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Original Paper

Biomass thermal decomposition induced hydrogen sulfide blooming in thermal recovery reservoirs

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ABSTRACT

High concentration of secondary hydrogen sulfide (sH_2S) in thermal recovery reservoirs of Liaohe Oilfield, NE China was concluded to originate from thermochemical sulfate reduction (TSR), and no biotic source of H_2S under abundant biomass has been reported in these presumed steam sterilized reservoirs ever before. In this study, we propose a new mechanism, biomass thermal decomposition for sulfur compounds (BTDS), to interpret the increasing of sH_2S. Sulfur of cells' dry weight took 0.20%–1.92% of the active strains isolated from the *in-situ* thermal recovery reservoirs of Liaohe Oilfield. When microbial organic sulfur compounds (MOSC) in biomass were exposed to injected steam, it resulted in the BTDS process. The isolated *Bacillus subtilis* D3 (G+) and *Pseudomonas aeruginosa* XJ14 (G−) were chosen to simulate this process. About 36% of sulfur in MOSC emitted as H_2S in steam chamber by BTDS. The $\delta^{34}S$ of H_2S from produced gas ranged from 8.7‰ to 17.0‰, close to the $\delta^{34}S$ of H_2S 11.2‰ from BTDS simulation experiment. It provides new insight into the contribution and sulfur cycle made by subterranean microorganisms on H_2S formation.

1. Introduction

Previous studies concluded that the main origins of H_2S in oil or gas deposits were thermochemical sulfate reduction (TSR) (Machel et al., 1995), biological sulfate reduction (BSR) (Bastin, 1926), and/or thermal decomposition of sulfur compound (TDS) (Ritchie et al., 1985).

The recorded concentration of primary H_2S in Liaohe Oilfield as the biggest heavy oil resources basement in China, is less than 4 mg/m^3 in 1982 (Dai, 1985), while it gradually increased to 46000 mg/m^3 in 2006 after years of thermal recovery (Zhu et al., 2010). TSR was proposed to be the main cause of secondary H_2S (sH_2S) blooming where TDS and BSR were both considered not realizable for oils with low sulfur contents (<0.3%) and reservoirs presumed steam sterilized, respectively (Zhu et al., 2010). Until the microbial diversity and abundance were discovered in decreasing gradient-temperature fields (DGTF) of thermal recovery oil reservoirs (Tian et al., 2020, Fig. S1), the role of microorganisms in the sulfur cycling was not recognized. Apart from the H_2S produced by sulfate reducing bacteria, the microbial organic sulfur compounds (MOSC) exposed to higher temperature steam can be another possible source of H_2S in thermal recovery reservoirs. Unfortunately, microbial biomass thermal decomposition for sulfide (BTDS) has not been considered so far.

In this study, based on the discussion of microbial diversity and abundance induced by thermal recovery, a novel BTDS process was simulated thoroughly by type species *Bacillus subtilis* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative) as the most important species of the two biggest phyla *Bacillota* (*Firmicutes*) and *Pseudomonadota* (*Proteobacteria*) in reservoirs, respectively, in order to explore the mechanism of MOSC on the emission of H_2S and other volatile sulfur compounds (VSCs). The signs of sH_2S originating from BTDS in reservoirs were also proposed.

2. Materials and experiments

2.1. Background of study area

The study area locates at Du-84 block of Liaohe Oilfield, NE China. It is a continental deep heavy oil reservoir with substantial heterogeneity (Wan et al., 2023). The burial depth of oil reservoir is

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about 650–670 m for Guantao Formation, and 670–782 m for Xingping Formation (Xing I, Xing III and Xing VI). The original temperature of the heavy oil reservoir used to be 21 °C, but it turned to 250 °C in steam chambers for steam soak (SS) and steam-assisted gravity drainage (SAGD) development successively (Table 1). Six years (SDH3001) were the shortest developing history in this block. Well SD10 and SD11 had been developed by steam injection (SS and SAGD) for more than 10 years.

The crude oil was regarded as low sulfur level. The ions were detected by ion chromatograph (Tian et al., 2020) and the concentration of sulfate in formation water was 7.7–22.4 mg/L with the pH of 6.7–7.2 at 25 °C (Table S1).

2.2. Produced gas and biomass

Each produced gas was sampled separately from 9 wells (Table 1) by 1-L pre-vacuumed gas collection bags (Dalian Pulaite Gas Packing Co., China) in Sep. 2014. Produced fluid of each well was sampled by sterile procedures from the SAGD developing wells (Wan and Dong, 2014). The measurement of DNA extraction and sequencing was conducted as our previous report (Tian et al., 2020).

Eleven strains (four Gram-positive: *Bacillus amyloliquefaciens Bam*_LH, *B. tequilensis Bte*_LH, *B. subtilis* D3, *Brevibacillus formosus Bfo*_LH; seven Gram-negative: *Acinetobacter johnsonii Ajo*_LH, *Pseudomonas aeruginosa* XJ14, *P. balearica Pba*_LH, *P. stutzeri Pst*_LH, *Shewanella seohaensis Sse*_LH, *Stenotrophomonas rhizophila Srh*_LH, *St. maltophilia Sma*_LH) isolated from the produced fluid were chosen for experiments. All strains were cultured in the corresponding liquid media at optimal conditions, collected, lyophilized as previous described individually (Wan and Dong, 2014; Wan et al., 2020, 2022), and then kept in 4 °C fridge for further study. Two strains *B. subtilis* D3 (G+) and *P. aeruginosa* XJ14 (G−) were used to simulate the BTDS process.

2.3. BTDS experiments, sulfur compounds separation

About 20 mg microbial biomass was loaded in an alumina porcelain crucible, and then it was moved to the microbalance of a thermal gravimetric analyzer (ZRT-1, Hua Ke Jing Yi, Beijing, China) for BTDS simulation (Wan et al., 2015). Batch samples were simulated to explore the effect of reservoir temperature on MOSC decomposition. The temperature programs of BTDS started from room temperature, heat to 100 °C with the heating rate of 20 °C/min and maintained for 30 min, then kept heating to different final temperatures (250, 300 or 350 °C) with 20 °C/min, all final temperatures were maintained for another 30 min. Among the final temperature 250 °C, different heating rates (5, 10, 20 or 30 °C/min) were also simulated individually. Pure nitrogen (≥99.999%) was used as carrier gas with the flow rate of 30 mL/min. Each purged gases were collected by pre-vacuumed gas collection bags.

