerobes (Kushkevych and Jampilek, 2022), were probably found in the original

inoculum as this was obtained from a CSTR operated at an $NO_3^-/S^{2-}=0.6$. Under this condition, the limited availability of NO_3^- created a redox potential at the boundary between anoxic and anaerobic states. After operating the MBBR at $NO_3^-/S^{2-}=1.6$, these anaerobes decreased sharply from 49.4% to below 0.1%, while SO-NR bacteria became dominant, reflecting their strong competitive advantage when NO_3^- is available (Callbeck et al., 2021).

At $NO_3^-/S^{2-} = 1.6$, *Sulfurimonas*, *Aequorivita*, *Alishewanella*, and *Aliidiomarina* were enriched in the biofilm, while *Thiobacillus* and *Paracoccus* were more abundant in the planktonic biomass. These genera include organisms reported to participate in sulfur oxidation coupled with NO_3^- or NO_2^- reduction (Cui et al., 2019a; Li et al., 2023; Wu et al., 2023). Their dominance coincided with complete sulfide and nitrate removal and is therefore consistent with effective sulfur-based denitrification. Mixotrophic genera such as *Chiayiivirga* and *Chryseobacterium* also increased from < 1% in the inoculum to 10.7–16.0% in the biofilm and 9.2–11.2% in the planktonic biomass. This enrichment demonstrates that these taxa were substantial community members. Their reported ability to use EPS- and biomass-derived organics supports the possibility of concurrent heterotrophic/mixotrophic denitrification, consistent with the residual S^0 distribution and the electron-balance interpretation in Section 3.4.1.

Distinct microbial community compositions were observed at the sampled NO_x^-/S^{2-} operating phases and under the different electron acceptors. At $NO_3^-/S^{2-}=2.2$, *Sulfurimonas* reached its highest relative abundance in both biofilm (41.8%) and planktonic biomass (45.3%). This growth corresponded with the highest SO_4^{2-} concentration observed at this ratio, indicating that NO_3^- supported a greater extent of S^{2-} oxidation to SO_4^{2-} . Additionally, *Gelidibacter* and *Mucilaginibacter* were also enriched, consistent with previous works reporting that these EPS-producing genera thrive under nitrogen-rich conditions (Huang and Cui, 2025), as they obtain greater energy from denitrification to support EPS synthesis (Ma et al., 2024). Zhou et al. (2026) reported increased *Paracoccus* abundance and N_2O emissions at in a S^0 -packed bed

