Characteristics of cultivation and domestication of a sort of SRB isolated from landfill leachate under sulfite condition

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Keywords: SO_2 purification, Sulfite, Desulfurization, SRB, Landfill Leachate

Abstract. SO_2 is one of the most common pollutants in the country where fossil fuel as the main energy for industrial manufacture. Nowadays biological fume gas SO_2 desulfurization would be a most potential way to transform it to element S to realize S resources recycling. The initial step is to produce sulfide from SO_2 effectively. In the research a sort of SRB was selected from landfill leachate and results show that almost all the sulfite could be transformed at first 3 days and at least a third of them could be reduced to sulfide in 10 days. The characters would apply a promising basic prerequisite to reduce the gas fume SO_2 synergized with landfill leachate by SRB and form element S to realize the S resources recycling finally.

1 Introduction

A Paper named “Environmental toll” [1] indicated that most death of the organisms owns to air pollution, which show that the treatment of air pollution is beyond our thinking. SO_2 as one of the most common pollutants should be controlled as well for its forming acid rain and harmful for human beings described in article [2]. As usual, SO_2 came from the combustion of fossil fuel such as coal or petroleum in order to heating in thermal power plant or metallurgical industry and so on. [3-5] pointed out that the biological methods of SO_2 treatment can be divided into two pathways. One is to transform SO_2 to sulfate, and the other is to transform SO_2 to sulfide, and then to produce S as a cycle resource. As a result of SO_4^{2-} in wastewater is also harmful to environment indirectly described in [6], the second way is the most potential biological way to control SO_2, and the mot important first step is the forming of sulfide.

Sulfate reducing bacteria could use sulfate or sulfite as a electronic acceptor to reduce them to sulfide. However, as to wet flue gas desulfurization, SO_2 emitted into desulfurization devices could be dissolved and then form sulfite to a large extent. So the desulfurization of sulfite by SRB could be meaningful. The paper report some researches on characteristics of a sort of SRB isolated from landfill leachate and its desulfurization of sulfite to apply theoretical bases for Synergism purification of flue gas SO_2 with landfill leachate, realizing recycle and regeneration of sulfur resource finally.

2 Methods and Materials

2.1 SRB sampling procedure

SRB was selected from a ditch near Anju Road in Wuhan China. The ditch store and purified the landfill leachate emitted form a refuse transfer station besided. A turners with 3 m long handlebar

  DOI: 10.25236/acete.2019.111

was used to obtain the black mud in the bottom of the ditch into 250 mL purified dry hermetically sealed reagent bottle to store the bacteria.

2.2 Culture medium

Improved Postgate's C medium [7] was used and it contains 0.125 $g \cdot L^{-1}$ KH_2PO_4, 0.25 $g \cdot L^{-1}$ NH_4Cl, 0.015 $g \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$, 0.0025 $g \cdot L^{-1}$ $FeSO_4 \cdot 7H_2O$, 0.2175 $g \cdot L^{-1}$ $CaCl_2$, 2.5 $g \cdot L^{-1}$ Na_2SO_3, 0.875 $g \cdot L^{-1}$ sodium lactate, 0.25 $g \cdot L^{-1}$ yeast extract, 0.075g L^{-1} sodium citrate, which was prepared with distilled water. All the components were added into flask except $FeSO_4 \cdot 7H_2O$, sodium citrate, and Na_2SO_3. Then 200mL medium was taken into 250 mL anaerobic bottle. After that it was deoxidized by N_2 for 10-15 min. Then $FeSO_4 \cdot 7H_2O$, sodium citrate and Na_2SO_3 were added into the anaerobic bottle in proportion. Then its pH was adjusted to 7 by HCl (1:1 v/v). At last, it was deoxidized by N_2 for 10-15 min again then sealed.

