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A method of simultaneous registration of dissolved oxygen, Redox potential, hydrogen ion concentration and relative turbidity of bacterial cultures.

by H. Zeidler and U. Taubeneck.

Zentralblatt f. Bakt. 2. Abt. Orig. 109: 516-523 (1956).

The metabolic action within bacterial cultures may be characterized in toto by certain factors such as pH, Redox potential and dissolved oxygen content. Measuring instruments that permit continuous registration of all essential factors throughout the duration of the culture will be of particular value. As a complement to such measurements, the simultaneous registration of relative turbidity is informative, since this allows the assignment of an appropriate developmental phase to the corresponding physico-chemical condition of the culture. In this manner the problem of the effect of inhibitive materials, for instance, or the relation between oxygen concentration and growth intensity, etc., could be investigated with a measure of success.

In the present paper an experiment is described which permits exact simultaneous registration of the factors discussed, and meets the special requirements of microbiological investigation (sterilizability of the apparatus, prevention of foreign infection during the test, etc.)

Results of tests presently being conducted with this apparatus shall be described in future reports.

1. Electro-chemical measurement and registration of dissolved oxygen.

Dissolved oxygen is often determined with a dripping mercurial electrode (Bruns et al. 1954; du Buy and Olson, 1940; Dirscherl and Bergmeyer, 1950; Skerman and Millis, 1943; Wise, 1950). Due to the disturbing effect of mercury, such measurements may be conducted in microbiological cultures only if a sample is removed from the culture vessel and discarded after measurement. It must be noted also in the case of mercurial electrodes that the hydrogen overvoltage in undiluted media containing broth and peptone is shifted to positive values by the protein content. We were able to establish that already at -0.71 V (Tangent potential. Against a saturated Kalomel electrode) a reduction in hydrogen ions takes place under conditions favorable to bacterial growth (37°C; pH 7). It may be shifted to even more positive values, since according to our observations the catalytic activity of the components containing -SH and -SS- groups does not remain constant after inoculation with certain bacteria.

Solid metal electrodes have the advantage in the determination of dissolved oxygen in microbiological cultures of measurement without the removal of samples. For the maintenance of suitable diffusion it is necessary to stir the culture solution evenly or to rotate or vibrate the

electrode.

We used vibrating gold electrodes in our investigations in order to avoid moving the culture by means of agitators. We modified the vibrating electrode described by Oehme and Noack (1955) by installing a rubber cap on the pivot point (Fig. 1) and were thus able to introduce it into the test vessel, thereby meeting the requirement of sterility.

Fig. 1. Vibrating electrode.

K = contact for the electrode.
F = spring with variable tension.
A = anchor in the form of a clamping collar.
SP = exciting coil.
G = rubber cap.
NS = standard grind for insertion in the measuring cell.
E = gold electrode in a glass stem. Hollow space filled with polymerized metacrylate.

Registration requires extremely uniform vibration. The diffusional flow is dependent, among other things, upon the electrode's speed, i.e. the frequency and amplitude of vibration. It is necessary, therefore, to use a stabilized alternating current (50 cycles) for the iron core coil.

In the case of registration in smaller containers, the potential may not be applied to the electrodes constantly over extended periods, since this would cause considerable reduction in oxygen. (At atmospheric saturation, depolarizational currents up to 140 microA are flowing). In order to maintain the oxygen loss due to measurement within reasonable bounds, we have installed a complementary tilt relay in the multi-color point recorder (1) used in the registration, causing the circuit to be completed for a few seconds about once every minute for the purpose of oxygen determination (Fig. 2). Such an intermittent method of measurement presupposes a certain electrode speed (Dirscherl and Otto, 1953). A gold wire with a diameter of 1 mm (electrode surface 42 mm^2) fused into standard glass is used as electrode. For the determination of suitable potential at a given pH value, cell resistance and electrode metal, current potential curves (Damaschke et al., 1955) should be consulted. In our case a potential of -0.75 V was indicated. At more positive potentials the calibration curve (oxygen concentration/current) is not linear.

Fig. 2. Circuit schematic for oxygen registration.

a = storage battery
b = potential distributor
c = tilt relay, steered from the synchronous motor in the drop-stylus recorder.
d = Kalomel electrode
e = vibrating electrode
f = shunt
g = drop-stylus recorder.

