Marine Biology 73, 293–299 (1983)

Marine Biology
International Journal on Life in Oceans and Coastal Waters
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Microbial Activities in the Emitted Hydrothermal Waters of the Galápagos Rift Vents *

J. H. Tuttle [1, **], C. O. Wirsen [2] and H. W. Jannasch [2]

[1] **Department of Biology, The University of Texas at Arlington; Arlington, Texas 76019, USA**
[2] **Department of Biology, Woods Hole Oceanographic Institution; Woods Hole, Massachusetts 02543, USA**

Abstract

In-situ measurement of chemolithotrophic and some heterotrophic microbial activities were made in the immediate vicinity of actively discharging hydrothermal vents of the Galápagos Rift region at depths of 2 500 to 2 600 m. The CO_2-assimilation or chemosynthesis productivity in the emitted vent waters, freshly mixed with oxygenated ambient seawater of 2 °C, was minor compared to the bacterial biomass produced within the subsurface vent system prior to emission. Uptake of acetate and glucose indicated the presence of mixotrophic or facultatively chemolithotrophic bacteria in the emitted vent waters in agreement with isolations. Demonstration of ribulose bisphosphate carboxylase and phosphoenol pyruvate carboxylase in cultures of thiobacilli isolated from these vent water supports the notion that chemoautotrophic sulfur-oxidizing bacteria are one of the sources of primary production in the form of particulate organic carbon for filtering organisms in the deep sea hydrothermal environment. The rates of bacterial metabolic activities in emitted vent water are too low for the amount of invertebrate biomass and the rate of its growth and maintenance. Therefore, the larger portion of chemosynthetic sustenance of deep sea vent ecosystems appears to be based on symbiotic associations between bacteria and invertebrates and on surface attached bacteria.

Introduction

Chemoautotrophic sulfur-oxidizing bacteria are uncommon in offshore marine environments, including anoxic marine basins such as the Black Sea and the Cariaco Trench where oxidizable inorganic sulfur is abundant (Tuttle and Jannasch, 1972, 1973). Enrichment culture techniques (Tuttle and Jannasch, 1972, 1973; Kuenen, 1975), measurements of ribulose bisphosphate carboxylase (RuBPCase) activities (H. Glover, personal communication), and observations of thiosulfate or sulfide stimulation of CO_2 assimilation in the dark (Sorokin, 1964, 1972; Tuttle and Jannasch, 1973, 1977, 1979) have demonstrated only a minor role for these organisms in oceanic waters and the deep sea.

Exceptions to this general picture are the sites of luxurious assemblages of animal life discovered at hydrothermal vents on the ocean floor (Corliss *et al.*, 1979) at depths too deep to envision support by phytoplankton production at the sea surface. These vents at depths of 2 500 to 2 600 m are located at plate spreading centers of the East Pacific Rise and off the Galápagos Islands. Warm or hot sulfide- rich water is emitted into the surrounding cold and oxygenated seawater resulting in a number of characteristic chemical transformations (Edmond *et al.*, 1979; Mottl *et al.*, 1979). It has been suggested that the dense animal communities associated with the vents are sustained by primary production of organic carbon by chemosynthetic bacteria, using reduced inorganic compounds, primarily hydrogen sulfide, as the source of energy (Jannasch and Wirsen, 1979; Karl *et al.*, 1980; Jannasch and Wirsen, 1981; Ruby *et al.*, 1982).

Bacterial cell numbers counted in freshly collected vent waters by epifluorescence microscopy ranged from 5×10^5 to 10^6 ml^{-1} (Karl *et al.*, 1980) and 10^8 to 10^9 ml^{-1} in a glutaraldehyde preserved sample (Corliss *et al.*, 1979). The large differences have been attributed to fluctuating dilution of vent water with ambient seawater and to the common occurrence of bacterial cells in clumps (Karl *et al.*, 1980). The latter is supported by the observation that more than 60 percent of the total biomass measured as adenosine-5′-triphosphate (ATP) in vent water is associated with particulate material > 12 μm in diameter (Karl *et al.*, 1980).

