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Progress Report

OXYGEN TRANSFER MECHANISMS IN THE GLUCONIC ACID FERMENTATION BY *Pseudomonas ovalis*

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ABSTRACT

Submerged aerobic fermentations are important in the industrial production of antibiotics, yeast cells and vitamins as well as in waste treatment. In these fermentations, oxygen is an essential nutrient that must be continually supplied to the microorganisms.

Many workers have evaluated the oxygenating capacity of fermentors under conditions of aeration and agitation. Others have studied oxygen uptake by cells in the quiescent chambers of polarographs. Little data, however, have been published on the separate effects of agitation and aeration in an active fermentation, because it is difficult to design experiments to separate these two variables.

The fermentation of glucose to gluconic acid by cells of Pseudomonas ovalis suspended in a nitrogen-free medium was first used to measure the rate of oxygen transfer in a sparged and agitated system. Then, using the same system, measurements were made of the rate of transfer of dissolved oxygen to cells suspended in a liquid that was being stirred but not aerated. The effect of aeration on the rate of oxygen transfer was the difference between the rates found by the two methods.

It was found that the oxygen transfer rate measured by the electrode was less than the rate measured by the rate of gluconic acid production in a sparged system. Agitation rate had no effect on the oxygen transfer rate in the absence of aeration. However, in the sparged and aerated system, an increase in either the air rate or the agitation rate increased the rate of oxygen uptake by the

cells, even though the dissolved oxygen concentration in the medium was high.

These data suggested that an additional mechanism for oxygen transfer was present in addition to the direct supply from dissolved oxygen in the liquid. This second pathway was encountered when cells were adsorbed on the surface of bubbles. Adsorption merged the liquid films which surrounded the bubbles and the cells, resulting in a shorter path for the oxygen transfer.

The effect of viscosity has been investigated. It was found that long-chain carboxymethyl cellulose derivatives, which increase the viscosity when added to water in small amounts, did not affect the rate of oxygen transfer in the Pseudomonas ovalis system. However, these same compounds, as well as glycerol and sucrose, did reduce the oxygen transfer rates when these rates were measured by a chemical technique or with the oxygen electrode.

The effect of environmental conditions on the rate of gluconic acid production by resting cells of Pseudomonas ovalis was investigated. The maximum rate was found to occur at pH 7.35 and 37°C. An activation energy of 9,600 cal/mole was calculated for this fermentation. The critical dissolved oxygen concentration for resting cells of Pseudomonas ovalis was 1.1 mg/l.

I. INTRODUCTION

1. General Considerations

Submerged aerobic fermentations are used industrially to produce antibiotics, chemicals, yeast cells and vitamins, and are also used in waste treatment. These fermentations require that large amounts of oxygen be supplied to microorganisms submerged in liquids in order to support growth of the cultures.

The microorganisms must be continually supplied with all essential nutrients at rates sufficiently high to prevent the limitation of their metabolic activity. At the same time, metabolic products must be swept away to prevent inhibition of growth processes of the cells.

In aerobic fermentations, oxygen becomes an essential nutrient. Not only does it have a long, difficult path to travel from the air to reaction sites in the cell, but it also has a very low solubility in the medium.

Bartholomew *et al.* (3) have separated the oxygen transfer process into a number of steps, each with its own resistance. These resistances, shown diagramatically in Figure 1, are as follows:

(1) Gas-film resistance, between the gas and the gas-liquid interface

(2) Interfacial resistance, between the gas and liquid films

(3) Liquid-film resistance, between the gas-liquid interface and the bulk of the liquid

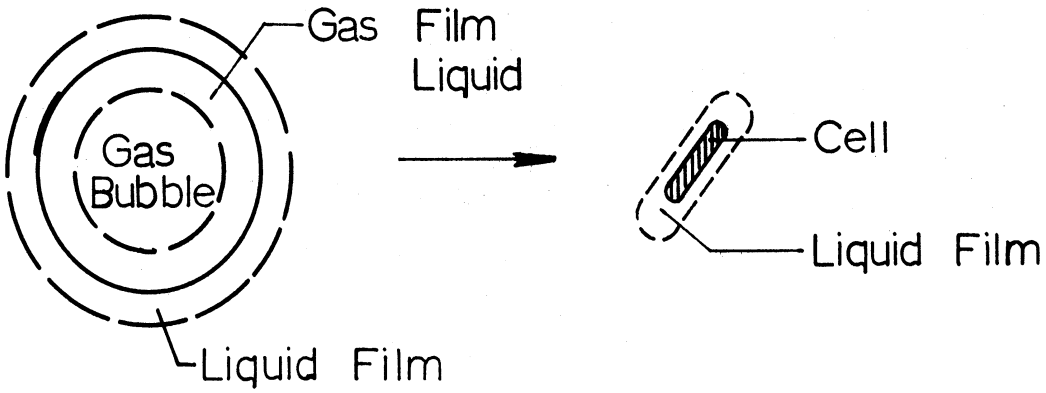

Figure 1. Path of oxygen transfer in submerged aerobic fermentations.

(4) Bulk liquid resistance

(5) Liquid film resistance, around the cell

(6) Internal resistance, associated with reactions of oxygen and respiratory enzymes of the cell

In addition to the path represented by the preceding resistances, Bartholomew and his co-workers presented evidence suggesting a shorter, but parallel path between the same concentration limits, by direct contact between the cells and the gas-liquid interface. This path, shown diagramatically in Figure 2, eliminates the bulk and liquid film resistances, since the liquid film surrounding the bubble is the same liquid film as that surrounding the cell. This is an important concept that appears to have received little experimental attention.

The total amount of oxygen transferred by means of this alternate path depends on the number of bubbles contacting with the gas-liquid interface. The amount of contact will depend on the area of gas available to the cells. The area of the air surface available per unit time for oxygen transfer, in turn, is a function of the bubble size, the length of the path of bubbles in the liquid, and the relative velocity between the bubbles and cells. This path for direct transfer is favored by a large number of small bubbles and by a long retention time.

Since Bartholomew *et al.* (3) lacked quantitative data, they were only able to evaluate this new path on a qualitative basis. They observed that *Streptomyces griseus* and

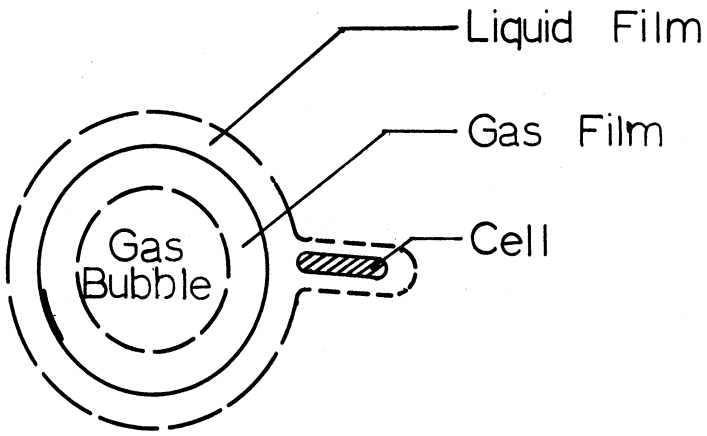

Figure 2. Path of oxygen transfer with micro-organisms adsorbed on the surface of a bubble.

Penicillium chrysogenum adhered to and had mobility on the surface of bubbles. It is known (73) that microorganisms can form an intimate adsorptive contact with air bubbles, much as that occurring with froth flotation of minerals. In fact, froth flotation has been used (36) to concentrate microorganisms.

The relative importance of each resistance is different in any given transfer system. In the first step, the oxygen encounters the resistances of the gas film, the interface and the liquid film. Between the liquid films surrounding the bubble and the cell, the oxygen must overcome diffusion resistance of the bulk liquid. At the cell, it meets the resistance of the liquid film surrounding the cell and finally, it encounters the reaction resistance due to the respiratory enzymes of the cell.

For the transfer of oxygen, one may write:

$$\frac{dC}{dt} = K_L A \, (C_g - C) \qquad (1)$$

In this equation, the first three resistances have been combined into an overall mass transfer coefficient, $K_L A$, where A is the interfacial area. The driving force is the difference between the concentration of oxygen in the liquid in equilibrium with the existing partial pressure of oxygen in the air and the instantaneous dissolved oxygen concentration in the liquid.

The other resitances apply, according to Richards (66), not only to oxygen transfer, but also to the transfer of

other substances such as nutrients. The resistance to passage of such substances through the bulk liquid should be minor if sufficient mixing is provided to maintain homogeneity.

There is a liquid film around the cells which could be affected by agitation. In the production of gluconic acid by suspended cells of Pseudomonas ovalis, Tsao and Kempe (94) proposed that the rate-limiting step occurred at this interface. Finn (32), however, has theoretically calculated the magnitude of this resistance and found it too small to be significant. He stated that agitation in excess of that required to suspend single cells uniformly will not markedly improve oxygen transfer from the liquid to the cell wall, because the cells move with the liquid: improvement can only be achieved by increasing relative movement between the liquid and the cell.

Even though cells do move with the liquid, there is still an increasing resistance to their movement as the rotational speed increases. Barker and Treybal (2) have shown that the mass transfer coefficient for solids, suspended in agitated liquids, could be correlated with a Reynolds number calculated for each tank.

The internal resistance of the microorganism to oxygen uptake is independent of stirring unless it is insufficient to remove metabolic products. The activity of these respiratory enzymes depends upon the method used for growing the cells among other factors.

Working with Penicillium chrysogenum, Rolinson (67) found

that the oxygen demand rate was considerably higher in stirred fermentors than in shaken flasks. He suggested that respiration was less active in systems where aeration was less intensive. He believed that aeration intensity could affect the kind and quantity of enzymes present in the cells. He reported that mycelium, grown under the conditions of intensive aeration found in a stirred fermentor, maintained its high oxygen utilization rate when transferred to the less active system found in a shaken flask.

Terui *et al.* (92) found a distinct difference in physiological properties between mycelia grown as surface and mycelia grown in submerged cultures. Expecially notable was the behavior of these two different cultures toward oxygen provided as gaseous or dissolved oxygen. They attributed this difference, in part, to differences in enzyme systems. *Aspergillus oryzae* grown in submerged cultures contained much more cytochrome oxidase and cytochrome c, but less catalase and flavins (FMN and FAD) than cells grown on a surface.

Winzler (100) has shown that the rate of oxygen consumption in a submerged culture is independent of the actual dissolved oxygen concentration, provided that the oxygen level exceeds a concentration known as the critical concentration, C_c. Finn (32) stated that critical concentrations are very low for unicellular organisms. Several of these critical concentrations have been reported in the literature and are listed in Table I.

TABLE I

CRITICAL DISSOLVED OXYGEN CONCENTRATIONS FOR MICROORGANISMS IN THE PRESENCE OF SUBSTRATE

Organism	Critical Dissolved Oxygen Concentrations, mg/liter	Reference
Luminous bacteria	0.31	(32)
Azotobacter Vinelandii	0.56 - 1.53	(32)
Yeast	0.10 - 0.11	(32)
Yeast	0.25	(67)
Penicillium chrysogenum	0.66	(32)
Escherichia coli	0.26	(32)
Saccharomyces cerevisiae	0.20	(86)
Pseudomonas sp.	0.2	(89)
Pseudomonas ovalis	0.7 ± 0.2	(1)

Many efforts have been made to measure aeration efficiency. Most often, the rate of oxygen transfer has been measured by the sulphite method (14, 29, 34, 98, 101) developed by Cooper *et al.* (19).

A second technique, employed with larger vessels, is an unsteady state process. The oxygen concentration in the liquid is reduced to zero by adding sodium sulphite, by boiling, or by stripping with nitrogen. The rate of reaeration is then measured polarographically (49) or chemically (42, 102).

Recently, instruments have become available for measuring the oxygen concentration in gaseous streams (75, 84). These instruments utilize the paramagnetic quality of oxygen. This method is fairly accurate (61), since the measurement of oxygen uptake is based upon the difference in oxygen concentrations between the inlet and outlet streams.

Shu (74) used a recording manometric technique. He described an especially designed fermentor attached to a shaker in which the uptake of oxygen during a fermentation was recorded automatically. This was possible since a completely closed system was employed.

Recently, considerable interest has been shown in measuring the dissolved oxygen contents of the fermentation medium. Many instruments are now available to measure dissolved oxygen directly: these include platinum electrodes (30, 59, 82, 85), diffusion tubing (59), dropping mercury electrodes (40, 55), and polarographs (92).

The platinum electrode is small, so it can be con-

veniently placed in a laboratory-size fermentor. Phillips and Johnson (59) stated that it measured dissolved oxygen concentrations; other authors (31, 78, 85) agree that oxygen activity or the fraction of oxygen saturation of the solution is measured.

Progress in instrumentation has now made it possible to record both oxygen uptake rates and oxygen concentrations in actual fermentations. Previously, it was only possible to calculate one from the other, but workers were not able to experimentally determine both values in actual fermentation systems.

2. Effect of Agitation

The effects of agitation and aeration have not been studied separately in fermentation systems because it is difficult to design suitable experiments to separate them (99). Phillips and Johnson (60) reported an effect of agitation on mold fermentations, but concluded that the effect was only that of mixing or distributing oxygen to various locations in the fermentor. In mold fermentations, it was found that intraclump resistances could be reduced by adequate agitation (99).

Dion et al. (24) studied the effect of mechanical agitation on the morphology of Penicillium chrysogenum in stirred fermentors. When the agitation was mild, the hyphae were long with a little branching; shortening of the hyphae and more branching occurred with vigorous agitation. Vigorous agitation also increased the penicillin yield.

Donovick (27) has presented data showing increased penicillin production with increasing power input to the agitator.

Camposano et al. (18) found that mechanical agitation and aeration, sufficient to produce saturated oxygen conditions, reduced the amount of Kojic acid produced from glucose by Aspergillus flavus in submerged fermentations. Physical damage can be done to organisms by mixing them in baffled shaker flasks. Such results have been reported by Vondrackova (96) and Smith (77).

As stated in the discussion of controlling resistances, Tsao and Kempe (94) presented data suggesting a significant effect of agitation on mass transfer at the cell-liquid interface, while Finn (31) disputed this view. Phillips and Johnson (60) worked with a variety of organisms, measuring both the oxygen transfer rates and the concentration of dissolved oxygen in the media. They concluded that variations in dissolved oxygen concentrations were not important as long as these concentrations remained above the critical.

A different view has been expressed by Steel and Maxon (83) who worked with the novobiocin fermentation. Here the rate of oxygen transfer was increased when the concentration of dissolved oxygen was increased by more vigorous agitation, even though the dissolved oxygen concentration was well above the critical throughout the experiment. They concluded that the liquid-cell resistance becomes the controlling factor for oxygen transfer in viscous, non-Newtonian fermentations like the novobiocin fermentation. They did not indicate that

this applied to low viscosity broths, asserting that the gas-liquid film still controlled in this instance.

3. Effect of Viscosity

In antibiotic fermentations and also in bacterial fermentations which develop products of high molecular weight, such as dextran and polyglutamyl peptide, the rheological properties of the medium change as the fermentation progresses. Very little has been published concerning the aeration and agitation characteristics of non-Newtonian broths. Most published data are concerned with mold mycelium and its effects on the oxygen transfer rate.

For Newtonian fluids, the shear stress τ is directly proportional to the rate of shear γ; the viscosity μ is independent of the shear rate.

$$\tau = \mu \gamma \qquad (2)$$

Any fluid not having this simple relationship is called non-Newtonian. A pseudoplastic fluid is one whose apparent viscosity decreases instantaneously with increasing shear (Figure 3). The following expression has been proposed for this case by Ostwald (56) and has since been fully described by Reiner (64):

$$\tau = k_v \gamma^n \qquad (3)$$

The apparent viscosity at any point is

$$\mu_a = \tau/\gamma \qquad (4)$$

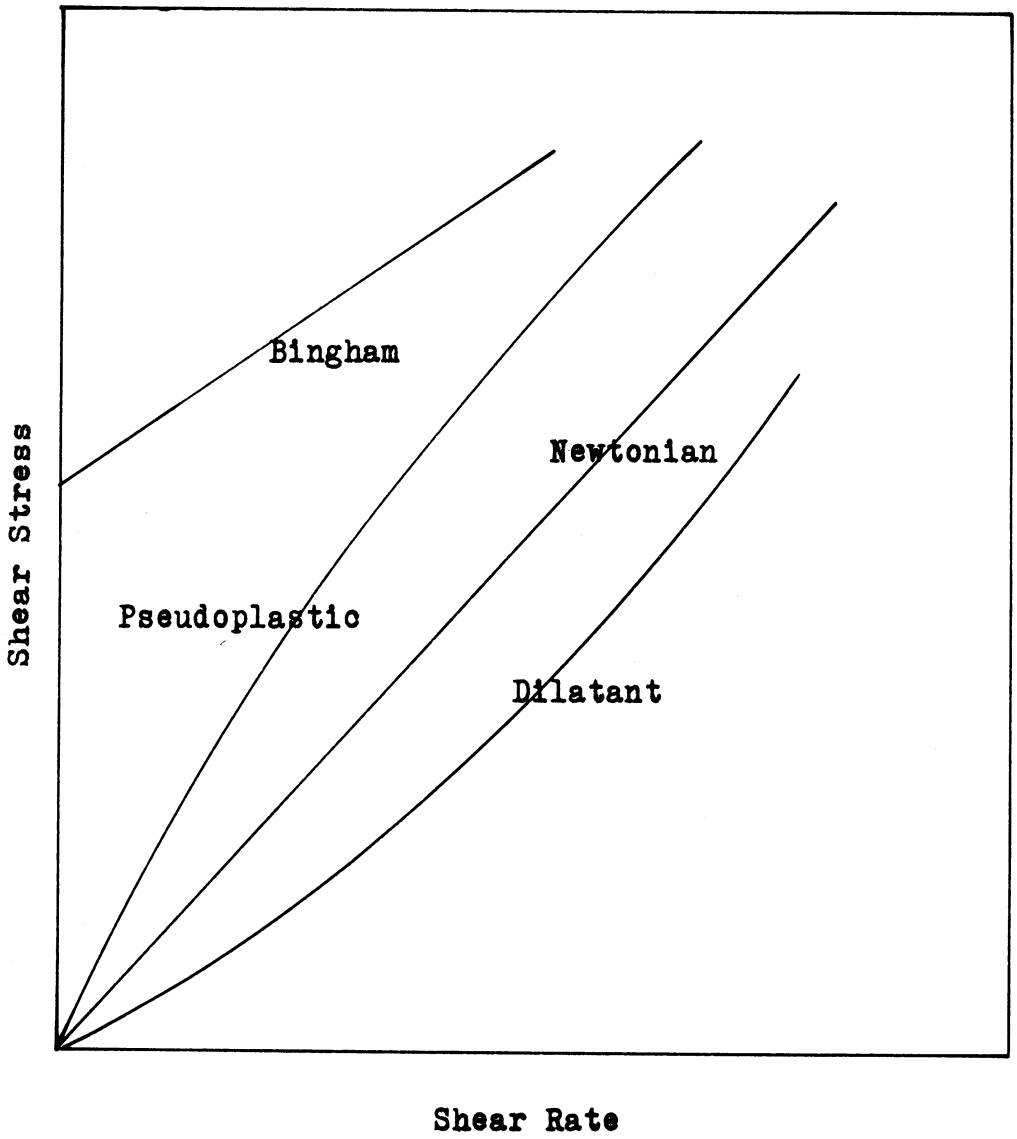

Figure 3. Flow curves for various types of time-independent, non-Newtonian fluids.

$$\therefore \quad \mu_a = k_v \gamma^{n-1} \qquad (5)$$

Thus, a plot of log τ versus log γ would give n as a slope and k_v as the intercept on the y axis. Such a plot is shown in Figure 4.

Deindoerfer and Gaden (21) showed that the oxygen uptake rate was markedly dependent on broth rigidity and mycelium concentration. They used killed, reconstituted Penicillium broth and measured oxygen concentration polarographically. An 85% reduction in the absorption rate occurred at a mycelial concentration of 13.4 g. dry tissue per liter. This concentration of tissue produced a rigidity of 180 centipoises in the Bingham plastic broth.

Chain and Gulandi (12) showed that 1.35 percent of dead mold mycelia reduced the sulphite absorption rate by one-half. Solomons (78) reported that a 45% solution of sucrose, which had a viscosity of 10 centipoises, reduced the oxygen transfer rate by a factor of 6 when measured by the sulphite method and by 12 when the polarographic method was used. Yoshida *et al.* (101) discussed the effect of increased viscosity due to the addition of glycerol on the rate of oxygen uptake in stirred sulphite solutions; at 300 RPM, an increase in viscosity from 1 to 3.6 centipoises lowered the oxygen transfer rate by a factor of 2.4.

With mold mycelium, Solomons and Perkin (79) found significant reductions in the oxygen transfer rate (OTR) with increasing viscosities. Their viscosity data were based on a shear rate of 1 sec.$^{-1}$; for apparent viscosities of 1, 10,

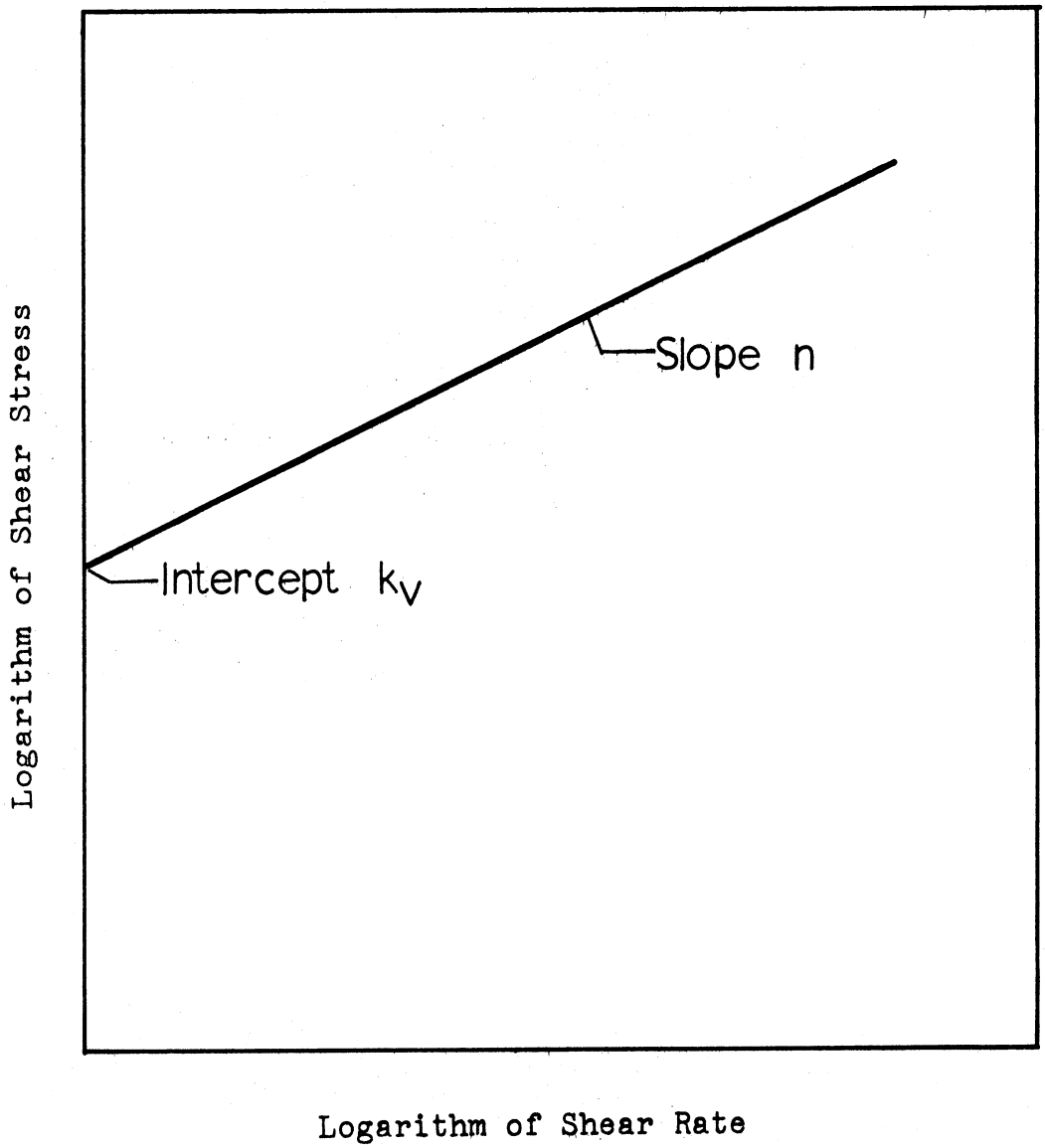

Figure 4. Shear flow diagram for a pseudoplastic fluid, showing the flow behavior index, n, and the consistency index, k_v.

100 and 1000 centipoises, they found OTR's of 58, 42, 30 and 19 millimoles/(l)(hr.), respectively. Solomons and Weston (80) furthered this work and found the OTR was a function of shear at the impeller according to the relationship:

$$\gamma = (IO)N \qquad (6)$$

Bowers (7) simulated mycelia using shredded paper pulp suspended in sulphite solutions. Two percent of pulp decreased the sulphite OTR by 43 to 96%. Brierly and Steel (9) found as high as 90% reduction in OTR when 20 g. of dry, filamentous Aspergillus niger mycelia were suspended in a liter of aqueous salt solution, and 85% reduction when shredded paper pulp was suspended at the same concentration level. Sago pellets, at 3% concentration, had little effect on the rate of oxygen uptake. At this concentration, the pellets had little effect on viscosity.

Bartholomew, et al. (3) found a decrease in specific oxygen transfer, i.e., oxygen uptake per cell, with increasing mycelial concentrations. The rates of oxygen transfer in their studies were determined by polarographic methods. Phillips and Johnson (60), using Aspergillus niger, noted that oxygen deficiency in the fermentation occurred shortly after the culture began to exhibit a non-Newtonian viscosity behavior.

Recently, Steel and Maxon (84) have reported on oxygen transfer studies in novobiocin fermentations. They found an

initial decrease in the OTR as the apparent viscosity of the beer increased; then the OTR became independent of further change in viscosity. Concurrently, with the increase in viscosity, an increase occurred in the amount of gas retained by a sample of the beer.

This summary of the published data shows that the effect of increasing viscosity generally was examined in one of two ways. In the first method, oxygen transfer rates were measured in growing mold cultures and the change in OTR was followed with time. In these systems, the increasing concentration of hyphae caused the viscosity to increase as the fermentation progressed.

The second method was to measure the OTR in a system in which the viscosity was established at a constant value with suspended, dead mycelia, or solutions of glycerol or sucrose. Either polarographic techniques or sulphite methods were employed in this case to measure the OTR.

It can be seen that, in all of the cases reported here, the OTR fell as the viscosity increased. However, each method experienced some side effects. In mold fermentations, there was the added resistance of diffusion into the pellets. Even though the oxygen concentration is high in the medium, it can be zero in the center of these pellets. Also, as the fermentation progressed, both the viscosity of the medium and the characteristics of the culture changed.

The second method used a non-biological system and has been criticized as not truly representing living systems.

The shortcomings of the sulfite system as a method for measuring fermentation rates arise, according to Blakebrough *et al.* (6), from two sources: essential differences between the processes which consume oxygen in sulphite solutions and in suspensions, and the physical properties of solutions. These authors also point out that the sulfite method measures oxygen which has reacted while the polarographic method measures oxygen in solution awaiting reaction.

4. Statement of the Problem

A method for measuring the rate of oxygen uptake using *Pseudomonas ovalis* cells suspended in a nitrogen-free medium, was proposed by Tsao and Kempe (94). In the absence of nitrogen, growth was arrested and the cells utilized one-half mole of oxygen and one mole of glucose to form one mole of gluconic acid. Hence, the rate of gluconic acid production as measured by the amount of alkali required to control the pH at a constant value, was proportional to the oxygen utilization rate of the cells.

This method has many unique features when compared to other methods. Since living cells are used, conditions more closely approximate those in commercial fermentation units than does the sulfite system. Also, this system does not require sampling of the medium and measurement of oxygen transfer externally without aeration, as required by the polarographic method.

The gluconic acid method is, however, limited to small scale operations, since it is impracticable to spin down

and resuspend large volumes of cells.

Tsao and Kempe (94) found that an increase in the agitation rate increased the OTR. There was evidence to suggest that the increase in the OTR occurred even when the oxygen concentration in the liquid was high. Assuming this, they concluded that the increase in the OTR resulted from a reduction in the controlling resistance for oxygen transfer, which they postulated as being the liquid surrounding the cell.

This assumption of a high concentration of dissolved oxygen in the liquid was important. If the dissolved oxygen concentration were below the critical value rather than above it, then the effect of increased agitation would more likely have been upon the gas-liquid portion of the overall oxygen transfer resistance than upon the cell-liquid portion, as they proposed.

At the time this work was done, suitable instruments were not available to measure dissolved oxygen concentrations in the liquid. In view of the critical aspect of this measurement for understanding more about the mechanism of oxygen transfer, it was important to further investigate the dissolved oxygen concentration during experiments of the type that Tsao and Kempe reported (94).

An additional mechanism for oxygen transfer, besides that occurring directly from oxygen dissolved in the liquid, has been reported. Bartholomew *et al.* (3) proposed that oxygen transfer took place by two separate paths but were

unable to experimentally evaluate their theory at that time.

With the bacterial fermentation of glucose to gluconic acid, a method for measuring oxygen transfer in living systems was available. With the development of the oxygen electrode, the means of measuring dissolved oxygen concentration in the liquid was at hand. If both of these methods were used together, it appeared that these mechanisms of oxygen transfer could be evaluated separately.

The effect of viscosity upon oxygen transfer has received little attention. It is well known that long chain carboxymethyl cellulose (CMC) derivatives, glycerol and sucrose reduce OTR in non-biological systems. Some work with mold fermentations has shown that as fermentations progress, the increasing concentration of filamentous material reduces the OTR. However, the effect of viscosity in bacterial systems has essentially been neglected. The gluconic acid fermentation seemed admirably suited to this investigation. In place of growing mycelia to increase viscosity, CMC derivatives could be used to give constant and high viscosities.

II. MATERIALS AND METHODS

1. The Fermentor

The equipment centered around a New Brunswick five liter fermentor.[1] This unit consisted fundamentally of a Pyrex glass jar six inches in diameter and twelve inches high, and a head plate assembly inserted into the jar. Four baffles were attached to the head plate. The baffles were 0.625 inches wide and were positioned with their surfaces perpendicular to the walls of the jar. They extended from the top of the jar to within 0.125 inches of the bottom where they were connected by a circular band 0.625 inches high.

An agitator shaft passed through a bearing on the head plate. Two impellers, 0.625 inches wide and 0.625 inches long were attached to the shaft with their flat surfaces perpendicular to the vessel's bottom. The distance from the tip of the blade to the center of the shaft was 1.50 inches. All of the components of the head plate, baffles and agitator were constructed of stainless steel.

