Comparative analysis of microbial communities from different full-scale haloalkaline biodesulfurization systems

Suyash Gupta1,2 · Caroline M. Plugge1,3 · Johannes B. M. Klok1,4 · Gerard Muyzer2

Received: 18 June 2021 / Revised: 17 December 2021 / Accepted: 6 January 2022 / Published online: 11 February 2022
© The Author(s) 2022

Abstract
In biodesulfurization (BD) at haloalkaline and dO2-limited conditions, sulfide-oxidizing bacteria (SOB) effectively convert sulfide into elemental sulfur that can be used in agriculture as a fertilizer and fungicide. Here we show which bacteria are present in this biotechnological process. 16S rRNA gene amplicon sequencing of biomass from ten reactors sampled in 2018 indicated the presence of 444 bacterial Amplicon Sequence Variants (ASVs). A core microbiome represented by 30 ASVs was found in all ten reactors, with Thioalkalivibrio sulfidiphilus as the most dominant species. The majority of these ASVs are phylogenetically related to bacteria previously identified in haloalkaline BD processes and in natural haloalkaline ecosystems. The source and composition of the feed gas had a great impact on the microbial community composition followed by alkalinity, sulfate, and thiosulfate concentrations. The halophilic SOB of the genus Guyparkeria (formerly known as Halothiobacillus) and heterotrophic SOB of the genus Halomonas were identified as potential indicator organisms of sulfate and thiosulfate accumulation in the BD process.

Key points
• Biodesulfurization (BD) reactors share a core microbiome
• The source and composition of the feed gas affects the microbial composition in the BD reactors
• Guyparkeria and Halomonas indicate high concentrations of sulfate and thiosulfate in the BD process

Keywords Core community · Full-scale biodesulfurization processes · Haloalkaliphilic bacteria · Sulfide oxidation · Sulfide-oxidizing bacteria (SOB) · Thioalkalivibrio

Introduction
Hydrogen sulfide (H2S) is a malodorous, toxic, and corrosive gas, which can be harmful to all forms of life. This gas is produced in nature by sulfate-reducing bacteria found in anaerobic habitats, such as marine and freshwater sediments, as well as man-made processes (Muyzer and Stams 2008). Naturally, geothermic activity also releases H2S in the atmosphere. Nowadays, the petrochemical industry is the largest anthropogenic producer of SO2, produced upon combustion of H2S. The other sources include paper mills, the textile industry, biogas plants, landfills, and the rendering industry (Mahmood et al. 2007; Janssen et al. 2009). Conventional methods to remove H2S on-site include physical and chemical processes. Alternatively, more environmentally friendly and cost-effective haloalkaline BD processes employ haloalkaliphilic SOB to convert sulfide into elemental sulfur at haloalkaline conditions and low redox potential (Janssen et al. 2001). In these processes, H2S is first absorbed into an alkaline solution as a predominantly soluble bisulfide (HS−). The HS−-loaded solution is subsequently directed to a bioreactor whereby SOB oxidizes it to elemental sulfur at a low redox potential of around −300 mV (Sorokin et al. 2008; Janssen et al. 2009). The produced sulfur is harvested from...
a settler and can be further reused as fertilizer or fungicide. With this BD process, 99.84% of sulfide can be removed (de Rink et al. 2020). Besides sulfur, thiosulfate and sulfate are formed as side products through chemical oxidation of polysulfide and complete oxidation of reduced sulfur compounds mediated by SOB, respectively. Both compounds are undesirable, as their formation (i) increases acidification and consequently, the need for extra caustic supply, (ii) increases the demand of oxygen supply (= energy consumption), and (iii) decreases the sulfur selectivity of the process (van den Bosch et al. 2007). The rate of formation and removal of sulfate and thiosulfate from the process determines the concentration of these compounds in the reactor system and can be regarded as indicators of a less efficient process. We hypothesize that these indicators are directly linked to the activity of particular bacteria in the reactor.

Aerobic sulfur oxidation is largely performed by chemolithoautotrophic SOB along with facultative autotrophs, lithoautotrophic, and obligate heterotrophs. The chemolithoautotrophic SOB possesses the ability to conserve energy from the oxidation of reduced sulfur compounds, such as sulfide, polysulfides, sulfur, thiosulfate, and polythionates with oxygen or nitrate as electron acceptor (Muyzer et al. 2013). They are members of four genera of haloalkaliphilic Gammaproteobacteria: Thiomicrospira: Thiobalkalimicrobiurn reclassified as Thiomicrospira (Boden et al. 2017), Thiobalkalispira, Thiobalkalivibrio, and Thiobalkalibacter (Sorokin et al. 2013, 2015). The haloalkaliphilic Thiomicrospira and Thiobalkalispira species can grow with thiosulfate or sulfide forming mostly sulfate as a product but can produce elemental sulfur under oxygen-limiting conditions (Sorokin et al. 2001; 2002). The genus Thiobalkalivibrio includes multiple isolates (more than a hundred) from natural soda lakes and haloalkaline bioreactors that can oxidize various reduced sulfur compounds to sulfur or sulfate using oxygen or nitrate as an electron acceptor (Berben et al. 2019). Until today, ten species of Thiobalkalivibrio have been well-characterized and a variety of other species have been identified based on their genomes (Ahn et al. 2017; Berben et al. 2019). Other SOBs from haloalkaline habitats are members belonging to the Alkalilimnicrobiurn–Alkalilimnicola group, i.e., Alkalilimnicrobiurn halodurans, Alkalilimnicola ehrlichii MLHE-1 (Hoef et al. 2007), Alkalilimnicrobiurn mobile (Rijkenberg et al. 2001), and several unclassified members. Members of the Alkalilimnicrobiurn–Alkalilimnicola group are facultatively autotrophic genera that can utilize CO as an additional e-donor (Sorokin et al. 2006). Other bacteria that might be present in the haloalkaline bioreactors are members of the genus Halomonas, which are heterotrophic bacteria that can oxidize thiosulfate to tetrathionate, which, in turn, can chemically oxidize sulfide to sulfur with the regeneration of thiosulfate, thus, potentially contributing to the overall sulfide oxidation to sulfur (Sorokin, 2003; Sorokin et al. 2008).

Multiple 16S rRNA sequence analyses of bacterial communities from some full- and pilot-scale BD processes has revealed the presence of both chemolithoautotrophic and heterotrophic SOB belonging to the Gammaproteobacteria, including Thiobalkalivibrio, Thiomicrospira, and Halomonas species (de Graaff et al. 2011; Sorokin et al. 2012; Roman et al., 2016; Kiragosyan et al. 2019). Recently, the BD process has been improved by the addition of an anoxic reactor in series (Klok et al. 2017, 2018). In this BD concept, SOB was exposed to alternating conditions of subsequent anaerobic-sulfidic and microaerobic conditions. In the new process, line-up selectivity for sulfur formation of 96.5% was achieved, which considerably decreases NaOH consumption and bleed stream formation compared to the line-up without the anoxic reactor. Correspondingly the dominance of the facultative chemolithoautotrophic SOB Alkalilimnicola also increased in the novel BD process (de Rink et al. 2019).

Although the aforementioned studies provide insight into the microbial community composition of some BD processes, detailed comparative information on the microbial community composition in different full-scale plants is still lacking. Besides, the presence of a possible core community in the BD process has not been studied before.

In this paper, we analyzed the microbial communities from eight full- and two pilot-scale biodesulfurization reactors along with a variety of physicochemical parameters. We determined the microbial diversity in these bioreactors and defined the presence of a core community. Moreover, we revealed a relation between the presence of specific community members at particular environmental conditions, such as alkalinity. We also showed that there are certain bacteria that can be linked to an increase in the undesired production of thiosulfate and sulfate.

