APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1980, p. 516-521
0099-2240/80/09-0516/06$02.00/0
Vol. 40, No. 3

Organic Carbon Utilization by Resting Cells of Thiosulfate-Oxidizing Marine Heterotrophs

JON H. TUTTLE

The University of Texas at Arlington, Arlington, Texas 76019

Two thiosulfate-oxidizing marine heterotrophs, strains 12W and 16B, were tested for utilization of [^{14}C]glucose and [^{14}C]acetate, respectively, in the presence or absence of thiosulfate. Thiosulfate oxidation caused an increase in organic carbon incorporation and a corresponding decrease in respiration at pH 6.5, near the optimum pH for thiosulfate oxidation and thiosulfate-stimulated growth in these bacteria. The amount of glucose or acetate metabolized remained virtually unaffected by thiosulfate oxidation. The metabolic shift in carbon utilization was diminished by increasing the initial pH to 8.0. The results indicate that marine heterotrophs 12W and 16B exhibit a type of mixotrophic metabolism which differs from that observed in the thiobacilli.

The microbial oxidation of reduced sulfur compounds in aerobic environments is usually attributed to the activities of chemolithotrophic bacteria whose metabolism of both sulfur and carbon is typified by species of the genus *Thiobacillus* (3, 7, 10, 13, 14, 21, 29). It has long been recognized, however, that a variety of heterotrophic bacteria also oxidize reduced, inorganic sulfur compounds (15, 16, 19), but thiosulfate is the usual substrate for an incomplete oxidation of inorganic sulfur. The heterotrophic thiosulfate-oxidizing bacteria appear to be a diverse taxonomic group and have been recovered from a wide variety of habitats, including soils (5, 15-17, 20), freshwater (1, 19, 28), and both inshore and offshore marine environments (23, 24), including the Galopagos Islands rift-vent upwellings (J. H. Tuttle, H. W. Jannasch, and C. O. Wirsen, unpublished data). Activities of these bacteria have been demonstrated in meromictic lakes (28), soils (2, 18), anoxic marine basins (24, 27a), inshore marine waters (27), and even at high pressures and low temperatures in the deep sea (26), but their ecological role with respect to other aerobic, thiosulfate-oxidizing bacteria is still unclear.

The metabolism of heterotrophic thiosulfate-oxidizing bacteria distinguishes them from the thiobacilli and other chemolithotrophs in that (i) thiosulfate is never completely oxidized to sulfate, but to tetrathionate or other polythionates with a corresponding increase in the pH of the medium, (ii) growth is never wholly dependent upon the oxidation of thiosulfate or other reduced sulfur compounds, and (iii) organic carbon is always required for growth under any culture conditions. Early investigations on thiosulfate oxidation and growth in batch cultures of thiosulfate-oxidizing heterotrophs failed to demonstrate increased growth rates or cell yields in the presence of thiosulfate (15, 16, 20). These results led to the conclusion that incomplete, heterotrophic thiosulfate oxidation is an incidental reaction serving no useful function in bacterial growth. It was later demonstrated, however, that the growth rates of three marine heterotrophs in pH-controlled batch cultures are stimulated by the oxidation of thiosulfate to tetrathionate (22). Failure of previous workers to find thiosulfate-stimulated growth was suggested to be due to the high ratio of organic carbon/thiosulfate in the various employed media, which would cause masking of the stimulatory effect.

Despite the demonstration of carbon dioxide assimilation coupled with thiosulfate oxidation in resting cells of marine pseudomonad 16B (27) and of simultaneous, additive oxygen uptake by three marine heterotrophs in the presence of thiosulfate and organic compounds (22), the physiological basis for thiosulfate-stimulated growth of heterotrophic bacteria has remained unclear. This investigation was undertaken to determine the influence of thiosulfate oxidation on the utilization of ^{14}C-labeled organic carbon in resting cells of two unrelated strains of thiosulfate-oxidizing marine heterotrophs.

MATERIALS AND METHODS

Bacteria. The microorganisms chosen for this study were facultatively anaerobic pseudomonads 12W and 16B, isolated from Eel Pond (Woods Hole, Mass.) and the oxygen-sulfide interface of the Black Sea, respectively, as previously described (23). Growth of both strains in organic carbon-containing media has been shown to be stimulated by thiosulfate oxidation (22). Detailed descriptions of strains 12W and 16B appear elsewhere (22, 25). Stock cultures were maintained on TB agar (23).

