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# Bioremediation of non-point hydrogen sulfide emissions using bacterial cellulose/activated carbon membrane

Mingbo Yang[1†], Yan Zhang[1†], Xueqing Zhao[1], Ge Gao[1], Yucheng Shi[1], Yifan Wang[1], Mengyue Duan[1], Ziye Guo[1], Xiaodong Ma[3], Ting Ma[1,2*] and Guoqiang Li[1,2*]

## Abstract

**Background** Hydrogen sulfide ($H_2S$) gas, characterized by its low odor threshold and toxicity, poses significant challenges in non-point source odor management. Traditional biotechnologies are effective in removing malodorous gases from point sources but they are limited for non-point source odor control.

**Results** In this study, the *sqr* and *pdo* genes from *Cupriavidus pinatubonensis* JMP134 were introduced into the bacterial cellulose-producing strain *Kosakonia oryzendophytica* FY-07. This genetic modification enhanced the strain's sulfur oxidation capacity, which increased over time, with an average transformation capacity of approximately 275 $mg{\cdot}L^{-1}{\cdot}day^{-1}$. By incorporating 1% activated carbon, an efficient, naturally degradable bio-composite membrane was developed, achieving a maximum $H_2S$ adsorption capacity of 7.3 $g{\cdot}m^{-3}{\cdot}day^{-1}$. FY-07 remained stable in soil and improved the microbial community for $H_2S$ treatment.

**Conclusion** The resulting bio-composite membrane is environment-friendly and efficient, making it suitable for emergency odor control in landfills. This study offers recommendations for using membrane materials in managing non-point hydrogen sulfide emissions.

**Keywords** $H_2S$ gas, Bacterial cellulose, $H_2S$ adsorption, Natural degradation, Emergency odor control

## Introduction

In recent years, the global generation of municipal solid waste (MSW) has surged, reaching approximately 2.1 billion tons annually [1]. Due to its cost-effectiveness and operational simplicity, landfill disposal remains the most widely adopted method for global urban solid waste management [2]. However, the decomposition of organic matter in landfills releases malodorous gases, which pose significant health risks to both workers and residents in nearby areas [3]. Among these gases, hydrogen sulfide ($H_2S$) is a primary contributor to the malodor. Being denser than air, $H_2S$ tends to accumulate in poorly ventilated spaces, increasing the risk of poisoning

†Mingbo Yang and Yan Zhang contributed equally to this article and are co-first authors.

*Correspondence:
Ting Ma
tingma@nankai.edu.cn
Guoqiang Li
gqli@nankai.edu.cn
[1]Key Laboratory of Molecular Microbiology and Technology, College of Life Sciences, Ministry of Education, Nankai University, Tianjin 300071, China
[2]Tianjin Engineering Technology Center of Green Manufacturing Biobased Materials, Tianjin 300071, China
[3]School of Energy and Environmental Engineering, Hebei University of Technology, Tianjin 300401, China

due to its extremely low odor threshold (approximately 1.83 μg·m$^{-3}$) and high toxicity [4, 5].

Conventional treatment methods such as scrubbers, photocatalytic oxidation, ozone oxidation, and biofilters are not suitable for landfill odor management due to the large area and non-point source characteristics of $H_2S$ emissions [6]. Thus, landfill cover layers are considered an effective method to reduce the release of odorous gases [7]. Qin et al. reported sludge-modified biochar achieving 95.43-100% $H_2S$ removal efficiency, representing a four-fold enhancement over conventional landfill soil [8]. He et al. reported that both waste biocover soil and landfill cover soil achieved $H_2S$ removal rates exceeding 90%, with faster biotransformation rates of sulfides to sulfates in waste biocover soil compared to landfill cover soil [6]. Chen et al. further demonstrated the potential of bio-stabilized leachate-modified, showing 1.25-fold increased $H_2S$ removal capacity (1.74 μmol $g^{-1}$ $h^{-1}$) compared to untreated loess [9]. Although these cover materials effectively remove $H_2S$ emissions, their material sources are not easily accessible and the time required for microbial colonization make them unsuitable for large-scale landfill applications. Systematic evaluation of odor treatment approaches (Table 1) highlights the strategic advantages of biofilm-based systems. Therefore, developing a biofilm materials for the removal of $H_2S$ is urgent. Zhao et al. prepared a bacterial cellulose/bentonite/polyethyleneimine bio-composite membrane material for wastewater treatment. In wastewater environments containing coexisting dyes and metal ions, such as those from textiles, coatings, agricultural wastewater, and household laundry, the membrane improved overall water treatment efficiency and safety by effectively adsorbing pollutants [10]. This demonstrates the excellent performance of bacterial cellulose composite materials in environmental applications. Bio-composite membrane materials can be flexibly scaled up or down according to treatment requirements, making them suitable for various environments. Additionally, the modular design enables rapid assembly and transformation of the biofilm materials, significantly reducing the treatment cycle. Collectively, these advanced features position bio-composite membrane materials as a technologically advantageous solution for sustainable environmental management.

$H_2S$ treatment by cover materials involves two steps: $H_2S$ adsorption onto a solid medium, followed by microbial biodegradation of pollutants within the material [11]. Microorganisms play a crucial role in this biodegradation and gas deodorization process [12]. In landfill $H_2S$ management, sulfide-oxidizing bacteria (SOB) are essential for the biological oxidation of sulfides and intermediate sulfur compounds [5]. The Sulfide: Quinone Oxidoreductase (SQR) and Persulfide Dioxygenase (PDO) oxidation system and Sox system are two primary pathways in SOB for oxidizing $H_2S$. SQR, which relies on the cofactor Flavin Adenine Dinucleotide (FAD), oxidizes $H_2S$ to polysulfides, which are then further converted by PDO to sulfite and thiosulfate [13–15]. The Sox system, a multifunctional enzyme system, is capable of oxidizing a range of reduced sulfur compounds, including sulfides, elemental sulfur, and sulfite, into sulfate. However, the Sox system must be coupled with the flavocytochrome c sulfide dehydrogenase (FCSD) system to effectively oxidize $H_2S$, and it is typically most effective at low concentrations of $H_2S$ [16, 17]. Thus, the SQR/PDO oxidation system is considered the primary oxidation pathway in most cases and plays a significant role in $H_2S$ management strategies [18].

Bacterial cellulose (BC) is an extracellular polymer synthesized by specific bacteria, characterized by high mechanical strength, excellent water retention, a three-dimensional nano-network structure, and biodegradability. BC holds significant potential as a landfill cover material [19]. Traditional cellulose-producing bacteria, such as *Komagataeibacter*, *Azotobacter*, *Pseudomonas*, *Salmonella*, *Sarcina*, and *Agrobacterium*, are aerobic and typically require oxygenated conditions for fermentation. In contrast, *K. oryzendophytica* FY-07 is currently the only reported strain that can produce BC under various oxygen conditions. This strain offers advantages such as rapid production, strong environmental competitiveness and broad substrate utilization. Its unique deep fermentation mode provides notable benefits, including enhanced uniformity and modification degree for in-situ BC modification [20–22]. However, FY-07 has limited sulfur oxidation ability. Thus enhancing its sulfur oxidation capability and improving the performance of the BC

**Table 1** Comparison of the characteristics of biocomposite film materials, biofilters, and Biochar

| Feature | Biocomposite Film Materials | Biofilters | Biochar |
|---|---|---|---|
| Adsorption Capacity (mg/g) | 0.8-2.0 | 0.3-1.0 | 1.5–3.5 |
| Removal Efficiency | 70-95% | 50-85% | 60-90% |
| Application Range | Suitable for both low and high concentrations of hydrogen sulfide removal | Suitable for treating hydrogen sulfide in low concentration waste gases | Primarily used for adsorbing high concentration hydrogen sulfide gas |
| Environmental Impact | Biodegradable, no secondary pollution | Filter material treatment may cause secondary pollution | Biochar treatment may generate waste materials |

it produces would be advantageous for controlling the odor of landfill gas.