Materials and methods

Description and sampling of full-and pilot-scale BD reactors

To determine the microbial community composition of BD reactors comprehensively, process solutions of eight full-scale BD reactors and two runs of a pilot-scale BD reactor were analyzed in 2018. Full-scale BD reactors studied were from industries that treated feed gas from different origins (Table 1). The pilot-scale BD reactors consisted of an extra reactor, namely an anoxic reactor in front of the oxic reactor. The difference between the pilot-scale and full-scale was the composition of the feed gas; for the pilot scale BD reactors, a synthetic gas with no contaminants was used, while the full-scale plants have organic sulfur compounds and
### Table 1 Description of biodesulfurization plants

| Reactor name      | Denotation | Plant description | Industry Feed gas type | Location | Sulfur separation | Additional components in the feed gas | Physicochemical parameters | ORP at source (mV) | H₂S/CO₂ | pH     | Conductivity (mS/cm) | Salinity (Na⁺, M) | Alkalinity (M) | Sulfate (M) | Thiosulfate (M) |
|------------------|------------|-------------------|------------------------|----------|-------------------|----------------------------------------|---------------------------|-------------------|---------|--------|---------------------|------------------|--------------|-------------|-----------------|                |
| Cadaver          | Cad1 (a, b, c) | Rendering Biogas | Son, NL | Effluent settler | Ammonia | -347* | 0.04 | 8.67 | 78.64* | 1.55* | 0.18* | 0.53* | 0.16* |
| Landfill         | LF1 (a, b, c) | Landfill waste Biogas | Amersfoort, NL | Effluent settler | None | -283 | 0.01 | 8.40 | 40.68 | 0.90 | 0.71 | 0.06 | 0     |
| Pilotunit_01     | Pil1 (a, b, c) | None Synthetic gas | Wageningen, NL | Decanter centrifuge | None | -370** | 0.02 | 8.14 | 49.49 | 1.02 | 0.42 | 0.29 | 0.02 |
| Pilotunit_02     | Pil2 (a, b, c) | None Synthetic gas | Wageningen, NL | Decanter centrifuge | Oxygen | -370** | 0.02 | 8.59 | 40.14 | 0.81 | 0.55 | 0.14 | 0.02 |
| Papermill_01     | PM1 (a, b, c) | Papermill Biogas | Eerbeek, NL | Effluent settler | None | -389 | 0.06 | 8.77 | 64.08 | 1.48 | 0.40 | 0.38 | 0     |
| Papermill_02     | PM2 (a, b, c) | Papermill Biogas | Roermond, NL | Effluent settler | None | -377* | 0.06 | 8.80 | 44.07* | 0.78* | 0.55* | 0.1* | 0.02* |
| Papermill_03     | PM3 (a, b, c) | Papermill Biogas | Cuijk, NL | Effluent settler | None | -360 | 0.06 | 8.66 | 54.72 | 0.97 | 0.29 | 0.32 | 0     |
| Papermill_04     | PM4 (a, b, c) | Papermill Biogas | Zulpich, DE | Effluent settler | None | NA | 0.06 | 8.89 | 62.16 | 1.17 | 0.57 | 0.28 | 0.05 |
| Oil&Gas_01       | OG1 (a, b, c) | Oil and gas Associated gas | Southern Illinois, USA | Decanter centrifuge | Thiols | NA | 1.67 | 9.03 | 50.66 | 0.89 | 0.49 | 0.03 | 0.16 |
| Oil&Gas_02       | OG2 (a, b, c) | Oil and gas Amine acid gas | Texas, USA | Decanter centrifuge | BTEX | NA | 0.02 | 8.2* | 85* | 1.5 | 0.85* | 0.36 | 0.03 |

*These measurements were taken from (Mol et al. 2020), + The value represents the average operational value, ** ORP set point of the BD reactor, NA These values are not available as the samples were not collected at the source.
hydrocarbons in the feed (Table 1). The process solution was collected from full-scale bioreactors sampling points. The solution was used to analyze the physical-chemical parameters and to extract genomic DNA for amplicon sequencing. For the pilot-scale BD reactors, the samples were collected from the sludge preserved at 4 °C after the process operation.

**Physicochemical analyses of the bioreactor samples**

To investigate the physical-chemical parameters of the collected samples, the samples were processed in the laboratory as early as possible. Prior to the analysis, the samples were kept at 4 °C and were filtered over a 0.45 µm membrane filter (HPF Millex, Merck, the Netherlands). Thiocyanate, sulfate, and sodium were measured by ion chromatography as described by Roman et al. (2015). Total inorganic carbon was quantified using a TOC-VCPH/CPN analyzer (Shimadzu, The Netherlands). Carbonate and bicarbonate ions concentration were estimated from the total inorganic carbon using the Henderson-Hasselbalch equation (Po and Senozan 2001) and further used to express the alkalinity of the system. The pH and conductivity of the well-mixed unfiltered samples were measured using the kit from Mettler Toledo, USA.

**DNA extraction and 16S rRNA gene amplicon sequencing**

For the microbial community composition analysis, genomic DNA was extracted from samples collected from the BD reactors in triplicate from each bioreactor sample. Twenty milliliters of sludge was centrifuged at 10,000 × g for 10 min at room temperature. The supernatant was discarded, and the pellet was suspended in a buffer containing 1M NaCl and phosphate-buffered-saline (PBS) to maintain the osmotic balance of the cells. The biomass was centrifuged again, and the obtained mixture of biomass and sulfur was used for the DNA extraction. The DNA was extracted using DNeasy PowerLyzer PowerSoil Kit (Qiagen, Germany) following the manufacturer’s instructions. The DNA was purified with DNA Clean & Concentrator-5 kit (Zymogen, USA). The integrity of the extracted genomic DNA was visualized by gel electrophoresis. PCR with 16S rRNA gene primers 27F and 1492R was performed, and amplicons were visualized by gel electrophoresis to verify the relative intensity of the amplicons. The extracted DNA was quantified using the Quantifluor dsDNA system on a Quanton fluorometer (Promega, USA). Samples were diluted to a final concentration of 30 ng/µL of DNA and sent for Illumina MiSeq sequencing performed at commercial sequencing company MR DNA (www.mrdnalab.com, Shallowater, TX, USA). At the facility, samples were first amplified in triplicate using primers 515f (5'-GTGYCAGCMGCC GCGGTAA-3') and 926r (5'-GGYCAATTYMTTRAG TTT-3') with a bar-coded forward primer. This primer set covers the 16S rRNA gene V4–V5 variable region (Parada et al. 2016). Amplification was done three times using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 53 °C for 40 s, and 72 °C for 1 min, with a final elongation step at 72 °C for 5 min. After amplification, the PCR products of three separate reactions of each sample were pooled, and PCR products were checked on a 2% (w/v) agarose gel to determine the success of amplification and the relative intensity of the bands. Then, multiple samples were pooled together in equimolar amounts based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads (Beckman Coulter, Indianapolis, IN, USA) and used for sequencing on a MiSeq system following the manufacturer’s guidelines. The sequence data has been submitted to the ENA database under study number PRJEB43104.

**Amplicon sequence analysis**

The 2 × 300 bp paired-end reads were generated by Illumina MiSeq sequencing. The reads generated were converted to paired-end format from a mixed paired format using the fastq processor software provided by MR DNA which removes the barcodes, primers and corrects the orientation of the reads. Microbiome analysis was performed with QIIME-2 version 2020.2 (Bolyen et al. 2019). Raw sequence data were demultiplexed and quality filtered using the q2-demux plugin followed by denoising with DADA2 (Callahan et al. 2016) (via q2-dada2) to remove the errors and chimeric sequences and identify all observed amplicon sequence variants (ASVs) (100% operational taxonomic units). All amplicon sequence variants (ASVs) were aligned with MAFFT (Katoh and Standley 2013) (via q2-alignment) and used to construct a phylogeny tree with fasttree2 (Price et al. 2010) (via q2-phylogeny). The taxonomy was assigned to the ASVs using the q2-feature-classifier (Bokulich et al. 2018), the classify-sklearn naive Bayes taxonomy classifier (Pedregosa et al. 2011) trained on nearly complete 16S rRNA sequences from the SILVA database version 138 (Quast et al. 2013; Yilmaz et al. 2014).