Media and culture conditions. Both strains were cultured in thiosulfate-basal salts medium containing the following: $Na_2S_2S_2O_3 \cdot 5H_2O$, 2.5 g; KH_2PO_4, 0.5 g; K_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $(NH_4)_2SO_4$, 3.0 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; NaCl, 20 g; and distilled water, 1 liter. Organic carbon consisted of 0.1% (wt/vol) glucose (strain 12W) or 0.1% (wt/vol) sodium acetate (strain 16B). Strain 16B is unable to utilize glucose as a sole carbon and energy source (22). The pH of the complete medium was adjusted to 7.5 with NaOH, followed by filter sterilization with 0.22-μm membrane filters (Millipore Corp., Bedford, Mass.).

The cultures (1-liter volume) were grown in 1-liter reaction kettles, mixed by a magnetic stirring bar, and aerated with sterile air passed through gas dispersion tubes. The pH was maintained at 7.5 throughout growth by the automatic addition of 0.5 N HCl controlled by a Radiometer TTT-2 titrator (The London Co., Cleveland, Ohio). Inocula were 10-ml portions of precultures grown in the appropriate medium for 24 h. All cultures were incubated at ambient temperature (25 ± 2°C).

The cultures were harvested after 24 to 28 h of growth by centrifugation at 10,000 × *g* and 5°C. The cell pellets were washed twice with and then suspended in sterile pH 8.0 artificial seawater (Seven Seas Marine Mix; Utility Chemical Co., New York) to a concentration of 0.04 g (wet weight) of cells per ml of suspension. The cell suspensions were stored aseptically at 4°C until used for experiments, but never for longer than 6 days. The rate of thiosulfate oxidation remained unaffected by storage.

Chemicals and chemical determinations. All chemicals used were of reagent quality or better. Radiolabeled glucose and sodium acetate were purchased from New England Nuclear Corp., Boston, Mass. Tetrathionate was determined by the method of Kelly et al. (6), protein was estimated by the method of Lowry et al. (9) with bovine serum albumin as the standard, and pH was measured with a Metrohm expanded-scale pH meter (Brinkmann Instruments, Inc., Westbury, N.Y.).

Measurement of glucose and acetate utilization. Incorporation and respiration of [*U*-^{14}C]glucose (strain 12W) and sodium [2-^{14}C]acetate (strain 16B) by appropriately diluted resting cell suspensions were determined at pH 6.5 and 8.0. Reaction mixtures contained 250 μmol of K_2HPO_4, 250 μmol of KH_2PO_4, and 12.5 μmol of either glucose (0.023 μCi/μmol) or sodium acetate (0.023 μCi/μmol). The above were added to 25-ml Erlenmeyer flasks as 4.0-ml portions of a common, sterile solution prepared in 2% (wt/vol) NaCl and adjusted to the appropriate pH with NaOH or HCl. Identical reaction mixtures were used for the measurement of tetrathionate formation, except that only unlabeled carrier glucose or acetate replaced radiolabeled carbon. Thiosulfate was added, when appropriate, as a 0.5-ml portion of 100 mM $Na_2S_2O_3 \cdot 5H_2O$ in artificial seawater. When thiosulfate was omitted, it was replaced by 0.5 ml of artificial seawater. Noncell controls were prepared as above, except that they contained an additional 0.5 ml of artificial seawater in place of the cell suspension.

After the above conditions, the flasks were tightly stoppered with rubber serum caps fitted with 0.5-ml polypropylene cups which were suspended through the flask neck. The cups contained 0.2 ml of Hyamine hydroxide (New England Nuclear Corp.) and a strip (3 by 0.3 cm) of filter paper.