Freshwater species of the genus *Beggiatoa*, such as *B. leptomitoformis* and *B. alba*, typically inhabit environments without a constant supply of hydrogen sulfide [23]. A PDO from the Gram-positive bacterium *Staphylococcus aureus* utilizes low-molecular-weight persulfides (RSSH and RSS-), rather than GSSH, as substrates [24]. Furthermore, some bacteria lack a complete enzymatic system, possessing only SQR or PDO, and thus rely on cooperative interactions to oxidize sulfide to thiosulfate [15]. In *Cupriavidus pinatubonensis* JMP134, the *sqr* and *pdo* genes are located adjacently on the chromosome, facilitating genetic manipulation. Compared to other strains, *C. pinatubonensis* JMP134 exhibits unique metabolic capabilities and superior enzymatic activity [25]. The SQR encoded by the *sqr* gene in *C. pinatubonensis* JMP134 demonstrates higher catalytic efficiency and stability than those of most microorganisms, while the PDO encoded by the *pdo* gene shows broad substrate specificity and efficient degradation capabilities. Additionally, the complete genome and well-characterized metabolic pathways of *C. pinatubonensis* JMP134 make it an excellent model for studying sulfur oxidation, with significant potential for applications in environmental remediation and industrial processes [26].

In this study, we aim to enhance the sulfur oxidation capacity of FY-07 and combine it with in-situ modification strategies to significantly improve $H_2S$ treatment, enabling its application in gas treatment at landfills. The *sqr* and *pdo* genes from *C. pinatubonensis* JMP134 were introduced into FY-07 to confer sulfur oxidation capabilities [27]. The engineered strain was then inoculated into a fermentation medium containing activated carbon (AC) for in-situ modification of BC, resulting in a novel bio-composite membrane for in-situ $H_2S$ degradation. Compared to existing methods, this bio-composite membrane material can form films directly on soil without the need for extensive equipment. The engineered strains not only produce membrane materials for $H_2S$ adsorption but also oxidize the adsorbed $H_2S$ into other harmless sulfides. Moreover, the produced composite membrane material is naturally degradable, eliminating the need for additional post-treatment. The $H_2S$ removal capability of the composite membrane was evaluated, and the microbial community composition during the remove process was analyzed, providing valuable insights into $H_2S$ odor control in landfill environments.

## Results and discussion

### Construction and characterization of engineered strain

FY-07 can rapidly produce BC, which efficiently blocks $H_2S$ release. However, it lacks an intrinsic $H_2S$ metabolic pathway. To address this, we introduced the SQR/PDO oxidation system from *C. pinatubonensis* JMP134 into FY-07, generating the FY-07 pBBR1-MCS2-*sqrpdo2* strain. Figure 1A illustrates the metabolic pathway for hydrogen sulfide oxidation in the SQR/PDO system. The engineered strain and the wild-type strain were inoculated in hydrogen sulfide production medium. After 15 days of fermentation at 30 °C, $H_2S$ production was detected using lead acetate paper. As shown in Fig. 1B, the wild-type strain produced significant levels of $H_2S$, whereas no $H_2S$ production was detected in the engineered strain. This result indicates that the introduced SQR/PDO system successfully enhances sulfur oxidation in FY-07. Furthermore, FY-07 pBBR1-MCS2-*sqrpdo2* exhibited growth and cellulose production levels comparable to the wild-type, suggesting that the SQR/PDO system did not affect the growth of the strain and its ability to produce bacterial cellulose. (Fig. 1C, D). Therefore, the construction of the FY-07 pBBR1-MCS2-*sqrpdo2* strain was in line with the expected design.

To further characterize the sulfur-oxidizing ability of the FY-07 pBBR1-MCS2-*sqrpdo2* strain, a sulfur oxidation experiment was conducted in anaerobic bottles. Air was introduced at 6-hours and 12-hours intervals to simulate low-oxygen conditions, which are necessary for the sulfur oxidation system to function and to evaluate the $H_2S$ degradation efficiency under reduced oxygen levels. As shown in Fig. 2A, the $H_2S$ concentration in the experimental group (85.3 $mg{\cdot}m^{-3}$) was significantly reduced by 17.4-fold compared to the control group (1480.7 $mg{\cdot}m^{-3}$) after 24-hours of inoculation. This indicated that FY-07 pBBR1-MCS2-*sqrpdo2* exhibits much stronger $H_2S$ degradation capability than the wild-type strain.

SQR in the cytoplasm oxidizes $H_2S$ to polysulfides and transfers them to a receptor. Glutathione (GSH) can react with these polysulfides to form persulfide glutathione (GSSH and GSS-) [15]. PDO then oxidizes GSSH to sulfite, which spontaneously reacts with polysulfides to form thiosulfate, the final accumulated product (Fig. 1A) [28]. The reduced $S^{2-}$ may also be converted into polysulfides, persulfide glutathione, and other sulfur species within microbial cells. Consequently, the concentrations of $SO_3^{2-}$ and $S_2O_3^{2-}$ in the solutions were measured. As shown in Fig. 2B and C, the concentrations of $SO_3^{2-}$ and $S_2O_3^{2-}$ in the experimental group (0.179 $mg{\cdot}L^{-1}$ and 138.9 $mg{\cdot}L^{-1}$) were significantly higher than those in the control group (0.110 $mg{\cdot}L^{-1}$ and 112.4 $mg{\cdot}L^{-1}$). The lower $SO_3^{2-}$ levels suggest it is an intermediate product, while the higher $S_2O_3^{2-}$ concentration indicates that the FY-07 pBBR1-MCS2-*sqrpdo2* strain has a much stronger sulfur-oxidizing ability.

These results demonstrated the feasibility of introducing the SQR/PDO oxidation system from *C. pinatubonensis* JMP134 into *K. oryzendophytica* FY-07. Furthermore, bacteria containing both SQR and PDO