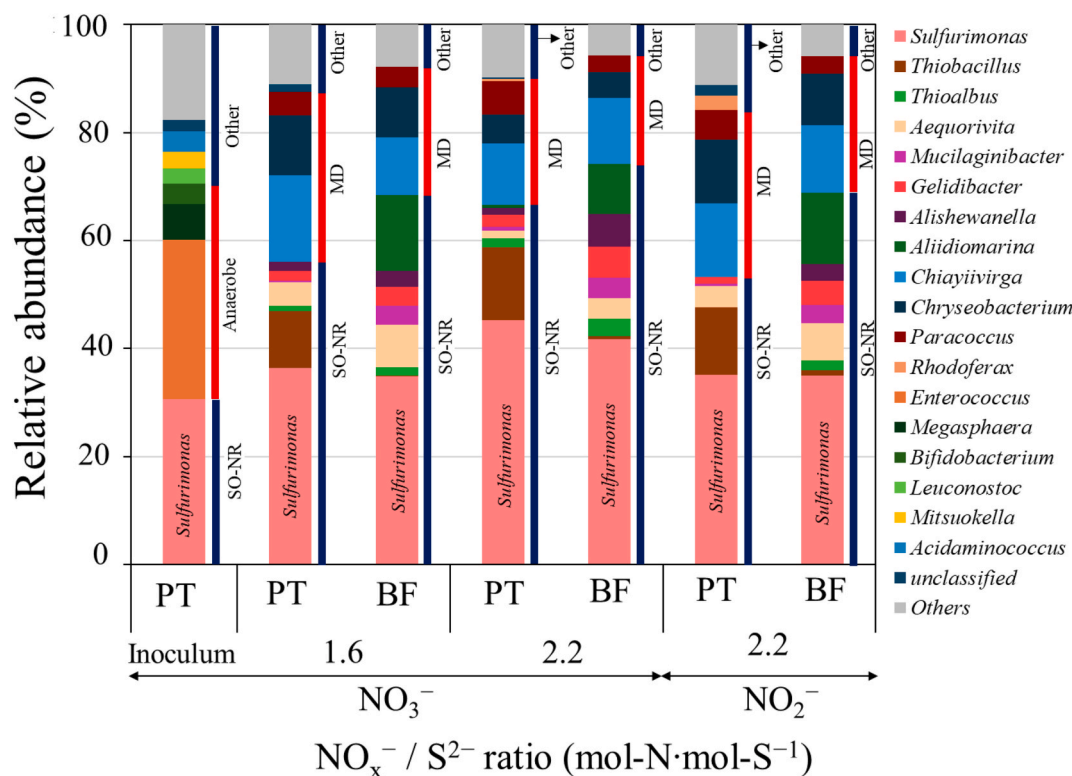


Fig. 3. Microbial community compositions in planktonic (PT) and biofilm (BF) biomass in original inoculum, during MBBR operation at NO_3^-/S^{2-} of 1.6 and 2.2 with NO_3^- , and at an NO_2^-/S^{2-} of 2.2 with NO_2^- . Note: SO-NR and MD mean SO-NR bacteria and mixotrophic denitrifier, respectively.

bioreactor at ratio above 1.7, with an N_2O emission of $0.09 \text{ mmol-N-L}^{-1} \cdot \text{d}^{-1}$ (0.5% of NO_3^- in feed), supporting its potential involvement in N_2O turnover.

At $\text{NO}_2^-/\text{S}^{2-}=2.2$ under NO_2^- feeding, *Chryseobacterium* was more abundant in both biofilm and planktonic communities than at the same ratio with NO_3^- . Du et al. (2023) reported that this genus preferentially utilizes NO_2^- as electron acceptor in denitrification and predominantly exists in biofilms, which enhances its resistance to NO_2^- inhibition. Meanwhile, *Sulfurimonas* declined due to the lower energy yield from S^{2-} oxidation to SO_4^{2-} after utilizing NO_2^- instead of NO_3^- (Supplementary Material, Eqs. S3 and S8). Overall, the $\text{NO}_x^-/\text{S}^{2-}$ ratio, together with electron-acceptor type, was a key operational driver shaping the microbial community structure in the MBBR, and these community shifts influenced sulfur-product distribution and nitrogen-transformation pathways.

3.3.2. Differences between biofilm and planktonic communities

A detailed comparison of biofilm and planktonic biomass across all conditions (1.6 (NO_3^-), 2.2 (NO_3^-), and 2.2 (NO_2^-)) is shown in Fig. 3. *Sulfurimonas*, *Thioalbus*, *Aequorivita*, *Alishewanella* and *Aliidiomarina* were more abundant in the biofilm. These genera grew better at $\text{NO}_3^-/\text{S}^{2-}=2.2$ than 1.6 (Section 3.3.1), as NO_3^- diffuses more effectively into the biofilm than sulfide ($\text{D}_{\text{NO}_3^-}/\text{D}_{\text{S}_2}=2.6$), allowing attached cells to access sufficient electron acceptors and thus develop more successfully than planktonic biomass. Moreover, *Gelidibacter* and *Mucilaginibacter* were detected in the biofilm due to their strong biofilm-forming ability. Wen et al. (2024) reported that higher proportions of *Gelidibacter*

reduced NO_2^- and N_2O inhibition because EPS on the biofilm layer acts as a barrier limiting direct exposure of microbial cells to NO_2^- and N_2O .

However, *Thiobacillus* and *Paracoccus* were more abundant in planktonic biomass, accounting for 10.6–13.6% and 4.3–6.2%, respectively, whereas their relative abundances in biofilm biomass were only 3.80% and 1.04%. Both are weak EPS producers with limited attachment ability (Cui et al., 2019a), resulting in low surface adhesion and a tendency to remain suspended. In addition, both genera have been reported to favor S^0 accumulation under NO_3^- -limited conditions (Polizzi et al., 2022), which is consistent with the residual S^0 observed in the MBBR effluent. The preferential enrichment of *Paracoccus* in the planktonic biomass also suggests that the suspended community may have contributed to N_2O turnover and residual N_2O emissions in the MBBR.

