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THE EFFECTS OF POWER INPUT, AGITATION, AND AIR FLOW ON THE RATE OF OXYGEN TRANSFER IN FERMENTATION SYSTEMS

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NOMENCLATURE

A	Transferring area per unit volume, (L^2/L^3)
d	Impeller diameter, (L)
E	Air held-up in volume % of air based upon air-free liquid volume
f_g	Transfer potential of the gas phase
f_ℓ	Transfer potential of the liquid phase
f_s	Transfer potential of the solid phase
G	Air flow rate in volume of air per volume of air-free liquid per minute, (1/T)
k_1, k_2, etc.	Exponential and pre-exponential coefficients
K	Mass transfer coefficient in mass per unit area per unit time per unit volume of air-free liquid
s	Total liquid volume increase due to air held-up, (L^3)
S	Total volume of air-free liquid, (L^3)
N	Agitator speed in rpm., (1/T)
P	Power input in horsepower per liter of air-free liquid, (M/LT^3)
R	Rate of mass transfer in mass per unit time per unit volume of air-free liquid.
x_ℓ	Mass fraction of O_2 in the liquid phase
y_g	Mass fraction of O_2 in the gas phase
σ	Surface tension of the air-free liquid (M/T^2)
ρ	Density of the air-free liquid (M/L^3)
μ	Viscosity of the air-free liquid (M/LT)

CHAPTER I

INTRODUCTION

Microorganisms require a variety of nutrients for their growth. Supply of the nutrient materials involves transfer of matter to the microbial cells. The overall process in which the nutrients are consumed includes mass transfer as one of the intermediate steps. Therefore, the rate of mass transfer can be a limiting factor in microbial activities. The present work is primarily concerned with achieving a better understanding of the mass transfer of oxygen in fermentation systems. However, from these studies it may be possible to deduce valuable information about the transfer of other substances as well.

The rate of oxygen supply is an important factor in aerobic fermentations. In many microbiological systems, the amount of oxygen available for the microbial cells alters the predominant metabolic pattern. Even if a system is dominated by one set of consistent metabolic functions, oxygen supply may still control the rate of formation of certain metabolic end products. The importance of dissolved oxygen for altering cellular metabolism and promoting growth has been known since the time of Pasteur.(11) However, most of the researches on oxygen transfer were done after development of the submerged culture technique in the antibiotics industry. The submerged process is also used in the manufacture of vitamins, yeast cells, alcohols, acids, antibiotics and many other valuable substances.

The submerged fermentation process can be very briefly described as a process in which the fermenting broth is continuously agitated and sparged with air in such a way that the organism can grow even when it is

submerged in the deep bulk of the broth. In this situation, the oxygen consumed by the organisms must be transferred from air bubbles to the microbial cells. The overall oxygen transfer can be visualized as consisting of the following consecutive steps:

(1) Dissolution of oxygen from the gas phase across the gas-liquid interface into the liquid.

(2) Transport of dissolved oxygen to the vicinity of the cells by diffusion and mixing, and

(3) Transfer of oxygen across the boundary between the liquid and the cell wall and thence into the cells.

The rate of oxygen transfer can be influenced at any or all of these stages. In a continuously agitated fermentation broth, the second step is usually considered to be sufficiently fast that it is not rate controlling. At the third stage, the oxygen obtained by the microbial cells are used in certain biochemical reactions which are part of the whole scheme of the culture's metabolism. The rates of such reactions are probably governed by the number of reaction sites, local concentration of oxygen, temperature and other factors. When these factors are in ranges such that the chemical reactions which involve oxygen are not rate limiting, the overall rate of oxygen utilization by the system is controlled either by the rate of oxygen transfer across the gas-liquid interface or across the liquid-cell wall boundary. From this viewpoint, the present study becomes essentially a special problem involving mass transfer across biphasic boundaries. This has been thoroughly and extensively studied in the physical and engineering sciences; however, in connection with microbiological systems, it has been explored much less thoroughly.

The best way to secure a thorough understanding of oxygen transfer would involve observations and measurements made directly at the biphasic boundaries. A complete picture of mechanism of oxygen transfer across these boundaries can only be drawn with knowledge concerning factors, such as local oxygen concentration at the interface, properties of the boundaries, and so forth. However, due to the limitations of present experimental techniques, such observations and measurements cannot be made. In the present work, therefore, indirect methods were used and the rate of oxygen transfer was measured on an overall basis. The experiments and methods used were selected accordingly. These were considered to be entirely satisfactory, since the purpose of the present study is an examination of the factors controlling the overall oxygen supply to fermentation systems rather than the mechanism by which the oxygen is transferred.

Three working systems were used. They were: sulfite oxidation, Penicillium chrysogenum respiration, and Pseudomonas ovalis respiration. In the sulfite oxidation system, the oxygen is absorbed by sulfite and converted into sulfate ions. The concentration of sulfite ions is measurable. In the Penicillium chrysogenum system, respiration of cells converts oxygen into carbon dioxide. The percent of carbon dioxide in the exhaust gas from the fermentor was measurable so the oxygen transfer rate was indirectly determined. Pseudomonas ovalis respiration involves the oxidation of glucose into gluconic acid. The rate of gluconic acid formation was selected as the experimentally measured variable. Thus the oxygen transfer rate in the Pseudomonas ovalis system was also determined in an indirect manner.

The rate of oxygen transfer crossing biphasic boundaries in submerged fermentations is a function of many factors, such as, agitator speed, air flow rate, viscosity, surface tension, and many others. In the present work, the agitator speed and air flow rate were studied in detail. They are also the two most important factors in the design and control of fermentation processes utilizing submerged culture. The combined effect of agitation intensity and air flow rate on oxygen transfer was studied in order to achieve a better understanding of transport phenomena in submerged culture fermentations.

CHAPTER II

HISTORICAL REVIEW

In submerged fermentations, oxygen consumed by microbial cells must be transferred progressively from gas to liquid, through the bulk of the liquid medium and then through the cell wall in order to reach the reaction sites within the cells. It is evident that the rate of oxygen transfer might be a rate limiting mechanism for metabolism, depending upon various internal and external cellular conditions.

For most aerobic organisms, there is a critical level of the rate of oxygen supply below which the overall metabolism of the organism is limited. Finn[11] has collected data concerning the critical level of oxygen supply for a few organisms.

Most of the work reported before 1940 concerning oxygen uptake by cells and tissues appears in the reviews by Goddard[17], Tang[44,45] and Kempner[24]. These reports reveal quite divergent viewpoints among different groups of researchers concerning the limiting portion of the overall oxygen consumption process when the rate of oxygen supply is below the critical level. Enzyme surface unsaturation and slow rate of oxygen diffusion were two major theories postulated to explain the rate limiting mechanism.

In 1941, Winzler[49], working with the yeast cells in carbon monoxide and oxygen mixtures, found a falling respiration rate at oxygen tensions so high that diffusion of oxygen could not possibly have been rate limiting. More recently, Longmuir[27] reopened the question in regard to bacteria. He studied the rate of oxygen uptake by bacteria having a

wide range of cell sizes and reported that larger bacteria exhibit a higher critical oxygen level. This supported the diffusion controlling postulate.

Ever since the development of submerged culture technique in the fermentation industry, oxygen transfer study has received attention due to the participation of a new group of workers from the field of industrial technology. In 1944, Cooper, Fernstrom, and Miller[7] published an article concerning the performance of agitated gas-liquid contactors. They used the sulfite oxidation technique. This method, along with the dropping mercury technique[15], has been extensively used in oxygen transfer study since that time.

Silcox and Lee[40] discussed the development of fermentation technology and drew attention to the need for fundamental studies of various phases of the general fermentation process from a combined biological and engineering viewpoint. Two articles, one by Hixon and Gaden[20], the other by Bartholomew, Karow, Sfat, and Wilhelm[1] appeared in a fermentation symposium in 1950. They analyzed the overall oxygen consumption as a sequence of individual steps:

(1) Dissolution of oxygen from gas into liquid.

(2) Oxygen transfer through the bulk of liquid to the vicinity of microbial cells.

(3) Oxygen transfer to the microbial cells.

(4) Chemical, probably enzymatic, reaction converting oxygen into metabolic end products.

They considered the possibility that any one of the above could be rate limiting in the overall process, depending upon properties of the organism

and the environmental conditions. The arguments of enzyme surface unsaturation and slow rate of oxygen diffusion of the early years seemed to be placed into a single, non-contradictory cord.

The concept of a "master reaction" has been discussed by Hinshelwood[19]. Based upon this concept, oxygen transfer in fermentation systems becomes essentially a special phenomenon of mass transfer in cases where the chemical reactions are not limiting. Use of this concept permits information obtained from non-biological systems for evaluation of mass transfer in fermentations.

Sulfite oxidation has been extensively studied by many fermentation researchers. Discrepancies between biological and non-biological systems are naturally unavoidable. However, because the sulfite oxidation reaction is relatively simpler to carry out, it has been widely investigated for obtaining information concerning aerobic fermentations.

In sulfite oxidation, sulfite ions are converted into sulfate in the presence of suitable catalysts such as cobalt or copper. By iodometric titration of sulfite ions, the rate of oxygen transfer to the system can be determined.[7] This reaction proceeds at a rate independent of sulfite concentration over ranges down to approximately 0.008M[32]. Because of this, and because the reaction occurs so rapidly, the reaction rate does not become the controlling factor. Thus, oxidation of sodium sulfite by dissolved oxygen is a convenient system for studying oxygen transfer in the air-water system as shown by Cooper, Fernstrom and Miller[7]. Their study has great historical value and is the basis of many current studies. The same basic process and procedure have been used to specify the capability

of a fermentor to make oxygen available to cultures in fermentation mashes. Such work has been reported by many fermentation workers [2,9,10,12,28,32,34].

Though the amount of work done with the sulfite oxidation reaction is enormous, the value of the sulfite results for elucidating the characteristics of oxygen transfer in actual fermentation systems is still debatable. In a recent article, Phillip and Johnson[32] stated that sulfite results only indicate the maximum amount of oxygen available for microorganisms in the fermentation systems. Therefore, a valid understanding of oxygen transfer in actual fermentations requires further study. Oxygen transfer in fermentation mashes has been reported by Hixon and Gaden[20], Wise[50], Bartholomew and co-workers[1], and many others[8,18,33].

The reported results of the studies on sulfite oxidation and actual fermentations are briefly described in the following paragraphs.

Cooper, Fernstrom and Miller[7] studied the performance of agitated gas-liquid contactors with the sulfite method. At constant air flow rate, they were able to correlate their results with an empirical equation:

$$K_V = k\ P^{0.95} \qquad \text{(II-1)}$$

where,

K_V = volumetric absorption coefficient, lb mole/cuft./hr./atm.

k = constant

P = power input per unit volume.

With another plot, they determined an equation, for constant power input:

$$K_V = k'\ V_s^{0.67} \qquad \text{(II-2)}$$

where,

k' = constant

V_S = superficial gas flow rate.

Then they attempted to correlate the three factors, K_V, P, and V_S, but the results followed by a curvilinear relationship rather than a linear one on a logarithmic plot. They did not record the amount of expansion occurring in the liquid due to the combined air flow and agitation effects.

Rushton, Gallagher and Oldshue[37] also used the sulfite oxidation reaction to measure mass transfer rates between gas and liquid during mixing. They found linear relationships existing between the transfer coefficient and power input when they were plotted on logarithmic coordinates. Rushton's group also studied the effects of number and spacing of impellers on the mass transfer coefficient and power requirement. They stated that with single turbines mass transfer rates may be less than, equal to, or greater than those occurring when dual turbines were used. The exact percentage increase or decrease was found to be a function of the turbine spacing and air flow.

Friedman and Lightfoot[12] studied sulfite oxidation in a fermentor having a diameter of six inches. They attempted to obtain an expression relating the oxygen transfer coefficient and power input in an equation similar to that given by Cooper, Fernstrom, and Miller[7], but their data points resulted in a curve on logarithmic coordinates. No measurement of liquid volume expansion was reported.