The separative and quantitative method (Wan et al., 2015) was applied to assess the sulfur compounds of BTDS simulation. Briefly, the purged gases were bubbled into 4 connected gas washing bottles, the bottles were filled with $AgNO_3$ and $CdCl_2$ to collect H_2S, mercaptans (thiols) successively. After the collection, H_2S was determined by the precipitated Ag_2S, and mercaptans were quantified by iodometric titration. All the BTDS simulation experiments with same conditions (same final temperature and heating rate) were conducted in triplicate. The precipitated Ag_2S and mercaptans from parallel experiments were combined and quantified together for trace amount of sulfur in biomass. H_2S samples from produced gases were also collected by bubbling into $AgNO_3$ solution for sulfur isotopic analysis.

2.4. Characterization of sulfur in microbial biomass and BTDS residues

The sulfur elemental content of the microbial biomass and their residues after BTDS were measured by Vario EL cube (Elementar, Germany). Sulfanilic acid (Sigma-Aldrich, Germany) was used as the reference material. X-ray photoelectron spectroscopy (XPS) tests of biomass and their residues after BTDS were carried on ESCALAB Xi+ (Thermo Scientific, USA), with pass energy of 30 eV and energy step size of 0.1 eV. The S-containing functional groups in spectra were considered into four peaks: C–S–H (162.4 eV), C–S–C (163.7 eV), $R_2S{=}O$ (166.6 eV) and $-SO_2$ (168.1 eV) (Leng et al., 2022) with the software Avantage v5.9931.

2.5. Characterization of produced gases and BTDS purged gases

Each collected BTDS produced gas or purged gas was pushed into a gas sample loop (volume 1 mL, temperature 50 °C), which was connected to a split injector of a gas chromatography (SCION GC-456, Bruker, Netherlands). The inlet temperature was maintained at 220 °C. A PLOT-Q column (30 m × 0.53 mm × 20 μm) was used to separate the VSCs. A pulsed flame photometric detector (PFPD) with linearized mode (i.e. with the square root turned on) and a thermal conductivity detector (TCD) were equipped to detect sulfur compounds. The carrier gas was helium (≥99.999%) with the flow rate of 2 mL/min. The oven temperature started at 60 °C, heat to 160 °C with the heating rate of 20 °C/min and maintained for 5 min, then heat to 190 °C with the rate of 5 °C/min, finally heat to 220 °C with 30 °C/min and maintained for another 7 min, the

Table 1
The developing history of wells in Du-84 block of Liaohe Oilfield.

No.	Well No.	Formation	Depth, m	Extraction method[a]	History[b], Year/Month
1	SD10	Guantao	650	SAGD pilot text	03/12 04/9 05/9 14/11
2	SD11	Guantao	653	SAGD pilot text	03/12 05/2 14/11
3	SD16	Guantao	670	SAGD I	03/12 05/9 09/1 14/11
4	SD17	Guantao	674	SAGD I	03/12 05/9 09/1 14/11
5	SD42	Xing VI	757	SAGD I	03/12 05/3 11/11 14/11
6	SD44	Xing VI	780	SAGD pilot text	03/12 04/8 06/10 14/11
7	SD47	Xing VI	782	SAGD I	03/12 05/7 07/5 14/11
8	SD281	Xing I	678	SAGD I	03/12 05/5 12/6 14/11
9	SDH3001	Xing III	705	SS	03/12 08/8 14/11

[a] SS, steam soak; SAGD, steam-assisted gravity drainage; SAGD I, steam assisted gravity drainage industrialization phase I.
[b] ▬, period of wells developed by SS; ▬, period of wells developed by SAGD; records end in Nov. 2014.

analysis totally cost 24 min for each sample. A standard VSCs sample (AP BAIF Gases Industry Co. Beijing) mixed nitrogen with six compounds (H_2S, CH_3SH (methyl mercaptan), CS_2 (carbon disulfide), COS (carbonyl sulfide), CH_3SCH_3 (dimethyl sulfide, DMS) and CH_3SSCH_3 (dimethyl disulfide)) with each concentration of 50 mg/m^3, was used for calibration in GC analysis. The concentration of VSCs in produced gases was calculated by the formula:

$$C_s = (C_{std} / A_{std})\, A_s \times (R_s + 1) \;/\; (R_{std} + 1)$$

where C_s and C_{std} are the concentration of VSC in samples and standard, respectively; A_s and A_{std} represent the peak area of samples and standard, respectively; R_s is the split ratio of GC analysis for samples, and R_{std} is the split ratio of GC analysis for standard.

The H_2S produced by BTDS was calculated by the formula:

$$wt_{H_2S} = n_{cell} \times wt_{cell} \times S_{cdw} \times R_{H_2S} \times 34/32$$

In which wt_{H_2S} is the weight of H_2S; n_{cell} is the number of cells; wt_{cell} is the average dry weight of microbial cells; S_{cdw} is the average sulfur content of cell dry weight; R_{H_2S} is the conversion rate of sulfur to H_2S in microbial cell after BTDS.

The sulfur isotope against the international standard V-CDT (Vienna Canyon Diablo Troilite) $\delta^{34}S$ of H_2S was carried out by an isotope mass spectrograph (MAT-253, Finnigan MAT Company, USA). It was conducted with the precipitated Ag_2S from produced gases and two strains' BTDS simulation experiments.

3. Results

3.1. Sulfur content in microbial biomass and its pyrolysis residues

The sulfur contents of strains (biomass) isolated from *in-situ* Liaohe Oilfield ranged from 0.20% to 1.92% (Table 2). The strains isolated from other oilfields also shared the same sulfur content range (Table S2).

