

Bioaugmentation Strategy for Treatment of Sulfur Black Wastewater Through Sequential Fenton Oxidation and Biological Process by Two Sulfide-oxidizing Strains

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Abstract: In order to develop an affective bioaugmentation strategy for the removal of sulfur black and increase sulfide-oxidization capability in biological treatment, bioaugmentation strains with higher sulfide-oxidizing capability, *Acinetobacter* sp. DS-9 and *Aspergillus* sp. DS-28, were isolated from a municipal wastewater (WW) treatment plant and selected to treat textile sulfur dyeing WW combined with Fenton oxidation. The sequential WW treatment process was evaluated in a bench-scale activated sludge tank. The performance of the bioreactor demonstrated the feasibility of bioaugmentation by strain DS-9 and DS-28 in terms of almost sulfur black removal, COD and color removal, significant sulfide removal in activated sludge. The effect of Fenton oxidation process, additional carbon source, bioaugmentation strains composition etc. was investigated. The bioaugmented process after Fenton oxidation and inoculation of DS-9 and DS-28 could maintain stable performance in terms of COD, color and sulfur removal from the WW. The capability of color and COD removal by bioaugmentation strains were greater than that by the original activated sludge from WW treatment plant. Sulfate concentration increased significantly from 140.5 to 485 mg L⁻¹. The outlet color and COD value reach 5 and 46.52 mg L⁻¹ after the sequential Fenton oxidation and bioaugmentation treatment.

Keywords: Sulfur Black Wastewater, Fenton Oxidation, Biodegradation, Bioaugmentation, Sulfide Bio-oxidization

1. Introduction

Sulfur dyes have a group of sulfur-containing, complex synthetic organic dyes applied from an alkaline solution of sodium sulfide to cellulose, where they become substantive to the fibre [18, 22, 32]. It is one of the largest variety of dyes in China and widely used in denim dyeing [5, 14, 18, 19, 22, 32]. Sulfur black is one of high-sulfur polymer compounds widely applied in dyeing cotton textiles. Its molecular structure was not confirmed because of the uncertain sulfur number in sulfur black (Figure 1) [5, 13, 18, 22]. During the dyeing process including dyeing agent, pre-treatment and post-cleaning, etc., a large amount of wastewater was discharged [7, 11, 18, 32]. Water consumption per ton of textile printing and dyeing is

100-200 tons, 80-90% of which is wastewater [9, 11]. The dyeing WW has high chroma and COD value, and difficult to treat and decolorize [6, 8, 18, 19, 22]. The untreated WW has mutagenic and carcinogenic effects on microorganism, plants and animals [4, 12, 20, 26].

Physicochemical methods, such as adsorption, membrane filtration, photocatalyst, Fenton oxidation and advanced ozone oxidation, etc., have been studied used in the pre-treatment process before biological treatment, because the WW containing sulfur dyes has poor biodegradability and low dissolved oxygen. Then biological treatment, such as membrane bio-reactor (MBR), sequencing batch reactor activated sludge process (SBR) etc. was used in WW treatment plant [4, 6-8, 10, 13, 14, 17, 19, 22, 29]. Biological

treatment often produce a large amount of activated sludge, the dried sludge after pressure filtrated was often used for incineration materials mixed with coal for power generation. However, the dried sludge contained a certain amount of sulfur from sulfur dyes, which lead to a large amount of SO_2 emission into air and SO_2 could cause serious acid rain.

In order to decrease sulfur content in activated sludge, *Aspergillus* sp. DS-28 and *Acinetobacter* sp. DS-9 have higher sulfur bio-oxidation capability, sulfates (SO_4^{2-}) and sulfuric acid (H_2SO_4) are generated during biological treatment using

the strain DS-28 and DS-9. In this study, sequential Fenton oxidation and biological process using the strain DS-28 and DS-9 were combined and used for efficient treatment of textile dyeing WW containing sulfur black. To our knowledge, no study has been reported on sequential Fenton oxidation and sulfur bio-oxidation processes for treatment of WW containing sulfur black dyes in order to decrease sulfur concentration in sludge, which can improve efficiency of comprehensive utilization of sludge and prevent secondary pollution.

