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Oxidization and biodegradation of sulfur black by a newly isolated strain *Acinetobacter* sp. DS-9

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ABSTRACT

Sulfur black is a high-sulfur polymeric compound, mainly used for dyeing cotton textiles. It is part of the most widely used sulfur dyes in China. Wastewater containing sulfur dyes is sometimes difficult to treat, due to its high Chroma, many suspended solids and poor biodegradability. The aim of this study was to screen microorganisms with high-efficiency sulfur-oxidizing and sulfur black degrading capabilities. The DS-9 strain, with effective sulfur black degrading capability, was isolated from agricultural soil. Physio-biochemical identification and sequence alignments of 16S rRNA illustrated that this strain belongs to the genus of *Acinetobacter*. Under the initial degradation conditions, the SO_4^{2-} content in the culture medium containing *Acinetobacter* sp. DS-9 was 15.22% higher than that of the control test on the fifth day. Single-factor optimization and orthogonal design were used to improve the desulfurization efficiency of DS-9 in a medium containing 100 mg L^{-1} sulfur black. The optimal degradation system was as follows: soluble starch 500 mg L^{-1}, 400 mg L^{-1} NH_4Cl, 7 g L^{-1} Tween 80, 28°C, 200 rpm, 5% inoculum volume, and pH 7.2. Under the optimal degradation conditions, the content of SO_4^{2-} in the culture medium increased by 52.06% over 4 d compared with the CK.

Keywords: Sulfur black; Biodegradation; *Acinetobacter* sp.DS-9; Desulfurization efficiency; Sulfate

1. Introduction

China is among the world's largest producers of dyes, yielding more than 900,000 tons of various synthetic dyes annually. Its output has been responsible for more than half of the world's total dye production for many years [1]. Sulfur dyes, used widely in the denim industry, are one of the largest varieties of dyes available because they are simple and inexpensive to synthesize. Although the dye industry itself does not produce wastewater, the environmental contamination caused by these pervasive dyes can be overlooked. Wastewater from its upstream and downstream companies is considerable, especially in the downstream printing and dyeing industry. The printing and dyeing factories not only use dyes in the dyeing process but also need the dyeing agent, pre-treatment, post-cleaning and other processes, resulting in a large amount of wastewater discharge [2,3]. Since dyes are designed to be chemically stable and photolytic stability, they are highly persistent in the natural environment [4]. Depending on statistics, each ton of textile printing and dyeing consumes 100–200 tons of water, of which 80%–90% is discarded as wastewater, during the treatment of wastewater contaminated with sulfur dyes, many sulfides are deposited in the activated sludge. After a period of time, the activated sludge at the bottom of the biochemical tank is replaced, and the original sludge is dried and used for the incineration of power generation materials. A considerable amount of SO_2

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is emitted by combustion, causing serious acid rain problems. Increasingly stringent legislation makes the search for appropriate treatment technology a significant priority [5].

Sulfur black, a black scaly solid, is a high-sulfur polymer compound utilized primarily for dyeing cotton textiles. It is formed by the reaction of 2,4-dinitrophenol and sodium polysulfide in an aqueous solution [6]. This type of sulfur black dye is part of the most productive dyes in China. Its main structure is still not affirmed. Some scholars believe that when the vulcanization temperature reaches 110°C, the structure is shown in Fig. 1 [7]. Recently, the color index online version has registered a new sulfur dye of C.I. Leuco Sulfur Black 19, trade name: EcoSol Liq Cloud Grey ISG. This product is utilized for dye gray, and it is rare in the series of sulfur dyes that are known for their deep dark colors [2]. Sulfur black dyes have huge future market application demands. Sulfur dye wastewater has characteristically high Chroma (500~500,000), low dissolved oxygen, high suspended solids, poor biodegradability, high heavy metal and salt content, high refractory organic pollutants content, large pH range (6~13) and high chemical oxygen demand (10,000~75,000 mg L^{-1}) [8]. Presently, physicochemical methods such as coagulation sedimentation, adsorption, membrane filtration, and advanced oxidation are commonly used [7,9–13]. These methods have disadvantages including complicated operation, high equipment costs, numerous personnel requirements, and the formation of hazardous by-products [14]. Owing to these drawbacks, economical and eco-friendly methods for the treatment of industrial effluent are urgently needed.

This study focuses on screening a highly efficient sulfur-oxidizing microbial strain from the environment and being used for the biological treatment of dyeing wastewater containing sulfur black. To our knowledge, there is not any report on the use of microorganisms to treat sulfur-containing black dye wastewater. The parameters of the reaction were optimized using single factor optimization and orthogonal design. We have further identified the degradation intermediates of sulfur black using liquid chromatography-tandem high-resolution mass spectrometry (LC-MS/MS) in an aqueous system.

2. Materials and methods

2.1. Sampling sites and powdered sulfur black dye

At different sites in the southern suburbs of Changzhou City (Jiangsu, China), 10 g soil samples with a depth of 5~10 cm and activated sludge from the bottom of the aeration tank and biochemical pool of sulfur black wastewater (Wastewater Treatment Plant, Black Peony Group, Changzhou) were collected in sterile 20 mL sealed bags and stored at 4°C in the dark until the sulfur black degrading bacteria were isolated. Powdered sulfur black dye was provided by Black Peony (Group) Co., Ltd., Changzhou, Jiangsu, China.