* Contribution No. 1398, Center for Environmental and Estuarine Studies of the University of Maryland. Contribution no. 5160 of the Woods Hole Oceanographic Institution. Contribution no. 38 of the Galápagos Rift Biology Expedition

** Present address: Chesapeake Biological Laboratory, Center for Environmental and Estuarine Studies, University of Maryland; Solomons, Maryland 20688, USA

The more or less constant output of bacterial cell suspensions of most of the observed vents indicated active growth within the subsurface vent system. The free oxygen required for the chemosynthetic oxidation of hydrogen sulfide is provided by a counterflow of seawater downward into the highly porous pillow lava (Edmond *et al.,* 1979). Another site of active biomass production is microbial mats overgrowing indiscriminately all those surfaces that are intermittently exposed to sulfide- or oxygen-containing seawater (Jannasch and Wirsen, 1981). An important site of chemosynthetic production, however, appears to take place in symbiotic associations (Cavanaugh *et al.,* 1981) with the newly described giant clams (*Calyptogena magnifica,* Boss and Turner, 1980) and vestimentiferan tube worms (*Riftia pachyptila,* Jones, 1981).

The question arises how much microbial activity occurs or remains within the plume emitted vent water. Substantial activity is suggested by elevated ratios of guanosine-5-triphosphate (GTP):ATP and from a qualitative examination of CO_2-assimilation *in situ* (Karl *et al.,* 1980; Jannasch and Wirsen, 1979). We report here quantitative determination of CO_2-assimilation and the potential uptake of acetate and glucose in Galápagos Rift vent waters.

Materials and Methods

Samples. Our experimental work was conducted as part of the joint Galápagos Rift Biology Expedition which took place during January and November–December, 1979. The Galápagos Rift is located about 640 km west of Ecuador and 330 km northeast of the Galápagos Islands. Vent water was obtained from vent areas designated Mussel Bed (January cruise) and Rose Garden (November–December cruise).

Samples for enrichment cultures and shipboard rate determinations were collected by the DSRV "Alvin" using sterile Niskin bag-type samplers. These were attached to the submersible basket and hence collected water above the vents (approximately 0.5 m) which had mixed to a degree with ambient seawater. Additionally, smaller samples (50 ml) for enrichments were collected directly at the vent opening with sterile syringe poker devices (Jannasch and Wirsen, 1979) which allow sampling of unmixed vent water.

Vent water for *in-situ* rate determinations was collected and incubated without decompression in sterile (120-ml volume) syringe sampler arrays (SSAs) or self-filling 120-ml serum bottles. The design and deployment of the SSAs (Jannasch and Wirsen, 1979) and the serum bottle procedure (Jannasch and Wirsen, 1973) have been described elsewhere.

CO_2 Assimilation Measurements. Dark assimilation of CO_2 was measured essentially according to procedures described previously (Tuttle and Jannasch, 1977, 1979). For *in situ* determinations, sterile syringes of SSAs or self-filling serum bottles were preloaded with sterile solutions of $NaH[^{14}C]O_3$ (20 or 50 μCi) and $Na_2S_2O_3 \cdot 5H_2O$, Na_2S, or $K_2S_4O_6$ as appropriate just before the deployment dive. The total volume of additions did not exceed 3 ml. The serum bottles were also flushed with N_2 to prevent hyperbaric oxygen conditions from developing upon filling on the ocean floor. SSAs and serum bottle racks were filled directly over the vents and then placed for incubation a few meters away from the vents to prevent their disturbance during subsequent dive operations. Incubation temperature was 3.6 °C.

Following retrieval, the contents of each syringe and bottle were fixed with 2 ml of 0.2% $HgCl_2$ and then filtered through 0.2-μm membranes. The filters were washed, fumed with HCl, and counted as previously described (Tuttle and Jannasch, 1977). DPM values were converted to CO_2 assimilated on the basis of ΣCO_2 data collected by Edmond *et al.* (1979).

One atmosphere controls consisted of water collected with Niskin samplers at the time of SSA deloyment and incubated on the ship in 120-ml serum bottles at 3 °C over the same time period as the SSAs. The amounts of $NaH[^{14}C]O_3$, thiosulfate, and tetrathionate used were identical to the *in-situ* determinations.