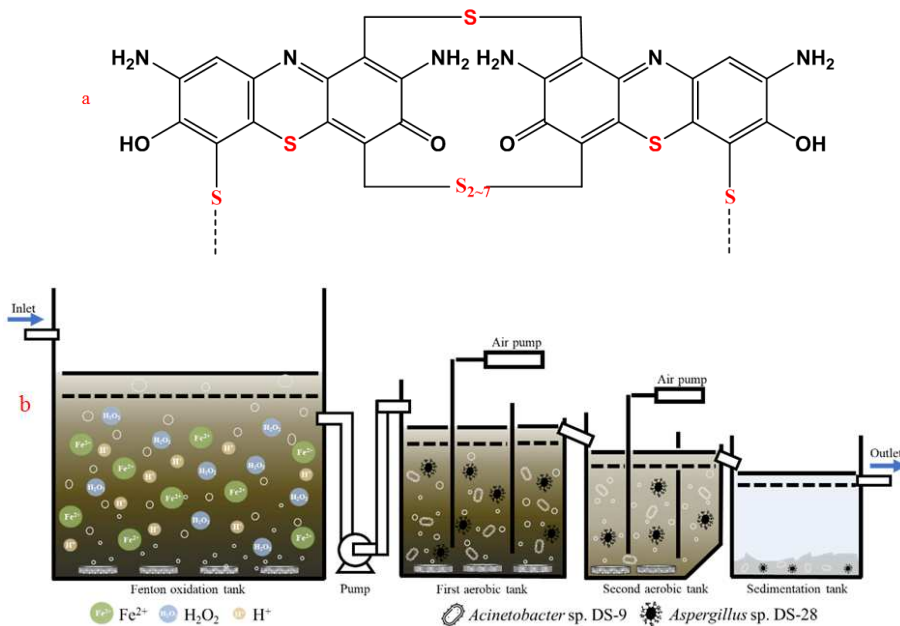


Figure 1. Molecular structure of Sulfur Black (a) and Schematic layout of sequential Fenton-biological process (b).

2. Materials and Methods

2.1. Chemicals and Microorganism

Dye of sulfur black (Figure 1a) was obtained by Black Peony (Group) Co., LTD., Changzhou, Jiangsu, China. WW was obtained from Yixing Lucky Textiles Group Co. Ltd. and WW Treatment Plant, Black Peony Group, Changzhou, China. Other chemical reagents were purchased from Sinoreagent Company and Sangon Biotech (Shanghai) Co. Ltd, and were of analytical grade and of highest purity available. Microorganisms (*Acinetobacter* sp. DS-9 and *Aspergillus* sp. DS-28) with higher capability of sulfur oxidation, were isolated from activated sludge in aerobic tank and stored in the Lab. of Applied Microbiology and Biotechnology, Changzhou University.

2.2. Fenton Oxidation Experiments

Fenton oxidation experiments were performed in 2 L tank reactor at room temperature, 100 rpm for 0.5. 1000 mL of dye WW was added in glass reactor. The solution was acidified to pH 3.0 using 1 M H_2SO_4 . The appropriate amount of $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ was added and the reaction was started when the H_2O_2 was added. In order to achieve the best decolorization and COD removal effect, different ratio of

$\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ and H_2O_2 was studied and optimized.

2.3. Decolorization and Sulfur Biological Oxidation Experiment

Sequential decolorization and sulfur biological oxidation experiments were performed with 2 L tank, two aerobic tanks and one sedimentation tank (Figure 1b). The strains of *Acinetobacter* sp. DS-9 and *Aspergillus* sp. DS-28, had higher decolorization and sulfur oxidation capability, were selected for decolorization and sulfur black degradation experiments. The inoculum was 3 g L^{-1} . The blank experiment was carried out at the same time.

The strain D-9 and DS-28 were activated with LB medium for 24 h, then centrifuged at 6000 rpm for 10 min at 4°C . The cells were washed twice with the sterile NaCl solution (0.85%). Cells were inoculated and in the tank.

The sequencing decolorization experiment was adjust pH value to 7.0 and stayed for 2 h at room temperature after Fenton oxidation. The WW from Yixing Lucky Textiles Group Co. Ltd contained COD concentration of 1800 mg L^{-1} and color chroma of 400 times, respectively. After 48-72 h, the cell grew up and the first decolorization process was finished, the WW after Fenton oxidation was spilled into the second aerobic tanks. Aeration intensity was 10 mL min^{-1} in the aerobic tank. The two reactors were joined up after the

bacterial population had been able to degrade the sulfur black WW. Last step was sedimentation tank and the effluent from sedimentation tank was collected to measure the value of chroma, COD and sulfate concentration.

2.4. Kinetic Analysis

In order to estimate the decolorization and COD removal characteristics of bioaugmentation bacterial strains, Michaelis-Menten kinetics Eq. (1) was used to calculate the V_{max} and K_m .

$$v = \frac{v_{max} S}{S + K_m} \quad (1)$$

where v is specific degradation rate of substrate COD (h^{-1}), S is the substrate COD concentration (mg l^{-1}), v_{max} is the maximum specific degradation rate (h^{-1}), K_m is the Monod saturation constant (i.e. substrate concentration at half v_{max} , mg l^{-1}).

$$\frac{1}{v} = \frac{K_m}{v_{max}} \times \frac{1}{S} + \frac{1}{v_{max}} \quad (2)$$

Equation 2 is the linear equation, In order to estimate the degradation characteristics of bioaugmentation bacterial strains, v_{max} and K_m could be calculated using equation 2.