Roxburgh, Spencer and Sallans[34] studied the effects of agitation and air flow on the rate of sulfite oxidation. They were primarily interested in correlating the oxygen transfer rate obtained from the sulfite

reaction with the rate of ustilagic acid production by an organism Ustilago zeae fermenting at the same operational conditions. This is a typical example of a system in which sulfite results were used as an index to specify the amount of oxygen available for a fermentation process.

Elsworth, Williams, and Harris-Smith[9,10] also used sulfite results to characterize a fermentor used for the Aerobacter cloacae fermentation. They presented their oxygen transfer results in several graphs.

Olson and Johnson[31] and Maxon and Johnson[28] also compared the results of sulfite oxidation and an actual fermentation. They found that the actual rate of oxygen utilized by propagating yeast cells was approximately equal to the rate of oxygen absorption predicted by the sulfite results. This is one of the rare cases where the rates of oxygen uptake in the sulfite system and in an actual fermentation system are approximately equal.

Hixon and Gaden[20] used a polarographic method for measuring oxygen transfer in actual fermentation systems. They obtained an equation relating the oxygen transfer rate and air flow rate in the yeast fermentation.

$$K_V = k\, V_S^{0.67} \qquad \text{(II-3)}$$

Their exponential coefficient agreed with that of Cooper et al.[7].

The polarographic technique has been used by many workers to observe oxygen uptake rate in different actual fermentations. Wise[50] applied the technique to measure the oxygen supply in Penicillium and Streptomyces broths. Rolinson[33] studied penicillin production in

relation to oxygen transfer. Similar researches were reported by Bartholomew and co-workers[1], Deindoerfer and Gaden[8], and Gondhalakar and Phadke[18].

The polarographic method was used for the oxygen transfer determination has been discussed in detail by Kolthoff and Lingane[25]. As applied in fermentation systems it usually involves the removal of oxygen in the broth by stripping with nitrogen followed by reoxygenation of the broth. The oxygen transfer rate was related to oxygen concentration change in the broths during the reoxygenation period. Therefore, this method measured the oxygen transfer rate during this unsteady stage rather than during the actual steady stage of fermentation. If the nitrogen stripping procedure affects the organism involved, the polarographic results will not reveal the actual oxygen transfer phenomena in the active fermentation systems.

Wise[50] reported that the polarographic results were considerably lower than those found by sulfite oxidation. However, agreement between the two methods was obtained by Chain and Gaulandi[5]. Solomons[41], Solomons and Perkin[42], Steel and Brierley[43], and Brierley and Steel[2] compared the rates of oxygen transfer in fermentors using the sulfite and polarographic methods in parallel. For sulfite oxidation, Steel and Brierley reported that,

$$K_L A = k\, G^a \qquad \text{at constant N} \qquad \text{(II-4)}$$

$$K_L A = k' N^b \qquad \text{at constant G} \qquad \text{(II-5)}$$

where,

G = air flow rate in VVM,

N = agitator speed in RPM,

K_L = mass transfer coefficient (mass/unit time/unit area)/ (mass/unit volume),

A = transfering area (area/unit volume),

k and k' = constants,

a varied between 0.4 and 0.7,

b varied between 2.5 and 3.2,

and, for the polarographic determination,

$$K_L A = k\, G^{0.53} \quad \text{at constant N} \tag{II-6}$$

$$K_L A = k'\, N^{1.0} \quad \text{at constant G} \tag{II-7}$$

Rushton and his co-workers[35,36] were primarily interested in the performance of various mixers. After extensive studies of mixing characteristics over wide ranges of different variables, they developed several equations to relate the power input into the mixers as functions of agitator speed and other variables. Since the power input and agitator speed are two factors affecting oxygen transfer in fermentation systems, their results are briefly reviewed. Rushton's group found that,

$$P = k\, \mu N^2 d^3 \quad \text{for viscous flow} \tag{II-8}$$

$$P = k'\rho N^3 d^5 \quad \text{for turbulent flow} \tag{II-9}$$

where,

μ = viscosity of the fluid

ρ = density of the fluid

d = diameter of the impellers.

In the transition range, the exponential constant of N varied between two and three. Rushton and his co-workers also found an empirical equation relating power input with air hold-up in the liquid, the superficial air flow rate, and the total liquid volume. Their equation has the following form,

$$(h/L) = c\ (P/V_s\ L)^{0.67} \qquad \text{(II-10)}$$

where,

h = air hold-up per unit depth of bulk liquid

L = total air-free liquid volume

P = power input per unit volume of liquid

V_s = superficial air flow rate

c = constant.

Friedman and Lightfoot[12] studied sulfite solutions in a fermentor having a diameter of six inches. At the superficial air flow rate below 45 feet per hour, they found the following relation between power input and agitator speed:

$$P = k\ N^{2.1} \qquad \text{(II-11)}$$

At the higher air flow rates, the exponential coefficient was changed to 2.4. Elsworth, William and Harris-Smith[9,10] worked with a similar system and obtained

$$P = k\ N^{3.1}\ d^{3.8} \qquad \text{(II-12)}$$

The results of studies of oxygen transfer in fermentation systems reported in the literature can be summarized as follows:

(1) The importance of oxygen transfer study for a better understanding of aerobic fermentation processes has been established.

(2) Sulfite oxidation and polarographic methods are widely used. However, an effective method for measuring the oxygen transfer rates in actual fermentations has not been developed.

(3) The results obtained by sulfite and polarographic techniques are usually expressed as functions of one of the three factors, power input, agitator speed and air flow rate, holding the rest of the variables constant.

(4) Equations relating oxygen transfer rate, power input and air flow rate in one correlation have not been developed. The volume expansion of the liquid has been observed in a few cases, but its true importance on oxygen transfer in fermentation systems has not been recognized.

CHAPTER III

MATERIALS AND METHODS

A. A General Description of the Systems Under Study

The study of oxygen transfer in fermentation systems is still at the early stage of its development. It seems more fruitful to study the phenomenon from a general viewpoint than from a specific one. At the present time, it seems more beneficial to study several systems with wide ranges of variations in properties than to study the detailed mechanism of oxygen transfer for a specific fermentation system. The systems and methods included in the present research were selected accordingly.

The organisms involved in submerged fermentations may be bacteria, molds, yeasts, or others. The properties of different types of fermentation broths vary over very wide ranges. Therefore, it is a difficult but important task to select representative systems for study so that the results may have general significance.

Three systems have been studied in the present program: Penicillium respiration, Pseudomonas respiration, and sulfide oxidation. The medium used for the Penicillium respiration system was prepared from pure inorganic and organic compounds along with corn steep liquor concentrate which is recovered as a by-product from the corn starch industry. Therefore, the medium used is chemically undefined. The Penicillium broth contains mycelium which represents a large amount of cell solids. Hydrodynamically, the broth is considered to be non-Newtonian; this is characteristic of mold fermentation broths.

Pseudomonas ovalis was selected for the second respiration study. These bacteria are short rods and exist as discrete single cells. The suspensions of resting cells of Pseudomonas ovalis used for the research program were prepared in a solution of glucose and inorganic phosphate. Chemically speaking, this solution is simple and well defined in composition.

The Penicillium and Pseudomonas systems studied involved microbial cells. In the Penicillium broths, the cells were continuously growing. Because the culture was in a stationary phase, the growth was relatively slow. In the experiments involving Pseudomonas ovalis, bacterial cells were centrifuged and then re-suspended in a nitrogen free solution which consisted only of glucose and inorganic phosphate. Therefore, the oxygen transferred into this system was consumed by resting cells of Pseudomonas ovalis.

The sulfite oxidation system is purely chemical. No biological factor is involved. It does not contain solid material. Gas and solution make the system a biphasic one. As described in the historical review, sulfite oxidation has been studied intensively as a possible system for characterizing oxygen transfer in fermentation systems. Study of the sulfite system permits a comparison of the present work and results already reported in the literature.

In Penicillium fermentation, once a stationary growth phase has been established, oxygen is stoichiometrically converted into carbon dioxide as a result of terminal respiration of this organism. Johnson[21] utilized the carbon dioxide content of the effluent air from tank fermentations as a guide to the amount of growth and metabolic activity of Penicillium

culture. Rolinson[33] used the manometric technique and observed a constant ratio between the amount of oxygen consumed and the amount of carbon dioxide generated by Penicillium chrysogenum. He measured the respiration quotient of the culture and found it to be approximately one.

In the present research, the carbon dioxide content of the exhaust gas from the Penicillium broths was accurately measured by infra-red technique. This technique has the desirable characteristic that the sensitivity of the infra-red response varies inversely with the carbon dioxide concentration in the gases. In the Penicillium broths studied, the carbon dioxide content of the exhaust gases was generally below one percent.

The specific strain of Pseudomonas ovalis used converts oxygen and glucose into gluconic acid. The growth of this specific strain was studied by Lockwood, Tabenkin, and Ward[26]. They found an 85% conversion of glucose into gluconic acid. A similar result was also reported by Ueda, Hayashi and Asai[47].

The technique for measuring the rate of acid formation in a fermentation system has been well developed by Kempe[23], Gilles[16] and West[48]. The rate of oxygen transfer in the Pseudomonas system was determined as a function of the rate at which acid was produced.

The rate of oxygen absorption by sulfite solution was measured by iodometric titration using the same technique pioneered by Cooper, Fernstrom, and Miller[7].

B. Derivation of the Empirical Equations for Correlating The Experimental Results

In fermentation systems, oxygen transfer is concerned with both the gas-liquid and the liquid-solid boundaries. It has already been pointed

out that the present research deals essentially with the phenomenon of mass transfer. A transfer equation is usually written in the following manner:

$$R = KA\,\Delta f \qquad \text{(III-1)}$$

where,

R = rate of oxygen transfer

K = oxygen transfer coefficient associated with either gas-liquid or liquid-solid interface

A = transferring area associated with either gas-liquid or liquid-solid interface

Δf = potential difference associated with either gas-liquid or liquid-solid interface.

When this equation is applied to a gas-liquid boundary, oxygen transfer can be expressed in the following way,

$$R = KA\,(f_g - f_\ell) \qquad \text{(III-2)}$$

where,

f_g = oxygen transfer potential of the gas phase

f_ℓ = oxygen transfer potential of the liquid phase.

In an overall process, if the rate limiting factor is the gas-liquid boundary resistance, Equation (III-2) can be approximated by Equation (III-3) or Equation (III-3a).

$$R = KA\,f_g \qquad \text{(III-3)}$$

$$R = KA\,y_g \qquad \text{(III-3a)}$$

where y_g is the mass fraction of oxygen in the gas phase. In the cases where the partial pressure of oxygen in the gas is substantially constant,

the transfer rate is roughly proportional to the product of a transfer coefficient and the transferring area at the gas-liquid inteface.

Similarly, for oxygen transfer across a liquid-solid boundary, the relationship can be expressed as Equation (III-4).

$$R = KA\ (f_\ell - f_s) \qquad \text{(III-4)}$$

where f_s is the oxygen transfer potential of the solid phase. If the overall rate is controlled by the liquid-solid boundary resistance, it can be approximated by Equation (III-5) or Equation (III-5a).

$$R = KA\ f_\ell \qquad \text{(III-5)}$$

$$R = KA\ x_\ell \qquad \text{(III-5a)}$$

where x_ℓ is the mass fraction of oxygen of the liquid. Since x_ℓ is essentially constant, the rate of transfer across this boundary can also be considered to be proportional to the product of a transfer coefficient and transferring area at the solid-liquid interface.

For transport phenomena, a rigorous theoretical approach is still in the development stage. Except for a few simplified models, most transport results are expressed in an empirical manner and dimensional analysis is the technique most often applied for development of correlations.

In a submerged fermentation system, both gas-liquid and liquid-solid boundaries are involved in oxygen transfer. For either boundary, if KA is considered a function of the factors in Equation (III-6),

$$KA = F\ (N,G,\rho,\mu,d,S,\sigma,s) \qquad \text{(III-6)}$$

where,

N = agitator speed in RPM, (1/T),

G = air flow rate in VVM, (1/T),

ρ = density of the liquid, (M/L^3),

μ = viscosity of the liquid (M/TL),

d = impeller diameter (L),

S = liquid volume (air free), (L^3)

σ = surface tension (M/T^2),

s = total liquid volume increase due to air hold-up (L^3),

a relation can be obtained by using dimensional analysis,

$$KA = k_1 N\left(\frac{G}{N}\right)^{k_2}\left(\frac{S}{d}\right)^{k_3}\left(\frac{d\mu N}{\sigma}\right)^{k_4}\left(\frac{d^2 N\rho}{\mu}\right)^{k_5}\left(\frac{s}{S}\right)^{k_6} \qquad \text{(III-7)}$$

The groups in the parentheses are all dimensionless.