Time course measurements were made in 75-ml serum bottles completely filled with vent water and containing 20 μCi $NaH[^{14}C]O_3$ with 1 mM thiosulfate or tetrathionate as appropriate. Incubation was at 3 °C and was stopped at 0, 4, 8, 12, 16, and 24 h by immediately filtering the samples. Rates of CO_2 assimilation were determined by linear regression analysis of the data.

Heterotrophic Activity Measurements. In-situ determinations were made in 120-ml self-filling serum bottles prepared, filled, and incubated as described above, except that sterile UL-[^{14}C]-glucose or Na-2^{14}[C]-acetate replaced $NaH[^{14}C]O_3$. For experiments at Mussel Bed, each bottle contained 1.1 μCi of glucose and 1.5 μCi of acetate. At Rose Garden, the amounts of added label were 1.0 and 2.7 μCi for glucose and acetate, respectively. Initial substrate concentrations were 10 mg l^{-1} throughout.

Upon retrieval, the samples were fixed with $HgCl_2$ as described above and 20 ml was filtered through 0.2-μm membranes which were then washed with two successive 10-ml portions of sterile seawater. Equal volumes of sterile seawater containing the same amount of radiolable were also filtered. Counts on these filters, due to adsorption of radiolabel, were subtracted from the experimental values. The filters were counted in Bray's solution and the values obtained taken as the amount of organic material incorporated into cells.

Labelled substrate respired to CO_2 was collected from sample aliquots following the procedure of Wirsen and Jannasch (1974) with the final values corrected for volatilization of radiolabeled substrate as determined in sterile controls. Metabolism is defined as the sum of incorporated and respired organic carbon.

Time-course shipboard determinations were made only with Rose Garden vent water. Uptake and respiration

of a UL[^{14}C] amino acid mixture was measured in addition to glucose and acetate metabolism. The specific activities and initial concentrations of the labelled substrates were: glucose, 0.01 mCi mg^{-1} and 1.03 mg l^{-1}; acetate, 0.75 mCi mg^{-1} and 0.083 mg l^{-1}; amino acid mixture, 4.17 mCi mg C^{-1} and 0.024 mg C l^{-1}. A labelled substrate was added to 170 ml of freshly collected vent water and 50-ml portions of the mixture were placed into each of three, 75-ml serum bottles for incubation. One bottle was supplemented with 1 mM thiosulfate, a second with 1 mM tetrathionate, and the third was unamended. The remaining 20 ml of solution was reserved for an initial filter control, a zero time CO_2 evolution control, and for checking the total radioactivity of the mixture.

The reaction bottles were incubated at 3 °C and sampled at 2-, 4-, 6-, and 8-h time intervals. Both incorporated and respired carbon were determined as described above, except that 10-ml rather than 20-ml portions were filtered. Rates were calculated from linear regression fits of the data.

Media and Culture Conditions. Enrichment cultures and enumerations were made in liquid TB medium (Tuttle and Jannasch, 1972) modified as follow: thiosulfate was decreased to 5 g l^{-1}, seawater increased to 80% (V/V), and 10 mg of phenol red l^{-1} added as a pH indicator. The initial pH was adjusted to 7.2. Cultures were incubated without shaking at room temperature (20 °–25 °C). Bacteria were isolated on TB agar and maintained on the same medium.

Enumerations were made by a vanishing dilution technique. The cultures were incubated for 3 wk and scored positive by an increase or decrease in the pH of the medium compared to an uninoculated control. Subcultures on TB agar were made to confirm the results.

Bacteria and Mass Cultures. Strains TB 49r, TB 49s, and TB 49c were isolated from enrichments made with Mussel Bed vent water as described above and have been classified as *Thiobacillus* sp. (A. Jump and J. Tuttle, Abstr. Annu. Meet. Am. Soc. Microbiol., 1981, N83, p 187). Mass cultures were prepared in 500 ml portions of filter sterilized (0.2-μm membrane) TL medium composed of: K_2HPO_4, 0.25 g; KH_2PO_4, 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; NH_4Cl, 0.5 g; $NaHCO_3$, 0.5 g; NaCl, 30 g; $Na_2S_2O_3 \cdot 5H_2O$, 10 g; sodium lactate, 6 mM; deionized water, 1l. Sodium lactate was not utilized by the bacteria, but rather served to decrease the redox potential of the medium (Jump and Tuttle, unpublished data). Shaken cultures lacking lactate, or some other organic compound such as yeast extract or ascorbic acid failed to develop. The initial pH was set at 7.5. Inocula were 10-ml portions of 3-d cultures prepared in the same medium.

