Advances in Environmental Technology 11(4) 2025, 414-425.

Iranian Reasearch Organization for Science and Technology (IROST)

Advances in Environmental Technology

Journal home page: https://aet.irost.ir

Isolation and characterization of sulfur-oxidizing bacteria at the Ilam gas refinery in Iran

Tayebe Karimi[a], Fazel Pourahmad[a]*, Ehsan Noorollahi[b]

[a] *Department of Microbiology, Faculty of Veterinary Sciences, Ilam University, P. O. Box: 69315516, Ilam, Iran.*

[b] *Higher Education Institute of Safir Danesh, Ilam, Iran.*

ARTICLE INFO

Document Type:
Research Paper

Article history:
Received 09 March 2025
Received in revised form
25 June 2025
Accepted 25 June 2025

Keywords:
Biodesulfurization
Sulfur-oxidizing bacteria
Natural Gas Sweetening
16S rRNA gene sequencing
Ilam gas refinery
Bioremediation

ABSTRACT

Natural gas is a critical energy resource, but sour gas, characterized by high hydrogen sulfide (H_2S) content, poses significant environmental and operational challenges, including corrosion, toxicity, and air pollution. Conventional desulfurization methods, such as hydrodesulfurization (HDS), are energy-intensive and environmentally taxing. Biodesulfurization (BDS) using sulfur-oxidizing bacteria (SOB) offers a sustainable alternative. This study, the first to characterize SOB from the Ilam Gas Refinery in Iran, aimed to isolate and identify SOB from soil samples to explore their potential for biodesulfurization and bioremediation. Soil samples were collected from various locations within the Ilam Gas Refinery, and 16 bacterial isolates were obtained using media enriched with sulfur compounds and sulfur-enriched media. The isolates were purified and characterized through Gram staining and molecular identification using 16S rRNA gene sequencing. Phylogenetic analysis was conducted to understand the evolutionary relationships among the isolated bacteria. The isolates were purified, characterized through Gram staining, 16S rRNA gene sequencing, and phylogenetic analysis. Sixteen bacterial isolates were cultivated, with 11 successfully identified through 16S rRNA gene sequencing. The identified species included *Achromobacter xylosoxidans*, *Sphingomonas paucimobilis*, *Streptomyces babili*, and *Priestia megaterium*. These species, particularly *S. babili* and *P. megaterium*, are less commonly associated with gas refinery environments, highlighting the novelty of this study. Statistical analyses confirmed a significant predominance of Gram-negative bacteria ($p < 0.05$). The study also identified the potential of these bacteria in the bioremediation process. The inability to amplify the *soxB* gene suggests alternative sulfur oxidation pathways, warranting further investigation. The findings provide a foundation for developing microbial-based solutions that are efficient, cost-effective and environmentally sustainable.

*Corresponding author Tel.: +98 (0)841- 2224308
E-mail: f.pourahmad@ilam.ac.ir
DOI: 10.22104/AET.2025.7467.2081

1. Introduction

Natural gas is a vital fossil fuel that serves as a primary energy source across residential, industrial, and commercial sectors due to its cleaner combustion and lower greenhouse gas emissions than other fossil fuels; it is valued for its cleaner combustion and lower greenhouse gas emissions compared to coal and oil [1]. It supports residential heating, industrial processes, and power generation, making it indispensable for energy security. It is widely used for heating, cooking, and power generation, making it an indispensable component of the global energy mix. However, sour natural gas, which contains high levels of hydrogen sulfide (H_2S), poses significant environmental and operational challenges. H_2S is highly corrosive, especially in the presence of water, leading to substantial damage to pipelines and equipment, resulting in costly repairs and increased maintenance [2]. Additionally, H_2S is a toxic gas that poses serious health risks to workers and surrounding communities, with exposure potentially leading to respiratory issues and even fatalities, including respiratory distress and potential fatalities [3]. During combustion, H_2S is converted into sulfur dioxide (SO_2), a major contributor to air pollution and acid rain, adversely affecting ecosystems and human health [2]. These issues underscore the necessity for effective desulfurization methods to mitigate the risks associated with sour gas.

Conventional desulfurization techniques, such as hydrodesulfurization (HDS), are widely used in the industry but are energy-intensive and environmentally taxing. HDS requires high temperatures (300–400°C) and pressures (30–130 atm), leading to significant energy consumption and operational costs, particularly when aiming for ultra-low-sulfur fuels and environmental impact [4]. Moreover, the HDS process can produce waste and emissions that contribute to environmental pollution, and it has limitations in effectively removing generated waste and emissions. It struggles to remove certain sulfur compounds, especially those with complex structures, such as dibenzothiophene, limiting its efficacy for ultra-low-sulfur fuels [5]. These drawbacks have prompted the exploration of more sustainable alternatives, such as biodesulfurization (BDS), which utilizes microorganisms to selectively remove sulfur compounds from fossil fuels under mild conditions.

BDS has emerged as a promising solution, particularly through the use of sulfur-oxidizing bacteria (SOB). SOB are capable of oxidizing reduced sulfur compounds, such as H_2S and elemental sulfur, into less harmful forms like sulfate, making them ideal for natural gas sweetening [6]. This process reduces the environmental impact of sulfur emissions and offers a cost-effective and energy-efficient solution for natural gas sweetening [6]. Beyond BDS, SOB are also widely used in other industrial applications, including biomining, bioremediation, and biofertilizer production, due to their ability to degrade pollutants and enhance soil fertility [7, 8]. In biomining, SOB oxidize insoluble metal sulfides to soluble metal sulfates, facilitating metal extraction from ores [7]. In bioremediation, SOB is crucial in detoxifying environments contaminated with sulfur compounds, making them valuable for environmental cleanup [8]. In biomining, they oxidize metal sulfides to extract metals, while in bioremediation, they detoxify sulfur-contaminated sites [7, 8].