Experiments were initiated by injecting 0.5 ml of an appropriately diluted resting cell suspension (usually 1:5 for isolate 12W and 1:10 for isolate 16B) through the serum stopper. The flasks were shaken throughout the duration of the experiments and the CO_2-trapping procedure at 150 rpm on a rotary shaker at room temperature (25 ± 2°C). Experiments were terminated by the injection of 0.07 ml (pH 6.5 experiments) or 0.14 ml (pH 8.0 experiments) of 4 N H_2SO_4 into the reaction mixtures. These volumes of acid were sufficient to decrease the pH of the reaction mixtures to approximately 2.5 and to stabilize tetrathionate formed during thiosulfate oxidation.

Flasks were shaken for an additional 15 min to permit trapping of $^{14}CO_2$ into the Hyamine hydroxide-soaked filter paper. The stoppers were then removed, and the contents of the polypropylene cup were rinsed into a scintillation vial with 10 ml of Bray solution. The CO_2-trapping procedure was found to be 98 to 101% effective based upon the recovery of 1.5×10^5 dpm of $NaH^{14}CO_3$ added to the various reaction mixtures in place of [^{14}C]glucose or [^{14}C]acetate, followed by the injection of the appropriate volume of 4 N H_2SO_4.

^{14}C-labeled cells were removed by filtering 2.5 ml of the acidified reaction mixtures through 0.22-μm membrane filters (Millipore Corp.) prewetted with artificial seawater. The filters were washed twice with 10-ml portions of artificial seawater, placed into scintillation vials, and dissolved in 10 ml of Bray solution.

All samples were allowed to stand for at least 24 h and were then counted to at least a 2% or 2σ error (10,000 counts) with an SL-20 liquid scintillation spectrometer (Teledyne Analytical Instruments Div., Teledyne, Inc., San Gabriel, Calif.). The results were corrected for background, for counting efficiency by the channels ratio method, for counts adhering to the filters by the subtraction of counts on time zero control filters, and for volatilization of [^{14}C]acetate into Hyamine hydroxide by the subtraction of values obtained for noncell controls. The data are expressed as total micrograms of glucose or acetate, based upon the average of triplicate determinations of disintegrations per minute, added to the reaction mixtures in each experiment.

Radioactivity collected on the filters and corrected to the total volume of the reaction mixture is hereafter referred to as incorporation. Radioactivity absorbed into Hyamine hydroxide is termed respiration. The sum of incorporation and respiration is referred to as metabolism. The metabolic ratio is defined as metabolism/incorporation.

RESULTS

Effect of acid treatment on glucose and acetate incorporation. Acidification of samples to stop organic carbon uptake and to collect respired CO_2 in heterotrophic organic carbon uptake experiments with natural bacterial populations has been found to decrease filter-

trapped radioactivity compared with unacidified and filtered samples (4, 11). This loss has been attributed to the release of unmodified radiolabeled substrate pools (4). This possibility was examined by exposing the bacterial suspensions to [^{14}C]glucose or [^{14}C]acetate for 2 h, followed by immediate filtration or acidification and additional incubation for time intervals of up to 20 min before filtration. Compared with unacidified, immediately filtered reaction mixtures, the acid-treated samples exhibited a rapid loss of radioactivity within 30 s of acid addition. Incorporated radioactivity then remained constant at 52% (strain 12W, glucose) and 63% (strain 16B, acetate) of unacidified incorporation values throughout the remainder of the incubation period and independent of the presence or absence of thiosulfate in the reaction mixtures. This finding suggests that the reported incorporation values represent true incorporation rather than an accumulation of radiolabeled substrate into glucose or acetate pools. Microscopic examination of acid-treated cells confirmed the absence of obvious cellular disruption.

Acetate utilization by strain 16B. In the presence of thiosulfate at pH 6.5, acetate incorporation increased and acetate respiration decreased compared with the cells given acetate alone (Fig. 1a and b), whereas the total amount of acetate utilized remained virtually unchanged (Fig. 1c). The changes in acetate utilization throughout the experiment are illustrated by a comparison of the metabolic ratio values (Fig. 1d). The initially starved cells (held in refrigerated seawater) had a relatively high metabolic ratio which had begun to decrease at the first sampling interval. The metabolic ratio in the thiosulfate-supplemented cell suspension had already decreased below the lowest value for the acetate-alone suspension which was reached by 1 h and remained constant throughout the experiment. Rates of acetate incorporation and respiration in the absence of thiosulfate also remained unchanged (Fig. 1a and b). In the presence of thiosulfate, however, the metabolic ratio began to increase as thiosulfate oxidation decreased (Fig. 1d), the ratio change resulting from a decreased incorporation rate and an increased respiration rate (Fig. 1a and b). These data, and the observations that strain 16B oxidizes thiosulfate stoichiometrically to tetrathionate but does not metabolize the latter, suggest that thiosulfate oxidation and not the mere presence of thiosulfate or tetrathionate accounted for the observed changes in acetate utilization. This conclusion is supported by the constant rates of acetate incorporation and respiration which were found at pH 8.0 at a nearly unchanged rate of thiosulfate oxidation throughout