For all runs, except the non-Newtonian viscosity series, one impeller was 2.5 inches and the other 6.5 inches above the bottom of the vessel. This resulted in the lower impeller being immersed in the liquid while the upper one contacted only the gas phase. For the series of runs with non-Newtonian broths, the upper impeller was lowered to

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1. Model F-05, New Brunswick Scientific Co., New Brunswick, N. J.

3.675 inches above the bottom of the vessel. This was done because it was necessary to have both impellers in the aqueous phase to obtain higher oxygen transfer rates and better mixing in the high viscosity broths.

Compressed air was taken from the building supply. It was passed through a cotton filter and a rotameter.[2] A calibration curve for the rotameter is given in the Appendix. The air was humidified by bubbling it through water. From the bubbler, the air entered a plenum chamber, and then a sparger, which was attached to the head plate. The sparger consisted of a single orifice, centrally located and facing upwards 0.5 inches below the lower impeller.

For one series of runs, the air used for sparging was diluted with nitrogen. To accomplish this, two more rotameters and control valves were required. The final flow rate of the mixed nitrogen and air was kept constant by varying the relative amounts or the other two flows.

Control of the pH was effected with a pH meter[3] and a recorder-controller.[4] A set of glass and reference electrodes, of the type normally used with a Beckmann model H-2 pH meter, was installed in the fermentor. As the pH of the medium fell below a set value, the controller made contact through a micro-switch. This activated a motor which

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2. Model 2F 1/4 20-5, Fischer and Porter Co., Hatboro, Penn.
3. Model W industrial pH meter, Beckmann Instruments, Inc., Fullerton, Cal.
4. Wide-strip Dynamaster Millivolt Meter, Bristol Co., Waterbury 20, Conn.

powered a Sigmamotor pump that pumped alkali from the burette until the pH rose to the set point. The alkali was introduced into the medium in the fermentation vessel through an entry port in the head plate.

Sodium hydroxide was used as the neutralizing alkali. Depending upon the situation, its concentration was varied from 0.1 to 1.0 N. It was standardized by titration against potassium acid phthalate, using phenolphthalein as the indicator.

An electrical relay was installed to reduce the electrical load on the micro-switch, since the initial power surge was large when the motor started. When no relay was used, the micro-switch often stuck.

The use of a 50 cc burette allowed accurate determinations of the amount of alkali added. This, coupled with a small diameter tube in the pump, provided pH control within ± 0.05 pH units.

The fermentor was immersed in a constant temperature bath that controlled the temperature of the fermentation broths within ± 0.5°C of the desired value. A temperature compensator was immersed in the bath to correct pH readings on the recorder-controller for variations in temperature.

Power for stirring was supplied by a converted drill press. The transmission was effected through a V-Belt and horizontal pulleys, one being mounted on the drill press and the other on a frame above the constant temperature bath. The stirring speed was quickly and easily altered by changing

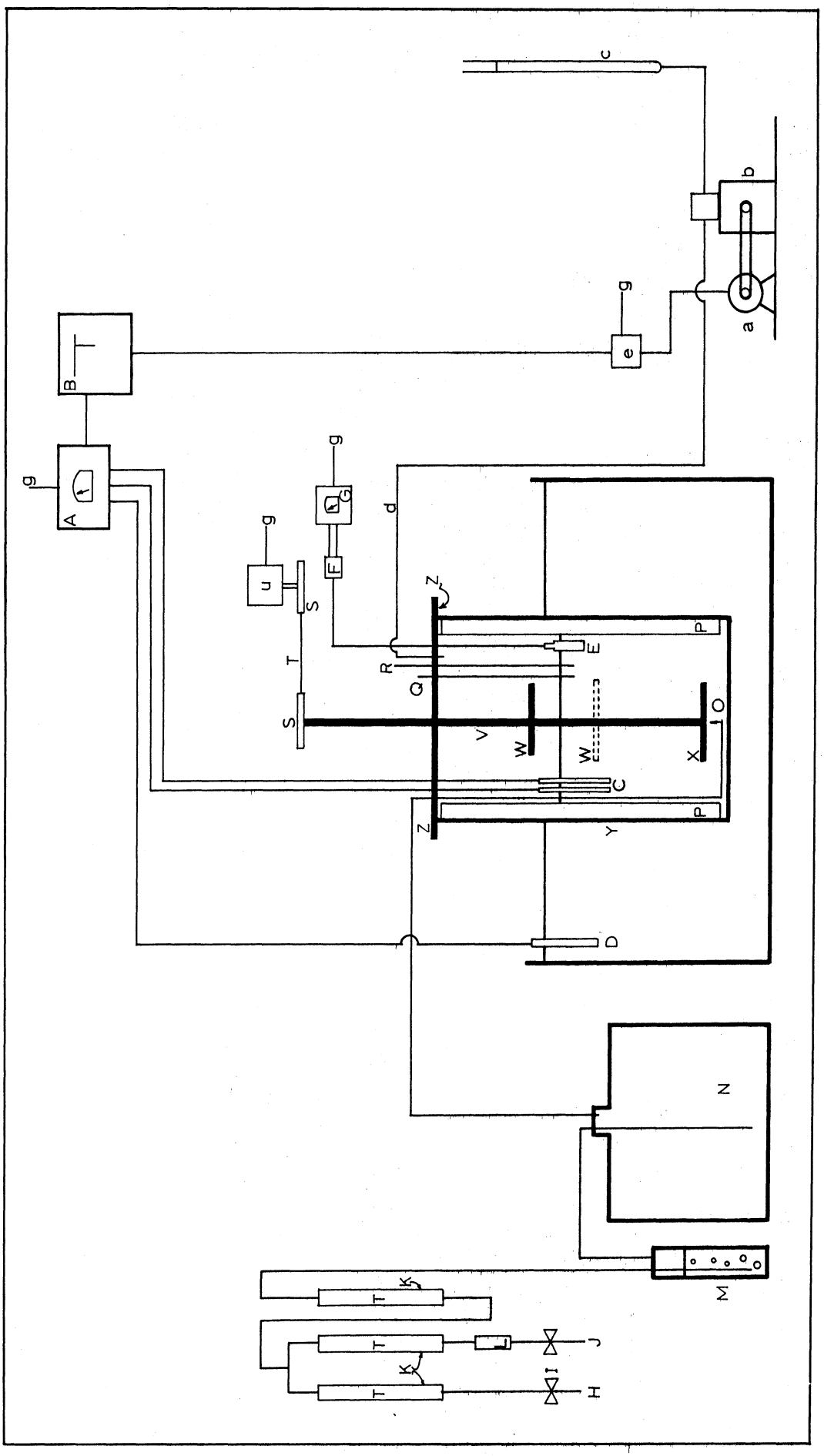

Figure 5. Equipment used in the study of the rates of oxygen transfer.

Legend for Figure 5

A. Indicating pH meter
B. pH recorder and controller
C. Electrodes
D. Temperature compensator
E. Oxygen electrode
F. Adapter for oxygen electrode
G. pH meter
H. Nitrogen tank
I. Valve
J. Air supply from building
K. Flow-meter
L. Cotton filter
M. Bubbler containing distilled water
N. Plenum chamber
O. Sparger
P. Baffle
Q. Sample port
R. Thermometer
S. Pulley
T. V-belt drive
U. Motor
V. Agitator shaft
W. Two positions of upper impeller
X. Lower impeller
Y. Fermentor, glass jar
Z. Head plate

a. Sigma pump
b. Driving motor for Sigma pump
c. Burrette for sodium hydroxide
d. Sodium hydroxide feed line
e. Relay
f. Constant temperature bath
g. Power source, 110V

the diameter of the pulleys. The stirring speed was measured with a revolution counter and a stop watch.

2. The Oxygen Electrode

Since the time when Clark (15) first introduced his oxygen electrode, it has been used in a number of biological systems (57, 82, 97). The oxygen measuring device used in this study[5] is shown in Figure 6. It consisted of a silver anode and a platinum cathode covered with a polyethylene membrane two to four mils in thickness. The cathode and the silver anode were immersed in an electrolyte of 3% KCL. The electrodes were encased in a dip-cell made of glass; the membrane was held securely against the cathode by a plastic sleeve. This sleeve was altered so that it slipped completely up, onto the body of the cell; no over-hanging edges were present to trap air bubbles.

The electrical circuit was such that a potential of 0.6 to 0.7 volts was supplied to the electrode by a size D dry cell, housed in an adapter unit. The adapter was connected to a pH meter.[6] At this operating voltage, dissolved oxygen was the chief substance reduced at the cathode.

The reactions taking place were:

$$2\ Cl^- + 2\ Ag \longrightarrow 2\ AgCl + 2e^- \quad \text{(anode)}$$

$$\tfrac{1}{2}\ O_2 + H_2O + 2e^- \longrightarrow 2OH^- \quad \text{(cathode)}$$

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5. No. 11098 Oxygen Electrode and No. 18902 Adapter unit, Beckmann Instruments, Inc., Fullerton, Cal.
6. Model H-2, Beckmann Instruments, Inc., Fullerton, Cal.

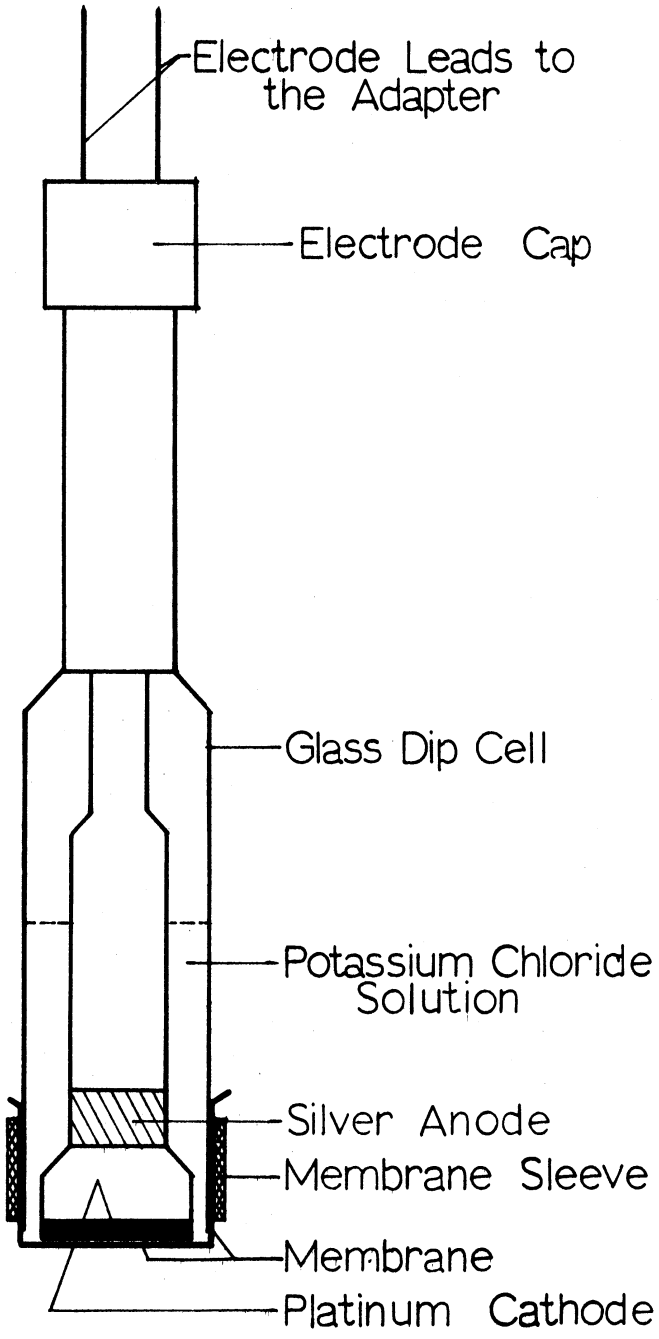

Figure 6. Schematic drawing of the oxygen electrode.

Thus, the current flowing in the system was a function of the number of oxygen molecules being reduced at the surface of the cathode. The potential drop of this current flowing through a known resistance was indicated on the pH meter. This potential drop, in millivolts, was therefore a measure of the concentration of dissolved oxygen in the solution.

It is generally agreed (31, 78, 85) that the oxygen electrode measures oxygen activity in solution rather than concentration of dissolved oxygen. The electrode cannot be calibrated by observing the response when placed in various air-saturated salt solutions, as is done with dropping mercury electrodes.

To test this statement by Finn (31), the electrode was placed in distilled water which had been saturated with oxygen by bubbling air through it for one hour. Then the electrode was placed in a solution of sodium chloride or sucrose which had similarly been saturated with oxygen. All of these solutions were at the same temperature. The electrode reading was independent of the concentration of the additive and did not change from its initial reading in the pure water.

To determine the dissolved oxygen concentration of a solution, it was first necessary to standardize the electrode. With the electrode disconnected, the pH meter was set at zero on the 0-800 millivolt scale. Then the cell was placed in water at 25.0°C which had been saturated with oxygen by bubbling air through it. The reading on the 600-1400 milli-

volt scale was adjusted to 1400. After the meter reading remained steady for 5 to 10 minutes, the electrode was disconnected and fastened to one of the baffles in the fermentor, so that the membrane was approximately one inch below the liquid surface during sparging and stirring of the liquid.

The percentage of oxygen saturation was calculated by dividing the pH meter output, in millivolts, by the full-scale value for saturation. Occasionally, the set point for a saturated solution was less than 1400 mv. In these cases, the reading at saturation, rather than 1400, was used to calculate the percentage of saturation. The manufacturer reported a linear relation between the instrument reading and the dissolved oxygen concentration. This claim was checked by placing the probe in solutions containing various concentrations of dissolved oxygen.

By mixing saturated and boiled water, the amount of dissolved oxygen in solution was varied. The oxygen electrode reading, in millivolts, was recorded and plotted against the dissolved oxygen concentration determined by the Winkler method (81). The data showing linearity, as reported, are recorded in Appendix B.

To measure the rate of oxygen uptake with the electrode, the air supply to the fermentor was stopped. Then the rate of change of the dissolved oxygen concentration in the liquid was recorded. The slope of the plot of dissolved oxygen concentration against time then became the apparent rate of

oxygen uptake by the cells. Before calculation of the oxygen utilization rate, however, it was necessary to account for surface reaeration. Reaeration occurred when oxygen diffused through the surface of the liquid into the medium at the same time as the cells were removing oxygen from the solution.

Blanketing the surface of the liquid with nitrogen did not stop surface reaeration. To completely purge oxygen from the space above the liquid took about 15 minutes, during which time the solution was exposed to a gas of varying oxygen concentration. When pure nitrogen finally overlaid the surface, there was counter-diffusion of oxygen from the liquid surface into the gas. Hence, there was a two-fold loss of oxygen to the cells and to the atmosphere.

It seemed best to allow air to cover the liquid surface and to correct for surface reaeration. To determine the amount of oxygen supplied through the surface, the fermentor was charged with a glucose-phosphate solution at 25°C. The liquid was then stripped of oxygen by sparging nitrogen through it.

When the solution was free of oxygen, the nitrogen flow was stopped, the nitrogen above the surface of the liquid was replaced by air and oxygen was allowed to diffuse into the solution through the surface. The short time taken to replace nitrogen with air was not critical in this case, since reaeration continued for at least an hour before the liquid became saturated with oxygen. The oxygen concentration in the liquid was recorded as a function of time.

From these data, it was possible to calculate a mass transfer coefficient for reaeration. The method of calculation of the coefficient is given in a later section.

Using an electrode of their own design, Phillips and Johnson (59) found a significant lag in meter response to changes in dissolved oxygen concentration. To check this, the electrode previously described was placed in a saturated solution of oxygen in water which was being stirred. A mixture of sodium sulfite and copper sulfate was added in order to quickly reduce the dissolved oxygen concentration to zero. The meter reading fell from 1400 to 100 in 18 seconds and to 0 in 30 seconds. This test was repeated with a sodium sulfite solution containing cobalt ions with similar results.

3. The Rotational Viscometer

Since sugar and glycerol form Newtonian solutions, their viscosities were measured with a capillary tube viscometer at 25.0°C. Carboxymethyl celluloses (CMC), however, form non-Newtonian solutions. Because their flow curves are not linear, another instrument was necessary to measure the viscosity of the CMC solutions.

For this purpose a rotational viscometer (70) was used. The material was confined between long vertical coaxial cylinders. The outer one could be rotated at various speeds, while the inner one was attached to a resistance wire strain gauge that converted the shear force caused by contact of the fluid and the fixed internal cylinder into a direct current electrical signal. A tachometer generator geared to

the drive of the outer cylinder provided direct current signals proportional to the rate of shear. These two currents were fed to an X-Y recorder, which plotted the shear stress from the strain gauge, as a function of the shear rate from the tachometer generator. Thus, a continuous flow curve was plotted for the sample as the speed of the rotating cup was progressively increased.

4. Bacterial Species

A strain of Pseudomonas ovalis, NRRL B-8S was obtained from the Northern Utilization Research and Development Branch of the United States Department of Agriculture located at Peoria, Illinois. Lockwood et al. (47) had previously reported that this strain oxidized glucose to gluconic acid without the development of side products. This organism was also used by Tsao and Kempe (44) to measure oxygen uptake by Pseudomonas ovalis in resting cell suspensions.

5. Media

A medium of the following composition was used for cultivating the microorganism:

Glucose	50	grams
Yeast extract	5.0	grams
Urea	2.0	grams
KH_2PO_4	0.6	grams
$MgSO_4 \cdot 7H_2O$	0.25	grams
Distilled water	1.0	liter

When a solid medium was required, 2% agar was added. About

ten ml. of the agar medium were slanted in each test tube. Twelve hundred ml. of the liquid culture were placed in a two-liter Erlenmeyer flask which was stoppered with a cotton plug. Both the slants and the liquid culture were autoclaved at 15 pounds gauge pressure for 25 minutes.

To resuspend the cells, a nitrogen-free medium of the following composition was employed:

Glucose	50	grams
K_2HPO_4	0.9	grams
KH_2PO_4	0.6	grams
Polyglycol P-2000[7]	0.025	cc
Distilled water	2.0	liters
pH	7.0	

Since there was no need to maintain aseptic conditions, this medium was not autoclaved.

6. Stock Cultures

In order to preserve the original culture, the organism was grown in a liquid medium in test tubes for 24 hours at 30°C, after which the cultures were frozen in a dry ice-alcohol bath and stored at -10°C. Every three months, one of these cultures was quickly melted and used to inoculate fresh tubes of sterile media. After incubation, these cultures were added to the collection, which was stored at a low temperature.

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7. Dow Chemical Company, Midland, Mich.

7. General Experimental Conditions

The organism was carried on agar slants which were incubated at 30°C ± 2°C. Daily transfers were made from a 24-hour slant to a fresh slant.

In preparation for a run, a slant culture was streaked and incubated for 24 hours. About ten ml. of a sterile liquid medium were aseptically poured into the slant tube. The cells on the surface of the agar were mixed into this medium with a sterile transfer needle, and the suspension was then poured into a two-liter Erlenmeyer flask that contained 1200 ml. of fresh medium.

The inoculated medium was incubated for 24 hours at 30°C on a rotary shaker.[8] A setting of 3.0 on the shaker dial resulted in a shaking action of 180 RPM with a circular motion 1.5 inches in diameter.

8. Physical and Chemical Measurements

All measurements were made on cells grown in liquid medium at 30°C for 24 hours. The dimensions of 10 cells were measured with an American Optical AO Spencer microscope using a 10X eyepiece and a 97X, oil immersion, objective. The ocular micrometer in the instrument was calibrated against a stage micrometer. One slide was made from a 10^{-2} dilution of the cells in physiological saline. It was fixed with heat, stained with crystal violet and examined under oil immersion. Another smear of cells in their growth medium was not heat

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8. Model VS, New Brunswick Scientific Co., New Brunswick, N. J.

fixed, but was also stained with crystal violet and examined on a wet mount. A third measurement of cell size was made using unstained cells. In this instance, the growth medium was diluted with physiological saline and the cells were examined on a wet mount.

The moisture content of cells, harvested in a Sharples continuous centrifuge, was determined by placing about one gram of packed cells in tared, dry, aluminum-foil dishes. The cells were dried at 110°C until a constant weight was obtained.

To measure the ash content, about one gram of packed cells was placed in a tared, dry crucible. After combustion for one hour at 1800°F, the crucible was cooled in a desiccator and reweighed.

In the following four chemical analyses, a stock solution of 10% by weight of cells in distilled water was used. The stock solution was further diluted to 1:100, 1:1000 and 1:10,000 to give readings within limits of the tests.

To analyze for protein, the Folin phenol method (48) was employed. The method was a colorimetric one, in which the sample was treated with an alkaline copper solution and then with the Folin reagent. The intensity of color of the blue solution which developed was determined in a colorimeter using a red, 660 mμ filter. The actual amount of protein was then obtained when the reading was compared to a standard curve prepared using crystalline, bovine albumin. Distilled

water was used to set the zero reading on the colorimeter.

The diphenylalanine method (72) was employed to analyze for deoxyribonucleic acid. Here the sample was extracted with cold 5% trichloroacetic acid, centrifuged and the supernatant discarded. The precipitate was treated with diphenylalanine reagent and the solution boiled. After the solution was cooled, the intensity of the color was determined with the colorimeter, using a green, 540 mμ filter. Distilled water was used to set the zero reading on the colorimeter. The amount of deoxyribonucleic acid was obtained when the reading was compared to a standard curve prepared using Herring sperm DNA which was run concurrently with the Pseudomonas ovalis sample.

The orcinol method (16) was used for the determination of the ribonucleic acid content of the cells. This technique involved an initial extraction with cold, 50% acetic acid followed by centrifugation and treatment of the precipitate with an HCl - $FeCl_3$ reagent and an alcoholic orcinol solution. The solution was boiled, cooled and the intensity of the color evaluated in a colorimeter using a red 660 mu filter. Distilled water was used to set the zero reading on the colorimeter. The readings were compared with a series of solutions of known concentrations that were run concurrently, and the amount of ribonucleic acid determined.

The amount of carbohydrate was determined by the anthrone-sulfuric acid method (71). The cells were boiled with anthrone-sulfuric acid reagent, cooled and the color

intensity was determined with a colorimeter. A red, 660 mμ filter was employed. The blank used to set the zero reading on the colorimeter was a sample of distilled water carried through the test concurrently. Results were obtained when these readings were compared to standard glucose solutions tested at the same time.

The growth characteristics for Pseudomonas ovalis in a shaken culture were determined at 30°C. The test was performed with 100 ml. of medium in a 250 ml. Erlenmeyer flask, to which a side-arm colorimeter tube had been attached. The flask was inoculated with 0.01 ml. of a 24 hour liquid culture. This inoculum was obtained by aseptically diluting one ml. of a 24 hour broth in 100 ml. of fresh, sterile media.

As growth occurred, turbidity and protein content were frequently determined. Viable and total cell counts were made at only a few points.

To analyze for protein, one ml. of the culture was aseptically removed and centrifuged[9] for 15 minutes at about 1800 RPM. The supernatant was discarded; the cells were resuspended and recentrifuged under the same conditions. One ml. of water was added to the drained cell pack and the cells were resuspended and stored at 0°C for a period not exceeding 24 hours. Storage at such low temperatures does not affect the protein content (28). The Folin reagent method described

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9. Model CL, International Clinical Centrifuge, International Equipment Co., Boston, Mass.

previously, was used to determine the protein content of the cells.

To measure the rate of thermal killing of the micro-organism, one ml. of a 24 hour culture of Pseudonomas ovalis was placed in 99 ml. of a gelatin-saline solution. The saline in each dilution bottle was equilibrated with the water in a constant temperature bath. Temperatures employed were 25.0, 48.0, 53.5 and 60.5°C (± 0.5°C). The decrease in the number of viable cells was determined at each temperature by making pour plates with trypticase soy agar. These plates were incubated in an inverted position at 35°C for 24 hour and counted.

9. Procedure for a Run

The culture was removed from the shaker, and about one ml. of 1.0 N sodium hydroxide was added per 100 ml. of broth to raise the pH from 4.3 to 7.0. Then the cells were vigorously aerated for 30 minutes by means of air blown through a sintered glass sparger. After this neutralization and aeration process, the cells were separated from the broth by centrifuging[10] at 1300 G for 20 minutes. The supernatant was discarded and the cells resuspended in two liters of the nitrogen-free glucose medium described previously.

This cell suspension was poured into the fermentor, the agitator and air flow were started and a sample of the medium taken. The pH of this initial sample was determined with a

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10. Model V, Size 2, International Centrifuge, International Equipment Co., Boston, Mass.

Beckmann model H-2 pH meter and used to standardize the recorder-controller. This sample was also used for measuring the initial turbidity.

The rate of gluconic acid production was obtained from the rate of sodium hydroxide addition. The level of sodium hydroxide in the burette was read every 5 to 10 minutes; readings were continued until a steady rate of acid production was established which usually required about one hour.

When the concentration of dissolved oxygen in the liquid medium was desired, the agitator was stopped and the head plate removed. The oxygen electrode was then mounted on one of the baffles and the run started again. A steady reading for the oxygen concentration in the solution usually established itself within 5 minutes.

To measure the rate of oxygen utilization with the electrode, the air was shut off, but the agitator was not. As the dissolved oxygen was used by the cells, the level in the fermentor fell; then the data for the concentration of dissolved oxygen in the solution was plotted against time to determine the rate of removal.

10. Bacterial Densities

The concentration of bacteria was measured with a Klett-Summerson photoelectric colorimeter[11] using a blue, 420 mμ filter, against a distilled water blank. In order to compare data from several runs, it was desirable to find a correlation

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11. Klett-Summerson Photoelectric Colorimeter, Klett Manufacturing Co., New York.

between cell concentrations and turbidity.

A concentrated suspension of Pseudomonas ovalis, in the nitrogen-free medium described previously, was diluted with an equal volume of the same solution. The turbidity of the suspension was read with the colorimeter before and after dilution. The diluted sample, in turn, was mixed with an equal volume of solution, and the turbidity was determined again. This process was repeated until the suspension was almost clear.

To relate turbidity and viable cell numbers, counts were made at two different cell concentrations of Pseudomonas ovalis in the buffered medium. The cells in the glucose-phosphate medium were spread on the surface of trypticase soy agar, inverted and incubated for 24 hours at 35°C.

Dry weights were also determined for several cell concentrations. Twenty-five ml. of the cell suspension in phosphate solution at pH 7.0 were pipetteted into tared aluminum dishes. The phosphate solution contained 0.9 g. K_2HPO_4 and 0.6 KH_2PO_4 per liter of distilled water. The dishes were placed in an oven at 105°C and reweighed after 24 hours. There were three samples for each concentration including the phosphate solution used as the buffering agent. The weight of the blank was subtracted from the dry weight.

11. Alteration of Viscosity

For these studies it was desirable to use a non-Newtonian fluid having an easily varied viscosity. It was also desirable to add as little solid material as possible

to the water so as not to alter the oxygen solubility or the osmotic pressure.

The materials chosen were Natrosol 250 H[12] and Methocel 65 HG, 4000 CP[13]. Both of these compounds are high molecular-weight cellulose ethers. They are readily soluble in water. Small amounts of them increase the viscosity markedly, and neither is affected by moderate concentrations of acids, bases, or salts. They are fairly resistant to microbiological degradation, and their viscosities show no time dependency up to 2% by weight of the dry material in water. The density of these solutions is approximately 1.0 g/ml.

Natrosol is cellulose etherified with hydroxyethyl groups. In a 2% solution, the viscosity measured by a Brockfield viscometer may be as high as 25,000 centipoises at 25°C (39). Methocel is a propylene glycol ether of cellulose. A 2% solution of 65 HG results in a viscosity of about 4000 centipoises when measured at 25°C.

To initiate a run, the cells were grown and harvested in a manner described previously. Since turbidimetric readings were affected by the viscosity additives, a suitable way to control cell concentration was to spin down the same volume of a 24 hour broth culture each time. About 1.2 liters of a 24 hour cell suspension were used for each run; the Klett reading was about 100 if the cells were re-suspended in 2 liters of distilled water.

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12. Hercules Powder Company, Wilmington, Delaware.
13. Dow Chemical Company, Midland, Michigan.

For a run, the cell pack was resuspended in a small volume of distilled water. This concentrated cell suspension and a solution of 50 grams of glucose in water were added to a solution of the viscosity agent which had been prepared the night before. The total volume was made up to 2.00 liters.

This suspension was poured into the fermentation vessel, and the rate of gluconic acid production determined using a stirring speed of 570 RPM and an air rate of 1.77 VVM in order to maintain a high dissolved oxygen level in the liquid. By placing the oxygen probe into the medium at the end of the run, it was possible to determine the dissolved oxygen content of the broth.

A sample of the viscous broth of each run was saved and stored in a refrigerator in order to determine its viscosity at a later time. The viscosity test required about 300 ml. which were placed in the central compartment of the rotational viscometer and allowed to equilibrate at $30.0 \pm 0.1^{\circ}C$. During the viscosity test, the speed of the outer cylinder was increased automatically in steps from 32 to 438 RPM. After reaching maximum velocity, the process was reversed. A curve of shear rate against shear stress was obtained.

12. Sulfite Oxidation

Many oxygen transfer studies have been conducted using oxidation of sodium sulfite to sulfate in the presence of a suitable catalyst, such as cupric or cobalt ions. The method

was introduced by Miyamoto *et al.* (53, 54), and adopted for the evaluation of agitated gas-liquid contactors by Cooper *et al.* (19).

In this series, no living cells were employed. Three liters of an aqueous solution containing 0.25 mole of sodium sulfite per liter were placed in the fermentor. Air was bubbled through the liquid at a constant rate of 1.0 VVM. The agitator was set at 300 RPM, and a solution of $CuSO_4$ was added to provide a concentration of cupric ions of 10^{-3} moles/l.

Ten ml. samples were withdrawn at appropriate intervals and pipetted into 25 ml. of an iodine solution of known concentration. The excess iodine was back-titrated with a sodium thiosulfate solution. In this way, the concentration of the sulfite was determined; the rate at which sodium sulfite disappeared was a measure of the rate of oxygen transfer. Details of this method, including equations for the chemical reactions involved, have been described more fully by Tsao (93).

The viscosity was increased by incorporating sucrose or glycerol into the original three liters. The viscosity of the medium was measured at 25.0°C with an Ostwald viscometer calibrated against liquids of known viscosity.

13. Measurement of Oxygen Transfer Rates Using the Oxygen Electrode

The rate of oxygen transfer to cell-free solutions was determined by measuring the increase in the dissolved oxygen

concentration in the liquid with the oxygen electrode. Before each aeration test, the water was deoxygenated; in the series of runs in which the agitation rate was studied, nitrogen was bubbled through the solution; in the series to determine the effect of viscosity and air flow rate, a small amount of sodium sulfite was added. All tests were conducted at 25.0°C. No changes were made in stirring speed, air rate or medium composition during each test.

In the investigation of the effect of agitation, no air was supplied to the sparger. The only supply of oxygen to the liquid was from air overlaying the surface. The liquid medium was the same glucose-phosphate solution used to re-suspended the Pseudomonas ovalis cultures.

