



Use of an Isolated Sulfate Reducing Bacterium Desulfuricant Strain for Decomposition of Sulfite

YINAN YAN¹, JIANBIN ZHANG^{1,2,*}, YONGFENG ZHANG^{1,2,*}, ZHANYING LIU^{1,2} and JIE BAI^{1,2}

¹College of Chemical Engineering, Inner Mongolia University of Technology, Huhhot 010051, P.R. China

²Institute of Coal Conversion & Cyclic Economy, Inner Mongolia University of Technology, Huhhot 010051, P.R. China

*Corresponding authors: Tel/Fax: +86 471 6575722; E-mail: tadzhang@pku.edu.cn; environzyf@sina.com; 10510892@pku.edu.cn

(Received: 25 May 2013;

Accepted: 25 November 2013)

AJC-14440

In this work, the use of a desulfurization strain SRB-1, which isolates from sewage sludge and belongs to the sulfate reducing bacterium (SRB), for the decomposition of sulfite is reported. The strain can utilize sulfite as sulfur source. The strain cells of SRB-1 are motile and Gram negative and belongs to facultative anaerobes. For SRB-1, the optimum growth temperature is determined at 45 °C, the optimum pH is found at 7.5 and the optimum inoculum concentration is ascertained to 8 %. As a method to eliminate sulfite, the advantages of biological flue gas desulfurization technology are a simple process, low cost and no secondary pollution. These all results suggest that the strain has potential application in the regeneration processes of the industrial solution containing sulfite.

Key Words: Biological flue gas desulfurization technology, Sulfate reducing bacteria, Sulfite.

INTRODUCTION

Combustion of sulfur-containing fuels, such as coal and oil, results in formation of SO₂¹. To meet the environmental requirements, flue gas desulfurization (FGD) systems are necessary^{2,3}. Many industrial waste and process water contain high concentrations of sulfite (SO₃²⁻), which is formed by the reaction of SO₂ with water. The absorption of SO₂ into water is a process of simultaneous mass transfer with instantaneous reversible reaction⁴.

SO₃²⁻ may be removed by using sulfate reducing bacteria (SRB)⁵, which can be found at anaerobic environment^{6,7}, such as oil field water, bottom silt, hot spring and sewer. Compared with traditional chemical desulfurization methods, biological process-based desulfurization shows many advantages, such as low overall costs and no gypsum waste, which has become a major environmental concern in recent years⁸.

EXPERIMENTAL

The sample came from sludge in sewer from Inner Mongolia University of Technology, Huhhot, China.

Culture medium: The enrichment culture medium was composed of NaCl 2 g, K₂HPO₄ 0.5 g, MgSO₄·7H₂O 2 g, FeSO₄·(NH₄)₂SO₄·6H₂O 0.5 g, yeast extract 1 g, NH₄Cl 1 g, Na₂SO₄ 0.5 g, CaCl₂ 0.1 g and 70 % lactate 5 mL per distilled water 1 L with pH 7.

The identification culture medium was composed of NaCl 1 g, K₂HPO₄ 0.5 g, MgCl₂ 2 g, FeCl₂ 0.5 g (selected under condition), yeast extract 1 g, NH₄Cl 1 g, Na₂SO₃ 3 g, CaCl₂

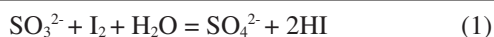
0.1 g and 70 % lactate 1.75 mL per distilled water 1 L with pH 7. Solid culture medium was added 2% agar into enrichment culture medium referring to before.

Isolation and purification: Shake 3 g of sludge well with 30 mL sterile water. After setting, the 6 mL of strain was inoculated on the enrichment culture medium 100 mL for 5 d at 37 °C. Meanwhile, the growth processes of the strain were observed and recorded. When the single colony was developed on the enrichment culture medium, isolated on the enrichment culture medium and transferred to solid culture medium for cultivation, the unanimous characteristics of the colony, figures, sizes and Gram's characteristics were presented. Then the strain was conserved as pure strain and named as SRB-1, maintained at 37 °C.