The cultures were incubated for 3 d on a rotary shaker at 60 rpm and 22 °C, neutralized with sterile saturated $NaHCO_3$, and incubated an additional day. Cultures were then harvested by centrifugation at 10 000 × g and 4 °C for 20 min and resuspended in 40 ml 0.01 M MOPS-KOH, pH 7.8, containing 1.0 mM Na_2EDTA and 2.5% (W/V) NaCl. The resulting suspension was treated 5–10 strokes with a tissue homogenizer, precipitated sulfur removed by centrifugation at 1 000 × g for 3 min, and the supernatant recentrifuged 10 000 × g for 10 min. The cell pellet was washed again with MOPS-KOH buffer, and the pellet resuspended in 0.1 M MOPS-KOH, pH 7.8 containing 10 mM Na_2EDTA and 2.5% NaCl to an absorbance (660 nm–1 cm light path) of 2.0 (equivalent to ca 0.4 mg dry weight of cells ml^{-1}).

Enzyme Assays. RuBPCase and phosphoenol pyruvate carboxylase (PECase) were analyzed according to procedures modified from the intact cell technique of Tabita *et al.* (1978). Two ml of the final cell suspension described above were carefully layered with 1.0-ml toluene. The toluene-layered cell suspension was gently mixed for 3 min and placed into an ice bath for 10 min. The toluene layer and drops of toluene adhering to the tube walls were then carefully removed with a Pasteur pipet.

To begin the assay, 100 μl of toluene treated cell suspension was placed into a 12 × 75 mm test tube. To the suspension was added 100 μl of radiotracer solution [0.15 M MOPS-KOH, pH 7.8, containing 25 mM $MgCl_2$, 2.5% (W/V) NaCl, and 50 mM NaH[^{14}C]O_3 (0.25 mCi/mmol)] and the mixture was incubated in a 30 °C water bath in a fume hood for 15 min. The reaction was started by addition of 50 μl of 4 mM ribulose 1–5 bisphosphate, pH 6.5 (RuBP, disodium salt) for RuBPCase measurements, 50 μl of 4 mM phosphoenol pyruvate, pH 6.5 (PEP, sodium salt) for PEPCase determinations, or 50 μl deionized water for endogenous CO_2 assimilation controls. The reaction was allowed to proceed at 30 °C and terminated at appropriate time intervals by the addition of 100 μl of 60% (W/V) trichloroacetic acid. The tubes were held in the hood for an additional 2 h to permit escape of $^{14}CO_2$ and then centrifuged at maximum speed for 15 min in a Serofuge (Clay-Adams, Parsippany, N.J.). A 200-μl portion of the resultant supernatant was added to 10 ml of Bray's solution and counted with a Beckman 8 000 liquid scintillation spectrometer. Quench was corrected by the channels ratio method. The activity of $NaH^{14}CO_3$ was determined by counting duplicate 20-μl portions of radiotracer mixture in 10 ml of Bray's containing 0.2 ml of Hyamine hydroxide (New England Nuclear). RuBPCase and PEPCase values were corrected for endogenous controls and all measurements were corrected for zero time residual radioactivity.

Chemicals. All reagents used were of reagent grade quality or better. RuBP, PEP, and MOPS buffer were purchased from Sigma Chemical Co. (St. Louis). Radiolabelled materials were obtained from New England Nuclear Corp. (Boston). NaH[^{14}C]O_3 was sterile prefiltered through a 0.2-μm membrane before use. $K_2S_4O_6$ was prepared from $Na_2S_2O_3 \cdot 5\,H_2O$ by the cold iodine oxidation method of Roy and Trudinger (1970).