2.2. Analysis of SO_4^{2-} in the degradation system

Barium chromate spectrophotometry was used to assess the sulfate content [15,16]. The specific operation method can be discovered in the Environmental Protection Industry Standard of the People's Republic of China (HJ/T 342–2007) [17]. Fermentation broth (5 mL) was centrifuged and the supernatant was done to an appropriate concentration. Hydrochloric acid (1 mL, 2.5 mol L^{-1}) was added, boiled for about 5 min, combined with 2.5 mL of strontium chromate suspension of heat, and then boiled for another 5 min. After chilling, 50% ammonia water was added dropwise to the Erlenmeyer flask until the solution was lemon yellow. After the solution cooled, it was filtered through slow qualitative filter paper (the pore size is 10~15 μm), and collected in a colorimetric tube and brought up to 50 mL in volume with distilled water. The absorbance was measured at 420 nm wavelength, and the standard curve for the relationship between absorbance and sulfate concentration was used to calculate the sample sulfate contents. The SO_4^{2-} concentration in solution was calculated using the following equation:

$$C = \frac{m}{v} \times n \times 1{,}000 \quad (1)$$

where C is the concentration of sulfate (mg L^{-1}), m is the mass of sulfate (mg) in the water sample calculated according to the standard curve, v is the water sample volume (mL), and n is the sample dilution factor.

2.3. Isolation and purification of sulfur black degrading bacteria

Lysogenic broth (LB) medium, mineral salt medium (MSM) and agar plate/slant medium were used to isolate and purify the strains (Table S1). The initial enrichment medium contained 50 mg L^{-1} of sulfur black, and the subsequent concentration in the media was varied according to experimental needs. Samples (3 g) of the soil and activated

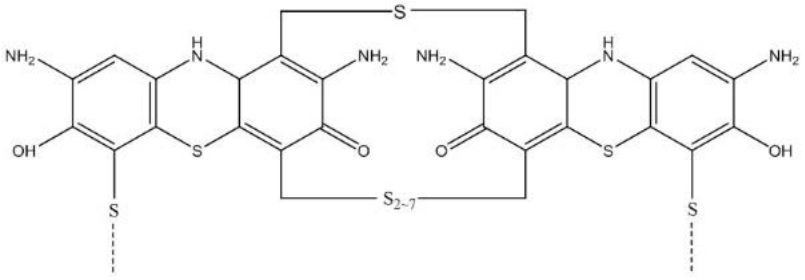

Fig. 1. Sulfur black molecular structure.

sludge were mixed into 250 mL Erlenmeyer flasks containing 50 mL of sterile saline and were allowed to stand for 30 min after being fully mixed by shaking. Enrichment cultures were performed in 250 mL Erlenmeyer flasks containing 50 mL of LB medium and 2 mL of the supernatant. These flasks were incubated at 28°C and 120 rpm for 2 d in a rotary shaker. Then, 5 mL of the inoculum was transferred to fresh MSM contained 50 mg L^{-1} of sulfur black for subsequent subculture. After 5 subcultures, 200 μL of the inoculum in the flask was streaked on MSM. The carbon and sulfur source in the solid medium was 100 mg L^{-1} sulfur blacks. Phenotypically different colonies were isolated and strains were streaked for purity. Isolated purebred strains were preserved at 4°C on LB agar slants and at –80°C in LB broth supplemented with 25% sterilized glycerol [18].

2.4. 96-well tissue culture primary screenings and shake flask re-screening

Isolated strains obtained through enrichment cultures were inoculated in 96-well tissue culture plates [19] with 5% of inoculum (V/V), and cultured at 28°C while shaking at 160 rpm for 3 d. The absorbance of the fermentation broth was measured at 600 nm with a microplate reader (Spark, TECAN, Switzerland). Strains with higher and lower absorbance than average were selected for shake flask rescreening. The SO_4^{2-} content in the degradation system was tested to identify a strain with augmented desulfurization capability.

2.5. Identification of a sulfur black degrading strain

The morphological characterization of the bacterial strain was conducted by gram staining and optical microscopy [20]. The specificity of bacterial strain synthesis and catabolic products was determined by a series of physio-biochemical experiments [21]. Cell morphology was observed by a cold field emission scanning electron microscope (SEM, Hitachi, Japan). The 16S rRNA gene sequence analysis of the sulfur black degrading bacteria strain was performed. The gene fragment was sequenced by Shanghai ShengGong Biological Engineering Technology Service Co., Ltd., (Table S2). A 16S rRNA nucleotide blast search was performed utilizing the National Center for Biotechnology Information (NCBI) sequence database. Sequences were utilized to generate phylogenetic trees using Molecular Evolutionary Genetics Analysis (MEGA, version 6.0) [22,23].

2.6. Optimization of degradation conditions of sulfur black in an aqueous system

Biodegradation studies were made in a series of 250 mL sterile Erlenmeyer flasks. A total of 2.5 mL of inoculum was transferred aseptically to each glass flask containing 50 mL of MSM supplemented with varying amounts of sulfur black as the only carbon and sulfur source. The pH of the broth was monitored with a precision pH meter (Sartorius Group, Germany). The control test was made in the same manner as above but without bacteria. Initial degradation conditions contained 100 mg sulfur black per liter of liquid MSM medium [24,25].

We have investigated the impacts of different types of carbon and nitrogen sources on the desulfurization effect of sulfur black through single-factor experiments. The earlier reports demonstrated that the surfactant Tween 80 could improve the bio-desulfurization ability [26–28]. Tween 80 (report volume) was in addition to the medium to observe its effect on the desulfurization by the strain. Based on the three conditions above combined with inoculation quantity, temperature, rotation speed and pH, the orthogonal test design of 7 factors and 3 levels was performed to obtain the optimal conditions for the degradation of sulfur black. All tests were conducted in triplicate.