3.4. Functional activity and biomass activity

3.4.1. Activity between SO-NR bacteria and heterotrophic denitrifiers

The activity of SO-NR biomass was determined from influent-effluent sulfur and nitrogen species concentrations and converted into equivalent electron-transfer units. Heterotrophic denitrification activity was then estimated from the difference between total nitrogen-based and sulfur-based equivalent electron transfer. The oxidation of S^{2-} to S^0 and S^0 to SO_4^{2-} donates two and six moles of electrons, respectively ($\text{S}^{2-} \rightarrow \text{S}^0 + 2\text{e}^-$ and $\text{S}^0 \rightarrow \text{SO}_4^{2-} + 6\text{e}^-$), while the NO_3^- reduction to NO_2^- and NO_2^- to N_2 consume two and three moles of electrons, respectively ($\text{NO}_3^- + 2\text{e}^- \rightarrow \text{NO}_2^-$ and $\text{NO}_2^- + 3\text{e}^- \rightarrow \text{N}_2$). Electron donation was

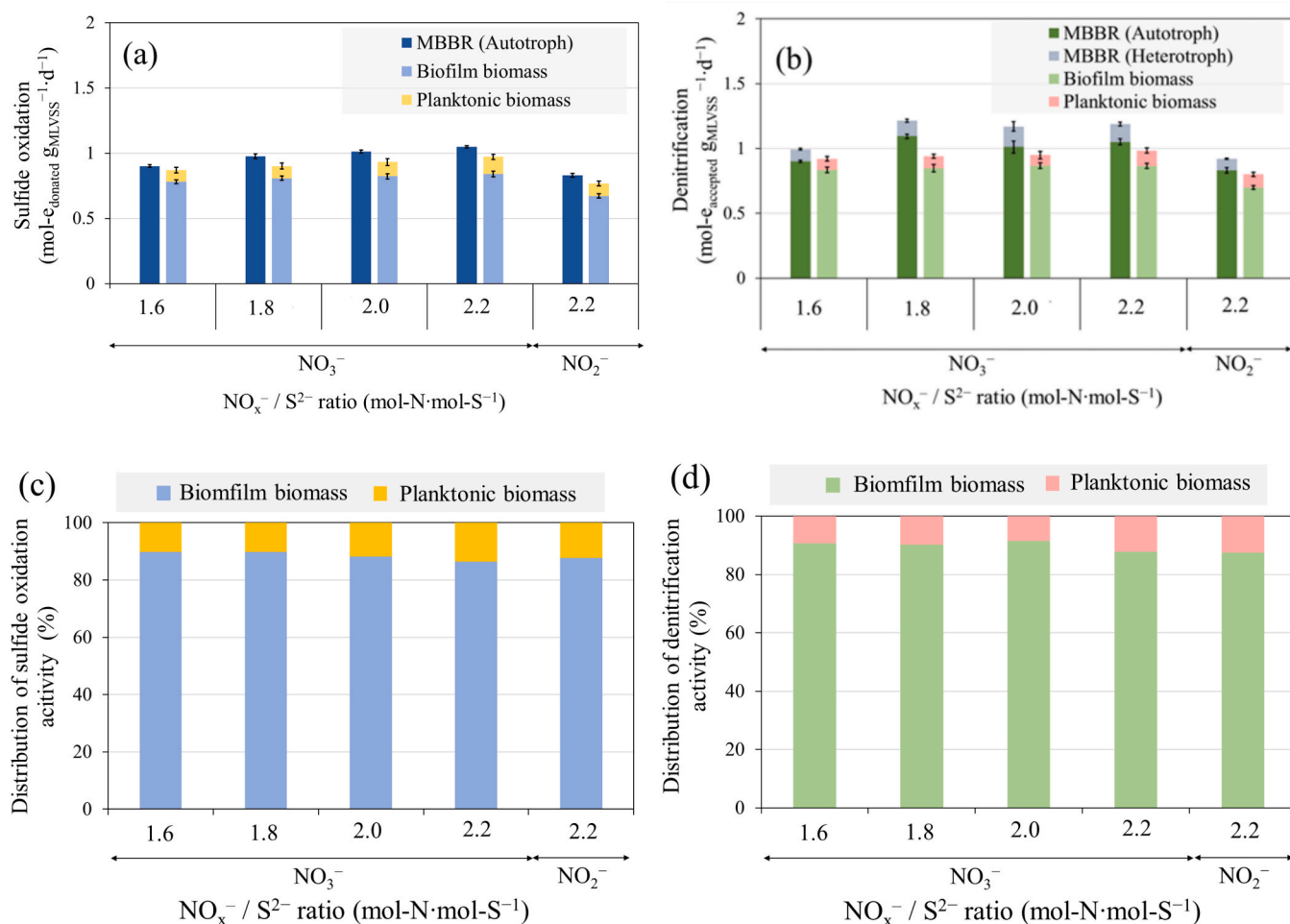


Fig. 4. Comparative distribution of electron transport between biofilm and planktonic biomass under varying $[\text{NO}_x^-]/[\text{S}^{2-}]$ ratios and electron acceptor types for (a) S^{2-} oxidation and (b) $\text{NO}_3^-/\text{NO}_2^-$ denitrification, and activity distribution for (c) S^{2-} oxidation and (d) $\text{NO}_3^-/\text{NO}_2^-$ denitrification.

calculated from the concentrations of S^0 and SO_4^{2-} produced via S^{2-} oxidation at steady-state during each operational condition along the operation of the MBBR (Supplementary Material, Fig. S5 and Table S1).

MBBR results showed that when NO_3^- served as the electron acceptor, total electron donating (r_S) and accepting (r_N) rates at NO_3^-/S^{2-} ratios of 1.6 and 2.2 ranged from 0.90–1.05 mol-e_{donated}·gVSS⁻¹·d⁻¹ and 0.99–1.19 mol-e_{accepted}·gVSS⁻¹·d⁻¹, respectively (Fig. 4a and 4b). In contrast, using NO_2^- as the electron acceptor in the MBBR, r_S and r_N were lower by approximately 20.0% and 18.7% (Fig. 4a and 4b), respectively, owing to