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Original Research Paper

Oxidation of Reduced Inorganic Sulphuric Compounds in Simulated Desulphurization Wastewater by *Thiobacillus Thioparus*

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ABSTRACT

This paper is aimed to investigate the oxidation ability of the bacteria (*Thiobacillus thioparus*, Z-2) and the biological oxidation mechanism and oxidation kinetics on the reduced inorganic sulphuric compounds in sodium-process desulphurization wastewater. Results showed that the Z-2 bacteria have high oxidation activity in the high-salt environment after acclimation, and the oxidation percentage of reduced inorganic sulphuric compounds reached 95% and the chemical oxygen demand (COD) reduced from 18000mg/L to 2000 mg/L in the high-salt environment. This showed that the Z-2 bacteria have strong salt resistance and high oxidation activity. In addition, ion chromatography analysis confirmed that Z-2 bacteria were beneficial for the oxidation of reduced sulphur. A biological oxidation model was constructed by measuring the enzyme activity of the oxidation process. The results indicated that direct and indirect oxidation processes were both involved in biological oxidation. The kinetic parameters of V_{max} (21.38 μM/min) and K_m (79.04 μM) were obtained through biological oxidation kinetic experiments.

INTRODUCTION

Sulphur dioxide emissions from coal-burning has increased with the rapid development of the Chinese economy, which results in acid rain and the more serious sulphur dioxide pollution (Sinton et al. 2004, Liu et al. 2013). Industries widely use sodium-process flue gas desulfurization technology to treat the problem of sulphur dioxide pollution, such as coal-fired power plants (Nolan et al. 2004, Jeery et al. 2005). Sodium-process desulfurization technology has numerous advantages, such as its high efficiency, low operation cost and great economic benefits. However, wastewaters containing partially reduced inorganic salts generated by sodium-process desulfurization are difficult to manage because of their high-salt contents and high concentration of suspended solids (Dahl et al. 1997, Glass et al. 1999). Moreover, the increasing emission of sodium-process desulphurization wastewater, causes tremendous pressure on the environment.

Direct aeration oxidation is the most commonly used methods to treat sodium-process desulphurization wastewater. However, this method is limited by its poor oxidation efficiency and low COD removal rate. Chemical oxidation method has the disadvantage of its high operational cost (Liu et al. 2011, Hu et al. 2008, Hu et al. 2007). Synthetic adsorbent materials have been used to adsorb the pollutants in sodium-process desulphurization wastewater. However, the adsorbent regeneration and the secondary pollution caused by the regeneration solution are difficult to solve (Barron & O'Hern 1966, Mishra & Srivastava 1976). The biological oxidation method has been given much attention because of its evident advantages, including lower operational costs and improved treatment effects (Sugio et al. 2013). However, the high salinity of the sodium-process desulphurization wastewater may cause high extracellular osmotic pressure, which dehydrates bacterial cells. To date, only a few studies have successfully used the bio-oxidation method to treat the sodium-process desulphurization wastewater, and none of these methods has been used in practice. Thus, further study of oxidizing inorganic sulphur compounds and COD by the biological oxidation is required.

The novelty of this study is the application of microorganisms to oxidize reduced sulphur and to remove the COD. Z-2 bacteria obtained through strain filtration, separation and various stages of acclimation are resistant to high-salt environments and can perform efficient oxidation of reduced sulphur. The biological oxidation of the reduced inorganic sulphur compounds in the simulated desulfurization waste-water by Z-2 bacteria as well as its salt tolerance is studied. The biological oxidation mechanism and the oxidation kinetics are discussed in this paper.

MATERIALS AND METHODS

Microbial strain and culture conditions: *Thiobacillus thioparus* (referred to as Z-2), isolated from the sludge near the thermal power plant outfall in Shandong Province, was obtained by selection, separation and various stages of acclimation (Lin et al. 2013). The optimal cultivating conditions for the bacteria were set at a temperature of 35°C, an initial pH value of 7.5 and a rotation speed of 120rpm. The culture medium consisted of the following compounds: $(NH_4)_2SO_4$ 2.0g/L, K_2HPO_4 1.4 g/L, KH_2PO_4 0.6g/L, $MgSO_4$ 0.1g/L, $CaCl_2$ 0.1g/L, Na_2SO_4 10g/L, $Na_2S_2O_3$ 10g/L, Na_2SO_3 70g/L.