2.7. Analysis of sulfur black biodegradation products

The culture was centrifuged at 8,000 rpm for 5 min, and the supernatant was to be sent through a 0.45 μm aqueous filter. We found that the fermentation broth has a gradual but significant rise in the absorption peak at 260 nm over the first 5 d by sampling the fermentation broth day for full-wavelength scanning (Fig. S3). Biodegradation intermediates were monitored by high-performance liquid chromatography (HPLC; Agilent 1260, Agilent Technologies, Santa Clara, CA). A UV detector was operated at 260 nm, with an Agilent ZORBAX Eclipse Plus C18 (5 μm, 4.6 mm × 250 mm) and 30°C column temperatures. Pure water (HPLC grade) was elected at a flow rate of 0.8 mL min^{-1}.

Agilent 1260 HPLC systems coupled with Agilent G6230 TOF LC/MS with an electrostatic ionization (ESI) source were used for LC-MS/MS analysis. The separate conditions were in line with those used for HPLC analysis. The sheath gas temperature was controlled at 250°C, and the capillary voltage was –3.5 kV. The analysis mode of ionization was ESI (positive). The operation conditions were as follows: nozzle voltage, 500 V; nebulizer, 45 psi; dry gas, 5 L min^{-1}; and a dry temperature, 300°C. The continuous full scanning of m/z 50–1,000 Da was performed in positive-ion mode.

3. Results and discussion

3.1. Isolation and characterization of sulfur black degrading strains

Thirty-five strains of bacteria and molds with distinct phenotypes were obtained by enrichment culture, separation and purification. These strains were antiquated into 96-well tissue culture plates with mineral salt medium containing sulfur black. After 3 d of culture, the 96-well tissue culture plates primary screening results were obtained and are shown in Table 1.

The DS-9/31/23/27 strains have the higher and DS-4/24/11/1 strains have the lower concentrations than the bacteria. It shows that the ability of different bacteria to use sulfur black is also variable. Because sulfur black is the only carbon source in MSM, the strains with elevated concentrations of bacteria indicate that they can use sulfur black as the sole carbon source for their own growth efficiently, otherwise they are inefficient. The DS-2/28 to strong decolonizing ability mycelium grew vigorously and sank into the bottom of the 96-well tissue culture plates, which

Table 1
96-well plate primary screening results

Strain	OD_{600}	Strain	OD_{600}	Strain	OD_{600}
DS-1	0.4645	DS-13	0.6832	DS-25	0.7105
DS-2	0.4953	DS-14	0.6671	DS-26	0.5315
DS-3	0.7179	DS-15	0.7364	DS-27	0.8303
DS-4	0.3515	DS-16	0.5644	DS-28	0.9959
DS-5	0.6853	DS-17	0.7331	DS-29	0.7881
DS-6	0.5022	DS-18	0.7408	DS-30	0.3973
DS-7	0.7778	DS-19	0.9433	DS-31	1.0119
DS-8	0.7164	DS-20	0.7440	DS-32	0.5007
DS-9	1.0754	DS-21	0.5051	DS-33	0.7348
DS-10	0.7121	DS-22	0.4515	DS-34	0.6275
DS-11	0.4510	DS-23	0.8511	DS-35	0.5145
DS-12	0.5620	DS-24	0.4200	CK	0.2289

made it impossible to measure the absorbance at 600 nm. However, the different growth rates of strains in mineral salt medium containing sulfur black do not indicate the ability to oxidize and desulfurize sulfur black. Therefore, the above 10 strains were chosen to undergo shake flask re-screening experiments and to directly measure sulfate. The sulfate product content directly represents the strength of desulfurization capacity for each strain.

Ten strains were cultured in shake flask degradation systems for 7 d, and the sulfate content in the fermentation broth was measured and is presented in Fig. 2. The SO_4^{2-} content in the control test was 1,785.96 mg L^{-1} while the amount of SO_4^{2-} was the highest in the DS-9 strain shake flask degradation system, reaching 2,063.52 mg L^{-1}. Report to the control, the desulfurization capacity of DS-9 is increased by 16%. Therefore, this strain was selected as the initial strain for the ensuing study. There is a definite relationship between the type of bacteria and the type of carbon source used for screening. Nguyen et al. [29] used sulfur blue as the sole carbon source to screen out a strain of bacteria (*Acidithiobacillus thiooxidans*), this bacterium can degrade sulfur blue into sulfuric acid, and it has high-efficiency adsorption capacity, which can adsorb colonies to the surface of sulfate particles.

Gram staining and SEM showed that DS-9 had no flagella, gram-negative, short rod-like, and often in the distribution of 2 or 4 (Figs. S1 and S2). Starch hydrolysis and catalase tests were positive, while the gelatin hydrolysis, methyl red (MR), VP, indole, citrate, phenylalanine deaminase, H_2S production, and oxidase tests were all negative (Table S3).