the limited availability of electron acceptors for the oxidation of S^0 to SO_4^{2-} . Notably, r_N exceeded r_S under all tested conditions by approximately 10.7–15.6%, indicating an electron-balance mismatch between nitrogen reduction and sulfide-derived sulfur oxidation. The calculated SO-NR activity in the MBBR reached up to 84.4%, confirming that SO-NR bacteria were the dominant contributors to electron transfer. However, the remaining electron demand for nitrogen reduction could not be fully explained by sulfide-derived sulfur oxidation alone. This difference indicates an additional contribution from HDN, most likely endogenous organic carbon released from EPS turnover and biomass decay (Cui et al., 2019a).

This interpretation is supported by the increase in heterotrophic/mixotrophic denitrification activity from 0.09 to 0.16 mol-e_{accepted}·gVSS⁻¹·d⁻¹ (Fig. 4b) when the NO_3^-/S^{2-} increased from 1.6 to 2.2 (Fig. 4b), consistent with the enrichment of mixotrophic denitrifiers in Fig. 3. Together with residual S^0 under all tested NO_3^-/S^{2-} ratios, these results indicate competition for NO_3^-/NO_2^- between SADN and heterotrophic/mixotrophic denitrification, contributing to residual S^0 accumulation in the MBBR.

3.4.2. Activity partitioning between biofilm and planktonic biomass

In batch assays, electron donation and acceptance were calculated from S^0 and SO_4^{2-} generated by S^{2-} oxidation and from NO_2^- and N_2 produced by denitrification along each batch test (Supplementary Material, Fig. S5 for a $NO_3^-/S^{2-}=1.6$ and Table S1). The major contribution to electron transport was provided by biofilm biomass, with r_S and r_N ranging from 0.78–0.84 mol-e_{donated}·gVSS⁻¹·d⁻¹ and 0.83–0.87 mol-e_{accepted}·gVSS⁻¹·d⁻¹, respectively. These values corresponded to biofilm activity, with $A_{BF,d}$ and $A_{BF,ac}$ accounting for 86.5–89.9% and 87.7–91.5% of the total electron donation and acceptance, respectively (Fig. 4c and 4d).