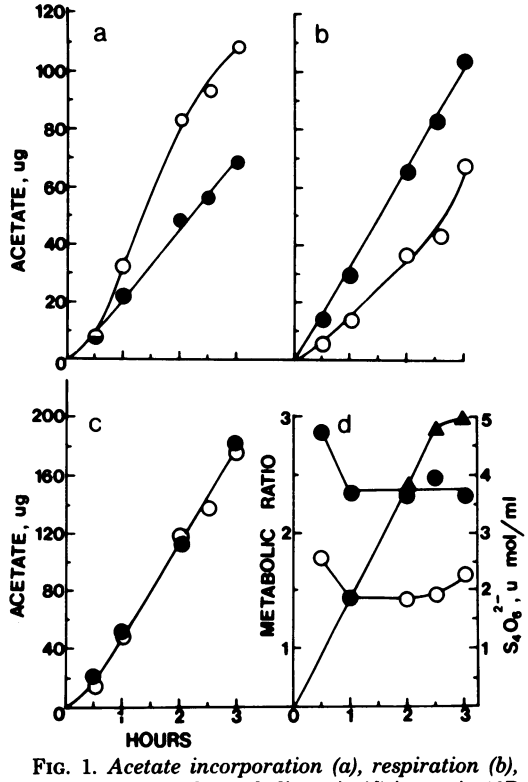

FIG. 1. *Acetate incorporation (a), respiration (b), metabolism (c), and metabolic ratio (d) in strain 16B (0.18 mg of cell protein) at an initial pH of 6.5. Cells were stored for 1 day. The final pH was 6.5 in thiosulfate-containing reaction mixtures and 6.4 with thiosulfate absent. Symbols: ●, 2.5 mM acetate; ○, 2.5 mM acetate plus 10 mM thiosulfate; ▲, tetrathionate concentration.*

the experiment (Fig. 2a through d).

The decreased effect of thiosulfate oxidation on acetate utilization at pH 8.0 compared with that at pH 6.5 and the increased metabolism of acetate at pH 8.0 in the presence or absence of thiosulfate are consistent with previously determined pH optima for acetate utilization, thiosulfate oxidation, and thiosulfate-stimulated growth in strain 16B (22). Acetate metabolism at pH 8.0 was not greatly increased by thiosulfate oxidation (Fig. 2c). Metabolic ratios at 0.5 h and pH 8.0 higher than those at pH 6.5 at the same time interval probably reflect cell storage for an extra day in the pH 8.0 experiment. The increased, constant metabolic ratio in the presence of thiosulfate at pH 8.0 compared with that a pH 6.5 suggests that thiosulfate-mediated differences in acetate utilization are dependent upon the rate of thiosulfate oxidation. At pH 8.0, the phosphate buffer was not as effective at maintaining the initial pH as it was at pH 6.5, particularly in the absence of thiosulfate. However, constant rates of acetate incorporation and