The isolated strain *P. aeruginosa* XJ14 and *B. subtilis* D3 had sulfur content of 0.33% ± 0.02% and 0.25% ± 0.01% of their cell dry weight, respectively. The sulfur contents in their residues changed with different final temperature or heating rate of BTDS (Fig. 1).

The S 2p peaks of raw microbial biomass (Fig. S2) were discomposed into four components at binding energy (BE) of 162.4, 163.7, 166.6 and 168.1 eV, assigned to C–S–H, C–S–C (C–S–S–C), $R_2S{=}O$ (sulfoxide) and $-SO_2$ (sulfone), respectively (Table 3). Part spectra of sulfur could not be fitted for scarce sulfur content in the residues after BTDS (Figs. S3 and S4).

3.2. VSCs in BTDS produced gases and purged gases

H_2S and CH_3SH composed the main VSCs of strain XJ14 and D3 in BTDS purged gases (Figs. 2, S5 and S6). COS, CS_2, and CH_3SSCH_3 was not detected (Fig. 2).

The sampled produced gases contained H_2S, CH_3SH, COS, CS_2 and DMS (Fig. 3). H_2S was detected in all wells with the highest concentration of 959.0 mg/m^3 in well SD11 and the lowest concentration of 1.6 mg/m^3 in well SD16. CH_3SH was detected in all H_2S containing wells, except SD10 and SDH3001. COS was found in every produced gas sample, while DMS was only detected in wells SD10, SD11 and SD281. CH_3SSCH_3 was not detected in all samples (Fig. 3). SD16, SD17 and SD44 had lowest concentration of VSCs.

The $\delta^{34}S$ results of H_2S in produced gases and BTDS purged gas are listed in Table 4. It ranged from 8.7‰ to 17.0‰, and the $\delta^{34}S$ value of H_2S was 11.6‰ in BTDS purged gases (Table 4). H_2S produced from the two strains was combined because of their limited biomass and even smaller amount of sulfur compounds.

4. Discussion

4.1. Microbial diversity and abundance induced by DGTF

It was believed the H_2S collected from BTDS originating in *in-situ* reservoirs was from a variety of strains as well.

High diversity microbial assemblages were discovered in DGTF. Based on our previous research, totally 27 phyla and 414 genera microorganisms were sequenced (Tian, 2017), including sulfur functional bacteria, nitrogen functional bacteria and iron oxidizing bacteria. Sulfate reducing bacteria (*Desulfurella*, *Desulfobulbus*, *Cupidesulfovibrio* (Wan et al., 2021)) and sulfur oxidizing bacteria (*Acidithiobacillus*, *Thiohalobacter*, *Sulfurospirillum*) compose the players of sulfur dissimilatory cycle. Nitrogen cycling bacteria like *Nitrosomonas* and *Nitrosospira* are ammonia oxidizing bacteria; *Nitrobacter* and *Nitrospira* are able to oxidize nitrite to nitrate; *Azotobacter* can fix nitrogen independently. *Thermomonas, Hyphomicrobium and Pseudomonas* are typical iron oxidizing bacteria.

The *in-situ* microbial diversity was more than the result of high-throughput sequencing (HTS). The genus *Brevibacillus* and strain *B. formosus Bfo*_LH were home-lab detected and cultured but not in the sequenced genera, as a case that culture-dependent method is a complement of sequencing for its limitations and biases (Brooks et al., 2015; Wan et al., 2021). Significantly, most of subterraneous strains have hitherto not been isolated and pure cultured yet (Luef et al., 2015). More than a hundred genera, thousand species and ten thousand strains of DGTF-adaptive microorganisms have been found, isolated and cultured in our Ministry of Education-based cell bank (UPRI, 2019).

Table 2
Sulfur contents of strains isolated from production fluid of Liaohe Oilfield.

No.	Strains	Gram[a]	Motility	Grown temperature, °C	Sulfur content, wt%[b]
1	*Acinetobacter johnsonii Ajo*_LH	G−	immotile	28–37	0.22 ± 0.01
2	*B. subtilis* D3	G+	motile	15–50	0.25 ± 0.02
3	*B. amyloliquefaciens Bam*_LH	G+	motile	25–50	0.32 ± 0.02
4	*B. tequilensis Bte*_LH	G+	motile	25–55	0.20 ± 0.03
5	*Brevibacillus formosus Bfo*_LH	G+	motile	30–35	0.43 ± 0.02
6	*P. aeruginosa* XJ14	G−	motile	10–45	0.33 ± 0.01
7	*P. balearica Pba*_LH	G−	motile	25–50	0.29 ± 0.01
8	*P. stutzeri Pst*_LH	G−	motile	28–35	0.33 ± 0.01
9	*Shewanella seohaensis Sse*_LH	G−	motile	30–35	0.81 ± 0.01
10	*Stenotrophomonas rhizophila Srh*_LH	G−	motile	5–37	0.22 ± 0.01
11	*St. maltophilia Sma*_LH	G−	motile	25–41	1.92 ± 0.01

[a] G+, Gram-positive; G−, Gram-negative.
[b] Sulfur content of cell dry weight.

