Calibration of Laboratory Aeration Apparatus[1]

R. E. Ecker[2] and W. R. Lockhart

Department of Bacteriology, Iowa State College, Ames, Iowa

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Aeration of some sort is frequently provided during laboratory cultivation of bacteria. Since metabolic responses and the nature of fermentation products may depend upon the amount of available oxygen (Pirt, 1957), it is important to have some adequate measure of this variable. Finn (1954) has pointed out that gassing rates alone are without significance; it is necessary to determine the rate at which oxygen actually goes into solution and becomes available to the cells during growth in a given culture apparatus. Of several methods available for measuring such oxygen absorption rates (OAR), the sulfite oxidation procedure (Cooper *et al.*, 1944) is convenient and requires little equipment.

While reports have been made outlining sulfite procedures for determining oxygen absorption rates in industrial reactors (Cooper *et al.*, 1944) and laboratory shake flasks (Corman *et al.*, 1957), many investigators make use of small volume culture apparatus employing a constant flow air pump or a laboratory compressed air line. Although such work may involve aerating culture volumes as small as 5 or 10 ml, accurate calibration of the apparatus for OAR is still essential.

Materials and Methods

The aeration apparatus described by Lockhart and Ecker (1958) was used in these experiments. The apparatus consists essentially of a controlled source of air under pressure which is led, via a manifold, to a series of needle valve outlets monitored by individual flow-rate meters. Each outlet is then connected to a gas delivery tube immersed in 10 ml of culture medium contained in an optically calibrated 18 by 150 mm culture tube. The metered air is presaturated with water vapor, prior to delivery to the culture tube, by bubbling it through a depth of 50 mm of distilled water. Both culture tubes and water saturation tubes are held in a water bath at a constant temperature.

For determining OAR at various flowmeter settings, 10.0 ml of 0.15 N Na_2SO_3 in distilled water are pipetted into each culture tube. This solution contains 10^{-4} M $CuSO_4$ (necessary as a catalyst in the oxidation of sulfite), and has been adjusted to pH 7.0 with 1 N H_2SO_4. To one such tube are added immediately 10.0 ml of 0.16 N I_2 in 5 per cent aqueous KI. This becomes the blank. The remaining tubes are aerated at measured rates of flow for a given time using fixed conditions of temperature, gas delivery tube diameter and depth of the delivery tube below the surface of the reaction mixture. After aeration, 10.0 ml of the iodine solution are added to each tube and mixed thoroughly to react with any sulfite which remains unoxidized. The difference thus obtained between the unreacted iodine in the blank and that in each of the aerated culture tubes represents the amount of oxygen absorbed by sulfite according to the following relationship:

$$\frac{\text{mEq } I_2 \text{ (difference)}}{4} = \text{mmoles } O_2$$

When the volume of reactant and the time of exposure to the gas are taken into consideration, the oxygen absorption rate can then be determined and expressed as mmoles of oxygen absorbed per L of reactant per hr.

The excess iodine may be determined by standard iodometric methods, titrating to the starch end point or to colorlessness with standardized $Na_2S_2O_3$. This, however, proves to be a cumbersome and time-consuming procedure if a large number of determinations must be made. To use a colorimetric determination of iodine for this purpose, it is necessary that it be possible to detect concentrations in the range 0.005 N to 0.10 N without dilution. Using a Coleman Universal spectrophotometer,[3] standard absorption curves were determined for several iodine concentrations over the visible spectrum. It was found that wave lengths below 650 mμ fail to provide the necessary concentration range and those above 650 mμ do not yield sufficient sensitivity over this range. However, using 650 mμ and a Coleman 14-214 filter, the required concentration range may be detected between 20 and 95 per cent transmittance when read against distilled water.

The standard curve of concentration against optical density at 650 mμ is shown in figure 1. Although this curve is quite reproducible, there is no direct, linear relationship between these quantities over the entire concentration range. The second curve in figure 1 shows

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[2] Predoctoral Research Fellow of the National Science Foundation.

