Electrolysis as a Means of Aerating Submerged Cultures of Microorganisms[1]

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The industrial importance of aerobic microbial processes carried out in large, stirred tanks has placed new emphasis on the problems of oxygen transfer from the gas to the liquid phase. Because oxygen is with difficulty soluble in water, the rate of its solution during the course of a fermentation is often the principal factor affecting the yield of cells or cell products. The physical factors which affect the rate solution of oxygen in aqueous systems have been studied thoroughly and have been reviewed by Finn (1954). In general, it appears that high rates of oxygen transfer are attained by creating large interfacial areas between the gas and liquid phases. With modern agitation-aeration equipment, this is usually achieved by sparging or agitation or a combination of the two techniques. The methods used to create a large interfacial area between gas and liquid can be compared to methods which are used to prepare colloids; these can be broadly classified as either dispersion or condensation methods. All of the conventional aeration practices correspond to the dispersion techniques in which large volumes of air are mechanically broken up or dispersed to form small bubbles.

A radically different method for generating fine oxygen bubbles involves the electrolytic cleavage of water into molecular oxygen and the subsequent aggregation or "condensation" of the molecules. The advantages which could accrue from the use of electrolysis as an aeration technique prompted the present investigation. A major goal was to find out if electrolysis would satisfy the oxygen demand of a submerged culture of aerobic microorganisms; the criterion of success selected was growth equal to that of aerated control cultures.

Materials and Methods

The organism used in this research was a strain of *Pseudomonas fluorescens* obtained from a culture collection of the Department of Bacteriology, University of Illinois. It was selected because it is an obligate aerobe, and therefore its growth response serves as a measure of oxygen availability.

For the growth of the organism under electrolysis a glucose-mineral salts medium was used, adjusted to pH 7.0 and containing, in per cent: $(NH_4)_2SO_4$, 0.3; glucose, 0.3; sodium hexametaphosphate, 0.3; yeast extract, 0.2; $MgSO_4$, 0.1; $FeSO_4$, 0.0005; Na_2SO_4, 0.5; and distilled water.

This medium was originally prepared with orthophosphate, but the high local concentration of hydroxyl ions generated at the cathode brought about the precipitation of $Mg(NH_4)PO_4$. This precipitate interfered with the turbidimetric determination of cell concentration; and it also deposited on the electrodes, thereby altering their surface properties. The presence of sodium hexametaphosphate in the medium prevented phosphate precipitation by sequestering the Mg^{++} ions. Sodium sulfate was added to increase the concentration of electrolytes and thereby to reduce the electrical resistance of the fermentation medium.

Fermentation Apparatus

The electrolysis cell. The vessel used for these growth studies was constructed from a 1-quart wide-mouth fruit jar, 3.7 in in diam and 7.3 in high. A screwed-on ring closure retained a brass cover, $\frac{1}{16}$ of an in thick, which was fitted with 4 ports. Through these, electrical and gas connections were made and the fermentor was stirred. Two strips of $\frac{1}{16}$-in Lucite sheet, $\frac{1}{2}$ in wide by $4\frac{1}{2}$ in long, were inserted in the vessel and held perpendicularly to the walls by small Lucite rods extending across the diameter of the jar. These strips provided full baffling, as based on the work of Mack and Kroll (1948), and not only eliminated vortexing but also provided maximum agitation for a given power input.

The contents, which were about 700 ml in most experiments, were stirred with a 1-in glass propeller (down draft) located "off center." The propeller was powered by a series-wound laboratory stirring motor and its speed could be varied over wide ranges by the use of a 1-Kw variable transformer. Agitator speeds were measured to within 1 per cent accuracy by the use of a stroboscope.[4]

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[4] Strobotac Type 631-B, General Radio Co., Cambridge, Massachusetts.

All electrolysis experiments were conducted at 30 C in a temperature-regulated water bath. The potential of the direct current applied to the electrodes was controlled by a voltage divider; a multirange voltmeter and a milliameter were used to measure the applied voltage and the current respectively.

