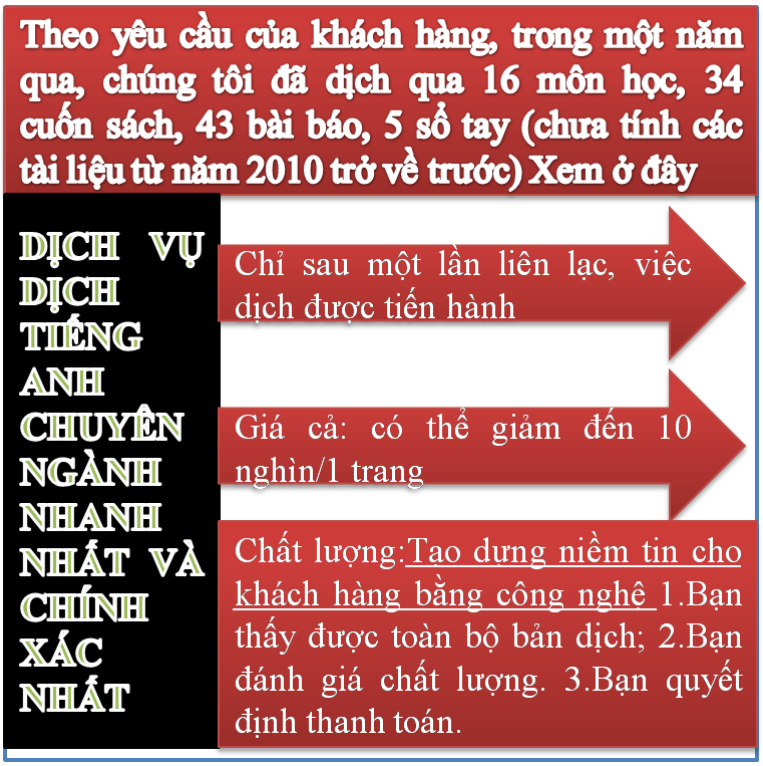


Tài liệu này được dịch sang tiếng việt bởi:

www.mientayvn.com

Tìm bản gốc tại thư mục này (copy link và dán hoặc nhấn Ctrl+Click):

https://drive.google.com/folderview?id=0B4rAPqlxIMRDSFE2RXQ2N3FtdDA&usp=sharing

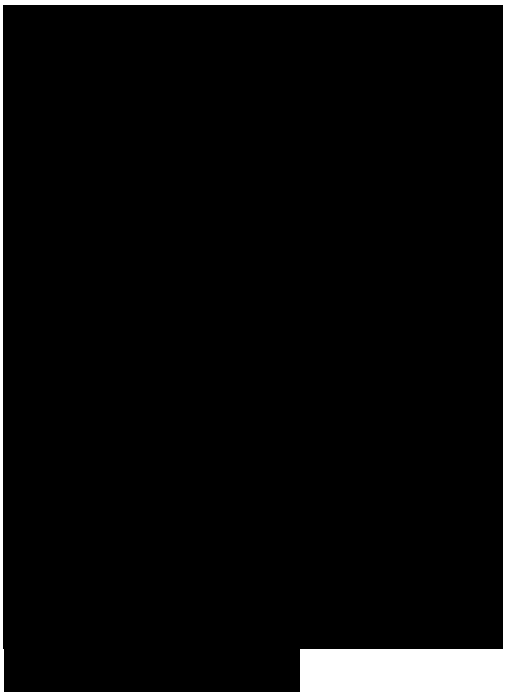
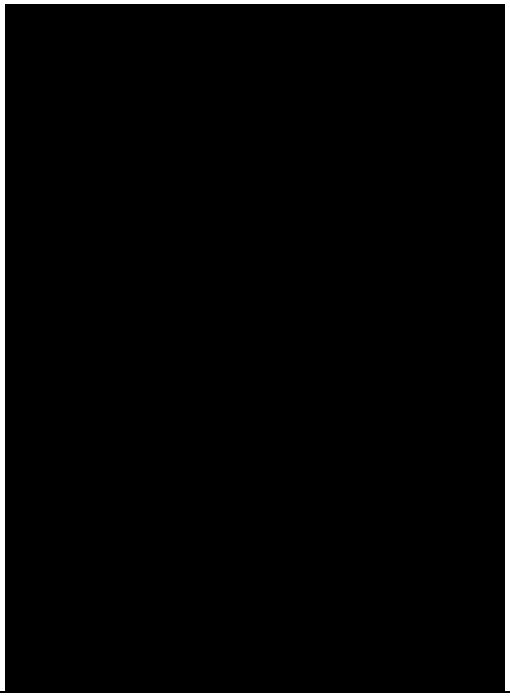
Liên hệ để mua:

thanhlam1910_2006@yahoo.com hoặc frbwrthes@gmail.com hoặc số 0168 8557 403 (gặp Lâm)

Giá tiền: 1 nghìn /trang đơn (trang không chia cột); 500 VND/trang song ngữ

Dịch tài liệu của bạn: http://www.mientayvn.com/dich_tieng_anh_chuyen_nghanh.html

