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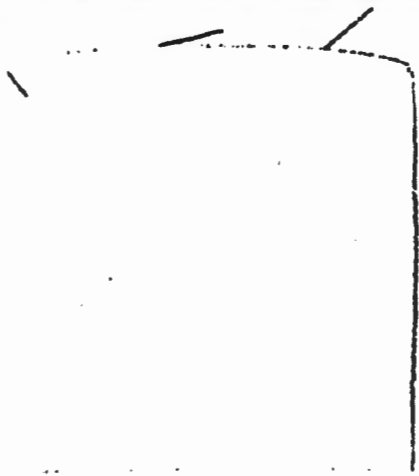


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ELECTROCHEMICAL OBSERVATIONS IN MICROBIOLOGICAL PROCESSES. I.

GROWTH OF THIOBACILLUS THIOOXIDANS



SUMMARY

The growth of Thiobacillus thiooxidans utilizing sulfur in three media was studied by observing changes in half-cell emf, bacterial cell count and production of acid as a function of time. A comparison of the biological half-cell emf with comparable control half cells reveals that T. thiooxidans makes an electrochemical contribution to half-cell voltage. A change from the more complex medium of Skerman's mineral salts to A.T.C.C. allowed a clearer delineation of T. thiooxidans' ability to make an electrochemical contribution.

Reproducible biological half-cell emf's were obtained when the ferrous sulfate was removed from the A.T.C.C. medium. One half cell comprising T. thiooxidans utilizing sulfur in A.T.C.C. was observed over a 111-day period. During this time the initial half cell voltage of -0.35 volts, decreased to a negative value of -0.64 volts (hydrogen emf series). T. thiooxidans in utilizing sulfur produces only sulfate ion, thereby simplifying the identification of an electrochemical contribution during growth.

I. INTRODUCTION

The concept of converting chemical energy from natural occurring fuels into electrical energy by biochemical reaction has intrigued man for many years. Potter¹ in 1911 was the first to conduct experiments with biochemical galvanic cells. He observed that "the disintegration of organic compounds by microorganisms is accompanied by the liberation of electrical energy." His experiments were conducted primarily with the yeast-glucose system which gave open circuit voltages between 0.3 and 0.5 V. These exploratory experiments led to investigations in 1931 by Cohen,² who studied several bacterial cultures as electrical half cells. More recently, Bean,^a Canfield,^b Bitterley^c and their co-workers have been working on various aspects of bioelectricity for the National Aeronautics and Space Administration. Emphasis in their investigations was placed on the utilization of organic foodstuffs as an energy source.

In order to gain a better understanding of voltages developed in biological oxidations, a decision was made by the authors to investigate some of the autotrophic bacteria. Autotrophic bacteria, because of their ability to utilize inorganic substrates as an energy source

^a Philco Corporation, Newport Beach, California.

^b Magnē Corporation, Anaheim, California.

^c The Marquardt Corporation, Van Nuys, California.

and carbon dioxide for their carbon requirements, offered a different and perhaps a simpler approach to associating electrochemical potentials with metabolic activity of bacteria. The sulfur oxidizing bacteria, Thiobacillus, were chosen since they were among the most metabolically active autotrophs. In these studies, primary emphasis was placed on T. thiooxidans.

The electrochemical investigations reported in this paper assume that sulfate ion is the only metabolic product associated with the oxidation of sulfur by T. thiooxidans. This assumption has the support of earlier workers such as Starkey,^{3,4} Starkey, Jones and Fredrick,⁵ Vogler and Umbreit,⁶ and Parker and Prisk.⁷

II. ELECTROCHEMICAL ACCESSORIES

Carbon (UP-62-R) from the United Carbon Company, Bay City, Michigan, was cut into electrodes. The ends of the electrodes were plated with copper from a CuSO_4 solution. Copper leads were soldered to the plated surfaces. The leads and their contact with the carbon were treated with paraffin to eliminate ~~leaking~~ and direct contact between the copper and nutrient.

Platinum electrodes were prepared from platinum gauze (45 mesh, 0.0078 in diameter) obtained from J. Bishop and Company. This gauze was cut into 2-in lengths, approximately 5/16 in wide. Copper leads were soldered to one end of the gauze. To prevent possible

oxidation of the copper, the lead wires were covered with plastic tubing. This tubing was then anchored to the copper-platinum solder joint by coating the end of the tubing and the junction with an epoxy resin. To further prevent any possible diffusion of water to and through the plastic tubing and the epoxy-covered junction, the lead wires were kept above the biological half-cell liquid level. The thermocouple effect for these copper-platinum electrodes was found to be negligible ($5 \mu\text{V}/^\circ\text{C}$).

These biological half-cell investigations involved maintaining an air atmosphere above the media in the cells. The electrochemical effect of supplying fresh air above versus bubbling it directly into the stirred media was negligible as long as gaseous concentration gradients did not exist within the media (Table I).

TABLE I

DYNAMIC AND STATIC AEROBIC CONDITIONS FOR STIRRED
ELECTROCHEMICAL HALF CELLS

<u>Medium</u>	<u>Aerobic Atmosphere</u>	<u>Potential, V</u>		
		<u>Static</u>	<u>Dynamic (Bubbling)</u>	<u>δ</u>
Sterile distilled water	Air	-0.323	-0.329	0.006
Skerman's	Air	-0.365	-0.365	0.000
A.T.C.C.	Air	-0.390	-0.390	0.000
A.T.C.C. (minus FeSO_4)	Air	-0.420	-0.420	0.000

Since composition gradients were known to be generated through utilization of substrate by the uneven suspensions of bacteria, it was considered necessary to uniformly stir the half cells. The electrochemical effect of turning off the stirrer was checked for the uninoculated media in which such composition gradients were absent. Table II reveals that the effect of not stirring was appreciable in the sterile distilled water. It became negligible when conducting nutrients were added to the water. In all large control and biological half cell experiments reported in this paper, the cell constituents were stirred and atmospheric air with its carbon dioxide was available to the media through sterile cotton plugs.

TABLE II

EFFECT OF NOT STIRRING LARGE ELECTROCHEMICAL HALF CELLS
(AIR BUBBLING INTO CELL)

<u>Medium</u>	<u>Potential, V</u>		
	<u>On</u>	<u>Off</u>	<u>δ</u>
Sterile distilled water	-0.329	-0.240	0.089
Skerman's	-0.365	-0.363	0.002
A.T.C.C.	-0.390	-0.388	0.002
A.T.C.C. (minus FeSO_4)	-0.420	-0.418	0.002

During the course of experimentation three basic biocell designs were implemented. The first design involved combining a biological half cell with either a control or a reference half cell in a U-tube. An agar plug in the bend of the tube separated the two half cells. Although positive results for associating emf's with growth of T. thiooxidans were obtained with this initial design, a modification (Fig. 1), which made a separate opening to the agar bridge for connecting a reference calomel cell, was desired. Each of the original half cells, the biological and control, could now be monitored individually with the calomel half cell. Experiments with this modified U-tube were satisfactory, however it was deficient in size and allowed concentration gradients to form so a new large biological half cell was designed.

The above cell designs limited the amount of liquid to be used to about 20 ml of nutrient. Furthermore, the long narrow tubes presented little opportunity for changes in electrode design, stirring, and continuous measurement of pH. Therefore, the new design used a large three- and, later, a five-necked 1 000 ml round-bottom flask (Fig. 2). A Teflon stirrer was suspended through the center neck surrounded with a glass bearing. Its action diminished acid and mineral concentration gradients and insured uniform suspension of bacteria for population density determinations. An agar salt bridge and calomel cell were mounted in one opening and a glass and a platinum electrode were placed in the third neck. The calomel and glass electrodes were

used for recording the pH values. This entire biological half cell was mounted in a constant temperature bath (29°). Prior to use of this bath the electrochemical voltages were observed to fluctuate in a cyclic manner with the room temperature whenever T. thiooxidans was present. This behavior was especially evident when platinum electrodes were used.

The agar salt bridge, mounted in one opening of the round-bottom flask, was led to a test tube containing saturated KCl maintained at the same temperature as the biological half cell. A standard calomel electrode was mounted in the test tube as a reference half cell. Leads from the complete cell were connected to a K-3 potentiometer and a pH meter.

All parts of the above cells which could withstand high temperatures were sterilized by autoclaving. The other parts were sterilized by rinsing in ethanol followed by three rinses with sterile distilled water. Before sterilization the electrodes were cleaned with concentrated sulfuric acid and then washed with distilled water.