Alpha-diversity metrics (observed OTUs and Shannon), beta diversity metrics (Bray–Curtis dissimilarity), and Principal Coordinate Analysis (PCoA) were estimated using q2-diversity after samples were rarefied (subsampled without replacement) to 33,340 sequences per sample.
Identification and phylogenetic analysis of the core community members

The core ASVs were identified using the QIIME 2 feature table core features plugin. To create a visual diagram depicting the core community, the ASV table was filtered based on industry type to generate five ASV tables specific to each industry type using QIIME 2 feature table filter samples plugin. The core ASVs for each industry type were identified using the QIIME 2 feature table core features plugin. The counts of core ASVs were used to create a Venn diagram to show the shared core community of all the BD reactors. The Venn diagram was created using the “InteractiVenn” tool (Heberle et al. 2015) whereby, each industry type is represented as one set. Next, the core ASV table for the whole data set was created using QIIME 2 feature table core features plugin. The representative sequences were filtered based on the core ASV tables using QIIME 2 filter sequences tool. The identified core sequences were aligned in SINA alignment (Pruesse et al. 2012) imported into ARB (Quast et al. 2013; Yilmaz et al. 2014) to create a phylogenetic tree showing the core ASVs and their close relatives.

Prediction of the functional potential of the core microbiome

The functional potential of the core community was predicted using the software program Tax4Fun2 that can predict the functional capabilities of the microbial communities based on 16S rRNA datasets (Wemheuer et al. 2018). The genomes of bacteria that were closely affiliated to the core community members were also included in the analyses as described in the pipeline (https://github.com/bwemheu/Tax4Fun2).

Co-occurrence network analysis

Co-occurrence networks were calculated with the software program GenePiper (Tong and Chan 2020) using the raw read counts as abundance type. In addition, we used the person correlation method; the correlation filter was set to 0.8 and the P-value to 0.05, and agglomeration was performed at the genus level.

Statistical analysis of the data

To identify the difference in microbial community composition between samples and different industry types, PCoA analysis was performed using the Bray Curtis similarity distances. Redundancy Analysis (RDA) was performed to determine which parameters among pH, salinity, alkalinity, conductivity, H₂S/CO₂ ratio, and concentrations of thiosulfate and sulfate significantly explain variation in microbial community composition. The analysis was performed in an R software environment (version 3.6.3) using the vegan package. The ASV table, tree, and taxonomy files created in QIIME 2 were imported in R for this analysis. As a prerequisite to RDA, the species frequency is converted to abundance data. Variance inflation factors were analyzed for all parameters to confirm the non-collinearity using package car (version 3.0-8). The significance of explanatory variables was analyzed using ANOVA-like analysis with 999 permutations, and a tri-plot was generated using the ggplot2 package in R.

In addition to the relationship, we were also interested in the differential abundance of ASVs/taxa with changing concentrations of sulfate, thiosulfate, and alkalinity. The differentials were generated using the q2-songbird plugin in QIIME 2 (Morton et al. 2019) by fitting environmental parameters to the multinomial regression model. The good fit was determined using the factor $Q^2$. A value closer to 1 indicates a good fit and therefore represents the significant differential abundance of species due to change in parameter values. A value closer to 0 indicates a poor fit and no conclusion can be inferred from the model.

Results

Physicochemical analyses

Several physicochemical parameters of the full-scale BD-processes, such as pH, salinity, conductivity, alkalinity, and concentrations of sulfate and thiosulfate, were determined and are presented in Table 1. The BD-reactor treating biogas from the rendering industry had the highest measured sulfate and thiosulfate concentrations and the lowest alkalinity. In contrast, the BD-reactor that was treating gas from a landfill had low sulfate and thiosulfate concentrations but high alkalinity. In the BD processes that were treating gas from the petrochemical industry, only sulfate was detected. For all samples, the salinity varied between 0.8 and 1 M Na⁺, and the conductivity was between 40 and 78 mS/cm. The alkalinity ranged from 0.18 to 0.71 M and the pH from 8.1 to 9, indicating haloalkaline conditions in all reactors. The oxygen supply in all plants was controlled using ORP (van den Bosch et al. 2007). The measured range of the ORP at the installation ranged from −389 to −283 mV.

16S rRNA gene-based amplicon sequencing and microbial diversity analyses

Demultiplexing and denoising of the raw Illumina MiSeq sequences from the 30 biomass samples of the BD-reactors resulted in 33,342 to 73,228 reads and a total of 444 ASVs were assigned (Supplementary Table S1). Alpha diversity,
which is indicative of the mean species diversity, based on both observed ASVs and Shannon indices varied significantly among the different BD reactors (Supplementary Fig. S1, Kruskal–Wallis, $H = 17.81, p = 0.03$ for observed ASVs and $H = 27.64, p = 0.001$ for Shannon).

The beta diversity, indicative of the difference between microbial communities from different samples, was significantly different among the samples (Bray Curtis distance; PERMANOVA: $F = 13.9778, p = 0.001$, permutations = 999). The Bray Curtis dissimilarity distances based on PCoA analysis (Fig. 1) showed great similarity in the microbial community composition of reactor samples from the paper mill industry and landfill gas treating plants, while the community composition of plants from the rendering industry and the petrochemical industry were more distinct. The BD reactors with no contaminants such as hydrocarbons and organic sulfur compounds in the feed gas had a less diverse community as compared to the BD reactors with a larger organic matter input. This was also observed for the two pilot plants that had an additional anoxic reactor. Two BD reactors employed in Oil and Gas industries had different contaminants in their feed gas, such as BTEX and thiols (Table 1), and had a different microbial community composition. The BD reactor of the rendering industry had high ammonia content and a very different microbial community composition than the other BD-reactors. The $\text{H}_2\text{S}/\text{CO}_2$ ratio in the feed gas and sulfur separation method did not have much effect on the microbial diversity among different BD reactors (Supplementary Fig. S2).

**Identification of a core microbial community in the BD reactors**

The core community of the bioreactor samples was defined based on the ASVs that were present in all BD reactors. A total of 30 ASVs were present in all samples indicative of the “core microbial community.” The Venn diagram shown in Figure 2A indicates the distribution of all 444 ASVs among the samples grouped based on the industrial waste they treat and the 30 ASVs that are common to all BD-reactors studied. Interestingly these 30 ASVs together account for 72 to 90% of total microbial diversity in BD reactors (Fig. 2B).

**Taxonomy-based analyses**

Taxa assignment to identify ASVs revealed that microbial community members from the class Gammaproteobacteria had the highest relative abundance. These included the genera Thioalkalivibrio, Alkalilimnicola, Guyparkeria, Halomonas, Alkalispirillum, Vibrio, and Thiimicrospira.

The core microbial community with 30 ASVs consisted of 16 members from the class Gammaproteobacteria with known SOBs Thioalkalivibrio sulfidiphilus, Thioalkalivibrio halophilus, Thioalkalivibrio sp.
K90mix, *Alkalilimnicola ehrlichii*, *Halomonas*, *Thiomicspira*, and *Guyparkeria* along with *Nitrincola* and *Wenzhouxiangella*. Four members belonged to the class *Alphaproteobacteria* that includes members of genera *Roseinatronobacter* (a known haloalkaliphilic lithoheterotrophic SOB), *Stappia*, and one unclassified member of the family *Rhodobacteraceae*. The other four belonged to class *Bacteroidia* that includes members of genera *Planktosalinus*, *Psychroflexus*, *Lentimicrobium*, and Blvii28_wastewater-sludge_group. The next three members belonged to class *Bacillia* which includes members of genera *Acholeplasma* and *Izimaplasma*.

The latter was also present among the abundant metagenomes recovered recently from Siberian soda lakes (Vavourakis et al. 2019). The final three members of the core community belonged to the class *Clostridia*, class *Desulfuromonadia*, and class *Chrysiogenetes*, which includes *Peptostreptococcaceae-Tissierellales*, *Desulfuromusa*, and *Desulfurispirillum*, respectively. The relative abundance of each core community member is shown in Fig. 2B. The most abundant bacterium was *Thioalkalivibrio sulfidiphilus* with a relative abundance of up to 60%.
To visualize the phylogenetic affiliation of the core community members, a phylogenetic tree was created with the 30 ASVs with their cultured and uncultured close relatives (Fig. 3). The tree shows that the members of the core community are affiliated with various phyla.