Water sample: The simulated wastewater sample containing sodium sulphite was prepared. The COD values of the water samples containing different concentrations of sodium sulphite were determined. Based on the testing data, the linear equation of the COD value (y) on the sodium sulphite concentration (x) was obtained, as shown in Fig. 1. Based on this linear equation, given the COD value (18000 mg/L) of the actual sodium desulphurization wastewater, the sodium sulphite dosage in the simulated water sample was calculated as 140g/L.

Determination of enzyme activity involved in sulphur metabolic pathways: The bacteria culture in the logarithmic growth phase was centrifuged at centrifugal force of 10000g for 20 min. The supernatant was decanted to retrieve the solid cells, which were washed with distilled water and centrifuged again at centrifugal force of 10000g for 20 min. These cells were suspended in a buffer solution precooled in an ice bath, and disrupted in an ultrasonic breaker. The supernatant was centrifuged at centrifugal force of 100000g and 3°C for 60 min, and the supernatant was collected to determine the amount of crude enzyme in the cytoplasm. The remaining solid cells were suspended in a buffer solution of 0.1mol/L tris-HCl (pH 7.5). Subsequently,

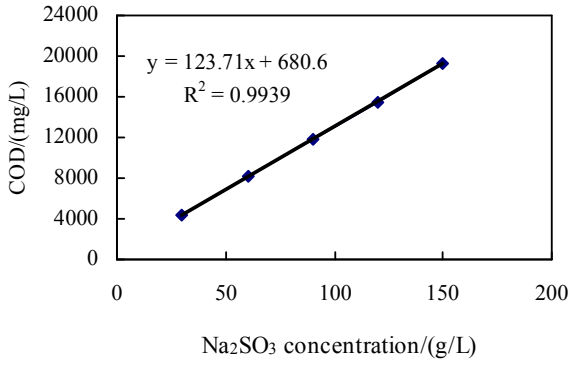

Fig. 1: Relationship of the Na_2SO_3 concentration and COD values in the simulated wastewater.

1% Triton X-100 at 4°C was added to the suspension, and the mixture was incubated for 24 h. Finally, the suspension was centrifuged at centrifugal force of 120000g and 3°C for 60 min. The supernatant was obtained to determine the crude enzyme activity in the cell membrane.

The amount of sulphite acceptor oxidoreductase (SAOR) and adenosine phosphosulphate (APS) reductase were measured using the optical density of reduced potassium ferricyanide at 420 nm. The amount of ATP sulfurylase was measured using the optical density of ATP at 280 nm. The standard reaction system of determining enzyme activity is given in Table 1 (Zimmermann et al. 1999).

Ion chromatography test: The change of the SO_3^{2-} concentration was determined by ion chromatography (Yin et al. 2009). The column was IonPac AS14A (250 nm×4 nm). The mobile phase was 14mmol/L NaOH-12mmol/L Na_2SO_3 (pH = 11.7). The current velocity and injection volume were 1.2 mL/min and 25 μL, respectively. The three-electrode system was introduced to test the performance, which had a titanium electrode as the counter electrode, a platinum electrode as the working electrode and Ag/AgCl as the reference electrode.

Analytical methods: The COD value was determined by the digestion method and SO_4^{2-} concentration was determined using barium chromate spectrophotometry (Chi et al. 2011, Zhang et al. 2006). The cell concentration was measured by its absorbance value at wavelength of 600 nm with deionized water as a reference. The oxidation percentage of reduced inorganic sulphur compounds in wastewater samples were calculated using the equation:

$$r\ (\%) = \frac{C_1}{C_0} \times 100\%$$

Where r is the oxidation percentage of the reduced inorganic sulphur compounds, C_1 is the SO_4^{2-} concentration in the water samples after treatment, and C_0 is the concentration of SO_4^{2-} before treatment.

RESULTS AND DISCUSSION

Salt acclimation of Z-2 bacteria: Z-2 bacteria were inoculated in the culture media with different mass concentrations of NaCl and 140 g/L of sodium sulphite. The growth of Z-2 bacteria and its biological oxidation percentage of reduced sulphur were determined at one-day intervals. The results are shown in Fig. 2. When the mass concentrations of NaCl were 20, 30 and 40g/L in the cultures, the growth of Z-2 bacteria was affected and the steady growth stage was delayed for 1, 2 and 3 d, respectively. These results indicated that Z-2 bacteria have an adaptive phase of adjusting the metabolism, to adapt the environment with an

Table 1: Standard reaction system for determining enzyme activity.