A saturated Kalomel electrode with a mercurial surface of about 3 cm^2 is used as anode. The electrolytic connection between anode and cathode space is made through a glass tube containing KCl-agar (6% agar, 6% KCl)(Fig. 3). Such a transfer has several advantages over coherers, clay pins and asbestos fusions in measurements of biological systems. A leak of anode space fluid into the culture solution is positively prevented, without having to consider an appreciable elevation in cell resistance.

The circuit plan of the point recorder in oxygen registration is depicted in Fig. 2. If the recorder's measuring apparatus is sufficiently sensitive, a booster will not be required.

In registration tests according to Toedt's residual current method (Damaschke et al. 1955; Toedt, 1955; Toedt et al. 1952, 1954) we noted that the potential constancy required over the space of several hours is very difficult to maintain with the anode arrangement customary in this method.

Kuester (1955) tries to alleviate this phenomenon, which already exerts a disturbing influence at non-registering oxygen measurements, by immersing the anode metal in agar. Manecke (1955), in his oxygen determinations after Toedt's residual current method, agitates the anode space fluid constantly by the introduction of nitrogen.

Fig. 3. Overall view of the measuring cell.

V = vibrating electrode
G = glass electrode with a symmetrical lead
K = Kalomel electrode
A = connective piece for the Kalomel electrode filled with KCl-agar
R = Redox electrode
S = connection with G4 coherer as stopper and for inoculation.

2. Registration of Redox potential and pH value.

Redox electrodes are relatively easy to polarize (Kordatzki, 1953; Wartenberg and Rummeni, 1952). Metric boosters with a high initial resistance must therefore be used in registrations, so that a practically currentless measurement is assured. The initial resistance of a booster with a mechanical circuit breaker generally is lower than that of one with a direct current booster (Cruse and Fritze, 1955). The former are better suited to registration, however, due to their high zero constancy.

Lange's (1950) acidometer was used by us both as a metric booster for the registration of the Redox potential and the registration of pH, with the measuring instrument of a drop-stylus recorder connected in place of the built - in microampere meter.

In sterile broth media, in Redox potential measurements with a saturated Kalomel electrode as source system, the precious metal electrode may be the positive pole of the chain. After inoculation with certain bacteria (e.g. Proteus vulgaris) it becomes the negative pole. For the shifting of the zero indication on the meter, the incorporation of positively adjustable auxiliary potentials into the metric circuit becomes necessary. In the case of pH registration with glass electrodes, the zero point may be adjusted by changing the internal buffer. The inclusion of an additional, variable potential is advantageous.

In the case of the precision direct current booster after Knick (1953) the counter potential may be derived from a built-in standard element.

Our Redox electrodes consist of platinum wires (2 cm long, 0.5 mm in diameter). They are covered with approximately 2.5 cm of culture solution.

In order to obtain a reliable potential reading, particular stress must be placed on an appropriate pre-treatment of the electrodes. Data thereon are found in Kordatzki (1953), Geller (1940) and Flaig et al. (1955). We treat the platinum electrodes with hot concentrated nitric acid, rinse well and insert them in the test vessel prior to sterilization in the autoclave (45 min. at 130°C). The electrode is thus under water or in the broth medium. A reproducible potential reading in parallel tests, even with electrodes of various sizes, is assured by this pre-treatment.

The question concerning the influence of dissolved oxygen on the measurable Redox potential may be answered if both are registered simultaneously. We found that the potential, measured at pH 7 in the buffered broth nutrients prior to inoculation, is not influenced noticeably by dissolved oxygen. The assumption that the development of negative potential is caused by the removal of dissolved oxygen by the bacteria and that it is indicated after some delay owing to certain electrode properties, cannot be substantiated. We have conducted registration tests with bacterial types in which the dissolved oxygen is used up after inoculation, without evidence of a change in potential.

pH registration was conducted with low-ohm electrodes (Schott and Gen., Jena, Order #9320 and 9340). In order to test the precision of the pH reading of grown cultures, we have examined electrode functions with various buffer solutions before and after weeks of immersion in grown cultures. No changes were observed.

Due to the high buffering capacity of many nutrients (owing to the content of phosphates and various proteins), metabolically induced changes in pH are inconsiderable, especially in sugar-free media with numerous bacterial types. By adjusting the sensitivity so that the width of the point recorder's paper corresponds to a potential change of 12 mV, it is possible to register characteristic pH changes due to metabolic actions in buffered solutions, provided the temperature is constant.