All experimentation was oriented towards obtaining zero-current potentials of complete as well as half-cell reactions. The initial electrochemical measurements were made with a Model K-3 Leeds-Northrup potentiometer. This instrument gave accurate voltage determinations when zero current conditions were established. However, while balancing the galvanometer to obtain zero current conditions,

power was drawn initially from the cell causing, in some cases, a loss of voltage. Since it was desirable to eliminate this probable contribution to variable results, a specially designed vacuum tube voltmeter was obtained for use with a recorder. Open circuit conditions were maintained by use of this vacuum tube voltmeter and continuous emf measurements could be taken with the recorder.

III. MICROBIOLOGICAL TECHNIQUES AND OBSERVATIONS

The application of microbiological techniques to support the electrochemical investigations was focused on two areas of study. The first involved obtaining reproducible bacterial growth. After such growth was established, less complex media were sought by removing individual constituents from the more complex formulae. The second area of study involved developing techniques for determining bacterial counts in the uniform biological half cell suspensions.

A. Growth of *Thiobacillus thiooxidans*

Successful growth of *T. thiooxidans* was studied primarily in three media. At first, reproducible growth of *T. thiooxidans* was obtained with shaker cultures using Skerman's⁸ basic mineral salts (17 salts). One per cent of sterile powdered sulfur was suspended in this medium. High yields of 10^9 organisms/ml were obtained with mature cultures (maximum population density) after five days incubation.

Since the interpretation of the emf measurements in the Skerman's medium was exceedingly difficult, a simpler medium was sought. A.T.C.C. medium,^d containing five salts plus 1 per cent sulfur, was studied as a growth nutrient. This medium gave mature cultures with populations of 10^8 bacteria/ml. A further consideration of reducing the salts, comprising A.T.C.C., brought about the removal of ferrous sulfate. The concentration of T. thiooxidans in mature cultures in this medium, A.T.C.C.(-), was approximately 1×10^7 organisms/ml.

The bacteria to be used with the biocells usually were taken from five-day-old mature shaker cultures. These cultures were grown in 250 ml Erlenmeyer flasks with 30 ml of medium in each flask. Incubation was either at 29° or at room temperature.

The mature cultures were harvested from the shaker flasks by centrifuging the organisms at 9,000 rpm for 5 min in a Lourdes centrifuge. After decanting the supernatant, the cells were then washed twice with sterile medium and were suspended in the various volumes of the sterile medium for the particular bacterial concentration to be used for biological half-cell studies.

^d Milligrams/100 cc: $(\text{NH}_4)_2\text{SO}_4$, 20.0; $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 50.0; CaCl_2 , 25.0; FeSO_4 , 5.0; KH_2PO_4 , 300.0.

B. Bacterial Counts

Two methods were applied for obtaining the bacterial populations of the cultures in the preparation and operation of the biocells. These methods were the Petroff-Hausser chamber count and the micro-Kjeldahl analysis⁹ for total nitrogen content of the bacteria.

T. Thiooxidans was removed from the medium by filtration before a modified micro-Kjeldahl analysis was applied. Turbidity determinations for bacterial counts were not practical because of the presence of powdered sulfur. Pour and spread plate counts were discarded after obtaining irregular and time consuming results. The Petroff-Hausser chamber counts were used to calibrate the nitrogen content from the bacterial with their concentration in the medium.

The micro-Kjeldahl technique was only used when appreciable volumes of samples were available and concentrations of bacteria were approximately 1×10^7 /ml or greater. Use of the Petroff-Hausser counting chamber technique was preferred for lower concentrations of bacteria and experiments where less than 1 ml of sample was available. The latter technique was adopted completely after the earlier phases of investigation in order to minimize disturbing the biological half-cell ecology. The total amount of liquid required for the samples by this technique was negligible compared to the large biocell volume.

C. General Microbiological Observations

Mature populations obtained in the large biological half cell (Table III) with Skerman's medium were consistently less than observed in the shaker cultures. Subsequent experimentation with growth of T. thiooxidans in less complex media showed that this difference in population density decreased with A.T.C.C. and disappeared when A.T.C.C.(-) was used.

TABLE III

TYPICAL MATURE POPULATION DENSITIES

<u>Medium</u>	<u>Shaker Culture</u> <u>(organisms/ml)</u>	<u>Large Biological</u> <u>Half Cell</u> <u>(organisms/ml)</u>	<u>Number of</u> <u>Salts</u>
Skerman's	1×10^9	1×10^8	17
A.T.C.C.	1×10^8	1 to 5×10^7	5
A.T.C.C.(-)	1 to 5×10^7	1 to 5×10^7	4

Experiments with growth of T. thiooxidans in both shaker and biocell cultures using Skerman's and A.T.C.C. as growth media showed that the reduction in the number of mineral salts caused a decrease in the mature population density. Two typical biocells (Fig. 3) were started with similar inoculi and similar volumes of medium. The temperature for both biocells was held at 29°. Similar lag periods were observed during the first day, followed by a rise in bacterial concentrations during the second day. During the third day of incubation,

the bacteria concentrations were observed to rise more rapidly in Skerman's than in A.T.C.C. After this period the population density appeared to stabilize at 1×10^8 for Skerman's and 4×10^7 bacteria/ml for the A.T.C.C. media.

IV. ELECTROCHEMICAL OBSERVATIONS WITH T. THIOOXIDANS

The initial investigations were concerned with establishing that an electrochemical potential, different from that of a control cell, exists when T. thiooxidans utilizes sulfur. The U-tube was chosen for these studies. Each side of the U-tube comprised a half cell, one biological and the other an oxygen-carbon reference electrode. Duplicate U-tubes were prepared with only one difference. T. thiooxidans was present in the arm of one of them. The other half cell had only sulfur suspended in Skerman's medium. Figure 4 shows that a significant difference exists between the complete cell emf's of the inoculated and control cell. Such results were typical both when carbon and when platinum electrodes were used in the cells. The increase in the difference with time suggests that after acclimation to the cell, T. thiooxidans became active and started to utilize the sulfur which in turn changed the electrochemical nature of the half cell.

Biological and control half cell emf's, using platinum electrodes, were measured as a function of time in the modified U-tube design (Fig. 1). The biological half cells consistently gave voltages

which were lower than their controls, however, leaks through the agar plug at various times after cell preparation encouraged pursuing experimentation with a better cell design.

The remaining experiments were conducted with large biological half cells using platinum in preference to the slower responding carbon electrodes. Since quantitative data were expected from the use of this new cell design, a check was made on how increases in hydrogen and sulfate ion would affect the half cell electrochemical emf as measured by this electrode. Sulfuric acid was added separately in approximately 20 increments to 1 per cent suspensions of powdered sulfur in each of the three media under consideration for studying growth of T. thiooxidans. The initial pH values of approximately 5.0 gave way upon additions of the acid to values of 1.0. Each addition of acid simulated production of sulfuric acid by T. thiooxidans. The maximum variation in half cell emf's with Skerman's medium experiencing these changes in pH was 0.012 V. Subsequent experimentation with A.T.C.C. and A.T.C.C.(-) media gave a smaller maximum variation for the same total change in pH. Thus, the electrochemical background fluctuations to be expected when sulfate ion is produced by T. thiooxidans were identified.

A. Medium Effect on Biocell Activity

Shaker cultures of T. thiooxidans were grown in Skerman's, A.T.C.C., and A.T.C.C.(-) media under similar conditions. The preparations for centrifuging, washing, and resuspension in fresh sterile

media, were planned to give populations having a concentration of 1×10^7 bacteria/ml. However, the indeterminate losses in the transfers gave rise to a slight variance in initial bacterial suspensions in the large biological half cells. The suspension in A.T.C.C.(-) was 1.2×10^7 . The value for Skerman's was 1×10^7 and for A.T.C.C., 2×10^6 bacteria/ml.

After inoculation, a lag phase was observed to take place during growth in each medium (Fig. 5). The values for bacterial population counts under the dotted line in Fig. 5 were below the micro-Kjeldahl analysis so they were estimated (Petroff-Hausser Count). After 70 hr, the population in the Skerman's salts, which was initially similar to the other two media, was now greater. Subsequent bacterial counts showed that the populations stabilized and after eight days the Skerman's medium had a population of 1×10^8 while both A.T.C.C. media had approximately 5×10^7 bacteria/ml. These saturation populations were typical of large biocell experiments with these three media (Table III).

If the variation in the initial concentration in bacteria can be ignored the amount of total acid produced by T. thiooxidans seemed to depend upon the medium in which it grew. Since it was not possible to wash residual amounts of acid from the centrifuged cells, the initial pH values differed. Subsequent accumulation of acid is shown in Fig. 6. Of particular interest was the fact that T. thiooxidans produced less

acid (0.73 mmole) in the Skerman's medium in attaining a greater cell population, 7×10^7 bacteria/ml than in the other two media (3.66 mmole for A.T.C.C.(-) and 1.89 mmole for A.T.C.C.) for the initial 70-hr growth interval.