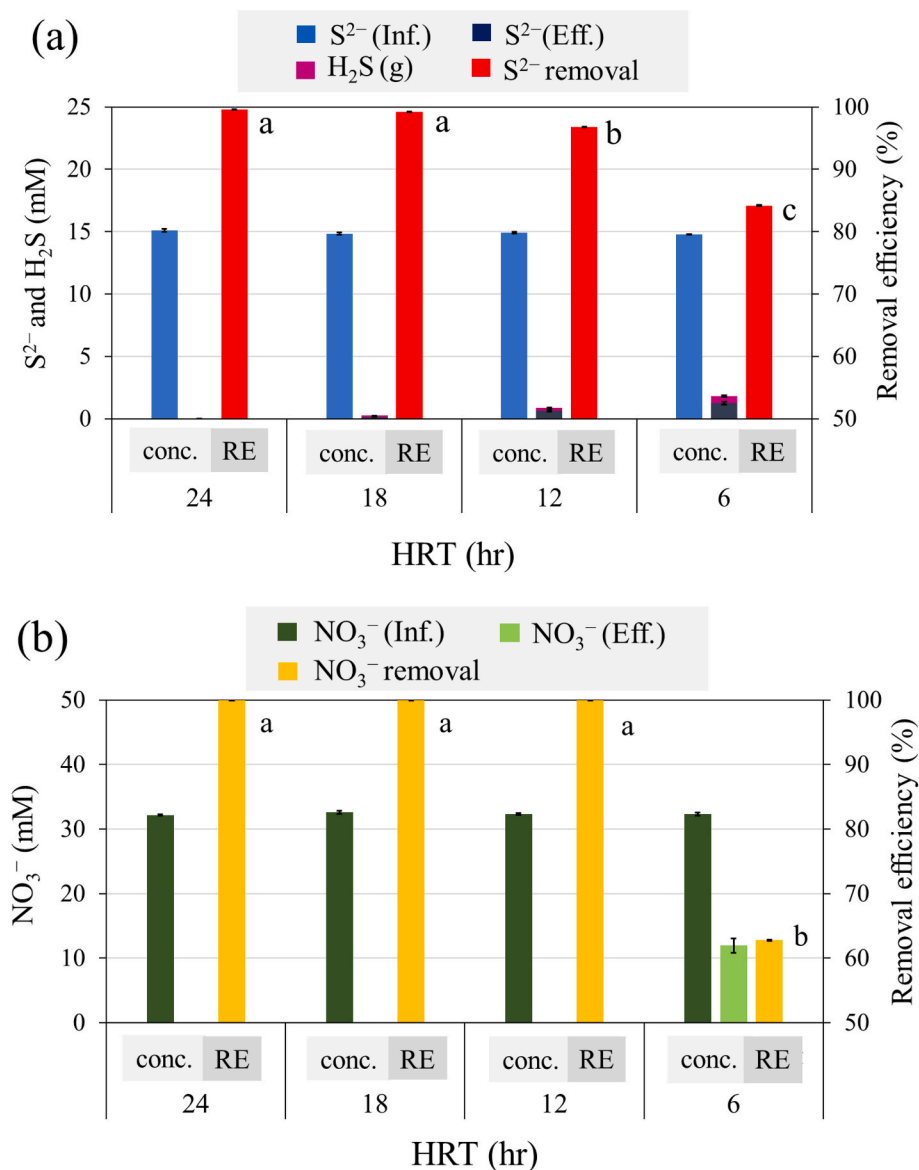


Fig. 5. Comparison of S^{2-} and NO_3^- concentrations and removal efficiencies of MBBR under different HRTs; (a) S^{2-} concentration and removal efficiency, and (b) NO_3^- concentration and removal efficiency. Different letters indicate significant difference of the means at $\alpha = 0.05$.

Moreover, MBBR activity exceeded the sum of individual biofilm and planktonic biomass activities (Fig. 4a and 4b), indicating synergistic interactions. Huang et al. (2017) and Li et al. (2021) reported that planktonic biomass in MBBRs plays a crucial role in alleviating diffusion limitations helping reduce steep concentration gradients at the interface between the bulk liquid and the biofilm surface. Such interactions could facilitate the delivery of S^{2-} and NO_3^- to biofilm-associated microbial communities, potentially enhancing the overall process efficiency. It is worth mentioning that, according to the diffusion coefficients of S^{2-} and NO_3^- in water, $0.73 \times 10^{-9} \text{ m}^2\text{s}^{-1}$ and $1.90 \times 10^{-9} \text{ m}^2\text{s}^{-1}$ (Parkhurst and Appelo, 1999), respectively, formation of biosulfur inside the biofilm was eventually minimal. The faster diffusion of NO_3^- compared with S^{2-} leads to a higher NO_3^-/S^{2-} ratio than that in the bulk liquid as both ions penetrate in the biofilm, thus promoting further oxidation of S^{2-} to SO_4^{2-} for SO_4^{2-} to become the dominant sulfur product within the biofilm. These findings indicate that MBBR performance was primarily governed by biofilm biomass with minimal biosulfur production, which helps improve MBBR stability in the long term as biosulfur accumulation is prevented. However, results indicate that planktonic biomass significantly participated in sulfur and nitrogen conversions as it likely supported the overall process by enhancing mass transfer, dispersing cells from biofilms, and promoting community stability.

