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Thiosulfate Utilization by *Thiobacillus thiooxidans* ATCC 8085

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The "Waksman" strain of *Thiobacillus thiooxidans* (*T. thiooxidans* ATCC 8085) was reported to be incapable of growth when thiosulfate is supplied as the energy source (W. I. Schaeffer, Ph.D. Thesis, Rutgers Univ., New Brunswick, N.J., 1963). Schaeffer noted, however, that the organism possesses the ability to oxidize thiosulfate. S. C. Rittenberg and R. P. Grady (J. Bacteriol. **60**:509, 1950) were successful in growing this organism (apparently the same strain) in a thiosulfate medium, and this has also been our experience (J. M. Shively and A. A. Benson, J. Bacteriol. **94**:1679, 1967). In view of these inconsistencies, a more thorough study of the growth of *T. thiooxidans* ATCC 8085 in a thiosulfate medium was undertaken.

The media used in this experiment contained (per liter of distilled water): either $Na_2S_2O_3 \cdot 5H_2O$, 5.0 g or sublimed sulfur, 10 g; KH_2PO_4, 3.0 g; $(NH_4)_2SO_4$, 0.4 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCl_2 \cdot 2H_2O$, 0.25 g; $FeSO_4 \cdot 7H_2O$, 0.01 g. The *p*H was 4.5 for the thiosulfate medium and 4.0 for the sulfur medium. The media (50 ml) were dispensed into 250-ml Erlenmeyer flasks and were sterilized by autoclaving at 121 C for 15 min. Sulfur was sterilized separately by steaming 0.5-g quantities for 1 hr on 3 successive days, and these portions were added aseptically to 50 ml of the autoclaved medium. Autoclave sterilization of the thiosulfate medium in its complete form did not visibly alter the medium nor hinder the growth of the culture.

All incubations were made at 30 C on a rotary shaker (New Brunswick Incubator Shaker Model G-27, New Brunswick Scientific Co., New Brunswick, N.J.) adjusted to 180 rev/min. The culture was routinely carried in the sulfur medium by transferring 0.5 ml to fresh medium every 7 days. Sulfur cultures were incubated in a stationary condition for 3 days before shaking.

Initial inoculations into the thiosulfate medium were made by allowing the sulfur to settle from the medium of a 5-day culture, carefully removing 10 ml of fluid, centrifuging, resuspending the cells in 10^{-5} N H_2SO_4, and inoculating with 0.5 ml of this suspension. The initial thiosulfate culture required 20 days before growth occurred. The second transfer resulted in a culture which grew to maximal turbidity in about 5 days. Subsequent transfers resulted in cultures which reached the maximal stationary phase of growth in about 45 hr. When serial dilutions (sulfur culture) containing from 10^2 to 10^6 cells were used for the inoculum, all of the resulting cultures grew in approximately 20 days. This tended to eliminate the possibility of thiosulfate utilizing contaminants or mutants. The thiosulfate-adapted culture retained its ability to utilize sulfur as an energy source.

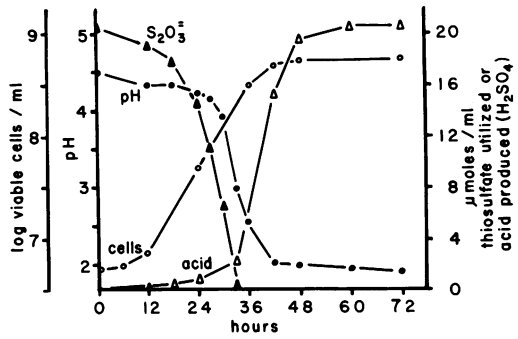

FIG. 1. *Growth of Thiobacillus thiooxidans ATCC 8085 in a thiosulfate medium.*

The growth characteristics of the thiosulfate-adapted culture were determined by use of several parameters. Cells were enumerated by use of the "most probable number" technique (J. M. Shively and A. A. Benson, J. Bacteriol. **94**:1679, 1967), and acid production was measured by determining *p*H and by titrating (to the original *p*H of the medium) 10-ml samples with standardized NaOH. Thiosulfate utilization was determined by iodine titration (A. R. Colmer, J. Bacteriol. **83**:761, 1962).

The culture reached the maximal stationary phase in about 45 hr (Fig. 1), with a generation time of about 4 hr. The *p*H dropped to about 1.9, and the thiosulfate was depleted in about 33 hr.

The ability of *T. thiooxidans* ATCC 8085 to grow in a thiosulfate-containing medium has been established. The reason for the long adaptation period requires further clarification.

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