The growth of T. thiooxidans was followed by electrochemical measurements. The fact that the control half cells (Fig. 7) started at exactly the same emf was coincidental. They usually differed by small amounts. T. thiooxidans was observed as a contaminant (Fig. 7) in the control half cell for the Skerman's medium after the 50-hr measurements. The visual presence of this microorganism was supported by a corresponding change in pH due to acid production. This microbiological activity caused a decrease in half-cell voltage as indicated by the values at the 70-hr interval.

The change from the initial emf's for the Skerman's and A.T.C.C. half cell controls were typical for these media. Since their half-cell voltages usually stabilized after 20 hr, subsequent experimentation involved preparation and operation of two control half cells until voltage stability was observed. Then, one of the half cells was inoculated for comparison of their behavior as a function of time. These complications were minimized when working with the A.T.C.C.(-) medium. Its half-cell emf was less erratic and stabilized quite readily.

The inoculated A.T.C.C.(-) (Fig. 8) half cell had the same initial voltage as its control half cell, whereas the other inoculated half cells were higher than their controls. After 33 hr each of the inoculated half cells had voltages more negative than their control cells. The behavior of the inoculated A.T.C.C. and A.T.C.C.(-) half cells was comparable after 29 hr of operation. These cells gave lower voltages than the inoculated Skerman's half cell. The relatively small difference between the inoculated and control half cells comprising Skerman's medium pointed out the need to have a less complex medium for intimately following growth of T. thiooxidans electrochemically. Thus, subsequent studies were conducted with A.T.C.C. and A.T.C.C.(-) media. The biological half cell emf's with these media were found to be more stable and further removed from their control half-cell values. The removal of the ferrous sulfate from the A.T.C.C. medium offered additional improvement in control and biological half-cell stability and reproducibility.

B. Long Term Biological Half-Cell Activity

Special precautions were taken with the preparation of one experiment which was allowed to run for an extended period of time. Emphasis was placed on minimizing external contact with the medium to eliminate the possibility of contamination and to increase the probability of long life. The supply of oxygen and carbon dioxide for this

biological half cell came from the atmosphere through sterile cotton plugs placed in the small air gaps around the wires leading into the cell through the rubber stoppers. The large biological half cell was used with its Teflon stirrer, pH meter, and the platinum electrode attachments. It was filled with sterile A.T.C.C. medium, characterized (pH, emf, sterility) and then inoculated with T. thiooxidans to give an initial concentration of approximately 7×10^7 bacteria/ml. Sterile sulfur was used as the energy source in this medium. The cell count, pH and biological half-cell behavior for a 16-day period are shown in Fig. 9. Table IV describes the behavior of this cell approaching the 111th day. Between these times the emf and the bacterial counts fluctuated slightly with a fairly uniform accumulation of acid. The general trend of the half cell potential was to become more negative. After the 111 days, the biological half cell was observed to be contaminated with bacteria other than T. thiooxidans. The half-cell emf was observed to become more positive after becoming contaminated. This observation was consistent with that obtained from other inoculated biological half cells which became contaminated with foreign bacteria.

TABLE IV

SUPPLEMENTARY DATA FOR LONG TERM BIOLOGICAL HALF CELL(T. thiooxidans Utilizing Sulfur)

<u>Time Period</u> <u>(days)</u>	<u>Half-Cell emf</u> <u>(V)</u>	<u>pH</u>	<u>Bacteria/ml</u>
72	-0.61	1.18	5×10^7
76	-0.62	1.09	5×10^7
79	-0.61	1.05	3×10^7
83	-0.62	0.97	4×10^7
90	-0.63	0.92	4×10^7
99	-0.63	0.91	6×10^7
106	-0.64	0.95	4×10^7
111	-0.64	0.92	3×10^7

Several interesting features were worth noting in this long term experiment. The initial inoculation gave a population of 7×10^7 T. thiooxidans/ml. Death occurred lowering the population below the level of detection by the micro-Kjeldahl analysis. At the end of the initial day of operation, the population was observed to increase to 1.5×10^7 . Fluctuations between this value and 3×10^7 were observed throughout the 17-day interval shown in Fig. 9. Between the end of this period and the 111th day, a maximum count of 5×10^7 was obtained. The population dropped to 3×10^7 bacteria/ml towards the end of the experiment. The ecological factors effecting bacterial growth kept the cell population in this range. The pH of the medium changed from an initial value of 4.85 to about 1.7 units after 15 days. Changes in its value after this time were small since appreciable amounts of acids had to be

produced relative to the total amount present in order to bring about a change in pH. The fact that T. thiooxidans was not increasing its population suggests inactivity but the increase in sulfuric acid concentration indicates that a constant cell division and death rate existed. The general trend in the half-cell emf throughout this time period was to become more negative.

The consistent trend in the curves obtained in this experiment indicated that many of the past variations in half cell potentials were no longer present and that reproducibility of subsequent biological half cells should be expected. One exception to this consistent trend toward a lower half cell potential may be significant. A noticeable decrease in cell population appeared to take place after the eighth day. This dip and later recovery seemed to cause a simultaneous change in the electrochemical potential.

C. Reproducibility of Biological Half Cell

Several biological half cells were prepared with T. thiooxidans utilizing sulfur in the A.T.C.C.(-) medium to determine reproducibility of results. Figure 10 records their biological half-cell emf for a five-day period. Initially, the cells started at approximately the same voltage followed by a slight rise and then a decrease. A slight divergence in values then takes place for one and one-half days. After this interval they gave equivalent results. The effect

of higher temperature is shown in Series XLVI when after three days the emf decreases markedly. At this time the thermostat for its bath malfunctioned and temperatures exceeding 60° were probably experienced during the evening period. This irreparable damage caused formation of a lower half-cell emf, measured upon return and subsequent control at 29°.

Figure 11 records the change in pH of each of these half cells. One can readily see that after the initial inoculation, a slight divergence takes place. This divergence disappears after about one day, giving rise to similar amounts of acid formed in each of the cells.

The divergence in the microorganisms' contribution to emf and acid formation between the one-fourth and one and one-half day period appears to be consistent with the microorganism experiencing a lag phase in each of the biological half cells throughout this time interval. Figure 12 shows the bacterial cell count per milliliter as a function of time. A lag phase existed in each cell with a slight inconsistency in the recovery time. In general, the cell populations duplicated themselves in each of the cells. The last two values for the cell count in Series XLVI reflect the damage caused by the temperature of the bath exceeding the control value.

V. DISCUSSION

The existence of an electrochemical contribution from T. thiooxidans was established during the initial investigations with combined half cells comprising biological and control half cells with either a calomel or oxygen-carbon electrode. The cell comprising T. thiooxidans utilizing sulfur made contributions to an electrochemical potential which reflected microbiological activity. Subsequent experiments with well defined biological half cells, comprising emf, pH, and bacterial count determinations, supported the initial observations. The interaction of T. thiooxidans with the various nutrients as well as sulfur gave half cell emf's which were distinctly different than control half cells operated for comparable periods of time under identical experimental conditions. The reduction in the number of nutrient ions for growth of T. thiooxidans improved one's ability to measure its more intimate contributions to an electrochemical emf. However, since the measured half cell emf is an algebraic resultant of each electrochemical contribution in the half cell, it is not possible at this time to say how T. thiooxidans is implicated. If sufficient activity coefficients at these ionic concentrations would be available, the theoretical contributions of the constituents of the media could be calculated so the remaining biological contribution could be identified. This calculation is much too complicated at present. However, as further