2.5. Analytical Method

All analyses were carried out in accordance with Standard Methods for the Examination of Water and wastewater [25]. COD, pH, chroma, decolorization ratio and dye concentrations were monitored. Barium chromate spectrophotometry and the method of HJ/T 342-2007 were used for determining the sulfate content [21, 30]. Chemical oxygen demand (COD) was measured with potassium dichromate method [21, 30]. UV-vis spectrophotometer (Gold Spectrumlab 53, Shanghai, China) was used to determine the absorbance (A) and transmittance (T) of the solution before and after reaction, measuring the absorbance of the solution at the maximum absorption wavelength. The decolorization rate was calculated as the formula:

$$\text{decolorization rate (\%)} = \frac{A_0 - A_t}{A_0} \times 100 \quad (3)$$

where, A_0 : initial absorbance of the solution, A_t : absorbance of the solution at any time interval after reaction. Each decolorization experiment was performed in triplicate and mean of decolorization rate were reported.

3. Results and Discussion

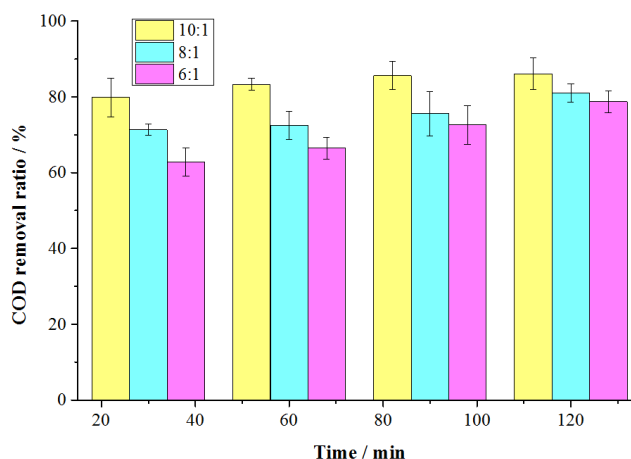
3.1. Fenton Oxidation of Dyeing WW Containing Sulfur Black

As shown in Figure 2, the biodegradability of raw dyeing WW was poor due to the higher concentration of sulfur black

and COD. The ratio of BOD_5/COD (0.28) was relative lower than the limitation value for WW biodegradation of 0.4, as described in previous reports [3]. Several publications showed that different factors affected COD removal ratio and decolorization efficiency of Fenton oxidation in the following order: $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ molar ratio > H_2O_2 concentration > initial pH > reaction time > temperature [28]. Thus H_2O_2 concentration, the $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ molar ratio and reaction time were investigated during the process of Fenton oxidation in this study.

Under the condition of $\text{H}_2\text{O}_2/\text{COD}$ concentration ratio was 1.5, 1.0 and 0.5, the $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ molar ratio was 10:1, 8:1 and 6:1, results of COD removal ratio was showed in Figure 2. Higher $\text{H}_2\text{O}_2/\text{COD}$ concentration ratio and $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ molar ratio gained higher COD removal and decolorization percent, and also higher processing cost. Based on the results and chemical cost, the optimized results of Fenton oxidation were achieved with $\text{H}_2\text{O}_2/\text{COD}$ concentration ratio of 1:1 (w/w), a molar ratio of H_2O_2 to Fe^{2+} of 8:1, initial pH 3.0 and reaction time of 60 min. Through the Fenton oxidation process, the COD concentration decreased from 1800 mg/L to 399.16 mg/L, chroma descended from 500 to 123, respectively. COD removal ratio and decolorization rate reach 77.83% and 75.40%, respectively. BOD_5/COD increased significantly from 0.28 to 0.76. The results indicated that the dyeing WW containing sulfur black could be effectively pretreated using Fenton oxidation to enhance sulfur black biodegradability.

During the Fenton-oxidation process, H_2O_2 is almost sole source of HO^\bullet and plays a very important role in the treatment of WW [28]. The optimum H_2O_2 dosage and $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ molar ratio must be determined experimentally. Insufficient H_2O_2 dosage or relative low $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ molar ratio can result in a decrease in WW treatment efficiency due to a deficiency of HO^\bullet generated. However, excessive H_2O_2 dosage or $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ molar ratio is also not encouraged because a massive amount of H_2O_2 could have a negative effect on the microbial reactivity in the subsequent bio-treatment process, and excessive H_2O_2 dosage also increase the treatment cost [28, 31].