Sulfur Metabolism in Beggiatoa alba Checked 29/7 The metabolism of sulfide, sulfur, and acetate by Beggiatoa alba was investigated under OXỈC and anoxic conditions. B. alba oxidized acetate to Cacbon điôxít with the stoichiometric reduction of oxygen to water. In vivo acetate oxidation was suppressed by sulfide and by several classic respiratory inhibitors, including dibromothymoquinone, an inhibitor specific for ubiquinones. £ệ alba also carried out an oxygen-dependent conversion of sulfide to sulfur, a reaction that was inhibited by several electron transport inhibitors but not by dibromothymoquinone, indicating that the electrons released from sulfide oxidation were shuttled to oxygen without the involvement of ubiquỉnones Intracellular sulfur stored by B. alba was not oxidized to sulfate or converted to an external soluble form under aerobic conditions. On the other hand, sulfur stored by filaments of Thiothrix nivea was oxidized to extracellular soluble oxidation products, including sulfate. Sulfur stored by filaments of B. alba, however, was reduced to sulfide under short-term anoxic	Quá trình chuyển hóa lưu huỳnh trong Beggiatoa alba In vivo: quá trình diễn ra trong cơ thể sinh vật sống Chúng tôi khảo sát quá trình chuyển hóa sunfua, lưu huỳnh, và acetate của Beggiatoa alba trong điều kiện có oxy và thiếu oxy. B. alba oxy hóa acetate thành cacbon điôxít cùng với việc khử theo đúng tỷ lệ hợp phần oxy thành nước. Quá trình oxy hóa acetate In vivo bị hạn chế do sunfua và do một số chất ức chế chuỗi truyền điện tử cổ điển, kể cả dibromothymoquinone, một chất ức chế đặc trưng cho các ubiquinone. …alba cũng thực hiện quá trình chuyển đổi sunfua thành lưu huỳnh phụ thuộc oxy, một phản ứng bị ức chế bởi một vài chất ức chế truyền điện tử, ngoại trừ dibromothymoquinone, cho thấy rằng các electron được giải phóng từ quá trình oxy hóa sunfua được chuyển vào oxy mà không có sự tham gia của enzyme ubiquinone. Lưu huỳnh nội bào được dữ trữ bởi B. alba không bị oxy hóa thành sunfat hoặc chuyển đổi sang một dạng có thể hòa tan bên ngoài trong điều kiện hiếu khí. Mặt khác, lưu huỳnh được dự trữ bởi các sợi Thiothrix NIVEA được oxy hóa thành các sản phẩm oxy hóa có thể hòa tan bên ngoài tế bào, kể cả sunfat. Tuy nhiên, lưu huỳnh được dự trữ bởi các sợi B. alba	

conditions. This anaerobic reduction of sulfur was linked to the endogenous oxidation of stored carbon and to hydrogen oxidation.	bị khử thành sunfua trong điều kiện thiếu oxy ngắn hạn. Quá trình khử lưu huỳnh kỵ khí này có liên quan đến quá trình oxy hóa nội sinh của carbon được lưu trữ và quá trình oxy hóa hydro.	
All freshwater Beggiatoa strains thus far tested have the ability to grow heterotrophically on acetate in the presence of oxygen (14, 25, 26). Members of the genus Beggiatoa, however, lack catalase (3, 26), so aerobic growth under highly oxygenated conditions is apparently limited. More-over, lowered oxygen tension and the presence of sulfide have been shown to have beneficial effects on the growth and metabolism of Beggiatoa spp. (13, 15, 17, 18, 26).		
Oxidation of sulfide may eliminate the need for catalase by detoxifying metabolically formed hydrogen peroxide (3). Alternatively, oxidation of sulfide may simply alleviate the toxic effects of sulfide in the environment. Sulfide oxidation may supplement energy for growth on acetate by freshwater strains of Beggiatoa (6, 27) and is the sole energy source for the chemolithoautotrophic growth of at least one marine Beggiatoa strain (15, 16). Whatever the effect of sulfide and its oxidation		

might be, marked concentrations of Beggiatoa filaments in nature virtually always coincide with the presence of hydrogen sulfide (26).

Recently we showed that Beggiatoa alba B18LD and other strains contain multiple electron transport system components (29). In this study, the functioning of the respi-ratory electron transport chain in the presence and absence of sulfide was investigated with the use of electron transport inhibitors. It was found that under aerobic conditions, sul-fide slightly suppressed acetate oxidation and was converted to elemental sulfur, with oxygen serving as an electron acceptor. Under anaerobic conditions, sulfur was reduced either by endogenous substrates or by added hydrogen gas.

MATERIALS AND METHODS

Strains and growth conditions.

B. alba strains B18LD, B25RD, and B15LD (12), Beggiatoa sp. strains 75-2a (13,14) and SM-1 (S. Maier, unpublished), Vitreoscilla beggiatoides B23SS (28), Vitreoscilla filiformis ATCC 15551 (28), Thiothrix nivea JP3 (10), and Chromatium

vinos urn (7) were used in this study.

The media were prepared in a basal salts solution (BSS) consisting of 4.7 mM NH4C1, 1 mM CaCl2, 73.5 |xM KH2PO4, 40 |aM MgS04 • 7H20, and 5 ml of a microelement solution per liter (34). BH (heterotrophic) medium consisted of BSS plus 6.1 mM sodium acetate (pH 7.3). BSO (suifide oxidation) medium contained BSS, 6.1 mM sodium acetate, and 2 mM neutralized sodium sulfide, with all components added before autoclaving (24).

Cultures of Beggiatoa and Thiothrix strains were grown at 23°c in 2-liter flasks that contained 1 liter of medium. The flasks were inoculated with approximately 100 ml of a stationary-phase culture and shaken at 100 rpm. The cultures were harvested after 24 h of incubation by centrifugation at 6,0 rpm and washed once in BSS. Concentrated cell suspensions were prepared by suspending the pellet to a density of ca. 0.3 to 0.4 mg of cell protein per ml in the appropriate buffer.

