[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE CUDAHY PACKING CO.]

A BACTERIOLOGICAL CONDUCTIVITY CULTURE CELL AND SOME OF ITS APPLICATIONS

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Introduction

It has been shown in previous investigations[1] that putrefactive anaerobes grown in gelatin or other suitable liquid media cause changes in specific conductance which are proportional to the ammonia production. The latter is an end product resulting from the proteolytic action of these bacteria and hence furnishes a convenient index of biochemical activity and growth.

It appeared that the new method of conductance measurement could be made doubly valuable in studies of the growth of certain anaerobes under various environmental conditions if a conductance cell could be made to function also as a growth vessel. After several trials a type of cell was devised which has been in constant use for a period of two years with satisfactory results.

The method developed has distinct advantages over the usual procedures since a number of consecutive observations may be readily made upon a single culture, sealed in glass, thus eliminating all possibility of evaporation, change in anaerobic conditions or chance contamination. The purpose of this paper is to present a description of the cell and to illustrate several applications to definite biochemical problems.

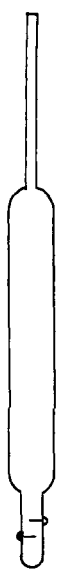


Fig. 1.—The conductivity culture cell.

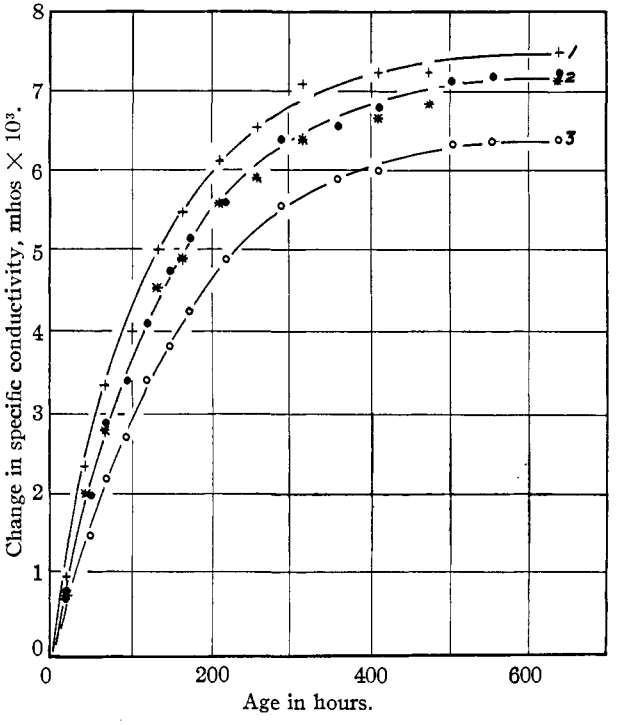
The Conductivity Culture Cell

The cell, Fig. 1, is made of 25-mm. tubing constricted at the lower end to a portion 12 mm. × 40 mm. Electrodes of No. 18 B. and S. gage platinum wire are sealed into the latter so that about 6 mm. of wire projects into the tube. Electrodes are placed 8 to 10 mm. apart. A piece of 8-mm. tubing sealed to the upper portion serves to introduce medium and for inoculation. After inoculation this tube is constricted, evacuated and sealed off. By sealing on 8-mm. tubing, when necessary, the cell can be used indefinitely. The volume of the completed cell is approximately 75 cc. The cell is designed for 5 cc. of culture medium. The large free space is required because most proteolytic organisms evolve considerable quantities of gas. Electrodes must be kept well platinized. Cell constants vary from 1.5 to 2.5. Constants are determined at the end of each run and seldom vary more than 2% from former determinations, showing that repeated sterilization does not affect the cell. It has been found convenient to immerse the cells in 30-mm. tubes containing paraffin oil which are in turn immersed in a water thermostat. The copper lead wires are thus satisfactorily insulated. The latter are arranged to dip into mercury cups making contact with the leads from the bridge.

[1] Parsons and Sturges, *J. Bact.*, **11**, 177 (1926); **12**, 267 (1926).

Reproducibility of Results

In Fig. 2 curves are plotted showing the change in conductivity with time for *C. putrefaciens* in gelatin at 25°. The data were obtained from two separate runs of five cells each. Run 1 is shown by crosses and Run 2 by circles. Curve 1 represents the maximum rate and Curve 3 the minimum rate observed in the 10 individual cells used. The averages of Runs 1 and 2 show practically perfect agreement and are represented by Curve 2.



○, Minimum, series I and II; ●, average, series I; +, maximum, series I and II; *, average, series II.

Fig. 2.—Reproducibility of results. *C. putrefaciens* in 10% gelatin at 25°.

It has been found that in general the average results obtained with any putrefactive organism using 5 cells per run can be satisfactorily checked in subsequent runs under similar conditions.