Identification of the isolate: The identifications of isolate were performed by conventional and chemo-taxonomic analyses, including microscope and SEM (Hitachi, S-3400N).

Preferred experiments of cultivated conditions. 12 mL cell solution was added into 150 mL medium was used to perform the preferred experiments by changing the conditions of pH and temperature. Then change the inoculum concentration under the fixed pH, temperature and 150 mL medium. The prepared cell solution was conserved under -25 °C for the following experiments.

Calculation on the concentration of sulfite: In this work, the concentration of I₂ was 0.1110 mol/L. Starch solution was selected as indicator. The concentration of SO₃²⁻ was tested with the reaction:



$$C_{\text{SO}_3^{2-}} = 0.1110 \times V_{\text{I}_2} \times 80.06 / V_{\text{SO}_3^{2-}} \quad (2)$$

RESULTS AND DISCUSSION

Identification of strain: The strain was named as SRB-1 and its characteristics have been ascertained through the physiological and biochemical experiments and motive experiment. The colony's diameter of the strain on the solid medium is (1.5-2) mm and gray-white. Fig. 1 showed that the colony is short stick with the motive of flip-flop movement. The cells of SRB-1 are Gram negative. The strain can utilize sulfite as sulfur source and utilize various organic carbon source.

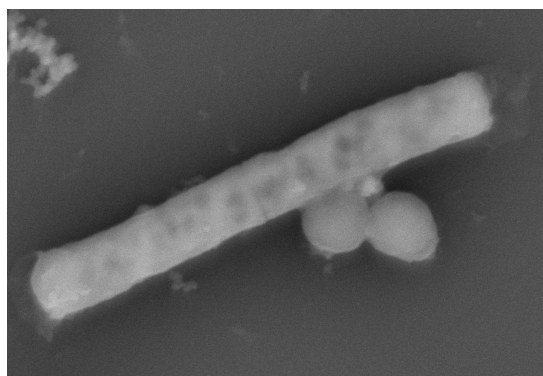


Fig. 1. SEM of SRB-1

Blank experiment: The decomposition of SO_3^{2-} in the absence of bacteria was performed as blank experiment in the optimum conditions. The result shows that the concentration of SO_3^{2-} in the media keeps almost invariable in 28 h.

Growth temperature: The strain was inoculated and cultivated at 25, 30, 37, 45 and 50 °C; meanwhile, the strain's growth processes were recorded. So the step of 5 °C was confirmed to optimize experimental temperature and 37 °C was used as the basic temperature.

The effects of growth temperature on the strain are shown in Table-1 and Fig. 2. It shows that with the time going, the concentration of SO_3^{2-} in different temperatures are all decreasing. The optimized temperature is 45 °C.

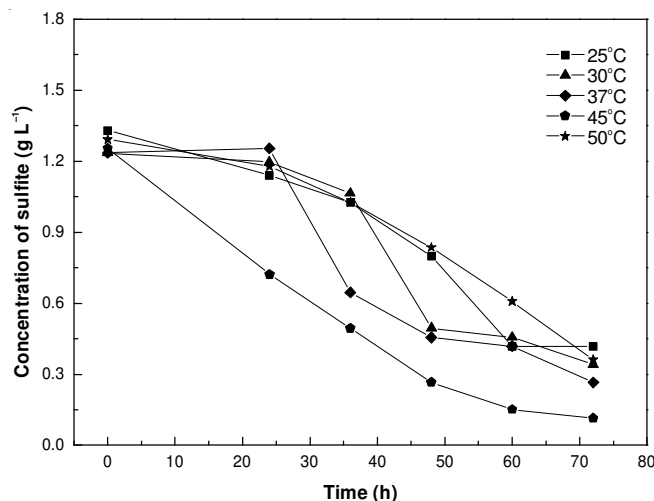


Fig. 2. Effect of growth temperature on the sulfite concentration

TABLE-1
EFFECT OF GROWTH TEMPERATURE ON THE
SULFITE CONCENTRATION (g/L)

Time (h)	Temperature (°C)				
	25	30	37	45	50
0	1.330	1.235	1.235	1.254	1.292
24	1.140	1.197	1.254	0.722	1.178
36	1.026	1.064	0.646	0.494	1.026
48	0.798	0.494	0.456	0.266	0.836
60	0.418	0.456	0.418	0.152	0.608
72	0.418	0.342	0.266	0.114	0.361

Experiments of pH: Based on the decomposition of medium, a series of media were prepared with pH step of 0.5 from 6 to 9. The effects of growth pH on the strain are shown in Table-2 (Fig. 3).

