Science in China Series B: Chemistry

www.scichina.com
chem.scichina.com
www.springerlink.com

Isolation and identification of the *thermophilic alkaline desulphuricant* strain

ZHANG JianBin[1†], ZHANG Tong[2], MA Kai[2], CHEN GuoHua[2], ZHANG DongYan[2] & WEI XiongHui[1†]

[1] Department of Applied Chemistry, College of Chemistry & Molecular Engineering, Peking University, Beijing 100871, China;
[2] College of Chemical Engineering, Inner Mongolia University of Technology, Huhhot 010051, China

A desulfurization strain that belongs to the *thermophilic alkaline desulphuricant* is designated as strain *GDJ-3* and isolated from Inner Mongolia, China. The colony of the strain shows tiny, yellow, or white-yellow, and it becomes henna with the protracting of cultivated time. The cells are bacilliform (0.3 −0.6 × 1.0−1.2 μm), motive, and Gram negative. The strain *GDJ-3* is able to utilize respectively the thiosulphate, sulfate, sulfite, or sulfide as sulfur source, utilize the carbon dioxide as the carbon source, and utilize the ammonium or nitrate as the nitrogen source. According to GenBank data, 16s RNA results of *GDJ-3* are in good agreement with *Alpha proteobacterrium sp*. (97%) and *Ochrobactrum sp*. (98%). For *GDJ-3*, the optimum growth temperature is at 45℃, the optimum pH is at 8.5−8.8, and the optimum rocking speed of sorting table is at 150 r/min. Under the optimum culture condition, the cells of the strain can live for about 18 h. In the desulfurization solution, which is prepared according to the composition of DDS solution, the objectionable constituents of sodium thiosulphate and sodium sulfide were added factitiously, and the bacterial cell concentration was set at 10^7/mL. After the regeneration of the above desulfurization solution by the strain cells, the concentration of sodium thiosulphate was decreased by 14.75 g/L (percentage loss of content 13.21%), the concentration of sodium sulfide was decreased by 0.76 g/L (percentage loss of content 87.36%) in the desulfurization solution in 9.5 hours, and sulfur appeared. Maybe, this kind of strain can be used as the regeneration's bacterial source of DDS solution.

thermophilic alkaline desulphurican, thiobacillus, strain identification, DDS catalyst

1 Introduction

With the rapid development of industry, there is a trend that sulfur compounds' emission keeps on a steady growth in recent years. The emission of huge amounts of sulfur compounds to the atmosphere makes a great deal of environmental pollution and diseases, relating to acid rain, acidification of buildings, breathing diseases, and impacts the human being's health[1]. Therefore, these desulfurization processes of flue gases, industrial process gases, and other waste gases have attracted great attention in recent years. And the researchers in various countries have developed in-depth researches on the desulfurization of industrial gases and other waste gases and accumulated a great amount of data[2−9].

As a new desulfurization method, the microorganism desulfurization way drew more attention in various countries[10−18], because it had appeared to have lower cost, higher yield of second-produces, and higher desulfurization efficiency. However, it had some shortages, such as longer desulfurization time, higher possibilities of aberrance in desulfurization processes, and more complicated desulfurization mechanism in desulfurization processes. With the rapid development in gene engineering technology, the microorganism with the performances relating to the stable desulfurization capacity, the high speed propagate, and the rapid recycled

Received September 23, 2006; accepted August 29, 2007
doi: 10.1007/s11426-008-0008-5
†Corresponding author (email: tadzhang@pku.edu.cn; xhwei@pku.edu.cn)

metabolism in desulfurization processes has become one of the evolution directions of microorganism desulfurization processes.

The "Desulfurication & Decarburization Solution Activities" (DDS catalyst, or bio-chemical Fe-basic solution desulfurization technology)[19,20] is a biochemical wet desulfurization technology and was confected by the DDS catalyst, hydroxybenzene complexes, the activated ferrous carbonate, and the aerobic *Thermophilic Alkaline Desulphurican* in sodium carbonate or other alkali aqueous solution. DDS catalyst (DDS-Fe), which is stable and cannot be decomposed in alkali solution, is a kind of polymeric complex or chelate complex. DDS solution can absorb H_2S and COS; then, DDS solution containing H_2S and COS is regenerated by air and catalyzed by DDS catalyst and hydroxybenzene, and the by-product of sulfur is produced, and the regenerated DDS solution is recycled during the whole desulfurization processes. In the processes for absorption and regeneration, because a little DDS-Fe was decomposed and some second-reactions happened and some second-salts, such as $Na_2S_2O_3$ and Na_2S were produced, the desulfurization capabilities of desulfurization solution were decreased rapidly. At the same time, the undissolved ferric salts and the activated ferrous carbonate were changed into DDS-Fe under the help of the aerobic *Thermophilic Alkaline Desulphurican*, and the DDS-Fe could keep the concentration of ferrous in DDS solution stable. Meanwhile, the aerobic *Thermophilic Alkaline Desulphurican* could reduce the $Na_2S_2O_3$ and Na_2S into sulfur to release the second-reactions to make the desulfurization processes more complete and to enforce the absorption capacity of the DDS solution.