Enzyme activity	Materials in the reaction system
SAOR	2.0mM $K_3Fe(CN)_6$, 2.5mM Na_2SO_3, 2.5mM citric acid and enzyme
APS reductase	2.5mM Na_2SO_3, 2.0mM $K_3Fe(CN)_6$, 2.5mM AMP and enzyme
ATP sulfurylase	2.5mM Na_2SO_4, 2.0mM $K_3Fe(CN)_6$, 2.5mM ATP and enzyme

Table 2: Activity of the enzymes from Z-2 bacteria.

Enzyme activity	Reaction catalyzed	Enzyme activity in cytoplasm	Enzyme activity in cell membrane
SAOR	$SO_3^{2-} + 2Fe(CN)_6^{3-} = SO_4^{2-} + 2Fe(CN)_6^{4-}$	0.87	267.4
APS reductase	$AMP + SO_3^{2-} + 2Fe(CN)_6^{3-} = APS + 2Fe(CN)_6^{4-} + H^+$	43.58	0.12
ATP sulfurylase	$APS + HP_2O_7^{3-} = ATP + SO_4^{2-} + H^+$	45.82	0.39

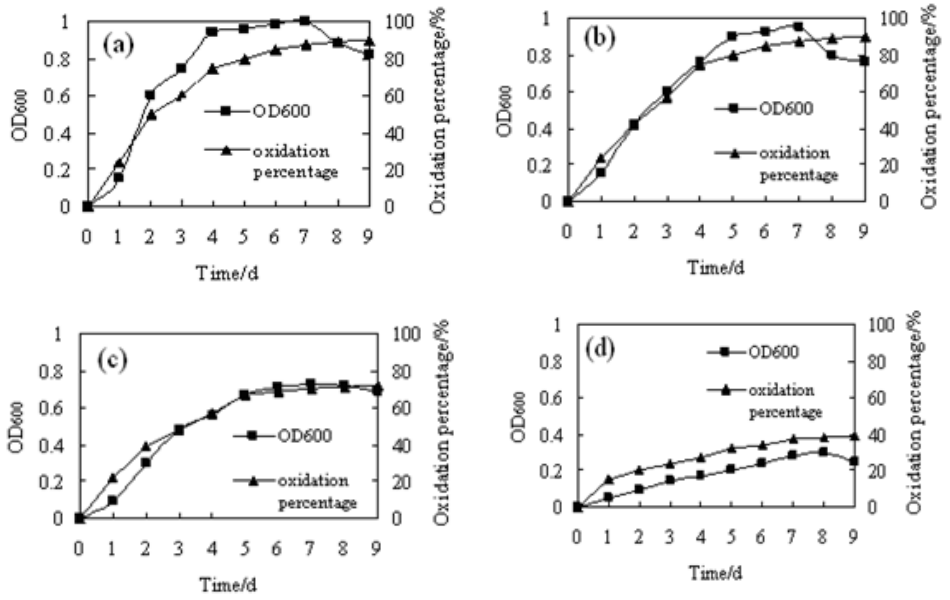

Fig. 2. Effects of different NaCl mass concentrations on the growth of Z-2 bacteria and the biological oxidation percentage of reduced sulfur (a) 10g/L NaCl; (b) 20g/L NaCl; (c) 30g/L NaCl; (d) 40g/L NaCl

increasing NaCl concentration. When the mass concentrations of NaCl were 10 and 20g/L, the oxidation ability of Z-2 bacteria was not affected and the oxidation percentage of reduced sulphur reached approximately 90%. Moreover, the oxidation percentage of reduced sulphur was 70% when the mass concentrations of NaCl was 30g/L. When the mass concentration of NaCl further increased to 40g/L, the growth and oxidation ability of Z-2 bacteria were inhibited greatly. The bacterial concentration and the oxidation percentage of reduced sulphur were both low. Guo et al. (2008) indicated that the oxidation percentage of reduced sulphur was 70%, caused by the colourless sulphur bacteria grown in media with 20 g/L NaCl. Z-2 bacteria have higher oxidation ability, as compared with the colourless sulphur bacteria at the same NaCl concentration. These phenomena demonstrate that Z-2 bacteria have a high oxidation activity in the high-salt environment.

Oxidation of Z-2 bacteria on reduced inorganic sulphuric compounds from simulated wastewater: The simulated wastewater sample with the initial COD of 18000 mg/L was prepared containing 20 g/L NaCl and 140 g/L Na_2SO_3. The growth of Z-2 bacteria in the simulated wastewater sample and the biological oxidation percentage of sodium sulphite is shown in Fig. 3. The results indicate that Z-2 bacteria could grow well in an environment with high concentrations of salt and reduced sulphur compounds.