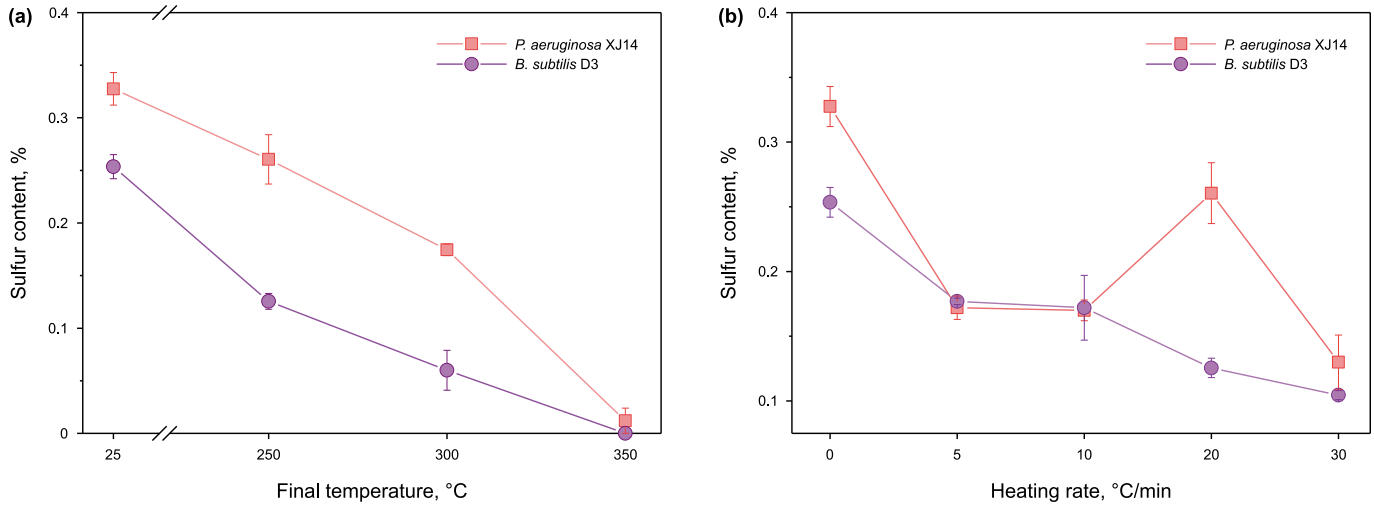

Fig. 1. Effect of final temperature **(a)** or heating rate **(b)** in BTDS on Sulfur content of biomass. (a) Heating rate = 20 °C/min; (b) Final temperature = 250 °C.

Table 3
Deconvolution results of XPS S 2p peaks of microbial biomass before and after BTDS.

BTDS condition, °C - °C/min[a]	Biomass sulfur fraction, %							
	B. subtilis D3				*P. aeruginosa* XJ14			
	C–S–H	C–S–C[b]	$R_2S{=}O$[c]	$-SO_2$	C–S–H	C–S–C[b]	$R_2S{=}O$[c]	$-SO_2$
Raw	15.75	45.21	19.16	19.89	18.02	43.63	28.18	10.17
250-5	0	46.48	0	53.52	0	57.66	0	42.34
250-10	0	62.91	0	37.09	0	71.46	0	28.54
250-20	0	60.61	0	39.39	0	68.28	0	31.72
250-30	0	63.00	0	37.00	0	65.82	0	34.18
300-20	0	57.45	0	42.55	0	65.29	0	34.71
350-20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[a] Finally temperature-heating rate.
[b] Typical compounds like thiazole and thiophene; disulfide (C–S–S–C) is also included for raw biomass.
[c] R, stands for a grouping of carbon and hydrogen atoms; N/A, Spectra not fitting for trace sulfur content.

The isolated strains were psychrophiles, mesophiles or thermophiles (Wan and Dong, 2023) with motility including the sulfate reducing bacterium *C. liaohensis* XJ01^T (CGMCC 1.5227^T; DSM 107637^T) (Wan et al., 2021) growing on 5–55 °C (Table 2). Hyperthermophiles such as *Thermotoga* grow at temperature higher than 75 °C; a strain of genus *Hydrogenobaculum* grown between 73.8 and 96 °C (Tian et al., 2020) was isolated and identified by 16S rRNA gene sequencing once in our lab (data not shown) but could not be re-cultured unfortunately.

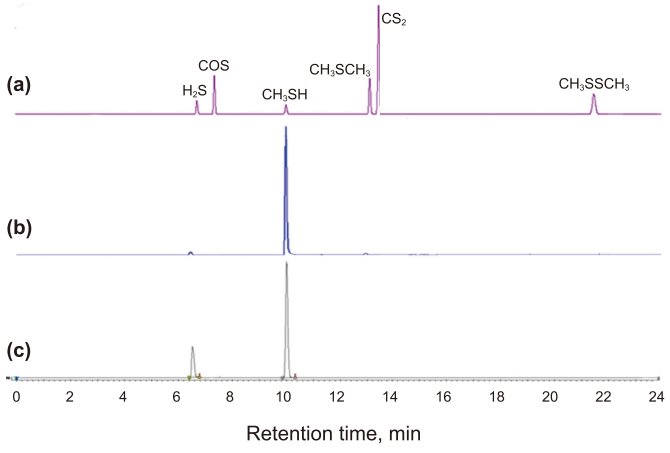

Fig. 2. Volatile sulfur compounds in the BTDS purged gas. **(a)** Standard samples; **(b)** *B. subtilis* D3 BTDS purged gas at 250 °C at the heating rate of 30 °C/min; **(c)** *P. aeruginosa* XJ14 BTDS purged gas at 250 °C at the heating rate of 20 °C/min.

The microbial abundance of all produced liquid in Liaohe Oilfield was confirmed by microbial vials, UV spectrophotometry and qPCR (Wan et al., 2023). In DGTF of Liaohe Oilfield, the concentration of 16S rRNA gene in produced fluid ranged from 2.242×10^7 to 3.249×10^7 copies/mL (Tian et al., 2020), about 4.152×10^6 to 6.016×10^6 prokaryotic cells in every milliliter (cell/mL) of produced fluid. This was larger than the reported prokaryotic cell number 0.95×10^6 cell/mL in unconsolidated subsurface sediments (Whitman et al., 1998) or 10^3–10^6 cell/mL in water from petroleum deposits with approximate depth (Pedersen, 1993). It should be pointed out that the actual quantity of microorganisms must be larger than the determined number by culturing-independent because some isolates as mentioned above could not be detected by HTS. Besides the remained cells in produced water of wells (Table 1) were ones that had not decomposed when crossing steam chambers, meanwhile they were diluted by steam condensate water continuously. It can be inferred that DGTF harbors large numbers of prokaryotes.