Results

CO_2 Assimilation. Rates of CO_2 assimilation measured in bottles or SSA *in situ* were highly variable, not only at the same vent sampled at different times, but even among replicate samples collected by the same device (Tables 1, 2). For example, CO_2 assimilation occurring in unsupplemented Rose Garden vent water collected by SSA 2 was 2 orders of magnitude greater than in one of the duplicate syringes of SSA 1, while the 2 duplicates themselves differed 10-fold (Table 2). Likewise, considerable variation was also observed among replicate serum bottles (Table 2), which were deployed in the same pressure cylinder and thus filled at about the same time. We attribute these differences to incomplete mixing of vent water with ambient cold ocean bottom water and to uneven distribution of particulates within the vent plume.

Maximum observed rates of CO_2 assimilation in unsupplemented vent water were 3.6 and 4.8 mg C m^{-3} d^{-1} for the Mussel Bed and Rose Garden vents, respectively. These values are similar to a maximum rate of 3.1 mg C m^{-3} d^{-1} measured at the oxygen-sulfide interface of the Cariaco Trench (Tuttle and Jannasch, 1979) but are not strikingly high compared to a maximum rate of 11.7 mg C m^{-3} d^{-1} found in the Black Sea (Tuttle, unpublished data).

The addition of l-or 10-mM thiosulfate increased CO_2 assimilation compared with unamended vent water in some samples only (Tables 1, 2). This variability is attributed to the same reasons discussed above. Thiosulfate at 1 mM increased average (all values) CO_2 assimilation by only 0.4 mg C m^{-3} d^{-1}. Stimulation of CO_2 assimilation is indicative of the activities of sulfur-oxidizers, but does not necessarily mean that the responsible microorganisms are chemoautotrophs (Tuttle and Jannasch, 1977, 1979). Thiosulfate added at 0.1 mM had little effect on CO_2 assimilation in Mussel Bed water (Table 1), but decreased assimilation when added to Rose Garden water (Table 2). Insufficient data exist to evaluate the effect of additional sulfide (Table 2).

Shipboard controls incubated simultaneously with *in-situ* experiments at the Rose Garden vent suggest at most a 47% decrease in CO_2 assimilation at *in-situ* pressure (Table 3, thiosulfate supplemented). The results also show that 1-mM tetrathionate is clearly inhibitory to CO_2 assimilation in Rose Garden vent water. The 28% decrease in CO_2 incorporation *in situ* compared to 1 atm for thiosulfate-containing samples agrees well with data for *Thiomicrospira* sp. isolated from vent samples (Ruby and Jannasch, 1982).

CO_2 assimilation in Rose Garden vent water was also measured in time course experiments conducted over a 24-h incubation period (Table 4). The units have been changed to facilitate comparison with amino acid uptake measurements done time course over an 8-h period with the same water samples. The results show that actual rates of CO_2 assimilation may be even lower than those calculated from *in-situ* incubation (Tables 1 and 2) or the long-term shipboard end point measurements (Table 4). Com-

Table 1. Dark assimilation of CO_2 in undecompressed Mussel Bed water incubated *in situ*

Additions	CO_2 incorporated[a], nmol l^{-1} d^{-1}
None	76.1
None	299.1
1 mM $S_2O_3^{2-}$	271.1
1 mM $S_2O_3^{2-}$	384.2
0.1 mM $S_2O_3^{2-}$	233.2
0.1 mM $S_2O_3^{2-}$	284.4

[a] Incubation was for 23 d in 120-ml syringes

Table 2. Dark assimilation of CO_2 in undecompressed Rose Garden water incubated *in situ*

SSA no.	Incubation chamber	Additions	CO_2 incorporated[a], nmol l^{-1} d^{-1}
1	Syringe	None	33.2
1	Syringe	None	3.4
2	Syringe	None	398.1
3	Syringe[b]	None	21.0
3	Syringe[b]	None	21.3
	Serum bottle	None	146.1
	Serum bottle	None	2.7
	Serum bottle	None	26.7
1	Syringe	1 mM $S_2O_3^{2-}$	79.6
1	Syringe	1 mM $S_2O_3^{2-}$	134.2
2	Syringe	1 mM $S_2O_3^{2-}$	70.2
2	Syringe	1 mM $S_2O_3^{2-}$	97.2
3	Syringe[b]	1 mM $S_2O_3^{2-}$	21.7
3	Syringe[b]	1 mM $S_2O_3^{2-}$	37.3
1	Syringe	0.1 mM $S_2O_3^{2-}$	12.2
1	Syringe	0.1 mM $S_2O_3^{2-}$	9.2
2	Syringe	0.1 mM $S_2O_3^{2-}$	10.9
2	Syringe	0.1 mM $S_2O_3^{2-}$	2.5
	Serum bottle	10 mM $S_2O_3^{2-}$	154.0
	Serum bottle	0.5 mM Na_2S	140.8

