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ANAEROBIC REDUCTION OF ELEMENTAL SULFUR
BY *CHROMATIUM VINOSUM* AND *BEGGIATO A ALBA*

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Introduction

Chromatium vinosum, a flagellated photosynthetic rod bacterium, and *Beggiatoa alba*, a gliding filamentous bacterium, both oxidize sulfide to sulfur, which is stored inside their cells in the form of sulfur globules. Although these bacteria are morphologically and physiologically distinct, they both belong to the purple bacterial group as determined by their 5S rRNA sequences (Krieg, 1984).

Filaments of *Beggiatoa* glide through sediments so that they are situated at the oxic/anoxic interface where sulfide and oxygen coexist. At this interface, sulfur is accumulated by the cells. Since this interface rises at night and falls during the day, *Beggiatoa* filaments must be able to generate energy under both oxic and anoxic conditions as they glide towards the interface. The aerobic oxidation of acetate by *Beggiatoa* has been well documented and shown to be responsible for energy generation (Strohl, 1981). However, there is no known mechanism by which *Beggiatoa* can generate energy in the absence of oxygen.

Chromatium is found in the anoxic layers of lakes. Electrons released from the oxidation of sulfide are used in anoxygenic photosynthesis. The sulfur that accumulates from this reaction increases the buoyant density of the cells. As sulfur globules accumulate, the cells sink out of the photic zone if the bacterial layer is concentrated enough to limit the light available for photosynthetic energy production. Once below the photic zone *Chromatium* gains maintenance energy from the oxidation of polyglucose to poly- β -hydroxybutyric acid (PHB) and the reduction of sulfur to sulfide (van Gemerden, 1968). Decrease in the amount of intracellular sulfur globules reduces the buoyant density of the cell and may permit cells to return to the photic zone.

This project examined:

- 1) The effect of sulfur globules on the buoyant density of *Chromatium vinosum* and *Beggiatoa alba*,
- 2) The potential use of sulfur as a terminal electron acceptor in the anaerobic metabolism of *Beggiatoa alba*, and
- 3) The effect of the reduction of intracellular sulfur during dark metabolism on the buoyant density of *C. vinosum*.

Culturing Freshwater Strains of *Beggiatoa*
(Type strain: *B. alba* B1BLD)

STOCK SOLUTIONS

BASIC GROWTH MEDIUM

| | |
|--|----------|
| Modified Pringsheim's Microelement Solution | 5 ml/l |
| NH ₄ Cl (5 percent) | 5 ml/l |
| CaCl ₂ (15 percent) | 5 ml/l |
| MgSO ₄ ·7H ₂ O (1 percent) | 1 ml/l |
| KH ₂ PO ₄ (1 percent) | 1 ml/l |
| Sodium acetate | 0.5 g/l |
| Sodium sulfide | 0.24 g/l |

Adjust the pH of the medium to 7.4 with 1N NaOH and autoclave. Add neutralized and sterilized sulfide to a final concentration of 1 mM. Use a 10 to 15 percent inoculum and gently agitate the culture for best growth. Cultures can be grown in the presence or absence of sulfide.

MODIFIED PRINGSHEIM'S MICROELEMENTS

H₃BO₃ - 0.001 percent
Na₂MoO₄·7H₂O - 0.0001 percent
FeSO₄·7H₂O - 0.07 percent
MnSO₄·7H₂O - 0.0002 percent
CoCl₂·6H₂O - 0.0001 percent
ZnSO₄·7H₂O - 0.001 percent
CuSO₄ - 0.0000005 percent
EDTA - 0.2 percent

Prepare in ddH₂O and add 1 ml/l HCl to prevent precipitation of iron.

Maintaining Cultures on Agar Plates

Cultures can be maintained for two to three weeks on agar plates. Prepare the growth medium as described above except lower the acetate concentration to 0.1 g/l and add 0.1 g/l yeast extract. Sulfide should be added to 1 mM after autoclaving.

Transfer plate cultures every two to three weeks by cutting out a slab of agar and gently sliding it over the surface of a new plate.

Starting Suspension Cultures from Agar Plates

Prepare 100 ml of 2 percent agar in a 250 ml flask. Autoclave, then add neutralized and sterilized sulfide to a final concentration of 1 mM. After this solidifies, add 100 ml of basic growth medium (+ sulfide) and inoculate the flask with a section of the agar culture. Agitate gently. Growth should occur in two days. The agar plug will provide a continuous release of sulfide into the medium. *Beggiatoa* survives best under microaerophilic conditions.