**Prediction of the functional potential of the core community**

Using Tax4Fun2, we focused on the genes involved in the oxidation of reduced sulfur compounds. The predictions indicate the presence of all genes needed for complete sulfide oxidation to sulfur (Fig. 4; Table S2). The sulfide-oxidizing genes sqr, fccAB (Griesbeck et al. 2000; Trüper et al. 2002), and thiosulfate-oxidizing genes soxABXYZ were found to be highly abundant in the reactor samples from the paper mill (PM) and from the landfill (LF). We also searched for genes involved in the conversion of contaminants such as ammonia, thiols, and BTEX. Only BTEX degrading genes were found to be present in low numbers in oil and gas plants and pilots plant (Table S2).

**Co-occurrence analysis**

To understand the interaction among the members of the core community, a co-occurrence network was created. Figure 5 shows the co-occurrence of the core community members that have significant interactions. The network shows a negative correlation between Thioalkalivibrio sulfidiphilus and Alkalilimnicola ehrlichii genera, a positive correlation between Alkalilimnicola ehrlichii and Halomonas sp. Likewise, Lentimicrobium, Acholeplasma, and Rhodobacteracea were found to have significant positive correlations in their occurrence. This relationship was also found for the Blvii-wastewater sludge group, Candidatus “Izimaplasma,” and members of the family Peptostreptococcaceae-Tissierellales.
Relation between physicochemical parameters and microbial community composition

Redundancy analysis was performed to find a relationship between the microbial community composition and the physicochemical parameters. The parameters that were significant explain the microbial composition with thiosulfate ($R^2 = 0.24$, $p < 0.05$), sulfate ($R^2 = 0.30$, $p < 0.05$) and alkalinity ($R^2 = 0.46$, $p < 0.05$). As shown in Fig. 6, Guyparkeria, Nitrinicola, and Halomonas were associated with the presence of more sulfate and thiosulfate in the samples while the presence of Tv. halophilus and Alkalilimnicola were more aligned with alkalinity. On the other hand, Roseinatronbacter, Tv. sulfidiphilus, and Thiomicrospira were not associated with any of the tested parameters.

To find the differential abundance of the microbial population with respect to industry type, alkalinity, sulfate, and thiosulfate, the multinomial regression model was fitted with these variables. A fit value of $Q^2 = 0.636$ was obtained. However, when the model was fitted with only alkalinity, sulfate, and thiosulfate, a fit value of $Q^2 = 0.09$ was obtained suggesting that these factors alone cannot explain the differential abundances of microbial community members across the samples.
Fig. 5 Co-occurrence network of the core community members (ASVs) with a significant Pearson correlation \( (p > 0.05) \). The positive correlations are shown with green lines and negative correlations with red lines. The thickness of the lines corresponds to the correlation coefficients.

Discussion

Physicochemical analyses

In this paper, we have studied several operational and physicochemical parameters associated with BD reactors. Table 1 clearly indicates distinct variability of parameter values in all reactors and served as a good data set for studying the microbial composition across the plants. For the process, a higher rate of accumulation of sulfate and thiosulfate can be seen as an indicator of a decrease in sulfur selectivity (de Rink et al. 2019). For example, the measured levels of thiosulfate levels are indicators for dominating side-product oxidation pathways. Typically, thiosulfate formation cannot be avoided as, when dissolved oxygen and sulfide will chemically react to thiosulfate (de Graaff et al. 2012; Klok et al. 2013). Increased thiosulfate levels indicate that significant amounts of sulfide are chemically oxidized. Furthermore, the absence of thiosulfate indicates that all thiosulfate which is formed is subsequently oxidized to sulfate. While the analyzed concentrations of accumulated ions cannot directly be translated to the rate formation, these results can still be directly related to BD plant performance. Hence, these values are useful to explain the microbial community composition of the plants. For future analyses, the ratio of \( S_2O_3^{2-}\)/\( SO_4^{2-}\) can also help in explaining the presence of SOB that are sulfide specialized and/or more generalists in the BD reactors. Higher \( S_2O_3^{2-}\)/\( SO_4^{2-}\) reflects more chemical oxidation and can also suggest less thiosulfate oxidizing bacteria in the reactor. Additionally, the sampling and physicochemical analyses can be extended to have higher sampling frequencies to visualize the fluctuations in the reactors.

The ORP is considered to play an important role in determining sulfur selectivity. However, recently it has been shown that a constant redox potential does not lead to the highest sulfur selectivity, and a stoichiometry-driven feed-forward controlling of oxygen has been suggested to lead to a high sulfur selectivity (Kiragosyan et al. 2020b). Nevertheless, microbial community composition can influence or can be influenced by the ORP and could indirectly affect the sulfur selectivity.

Microbial diversity analysis

The alpha diversity analyses have indicated that the two pilot plant samples (Pil1 and Pil2) have more diversity as compared to the full-scale BD reactors. This suggests that the addition of an anaerobic bioreactor might provide a niche to specific bacteria, for example, facultative anaerobes (de Rink et al. 2019) that were not present in the full-scale reactors.
contaminants in them. Other studies have shown that the presence of thiols and BTEX have an effect on the microbial community composition in BD reactors (Roman et al., 2016; Kiragosyan et al. 2020a).

The microbial community was found to be less diverse in the BD-reactors that treat feed gas from the same source industry type suggesting the huge impact of the feed gas source. For example, *Tv. sulfidophilus* was abundant in all paper mill and landfill plants (PM1–4, LF1), while *Thiomicrospira* was more abundant in pilot plant samples (Pii1 and 2). Other process parameters such as the sulfur separation method and the H₂S/CO₂ ratio have little effect on the microbial community (Supplementary Fig. S2). However, analysis with more BD reactors could help to get more conclusive results.

The core microbial community of BD reactors

The microbial diversity analysis of ten BD reactors clearly reveals a shared microbial community composition that is variably abundant across the plants. The 30 ASVs can be defined as the core microbial community because they are shared by all the plants and has no bias of their geographical location, the industrial gas streams they treat, or the differences in the physicochemical parameters. The possible reason for the presence of a core community is the haloalkaline environment in all the BD reactors. The microbiome of soda lakes, a natural haloalkaline environment, also has a core microbiome in lakes located across the continents (Zorz et al. 2019). A metagenome-based study of soda lakes has also highlighted the presence of similar phyla in the soda lakes that were found in BD reactors (Vavourakis et al. 2016). Interestingly, these 30 core community members are not only common but together also comprise a very large proportion of the total microbial diversity of BD reactors (Fig. 2B).

Taxonomy-based and phylogenetic analyses

Taxonomy-based assignment of the 30 core ASVs (Figs. 2B and 3) indicated that they could be assigned to the *Gammaproteobacteria* (16), *Alphaproteobacteria* (4), *Bacteroidia* (4), *Bacilli* (3), *Clostridia* (1), *Desulfuromonadida* (1), and *Chrysiogenetes* (1). It is worth noting that within 30 core ASVs, there are ASVs that were assigned similar phylogenies even though they have different sequences. BLASTn analysis of these sequences has revealed that sequences vary in identity (96 to 100%) and coverage (90 to 100%). Based on this analysis, it is possible that they represent different species of bacteria or different strains of the same species. However, considering the fact that most common SOB such as *T. sulfidophilus* and *A. ehrlichii* are not very phylogenetically distant (Ahn et al. 2017), we have considered all 30 ASVs as individual bacteria.

In general, several known SOBs belong to the class *Gammaproteobacteria* such as species of *Thioalkalivibrio*, *Thiomicrospira*, *Alkalimicrobium*, *Gyaparkeria*, *Thioalkalibacter*, and *Alkalispirillum* (Rijkenberg et al. 2001; Sorokin et al. 2001, 2012; Hoeft et al. 2007; Banciu et al. 2008; Boden 2017). The first four were also part of the core community, indicative of their important role in sulfide oxidation in the BD process (Fig. 7), which was confirmed by the predicted abundance of the sqr and fccAB genes (Fig. 4). The later ones were only abundantly present in a few reactors. In addition, the core community also consisted of other members assigned as *Ectothiorhodospira*. Members of this phototrophic genus can oxidize sulfur compounds. Other genera belonging to a group of 16 ASVs contained *Halomonas*, *Nitrincola*, and *Wenzhouxiangella*. *Halomonas* are known organotrophs but can also oxidize sulfur compounds incompletely to tetrathionate (Sorokin 2003). Some of the denitrifying species have the potential to oxidize sulfide and thiosulfate anaerobically (Sorokin 2003; Wang and Shao 2021). *Nitrincola* is a facultatively anaerobic, chemo-organotrophic bacterium capable of nitrate reduction but not known for sulfide oxidation. But they contain potential genes for thiosulfate dehydrogenase yielding tetrathionate (Vavourakis et al. 2016; Borsodi et al. 2017). A novel haloalkaliphilic species of the genus *Wenzhouxiangella* sp. has recently been enriched and isolated from Siberian soda lakes using cells of Gram-positive cocci as substrate and was proven to be strongly proteolytic with the ability to feed on microbial biomass (Sorokin et al. 2020). In all, these obligate heterotrophic haloalkaliphiles can be considered a “satellite” population of the autotrophic SOB feeding on their organic products with an ability to marginally contribute to sulfur cycling, such as by the production of tetrathionate.