Bacteria strains were classified according to the best 16S rRNA match in the Gen Bank database. Isolated bacteria strain DS-9 had the highest similarity of all the sequences closely related to *Acinetobacter* sp. MO (99% identity). Draw a phylogenetic tree of bacterial strains based on 16S rRNA through the crystal and neighbor connection algorithm of MEGA6.0 (Fig. 3)

3.2. Optimization of degradation conditions of an isolated strain

MSM with 100 mg L^{-1} of sulfur black was inoculated with the isolated DS-9 strain and cultured under the condition of 28°C and 200 rpm for 7 d in a rotary shaker. The mass of SO_4^{2-} in the cultures and the pH were recorded daily. The dynamic curve of sulfate and pH over time is plotted in Fig. 4. The sulfate content in the fermentation liquid reached the highest on the fifth day, so the culture time in the subsequent optimizations was set to 5 d. In the initial 5 d, the pH of the fermentation broth gradually decreased, as the concentration of SO_4^{2-} increased. Notably, the content of SO_4^{2-} decreased after the fifth day, while the

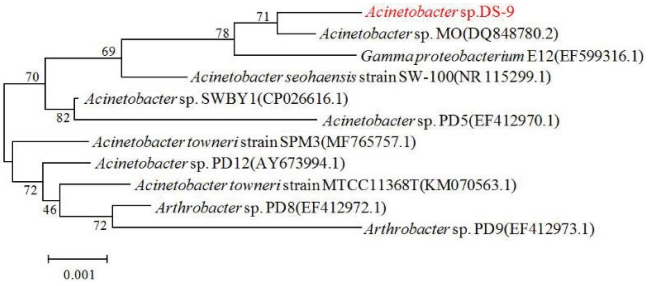

Fig. 3. Phylogenetic tree of strain DS-9.

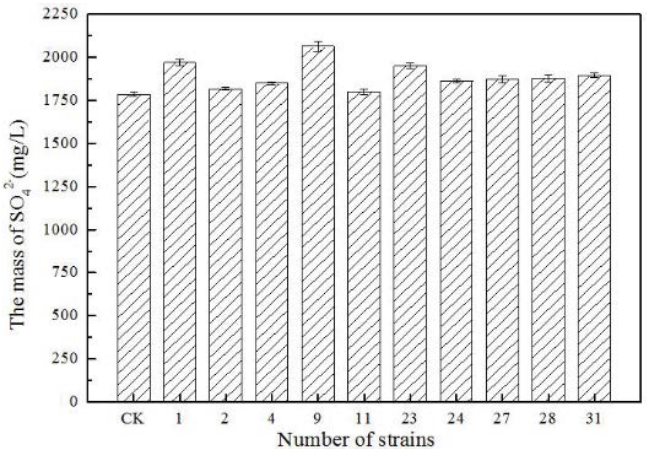

Fig. 2. Shake flask re-screening results.

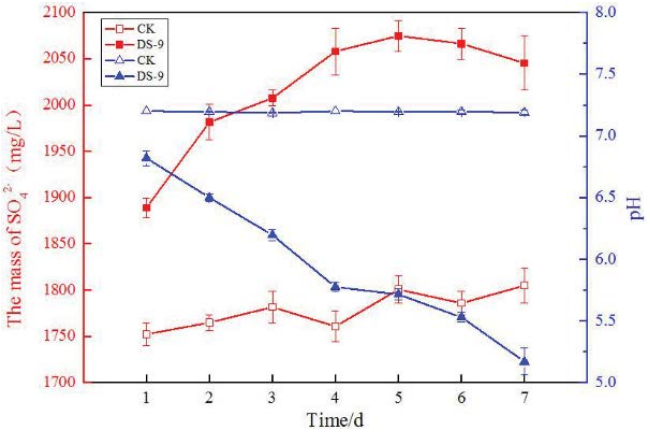

Fig. 4. The SO_4^{2-} and pH change curves of DS-9 in the initial MSM for 7 consecutive days.

pH continued to decrease. These results suggest that the lower pH of the environment may cause bacterial cell lysis, releasing intracellular substances, thereby affecting the ability of bacteria to decarburize.

After the addition of the carbon source (soluble starch), nitrogen source (NH_4Cl) and Tween 80, the desulfurization capacity of DS-9 improved as shown in Fig. 5. Report to the control test, the sulfate concentration in the experimental group increased by 34.76%, 29.41% and 32.18%, respectively.

The purpose of supplementing the carbon and the nitrogen sources is to allow the bacterial cells to grow and multiply in the initial degradation stage. This allows cultures to reach a higher cell concentration, beneficial for efficient biological desulfurization of sulfur black. Tween 80 is a non-ionic surfactant whose use significantly improves the biological desulfurization ability of DS-9. The mechanism may be that Tween 80 acts as a dispersing agent so that the bacterial cells are more divided up in the medium. Secondary, the permeability of the cellular membrane is improved, and the enzymes in the microbial cells can be easily secreted through the cellular membrane to act on the sulfur bond in the sulfur black. The improved permeability of the cellular membrane is also beneficial to the chemical substances entering and leaving the cell, strengthening the biological oxidation ability of the dye. Lastly, improved permeability enhances the dissolution and absorption properties of the dye, and also improves the biological desulfurization efficiency.

Seven factors and three levels of orthogonally designed experiments were performed by single factor tests combined with inoculum amount, temperature, rotation speed and initial pH factors on desulfurization efficiency. The precise factors and levels of the orthogonal test are provided in Table 2. The test was outlined with the orthogonal table designed by Orthogonal Design Assistant II (Latin, version 3.1). The test results are given in Table 3. Optimal degradation conditions for strain DS-9 were 5% inoculum, NH_4Cl 400 mg L^{-1}, 200 rpm, 500 mg L^{-1} soluble starch, 28°C, initial pH 7.2, and 7 g L^{-1} Tween 80.