If power input into the system, through the agitator, is used in place of rotational speed, Equation (III-6) becomes

$$KA = F(P,G,\rho,\mu,d,S,\sigma,s) \qquad \text{(III-8)}$$

where P refers to power input per unit volume of air free liquid

(M/LT^3)

and another equation can be derived as below,

$$KA = k_7 \rho G\left(\frac{S}{d}\right)^{k_8}\left(\frac{G\rho d^2}{\mu}\right)^{k_9}\left(\frac{Pd}{G\sigma}\right)^{k_{10}}\left(\frac{\sigma}{d^3 G^2 \rho}\right)^{k_{11}}\left(\frac{s}{S}\right)^{k_{12}} \qquad \text{(III-9)}$$

In the present experiments, conditions were arranged so that the terms, S,d,μ,ρ, and σ, were not varied in a given series of experiments. The equations, therefore, reduce to

$$KA = k_{13} N^{k_{14}} G^{k_{15}} E^{k_{16}} \qquad \text{(III-7a)}$$

$$KA = k_{17} P^{k_{18}} G^{k_{19}} E^{k_{20}} \qquad \text{(III-9a)}$$

where,

$$E = (^{s}/S) \times 100$$

The results obtained from the present experiments were fitted with the derived equations. Meanwhile, the pre-exponential and the exponential constants were determined by graphical trial and error analysis.

It should be noted that the derived equations are of empirical nature; hence, they can only be used to approximately relate experimental results which might be better correlated by equations of different, but as yet unknown forms.

C. Sulfite Oxidation

The first oxygen transfer study using the sulfite oxidation method was reported by Miyamota, Kaya, Nakata[29,30] in 1930. In 1944, Cooper, Fernstrom and Miller[7] extended this technique to characterization of the performance of agitated gas-liquid contactors. Ever since the publication of the results of this study, sulfite oxidation has been used for evaluation of the aeration efficiency of aerobic fermentors.

In the sulfite oxidation reaction, sulfite ions are converted into sulfate in the presence of a suitable catalyst such as cupric ions. The overall reaction consists of many intermediate steps which are not completely understood at present. However, the reaction is usually presented in the following overall form:

$$SO_3^{=} + 1/2\ O_2 \xrightarrow{Cu^{++}} SO_4^{=}$$

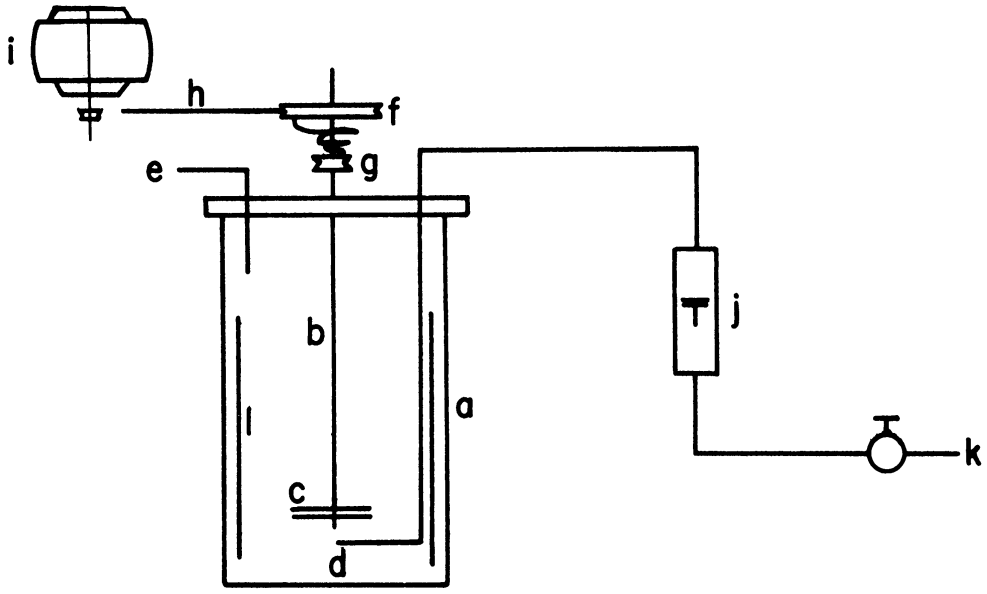
The oxygen transfer rate can be indirectly calculated by determining the change of sulfite ion concentration. A most remarkable characteristic of

this reaction is that it proceeds at a rate independent of sulfite ion concentration over a wide range of concentrations[7,14,32]. Because of this desirable property, sulfite oxidation can be employed as a convenient method for measuring oxygen absorption.

1. Equipment

Two different agitated fermentors were used. One of the vessels had a total volume of 44.5 liters and was made of glass. It was cylindrical, one foot in diameter, two feet high and was baffled with four, flat, stainless steel plates 1.25 inches in depth and 12.5 inches high, located 90° apart from one another. These plates were tied together with two stainless steel rings, one located at each end of the plates. For use, the baffle ensemble was placed in the vessel with one end resting on the bottom of the glass jar and with the surface of the plates perpendicular to the vessel wall. The glass vessel was covered with a steel head plate through which a central agitator shaft was passed. An impeller was fixed at the end of the shaft for agitation purposes. This impeller had four blades spaced at right angles. Each blade was three inches in length, with the blade surfaces perpendicular to and 1.5 inches above the bottom of the fermentor. A pipe was fixed on the head plate; the tip of the pipe had a single-hole sparger attached to serve as air supply device. The pipe was equipped with an elbow and a coupling in such a way that the air sparger was set immediately below the central agitation shaft. The opening of the sparger faced upwards. A rotometer was used to measure air flow rates. The equipment is shown in Figure 1.

Besides the 44.5 liter fermentor, another mechanically similar vessel of a different size was also used for the sulfite oxidation tests.



a : Fermentor
b : Agitator shaft
c : Impeller
d : Air sparger
e : Exhaust gas line
f : Pulley
g : Dynamometer
h : V-belt
i : Motor
j : Flow meter
k : Air line
l : Baffle plates

Figure 1. Diagram of the 44.5 liter Fermentor and its Accessories.

This second vessel was also cylindrical but smaller, having a total volume of five liters. It was six inches in diameter and twelve inches high. Four stainless steel baffle plates were fixed on the head plate which was also equipped with a bearing for the central agitator shaft. The baffle plates were placed at right angles, with their surfaces perpendicular to the vessel wall and their lower ends 0.5 inch above the bottom of the vessel. Two impellers of the same type and geometry were fixed on the central agitator shaft, one was 2.5 inches and the other 6.5 inches above the bottom of the vessel. The impellers were made of stainless steel, with four blades 90° apart from one another. They were 0.625 inch wide and 0.625 inch long with their flat surfaces perpendicular to the vessel bottom. An air pipe was passed through the head plate; at its tip a single hole sparger was attached with the aperture facing upwards, 0.5 inch below the lower impeller.

The air flow rate for both of the fermentors described above was determined with a rotometer. A spring type dynamometer was used to measure the power input. The detailed construction and manner of operation of the dynamometer are described in Appendix I. The volume of liquid in the fermentors was measured by eye using a scale marked on the outside of the glass vessel for reference.

2. Experimental Procedure

An aqueous solution containing 0.25 mole of sodium sulfite per liter was placed in the fermentors, fifteen liters being used with the 44.5 liter vessel and three liters with the five liter vessel. The rest of the experimental procedure was exactly the same for the two fermentors.

After the head plate was mounted, air was bubbled through the liquid at a constant rate, and the agitator was started. Due to the absence of catalyst, the sulfite ions in the solution did not oxidize appreciably during this period.

Next, a ten percent cupric sulfate solution was added in sufficient quantity to produce a concentration of approximately 0.001 mole of copper ions per liter. After about one minute, a 10 ml. sample was taken from the liquid in the fermentor. The sulfite concentration in the sample was immediately determined by titration with a thiosulfate solution. A detailed description of this analytical method is given in Appendix II.

Ten minutes after the first sampling, a second sample was taken and analyzed in the same manner. From then on, samples were taken from the fermentor and analyzed every ten minutes. The rate of change of sulfite ion concentration was thus determined. From the rate of change of sulfite ion concentration, the rate of oxygen transfer into the solution was calculated by a multiplication factor. Usually four or five samples were taken for each run, the actual number depending upon the agitation level and the rate of air flow. The rate of change of the sulfite ion concentration was constant until the sulfite ions were almost completely exhausted from the solution. The declination in the rate of change in sulfite concentration signaled the termination of a run of the experiment.

The volume expansion of the liquid due to air entrainment was read from the scale marked on the outside of the glass vessel during each run. Only one agitator speed and one air flow rate was studied in each run. Parallel runs were made for different sets of agitator speeds and

air flow rates. The range of agitator speeds extended from 200 to 660 revolutions per minute; the air flow rates ranged from 0.3 to 2.0 volumes of air per volume of solution per minute.

D. Pseudomonas ovalis Respiration

A specific strain of Pseudomonas ovalis NRRL-B-8 converts glucose and oxygen into gluconic acid as its terminal metabolic product. This strain has been thoroughly studied by Lockwood, Tabenkin, and Ward[26]. They reported conversion of glucose into gluconic acid of more than 85%. The reaction can be expressed in the following form:

$$C_6H_{12}O_6 + 1/2\ O_2 \longrightarrow CH_2OH(CHOH)_4COOH$$

A continuous recording technique was developed by Kempe[23], Gilles[16], and West[48] to measure the instantaneous rate of acid formation in the lactic acid fermentation. The same technique was used in the present work to measure the rate at which gluconic acid was produced by the resting cells of Pseudomonas ovalis NRRL-B-8. The rate of oxygen transfer into the broth containing the resting cells of Pseudomonas ovalis was expressed in terms of the rate of acid formation. It was assumed that a constant proportion of oxygen was converted to gluconic acid and any acid by-products during the period of a single run.