Well SD10 (Table 1) has DMS with the concentration of 924.1 mg/m^3, and 959.0 mg/m^3 DMS was detected in well SD11, which was significantly higher than other wells (Fig. 3). A strong correlation between DMS and high biomass (Table S3) was found. DMS has no natural abiotic source ever been identified on Earth and is considered a robust biosignature (Hänni et al., 2024). DMS was

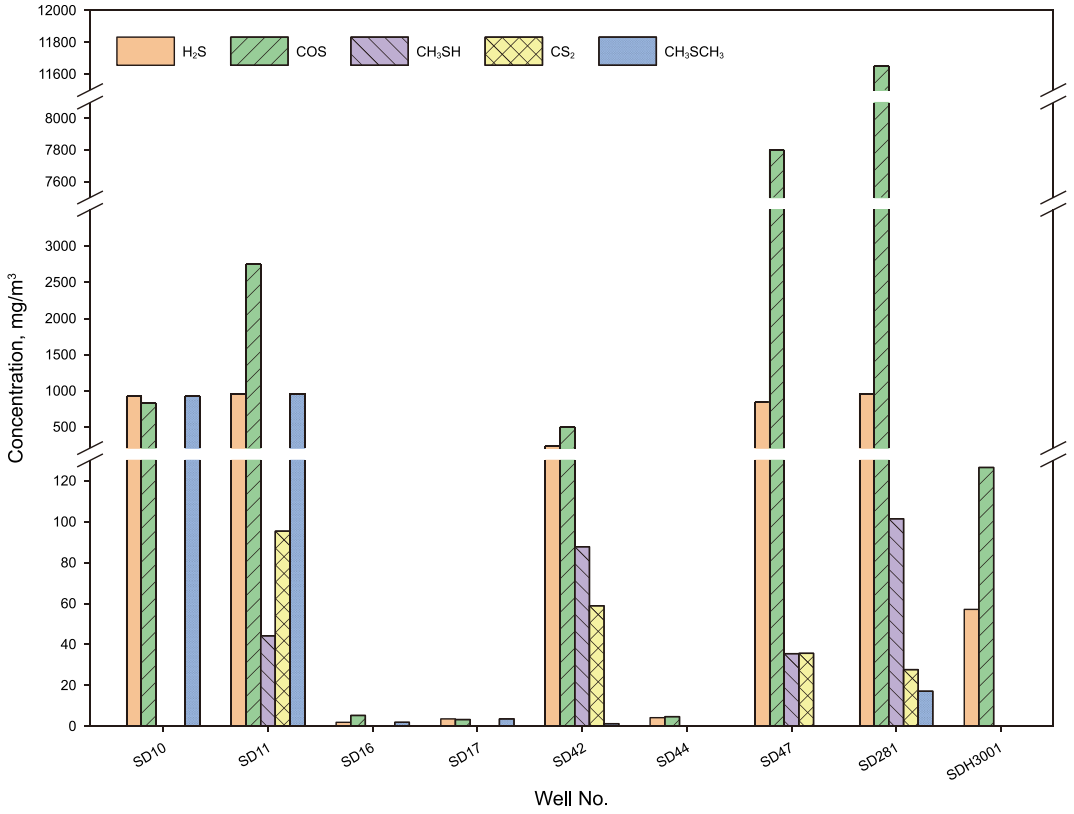

Fig. 3. Volatile sulfur compounds in produced gas of Liaohe Oilfield.

Table 4
Sulfur isotopic values of H_2S in produced gases and BTDS purged gas.

Sample	Biomass[a]	SD10	SD11	SD16	SD17	SD42	SD44	SD47	SD281	SDH 3001
$\delta^{34}S$, ‰	11.6	12.8	11.3	9.3	8.7	12.0	10.3	14.3	17.0	-

[a] H_2S precipitated by $AgNO_3$ from two strain's BTDS simulation experiments. "-" represents no data.

almost non-existent in thermal maturation and simulation experiments (Kutuzov et al., 2023), either.

Based on the culture-dependent and -independent results, and DMS as molecular indicator of microbial activity, it is confirmed that microorganisms can settle and keep thriving in the artificial DGTF of thermal recovery reservoirs in Liaohe Oilfield, supplying biomass continuously. This phenomenon was not unique in thermal recovery oilfields, by water flooding with high microbial abundance forming in hot reservoirs (Zhang et al., 2012a) can be considered just as a result of DGTF.

4.2. BTDS mechanism

Usually, sulfur makes up 0.5%–1% of the microbial cells' dry weight. Part of the sulfur content of strains isolated from *in-situ* oilfield fell in this range (Table 5). *Pseudomonas* sp. and *Bacillus* sp. have sulfur content no larger than 0.5% of their dry weight. While *St. maltophilia* has 1.92% sulfur of its cell dry weight; *Beggiatoa* sp. is capable of oxidizing H_2S to sulfur and deposits in cells, it has the sulfur content up to 25% of its dry weight (Nelson and Castenholz, 1981). Though with different sulfur content, the MOSC primarily are amino acids (cysteine and methionine), enzyme cofactors, and building blocks for redox reactions.

With the continuous steam injection and expanding of steam chamber, the proliferous abundant microbial biomass in DGTF gradually suffered to the high-temperature steam because of heat transfer, radiation and/or convection (Tian et al., 2020). *B. subtilis* D3 and *P. aeruginosa* XJ14 are able to metabolize inorganic sulfur such as SO_4^{2-}, SO_3^{2-}, $S_2O_5^{2-}$, $S_2O_6^{2-}$ and S^{2-} (Schook and Berk, 1978; Villarejo and Westley, 1966) into MOSC (Table 5). At the same time, as the most representative species of the two biggest phyla *Bacillota* (*Firmicutes*) and *Pseudomonadota* (*Proteobacteria*) in reservoirs, respectively, the two strains were chosen to simulate BTDS process. Until now, it was the first time to report the composition and functional groups of sulfur (Table 3 and Figs. S2–S4), instead of carbon, nitrogen and oxygen described merely in *B. subtilis* (Leone et al., 2006).