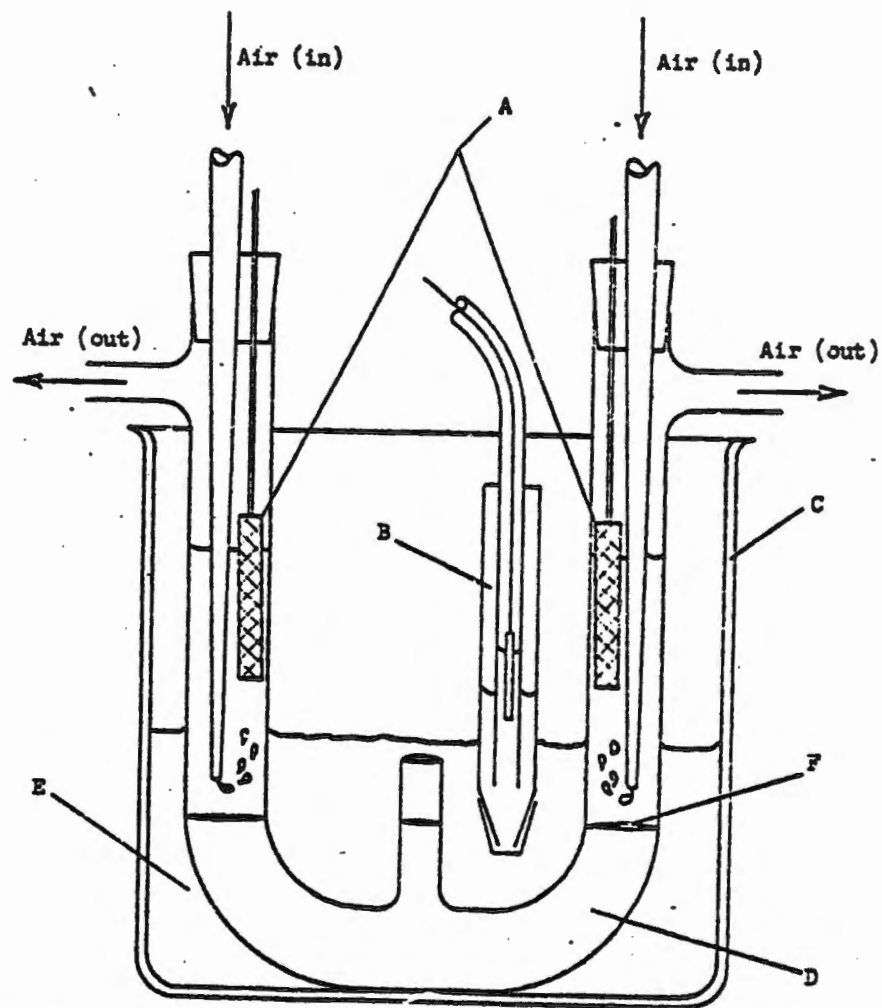
experimentation progresses towards finding the minimum number of minerals necessary for growth of T. thiooxidans utilizing sulfur, the possibility of calculating the actual contribution of each constituent and thereby the specific contribution of T. thiooxidans becomes much greater. The premise that the study of electrochemical behavior of autotrophs, especially Thiobacillus sp., may be simpler than heterotrophs has not been resolved in these investigations. Additional study with both types of species will be needed before a conclusion can be made that one or the other will offer the simpler approach to gaining a better understanding of electrochemical processes that take place during microbiological growth.

Acknowledgment

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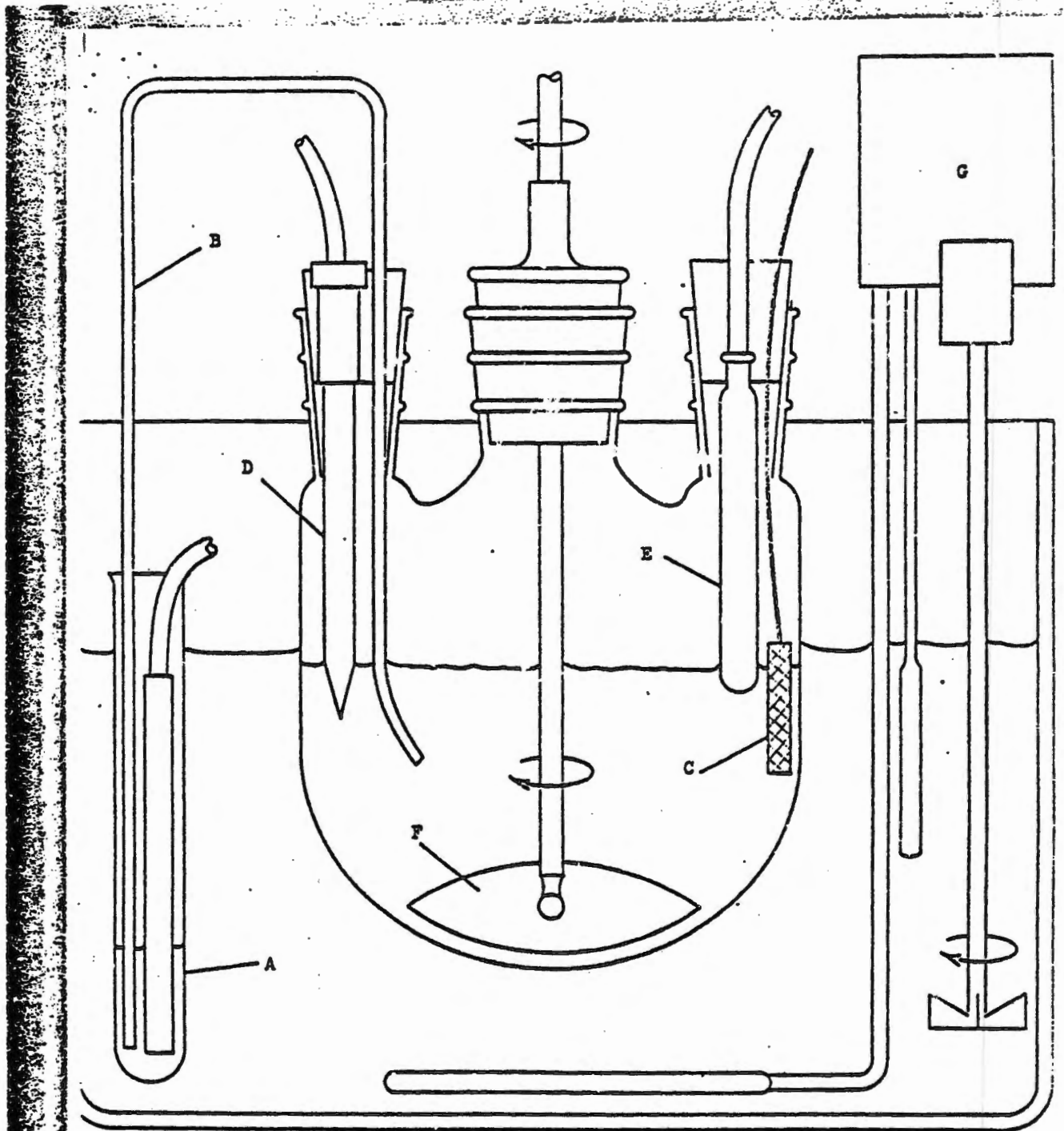
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U-Tube Biocell Key:

- A - Platinum or carbon electrodes
- B - Calomel reference electrode
- C - 1,000 ml beaker
- D - Agar salt bridge
- E - Saturated KCl solution
- F - Bridge - biocell interface

Fig. 1 - Modified U-Tube for Use with Calomel Electrode



Key for Large Biological Half Cell

A - Calomel reference electrode
 B - Agar salt bridge
 C - Platinum electrode
 (biological half cell)

D - Glass electrode (pH meter)
 E - Calomel electrode (pH meter)
 F - Teflon stirrer
 G - Constant temperature apparatus