In the other two tests also conducted at 25.0°C, the agitator was constantly turning at 300 RPM. Air was sparged into the liquid at the rate of 0.2 VVM in the series in which the effect of viscosity was examined. In these tests, Methocel was dissolved in distilled water.

In the last series, the air rate was altered. The medium contained 2.5 g/l of Methocel dissolved in distilled water.

The operation of the electrode has been described previously in detail. In this series, it was set at a zero reading in the deoxygenated liquid and at a full scale deflection of 1400 millivolts in water saturated with oxygen when in contact with air. Both of these settings were accomplished at 25.0°C.

III. EXPERIMENTAL RESULTS

A. Preliminary Experiments

1. Physical and Chemical Measurements

Microscopic observation of the cells showed them to be rods with rounded ends. The dimensions, which varied somewhat according to the methods of staining and fixing, were:

1.6 x 0.5 microns when stained with methylene blue and the cells were dry mounted

2.5 x 0.5 microns when not stained and the cells were wet-mounted

2.3 x 0.7 microns when crystal violet was added and the cells were wet-mounted.

These data agree with those of Aiba *et al.* (1), who reported values ranging from (1.4 to 2.5) x 0.6 microns for his 8-hour cultures of *Pseudomonas ovalis*. An electron photomicrograph of these bacteria magnified 10,000 X is shown in Figure 7.

Chemical analyses of the bacteria are reported as percentages of the dry weight in Table II; moisture content of packed cells was found to be 71.4% of the wet weight.

2. Growth Curve

Readings of turbidity and protein content defined the growth curve. Viable cell counts, at a limited number of points, provided date for demarcation of the stationary and declining phases.

The generation time was found to be 42 minutes during the exponential growth period. This value for the generation

TABLE II

CHEMICAL ANALYSIS OF *Pseudomonas ovalis*

Determination	Percentage by Weight (dry base)
Protein	34
Carbohydrate	20
Ribonucleic acid	9.1
Deoxyribonucleic acid	7.8
Ash	6.7

Figure 7. Electron micrograph of Pseudomonas ovalis NRRL B-8S, grown for 24 hours on an agar surface (X 10,000).

time is in general agreement with reported values of 30-40 minutes for most microorganisms. The initial lag phase lasted 7.5 hours. A maximum viable population of 1.6×10^9 cells/ml. occurred at about 12 hours, following inoculation.

From these data, it can be seen in Figure 8 that the cells entered the stationary phase after approximately twelve hours. There was no significant decline in the number of viable cells at 24 hours; however, at this point the culture was in the stationary phase of growth. In the stationary period, there should be very little change in the characteristics of the cells with time; this should result in good reproducibility between runs in which such cells are used for the conversion of glucose to gluconic acid as described previously.

3. Thermal Death Rate

The thermal destruction of bacteria is generally considered to be caused by coagulation of cellular protein (43). As such, the death rate can be represented by a first order reaction, where the rate of death is directly proportional to the number of living cells present at the beginning of any interval of time:

$$\frac{dN_o}{dt} = -kN_o t \qquad (7)$$

where N_o is the number of bacteria in the volume under consideration, t is the time of exposure to the killing temperature and k is the rate constant for thermal destruction.

Figure 8. Growth of Pseudomonas ovalis, NRRL B-8S in a 5% glucose medium at 30°C.

TABLE III

GROWTH OF *Pseudomonas ovalis* NRRL B-8S IN A 5% GLUCOSE MEDIUM AT 30°C

Time Hours	Klett Reading	Protein Contents µg/ml.	Viable Cells number/ml.
0	28	-	1.1×10^5
4.5	28	-	1.0×10^6
8.5	49	22	6.8×10^6
9.2	67	33	-
9.5	80	44	-
9.9	105	60	-
10.5	140	84	7.0×10^8
11.0	167	118	-
12.3	212	166	1.6×10^9
12.5	218	-	-
22.8	350	233	1.3×10^9
23.3	355	-	-
24.3	360	233	1.3×10^9
30.5	385	259	-
47.0	420	290	-
52.0	425	300	1.9×10^8

At room temperature, there was no measurable decrease in the concentration of viable cells. At the other four temperatures, the number of cells decreased quickly. The data for cell numbers were plotted in Figure 9, and a slope, k, was determined for each temperature. Q_{10} values, which are defined as the ratio of the velocity constant at one particular temperature to the velocity constant at a temperature ten degrees lower, were calculated using the formula:

$$\log Q_{10} = \frac{10}{t} \log \left(\frac{k_{T_2}}{k_{T_1}} \right) \qquad (8)$$

These Q_{10} values are found in Table VI. Lamanna and Mallette (46) reported that the Q_{10} values of spores were in the range 3.8 - 10.7, with higher values occurring as the temperature of killing increased. Comparable values for vegetative cells may reach 70. Both Deindoerfer (20) and Rahn (63) reported that the Q_{10} values decrease as the temperature decreases, as was found in this work.

Rahn (63) gave several values for temperature coefficients in the range 55 - 60°C. These rose as high as 42 for Salmonella scottmuelleri, but usually were around 20 for the Salmonella species. Salmonella scottmuelleri, like Pseudomonas ovalis, is a gram negative rod.

In any given environment, k values for a species generally vary with temperature in a fashion which can be represented by the Arrhenius equation. This equation is usually used to represent the large changes in velocity of chemical reactions with temperature (4):

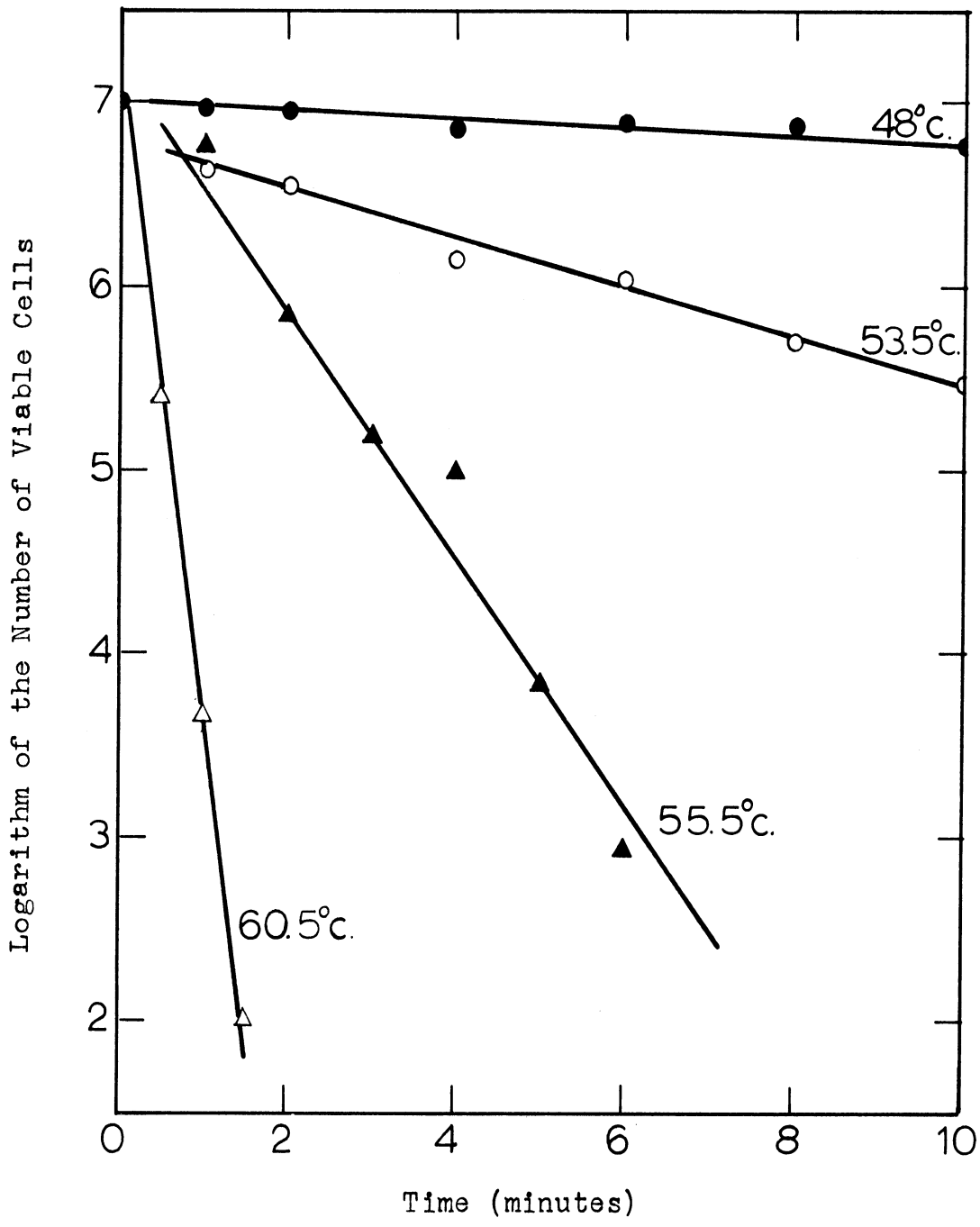


Figure 9. Effect of temperature on survival of Pseudomonas ovalis in a gelatin-saline medium.

TABLE IV

EFFECT OF TIME AND TEMPERATURE ON THE NUMBER OF VIABLE CELLS OF A 24 HOUR CULTURE OF *Pseudomonas ovalis* CELLS IN GELATIN-SALINE BROTH

Time of Exposure minutes	Number of Viable Cells at the Following Temperatures of Exposure (°C)				
	25.0	48.0	53.5	55.5	60.5
0	7.8×10^6				
1/2					2.5×10^5
1		9.5×10^6	4.3×10^6	6.0×10^6	4.5×10^3
1 1/2					1.0×10^2
2		9.0×10^6	3.6×10^6	7.0×10^5	0
3				1.6×10^5	
4		7.6×10^6	1.5×10^6	1.0×10^5	
5	9.6×10^6			7.2×10^3	
6		7.8×10^8	1.1×10^6	9.0×10^2	
8		7.5×10^6	5.0×10^5		
10	8.0×10^6	6.0×10^6	3.0×10^5		
	Av. 8.5×10^6				

TABLE V

EFFECT OF TEMPERATURE ON THE THERMAL DEATH RATE CONSTANT FOR A 24 HOUR CULTURE OF Pseudomonas ovalis IN GELATIN-SALINE BROTH

Temperature	k*
48	0.022
53.5	0.18
55.5	0.66
60.5	3.9

*k is the thermal death rate constant for Equation 7, which relates number of surviving cells to time.

TABLE VI

TEMPERATURE COEFFICIENTS FOR THERMAL DEATH RATES OF A 24 HOUR CULTURE OF *Pseudomonas ovalis* IN GELATIN-SALINE BROTH

Temperature Range °C	Q_{10}
48 - 60.5	96
53.5 - 60.5	82
55.5 - 60.5	35

$$k = A_A e^{-E_A/RT} \qquad (9)$$

where A_A is the frequency factor, R the universal gas constant, T the absolute temperature and E_A is an apparent activation energy for heat of destruction of the microorganism. Taking logarithms:

$$\log k = \log A_A - \frac{E_A}{2.3\ RT} \qquad (10)$$

For all practical purposes, log A_A may be treated as a constant (20). Then the equation is of the form y = mx + b and a plot of log k versus 1/T for any species should yield a straight line with a slope of $-E_A/2.3$ RT.

The data were plotted in Figure 10. Using the slope of the resulting straight line, the activation energy was found to be 90,000 calories when the data for the thermal death rate of Pseudomonas ovalis were examined by the method of least squares. The intercept, log A_A, was found to be 62.2. Chick and Martin (13) reported values for protein denaturation and the lethal action of heat on bacterial spores to be 60,000 and 135,000 respectively. Pfeiffer and Vojonovich (58) reported the range of Arrhenius activation energies to be 50,000 to 100,000 cal/mole.

4. Products of the Fermentation of Glucose by Pseudomonas ovalis

Pseudomonas ovalis has been reported (47) to yield only gluconic acid when supplied with glucose and oxygen as nutrients. The equation for the reaction has been given by

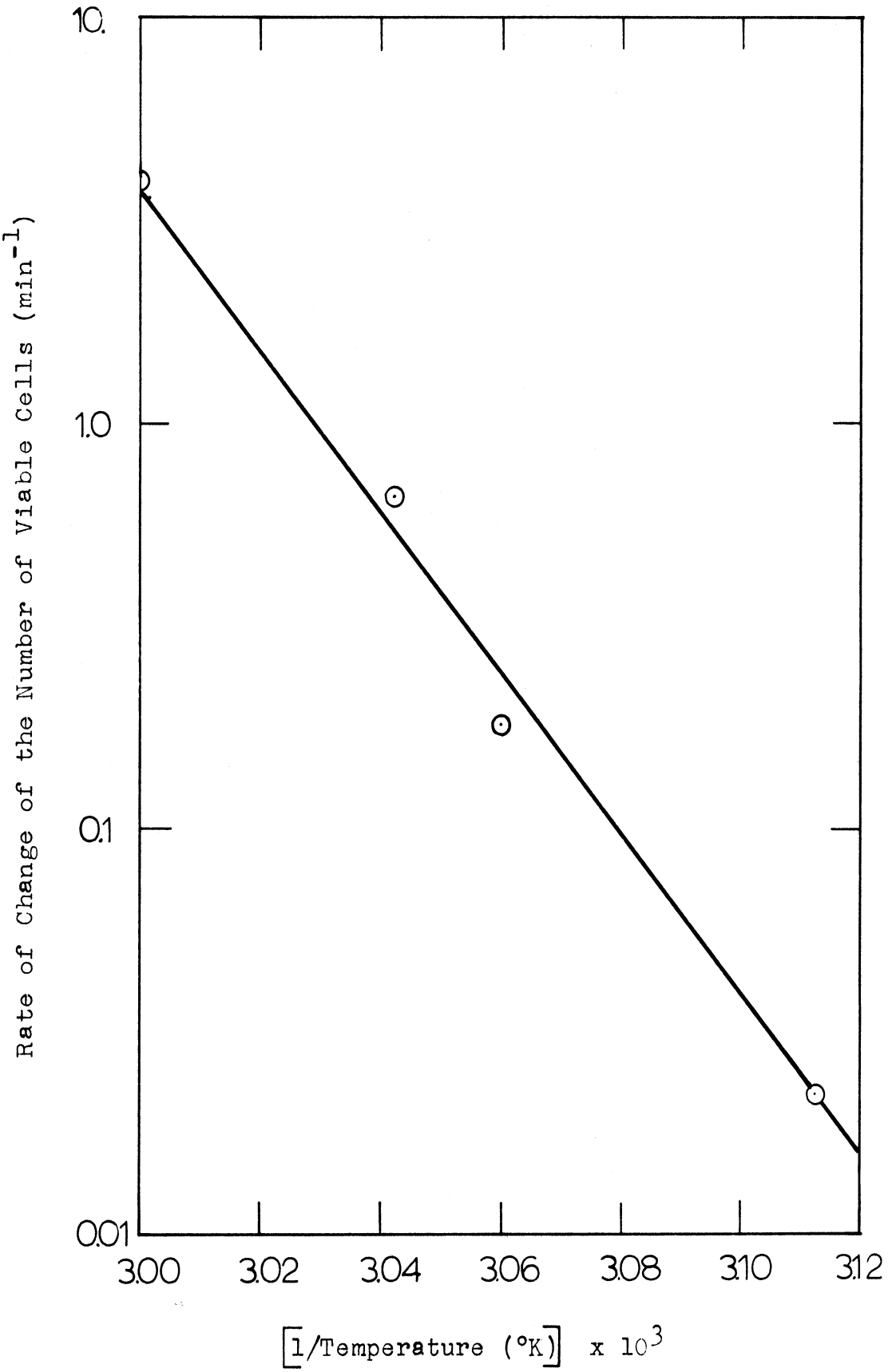


Figure 10. Arrhenius plot for the effect of temperature on the survival of Pseudomonas ovalis in a gelatin-saline medium.

Tsao (93):

$$C_6H_{12}O_6 + \tfrac{1}{2} O_2 \longrightarrow C_6H_{12}O_7$$

glucose — gluconic acid

Tsao (93) reported conversion of 99% of the glucose to acid at pH 5.5. Aiba et al. (1) found 95% conversion at pH 6.0 in a resting cell suspension. A resting cell suspension of Pseudomonas ovalis was produced by separating cells from their growth medium by centrifugation. The liquid was discarded and the cell pack was resuspended in a glucose solution containing no nitrogen. The absence of nitrogen prevented the cells from reproducing. Therefore, the only process taking place was respiration, and all oxygen utilized was used for this purpose. A suspension of this type will be referred to as a "resting" cell suspension.

The rates of glucose utilization and of acid production were measured in resting cell fermentations at pH 5.5 and 7.0. The concentration of glucose was measured by the anthrone method; the acid formation rate was determined by measuring the rate of sodium hydroxide addition. Both values were plotted against time with the degree of conversion being taken as the slope of the glucose utilization rate divided by the slope of the acid production rate.

A conversion of 96% was found for the single run made at pH 5.5. At pH 7, three runs were made with 93, 98, and 100% conversions being found.

To determine the pK_a of the acid produced in the fermen-

tation of glucose by Pseudomonas ovalis, cells were resuspended in a solution of glucose and water. The phosphate buffer was used. The cells were allowed to ferment the sugar until approximately 0.08 moles acid had been produced.

This suspension was centrifuged to remove the cells; the supernatant liquid was decanted and acidified. It was then titrated with sodium hydroxide from its initially low pH of 3 to a pH of 11.

The buffer index, β , was determined for each addition of base by dividing the amount of base added by the change in pH resulting from that addition. Van Slyke (95) has shown that the pK_a is the value of the pH at the maximum value of the buffer index, i.e., the point at which the addition of either acid or base results in a minimum pH change. The supernatant liquid from the experimental run yielded a value of 3.56. The Merck Index (50) lists the pK_a value for gluconic acid as 3.60. The pK_a values for reagent grade sodium and potassium gluconate were also tested. They were found to be 3.58 and 3.70 respectively.

5. Turbidity and the Estimation of Bacterial Populations

The most widely used procedure for the measurement of bacterial density is the determination of transmitted or scattered light (46). Data for a series of two-fold dilutions of a concentrated suspension of Pseudomonas ovalis with a glucose-phosphate medium are recorded in Table VII.

The turbidity, measured in Klett units, has been plotted against cell concentration in Figure 12. Arbitrarily, the

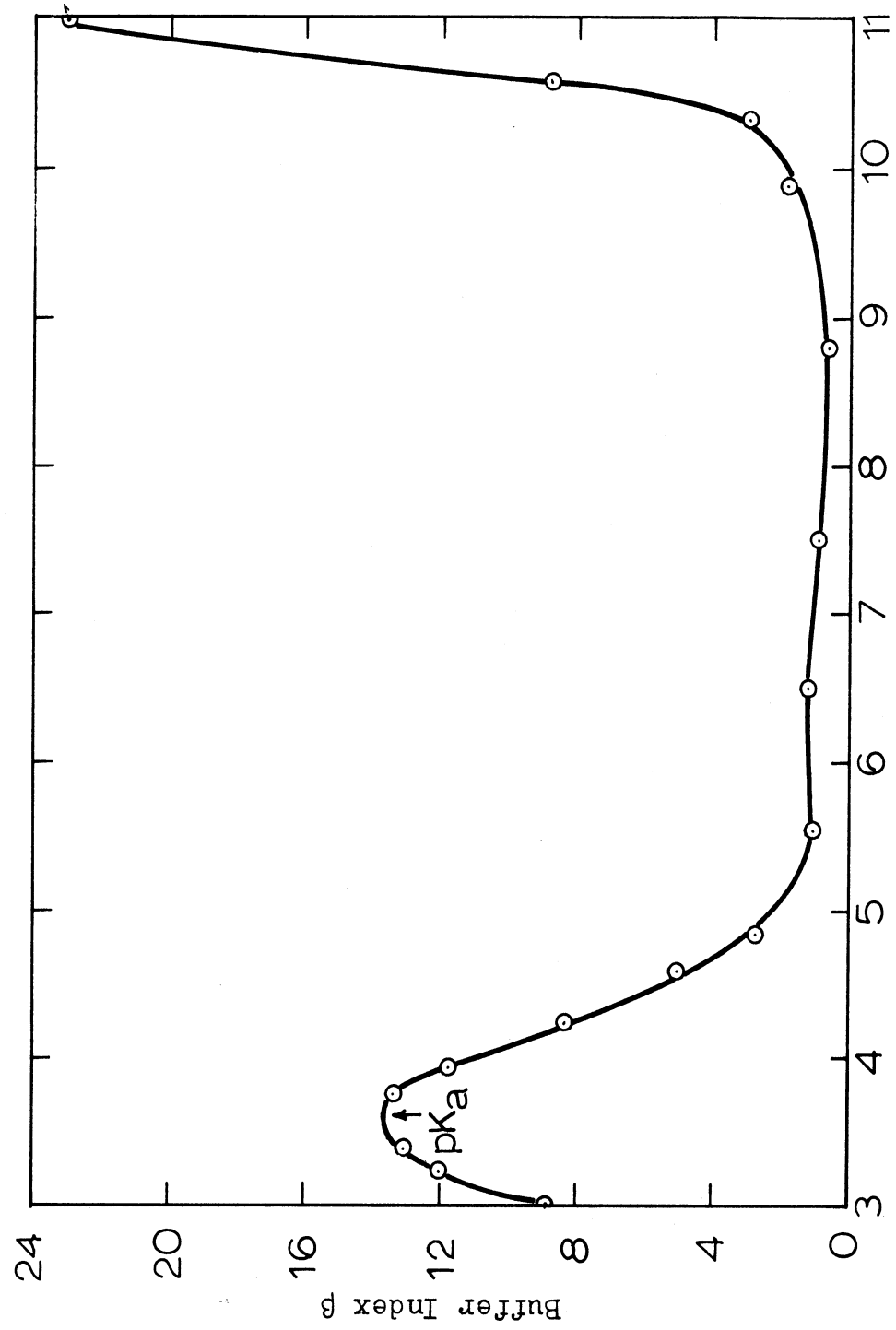


Figure 11. Graphical determination of the pK_a of the acid formed during the fermentation of glucose by Pseudomonas ovalis, NRRL B-8S in a resting cell suspension.

TABLE VII

RELATIONSHIP BETWEEN TURBIDITY AND THE LOGARITHM OF THE MULTIPLE OF DILUTION OF A SUSPENSION OF *Pseudomonas ovalis* WITH A GLUCOSE-PHOSPHATE MEDIUM

Logarithm of the Multiple of Dilution to the Base Two	Turbidity of the Cell Suspension	Cell Concentration	Fractional Transmittance of Light	Metameters of D (Optical Density)
	Klett Reading	Arbitrary Units	D	-log D or 0.6+log (1-D)
0	975	512	0.0167	1.777
1	810	256	0.0245	1.611
2	630	128	0.0549	1.260
3	465	64	0.112	0.951
4	333	32	0.216	0.666
5	206	16	0.387	0.412
6	85	8	0.667	0.109
7	46	4	0.810	-0.121
8	24	2	0.896	-0.383
9	10	1.0	0.955	-0.747
10	7.5	0.5	0.966	-0.869
11	5.0	0.25	0.9775	-1.048

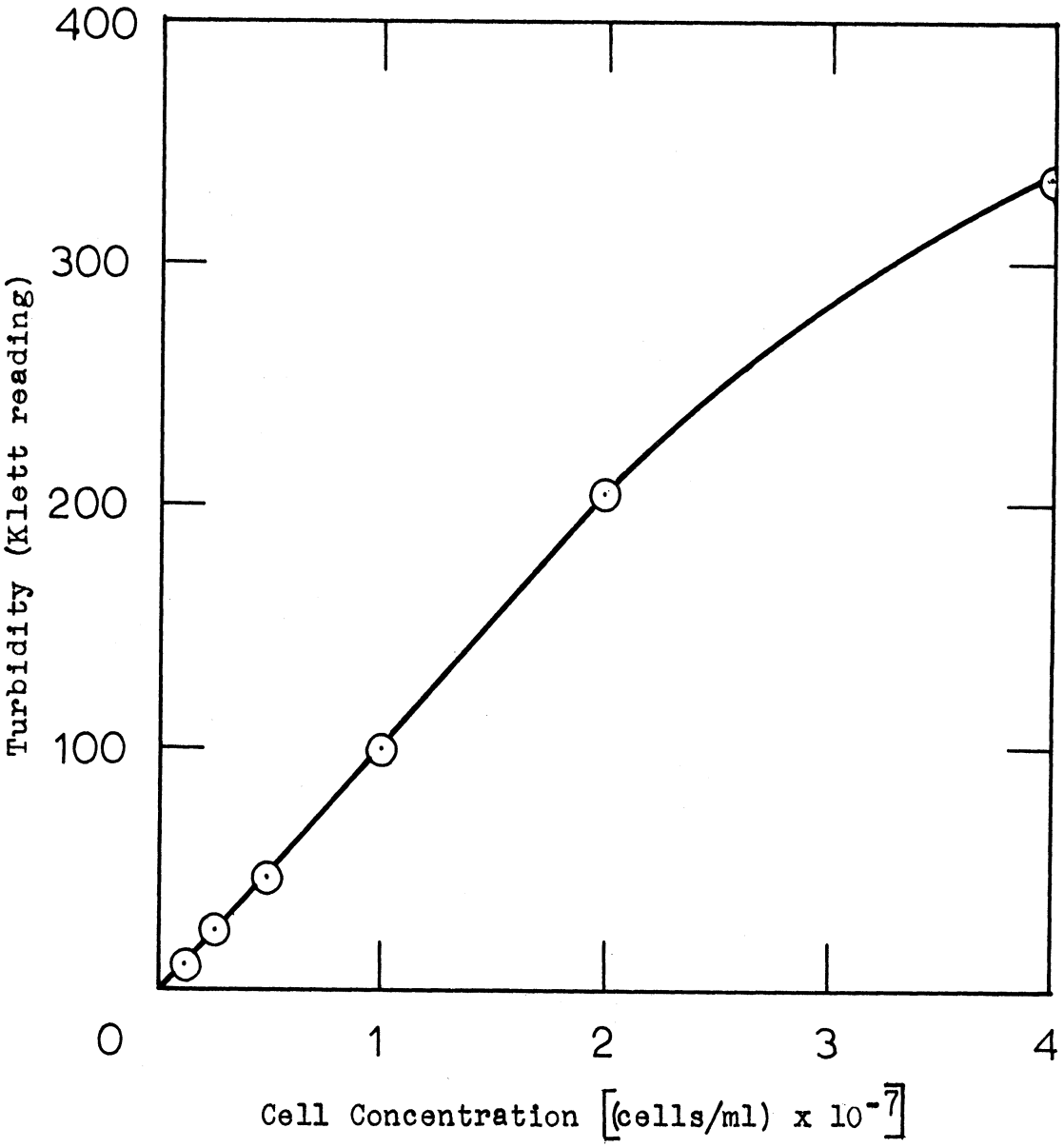


Figure 12. Relationship between turbidity and cell concentration for a suspension of Pseudomonas ovalis in glucose-phosphate medium.

cell suspension having a Klett reading of 10 was taken as a concentration of cells equal to 1. Each succeeding measurement had a cell concentration twice that of the previous one. In the lower range of cell concentrations (with the Klett reading less than 100), the graph was linear, indicating that Beer's Law was obeyed.

Beer's Law is an expression which relates the absorbance of light to the density of a given bacterial suspension. It can be expressed as:

$$\log_{10}\left(\frac{I_o}{I}\right) = \eta\, O \qquad (11)$$

where I is the intensity of an incident beam of light striking a solution, I_o the intensity after passing through the solution, η the turbidity coefficient and O is the number of microorganisms in cells/ml. When Beer's Law is followed, as is the case for lower concentrations of Pseudomonas ovalis, a plot of log (I_o/I), or Klett reading which is directly related to it, is linear.

In this range, all corrections for deviations in cell concentration were made using Klett readings directly. For example, in the study of the effect of pH on the rate of production of gluconic acid, all rate data were corrected to a cell concentration corresponding to a Klett reading of 100. If in one run the Klett reading was 110 and acid was produced at the rate of X meq/(l)(hr.), then the corrected acid rate for a run having a cell concentration corresponding to a Klett reading of 100 would be (100/110) X meq/(l)(hr.).

At higher cell concentrations (greater than a Klett reading of 200) a considerable departure from a straight line occurred. Hence, the usual method to determine the number of cells in a medium containing a high cell population is to dilute the sample until a more suitable range is reached.

Kurokawa *et al.*, (45) however, showed that the data for turbidity could be represented by a linear plot over the whole range of measurable values, if (-)log D for ($0 \leq D \leq 0.5$) and $0.6 + \log (1-D)$ for ($0.5 \leq D \leq 1.0$) were plotted against the logarithm to the base two of the multiple of dilution of the suspension. The term (-)log D has been defined as optical density, and D is the fractional transmittance of light. These data are plotted in Figure 13.

Corrections for deviations in cell concentrations at higher levels can be made using this plot. For example, it can be seen that a medium which has a turbidity corresponding to a Klett reading of 333 has twice as many cells/ml. as one having a Klett reading of 206. For intermediate values, corrections were made by changing the Klett reading to transmittance, calculating (-)log D or $0.6 + \log (1-D)$ and getting the arbitrary concentration value which could be related to any other Klett reading.

In order to relate these arbitrary cell concentrations to some definite value, several cell suspensions were evaporated to dryness. The data relating dry weight and Klett reading are found in Table VIII.