Acetate, sulfide, and thiosulfate oxidation. Acetate-de-pendent oxygen consumption by B. alba

B18LD was mea-sured with the Warburg respirometer as described by Umbreit et al. (32). Two milliliters of a concentrated cell suspension from BH medium were dispensed into Warburg flasks. The center well of the flasks contained pleated filter paper (5 by 25 mm) saturated with 0.2 ml of 20% potassium hydroxide. The side arm of each flask contained 222 |xl of 60 mM sodium acetate in BSS. Inhibitors were incubated with the cell suspension at the concentrations designated in Table 1 for 15 min before the addition of sodium acetate. Mano- metric readings were taken every 15 min. The effect of inhibitors on the rate of [2-14C]acetate oxidation was measured in 25-ml sidearm reaction flasks (no. 882360; Kontes, Vineland, N.J.) by adding 333 |xl of [2- 14C]acetate (1 ịxCi; 0ẻl |xCi/|xmol) to the concentrated cell suspension as described previously (35). Inhibitors were added to the reaction flasks 15 min before the addition of substrate. 14C02 was collected on KOH-saturated filter paper and counted with a Beckman LS-6800 scintillation counter. Data were corrected for quenching by using an internal standard of [methyl-14C]toluene.	[redacted]	

Sulfide-dependent oxygen consumption by B. aiba B18LD was measured in the Warburg apparatus by the method described for acetate oxidation, except that the side arm of each flask contained 333 ịú of freshly prepared and neutralized 10 mM sodium sulfide in place of sodium acetate. Duplicate measurements of the chemical oxidation of sulfide were made with respirometer flasks containing 2 ml of BSS without cells. The oxidation of radiolabeled sulfide to labeled "intracellular" sulfur (24, 27, 35) was measured concurrently with identical cell suspensions. Samples (10 mi) of the concentrated cell suspension were dispensed into 50-ml Erlenmeyer flasks, and the vessels were shaken at 120 strokes per min in á Dubnoff metabolic shaker at 23°c. When anoxic conditions were required, oxygen was removed from the flasks by flushing with nitrogen for 10 min, and then the flasks were carefully capped with a rubber stopper (35). Sodium [35S]sulfide (0.425 jxCi/|xmol) was added to each flask to a final concentration of 1 mM. The autooxidatiòn of sulfide and the sorption of sulfide to the cells were measured in control flasks

with autoclaved cells. The effect of respiratory inhibitors or 6 mM acetate on sulfide oxidation by B. alba B18LD was measured by adding the affectors to the cell suspension 15 min before the addition of sulfide. Samples (200 |XỈ) were removed at timed intervals and filtered through Whatman glass fiber filters. The filters with cells containing 35s inclusions were washed with BSS at pH 3 to remove any externally bound label (24, 27) and then dried at 60°c for 2 h. The dried filters were counted in the scintillation cocktail described previously (25). Quench corrections were made with [14C]toluene as described previously. To confirm that the cell-bound product of [35S]sulfide oxidation was 35s, replicate samples were filtered through Gelman GA-3 glass fiber filters (Gelman Sciences, Inc., Ann Arbor, Mich.) and washed with 2 ml of either BSS (pH 3), ẽthanol, benzene, acetone, or 5% aqueous trichloroacetic acid.

Oxidation of cellular sulfur. In an attempt tò deplete the cellular reserves of sulfur, B. alba B18LD and T. nivea JP3 were grown to late log phase in BSO medium, harvested, and then suspended in sterile BSS to the same volume and incubated at

23°G on a rotary shaker at 150 rpm. After 12 h, the cells wiere examined microscopically for the presence of sulfur inclusions and prepared for respirometiy as described previously. Cells that were harvested from BSO medium and not starved for sulfur were also prepared for respirometry.

To measure oxidation products obtained from intracellular 35s stores, B. alba B18LD and T. nivea were grown in BSO mediuni containing 1 mM [35S]siilfide (0.4 |xCi/|imol). Filaments harvested from the radioactive medium were washed twice in BSS and suspended to their original density in one of the following: normal BSS, BSS with chloride salts substituted for all sulfate salts, BSS with chloride salts plus 1 mM freshly prepared and neutralized sodium sulfide, BH medium, or BSO medium (all solutions were adjusted to pH 7.2).

The cultures were incubated aerobically in 500-ml flasks shaken at 200 rpm on a rotary shaker for periods of 12 h to 4 days, depending on the experiment. Typically, four 200-|xl samples were taken from each flask at each time point. Two of the samples were filtered

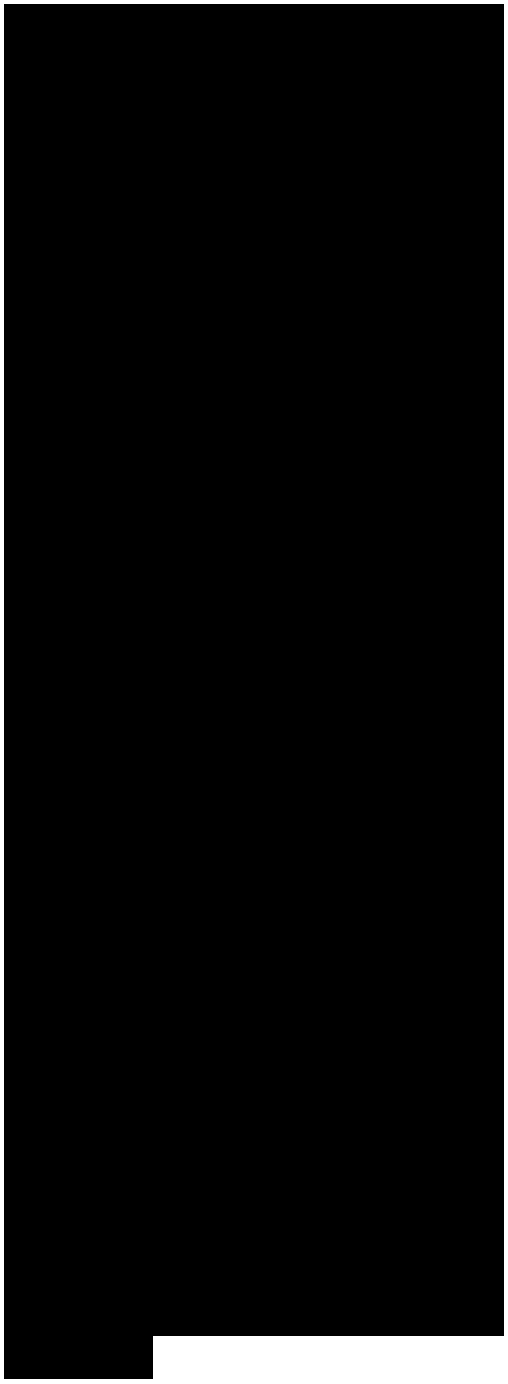
through Gelman glass fiber filters and washed with 2 ml of BSS at pH 3.0. The radioactivity on the filters, measuring the intracellular sulfur, was counted as described previously. To measure the amount of 35S-labeled soluble compounds released from the filaments, the other two samples were centrifuged for 2 min in a microfuge, from which 100-|xl samples of the supernatant were added to a toluene-based scintillation cocktail containing 5 g of PPO (2,5-diphenyloxazole) and 50 mg of POPOP [l,4-bis(5-phenyloxazolyl)benzene] per liter in 33% Triton X-100 in toluene (21) and counted as described previously.