Electrodes. The use of electrolysis to supply the oxygen for microorganisms growing in submerged culture introduces a problem in the choice of anodic materials. Iron and nickel are passive under alkaline conditions; but during the electrolytic evolution of oxygen, the area surrounding the anode becomes acidic and there is dissolution of the metal. It was found that graphite anodes cannot be employed because carbon monoxide is evolved during electrolysis in amounts sufficient to inhibit the growth of the organism. Lead dioxide possesses the required passivity and is an electrical conductor by virtue of its imperfect crystal lattice. The presence of chloride ions in the electrolyzed medium, however, might have an undesirable effect on anodes composed of this material. To insure that no extraneous electrode reactions occurred, platinum anodes were used in this research.

Preliminary experiments were conducted using 2 electrodes of shiny platinum foil. These were 2.7 cm wide and 4.3 cm long and were sealed into glass tubes through which the electrical connections were made. The electrodes were mounted parallel to each other and 0.5 cm apart. The glass tubes protruded through a 2-holed stopper which in turn was fitted into one of the ports in the cover. The electrode assembly was modified later so that the platinum electrodes were mounted between 3 graphite cathodes or, alternatively, between 3 iron cathodes; the anode-cathode spacing was maintained at 0.5 cm, however.

Analytical Procedures

Determination of cell concentration. Cell concentrations were measured turbidimetrically either in a Coleman Model 9 Nepho-Colorimeter at 655 mμ or in a Klett-Summerson Photoelectric Colorimeter using a #64 filter. Both instruments had been calibrated by correlation of the optical absorbance of washed suspensions of *P. fluorescens* with the corresponding dry weight of cells. Cell concentrations in grams dry weight of cells per liter could be expressed by the following equations:

$$c_{cell} = \frac{\text{Klett reading}}{225} \text{ (to Klett values of 200), and} \quad (1)$$

$$c_{cell} = \frac{\text{Absorbance}}{1.56} \text{ (to absorbance values of 0.80).} \quad (2)$$

Polarography. Polarography was employed in the present research to determine rates of oxygen uptake by microorganisms and also to measure the concentration of dissolved oxygen and hydrogen peroxide.

The polarographic determination of oxygen is based on its reduction at a mercury cathode, with the formation of hydrogen peroxide and hydroxyl ions. The oxygen determinations were made by the single-potential method of Lewis and McKenzie (1947) using an Elecdropode polarographic analyzer[5] (manually operated) and a dropping-mercury electrode. The instrument was calibrated using sodium chloride solutions for which Seidell (1940) had reported the concentrations of oxygen in equilibrium with air. The H-cell type of polarographic cell described by Lingane and Laitinen (1939) was employed because of the advantages inherent in the "built-in" reference electrode and in the elimination of a mercury pool as anode. At −0.5 volts vs. SCE (saturated calomel electrode), the oxygen concentration was directly proportional to the measured diffusion current.

Oxygen uptake rates of bacterial suspensions were obtained by observing the time course of oxygen depletion in the suspension. The premise was made that the respiratory activity of the cells constituted the only oxygen demand. Samples were withdrawn from the cultures, placed in the polarographic cell, and aerated. Then aeration was stopped and readings of the diffusion current were taken at intervals after turbulence in the sample arm of the H-cell had subsided. From these readings, uptake rates were calculated.

The oxygen concentrations in the bacterial cultures were determined by a modification of the above technique. As a sample was withdrawn from the culture the time was noted and the concentration of oxygen in solution was determined at 5-sec intervals; extrapolation to zero time yielded the initial concentration. The manipulations and the instrument lag for this procedure occupied at least 30 sec. In many cultures the demand was so great that the dissolved oxygen present at the time of sampling was almost depleted in 30 sec. In these cases, only one determination of the oxygen concentration could be made, and the rate of depletion was therefore established after reaerating the culture. The resulting curve, when extrapolated to zero time, gave the initial oxygen concentration in the culture.

