

# 我国盐湖沉积物中的硫酸盐还原 细菌及其地球化学作用

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**内容提要** 本文对我国某些盐湖和苦水湖沉积物中的硫酸盐还原细菌进行了分离和鉴定。讨论了它们所处的耐盐度、温度、pH等生态条件。从而探讨了它们对沉积物中有机质的富集和保存所起的作用。同时,由于它们在还原硫酸盐时产生大量的硫化氢,导致沉积物中的金属离子以硫化物形式大量地沉淀和富集,这对次生硫化矿沉积物的形成也起着较大的作用。

**主题词** 盐湖沉积物 硫酸盐还原细菌 硫化氢 次生硫化矿

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## SULFATE-REDUCING BACTERIA AND THEIR GEOBIOCHEMICAL ROLE OF SALT LAKE SEDIMENTS OF CHINA

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### PREFACE

Sulfate-reducing bacteria as a biogeochemical factor possess considerable functions for petroleum formation, secondary sulfide ore formation and gypsum ore destruction, soil alkalization and salification, and even for iron-steel corrosion. The bacteria also effect the kinds and growth of organiams in aquatic environments. Sulfate-reducing bacteria, therefore, are very important in economic fields. For many years, scientists have investigated their ecology, physiology and biochemistry etc. and accumulated considerable amount of data. This paper deals initially with sulfate-reducing bacteria from Dachaidan Salt Lake and Huining Saline Lake in China.

## ENRICHMENT CULTIVATION AND ISOLATION OF SULFATE-REDUCING BACTERIA

Black-coloured and H<sub>2</sub>S-smelled sedimentary samples were collected from Dachaidan Salt Lake and Huining Saline Lake sealed in test tubes and preserved in refrigerator. According to Butlin, Adams and Thomas(1), enrichment cultivation happened under 30°C within one week 2-3g of non-contaminated samples were inoculated to a 250ml ground Erlenmayer flask, in which Baars medium and 25ml salt-lake or saline-lake water were present and 3% Na<sub>2</sub>SO<sub>3</sub> was used as selected reagent. After 7 days, great amount of black precipitate of ferrous sulfide occurred in the cultures, in which the vibrio bacteria grew absolutely dominately. Two pure cultures were obtained from two lake samples seperately by single cell seperation after enrichment cultivation for 2-3 times as above mentioned by the method of Yin Minshan, et al. (2) Both of the strains were Gram negative, curve or vibrio with 0.3-0.6×1.4-2.0μ, motile vigorously with polar fragellum, anaerobic, non-sporing and non-capsule. Organic compounds such as peptone, asparagine and lactate were used and reduced sulfate to H<sub>2</sub>S. Although the salinities of the two strains were different, the results of their mophology and physiological examinations were basically similar. We tried, therefore, to distribute both of the strains into the genus of Desulfovibrio sp.

### ECOLOGICAL ENVIRONMENT OF SULFATE-REDUCING BACTERIA

(1) Salt tolerance. In general, the salt tolerance of sulfate-reducing bacteria isolated from soil, polluted water and sediments of freshwater lakes is 0.85% NaCl but the bacteria will be inhibited from growing when the concentration of NaCl in the medium enhances to 1.5-3.0%. We added 1, 5, 10, 15, and 20% NaCl in each medium to cultivate sulfate-reducing bacteria isolated from the sediments of saline lake, and determined the amount of H<sub>2</sub>S producing for the series of cultures after 6, 11, 18, 30 and 35 days. The results shown in Fig. 1 are that the optimum NaCl conc. for the strain is 1-5%, 10% NaCl reduces its growth rate, on growth occurs when the NaCl conc. enhances to 15% or when NaCl is absent in the medium as a control. Therefore we conclude the strain belongs to slight halotrophic bacteria. The parallel work carried under the same conditions was to add 10, 20, 30, and 40% NaCl in the medium of the salt lake cultures. After 15 days, it showed that the optimum was 20% NaCl, growth was slow in 30% and no growth occurred both in 40% NaCl and in the control (no NaCl added). we conclude this strain belongs to extreme halotrophic bacteria. Kimata et al. have described the relationship of transformation in slight, intermediate and extreme halotrophic bacteria is based on the environments of their haditats.