[a] Incubation was for 6.2 d
[b] $NaH[^{14}C]O_3$ was increased from 20 to 50 μCi

Table 3. Comparison of dark assimilation of CO_2 in Rose Garden water incubated at 1 atm. and *in situ* at 245 atm

Pressure atm	Temp. °C	Additions	CO_2 incorporated[a] nmol l^{-1} d^{-1}
1	3.0	None	35.7
245	3.6	None	21.0
245	3.6	None	21.3
1	3.0	1 mM $S_2O_3^{2-}$	41.0
245	3.6	1 mM $S_2O_3^{2-}$	21.7
245	3.6	1 mM $S_2O_3^{2-}$	37.3
1	3.0	1 mM $S_4O_6^{2-}$	7.0
245	3.6	1 mM $S_4O_6^{2-}$	7.7
245	3.6	1 mM $S_4O_6^{2-}$	8.3

[a] Incubation was for 6.2 d

parison of the values for time course and end point determinations made with water collected on Dive 984 suggests that bottle effects occurred at long incubation periods. *In-situ* time course measurements were not technically possible during either cruise.

Table 4. Assimilation of CO_2 and amino acid incorporation in Rose Garden water determined from time course measurements made aboard ship. CO_2 assimilation and amino acid incorporation were measured over 24-h and 8-h time periods respectively. The r values for regression were 0.95 or greater

Dive no.	Addition	Assimilation, ng C l^{-1} d^{-1}	
		$^{14}CO_2$	^{14}C-amino acids
984	None	12.0 (428.4)[a]	45.6
	1 mM $S_2O_3^{2-}$	3.9 (492.0)	79.7
	1 mM $S_4O_6^{2-}$	7.7 (84.0)	44.2
988	None	1.3	48.0
	1 mM $S_2O_3^{2-}$	8.6	62.4
	1 mM $S_4O_6^{2-}$	2.2	28.1

[a] Values obtained from the same water incubated for 6.2 d at 1 atm

Table 5. Heterotrophic activity in Mussel Bed vent water

Additions[a]	Incorporation (μg l^{-1} d^{-1})	Respiration (μg l^{-1} d^{-1})	Metabolism (μg l^{-1} d^{-1})	%Organic Substrate used[b]
Acetate	0.02	0.47	0.49	1.6
Acetate	0.10	1.84	1.94	6.4
Acetate + $S_2O_3^{2-}$	1.05	7.74	8.79	28.9
Acetate + $S_2O_3^{2-}$	0.97	15.19	16.16	53.2
Glucose	0.04	0.90	0.93	3.1
Glucose	0.05	0.74	0.79	2.6
Glucose + $S_2O_3^{2-}$	0.79	3.75	4.55	15.0

[a] Initial glucose or acetate conc. = 10 mg l^{-1}, initial $S_2O_3^{2-}$ conc. = 1 mM

[b] Based upon initial glucose or acetate conc. *In-situ* incubation was for 329 d

Table 6. Heterotrophic activity in Rose Garden vent water

Additions[a]	Incorporation (μg l^{-1} d^{-1})	Respiration (μg l^{-1} d^{-1})	Metabolism (μg l^{-1} d^{-1})	%Organic Substrate used[b]
Acetate	0.42	140.06	140.48	8.7
Acetate	0.66	133.45	134.11	8.3
Acetate + $S_2O_3^{2-}$	12.53	251.08	263.61	16.3
Acetate + $S_2O_3^{2-}$	2.00	174.16	176.16	10.9
Glucose	21.08	291.45	312.53	19.4
Glucose	6.92	421.95	428.87	26.6
Glucose + $S_2O_3^{2-}$	165.44	330.57	496.00	30.8
Glucose + $S_2O_3^{2-}$	2.52	399.03	401.55	24.9