An alternative method for obtaining B. alba B18LD labeled sulfur inclusions was also used. B. alba was grown in 500 ml of BH medium for 16 h, after which 40 jjiCi of Na235s (1 ^Ci/mmol) was added. The filaments were incubated with the labeled sulfide for 6 h, pelleted aseptically by centrifugation, washed twice with BSS, suspended in 50 ml of BH medium, and incubated aerobically on a rotary shaker (250 rpm) for 72 h (25°C). Samples were taken as described above to determine the amount of label remaining in the filaments and the amount

released into the medium.

Microelectrode studies A 5% inoculum of alba Ð18LD was introduced into BSO medium, which upon incubation yielded visible tufts of the organism in early exponential growth. Individual tufts were removed from the medium, rinsed gently in BSS, and embedded in a 2% agar cube measuring approximately 3 mm per side. The agar cube containing the tuft of £È alba filaments was suspended in the middle of a 500-ml glass bowl filled with BSS by using capillary tubes that were anchored to the bottom of bowl. Air, nitrogen, or hydrógen was bubbled through the BSS solution at ambient temperature (cá. 23°C). Microelectrodes, made according to the methods described by Revsbech et al. (23) , were positioned with a micromanipulator to measure sulfide and oxygen gradients around and through the tuft of B. alba filaments embedded in agar while the vessel was bubbled with air. The electrodes were then relocated at the surface of the tuft of filaments in the agar cube, and either nitrogen or hydrogen was bubbled through the reaction vessel.

Measurement of sulfide production. Reduction of sulfur to sulfide by mid-exponential-phase cells of B> alba B18LD



was measured in flasks which were continuously flushed with high-purity nitrogen. Sulfide was trapped in two serial tubes, each containing 10 ml of 2% zinc acetate solution, which was assayed for sulfide by the method of Kline (9) at intervals. Prior to incubation, cells grown in either BH or BSO medium were washed and suspended in BSS to their original density. This procedure effectively eliminated the accumulation of sulfide in cell suspensions.

Hydrogen evolution and uptake assays. To measure hydrogen evolution in Beggiatoa and Vitreòscilla spp., concentrated cell suspensions of o.i to 0.3 mg of cell protein per ml were prepared in BSS also containing 25 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid) buffer (pH 7.2). Two. milliliters of each suspension was dispensed into 9-ml serum bottles (Wheaton Scientific, Millville, N.J.) which were sealed with rubber stoppers and aluminum caps. Forty microliters of 100 mM methyl viologen in 10 mM phosphate buffer (pH 7.2) was added to each flask. The bottles were flushed with nitrogen for 15 min, and the hydrogen evolution assay was initiated by the addition of 0.1 ml of 100 mM

sodium dithionite (in distilled water) to a final concentration of 10 mM. The bottles were shaken at 120 strokes per min at 23°c in a reciprocal metabolic shaker. Headspace gas samples of 100 |xl were withdrawn from the bottles every 30 min and analyzed by injection into a Varian Aerograph 3700 gas chromatograph equipped with a 3-m stainless steel column packed with molecular sieve 5A (30-40 mesh). The following temperatures were used: injector, 100°C; column, 30°C; thermal conductivity detector, 150°C; and filament, 300°c. Nitrogen, at a flow rate of 30 mi/min, served as the carrier gas. The output from the gas chromatograph was recorded on a model 252A strip chart recorder (Linear Instruments Corp., Costa Mesa, Calif.). Peak heights were measured and compared with a standard curve prepared from the heights measured for hydrogen standards in nitrogen. Hydrogen consumption was measured by dispensing 1.5 ml of concentrated filament suspension into 9-ml serum bottles. After the bottles were flushed with nitrogen for 15 min, 4% of the headspace was replaced with 1 atm (101.29 kPa) of hydrogen. The bottles were incubated as above. The headspace was sampled at 60-	[redacted]	

min intervals and analyzed by gas chromatography for remaining hydrogen as described above.

Protein determinations. The concentration of total cell protein was estimated by the method of Lowry et al. (11) after extraction of the sulfur with 95% ethanol for 1 h and digestion of the cells by heating the samples at 90°c in 1 N NạOH for 10 min, followed by reneutralization of the solutions with 1 N HC1. Bovine serum albumin was used as a protein standard.

Chemicals. Radiochemicals were obtained from Amer- sham Corp. (Arlington Heights, 111.). Sodium [35S]sulfide, prepared similarly to the procedure described by Vargas and Strohl (35), was dissolved in deoxygenated water containing unlabeled sulfide so that the final concentration of sulfide was 20 mM at pH 7.2. Unlabeled crystals of sodium sulfide were washed in distilled water and dried before being weighed. The sulfide solution was stored under a nitrogen atmosphere at 4°c. The nitrogen atmosphere in the labeled sulfide stock solutions was replenished after each use.