In the desulfurization processes of the DDS solution, the microorganism can activate the regeneration of the DDS solution containing sulfides with high effectiveness and activeness to decompose $S_2O_3^{2-}$ and (or) S^{2-} into sulfur and to hold the desulfurization capacity of the DDS solution; therefore, the microorganism has become an important part in the DDS solution. In addition, it has been applied in the desulfurization processes of industrial gases for several years in more than one hundred companies to remove the sulfur compounds from the industrial gases to below 1 $mg \cdot m^{-3}$; furthermore, the DDS desulfurization method did not bring the second pollution and it makes the biochemical technology become an actual technology. The regeneration process by microorganism is an important part in the DDS desulfurization technology. Thus, to screen out the high effective microorganism for the regeneration of the DDS solution, to determine the cultivated condition of the strain, to make the identification of the strain, and to keep the stabilities of the aerobic *Thermophilic Alkaline Desulphurican* are shown to be very important to the DDS desulfurization technology.

In the past years, the reports on microorganism regeneration of the DDS solution were rare; therefore, this work can provide some reference data for the microorganism regeneration processes and microorganism desulfurization processes.

2 Materials and methods

2.1 Sample Source

The sample for the isolation of desulfurization strain was extracted from the neutral soil about 15 cm under the earth's surface.

2.2 Culture medium (sulfur synthetic medium, g/L)

$Na_2HPO_4 \cdot 7H_2O$ 7.9, metal salt solution 5.0 mL, KH_2PO_4 1.5, NH_4Cl 0.3, $Na_2S_2O_3 \cdot 5H_2O$ 10.0, $MgSO_4 \cdot 7H_2O$ 0.1, distilled water 1.0 L, and pH 8.5—8.8 (controlled by 10% Na_2CO_3).

The strain was maintained on nutritive medium added agar 16 $g \cdot L^{-1}$ (15%—20%), which was sterilized at 121℃ for 20 min, and kept at 4.0℃.

2.3 Pretreatment of the sample

10 g sample was put into one neck flask containing asepsis water, broken by glass balls, controlled pH between 8.5 and 9.5 using the NaOH or Na_2CO_3 aqueous solution, and kept for 8—10 d. Treated in the same way, the sample at the natural pH was used as the contrast sample (no bacteria could be cultivated from the contrast sample).

2.4 Multiplication culture

5 mL clear liquid extracted from the above sample of pretreatment was put into the flask containing 120 mL culture medium to produce the original bacteria sample. The enriched cell solution was obtained by adding the original bacteria sample into the culture medium for 3—5 d cultivation under the condition of 45℃ and 150 r/min.

2.4.1 Isolation and purification. The strain was inoculated on the solid culture medium and cultivated for 2—7 d at 45℃. Meanwhile, the growth processes of the strain were observed and recorded. When the single colony was developed on the solid culture medium, and the single colony was isolated from the solid culture medium and transferred to polybasic culture medium solution for cultivation, the unanimous characteristics of the colony, figures, sizes, and Gram's characteristics appeared. Then this strain was conserved as the pure strain and named GDJ-3 which was cultivated on the inclined solid culture medium, maintained in the icebox at 4℃, and inoculated after 1—2 months.

2.4.2 Re-screening method. In order to cultivate useful stains for the DDS solution to make known the regeneration mechanism of the DDS solution activated by bacteria, the experimental DDS solution sterilized at 121℃ for 20 min (no interaction among various compounds at 121℃) was confected according to the industrial DDS solution. The strain was inoculated in the experimental DDS solution and cultivated at the condition of 45℃ and 150 r/min. The analytical method and the experimental data of the bacterial regeneration of the experimental DDS solution will be published elsewhere.