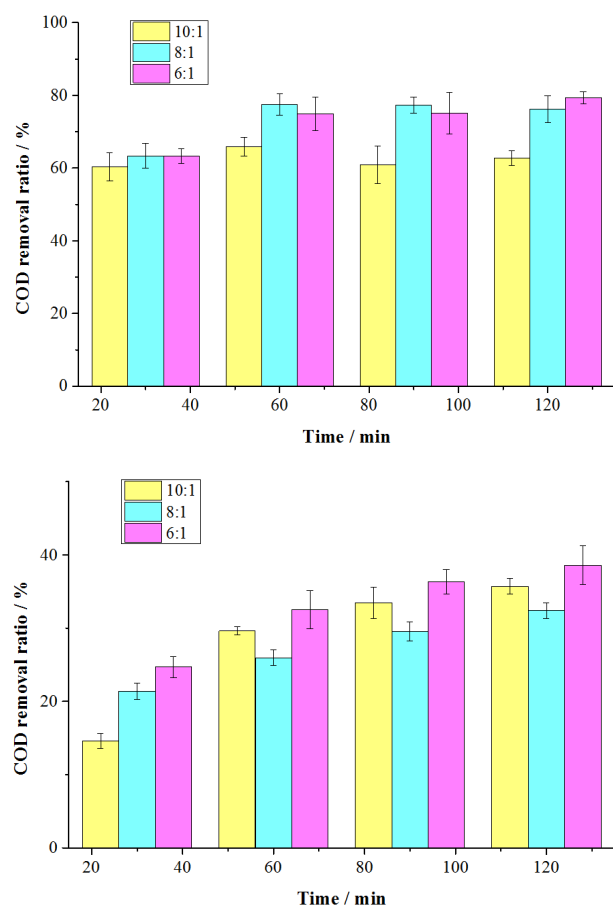


Figure 2. The effect of H_2O_2/Fe^{2+} molar ratio on COD removal ratio (H_2O_2/COD concentration ratio 1.5 (a), 1.0 (b) 0.5 (c)). Phylogenetic tree of strain ds-28 based on the 18S rRNA gene homology.

3.2. Biological Treatment of Dyeing WW

3.2.1. The Effect of Additional Carbon Source on Sulfur Oxidation Efficiency

In order to determine the characteristics of WW containing sulfur black biodegradation, especially additional carbon source, sulfate content, the change of sulfur black molecule structure, optimal operation parameters with dyeing WW biological treatment by sulfur-oxidizing strains, *Acinetobacter* sp. DS-9 and *Aspergillus* sp. DS-28, etc. were investigated.

Three kinds of additional carbon source (glucose, sucrose and soluble starch, 500 mg L⁻¹) was added into dyeing WW, respectively. The strain DS-9, DS-28 and bioaugmentation strains (the mixture of DS-9 and DS-28, 1:1, w/w) were also inoculated in WW, respectively. Additional carbon source (glucose, sucrose and soluble starch) can accelerate cell growth as well as the decolorization rate as shown in Figure 3. The carbon source from sulfur black was difficult to use for cell growth compared with additional carbon source in this experiment, glucose, sucrose and soluble were easily oxidized in respiration and produced ATP and other products through glycolysis and the Krebs cycle [1, 2, 23, 26]. Several reports showed that addition of significant organic carbon is desirable co-substrates for bacterial dye decolorization process [1, 2, 7, 23, 26]. In this study, the decolorization percentage of sulfur black was higher than the control and non-additional group.

Soluble starch was the best additional carbon source for the strain DS-28, and glucose was the best additional carbon source for the strain DS-9. Based on the experimental results, bioaugmentation strains showed higher decolorization capability and sulfur oxidation efficiency. The maximal COD removal ratio reach 85.47% and sulfur oxidation efficiency increased 38.85% from 1520 mg L⁻¹ to 2111mg L⁻¹ after bioaugmentation strains inoculation. However the decolorization ratio, COD removal and sulfur oxidation efficiencies were relative lower under the condition of inoculation of DS-28 or DS-9 (Figure 3).