The ecological importance of SOB lies in their role in the sulfur cycle, where they facilitate the oxidation of toxic sulfides into sulfate, contributing to the detoxification of contaminated environments [9]. This process is essential for maintaining the balance of sulfur in various ecosystems and contributes to the detoxification of environments contaminated with sulfur compounds and supports ecosystem health [7, 9]. SOB are also involved in biogeochemical cycling, influencing nutrient availability and ecosystem health in soil and aquatic environments [10]. Their metabolic versatility allows them to thrive in diverse environments, making them valuable for both environmental and industrial applications.

Despite their potential, the diversity and metabolic pathways of SOB in sulfur-rich environments, such as gas refineries, remain underexplored. Previous studies have identified various SOB, such as *Rhodococcus erythropolis*, which have demonstrated promising capabilities as effective for BDS, desulfurizing fuels while preserving hydrocarbon structures [11]. However, the specific

mechanisms and pathways employed by SOB in different environments, particularly in industrial settings like gas refineries, require further investigation. Recent studies (2020–2024) have advanced our understanding of SOB diversity and sulfur oxidation pathways. For instance, *Acidithiobacillus* species employ multiple pathways, including the tetrathionate intermediate pathway and the reverse dissimilatory sulfite reductase (rDsr) pathway, which offer superior energy efficiency [8, 12]. Studies on SOB in wastewater treatment and bioremediation highlight their ability to reduce sulfur emissions and detoxify heavy metal-contaminated sites [13, 14].

This study is the first to characterize SOB from the Ilam Gas Refinery in Iran, a sulfur-rich industrial site. The identification of species such as *Streptomyces babili* and *Priestia megaterium*, which are less commonly associated with gas refinery environments, adds novelty to the findings. These isolates demonstrate potential for bioremediation due to their ability to degrade hydrocarbons and heavy metals, offering unique insights into microbial solutions for sulfur-related challenges. This study focused on isolating and characterizing SOB from soil samples collected at the Ilam Gas Refinery. The objective was to identify bacterial species capable of oxidizing sulfur compounds and to explore their potential for biodesulfurization. This research aimed to advance the development of sustainable BDS technologies and enhance our understanding of SOB's ecological and industrial roles by integrating microbiological, molecular, and phylogenetic techniques.

2. Materials and Methods

2.1. Study site and sample collection

Soil samples were collected from various locations within the Ilam Gas Refinery during the fall of 1403. Ten soil samples were collected from both the surface and subsurface layers (5–30 cm deep) at carefully chosen sites and the main refinery, covering all directions (north, south, east, and west). The samples were mixed thoroughly, homogenized, transferred to sterile Falcon tubes, and stored at 4°C until further processing within 48 hours to preserve microbial viability.

2.2. Bacterial isolation and purification

For bacterial isolation, a 1:10 dilution was prepared by dissolving 1 g of sieved soil (to remove stones and coarse particles) in 10 mL of sterile distilled water. Serial dilutions (1:100, 1:1000, and 1:100,000) were prepared, and 100 µL from the 1:1000 and 1:100,000 dilutions were inoculated into M701 and M789 broths, which are selective media for sulfur-oxidizing bacteria selective media enriched with sulfur compounds for SOB growth. The broths were incubated in a shaking incubator at 25°C (150 rpm) until turbidity indicated bacterial growth (typically 3–5 days). Subsequently, 100 µL of the bacterial culture was spread onto M701, M789, and 9K agar plates and incubated for approximately one week at 25°C for 7–10 days. Visible colonies were subcultured sequentially on 9K, M701, and M789 media to obtain pure cultures. Gram staining was performed to confirm the purity of the isolates and to classify them as Gram-positive or Gram-negative based on their morphology and staining characteristics.

2.3. Molecular identification of bacterial isolates

DNA extraction was performed using a simple boiling method as described in [15]. Briefly, bacterial pellets from 1 mL of the overnight cultures were resuspended in 200 µL of sterile distilled water, heated at 95°C for 10 minutes, cooled on ice for 5 minutes, and centrifuged at 13,000 ×g for 5 minutes. The supernatant containing DNA was collected and stored at -20°C.

The 16S rRNA gene was amplified using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). The PCR reaction mixture (25 µL) contained 13.5 µL of master mix (Amplicon, Denmark), 1 µL of each primer (10 µM), 3 µL of template DNA, and 7.5 µL of DNase/RNase-free distilled water. The thermal cycling program was performed in a Bio-Rad T100 Thermal Cycler with an initial denaturation at 96°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 1.5 minutes, with a final extension at 72°C for 7 minutes.

For the *soxB* gene amplification, the *soxB* gene, a marker for sulfur oxidation, was amplified using primers SoxB693F (5′-ATCGGNCARGCNTTYCCNTA-3′) and SoxB1446R (5′-CATGTCNCCNCCYTGYTG-

3′). The PCR conditions were similar to those for the 16S rRNA gene, with variations in the annealing temperature (51–55°C) and the number of cycles (35-40 cycles). The PCR mixture was identical to that for 16S rRNA, with an annealing temperature range of 51–55°C (optimized via gradient PCR) and 35 cycles. Positive controls utilized *Acidithiobacillus ferrooxidans* DNA, while negative controls excluded template DNA.

2.4. Sequencing and phylogenetic analysis

PCR products were purified using a PCR purification kit (Yekta Tajhiz Co., Iran) following the manufacturer's protocol. The quality of the purified PCR products was confirmed and verified by 1% agarose gel electrophoresis with ethidium bromide staining.