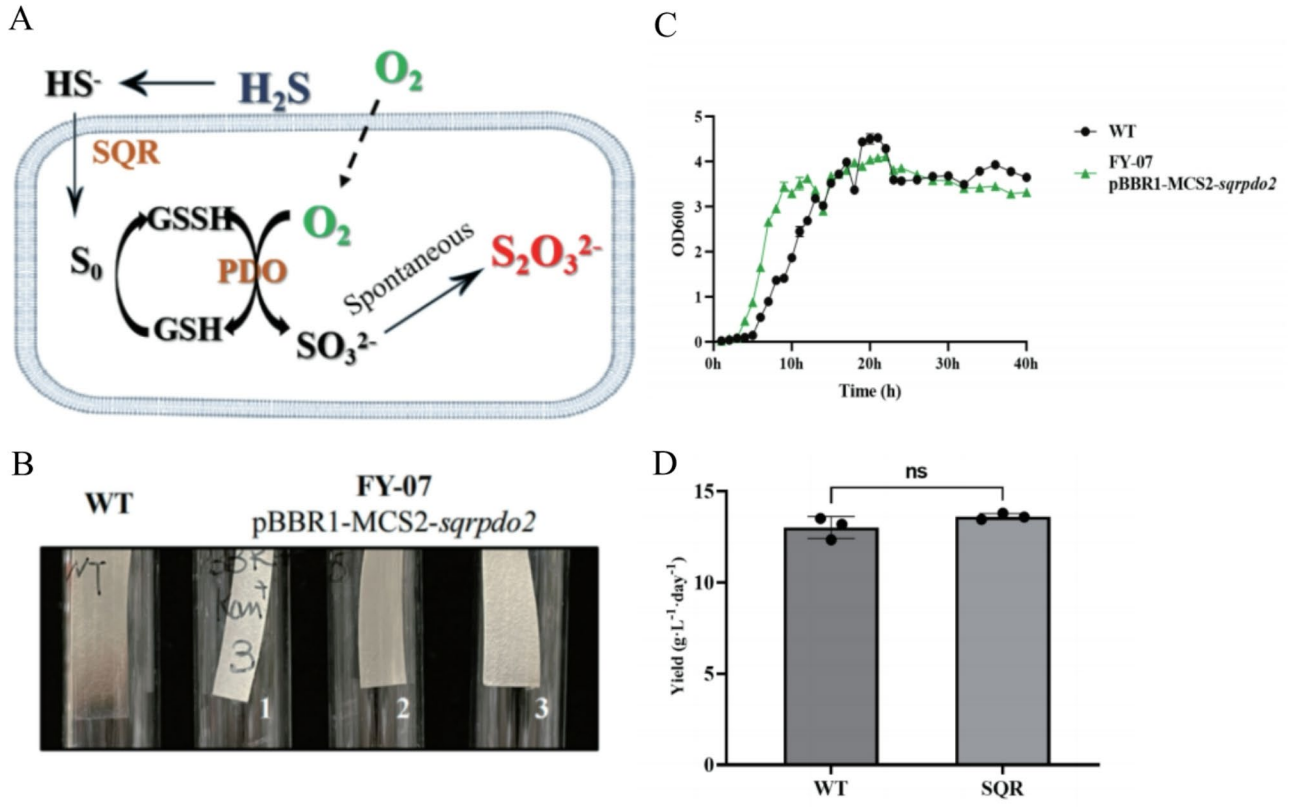


**Fig. 1** The $H_2S$ oxidation pathway mediated by the SQR/PDO oxidation system in microorganisms. (**A**), Characterization of FY-07 wild-type and FY-07 pBBR1-MCS2-sqrpdo2 on lead acetate test paper (**B**), Growth curves of FY-07 wild-type and FY-07 pBBR1-MCS2-*sqrpdo2* (**C**) and Comparison of bacterial cellulose production between FY-07 wild-type and FY-07 pBBR1-MCS2-*sqrpdo2* (**D**)

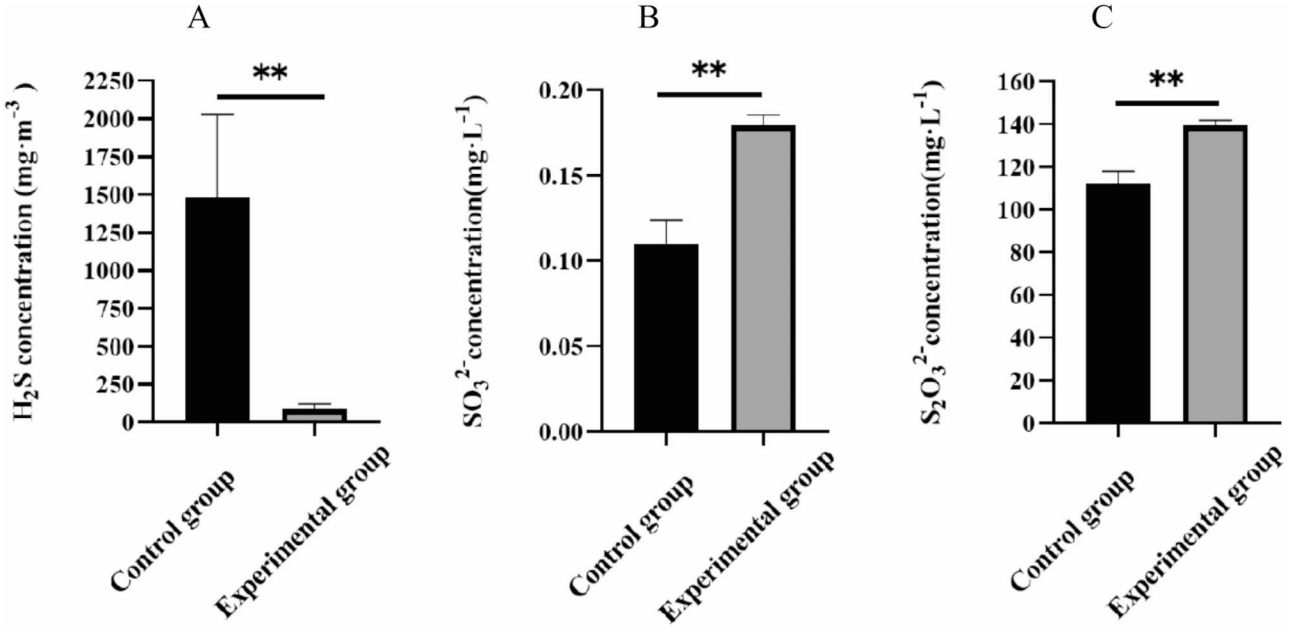


**Fig. 2** Degradation efficiency of $H_2S$ (**A**) and production rates of $SO_3^{2-}$ (**B**) and $S_2O_3^{2-}$ (**C**) under microaerobic conditions for FY-07 wild-type and FY-07 pBBR1-MCS2-*sqrpdo2*.**$p < 0.01$

preferentially convert sulfide into thiosulfate, which contains two sulfur atoms, producing less acid compared to sulfite or sulfate as the final product [25]. This results in a more alkaline solution, which promotes the growth and BC production of FY-07.

#### Evaluation of composite membrane materials

The novel bio-composite membrane materials were developed by inoculating the engineered strain FY-07 pBBR1-MCS2-*sqrpdo2*, which has enhanced sulfur oxidation capabilities, into a fermentation medium containing activated carbon for in-situ modification. These membrane materials exhibited significant $H_2S$ adsorption capacity, with adsorption levels positively correlating to the amount of activated carbon (AC) particles added in the fermentation medium. At an AC particle addition of 1%, the composite membrane was able to completely absorb hydrogen sulfide released from a 10 mL solution of 12.8 mM $Na_2S$ (pH = 6.0) (Fig S1). The maximum adsorption capacity reaches 7.3 $g{\cdot}m^{-3}{\cdot}day^{-1}$, surpassing the volumetric adsorption capacity of standalone AC particles and BC alone, with a rapid adsorption rate observed within 24 h. This remarkable adsorption capacity is attributed to the unique properties of the materials. BC has excellent water-holding capacity, forming a water film on its surface. As $H_2S$ is absorbed by the water film, it dissociates into $HS^-$, reducing the transfer rate of $H_2S$ from the water film to the gas phase. Combined with activated carbon adsorption, the BC/AC composite membrane effectively enhances $H_2S$ treatment efficiency, achieving purification levels of up to 100% [6].

The bio-composite membrane should be maintained within a temperature range of 20–40 °C during the first day to ensure the engineered strain FY-07 pBBR1-MCS2-*sqrpdo2* produces bacterial cellulose. After this initial period, temperature no longer becomes a limiting factor. However, if the temperature exceeds 35 °C or the relative humidity drops below 20% RH, the membrane may lose moisture, leading to reduction in its adsorption and conversion capabilities. In such cases, water should be sprayed to maintain the bio-membrane moist. Other odorous gases in the air may also affect the membrane's ability to adsorb $H_2S$. However, due to the strain's sulfur oxidation abilities, it can rapidly oxidize the adsorbed $H_2S$, reducing the $H_2S$ concentration in odorous areas below the olfactory threshold. As shown in Figure S2, the bio-composite membrane can form on brick soil, humus soil, and desiccated soil, demonstrating a broad application range of potential applications under various soil conditions. Furthermore, the bio-composite membrane is capable of self-degradation without maintenance (Figure S3).