3. Registration of turbidity (2).

The growth curves of different microorganisms may be established by means of scattered light measurements if a proportional correlation exists between Tyndall scattered light and the number of bacteria. Turbidity measurements and registrations of static solutions are significant only if the cultures are overgrown with bacteria that cause uniform turbidness over extended periods of time. This is the case with some types, and a record of turbidity may be evaluated as a supplement to other measurements.

For the purposes of our registration of turbidness, we placed the entire culture vessel with the metric instrumentation into the water chamber of a turbidity attachment (an accessory to the Pulfrich photometer). A well-stabilized potential is used for the production of the primary light. A selenium photo element (20 mm in diameter), attached directly behind the scattered light converging lens, is used as a scattered light receptor. The photo current is transmitted to a self-rectifying photo cell compensator in a Lindeck-Rothe hookup. The electrodes in the culture vessel must be placed in such a way that the turbidity registration is not disturbed optically by them.

Up to a certain turbidity, which we do not achieve in our examinations, linear relations exist between the intensity of scattered light and the density of germs. Rising gas bubbles in gassed cultures cause the intensity of scattered light to fluctuate.

4. Sterilization, inoculation and temperature adjustment.

The metric functions described above were assembled in the manner depicted in Fig. 3.

The vessel, filled with the nutrient, may be sterilized in the autoclave in its totality. Solely the KCl-agar connective piece for the Kalomel electrode and the glass electrode are sterilized separately and installed subsequently.

The glass electrodes lose their electrode properties completely upon sterilization in the autoclave. Sterilization with formaldehyde vapor, as described by Kratz (1950), has proved feasible.

The inoculation of the medium takes place through the connection S (Fig. 3). In order to achieve a uniform distribution of bacteria after inoculation, the medium is briefly gassed with sterile air or sterile nitrogen. For this purpose a gas tube terminating in a coherer is attached to the culture vessel. This arrangement also permits the airing of the culture or, by use of nitrogen or another suitable gas, the removal of dissolved oxygen from the nutrient. The entire apparatus is placed in an incubator adjusted to 37°C, so that all measurements are made at a constant temperature. In order to prevent a temperature elevation by the 30 W primary light bulb during turbidity registration, the turbidity attachment is connected to a thermostat which is adjusted in such a manner

that the immersion fluid in the water chamber invariably remains at the temperature of the incubator.

The described test arrangement allowed the simultaneous registration of dissolved oxygen, hydrogen ion concentration, Redox potential and turbidity in growing cultures. The results of measurements of Proteus vulgaris SG 2 and Micrococcus pyogenes var. aureus SG 511 cultures are reproduced in Fig. 4 and 5. (Without registration of the hydrogen ion concentration. The deviations were less than 0.2 pH).

It should be pointed out at this time that a strong development of negative potential takes place in the case of Proteus vulgaris, while this is not true in connection with Micrococcus pyogenes var. aureus, not even after the consumption of dissolved oxygen. The remaining metric values, on the other hand, are principally equal.

Fig. 4. Results of the registration of a bacterial culture.

Proteus vulgaris SG 2 in buffered broth medium at 37°C. The instant of inoculation is recognizable by the sudden change in relative turbidity.

Stunden = hours.
Luftsättigung = atmospheric saturation.
relative trübung = relative turbidity.
Gelöstsauerstoff = dissolved oxygen.

Fig. 5. Results of the registration of a bacterial culture.

Micrococcus pyogenes var. aureus SG 511 in buffered broth medium at 37°C.

SUMMARY

Simultaneous registration of dissolved oxygen, Redox potential, hydrogen ion concentration and turbidity in growing bacterial cultures was carried out during the entire duration of culture. The metric-technical principles are described in terms of microbiological conditions.

NOTES

(1) Developed by the thermotechnical division of the Institute for applied silicate research, Jena. Sensitivity: 3 microA at full declension.

(2) For very precise and extensive measurements of the turbidity of bacterial cultures our institute has developed, according to the data of H. Knoell, in conjunction with the firm of Carl Zeiss, Jena, a recording light-electric turbidimeter (Leiterer, 1954). A registration of other actions -- parallel to measurements of turbidity -- is not possible with this instrument at this time.