RESULTS

Acetate oxidation. Acetate-dependent oxygen consump-tion by Beggiatoa alba B18LD was linear for 90 min at a rate of 3.60

|xl of c>2 per min per mg of protein. This consumption was equivalent to a rate of 160 nmol of 02 per min per mg of protein. The initial rate of 14C02 evolution from [2- 14C]acetate was 65 nmol of 14C02 per min per mg of protein. The acetate oxidation was effectively inhibited by dibro- mothymoquinone, 8-hydroxyquinoline, 1,10-phenanthrol- ine, 4-Af-hydroxyquinoline-rt-oxide, KCN, NaN3, and 2,4-dinitrophenọl as determined by measuring the rates of both acetate-dependent oxygen consumption and the release of 14C02 from [2-14C]acetate (Table 1). The rate of acetate oxidation, measured isotopically, was decreased ca. 18 to 20% by the presence of 2 mM sulfide.

Sulfide oxỉdatỉonề The initial rate of [35S]sulfide assimilation by B. alba in BSS minus iron salts was 35 to 65 nmol/min per mg of protein in the presence of oxygen; in the absence of oxygen there was no significant measurable sulfide uptake above background (sorption of [35S]sulfide by autoclaved cells; Table 1). Sulfide uptake by T. nivea was also oxygen dependent, and the rates were similar to those displayed by B. alba (27). Approximately 90% of the labeled internal sulfur

accumulated by B. alba B18LD and T. nivea in the presence of oxygen was soluble in acetone, benzene, or ethanol (data not shown). Treatment of 35S-containing filaments collected on filters with 5% aqueous ice-cold trichloracetic acid always resulted in recovery of ca. 10% more counts than measured with filaments washed with BSS (data not shown).

The relative effects of several electron transport inhibitors on oxygen-dependent sulfide assimilation by B. alba B18LD are shown in Table 1. The inhibitors thenoyltrifluoroacetone, 8-hydroxyquinoline, KCN, and NaN3 suppressed sulfide oxidation to sulfur by more than 60%. Dibromothymo-

TABLE 1. Effect of electron transport inhibitors on acetate and sulfide oxidation in Beggiatoa alba B18LDa

Three different methods were used to obtain these data, as detailed in the Materials and Methods section. Control values: acetate-dependent oxygen consumption, 162 nmol/min per mg of protein; [2-14C]acetate oxidation to 14cc>2, 65 nmol/min per mg of protein; Na235s oxidation to 35s°, 53 nmol/min per mg of protein. All values are averages of at least duplicate experiments and

represent the net values after autoclaved-cell control values were subtracted from the original gross data.

b Abbreviations: TTFA, thenoyltrifluoroacetone (flavoprotein inhibitor); DBMIB, dibromothymoquinone (ubiquinone inhibitor); 8-HQ, 8-hydroxyqui- noline (inhibitor of b-type cytochromes); PHEN, o-phenanthroline (inhibitor of b-type cytochromes); HOQNO, 4-jY-hydroxyquinoline-/2-oxide (inhibitor of cytochrome ^cytochrome c couple). c —, Not done.

d The data for inhibition of sulfide oxidation by malate are courtesy of V. A. Vinci.

' The sulfide was neutralized to pH 7.2 just prior to the experiment.

quinone at concentrations five times the amount necessary to inhibit acetate oxidation essentially had no effect on sulfide oxidation (Table 1). Sodium acetate at a final concentration of 6 mM reproducibly inhibited sulfide oxidation by ca. 50%, and 1 to 2 mM malate strongly inhibited sulfide oxidation (Table 1).

To determine the stoichiometry between sulfide oxidation and oxygen reduction, the rates of sulfide-dependent oxygen consumption and [35S]sulfide

oxidation were determined concurrently in duplicate flasks for each measurement. The rate of sulfur accumulation, with cells grown in either BH or BSO medium, averaged 38 nmol/min per mg of protein in this experiment. The concomitant oxygen consumption was 0.96 ịxữmin per flask. After accounting for the oxygen consumption due to chemical sulfide oxidation (0.35 |xl/min per flask) and endogenous oxygen consumption (0.30 |xl/min per flask), the rate of sulfide-dependent biological oxygen consumption was 0.31 \XẢ of 02 per min per flask. Each flask contained 0.8 mg of cell protein. The rate of sulfide-dependent oxygen consumption was calculated from these data to be 14 nmol/min per mg of protein.

Sulfur oxidation Microscopic observation of filaments of B. alba B18LD and rẽ nivea JP3 grown in BSO medium revealed the presence of numerous refractile sulfur inclu-sions. After such filaments had been harvested, washed, and incubated in BSS for 12 h, the phase-bright inclusions were depleted from filaments of T. nivea but not from filaments of B. alba B18LD. Moreover, numerous sulfur inclusions in

TABLE 2. Release of

radiolabeled sulfur from aerobically incubated filaments of B. alba B18LD Time at which the measurements were taken after initiating incubation of filaments containing 35s inclusions in the media described. b Abbreviations for media: BHA, BH medium (see text) plus 0.05% asparagine; BSS-Ac, BSS plus 0.001% sodium acetate; BSS-Ac-Na2S, BSS- Ac plus 0.03% neutralized Na2S; BH-no SO42- and BSO-no SO42-, BH and BSO media with cr salts replacing all sulfate salts. filaments of B. alba B18LD were still visible 72 h after the filaments were removed from sulfide. Endogenous respiration by B. alba containing sulfur in-clusions (pregrown in BSO) was ca. 0.50 |xl of 02 per min per mg of cell protein. Endogenous respiration of B. alba lacking sulfur inclusions (pregrown in BH medium) was 0.48 |jl1 of 02 per min per mg of cell protein. On the other hand, sulfur- containing filaments of T. nivea consumed oxygen at rates 20- to 25-fold greater than sulfur-starved filaments of T. nivea (data not shown). The oxidation of intracellular sulfur by B. alba and T. nivea was also determined by	[redacted]	