Under industrial conditions, $H_2S$ levels can fluctuate dramatically, leading to toxic effects on microbial communities and hindering mass transfer. To evaluate the membrane material's ability to handle sudden increases in malodorous environments, we simulated such conditions by adding 10 mL and 20 mL of 12.8 mM $Na_2S$ at 48 and 72 h, respectively. The concentrations of $H_2S$ within the bottles were measured 24 h after each addition. As shown in Fig. 3A, the concentration of $H_2S$ in the experimental group did not increase significantly when $Na_2S$ was added at different time points. The results indicated that the BC/AC bio-composite membrane material performed excellent well in adsorbing $H_2S$ from malodorous environments. Although some fluctuations occurred as $H_2S$ levels increased, the treatment efficiency remained nearly 100% in the short term, with a maximum degradation rate of 35 $mg{\cdot}L^{-1}{\cdot}h^{-1}$. The material demonstrated

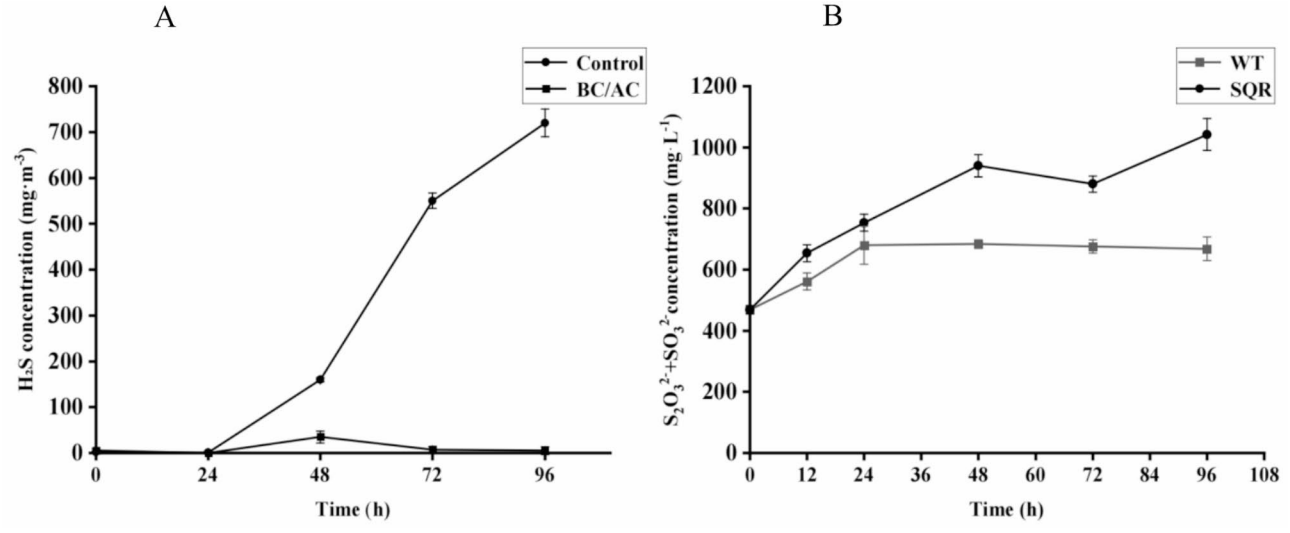


**Fig. 3** Effectiveness of Composite Membranes in $H_2S$ Gas Removal (**A**) and Sulfur Compound Generation (**B**) under $H_2S$ Shock Conditions

significant resilience to shock loads, with a 1% activated carbon addition showing remarkable stability.

The accumulation of $SO_3^{2-}$and $S_2O_3^{2-}$ was also measured after grinding, washing, and dilution the BC/AC bio-composite membrane. As shown in Fig. 3B, the composite membrane prepared with the wild-type FY-07 strain was able to oxidize some of the $H_2S$, likely due to oxidation reactions occurring within the micropores of the activated carbon or the wild-type FY-07 utilizing $H_2S$ as a sulfur source. In contrast, the engineered strain FY-07 pBBR1-MCS2-*sqrpdo2* exhibited a significantly higher sulfur oxidation capacity, which increased with longer cultivation times, with an average conversion rate of approximately 275 $mg{\cdot}L^{-1}{\cdot}day^{-1}$. This strain's enhanced sulfur oxidation ability makes it more suitable for emergency treatment of $H_2S$ gas compared to modified activated carbon sludge and activated carbon-amended soil, as it offers faster startup times without the need for extensive pre-treatment [8, 29]. Unlike biotrickling filters used for hydrogen sulfide removal, this composite membrane does not require additional large equipment [30]. Furthermore, compared to composting materials and landfill biocover materials, the biocomposite membrane naturally degrades in the soil without the need for post-treatment, offering the advantage of ease of use [31, 32]. These results demonstrate the significant potential of the BC/AC bio-composite membrane in the treatment of $H_2S$.

#### Microcosm experiments

Building on the laboratory-scale demonstration of the membrane's ability to treat $H_2S$, we further evaluated its effectiveness in a simulated landfill environment using microcosm experiments. These experiments were designed to assess the $H_2S$ treatment capacity of BC/AC bio-composite membrane under simulated landfill conditions. During fermentation of the engineered FY-07 pBBR1-MCS2-*sqrpdo2* strain in a medium containing 1% activated carbon (XGK/AC), a 1 cm thick membrane composite formed on the microcosm, with activated carbon particles uniformly embedded within the membrane (Fig. 4A). This composite material created a biohybrid material consisting of BC, activated carbon, and soil, with the upper layer consisting of BC and activated carbon, while the lower layer was a BC and soil composite membrane (Fig. 4B).

In the assembled container model, periodic sampling of gas samples from both ends revealed no detectable release of $H_2S$ from the outlet over a 5-day monitoring period, achieving a complete degradation efficiency of 100% (Fig. 5A). To quantify the microbial content of *K. oryzendophytica* FY-07 within the samples, the gene AKI40_0895, known to be involved in BC production by FY-07, was selected as a marker gene for qPCR analysis. As shown in Fig. 5B, FY-07 was able to establish growth and maintain stability throughout the domestication process after being introduced into soil, demonstrating the strong adaptability and stability of the engineered strains in the environment. These results indicate that the BC/AC composite membrane material removes $H_2S$ primarily through chemical adsorption and microbial oxidation [33]. The medium for FY-07 pBBR1-MCS2-*sqrpdo2* is alkaline, which facilitates the adsorption of $H_2S$ by the activated carbon embedded in the biofilm through acid-base neutralization reactions. The strain's inherent sulfur oxidation capability further enhances the bio-composite