Materials and Methods

Pure cultures of *Chromatium vinosum* and *Beggiatoa alba* were grown under conditions where elemental sulfur was accumulated by the cells. The cultures were then transferred to the apparatus diagrammed in Figure II-17. In the experiments with *Beggiatoa*, the culture was centrifuged and washed twice in basal salts (Strohl and Schmidt, 1984) before being transferred to the apparatus. Nitrogen was flushed through the system and the outflowing gas was bubbled through two test tubes that each contained 10 ml of 2 percent zinc acetate to precipitate sulfide. The zinc acetate tubes were changed every hour during the course of the experiments. This apparatus permitted the continuous removal of sulfide so that sulfide toxicity did not limit metabolic reduction of sulfur globules in the cell (Table II-3). *C. vinosum* and *B. alba* reduced the intracellular sulfur to sulfide at similar rates (Fig. II-18). *Beggiatoa* filaments that lacked sulfur inclusions produced no sulfide, suggesting that sulfur-containing amino acids were not the source of the sulfide measured.

The buoyant density of *Chromatium* cells was increased to 1.130 g/cm by providing illuminated cells with 1 mM sulfide 4 hours before the density measurement. This ensured that the sulfur to sulfide during six hours did not make a significant change in the buoyant density of the cells. Although sulfur reduction to sulfide with correlated decrease in buoyant density is not a mechanism by which *Chromatium* returns to the photic zone after 6 hours, it may be important to cell maintenance as the sulfur continues to decrease for several days.

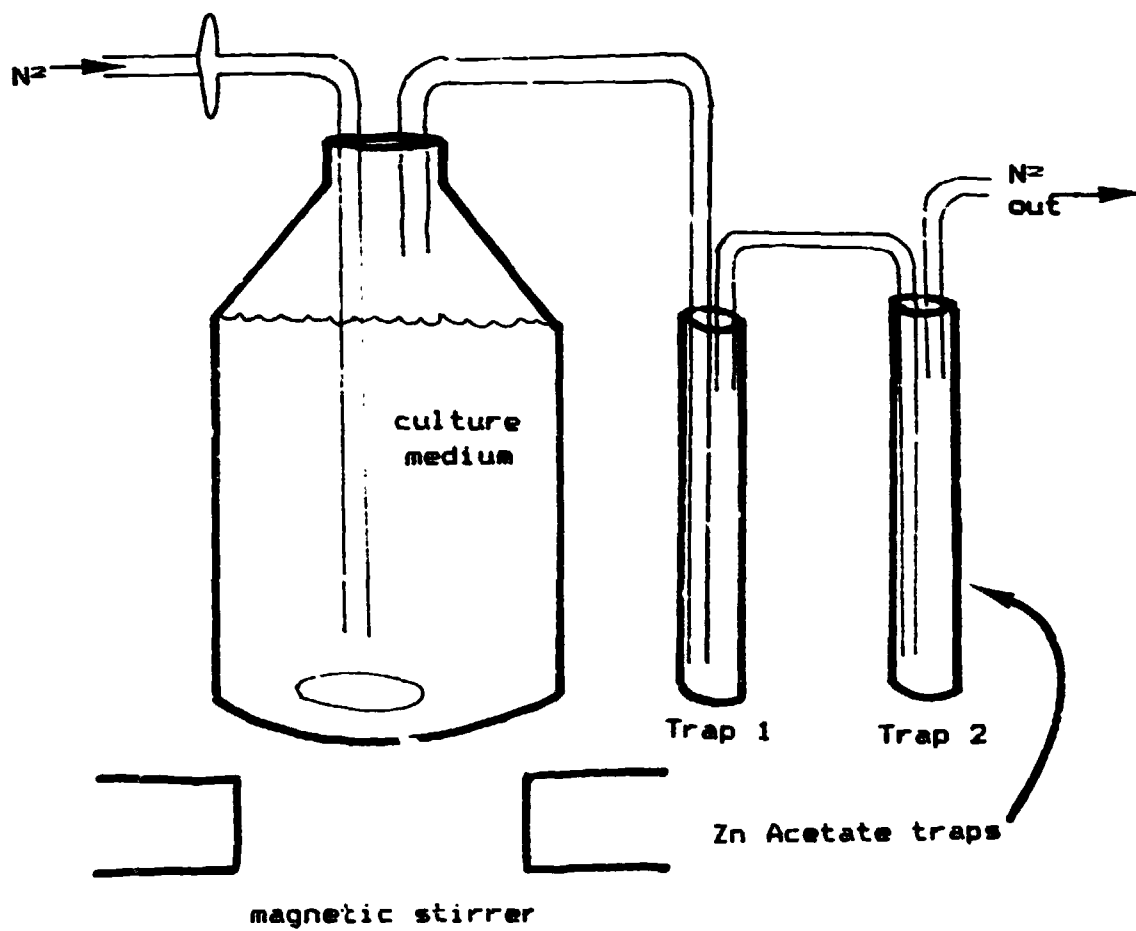
Since *Beggiatoa* can also reduce sulfur to sulfide, we hypothesized that sulfur to sulfide is part of an anaerobic energy-generating system. This pathway was previously suggested to exist in *Beggiatoa* (Nelson and Castenholz, 1981). Our work provides evidence to confirm such a suggestion. Carbon stored as PHB may be oxidized with the concomitant reduction of sulfur to sulfide. Additional research is required to determine whether the oxidation of PHB using sulfur as a terminal electron source provides energy in *Beggiatoa* and whether this is sufficient to maintain cells and provide energy for movement back into the oxic zone.