Every Gram-negative and Gram-positive strain showed different weight loss and volatile substances produced (including sulfur-containing compounds) at different pyrolysis temperatures (Wan et al., 2022). The GC results indicated that H_2S and CH_3SH were the main VSCs during BTDS (Figs. 2, S5 and S6), in agreement with the reported pyrolyzates (Boon et al., 1981).

Briefly, the BTDS mechanism was proposed as Eq. (1) at 250 °C:

$$\text{Cell} - \text{S} \xrightarrow[350^\circ\text{C}]{\text{BTDS}} \text{H}_2\text{S} + \text{CH}_3\text{SH} + \text{DMS} \quad (1)$$

The simulation experiments indicated that final temperature

Table 5
Sulfur content in cell of each strain (dry weight).

Genus	Species	Sulfur content, wt%	Reference
Agrobacterium	*A. tumefaciens*	0–0.3	Spector (1956)
Bacillus	*B. subtilis*	0.25 ± 0.02	this study
	B. amyloliquefaciens	0.32 ± 0.02	
	B. tequilensis	0.20 ± 0.03	
Beggiatoa	*B.* sp.	25	Nelson and Castenholz (1981)
Brevibacillus	*B. formosus*	0.43 ± 0.02	this study
Brucella	*B. melitensis*[a]	0.4–0.6	Spector (1956)
Corynebacterium	*C. diphtheriae*	1.4	
Escherichia	*E.coli*	0.1	Spector (1956)
		0.41–0.58	Taymaz-Nikerel et al. (2010)
Malleomyces	*M. mallei*[b]	1.0	Spector (1956)
Mycobacterium	*M.tuberculosis*	0.2–1.4	
Neisseria	*N.gonorrhoeae*	7.2–8.9	
Pseudomonas	*P. aeruginosa*	0.33 ± 0.01	this study
	P. balearica	0.29 ± 0.01	
	P. stutzeri	0.33 ± 0.01	
	P. putida	0.41–0.45	Beil et al. (1996)
Shewanella	*S. seohaensis*	0.81 ± 0.01	this study
Stenotrophomonas	*St. rhizophila*	0.22 ± 0.01	
	St. maltophilia	1.92 ± 0.01	

[a] *Brucella abortus* and *B. suis* was were combined to *B. melitensis* and treated as heterotypic synonym of *B. melitensis* since 1920 (Meyer and Shaw, 1920) and 1931 (Huddleson, 1931), respectively.
[b] *M. mallei* was renamed as *Burkholderia mallei* since 1992 (Yabuuchi et al., 1992).

and heating rate had significant effect on the amount of MOSC cracking into H_2S and CH_3SH in BTDS. While the heating rate was affected by plenty of parameters: steam injection rate, the depth of well, the length of the hot steam injection pipelines and heterogeneity in thermal recovery reservoirs.

4.2.1. Effect of temperature on BTDS

After BTDS process with final temperature of 350 °C, the sulfur content in XJ14 and D3 (Fig. 1(a)) decreased to 0.01% and 0.00%, respectively. Almost no sulfur remained in the residues after BTDS at temperature 350 °C, this was different from the plant biomass (Leng et al., 2022). It also indicates that MOSC are not thermally stable as organic sulfur compounds in kerogen or fossil fuel (Kutuzov et al., 2023).

The XJ14's sulfur elemental fraction in H_2S (S_{H_2S}) increased from 15% to 30% with the BTDS final temperature increased from 250 to 300 °C, and decreased to 24 % after BTDS at 350 °C (Fig. 4(a)). In D3's BTDS simulation, S_{H_2S} increased from 24% to 36% with higher final temperature (Fig. 4(b)). Generally, higher temperature led to the production of more H_2S in BTDS, while XJ14 tended to produce CH_3SH (6% of sulfur at 250 °C, 21% of sulfur at 350 °C) rather than H_2S with temperature changing from 250 to 350 °C.

For biomass after BTDS at 250 °C, sulphydryl and sulfoxide functional groups in microbial biomass disappeared (Table 3). This was aroused by the sulfur-containing amino acid cracking into gaseous phase like H_2S and mercaptans, or reacting with reduced saccharides into aromatic sulfur compounds like thiazole (Maillard reaction) (Zhang et al., 2020). Thiophene and sulfone function groups were also not detectable in the residues after BTDS at 350 °C, which suggested that elevated temperature enhanced the cleaving of thiophene and sulfone. More H_2S produced at 350 °C than 250 °C (Fig. 4), for part of remained MOSC decomposed into