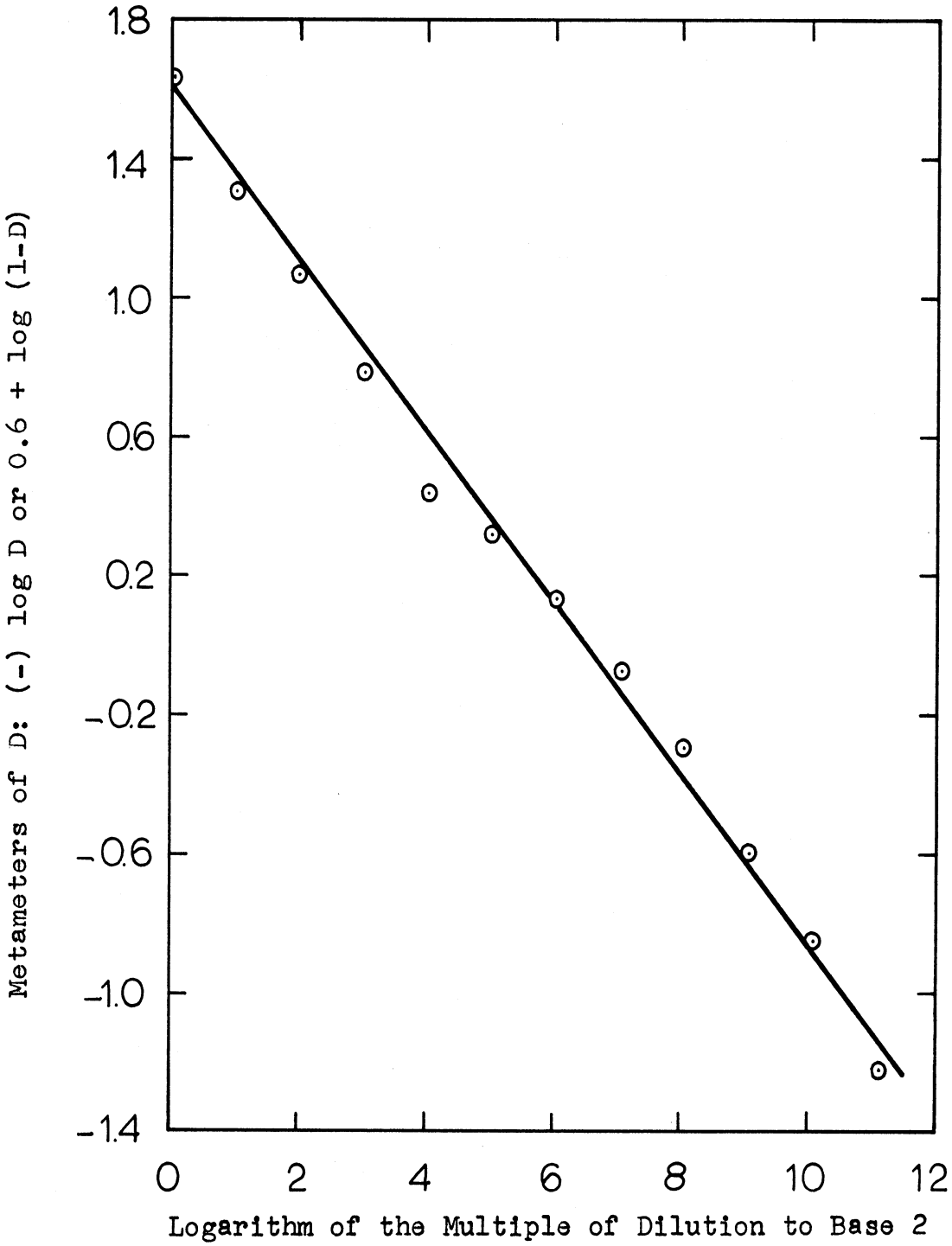


Figure 13. Relationship between transmittance (D) or its metameters and the logarithm of the multiple of dilution of a suspension of Pseudomonas ovalis with glucose-phosphate medium.

TABLE VIII

RELATIONSHIP BETWEEN TURBIDITY AND DRY CELL WEIGHTS FOR *Pseudomonas ovalis*

Klett Reading	Dry Weight g/liter
97	0.22
100	0.23
206	0.53
206	0.57
286	0.78
317	0.83
406	1.54

Numbers of viable cells were also measured as a function of turbidity. At Klett readings of 159 and 233, the number of viable cells/ml. was 1.6×10^7 and 3.5×10^7 respectively.

B. Effect of pH, Temperature and Concentration of Cells on the Rate of Production of Gluconic Acid by Pseudomonas ovalis

1. Effect of pH

Two series of runs were made. The first series used cells still in their growth medium while, in the second, the cells were centrifuged from the growth medium and then resuspended in the nitrogen-free glucose-phosphate medium previously described.

Two liters of media inoculated 24 hours previously and incubated on the shaker at 30°C were placed in the fermentor with 50 grams glucose and 0.025 cc of the antifoam agent. The suspension was agitated at 300 RPM and sparged with air at a rate of 1.16 VVM.

To determine the cell concentration, 10.0 ml. of the suspension were centrifuged in an International Clinical Centrifuge, model CL, at approximately 4000 RPM for 15 minutes. The clear supernatant liquid was discarded, and 10.0 ml. of distilled water were used to resuspend the cells. The turbidity of the suspension was determined with a Klett colorimeter.

Several runs were made with the pH being held at a constant value in each run. Initially, the pH of the medium

was about 4.3 due to acid production during growth of the cells. Sodium hydroxide, 1.0 N, was used to raise the pH to the desired value for the run. During each run, the pH was kept constant. Determinations of the rate of acid production were made at pH values ranging from 5.5 to 10.0.

Concentration of the base used for control of the pH was varied from 0.1 to 1.0 normal. The choice of concentration was governed by the fact that dilute alkali increased the precision of the determination of the rate of acid production. However, too dilute a solution altered the volume of the liquid in the fermentor beyond an acceptable value.

It was necessary to apply two corrections to the data; one accounted for variations in the cell concentration, while the other corrected for the effect of undissociated acid.

The Kurokawa plot (Figure 13) relating turbidity and cell concentration was used for the first correction. The second, and minor correction was developed for weak acids in solution as follows: in solution, weak acids ionize to give:

$$HA \rightleftharpoons H^{+} + A^{-} \qquad (12)$$

for which an ionization equilibrium constant can be written

$$K_a = \frac{[H^+]\ [A^-]}{[HA]} \qquad (13)$$

Taking derivatives at a constant pH,

$$\frac{d[HA]}{dt} = \frac{[H^+]}{K_a}\ \frac{d[A^-]}{dt} \qquad (14)$$

From equation 14, one can see that the rate of production of dissociated acid is proportional to the total acid production.

The rate of production of gluconic acid, both dissociated and undissociated, can be calculated when both the pH and ionization constants are known. For gluconic acid, the correction becomes only important below a pH of 6. For a sample calculation of these corrections, see Appendix D.

The data for variation in acid rate with pH are plotted in Figure 14. The maximum rate of acid production was 14.5 meq/(l)(hr.) at a pH of 7.15. The Michaelis-Menten equation can be used to examine the data (10):

$$\frac{R_m}{R_A} = 1 + \frac{1}{K}\left(\frac{[H^+]}{[H_o^+]} + \frac{[H_o^+]}{[H^+]}\right) \qquad (15)$$

Here, R_A is the velocity of acid production at a hydrogen ion concentration of $[H^+]$, and R_m is the maximum acid production rate at a hydrogen ion concentration of $[H_o^+]$. Using the optimum values given above for R_m and $[H_o^+]$, K was found to be 15. The resulting equation represents the data with an average arithmetical deviation of 3.1% over the pH range from 5.75 to 8.0.

Suspended cells were used in a similar series. In this case, the initial pH was established by adding either 1.0 N hydrochloric acid or 1.0 N sodium hydroxide. The air rate, agitation speed and temperature were the same as those used for the cells when they were prepared in their growth medium. Turbidity of the suspended cells, which was usually within 10 Klett units of 100, was measured before the pH was adjusted.

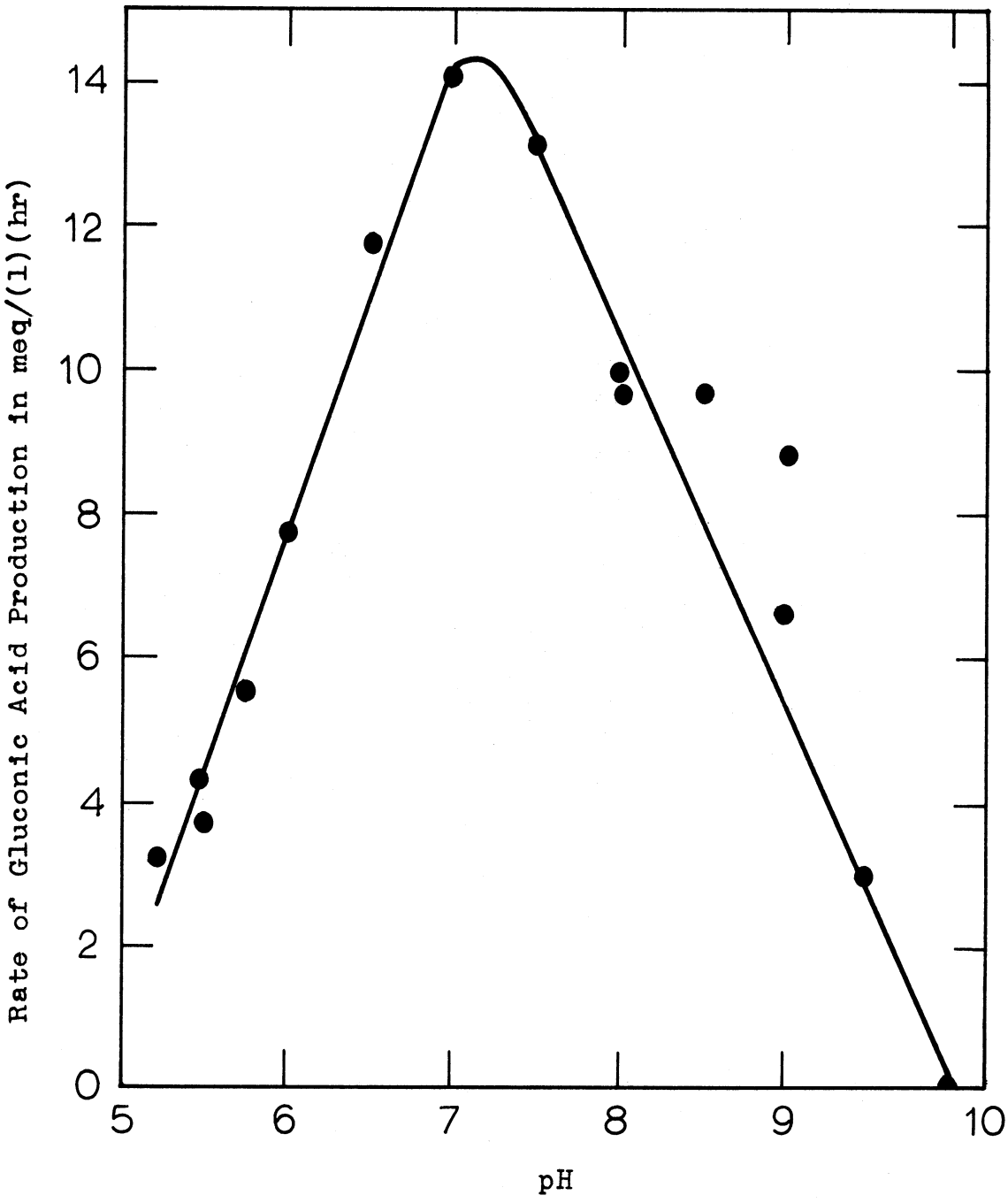


Figure 14. Effect of pH on the rate of production of gluconic acid by Pseudomonas ovalis in a growth medium at 25°C and Klett reading 300.

These data, shown graphically in Figure 15, may also be represented by the Michaelis-Menten equation (15). For a maximum rate of acid production of 2.85 meq/(l)(hr.) at a pH of 7.35, K is 18.4. The Michaelis-Menten equation represents the data with an average arithmetical deviation of 8.5% over the range of pH values from 5.85 to 8.5.

When the two plots for the rate of acid production are examined, it is seen that cells resuspended in a nitrogen-free medium become inactive at pH 5 and 9.7, while cells in their original growth medium will still produce acid. For suspended cells, the K value in Equation 11 is larger. The larger value of K indicates a greater effect of pH on the reaction. This is reasonable, since the cells in their growth medium would be better protected by components of the medium such as yeast extract which acts as a buffer.

2. Effect of Temperature

To study the effect of temperature on the rate of acid production, Pseudomonas ovalis cells were suspended in the nitrogen-free medium described previously. The rate of agitation was 300 RPM and the liquid was sparged with air at the rate of 1.16 VVM.

The cell concentration was measured with the Klett photoelectric colorimeter. Usually, the reading on this instrument for a sample of the liquid from the fermentor was close to 100. To account for any deviation in concentration from this figure, a correction was applied to the rate of gluconic acid production of the cells by multiplying

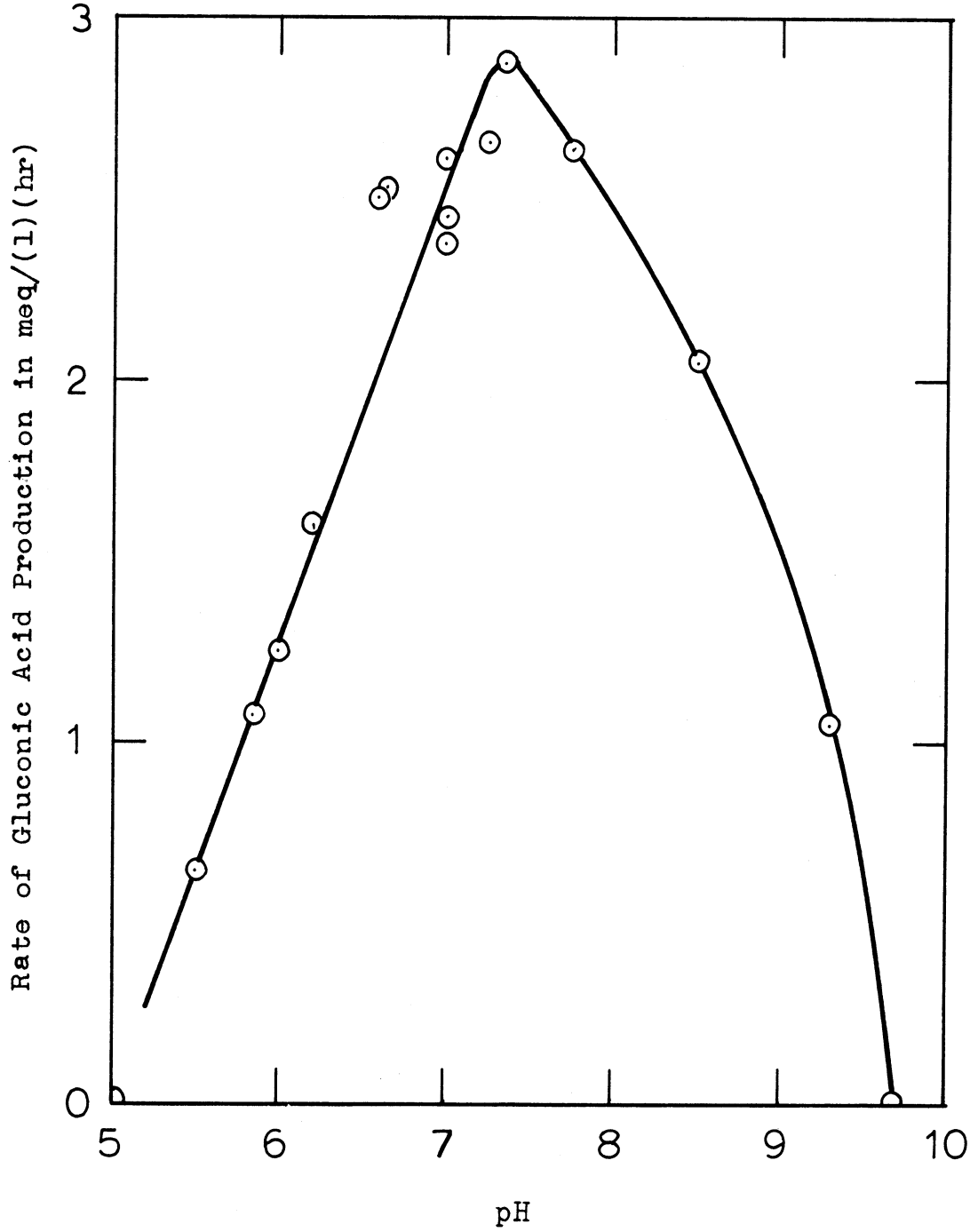


Figure 15. Effect of pH on the rate of production of gluconic acid by Pseudomonas ovalis in a nitrogen-free medium at 25°C and Klett reading 100.

it by 100/Klett reading.

Runs were made at temperatures between 9°C and 40°C, with the rate of acid production being measured (Figure 16). The data can be correlated using the Arrhenius equation. In Figure 17, the activation energy was found to be 9,600 cal/mole while the Q_{10} values for these runs are reported in Table X and range between 1.7 and 1.8.

These data agree fairly well with those reported in the literature. Sizer (76) stated that Q_{10} values for enzymes vary from 1.3 to 3.5 while Sumner and Somers (88) reported that the Q_{10} values vary between 1.1 and 2.0.

Sultzer (87) reported activation energy values for the oxidation of saturated fatty acids by Pseudomonas species and Pseudomonas geniculata ranging from 6,870 to 14,666 cal/mole. Ingraham and Bailey (41) studied the effect of temperature on the rate of oxidation of glucose by Pseudomonas species and Pseudomonas perolens. They found values of 11,600 and 9,040 cal/mole for the respective activation energies. Kempe et al. (44) also used a controlled pH to study the rate of acid production by Lactobacillus delbrueckii. From steady-state operation at various temperatures, they calculated values of 17,000 cal/mole for the activation energy in the conversion of glucose to lactic acid.

3. Effect of Cell Concentration

The effect of cell concentration on the rate of production of gluconic acid was investigated over a 30-fold

TABLE IX

EFFECT OF TEMPERATURE ON THE RATE OF GLUCONIC ACID PRODUCTION BY *Pseudomonas ovalis* IN A NITROGEN-FREE MEDIUM AT pH 7.0, WITH AN AGITATOR SPEED OF 300 RPM, AN AIR SPARGING RATE OF 1.16 VVM AND A KLETT READING OF 100

Run Number	Temperature °C	Acid Production Rate meq/(l)(hr.)
T 10	9.0	1.09
T 7	14.0	1.67
T 8	19.5	1.92
T 6	24.9	2.51
T 5	30.0	3.69
T 1	30.0	4.20
T 6	34.8	5.20
T 3	34.3	4.00
T 4	40.0	2.82

TABLE X

Q_{10} VALUES FOR THE RATE OF PRODUCTION OF GLUCONIC ACID BY RESTING CELLS OF *Pseudomonas ovalis* AT pH 7.0 WITH AN AGITATOR SPEED OF 300 RPM AND AN AIR SPARGING RATE OF 1.16 VVM

Temperature Range °C	Q_{10}
10 - 20	1.80
15 - 25	1.76
20 - 30	1.73
25 - 35	1.70

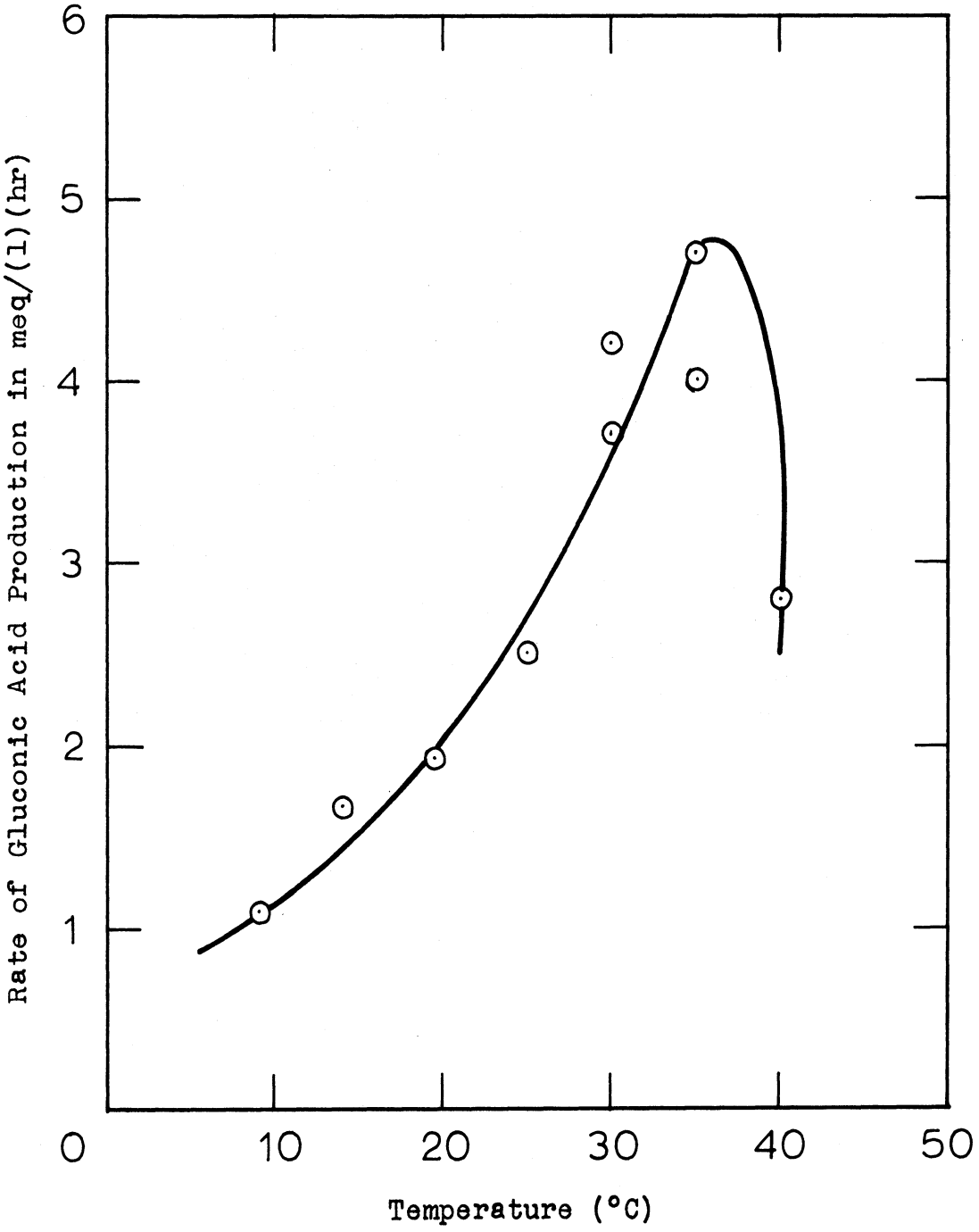


Figure 16. Effect of temperature on the rate of gluconic acid production by Pseudomonas ovalis in a nitrogen-free medium.

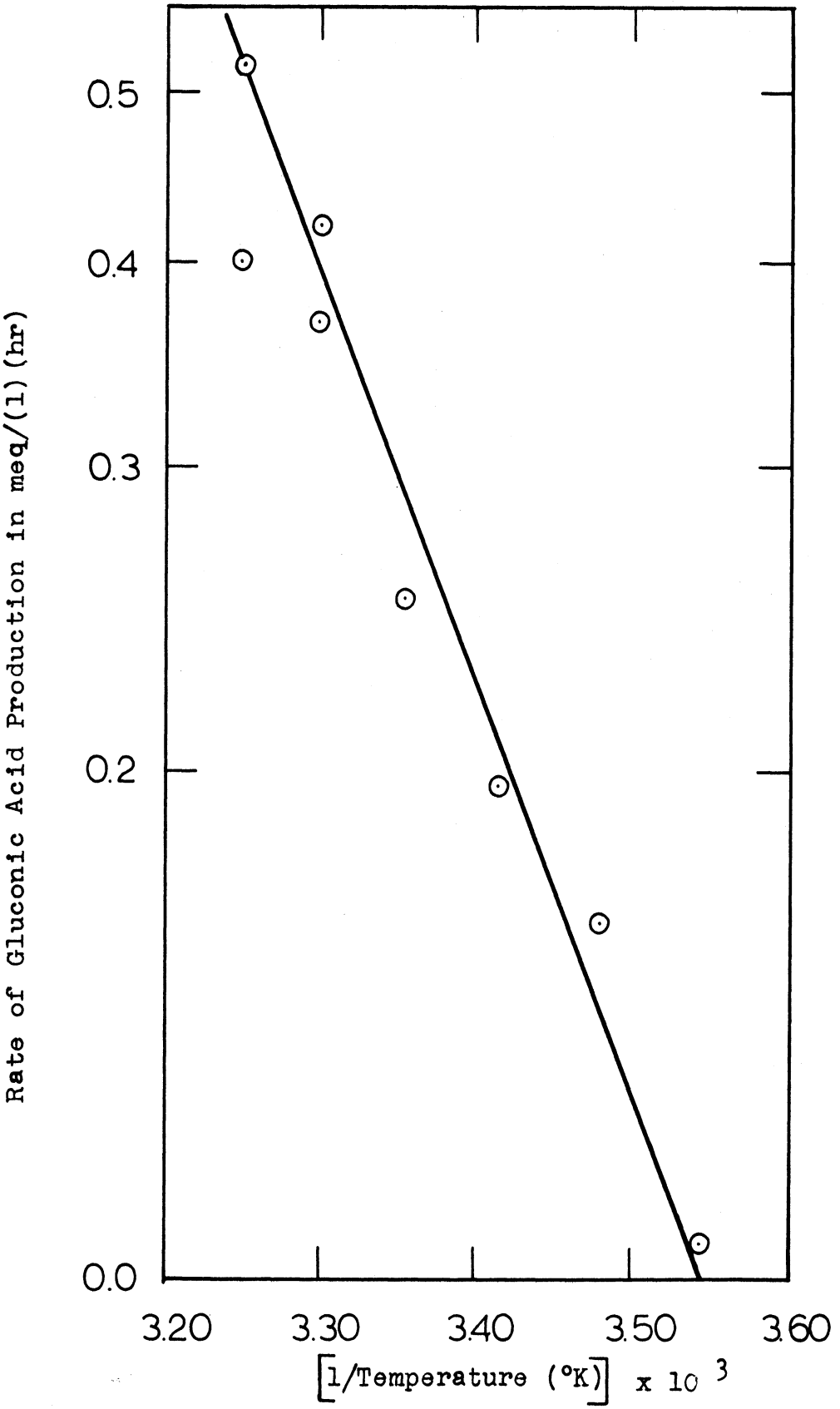


Figure 17. Arrhenius plot for the effect of temperature on the rate of gluconic acid production by Pseudomonas ovalis in a nitrogen-free medium.

range of concentrations. At a constant stirring speed and rate of air flow, acid production of Pseudomonas ovalis cells was proportional to cell concentration over a very wide portion of this range.

The scale on the abscissa on Figure 18 is the concentration of cells in number of viable cells per ml., obtained from turbidity values using a Klett-Summerson colorimeter and plate counts. From this plot, it can be seen that cell concentration increased as the oxygen concentration decreased, or vice versa depending upon which was designated as the independent variable. The values of the rate of gluconic acid production began to deviate from the straight line when the oxygen concentration in the medium fell to 40% saturation.

C. Direct Use of the Oxygen Electrode

1. Measurement of the Critical Oxygen Concentration

All respiration, according to Finn (32), proceeds at a rate which is independent of the dissolved oxygen concentration so long as the latter remains above a critical value. Above this critical oxygen concentration (C_c), the rate of oxygen uptake by cells is unaffected by an increase or a decrease in dissolved oxygen concentration.

Most measurements of the critical oxygen concentration are made by withdrawing samples from the fermenting medium and placing them in a polarographic cell. As the cells respire, the oxygen content of the liquid falls. The rate of oxygen utilization is constant until the oxygen concen-

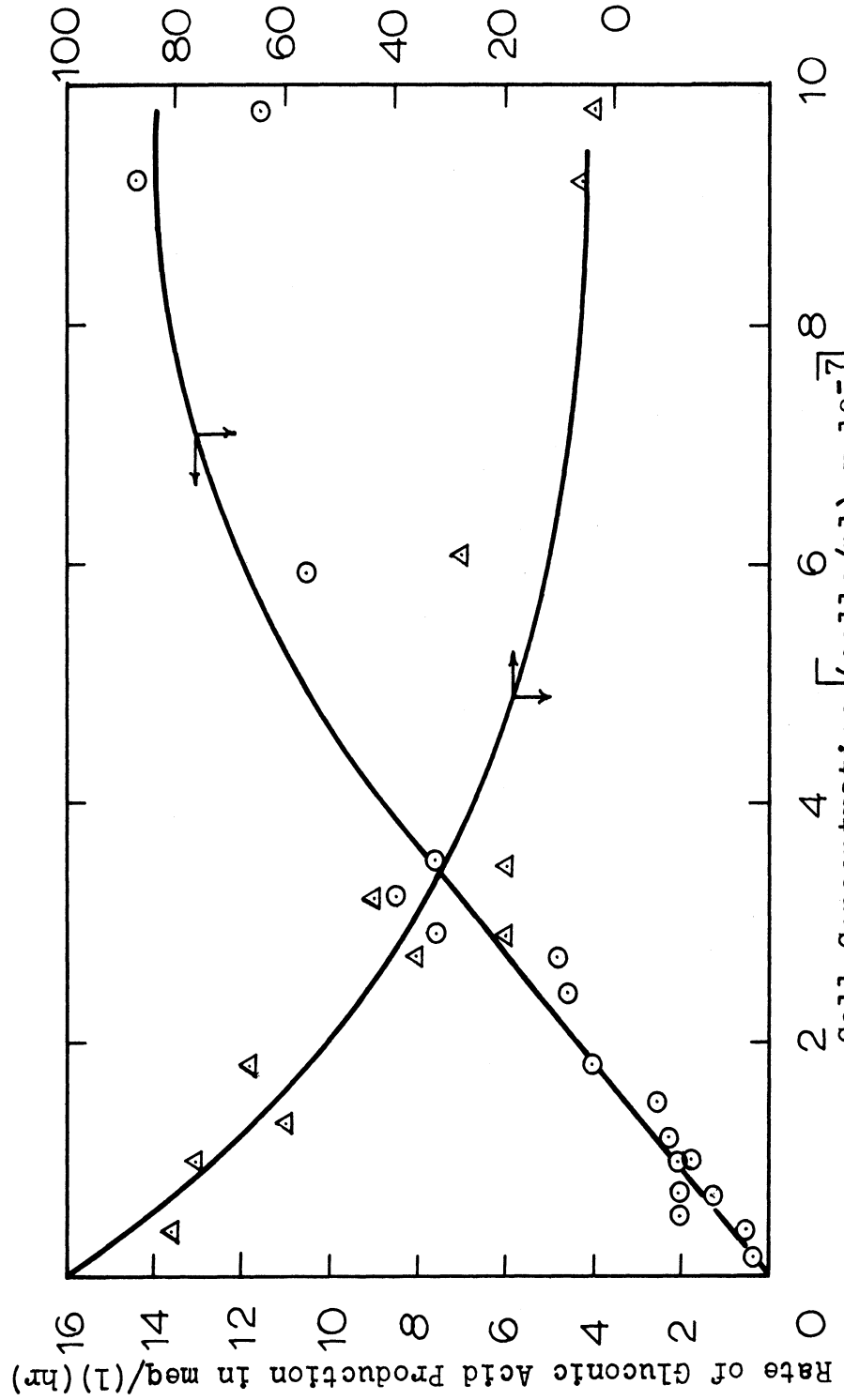


Figure 18. Effect of cell concentration on the rate of gluconic acid production and on the equilibrium oxygen concentration of the liquid during the fermentation of glucose to gluconic acid by resting cells of Pseudomonas ovalis.

tration reaches a very low value (3, 32).

In this work, a direct measurement was made without sampling, since the electrode was immersed in the medium which was being aerated and agitated. The value of $\underline{C_c}$ was obtained by stopping the air flow to the fermentor while still continuing to stir. The value of dissolved oxygen concentration, measured by the electrode, was recorded as a function of time.

