Growth of *Desulfovibrio desulfuricans* Under Heterotrophic and Anaerobic Conditions

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ABSTRACT

ALICO, ROBERT K. (St. Bonaventure University, St. Bonaventure, N.Y.), AND FRANCIS W. LIEGEY. Growth of *Desulfovibrio desulfuricans*, under heterotrophic and anaerobic conditions. J. Bacteriol. 91:1112-1114. 1966.—Growth of *Desulfovibrio desulfuricans* was investigated under heterotrophic and anaerobic conditions. For initial growth to occur, it was found that the E_h or redox potential must be at least 0 mv. Some carbon sources were tested, and those which could be metabolized by *D. desulfuricans* were pyruvate, lactate, glycerol, glyceraldehyde, and ribose. Observations were also made on the sulfate used during growth. Various amounts of sulfate were added and depleted within 48 hr. This may be correlated with the decline in growth. As the terminal electron acceptor was exhausted the organisms could not respire, and, with the subsequent energy depletion, the population decreased.

The nutritional requirements of the sulfate-reducing anaerobe *Desulfovibrio desulfuricans* appear to be rather diverse. Various reports have described both a heterotrophic and an autotrophic spectrum, which are reviewed by Butlin, Adams, and Thomas (4) and Postgate (8). Since our study encompassed the heterotrophic spectrum only, this report is concerned solely with the heterotrophic mode of nutrition.

Growth is faster and more abundant under heterotrophic conditions as shown by the early investigations of Baars (Ph.D. Thesis, Technische Hogeschool te Delft, Delft, The Netherlands, 1930) and Starkey (9). A good yield is obtained with a medium containing lactate, yeast extract, sulfate, and trace amounts of ferrous iron salts. Growth patterns were correlated with anaerobic requirements and sulfate reduction so that more could be learned about the basic physiology of *D. desulfuricans*.

Because anaerobic conditions were essential, E_h was considered an important factor in these studies. Various reducing agents have been used to lower the E_h , and these are reviewed by Postgate (8). Part of this study concerns E_h measurements, with the purpose of clarifying conflicting reports on the oxidation-reduction potential requirements.

A unique aspect of the metabolism of this organism is its ability to use sulfate as an electron

acceptor, reducing it to sulfide. Although *D. desulfuricans* is a strict anaerobe, its sulfate-reducing ability is suggestive of an aerobic mechanism. The sulfate needed for optimal growth ranges from 10 mg per liter (10) to 100 mg per liter (6). Since there is a direct relation between biosynthesis and sulfate reduction, this criterion can be used as a measure of the physiological state of a population. Our study included measurements of sulfate reduction in conjunction with the growth of cell populations.

MATERIALS AND METHODS

Organisms. Three strains of *D. desulfuricans* were studied. The strains were obtained from the following sources: strain 7757, American Type Culture Collection; 8303 (Hildenborough) and 8326 (E1 Aghelia Z), from J. R. Postgate. Continuous checks for purity were made according to the general method of Butlin et al. (4) and Postgate (7). Stock cultures were maintained in semisolid agar tubes in the medium described below.

Media. The medium used in these studies was a basal type, modified according to Allred, Mills, and Fisher (1). The modified medium contained: $MgSO_4 \cdot 7H_2O$, 0.2 g; K_2HPO_4 , 0.01 g; $Fe(NH_4)_2(SO_4)_2$, 0.1 g; NaCl, 10.0 g; Na_2SO_4 (anhydrous), 1.5 g; sodium lactate (60% solution), 4.0 ml; yeast extract, 1.0 g; in 1 liter of distilled water. The pH of the modified medium was adjusted to 7.8 before sterilization at 121 C for 20 min. After sterilization, the pH tested approximately 7.2. Some of the work required the addition of agar to the medium. Three concentrations of agar were used: semisolid agar, 1.75 g; semihard agar, 4 g; and hard agar, 17.5 g; in 1 liter of the above-

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mentioned medium. Semisolid agar was used to maintain stock cultures and also for the carbon utilization studies. The semihard agar was used in serial dilutions for the isolation of discrete colonies in agar deeps, and hard agar was used in media for all colony counts made in the viable-growth studies.

Methods. Various organic substrates were tested to determine whether they could support growth. Each substrate was added in concentrations of 0.5 g per liter to the modified medium minus sodium lactate and yeast extract. This basal medium provided the necessary salts for maintenance of ionic and osmotic equilibria. Growth was ascertained by microscopic examination and by the production of the characteristic iron sulfide precipitate.