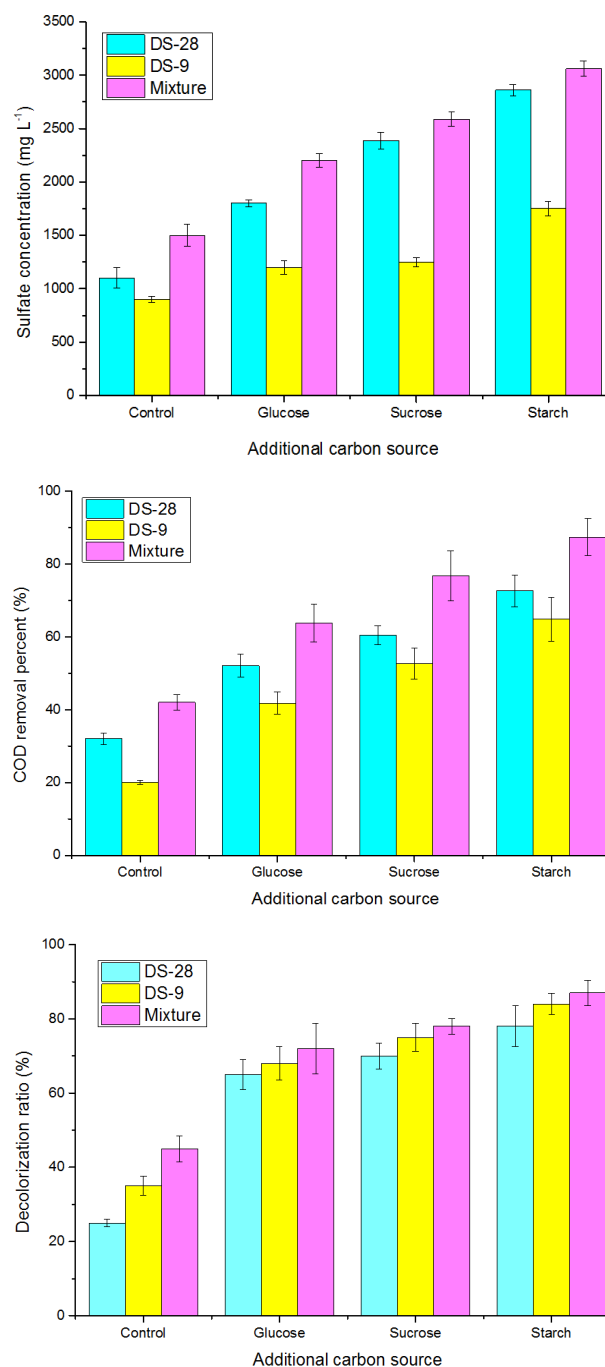


Figure 3. Effect of adding carbon source on sulfate concentration (a), COD removal percent (b) and decolorization ratio (c) by the strain DS-28, DS-9 and bioaugmentation strains.

3.2.2. Composition of Bioaugmentation Strains on WW Treatment

Several reports showed that bacteria composition and their ratio could affect COD removal ratio and decolorization efficiency etc. [6, 16, 24]. In this study in order to investigate the effect of the composition of bioaugmentation strains (ratio of strain DS-9/DS-28, w/w, 1:1, 2:1, 3:1, 1:2, 1:3) on decolorization ratio, COD removal and sulfur bio-oxidation efficiency, temperature, pH, and starch concentration were kept constant at 28 °C, 7.2, and 500 mg L⁻¹, respectively, for 5 days, while different ratio of strain DS-9 to strain DS-28 was inoculated into the reactor. Figure 4 depicted that as the sulfur oxidation strains composition (DS-9:DS-28, w/w, 1:1, 2:1, 3:1) changed from 1:1 to 3:1, sulfate concentration increased significantly from 4201.8 mg L⁻¹ to 4661.2 mg L⁻¹, while it was only 3100 mg L⁻¹ in the control group. The cell growth rate was relatively rapid under the condition of 3:1 composition (data not shown), and COD removal percent reach maximum, however, decolorization ratio was only 90%. When the strain DS-9/DS-28 ratio was 1:2, COD removal percent and sulfate concentration decreased sharply, and

decolorization ratio increased. Similar observations have been recorded earlier for decolorization, strain DS-9 was belonged to the family of *Acinetobacter* sp., strain DS-28 was *Aspergillus* sp. [16, 24]. The content and ratio of *Aspergillus* sp. DS-28 increased, the capability of absorb dye also increased, therefore the decolorization ratio increased, and COD removal percent and sulfate concentration decreased. The figure 5 showed that the strain DS-28 could absorb sulfur black during decolorization process. Many reports showed that fungus could absorb dyeing during dyeing WW bio-treatment [15, 16], at the meanwhile, fungus also can express and secrete dye degradative enzymes to degrade dyes [6, 15, 16]. Therefore, selection of these two species for bioaugmentation was beneficial for enhancement of biodegradation of sulfur black. Several intermediates of sulfur black were also monitored and identified by LC-LC/MS (Figure 6) and the biodegradation pathway was proposed (Figure 7), revealing that the bioaugmentation bacteria was superior to the non-augmented for all effluent quality parameters analyzed (Figures 2, 3, 4, Tables 1, 2).