Effect of P_H on the Biochemical Activity of *C. Sporogenes*

The conductivity culture cell is well adapted to the study of the effect of P_H on the growth and biochemical activity of anaerobes. In Fig. 3 are presented data on the effect of initial P_H upon the activity of *C. sporogenes*, which is the most common putrefactive organism. The medium

was 10% nutrient gelatin adjusted to the required P_H by the addition of acid or alkali. P_H was measured electrometrically on portions of the sterilized inoculated medium. The cells were incubated at 37.5° and conductances measured at the intervals noted. The sharp maxima at the younger ages are especially noticeable. The tendency of the curves to become more symmetrical at older ages is noteworthy. The zones of optimum reaction at 115 and 211 hours are rather wide. This is due to the high ammonium salt accumulation tending to raise the P_H toward the optimum of 8.0 with consequent rapid growth of the organisms. It is evident that the most significant curves are those at 19 and 27 hours.

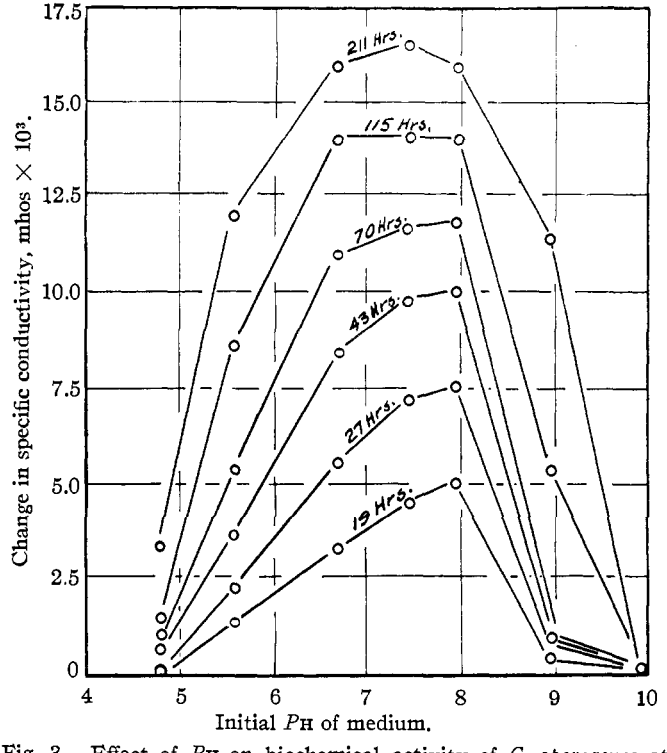


Fig. 3.—Effect of P_H on biochemical activity of *C. sporogenes* at 37.5° in 10% gelatin.

The Effect of Temperature on the Growth and Activity of *C. Putrefaciens*

The exact effect of temperature on the growth and biochemical activity of proteolytic anaerobes is easily investigated by means of the conductivity culture cell. In Fig. 4 curves are presented showing the averages of runs of 5 cells each at 25, 30, 35 and 40° for *C. putrefaciens* in gelatin medium at P_H 7.8.

Since the cells were incubated in separate thermostats and the con-

ductances were measured at the temperatures indicated, it was necessary to refer the data to some standard temperature for comparison. Twenty-five degrees has been selected as the comparison temperature and all observations have been calculated to this temperature, using experimentally determined temperature coefficients.

A distinct lag period always occurs in bacterial cultures before growth starts. If allowance is made for this period, the curves in Fig. 4 follow the monomolecular law very closely, that is, the rate of change of conductance is logarithmic. From large scale logarithmic plots the velocity