In a system in which microorganisms are respiring, the level of the dissolved oxygen concentration is a balance between the rate of removal of oxygen by the cells and the rate of supply. The supply comes from two sources: (1) the gas sparged below the surface, (2) oxygen dissolved through the surface.

When the air supply to the sparger was turned off, the major supply of oxygen stopped. As a result, the dissolved oxygen concentration fell. Unless the agitation rate was in excess of 400 RPM, there was insufficient oxygen diffusing through the surface to supply the needs of the cells, and the dissolved oxygen concentration fell to zero.

Figure 19 shows the change in dissolved oxygen concentration with time. The straight line indicates a constant rate of cell respiration. The slope of this line, the rate of change of oxygen concentration with time (dC/dt), is the apparent rate of oxygen uptake by the cells, and it is constant. The oxygen concentration at which this plot deviates from a straight line is the critical oxygen concen-

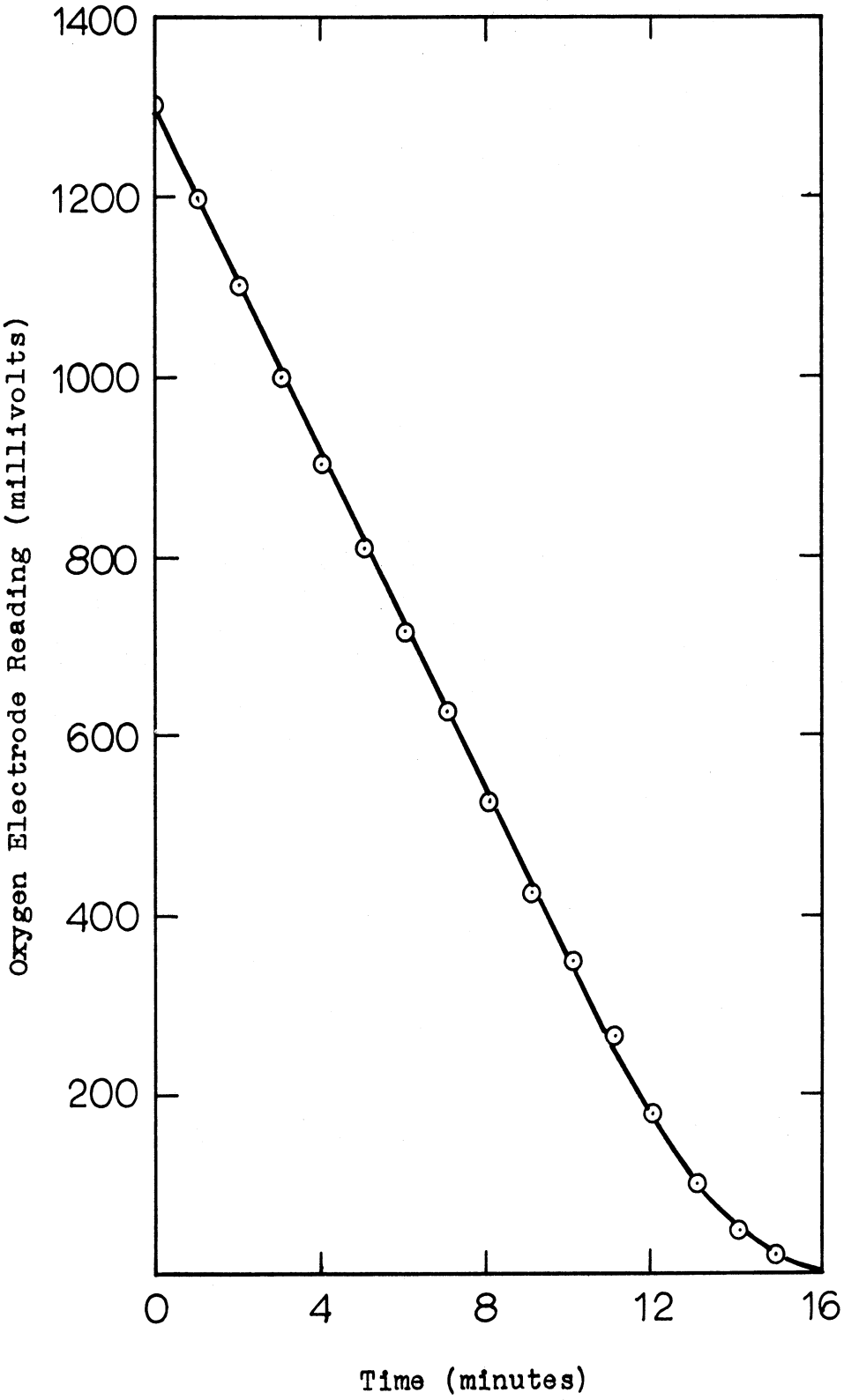


Figure 19. Measurement of the rate of oxygen utilization by resting cells of Pseudomonas ovalis using the oxygen probe with agitation at 114 RPM and no aeration.

tration.

Most previous workers used polarographic cells to measure the rate of change of oxygen concentration. In these cells, the surface of the liquid was covered or the surface area was small when compared to the volume. In both cases, the amount of oxygen dissolving into the liquid sample was probably small.

With the method of measurement of oxygen uptake described here, the amount of oxygen entering the liquid through the surface was significant. Moreover, this source of oxygen became more important as the turbulence of the surface increased due to greater agitation. In the present study, an attempt was made to blanket the surface with nitrogen to eliminate this surface supply. It was impossible to quickly eliminate all the oxygen from the head space of the fermentor by flushing the space over the liquid with nitrogen. Also, when pure nitrogen was involved, oxygen passed from the liquid into the gas by counter-diffusion.

Thus, a correction factor was applied to obtain the true oxygen uptake by the bacteria. This factor became more important as the stirring speed was increased and as the concentration of oxygen in the liquid decreased: at 114 RPM, the correction factor was very small.

The slope (dE/dt) of the curve in Figure 19 was plotted against oxygen concentration in Figure 20. The rate of oxygen uptake (dE/dt) was initially represented by a horizontal line which fell off rapidly to zero as the

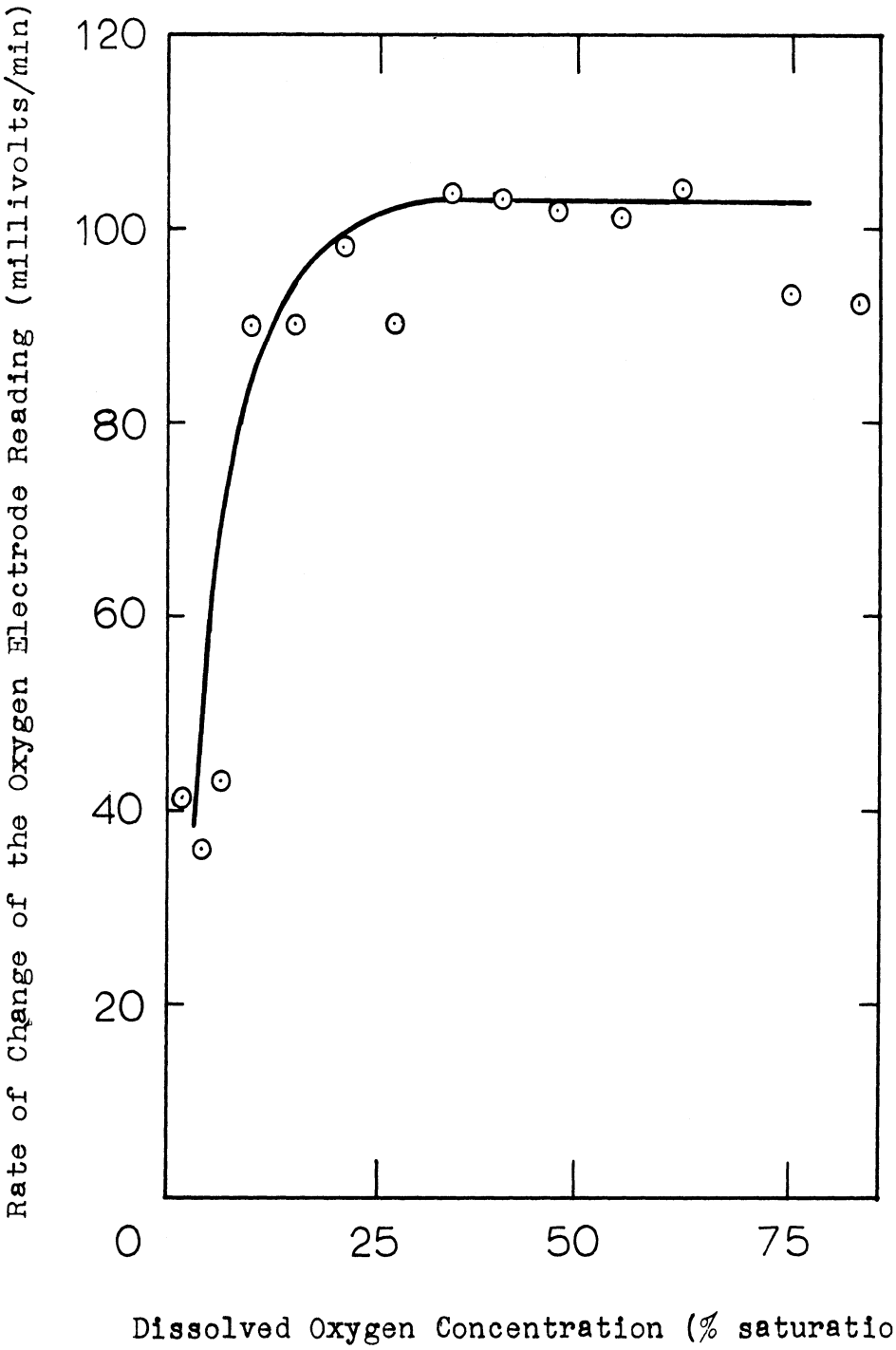


Figure 20. Effect of dissolved oxygen concentration on the rate of oxygen utilization by resting cells of Pseudomonas ovalis with no aeration at pH 7.0 and 25°C.

dissolved oxygen concentration approached zero. The point at which the oxygen utilization rate became dependent on oxygen concentration was the critical value, C_c.

C_c was determined in 15 separate runs; values were measured a total of 20 times. Stirring speed was kept constant for each determination; speeds employed were 114, 217 and 300 RPM. These data were analyzed statistically, and the critical oxygen concentration was found to be 1.1 ± 0.2 mg/l. It was not dependent on stirring speed. For comparison with these data, several values of C_c are listed in the introduction. They are lower than the value of 1.1 mg/l. reported here.

C_c, however, can vary widely for the same microorganism. Finn (14) has suggested that discrepancies may arise from differences in methods of measurement. Winzler (100) reported that the presence of a substrate such as glucose increases the critical level of dissolved oxygen. His data were restricted to one organism.

2. Measurement of the Rate of Oxygen Utilization by Pseudomonas ovalis

The slope of the curve, dE/dt, with appropriate corrections for transfer of oxygen through the surface, is a measure of the rate at which the microorganisms consume oxygen. Figure 19 represents oxygen uptake by Pseudomonas ovalis in an agitated, but unsparged, system.

Measurements of the two rates of oxygen utilization may be compared. The first rate was obtained by the continuous titration of the gluconic acid, while the second was calculated

from the oxygen electrode measurement.

The rate of oxygen utilization was first calculated from the data on the rate of production of gluconic acid:

$$R_G = \frac{N_B\,(dV_B/dt)}{V_F} \times \frac{1}{2} \quad \frac{\text{millimoles oxygen}}{\text{liter-hour}} \qquad (16)$$

The factor of one-half is necessary because the conversion of glucose to gluconic acid requires one-half mole of oxygen per mole of glucose.

Oxygen consumption for the cells without aeration of the liquid medium can be calculated from the electrode measurements:

$$R_E = \frac{(dE/dt)\,C_g}{(1/60)(32)(E_S - E_0)} \quad \frac{\text{millimoles oxygen}}{\text{liter-hour}} \qquad (17)$$

In the above expression, E is the measure of oxygen concentration expressed in millivolts. It is the direct reading from the pH meter. The difference in the reading on the millivolt scale of the pH meter between two oxygen electrodes placed in separate glucose-phosphate solutions of the same composition and temperature, one solution being saturated with oxygen from the air and the other being devoid of oxygen, was $(E_S - E_0)$.

The saturation concentration of dissolved oxygen in distilled water at 25°C has been reported to be 8.18 mg/l. (17). The medium used in this work contained glucose, sodium mono- and di- hydrogen phosphate, sodium gluconate,

antifoam and bacteria. The amount of oxygen needed to saturate the liquid was reduced by the presence of these solutes. Solomons (78) has reported data for variations in dissolved oxygen concentrations in water with various concentrations of glucose. These data have been used to predict the 6.8 mg/l. used as the saturation concentration for the medium in the work.

3. Effect of Oxygen Concentration on the Rate of Gluconic Acid Production

Preliminary experiments indicated that the rate of acid production might be affected by the concentration of dissolved oxygen in the medium as well as by the rate of air flow and stirring speed, even at values above C_c. This phenomenon was investigated in a separate series of runs.

It was shown that the acid production rate could be raised if agitation speed were increased from 114 to 400 RPM; changes in the air supply rate were unimportant. However, the reverse of this change could not be accomplished, i.e., lowering the stirring speed did not immediately reduce the rate of acid production. Whether the rate would eventually have dropped was not established.

The data recorded in runs D-3 to D-7 (Table XI) illustrate this effect. As the oxygen concentration was raised by changing the stirring speed to 400 from 114 RPM, the rate of and production increased. However, lowering the speed did not decrease the gluconic acid production rate within a reasonable length of time.

TABLE XI

EFFECT OF VARIATIONS IN THE AGITATION SPEED ON THE RATE OF GLUCONIC ACID PRODUCTION BY RESTING CELLS OF *Pseudomonas ovalis* AT pH 7.0 AND 25°C

Run	Turbidity of Cell Suspension, Klett Reading	Air Flow Rate VVM	Production Rate of Gluconic Acid at Low and High Agitation Speeds meq/(l)(hr.)			
			114RPM	400RPM	114RPM	400RPM
D-3	90	0.75	1.27	1.89	1.65	-
D-4	66	1.77	0.88	1.07	1.05	-
D-5	78	-	0.83	1.04	1.27	-
D-6	140	3.19	3.44	4.77	4.36	4.36
D-7	100	2.35	-	2.94	3.01	3.17
D-8	99	1.77	1.89	2.89	2.14	2.59
D-9	98	2.35	-	2.39	2.01	2.64

In an attempt to obtain this decrease in rate of acid production between the high and the low speeds, the following experiment was attempted: the fermenting medium was aerated and agitated at 400 RPM; the air was shut off for 30 minutes; the agitator was set at 114 RPM. As a result, the oxygen concentration fell to zero. During this period, the only oxygen supply to the liquid was by diffusion from air contacting the upper surface. Shutting off the air to the sparger, in this experiment, allowed the initial rate of gluconic acid production to be re-established (runs D-8 and D-9). In another run, D-10, several levels of dissolved oxygen were attained by altering both the stirring speed and the gas rate. A plot of rate of gluconic acid formation against the dissolved oxygen concentration is shown for this experiment in Figure 21.

At the end of this run, the "reducing technique" just described was tried, and a point was inserted on the plot at an acid production rate of 1.92 meq/(l)(hr.). It coincided with the curve drawn through the other data taken as the dissolved oxygen concentration was progressively increased.

This last run showed a direct relation between acid production and the concentration of dissolved oxygen. The alteration in oxygen concentration in the run was effected by changing the stirring speed and the rate of air supply to the cell suspension. To remove these two variables, three runs, D-12, D-13 and D-14 were conducted. In the first

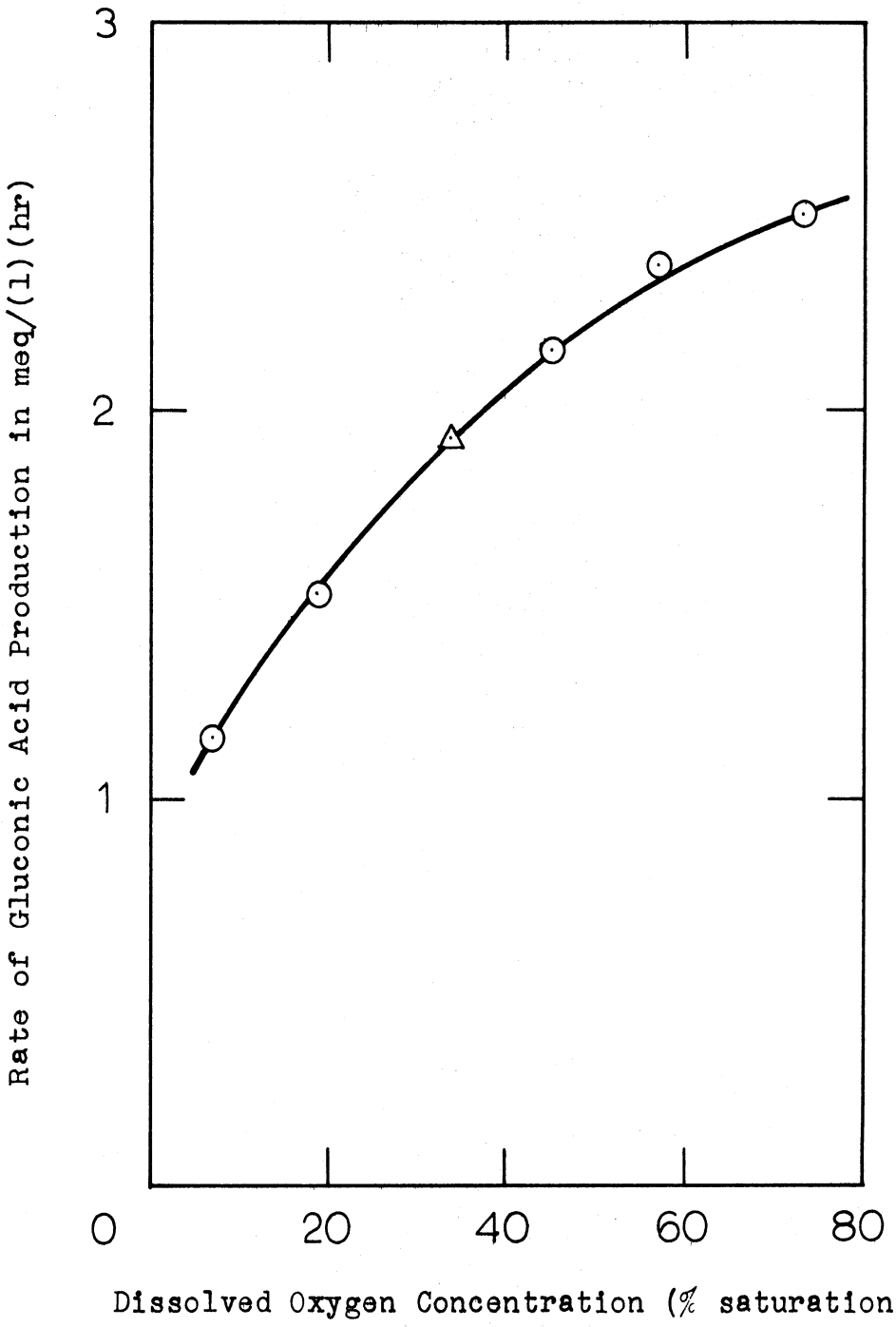


Figure 21. Effect of oxygen concentration on the rate of gluconic acid production (speed and air flow rate varied) by resting cells of Pseudomonas ovalis at pH 7.0 and 25°C.

two, the stirring speed was maintained at 300 RPM and the air was supplied to the fermentor at a rate of 2.0 liters per minute. However, the oxygen concentration in the gas stream was altered by mixing various ratios of air and nitrogen. The last run was similar to the first two, but the speed was increased to 400 RPM.

The rate of gluconic acid production at both 300 and 400 RPM increased with increasing oxygen concentration (Figure 22) until the concentration reached approximately 40% of saturation. The rate of gluconic acid production did not increase further with increases in dissolved oxygen concentration. It should be noted that the rate of acid production at 400 RPM exceeded the rate at 300 RPM.

Furthermore, the rate of acid production continued to rise with an increase in dissolved oxygen concentration, although the dissolved oxygen concentration was well above the critical. This last phenomenon has also been reported by Steel and Maxon (83). The slope of the curve showing the rate of gluconic acid formation at 400 RPM is steeper than the curve for 300 RPM.

Another run was made to determine the independent effect of stirring on the rate of gluconic acid production. In this case, the air sparging rate was held constant at 0.46 VVM, while the stirring speed was increased, in steps, from 114 to 570 RPM. Both the acid production rate and the oxygen concentration in the medium were measured during the fermentation; at the end of the run the rate of oxygen utilization

TABLE XII

EFFECT OF DISSOLVED OXYGEN CONCENTRATION ON THE RATE OF PRODUCTION OF GLUCONIC ACID BY RESTING CELLS OF *Pseudomonas ovalis* AT pH 7.0 AND 25°C

Run	Turbidity of Cell Suspension Klett Reading	Agitation Speed RPM	Rate of Air Flow VVM	Rate of Gluconic Acid Production meq/(l)(hr.)	Concentration of Dissolved Oxygen, % Saturation
D-10	104	114	1.00	1.14	7
		114	1.77	1.53	19
		157	2.35	2.16	45
		217	1.77	2.37	57
		300	0.23	2.49	73
		114	2.00	1.92	34
D-12	114	300	1.00	1.48	13
				1.87	29
				2.17	45
				2.22	60
				2.26	81
D-14	114	400	1.00	1.00	4
				1.87	14
				2.34	31
				2.61	43
				2.52	62
				2.52	73
				2.52	93
D-13	107	300	1.00	0.11	6
				0.88	15
				1.57	29
				1.90	50
				2.13	63
				2.24	82

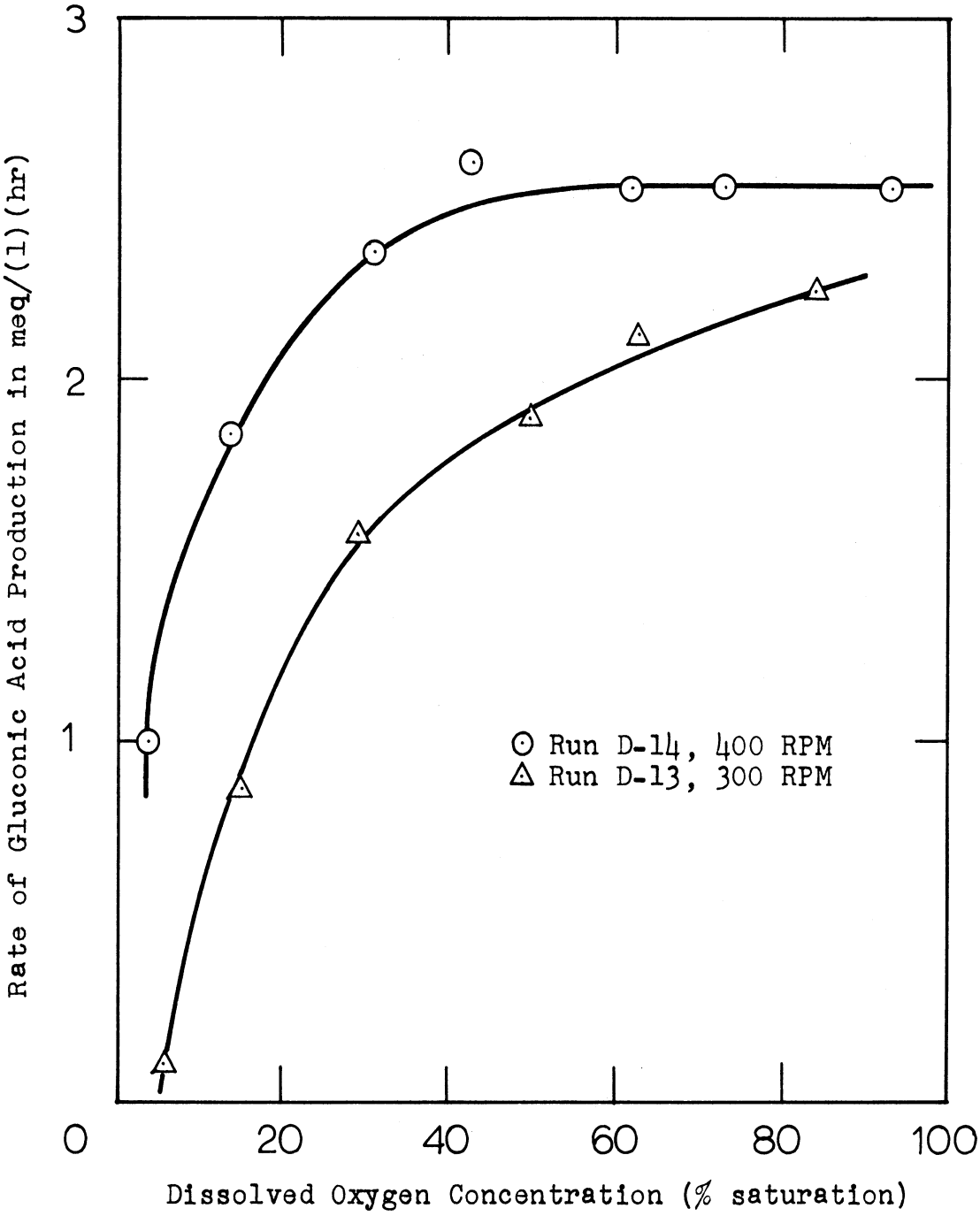


Figure 22. Effect of oxygen concentration on the rate of gluconic acid production by resting cells of Pseudomonas ovalis at 25.C, a constant air rate of 1.00 VVM and a pH of 7.0.

was determined using the oxygen probe.

It can be seen in Figure 23 that as the stirring speed was increased, both the concentration of oxygen and the rate of gluconic acid production increased. The lower, horizontal line represents the rate of oxygen uptake as measured by the oxygen probe. It is about 55% of the maximum oxygen transfer rate determined by measurement of the rate of acid production at 570 RPM.

Finally, the air flow rate to the medium containing suspended cells was varied to observe the effect on the dissolved oxygen concentration in a fermenting medium. Dissolved oxygen concentrations were measured in the medium at two agitation speeds. These data are recorded in Table XIV and are plotted in Figure 24. The results indicate that below 217 RPM, the air rate must be very high to maintain reasonable levels of dissolved oxygen in the liquid. Conversely, above 217 RPM only very low air flow rates are needed to supply the needs of resting cells of this concentration.

D. Effect of Viscosity

1. Sulfite System

Sucrose and glycerol, which develop Newtonian solutions in water (78), were used to conduct the initial experiments with increased viscosity. However, in order to attain significant increases in viscosity, large concentrations of these chemicals were needed.

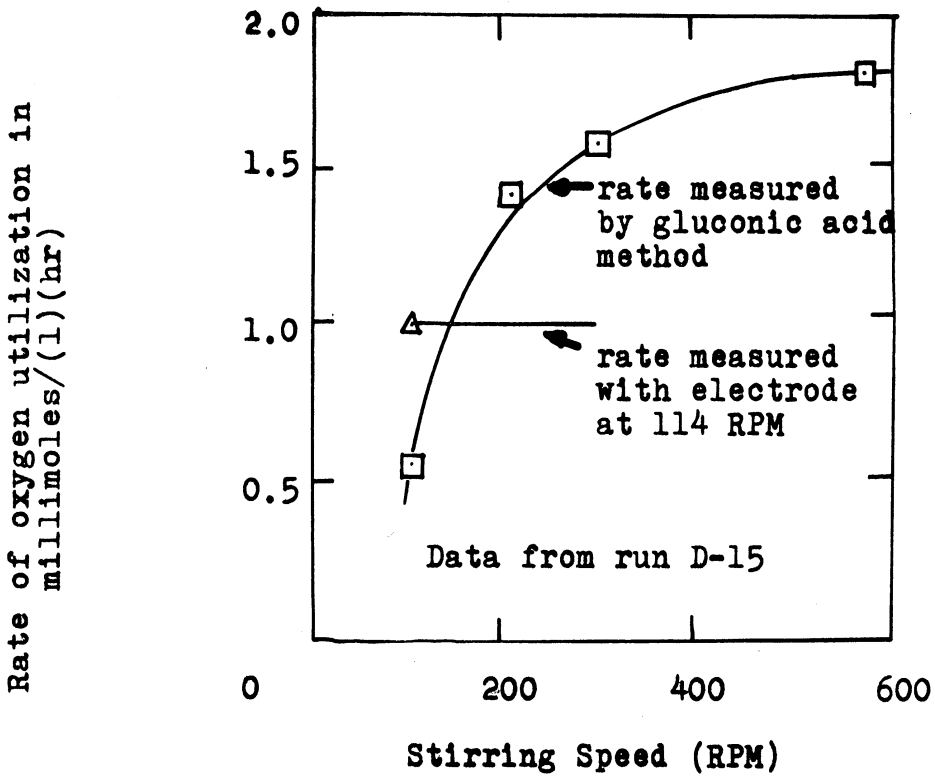
TABLE XIII

EFFECT OF AGITATION SPEED ON THE RATE OF OXYGEN UTILIZATION AND ON THE DISSOLVED OXYGEN CONCENTRATION: RESTING CELLS OF *Pseudomonas ovalis* IN A GLUCOSE MEDIUM AT 25°C, pH 7.0 AND AIR FLOW RATE 0.46 VVM IN RUN D-15

Agitation Speed	Rate of Oxygen Utilization	Concentration of Dissolved Oxygen
RPM	(mmoles/(l)(hr.)	% Saturation
114	0.56 (1)	6
204	1.39 (1)	79
300	1.63 (1)	95
570	1.82 (1)	100
114	0.99 (2)	decreasing

(1) Calculated from the rate of gluconic acid production

(2) Calculated from the oxygen electrode measurements



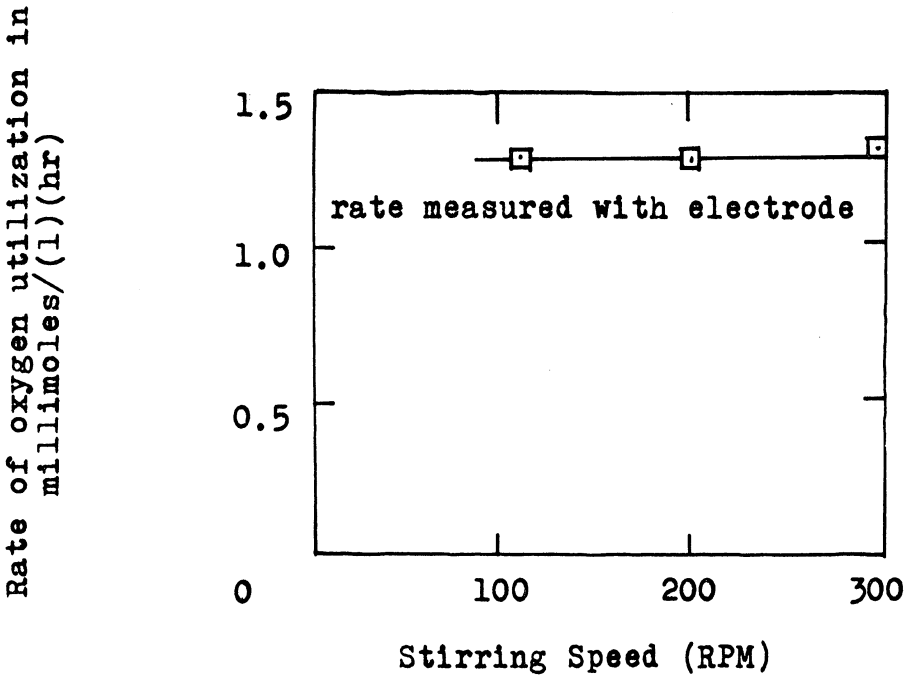


Figure 23. Effect of agitation speed on the rate of oxygen utilization by resting cells of Pseudomonas ovalis in a glucose medium at 25°C and pH 7.0. Rates determined with aeration (gluconic acid) and without aeration (electrode).