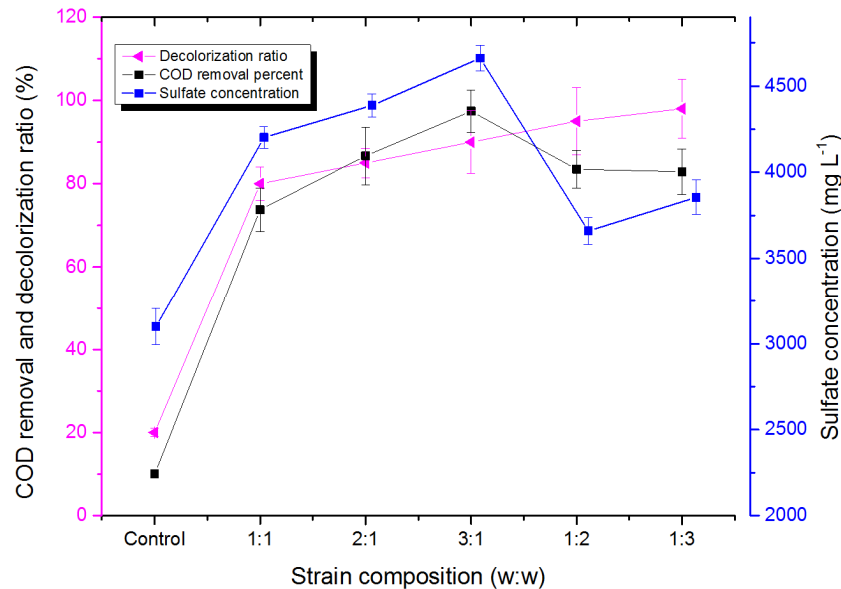


Figure 4. The Effect of Composition of bioaugmentation strains on wastewater biological treatment.

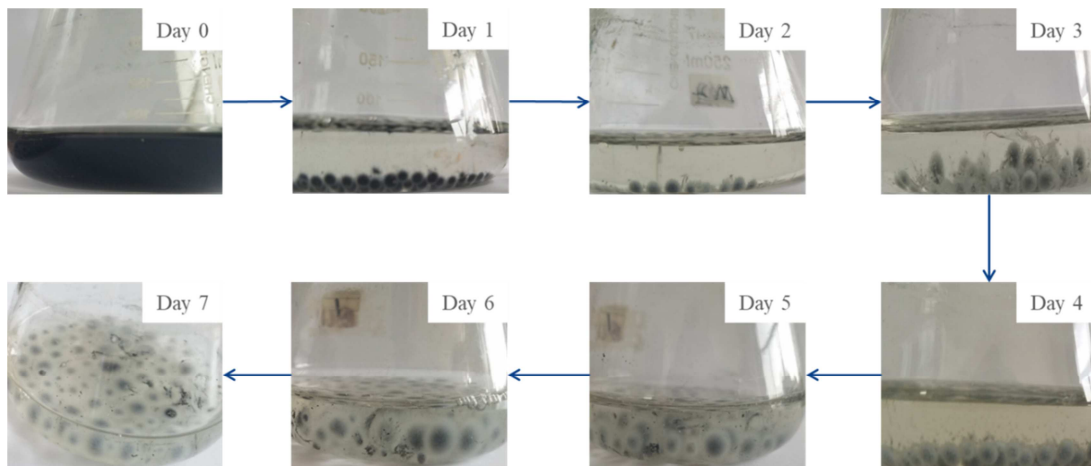


Figure 5. The Change of decolorization process by the strain of *Aspergillus* sp. DS-28.