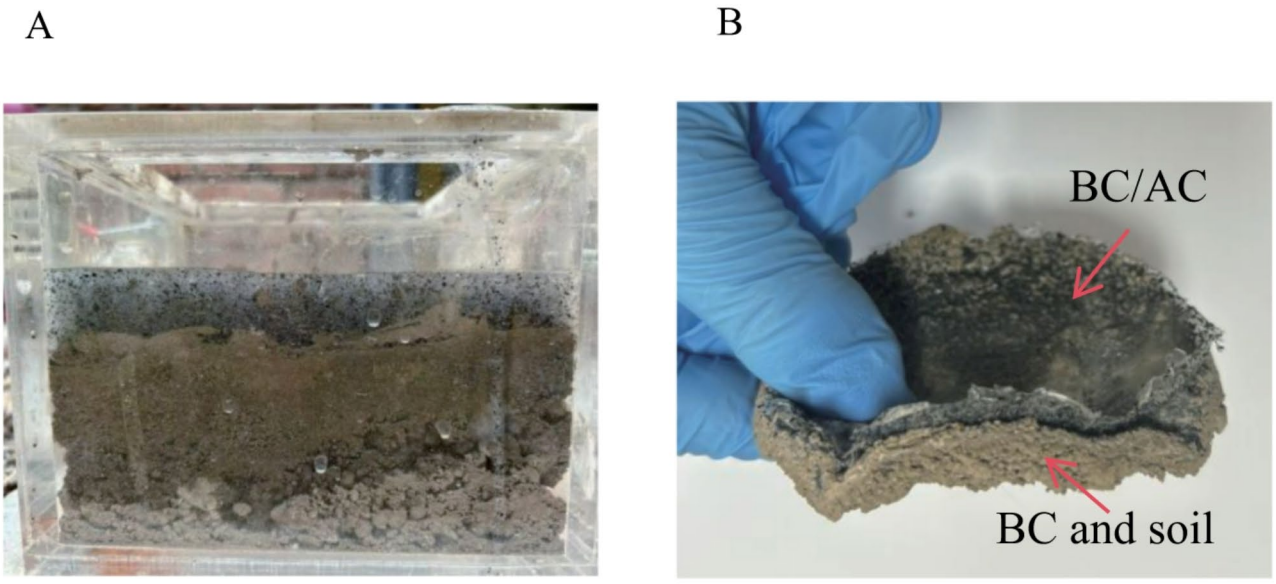


**Fig. 4** Formation (**A**) and Local State of Composite Membranes (**B**) in a Microcosm

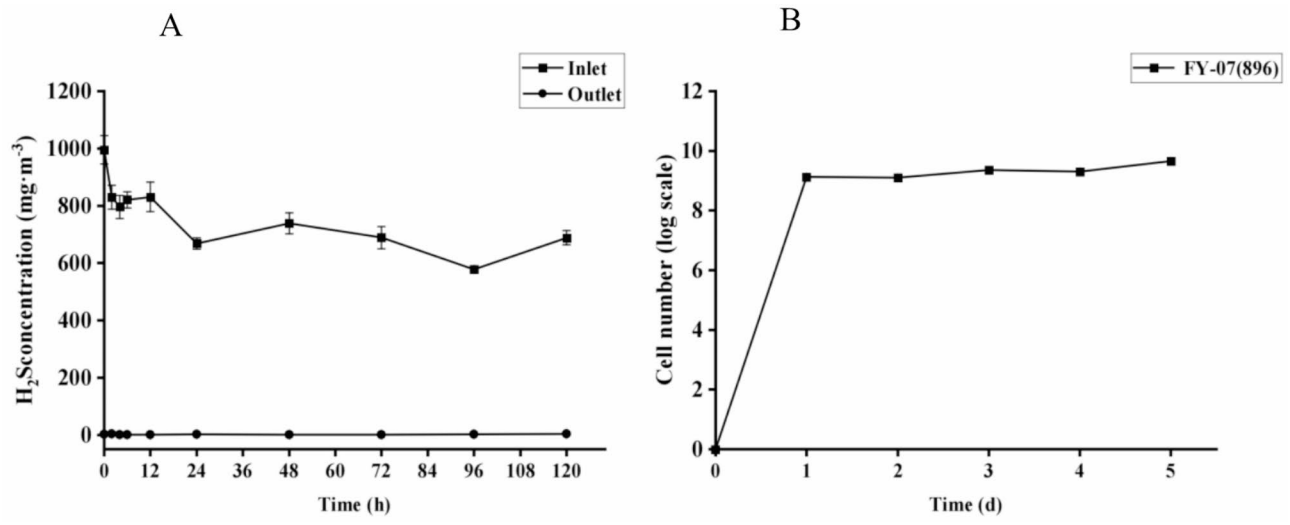


**Fig. 5** $H_2S$ Removal Efficiency in Microcosm Simulation (**A**) and Survival of FY-07 Strain in Soil (**B**)

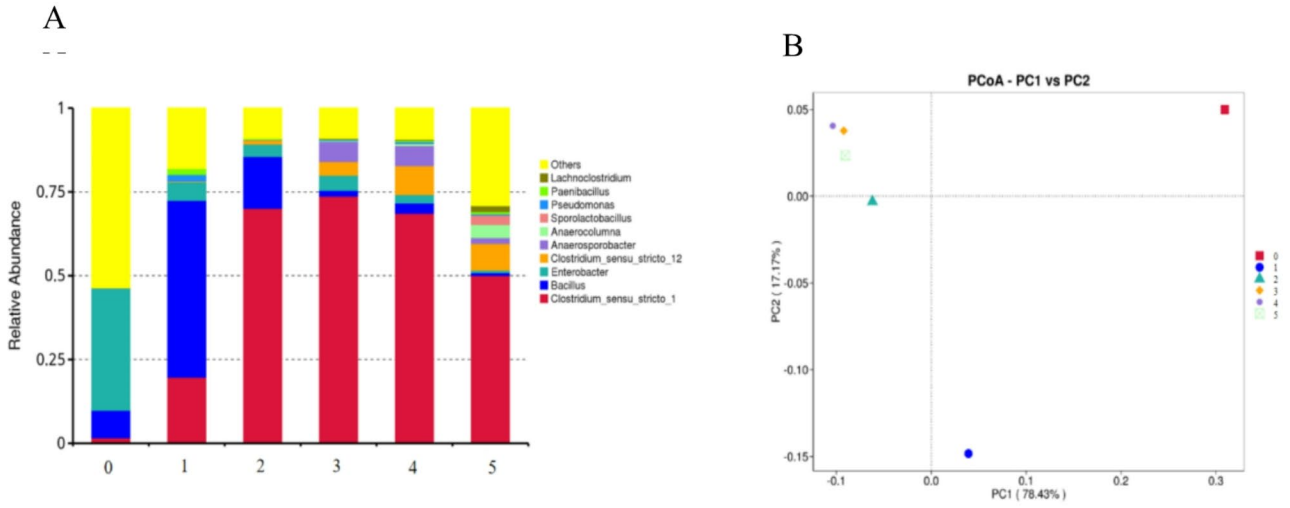


**Fig. 6** Genus-Level Community Analysis (**A**) and Principal Coordinate Analysis Based on Weighted UniFrac Distances (**B**)

membrane's capacity to treat $H_2S$. Additionally, it can work synergistically with soil sulfur-oxidizing bacteria to achieve a relatively high $H_2S$ removal efficiency in a short time.

### Analysis of microbial communities

Sampling is conducted daily in the microcosm experiment for 16s high-throughput sequencing to monitor changes in the relative abundance of microbial communities. As shown in Fig. 6A, the initial dominant genera in the soil film were *Enterobacter* (36.5%), *Bacillus* (8.2%), and *Clostridium_sensu_stricto_1* (1.7%), respectively. As the treatment period progressed, *Anaerosporobacter* gradually increased in abundance, reaching a peak of 6% on the fourth day, while *Clostridium_sensu_stricto_12* increased steadily throughout the process, eventually stabilizing. *Clostridium_sensu_stricto_1* rapidly enriched in the soil film and maintained absolute dominance throughout the entire treatment. On the fifth day, the top three bacterial genera in the soil film were *Clostridium_sensu_stricto_1* (50.1%), *Clostridium_sensu_stricto_12* (8.0%), and *Anaerocolumna* (3.9%), indicating significant changes in the prokaryotic communities during the composite membrane treatment. *Clostridium sensu stricto 12* and *Clostridium sensu stricto 1*, both belonging to the *Clostridiales* order, are widely recognized hydrolytic microorganisms capable of degrading complex organic compounds such as cellulose, starch, and proteins. These bacteria play crucial roles in microbial community interactions and balance in soil and aquatic ecosystems, contributing to ecosystem health and stability [34, 35].