Hydrogen peroxide can be prepared by the electrolytic reduction of oxygen. Because peroxide is known to be toxic to some microorganisms, it was necessary to consider the possibility of its formation in the electrolysis of bacterial cultures. Hydrogen peroxide was determined polarographically by the method outlined by Aussen *et al.* (1950).

Determination of hypochlorite. The concentration of hypochlorite ion in the uninoculated medium was determined spectrophotometrically in a Cary Model 11 automatic spectrophotometer. The ion absorbs at 290 mμ and has a molar extinction coefficient of 310.

[5] Fisher Scientific Co., Pittsburgh, Pennsylvania.

Experiments and Discussion of Results

Preliminary Electrolytic Growth Experiments

Experiments in which the bacterial cells utilized oxygen from electrolysis were performed in the following manner:

A culture of *P. fluorescens* whose total volume was 1 L was grown in shake flasks to approximately 0.2 g dry weight per L; 700 ml were introduced into the fermentor, and the remaining 300 ml were maintained as an aerated control in a shake flask. The contents of the electrolysis vessel were stirred at 1100 rpm, and nitrogen was passed continuously into the gas space above the culture to eliminate atmospheric oxygen.

In early experiments, the electric current (which in turn fixed the rate of oxygen generation) was held constant throughout each run. Oxygen uptake rates and cell densities were observed every 15 min in electrolyzed cultures and every 30 min in aerated control cultures. Figure 1 summarizes the results of preliminary growth experiments. Initial growth rates were equal to those of control cultures for currents up to 300 ma, but after a time growth proceeded at a slower rate. The onset of such "growth inhibition" occurred at various population levels depending on the applied current (see figure 1). At the highest current density (430 ma total current), the organism grew at approximately one-half the growth rate of control cultures for a short period, and then growth was completely inhibited.

The inhibition which occurred at low current values (100 and 200 ma) was probably due to insufficient oxygen since an increase in the current produced an increase in the cell population. At the high current

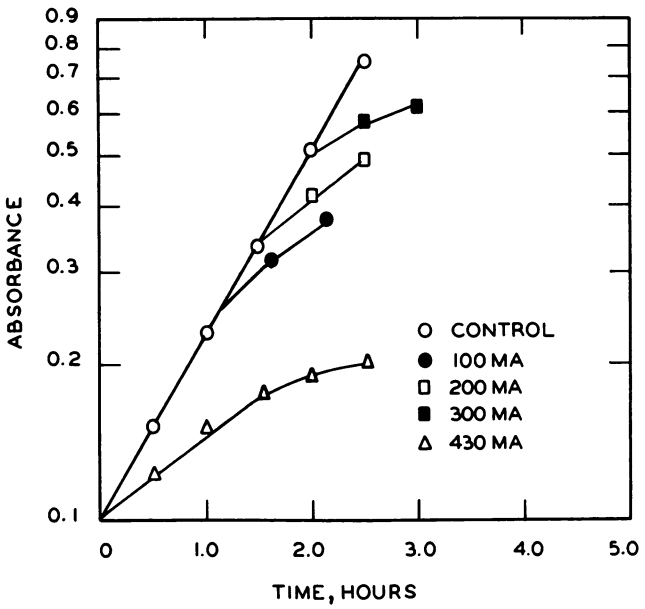

Fig. 1. The growth response of cultures of *Pseudomonas fluorescens* which had been electrolyzed at various current levels.

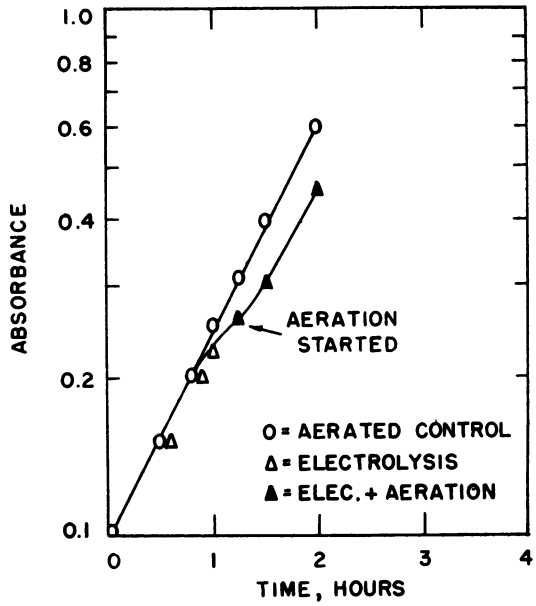

Fig. 2. The growth response of a culture of *Pseudomonas fluorescens* which had been electrolyzed at 200 ma. After inhibition had been established, the culture was aerated as well as electrolyzed, and the normal growth rate was resumed.