3.5. Reactor performance under shortened HRT and increased sulfur and nitrogen loading rates

Fig. 5 shows the influent and effluent S^{2-} and NO_3^- concentrations and their removal efficiencies at HRTs of 24, 18, 12, and 6 h. Influent concentrations were fixed at 14.9 mM S^{2-} and 32.8 mM NO_3^- ; consequently, shortening HRT increased SLR from 14.9 to 59.6 mmol-S $\cdot$ L $^{-1}\cdot$ d $^{-1}$ and NLR from 32.8 to 131.2 mmol-N $\cdot$ L $^{-1}\cdot$ d $^{-1}$. The observed response therefore represents the combined effects of reduced contact time and increased volumetric loading. Removal of both compounds exceeded 99% at 24 and 18 h. At 12 h, S^{2-} removal decreased to 96.3%, while NO_3^- removal was not significantly affected ($\alpha = 0.05$); thus, 12 h was the shortest tested HRT maintaining > 95% removal of both compounds while accommodating twice the volumetric loading applied at 24 h. At 6 h, S^{2-} and NO_3^- removal decreased to 87.3% and 62.8%, respectively. This decline in efficiency may reflect both insufficient reaction time and overloading, and the present design does not allow their individual effects to be separated.

In comparison, Fajardo et al. (2012) reported an SBR with planktonic-growth biomass at 24 h HRT that achieved 99.9% S^{2-} removal but only 46.8% NO_3^- removal at similar influent levels (10.9 mM S^{2-} , 31.2 mM NO_3^-), corresponding to volumetric removal rates of 0.45 and 0.61 mmol-L $^{-1}\cdot$ h $^{-1}$ for S^{2-} and NO_3^- . Likewise, Zhao et al. (2025) demonstrated that a UBF with granular sludge achieved equivalent S^{2-} removal (99.9%) but exhibited even lower NO_3^- removal (59.4%), with removal rates of 0.12 mmol-L $^{-1}\cdot$ h $^{-1}$ and 0.37 mmol-L $^{-1}\cdot$ h $^{-1}$ for S^{2-} and NO_3^- under the same HRT, with influent concentrations as low as 3.9 mM S^{2-} and 15.0 mM NO_3^- . These comparisons highlight the superior NO_3^- removal and operational flexibility of the MBBR, particularly under higher influent loads and shorter HRTs. However, Cui et al. (2019b) reported an MBBR achieving 99.9% S^{2-} and NO_3^- removal at 24 h with influent 14.1 mM S^{2-} and 17.9 mM NO_3^- (0.59 and 0.75 mmol-L $^{-1}\cdot$ h $^{-1}$, respectively).

This is because planktonic biomass takes up S^{2-} and NO_3^- independently through dispersed cells, resulting in less synchronized metabolic activity (Huang et al., 2021). Granular biomass offers better synchronized metabolic activity than planktonic biomass, but its dense structure imposes internal diffusion resistance, slowing substrate penetration across layers (Yang et al., 2016). However, biofilms have high surface-area-to-volume ratios, enabling efficient diffusion of S^{2-} and NO_3^- into biofilm (Cui et al., 2019c), leading to higher synchronized activity than planktonic and granular biomass.

4. Operational tradeoffs and practical implementation of SADN bioscrubbers

SADN provides an environmentally sound and cost-effective pathway for integrated waste treatment in facilities where biogas desulfurization and nitrogen-rich wastewater treatment are both required. Such conditions are common in latex processing, agro-industrial plants, livestock farms, and meat-processing industries. In these systems, biogas scrubbing generates sulfide-rich absorbents, while wastewater streams contain excess NH_4^+ . Through nitrification, NH_4^+ is converted to NO_3^- or NO_2^- , which serve as electron acceptors for SADN in the biological stage of a bioscrubber (Supplementary Material, Fig. S1), enabling simultaneous H_2S and NH_4^+ removal and transforming two waste streams into a synergistic loop.