2.5 Preferred experiments of cultivated condition

2.5.1 Experiments of temperature for the growth of the strain. The cell solution was prepared and conserved under 4℃ for the following preferred experiments. 0.2 mL of cell solution was coated on the flat panel and signed with date or serial numbers. The strain was inoculated and cultivated with the step of 5℃ from 20 to 65℃; meanwhile, the processes of the strain growth were recorded in detail.

2.5.2 Experiments of pH for the growth of the strain. Based on the composition of the sulfur synthetic medium, a series of culture media were prepared with the step of 0.5 pH from 4 to 12 detected by a PHS-3 exact acidometer (Leici, Shanghai) and sterilized. Also, 0.2 mL bacterial solutions was respectively coated on the above different solid culture media and then cultivated for 2—5 days at 45℃, and the processes of the strain growth were recorded in detail.

2.5.3 Preferred experiments of cultivated conditions. 120 mL culture medium added with 10 mL cell solution was used to perform the preferred experiments of cultivated conditions by changing the temperature, the rocking speed of sorting table, and pH.

2.6 Test of nutrition

The test of sulfur source was respectively performed by using Na_2S 1%, Na_2SO_4 1%, Na_2SO_4 1%, or S^0 1% to replace $Na_2S_2O_3$; and the physiological-biochemical identification was performed at the same time.

2.7 Determination of growth curve

The cell concentration of the strain was counted by a hemocytometer under the amplification of 640 times. Then the growth curve was plotted on the counted data of cell concentration.

2.8 Test of desulfurization capacity of the strain

The compounds, which can be oxidated by iodine aqueous solution, including $Na_2S_2O_3$, Na_2S, and NaHS in the DDS solution, are named the reducing compounds. When the concentration of the reducing compounds in the DDS solution is over the limited condition, the absorption capacity of the DDS solution for the sulfur compounds from gases will be badly affected, and the industrial operation will be seriously out of control. Therefore, the concentration of the reducing compounds must be determined and controlled continually in the whole desulfurization processes. In this work, the concentration of reducing compounds in the DDS solution was determined by the residual titration method and calculated by the relation of equivalent proportions. The sulfide in the DDS solution was deposited by cadmium chloride, and the concentration of $Na_2S_2O_3$ in the DDS solution without sulfide was also analyzed by the residual titration method. In addition, the remnant sulfide in the DDS solution was calculated by the difference between the concentration of reducing compounds and the concentration of $Na_2S_2O_3$.

3 Results and discussions

3.1 Identification of the strain[22]

3.1.1 TEM results of GDJ-3 strain. The characteristics of GDJ-3 strain, which was prepared by negative staining, were observed on the high resolution transmission electron microscope (TEM, JEM-2010, made in Japan). The TEM photos of GDJ-3 strain are shown in Figure 1.

3.1.2 Description of the strain. The characteristics of the strain have been ascertained through the physiological and biochemical experiments and motive experiment.

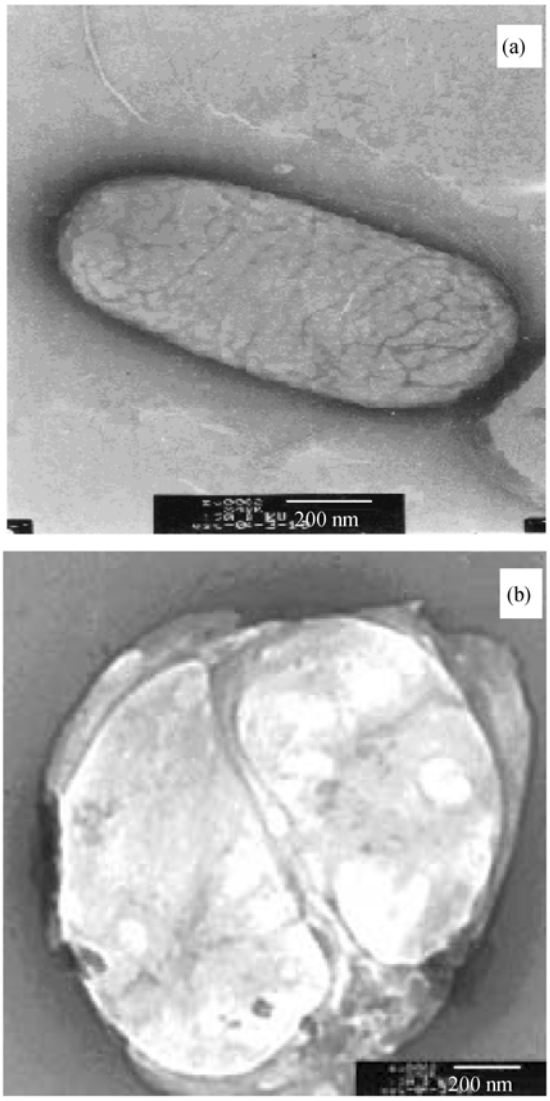

Figure 1 Single cell's TEM photos of *GDJ-3* ((a) and (b)).