The SO_4^{2-} concentration and pH in the degradation system were measured for 7 d under the optimal degradation conditions. The curve of sulfate content and pH with time is illustrated in Fig. 6. On the fourth day, the sulfate concentration (2773.34 mg L^{-1}) in the optimal degradation system was greater than that in the control test (1823.81 mg L^{-1}). The sulfate concentration increased by 52.06%, and the optimization goal of the final desulfurization condition was ultimately achieved. The degradation ability of sulfur-oxidizing bacteria is linked to the fermentation conditions. Under the optimal fermentation conditions, sulfur oxidative degradation ability can reach the optimal value. Under optimal fermentation conditions (initial pH 11.7, initial sulfur blue dye concentration of 300 mg L^{-1} and initial biomass concentration of 1.0 g L^{-1}), the sulfur blue dye was degraded by 50% on the fourth day [29].

In addition, some researchers also use physical and chemical methods to degrade sulfide. Li et al. [30] used environmentally-friendly reducing agents to degrade the sulfur in sulfur black dye wastewater, decreasing the mass fraction of reducing sulfur in the wastewater by 32%. A combination of multiple methods may better treat dye wastewater,

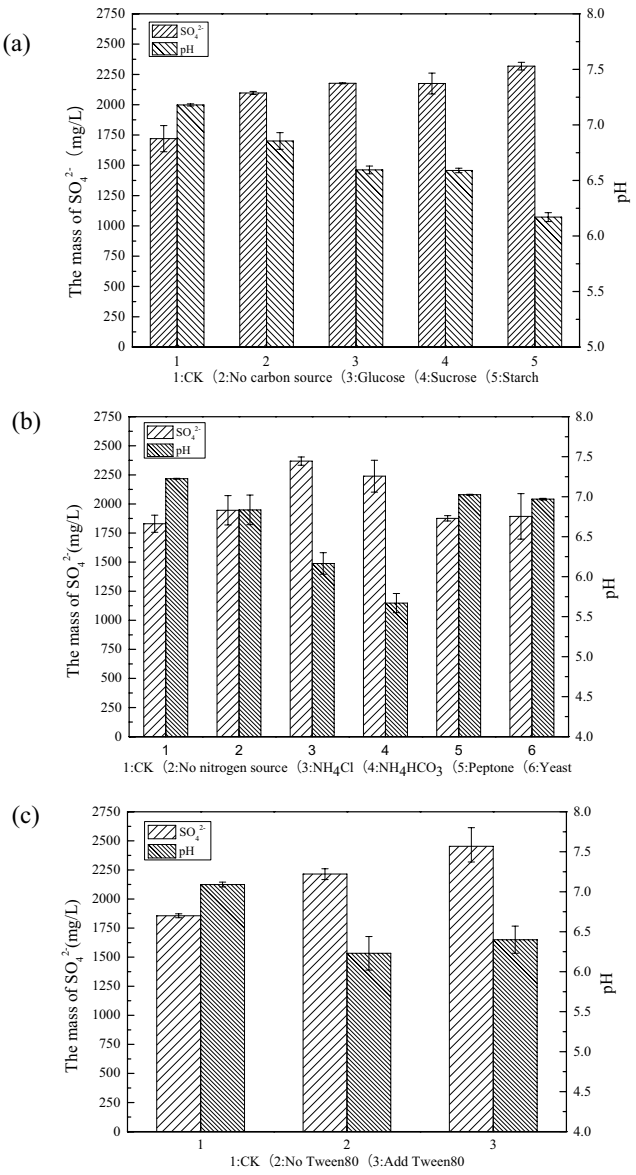

Fig. 5. (a) Effect of adding carbon source on desulfurization efficiency and final pH in initial MSM containing sulfur black 100 mg L^{-1} after 5 d of culture. (b) Effect of adding nitrogen source on desulfurization efficiency and final pH in initial MSM containing sulfur black 100 mg L^{-1} optimized for carbon source after 5 d of culture. (c) Effect of adding Tween 80 on desulfurization efficiency and final pH in initial MSM containing sulfur black 100 mg L^{-1} optimized for carbon and nitrogen source after 5 d of culture.

some researchers are combined two methods to degrade dyes in wastewater. Thanavel et al. [31] use a combination of the oxidation process and biological treatment to degrade dyes in dye wastewater. The separate oxidation process method and biological treatment method were reacted for 9 h under optimal reaction conditions respectively to degrade the dye (100 mg L^{-1}) by 90% and 63.07%. When the two methods are used to treat wastewater, only the dye degradation rate reached 100% in 4 h.

Table 2
Orthogonal test factor level

Level	Factors						
	A Glucose	B NH_4Cl	C Tween 80	D Temp.	E rpm	F Initial	G Inoculation quantity
	mg L^{-1}	mg L^{-1}	g L^{-1}	°C	r min^{-1}	pH	%
1	200	100	2	23	150	6.7	1
2	500	400	4	28	200	7.2	5
3	800	700	6	33	250	7.7	9