[a] Initial glucose or acetate conc. = 10 mg l^{-1}, initial $S_2O_3^{2-}$ conc. = 1 mM

[b] Based upon initial glucose or acetate conc. *In-situ* incubation was for 6.2 d

Heterotrophic Activity. Thiosulfate stimulated acetate incorporation an average of 17-fold and respiration 10-fold in Mussel Bed water incubated *in situ* (Table 5). Similarly, glucose incorporation was about 18 times greater in the presence of thiosulfate while respiration was increased only 5-fold. The unavoidably long incubation period (329 d) was caused by failure to locate and retrieve the test bottles during the first expedition cruise.

Experiments at Rose Garden vent in which the bottles were retrieved after a much shorter incubation period yielded qualitatively similar results (Table 6). The major stimulatory effect of thiosulfate was exerted on acetate incorporation rather than respiration. This effect was not conclusive with glucose. However, in all but one of the bottles (glucose plus thiosulfate), respiration remained relatively high with respect to incorporation, perhaps indicating stress on the microbial populations.

In agreement with CO_2 assimilation measurements (Table 4), rates of heterotrophic activity were much lower when estimated from shipboard time course experiments rather than *in-situ* end point determinations. Respiration of amino acids, acetate, and glucose as well as incorporation of the latter two substrates were undetectable in the 8-h time course experiments by the methods employed.

Bacterial Counts. Counts of thiosulfate-oxidizing bacteria were made with five different Rose Garden vent water samples, two of which were collected with syringe pokers and three with Niskrin samplers. Counts ranged from 10^2 to 10^3 ml^{-1} in water collected over the vents to 10^4 to 10^5 ml^{-1} in water sampled at the vent opening with syringe pokers. Both samplers recovered heterotrophic thiosulfate-oxidizing microorganisms, but acid-producing strains were collected only with the syringe pokers. When cultured on TB or TL media and examined microscopically, the acid-

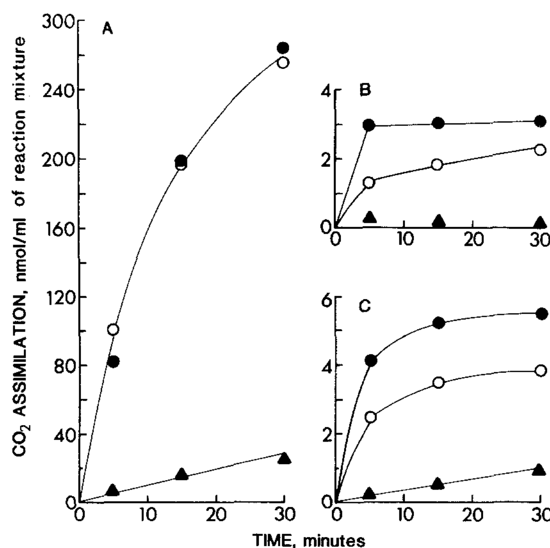

Fig. 1 CO_2 assimilation by toluene treated cell suspensions of rift vent *Thiobacillus* spp. in the presence of (A) 0.8 mM final concentration of RuBP, (B) deionized water, and (C) 0.8 mM final concentration of PEP. Symbols: (●) strain TB 49r; (○) strain TB 49s; (▲) strain TB 49c

producing bacteria resembled *Thiobacillus* spp. and *Thiomicrospira* sp. previously reported to inhabit the hydrothermal vent environment (Ruby *et al.,* 1981).

RuBPCase and PEPCase. All three *Thiobacillus* spp. exhibited RuBPCase activity (Fig. 1). This finding firmly establishes the existence of true chemoautotrophic sulfur bacteria at deep sea hydrothermal vents. PEPCase activity was also detected above endogenous CO_2 assimilation in each of the three isolates, but in TB 49r and TB 49s suspensions, activity tailed off rapidly after 5 min of incubation. Endogenous activity in these strains may have been due to sulfur which precipitated in the cultures and could not be completely removed from the cell suspension. Cultures of TB 49c did not precipitate large amounts of sulfur.