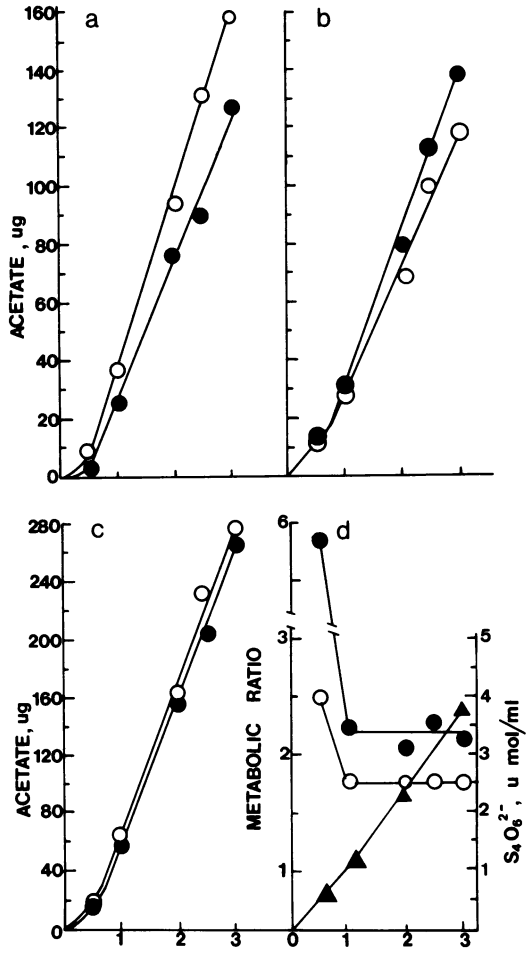

FIG. 2. *Acetate incorporation (a), respiration (b), metabolism (c), and metabolic ratio (d) in strain 16B (0.18 mg of cell protein) at an initial pH of 8.0. Cells were stored for 2 days. The final pH was 7.7 in thiosulfate-containing reaction mixtures and 7.3 with thiosulfate absent. Symbols: ●, 2.5 mM acetate; ○, 2.5 mM acetate plus 10 mM thiosulfate; ▲, tetrathionate concentration.*

respiration, consistent differences in the metabolic ratios between thiosulfate-supplemented and unsupplemented cell suspensions, and the fact that acetate utilization rates determined from oxygen uptake experiments are hardly affected by pH changes within the pH range of this experiment (22) indicate that the pH differences cannot account for the observed changes in acetate utilization.

The average rate of thiosulfate oxidation at pH 6.5 during maximum thiosulfate oxidation (1- to 2-h time intervals) was 112.5 ± 7.7 μmol of thiosulfate oxidized per h per mg of cell protein (95% confidence limit, five experiments), which resulted in a 33% increase in acetate incorporation but only a 10% increase in acetate metabolized when compared with acetate utilization in the absence of thiosulfate. At pH 8.0, comparative values were 67.2 ± 8 μmol of thiosulfate oxidized per h per mg of cell protein (95% confidence limit, four experiments), a 15% increase in acetate incorporation, but no change in acetate metabolized.

Glucose utilization by strain 12W. Glucose utilization by strain 12W at an initial pH of 6.5 in the presence or absence of thiosulfate (Fig. 3a through d) agrees qualitatively with acetate utilization by strain 16B at the same pH values. Increasing rates of glucose incorporation and respiration are due to an increase in cell protein during the experiment, an increase which was not observed with strain 16B. The effect of thiosulfate on glucose respiration decreased greatly as thiosulfate oxidation decreased. The increase

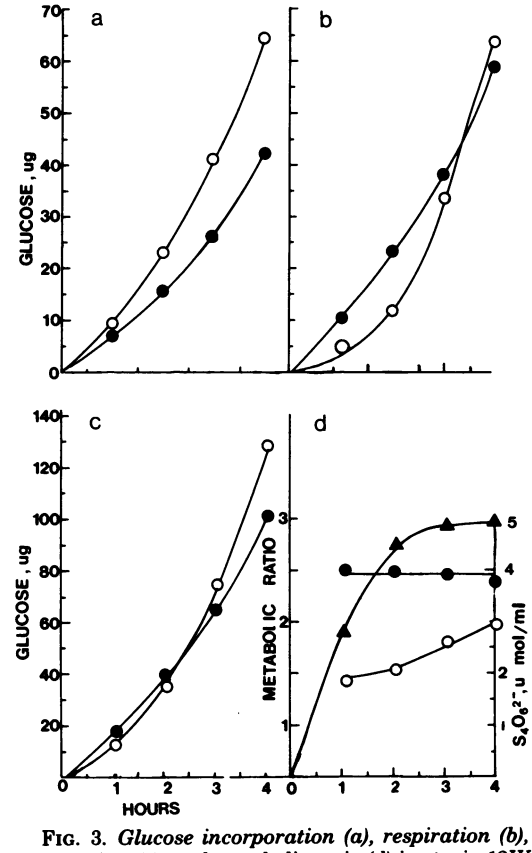

FIG. 3. *Glucose incorporation (a), respiration (b), metabolism (c), and metabolic ratio (d) in strain 12W (0.30 mg of cell protein) at an initial pH of 6.5. Cells were stored for 2 days. The final pH was 6.5 in thiosulfate-containing reaction mixtures and 6.3 with thiosulfate absent. Symbols: ●, 2.5 mM glucose; ○, 2.5 mM glucose plus 10 mM thiosulfate; ▲, tetrathionate concentration.*