The greater part of this study was concerned with the growth patterns of *D. desulfuricans*, along with the measurement of oxidation-reduction potentials and the reduction of sulfate during growth. A satisfactory growth chamber proved to be a 1-liter volumetric flask, since the long narrow neck minimized the surface area and the diffusion of atmospheric oxygen. A Teflon-coated magnetic stirring bar was placed inside along with some glass beads. Since organisms grown in lactate tend to become stringy, this mechanical stirring device readily broke up cell clumps.

Growth methods. All growth cycle studies were carried out with strain 7757. A single temperature of 30 C was maintained for all tests, and the environment was strictly anaerobic. A lowered E_h was maintained by adding 0.15% anhydrous sodium sulfite to the medium. The standard inoculum was 2 ml of a young broth culture added to 1 liter. Two types of counting were employed in measuring bacterial growth; these were total- and viable-count methods. Total counts were made microscopically by use of a hemocytometer, and samples of bacteria diluted in an iodine solution were counted. These direct counts were converted to cells per milliliter with a standard conversion formula. Viable counts were made by serial dilutions in tubes (150 by 16 mm) containing complete medium and hard agar. The tubes were sealed with a plug of sterile hard agar. Colonies were counted after 3 to 4 weeks of incubation.

E_h measurements. The oxidation-reduction potential was determined with the Beckman model G pH meter equipped with a platinum electrode. Measurements were corrected by adding the calibrated reference potential to the sample readings.

Sulfate utilization. The sulfate was measured by a method described by the American Public Health Association (2). The basis of this procedure was the reaction of sulfate ions with barium ions to form a white precipitate. The precipitate was quantitatively measured with a Photovolt (model 402-E) lumetron colorimeter (Photovolt Corp., New York, N.Y.) equipped with a 515-m μ filter. The amount of sulfate was determined by comparing each sample to a standard curve. All sulfate determinations were carried out at 30 C.

RESULTS

Carbon sources for growth. Strains 7757, 8303, and 8326 were tested for their ability to grow on

various carbon sources. The basal medium previously described was used for all tests, with various carbon sources substituted for lactate. All strains grew on pyruvate, lactate, glycerol, glyceraldehyde, and ribose. No growth was obtained on glucose, fructose, fructose-1,6-diphosphate, lactose, galactose, erythrose, or sedoheptulose. This confirmed the findings of Postgate (*unpublished data*) and Baker, Papiska, and Campbell (3).

Growth cycle studies. All population measurements were made with strain 7757 grown at 30 C on the modified medium containing lactate. Growth measurements were made by total- and viable-count methods and are represented in Fig. 1. The lag phase lasted about 12 hr, and, after growing exponentially for about 30 to 35 hr, the population entered the stationary phase after 42 to 47 hr of incubation. The E_h decreased to -180 mv as the culture grew exponentially

FIG. 1. Growth of *Desulfovibrio desulfuricans* as measured by the total- and viable-count methods. The broken line represents E_h values during growth.

FIG. 2. Sulfate reduction by *Desulfovibrio desulfuricans* during growth.

The final E_h did not decrease below -200 mv during the 90-hr cycle.

Sulfate studies. Because sulfate reduction is a distinguishing characteristic of this organism, some studies were made on the utilization of sulfate. Different amounts of sulfate were added to the growth medium, and its depletion was measured at intervals (Fig. 2). All available sulfate was used by 48 hr.

DISCUSSION

Various growth and nutritional aspects of *D. desulfuricans* were studied. A reducing environment was found to be essential for the initiation of growth (5, 8). Viable counts required an E_h of zero and below or such quantitative measurements would not be valid. The addition of sulfite to the medium proved satisfactory in lowering the E_h .

The basal medium used had the necessary inorganic elements plus sulfate and minimal amounts of yeast extract. The carbon sources which were capable of supporting growth included pyruvate, lactate, glycerol, glyceraldehyde, and ribose.

To clarify some of the disparity found in the literature (8), much of our work was concerned with the growth cycle. The results show that both total- and viable-count methods coincided closely in the growth phases. The E_h measurements during growth ranged from about 0 to -200 mv.

The utilization of sulfate was also measured. During the early phases of growth, sulfate was used rather rapidly, and its depletion appears to

be a significant factor contributing to the decline of the population.

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