

DEVELOPMENT OF A BIOCHEMICAL DESULFURIZATION PROCEDURE FOR FUELS

A. J. Davis and T. F. Yen
Departments of Biological Sciences and Chemical Engineering
University of Southern California, University Park
Los Angeles, California 90007

INTRODUCTION

The removal of sulfur from high-sulfur containing petroleum has long presented a problem. With the depletion of so-called "sweet" oil (i.e., oil low in sulfur content), the problem has escalated into major proportions to contend with a clean and livable environment of our biosphere. The development of practical methods of decreasing, or eliminating the sulfur content of petroleum has repeatedly proved an economical obstacle to the industry. The amount of dollars spent on corrosion technology alone is phenomenal (1).

Rudimentary investigations of microbial desulfurization have received little attention in the literature at this time (2). A successful example is the removal of pyrite from coal by *Thiobacillus* sp. and *Ferrobacillus* sp. (3). While studies of the complex hydrocarbon-sulfur systems, being closer to in situ reality are of great value, investigations of pure systems should form the foundations of these more detailed investigations.

Our present study was intended to explore the ability of a micro-organism as a possible desulfurizing agent. To this end, a pure system was employed that would give us some idea as to the ease with which such an agent could abstract organically-bound sulfur. While this program is preliminary in scope, it is hoped that future investigations may lead to a feasible industrial application.

MATERIALS AND METHODS

a) Media and Culture

The strain of *Thiobacillus thiooxidans* used in these experiments was originally obtained from the National Type Cultures Collection. Inoculate strain was obtained by further culture in this laboratory (4). For growth of the organisms, a variation of Waksman's medium was prepared; it contained the following concentrations of salt in grams per liter (all Mallinckrodt "AR" grade): $(\text{NH}_4)_2\text{SO}_4$, 0.20; KH_2PO_4 , 3.00; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.50; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.25. The medium was prepared with distilled water. In place of the elemental sulfur substrate of Waksman's medium, different symmetric organic sulfides of the tertiary butyl class with a normalized sulfur equivalent of 10.0g/liter were utilized. In order to provide a greater surface area, the normally viscous sulfides were emulsified with 1-2 drop(s) of Triton X-100 surfactant (J. T. Baker Chemical Co.) added to the medium. The pH was then adjusted to 3.5 with 1.0 M H_3PO_4 (Mallinckrodt "AR" grade). Sterilization of the media was accomplished by autoclaving and by membrane filtration.

b) Sulfide Samples

The tertiary butyl sulfide and the tertiary butyl disulfide used in these experiments were obtained from the Aldrich Chemical Company. The purity of the monosulfide, molecular weight: 146.30, was in excess of 97%. The disulfide, molecular weight: 178.36, being "technical" grade was assayed to be 88% pure disulfide with the remainder consisting of tertiary butyl trisulfide.

The tertiary butyl polysulfide employed was kindly supplied by Phillips Petroleum Company. The purity of the polysulfide whose molecular weight averaged out to be 190.00 was greater than 90%. The polysulfide contained an average of four and five sulfur atoms per molecule. No pre-treatment of the sulfides was performed.

c) Analytical Methods

Insoluble barium sulfate precipitate, one of the two criteria by which growth of the bacteria was established was determined by standard gravimetric methods. The amount of barium sulfate is directly proportional to the amount of sulfide that has been oxidized by the sulfur bacteria.

Hydrogen ion concentration (pH) was the second criterion by which growth of the bacilli was established. Sulfuric acid is a natural metabolic by-product of the acidophilic *Thiobacillus thiooxidans* (5); and as sulfur is utilized, acid build-up in the medium is to be expected---thus lowering the pH. Studies done in this laboratory has shown that the bacteria grow well in a pH as low as 0.5.

Fernbach culture flasks (Pyrex, 2800 ml) containing 1.0 liter of modified Waksman's medium were prepared in duplicate. The first pair of flasks received 49.0 ml t-butylsulfide and one drop Triton X-100 each. The second pair of flasks received 28.0 ml t-butyl disulfide and one drop of Triton X-100 each. The third pair of flasks received 17.0 ml t-butyl-polysulfide and two drops of Triton X-100 each. In addition, flasks of elemental sulfur and flasks of organic sulfur substrates were prepared to act as controls. After sterilization, all flasks containing sulfur or organic sulfur compounds with the exception of the organic sulfur control flasks, were inoculated with 10.0 cc *T. thiooxidans* suspensions. All flasks were incubated at room temperature.