Certain bacteria like *Clostridium sensu stricto 12* and *Anaerosporobacter* may be involved in sulfur cycling in the environment, participating in the transformation of

sulfur compounds [36, 37]. This potential application is significant for environmental remediation and pollution treatment. Principal Coordinate Analysis (PCoA) results show that the introduction of FY-07 pBBR1-MCS2-*sqrpdo2* alters the soil microbial community, stabilizing into a new community structure by the third, fourth, and fifth days. This new structure favors both bacterial cellulose and $H_2S$ gas degradation (Fig. 6B). This functional shift in the community structure enhanced its capability for specialized functions. In summary, these results indicate that the BC/AC composite membrane drives a shift in community structure towards one more favorable for $H_2S$ treatment.

## Conclusions

In this study, the SQR/PDO system was introduced into the BC producing strain *K. oryzendophytica* FY-07, resulting in the fabrication of a novel bio-composite membrane material through in-situ modification with activated carbon and xanthan gum. The bio-composite membrane exhibited excellent $H_2S$ adsorption and oxidation capabilities in both anaerobic bottles and simulated landfill facilities. Moreover, the engineered strain remained stable in the soil, altering the soil's microbial community structure to a state more favorable for $H_2S$ treatment, with stability achieved over time. Further optimization of microbial strains and culture medium formulations is needed to develop a high-efficiency and low-cost production process for cellulose composite membranes. The long-term stability and adaptability of engineered strains should be tested in real-world environments, such as landfill sites, and their potential ecological impacts must be assessed to ensure safety, facilitating the practical application of bacterial cellulose composite membrane materials. Despite the broad application potential of bacterial cellulose composite membranes, several challenges remain in their practical use. For instance, the adaptability and stability of engineered strains in complex real-world environments may be insufficient, necessitating further optimization of their environmental tolerance and functional stability. Additionally, the performance of membrane materials may degrade over time due to fouling, aging, or mechanical wear, underscoring the need for research to improve their anti-fouling properties and durability. To overcome these limitations and advance the practical application of bacterial cellulose composite membranes, the biodegradability and environmental compatibility of the materials must be studied to ensure they do not cause secondary pollution after use. By integrating nanotechnology, bioengineering, and materials science, multifunctional composite membrane materials with adsorption, catalytic, and mechanical stability can be developed. Research on the structure-performance relationship of membrane materials should be conducted to optimize their functional characteristics. Through optimization of production processes, development of multifunctional materials, and promotion of interdisciplinary collaboration, the large-scale application of this technology can be achieved in the future, offering efficient and sustainable solutions for environmental pollution control.

## Materials and methods

### Material

Glucose, yeast extract, tryptone, and anhydrous disodium phosphate were purchased from Oxoid Ltd (UK). *Kosakonia oryzendophytica* FY-07 (*Enterobacter sp.* FY-07, CGMCC No. 6103, FY-07) was cultured at 30 °C to produce bacterial cellulose (BC) [38]. Hydrogen Sulfide Production Medium: 1% tryptone, 1% beef extract, 0.5% NaCl, 0.05% cysteine, adjust the pH to 7.2 ~ 7.4. Hydrogen Sulfide Medium: 0.1% $Na_2S$, 0.2% $K_2HPO_4$, 0.2% $KH_2PO_4$, 0.04% $NH_4Cl$, 0.02% $MgCl_2{\cdot}7H_2O$, 0.001% $FeSO_4{\cdot}7H_2O$ [39]. XGK Medium: 0.75% yeast extract, 1% tryptone, 1% $Na_2HPO_4$, 2.5% glucose, 0.6% xanthan gum [40]. Activated carbon particles were purchased from Sinopharm Chemical Reagent Co., Ltd (China). Unless otherwise stated, all chemicals were of analytical grade.

### Construction of strain FY-07 pBBR1-MCS2-*sqrpdo2*

The SQR/PDO gene sequence of the *C. pinatubonensis* JMP134 strain was optimized and synthesized by Suzhou GENEWIZ Biotechnology Co., Ltd. The *sqrpdo2* fragment was then assembled with pBBR1-MCS2 using the MultiF Seamless Assembly Mix (ABclonal). The selected antibiotics were ampicillin (100 $\mu g{\cdot}mL^{-1}$) and kanamycin (50 $\mu g{\cdot}mL^{-1}$). The recombinant plasmid was subsequently introduced into *K. oryzendophytica* FY-07 via the S17-mediated conjugation transfer method to construct the strain FY-07 pBBR1-MCS2-*sqrpdo2* [41]. The primers used in this study are listed in Table S1.

### Performance evaluation of the engineered strain

To initially evaluate the strain, both FY-07 pBBR1-MCS2-*sqrpdo2* and the wild-type strain were tested in test tubes containing a hydrogen sulfide-producing medium. Additionally, a bio-oxidation experiment of $S^{2-}$ was conducted to further assess the strain's capabilities. This experiment involved using 500 mL anaerobic bottles containing a hydrogen sulfide medium, with air introduced intermittently. One group served as the experimental group, while the other served as the control group. Each group consisted of three parallel experiments. In the reaction setup, the experimental group was inoculated with strain FY-07 pBBR1-MCS2-*sqrpdo2* at a 1% inoculation volume into the hydrogen sulfide medium, while the control group was inoculated with the wild-type strain at a 1% inoculation volume into the same medium. After

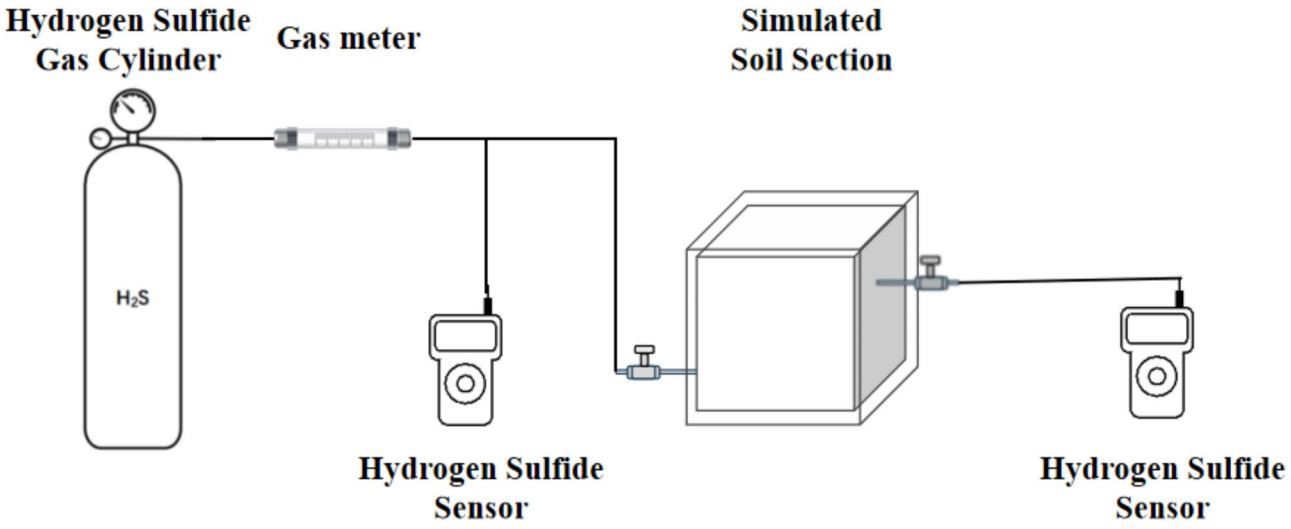


**Fig. 7** Diagram of Microcosm Experimental Setup (Hydrogen sulfide cylinders and flow meters control gas flow, while the simulation section mimics odorous gas environments in soil. Two sensors detect hydrogen sulfide concentration.)