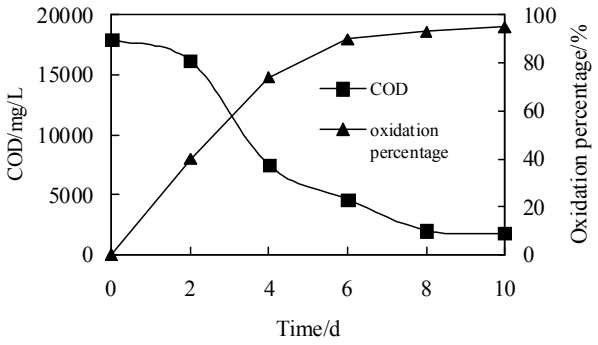

Fig. 3: COD value of water sample and oxidation percentage of reduced sulfur vs. the biological treatment time.

The OD_{600} of bacterial liquid reached up to 1.109 and then remained constant after 5 d. The oxidation percentage of sodium sulphite reached 85% after 5 d, then gradually increased and reached 95% after 10 d. These results show that Z-2 bacteria had efficient oxidative activity and high resistance to high-salt conditions. The COD value of the simulated wastewater sample treated by Z-2 bacteria is shown in Fig. 3. After 8 d, the COD reduced to 2000 mg/L and the COD removal rate reached to about 89%.

Oxidation Mechanism and Kinetics

Ion chromatography analysis: Water samples containing 12.5, 25, 50, 75 and 100 mg/L of SO_3^{2-} were initially prepared. The ion chromatography tests of these water sam-

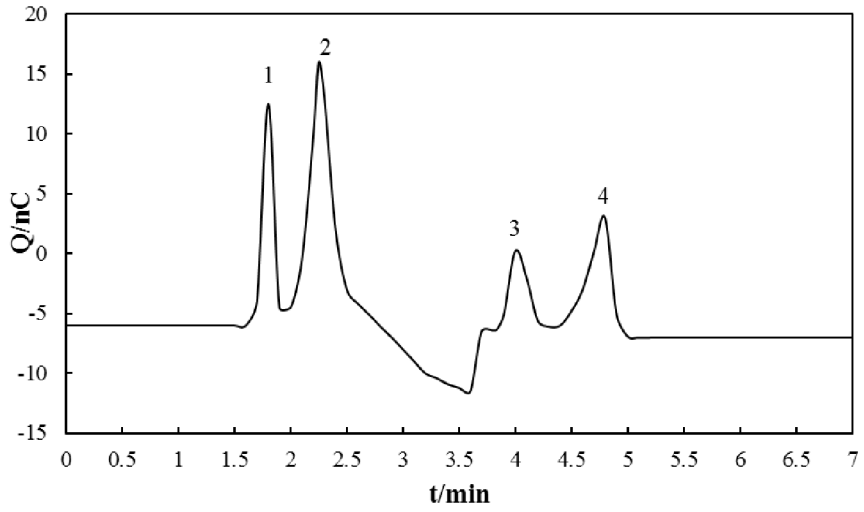

Fig. 4: Ion chromatogram of the water sample with the SO_3^{2-} concentration of 12.5 mg/L.

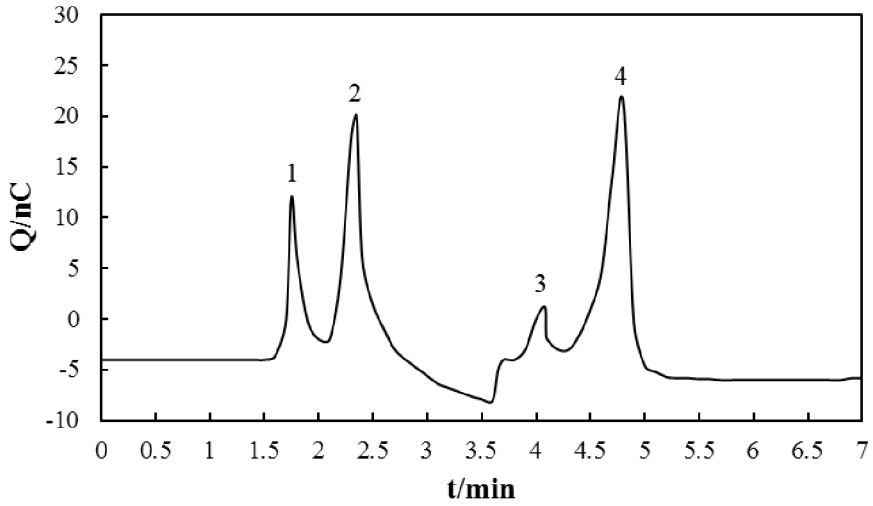

Fig. 5: Ion chromatogram of SO_3^{2-} in the water sample after biological oxidation treatment.