Samples for the barium sulfate determinations were prepared by removing a 5.0 ml aliquot portion from each flask and then submitting these to centrifugation on an Adams centrifuge for 15 minutes at 3,000 rpm. To the supernatant of each was added two drops concentrated HCl, an acid buffer; and eleven drops of a saturated solution of barium chloride (Mallinckrodt "AR" grade). The precipitated was filtered on a "Millipore" apparatus (Pyrex); washed with distilled water and then allowed to dry three days in an evacuated dessicator. The samples were then ignited and the weight of barium sulfate established. The organic sulfur controls were handled in the same manner to avoid discrepancy. The amount of barium sulfate produced from the organic sulfides less the controls was averaged and then plotted (Fig. 1). The pH was read directly with a Beckman "Zeromatic," SS-3 pH meter (Fig. 2).

PROCEDURE

Modified Waksman's medium was prepared in a Fernbach culture flask. An amount of organic sulfide normalized to an equivalent sulfur content of the standard medium (10g/liter) was added; followed by the addition of an emulsifier. The medium was then autoclaved for 30 minutes at 15 psi (121°C) or subjected to membrane filtration. Upon cooling, the medium was inoculated with 10 cc pure strain *Thiobacillus thiooxidans*. The culture's initial pH value was read, and an initial gravimetric sulfate assay was performed on it. Thereafter, pH and sulfate values were determined at two day intervals for a period of 25 days.

The model was so designed as to study the ability of sulfur-oxidizing bacteria to utilize the organically bound sulfur as substrate. The symmetry of the sulfides provided an insight into the sulfur abstracting process of *T. thiooxidans*.

It can be seen that the oxidizing potential of the organism is enhanced by the availability of unshielded sulfur in the molecular structure. The sulfur-sulfur bonds of the di- and poly-sulfides are easily disrupted while the sulfur-carbon bond of the mono-sulfide seems questionable. While some sulfate ion is produced by the organism on the monosulfide, it is unknown at this point how much of the sulfate is due to residual sulfur previously incorporated by the bacteria. The values of pH seem to bear this out. There is no question that the organism is growing on the di- and poly-sulfides, as shown by sulfate ion and pH values.

DISCUSSION

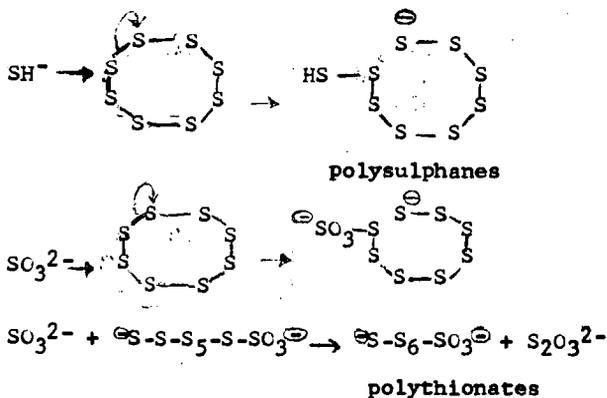
This study indicates that the sulfur of our sulfide samples was susceptible to bacterial attack in two of the three cases. The aliphatic sulfides could be ranked in the order of their ease of oxidation as: di-*n*-butyl polysulfide > di-*n*-butyl disulfide > di-*n*-butyl sulfide.

Seemingly, *T. thiooxidans* is able to attack the sulfur-sulfur bond quite readily and the sulfur-carbon bond with more time and difficulty. Since a large portion of the sulfur compounds in petroleum are of the monosulfide-aromatic type more attention will be paid to this facet.

The simplest scheme of the bacterial oxidation would be:



A few mechanisms have been postulated for this reaction (6). No matter what mechanism is considered, the oxidation of elemental sulfur or thio-sulfate is accompanied by reductive cleavage of the sulfur-sulfur bridges. In the case of sulfur, the intermediate involved is a cyclic form of sulfur, probably S₈; although there is little difference observed for different allotropic forms of sulfur, such as rhombic, precipitated, and amorphous. These cyclic sulfides form the basis of polysulphanes as well as polythionates which could be metabolized readily by Thiobacilli.