(2) Growth temperature. In general, the growth temperature for sulfate-reducing bacteria is 0-100°C. The optima for the intermedium type are 25-40°C and for the thermophilic type are 40-65°C. Temperature of sediments at sea bottom is lower than 5°C in general. Rittenberge has reported the highest cultivative

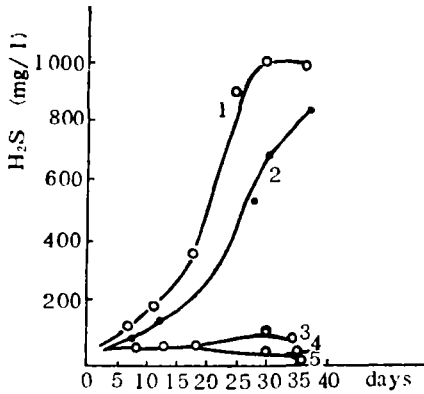


Fig.1 Effect of the NaCl conc. on the growth of sulfate-reducing bacteria isolated from the sediments of saline lake

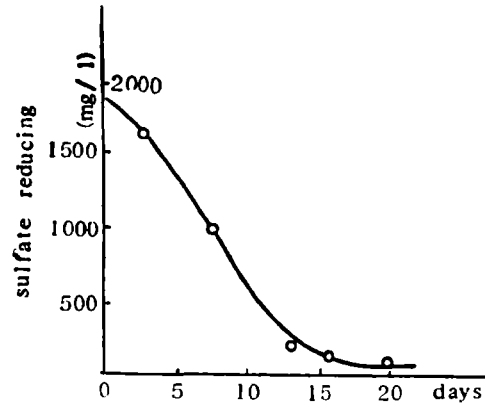


Fig.2 Sulfate reduced by sulfate-reducing bacteria in saline water

temperature for sulfate reducing bacteria is 40-45°C but can fall with the decrease of the NaCl conc. in medium. For example, the cultivative temperature for sulfate-reducing bacteria would decrease from 35-40°C to 30-35°C, if seawater is diluted with freshwater by twice or five as much in media. We examined the growth temperature of sulfate-reducing bacteria isolated from the sediments of saline lake and salt lake with 5, 10, 15, 20, 25, 35, and 45°C in 1% and 20% NaCl media respectively. The optimum temperature for the former is 25-35°C and no growth presents under 45°C, 30°C for the later. So that, we conclude both of them are the intermedium type.

(3) pH and Eh. ZoBell et al. reported the ranges of pH and Eh for sulfate-reducing bacteria reproducing in sediments at sea bottom were 6.4-9.5 and +350-500mv separately and pH was 4.2-10.4 in soil, swamp and polluted water. But Starkey and Wight considered neutral or slight alkaline environment, i.e. pH 5.5-8.5 was the optimum for sulfate reducing bacteria, acidic conditions would lower the growth rate. Bass Becking and Wood reported Eh, compositions of media and even other factors were great effects on the pH range for the growth of sulfate-reducing bacteria; pH 6.2-7.9 and Eh-50-150mv were the best. In the process of the bacteria cultivation, Starkey et al. determined Eh which was +300-+400mv in the initial and -200-300mv after 3-4 days. They also reported that H<sub>2</sub>S produced from sulfate reduced by bacteria in the cultivation period might result in pH rising and Eh lowering distinctly, which created a good reducing environment. In our experiments, sulfate-reducing bacteria isolated from the sediments of saline lake and salt lake were cultivated in pH 6.0, 6.5, 7.0, 7.5 and 8.0 media. The results are known pH 7.0-7.5 and Eh-50-15mv are the optima for them.

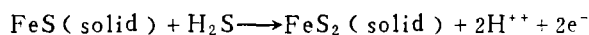
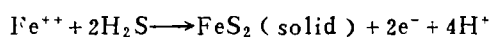
In short, sulfate-reducing bacteria can reduce sulfate to produce a great amount of H<sub>2</sub>S, and then cause Eh lowering and a stronger reducing environment.