Discussion

The results of measuring *in-situ* chemolithotrophic and heterotrophic bacterial activities within the plume of emitted vent waters demonstrate patchiness and relatively low metabolic rates at 3 °C. Therefore, the relatively high numbers of bacteria in the emitted vent water (Corliss *et al.,* 1979; Karl *et al.,* 1980) must have originated at higher temperatures within the vent system.

No direct observations or measurements on microbial growth within the upper pillow lava interstices have been possible at the Galápagos vents. The intrusion of oxygenated seawater into these layers has been found and discussed by Lister (1977), Mottl *et al.* (1979) and Edmond *et al.* (1979). The types of cell aggregations found in the emitted waters suggest a sloughing of microbial mats from rock surfaces within cracks and interstices of the highly fractured pillow lava at an undetermined depth below the sea floor. This notion is supported by the fact that "syringe poker" samples, taken as deep as possible within vent openings (unpublished data), yielded strains of acidophilic sulfur-oxidizing bacteria. These apparently find favorable growth conditions in dense microbial mats rather than suspended in well buffered vent water. Furthermore, none of the sulfur bacteria isolated from vent waters were psychrophilic (Ruby *et al.,* 1981; A. Jump and J. Tuttle, Abst. Annu. Meetg. Am. Soc. Microbiol., 1981, N83, p 187). In contrast, thermophilic bacteria have been obtained only from the hot vent waters of the 21° N East Pacific Rise ocean spreading center (Baross *et al.,* 1982; Leigh, in preparation).

Growth and metabolic rates of microorganisms in emitted vent waters can be expected to slow considerably as the warm vent waters mix with the ca 2 °C ambient seawater. The vent plume, representing the zone of temperature transition from ca 25 ° to 2 °C, is insignificant in size in most of the vents observed at the Galápagos Rift region. In a distance of 1 to 2 m from a vent opening, the temperature was commonly found to be equal to ambient. As the emitted bacterial cell suspension is diluted, the large portion not harvested by filtering benthic animals becomes available to planktonic organisms. It is likely, however, that most of the bacterial biomass emitted is lost from the vent community into the surrounding waters.

This leads to the question of the major site of chemosynthetic primary productivity. The largest portion of animal biomass at the vents appears to be in the form of vestimentiferan tube worms (*Riftia pachyptila,* Jones 1980) and giant clams (*Calyptogena magnifica,* Boss and Turner, 1980). Although clusters of these animals tend to live near vent openings, their occurrence appears to depend more on the presence of hydrogen sulfide, and possibly methane and hydrogen in the seawater, rather than on turbidity (bacterial cell suspensions) or elevated temperature. The implication of their symbiotic mode of life (Cavanaugh *et al.,* 1981; Felbeck, 1981; Felbeck *et al.,* 1982) corroborates this observation. The evidence for this new and quite spectacular case of symbiotic associations between invertebrates and chemosynthetic bacteria is based on morphological and enzymatic studies only, but experimental studies with freshly collected and recompressed animals at *in-situ* temperature are underway.

In conclusion, the emitted vent water can be described as the carrier of (i) particulate organic carbon for filter-feeding animals, (ii) possibly dissolved organic carbon for further microbial growth (e.g. heterotrophic and methylotrophic bacteria), and (iii) residual hydrogen sulfide as the source of energy for the symbiotic sustenance of *Riftia pachyptila* and *Calyptogena magnifica.* The stimulatory effect of thiosulfate on heterotrophic carbon metabolism is indicative of the activity of mixotrophic sulfur bacteria (Tuttle, 1980). Although these organisms represent the majority of the sulfur bacteria isolated from the vents (Ruby *et al.,* 1981), their actual role is yet to be determined.

Acknowledgements. We thank A. A. Jump for technical assistance. J. H. Tuttle wishes to thank W. C. McDonald whose help with teaching responsibility enabled him to participate in the Galápagos expedition. This work was supported by grants OCE 78-10457, OCE 80-24253 and OCE 81-14909 from the National Science Foundation.

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Date of final manuscript acceptance: January 17, 1983.
Communicated by S. K. Pierce, College Park