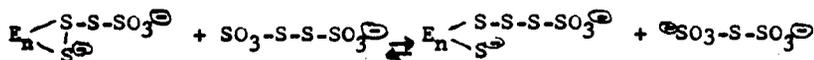
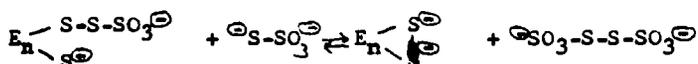


Actually, oxidation of sulfur begins with its reduction, in which the glutathione-sulhydryl groups located near the cell surface take part

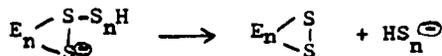
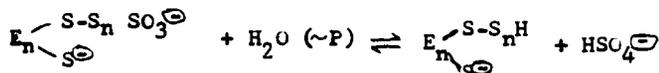


The SH^{\ominus} involved or the SO_3^{2-} performs the attack and the cleavage.

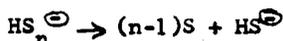
For the thiosulfate oxidation, it is hypothesized that an enzyme system on the cell surface initiates the formation of polythiosulfonic acid and consequently splits the terminal SO_3^- as sulfate. This process may evolve the intermediate of mixed anhydride $-\text{S-O-PO}_4^-$ from phosphorylation:



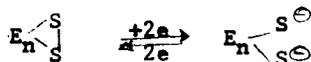
Therefore



polysulfanes



The overall reaction is one of electron transfer in the enzyme system:



The critical step in the utilization of multiple sulfur linkages is the availability of HS^{\ominus} from the reduced enzyme system. The HS^{\ominus} could possibly attack the cyclic or the acyclic multiple sulfur linkages through a nucleophilic mechanism. The sulfide linkages thus cleaved will undergo oxidation to sulfite or thiosulfate. This holds true for the present case of disulfide and polysulfide.

Finally, removal of the sulfate ion creates a new problem of contamination. In connection with this, an examination of sulfate-reducing bacteria for the complete removal of sulfur is being undertaken. The conversion of the organic sulfide into inorganic sulfide will be one future objective.

ACKNOWLEDGEMENT

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REFERENCES

1. T. A. Bertness, Personal Communication, Mobil Oil Corp., 1973.
2. D. L. Isenberg, Microbial Desulfurization of Petroleum, Ph.D. Dissertation, Louisiana State University, 1961.
3. M. P. Silverman, M. H. Rogoff, and I. Wender, Removal of Pyritic Sulfur from Coal by Bacterial Action, *Fuel*, **42**, 113-124 (1963).
4. J. E. Findley, M. D. Appleman, and T. F. Yen, Papers delivered at ACS, Div. of Microbial Chemistry and Technology, Chicago, 1973.
5. A. B. Roy and P. A. Trudinger, *The Biochemistry of Inorganic Compounds of Sulfur*, Cambridge University Press, 1970, pp. 207-248.
6. G. A. Sokolova and G. I. Karavaiko, *Physiology and Geochemical Activity of Thiobacilli*, Israel Program for Scientific Translations, pp. 48-55, 1968.