Fig. 2 - Large Volume Biological Half Cell

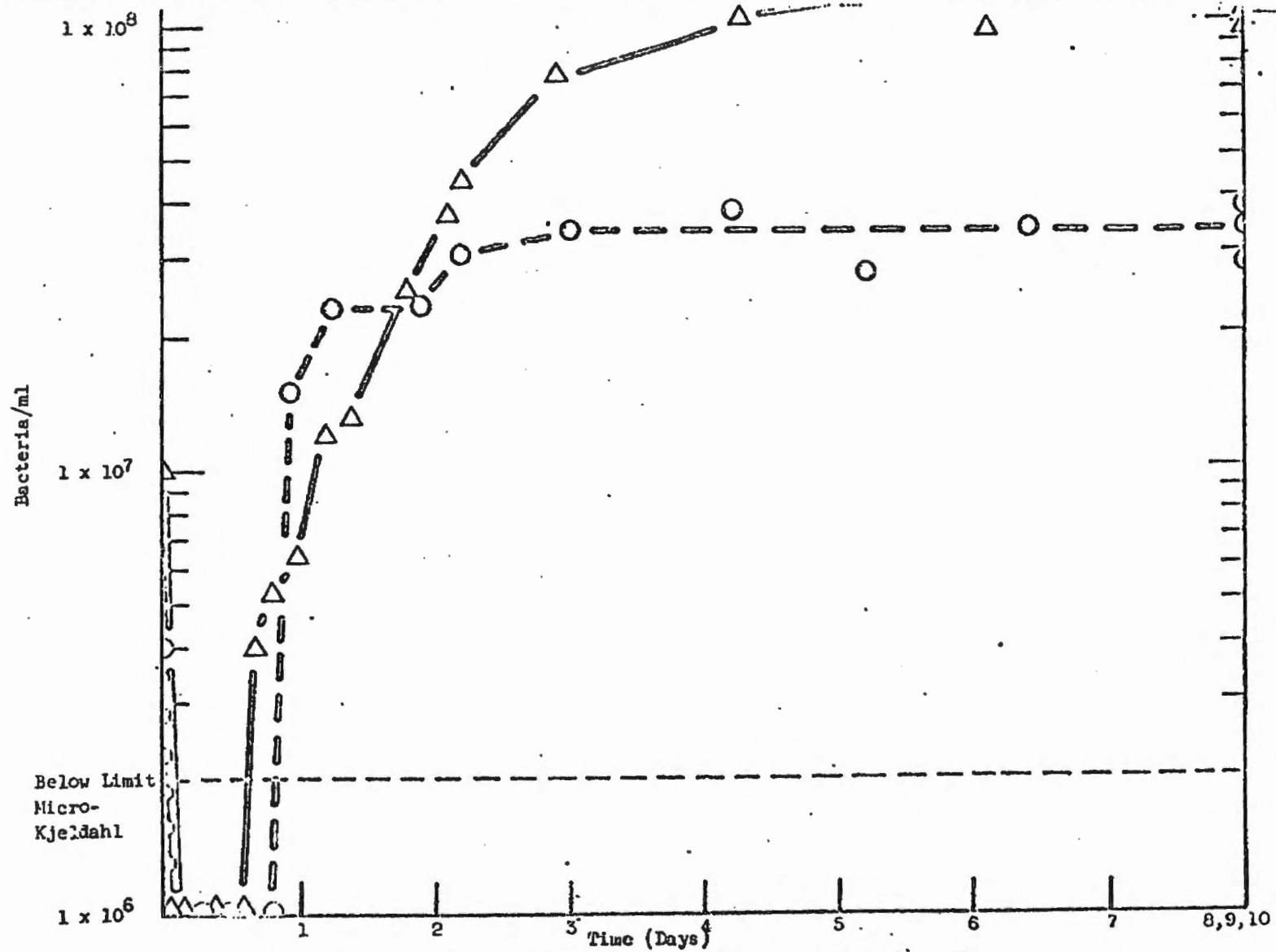


Fig. 3 - Bacterial Growth in A.T.C.C. and Skerunn's Media

○-----○ A.T.C.C.
 △-----△ Skerunn's

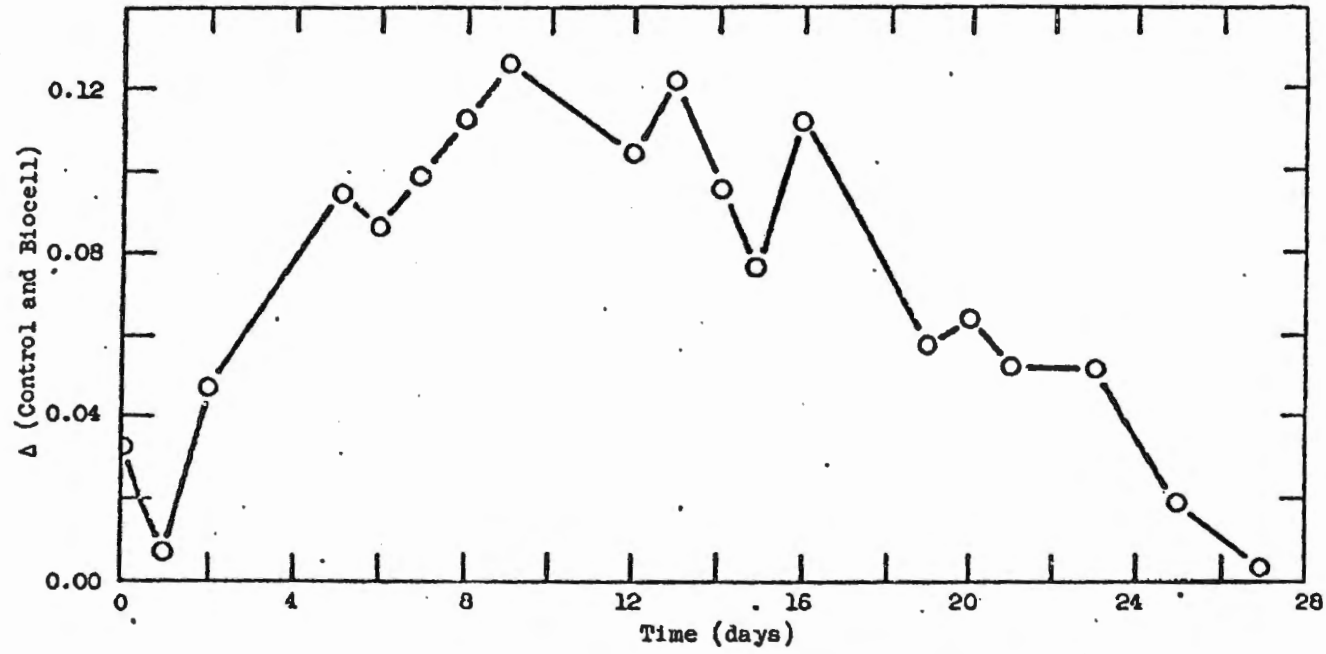


Fig. 4 - Voltage Difference Between Control and Biocell, U-Tubes

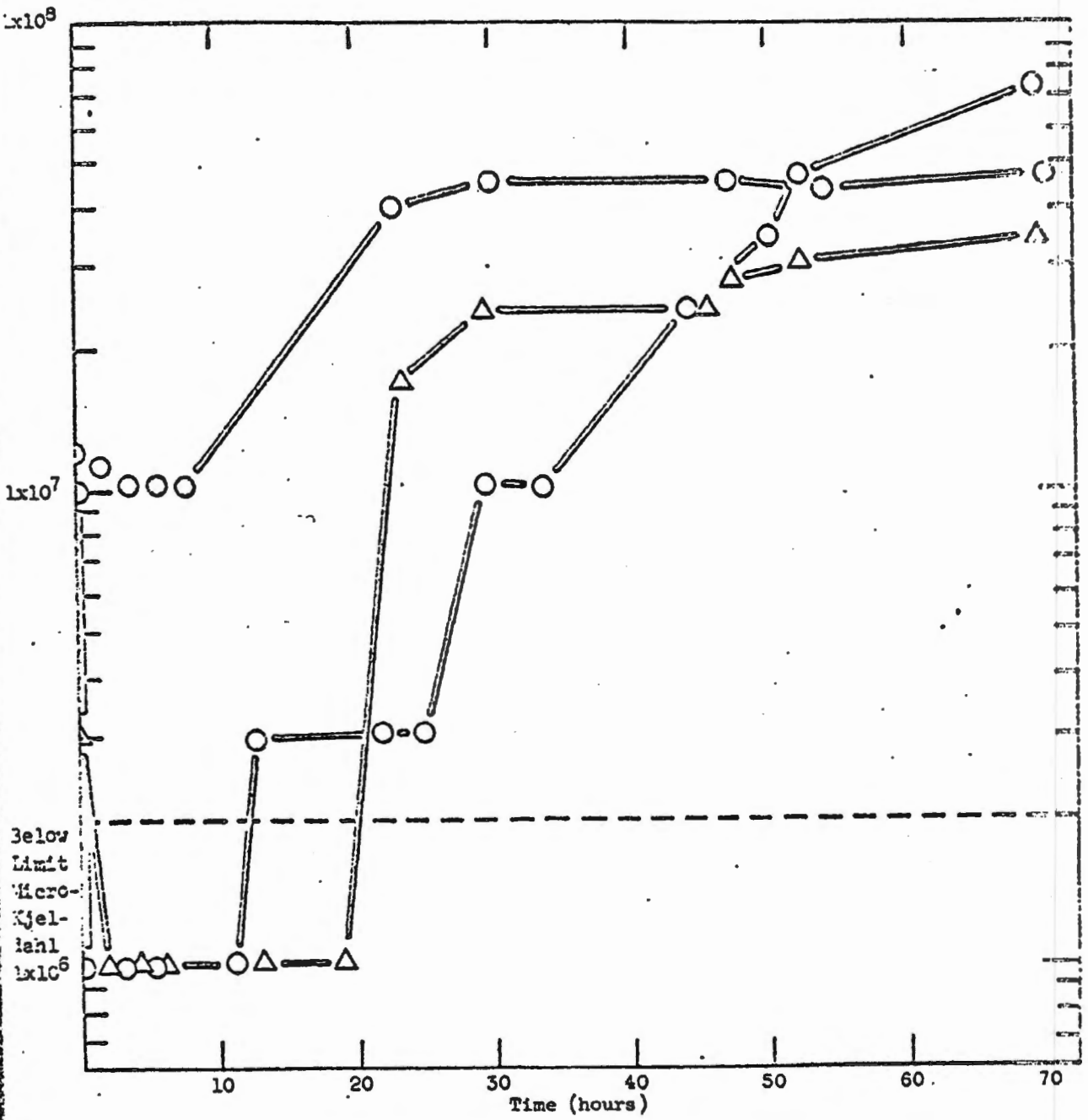


Fig. 5 - Bacterial Growth in Different Media
(*T. thiooxidans* utilizing sulfur)

○ — ○ A.T.C.C. (-)
 △ — △ A.T.C.C.