level of 430 ma, however, inhibition was attributed to an electrochemical discharge of some toxic material; this could easily occur because a relatively high voltage was needed to achieve the highest current employed (5.3 v). Both hypotheses were tested.

Test for Limiting Oxygen

Electrolytic growth experiments were performed using currents of 200 ma. After growth inhibition had been well established in electrolyzed cultures, the cultures were aerated as well as electrolyzed. If the inhibition at low levels of current was due solely to an oxygen deficiency, the cultures could be expected to grow normally when oxygen was supplied by aeration plus electrolysis. This was the case, and the results of one such experiment are shown in figure 2.

Determination of the Presence of Toxic Materials

Hydrogen peroxide could not be detected in uninoculated growth medium which had been electrolyzed at 5.3 v for 2 hr. The polarographic technique which was previously described could detect hydrogen peroxide concentrations of less than 5 ppm; therefore it was concluded that, in the electrolysis, the rate of formation of this material was essentially zero.

In the same experiment, aliquots of the medium were taken, and the presence of hypochlorite ion was established spectrophotometrically. Subsequent studies showed that dosages of hypochlorite as low as 0.03 mg. OCl^- per L per min had a deleterious effect on the growth of *P. fluorescens*. Hypochlorite poisoning could not be prevented by the incorporation of thiosulfate into the growth medium.

In view of the above results, it was mandatory to prevent the discharge of chlorine if the electrolytic process were to be successful. This could be accomplished in either of two ways: (a) by reducing the concentration of chloride ions in the medium,[6] or (b) by increasing the electrode surface so as to decrease the current density for a given total current. The latter change would decrease the polarization potential of the system, that is, it would permit the discharge of sufficient oxygen for growth at a voltage so low that hypochlorite would not be formed.

It was not feasible to try to lower the chloride content of the medium since the latter contained only 0.018 per cent (180 ppm chloride). Yeast extract contributed the major portion of the chloride, and it was felt that all common laboratory and commercial mediums would contain at least this much chloride. Therefore, the electrode assembly was modified. Two platinum anodes were mounted in parallel between 3 graphite cathodes resulting in a total anode surface of 46.4 cm^2 or 0.066 cm^2 per ml of electrolyzed medium. With these electrodes, discharge of chlorine could not be detected below 5.3 v.

Oxygen Uptake Rates of Pseudomonas fluorescens

In order to avoid oxygen poisoning of the test organism and yet satisfy its metabolic needs, the level of the electrolysis current had to be based on the oxygen demand of the culture during the course of its growth. It was therefore necessary to study the oxygen requirements of cultures of *P. fluorescens* as a function of cell concentration and age.

A liter of growth medium was inoculated with 40 ml of a culture of *P. fluorescens* which was growing exponentially. Aliquots of the inoculated medium (25 ml) were distributed aseptically into sterile 250-ml Erlenmeyer flasks and shaken at 30 C. At half-hour intervals, turbidimetric determinations were made of cell concentrations, and oxygen uptake rates were measured polarographically. Figure 3 shows the course of growth, the specific rate of oxygen uptake, and the total oxygen demand of the culture. Specific oxygen uptake was based on 1 g of dry cells.

The growth response of *P. fluorescens* on glucose-mineral medium typifies that which is obtained when aerobes are grown with usual laboratory aeration procedures. Only 40 per cent of the total cell crop had been attained at the end of the exponential growth phase. The maximum total oxygen demand of the culture occurred a short time later and was followed by a rapid decrease in this demand. An oxygen deficiency in the bulk of the culture could have led to an impairment of the cells' metabolic system which resulted in the lowered oxygen demand. Such a possibility is supported

[6] An increase in discharge potential of 59 mv occurs for each 10-fold decrease in concentration.