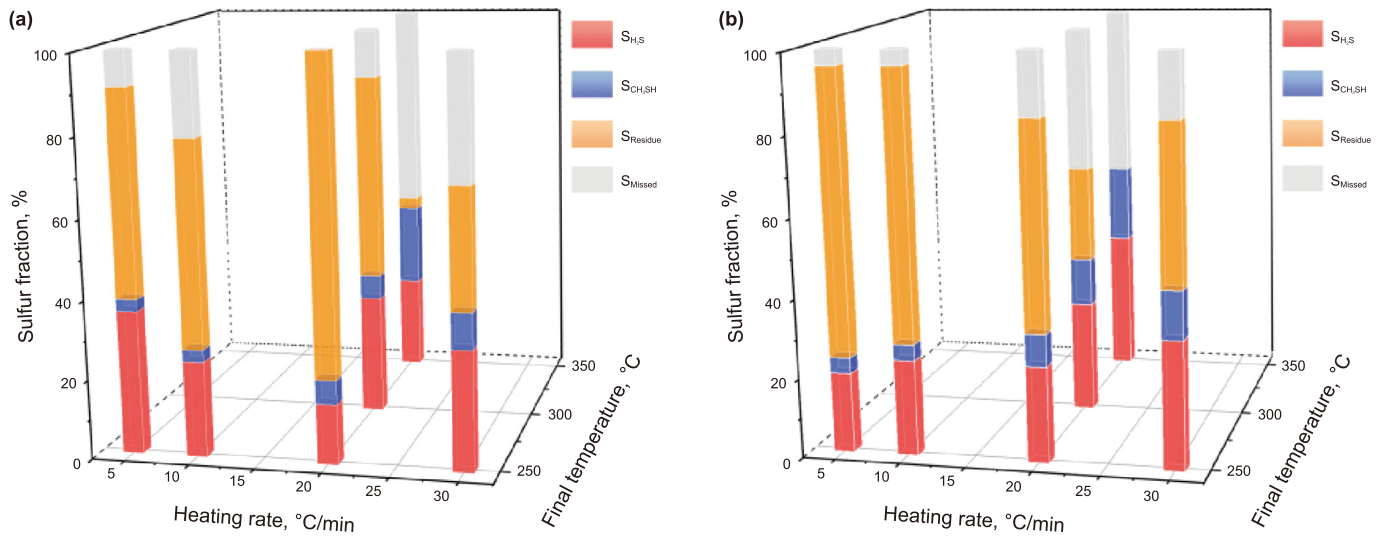

Fig. 4. Sulfur elemental fraction of *P. aeruginosa* XJ14 **(a)** and *B. subtilis* D3 **(b)** in BTDS treated by various final temperatures and heating rates.

H_2S and other compounds.

4.2.2. Effect of heating rate on BTDS

Strain D3's sulfur content decreased to 0.10% with the heating rate of 30 °C/min at 250 °C, it was lower than 0.13%, 0.17% and 0.18% with heating rate of 20, 10 and 5 °C/min, respectively; Sulfur content of strain XJ14 shared almost the same trend in BTDS simulation, except for the experiment conducted with heating rate of 20 °C/min (Fig. 1(b)). Both strains' sulfur content changes indicated that the fast heating rate also could accelerate the decomposition of MOSC.

In the BTDS experiments on strain XJ14, S_{H_2S} fluctuated with different heating rates at 250 °C, it declined from 36% to 15% when the heating rate changed from 5 to 20 °C/min, then increased to 30% with the heating rate of 30 °C/min (Fig. 4(a)). The results indicated that the lower heating rate resulted in the formation of more H_2S during XJ14's BTDS. One possible reason was that most sulfur-containing amino acids started cracking into H_2S at 178 °C (Johansen et al., 2011), before it reacted with reduced sugars in lipopolysaccharide outside of Gram-negative strains (XJ14) at 200 °C (Zhang et al., 2020). This also could express the fact that BTDS residues of strain XJ14 had larger percent of C–S–C function groups than D3 (Gram-positive) (Table 3). For strain D3, S_{H_2S} increased from 20% to 32% with faster heating rate (Fig. 4(b)), more H_2S formed in BTDS of Gram-positive strains with faster heating rate. Certainly, the temperatures of steam chambers were actually kept around 250 °C (Tian et al., 2020) in Liaohe Oilfield, though higher temperature was on the schedule of the oilfield.

Theoretically, the majority of cells had decomposed crossing steam chambers, while the remained MOSC still could produce at least 23.0 mg H_2S with 6.016×10^{12} cells in every cubic meter of produced fluid, for lower solubility of H_2S in hot environment, based on the average dry weight of microbial cells about 10^{-12} g per cell (Wan and Dong, 2014), 1% average sulfur content of cell dry weight, 36% conversion rate of sulfur to H_2S in microbial cell after BTDS.

It was remarkable that the higher final temperature or faster heating rate, the more lost sulfur (Fig. 4), which probably turned into other sulfur compounds, such as SO_2, DMS (Figs. S5 and S6) or unclear matters (Fig. S7) as Eq. (2):

$$\text{Cell} - \text{S} \xrightarrow[350^\circ\text{C}]{\text{BTDS}} \text{H}_2\text{S} + \text{CH}_3\text{SH} + \text{DMS} + \text{UCM} \quad (2)$$

UCM, unclear matters.

4.3. Signs of BTDS in sH_2S

In Du-84 block of Liaohe Oilfield, the crude oil was characterized by low sulfur content (<0.3%), while the concentration of sH_2S was still as high as 1000 mg/m^3 (Fig. 3) even after 9 years of thermal recovery (Table 1). Then TDS was not the main origin of sH_2S. Obviously, the previous concerned organic sulfur compounds of crude oils in TDS (Kutuzov et al., 2023; Yang et al., 2006) were structurally different from those of MOSC. Meanwhile DGTF were artificially built around the steam chambers, where abundant biomass proliferated and decomposed as discussed above as signs contributed to the BTDS process and sH_2S.

The $\delta^{34}S$ of H_2S in Du-84 block of Liaohe Oilfield had a range between 8.7‰ and 17.0‰, which was a sign of biotic origin. Because microorganisms mainly account for the decline of $\delta^{34}S$ in sH_2S, though sulfur isotopic fractionation was affected by plenty of parameters (Sim et al., 2023), usually resulted in low (even negative) and variable $\delta^{34}S$ of H_2S (Table 6, Nos. 1–8). Oppositely, primary H_2S forming by TSR during long geological time scale or TDS of abiotic reduced sulfide had a value of $\delta^{34}S$ not less than 20‰ and usually appeared in a narrow range (Table 6, Nos. 9–12), because no significant sulfur isotope fractionation occurred in TSR (Cai et al., 2022).

BTDS interpreted the main origin of the sH_2S each well in Du-84 block of Liaohe Oilfield. The $\delta^{34}S$ of H_2S in SD10 and SD11 was 12.8‰ and 11.3‰, respectively. They were close to the $\delta^{34}S$ of sH_2S collected in BTDS (11.6‰), besides produced gases collected from SD10 and SD11 contained high DMS concentration (Fig. 3), implying large microbial abundance in these two wells. Both signs featured the BTDS origin. High concentration of H_2S and COS was detected in SD47 and SD281, with the relative heavier sulfur isotopic values in H_2S of 14.3‰ and 17.0‰, respectively. Though few DMS was detected in SD47 and SD281, the main source of sH_2S in these two wells was not TSR. Because the steam chamber did not reach the temperature for uncatalyzed TSR (He et al., 2019; Zhang et al., 2012b) with pH of 7.2 and low $[MgSO_4]_{CIP}$ (contact ion pairs) concentration (Table S1); and once if TSR was initiated, the catalyzed TSR with sufficient sulfate should have produced increasing concentration of H_2S. Well SDH3001 was developing by SS with the steam chamber temperature of 104.0 °C, the H_2S mainly generated from microbial process during steam injection period of SS.