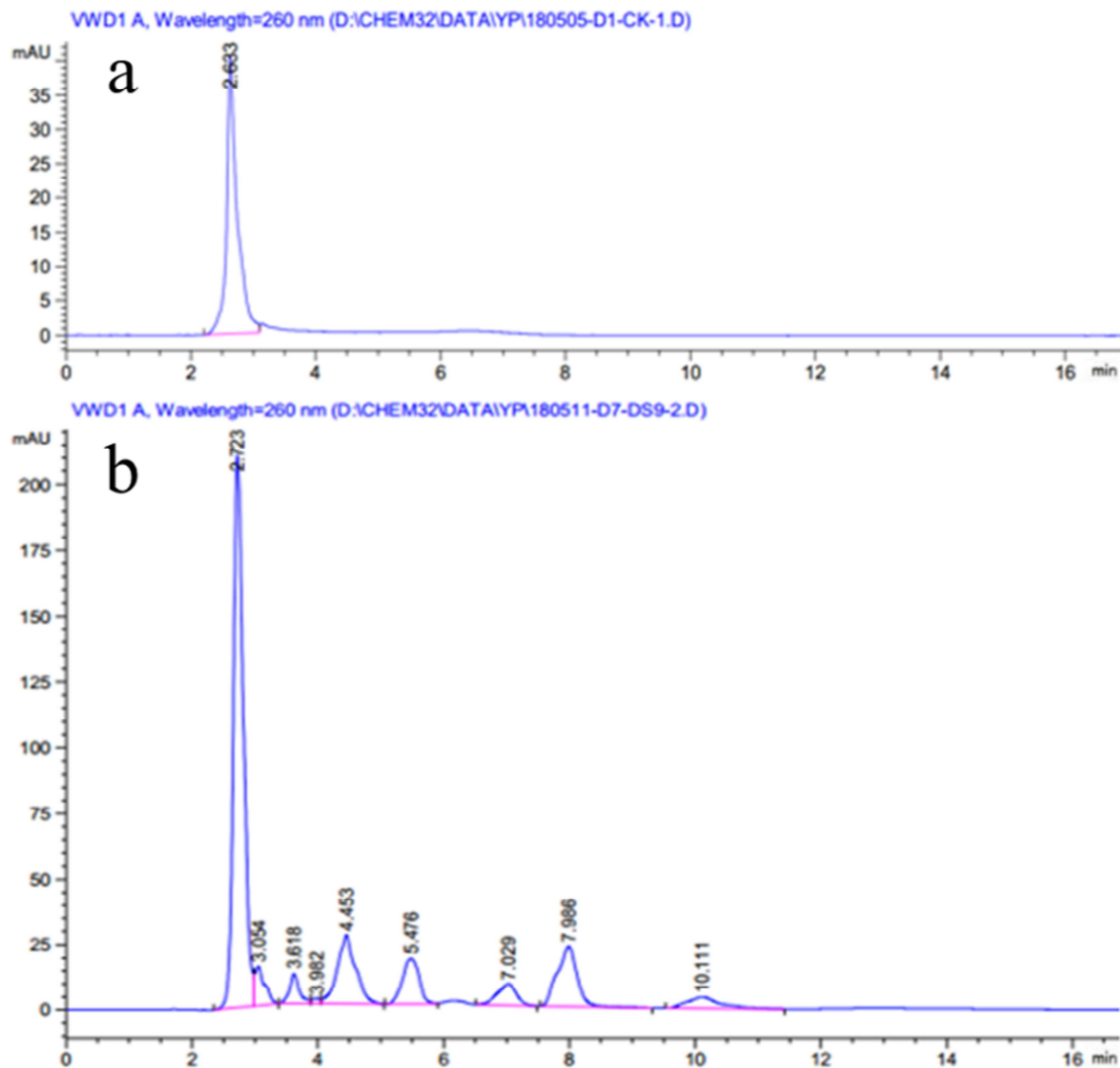


Figure 6. LC-MS / MS diagram of sulfur black before (a) and after (b) treatment biological treatment.

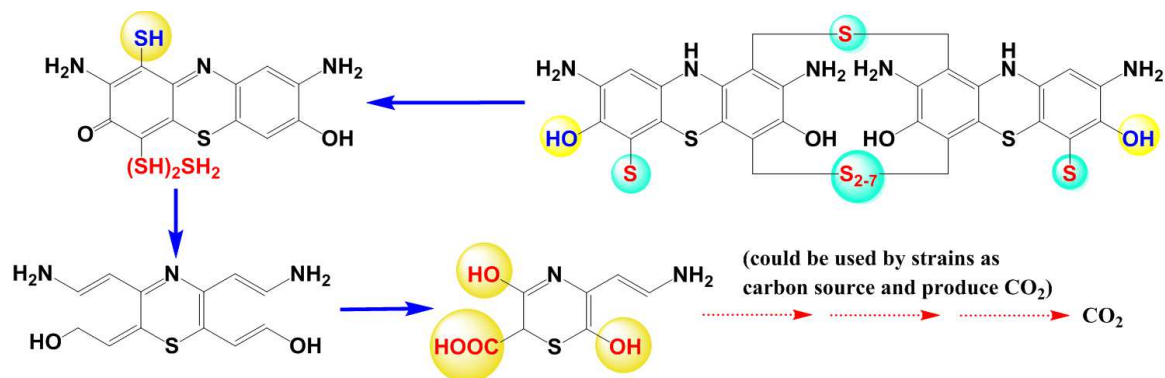


Figure 7. The proposed biodegradation pathway by the bioaugmentation strains.

Table 1. The results of dye wastewater containing sulfur black sequential Fenton-Biological treatment.