Table 3
Orthogonal test results

Run no.	A	B	C	D	E	F	G	SO_4^{2-} (mg L^{-1})
1	1	1	1	1	1	1	1	1,703.95
2	1	2	2	2	2	2	2	2,369.11
3	1	3	3	3	3	3	3	2,129.39
4	2	1	1	2	2	3	3	2,070.51
5	2	2	2	3	3	1	1	1,755.09
6	2	3	3	1	1	2	2	2,055.11
7	3	1	2	1	3	2	3	1,790.16
8	3	2	3	2	1	3	1	1,969.57
9	3	3	1	3	2	1	2	2,095.74
10	1	1	3	3	2	2	1	1,881.25
11	1	2	1	1	3	3	2	2,088.76
12	1	3	2	2	1	1	3	1,999.01
13	2	1	2	3	1	3	2	1,856.02
14	2	2	3	1	2	1	3	2,078.25
15	2	3	1	2	3	2	1	1,805.55
16	3	1	3	2	3	1	2	2,116.77
17	3	2	1	3	1	2	3	2,398.54
18	3	3	2	1	2	3	1	2,055.11
Comprehensive scores of various factors								
K1	2,028.578	1,903.110	2,027.175	1,961.890	1,997.033	1,958.135	1,861.753	
K2	1,936.755	2,109.887	1,970.750	2,055.087	2,091.662	2,049.953	2,096.918	
K3	2,070.982	2,023.318	2,038.390	2,019.338	1,947.620	2,028.227	2,077.643	
R	134.227	206.777	67.640	93.197	144.042	91.818	235.165	

As shown in Fig. 6, the content of sulfate in the control test without DS-9 did not change notably with treatment time, and the sulfate concentration in the DS-9 experimental group gradually increased with time, reaching the maximum on the fourth day. Afterwards, they all had a tendency to remain stable. From the sixth day forward, the pH continued to drop while the sulfate concentration did not rise. These results are in agreement with the previous experimental results. As the strain grows and reproduces, the metabolites gradually accumulate, resulting in a gradual decrease in the pH value in the medium, which affects the growth and reproduction of the strain, so that the sulfate concentration no longer changes.

3.3. Identified sulfur black degradation intermediates

During the degradation of sulfur black in aqueous systems, two degradation intermediates were detected by

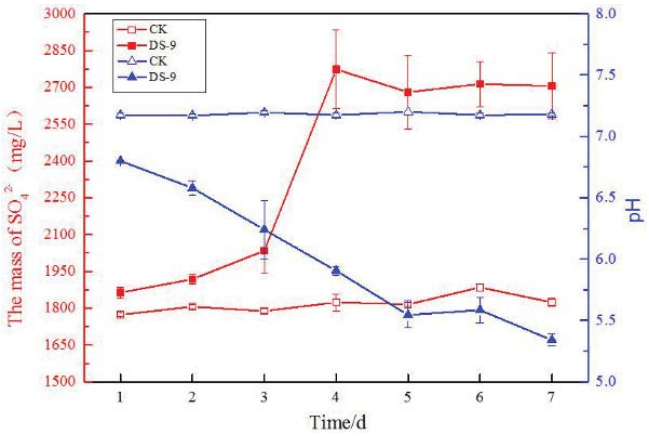

Fig. 6. The SO_4^{2-} and pH change curves of DS-9 were continuously cultured for 7 d in the optimized degradation system.