It would be harmful to aerobic bacteria and they might be gradually replaced by anaerobic bacteria. Furthermore,  $H_2S$  takes deadly damage to aerobic bacteria and their remains turn into nutrition for sulfate-reducing bacteria. In addition,  $H_2$  as a by-product of the metabolism of anaerobic bacteria promotes sulfate-reducing bacteria with hydrogenous to reduce sulfate. All of the facts much benefit the enrichment and preservation of organic matter in sediments.

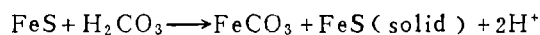
#### FUNCTIONS OF SULFATE-REDUCING BACTERIA IN SEDIMENTS

The surface layers of the samples collected from the sediments of salt lakes, are light yellow brown. Their inner layers are black and become grey immediately if exposed to dry air.  $H_2S$  is released if dilute HCl is dropped into the black samples. It is evident that the black sediments contain a great amount of FeS, which is a kind of  $FeS \cdot nH_2O$  amorphous hydrate. In sediments, sulfate-reducing bacteria reduce sulfate to  $H_2S$  when organic detritus are used as energy source. Wang has described the detail of the complex biological chains including sulfur cycle. [3] It can be found that a great amount of  $H_2S$  is produced everywhere in sedimentary reducing environments by sulfate-reducing bacteria. Lin and others have considered that  $FeS \cdot nH_2O$  is a precursor of pyrite forming.

In  $H_2S$  dominance,



in  $CO_2$  dominance,



We made an experiment to further prove that sulfate was reduced by sulfate-reducing bacteria in the sediments. We added Na-lactate 5g,  $K_2HPO_4$  0.5g,  $NH_4Cl$  1.0g and  $FeCl_3$  0.5g, pH=7.0-7.2 to saline lake water containing 1842 mg/l sulfate, inoculated 10 ml isolated, suspension of bacteria and cultivated under 28-30°C. After 7, 14 and 21 days, determined the sulfate content in the cultures separately (Fig.2). More than 90% original sulfate had been reduced after 14-days cultivation. Therefore, the reduction of sulfate-reducing bacteria to sulfate is very strong when the sediments enriched organic matter and great amount of sulfate.

Similar work was taken in saline water, containing 129.26 mg/l sulfate. We added all of components mentioned above and the suspension of sulfate-reducing bacteria in the saline well water. After 14-days cultivation compared the compositions of the upper liquid cleared by bacteria with its precipitate and the original saline water by spectrometer. The results are shown in Tab. 1.

In Table 1 it is shown that during the sulfate reduction and  $H_2S$  formation caused by the bacteria, the reaction between  $H_2S$  and metal anions in saline water, such as Cu, Bi, Ti, Mn and Fe etc. leads to sulfide precipitation. Thus, it plays a certain role to the formation of secondary sedimentary sulfide ores namely it can, in the form of sulfide, precipitate and enrich amounts of metal

anions deriving from lakewater and sediments.

Table. 1 Spectrometer analysis of the components of saline water and precipitate before/after the bacteria cultivation

elements samples	Ca	Al	Sr	Cu	Bi	Mg	Si	Mn	Ti	Na	Fe	K
	upper cleared liquid from culture	✓*	✓	✓			✓	✓			✓	
precipitate from culture	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
original saline water	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

\*presence of metal elements

#### Reference

1. Butlin, K.R. et al, 1949, The isolation and cultivation of sulfate reducing bacteria, *J. Gen Microbiol.*, V.3, p.46-59.
2. Yin Mingshan and Wang Dazhen, 1965, Isolation of sulfate reducing bacteria, *Acta Microbiologica Sinica*, V.11 N.2.
3. Wang Dazhen, 1983, Microbiological conversion of substances and energy in organic sedimentary environments, *Acta Sedimentological Sinica*, V.1 N.1
4. Lin Hejie et al., 1983, Discuss for the important meaning on the studies of auto-micro-spherical pyrite by scanning electromicroscopic technic, *Academia Sinica B*, N.1