BTDS can happen in reservoirs worldwide as well because microorganisms exist in deep intraterrestrial environments (Pedersen, 2000) such as oil reservoirs.

The main origin of sH_2S of the Qi40 and Xiaowa region as other thermal recovery reservoirs in Liaohe Basin was believed as TSR

Table 6
Reported sulfur isotopic values of H_2S in primary reservoirs.

No.	Basin	Oil, gas deposit	Depth, m	T[a], °C	H_2S, %	$\delta^{34}S$[b], ‰, CDT	Main mechanism[c]	Reference
1	Liaohe	Qi40	650–1050	150–300	3.27	3.10–22.68	TSR	Zhu et al. (2010)
2		Xiaowa	1279–1363	220–350	N/A	13.3–26.8	TDS	Lin et al. (2014)
3	Alberta	Halfway	1700–2200	50–90	4	6.2–23.6	BSR	Desrocher et al. (2004)
4		Montney	5500	175	1	13–15	TSR	
5		Halfway - Montney	2400	85	7.49	15.6–16.2	Mix	Kutuzov et al. (2021)
6	Bohai Bay	Es4	2603–2618	90	92	0	BSR	Cai et al. (2005)
7	Zagros	Dalan	4230	>100	0.1	−7.3–−11.8	TSR	Torghabeh et al. (2021)
8	Jianghan	Qianjiang Xingouzui	997–2067	56–81	0.06	0.5–13.2	BSR	Xiao et al. (2021)
9	Abu Dhabi	Khuff	>4300	>140	2–50	18	TSR	Worden and Smalley (1996)
10	Tarim	Halahatang	6076–7059	140–160	<8.5	21	TDS	Zhu et al. (2017)
11		Hetianhe	1035–2885	35–85	0.1	20.28–21.27	TSR	Zhu et al. (2019)
12	Qaidam	Ganchaigou	5451–5514	181–182	2.75	29–32.5	TSR	Tian et al. (2021)

[a] temperature of reservoirs in Liaohe Basin was manipulated by thermal recovery.
[b] $\delta^{34}S$ of H_2S.
[c] TSR, thermochemical sulfate reduction; BSR, biological sulfate reduction; TDS, thermal decomposition of sulfur compounds; Mix, TSR and BSR. N/A, no data.

(Zhu et al., 2010; Lin et al., 2014). Based on the wider range of $\delta^{34}S$ values of H_2S (Tables 4 and 6, Nos. 1 and 2), besides DGTF and abundant biomass (data not shown), the complicate H_2S origins, however, must mainly be BTDS to our aspect. In Albert Basin (Table 6, Nos. 3–5), BTDS, instead of TSR (Desrocher et al., 2004), should happen in deep Montney Formation for natural DGTF with microbial settlement built by faults or fractures (Montney Formation was connected to upper Charlie Lake Formation by Rycroft fault (Ardakani and Pedersen, 2022)). For Zagros Basin (Table 6, No. 7), it is suggested to consider the microbial contribution on H_2S increasing, instead of TSR (Torghabeh et al., 2021), after ten years of development for its light isotopic values of sulfur between −7.3‰ and −11.8‰. The explained TSR (Worden and Smalley, 1996, Table 6 No. 9) should be reconsidered as the mixed contribution including BTDS. Even the Hetianhe gas field (Table 6, No. 11) should be reconsidered for its biological role in the process of oil and gas migration from deep layers through fractures, rather than simply TSR.

5. Conclusion

In this study, a new generative mechanism of H_2S during thermal recovery process was systematically proposed. Microorganisms grow rapidly in DGTF reservoirs, and BTDS happens when microbial biomass exposed to the higher temperatures than their thermal decomposition. BTDS mechanism indicates that:

1) Generally, higher temperatures and faster heating rates are conducive to the decomposition of MOSC. H_2S and CH_3SH are the main products of VSCs in BTDS at 250–350 °C.
2) BTDS mechanism fills the sulfur isotopic gap of H_2S between BSR and TSR based on the isotopic studies. It breaks the traditional idea that only BSR can cause sulfur isotopic fractionation, BTDS also introduces the sulfur isotopic fractionation. The sH_2S with its $\delta^{34}S$ values show wide range and lower than dissolved sulfate in reservoirs with DGTF shall be considered as signs of BTDS origin.
3) For the first time, MOSC was treated and considered as an important role for H_2S prevention and control in primary and/or secondary (anthropogenic intervention) reservoirs. It is necessary to reconsider the reported source of H_2S in reservoirs where natural DGTF forms around fractures or faults by geothermal gradient, in which there are abundant microorganisms and BTDS likewise. Variously repeated field practice case investigation recently by us (data not shown) has shown that the prevention of sH_2S should be focused on the microbial sulfur circle by BTDS because of continuously anthropogenic intervention.

CRediT authorship contribution statement

Ying-Jia Zhu: Writing – original draft, Methodology, Formal analysis. **Yun-Yang Wan:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization, Validation, Methodology, Data curation. **Yan Tian:** Supervision, Resources, Methodology, Formal analysis. **Hong-Mei Mu:** Validation, Resources, Formal analysis. **Tong-Gang Zhang:** Conceptualization, Funding acquisition.

Declaration of interest statement

The authors imply that it has not been published previously, except a conference proceeding titled 10th Youth Academic Conference of China Petroleum Society in 2017 and a pending patent, not under consideration for publication in another journal, and that if accepted it will not published elsewhere in the same form in any other language, without the written consent of the publisher.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.petsci.2025.03.044.

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