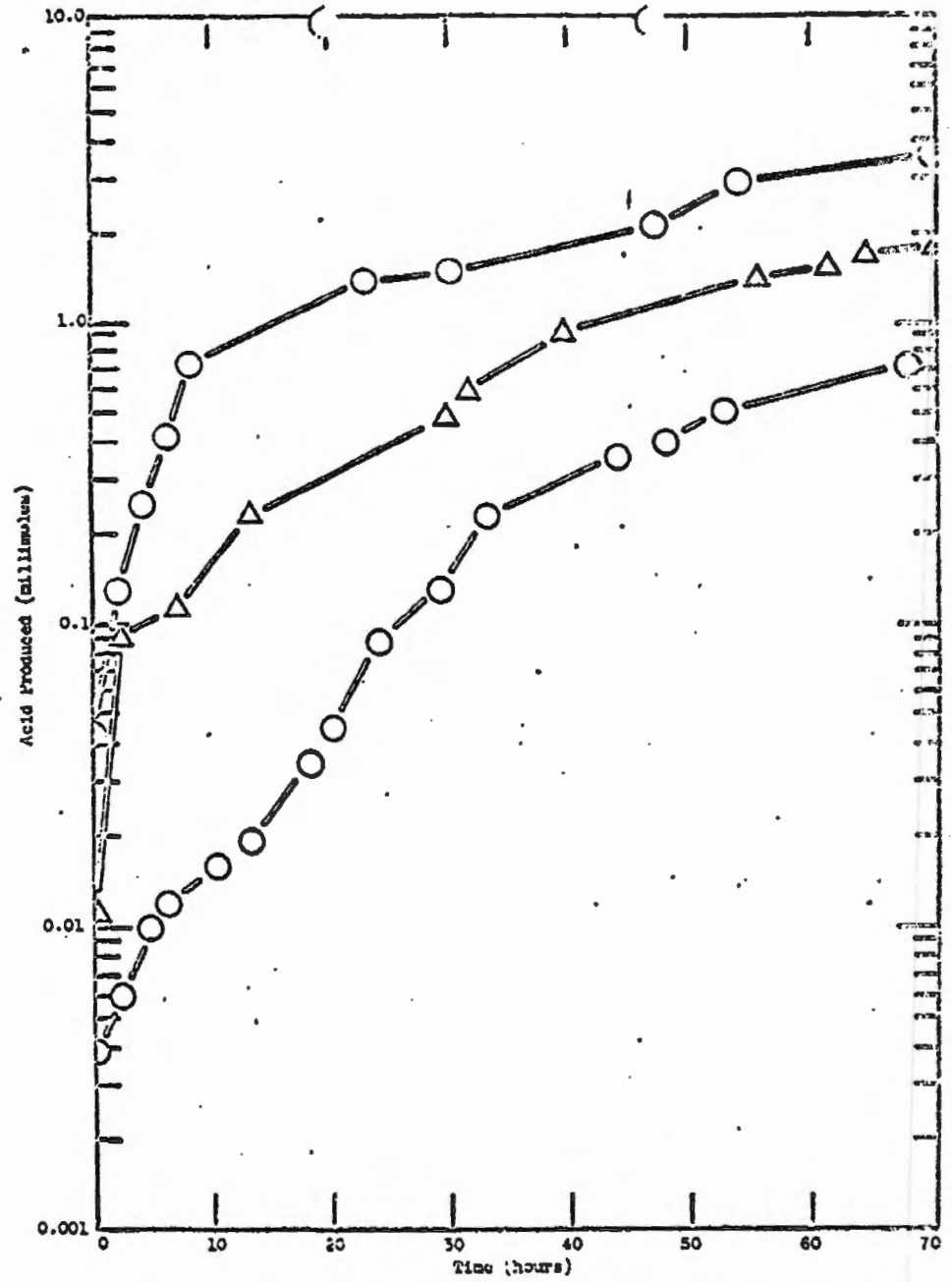


Fig. 6 - Acid Produced in Different Media
(*T. thiooxidans* utilizing sulfur)

○—○ A.T.C.C.(-)
 △—△ A.T.C.C.
 ○—○ Skerman's

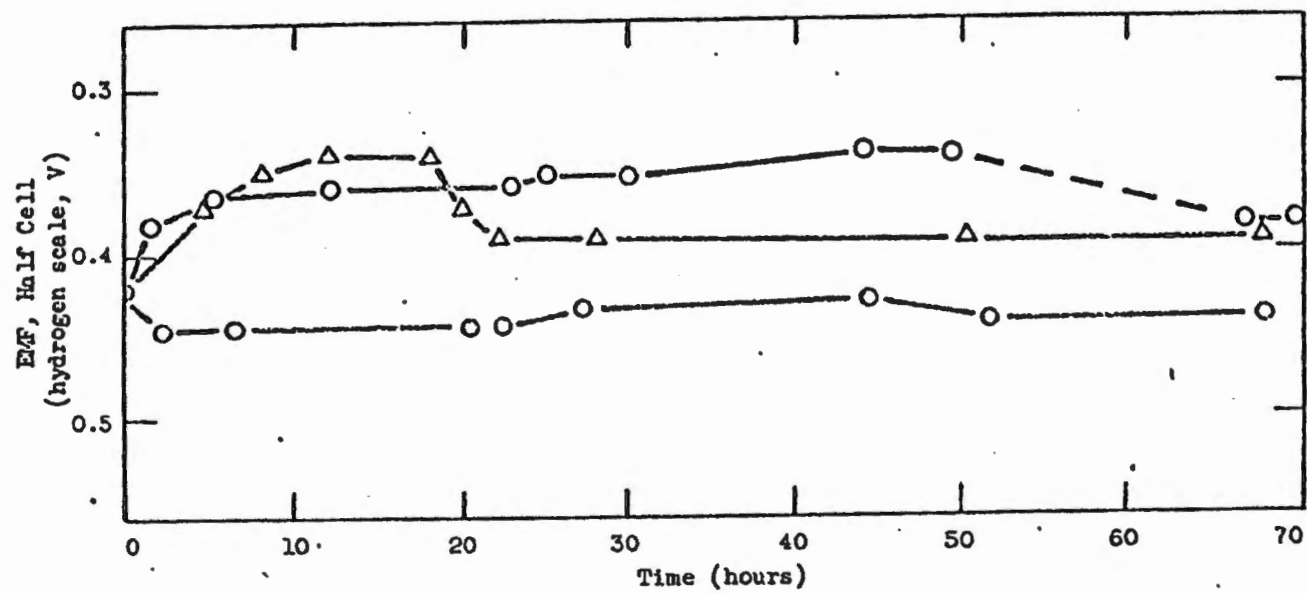


Fig. 7 - Control Half Cells with Different Media
(sulfur suspensions)

○—○ A.T.C.C. (-)
△—△ A.T.C.C.
○—○ Skerman's

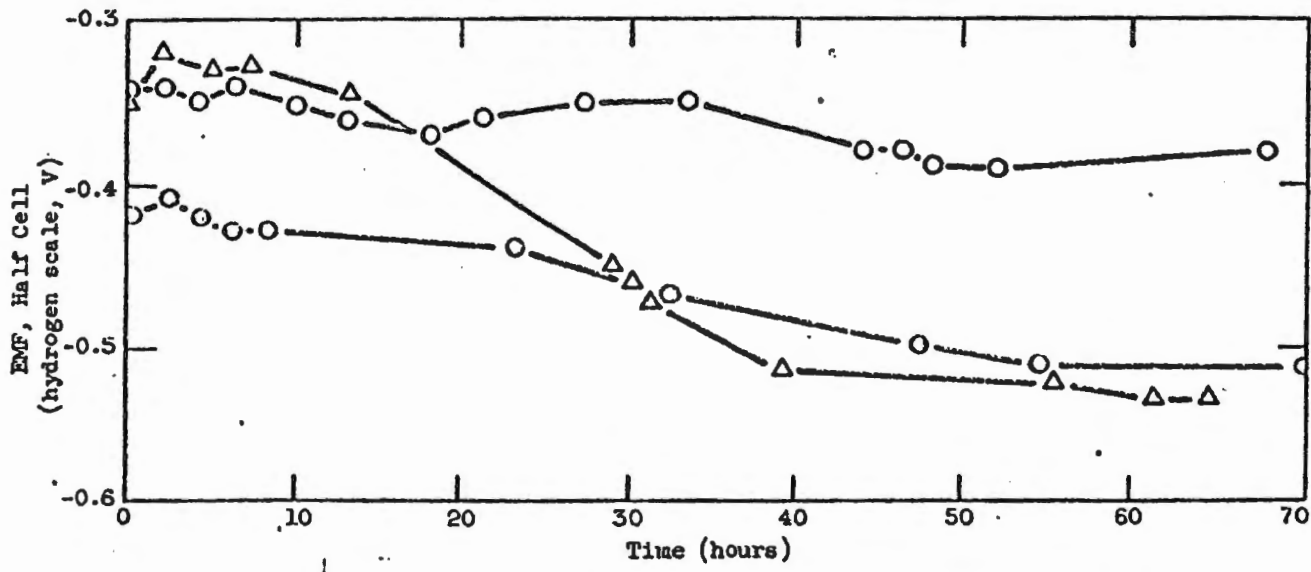


Fig. 8 - Inoculated Half Cells with Different Media
(*T. thiooxidans* utilizing sulfur)