in the metabolic ratio clearly indicates a return to thiosulfate-absent glucose utilization (Fig. 3d). The effect of thiosulfate on glucose metabolized was variable (Fig. 3c). On the basis of six similar experiments in which different cell batches were used, there was no more than a 10% increase in glucose metabolized during maximal thiosulfate oxidation. The average maximum thiosulfate oxidation at pH 6.5 was 78.5 ± 15.4 μmol/h per mg of cell protein (95% confidence limit). The corresponding average increase in glucose incorporation was about 30%, similar to the thiosulfate-mediated increase of acetate incorporation by strain 16B at pH 6.5. These data are also in agreement with the previously observed growth rate stimulation of strain 12W by thiosulfate at pH 6.5 and the pH optimum for thiosulfate oxidation in this bacterium determined from oxygen uptake experiments (22).

At an initial pH of 8.0, there appeared to be a slight increase in both glucose incorporation and glucose respiration (Fig. 4a and b), which led to an average (three experiments) increase of 12% in glucose metabolized in the presence of thiosulfate, but given the variability in rates of glucose utilization with time, this difference may not be significant in the experiments reported here. Except at the 4-h time interval, there was little difference in the metabolic ratios between thiosulfate-supplemented and unsupplemented cell suspensions (Fig. 4d). Metabolic ratio values calculated from three similar experiments were not significantly different at a 95% confidence limit. However, the rate of thiosulfate oxidation at pH 8.0 (15.5 ± 1.1 μmol of thiosulfate per h per mg of cell protein, 95% confidence limit, three experiments) was only 20% of the maximum rate at pH 6.5. This oxidation rate may simply have been too low to observe a significant change in glucose metabolism in strain 12W at pH 8.0.

DISCUSSION

Thiosulfate-stimulated growth of the marine heterotrophs 16B and 12W (22) appears to be based upon an increased efficiency in the utilization of organic carbon and energy sources which permits these bacteria to commit a greater portion of available organic carbon for biosynthesis rather than for respiration. This effect is greatest near the pH optimum for thiosulfate oxidation (Fig. 1 and 3), but also occurs to a lesser degree at pH values more representative of the natural seawater habitat (23, 25) of these bacteria (Fig. 2 and 4). It should be emphasized that organic carbon and thiosulfate concentrations used in experiments reported here were chosen to be similar to those used previously in batch culture growth experiments (22). It is likely that increased carbon utilization efficiency is even more important to bacterial survival and growth at the lower organic carbon concentrations found in natural marine environments (12). This discovery is supported by the results of carbon-limited continuous culture experiments in which the addition of 1 mM thiosulfate to strain 12W growing on 10 mg of glucose per liter at pH 7.3 resulted in a 10-fold increase in the steady-state population density (J. H. Tuttle, P. E. Holmes, and H. W. Jannasch, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, 9238, p. 59).

The finding that thiosulfate oxidation in strains 12W and 16B causes increased organic carbon incorporation with simultaneously decreased organic carbon respiration (Fig. 1 and 3) suggests that the oxidation of thiosulfate to tetrathionate is an energy-yielding reaction. It has recently been observed that starved suspensions of pyruvate-thiosulfate-grown 16B cells directly