24 h of inoculation, a handheld gas detector was used to measure the $H_2S$ gas in the anaerobic bottles, and fermentation liquid samples were centrifuged to obtain the supernatant. The concentrations of $S_2O_3^{2-}$ and $SO_3^{2-}$ in the samples were quantitatively analyzed using a Thermo Scientific ICS-1100 ion chromatograph equipped with a Dionex™ IonPac™ AS5 IC column. Results were obtained by comparison with standard concentration solutions, allowing for qualitative and quantitative calculations. Each group was tested in triplicate.

#### Construction of BC/AC composite membrane

The FY-07 pBBR1-MCS2-*sqrpdo2* strain was used for BC production in XGK medium. Initially, 0.6% xanthan gum (XG) was dissolved in the XGK medium (Figure S4) and continuously stirred for 30 min. Subsequently, various concentrations of AC (0.05%, 0.1%, 0.2%, 0.5% or 1%) were added and stirred until the medium became homogeneous, resulting in an activated carbon-containing fermentation medium.

For the preparation of the seed culture, FY-07 pBBR1-MCS2-*sqrpdo2* was cultured at 30 °C on slant medium (0.5% yeast extract, 1% tryptone, 0.5% NaCl, 1% glucose, and 1.5% agar, pH 7.2) for 24 h [42]. Single colonies were resuspended in 100 mL of 0.9% NaCl solution to form the seed culture. This seed culture was then inoculated at 2% into the various AC-containing fermentation media. The mixtures were fermented at room temperature for 24 h, resulting in the formation of BC/AC biocomposite membrane materials.

#### Performance evaluation of composite membrane materials

The preparation of the BC/AC composite membrane was carried out in 250 mL anaerobic bottles containing 50 mL of fermentation medium. After fermentation, 10 mL of 12.8 mM $Na_2S$ (pH = 6.0) was added to the BC/AC composite membrane material. A control bottle containing the same amount of sodium sulfide solution but without the BC/AC composite membrane was used for comparison. Both bottles were incubated at room temperature for 12 h. The residual $H_2S$ levels were measured using hydrogen sulfide gas detection tubes from the Beijing Labor Protection Institute.

Using the BC/AC composite membrane material as the medium, activated charcoal particles were added at an experimentally determined optimal dosage along with the optimal suspending agent. Experimental and control groups were established by inoculating 1 mL into 100 mL conical flasks containing 50 mL of fermentation medium. The cultures were statically incubated at 30 ℃. Samples were collected every 24 h, and the composite membrane was ground and soaked in distilled water. After thorough vortexing to elute the membrane components, the eluate was appropriately diluted. Quantitative measurement of sulfate ion content was performed using a Thermo Fisher ICS-1100 ion chromatograph to evaluate the membrane's effectiveness in $H_2S$ utilization.

#### Microcosm experiments

The simulation device consists of two parts: a simulated hydrogen sulfide ($H_2S$) release source and a simulated landfill site (Fig. 7). The $H_2S$ source consisted of a 40 L cylinder containing 830 $mg{\cdot}m^{-3}$ $H_2S/N_2$ mixture, purchased from Dongrun Gas Sales Co., Ltd. The simulated landfill site was constructed using custom rectangular acrylic containers, each with internal dimensions of 10 cm *10 cm* 9 cm, with 1 cm thick inner and outer layers. Each container was equipped with sampling ports on

opposite sides, a controllable inlet 3.5 cm from the bottom on one side, and a controllable outlet 7.5 cm from the bottom on the opposite side. The gas leak test was conducted to ensure the integrity of the setup, and then each container was filled with soil to a height of 6 cm. $H_2S$ gas was introduced at a flow rate of 5 mL·min$^{-1}$. Once the $H_2S$ concentration at the inlet and outlet stabilized to the same level, a 1 cm thick layer of the BC/AC composite membrane material was placed over the soil. The gas at both ends of the containers was monitored using a handheld gas detector for 5 consecutive days. The genomic DNA was extracted every 24 h from the soil-membrane interface. Part of the extracted DNA was used for fluorescent quantitative PCR to analyze the stability of FY-07 in the soil by detecting the FY-07-specific AKI40_0895 gene (sequence provided in Supplementary Information Table S2). The remaining DNA was used for High-throughput sequencing of 16 S rRNA genes by Beijing Novogene Co., Ltd.

## Supplementary Information

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Supplementary Material 1

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**Author contributions**
M.Y.and Y.Z.performed the experiments and wrote the main manuscript.G.L. and T.M. designed the experiments and modified the paper.X.Z.,G.G.and Y.S. assisted the experiments and modified the paper.Y.W. prepared Fig. 2 M.D. prepared Fig. 3. Z.G. analyzed the data. X.M. modified the paper. All authors reviewed the manuscript.

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**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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**References**

1. Yatoo AM, Hamid B, Sheikh TA, Ali S, Bhat SA, Ramola S, Ali MN, Baba ZA, Kumar S. Global perspective of municipal solid waste and landfill leachate: generation, composition, eco-toxicity, and sustainable management strategies. Environ Sci Pollut Res Int. 2024;31:23363–92.
2. Kaza S, Yao L, Bhada-Tata P, Woerden FV. What a waste 2.0: A global snapshot of solid waste management to 2050. Washington DC: The World Bank; 2018.
3. Kim K-H. Emissions of reduced sulfur compounds (RSC) as a landfill gas (LFG): A comparative study of young and old landfill facilities. Atmos Environ. 2006;40:6567–78.
4. Chan YH, Lock SSM, Wong MK, Yiin CL, Loy ACM, Cheah KW, Chai SYW, Li C, How BS, Chin BLF, et al. A state-of-the-art review on capture and separation of hazardous hydrogen sulfide ($H_2S$): recent advances, challenges and outlook. Environ Pollut. 2022;314:120219.
5. Fang Y, Du Y, Hu L, Xu J, Long Y, Shen D. Effects of sulfur-metabolizing bacterial community diversity on $H_2S$ emission behavior in landfills with different operation modes. Biodegradation. 2016;27:237–46.
6. He R, Xia FF, Bai Y, Wang J, Shen DS. Mechanism of $H_2S$ removal during landfill stabilization in waste biocover soil, an alterative landfill cover. J Hazard Mater. 2012;217–218:67–75.
7. Huang D, Du Y, Xu Q, Ko JH. Quantification and control of gaseous emissions from solid waste landfill surfaces. J Environ Manage. 2022;302:114001.
8. Qin L, Huang X, Xue Q, Liu L, Wan Y. *In-situ* biodegradation of harmful pollutants in landfill by sludge modified Biochar used as biocover. Environ Pollut. 2020;258:113710.
9. Chen J, Wang Y, Shao L, Lu F, Zhang H, He P. *In-situ* removal of odorous $NH_3$ and $H_2S$ by loess modified with biologically stabilized leachate. J Environ Manage. 2022;323:116248.
10. Zhao X, Yang M, Shi Y, Sun L, Zheng H, Wu M, Gao G, Ma T, Li G. Multifunctional bacterial cellulose-bentonite@polyethylenimine composite membranes for enhanced water treatment: sustainable dyes and metal ions adsorption and antibacterial properties. J Hazard Mater. 2024;477:135267.
11. Duan H, Yan R, Koe LC, Wang X. Combined effect of adsorption and biodegradation of biological activated carbon on $H_2S$ biotrickling filtration. Chemosphere. 2007;66:1684–91.
12. Zhu Q, Wu P, Chen B, Wu Q, Cao F, Wang H, Mei Y, Liang Y, Sun X, Chen Z. Improving $NH_3$ and $H_2S$ removal efficiency with pilot-scale biotrickling filter by co-immobilizing *Kosakonia oryzae* FB2-3 and *Acinetobacter baumannii* L5-4. Environ Sci Pollut Res Int. 2023;30:33181–94.
13. Holdorf MM, Owen HA, Lieber SR, Yuan L, Adams N, Dabney-Smith C, Makaroff CA. Arabidopsis ETHE1 encodes a sulfur dioxygenase that is essential for embryo and endosperm development. Plant Physiol. 2012;160:226–36.
14. Liu H, Xin Y, Xun L. Distribution, diversity, and activities of sulfur dioxygenases in heterotrophic bacteria. Appl Environ Microbiol. 2014;80:1799–806.
15. Xia Y, Lu C, Hou N, Xin Y, Liu J, Liu H, Xun L. Sulfide production and oxidation by heterotrophic bacteria under aerobic conditions. ISME J. 2017;11:2754–66.
16. Friedrich CG, Rother D, Bardischewsky F, Quentmeier A, Fischer J. Oxidation of reduced inorganic sulfur compounds by bacteria: emergence of a common mechanism? Appl Environ Microbiol. 2001;67:2873–82.
17. Xin Y, Gao R, Cui F, Lü C, Liu H, Liu H, Xia Y, Xun L. The heterotrophic bacterium *Cupriavidus pinatubonensis* JMP134 oxidizes sulfide to sulfate with thiosulfate as a key intermediate. Appl Environ Microbiol 2020, 86.
18. Reinartz M, Tschäpe J, Brüse T, Trüper H, Dahl C. Sulfide oxidation in the phototrophic sulfur bacterium Chromatium vinosum. Arch Microbiol. 1998;170:59–68.
19. Gao G, Liao Z, Cao Y, Zhang Y, Zhang Y, Wu M, Li G, Ma T. Highly efficient production of bacterial cellulose from corn stover total hydrolysate by *Enterobacter sp.* FY-07. Bioresour Technol. 2021;341:125781.
20. Gao G, Niu S, Liu T, Zhang Y, Zhao X, Shi Z, Chen S, Wu M, Li G, Ma T. Fabrication of bacterial cellulose composites with antimicrobial properties by modification utilizing the specific function-suspension containing water-insoluble Magnolol. Int J Biol Macromol 2023, 239.
21. Ma T, Ji K, Wang W, Wang J, Li Z, Ran H, Liu B, Li G. Cellulose synthesized by *Enterobacter sp.* FY-07 under aerobic and anaerobic conditions. Bioresour Technol. 2012;126:18–23.
22. Singhania RR, Patel AK, Tseng YS, Kumar V, Chen CW, Haldar D, Saini JK, Dong CD. Developments in bioprocess for bacterial cellulose production. Bioresour Technol. 2022;344:126343.
23. Rudenko TS, Trubitsina LI, Terentyev VV, Trubitsin IV, Borshchevskiy VI, Tishchenko SV, Gabdulkhakov AG, Leontievsky AA, Grabovich MY. Mechanism of intracellular elemental sulfur oxidation in *Beggiatoa leptomitoformis*, where persulfide dioxygenase plays a key role. Int J Mol Sci 2024, 25.