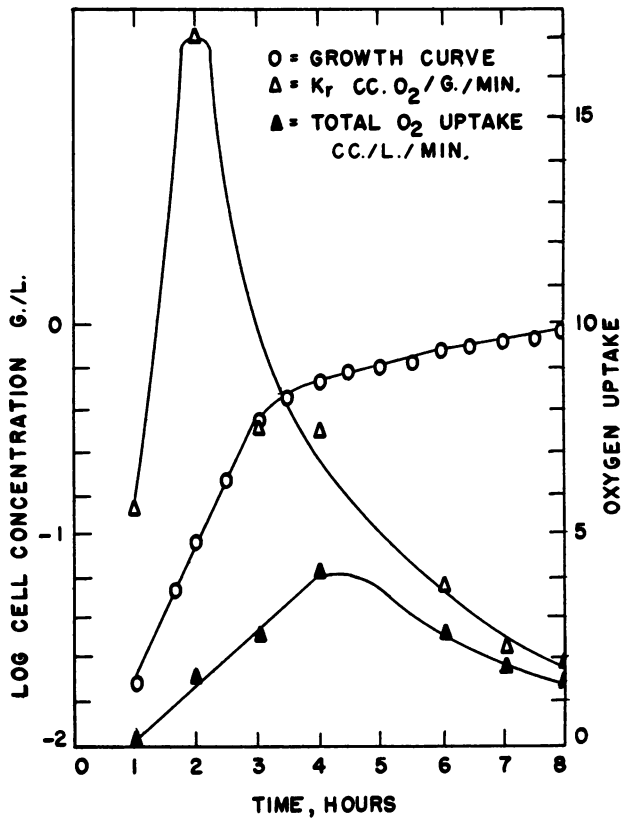

FIG. 3. The growth response of a shake culture of *Pseudomonas fluorescens* on glucose-mineral medium. Data for the oxygen uptake per unit weight of cells per unit time and total oxygen demand are also presented.

by the work of Rahn and Richardson (1942), who showed that moderately rapid death occurs when cultures of *P. fluorescens* containing 10^8 cells per ml or more are subjected to oxygen starvation.

The rate of oxygen uptake per unit weight of cells decreases rapidly from its maximum value in the exponential phase. The data are therefore in accord with the findings of Martin (1932), Clifton (1937), and Hershey and Bronfenbrenner (1938), all of whom presented data on the oxygen uptake per cell.

In the interval of growth chosen for the electrolytic studies, the bacterial concentration increased from 0.15 to approximately 0.80 g of cells (dry weight) per L. It is in this period that the total oxygen demand of shake-flask cultures reached a maximum, and therefore it is in this period that the most rigorous test could be made of the effectiveness of electrolysis.

Electrolytic Growth Experiments

In electrolytic growth experiments, the electrolysis current was increased as the oxygen demand of the culture increased. The average rate of oxygen uptake at the beginning of an electrolytic run was 0.75 ml oxygen per L per min for cultures containing 0.2 g dry weight cells per L. When cell concentrations of approximately 1.0 g dry weight cells per L had been attained,

2½ hr later, the average rate was 3.5 ml oxygen per L per min. The electrolytic growth experiments were therefore initiated at currents of 150 ma, a value calculated to provide sufficient oxygen for the 700-ml culture. A step-wise increase of 50 ma was made every 15 min until the final current was 550 ma. This current would induce the formation of 1.95 ml of oxygen per min. In the course of the experiment, 15-ml samples were withdrawn from the electrolysis cell every 15 min, resulting in a final volume of culture of 550 ml. The effective oxygenation rate for the 550-ma current was then 3.6 ml of oxygen per L per min.