References

- Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principles of protein dye binding, *Anal. Biochem.*, **72**:248-254.

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Figure II-17. Apparatus for continuous removal and measurement of sulfide.



| Organism | Sulfur inclusions | Buoyant density gm/cm^3 |
|-------------------------------|-------------------|----------------------------------|
| <i>B. alba</i> | + | 1.115 |
| <i>B. alba</i> | - | 1.095 |
| <i>C. vinosus</i> | + | 1.087 |
| <i>C. vinosus</i> | ++ | 1.130 |
| " | " | " |
| after 3 hours dark incubation | ++ | 1.130 |
| " | " | " |
| after 6 hours dark incubation | ++ | 1.130 |

Table II-3. Buoyant densities of *B. alba* and *C. Vinosus* with varying amounts of intracellular sulfur

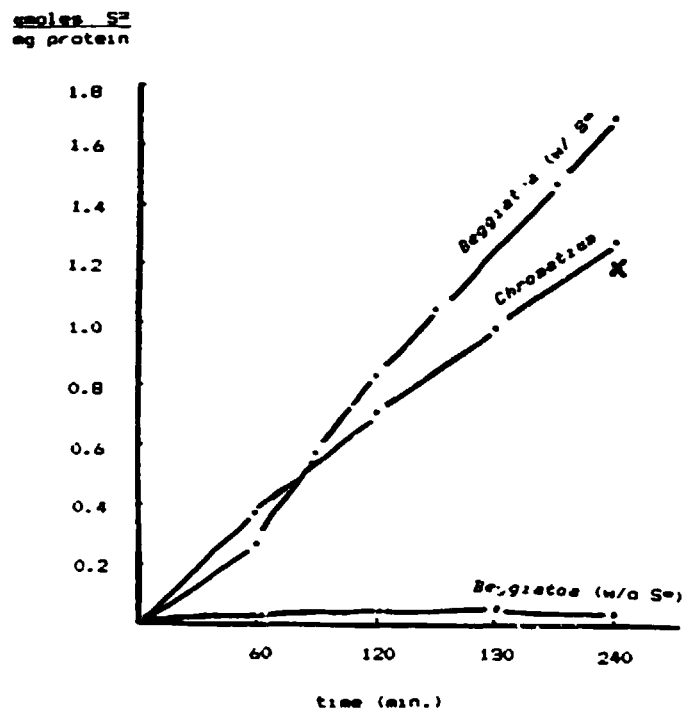


Figure II-18. Reduction of intracellular sulfur to sulfide.

- Cline, J., 1969. Spectrophotometric determination of hydrogen sulfide in natural environments. *Limnol. Oceanog.*, 14:454-459.
- Guerrero, R., Mas, J., and Pedros-Alio, C., 1984. Buoyant density changes due to intracellular content of sulfur in *Chromatium warmingii* and *Chromatium vinosum*, *Arch. Microbiol.*, 137:350-356.
- Krieg, N.R. and Holt, J.G., 1984. In *Bergey's Manual of Systematic Determinative Bacteriology*, Vol. 1, Williams and Wilkins, Baltimore, Md.
- Nelson, D.C. and Castenholz, R., 1981. Use of reduced sulfur compound by *Beggiatoa* sp. *J. Bacteriol.*, 147:140-154.
- Strohl, W. R., Cannon, G. C., Shivley, J. M., Gude, H., Hook, L. A., Lane, C. M., and Larkin, J. M., 1981. Heterotrophic carbon metabolism by *Beggiatoa alba*, *J. Bacteriol.*, 148:572-83.
- Strohl, W. R. and Larkin, J. M. 1978. Enumeration, isolation and characterization of *Beggiatoa* from freshwater sediments, *Appl. Environ. Microbiol.*, 36:755-70.
- Strohl, W. R. and Schmidt, T. M., 1984. Mixotrophy of the colorless, sulfide-oxidizing gliding bacteria *Beggiatoa* and *Thiothrix*. In *Microbial Chemototrophy* (W. R. Strohl and O. H. Tuovinen, eds.), Ohio State University Press, Columbus.
- van Gemerden, H., 1968. On the AIF generation by *Chromatium* in darkness, *Arch. Microbiol.*, 64:118-124.