2.3 Enrichment, domestication and Sulfite reduction

As the medium prepared, SRB was inoculated into the anaerobic bottle. Then it was put into the shaking table (HNY-2102C, Honour, Tianjin, China) to grow at the rotation speed 120 r/min and 35℃. 1 hour later and the next day, concentration of sulfite, pH, sulfide or OD_{600} was tested. After that every 2-3 days it was texted to determine the characters of the growth of SRB. At the very fist time, 3 g mud was taken into the anaerobic bottle as well, and then the mixture was deoxidized by N_2 for 10-15 min, sealed to grow. While later cultivation times, 20 mL bacteria solution was inoculated into 200 mL new prepared medium every 7 days to keep SRB growing well.

2.4 Analytical method

A certain volume mixture of the bacteria solution was taken into the centrifuge tube by injection syringe after shaking up the anaerobic bottle. If the gas volume produced by the bacteria was more than that volume, the sample was taken out directly. Otherwise, it must be replenished to that volume by N_2 through the injection syringe to make its pressure balance with atmosphere. pH value was texted by a pH meter (PHS-3C, INESA, Shanghai, China). Then the samples were centrifuged at 4000 r/min and 10min to separate solid from solution (TDZ4A-WS, Xiangli, Hunan, China). certain supernate was taken by a pipettor (Dragon-med, Beijing, China) to determine sulfite and sulfide by pararosaniline hydrochloride spectrophotometric methods at 550 nm [8] and Methylene blue spectrophotometric methods at 665 nm [9], respectively. Another certain solution was also taken out form the anaerobic bottle by a new injection syringe without shaking to determined OD_{600} using spectrophotometer (V-1200, AOE, Shanghai, China) at 600 nm.

3 Results and discussion

3.1 Characters of sulfite reduction during cultivation and domestication

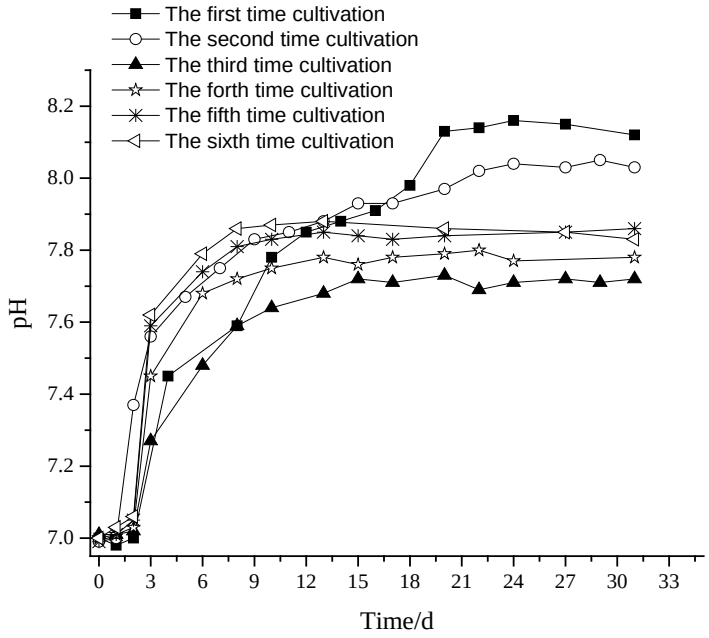

Fig. 1 pH variations in every time cultivation

At the first and second time cultivation pH increased more than others as shown in Fig. 1, besides, sulfide produced also much more than other others as shown in Fig. 2, which shows that initial SRB living condition could exist some factors to let SRB produce more sulfide and pH higher. So the black mud selected from bottom of landfill leachate was inoculated into the same medium except adding sulfite or sodium lactate to detect the reason why in the first time cultivation sulfide was produced more. However the result shows that sulfide was produced little in both tests, which indicates that SRB in the mud may grow with other nutrition matters rather than sodium lactate.