The colony's diameter of the strain on the solid medium is 1—2 mm, and the mid part of colony becomes henna after protracting the cultivated time. The cell of strain is single, double, or chained. The flagellum is in the top of cell (Figure 1(a)), and there is solid sulfur in the cell (Figure 1(b)). The cells are bacilliform (0.3—0.6 μm× 1.0—1.2 μm), and motive experiment shows that cells can move in the liquid medium under the microscope commendably. Gram experiment shows negative results in Figure 2. The strain can utilize thiosulphate or sulfide as sulfur source, and cannot utilize glutin, starch, and glucose as carbon source; maybe, the carbon source is due to carbon dioxide in the air. Then, the optimum cultivated conditions were determined as follows: the optimum temperature is at 45.0℃ and the strain can grow between 20.0 and 50.0℃ (there is slow growth below 20.0℃), the optimum cultivated pH condition is at 8.5—8.8, and the strain can grow at the pH between 7.5 and 10.0. When the NH_4Cl in the medium was replaced with nitrate by the same nitrogen mole fraction, the strain grew rapidly. Therefore, a conclusion can be obtained that the cell strain is chemoautotrophy.

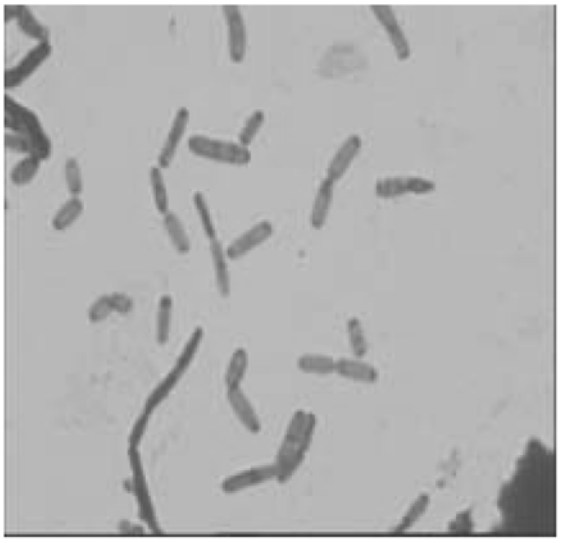

Figure 2 Cells' Gram micrograph of *GDJ-3*.

The identification results of 16s RNA were determined by Institute of Microbiology, Chinese Academy of Sciences (Beijing, China) as follows:

```
TGGGC GGCCG CCTGC AGACC AGGTC TAGAG     30
TTTGA TCCTG GCTCA GAACG AACGC TGGCG     60
GCAGG CTTAA CACAT GCAAG TCGAG CGCCC     90
CGCAA GGGGA GCGGC AGACG GGTGA GTAAC    120
GCGTG GGAAC GTACC TTTTG CTACG GAATA    150
ACTCA GGGAA ACTTG TGCTA ATACC GTATG    180
TGCCC GAAAG GGGAA AGATT TATCG GCAAA    210
GGATC GGCCC GCGTT GGATT AGCTA GTTGG    240
TGAGG TAAAG GCTCA CCAAG GCGAC GATCC    270
ATAGC TGGTC TGAGA GGATG ATCAG CCACA    300
CTGGG ACTGA GACAC GGCCC AGACT CCTAC    330
GGGAG GCAGC AGTGG GGAAT ATTGG ACAAT    360
GGGCG CAAGC CTGAT CCAGC CATGC CGCGT    390
GAGTG ATGAA GGCCC TAGGG TTGTA AAGCT    420
CTTTC ACCGG TGAAG ATAAT GACGG TAACC    450
GGAGA AGAAG CCCCG GCTAA CTTCG TGCCA    480
GCAGC CGCGG TAATA CGAAG GGGGC TAGCG    510
TTGTT CGGAT TTACT GGGCG TAAAG CGCAC    540
GTAGG CGGAC TTTTA AGTCA GGGGT GAAAT    570
CCCGG GGCTC AACCC CGGAA CTGCC TTTTG    600
ATACT GGAAG TCNTG AGTTA ATGGT AANAG    630
GTTGA GTGGG AATTC CGAGT GTANA GGTGA    660
AAATT CGTAA NATAT TTCGG AGGGA ACACC    690
AGTGG GCGAA AGGCG GCTCC ACTNG GAACC    720
ATTNA CTGGA CGCTT GAAGN TGCGG AAAAC    750
CGTGG GGGAG CNAAC ANGGA ATAAA ATACC    780
```

