intervals with a Coleman model 14 spectrophotometer at 650 mμ. Uninoculated media served as blanks. Doublings per hour were calculated from turbidity and viable-count (Panos et al., J. Bacteriol. **80**:336, 1960) data. Each point is an average of at least two duplications.

Figure 1 illustrates the comparative relationship of doublings per hour with temperature for the parent coccus and L form. No growth of either organism occurred at 45 C. It will be noted that an increase in temperature results in an increase in the growth rate for both organisms. However, the rates for the L form remain considerably lower than those of the coccus. A marked decrease in the growth rate of the L form at 40 C, as compared with the maximal rate attained by the coccus, points to a new variable which may be temperature-mediated. What this new variable may be indicative of is, at present, obscure. These observations, coupled with the extreme diversity of individual cell sizes consistently observed during growth of an L form, may prove useful in studies of cellular division.

This investigation was supported in part by research grant E-4495 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

METHOD FOR ELECTROLYSIS OF CULTURE MEDIUM TO INCREASE GROWTH OF THE SULFUR-OXIDIZING IRON BACTERIUM *FERROBACILLUS SULFOOXIDANS*

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Received for publication 7 November 1963

Autotrophic growth of *Ferrobacillus sulfooxidans* (Kinsel, J. Bacteriol. **80**:628, 1960) on iron as the energy source requires the oxidation of extremely large amounts of iron. Our experiments indicate a cell yield of only 1 to 2 g (wet weight) from the oxidation of 500 g of $FeSO_4 \cdot 7H_2O$. This is the harvest obtained from 20 liters of medium at a ferrous iron concentration of 5,000 ppm.

The following describes a procedure for the continual regeneration of reduced iron in culture medium which has been inoculated with iron-oxidizing autotrophs. This procedure enables one to obtain high yields of physiologically active cells with small volumes of medium.

The culture apparatus (Fig. 1) consists of a 500-ml wide-mouth Erlenmeyer flask containing 400 ml of inoculated culture medium, an aerator, and two platinum electrodes (A. H. Thomas Co., Philadelphia, Pa.; cathode electrode-8308, anode electrode-8306); one of the electrodes, the anode, is contained in a porous clay cup (Fisher Scientific Co., Pittsburgh, Pa.; 2-535-1). The clay cup is attached to the aerator with rubber bands; the other electrode, the cathode, surrounds the entire assembly. The assembly is held in a rubber stopper cut to permit exit of an air-outlet tube, a culture (or water)-addition tube, the aerator, and the two electrodes. The apparatus is sterilized prior to the introduction of the culture. Direct

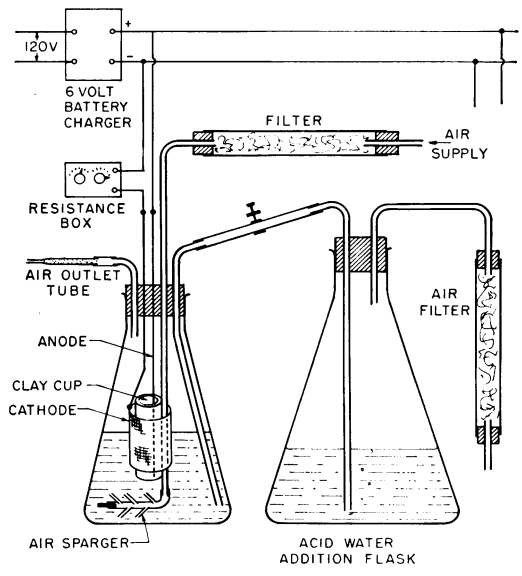

FIG. 1. *Apparatus for electrolysis of iron medium.*

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TABLE 1. *Yield and respiratory activity of cells of Ferrobacillus sulfooxidans grown in iron medium with and without electrolysis*

Expt no.	Current (ma at 47 ohms)	Iron oxidized (%)		Yield (μg of protein/10 ml of medium)	Activity (28 C)	
		Beginning	End (days)		Cell concn (μg of protein)	Q_{O_2} (protein)*
1	80 ± 10	15.9	100.0 (9)	375.00	107.77	1,648
	Control	6.8	100.0 (9)	65.65	106.48	1,437
2	86 ± 6	19.2	92.7 (6)	373.59	109.73	1,312
	Control	19.2	100.0 (6)	48.92	109.46	2,193
3	87 ± 7	19.2	91.5 (6)	334.46	110.68	1,409
	Control	19.2	100.0 (6)	53.04	112.56	1,919
4	84 ± 7	13.4	77.8 (6)	384.35	127.33	1,037
	Control	13.4	100.0 (6)	62.58	112.68	1,917

* Expressed as microliters of O_2 taken up per mg of cell protein (bovine albumin equivalents) per hour. Reaction flasks contained a total volume of 3.1 ml distributed as follows. The main compartment contained 3.0 ml of cells free from iron precipitate in water at pH 2.5 and 50 μmoles of Fe^{++}. The center cup contained 0.1 ml of 20% KOH. Protein concentration was determined colorimetrically with the Folin-Ciocalteau reagent (Oyama and Eagle, Proc. Soc. Exptl. Biol. Med. **91**:305, 1956). Experiments 1 and 2 were not performed aseptically.

current is supplied to the system by a 6-v battery charger (Sears, Roebuck and Co., Chicago, Ill.; 608.71511) and regulated by a variable resistor (Lafayette Radio Electronics Corp., Syosset, L.I., N.Y.; TE-17).

The system is operated as follows. The iron-oxidizing autotroph is grown in 500 ml of Leathen's iron medium (Leathen, McIntyre, and Braley, Science **114**:280, 1951) modified to contain 5,000 ppm of ferrous iron (25 g per liter, $FeSO_4 \cdot 7H_2O$) and adjusted to pH 2.5 to 3.0 (25 ml per liter, 0.1 N H_2SO_4) until 15 to 20% of the ferrous iron has been oxidized (ca. 3 days). A 400-ml amount of this culture is added to the apparatus through the culture-addition tube (suction is applied at the air outlet tube with the aerator clamped shut) and, by tilting the apparatus flask, to the clay cup. The flask which contained the culture is now replaced with a similar flask containing sterile acid water (distilled water acidified to pH 3.0 with 1 N H_2SO_4). This water is used to maintain the volume of liquid in the apparatus throughout the experiment and is added in a manner similar to that described for introducing the culture. (The water addition tube is clamped shut during aeration.) The electrodes are connected to the appropriate terminals (anode to positive, cathode to negative) on the battery charger, and electrolysis is begun. Air, admitted through the air sparger, is sterilized by passage through sterile glass wool. The rubber stoppers on all flasks are wired in place.

Representative results obtained with this apparatus are shown in Table 1. Cells harvested from electrolyzed medium showed respiratory activity of the same order of magnitude as cells from cultures without electrolysis. As indicated by protein determinations, the cell yield in electrolyzed medium was approximately sixfold greater. Other experiments not listed show that yield of cells could be increased as much as 20-fold by electrolysis with current at levels approaching 200 ma. The Q_{O_2} (protein) values of these cells ranged from 807 to 1,161, whereas control cells showed Q_{O_2} (protein) values of 1,919 to 2,193. At this current level, iron was usually precipitated on the reducing (cathode) electrode.

This work was supported by a Postdoctoral Research Fellowship (EPD-18,081) awarded to the senior author from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.