measuring the extracellular release of 35S-labeled compounds from radiolabeled sulfur inclusions under aerobic conditions. In seven separate experiments conducted with B. alba B18LD, never more than 5% of the 35s deposited in the filaments was released into the medium after 22 h (or 11% released after 60 to 72 h) of incubation under aerobic conditions (Table 2). This absence of significant sulfur oxidation was observed regardless of whether sulfate salts, phosphate salts, acetate, or sulfide was present or absent in the medium. Filaments of Tể nivea, on the other hand, released approximately 40% of their 35s label into the medium within 21 h of incubation (Fig. 1). The presence or absence of 1 mM sulfide or sulfate in the BSS solution had little effect on the solubilization of the label by T. nivea filaments (Fig. 1). Chemical measurements indicated that sulfate was a major product of the oxygen-dependent sulfur oxidation in 7. nivea, although stoichiometric values were not obtained (T. M. Schmidt, Ph.D. thesis, Ohio State University, Columbus, 1985).

Microelectrode studies While air was bubbled around a tuft of B. alba B18LD filaments embedded

in an agar cube, the endogenous metabolism lowered the concentration of dissolved oxygen to an undetectable level at the surface of the filament pellet (Fig. 2). At the point where the oxygen concentration neared zero, sulfide was detected and reached a concentration of 10 fjiM at a distance of 50 fxM inside the filament pellet. When nitrogen instead of air was bubbled through the microelectrode apparatus, a constant rate of sulfide production was measured at the surface of the cell pellet (Fig. 3). When the nitrogen was replaced with hydrogen, the rate of sulfide production increased (Fig. 3).

Endogenous anaerobic respiration. In a bubbling apparatus designed to provide short-term anoxic conditions with continuous removal of sulfide, sulfide was produced at a rate of 6.7 nmol/min per mg of protein by sulfur-containing filaments of B. alba B18LD (Fig. 4). B. alba filaments that lacked sulfur inclusions and had been grown in BH medium did not produce any detectable sulfide over a period of 4 h (Fig. 4). As a positive control, sulfide was produced at a rate of 5.0 nmol/min per mg of protein by dark-incubated cells of c. vinosum (Fig. 4), a purple phototrophic bacterium known to

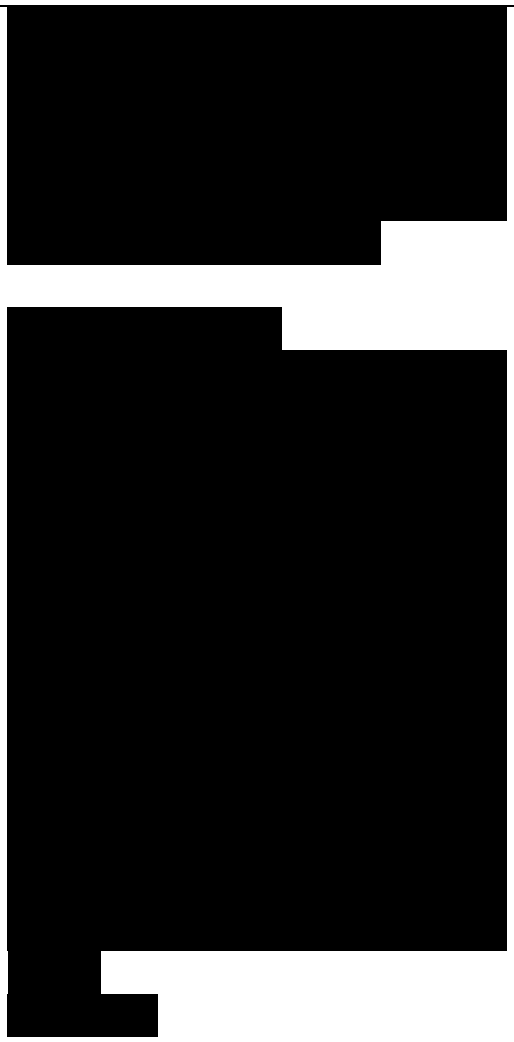
reduce sulfur to sulfide (33).

Anaerobic hydrogenase activity. Because hydrogen ap-peared to stimulate the anaerobic production of sulfide from sulfur-containing filaments of B. alba B18LD (Fig. 3), that organism was tested for hydrogenase activity. Sulfur-containing filaments of B. alba B18LD, incubated anaerobically, consumed hydrogen at a constant rate of 7.9 nmol/min per mg of protein. There was no detectable hydrogen consumption by B. alba filaments that lacked sulfur inclusions or by #Ệ alba filaments that were boiled for 2 min (not shown).

Hydrogen production by B. alba B18LD was detected only in the presence of both methyl viologen and dithioniteÊ The rate of methyl viologen- and dithionite-dependent in vivo hydrogen production by B. alba B18LD was 2.25 nmol/min per mg of cell protein and was insensitive to 50 fjiM carbonylcyanide-p-trifluoromethoxyphenylhydrazone. Filaments of B. alba B18LD exposed to sulfide alone or to sulfide in combination with either methyl viologen or dithionite produced no hydrogen.

A survey of several other strains of Beggiatoa and Vitreoscilla was carried out. The rates of in vivo methyl viologen- and

dithionite-dependent hydrogen production found were (in nanomoles per minute per milligram of cell protein): B. aiba B25RD, 6.1; B. alba B15LD, 4.9; Beggiatoa sp. clone 75-2a, 6.7; Beggiatoa sp.



TIME (HOURS)

FIG. 1. Release under aerobic conditions of 35S-labeled compounds from filaments of T. nivea JP3 containing 35s inclusions. Decrease of 35s label in the filaments (sulfur inclusions; solid symbols) and increase in soluble, acid-stable 35s label (open symbols) during incubation in the following media: BSS containing sulfate salts (■, □); BSS with chloride salts replacing sulfate salts (A, A); or BSS containing sulfate and 1 mM fresh neutralized sodium sulfide (pH 7.5) (•, O).