Purified 16S rRNA PCR products were sequenced using PCR primers via Sanger sequencing (Macrogen, South Korea). Sequences were assembled, trimmed using the SeqMan program in the Lasergen software package (DNAStar, Madison, WI, USA), and compared to the NCBI database using BLASTn to identify the closest matches. Reference sequences were selected based on >98% similarity, prioritizing type strains and sequences from sulfur-rich environments. The retrieved sequences were aligned with ClustalW in MEGA 11, and phylogenetic trees were constructed using the neighbor-joining method with the Kimura 2-parameter model. Bootstrap values were calculated from 1,000 replicates to assess tree robustness. Trees were visualized and annotated in MEGA 11.

2.5. Statistical analysis

A chi-square test compared the observed distribution of Gram-positive and Gram-negative isolates against an expected 50:50 distribution to determine whether the predominance of Gram-negative bacteria was significant. A one-way ANOVA assessed differences in species abundance based on 16S rRNA sequencing results, with post-hoc Tukey tests identifying specific differences. Statistical analyses were performed in R (version 4.3.2), using a significance threshold of $p < 0.05$.

3. Results and Discussion

3.1. Isolation and purification

Sixteen bacterial isolates were isolated and purified from soil samples collected at the Ilam Gas Refinery using selective media enriched with sulfur compounds (M701 and M789 broths) and sequential subculturing on 9K, M701, and M789 agar plates. The diverse morphological characteristics of the colonies indicated a wide range of bacterial species present in the sulfur-contaminated soil. This isolation highlighted the microbial diversity in sulfur-contaminated environments, as previously observed in similar studies [16, 17]. Sixteen bacterial isolates were obtained from soil samples using M701 and M789 broths, followed by subculturing on 9K, M701, and M789 agar plates. The diverse colony morphologies (e.g., size, color, and texture) indicated a broad range of bacterial species, reflecting the microbial diversity in sulfur-contaminated environments [16, 17]. The use of sulfur-enriched media ensured the selection of SOB, aligning with previous studies on sulfur-rich industrial sites [18, 19].

3.2. Gram staining and classification

Gram staining classified three isolates (18.75%) as Gram-positive and 13 (81.25%) as Gram-negative, based on the 16 isolates. A chi-square test confirmed a significant predominance of Gram-negative bacteria ($\chi^2 = 6.25$, $p = 0.012$), consistent with their prevalence in sulfur-rich habitats [16, 17]. The Gram-positive isolates predominantly exhibited bacilli morphology, while the Gram-negative isolates displayed a mix of bacilli and cocci forms. This distribution reflected the microbial diversity in the sulfur-rich environment of the refinery. Gram-positive isolates were primarily bacilli, while Gram-negative isolates included bacilli and cocci. The predominance of Gram-negative bacteria, particularly *Sphingomonas paucimobilis* and *Achromobacter xylosoxidans*, aligned with their known prevalence in sulfur-rich habitats. These bacteria are known for their metabolic versatility and ability to degrade hydrocarbons and heavy metals, making them promising candidates for bioremediation and biodesulfurization applications [16, 17]. Additionally, *A. xylosoxidans* thrives in

environments with high organic pollutants, aided by its unique fatty acid composition [20].

3.3. Molecular identification of bacterial isolates

Molecular identification through 16S rRNA gene sequencing revealed that 11 isolates belonged to three phyla: *Pseudomonadota*, *Actinomycetota*, and *Bacillota*, with genera including *Achromobacter*, *Sphingomonas*, *Streptomyces*, and *Priestia*. Eleven of the 16 isolates were identified via 16S rRNA gene sequencing, belonging to three phyla: *Pseudomonadota*, *Actinomycetota*, and *Bacillota*. *Sphingomonas paucimobilis* was the most frequently identified species (45.5%), followed by *A. xylosoxidans* (36.4%), *Streptomyces babili* (9.1%), and *Priestia megaterium* (9.1%). The similarity percentages of the 16S rRNA gene sequences to their closest type strains ranged from 99.37% to 100%, confirming the accuracy of the identification. Identified species included *Sphingomonas paucimobilis* (5 isolates, 45.5%), *Achromobacter xylosoxidans* (4 isolates, 36.4%), *Streptomyces babili* (1 isolate, 9.1%), and *Priestia megaterium* (1 isolate, 9.1%). Sequence similarities to the closest type strains ranged from 99.37% to 100%, confirming identification accuracy (Figure 1, Table 1). A one-way ANOVA revealed significant variation in species abundance ($F(3,7) = 5.82$, $p = 0.023$), with post-hoc tests indicating *S. paucimobilis* was significantly more abundant than *S. babili* and *P. megaterium* ($p < 0.05$).

The identification of *S. babili* and *P. megaterium* is novel for gas refinery contexts, as these species are less commonly reported in such environments compared to *Rhodococcus* or *Acidithiobacillus* [11, 12]. The identification of *S. babili* and *P. megaterium* adds to the diversity of sulfur-oxidizing bacteria and underscores their potential utility in industrial applications. *Streptomyces* species are renowned for producing bioactive secondary metabolites, including antibiotics and anticancer agents [17], while *Priestia* species have shown promise in agriculture due to their enhanced soil fertility, drought tolerance, and production of antimicrobial compounds, suggesting diverse industrial applications [21].