1. Equipment

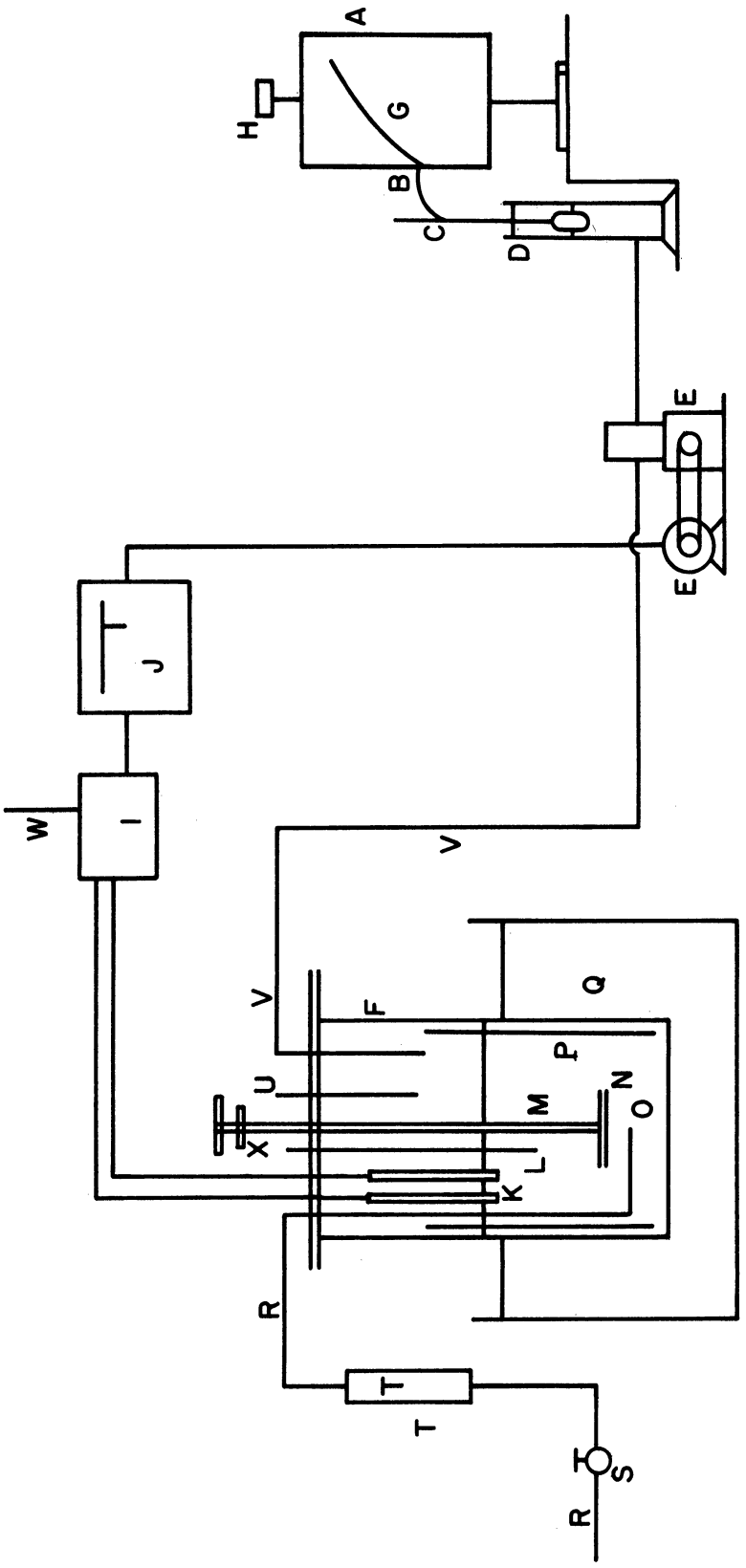
The five liter fermentor previously described in the sulfite oxidation section, was also used for the Pseudomonas ovalis respiration study. The arrangement of the baffles, the impeller and the air sparger were the same as in the sulfite work. The only difference in the physical

arrangement was that, in the Pseudomonas experiments, a calomel and a glass electrods were inserted in the broths to measure and control the pH value of the liquid in the fermentor.

The electrodes inserted into the liquid were connected to a Bristol pH-controller. During an experiment, the gluconic acid produced by the Pseudomonas ovalis cells decreased the pH value of the liquid. Once the pH reached the desired value, the controller started a pump which added a sodium hydroxide solution to the fermentor and thus raised the pH of the broth. The rate at which sodium hydroxide was pumped into the fermentor revealed the rate of gluconic acid formation. A diagram of the equipment used is shown in Figure 2.

The sodium hydroxide solution was contained in a one-liter graduate, D, which was connected to the fermentor, F, through the pump, E. A float, C, was placed on the surface of the sodium hydroxide solution. This float was fabricated from a glass bulb and a light wooden stick on which an ink-pointer, B, was attached with its tip pressed against the surface of drum, A. The drum was driven by a motor, H, at a speed of 24 or 12 hours per revolution as desired. During an experiment with Pseudomonas ovalis cells, the ink-pointer drew a curve on a sheet of paper mounted on the surface of the drum, A. The vertical coordinate indicated the volume of the sodium hydroxide solution in the cylinder, D; the horizontal coordinate indicated the time elapsed.

The rate at which the sodium hydroxide solution was pumped into the fermentor at any instant was obtained by graphical differentiation of this curve using a platen that was especially designed for this purpose.



A Kymograph drum
B Ink-pointer
C Float
D Cylinder (NaOH reservior)
E Sigma pump and its driving motor
F Fermentor
G Curve drawn
H Clock motor
I pH meter
J pH controller and recorder
K Electrodes
L Thermometer
M Agitator shaft
N Impeller
O Air sparger
P Baffle plates
Q Water bath
R Air line
S Valve
T Flow-meter
U Sampling tube
V NaOH feed line
W Power source
X Dynamometer

Figure 2. Equipment Used in the Pseudomonas ovalis Fermentation Study.

2. Preparation of Resting Cells of Pseudomonas ovalis

The strain of Pseudomonas ovalis NRRL-B-8 used in this study was obtained from the Northern Utilization, Research and Development Division of the United States Department of Agriculture at Peoria, Illinois. The culture was maintained on a slanted agar medium in an ice-box. The liquid medium used for culturing this organism was of the following composition:

Glucose	50 grams
KH_2PO_4	0.6 gram
$MgSO_4$	0.25 gram
Urea	2.0 grams
Yeast extract	5.0 grams
Distilled water	1.0 liter

When a solid medium was required, 2% of Bacto-agar was added. About ten milliliters of the agar medium were slanted in each test tube.

In preparation for an experiment, a slant culture was inoculated and incubated for 24 hours at 30°C. About ten milliliters of the liquid medium were sterilized in a separate test tube and poured over the 24 hour slant culture. The cells on the agar slant were then mixed into the liquid medium with a sterile transfer needle and the cell suspension thus obtained, was poured into one liter of fresh liquid medium contained in an 1.5 liter Florence flask. The liquid media used in both the test tubes and the 1.5 liter flasks were of the composition given above.

The incoulated media in the 1.5 liter flask was incubated at 30°C on a rotary shaker. This shaker rotated the flask at 200 revolutions

per minute in a circle with a one inch diameter. After 24 hours, the cells that developed during this incubation period were separated from the broth by centrifuging at 1300 gravities for 20 minutes. After decanting off the supernatant, the precipitated cells of Pseudomonas ovalis were resuspended in two liters of a buffered glucose solution of the following composition:

Glucose	50 grams
KH_2PO_4	1.2 grams
Distilled water	2.0 liters

No nitrogen was present in the solution. This resting cell suspension was used for the respiration study carried out in the five liter fermentor.

Most of the cell suspensions used in the experiments were made by harvesting the Pseudomonas ovalis cells from 880 ml. of 24 hour culture broths prepared on the rotary shaker as previously described and by resuspending the cells in two liters of the buffered glucose solution. The suspensions were checked with a photoelectric colorimeter. They gave an average reading of 60 with a brown filter, No.66. The instrument was of Model 800-3, manufactured by Klett-Summerson Company.

A series of experiments were conducted with the resting cells of Pseudomonas ovalis to study the effect of cell concentration on the rate of gluconic acid formation. In this series of experiments, solutions of different cell concentrations were used. These solutions were prepared by harvesting the cells from different amounts of 24 hour culture broths and resuspending them in two liters of the buffered glucose solution.

3. Experimental Procedure

Two series of experiments were conducted with resting cells of Pseudomonas ovalis. In the first series, the combined effect of agitation and air flow on the rate of oxygen transfer were studied; in the second series, the effect of cell concentration on the rate of oxygen transfer was investigated.

In the first series of experiments, 880 ml. of a liquid culture of Pseudomonas ovalis, that had been incubated for 24 hours on the shaker, were centrifuged and then resuspended in two liters of solution of the composition previously given. The cell suspension was placed in the five liter fermentor; four drops of an antifoaming agent, Dow polyglycol P-2000, were added; the head plate was mounted; then the agitator and air flow were started; finally the controller was set at a pH value of 5.5. The alkali liquid in the graduate was automatically recorded on Drum A, shown in Figure 2. This drum turned once in 24 hours.

In each run, the acid production rate started at a low value and increased gradually. After 30 to 60 minutes, a steady rate of acid production was established. This was indicated by a constant slope of the trace drawn on Drum A. Once a steady rate of acid production was established, the experiment was considered to be under way. It then was continued for one to two hours. This time was needed to allow the line drawn on the drum to become sufficiently long that its slope could be read accurately. The experiment was then terminated.

One level of agitator speed and air flow rate were studied during each run. Different runs were performed for different levels of agitation and air flow. These ranges covered 100 to 470 revolutions per minute for

the agitator speed, and 0.31 to 1.81 volumes of air per volume of liquid per minute for the air flow rate.

The second series of experiments was performed to study the effect of cell concentration on the rate of acid formation by Pseudomonas ovalis. For this purpose, the cell suspensions were prepared to contain different concentrations of bacterial cells as previously described. The effect of different cell concentrations on the rate of acid formation was then determined, following the experimental procedure described previously for the first series of experiments.

In all of the experiments, the volume expansion of the liquid due to air entrainment was recorded. Power input was also measured with the dynamometer as described in Appendix I.

E. Penicillium Chrysogenum Respiration

Rolinson[33] studied the terminal respiration of Penicillium chrysogenum and observed a respiration quotient of approximately one. Johnson[21] utilized the carbon dioxide content of the effluent air from tank fermentations as a guide to the amount of growth and metabolic activity of the fungus. In the present work, carbon dioxide, produced by a specific strain of Penicillium chrysogenum N329, was analyzed with the infra-red spectrophotometer. The rate of oxygen transfer was expressed in terms of the rate at which carbon dioxide was produced by the culture.

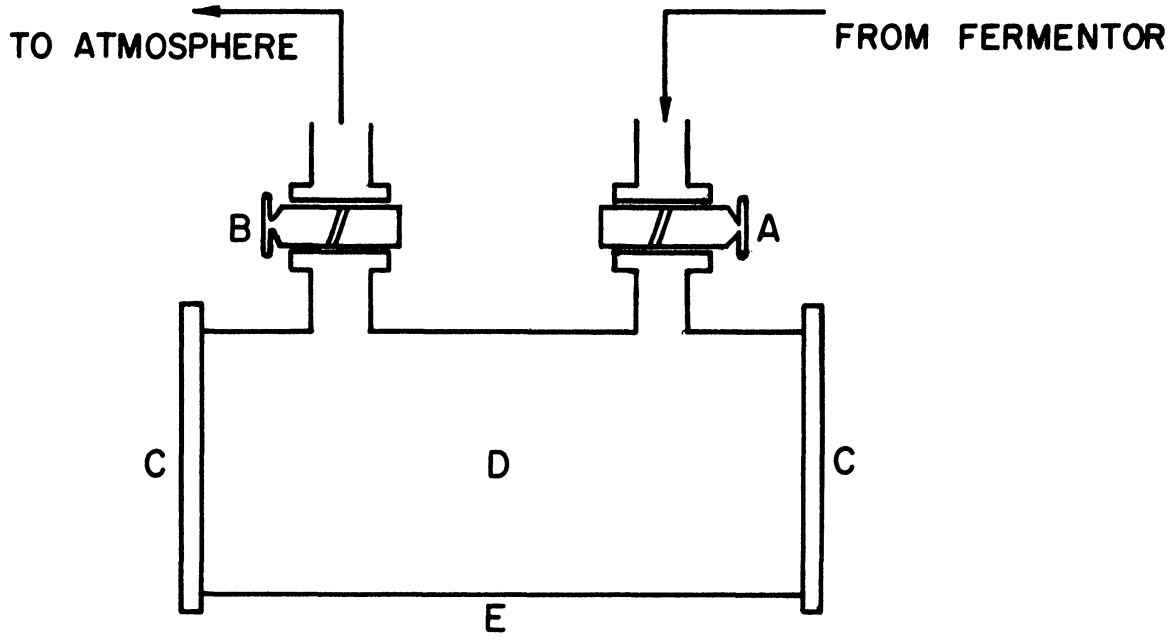
The Penicillium broths used for the respiration study were prepared in a separate container. Because the broths were prepared in this way, their consistency was controlled and the number of fermentations that could be carried out in a given period of time in one fermentor was increased.

1. Equipment

The five liter fermentor described in the sulfite oxidation section was also used for the Penicillium respiration study. The only difference in the physical arrangement of the equipment concerned the exhaust gas line. In the sulfite experiments, the exhaust gas from the fermentor discharged directly into atmosphere; in the experiments conducted with Penicillium chrysogenum, a part of the exhaust gas was collected in a gas cell for carbon dioxide analysis. This cell had sodium chloride windows and two glass stop cocks. A diagram showing the detailed construction of the gas cell is given in Figure 3 and the assembly of equipment in Figure 4.