DISTANCE (Mm)

FIG. 2. Microelectrode studies of oxygen (A) and sulfide (•) gradients resulting from the endogenous metabolism of a tuft of B. alba B18LD filaments. Distances are measured in micrometers from the outer edge of the tuft, which is designated zero on the abscissa. Values to the left of zero indicate a position inside the tuft of filaments, and values to the right represent positions in the agar just outside the tuft of filaments.

strain SMI, 2.2; V. filiformis

ATCC 15551, 2.3; and V. beggiatoides B23SS, 7.2.

DISCUSSION

Acetate oxidation. B. alba (25) and other freshwater Beg- giatoa strains (14, 29) utilize acetate as a sole carbon and energy source in respiratory metabolism. The rate of [2- 14C] acetate oxidation to 14C02 (65 nmol/miri per mg of protein) found in the present work agrees closely with the vmax value of 72 nmol/min per mg of protein determined by Strohl et al. (25). The methyl carbon is oxidized to C02 at a lower rate than the carboxyl carbon, accounting for only 34 to 40% of the acetate oxidized by beggiatoas (25, 29). Because the components of a typical aerobic, hetérotrophic bacterial metabolism are present (29), the following stoichi- ometry for the complete oxidation of acetate is proposed:

(160 nmol/min per mg of protein)
[95 nmol/min per mg of protein]
(65 nmol/min per mg of protein)

where the numbers given in parentheses are values obtained experimentally and the number given in brackets was calculated from the expected stoichiometry.

Several known respiratory inhibitors (8) reduced the rate of

acetate oxidation in cells of B. alba B18LD, as measured by either the rate of acetate-dependent oxygen consumption or the release of 14C02 from [2-14C]acetate. The suppression of acetate oxidation by sulfide is consistent with the results obtained by Strohl et al. (25) and suggests that perhaps acetate and sulfide metabolism are competing for oxygen.

TIME CMIN)

FIG. 3. Microelectrỏde experiments showing the effect of gassing tufts of B. alba B18LD filaments, embedded in agar, with nitrogen or hydrogen. In one experiment, the filaments were gassed with only nitrogen (•). In the second experiment (A), the filaments were initially gassed with nitrogen, and at the first arrow the nitrogen was replaced with hydrogen gassing. After 5 mill of gassing with hydrogen, the filaments were gassed with nitrogen again, arid at 10 min (second arrow; ■) they were gassed once again with hydrogen.

Sulfide oxidation. The oxidation of sulfide to sulfur is catalyzed by filaments of B. alba incubated in the presence of oxygen. We tested the possible involvement of electron transfer proteins in sulfide oxidation by using electron transport inhibitors that were shown to be effective

inhibitors of acetate oxidation in £é alba. Inhibitors of cytochromes or flavoproteins (1) suppressed sulfide oxidation, but the ubiquinone analog dibromothymoquinone (22, 30) was ineffective, suggesting that electrons from sulfide oxidation do not interact at the level of ubiquinone in B. alba B18LD.

If electrons enter an electron transport chain, the inhibitor data suggest that both a flavoprotein and cytochromes are involved.

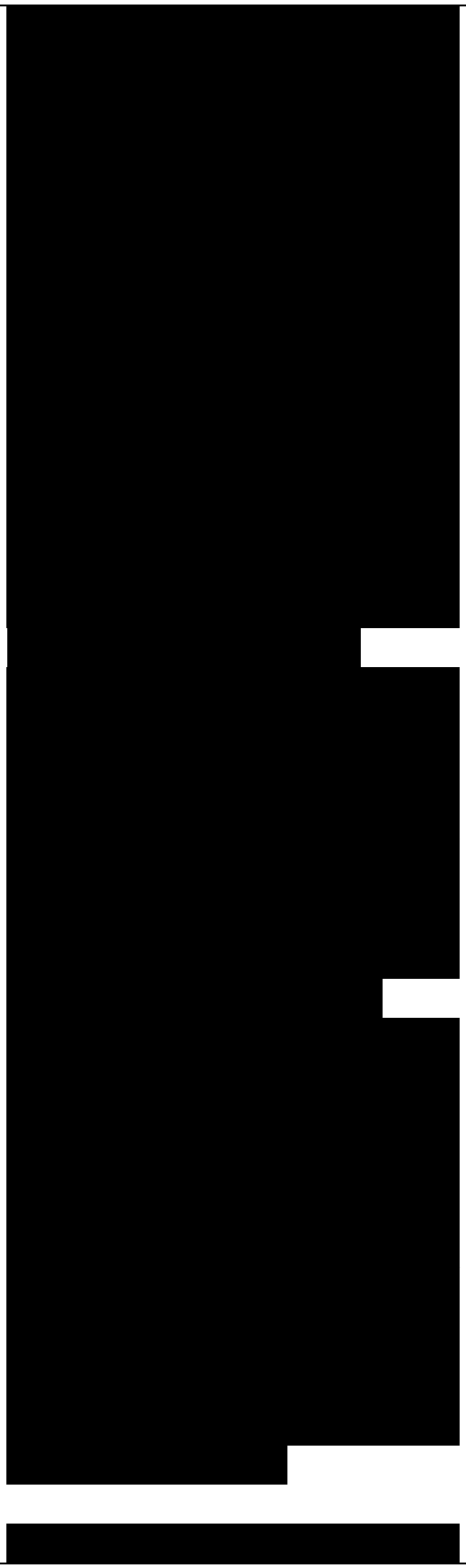


FIG. 4. Production of sulfide by filaments of B. alba B18LD containing (•) or lacking (A) sulfur inclusions incubated under anaerobic conditions. Anaerobic production of sulfide by dark-incubated, sulfur-containing cells of c. vinosum (■) was used as a positive control.