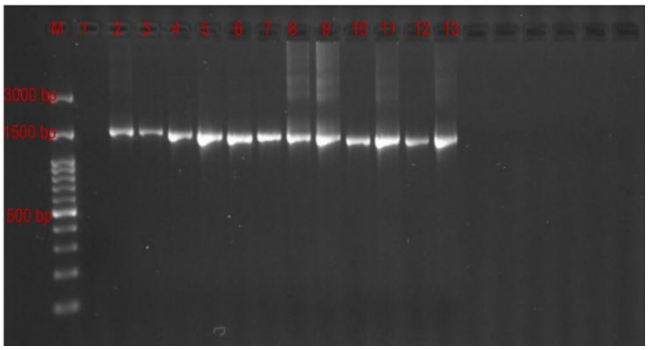

Fig. 1. Agarose gel electrophoresis of purified product PCR 16S rRNA gene. Lanes: M (Molecular size marker, 100 base pairs), 1) Negative control, 2) M789A1, 3) 9KA22, 4) 9KA24, 5) M701A71, 6) M701A72, 7) M789A73, 8) M789A75, 9) M789A77, 10) M701A81, 11) M789A84, 12) M789A92, 13) M789A101

3.4. Phylogenetic analysis

Phylogenetic trees showed clustering of the isolates into genera *Achromobacter*, *Sphingomonas*, and *Streptomyces*, with *Priestia* forming a separate branch (Figure 2). This classification confirmed the accuracy of the identification and highlighted the evolutionary relationships among these bacteria. Phylogenetic trees constructed using the neighbor-joining method clustered isolates into *Achromobacter*, *Sphingomonas*, *Streptomyces*, and *Priestia* genera, with bootstrap values >70% indicating robust branching. *Priestia megaterium* formed a distinct branch, reflecting its evolutionary divergence. Reference sequences from sulfur-rich environments (e.g., gas refineries and wastewater treatment plants) strengthened the analysis, confirming the ecological relevance of the isolates.

Several genera have adapted to high H_2S levels, showcasing diverse strategies to mitigate toxic sulfur compounds. For example, *Poecilia* fish have evolved mitochondrial pathways to detoxify H_2S, enabling survival in lethal conditions [22, 23]. Additionally, sulfate-reducing bacteria like *Desulfovibrio* and *Desulfuromonas* transform oxidized sulfur compounds into H_2S, demonstrating resistance to toxic environments [24]. These adaptations highlight the diverse mechanisms organisms employ to thrive in sulfur-rich environments.

Table 1. Results of Nucleic Acid Sequence Determination for the Studied Isolates

No	Isolate Name	Identity	Closest Type Strain	Similarity (%)
1	9KA33	Streptomyces babili	NRRB-1338	99.93
2	9KA33	Sphingomonas paucimobilis	NBRC 13935	100
3	M701A71	Sphingomonas paucimobilis	NBRC 13935	100
4	M701A73	Sphingomonas paucimobilis	NBRC 13935	100
5	M789A73	Achramobacter xylosoxidans	NBRC 15136	99.5
6	M789A75	Achramobacter xylosoxidans	NBRC 15136	99.5
7	M789A77	Achramobacter xylosoxidans	NBRC 15136	99.5
8	M701A81	Sphingomonas paucimobilis	NBRC13935	100
9	M789A83	Achramobacter xylosoxidans	NBRC 15136	99.5
10	M789A93	Sphingomonas paucimobilis	NBRC 13935	100
11	M789A101	Priestia megaterium	NBRC 15308	99.37

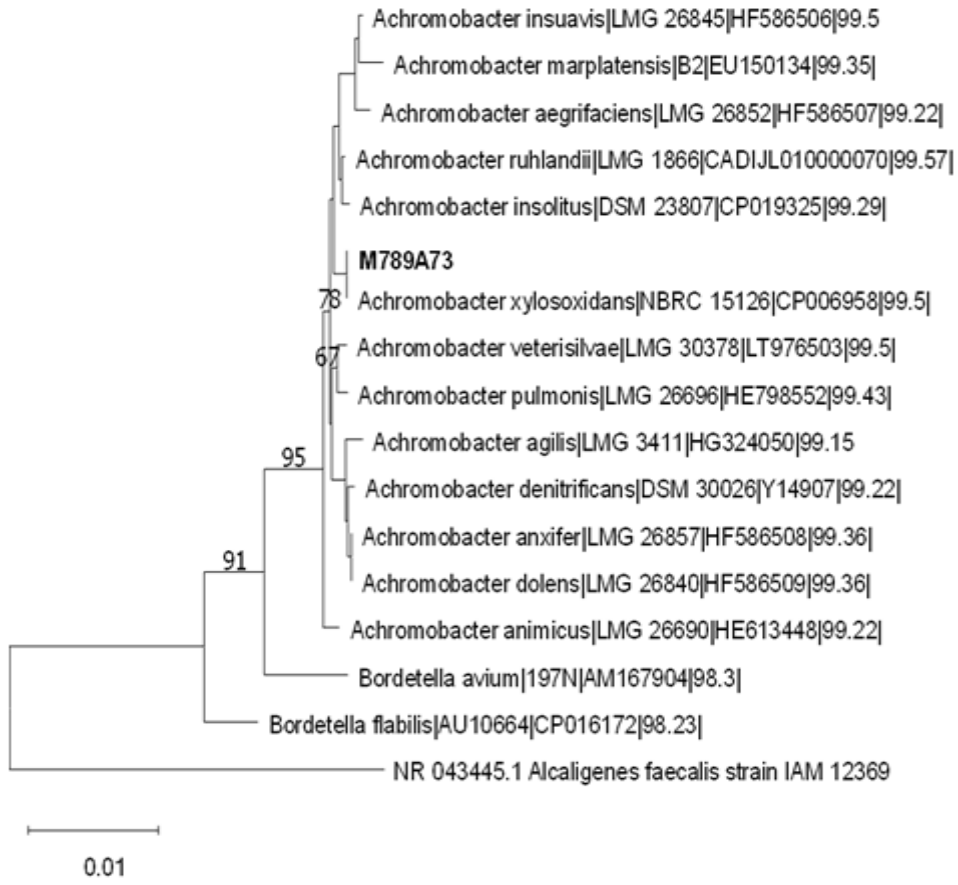

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequence for isolate M789A73

The identification of *Streptomyces* and *Priestia* species adds significant diversity and potential utility in industrial applications. The novel identification of *Streptomyces* and *Priestia* species in this context expands the biotechnological toolkit. *Streptomyces* is valuable for biotechnological applications due to its production of species that produce bioactive secondary metabolites [22], applicable in pharmaceuticals [17]. *Priestia*, particularly *P. filamentosa*, shows promise in agriculture for its drought tolerance and production of antimicrobial agents [25]. Both genera exhibit robust metabolic profiles, enabling them to improve agricultural productivity and degrade pollutants like hydrocarbons and pharmaceuticals, further highlighting their ability to support bioremediation potential [26, 27].