TABLE XIV

EFFECT OF AIR FLOW RATE AND AGITATOR SPEED ON THE DISSOLVED OXYGEN CONCENTRATION IN A GLUCOSE MEDIUM CONTAINING RESTING CELLS OF *Pseudomonas ovalis* AT 25°C, pH 7.0 AND KLETT READING 100

Rate of Air Flow	Dissolved Oxygen Concentration, % Saturation	
VVM	217 RPM	114 RPM
4.6	75	70
4.3	73	-
4.1	73	56
3.5	71	48
3.0	68	36
2.4	63	25
1.8	58	22
1.3	50	8
0.8	41	3
0.5	30	-
0.25	6	-

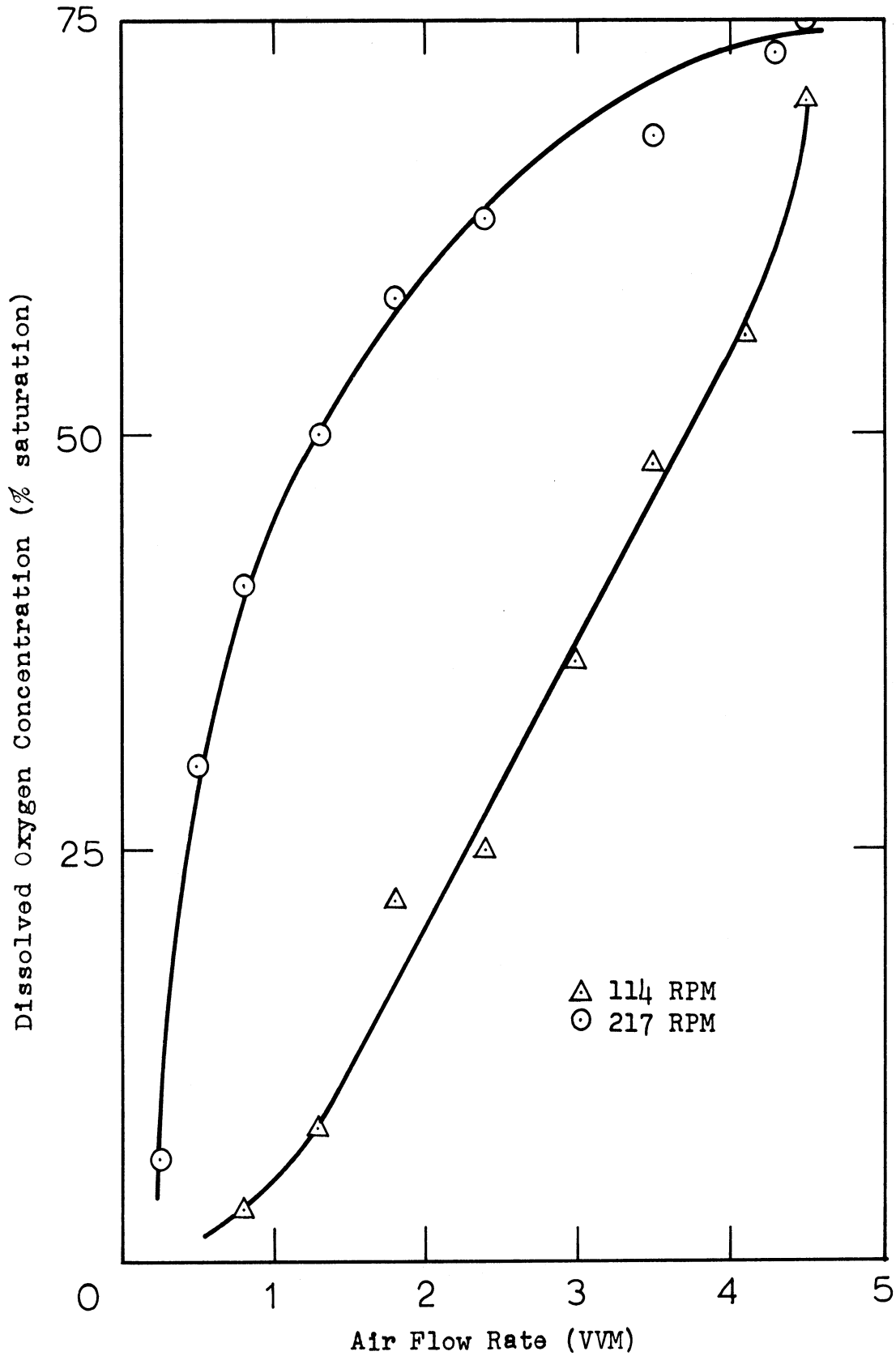


Figure 24. Effect of stirring speed and air flow rate on the dissolved oxygen concentration in a suspension of resting cells of Pseudomonas ovalis at 25°C, pH 7.0 and Klett reading 100.

Concurrent with the increase in viscosity was a large increase in osmotic pressure. If the osmotic pressure in the viscous medium was higher than in the cells, the microorganisms were dehydrated. It was, therefore, not possible to use Pseudomonas ovalis with high concentrations of glycerol and sucrose; thus, the chemical system in which sulfite is oxidized to sulfate was chosen.

The data for the effect of viscosity on the rate of oxygen transfer are recorded in Table XV and are plotted in Figure 25. Both glycerol and sucrose significantly reduced the rate of oxygen transfer. With sucrose solutions, reductions of 93% in the rate of oxygen transfer were accomplished by increasing the viscosity of the solution from 1 to 12 cp. Using glycerol, the OTR was decreased by 72% when the viscosity was raised from 1 to 4.75 cp.

These data are in agreement with published results. Solomons (78) found a 45% solution of sucrose, equivalent to a viscosity of 10 centipoises, reduced the OTR by a factor of 6 (84%) as measured by the sulfite method and 12 (92%) by the polarographic method. Yoshida et al. (101) found a 60% reduction in the OTR when the viscosity was increased to 3.6 centipoises with glycerol.

Plotted on logarithmic scales (Figure 26), the data for OTR against viscosity yielded straight lines for both substances. Slopes of these lines are (-) 1.11 for sugar and (-) 0.97 for glycerol, which means that the OTR varied inversely as the viscosity raised to the power 1.11 or 0.97, depending on the chemical added.

TABLE XV

EFFECT OF VISCOSITY ON THE RATE OF OXYGEN TRANSFER TO SPARGED, AGITATED SOLUTIONS OF SODIUM SULFITE: AIR FLOW RATE 1.0 VVM, AGITATION SPEED 300 RPM, TEMPERATURE 25°C

Run Number	Viscosity Centipoises	Amount of Viscosity Additive in 3 Liters of Solution	Oxidation Rate meq/(l)(hr.)
V10	4.78	glycerol-1500 ml.	27.5
V11	3.07	glycerol-1200 ml.	42.0
V12	2.34	glycerol-1000 ml.	55.8
V13	1.77	glycerol-750 ml.	73.9
V14	1.36	glycerol-500 ml.	89.5
V17	11.75	sucrose-1990 g.	7.1
V16	3.36	sucrose-1285 g.	26.4
V18	2.57	sucrose-1075 g.	36.4
V15	1.61	sucrose-1075 g.	50.8
V19	1.15	sucrose-380 g.	88.9
V9	0.89	-none-	151

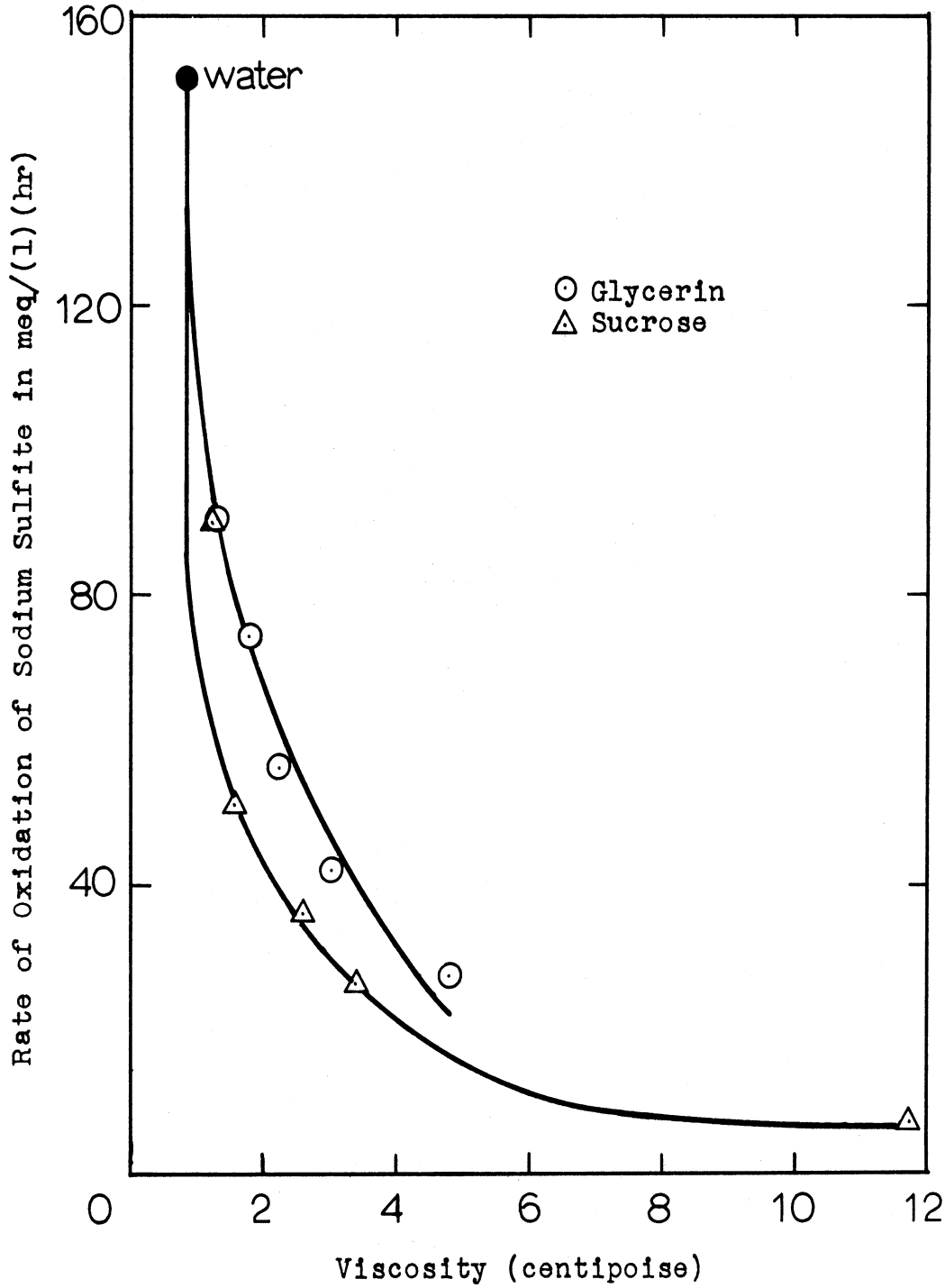


Figure 25. Effect of increasing viscosity, due to the addition of glycerin or sucrose on the rate of sodium sulfite oxidation. The liquid was sparged with air at a rate of 1.0 VVM and agitated at 300 RPM at 25°C.

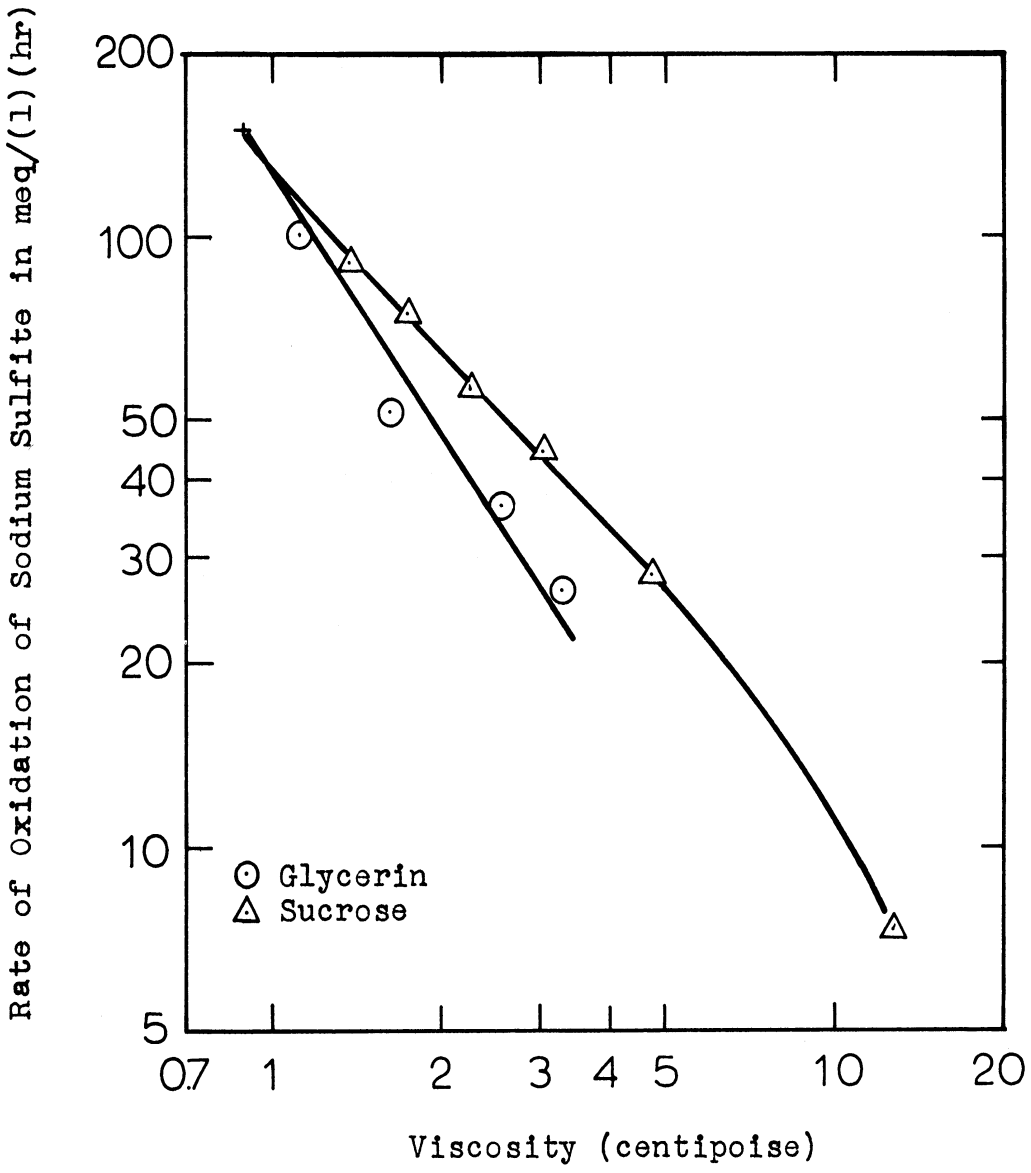


Figure 26. Logarithmic plot showing the effect of increasing viscosity, due to the addition of glycerin or sucrose, on the rate of sodium sulfite oxidation. The liquid was sparged with air at a rate of 1.0 VVM and agitated at 300 RPM at 25°C.

It is possible to estimate the effect of decreased oxygen solubility due to increasing sugar concentrations on the rate of mass transfer. Solomons (78) has published data for the equilibrium solubility of the oxygen from the air in glucose at 37°C. If it is assumed that oxygen concentrations would be equivalent in solutions of glucose and sucrose of the same molarity, then a correction can be made for oxygen saturation reductions. Although these data are for 37°C, the percent reduction in solubility is the same for all oxygen and sugar concentrations over a given range (81). Thus, these figures indicate that some mechanism other than solubility is important.

2. Production of Gluconic Acid in non-Newtonian Broths

Small concentrations of ether celluloses impart non-Newtonian characteristics to aqueous solutions. Two such commercial preparations were used in this study.

(1) Natrosol H; a non-ionic, hydroxyethyl ether of cellulose

(2) Methocel 65 HG, 4000 CP; a propylene glycol ether of cellulose

Initial experiments showed the necessity for increasing both the stirring speed to 570 RPM and air flow rate to 1.85 VVM in order to maintain the dissolved oxygen (D.O.) concentration at a level above the critical so that it did not influence the rate of respiration. In addition to increasing the air flow and stirring speed, the second impeller was lowered so that it, too, was immersed in the

TABLE XVI

SULFITE OXIDATION RATE IN SUCROSE SOLUTIONS CORRECTED FOR REDUCED SOLUBILITY OF OXYGEN IN EQUILIBRIUM WITH AIR

Run Number	Sucrose Concentration moles/liter	Equilibrium Oxygen Concentration ppm. 37°C	Corrected Oxidation Rate meq/(l)(hr.)(ppm)
V17	1.94	2.5	2.8
V16	1.25	3.8	6.9
V18	1.05	4.2	8.7
V15	0.73	4.7	11
V19	0.37	5.2	17
V9	0.00	6.9	22

liquid to improve gas dispersion and increase turbulence.

Several runs were made in which different concentrations of chemicals were used to alter viscosity. During each run, the rate of production of gluconic acid was measured, and the level of dissolved oxygen was determined. When each run was terminated, a sample was taken for viscosity measurement.

The flow curve for the medium used in the run was determined with a rotational viscometer. The recorder plotted shear rate against shear stress. An example curve as plotted by the machine is shown in Figure 27. Logarithmic plots of shear stress against shear rate were linear for all concentrations of Natrosol and Methocel. The slope of the straight line was n. A curve is shown for each compound in Figure 28.

These data do not agree with the published works of Godleski and Smith (39), who reported that Natrosol was a pseudoplastic fluid that did not follow the simple power law equation. Their flow curves were established with a Brookfield, synchoelectric viscometer.

Metzner and Otto (52) employed Natrosol in concentrations of 1.0% and 2.0%. The 2.0% solution gave a straight line when the logarithm of shear stress was plotted against shear rate. Although there was some deviation for the 1% solution from the straight line, that deviation, according to the authors, was less than the scattering of the data points. Accordingly, values of 0.32 and 0.40 were found for the exponent n.

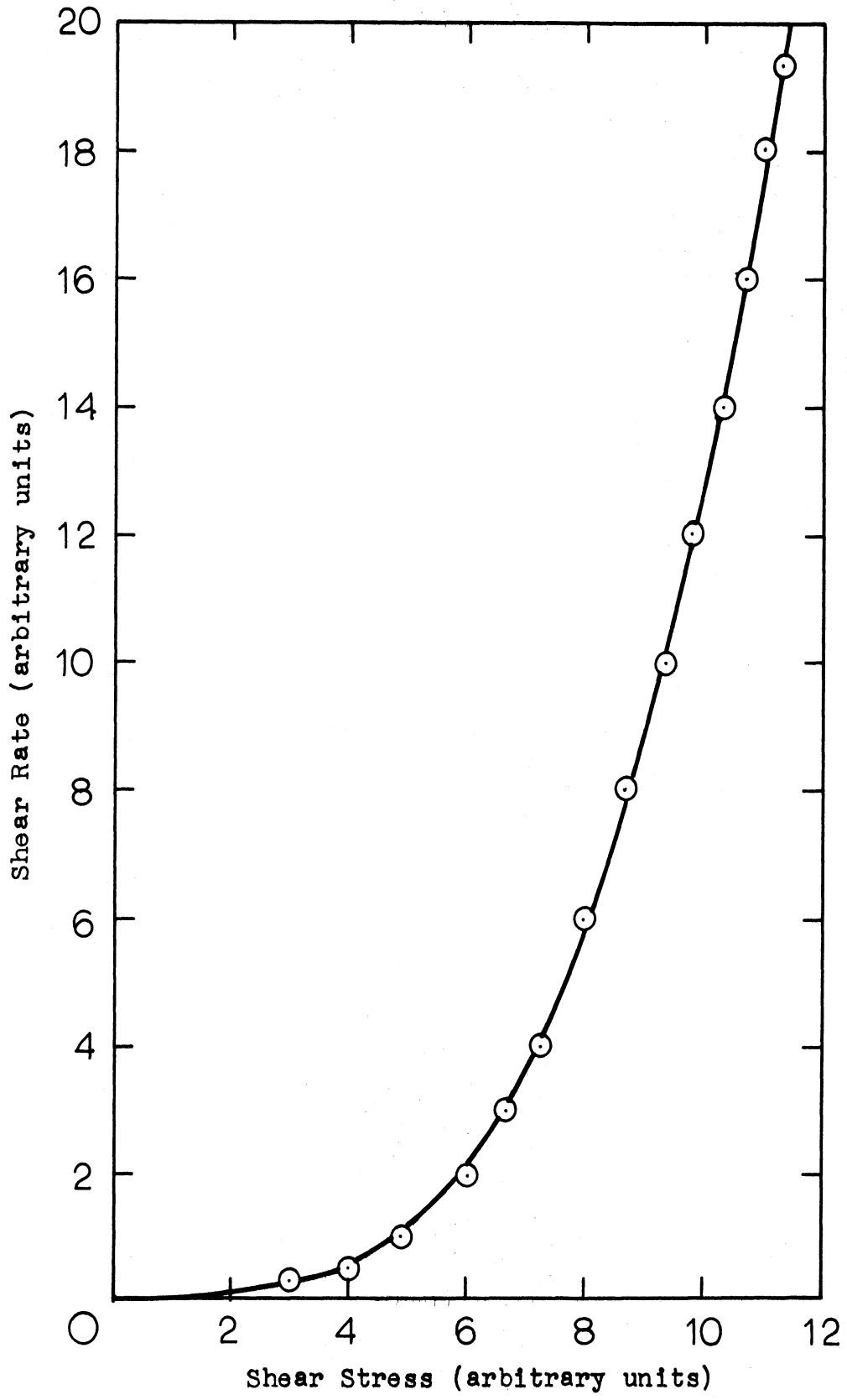


Figure 27. Flow curve for a medium containing 15 grams of Natrosol per liter. The plot of shear rate versus shear stress was determined with a rotational viscometer.

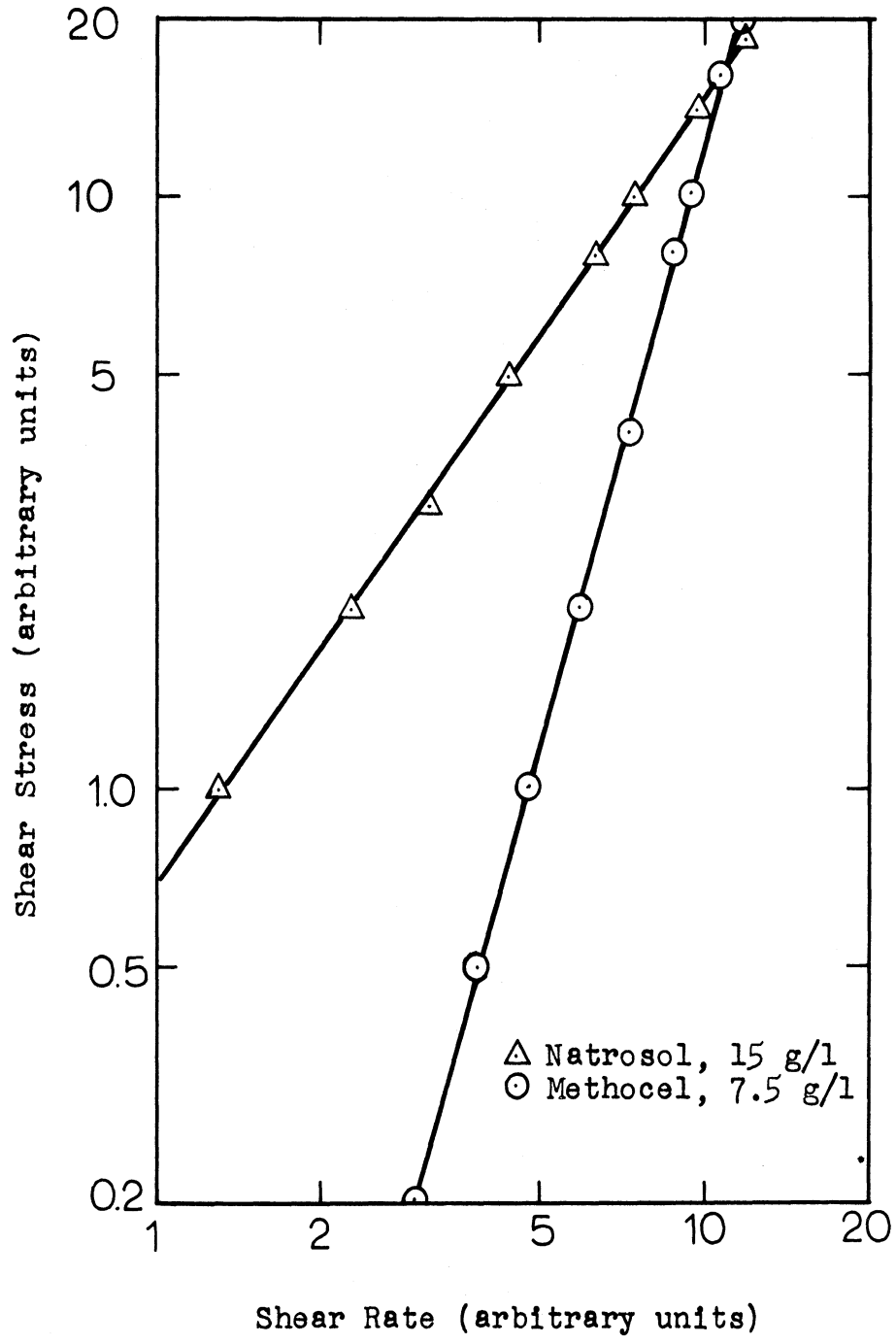


Figure 28. Relationship between shear stress and shear rate for Natrosol and Methocel.

TABLE XVII

VALUES OF THE EXPONENT n IN THE POWER LAW EQUATION FOR SEVERAL CONCENTRATIONS OF NATROSOL AND METHOCEL

Run Number	Concentration of Viscosity Agent grams/liter	Power Law Exponent n
E11	5 (Natrosol)	0.57
E12a	15 "	0.286
E12b	11 "	0.392
E12c	8.5 "	0.466
E14	10 (Methocel)	0.637
E13	20 "	0.426
M1	2.5 "	0.968
M2	5 "	0.885
M3	7.5 "	0.721
M4	10 "	0.616
M5	15 "	0.458

Calderbank and Moo-Young (11) have also reported on work with carboxymethyl cellulose. For several different concentrations of that substance, they reported values of $\underline{n}$ ranging from 0.44 to 0.64, indicating a linear plot of the logarithm of shear stress versus the logarithm of shear rate.

The power law exponent, $\underline{n}$, which is the slope of the logarithmic curve of shear stress versus shear rate, is a measure of the magnitude deviation from Newtonian solutions. As the solution became more concentrated, the viscosity rose and $\underline{n}$ decreased. A plot of $\underline{n}$ versus concentration of the viscosity agent (Figure 27), yielded straight lines for both Natrosol and Methocel.

Since the apparent viscosity of non-Newtonian fluids depends on the shear rate, the data were extrapolated to a standard of one sec.$^{-1}$, as suggested by Solomons and Perkin (79). The data are reported in Table XVIII.

These viscosity agents were then studied as components of the medium in the fermentor. As the viscosity increased, the turbulence of the liquid decreased, and the efficiency of gas distribution fell off markedly. Large bubbles formed and rose to the surface around the impeller. There was very little movement of the liquid near the baffles.

The oxygen electrode was installed behind one of the baffles; the values of the dissolved oxygen concentration indicated that the suspension, even at that point, was supplied with sufficient oxygen. It was noted that as the broth became more viscous, the bubbles rose much more slowly.

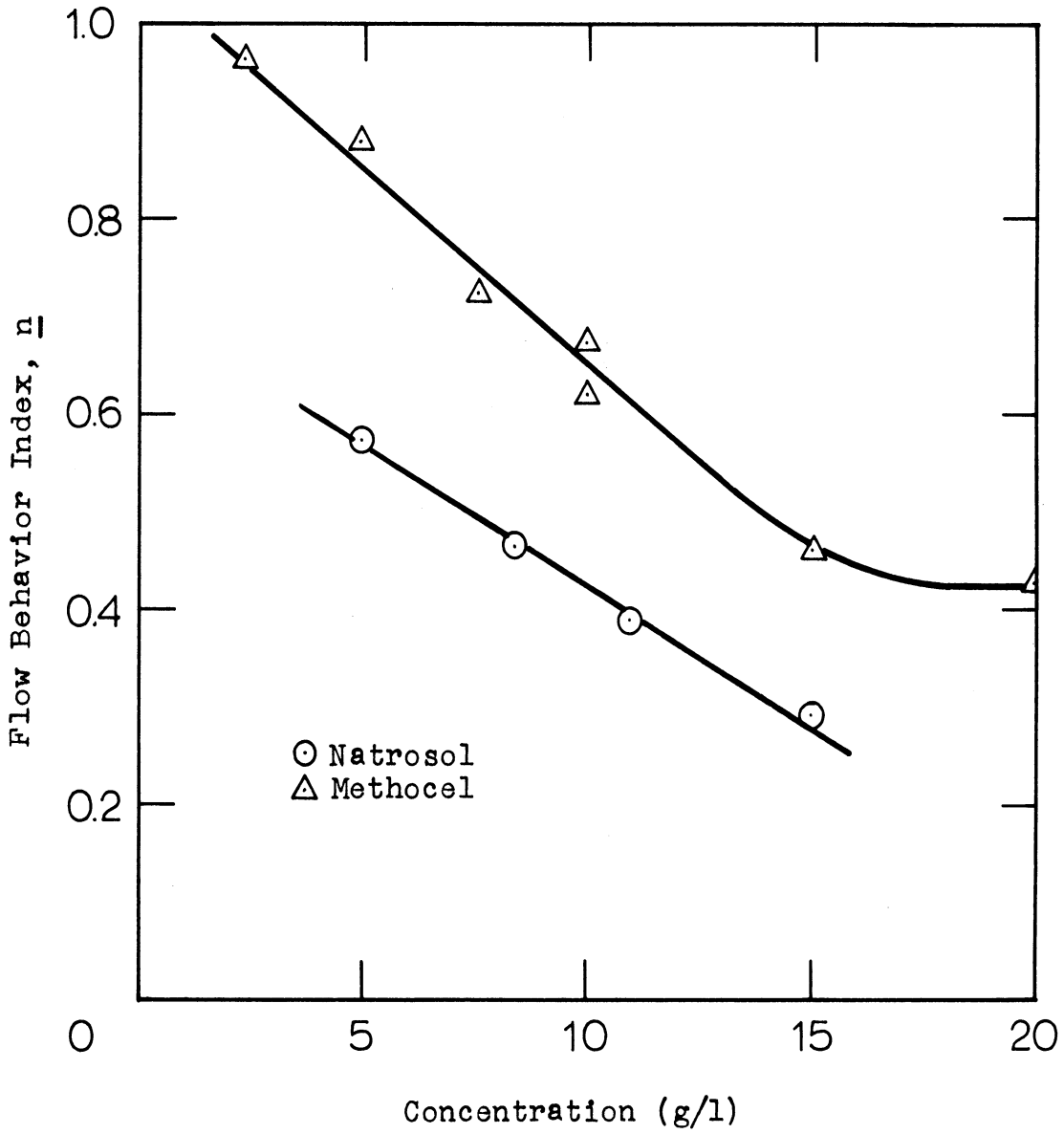


Figure 29. Relationship between the concentration of Natrosol and Methocel added to water to increase the viscosity and n, the slope of the logarithmic plot of the flow curve for that medium.