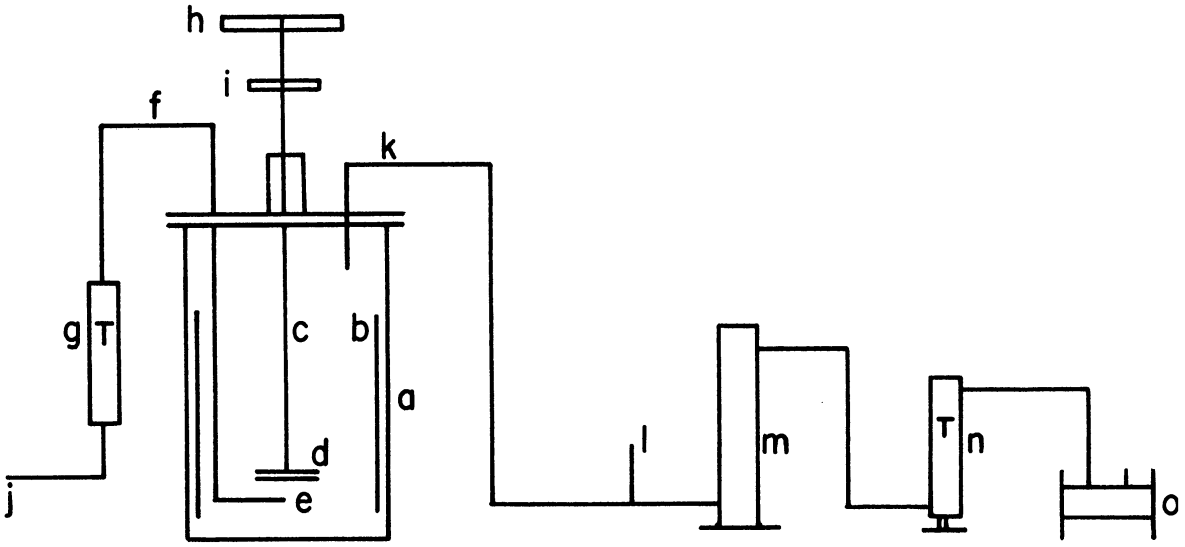
2. Preparation of Penicillium Broths

The slant culture of Penicillium chrysogenum N329 was kept in a refrigerator. For performance of a respiration experiment, the slant culture was first inoculated into 100 ml. of liquid medium contained in a 250 ml. Eryenmeyer flask. The methods used for preparation of the slant culture and the medium are given in Appendix III. The inoculated broth in the Eryenmeyer flask was incubated at 30°C on a rotary shaker which rotated the flasks in a 1 inch diameter circle at 200 revolutions per minute. After 48 hours, 10 ml. of the broth from the 250 ml. flask were poured into 100 ml. of fresh medium in a second 250 ml. flask which was then incubated at 30°C on the same shaker. Again, after 24 hours, 10 ml. of the broth from the second flask was inoculated into one liter of fresh medium in a 1.5 liter Florence flask. The third 250 ml. flask was incubated and used for further experiments in the same way as the second



A : Glass stop cock
B : Glass stop cock
C : Windows made of sodium chloride crystal
D : Gas chamber (200 ml. total volume)
E : Glass wall

Figure 3. Infra-red Gas Cell.



a Fermentor
b Baffle
c Central shaft
d Impeller
e Air sparger
f Air supply line
g Air flowmeter
h Driving pulley
i Dynamometer
j Valve
k Exhaust gas line
l By-pass
m Packed tower (packed with phosphorous pentoxide)
n Flowmeter
o Infra-red gas cell

Figure 4. Equipment Used in the Penicillium Chrysogenum Respiration Study.

250 ml flask. The culture in the 1.5 liter flask was incubated at 30°C for 48 hours on the same shaker and the broth was then ready for use in the respiration studies.

3. Experimental Procedure

Two liters of the Penicillium broth, prepared on the shaker, were first poured into the five-liter fermentor. After addition of four drops of antifoaming agent, Dow polyglycol P-2000, the head plate was mounted; then the agitator and air flow were started. After 45 minutes of operation, a part of the exhaust gas from the fermentor was passed through a tower packed with phosphorous pentoxide and then through the infra-red gas cell. This cell was evacuated twice before it was connected to the exhaust gas line shown in Figure 4. After 30 minutes of continuous passage of the exhaust gas, the stop cocks A and B of the gas cell were closed. A gas sample was thus collected in the cell; its carbon dioxide content was then determined in an infra-red spectrophotometer. A detailed description of the infra-red analytical method is given in the next section After completion of the analysis of the first gas sample, the gas cell was again evacuated and connected to the fermentor in order to collect a second gas sample. After 15 minutes of continuous passage of the exhaust gas through the cell, the stop cocks were closed and the second sample was analyzed. If the carbon dioxide contents of the two samples were identical, the experiment was terminated; if different, a third sample was taken. Usually two samples were enough to determine the steady rate at which carbon dioxide was being produced by the Penicillium broth. A run lasted about 110 minutes; when three gas samples were taken,

it took 135 minutes. To obtain the rate at which carbon dioxide was produced by the fermentation broth, the carbon dioxide content of the exhaust gas was multiplied by the air flow rate.

One level of agitator speed and air flow rate was studied in each run. Parallel runs were performed for different levels of agitation and air flow. The volume expansion of the broth was recorded in each run. Power input was measured with the dynamometer described in Appendix I.

4. Analytical Method: Infra-red Spectrophotometric Method

An infra-red spectrophotometer of double beam type was used for carbon dioxide analysis. The instrument was designated Model 21 by the manufacturer, the Perkin-Elmer Corporation. One of the two beams traversed the atmosphere and two layers of sodium chloride crystal; the other beam passed through the two sodium chloride windows of the gas cell and the sample of gas. A typical infra-red spectrum of the exhaust gas from the fermentor is shown in Figure 5. The height of peak A from m to n corresponds to the carbon dioxide content of the gas sample[6]. The numerical values of the carbon dioxide content were read from the calibration curve shown in Figure 6. This calibration curve was established by using mixtures of air and carbon dioxide of known compositions.

Because of the double beam arrangement, the infra-red method used showed the difference between the carbon dioxide content of the gas sample and that of the outside air entering the fermentor. Since the main interest of the present study involved the determination of the additonal carbon dioxide produced by the Penicillium broth, the double beam arrangement was admirably suited to the purpose.

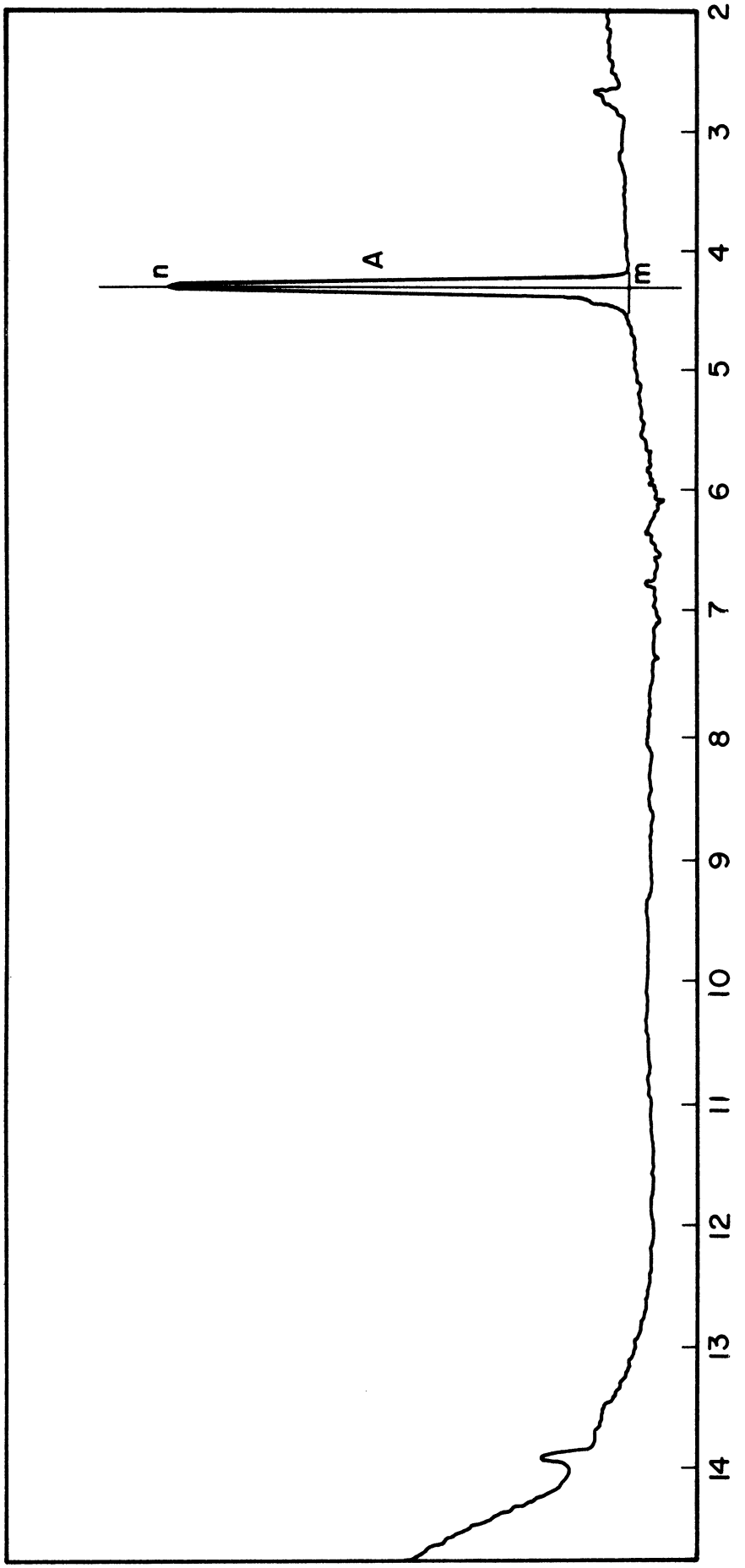


Figure 5. Infra-red Spectrum of Exhaust Gas from the Penicillium chrysogenum Respiration System.

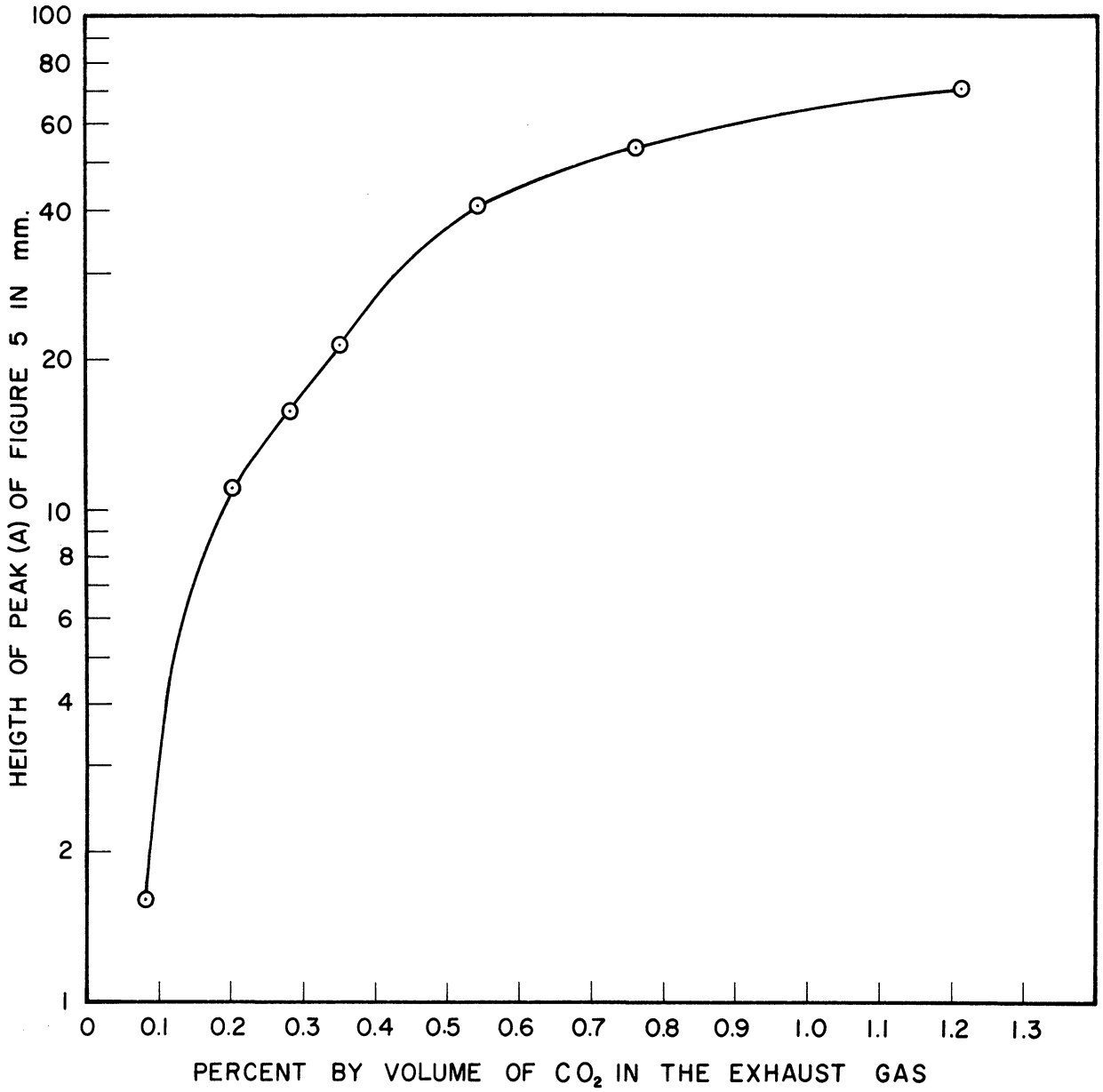


Fig. 6 Calibration Curve used in Infra-red Analysis for the Determination of Carbon Dioxide in the Exhaust Gas from the Penicillium chrysogenum Respiration System.