Members of the class *Alphaproteobacteria* and family *Rhodobacteraeae* such as *Roseinatronbacter* are aerobic sulfur-oxidizing lithoautotrophs that oxidize inorganic sulfur compounds to sulfate during organotrophic growth (Sorokin et al. 2000; Boldareva et al. 2007; Gorlenko et al. 2010). *Stappia* is well known to be involved in denitrification and CO oxidation (Weber and King 2007). However, it has genes to convert thiosulfate to sulfate (Huang et al. 2015a) and has been found in several sulfide-removing reactors (Huang et al. 2015b; San-Valero et al. 2019). Their role in BD reactors is shown in Fig. 7.

The ASVs assigned to *Bacteroidota* include *Planktosalminus*, *Lentinimicobium*, uncultured_Bacteroidetes/Chlorobi, and *Psychroflexus*. So far only one member of *Planktosalminus*, *P. lacus* has been characterized as a strict aerobic heterotroph (Zhong et al. 2016). For *Lentinimicobium*, *L. saccharophilum* is a strict anaerobe and cannot use any sulfur compounds as electron acceptors (Sun et al. 2016).
Uncultured_Bacteroidetes/Chlorobi assigned to the Blvii28 wastewater-sludge_group are members of the core community, and they are frequently detected in sulfide-oxidizing bioreactors (Vannini et al. 2008). However, the specific role of this Blvii28 wastewater-sludge_group is not clear. On the other hand, Chlorobi, the green sulfur bacteria, are known to perform sulfide oxidation (Fig. 7) (Visser et al. 1997; Ghosh and Dam 2009). Members of the genus Psychroflexus and, in particular, P. torquis are highly versatile bacteria that use an extensive range of carbon and energy sources, including carbohydrates, amino acids, organic acids, and odd-chain-length lipid oxidation products (Bowman et al. 1998).

Members of class Bacilli namely Acholeplasma and Candidatus “Izimaplasma” are not known to participate in the sulfur cycle but may play a role in degrading organic matter present in the BD reactors (Skennerton et al. 2016; de Rink et al. 2019; Zorz et al. 2019). Members of class Clostridia, order Peptostreptococcales-Tissierellales are also part of the core community. Some of these members are strictly anaerobic and can reduce elemental sulfur (Fig. 7) (Takai et al. 2019). Members of class Desulfochlorales, Clorobi, the green sulfur bacteria, are known to perform sulfide oxidation (Fig. 7) (Visser et al. 1997; Ghosh and Dam 2009). Members of the genus Psychroflexus and, in particular, P. torquis are very versatile bacteria that use an extensive range of carbon and energy sources, including carbohydrates, amino acids, organic acids, and odd-chain-length lipid oxidation products (Bowman et al. 1998).

Table 2 Biological and chemical conversions occurring in the BD reactors with the potential role of each core community member

| Biological conversions | Reaction | Core ASVs |
|------------------------|----------|-----------|
| **Sulfide oxidation**  |          |           |
| Complete sulfide oxidation HS⁻ → SO₄²⁻ | 1, 3 | Thioalkalivibrio, Guyparkeria, Thioalkalispira (formerly Thioalkalimicrobium), Ectothiorhodospiraceae, Rhodobacteraceae, Roseinatrobacter |
| Partial sulfide oxidation HS⁻ → SO₃⁻ | 1 | Alkalilimnicola |
| **Polysulfide oxidation** |          |           |
| Complete polysulfide oxidation HSₐ⁻ → SO₃⁻ | 2,3 | Thioalkalivibrio, Guyparkeria, Thioalkalispira (formerly Thioalkalimicrobium), Ectothiorhodospiraceae, Rhodobacteraceae, Roseinatrobacter |
| Partial polysulfide oxidation S₈⁻ → S₈ | 2 | Alkalilimnicola |
| **Thiosulfate oxidation** |          |           |
| Thiosulfate oxidation to sulfate S₂O₃²⁻ → SO₄²⁻ | 4 | Thioalkalivibrio, Guyparkeria, Thioalkalispira (formerly Thioalkalimicrobium), Ectothiorhodospiraceae, Rhodobacteraceae, Roseinatrobacter |
| **Sulfur reduction** |          |           |
| Sulfur reduction S₈→ HS⁻ | 5 | Desulfuromusa, Desulfurispirillum |
| **Heterotrophic growth** |          |           |
| Organic carbon assimilation by heterotrophs CHₓₙOₓₙN₀₂→ CO₂ | 6 | Nitrincola, Halomonas, Wenzhouxiangella, Planktosalinus, Psychroflexus, Lentimicrobium, Blvii28 wastewater sludge group, Acholeplasma, Izimaplasma, Peptostreptococcales-Tissierellales |
| **Chemical conversions** |          |           |
| Polysulfide formation HS⁻ + S₈→ S₈⁻ | a |           |
| Sulfide oxidation HS⁻ → S₈₂O₃²⁻ | b |           |
| Polysulfide oxidation S₈⁻ → S₂O₃²⁻ | c |           |
| Sulfite and sulfide reaction 2HS⁻ + 4HSO⁻ → 3S₂O₃²⁻ + 3H₂O | d |           |
important role in generating alternative substrates for SOB, like polysulfides and thiosulfate.

The core ASVs shape the major fraction of the total microbial community, but there are other species that were abundant only in some of the BD reactors (Fig. 2B). *Alkalisprillum* was abundant (14.3%) in Papermill_02, *Vibrio* (16.2%) in Papermill_03 and *Nitrincola* (13.8%) in Cadaver_01. *Alkalisprillum* is a close relative of *Alkalilimnicola* and is known to oxidize sulfide/polysulfide to elemental sulfur aerobically (Sorokin et al. 2006). Members of genera *Vibrio* are widely found in marine and haloalkaline environments (Simidu and Tsukamoto 1985; Mwirichia et al. 2010). Studies have predicted that members of the family *Vibrionacea* have the potential to oxidize thiosulfate to tetrathionate with heterotrophic growth (Sorokin 2003).
Prediction of the functional potential of the core community

Tax4Fun2 predicted the overall high abundance of genes involved in the oxidation of sulfide to sulfur (sqr; fccAB; Fig. 4). However, sqr and fccAB were variably present in different reactors suggesting that the mechanism of sulfide oxidation might differ among the different industry reactors. The possibility of variable mechanisms was also shown in the previous studies on the sludge from full-scale plants (Kiragosyan et al. 2019). Additionally, paper mill (PM1–4) and landfill-treating reactors (LF1) have a similar functional profile. These plants were dominated by *Thioalkalivibrio sulfidiphilus* which has fccAB genes and a sox gene system but lacks the sqr gene. Contrarily, the rendering waste treating reactor (Cad1) has a very different functional profile than others with a high abundance of sqr genes and less of fcc and sox genes. The pilot runs studied in this research have an abundance of both fcc and sqr. These results differ from the previous research where sqr was suggested to be most abundant (de Rink et al. 2019). All these predictions indicate the differences in the potential mechanisms of sulfide oxidation among the reactors. However, to make a definite conclusion, more information on the sulfide loads and sulfur selectivity along with gene expression results is needed.

Co-occurrence analyses

Co-occurrence analysis of the core community has revealed an antagonistic relationship between *Tv. sulfidiphilus* and *A. ehrlichii* (Fig. 5). Recent studies with the modified BD line-up have also indicated these dynamics in the reactors (de Rink et al. 2019). Being both a facultative autotroph and facultative anaerobe, *A. ehrlichii* is more versatile than *T. sulfidiphilus*, which is a strict autotroph. *A. ehrlichii* was found to co-occur with *Halomonas* sp., indicating the presence of organic compounds in the reactor. The analysis also shows that there are two sets of heterotrophic bacteria from different phyla co-occurring in the BD reactors.