A major operational challenge in anoxic desulfurization systems is biosulfur accumulation under NO_3^- -limited conditions, which can clog carriers, reduce mass transfer, increase pressure drop, and cause downtime (Wang et al., 2023). Table 3 summarizes the utility costs for the selected biogas desulfurization scenario. The cost analysis focused on the utility and operational costs considered most relevant to the operational tradeoffs governing SADN performance, rather than providing a comprehensive estimation of total OPEX. At $NO_3^-/S^{2-}=0.3$, nitrification energy demand was lowest and N_2O emissions were absent; however, severe S^0 accumulation required frequent cleaning, resulting in the highest annual cost (7,107 €-yr $^{-1}$). Increasing the NO_3^-/S^{2-} ratio reduced S^0 accumulation and extended cleaning intervals. At $NO_3^-/S^{2-}=1.6$, S^0 accumulation decreased to 16.5%, the cleaning cycle increased to 85 days, and total cost declined to 1,681 €-yr $^{-1}$, 76.3% lower than at $NO_3^-/S^{2-}=0.3$. Although higher ratios (1.8–2.2) further reduced cleaning frequency to 2–3 times per year, increased acid consumption, N_2O fees, and nitrification energy raised total costs to 2,060–2,611 €-yr $^{-1}$. At these higher ratios, gaseous N_2O became an additional consequence of increasing electron-acceptor supply: greater NO_3^- availability reduced residual S^0 but increased incomplete denitrification and its associated emission cost. The annual N_2O fee increased from 257.7 to 565.2 €-yr $^{-1}$ as the NO_3^-/S^{2-} ratio increased from 1.8 to 2.2, demonstrating its growing contribution to the operational trade-off. Thus, within this reference case, $NO_3^-/S^{2-}=1.6$ was the lowest-cost NO_3^- -fed condition. Normalized cost indices for NO_3^-/S^{2-} of 1.8, 2.0, 2.2, and 2.2(NO_2^-) were 1.23, 1.42, 1.55, and 1.29, respectively.

Sensitivity analysis confirmed that this economic preference remained robust within the tested uncertainty range. Varying nitrification electricity demand, acid cost, N_2O penalty, carrier-cleaning water use, and biogas-derived electricity loss by $\pm 30\%$ did not change the overall cost ranking. Acid cost and nitrification electricity demand were the most influential parameters, whereas water use and electricity loss during downtime had smaller effects, becoming relevant mainly under high S^0 accumulation and frequent cleaning. Although the N_2O -related cost was less influential than acid and nitrification electricity under the reference assumptions, it represents a direct climate-related penalty and may become more influential under higher emission-pricing scenarios.

The combined low- and high-cost scenarios further supported the same ranking. Although $NO_3^-/S^{2-}=2.2$ minimized residual S^0 and extended cleaning intervals, its higher acid demand, nitrification energy, and N_2O -related cost reduced its economic attractiveness. The NO_2^- -fed condition achieved complete denitrification without detectable N_2O , but higher residual S^0 increased cleaning-related costs. Therefore, within the tested range and reference-case assumptions, $NO_3^-/S^{2-}=1.6$ provided the preferred compromise for SO_4^{2-} -oriented S^{2-} removal, nitrogen control, N_2O avoidance, and operating cost.

Studies evaluating SADN with real wastewater, particularly for controlling biosulfur accumulation to prevent clogging, remain limited. Almenglo et al. (2016) demonstrated the feasibility of SADN for biogas upgrading using NO_3^- derived from nitrified municipal wastewater effluent and investigated the effect of NO_3^- concentration, which was directly linked to the NO_3^-/S^{2-} ratio, in a pilot-scale SADN biotrickling

Table 3

Main utility costs for desulfurizing bioscrubber under different $\text{NO}_3^-/\text{S}^{2-}$ ratios. Calculation basis: biogas flowrate of $250 \text{ m}^3\cdot\text{h}^{-1}$, 55% CH_4 , 20,450 ppm_v H_2S and MBBR at SLR of $1.034 \text{ kg}\cdot\text{S}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$.