CHAPTER IV

RESULTS

The experimental results obtained from the systems of sulfite oxidation, respiration of Pseudomonas ovalis and respiration of Penicillium chrysogenum will be presented in the following sections. The results are correlated with empirical equations developed by dimensional analysis.

A. Sulfite Oxidation

Sulfite solutions were agitated and sparged with air. The rate of oxidation was determined by iodometric titration. The experimental results obtained from the 44.5 liter and the five liter fermentors are given in Tables I and II, respectively.

Equation (III-7a) developed by dimensional analysis

$$KA = k_{13} N^{k_{14}} G^{k_{15}} E^{k_{16}} \qquad \text{(III-7a)}$$

involves three exponential coefficients, k_{14}, k_{15}, and k_{16}. In the ranges where these coefficients are constant, Equation (III-7a) suggests a straight line correlation when the suitable groups of variables are plotted on logarithmic coordinates. Due to the complex form of the equation, the exponential coefficients could not be determined by ordinary graphical methods. Therefore, a graphical trial and error method was followed.

In the trial and error method, a value of k_{16} was first assumed. Based upon the assumption that the exponential coefficients are constant within the present experimental ranges, a plot of $KA/E^{k_{16}}$ versus N at constant G on logarithmic coordinates would give a straight line.

The value of k_{14} was then determined as the slope of the line. Similarly, k_{15} was determined with a plot at constant N. With k_{14} and k_{15} thus determined, a plot of $KA/(N^{k14}\ G^{k15})$ versus E was made on logarithmic paper to yield a straight line. The slope of this line determined a value of k_{16}. If this determined k_{16} was different from the first assumed k_{16}, a second value of k_{16} was then assumed and the graphical procedure of the above was followed again; if the two values were in good agreement, the trial and error procedure was stopped.

The values of k_{14}, k_{15} and k_{16} thus determined are 2.5, 0.4 and -0.6, respectively, for the 44.5 liter fermentor and 2.5, 0.4 and 0, respectively, for the five liter fermentor. The equations correlating agitator speed, air flow rate, liquid expansion and the oxygen transfer rate for the sulfite system are given below:

$$KA = 5.11 \times 10^{-7} N^{2.5} G^{0.4} E^{-0.6} \qquad \text{44.5 liter fermentor} \qquad \text{(IV-1)}$$

$$KA = 0.26 \times 10^{-7} N^{2.5} G^{0.4} E^{0} \qquad \text{5 liter fermentor} \qquad \text{(IV-2)}$$

The pre-exponential coefficients were determined by the least square method. The cross plots with the finally accepted values of k_{14}, k_{15} and k_{16} are given in Figures 7, 8, and 9. The final plot of KA/E^{k16} versus $(N^{k14}\ G^{k15})$ on regular coordinates with the data obtained from the 44.5 liter and the 5-liter fermentors are given in Figures 10, and 11, respectively.

The method for measuring power input into the sulfite oxidation system has been previously described. The experimental results of power input are also given in Tables I and II.

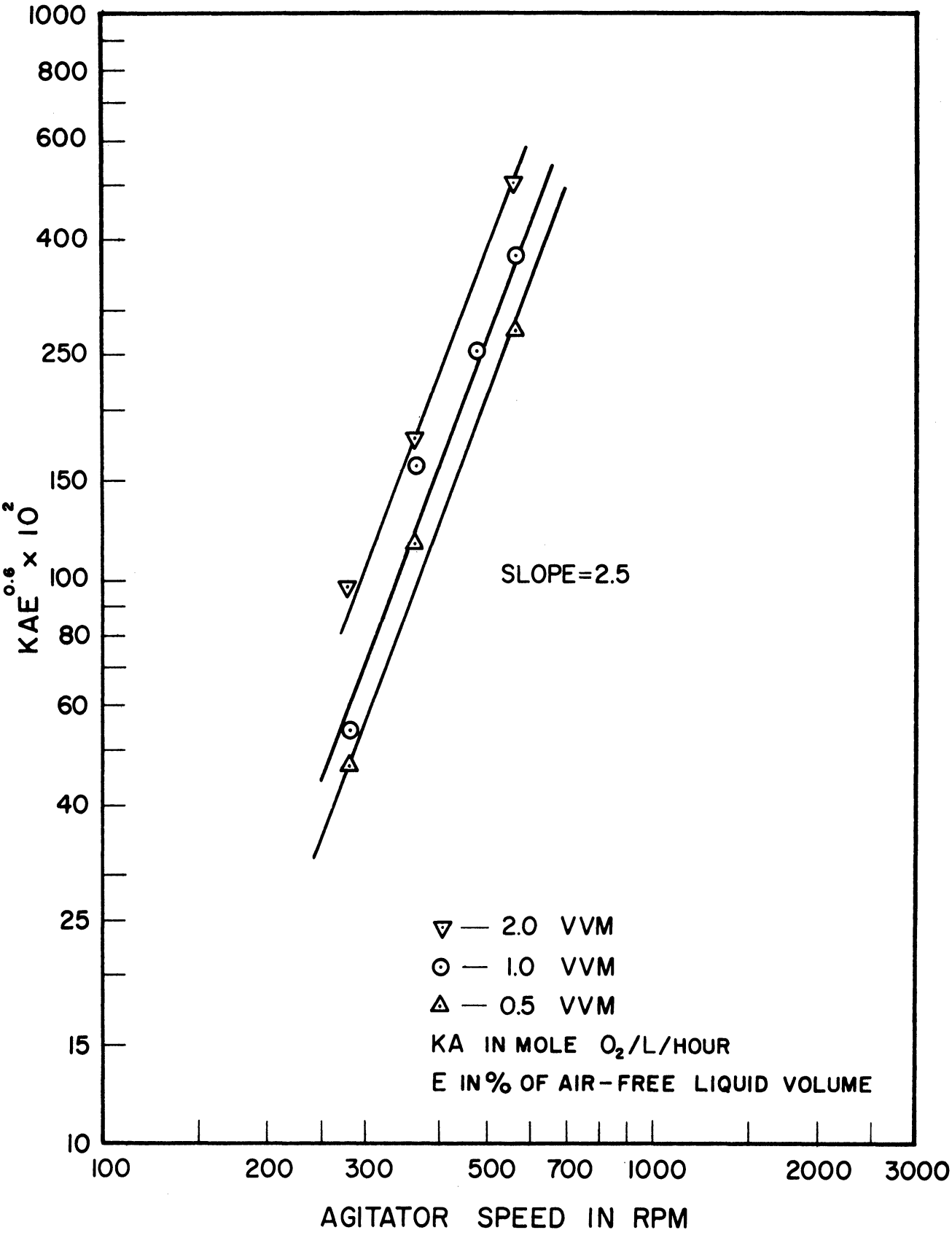


Figure 7. The Effect of Agitation on the Rate of Sulfite Oxidation in A 44.5 Liter Fermentor.

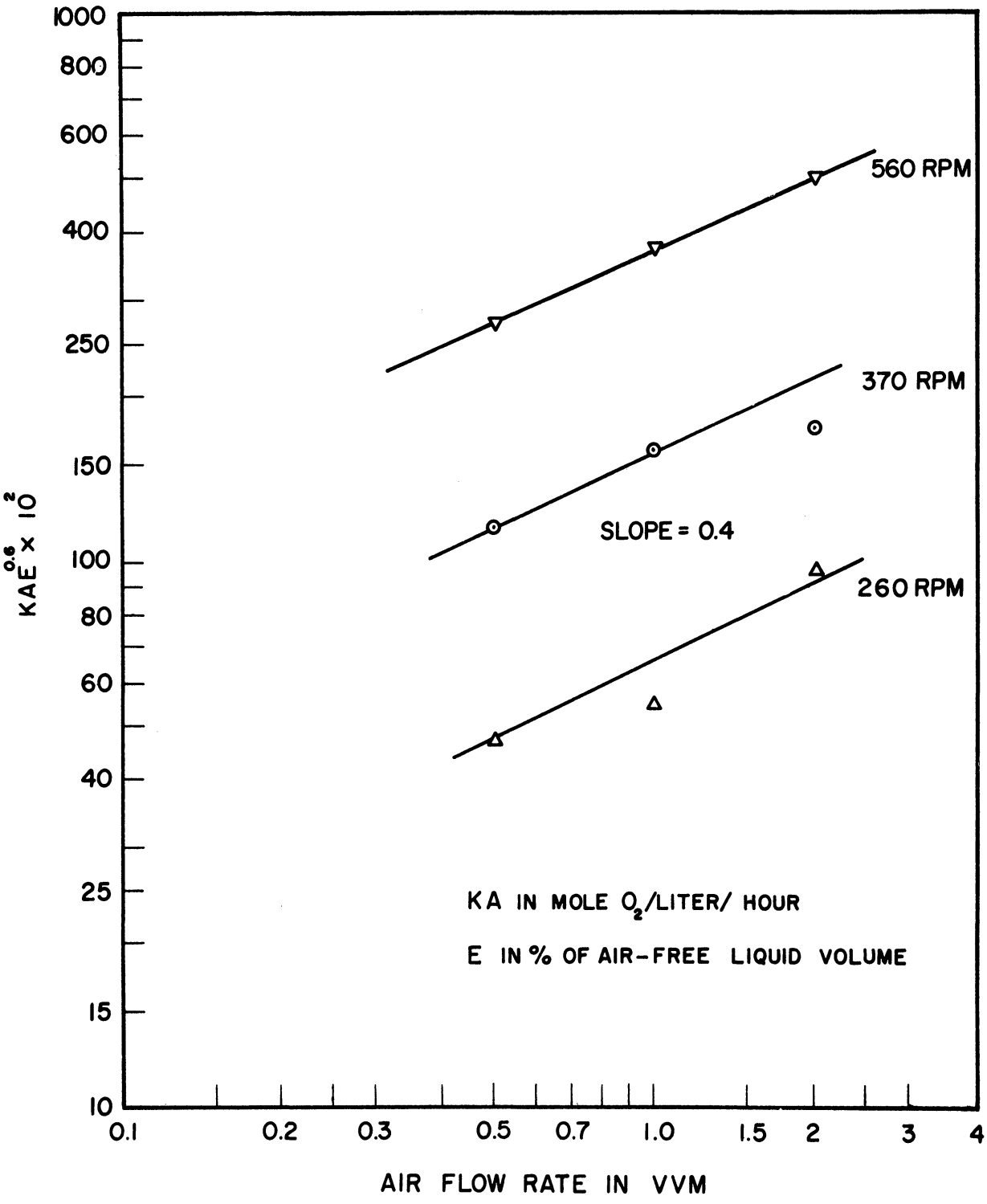


Figure 8. The Effect of Air Flow on the Rate of Sulfite Oxidation in A 44.5 Liter Fermentor.