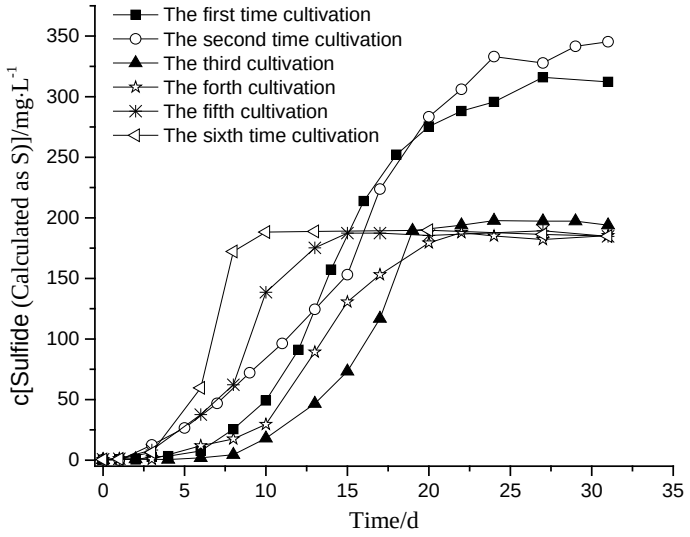

Fig. 2 Sulfide variations in every time cultivation

But after the third time cultivation as shown in Fig.1 and Fig. 2, the speed of pH increase and sulfide formation were both improved after early three times domestication. After six times enrichment and domestication of SRB, the variations of pH, sulfite and sulfide tended to

stabilization. So at least six generations cultivation and domestication were needed to enrich such a sort of SRB and keep sulfite reduced and sulfide produced fast and stably by such SRB. Besides after six times cultivation, pH could reach nearly 7.9 from 7.0 within 10 days as shown in Fig. 1.

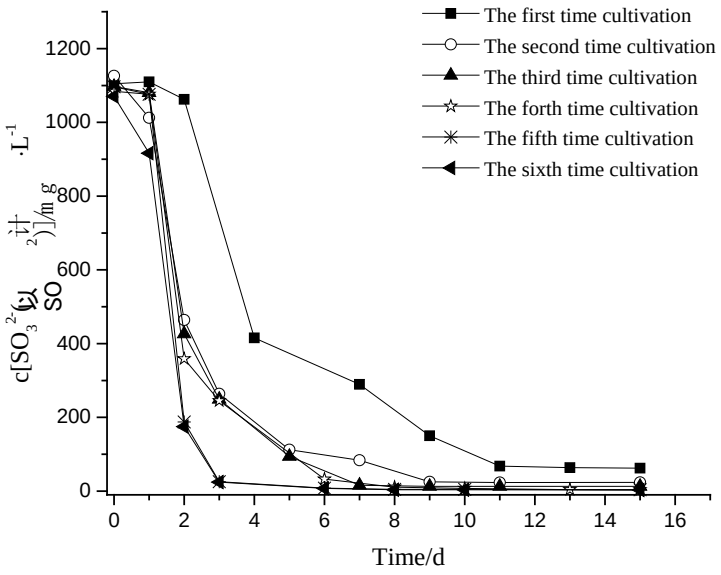

Fig. 3 SO_3^{2-} variations in every time cultivation with initial sulfite 1270 mg·L^{-1}

After first 3 days sulfite nearly were disappeared as shown in Fig. 3. In Fig. 2 sulfide were produced nearly 200 mg/L also within 10 days. The characters could apply some preliminary research for the possibility of purification of fume gas SO_2 synergized with landfill leachate.

3.2 Characters of sulfite reduction and sulfide formation

Sulfide was produced about 200 mg/L (in bacteria solution) calculated as S as shown in Fig. 2 while the initial sulfite was about 1270 mg/L as shown in Fig. 3. If sulfite was reduced absolutely, sulfide should be formed to about 635 mg/L. Only about a third of it was reduced. But sulfite disappeared after the third day cultivation, which seems that sulfite would transform to other sulfocompound in the low concentration of organic carbon resource. Barton L. L. [10] pointed out that the more electron exists , the more sulfide would be produced. If the electron concentration is low, trithionate and thiosulfate would be formed and sulfide was produced little. In the medium, sodium lactate was the electron donor, and its concentration may decide how much the sulfide was produced. So in the research, the other sulfite would be transformed to trithionate and thiosulfate by SRB. And increasing C/S would be a effective way to produce more sulfide by SRB.