[3] Coleman Instruments, Inc., Maywood, Illinois.

the result of a slight mathematical manipulation which simplifies use of the standard curve. Concentration is plotted against the quantity (log log 100 − log log %T). This quantity has been termed log density (*LD*).[4]

For the determination of OAR, it is necessary only to read the transmittance of the blank and the reaction tubes in a spectrophotometer against distilled water and calculate the OAR by use of the standard curve.

[4] Tables for conversion of per cent transmittance to $LD \times 10^3$ will be furnished by the authors on request.

The following relationships are used in deriving the conversion formula:

$$\text{mmoles } O_2/L/hr = \frac{I_2}{(4)(V_L)(t)}$$

$$= \frac{2(LD - LD_0)(V_m)}{(4)(V_L)(t)(b)}$$

$$= \frac{500(LD - LD_0)}{(b)(t)}$$

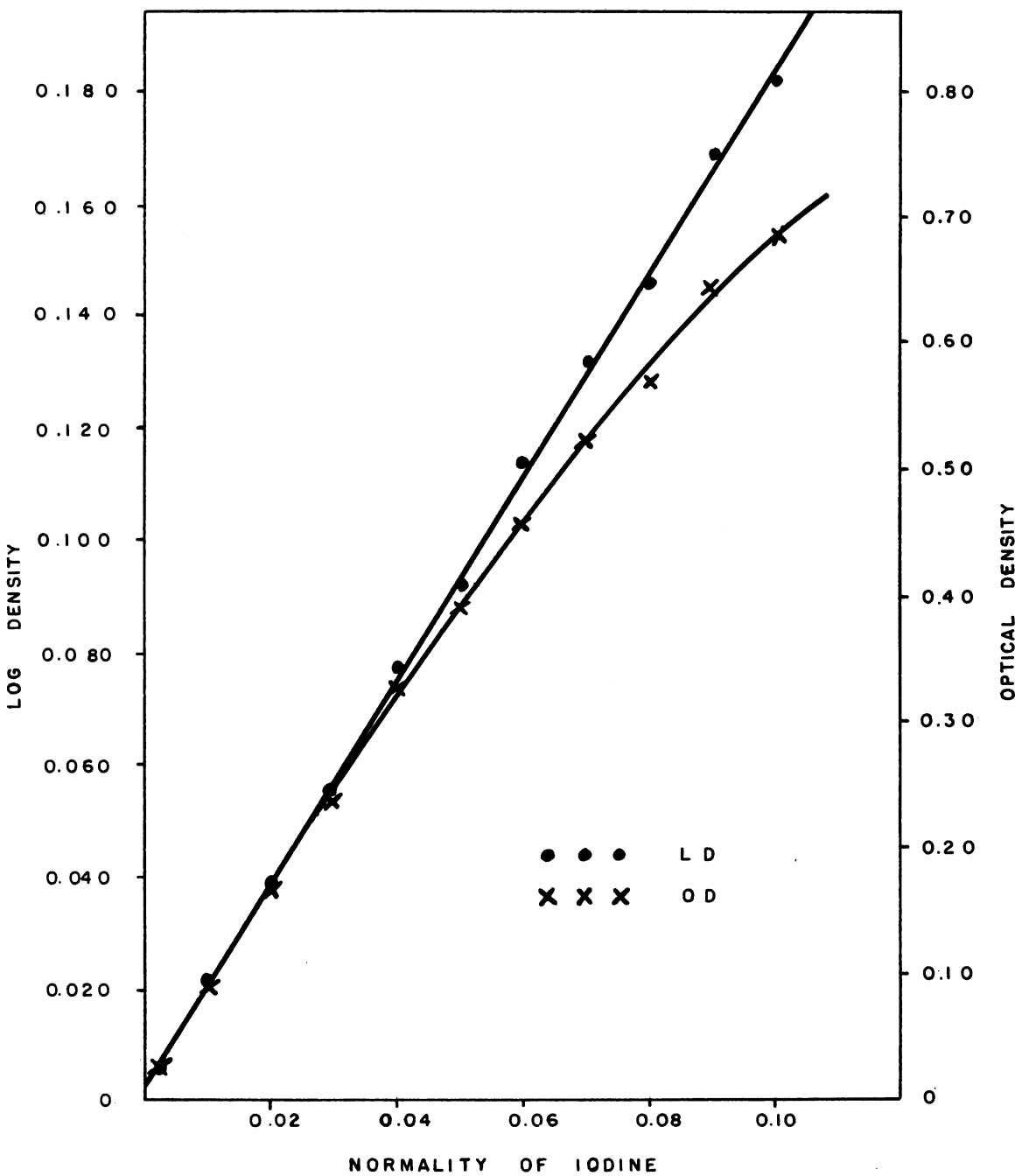

Figure 1. Standard curves for iodine determination in the Coleman Universal spectrophotometer at 650 mμ