Relation between physicochemical parameters and microbial community composition

Though the biological process of full-scale BD reactors is the same, there are several operational and design parameters that make them different. RDA analysis indicated that the concentration of thiosulfate, sulfate, and alkalinity in the BD reactors is related to the presence of certain bacteria. Figure 7 highlights that members of the core community have a different potential to oxidize sulfur compounds. The complete/incomplete biological sulfide oxidation can thus lead to the formation of sulfate/sulfur. Moreover, different SOBs, such as *Thioalkalivibrio* and *Thiromicrospira* have different growth strategies (Sorokin et al. 2003).

From the results of RDA analysis (Fig. 6), it is evident that *Guyparkeria* and *Halomonas* were found to be highly correlated with the increasing concentration of thiosulfate and sulfate in the BD process. *Guyparkeria* can oxidize several reduced sulfur compounds and oxidize them to sulfate (Sievert et al. 2000) and can decrease the alkalinity of a neutral environment. They are also capable to drive a neutral environment to less alkalinity (Whaley-Martin et al. 2019). This can be explained by an increase in H⁺ ions concurrently formed with sulfate production. On the other hand, *Alkalilimoncola* was more correlated with high alkalinity.

As mentioned before, in a BD reactor a high rate of thiosulfate and sulfate formation together with their accumulated concentration indicates a sub-optimal process performance. Nevertheless, considering only the accumulated concentration, *Guyparkeria* and *Halomonas* can be regarded as indicator organisms of high sulfate and thiosulfate accumulation in the process. High thiosulfate and sulfate formation directly corresponds to a decreased alkalinity and concomitantly an increase in the caustic demand, thereby decreasing the performance of the BD process and increasing costs.

To conclude, microbial community analysis of ten full-scale BD reactors treating gas streams from different industries demonstrated the presence of a core microbiome consisting of 30 ASVs with *Thioalkalivibrio sulfidiphilus* as the most abundant community member. The diversity is largely affected by the source and composition of the feed gas. Members of the genera *Guyparkeria* and *Halomonas* are related to an increase in sulfate and thiosulfate levels and as such can be regarded as indicator organisms for a suboptimal performance of the BD processes.

In this study, only a few physicochemical parameters such as pH, salinity, alkalinity, conductivity, thiosulfate, sulfate concentrations have been considered. In the future, it is recommended to include parameters such as ORP, sulfide load, airflow rate, and sulfur selectivity while performing microbial composition analysis for these reactors.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00253-022-11771-y.

Acknowledgements This work was performed in the cooperation framework of Wetsus, the European Centre of Excellence for Sustainable Water Technology (www.wetsus.nl). We thank all the full-scale biodesulfurization plant operators and Ricks de Rink for providing sludge for this study. We thank dr. Dimitri Y. Sorokin for critical discussions. We thank Pieter van Veen for his help with the data analysis in QiIME 2 and R.

Author contribution SG, CP, and GM conceived and designed the research. SG conducted the experiments. JK contributed in materializing the study by procuring samples and analyzing physicochemical
data. SG and GM analyzed the data. SG wrote the manuscript. All authors contributed to the manuscript.

**Funding** This work was performed in the cooperation framework of Wetsus, the European Centre of Excellence for Sustainable Water Technology (www.wetsus.nl). Wetsus is co-funded by the Dutch Ministry of Economic Affairs and Ministry of Infrastructure and Environment, the European Union Regional Development Fund, the Province of Fryslan, and the Northern Netherlands Provinces. This work is part of a project that has received funding from the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement no. 658784. The study was co-financed by Paqell B.V.

**Availability of data and material** All the data and material has been shared as part of the manuscript. The amplicon sequences of this study are available from the GeneBank database (accession numbers: MG183893-MG188952). Additional mapping and annotation are included in the article’s Supplementary Information document.

**Code availability** Not applicable.

**Declarations**

**Ethics approval** Not applicable.

**Conflict of interest** The authors declare no competing interests.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

**References**

Ahn AC, Meier-Kolthoff JP, Overmars L, Richter M, Woyke T, Sorokin DY, Muyzer G (2017) Genomic diversity within the haloalkaliphilic genus *Thioalkalivibrio*. PLoS ONE 12:1–23. https://doi.org/10.1371/journal.pone.0173517

Banciu HL, Sorokin DY, Tourova TP, Galinski EA, Muntyan MS, Kuenen JG, Muyzer G (2008) Influence of salts and pH on growth and activity of a novel facultatively alkaliphilic, extremely salt-tolerant, obligately chemolithoautotrophic sulfur-oxidizing Gammaproteobacterium *Thioalkalibacter halophilus gen. nov.*, sp. nov. from South-Western Siber. Extremophiles 12:391–404. https://doi.org/10.1007/s00792-008-0142-1

Berben T, Overmars L, Sorokin DY, Muyzer G (2019) Diversity and distribution of sulfur oxidation-related genes in *Thioalkalivibrio*, a genus of chemolithoautotrophic and haloalkaliphilic sulfur-oxidizing bacteria. Front Microbiol 10:160. https://doi.org/10.3389/fmicb.2019.00160

Boden R (2017) Reclassification of *Halothiobacillus hydrothermalis* and *Halothiobacillus halophilus* to *Guyparkeria gen. nov.* in the *Thioalkalibacteraceae fam. nov.*, with emended descriptions of the genus *Halothiobacillus* and family *Halothiobacillaceae*. Int J Syst Evol Microbiol 67:3919–3928. https://doi.org/10.1099/ijsem.0.02222

Boden R, Scott KM, Williams J, Russel S, Antonen K, Rae AW, Hutt LP (2017) An evaluation of *Thiomicrospira*, *Hydrogenovibrio* and *Thioalkalimicrobium*: reclassification of four species of *Thiomicrospira* to each *Thiomicrothrix* genus. gen. nov. and *Hydrogenovibrio*, and reclassification of all four species of *Thioalkalimicrobium* to *Thiomicrospira*. Int J Syst Evol Microbiol 67:1140–1151. https://doi.org/10.1099/ijsem.0.00855

Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Gregory Caporaso J (2018) Optimizing taxonomic classification of marker-genome amplicon sequences with QIIME 2’s q2-feature-classifier plugin. Microbiome 6:1–17. https://doi.org/10.1186/s40168-018-0470-z

Boldareva EN, Bryantseva IA, Tsapin A, Nelson K, Sorokin DY, Tourova TP, Boichenko VA, Stadnichuk IN, Gorlenko VM (2007) The new alkaliphilic *bacteria* *Thioalkali bacterium* *halophilum* sp. nov. from the hyper-saline soda Mono Lake (California, United States). Microbiol 76:82–92. https://doi.org/10.1134/S0022221707010112

Bolyen E, Rideout JR, Dillon M, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittiger K, Breyjardu A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodriguez AM, Chase J, Cope KE, Da Silva R, Dienzer C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gaulgitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Hutton J, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Bin KK, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciulek T, Kreps J, Langille MI, Lee J, Ley R, Liu YX, Lotfisfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf J, Morgan SC, Morton JT, Naimy AT, Navas-Molina JA, Nothias LF, Orphanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruess E, Rasmussen LB, Rabos LG, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft J, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld J, Zhang Y, Zhu Q, Knight R, Caporaso JG (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857. https://doi.org/10.1038/s41587-019-0209-9

Borsodi AK, Korponai K, Schumann P, Pröer C, Felföldi T, Máriageti K, Szili-Kovács T, Tóth E (2017) *Nitrincola aliciculacustris sp. nov.* and *Nitrincola schmidti sp. nov.*, alkaliphilic bacteria isolated from soda pans, and emended description of the genus *Nitrincola*. Int J Syst Evol Microbiol 67:5159–5164. https://doi.org/10.1099/ijsem.0.02437

Bowman JP, Mccammon SA, Lewis T, Skerratt JH, Brown JL, Nichols DS, Mcmeeken TA (1998) Psychrophilic species from Antarctic sea ice and reclassification of *Flavobacterium*. Microbiology 144:1601–1609