TABLE-2
EFFECT OF GROWTH pH ON THE SULFITE
CONCENTRATION (g/L)

Time (h)	pH					
	6.0	6.5	7.0	7.5	8.0	9.0
0	1.368	1.254	1.254	1.216	1.235	1.254
12	1.292	1.140	1.178	0.551	0.570	0.874
24	1.197	1.083	0.741	0.494	0.494	0.608
36	1.140	0.988	0.513	0.380	0.380	0.532
48	0.950	0.817	0.304	0.228	0.266	0.418
60	0.874	0.722	0.266	0.228	0.228	0.342
76	0.874	0.608	0.190	0.152	0.152	0.228

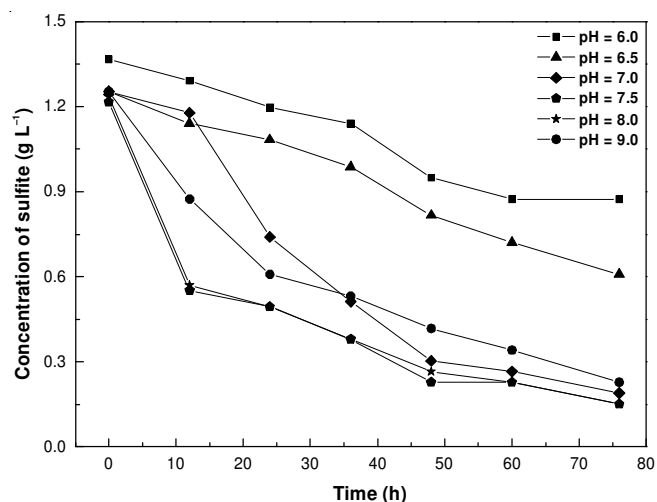


Fig. 3. Effect of growth pH on the sulfite concentration

From Table-2 and Fig. 3, the optimum growth pH for the strain was confirmed at 7.5. The results were attributed to the strain which can play commendably the role of enzyme at optimum acid condition, benefit the dissolution of CO_2 in culture medium and benefit the strain's metabolism for the carbon source. At higher pH condition, the physiological action of the amino acids in the cell was affected grievously because the basic complexes entered the cell under the effect of osmotic pressure when the concentration of basic complexes in the surrounding of cells was higher than in the cell. Under lower pH condition, on the contrary, the obvious decreasing dissolution capacity of CO_2 in culture medium affected the growth of the strain severely and the results explained

commendably the phenomenon that the strain cannot grow below pH 6.

Inoculum concentration: The strain was inoculated and cultivated at the inoculum concentration step of 2 % from 2 to 10 %. The temperature was confirmed at 37 °C and the pH was 7. The effects of inoculum concentration on the strain are shown in Table-3 (Fig. 4).

Time (h)	Inoculum concentration (%)					
	2	4	6	8	10	12
0	1.482	1.444	1.444	1.444	1.444	1.368
12	1.330	1.254	1.254	1.254	1.254	1.178
24	1.254	1.178	1.102	1.102	1.102	1.026
36	1.178	1.064	0.988	0.988	0.988	0.950
48	0.950	0.836	0.760	0.570	0.608	0.532
60	0.836	0.760	0.684	0.494	0.608	0.456
72	0.760	0.760	0.456	0.494	0.494	0.418