where I_2 is the difference in milliequivalents of iodine present between the blank and the reaction tube; t is time in hr of exposure to the gas mixture; V_L is the volume of the reactant in L; V_m is the volume of the reactant in milliliters; b is the slope of the standard curve; LD is the log density of the aerated tube and LD_0 is the log density of the blank. Further simplification can be made in the conversion formula by expressing the optical response as $LD \times 10^3$ and using a time of exposure of one-half hr. In this way the OAR can be determined simply as the quotient of the difference in $LD \times 10^3$ and the slope.

The conversion formula indicates that this relationship is independent of volume of reactant. It is thus possible to use any desired volume in the culture tubes and any size aliquot for determination as long as the volumes of sulfite reagent and of iodine are equal. When larger culture volumes are used, a sample of 5.0 or 10.0 ml can be removed for determination and placed directly into an equal volume of the iodine solution in an optically calibrated tube. No significant error has been detected when pipettes used for this transfer were not first flushed with an inert gas.

There was no absorption of light at 650 mμ by any other constituent or combination of constituents of the reaction mixture.

Results and Discussion

For a comparison of titrimetric and colorimetric methods of iodine determination, oxygen absorption rates were determined for three arbitrary flowmeter settings in five separate experiments. The aeration was conducted according to the procedure previously mentioned, with a volume of 10.0 ml in each culture tube. The air delivery tube was of 2.25 mm inside diameter and was placed so that the bubbles were released 50 mm below the surface of the liquid. The reaction temperature was 37 C and the time of exposure was one-half hr. Each tube was first read in the spectrophotometer and then immediately titrated to colorlessness with standardized $Na_2S_2O_3$.

TABLE 1

Comparison of titrimetric and colorimetric methods of determining oxygen absorption rates

Method	Titrimetric			Colorimetric		
Flowmeter Setting	A	B	C	A	B	C
Experiment	Oxygen absorption rates (mmoles/L/hr)					
I	12.3	14.9	24.6	12.9	16.4	25.1
II	11.3	15.2	23.0	12.1	15.6	22.8
III	11.0	16.1	24.8	11.3	15.9	24.7
IV	11.6	15.7	25.0	12.0	15.8	24.5
V	11.9	15.5	23.8	11.5	15.2	23.1
Mean	11.6	15.5	24.2	12.0	15.8	24.0

Results of the comparison experiments are shown in table 1. In each case the mean value of the colorimetric determinations is within the range of variation of the corresponding titrimetric determinations, and in no case is the range of variation within the colorimetric determinations significantly greater than that of the corresponding titrimetric values. The observed variation in experimentally determined OAR values seems to result from minor fluctuations in rate of gas flow at particular flowmeter settings rather than from errors in the respective analytical methods.

Using this method of aeration, we have detected no equilibration period for sulfite oxidation such as has been reported by Corman *et al.* (1957). Results when aeration was carried out for one-half hr agreed very closely with values obtained over longer periods of exposure.

Since air flow rate alone is a meaningless expression in aeration procedures, we have found it more convenient to express the calibration of aeration apparatus at any given flowmeter setting in terms of a "base rate" of oxygen absorption, specifying the particular conditions under which the calibrations were conducted. In the case of the apparatus used in these experiments, base rates are determined under the conditions used in the conduct of the comparison test. Thus table 1 reflects the base rates for the three flowmeter settings shown.