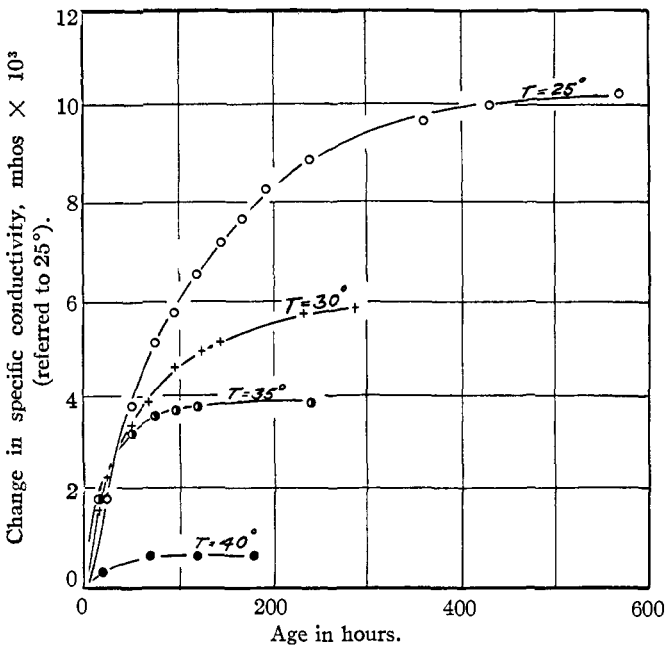


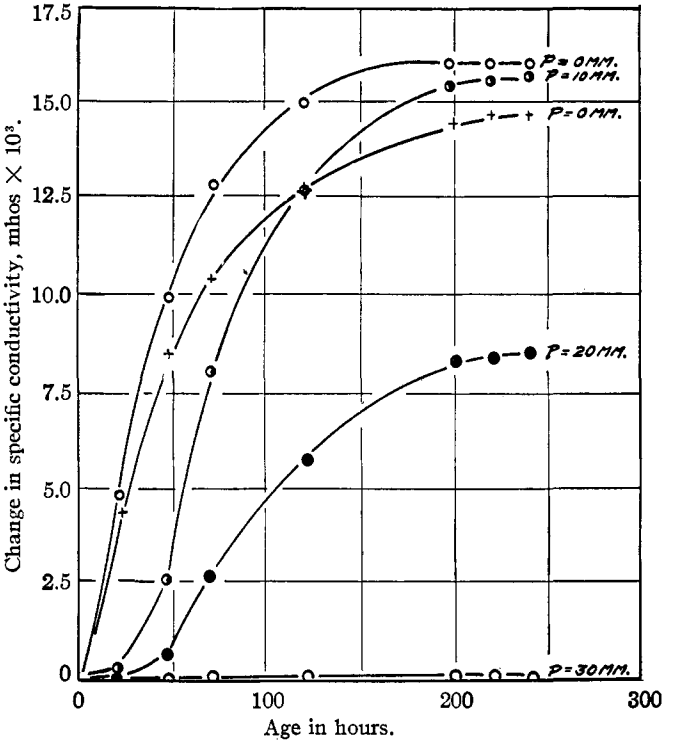
Fig. 4.—Effect of temperature on biochemical activity of *C. putrefaciens* in gelatin.

constants in terms of mhos $\times 10^3$/hours have been calculated to be at 25, 30 and 35°, respectively, 0.0034, 0.0066 and 0.0151. Thus the reaction is more rapid at the higher temperatures, although the quantities of ammonia finally accumulating are much smaller. The velocity is approximately doubled for each 5° increase in temperature, that is, 1.95 for 25–30° and 2.28 from 30–35°.

The Effect of Oxygen Tension on the Growth of *C. Sporogenes* at 37.5°

The conductivity culture cell is especially useful in the study of the effect of oxygen tension on the growth of anaerobes. The data presented in Fig. 5 were obtained on *C. sporogenes* by sealing the culture cells at

suitable air pressures to give the desired oxygen tensions. It was found that shaking was necessary to secure uniform results at the various oxygen concentrations. Presumably shaking insures saturation of the medium at the tension of oxygen present. A modification of the cell was made by sealing the neck at right angles to the cell. A shaker was arranged in the incubator at 37.5° so that about 15 excursions per minute were made. Since the long axis of the cell was in the horizontal direction a



+, Not shaken; all others shaken 15 r.p.m.

Fig. 5.—The effect of oxygen on *C. sporogenes* in 10% gelatin at 37.5°.

thorough exposure of the medium to the oxygen was obtained. Readings of conductance were made at 30°. This involved exposure of the cells to a lower temperature for about half an hour per day. As a check on the effect of shaking, a cell which was not shaken has always been included in the series. In all cases it has been found that greater increases in conductance occur in the shaken cell.

The results are uniform. The lag period becomes more noticeable the higher the oxygen tension, while between 20 and 30 mm. oxygen tension, *C. sporogenes* can no longer grow.

Possible Applications of the Cell

The preliminary results which have been presented show that the cell is peculiarly adapted to the study of such environmental factors as temperature, P_H and oxygen tension on the growth and activity of the proteolytic, ammonia-producing anaerobes. There is no reason why the cell might not be successfully applied to the effects of antiseptics, carbon dioxide tension, antagonistic action of salts or the stimulating effect of extractives on this type of organism.

The cell may possibly be applied to the study of any metabolic process involving the production of conducting substances from slightly or non-conducting substrates, for example, the rate of acid production from carbohydrates. It should also lend itself to investigations of the rate of disappearance of conducting substances as, for example, the conversion of nitrate into nitrogen by denitrifying bacteria.

Summary

A new method of studying biochemical activities of certain organisms involving a combined culture conductivity cell has been developed.

This has been successfully applied to the study of biochemical activities of anaerobes at different P_H's, temperatures and oxygen tensions.

Further possible applications of the new cell have been enumerated.

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THE PREPARATION AND STUDY OF TWO AMMONIUM MOLYBDOTELLURATES

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Considerable work has been done by Denigès[1] on the adaptation of the complex phosphomolybdate to the colorimetric determination of small amounts of phosphorus. His method depended upon the formation of a blue color upon the addition of stannous chloride to an acid solution of the phosphomolybdate. Since Gibbs[2] and Klein[3] have already mentioned the formation of complex molybdotellurites and tungstotellurites and Pechard[4] has isolated complex molybdoselenites, it was thought that a study of the possible combinations between telluric acid and molybdic acid might furnish the basis for a delicate determination of tellurium similar to the one used for small amounts of phosphorus. The following

[1] M. G. Denigès, *Compt. rend.*, **171**, 802 (1920).

[2] Gibbs, *Am. Chem. J.*, **17**, 177 (1895); *Ber.*, **18**, 1089 (1895).

[3] Klein, *Bull. soc. chim.*, **42**, 169 (1884).

[4] Pechard, *Compt. rend.*, **117**, 104 (1893).