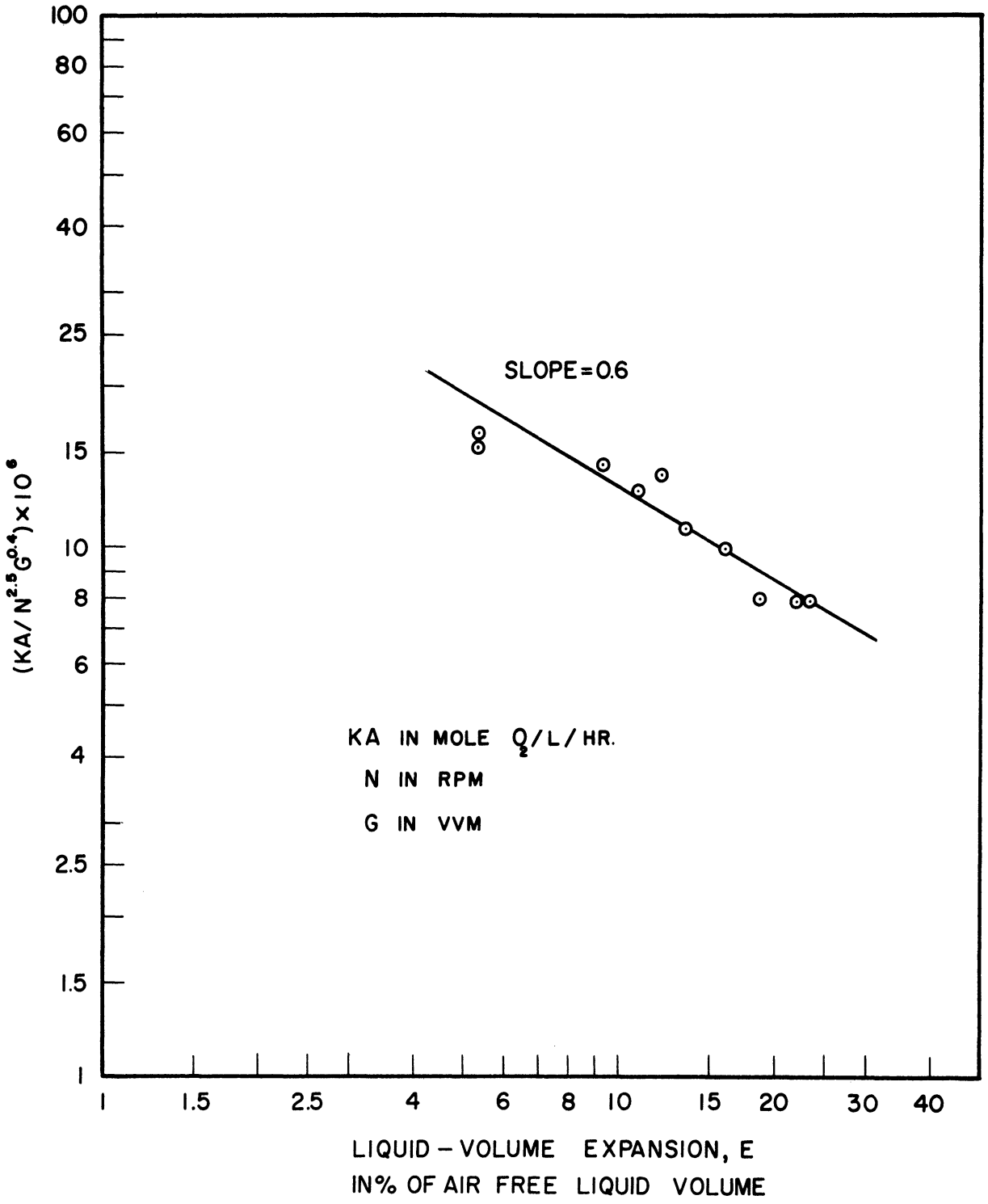


Figure 9. Effect of Liquid Volume Expansion on the Rate of Sulfite Oxidation in A 44.5 Liter Fermentor.

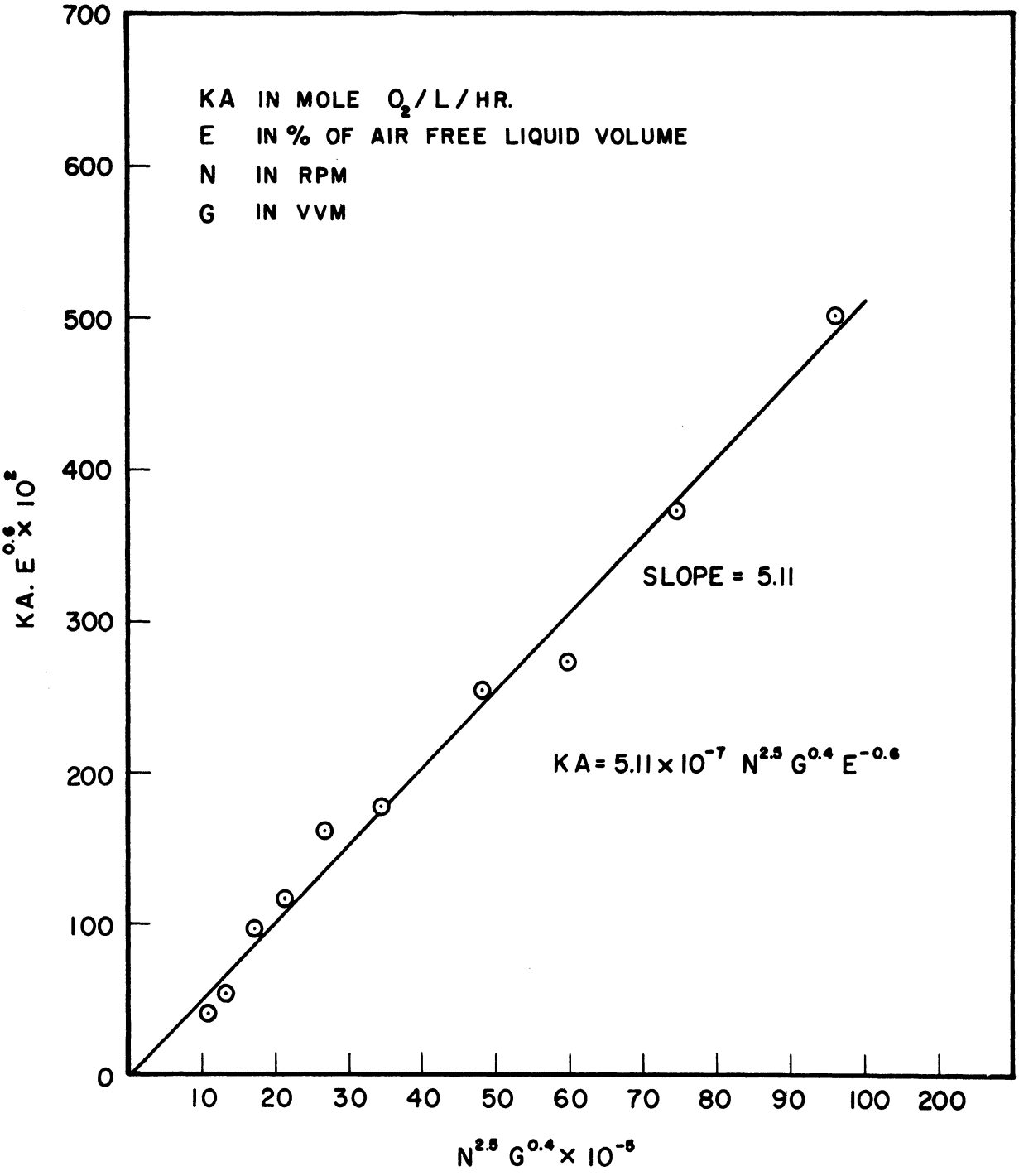


Figure 10. Combined Effect of Agitation and Air Flow on the Rate of Sulfite Oxidation in A 44.5 Liter Fermentor.

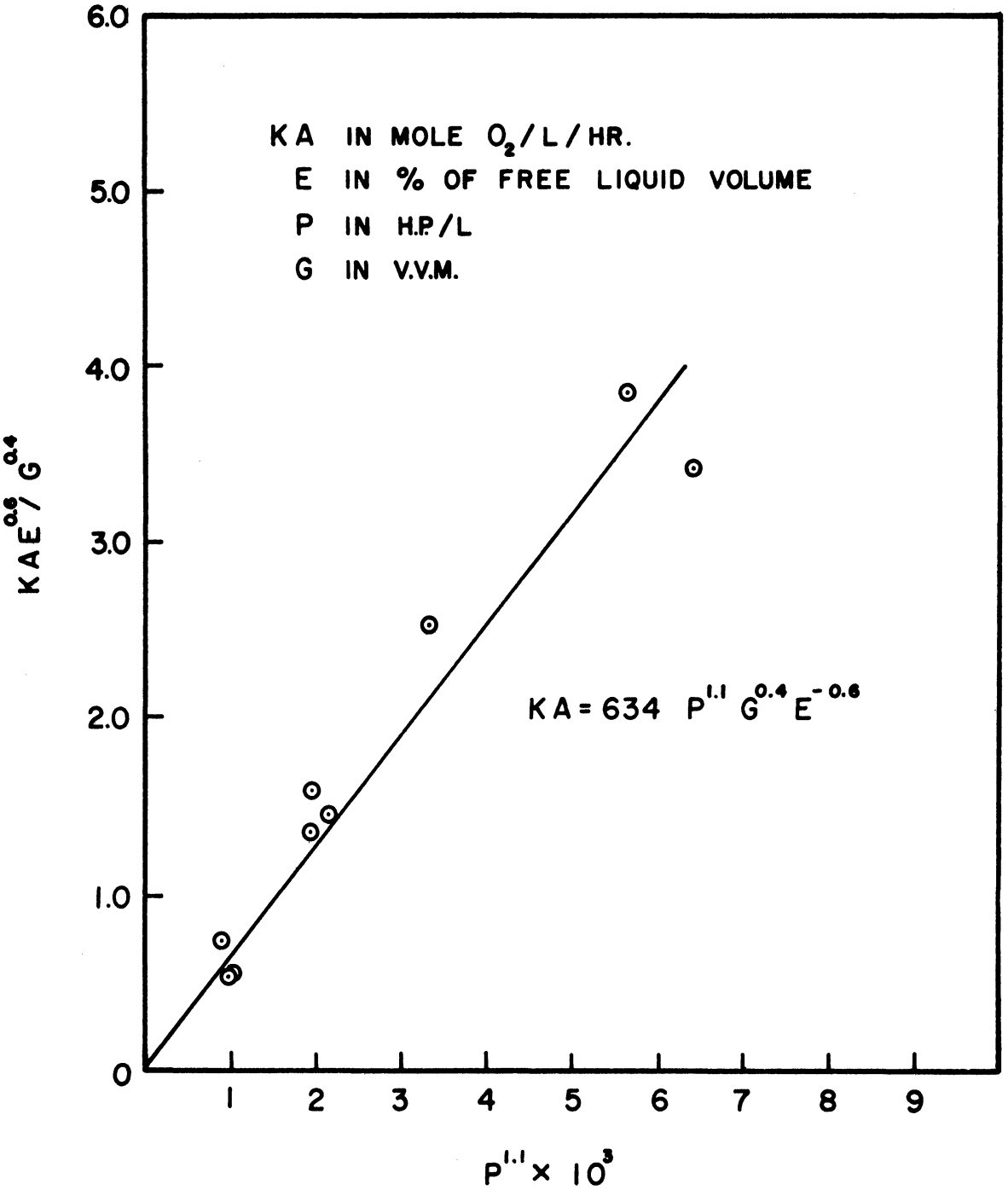


Figure 11. Combined Effect of Power Input and Air Flow on the Rate of Sulfite Oxidation in A 44.5 Liter Fermentor.