Employing some fundamental concepts, a number of general predictions can be made concerning oxygen absorption rates under varying conditions when the base rate is known. For example, the change in OAR can generally be considered to be inversely proportional to a change in the volume aerated as long as other factors are held constant. Thus it could be predicted that a culture volume of 25 ml, aerated at a base rate of 15.8 mmoles/L/hr and under conditions similiar to those used in calibration, would yield an OAR of 15.8/2.5 or 6.3 mmoles/L/hr. The experimental value obtained under these conditions is 6.6. However, when the change in volume results in large differences in surface-to-volume ratios or in marked differences in agitation by the gas bubbles, this relationship will not hold.

OAR can also be altered without changing the rate of air flow by increasing the gas-liquid interfacial area by means of a sparger. In this case the sparger can be calibrated using the base rate. For example, 25 ml aerated at a base rate of 15.8 mmoles/L/hr with an extra coarse fritted glass sparger gave an OAR of 31.8 mmoles/L/hr. Since it can be predicted that the actual OAR should be $\frac{2}{5}$ the base rate due to the increase in volume, the additional change can be attributed to the sparger, all other factors constant. Thus the sparger factor can be computed to be (5/2) (31.8/15.8) or 5.0. If 80 ml are aerated with the same sparger at base rates

of 24.0 and 12.0, using the sparger factor, OAR could be predicted to be 5.0(24.0)/8 = 15.0 and 5.0(12.0)/8 = 7.5 mmoles/L/hr, respectively. The respective experimental values for these rates are 17.4 and 6.9. These relationships again hold only if other factors are held relatively constant.

It is to be expected that most investigators will want to make accurate calibrations for such values as can be predicted in this way. However, these methods provide a handy means for estimating the order of magnitude of possible oxygen absorption rates attainable with various types of apparatus when only a limited number of air flow rates are available.

Summary

A method is described which provides a simple and convenient means for accurate calibration of laboratory aeration apparatus. A colorimetric determination of iodine concentration is incorporated to simplify determinations of oxygen absorption rates by the sulfite oxidation method, and comparison of this colorimetric procedure with the standard titrimetric method is made. Use of calibration data is discussed.

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Polymerized Organic Salts of Sulfonic Acids Used as Dispersing Agents in Microbiology

R. E. Marquis and P. Gerhardt

Department of Bacteriology, The University of Michigan, Ann Arbor, Michigan

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Polymerized organic salts of sulfonic acids of the alkyl-aryl type have been used extensively by the chemical industry to obtain highly stable dispersions and emulsions. These lignin compounds are not wetting agents like the "Tweens" but rather dissociate in water to give highly charged anions which, when adsorbed to particles of solids in suspension, impart their negative charge to each particle with resulting mutual repulsion. Brownian movement then keeps these charged particles in suspension for a longer time than usual. Some of these compounds also stabilize water-oil emulsions by electrokinetic action at the interface. They can withstand 175 C, have recommended pH ranges from pH 7.0 to 9.0, and act effectively with particles in the size range of microorganisms.

These dispersing agents, or dispersants, have been shown to be relatively nontoxic for mice (Latven *et al.*, 1952; Latven, 1957). Their use has recently been extended to obtain more uniform direct counts of human red blood cells following dilution (Latven, 1957). These findings suggested possible uses in microbiology, and this study was undertaken to assay their toxicity for some microorganisms, test their dispersing effect in a few of the possible applications, and indicate to microbiologists the existence and potential of this type of compound.

Experimental Methods and Results

Viability of bacteria in dispersant solutions. Representative dispersing agents were supplied by the sources indicated: Darvans 1 and 2,[1] Daxads 11 and 23,[2] and Marasperses C, CB, and N.[3] Viable counts in this and subsequent work were assayed by the surface-plate method; samples for 24-hr counts were stored overnight in the refrigerator. Table 1 shows the results of tests with *Serratia marcescens* and the seven dispersants incorporated in two representative diluents. The data indicate that the dispersants were not toxic to this organism. In distilled water, the presence of a dispersing agent actually increased the plate count; in gelatin-saline solution (8.5 g sodium chloride plus 1 g gelatin in 1 L distilled water), the addition of dispersants resulted in viability, at least as good as that obtained

[1] R. T. Vanderbilt Co., Inc., New York, New York.

[2] Dewey and Almy Chemical Co., Cambridge, Massachusetts.

[3] Marathon Corporation, Rothschild, Wisconsin.