24. Shen J, Keithly ME, Armstrong RN, Higgins KA, Edmonds KA, Giedroc DP. Staphylococcus aureus CstB is a novel multidomain persulfide Dioxygenase-Sulfurtransferase involved in hydrogen sulfide detoxification. Biochemistry. 2015;54:4542–54.
25. Xin Y, Liu H, Cui F, Liu H, Xun L. Recombinant *Escherichia coli* with sulfide:quinone oxidoreductase and persulfide dioxygenase rapidly oxidises sulfide to sulfite and thiosulfate via a new pathway. Environ Microbiol. 2016;18:5123–36.
26. Perez-Pantoja D, Donoso R, Agullo L, Cordova M, Seeger M, Pieper DH, Gonzalez B. Genomic analysis of the potential for aromatic compounds biodegradation in Burkholderiales. Environ Microbiol. 2012;14:1091–117.
27. Gao R, Liu H, Xun L. Cytoplasmic localization of sulfide:quinone oxidoreductase and persulfide dioxygenase of *Cupriavidus pinatubonensis* JMP134. Appl Environ Microbiol. 2017;83:01820–01817.
28. Hou N, Xia Y, Wang X, Liu H, Liu H, Xun L. $H_2S$ biotreatment with sulfide-oxidizing heterotrophic bacteria. Biodegradation. 2018;29:511–24.
29. Huang D, Yang L, Ko JH, Xu Q. Comparison of the methane-oxidizing capacity of landfill cover soil amended with Biochar produced using different pyrolysis temperatures. Sci Total Environ. 2019;693:133594.
30. Duan H, Koe LC, Yan R. Treatment of $H_2S$ using a horizontal biotrickling filter based on biological activated carbon: reactor setup and performance evaluation. Appl Microbiol Biotechnol. 2005;67:143–9.
31. Ding Y, Xiong J, Zhou B, Wei J, Qian A, Zhang H, Zhu W, Zhu J. Odor removal by and microbial community in the enhanced landfill cover materials containing biochar-added sludge compost under different operating parameters. Waste Manag. 2019;87:679–90.
32. Wang Q, Gu X, Tang S, Mohammad A, Singh DN, Xie H, Chen Y, Zuo X, Sun Z. Gas transport in landfill cover system: A critical appraisal. J Environ Manage. 2022;321:116020.
33. Xia FF, Zhang HT, Wei XM, Su Y, He R. Characterization of $H_2S$ removal and microbial community in landfill cover soils. Environ Sci Pollut Res Int. 2015;22:18906–17.
34. Lian T, Zhang W, Cao Q, Yin F, Wang S, Zhou T, Wei X, Zhang F, Zhang Z, Dong H. Enzyme enhanced lactic acid fermentation of swine manure and Apple waste: insights from organic matter transformation and functional bacteria. J Environ Manage. 2024;356:120573.
35. Ren W, Zhang Y, Liu X, Li S, Li H, Zhai Y. Peracetic acid pretreatment improves biogas production from anaerobic digestion of sewage sludge by promoting organic matter release, conversion and affecting microbial community. J Environ Manage. 2024;349:119427.
36. Stylianou M, Samanides G, Vyrides C, Agapiou I. A: High biodesulfurization efficiency of oil by aerobic (*Burkholderia Sp.* and *Serratia Sp.*) and anaerobic bacteria using various additives. Energy 2023, 282.
37. Zhuang X, Wang S, Wu S. Electron transfer in the biogeochemical sulfur cycle. Life (Basel) 2024, 14.
38. Gao G, Zhang Y, Niu S, Chen Y, Wang S, Anwar N, Chen S, Li G, Ma T. Reclassification of *Enterobacter Sp.* FY-07 as *Kosakonia oryzendophytica* FY-07 and its potential to promote plant growth. Microorganisms 2022, 10.
39. Lee EY, Lee NY, Cho KS, Ryu HW. Removal of hydrogen sulfide by sulfate-resistant *Acidithiobacillus thiooxidans* AZ11. J Biosci Bioeng. 2006;101:309–14.
40. Zhao X, Shi Y, Niu S, Wei X, Liu T, Yang M, Wu M, Gao G, Ma T, Li G. Enhancing wound healing and bactericidal efficacy: A hydrogel membrane of bacterial cellulose and Sanxan gel for accelerating the healing of infected wounds. Adv Healthc Mater. 2024;13:e2303216.
41. Liu AY, Koga H, Goya C, Kitabatake M. Quick and affordable DNA cloning by reconstitution of seamless ligation cloning extract using defined factors. Genes Cells. 2023;28:553–62.
42. Liu D, Cao Y, Qu R, Gao G, Chen S, Zhang Y, Wu M, Ma T, Li G. Production of bacterial cellulose hydrogels with tailored crystallinity from *Enterobacter sp.* FY-07 by the controlled expression of colanic acid synthetic genes. Carbohydr Polym. 2019;207:563–70.


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