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, A AJ (2016) Dada2. 13:581–583. https://doi.org/10.1038/nmeth.3869.DADA2

de Graaff M, Bijmans MFM, Abbas B, Euverink GJW, Muyzer G, Janssen AJH (2011) Biological treatment of refinery spent caustics under halo-alkaline conditions. Bioresource Technol 102:7257–7264. https://doi.org/10.1016/j.biortech.2011.04.095
de Graaf M, Klok JBM, Bijmans MFM, Muyzer G, Janssen AJH (2012) Application of a 2-step process for the biological treatment of sulfidic spent caustics. Water Res 46:723–730. https://doi.org/10.1016/j.watres.2011.11.044

de Rink R, Klok JBM, van Heerening GJ, Keessman KJ, Janssen AHJ, ter Heijne A, Buissman CJN (2020) Biologically enhanced hydrogen sulfide absorption from sour gas under haloalkaline conditions. J Hazard Mater 383:121104. https://doi.org/10.1016/j.jhazmat.2019.121104

de Rink R, Klok JBM, van Heerening GJ, Sorokin DY, ter Heijne A, Zeijlmaker R, Mos YM, de Wilde V, Keessman KJ, Buissman CJN (2019) Increasing the selectivity for sulfur formation in biological gas desulfurization. Environ Sci Technol 53:4519–4527. https://doi.org/10.1021/acs.est.8b06749

Ghosh W, Dam B (2009) Biochemistry and molecular biology of lithotrophic sulfur oxidation by taxonomically and ecologically diverse bacteria and archaea. FEMS Microbiol Rev 33:999–1043, https://doi.org/10.1111/j.1574-6976.2009.00187.x

Gorlenko VM, Buryukhaev SP, Matyugina EB, Borzenko SV, Namaraev ZB, Bryantseva IA, Boldareva EN, Sorokin DY, Namaraev BB (2010) Microbial communities of the stratified soda Lake Doroninskoe (Transbaikal region). Microbiology 79:390–401. https://doi.org/10.1134/S0026261710030161

Griesbeck C, Hauska G, Schutz M (2000) Biological sulfide oxidation: sulfide-quinone-reductase (SQR), the Primary Reaction, Vol. 4, Research Signpost, Trivandrum, India.

Heberle H, Meirelles VG, da Silva FR, Telles GP, Minhimg R (2015) InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. BMC Bioinformatics 16:1–7, https://doi.org/10.1186/s12859-015-0611-3

Hoeft SE, Blum JS, Stolz JF, Tabita FR, Witte B, King GM, Santini JM, Oremland RS (2011) Alkalimicrobium ehrlichii sp. nov., a novel, arsenite-oxidizing haloalkaliphilic gammagammaproteobacterium capable of chemotrophic or heterotrophic growth with nitrate or oxygen as the electron acceptor. Int J Syst Evol Microbiol 57:504–512. https://doi.org/10.1099/ijs.0.64576-0

Huang C, Li Z, Chen F, Liu Q, Zhao Y, Zhou J, Wang A (2015a) Microbial community structure and function in response to the shift of sulfide/nitrate loading ratio during the denitrifying sulfide removal process. Bioreasour Technol 197:227–234. https://doi.org/10.1016/j.biortech.2015.08.019

Huang C, Zhao Y, Li Z, Yuan Y, Chen C, Tan W, Gao S, Gao L, Zhou J, Wang A (2015b) Enhanced elementary sulfur recovery with sequential sulfate-reducing, denitrifying sulfide-oxidizing processes in a cylindrical-type anaerobic baffled reactor. Bioreasour Technol 192:478–485. https://doi.org/10.1016/j.biortech.2015.04.103

Janssen AJH, Lens PNL, Stams AJM, Plugge CM, Sorokin DY, Muyzer G, Dijkman H, van Zessen E, Buissman CJN (2009) Application of bacteria involved in the biological sulfur cycle for paper mill effluent purification. Sci Total Environ 407:1333–1343. https://doi.org/10.1016/j.scitotenv.2008.09.054

Janssen AJH, Ruitenbergh R, Buissman CJN (2001) Industrial applications of new sulphur biotechnology. Water Sci Technol 44:85–90. https://doi.org/10.2166/wst.2001.0471

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/msd010

Kiragosyan K, Klok JBM, Keessman KJ, Roman P, Janssen AJH (2019) Development and validation of a physiologically based kinetic model for starting up and operation of the biological gas desulfurization process under haloalkaline conditions. Water Res 4:100035. https://doi.org/10.1016/j.watres.2019.100035

Kiragosyan K, Picard M, Timmers PHA, Sorokin DY, Klok JBM, Roman P, Janssen AJH (2020a) Effect of methanethiol on process performance, selectivity and diversity of sulfur-oxidizing bacteria in a dual bioreactor gas biosulfurization system. J Hazard Mater 398:123002. https://doi.org/10.1016/j.jhazmat.2020.123002

Kiragosyan K, Roman P, Keessman KJ, Janssen AJH, Klok JBM (2020b) Stoichiometry-driven heuristic feedforward control for oxygen supply in a biological gas desulfurization process. J Process Control 94:36–45. https://doi.org/10.1016/j.jprocont.2020.08.007

Klok JBM, van Heerening GJ, de Rink R, Wijnbelt J (2017) Introducing the next generation of the THIOPAQ R&G process for biotechnological gas desulphurization: THIOPAQ-SQ. Sulfur 1–4

Klok JBM, de Graaff M, van den Bosch PLF, Boele NC, Keessman KJ, Janssen AJH (2013) A physiologically based kinetic model for bacterial sulfide oxidation. Water Res 47:483–492. https://doi.org/10.1016/j.watres.2012.09.021

Klok JBM, van Heerening GJ, de Rink R, Wijnbelt H, Bowerebank G (2018) Techno-economic impact of the next generation Thioaq O&G process for sulfur removal. In: Proceedings of the GPA-GCC 26th Annual Technical Conference. Muscat

Liesack W, Finster K (1994) Phylogenetic analysis of five strains of Gram-negative, obligately anaerobic, sulfur-reducing bacteria and description of Desulfuromusa gen. nov., including Desulfuromusa kysingii sp. nov., Desulfuromusa bakii sp. nov., and Desulfuromusa succinoxidans sp. Int J Syst Bacteriol 44:753–758. https://doi.org/10.1099/ijs.0.002771.44-4-753

Mahmood Z, Zheng P, Cai J, Hayat Y, Hassan M, Wu D, Hu B (2007) Sources of sulfide in waste streams and current biotechnologies for its removal. J Zhejiang Univ - Sci A 8:1126–1140. https://doi.org/10.1631/jzus.2007.A1126

Mol AR, van der Weijden RD, Klok JBM, Buissman CJN (2020) Properties of sulfur particles formed in biosulfidization of biogas. Minerals 10: https://doi.org/10.3390/min10050433

Morton JT, Marotz C, Washburne A, Silverman J, Zaremba LS, Edlund A, Zengler K (2019) Establishing microbial composition measurement standards with reference frames. Nat Commun 10. https://doi.org/10.1038/s41467-019-10656-5

Muyzer G, Kuenen JG, Robertson LA (2013) Colorless sulfur bacteria. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Stahl DA, Woese CR (eds) The Prokaryotes: Prokaryotic physiology and biochemistry. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp 555–588

Muyzer G, Stams AJM (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol 45:187–192. https://doi.org/10.1038/nrmicro1892

Mwirichia R, Muigai AW, Tindall B, Boga HI, Stackebrandt E (2010) Isolation and characterisation of bacteria from the haloalkaline Lake Elmenteita, Kenya. Extremophiles 14:339–348. https://doi.org/10.1007/s00792-010-0311-x

Parada AE, Needham DM, Fuhrman JA (2016) Every base matters: assessing small subunit tRNA primers for marine microorganisms with mock communities, time series and global field samples. Environ Microbiol 18:1403–1414. https://doi.org/10.1111/1462-2920.12302

Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Olivier G, Blondel M, Prettenhofer P, Weiss R, Dubourg V, Vanderplas O, Passos A (2011) Machine learning in Python. J Mach Learn Res 12:2825–2830