Treatment process	Inlet chroma (times)	Outlet chroma (times)	Color removal (%)	Inlet COD (mg L ⁻¹)	Outlet COD (mg L ⁻¹)	COD removal (%)	Inlet Sulfate concentration (mg L ⁻¹)	Outlet Sulfate concentration (mg L ⁻¹)
Fenton oxidation tank	500 ± 30	113 ± 10	77.40	1800 ± 120	399.16 ± 15	77.82	—	—
The first aerobic biological tank	113 ± 10	15 ± 4	86.73	399.16 ± 15	108.7 ± 9.5	72.76	140.5 ± 11	392 ± 25
The second aerobic biological tank	15 ± 4	5 ± 1	66.67	108.7 ± 9.5	46.52 ± 5	55.36	392 ± 25	485 ± 32

Table 2. Kinetic parameters evaluated for bioaugmentation strains of COD and sulfate concentration.

	K_m (mg L ⁻¹)	v_{max} (h ⁻¹)	R ²	Michaelis-Menten Equation
COD concentration	116.28	829.97	0.9832	$v = 829.97 \times S / (S + 116.28)$
Sulfate concentration	151.52	960.78	0.9991	$v = 960.78 \times S / (S + 151.52)$

3.3. Sequential Batch Treatment Through Fenton Oxidation and Biological Treatment

The overall color and COD removal ratio were above 95% through sequential Fenton oxidation and biological processes. Sulfate concentration also increased 70% from 120 mg L⁻¹ to 485 mg L⁻¹. During the Fenton oxidation treatment, H₂O₂/COD concentration ratio and molar ratio of H₂O₂ to Fe²⁺ were 1:1 (w/w) and 8:1, respectively, initial pH value was adjusted to 3.0 and reaction time was 60 min. The COD concentration decreased from 1800 mg/L to 399.16 mg/L, chroma descended from 500 to 113, respectively. COD removal ratio and decolorization rate reach 77.82% and 77.4%, respectively. Table 1 showed that the color removal, COD reduction and sulfate generation in first and second aerobic tank. In the first aerobic biological process, the outlet COD value decreased from 400 to 108 mg L⁻¹, COD removal reached 72.76%, sulfate concentration increased from 140.5 to 392 mg L⁻¹ by bioaugmentation bacterial strains. And in the second aerobic biological process, the COD of outlet water was only 46.52, color chroma was 5. The sulfate concentration reaches 485 mg/L.

The capability of color and COD removal by bioaugmentation bacterial strains were greater than that by original activated sludge from WW treatment plant. Sulfate concentration increased significantly, from 140.5 to 485 mg L⁻¹. The outlet color and COD value reach 5 and 46.52 mg L⁻¹ after the second biological process by the strains, which was sufficient meet China standards for discharge (a fixed emission standard of 60 mg L⁻¹ COD and 20 color is required in south of Jiangsu Province, China).

From the results obtained in this study, kinetic analysis was performed using Michaelis-Menten kinetics models. Decolorization removal and sulfate concentration increased are the main indicators, which were used to assess the efficiency of the decolorizing process. According Michaelis-Menten kinetics equation were calculated as in Table 2. It is clear that Michaelis-Menten kinetics model gives a good correlation and kinetic analysis was performed used Michaelis-Menten kinetics equation. The table 2 demonstrated that the K_m and v_{max} of COD and sulfate concentration were both good correlation. The K_m and v_{max} of COD concentration were 116.26 mg L⁻¹ and 829.97 h⁻¹, and K_m and v_{max} of sulfate concentration were 151.52 mg L⁻¹ and 960.78 h⁻¹, which illustrated that COD removal and sulfur bio-oxidation were occurred at almost same time, and the bioaugmentation strain (mixture of DS-28 and DS-9) had higher capability with sulfur bio-oxidation.

4. Conclusions

In the present study, a sequential Fenton oxidation and

bioaugmentation process was developed and employed for efficient treatment of textile dyeing WW containing sulfur black. The bioaugmentation bacteria (mixture of *Acinetobacter* sp. DS-9 and *Aspergillus* sp. DS-28) with higher capability of sulfur oxidation was used for bioaugmentation potential. The study of the effect of some factors of Fenton oxidation and biological treatment showed that increase in H₂O₂/Fe²⁺ molar ratio enhanced the decolorization efficiency and COD removal ratio. Amendment of dying WW with up to 500 mg L⁻¹ soluble starch increase decolorization efficiency, sulfur bio-oxidation capability and degradation rate, whereas further increase did not have any effect. The composition of bioaugmentation strains structure also can affect sulfur bio-oxidation and decolorization efficiency. The results in this study will provide technical support for sulfur dyeing WW deep and harmless treatment, and it also provide theoretical guidance for decreasing the sulfur content in activated sludge and other similar studied of other pollutants containing sulfur.

Conflict of Interest

All the authors do not have any possible conflicts of interest.

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