3.5. Sulfur oxidation pathways

Despite multiple attempts, the *soxB* gene, a key marker for the sulfur oxidation pathway, could not

be amplified in any of the isolates. Potential reasons include primer mismatch due to sequence variability, absence of the *soxB* gene, or reliance on alternative pathways, such as the APS or rDsr pathways [28, 29]. The use of sulfur-enriched media (M701, M789, 9K) ensured the selection of SOB, but direct biochemical assays for sulfur oxidation (e.g., sulfate production) and H_2S tolerance were beyond this study's scope due to time and resource constraints. These assays are planned for future work to quantify sulfur-oxidizing activity and H_2S tolerance under refinery-relevant conditions.

Research has shown that SOB, such as those in the genus *Acidithiobacillus*, possess multiple sulfur oxidation pathways, including the tetrathionate intermediate pathway and the sulfur dioxygenase pathway. Some species can oxidize thiosulfate to tetrathionate using alternative enzymes, demonstrating the diversity of sulfur oxidation mechanisms [30]. The limitation in amplifying the *soxB* gene opens new research avenues to investigate less-characterized sulfur oxidation mechanisms. For instance, some sulfur-oxidizing bacteria utilize the reverse dissimilatory sulfite reductase (rDsr) pathway, which shows superior energy conservation efficiency compared to the Sox system [31]. Understanding these adaptations could enhance our knowledge of microbial ecology and biogeochemical cycles in sulfur-rich environments. The failure to amplify *soxB* highlights the need for advanced genomic approaches, such as metagenomic sequencing, to uncover novel sulfur oxidation mechanisms [32].

3.6. Broader implications and applications broader implications

SOB play a crucial role in the biogeochemical cycling of sulfur, essential for processes like bioleaching, bioremediation, and biofertilizer production. Their ability to oxidize reduced sulfur compounds to less harmful forms makes them valuable in reducing environmental pollution, particularly in wastewater treatment [33, 34]. SOB are also utilized in biofilters and biosensors, enhancing their applicability in industrial settings [35]. Their metabolic versatility allows them to thrive in various environments, making them adaptable for specific industrial needs [36, 37]. The identified SOB offers significant potential for addressing sulfur-related challenges in gas refineries. By converting H_2S to elemental sulfur or sulfate, SOB can reduce corrosion, health risks, and environmental pollution, offering advantages over HDS, including lower energy consumption and fewer harmful byproducts [36]. Their ability to degrade hydrocarbons and heavy metals supports bioremediation applications, while their role in the sulfur cycle enhances ecosystem health [18].

The amplification and sequencing of the 16S rRNA gene are crucial for establishing a taxonomic framework for bacterial isolates. This method allows for the detection and identification of bacteria in various samples, enhancing diagnostic accuracy in clinical settings [37, 38]. Phylogenetic analysis based on 16S rRNA sequences provides insights into the evolutionary relationships among bacterial taxa, making it a fundamental tool in microbiology [39]. In bioleaching, SOB oxidize metal sulfides to extract metals, while in wastewater treatment, they reduce sulfur emissions [12, 13]. The novel identification of *Streptomyces babili* and *Priestia megaterium* expands their applicability, as these species produce bioactive metabolites and improve soil fertility, respectively [17, 18].

The presence of sour gas, characterized by high H_2S content, poses significant challenges, including corrosion of pipelines, health risks to workers, and environmental contamination [40, 41]. Addressing these challenges requires effective monitoring, advanced corrosion-resistant materials, and robust safety measures. The ability of SOB to convert H_2S into elemental sulfur or sulfate presents a promising biotechnological solution. This microbial approach offers advantages over traditional chemical methods, including lower operational costs, reduced energy consumption, and fewer harmful byproducts [42]. SOB processes operate under milder conditions, leading to significant energy savings, especially in large-scale applications [35].

SOB are essential for the sulfur cycle and play a significant role in detoxifying environments contaminated with reduced sulfur compounds. They facilitate the oxidation of toxic sulfides to less harmful sulfate, crucial for maintaining the ecological balance in sulfur-rich ecosystems [43].

SOB are utilized in various biotechnological applications, including bioremediation, where they help eliminate heavy metals from contaminated sites through processes like bioleaching [13]. Their ability to oxidize reduced sulfur compounds not only aids in detoxification but also enhances soil fertility and reduces environmental pollution [44]. The challenges faced in amplifying the *soxB* gene highlight the need for advanced genomic and transcriptomic techniques. The failure to amplify this gene suggests that alternative pathways may exist, which could be uncovered through more sophisticated methods such as metagenomic sequencing. This approach can provide a comprehensive view of microbial isolates' functional genes, revealing SOB's diversity and potential roles in various environments [45]. Additionally, the use of high-throughput sequencing techniques has shown promise in characterizing microbial communities and their functional potential, indicating that such methods could enhance our understanding of the soxB gene's role in sulfur oxidation [46]. These advancements could lead to better insights into the ecological functions of SOB and their contributions to biogeochemical cycles. The failure to amplify *soxB* underscores the need for advanced genomic and transcriptomic techniques, such as high-throughput sequencing, to characterize alternative sulfur oxidation pathways [47]. These advancements could enhance our understanding of SOB's ecological and industrial roles, paving the way for tailored microbial solutions.