Table 1 The similar microorganisms with GDJ-3 strain in 16s RNA

Data base	Serial number	Name	Homologous rate (%)
gi|56292117	AJ242584.2	*Ochrobactrum tritici*	98%
gi|93115169	DQ468102.1	*Ochrobactrum sp. Yw18*	98%
gi|97954784	AM231041.1	*Ochrobactrum sp. R-24468*	98%
gi|54873569	AY776289.1	*Ochrobactrum anthropi*	97%
gi|26324205	AY162056.1	*Alpha proteobacterium PII_GH1.2.A1*	97%
gi|30408111	AY274164.1	*Uncultured bacterium clone D8*	97%
gi|29825839	AF337864.2	*Uncultured gold mine bacterium D11*	97%

The resulting sequences are compared with those retrieved from GenBank (http://www.ncbi.nlm.nih.gov), and the similarity between the strains (%) was assessed. 16s RNA result is in good agreement with *Alpha proteobacterrium sp*. (97%) and *Ochrobactrum sp*. (98%). For *Alpha proteobacterrium sp*., Kathleen E. Duncan and co-workers[23] studied some strains, which can use a gas mixture of hydrogen sulfide and methyl mercaptan as the carbon and energy sources. Through strain's 16s RNA experiments, they discovered the strains' 16s RNA was in good agreement with *Alpha proteobacterrium sp*. However, the relevant reports about *Ochrobactrum sp.* with capabilities to decompose sulfur compounds have not been found up to now.

3.2 Results and discussions on the preferred experiments of cultivated condition

3.2.1 Effects of various pH on the growth of bacteria. According to the experiments of pH, the growth pH range of the strain was confirmed from 7.5 to 10.0, so the step of 0.5 was confirmed as the step of optimizing experiment of pH.

The experimental results showed that the optimum growth pH for the strain was confirmed at 8.5—8.8. Figure 3 shows that the cell concentration of the strain increases rapidly under the condition of the constant temperature and rocking speed and at 8.8 pH after cultivation for 12 h, and the growth proportion of the cell concentration of the strain arrives at $Y = n_{12}/n_0 = 1.414$ (n_{12} and n_0 represent the cell concentration of the strain at 12 h and 0 h, respectively). 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 acid 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 7.5 of pH.

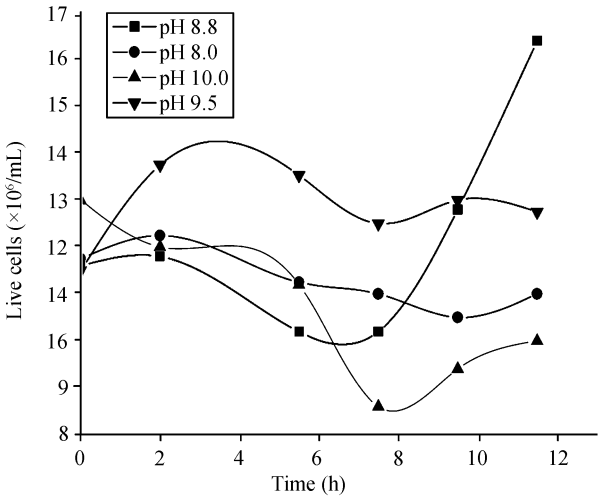

Figure 3 Growth curves of bacteria in different pH condition. Temperature at 45℃ and rocking speed at 150 r/min.

3.2.2 Effects of temperature on the growth of bacteria. Through the preferred experiments at various temperatures, the growth temperature range of the strain was confirmed from 20 to 55℃ (the strain can grow slowly below 20℃), so the step of 5℃ was confirmed in the optimizing experiment of temperature and 45℃ was used as the basic temperature.