ples were conducted. Based on the testing data, the linear equation of the peak areas (y) on SO_3^{2-} concentration (x) in the range from 12.5 mg/L to 100 mg/L was obtained as y = 0.0349x + 0.1746, and the correlation coefficient was 0.996. The ion chromatogram of the water sample with the SO_3^{2-} concentration of 12.5 mg/L is shown in Fig. 4. The peak marked No. 4 is the chromatographic peak of SO_3^{2-}, which corresponds to the retention time of 4.832 min.

The simulated wastewater sample containing 140 g/L of Na_2SO_3 was treated by Z-2 bacteria. After 5 d of treatment, the water sample was diluted 60 times with pure water and subjected to the ion chromatography test. The ion chromatogram of SO_3^{2-}, in the diluted sample produced by biological oxidation treatment is shown in Fig. 5. Based on the previously mentioned linear equation $y = 0.0349x + 0.1746$, the SO_3^{2-} concentrations in the diluted water sample was 69.8 mg/L and the SO_3^{2-} concentration in the water sample without dilution was calculated as 4.188 g/L. Therefore, the oxidation percentage of reduced sulphur reached 97%, thereby confirming a good oxidation ability of Z-2 bacteria.

Sulphur-oxidizing system of Z-2 bacteria: The enzyme activities of enzyme solution in the cytoplasm and cell membrane were determined and summarized in Table 2. The SAOR in the cell membrane was approximately 300 times more than that in the cytoplasm. Therefore, the SAOR mainly exists in the cell membrane of Z-2 bacteria. In contrast, APS reductase and ATP sulfurylase were mainly present in the cytoplasm of Z-2 bacteria (Kapplera & Dahlb 2001, Kapplera 2011). Therefore, the direct oxidation of sulphite and the indirect oxidation reduction reaction of APS are both present in the cell of Z-2 bacteria. These results are consistent with the research results of Zimmermann et al. (1999).

Oxidation kinetics of sodium sulphite: The SAOR of the systems with different concentrations of the sodium sulphite substrate were extracted at pH 7.5 and 35°C. The rates of the enzyme-catalysed reactions were determined and the dynamic characteristics were revealed. The Michaelis constant (K_m) of SAOR in the enzyme-catalysed reactions was calculated using the Lineweaver-Burk method. The results are presented in Fig. 6.

The biological oxidation of the sulphite followed Michaelis-Menten kinetics. The maximum reaction rate (V_{max}) and K_m of the enzyme-catalysed reactions were obtained as 21.38 μM/min and 79.04μM, which were calculated by the intercept of double reciprocal plot.

CONCLUSIONS

Z-2 bacteria have a high oxidation activity in the high-salt environment after salt acclimation. When the mass concentration of NaCl was 20 g/L, the growth and oxidation ability of Z-2 bacteria were not affected and the oxidation percentage of reduced sulphur exceeded 90%. The COD of the simulated wastewater sample reduced from 18200 mg/L to 3064 mg/L and the COD removal rate reached 85%.

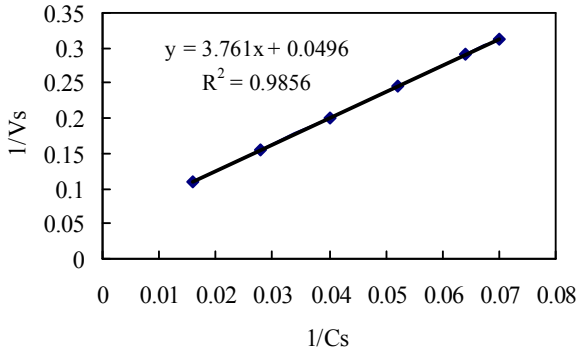

Fig. 6: Lineweaver-Burk graph of SAOR.

Ion chromatography of SO_3^{2-} in the water samples before and after biological oxidation treatment confirmed that Z-2 bacteria has desirable oxidation effects on the reduced sulphite. The biological oxidation reaction system of Z-2 bacteria were constructed by measuring the enzyme activity involved in the sulphur metabolism pathway. Results showed that direct and indirect oxidation processes were both involved in biological oxidation. The enzymatic oxidation reaction kinetics parameters were experimentally obtained as V_{max} = 21.38 μM/min and K_m = 79.04 μM.

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