- A.T.C.C. (-)
- △—△ A.T.C.C.
- Skerman's

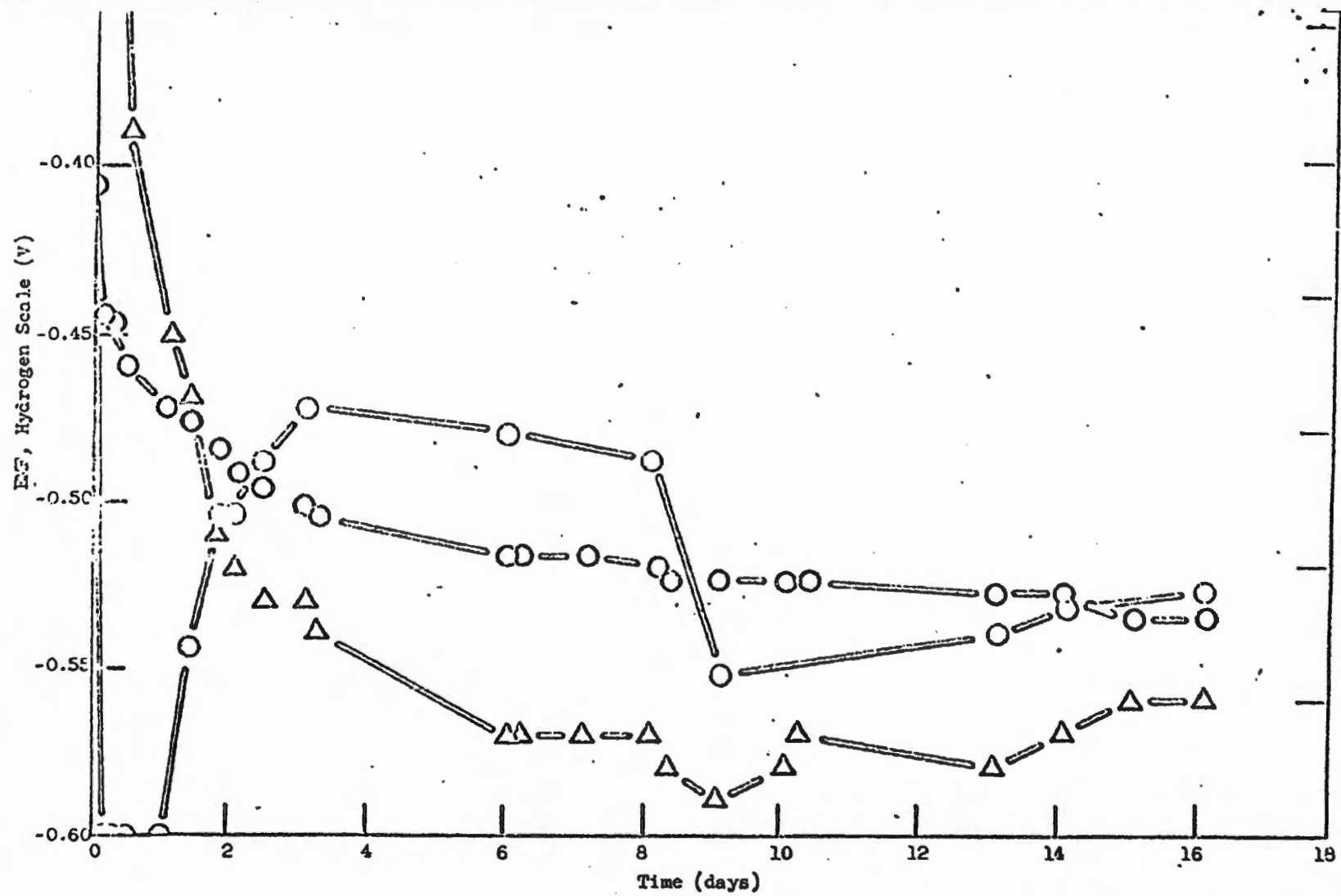


Fig. 9 - Long Term Biological Half Cell Behavior
(T. thiooxidans, Sulfur, A.T.C.C.)
○ — ○ pH
○ — ○ Bacteria/ml

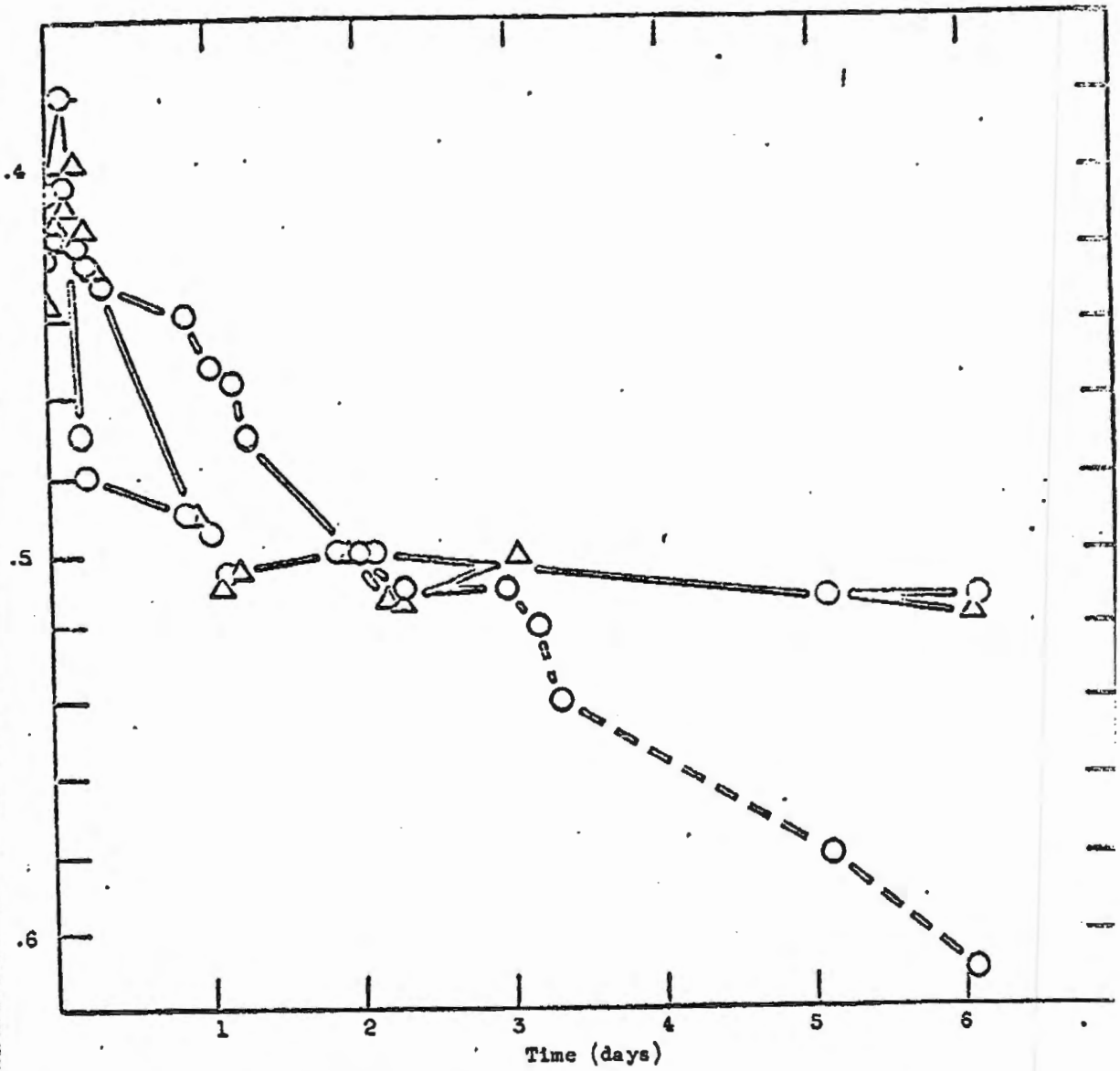


Fig. 10 - Reproducibility of Biological Half Cell, emf
(*T. thiooxidans*, A.T.C.C.(-), sulfur)