A pathway in which electrons from sulfide oxidation enter at cytochrome c via a flavocytochrome, as found in Chromatium spp. (5, 31), is possible for B. alba. This mechanism of sulfide oxidation differs from the proposed pathway for the cyanobacterium Oscillatoria limnetica, in which quinones are an essential part of the sulfide-oxidizing system (19).

The stoichiometry for the

chemical oxidation of sulfide to sulfur is 2:1 (H2S:02; equation 2). The rate of [35S]sulfide assimilation to internal 35s° (38 nmol/min per mg of protein) and the rate of sulfide-dependent oxygen consumption (14 nmol/min per mg of protein), determined simultaneously, indicate that the molar stoichiometry for the biological oxygen-dependent sulfide oxidation by B. alba was 2.7:1 (H2S:02; equation 3):

2H2S + 02^ 2S° + 2H20 (2)

2.7H2S + 02 2.7S0 + 2H20 + 1.4H+ (3)

Based on growth yields of a marine of Beggiatoa, Nelson et al. (16) showed that the stoichiometry of sulfide oxidation to sulfur for energy purposes alone should be 2:1 (H2S:02; equation 2), whereas the stoichiometry of oxygen-dependent sulfide oxidation to sulfur for energetic and reduction purposes would be 2.42:1 (H2S:02). That ratio would fall to approximately 0.5:1 if the sulfide were oxidized completely to sulfate (16). Our data indicate a molar ratio of 2.7:1, which, if compared directly with the autotrophic marine strain of Beggiatoa (16), should indicate that B. alba obtains both energy and reductive potential from the oxidation of sulfide. It is

unlikely, however, that B. alba B18LD requires reduction potential from the oxidation of sulfide, because it contains NAD(P)H dehydrogenase (29) and oxidizes acetate (25,29). These processes should generate all of the reduction potential required by B. alba, as differentiated from autotrophs, which do not generate reducing power from organic metabolism.

Sulfur oxidation by Beggùrúoa and Thiothrix spp. The initial observations on Beggiatoa by Winogradsky included descriptions of the depletion of sulfur inclusions in the fila- ments, which was attributed to the oxidation of intracellular sulfur to sulfate (36). This hypothesis was recently confirmed for a marine strain of Beggiatoa which was capable of autotrophic growth (16). On the other hand, the microscopic, respirometric, and isotopic data presented here indicate that the freshwater strain, B. alba B18LD, does not oxidize sulfur to sutfate. Experiments with B. alba B15LD have yielded comparable results (W. R. Strohl, unpublished data). It is possible, then, that other freshwater strains of Beggi- atoa are incapable of sulfur oxidation to sulfate, which would make them metabolically very different from the marine strains.

Our ability to detect sulfur oxidation to sulfate was checked by measuring sulfur oxidation by T. nivea JP3. Under the same experimental conditions as used for B. alba, we showed that sulfur in 7ã nivea was a transient compound that was converted to a soluble form under aerobic conditions. The resolubilization of sulfur in T. nivea was coupled with oxygen reduction (27), and sulfate has been measured as one of the oxidation products. Thus, it appears that the freshwater Thiothrix strain may have a sulfur metabolism more similar to that of marine Beggiatoa strains than to that of the freshwater strains.

Sulfur reduction. The inability of B. alba B18LD to oxidize sulfur leaves a potential source of electrons unused. This stored sulfur, however, might be used as a terminal electron acceptor under anoxic conditions. Sulfur-containing filaments of B. alba B18LD reduce sulfur to sulfide, and this reduction is apparently coupled to the oxidation of endogenous carbon reserves, possibly poly-p-hydroxybutyric acid (6, 25).

Desulfuromonas acetoxidans also linked anaerobic sulfur reduction with the oxidation of organic compounds such as ethanol and acetate (2).

Filaments of B. alba B18LD that lack sulfur inclusions do not produce sulfide under anoxic conditions, eliminating the possibility that the sulfide was a product of sulfate reduction or the degradation of sulfur-containing proteins. Anaerobic sulfide production by Beggiatoa spp. was first observed in strain 75-2a by Nelson and Castenholz (13) and was thought to be the means by which the cells survived periods without oxygen. It is possible that anaerobic reduction of sulfur may be common to all beggiatoas and certainly to the freshwater strains that do not oxidize sulfur to sutfate. When B. alba B18LD filaments were placed under short-term anoxic conditions, the presence of hydrogen stimulated sulfide production, suggesting that B. alba contained a hydrogenase and that hydrogen oxidation might be coupled with sulfur reduction. Anaerobic hydrogen consumption was detected only when B. alba B18LD filaments contained microscopically visible sulfur inclusions. Further experiments showed that the hydrogenase in B. alba B18LD was an uptake hydrogenase. The oxidation of hydrogen is coupled to the reduction of periplasmically located sulfur	[redacted]	

(27) to sulfide. The coupling of hydrogen oxidation to sulfur reduction has been observed with Desulfuromonas spp (4) and is also evident in experiments conducted with Chlorobium spp (20). It is not certain that hydrogen is available in the natural habitat of Beggiatoa spp., but since several strains of Beggiatoa contain nitrogenase (18), the hydrogenase may function in the recycling of hydrogen produced by a side reaction of the nitrogenase. The metabolic flexibility offered by the coupling of sulfur reduction to anaerobic oxidation of endogenous carbon reserves or hydrogen may be essential to an organism that exists in a changing environment such as that of Beggiatoa spp. (13,17). While the organism appears to grow best in the presence of low concentrations of oxygen, it is apparently capable of surviving at least short periods of anoxia (17). In nature this anaerobic respiration of sulfur may be the means by which filaments produce maintenance energy and the energy required to glide to the oxic-anoxic interface. …… …… ……..	[redacted] Trong tự nhiên, quá trình hô hấp kỵ khí này của lưu huỳnh có thể là một phương tiện để các sợi tạo ra năng lượng duy trì và năng lượng cần thiết để trượt đến các bề mặt phân cách oxy-thiếu oxy. …… …… ……..	