4. Conclusion

This study provides the first comprehensive characterization of sulfur-oxidizing bacteria from the Ilam Gas Refinery in Iran, identifying *Achromobacter xylosoxidans*, *Sphingomonas paucimobilis*, *Streptomyces babili*, and *Priestia megaterium*. The novel identification of *S. babili* and *P. megaterium* in this industrial context highlights their potential for biodesulfurization and bioremediation, particularly in degrading hydrocarbons and heavy metals. Statistical analyses confirmed a significant predominance of Gram-negative bacteria ($p = 0.012$), and phylogenetic analysis validated species identification. The inability to amplify the *soxB* gene suggests alternative sulfur oxidation pathways, a limitation to be addressed through future metagenomic sequencing and biochemical assays for sulfur oxidation and H_2S tolerance. These findings establish a robust foundation for developing sustainable microbial-based solutions to mitigate sulfur-related challenges in gas refineries, with applications in bioremediation, bioleaching, and environmental management. Future research should focus on the functional characterization of these isolates and the exploration of their metabolic pathways to optimize their industrial utility.

Acknowledgment

The authors express gratitude to the dedicated staff at the Ilam Gas Refinery for their invaluable support and contributions to this study.

Data availability

The datasets generated and analyzed during the current study are available in the GenBank repository, under accession numbers PQ857180–PQ857190.

Declaration of competing interests

The authors declare that they have no conflict of interest.

Authors contribution

T.K. and F.P. designed, analyzed, and wrote the manuscript. T.K. and E.N. designed and developed the protocol and secured funding. T.K., F.P., and E.N. participated in data and sample collection. T.K. and F.P. performed the bioinformatics analysis of 16S rRNA sequences. All authors participated in the critical review of the submitted manuscript. We give consent to publish and disseminate this study in Scientific Reports. All authors contributed to the critical review of the manuscript and approved its publication.

Funding

This research was partially supported by a grant from the Ilam Gas Refinery.

Declaration of using generative AI

We used Grammarly, an AI-based tool, to improve the manuscript's language and grammar. All content and ideas are the authors' original work, with the final manuscript reviewed and edited by the authors for accuracy.

References

[1] Almaktar, M., & Shaaban, M. (2021). Prospects of renewable energy as a non-rivalry energy alternative in Libya. *Renewable and Sustainable Energy Reviews, 143*, 110852. https://doi.org/10.1016/j.rser.2021.110852

[2] Molnár, É., Rippel-Pethő, D., Horváth, G., Hodai, Z., Bocsi, R., & Bobek, J. (2016). Benefits of jet reactor application in alkaline gas purification. *Periodica Polytechnica Chemical Engineering, 60*(2), 74-77. https://doi.org/10.3311/PPch.8261

[3] Tengku Hassan, T. N., Shariff, A. M., Mohd Pauzi, M. M., Khidzir, M. S., & Surmi, A. (2022). Insights on cryogenic distillation technology for simultaneous CO2 and H2S removal for sour gas fields. *Molecules, 27*(4), 1424. https://doi.org/10.3390/molecules27041424

[4] Sikarwar, P., Gosu, V., & Subbaramaiah, V. (2019). An overview of conventional and alternative technologies for the production of ultra-low-sulfur fuels. *Reviews in Chemical Engineering, 35*(6), 669-705. https://doi.org/10.1515/revce-2017-0082

[5] Wagle, D. V., Zhao, H., Deakyne, C. A., & Baker, G. A. (2018). Quantum chemical evaluation of deep eutectic solvents for the extractive desulfurization of fuel. *ACS Sustainable Chemistry & Engineering, 6*(6), 7525-7531. https://doi.org/10.1021/acssuschemeng.8b00224

[6] Martzoukou, O., Mamma, D., & Hatzinikolaou, D. G. (2023). Medium composition overturns the widely accepted sulfate-dependent repression of desulfurization phenotype in *Rhodococcus qingshengii* IGTS8. *Biotechnology and Bioengineering, 120*(10), 3092-3098. https://doi.org/10.1002/bit.28436

[7] Rawlings, D. E. (2002). Heavy metal mining using microbes. *Annual Review of Microbiology, 56*(1), 65-91. https://doi.org/10.1146/annurev.micro.56.012302.161052

[8] Nguyen, P. M., Do, P. T., Pham, Y. B., Doan, T. O., Nguyen, X. C., Lee, W. K., & Ngo, H. H. (2022). Roles, mechanism of action, and potential applications of sulfur-oxidizing bacteria for environmental bioremediation. *Science of the Total Environment, 852*, 158203. https://doi.org/10.1016/j.scitotenv.2022.158203

[9] Pokorna, D., & Zabranska, J. (2015). Sulfur-oxidizing bacteria in environmental technology. *Biotechnology Advances, 33*(6), 1246-1259. https://doi.org/10.1016/j.biotechadv.2015.02.007

[10] Valentin-Alvarado, L.E., Fakra, S.C., Probst, A.J., Giska, J.R., Jaffe, A.L., Oltrogge, L.M., West-Roberts, J., Rowland, J., Manga, M., Savage, D.F., & Greening, C. (2024). Autotrophic biofilms sustained by deeply sourced groundwater host diverse bacteria implicated in sulfur and hydrogen metabolism. *Microbiome, 12*(1), 15. https://doi.org/10.1101/2022.11.17.516901

[11] Hu, T., Yang, Y., Zhang, M., Gao, Y., Cheng, Q., & Ji, H. (2019). Biodesulfurization of coal using *Rhodococcus erythropolis* SX-12 and *Acidithiobacillus ferrooxidans* GF: A two-step approach. *Energy Science & Engineering, 7*(1), 162-169. https://doi.org/10.1002/ese3.266

[12] Ibáñez, A., Garrido-Chamorro, S., Coque, J. J., & Barreiro, C. (2023). From genes to bioleaching: Unraveling sulfur metabolism in *Acidithiobacillus* genus. *Genes, 14*(9), 1772. https://doi.org/10.3390/genes14091772