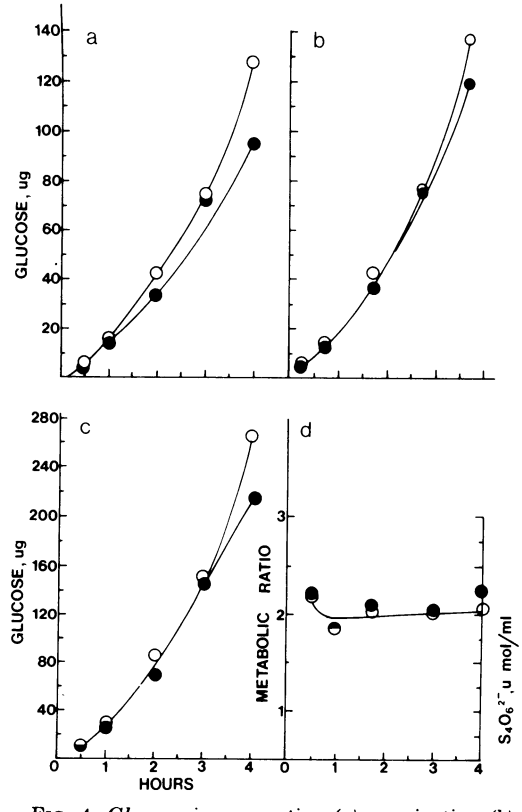

FIG. 4. *Glucose incorporation (a), respiration (b), metabolism (c), and metabolic ratio (d) in strain 12W (0.29 mg of cell protein) at an initial pH of 8.0. Cells were stored for 3 days. The final pH was 7.7 in thiosulfate-containing reaction mixtures and 7.6 with thiosulfate absent. Symbols: ●, 2.5 mM glucose; ○, 2.5 mM glucose plus 10 mM thiosulfate.*

produce adenosine triphosphate in the presence of thiosulfate but not in its absence (unpublished data). Thiosulfate-oxidizing bacteria, such as strains 12W and 16B, could be characterized as chemolithotrophic heterotrophs similar to *Thiobacillus perometabolis*, which is not capable of autotrophic growth (8, 10), except that the former are never metabolically dependent upon reduced sulfur compounds for growth and are incapable of complete thiosulfate oxidation. Thus, these strains should be termed thiosulfate-oxidizing heterotrophs.

The observed shift in carbon metabolism of strains 12W and 16B during thiosulfate oxidation appears to be much different from carbon utilization by thiobacilli under mixotrophic growth conditions (10). At relatively high concentrations of utilizable organic carbon, there is an antagonistic effect between organic carbon and inorganic sulfur metabolism in both *Thiobacillus intermedius* and *Thiobacillus novellus* (10). No effect has been found in the thiosulfate-oxidizing heterotrophs. However, *T. novellus* exhibits a twofold increase in steady-state biomass during mixotrophic growth in thiosulfate-limited chemostats when compared with glucose limitation alone (10). In agreement with Matin (10), experiments on the growth of various metabolic types of sulfur-oxidizing bacteria under conditions of nutrient limitation deserve further study.

ACKNOWLEDGMENTS

I thank S. Molyneaux for excellent technical assistance.

This work was supported by grants GA 29665 and DES 75-15017 (H. W. Jannasch, principal investigator) from the National Science Foundation and grant ORF 15-655 from the University of Texas at Arlington.