Factors	Unit	$\text{NO}_3^-/\text{S}^{2-}$ ratio (Electron acceptor)					
		0.3 (NO_3^-)	1.6 (NO_3^-)	1.8 (NO_3^-)	2.0 (NO_3^-)	2.2 (NO_3^-)	2.2 (NO_2^-)
Cleaning cycle	day	14	85	140	266	270	53
Cleaning frequency	$\text{time}\cdot\text{y}^{-1}$	27	5	3	2	2	7
Cost of pressure wash (Cleaning)	$\text{€}\cdot\text{y}^{-1}$	579.4	107.3	64.4	42.9	42.9	150.2
Cost of opportunity losses from biogas energy recovery during downtime	$\text{€}\cdot\text{y}^{-1}$	795.9	147.4	88.4	59.0	59.0	206.3
Fees of N_2O Emissions	$\text{€}\cdot\text{y}^{-1}$	0	0	257.7	407.3	565.2	0
Cost of acid consumption	$\text{€}\cdot\text{y}^{-1}$	5,645	931	1,089	1,257	1,258	1,306
Cost of energy from nitrification for biogas-upgrading	$\text{€}\cdot\text{y}^{-1}$	87.2	495.4	560.4	624.4	686.8	508.0
Total cost	$\text{€}\cdot\text{y}^{-1}$	7,107	1,681	2,060	2,390	2,611	2,170

filter. Although this study illustrated the potential for integration with upstream and downstream treatment processes, the nitrified effluent did not provide sufficient nitrogen for stable long-term operation. As a result, external NO_3^- supplementation was required to maintain stable reactor performance at a constant SLR of $95.0 \text{ gS}^{2-}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$. The applied $\text{NO}_3^-/\text{S}^{2-}$ ratio was 1.25, and 47% of the removed H_2S was converted to SO_4^{2-} . Approximately 53% of the produced S^0 remained attached to the packing media, and the system required 37 days of shutdown for accumulated S^0 removal during 323 days of operation.

Soreanu et al. (2008) further showed that nitrate availability strongly affected long-term desulfurization stability. At an inlet loading of $4.9 \text{ gS}^{2-}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$, at least $20 \text{ mgN}\cdot\text{NO}_3^-\cdot\text{L}^{-1}$, corresponding to an N/S ratio of 0.3, was required to maintain 99% H_2S removal. When NO_3^- supply was insufficient, H_2S removal decreased to 43% after 14 days because of S^0 deposition, whereas increasing NO_3^- supplementation to $30 \text{ mgN}\cdot\text{NO}_3^-\cdot\text{L}^{-1}$ restored 99% removal within 2 days. This rapid recovery indicates that SADN systems can tolerate short-term NO_3^- fluctuations, but only when electron-acceptor supply is restored before S^0 deposition becomes excessive.

Cui et al. (2019a) reported that biofilm thickness on the internal surface of MBBR carriers increased gradually to only $162 \mu\text{m}$ after approximately 360 days, a much slower timescale than S^0 accumulation. These findings suggest that, in real-wastewater SADN systems, operational instability is driven less by biomass overgrowth than by sulfur deposition linked to inadequate or fluctuating NO_3^- supply. Therefore, practical SADN implementation requires strategies to buffer NO_3^- supply, control the effective $\text{NO}_3^-/\text{S}^{2-}$ ratio, and manage S^0 deposition to sustain long-term carrier performance and reduce cleaning frequency.

5. Conclusion

Considerable improvements in bioscrubber performance were achieved by adjusting the $\text{NO}_3^-/\text{S}^{2-}$ ratio and HRT in the MBBR to jointly control sulfide removal, residual S^0 accumulation, and N_2O emissions. Increasing the $\text{NO}_3^-/\text{S}^{2-}$ ratio to 2.2 achieved 99.9% S^{2-} removal and minimized residual S^0 accumulation to 5.2%, which could reduce cleaning demand; however, this condition also promoted NO_2^- and N_2O accumulation. In contrast, $\text{NO}_3^-/\text{S}^{2-}=1.6$ provided the most balanced performance within the tested range, combining complete S^{2-} and NO_3^- removal, limited S^0 accumulation, no detectable N_2O emission, and the lowest operating cost under the reference scenario. An HRT of 12 h was the shortest tested HRT maintaining $> 95\%$ S^{2-} and NO_3^- removal under the corresponding increased loading rates while reducing the required reactor volume. Microbial and activity analyses showed that Sulfurimonas dominated sulfide oxidation, whereas heterotrophic/mixotrophic denitrifiers competed for NO_3^- using endogenous organic carbon, contributing to residual S^0 accumulation under NO_3^- -limited conditions. Biofilm biomass performed most electron transfer, while planktonic biomass supported reactor activity. Results demonstrated that MBBR-based SADN should be operated within a balanced window because

sulfur conversion, denitrification completeness, N_2O control, microbial competition, and cost are strongly interconnected.

CRedit authorship contribution statement

Worakan Chetawan: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sumate Chaiprapat:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **David Gabriel:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2026.135940>.

Data availability

Data will be made available on request.

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