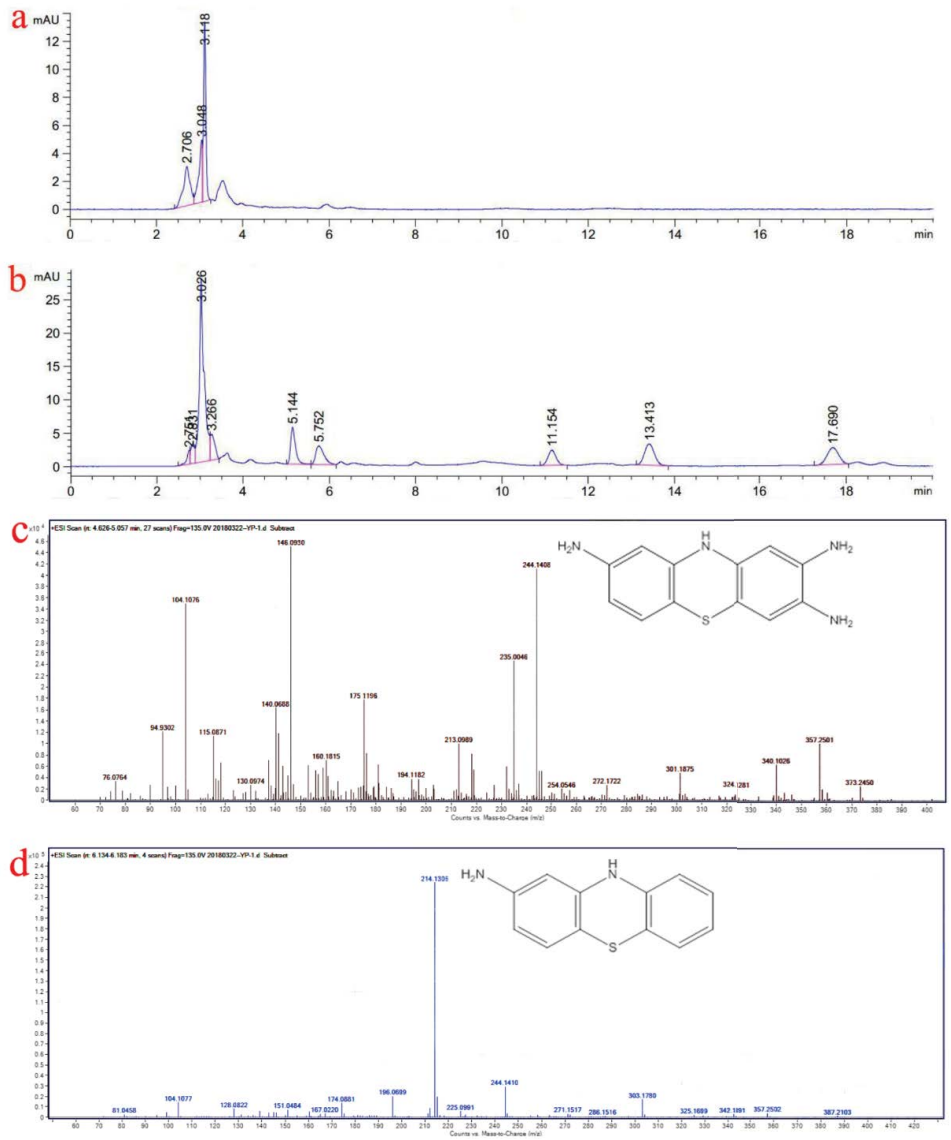

Fig. 7. (a) HPLC chromatogram of CK after 5 d of culture. (b) HPLC chromatogram of DS-9 after 5 d of culture. (c) Mass spectra of metabolites (I1) formed during sulfur black biodegradation in aqueous systems. (d) Mass spectra of metabolites (I2) formed during sulfur black biodegradation in aqueous systems.

HPLC analysis with a retention time of 5.1 min (I1) and 5.7 min (I2). These intermediates only occurred in the DS-9 degradation system, as shown in Figs. 7a and b. Two degradation intermediates of sulfur black were analyzed and identified by high-performance chromatography-time-of-flight mass spectrometry (LC-MS) and LC-MS/MS.

Intermediate I1 was identified as 10H-phenothiazine-2,3,8-triamine according to its LC-MS/MS and MS^2 spectra shown in Fig. 7c. The ion fragment of I1 was at m/z 244.1408 ($C_{12}H_{12}N_4S$). The Intermediate I2 was at m/z 214.1306 ($C_{12}H_{10}N_2S$). I2 likely corresponded to the loss of the group of 3,8-amine on I1 and was identified as 10H-phenothiazin-2-amine shown in Fig. 7d. The type of intermediate is tied to the source of sulfide. When Dai et al. [32] studied the single-chamber air cathode microbial fuel cell, he found that the intermediates produced during the degradation of sulfide by sulfate-reducing bacteria mainly included 3,4-diaminonaphthalene-1-sulfonic acid, sodium 4-amino-naphthalene-1-sulfonate and 4,4′-diamine biphenyl.

4. Conclusions

The present study was focused on the screening of biological desulfurization degrading bacteria for textile dye sulfur black and resulted in the isolation of *Acinetobacter* sp. DS-9 can be used for sulfur black biological desulfurization. The optimum medium and degradation conditions for the strain were found by single factor experimentation and orthogonal condition optimization. The results showed that in the DS-9 optimal degradation system, the desulfurization capacity for sulfur black improved by 52% and two intermediate degradation products were identified by HPLC and LC-MS/MS. In this study, the acquisition of strain DS-9 provides the

possibility of studying the biological desulfurization mechanism of textile dye sulfur black and industrial treatment of textile printing and dyeing sewerage with sulfur black.

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Supporting information

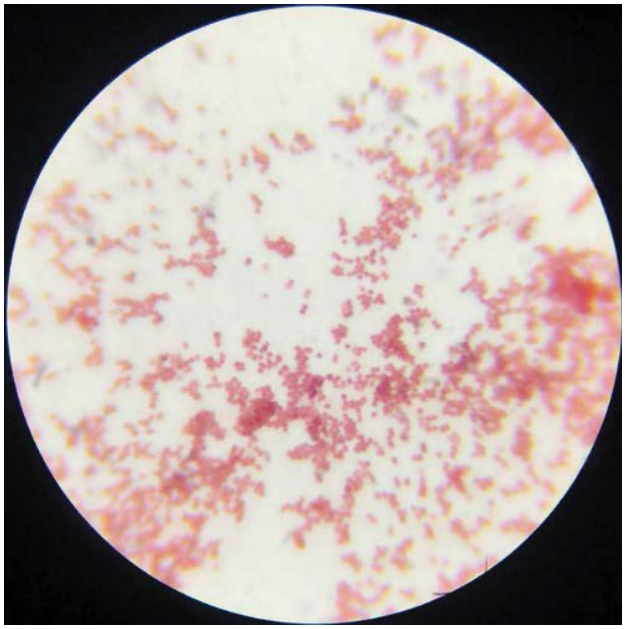

Fig. S1. Gram staining image of strain DS-9.

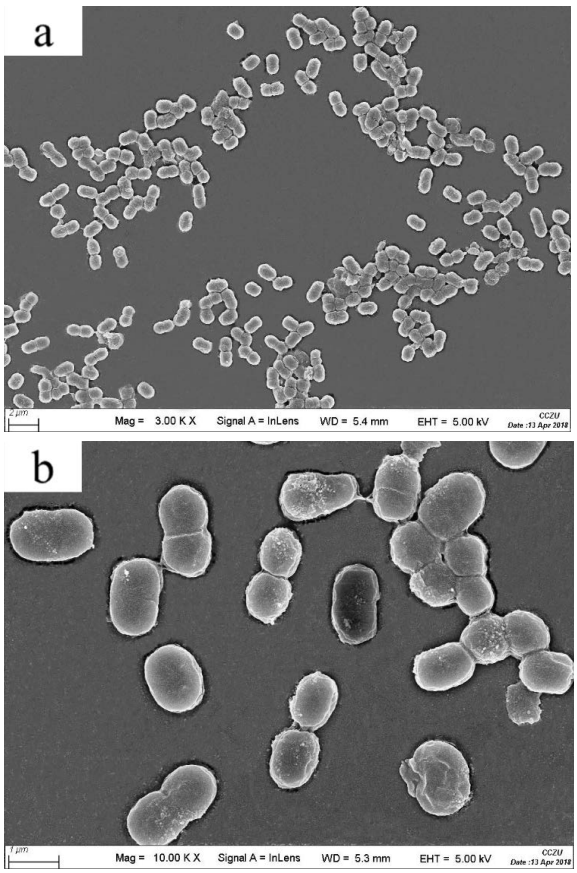

Fig. S2. SEM image of strain DS-9.