LITERATURE CITED

1. **Caldwell, D. E., S. J. Caldwell, and J. M. Tiedje.** 1975. An ecological study of the sulfur-oxidizing bacteria from the littoral zone of a Michigan lake and a sulfur spring in Florida. Plant Soil **43**:101-114.
2. **Gleen, H., and J. H. Quastel.** 1953. Sulphur metabolism in soil. Appl. Microbiol. **1**:70-77.
3. **Goldhaber, M. B., and I. R. Kaplan.** 1975. The sulfur cycle, p. 569-655. *In* E. D. Goldberg (ed.), The sea ideas and observations on progress in the study of the seas, vol. 5. John Wiley & Sons, Inc., New York.
4. **Griffiths, R. P., F. J. Hanus, and R. Y. Morita.** 1974. The effects of various water-sample treatments on the apparent uptake of glutamic acid by natural marine microbial populations. Can. J. Microbiol. **20**:1261-1266.
5. **Guittonneau, G., and J. Keilling.** 1932. L'évolution et la solubilisation du soufre élémentaire dans la terre arable. Ann. Agron. **2**:690-725.
6. **Kelly, D. P., L. A. Chambers, and P. A. Trudinger.** 1970. Cyanolysis and spectrophotometric estimation of trithionate in mixture with thiosulfate and tetrathionate. Anal. Chem. **41**:898-901.
7. **Kuenen, J. G.** 1975. Colourless sulfur bacteria and their role in the sulfur cycle. Plant Soil **43**:49-76.
8. **London, J., and S. C. Rittenberg.** 1967. *Thiobacillus perometabolis* nov. sp., a non-autotrophic *Thiobacillus*. Arch. Mikrobiol. **46**:329-337.
9. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
10. **Matin, A.** 1978. Organic nutrition of chemolithotrophic bacteria. Annu. Rev. Microbiol. **32**:433-468.
11. **Ramsay, A. J.** 1976. The effect of acidification on measurements of microbial uptake of radioactive glucose in freshwater. Limnol. Oceanogr. **21**:922-926.
12. **Redfield, A. C., B. H. Ketchum, and F. Richards.** 1963. The influence of organisms on the composition of seawater, *In* M. N. Hill (ed.), p. 26-77. The sea, vol. 2. Interscience Publishers, Inc., New York.
13. **Rittenberg, S. C.** 1969. The roles of exogenous organic matter in the physiology of chemolithotrophic bacteria. Adv. Microb. Physiol. **3**:159-196.
14. **Rittenberg, S. C.** 1972. The obligate autotroph—the demise of a concept. Antonie van Leeuwenhoek J. Microbiol. Serol. **38**:457-478.
15. **Starkey, R. L.** 1934. Cultivation of organisms concerned in the oxidation of thiosulfate. J. Bacteriol. **28**:365-386.
16. **Starkey, R. L.** 1934. The production of polythionates from thiosulfate by microorganisms. J. Bacteriol. **28**: 387-400.
17. **Starkey, R. L.** 1935. Isolation of some bacteria which oxidize thiosulfate. Soil Sci. **39**:197-219.
18. **Starkey, R. L.** 1950. Relations of microorganisms to transformations of sulfur in soils. Soil Sci. **70**:55-65.
19. **Trautwein, K.** 1921. Beitrag zur Physiologie und Morphologie der Thionsäurebakterien (Omelianski). Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 2 **53**:513-548.
20. **Trudinger, P. A.** 1967. Metabolism of thiosulfate and tetrathionate by heterotrophic bacteria from soil. J. Bacteriol. **93**:550-559.
21. **Trudinger, P. A.** 1967. The metabolism of inorganic sulphur compounds by thiobacilli. Rev. Pure Appl. Chem. **17**:1-24.
22. **Tuttle, J. H., P. E. Holmes, and H. W. Jannasch.** 1974. Growth rate stimulation of marine pseudomonads by thiosulfate. Arch. Microbiol. **99**:1-14.
23. **Tuttle, J. H., and H. W. Jannasch.** 1972. Occurrence and types of thiobacillus-like bacteria in the sea. Limnol. Oceanogr. **17**:532-543.
24. **Tuttle, J. H., and H. W. Jannasch.** 1973. Sulfide and thiosulfate-oxidizing bacteria in anoxic marine basins. Mar. Biol. **20**:64-70.
25. **Tuttle, J. H., and H. W. Jannasch.** 1973. Dissimilatory reduction of inorganic sulfur by facultatively anaerobic marine bacteria. J. Bacteriol. **115**:732-737.
26. **Tuttle, J. H., and H. W. Jannasch.** 1976. Microbial utilization of thiosulfate in the deep sea. Limnol. Oceanogr. **21**:697-701.
27. **Tuttle, J. H., and H. W. Jannasch.** 1977. Thiosulfate stimulation of microbial dark assimilation of carbon dioxide in shallow marine environments. Microb. Ecol. **4**:9-25.

27a. **Tuttle, J. H., and H. W. Jannasch.** 1979. Microbial dark assimilation of CO_2 in the Cariaco Trench. Limnol. Oceanogr. **24**:746-753.

28. **Veinstein, M. B.** 1976. On taxonomy of *Thiobacillus trautweinii*. Mikrobiologiya **45**:137-141.
29. **Vishniac, W., and M. Santer.** 1956. The thiobacilli. Bacteriol. Rev. **21**:195-213.