TABLE XVIII

PRODUCTION OF GLUCONIC ACID BY *Pseudomonas ovalis* IN SOLUTIONS HAVING PSEUDOPLASTIC VISCOSITY: AGITATION RATE 570 RPM, AIR RATE 1.85 VVM, TEMPERATURE 25°C

Run Number	Viscosity Agent	Apparent Viscosity at Shear Stress of 1 $sec.^{-1}$ Measured at 30°C	Oxygen Concentration	Rate of Gluconic Acid Production
	g/l	centipoises	% Saturation	meq/(l)(hr.)
E10	0	1	-	2.27
E4	0	1	-	2.13
E8	5(Natrosol)	-	88	2.45
E11	5(Natrosol)	460	-	2.12
E7	15(Natrosol)	-	-	2.82
E9	15(Natrosol)	-	-	3.14
E12	15(Natrosol)	17,000	39	2.43
E13	20(Methocel)	19,000	73	2.51
E14	10(Methocel)	738	94	2.07
			Average Value	2.44

This agrees with reasoning based on Stokes Law, which indicates that as the viscosity increases, bubble velocity decreases, and the contact time increases.

The rate of production of gluconic acid was not affected by viscosity. The data are reported in Table XVIII for 9 separate runs; concentrations of Methocel as high as 2% produced an apparent viscosity of 19,000 cp measured at a shear rate of one $sec.^{-1}$. The average rate of acid production for these runs was 2.44 meq/(l)(hr.) and all runs (except one) were within 20% of that figure.

3. Effect of Viscosity on the Rate of Oxygen Transfer in a Cell-free System

The rate of oxygen transfer to a solution from the atmosphere has been expressed as:

$$\frac{dC}{dt} = K_L A(C_g - C) \qquad (18)$$

If the oxygen concentration, C, is measured with the oxygen electrode, and if it is assumed that C is proportional to the meter reading, then

$$C = m(E - E_o) \qquad (19)$$

when m is a constant, E_o is the zero reading on the meter when no oxygen is present in the liquid and E is the meter reading at concentration C. The following expression is obtained if Equation 19 is differentiated:

$$\frac{dC}{dt} = m \frac{dE}{dt} \qquad (20)$$

Substituting the above expression in Equation 18, it is found that

$$\frac{dE}{dt} = K_L A \, (E_S - E) \qquad (21)$$

The reaeration method for measurement of OTR first involved stripping all of the dissolved oxygen from the solution. Then, as the oxygen was replenished by bubbling air through the medium or by diffusion of air through the surface of the liquid, the meter reading was recorded as a function of time.

Figure 30 gives the results of such an experiment. Initially, the meter reading, which was proportional to the dissolved oxygen concentration, was zero. As absorption proceeded, the oxygen concentration increased and asymptotically approached the saturation value, E_S.

The rate of oxygen transfer, at any time, is the slope of the curve in Figure 30. The rate decreased as oxygen uptake progressed. Equation 21 can be integrated (42) using $E = 0$ at $t = 0$ and $E = E$ at $t = t$ as limits, giving:

$$K_L A = \frac{2.3}{t} \log_{10} \frac{E_S - E_o}{E_S - E} \qquad (22)$$

allowing the mass transfer coefficient $K_L A$ to be evaluated.

Since the unit of $K_L A$ is the reciprocal time, it applies without alteration to both Equations 18 and 21. The values for the mass transfer coefficient have been measured for the transfer of oxygen to the glucose-phosphate medium used to resuspend the cells of *Pseudomonas ovalis*. The data

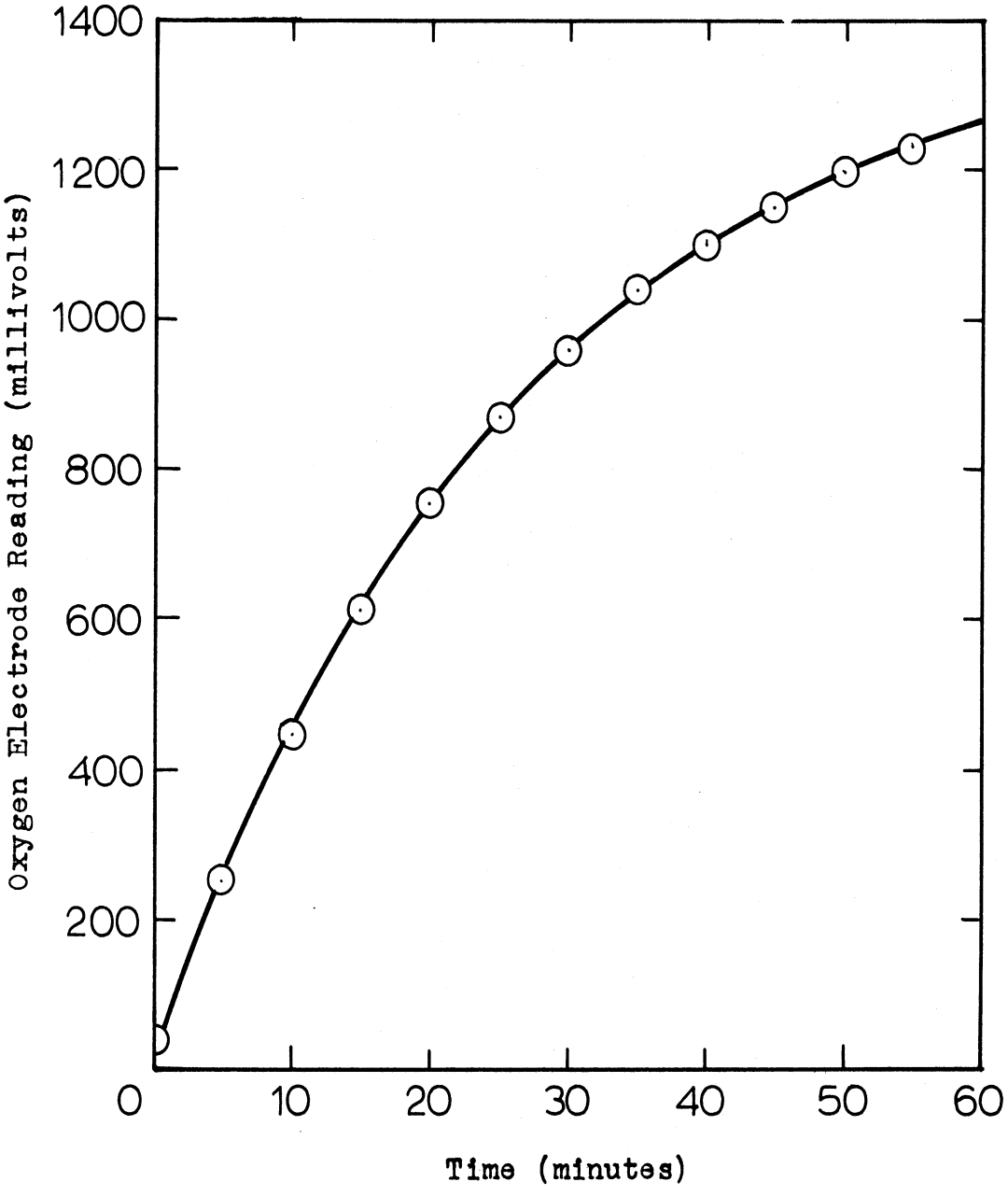


Figure 30. Oxygen transfer by diffusion through the surface of a glucose-phosphate medium stirred at 300 RPM at 25°C.

showing the effect of stirring speed on $\underline{K_L A}$ are found in Table XIX. These values have been used to calculate the rate at which oxygen diffused through the surface from the atmosphere when the electrode was being used to measure oxygen transfer rates. The correction for diffusion may be calculated for any concentration of dissolved oxygen according to the equation:

$$\left(\frac{dE}{dt}\right)_L = K_L A \quad (E_S - E) \qquad (23)$$

The net rate at which the cells consumed oxygen was the sum of the measured rate of removal plus the rate of oxygen diffusing through the surface:

$$\left(\frac{dE}{dt}\right)_{TOTAL} = \left(\frac{dE}{dt}\right)_E + \left(\frac{dE}{dt}\right)_L \qquad (24)$$

An example calculation is shown in Appendix E.

To determine the effect of viscosity on the rate of oxygen transfer, the oxygen electrode was employed to measure transfer rates in solutions containing different concentrations of a high molecular weight viscosity additive. As the concentration of Methocel was increased, a decrease in OTR occurred concurrently with the increase in viscosity.

From the data recorded in Table XX, it can be seen that the major reduction in OTR occurred when the Methocel concentration was increased from 5 to 10 grams per liter. This corresponds to the point at which the viscosity shows a significant change.

TABLE XIX

EFFECT OF AGITATION SPEED ON THE MASS TRANSFER COEFFICIENT FOR OXYGEN DIFFUSING THROUGH THE SURFACE OF A GLUCOSE SOLUTION. MEASUREMENTS MADE WITH THE OXYGEN ELECTRODE

Agitation Speed RPM	Mass Transfer Coefficient $(K_L A) \times 100\ min^{-1}$
114	1.07
217	1.88
300	3.78
412	16.6

TABLE XX

EFFECT OF INCREASING CONCENTRATIONS OF METHOCEL ON THE OXYGEN TRANSFER RATE MEASURED BY THE OXYGEN PROBE: AGITATION RATE, 300 RPM; AIR FLOW RATE TO SPARGER, 0.2 VVM, TEMPERATURE, 25°C

Run Number	Concentration of Methocel g/l	Mass Transfer Coefficient min^{-1}	Power Law Exponent n
1-C-1	0	0.55	1.0
2-B-2	2.5	0.48	0.968
2-C-2	5.0	0.40	0.885
2-D-2	10	0.092	0.616

The effect of the rate of air flow was determined by sparging air into distilled water. The data are recorded in Table XXI and the logarithmic plot, Figure 31, shows that for agitation at 300 RPM, the OTR varies at the 2.3 power of the air rate.

TABLE XXI

EFFECT OF THE AIR FLOW RATE ON THE OXYGEN TRANSFER RATE MEASURED BY THE OXYGEN ELECTRODE IN A FERMENTOR AGITATED AT 300 RPM. THE METHOCEL CONCENTRATION IN THE LIQUID WAS 2.5 g/l.

Run Number	Air Rate VVM	Mass Transfer Coefficient min^{-1}
2-B-1	0.05	0.31
2-B-2	0.2	0.51
2-B-3	0.75	0.59
2-B-4	1.25	0.73

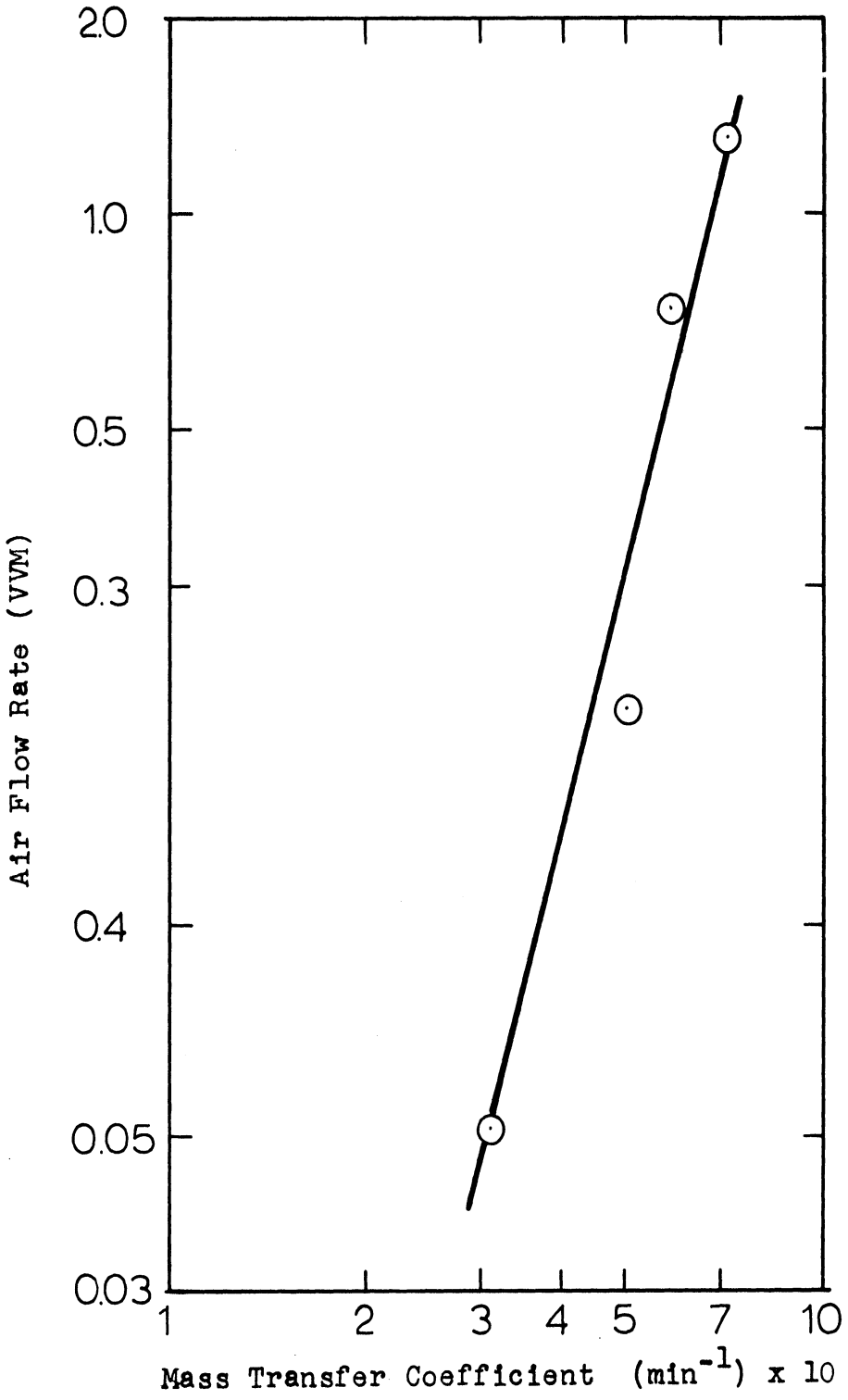


Figure 31. Effect of the air flow rate on the oxygen transfer rate measured by the oxygen electrode in a fermentor agitated at 300 RPM. The Methocel concentration in the liquid was 2.5 g/l.

IV. DISCUSSION

1. Mechanism of Cell Adsorption on Bubbles

The supply of oxygen to microorganisms, its subsequent reaction within the cells and the later removal of the metabolic products is carried out by an integrated series of physical, chemical and biochemical reactions. The resistances to oxygen transfer from sparged air to the respiring cells in the liquid are many, and the mechanism of transfer is still not completely understood. As an example of the controversy surrounding this subject, the multitude of articles about the seemingly simple oxidation of sodium sulfite to sodium sulfate can be cited; even in this cell-free system, the mechanism has not been clearly detailed.

Aeration and agitation are coupled. Indeed, Solomons (78) has stated that: "The effects of agitation independent of aeration have not been studied a great deal, since it is often difficult to devise experiments where it is possible to differentiate between agitation and aeration." The process of agitation promotes the transfer of oxygen through the surface and, in fact, vortex aeration (6) has been used as a means of supplying oxygen to fermentations. Vortex aeration occurs if a turbine impeller is employed in a unbaffled system. The liquid moves in wide, cylindrical paths, but when the vortex touches the impeller, air is distributed into the liquid as small bubbles: the system becomes an efficient gas-liquid contactor at high agitation speeds.

Similarly, aeration by sparged air provides agitation, since the passage of swarms of bubbles through the liquid aids in mixing besides replenishing the oxygen supply. Many studies have been made to determine oxygen transfer rates in non-biological systems, both with and without the use of sparged air. With respiring organisms, however, it has been very difficult to separate these two effects. Most studies of this type have relied upon removing a sample of the broth from the medium and measuring the rate of oxygen utilization in a polarographic cell. In this instance, the microorganisms are in a quiescent liquid that is being neither aerated nor agitated.

Tsao (93) developed a method of measuring the oxygen transfer rates in an actual fermentation by using the Pseudomonas ovalis conversion of glucose to gluconic acid. Since he knew that each mole of acid produced required one-half mole of oxygen, he was able to calculate the OTR, provided he measured the rate at which sodium hydroxide was supplied in order to keep the pH constant.

He concluded that the OTR was affected by the stirring speed and that the major resistance to oxygen transfer lay at the cell-liquid interface. His conclusions were based upon the fact that the OTR was proportional to cell concentration and, hence, to the area of the cell-liquid interface. He also noted that the OTR measured by the sulfite method was very much larger than that found by the Pseudomonas ovalis fermentation of glucose. Thus, he concluded that the

dissolved oxygen concentration in the liquid was high during his fermentations and that the cell-liquid resistance must control the OTR.

The present research supported his data and provided further proof of his conclusions, but the new data also suggested that an additional mechanism of oxygen transfer was operative in the overall transfer scheme. The oxygen electrode was used to measure dissolved oxygen (D. O.) concentrations in the medium as the cells respired. Indeed, as Tsao predicted, the D. O. levels were high for most cell concentrations and stirring speeds. Only at agitation speeds below 200 RPM did the D. O. concentration fall below the critical value.

The present research also confirmed Tsao's finding that the rate of acid production was proportional to the cell concentration. Figure 18 illustrates this fact, but the data presented in this thesis cover a thirty-fold range of cell concentrations, compared to the seven-fold range that Tsao investigated. It will be noted, however, that the concentration of D. O. fell below the critical value at rates of gluconic acid production well above the levels attained by Tsao. Both of these curves, one for the rate of gluconic acid production and the other for D. O. concentration, leveled out at about the same cell concentration.

By shutting off the air supply, the OTR was studied at various agitation rates without aeration. The oxygen probe was used to follow the fall of the D. O. concentration

in the medium as the cells removed it. The slope of this curve is a measure of the rate of oxygen consumption by the cells. Since oxygen also entered the liquid by diffusion through the surface of the liquid, it was necessary to apply a correction factor. This method of measuring the OTR was limited to stirring speeds equal to or less than 300 RPM since, above this speed, the reaeration rate by diffusion of oxygen through the surface of the liquid became excessively high. Above 300 RPM, the dissolved oxygen concentration value fell very slowly, and it was necessary to calculate the utilization rate mainly from the correction factor for surface reaeration, rather than from the slope of the curve of concentration versus time.

It was found that the dissolved oxygen content of the broth fell at a rate which was independent of stirring speed for 114, 217 and 300 RPM. This is evidence for the view that the controlling mechanism is not restricted to the liquid film around the cell. This conclusion is supported by Finn (31) and Aiba et al. (1), who have independently calculated the resistance of the film to be insignificant.

However, it is important to recognize that the rate of production of gluconic acid has been shown, in the present study, to rise with:

(1) an increase in air rate,

(2) an increase in dissolved oxygen concentration in the liquid, above the critical value,

(3) an increase in agitation speed.

Also, it is important to observe that in run D-10, a single batch of cells was employed to investigate the combined effect of stirring speed and air flow rate on the rate of production of gluconic acid. In the same manner, a single batch of cells was used in run D-15 to evaluate the effect of agitation rate alone on the rate of oxygen transfer. In this manner, differences in physiological characteristics between different batches of cells were eliminated.

It is to be noted in Figure 23, which contains the results of Run D-15, that the OTR increased with each increase in speed, even at D. O. concentrations well above the critical value. Steel and Maxon (83) have reported the same phenomenon in novobiocin fermentations. Moreover, in the study reported here, the rate of oxygen uptake was less when measured with the probe than when calculated from the rate of acid production.

The transfer of oxygen from air bubbles to a cell wall encounters a number of resistances which have been described in detail previously. These resistances are found at the gas-liquid and the cell-liquid interfaces, as well as in the bulk of the liquid. All of these restrict the availability of oxygen to the cells.

Another concurrent path was suggested by Bartholemew et al. (3), but direct evidence to support their model could not be obtained due to limitations of the system and equipment available at that time. They proposed that a shorter but parallel path for oxygen transfer existed when cells were

adsorbed on bubbles. Direct contact between the two would combine the two liquid films into one. This would eliminate the path through the bulk of the liquid as well as reduce the length of the diffusion path in the liquid films surrounding the bubbles and the cells.

The combination of films would result in a cell being exposed to high concentrations of oxygen across a shortened path. Such a system would be favored, according to the originators of the theory (3), "by a large population of small bubbles having a large interfacial area coupled with thorough mixing between bubbles and cells."

It was noted in Tsao's work (93) that increasing the stirring speed increased the gas holdup. In this research, it was found that increasing the air rate, or the stirring speed, increased the OTR.

Oxygen concentration was another variable whose effect on the rate of production of gluconic acid was measured. High oxygen concentrations in the gas phase led to higher rates of oxygen transfer as shown in Figure 22, even though the concentration of dissolved oxygen in the liquid was well above the critical value.

In this work, the OTR was measured by two methods: during aeration and agitation, it was calculated from the rate that sodium hydroxide was added to maintain the pH at a constant value; when the air flow was stopped, the rate of decrease of oxygen concentration was the measurement used to calculate the OTR. When the two rates were compared, it was found that

in Run D-15, the OTR, measured under conditions of no aeration, was 53% of the rate when the cells were being both aerated and agitated - i.e., the OTR was less when there was no direct bubble-cell contact.

Aiba, et al. (1), using the oxidation of glucose to gluconic acid as one method to measure OTR and a polarograph for the second measurement, agree with this finding. They found the OTR without aeration to be about 66% of the rate with aeration. Siegell and Gaden (75) also observed a similar phenomenon in yeast fermentations and stated that it was likely due to a change in the environment from active aeration and agitation in the fermentor to static conditions in the sample chamber of the polarograph.

Phillips et al. (59) have expressed dissatisfaction with the accepted theories and mechanisms that describe the effects of liquid behavior on the rate of absorption of a gas by a liquid. They decided that no single mechanism would explain their data. They proposed that oxygen transfer would result from normal diffusional processes together with the incorporation into the medium of an adsorbed layer of molecules.

The theories presented in this thesis also propose that no single mechanism is able to account for oxygen transfer. The view of Bartholomew et al. (3), that oxygen transfer occurs across the common film around a cell and a bubble, can be used to represent the data in both this work and that of the previous authors. Moreover, the "film" mechanism does

not rely upon getting more oxygen into solution in the bulk of the liquid, but assumes direct transfer from the air bubble to the solid cell adsorbed on the air bubble. The film joining the two acts as a path for direct transfer.

Strohm and Dale (85) compared oxygen transfer in the oxygen electrode and in living cells. They believed that a high resistance to diffusion existed at the polyethylene surface covering the oxygen electrode and that a similar resistance was provided by the common water film between an air bubble and the surface of the cell. Thus, the significant variable would be partial pressure of oxygen in the gas phase, rather than its concentration in the solution.

This presentation by Strohm and Dale (85) is consistent with the data reported here. Partial pressure of oxygen in the gas was important to the rate of gluconic acid production (Figure 22) when the cells were being aerated, because there was a direct transfer of oxygen across the film. When this shortened path was removed by ceasing aeration, the OTR dropped since oxygen was drawn only from that dissolved in the liquid. In this case, the rate of oxygen removal from the water was independent of the agitation rate, thus indicating that the major resistance lay at the cell membrane.

It is well known that solids tend to concentrate at interfacial boundaries. For example, froth flotation has long been used to concentrate metallic ores and to separate useful minerals from worthless rock (35). Air bubbles adhere to mineral particles in water and bring them to the surface

as a froth. It is also well known that microorganisms adhere to bubbles. Gaudin et al. (36) have presented data for froth flotation of Escherichia coli. Many other organisms, such as Staphylococcus albus, Schizosaccharomyces sp. (25, 26), Serratia marcescens (8), Bacillus subtilis var. niger spores (37, 38) and Bacillus cerceus var. terminalis spores (5) also have been concentrated by froth flotation. An experiment was also performed in the present study with Pseudomonas ovalis suspended in a nitrogen-free glucose phosphate medium, which showed that they, too, could be concentrated at the gas-liquid interface by this means.

2. Effect of Viscosity on the Oxygen Uptake Rate

Rheological properties of mold and actinomycete fermentation broths change as the fermentation progresses. During growth, yield stress increases with the aging of the culture, and the media tend toward plastic behavior due to the increase in the concentration of the organism (21). Steel and Maxon (84) also illustrated the change in rheological properties of a fermentation medium as the mold grew; they reported, however, that the apparent viscosity reached a maximum and then declined.

In studies of power requirements for gas-liquid contactors, the data have been correlated by the following equation:

$$\frac{Pg_c}{\rho N^3 D_i} = \phi \left(\frac{D_i^2 N \rho}{\mu_a} \right) \qquad (25)$$

This shows that the power number is a function of the Reynolds number. It is the same basic equation that is applied to Newtonian fluids, but the value of the viscosity used to calculate the Reynolds number in non-Newtonian systems is that value measured at the appropriate shear rate for the impeller. Calderbank and Moo-Young (11) give the following equation for the calculation of the rate of the amount of shear produced by an impeller in a stirred vessel containing either a Bingham or a pseudoplastic fluid:

$$\gamma = 10\ N \qquad (26)$$

Richards (65) found 1000 to be the lower limit for turbulent flow regions in which the power required is directly proportional to the fluid density and is independent of viscosity. He also found 10 to be the upper limit of the Reynolds number for viscous flow, although Godleski and Smith (39) used 40 for pseudoplastic fluids. Below this figure, the power input is directly proportional to the fluid density and independent of viscosity.

In the studies with non-Newtonian broths, which have been reported here, the Reynolds number varied from a high value of 33,000 for water, to a low of 51 with the maximum concentration of Natrosol. Detailed calculations for these figures are found in Appendix G. In the latter case, the Reynolds number was close to the boundary value for the viscous region; this was also indicated by visual observation. The air sparger emitted large bubbles which rose to the

surface and burst. There were, however, a large number of small bubbles immersed in the liquid; these rose very slowly. Samples of the viscous medium contained many small, finely dispersed bubbles, and these often persisted in the liquid for a period as long as one-half hour. This prolonged residence of small bubbles increased the volume of air held in the medium. Similar results were reported by Steel and Maxon (84), who found an approximate correlation between apparent viscosity and gas retention percentage.

The shear rate in the fluid falls rapidly with distance from the tip of the impeller. For this reason Solomons and Weston (80) believed that, in viscous mold broths, virtually all of the oxygen transfer took place within the impeller envelope and that little resulted from the slow-moving bubbles outside of this envelope.

It would be expected that the large increases in viscosity produced by carboxymethyl cellulose compounds would result in a decreased OTR. However, the data presented here show that there is little, if any, effect upon the OTR measured by the gluconic acid fermentation when the viscosity is raised by adding carboxymethyl cellulose.

The data for the effect of viscosity on the OTR, measured in this work by the sulfite method or by use of the electrode in a cell-free system, agree with published works. When the OTR was measured by the oxidation of sodium sulfite in the presence of glycerol and sucrose, the data for the decrease in the OTR duplicated that of Yoshida *et al.* (101).

When carboxymethyl cellulose was added to distilled water, the OTR decreased if measured with the oxygen electrode. Analogous results, indicating reductions in OTR with an increase in viscosity, were reported by a number of investigators. These include Chain and Gulandi (12), who used killed Penicillium chrysogenum and measured the OTR with a rotating platinum electrode; Solomons and Weston (80), who used Aspergillus mycelium inhibited by sodium azide to increase the viscosity and the platinum electrode to measure OTR; Deindoerfer and Gaden (21), who used the non-steady state polarographic technique to study OTR in a suspension of azide-inhibited Penicillium chrysogenum.

The effect of viscosity on the OTR has been measured in the novobiocin fermentation by Steel and Maxon (84). They reported an initial decrease in the OTR as the apparent viscosity rose. However, as such fermentations progressed, the cell weight and rigidity increased concurrently.

The resultant decrease of the OTR in media in which the viscosity increased, was due not only to the increased mycelial concentration, but also to the structure produced by intertwining of the hyphae. Much of the resistance to oxygen transfer in such a system is due to the slow diffusion of oxygen within the mycelial clumps. Bartholomew et al. (3) noted that this resistance could be a major factor in affecting diffusion into the center of such structures.

However, when cells are adsorbed on the surface of a bubble, the length of the path for oxygen transfer and total

resistance to such transfer is less than that for cells freely suspended in the liquid; the driving force, which is the difference in dissolved oxygen concentration between the almost saturated film surrounding the bubble and the very low concentration at the surface of the cell, is at a maximum. Agitation of the fluid will not affect the transfer of oxygen across the film between adsorbed cells and air in a bubble, except as agitation may increase the gas-liquid interfacial area upon which adsorption can occur. Thus, both sets of data, one showing reduction of the OTR with increasing viscosity, and the other showing no effect, are consistent with the concept of liquid-film controlled mass-transfer operations in which liquid turbulence or film thickness depends upon viscosity characteristics.

V. SUMMARY

The study of oxygen transfer rates in the Pseudomonas ovalis fermentation of gluconic acid has suggested that two independent paths of oxygen transfer are operative in aerobic fermentations. Conventionally, it is thought that oxygen dissolves in the liquid and then is transported through the bulk of the liquid to the cells, where it reacts with the enzymes. In this process, the oxygen encounters a number of resistances to its movement: these are the film on the inside of the bubble, the gas-liquid interface, the film on the outside of the bubble, the bulk of the liquid and the liquid film surrounding the cell.

Another path appears to exist and operate concurrently as a result of the adsorption of microorganisms on air bubbles. Due to this adsorption, the liquid films surrounding the cell and the bubble are united and oxygen transfer takes place directly across this common film from the air to the cell. The common film can be regarded as a membrane and the mechanism designated as direct oxygen transfer.