[13] Zhang, R., Wei, X., Hao, Q., & Si, R. (2020). Bioleaching of heavy metals from municipal solid waste incineration fly ash: Availability of recoverable sulfur prills and form transformation of heavy metals. *Metals, 10*(6), 815. https://doi.org/10.3390/met10060815

[14] Meyer, B., Imhoff, J. F., & Kuever, J. (2007). Molecular analysis of the distribution and phylogeny of the *soxB* gene among sulfur-oxidizing bacteria—evolution of the Sox sulfur

oxidation enzyme system. *Environmental Microbiology, 9*(12), 2957-2977. https://doi.org/10.1111/j.1462-2920.2007.01407.x

[15] Hajizadeh, M., Pourahmad, F., & Nemati, M. (2023). Isolation and screening of antibacterial activity of *Actinomycetota* from the medicinal plant, *Anthemis pseudocotula* Boiss. *Archives of Razi Institute, 78*(5), 1638-1646. https://doi.org/10.22092/ari.2023.78.5.1638

[16] Dees, S. B., & Moss, C. W. (1978). Identification of *Achromobacter* species by cellular fatty acids and by production of keto acids. *Journal of Clinical Microbiology, 8*(1), 61-66. https://doi.org/10.1128/jcm.8.1.61-66.1978

[17] Lacey, H. J., & Rutledge, P. J. (2022). Recently discovered secondary metabolites from *Streptomyces* species. *Molecules, 27*(3), 887. https://doi.org/10.3390/molecules27030887

[18] Yang, Z., Liu, Z., Sklodowska, A., Musialowski, M., Bajda, T., Yin, H., & Drewniak, L. (2021). Microbiological sulfide removal—from microorganism isolation to treatment of industrial effluent. *Microorganisms*, *9*(3), 611. https://doi.org/10.3390/microorganisms9030611

[19] Borse, K., Agnihotri, V., Bhandarkar, H., & Bhardwaj, S. (2021). Effective sulfur oxidizing bacterial isolation from process wastewater of food industry. *Plant Archives*, *21*(1), 1595-1604. https://doi.org/10.51470/PLANTARCHIVES.2021.V21.S1.252

[20] Strnad, H., Ridl, J., Paces, J., Kolar, M., Vlcek, C., & Paces, V. (2011). Complete genome sequence of the haloaromatic acid-degrading bacterium *Achromobacter xylosoxidans* A8. *Journal of Bacteriology*, *193*(3), 791-792. https://doi.org/10.1128/JB.01299-10

[21] Rodrigues-dos Santos, A. S., Rebelo-Romão, I., Zhang, H., & Vílchez, J. I. (2022). Discerning transcriptomic and biochemical responses of *Arabidopsis thaliana* treated with the biofertilizer strain *Priestia megaterium* YC4-R4: Boosting plant central and secondary metabolism. *Plants, 11*(22), 3039. https://doi.org/10.3390/plants11223039

[22] Greenway, R., Barts, N., Henpita, C., Brown, A. P., Arias Rodriguez, L., Rodríguez Peña, C. M., & Tobler, M. (2020). Convergent evolution of conserved mitochondrial pathways underlies repeated adaptation to extreme environments. *Proceedings of the National Academy of Sciences, 117*(28), 16424-16430. https://doi.org/10.1111/jeb.13727

[23] Kushkevych, I., Procházka, V., Vítězová, M., Dordević, D., Abd El-Salam, M., & Rittmann, S. K. (2024). Anoxygenic photosynthesis with emphasis on green sulfur bacteria and a perspective for hydrogen sulfide detoxification of anoxic environments. *Frontiers in Microbiology, 15*, 1417714. https://doi.org/10.3389/fmicb.2024.1417714

[24] Birkeland, N. K. (2005). Sulfate-reducing bacteria and archaea. In *Petroleum Microbiology* (pp. 35-54). https://doi.org/10.1128/9781555817589.ch3

[25] Murthy, S., Govardhana, M., Satyan, K. B., & Sharma, G. (2024). Whole genome sequencing and comparative genomic studies of *Priestia filamentosa* JURBA-X for its drought-tolerance, plant-growth promotion, and fluorescent characteristics. *bioRxiv*, 2024-04. https://doi.org/10.1101/2024.04.09.588649

[26] Saravanan, A., Kumar, P. S., Vo, D. V., Jeevanantham, S., Karishma, S., & Yaashikaa, P. R. (2021). A review on catalytic-enzyme degradation of toxic environmental pollutants: Microbial enzymes. *Journal of Hazardous Materials, 419*, 126451. https://doi.org/10.1016/j.jhazmat.2021.126451

[27] Medaura, M. C., Guivernau, M., Moreno-Ventas, X., Prenafeta-Boldú, F. X., & Viñas, M. (2021). Bioaugmentation of native fungi, an efficient strategy for the bioremediation of an aged industrially polluted soil with heavy hydrocarbons. *Frontiers in Microbiology, 12*, 626436. https://doi.org/10.3389/fmicb.2021.626436

[28] Ren, W., Zhong, Y., Ding, Y., Wu, Y., Xu, X., & Zhou, P. (2022). Mismatches in 16S rRNA gene primers: An area worth further exploring. *Frontiers in Microbiology*, 13, 888803. https://doi.org/10.3389/fmicb.2022.888803

[29] Hügler, M., Gärtner, A., & Imhoff, J. F. (2010). Functional genes as markers for sulfur cycling and CO_2 fixation in microbial communities of hydrothermal vents of the

Logatchev field. *FEMS Microbiology Ecology*, *73*(3), 526-537. https://doi.org/10.1111/j.1574-6941.2010.00919.x