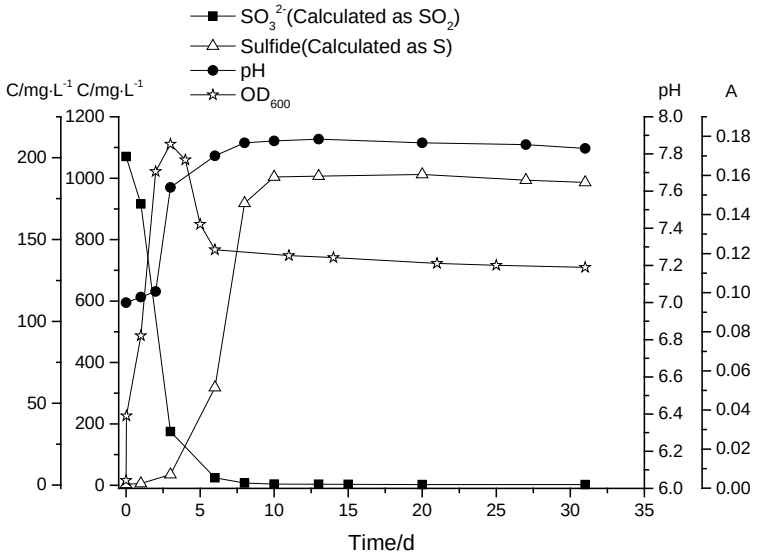

Fig. 4 All factors variations in the sixth time cultivation

The variations of OD_{600} in Fig. 4 shows that the growth rate of SRB reaches the top during first 3 days and on the 3rd day the amount of SRB reaches the top. While the decrease ratio of sulfite just right at the same period of time, which show that sulfite would be absorbed or transformed to other matters by SRB. In order to investigate whether it was absorbed or not, the mixture of bacteria solution without centrifugation was measured. The results was still the same with the initial one. So in this period of time, sulfite seemed to be transform to other matters, followed by Sulfide starting to be produced. The top rate of sulfide was detected on about the 6th day. Just about 3 days behind that of sulfite decrease and when the amount of SRB came to the most. But pH on the 2nd to 3rd increased dramatically, which shows that H^+ of the solution was decreased fast. From this point of view, sulfide should be formed fast at the same time, but we detected a little in solution, which indicates that the sulfide may be formed fast at 2-3 days and dissolve a little in bacteria solution while most of it was released above the solution in the anaerobic bottle. As time goes on, sulfide was dissolved more and more, the sulfide increased fast in the solution. That is to say the delay of sulfide increase in solution would probably own to the dissolution process which takes a certain period of time rather than delay of sulfide production by SRB.

4 Conclusion

A sort of SRB which could reduce sulfite and produce sulfide effectively from the mud at the bottom of landfill leachate in Wuhan China. The reduction cycle of sulfite by SRB is about 10 days and nearly all the sulfite could be transformed. The sulfite reduction and sulfide production by SRB almost occurred at the same time. The delay of sulfide detected probably owns to its delay of dissolution in solution. The time when the amount of the SRB reaches the top only needs as short as 3 days and sulfite was nearly disappeared at the same. Raising C/S would be a effective way to produce more sulfide, which could apply a promising basic prerequisite to reduce the gas fume SO_2 synergized with landfill leachate by SRB and form element S to realize the S resources recycling finally.

Acknowledgements

The authors are all grateful for the support of Science and Technology Research Project of Education Department of Hubei (B2016282).

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