Despite extreme care in avoiding excesses and maintaining an electrolytic oxygen supply commensurate with the metabolic needs of the organism, growth inhibition and decreased oxygen uptake rates were encountered in electrolytic growth. The impairment of growth was evident after the electrolysis had proceeded for 1 hr; and after 2 hr, oxygen uptake rates and succinic dehydrogenase activities of electrolyzed cells (Thunberg technique) were lower than the corresponding activities of aerated controls. The latter result indicated that the inhibition might be due to the leaching of heavy metals from the graphite electrodes or the poisoning of the cells by pure oxygen. Heavy metals were eliminated as a source of inhibition since normal growth occurred in aerated (nonelectrolyzed) cultures containing blocks of the electrode material.

When the graphite cathodes were replaced with iron, immediate success was obtained in the electrolytic growth of cultures of *P. fluorescens*, that is, electrolyzed cultures grew at the same rate and to the same final cell densities as aerated controls. The cell yields were in the order of 0.90 g (dry weight) per L. The inhibition which had been observed during electrolysis with graphite cathodes was possibly the result of the reduction of an unknown substance with the formation of a toxic material. The fact that the hydrogen overvoltage of the graphite is higher than that of the iron cathode lends support to this explanation.

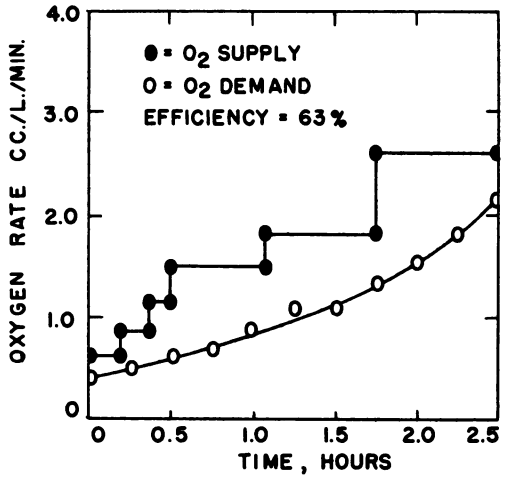

FIG. 4. The oxygen supply and demand for an electrolyzed culture of *Pseudomonas fluorescens*. The ratio of the areas under each curve is a measure of the average efficiency of oxygen utilization.

Oxygen Poisoning

Berghaus (1907) has reported an inhibition of cultures of *Pseudomonas pyocyanus* (*Pseudomonas aeruginosa*) in contact with oxygen at slightly more than one atmosphere. Because oxygen is released at the anode at approximately atmospheric pressure, an investigation was made of the possibility of oxygen toxicity in the electrolytic growth of microorganisms.

Cultures were grown in shake flasks to approximately 0.2 g per L dry weight cells, placed in the fermentor (no current flow) and oxygenated through a sintered glass sparger. The contents were stirred at 1100 rpm. The oxygen flow rates ranged from 25 to 140 ml of oxygen per min in a series of experiments so that various levels of dissolved oxygen could be maintained. Inhibition of cell growth and a diminution of succinic dehydrogenase activity were the criteria of the degree of oxygen poisoning. A general inhibition of the cultures of *P. fluorescens* appeared at dissolved oxygen concentrations somewhat above 4.65 ml per L of medium which, for the electrolysis medium, corresponds to a solution in equilibrium with gaseous oxygen at 0.27 atmospheres and 30 C. It would be difficult to attain or maintain this level of dissolved oxygen in an actively metabolizing culture of reasonable cell density. However, the possibility does exist that over-oxygenation could occur in the electrolysis of cultures of microorganisms in an early stage of growth when the oxygen demands were very low.

Oxygen Absorption Efficiency

Various schedules of current increases during electrolytic growth were attempted, and the resulting oxygen efficiencies for entire experiments were obtained by graphical integration. The results of one such experiment are given in figure 4 and indicate that close control of the electrolysis rate is necessary in order to obtain oxygen efficiencies approaching 100 per cent. The over-all utilization efficiencies for the electrolytic process were in the order of 50 per cent, which is higher than obtained with conventional aeration.

Oxygen Transfer Studies

The unusually high oxygen absorption led to a study of the factors affecting the rate of solution of oxygen in the electrolytic process.