[30] Wang, R., Lin, J. Q., Liu, X. M., Pang, X., Zhang, C. J., Yang, C. L., & Chen, L. X. (2019). Sulfur oxidation in the acidophilic autotrophic *Acidithiobacillus* spp. *Frontiers in Microbiology, 9*, 3290. https://doi.org/10.3389/fmicb.2018.03290

[31] Lin, X., Feng, X., Wang, X., Long, H., Crowe, S. A., & Luo, H. (2024). Sulfur oxidation through rDsr in a novel Sox-free marine *Roseobacter* lineage. *BioRxiv*, 2024-06. https://doi.org/10.1101/2024.06.14.599071

[32] Hügler, M., Gärtner, A., & Imhoff, J. F. (2010). Functional genes as markers for sulfur cycling and CO2 fixation in microbial communities of hydrothermal vents of the Logatchev field. *FEMS Microbiology Ecology, 73*(3), 526-537. https://doi.org/10.1111/j.1574-6941.2010.00919.x

[33] Jørgensen, B. B., Findlay, A. J., & Pellerin, A. (2019). The biogeochemical sulfur cycle of marine sediments. *Frontiers in Microbiology, 10*, 849. https://doi.org/10.3389/fmicb.2019.00849

[34] Zhuang, X., Wang, S., & Wu, S. (2024). Electron transfer in the biogeochemical sulfur cycle. *Life, 14*(5), 591. https://doi.org/10.3390/life14050591

[35] Boonyawanich, S., Prommeenate, P., Oaew, S., Pisutpaisal, N., & Haosagul, S. (2024). Hydrogen sulfide oxidizing microbiome in biogas-stream fed biofilter in palm oil factory. *Nature Environment and Pollution Technology, 23*(2). https://doi.org/10.46488/nept.2024.v23i02.017

[36] Seele, P., & Lock, I. (2017). The game-changing potential of digitalization for sustainability: Possibilities, perils, and pathways. *Sustainability Science, 12*, 183-185. https://doi.org/10.1007/s11625-017-0426-4

[37] Johnson, J. S., Spakowicz, D. J., Hong, B. Y., Petersen, L. M., Demkowicz, P., Chen, L., & Weinstock, G. M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications, 10*(1), 5029. https://doi.org/10.1038/s41467-019-13036-1

[38] Eamsakulrat, P., Santanirand, P., & Phuphuakrat, A. (2022). Diagnostic yield and impact on antimicrobial management of 16S rRNA testing of clinical specimens. *Microbiology Spectrum, 10*(6), e02094-22. https://doi.org/10.1128/spectrum.02094-22

[39] Sato, M., & Miyazaki, K. (2017). Phylogenetic network analysis revealed the occurrence of horizontal gene transfer of 16S rRNA in the genus *Enterobacter*. *Frontiers in Microbiology, 8*, 2225. https://doi.org/10.3389/fmicb.2017.02225

[40] Nair, S. R., & Wen, J. (2019). Uncertainties in sour natural gas dispersion modelling. *Chemical Engineering Transactions, 77*. https://doi.org/10.3303/CET1977060

[41] Sherar, B. W., Ellis, P., & Ning, J. (2021). Improved high-pressure, high-temperature (HPHT) materials qualification using dissolved H2S concentration as the sour service scalable metric. *Corrosion, 77*(11), 1218-1232. https://doi.org/10.5006/3867

[42] Peighami, R., Rasekh, B., Motamedian, E., Yazdian, F., & Khodaverdi, H. (2022). Investigating the ability of sulfur oxidizing strains in biodesulfurisation of sulfide-containing streams, screening the most capable strain and determining the optimum condition for elemental sulfur recovery from sulfide using response surface method. *Fuel, 309*, 121985. https://doi.org/10.1016/j.fuel.2021.121985

[43] Canfield, D. E., Stewart, F. J., Thamdrup, B., De Brabandere, L., Dalsgaard, T., Delong, E. F., & Ulloa, O. (2010). A cryptic sulfur cycle in oxygen-minimum-zone waters off the Chilean coast. *Science, 330*(6009), 1375-1378. https://doi.org/10.1126/science.1196889

[44] Pourbabaee, A. A., Koohbori Dinekaboodi, S., Seyed Hosseini, H. M., Alikhani, H. A., & Emami, S. (2020). Potential application of selected sulfur-oxidizing bacteria and different sources of sulfur in plant growth promotion under different moisture conditions.

Communications in Soil Science and Plant Analysis, 51(6), 735-745. https://doi.org/10.1080/00103624.2020.1729377

[45] Zhang, Y., Wang, X., Zhen, Y., Mi, T., He, H., & Yu, Z. (2017). Microbial diversity and community structure of sulfate-reducing and sulfur-oxidizing bacteria in sediment cores from the East China Sea. *Frontiers in Microbiology, 8*, 2133. https://doi.org/10.3389/fmicb.2017.02133

[46] Tian, H., Gao, P., Chen, Z., Li, Y., Li, Y., & Wang, Y. (2017). Compositions and abundances of sulfate-reducing and sulfur-oxidizing microorganisms in water-flooded petroleum reservoirs with different temperatures in China. *Frontiers in Microbiology, 8*, 143. https://doi.org/10.3389/fmicb.2017.00143

[47] Satam, H., Joshi, K., Mangrolia, U., Waghoo, S., Zaidi, G., Rawool, S., & Malonia, S. K. (2023). Next-generation sequencing technology: current trends and advancements. *Biology, 12*(7), 997. https://doi.org/10.3390/biology12070997

How to cite this paper:

Karimi, T., Pourahmad, F. & Noorollahi, E. (2025). Isolation and characterization of sulfur-oxidizing bacteria at the Ilam gas refinery in Iran. Advances in Environmental Technology, 11(4), 414-425. DOI: 10.22104/aet.2025.7467.2081