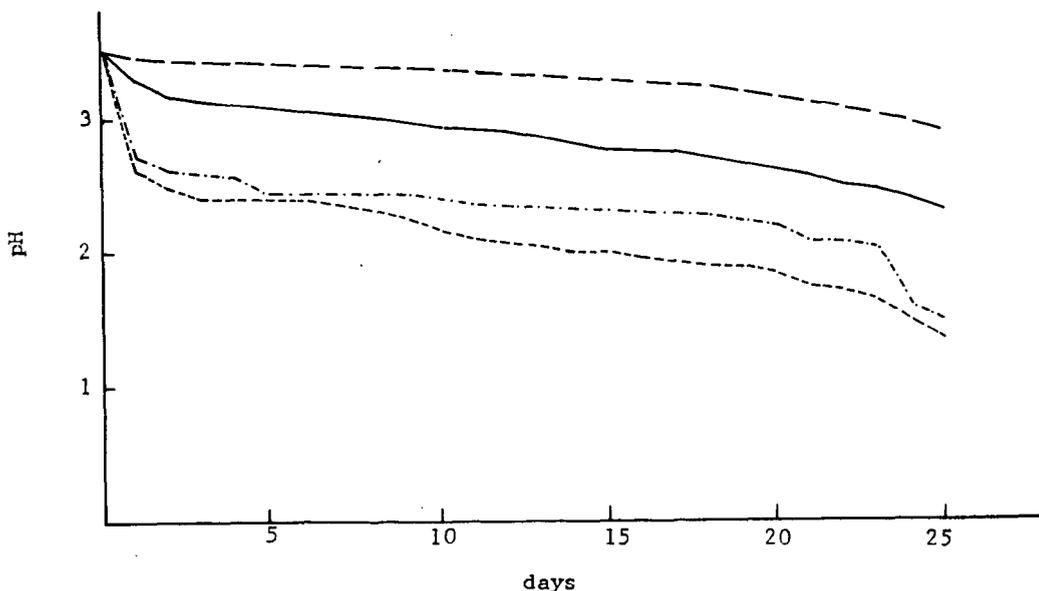


Fig. 2. Variation of pH after inoculation of sulfur bacteria: from top to bottom, the long dashed line represents di-t-butyl monosulfide; the solid line represents di-t-butyl disulfide; the dash-dot line represents di-t-butyl polysulfide, and the short dashed line represents control of elemental sulfur.

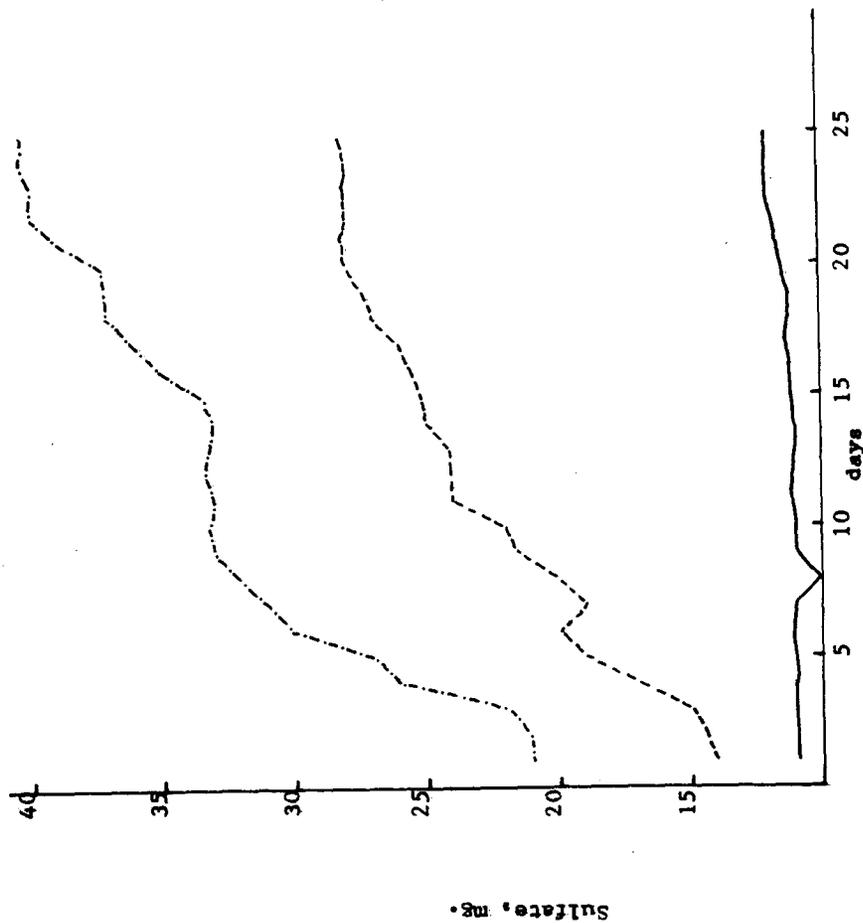


Fig. 1. Conversion of organic sulfide into sulfate in 25-day period. The amount of sulfate ion represents the difference of that produced in the inoculated flasks containing different organic sulfide and that in the controls containing the same organic sulfide without inoculation. From top to bottom, the --- represents di-t-butyl polysulfide, the - - - represents di-t-butyl disulfide and represents the di-t-butyl monosulfide.