Figure 4 shows that $Y = n_{12}/n_0$ is 1.414 at 45℃ and the cell concentration appears to increase at other temperatures, but the increasing quantity is evidently lower than at 45℃. This phenomenon was due to the fact that the strain can play perfectly the role of enzyme at this temperature. At higher temperature, the enzyme of the strain loses the physiological action so that the strain cannot grow above 60℃. At lower temperature, on the contrary, the enzyme of the strain cannot have the physiological action also, so the strain grows slowly below 20℃. In

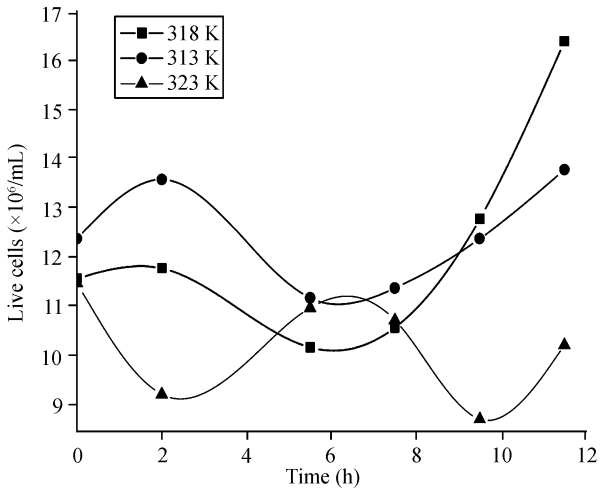

Figure 4 Growth curves of bacteria at different temperatures. Rocking speed at 150 r/min and pH at 8.8.

this work, the optimum temperature for the growth of the strain was ascertained; and it is very important to cultivate the strain and regenerate the DDS solution containing sulfides in the following regeneration processes.

3.2.3 Effects of various rotation speeds of sorting table on the growth of bacteria. Figure 5 shows that the $Y = n_{12}/n_0$ was confirmed for 1.414 when the rocking speed of sorting table was kept at 150 r/min. This phenomenon was attributed to the better dissolution capacity of oxygen and carbon dioxide at 150 r/min so that the strain can grow favorably under this cultivated condition. At higher rocking speed, the process benefits the mass transfer for the gases but does not benefit dissolution of oxygen and carbon dioxide in culture medium. At lower rocking speed, on the contrary, there is higher dissolution capacity for oxygen and carbon dioxide in culture medium, but lower dissolution capacity of oxygen and carbon dioxide in culture medium resulted from the lower rate of mass transfer. According to Figure 5, the rocking speed of sorting table at 150 r/min was confirmed as the better condition for the dissolution capacity of oxygen and carbon dioxide and the process of mass transfer in culture medium.

3.2.4 Determination of growth curve. Under the optimum cultivated condition, the strain was cultivated and the growth curve of the strain was plated in Figure 6. Figure 6 shows that the lag phase of the strain appears at 0—5 h, and the strain adapts themselves mainly to the cultivated condition in the lag phase. Exponential phase of the strain is presented at 5—11 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 11—14 h. Moreover, the medium cannot provide enough energy source for the growth of the strain, so the strain enters the death phase at 14 h and the cell concentration of the strain decreases obviously at this phase.

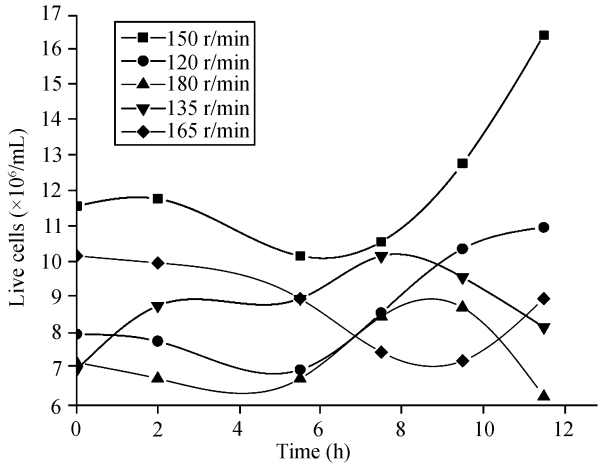

Figure 5 Growth curves of bacteria at different rocking speeds. Temperature at 45℃ and pH at 8.8.