○—○ Series XLVI
△—△ Series XLVII
- - - - -

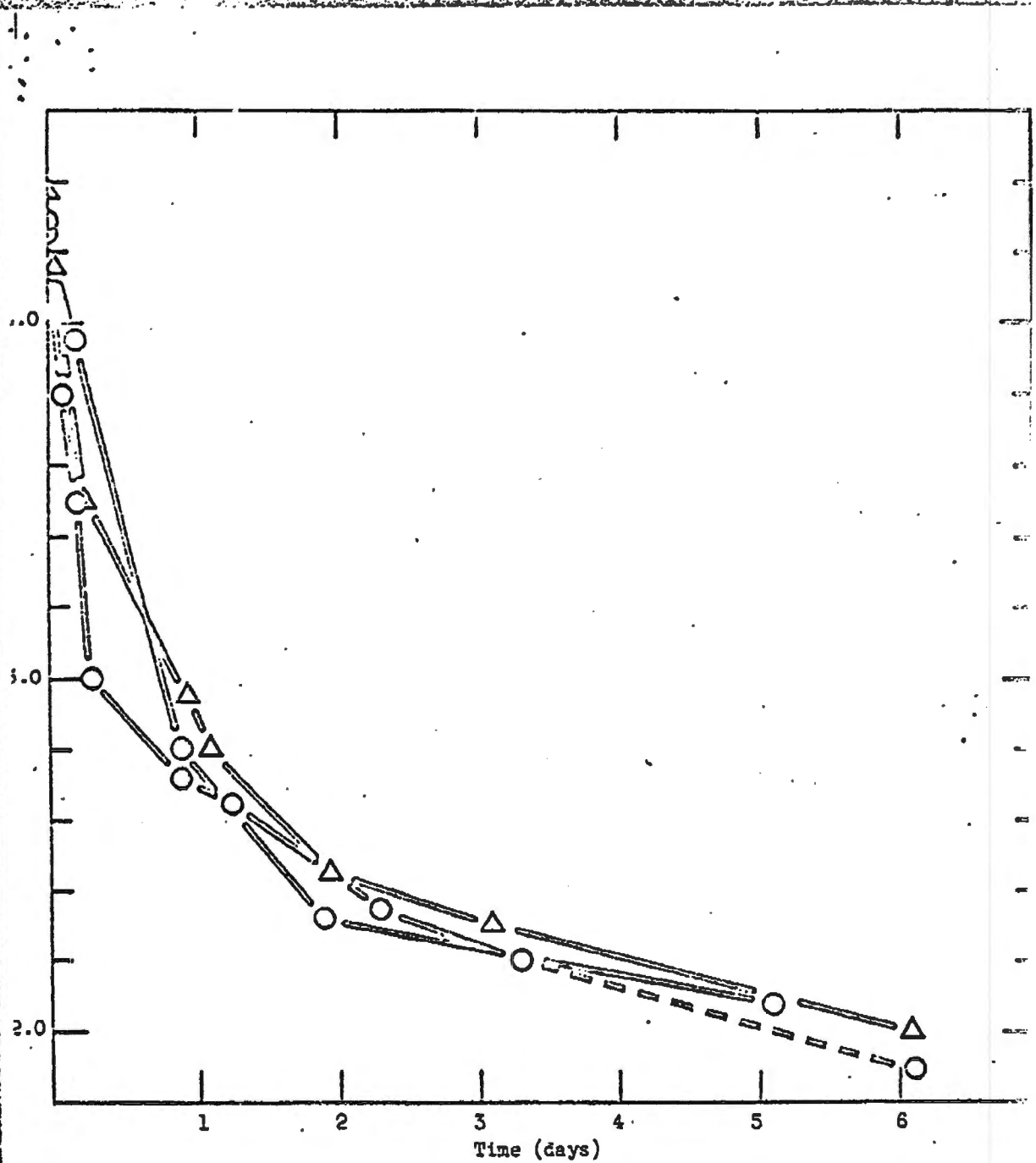


Fig. 11 - Reproducibility of Biological Half Cell, pH
(*T. thiooxidans*, A.T.C.3.(-), sulfur)

○—○ Series XLVI
△—△ Series XLVII
○—○ Series LII

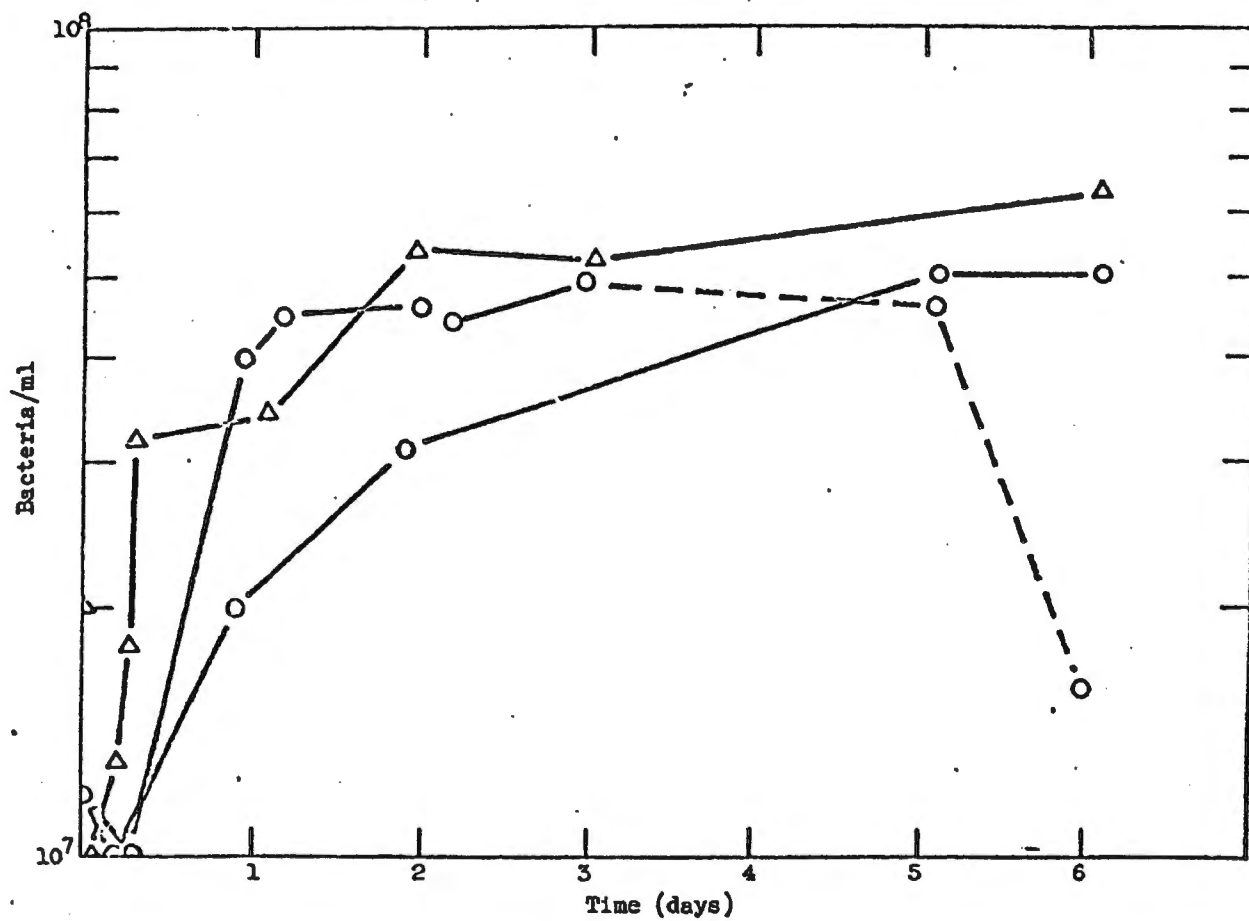


Fig. 12 - Reproducibility of Bacterial Growth in Biological Half Cell
(T. thiooxidans, A.T.C.C.(-), sulfur)

○—○ Series XLVI
 △—△ Series XLVII
 ○—○ Series III