Pikuta EV, Hoover RB, Bej AK, Marsic D, Detkova EN, Whitman WB, Krader P (2003) Tindallia californiensis sp. nov., a new anaerobic, haloalkaliphilic, spore-forming acetogen isolated from Mono Lake in California. Extremophiles 7:327–334. https://doi.org/10.1007/s00792-003-0326-7

Po HN, Senozan NM (2001) The Henderson-Hasselbalch equation: its history and limitations. J Chem Educ 78:1499–1503. https://doi.org/10.1021/ed078p1499
Price MN, Dehal PS, Arkin AP (2010) FastTree 2 - Approximately maximum-likelihood trees for large alignments. PLoS ONE 5. https://doi.org/10.1371/journal.pone.0009490

Pruess E, Peplies J, Glöckner FO (2012) SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28:1823–1829. https://doi.org/10.1093/bioinformatics/bts252

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:590–596. https://doi.org/10.1093/nar/gks1219

Rijkenberg MJA, Kort R, Hellingwerf KJ (2001) Growth physiology and competitive interaction of obligately organotrophic bacteria. Microbiology 72:641–653. https://doi.org/10.1099/mic.0.000008-0

San-Valero P, Penya-roja JM, Javier Álvarez-Hornos F, Buítrón G, Gabaldón C, Quijano G (2019) Fully aerobic bioscrubber for the application of sulfide oxidizing microorganisms able to withstand thiosulphate in gas biodesulfurization systems. Environ Sci Technol 50:12808–12815. https://doi.org/10.1021/acs.est.9b03404

Sorokin DY, Mosier D, Zorz JK, Dong X, Strous M (2020) Wenzhouxiangella strain AB-CW3, a proteolytic bacterium from hypersaline soda lakes that preys on cells of gram-positive bacteria. Front Microbiol 11:1–14. https://doi.org/10.3389/fmicb.2020.597686

Sorokin DY, Muntyan MS, Panteleeva AN, Muzyger Z (2012) Thioalkalivibrio sulfidophilus sp. nov., a haloalkaliphilic sulfur-oxidizing gammaproteobacterium from alkaliine habitats. Int J Syst Evol Microbiol 62:1884–1889. https://doi.org/10.1099/ijs.0.045040-0

Sorokin DY, Tourova TP, Kuznetsov BB, Bryantseva IA, Gorlenko VM (2000) Roseinatronobacter thiooxidans gen. nov., sp. nov., a new alkaliphilic aerobic bacteriochlorophyll a-containing bacterium isolated from a soda Lake. Microbiology 69:89–97. https://doi.org/10.1016/S0022-1230(99)00362-7

Sorokin DY, van den Bosch PLF, Abbas B, Janssen AJH, Muzyger Z (2008) Microbiological analysis of the population of extremely haloalkaliphilic sulfur-oxidizing bacteria dominating in lab-scale sulfide-removing bioreactors. Appl Microbiol Biotechnol 80:965–975. https://doi.org/10.1007/s00253-008-1598-9

Sorokin DY, Zhlinia TN, Lysenko AM, Tourouva TP, Spiridonova EM (2006) Metabolic versatility of haloalkaliphilic bacteria from soda lakes belonging to the Alkaliphilales. Front Microbiol 11:1–14. https://doi.org/10.3389/fmicb.2020.597686

Sun L, Toyonaga M, Ohashi A, Tourloussse DM, Matsuura N, Meng XY, Tamaki H, Hanada S, Cruz R, Yamaguchi T, Sekiguchi Y (2016) Lentimicrobiurn saccharophilum gen. nov., sp. nov., a strictly anaerobic bacterium representing a new family in the phylum bacteirodetaes, and proposal of Lentimicrobiurn bacteirodeteae fam. nov. Int J Syst Evol Microbiol 66:2623–2642. https://doi.org/10.1099/ijsem.0.001103-0

Takai K, Moser DP, Onstott TC, Speolstra N, Pfiffner SM, Dohnalkova A, Fredrickson JK (2001) Tenericutes/Alkalilimnicola ataka gen. nov., sp. nov., an alkaliphilic non-phototrophic member of the alkaliphilum gen. nov., spec. nov. Int J Syst Evol Microbiol 51:12808–12815. https://doi.org/10.1099/ijsem.0.0020713-5-3.1229

Timons LA, Villegas BM, O’Shea J, Roberts J, Shiff F, Jarett M (2009) Fully aerobic bioscrubber for the application of sulfide oxidizing microorganisms able to withstand thiosulphate in gas biodesulfurization systems. Environ Sci Technol 50:12808–12815. https://doi.org/10.1021/acs.est.9b03404

Trüper HG, Brüser T, Tschäpe J, Reinartz M, Dahl C (2002) Sulfide oxidation at halo-alkaline conditions in a fed-batch bioreactor. Extremophiles 6:363–370. https://doi.org/10.1007/s00792-003-0313-4

van den Bosch PLF, van Beusekom OC, Buismen CIN, Janssen AJH (2007) Sulfide oxidation at halo-alkaline conditions in a fed-batch bioreactor. Biotechnol Bioeng 97:1053–1063. https://doi.org/10.1002/bit.21326

Vannini C, Munz G, Mori G, Lubello C, Verni F, Petroni G (2008) Sulphide oxidation to elemental sulphur in a membrane bioreactor: performance and characterization of the selected microbial sulphur-oxidizing community. Syst Appl Microbiol 31:461–473. https://doi.org/10.1016/j.syamp.2008.07.003

Tong WM, Chan Y (2020) GenePiper, a graphical user interface tool for microbiome sequence data mining. Microbiol Resour Announc 9:13–15. https://doi.org/10.1101/sra.01195-19

Trüper HG, Brüser T, Tschäpe J, Reinartz M, Dahl C (2002) Sulfide oxidation in the phototrophic sulfur bacterium Chromatium vinosum. Arch Microbiol 170:59–68. https://doi.org/10.1007/s00203-00505615

van den Bosch PLF, van Beusekom OC, Buismen CIN, Janssen AJH (2007) Sulfide oxidation at halo-alkaline conditions in a fed-batch bioreactor. Biotechnol Bioeng 97:1053–1063. https://doi.org/10.1002/bit.21326

Vannini C, Munz G, Mori G, Lubello C, Verni F, Petroni G (2008) Sulphide oxidation to elemental sulphur in a membrane bioreactor: performance and characterization of the selected microbial sulphur-oxidizing community. Syst Appl Microbiol 31:461–473. https://doi.org/10.1016/j.syamp.2008.07.003
Varga D, Horváth AK (2007) Kinetics and mechanism of the decomposition of tetrathionate ion in alkaline medium. Inorg Chem 46:7654–7661. https://doi.org/10.1021/ic700992u

Vavourakis CD, Ghai R, Rodriguez-Valera F, Sorokin DY, Tringe SG, Hugenholtz P, Muyzer G (2016) Metagenomic insights into the uncultured diversity and physiology of microbes in four hypersaline soda lake brines. Front Microbiol 7:1–18. https://doi.org/10.3389/fmicb.2016.00211

Vavourakis CD, Mehrshad M, Balkema C, van Hall R, Andrei A-Ş, Ghai R, Sorokin DY, Muyzer G (2019) Metagenomes and metatranscriptomes shed new light on the microbial-mediated sulfur cycle in a Siberian soda lake. BMC Biol 17:1–20. https://doi.org/10.1186/s12915-019-0688-7

Visser JM, De Jong GAH, Robertson LA, Kuenen JG (1997) A novel membrane-bound flavocytochrome c sulfide dehydrogenase from the colourless sulfur bacterium Thiobacillus sp. W5. Arch Microbiol 167:295–301. https://doi.org/10.1007/s00203005447

Wang L, Shao Z (2021) Aerobic denitrification and heterotrophic sulfur oxidation in the genus Halomonas revealed by six novel species characterizations and genome-based analysis. Front Microbiol 12. https://doi.org/10.3389/fmicb.2021.652766

Weber CF, King GM (2007) Physiological, ecological, and phylogenetic characterization of Stappia, a marine CO-oxidizing bacterial genus. Appl Environ Microbiol 73:1266–1276. https://doi.org/10.1128/AEM.01724-06

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.