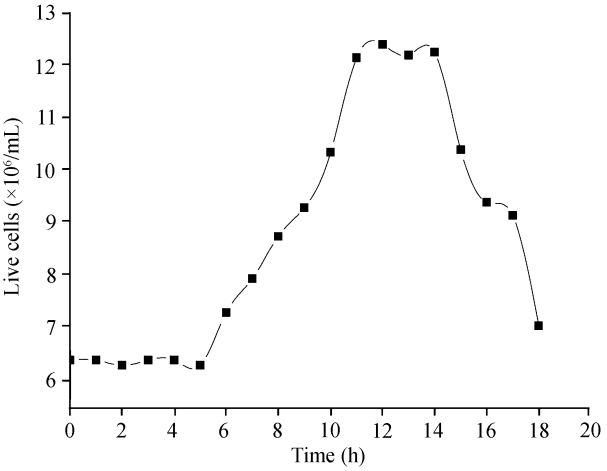

Figure 6 Growth curve of the strain at the optimum condition. Temperature at 45℃, pH at 8.8, and rocking speed at 150 r/min.

3.3 Desulfurization performance of the strain

The solution containing sulfides (SCS) was prepared according to the industrial DDS operation condition[24] as follows ($g \cdot L^{-1}$): $Na_2CO_3 \cdot 10H_2O$ 15.0, ferrous compound 8.4, $Na_2SO_4 \cdot 10H_2O$ 60.0, hydroquinone 1.2, $Na_2S \cdot 9H_2O$ 2.5, $Na_2S_2O_3 \cdot 5H_2O$ 200.0, distilled water 1.0 L, pH 8.5—9.0. The solutions of SCS were sterilized at 121℃ for 20 min before the regenerated experiments were carried out.

Setting the cell concentration of the strain in the SCS at 10^7/mL, the SCS was regenerated for 9.5 h, and the concentration of $Na_2S_2O_3$ and Na_2S in the SCS respectively decreased by 14.75 g/L and 0.76 g/L in Figure 7; meanwhile, the strain had decomposed the maleficent sulfur compounds into sulfur. The confirmation of the idiographic regenerating condition and the concentration of various compounds in the experimental DDS solution and the analytical methods of the concentration of various compounds will be published elsewhere.

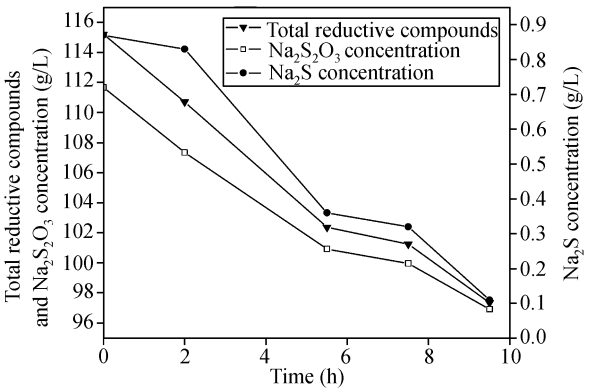

Figure 7 Changes of concentrations of different reducing compounds treated by the bacteria.

4 Conclusions

Research results showed that the pretreatment processes were very advantageous to the isolation of the strain. The optimum cultivated condition for the growth of the strain was confirmed by the preferred experiments as follows: temperature is at 45℃, pH is at 8.5－8.8, and rocking speed of sorting table is at 150 r/min. In addition, the growth curve of the strain at the optimum condition was plated; and this work provided the important reference data and the strain for the microbial regeneration of the DDS solution containing sulfides.

According to GenBank data, 16s RNA results of GDJ-3 are in good agreement with *Alpha proteobacterrium sp*. (97%) and *Ochrobactrum sp*. (98%). In the DDS solution which were prepared according to the composition of the industrial DDS solution, the objectionable constituents of sodium thiosulphate and sodium sulfide were added factitiously, and the cell concentration was set at 10^7/mL. After the regeneration of the above DDS solution, the concentration of sodium thiosulphate was decreased by 14.75 g/L (percentage loss of content 13.21%) and the concentration of sodium sulfide was decreased 0.76 g/L (percentage loss of content 87.36%) in the DDS solution in 9.5 h, and the sulfur appeared.

Thanks go to Profs. Hongcheng Gao and Wenting Hua (Peking University, China) for their suggestions on the preferred experiments. Thanks also go to Doctor Chengying Jiang (Institute of Microbiology, Chinese Academy of Sciences, China) for suggestions on the strain identification.