Recognition of the existence of the direct path in the gluconic acid fermentation was based upon a number of experimental observations, three of which are now noted:

(1) It was found that increasing the area of gas available for transfer either by sparging more air or by turning the agitator at a higher speed, increased the rate of oxygen transfer.

(2) Increasing the oxygen content of the gas used for

aeration increased the rate of oxygen transfer, even though the dissolved oxygen concentration in the liquid was well above the critical value.

(3) The rate of transfer of oxygen dissolved in the liquid to suspended cells, was unaffected by the agitator speed if the liquid was not being aerated.

The rate of oxygen transfer has been reported to decrease as the viscosity of a fermentation medium increased. Sucrose, glycerol and carboxymethyl cellulose were added to distilled water and the rate of oxygen transfer determined in a non-living system by the sulfite method or by means of the oxygen electrode. The Pseudomonas ovalis fermentation was used to study the effect of carboxymethyl cellulose on the oxygen transfer rate in a resting cell preparation.

In this living system, the viscosity had no effect on the rate of oxygen transfer. However, in the homogeneous chemical systems, the rate of oxygen transfer decreased as the viscosity of the medium increased.

The effect of environmental conditions on the rate of gluconic acid production by resting cells of Pseudomonas ovalis was investigated. The maximum rate was found to occur at pH 7.35 and 37°C. An activation energy of 9,600 cal/mole was calculated for this fermentation. The critical dissolved oxygen concentration for resting cells of Pseudomonas ovalis was 1.1 mg/l.

APPENDIX A

Calibration of Rotameter

The rotameter was calibrated as follows: The rate of air flowing through the rotameter at each reading was determined by passing the gas through a Precision Wet Test Meter. This meter recorded the total volume of air saturated with water at atmospheric pressure. The time taken for a given volume of air to flow through the meter was measured with a stop watch.

The wet test meter was calibrated by the volumetric displacement of water and found to be accurate to within $\pm$ 0.5%.

The data are found in Table XXII. The flow rate was read by noting the position of the top of the sapphire ball in the rotameter tube. The air flow rate was measured at 730.3 mm atmospheric pressure and 78°F while saturated with water.

TABLE XXII

CALIBRATION DATA FOR FISCHER AND PORTER FLOWMETER No. 2 F 1/4-20-5, SAPPHIRE FLOAT; CALIBRATED USING A WET TEST METER AT 78°F AND 730.3 mm PRESSURE

Rotameter Scale Reading	Rate of Air Flow l/min.
19.9	9.25
19.1	8.85
18.0	8.12
17.0	7.52
16.0	6.89
15.0	6.38
14.0	5.86
13.0	5.28
12.0	4.69
11.0	4.12
10.0	3.54
9.0	3.08
8.0	2.53
7.0	1.99
6.0	1.51
4.9	0.92
4.0	0.46
3.0	0.16

APPENDIX B

Calibration of the Oxygen Electrode

The following calibration was run to determine the relationship between the magnitude of oxygen activity in solution, which is read on the millivolt scale of the pH meter, and the chemically measured dissolved oxygen concentration in the liquid: several samples, each containing different concentrations of dissolved oxygen, were made by mixing boiled, distilled water which contained no oxygen and water saturated with oxygen from the air.

The meter was first set at zero by placing the electrode in the solution devoid of oxygen; the maximum reading of 1400 millivolts was set by placing the meter in the solution saturated with oxygen from the air. Then the electrode was placed in each of the samples and the reading was recorded. The samples were stirred during the five minute period required for the meter reading to become steady, and the temperature was maintained at $25.0 \pm 0.2^{\circ}C$. After the reading on the millivolt scale of the pH meter was determined, the dissolved oxygen concentration was determined chemically by the Winkler Test, as outlined in Standard Methods (81).

The data are given in Figure 32. A straight line has been drawn through the points for the plot of millivolt reading against dissolved oxygen concentration.

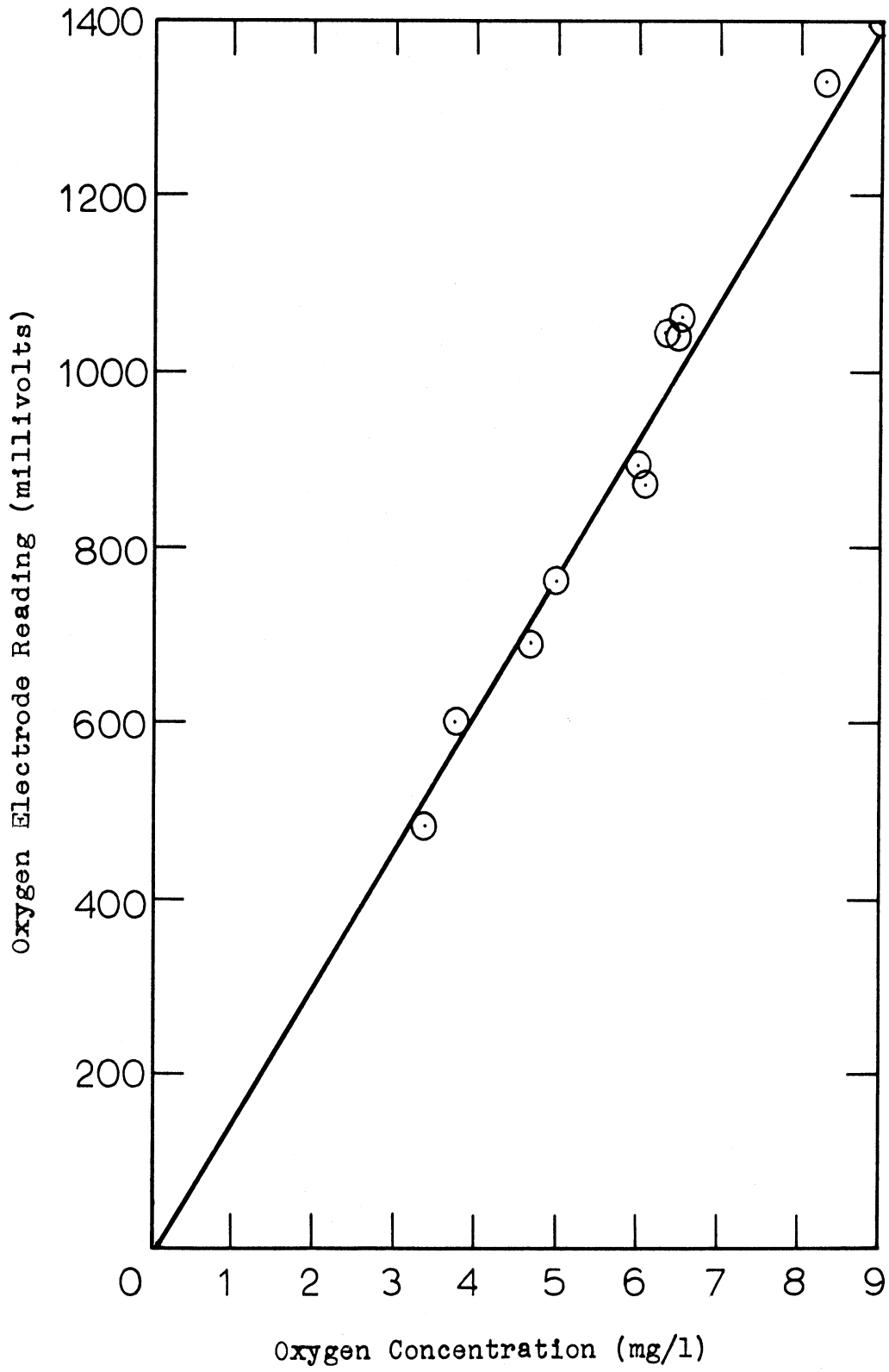


Figure 32. Relationship between the response of the oxygen electrode and the dissolved oxygen concentration in distilled water measured at 25°C by the Winkler method.

APPENDIX C

Effect of Temperature on the Oxygen Electrode

To study the effect of temperature on the measurement of dissolved oxygen concentration with the oxygen electrode, the following test was run: the electrode was placed in samples of distilled water saturated with oxygen from the air. The temperatures of these samples ranged from 13°C to 24°C and were controlled at the set value during the five minute period allowed for the electrode reading to become steady. At the end of this period during which the samples were stirred, the reading on the millivolt scale of the pH meter was recorded.

The data are plotted in Figure 33. This curve shows that a linear relationship exists between saturated dissolved oxygen concentration and temperature. There is approximately a 7% increase in reading per degree Centigrade rise in temperature. This figure agrees very closely with the results of Eye *et al.* (30), who measured the effect of temperature on the reading over a range from 13°C to 28°C.

Figure 33. Effect of temperature on the oxygen electrode response. The electrode was placed in distilled water saturated with oxygen from the air.

APPENDIX D

Calculation of the Correction for the Rate of Production Undissociated Gluconic Acid

During the presentation of the results showing the effect of pH on the rate of production gluconic acid, it was noted that some of the acid did not ionize as it was produced. As a result, no sodium hydroxide was needed to neutralize it. Equation 14 was developed to relate the rate of production of undissociated acid to the rate of actual addition of base needed to keep the pH constant:

$$\frac{d(HA)}{dt} = \frac{(H^+)}{K_a} \frac{d(A^-)}{dt} = \frac{(H^+)}{K_a} N_B \frac{dV_B}{dt} \quad (27)$$

In order to give an example calculation, the following data has been used:

Run	28A
pH	5.25
Rate of addition base (dV_B/dt)	14.2 ml/hr.
Strength of base (N_B)	0.500 N
Ionization constant of gluconic acid	2.4×10^{-4}

Thus the rate of production of undissociated acid was:

$$\frac{d(HA)}{dt} = \frac{(5.63 \times 10^{-6})}{(2.4 \times 10^{-4})}$$

$$(14.2)\ (0.500) = 0.16 \text{ meq/(l)(hr.)}$$

Then the corrected rate of production of gluconic acid was:

$$(14.2)\ (0.500) + 0.16 = 7.26 \text{ meq/(l)(hr.)}$$

APPENDIX E

Comparison of Oxygen Transfer Rate Measured by the Rate of Production of Gluconic Acid to the Rate Measured with the Oxygen Electrode

The rate of oxygen transfer, as measured by the rate of gluconic acid production, can be calculated by using Equation 17:

$$R_G = \frac{N_B dv_B/dt}{V_F} \times \frac{1}{2} \text{ mmoles/(l)(hr.)} \qquad (17)$$

In run D-15, the rate of addition of sodium hydroxide 0.2560N was 29.1 ml/hr. while the medium was agitated at 570 RPM and air flow rate was maintained at 1.86 VVM. The volume of medium in the fermentor was 2050 mls.

$$\therefore R_G = \frac{(0.256)(29.1)}{(2.05)(2)} = 1.82 \text{ meq/(l)(hr.)}$$

After the rate of addition of sodium hydroxide had attained a steady flow, the stirring speed was quickly changed to 114 RPM, and the air flow to the sparger ceased. The effect on the oxygen concentration has been shown in Figure 19, and data are given in Table XXIII.

The rate of decrease of oxygen concentration, dE/dt, was plotted in Figure 20, after a correction was added to account for leakage of oxygen through the surface during removal of dissolved oxygen from solution by the cells.

This correction was calculated in the following manner:

From t=5 to t=6 min., the reading changed from 810 to 715 mv. or a decrease of 95 mv/min. In this run, the reading for saturation was 1345 mv. and 0 at zero oxygen concentration. The leakage rate was:

$$\left(\frac{dE}{dt}\right)_L = K_L A \quad (E_S - E) \qquad (28)$$

$$= 1.07 \times 10^{-3} \left(1345 - \frac{810 + 715}{2}\right)$$

$$= 5 \text{ mv/min.}$$

$$\therefore \left(\frac{dE}{dt}\right)_{total} = 95 + 5 = 100 \text{ mv/min.}$$

Then Equation 17 was used to calculate the rate of oxygen utilization by the resting cells;

$$R_E = \frac{(dE/dt)\ C_g(60)}{(32)(E_S - E_o)} \text{ mmoles/(l)(hr.)} \qquad (17)$$

$$R_E = \frac{(100)(6.8)}{(1/60)(32)(1345)} = 0.96 \text{ mmoles/(l)(hr.)}$$

The ratio of the two rates was 0.96/1.82 = 0.53 showing that more oxygen was transferred in the sparged system.

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1. The value of $K_L A$ used in Equation 28 was obtained from Table XIX.
2. Rather than integrating, it was deemed sufficiently accurate to use an average value of E for this small interval in Equation 28.
3. The value of dE/dt substituted in Equation 24, was the slope of the curve plotted in Figure 19.

TABLE XXIII

CHANGE OF DISSOLVED OXYGEN CONCENTRATION IN A SUSPENSION OF RESTING CELLS OF *Pseudomonas ovalis* AGITATED AT 114 RPM AFTER THE AIR FLOW WAS STOPPED

Times minutes	Oxygen Concentration millivolts	Corrected Rate of Change of Oxygen Concentration mv/min.
0	1290	106
1	1185	87
2	1100	93
3	1010	104
4	910	105
5	810	101
6	715	102
7	620	103
8	525	104
9	430	95
10	345	98
11	258	90
12	180	90
13	103	43
13.5	67	36
14	38	41
15	16	

APPENDIX F

Calculation of the Parameters in the Power-Law Equation for Non-Newtonian Viscosity

The rotational viscometer plotted shear rate against shear. An example curve is shown in Figure 27. To interpret the data in terms of standard units, the following machine calibration constants were used: 33.3 grams force/unit for shear stress which was plotted on the y axis, 1028 sec.$^{-1}$/unit for shear rate, which was plotted on the x axis. To obtain shear stress or shear rate at any point, the scale reading was multiplied by these quantities. The apparent viscosity at any point was obtained by dividing the shear stress by the shear rate. An example of this calculation has been made at the point represented by shear stress 9.3 and shear rate 10:

$$\mu_a = \frac{\tau}{\gamma} = \frac{9.3 \times 33.3 \times 4.379 \text{ dynes/cm}^2}{1028 \times 10 \text{ sec.}^{-1}} = 133 \text{ cp.}$$

The data from Figure 27 were plotted logarithmically in Figure 28. The slope of the line in the figure was n, the exponent in the power-law equation.

APPENDIX G

Calculation of Reynolds Number for Agitation

The Reynolds number has been used in the following systems: to correlate power measurements (51), mixing efficiency (39), and scale-up calculations (69). If the Reynolds number is above 1000, turbulent flow is present; if below 40, viscous flow is present. In view of the importance of this quantity, calculations of the Reynolds number are shown below for the following systems:

(1) no carboxymethyl cellulose added; Newtonian solution in which the viscosity was 1 centipoise

(2) the most viscous solution employed; 15 g. Natrosol per liter; non-Newtonian

In both cases the solutions were stirred at 570 RPM.

(1) For Cells in Glucose-Phosphate Solution

$$N_{Re} = \frac{D_i^2 N \rho}{\mu_a} \qquad (29)$$

$$N_{Re} = \frac{(3/12)^2 (570/60) (62.4)}{(1.0)} \times 1488 = 33{,}000$$

(2) For Cells in Glucose-Phosphate plus 15 grams Natrosol per liter

In this case, the apparent viscosity must be the value measured at the appropriate shear rate for the impeller. Metzner and Otto (52) proposed that the average rate of shear in an agitated vessel varies linearly with the rotational speed of the impeller according to the equation:

$$\gamma = k_s N \qquad (30)$$

They originally suggested that $k_s = 13$ but recently altered this to 11. Calderbank and Moo-Young (11) found 10 to be a suitable value. A value of $k_s = 10$ was used in this calculation to compute the apparent viscosity.

Using the flow curve with $\gamma = 10N = 10(570/60) = 95$ sec.$^{-1}$, the apparent viscosity was found to be 722 centipoises. Calderbank and Moo-Young (11) have shown that the density of carboxymethyl cellulose compounds is close to 1.0.

$$\therefore \quad N_{Re} = \frac{(3/12)^2(570/60)(62.4)}{722} \times 1488 = 51$$

APPENDIX H

Nomenclature

A_A	Frequency factor in the Arrhenius equation - Equation 9
$[A^-]$	Concentration of anion in solution
C	Concentration of dissolved oxygen in a liquid solution
C_c	Critical concentration of dissolved oxygen
C_g	Concentration of oxygen in the liquid phase in equilibrium with air
D	Fractional transmittance of light
D_i	Diameter of impeller
log D	Optical density
E	Dissolved oxygen activity in solution as read on the millivolt scale of the pH meter
E_o	Reading of millivolt meter when electrode was placed in a solution devoid of oxygen
E_A	Apparent activation energy for heat destruction of the microorganisms
E_S	Reading a millivolt meter when electrode is placed in a solution saturated with oxygen from the air
g_c	Gravitational conversion factor
$[HA]$	Concentration of undissociated acid in solution
$[H^+]$	Hydrogen ion concentration at which the rate of gluconic acid production is R_A
$[H_o^+]$	Hydrogen ion concentration giving the maximum rate of gluconic acid production, R_M
I	Intensity of a beam of light striking a solution
I_o	Intensity of a beam of light after passing through a solution
K	Constant in Equation 15
K_a	Ionization constant for a salt in an aqueous solution

$K_L A$	Mass transfer coefficient for oxygen absorption, $sec.^{-1}$
k	Rate constant for the thermal destruction of bacteria in Equation 7
k_s	Constant in the shear rate equation, Equation 30
k_v	Constant in the power-law equation, Equation 3
m	Constant of proportionality in Equation 19
N	Agitator speed, RPM
N_B	Normality of sodium hydroxide used for maintaining constant pH in the fermentor
N_o	Number of viable cells per unit volume of medium
n	Constant in power-law equation for pseudoplastic materials, Equation 3
O	Total number of microorganisms per unit volume of medium
OTR	Oxygen transfer rate
P	Power input to an agitated fermentor
Q_{10}	Ratio of the velocity constant at one particular temperature to the velocity constant at another temperature ten Centigrade degrees lower
R	Universal gas constant
R_A	Rate of production of gluconic acid at any pH
R_E	Rate of oxygen utilization in meq/(l)(hr.) measured by the oxygen electrode
R_G	Rate of oxygen utilization, meq/(l)(hr.) measured by the rate of gluconic acid production
R_M	Maximum rate of production of gluconic acid at the optimum hydrogen ion concentration $[H_o^+]$
t	Time
T	Absolute temperature, ^oK
V_B	Volume of sodium hydroxide added to medium to maintain constant pH during the fermentation of glucose to gluconic acid

V_F	Volume of nitrogen-free glucose-phosphate medium in the fermentor
VVM	Volumes of air per volume of medium per minute
dC/dt	Rate of change of oxygen concentration with time
dE/dt	Rate of change of oxygen activity with time, millivolts/min.
$(dE/dt)_L$	Correction factor for oxygen leaking through the surface of the liquid during removal of dissolved oxygen by cells
$(dE/dt)_T$	Rate of fall of millivolt reading due to removal of dissolved oxygen by cells minus the supply through the surface of the liquid, as noted above
γ	Shear rate
η	Turbidity coefficient in Beer's Law, Equation 11
μ	Newtonian viscosity
μ_a	Apparent viscosity, ratio of shear stress to shear rate
ρ	Density of a solution
τ	Shear stress
N_{Re}	Reynolds number for agitation equal to $\frac{D_i^2 N \rho}{\mu_a}$
N_P	Power number equal to $\frac{Pg_c}{D_i^5 N^3}$

BIBLIOGRAPHY

1. Aiba, S., M. Hara, and J. Someya, Progress Report No. 21, Biochem. Eng. Lab., Inst. Appl. Microbiol., Univ. of Tokyo, Japan, 1962.

2. Barker, J. J., and R. E. Treybal, A. I. Ch. E. J., 6, 289 (1960).

3. Bartholomew, W. H., E. O. Karow, M. R. Sfat, and R. H. Wilhelm, Ind. Eng. Chem., 42, 1801 (1953).

4. Benson, S. W., The Foundations of Chemical Kinetics, McGraw-Hill Book Co., Inc., New York, N. Y., 1960.

5. Black, S. H., R. E. MacDonald, T. Hashimoto, and P. Gerhardt, Nature, 185, 782 (1960).

6. Blakebrough, N., G. Hamer, and M. W. Walker, Trans. Inst. Chem. Eng., 39, A71 (1961).

7. Bowers, R. H., J. Appl. Chem., 5, 542 (1955).

8. Boyles, W. A., and R. E. Lincoln, Appl. Microbiol., 6, 327 (1958).

9. Brierley, M. R., and R. Steel, Appl. Microbiol., 7, 57 (1959).

10. Calderbank, P. H., Trans. Inst. Chem. Eng., 37, 173 (1959).

11. Calderbank, P. H., and M. M. B. Moo-Young, Trans. Inst. Chem. Eng., 37, 26 (1959).

12. Chain, E. B., and G. Gulandi, Rendiconti Instituto Superiore Di Sanita, 17, 5 (1954).

13. Chick, H., and C. J. Martin, J. Physiol., (London), 40, 405 (1910).

14. Clark, D. S., Biotech. and Bioeng., 4, 241 (1962).

15. Clark, L. C., Jr., Trans. Am. Soc. Artificial Inst. Organs, 2, 41 (1956).

16. Colwick, S., and N. Kaplan, Methods in Enzymology, Vol. III, Academic Press, New York, N. Y., 1957.

17. Committee on Sanitary Eng. Rsch. of the Sanitary Eng. Div., JASCE, 86, 2556 SA4 (1960).

18. Camposano, A., E. B. Chain, and G. Gulandi, Proc. VIIth Int. Cong. Microbiol., Sweden, 1958.

19. Cooper, C. M., G. A. Fernstrom, and S. A. Miller, Ind. Eng. Chem., 36, 504 (1944).

20. Deindoerfer, F. H., Appl. Microbiol., 5, 221 (1957).

21. Deindoerfer, F. H., and E. L. Gaden, Jr., Appl. Microbiol., 3, 253 (1961).

22. Deindoerfer, F. H., and A. E. Humphrey, Ind. Eng. Chem., 53, 755 (1961).

23. Deindoerfer, F. H., and J. M. West, J. Biochem. Microb. Tech. and Eng., 2, 165 (1960).

24. Dion, W. M., A. Carilli, G. Sermonti, and E. B. Chain, Rendiconti Instituto Superiore Di Sanita, della Roma, English ed., 17, 187 (1954).

25. Dognon, A., Bull. soc. chim. biol., 23, 249 (1941).

26. Dognon, A., Rev. Scientifique, 79, 613 (1941).

27. Donovick, R., Appl. Microbiol., 8, 117 (1960).

28. Ecker, R. E., and W. R. Lockart, J. Bacteriol., 82, 511 (1961).

29. Elsworth, R., V. Williams, and R. Harris-Smith, J. Appl. Chem., 7, 261 (1957).

30. Eye, J. D., L. H. Reuter, and K. Keshavan, Water and Sew. Works, 108, 231 (1961).

31. Finn, R. K. 136th Meeting, ACS, Atlantic City, N. J., Sept. 1959.

32. Finn, R. K., Bacteriol. Revs., 18, 254 (1954).

33. Friedenwald, J. S., and G. D. Maengwyn-Davis, A Symposium on the Mechanism of Enzyme Action, W. D. McElroy, and B. Glass, Eds., The Johns Hopkins Press, Baltimore, Md., 1954.

34. Friedman, A. M., and E. N. Lightfoot, Jr., Ind. Eng. Chem., 49, 1227 (1957).

35. Gaudin, A. M., Flotation, 2nd ed., McGraw-Hill Book Co., New York, N. Y., 1957.

36. Gaudin, A. M., N. S. Davis, and S. E. Bangs, Biotech. and Bioeng., 4, 211 (1962).

37. Gaudin, A. M., A. L. Mular, and R. F. O'Connor, Appl. Microbiol., 8, 84 (1960).

38. Gaudin, A. M., A. L. Mular, and R. F. O'Connor, Appl. Microbiol., 8, 91 (1960).

39. Godleski, E. S., and J. C. Smith, A. I. Ch. E. J., 8, 617 (1962).

40. Hixson, A. W., and E. L. Gaden, Jr., Ind. Eng. Chem., 42, 1792 (1950).

41. Ingraham, J. L., and G. F. Bailey, J. Bacteriol., 77, 609 (1959).

42. Ippen, A. T., and C. E. Carver, Sew. and Ind. Wastes, 28, 813 (1954).

43. Kempe, L. L., Adv. Appl. Microbiol., 2, 313 (1960).

44. Kempe, L. L., R. A. Gillies, and R. E. West, Appl. Microbiol., 4, 175 (1956).

45. Kurokawa, M., M. Hatano, N, Kashiwagi, T. Saito, S. Ishida, and R. Homma, J. Bacteriol., 83, 14 (1962).

46. Lamanna, C., and M. F. Mallette, Basic Bacteriology, The Williams and Wilkins Co., Baltimore, Md., 1959.

47. Lockwood, L. B., B. Tabenkin, and G. E. Ward, J. Bacteriol., 42, 51 (1941).

48. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).

49. Mancy, K. H., and D. A. Okun, J. Water Pollution Control Federation, 32, 351 (1960).

50. Merck & Co., Inc., The Merck Index, 7th ed., Rahway, N. J., 1960.

51. Metzner, A. B., R. H. Feehs, H. L. Ramos, R. E. Otto, and J. D. Tuthill, A. I. Ch. E. J., 7, 3 (1961).

52. Metzner, A. B., and R. E. Otto, A. I. Ch. E. J., 3, 3 (1957).

53. Miyamoto, S. and T. Kaya, Bull. Chem. Soc. Japan, 5, 123 (1930).

54. Miyamoto, S., T. Kaya, and A. Nakata, Bull. Chem. Soc. Japan, 5, 229 (1930).

55. Morgan, P. F., and J. K. Bewtra, J. Water Pollution Control Federation, 34, 363 (1962).

56. Ostwald, W., Kollaidzschr., 38, 261 (1926).

57. Ough, C. S., and M. A. Amerine, Food Rsch., 24, 744 (1959).

58. Pfeifer, V. F., and C. Vojonovich, Ind. Eng. Chem., 44, 1940 (1952).

59. Phillips, D. H., and M. J. Johnson, J. Biochem. Microbiol. Tech. and Eng., 3, 261 (1961).

60. Phillips, D. H., and M. J. Johnson, J. Biochem. Microbiol. Tech. and Eng., 3, 277 (1961).

61. Phillips, K. L., H. R. Sallans, and J. F. T. Spencer, Ind. Eng. Chem., 57, 749 (1961).

62. Porter, J. R., Bacterial Chemistry and Physiology, John Wiley and Sons, Inc., New York, N. Y., 1946.

63. Rahn, O., Bacteriol. Revs., 9, 1 (1945).

64. Reiner, M., Deformation and Flow, Lewis, London, 1949.

65. Richards, J. W., Br. Chem. Eng., 6, 454 (1961).

66. Richards, J. W., Progress in Industrial Microbiology, Vol. III, D. J. D. Hockenhull, Ed., Interscience Publishers, New York, N. Y., 1961, p. 140.

67. Rolinson, G. N., J. Gen. Microbiol., 6, 336 (1952).

68. Rose, J. B., and G. F. Somers, Chemistry and Methods of Enzymes, Academic Press, New York, N. Y., 1953.

69. Rushton, J. H., E. W. Costich, and H. J. Everett, Chem. Eng. Prog., 46, 467 (1950).

70. Saymn, J. C. and A. M. Mattocks, J. Am. Pharm. Assoc. 46, 310 (1957).

71. Seifter, S., S. Dayton, B. Novic, and E. Muntwyler, Arch. Biochem., 25, 191 (1950).

72. Sevag, M. G., J. Smolens, and D. B. Lackman, J. Biol. Chem., 134, 523 (1940).

73. Shedlovsky, L., Ann. N. Y. Acad. Sci., 49, 279 (1948).

74. Shu, P., Agr. and Food Chem., 1, 1119 (1953).

75. Siegell, S. D., and E. L. Gaden, Jr., Biotech. and Bioeng., 4, 345 (1962).

76. Sizer, I. W., Advances in Enzymology, Vol. III, Interscience Publishers, Inc., New York, N. Y., 1943, p. 38.

77. Smith, C. G., Appl. Bacteriol., 8, 42 (1960).

78. Solomons, G. L., The Soc. of Chem. Ind., Monograph No. 12, 233.

79. Solomons, G. L., and M. P. Perkin, J. Appl. Chem., (London), 8, 251 (1958).

80. Solomons, G. L., and G. O. Weston, J. Biochem. Microbiol. Tech. and Eng., 3, 1 (1961).

81. Standard Methods for the Examination of Water and Waste Water, 11th ed., American Public Health Assoc., Inc., New York, N. Y., 1960.

82. Staub, N. C., J. Appl. Physiol., 16, 192 (1961).

83. Steel, R., and W. D. Maxon, 139th Meeting, ACS Convention, Atlantic City, N. J., Sept. 1962.

84. Steel, R., and W. D. Maxon, Biotech. and Bioeng., 4, 231 (1962).

85. Strohm, J. A., and R. F. Dale, Ind. Eng. Chem., 53, 760 (1961).

86. Strohm, J. A., R. F. Dale, and H. J. Peppler, Appl. Microbiol., 7, 235 (1959).

87. Sultzer, B. M., J. Bacteriol., 82, 492 (1961).

88. Sumner, J. B., and G. F. Somers, Chemistry and Methods of Enzymes, Academic Press, Inc., New York, N. Y., 1953.

89. Tengerdy, R. P., J. Biochem, Microbiol. Tech. and Eng., 3, 241 (1961).

90. Terui, G., and N. Konno, Tech. Reports of the Osaka University, 10, 889 (1960).

91. Terui, G., and N. Konno, Tech. Reports of the Osaka University, 11, 447 (1961).

92. Terui, G., N. Konno, and M. Sase, Tech. Reports of the Osaka University, 10, 527 (1960).

93. Tsao, G. T. N., Ph.D. Thesis, University of Michigan, Ann Arbor, 1959.

94. Tsao, G. T. N., and L. L. Kempe, J. Biochem. and Microbiol. Tech. and Eng., 2, 129 (1960).

95. Van Slyke, D. D., J. Biol. Chem., 52, 525 (1922).

96. Vondrackova, J., Ceskoslov. Microbiol., 2, 37 (1957).

97. Warshowsky, B., and B. J. Schantz, Anal. Chem. 26, 11 (1954).

98. Wasserman, A. E., and J. W. Hampson, Appl. Microbiol. Tech. and Eng., 1, 163 (1959).

99. West, J. M., and E. L. Gaden, Jr., J. Biochem. Microbiol. Tech. and Eng., 1, 163 (1959).

100. Winzler, R. J., J. Cell. Comp. Physiol., 17, 263 (1941).

101. Yoshida, F., A. Ikeda, S. Imakawa, and Y. Miura, Ind. Eng. Chem., 52, 435 (1960).

102. Zieminski, S. A., and R. L. Hill, J. Chem. Eng. Data, 7, 51 (1962).

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