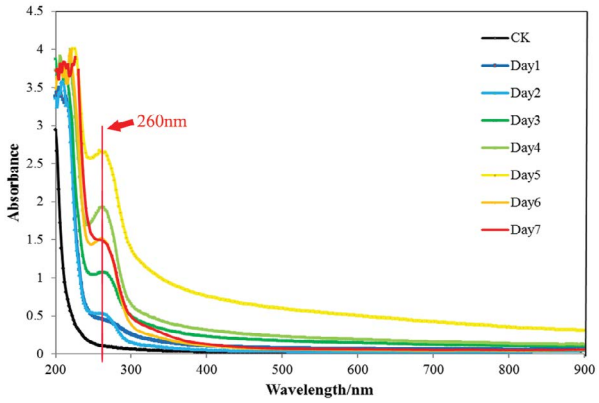

Fig. S3. Full-wavelength scanning image.

Table S1. Isolation and purification of sulfur black degrading strains

Type of medium	Carbon source	Effect
Lysogenic broth (LB)	Sulfur black	Enrichment culture
Mineral salt (MSM)	Sulfur black	Subculture
MSM Agar Plate	Sulfur black	Scribe separation and purification
LB Agar Plate/Slant	Sterilized glycerol	Preservation

Table S2. 16S rRNA gene sequence of DS-9

GGCAGGCTTAACACATGCAAGTCGAGCGGGGGAG
GTTGCTTCGGTAACTGAGCTAGCGGCGGACGGGTGAG
TAATGCTTAGGAATCTGCCTATTAGTGGGGGACAA
CATTCCGAAAGGGATGCTAATACCGCATACGTCCTAC
GGGAGAAAGCAGGGGCTCTTTATGACCTTGCGCTA
ATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGG
TAAAGGCCTACCAAGGCGACGATCTGTAGCGGGTCT
GAGAGGATGATCCGCCACACTGGGACTGAGACACGG
CCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
GACAATGGGGGGAACCCTGATCCAGCCATGCCGCGT
GTGTGAAGAAGGCCTTTTGGTTGTAAAGCACTTTAAGT
GGGGAGGAGGCTTACCTGGTTAATACCTGGGATAAGT
GGACGTTACCCACAGAATAAGCACCGGCTAACTCTGT
GCCAGCAGCCGCGGTAATACAGAGGGTGCGAGCGTTA
ATCGGATTTACTGGGCGTAAAGCGCGCGTAGGTGGT
TAATTAAGTCAAATGTGAAATCCCCGAGCTTAACTTG
GGAATTGCATTCGATACTGGTTAGCTAGAGTATGGGA
GAGGATGGTAGAATTCCAGGTGTAGCGGTGAAATGC
GTAGAGATCTGGAGGAATACCGATGGCGAAGGCAG
CCATCTGGCCTAATACTGACACTGAGGTGCGAAAG
CATGGGGAGCAAACAGGATTAGATACCCTGGTAGTC
CATGCCGTAAACGATGTCTACTAGCCGTTGGGGGATTT
GATCCTTTAGTGGCGCAGCTAACGCGATAAGTAGAC
CGCCTGGGGAGTACGGTCGCAAGACTAAAACTCAAAT
GAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGATGCAACGCGAAGAACCTTACCTGG
CCTTGACATAGTAGAAACTTTCCAGAGATGGAAAG
GTGCCTTCGGGAATCTACATACAGGTGCTGCATGGCT
GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTC
CCGCAACGAGCGCAACCCTTTTCCTTACTTGCCAG
CATTTCGGATGGGAACTTTAAGGATACTGCCAGTGA
CAAACTGGAGGAAGGCGGGGACGACGTCAAGTCAT
CATGGCCCTTACGGCCAGGGCTACACACGTGCTA
CAATGGTCGGTACAAAGGGTTGCTACCTCGCGAGAG
GATGCTAATCTCAAAAAGCCGATCGTAGTCCGGATC
GCAGTCTGCAACTCGACTGCGTGAAGTCGGAATC
GCTAGTAATCGCGGATCAGAATGCCGCGGTGAATAC
GTTCCCGGGCCTTGTACACACCGCCCGTCACACCAT
GGGAGTTTGTTGCACCAGAAGTAGGTAGTCTAACCG
CAAGGAGGACGCTTACCACGGTGTGGCCGATGACTGG
GGTGAAGTCGTAAC

Table S3. Physiological and biochemical characteristics of strain DS-9

Experiment	Experimental result
Starch hydrolysis	+
Catalase tests	+
Gelatin hydrolysis	–
Methyl red (MR)	–
VP	–
Indole	–
Citrate	–
Phenylalanine deaminase	–
H2S production	–
Oxidase tests	–