1 Krajick K. Long-term data show lingering effects from acid rain. Science. 2001, 292: 195－196
2 Al-Enezi G, Ettouney H, El-Dessoky H, Fawzi N. Solubility of sulfur dioxide in seawater. Ind Eng Chem Res, 2001, 40: 1434－1441
3 Shang H S, Ti O Y, Fan Y, Yuan K. A biomass-supported Na_2CO_3 sorbent for flue gas desulfurization. Environ Sci Technol, 2003, 37: 2596－2599
4 Butler A D, Fan M H, Brown R C, Cooper A T, Leeuwen J H V, Sung S H. Absorption of dilute SO_2 gas stream with conversion to polymeric ferric sulfate for use in water treatment. Chem Eng J, 2004, 98: 265－27
5 Shareefdeen Z, Herner B, Webb D. Hydrogen sulfide (H_2S) removal in synthetic media bio-filters. Envir Pr, 2003, 22(3): 207－213
6 Li K T, Hung Y C. Hydrogenation of sulfur dioxide to hydrogen sulfide over Fe/γ-Al_2O_3 catalysts. Appl Catal B: Environ, 2003, 40: 13－20
7 Iliuta M C, Larachi F, Grandjean B P A. Grandjean. Solubility of hydrogen sulfide in aqueous solutions of Fe(II) complexes of trans-1,2-cyclohexanediaminetetraacetic acid. Flu Ph Equi, 2004, 218: 305－313
8 Thanh D N, Block K, Bandosz T J. Bandosz. Adsorption of hydrogen sulfide on montmorillonites modified with iron. Chemosphere, 2005, 59: 343－353
9 Petrov K, Srinivasan S. Low temperature removal of hydrogen sulfide from sour gas and its utilization for hydrogen and sulfur production. Int J Hyd E, 1996, 21(3): 163－169
10 Selvaraj P T, Little M H., Kaufman E N. Bildesulfurization of flue gases and other sulfate/sulfite waste streams using immobilized mixed sulfate-reducing bacteria. Biotech Prog, 1997, 13: 583, 589
11 Lizama H M, Zielinski P A, Kerby L D, Abraham C C. Abraham. Comparison of biooxidation with carbon dioxide assimilation during bacterial growth on ferrous ion or elemental sulfur. Biotech Bioeng, 2002, 77(1): 111－117
12 Park D H. Hydrogen sulfde removal utilizing immobilized Thiobacillus, sp. IW with Ca-alginate bead. Biochem Eng J, 2002, 11: 167－173
13 Cha J M. Removal of organo-sulphur odour compounds by Thiobacillus novellas SRM, sulphur –oxidizing microorganisms. Proc Biochem, 1999, 34: 659－665
14 Stamford N P. Effect of sulphur imoculated with Thiobacillus on soil salinity and growth of tropical tree legumes. Biores Tech, 2002, 81: 53－59
15 Nakamura K. A microbial method using whole cells of Thiobacillus thiooxidans for measuring sulphate in waters. Appl Microbial Biotech, 1997, 48: 753－757

16 Michael A. The leaching of chromium from cement-based waste form via a predominantly biological mechanism. Adv Env Res, 2004, 8: 483－491
17 Li Y Q, Zhong H F. Isolation and characterization of moderately thermophilic sulfur-oxidizing bacteria. Biodivers Sci (in Chinese). 1993, 1(1): 9－15
18 Li Y Q, Xia W, Zhong H F. Biooxidation of absenic-contaning refractory gold concentrate with moderately thermophilic bacteria for gold recovery. Acta Microb Sin (in Chinese), 1996, 36(1): 42－47
19 Wei X H, Zou M H, Wei F H. Catalyzing Method of Gas Desulphurization, Decarburization, and Decyanation by Fe-basic Solutions. CN ZL99100596.1, 1999
20 Wei X H. Catalyzing Method of Gas Desulphurization by Biochemical Fe-basic Solutions. CN ZL02130605. 2, 2002
21 Hou Z X, Liu H Z, Luo M F, Li S, Xing J M, Liu J Y. Isolation and identification of specific desulfurization bacteria. Sci China B: Chem (in Chinese), 2002, 32(5): 397－405
22 Dong X Z. Manual of Systemic Determinative Bacteriology (in Chinese). Beijing: Science Press. 2001
23 Duncan K E, Sublette K L, Rider P A, Stepp A, Beitle R R, Conner J A, Kolhatkar R. Analysis of a microbial community oxidizing inorganic sulfide and mercaptans. Biotech Prog, 2001, 17: 768－774
24 Wei X H, Wang X Y, Liu X Q. DDS Desulfurization and its use. J Chem Fertil Ind (in Chinese), 2001, 28(6): 9－14