It has been stated that the rate of absorption of a gas in a stirred vessel is a function of: (a) the temperature; (b) the magnitude of the driving force, a function of oxygen solubility; (c) the area of the gas-liquid interface, a function of gas bubble size; (d) the time of

contact; and (e) the intensity of agitation. Several of these variables have been investigated and their interrelationships in the present system will be discussed in terms of the mass transfer coefficient.

Oxygen bubble diameter. Gas bubbles evolved electrolytically from a platinum anode (a wire 0.3 mm in diam. and 4.0 mm long) immersed in growth medium, were observed through a horizontally mounted microscope. The current was kept at approximately 1 ma so that the evolution of gas bubbles from various electrode areas could be followed and so that bubble size could be measured with a calibrated ocular micrometer. It was observed that bubbles were generated only at specific sites on the anode; they were of two distinct sizes, 55 and 80 μ in diam. Approximately 90 per cent of the bubbles were of the smaller size. The size of these bubbles was of the same order of magnitude as that for the hydrogen gas bubbles reported by Jenckel and Hammes (1937). They observed the chemical and electrolytic gas discharge from aluminum in acid solutions and found, for example, that hydrogen bubbles from an electrolysis at 50 ma per cm^2 have a diam of 20 μ.

In electrolytic growth experiments the currents ranged from 150 to 550 ma and, since the anode area was 46.5 cm^2, current densities were 3.2 to 11.9 ma per cm^2. Jenckel and Hammes report very little change in bubble diam over this range of current density. It is valid to assume, therefore, that the oxygen bubbles generated at the large anode actually used were about the same size (55 μ diam) as those observed at the tiny platinum wire.

There are no reports in the literature of mass transfer studies in which the interfacial area was as great as that which must have been attained in this research. In fact, few sparging devices are capable of producing such fine, uniform gas dispersions. The electrolytic technique of gas generation might, therefore, be advantageously employed in studies of mass transfer in agitated tanks.

Oxygen solubility. The solubility of oxygen in the bacteriological growth medium at one atmosphere pressure and 30 C was measured polarographically. The solubility of oxygen in pure water at these conditions is 29.10 ml per L (37.4 ppm oxygen), but in the medium it was only 17.45 ml per L (22.4 ppm oxygen).

During the electrolysis of uninoculated medium, the solubility of the oxygen must be affected by the presence of hydrogen. Although 2 volumes of hydrogen and 1 volume of oxygen are produced unmixed at the electrodes, each gas would tend to "scrub" or remove the other from solution, resulting in thorough mixing of the two gases. The solubility of oxygen in such a system could then be only one-third that of pure oxygen

The above considerations were tested by approaching the equilibrium solubility of oxygen from two directions; completely deaerated medium on the one hand, and oxygen-saturated medium on the other. These were electrolyzed and oxygen determinations were made by withdrawing 10-ml samples at intervals and introducing them into 20 ml of de-aerated medium in the H-cell. In order to minimize errors due to the pickup of atmospheric oxygen, the volumetric pipettes were flushed with nitrogen prior to sampling, and all liquid samples were introduced below the level of the medium in the H-cell. An equilibrium oxygen solubility of 5.82 ml per L, one-third that of the pure gas, was attained in approximately 20 min from either starting condition. The effective partial pressure of oxygen in the electrolysis was therefore only one-third of an atmosphere. It should be pointed out that the tests were made on uninoculated medium which has, essentially, no oxygen demand. Systems with a high oxygen demand could draw gas from the medium so rapidly that little scrubbing with hydrogen would occur. In this case, the effective partial pressure of oxygen might approach one atmosphere.

The determination of the mass transfer coefficient. The mass transfer coefficient, k_d, for the unsteady state transfer of oxygen into the uninoculated medium was determined by the "outgassing" technique of Bartholomew *et al.* (1950) and Wise (1951). This involves observations of the rate of solution of oxygen in a previously deaerated medium. An over-all mass transfer coefficient is then calculated from the slope of a plot of $\ln(c^*-c)$ versus time, where c^* is the ultimate solubility of oxygen and c is the instantaneous oxygen concentration in the liquid.