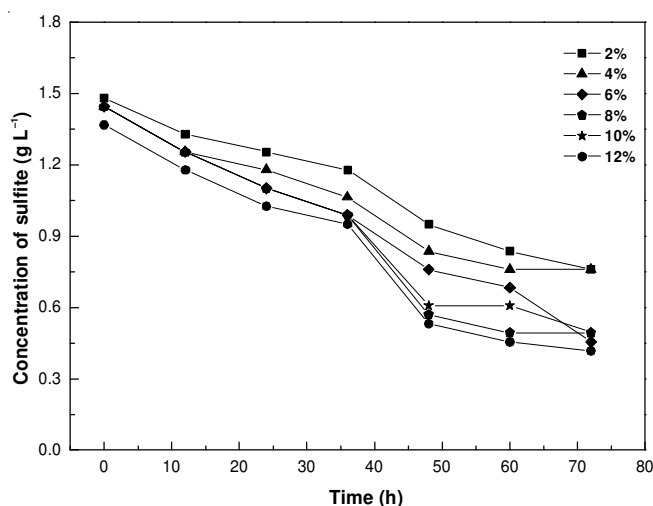


Fig. 4. Effect of inoculum concentration on the sulfite concentration

In Table-3 (Fig. 4), with the time go, the concentration of SO_3^{2-} was decreased in the all range of experiment. The optimized inoculum concentration was showed in 12 % and the rate of sulfite reducing was 47.02 %. 8 % was chosen as the best effect inoculum concentration.

Determination of growth curve: The cell concentration was counted by visible spectra at 600 nm. Under the optimum

cultivated condition, the strain was cultivated and the growth curve of the strain was plotted (Fig. 5).

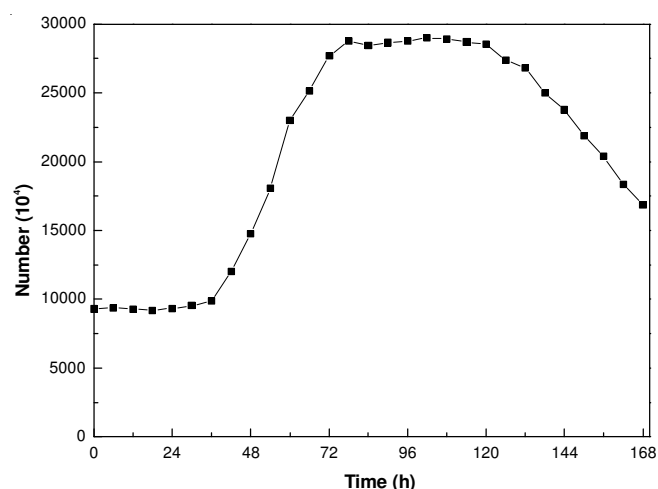


Fig. 5. Growth curve of SRB-1

From Fig. 5, the lag phase of the strain appears at 0-36 h and the strain adapts themselves mainly to the cultivated condition in the lag phase. Exponential phase of the strain is presented at 36-72 h and the strain utilizes the nutriment in the medium for growth and to reproduce rapidly. In the following process, the growth of the strain enters the stationary phase at 72-120 h. Moreover, the medium cannot provide enough energy source for the growth of the strain, so the strain enters the death phase at 120 h and the cell concentration of the strain decreases obviously at this phase.

REFERENCES

1. M.C. Macías-Pérez, A. Bueno-López, M.A. Lillo-Ródenas, C. Salinas-Martínez de Lecea and A. Linares-Solano, *Fuel*, **87**, 3170 (2008).
2. R.K. Srivastava, W. Jozewicz and C. Singer, *Environ. Prog. Sust. Energy*, **20**, 219 (2001).
3. L.M. Shi and X.C. Xu, *Energy Fuel*, **19**, 2335 (2005).
4. C.S. Chang and G.T. Rochelle, *Am. Inst. Chem. Eng.*, **27**, 292 (1981).
5. T. Jong and D.L. Parry, *Water Res.*, **40**, 2561 (2006).
6. R. Yamamoto-Ikemoto, S. Matsui, T. Komori and E.K. Bosque-Hamilton, *Water Sci. Technol.*, **37**, 599 (1998).
7. K. Ingvorsen, N.M. Yde and C. Joulain, *FEMS Microbiol. Ecol.*, **46**, 129 (2003).
8. Y. Sheng, H. Cao, Y. Li and Y. Zhang, *J. Hazard. Mater.*, **179**, 918 (2010).