Following deoxygenation of the medium with nitrogen, a potential of 4.2 V was applied between 2 platinum

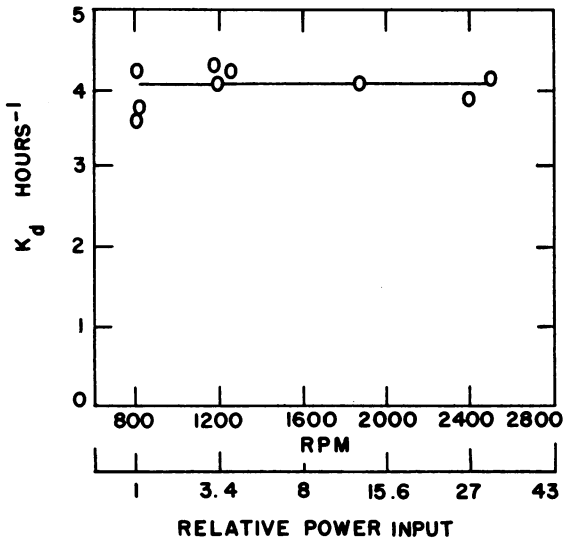

FIG. 5. The mass transfer coefficient, k_d, as a function of the degree of agitation of the electrolyzed medium. The power input varies as the cube of the agitator speed.

foil electrodes. This resulted in a current of 200 ma and brought about the evolution of 0.70 ml of oxygen per min or 2.1 ml of mixed gas per min. Mass transfer coefficients were calculated from data obtained at various agitator speeds from 800 to 2500 rpm and are presented in figure 5. The value of c^* for these calculations was taken as 5.82 cm^3 of oxygen per L of medium. Also indicated on the abscissa of figure 5 is the relative power input referred to 800 as unity.

In this investigation, the mass transfer coefficient was independent of the relative power input per unit volume in the turbulent range. (Reynolds number of 10,000 corresponds to 760 rpm.) This is in marked contrast to the relationship:

$$k_d = kP_v^{0.95} \quad (3)$$

which has been reported in the literature for stirred tanks, where a combination of sparging and mechanical shearing is responsible for the generation of gas-liquid interfacial area. It may be that a major portion of the effective horsepower input to a sparged fermentor is expended in creating new interface, and that once the bubbles are sufficiently small, further power is ineffective. These conclusions pertain, of course, to simple mediums containing yeasts or bacteria which do not form large clumps of cells.

SUMMARY

Electrolysis of the culture medium was used as a means of providing oxygen for the growth of a submerged culture of *Pseudomonas fluorescens*. Growth rates and cell yields equal to aerated cultures were obtained electrolytically when the dissolved oxygen concentration was not limiting. A cathode reaction which was inhibitory to growth occurred with graphite but did not occur when iron cathodes were used. The requirement that the applied voltage be maintained below the discharge potential of chlorine necessitated the use of relatively large electrode areas. In the present research, the anode area was equal to 66 cm^2 per L or approximately 40 sq in per gallon.

Moderate oxygen concentrations (above 4.65 ml oxygen per L of solution) inhibit the growth of *Pseudomonas fluorescens*. Hydrogen peroxide was not formed in the electrolysis in detectable amounts, but toxic quantities of hypochlorite could be formed at the anode under some experimental conditions.

The oxygen gas bubbles produced in the electrolysis were rather uniform and about 55 μ in diam. The discharge of hydrogen had a pronounced effect on the solubility of oxygen in the uninoculated growth medium.

The major portion of the effective power input to a sparged fermentor may be expended in the creation of new interface, and once the gas bubbles are sufficiently small, further power does not affect the rate of gas transfer.

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ERRATUM

In the paper by Snell et al., Volume 4, Number 1, January 1956, page 15, Table I, first column, under "Alcohol phase from n-butyl alcohol, acetic acid, H_2O" for "74:6:20" substitute "40:10:50".