TABLE I

EFFECTS OF POWER INPUT, AGITATION AND AIR FLOW ON THE RATE OF SULFITE OXIDATION IN A 44.5 LITER FERMENTOR

Agitator Speed N rpm	Air Flow Rate G vvm	Power Input P hp/l/hr	Rate of Oxidation mole O_2/l/hr	Liquid Volume Expansion E % by vol.
280	1.0	1.85×10^{-3}	20.0×10^{-2}	5.3
370	1.0	3.43 "	36.0 "	12.6
470	1.0	5.57 "	47.7 "	16.0
560	1.0	---	58.6 "	22.0
280	0.5	1.99 "	17.2 "	5.3
370	0.5	3.70 "	30.4 "	9.3
560	0.5	10.20 "	47.4 "	10.6
280	2.0	1.68 "	23.3 "	10.8
370	2.0	3.42 "	37.0 "	13.4
560	2.0	9.08 "	75.7 "	23.4

TABLE II

EFFECTS OF POWER INPUT, AGITATION AND AIR FLOW ON THE RATE OF SULFITE OXIDATION IN A 5 LITER FERMENTOR

Agitator Speed	Air Flow Rate	Power Input	Rate of Oxidation	Liquid Volume Expansion
N rpm	G vvm	P hp/l/hr	mole O_2/l/hr	E % by vol.
660	0.35	9.21×10^{-3}	13.55×10^{-2}	20
470	0.35	7.45 "	9.96 "	11.7
370	0.35	4.83 "	5.22 "	6.7
260	0.35	1.97 "	2.44 "	3.3
660	0.48	8.77 "	17.38 "	20
470	0.48	7.02 "	11.75 "	15.0
370	0.48	4.61 "	6.12 "	6.7
260	0.48	1.84 "	1.79 "	3.3
660	0.78	8.33 "	24.15 "	23.3
470	0.78	6.80 "	15.5 "	13.3
370	0.78	4.39 "	7.18 "	8.3
260	0.78	1.80 "	2.44 "	3.3
660	1.07	8.11 "	27.09 "	22
470	1.07	6.58 "	13.46 "	16.7
370	1.07	4.39 "	7.18 "	8.3
260	1.07	1.75 "	2.77 "	3.3
660	1.21	7.89 "	28.4 "	23.3
470	1.21	6.58 "	16.16 "	15
370	1.21	4.28 "	7.26 "	10
260	1.21	1.75 "	2.44 "	3.3

Equation (III-9a) is of the similar form as Equation (III-7a). By replacing N with P in the graphical trial and error method described previously, the exponential coefficients of k_{18}, k_{19} and k_{20} were determined.

$$KA = k_{17} P^{k_{18}} G^{k_{19}} E^{k_{20}} \qquad \text{(III-9a)}$$

The resulting equations for the sulfite oxidation system studied in the 44.5 liter and the five liter fermentors are given below:

$$KA = 634\ P^{1.1}\ G^{0.4}\ E^{-0.6} \qquad \text{44.5 liter fermentor} \qquad \text{(IV-3)}$$

$$KA = 17.8\ P^{1.4}\ G^{0.4}\ E^{0} \qquad \text{5 liter fermentor} \qquad \text{(IV-4)}$$

The two equations are also graphically presented in Figures 12 and 13.

B. Pseudomonas ovalis Respiration

The experiments for studying Pseudomonas ovalis respiration were conducted in the five liter fermentor. The rate of gluconic acid formation was measured by using the constant pH technique previously described. The rate of oxygen transfer was determined indirectly from the rate at which gluconic acid was produced.

The experimental results of Pseudomonas ovalis respiration are given in Table III. They are correlated with Equations (III-7a) and (III-9a) following the same graphical trial and error method described previously. The resulting empirical equations are given below:

$$KA = 3.43 \times 10^{-6}\ N^{1.2}\ G^{0.2}\ E^{-0.5} \qquad \text{(IV-5)}$$

$$KA = 0.174\ P^{0.8}\ G^{0.2}\ E^{-0.5} \qquad \text{(IV-6)}$$

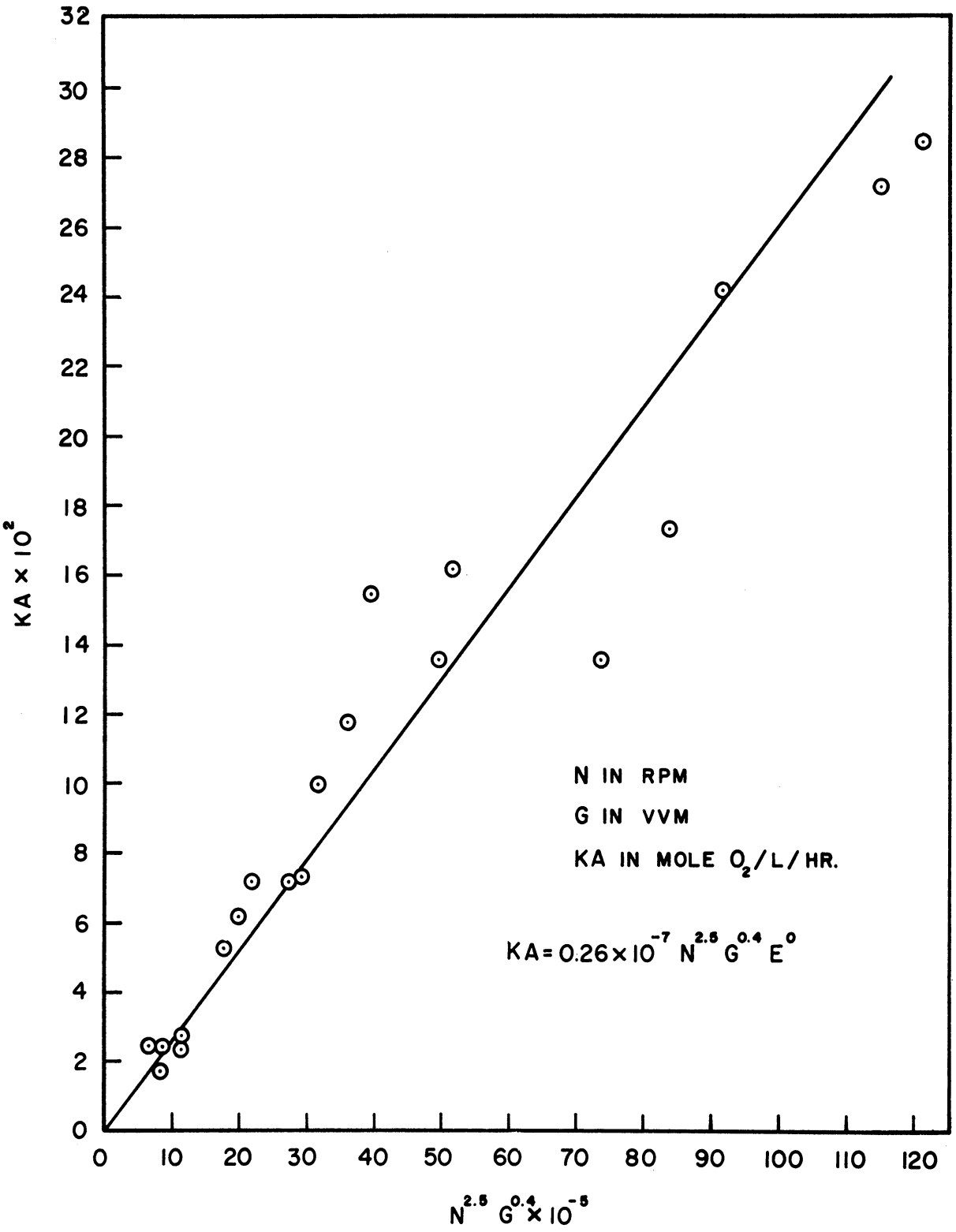


Fig. 12 Combined Effect of Agitation and Air Flow on the Rate of Sulfite Oxidation in a 5 Liter Fermentor.

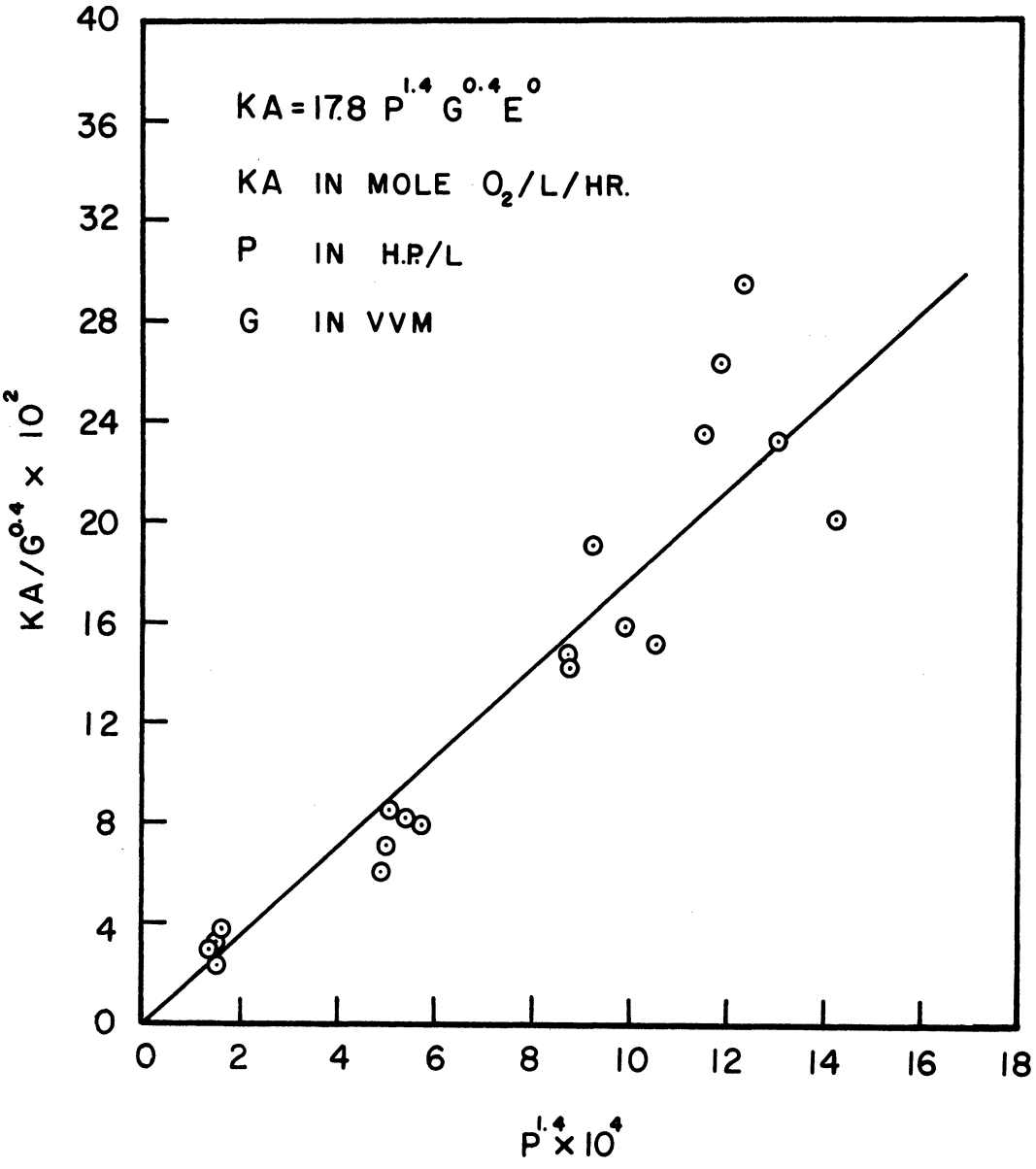


Figure 13. Combined Effect of Power Input and Air Flow on the Rate of Sulfite Oxidation in a 5 Liter Fermentor.

TABLE III

EFFECTS OF POWER INPUT, AGITATION AND AIR FLOW ON THE RATE OF GLUCONIC ACID PRODUCTION BY Pseudomonas ovalis NRRL-B-8

Agitator Speed	Air Flow Rate	Power Input	Rate of Acid Production	Liquid Volume Expansion
N rpm	G vvm	P hp/l/hr	mole/l/hr	E % by vol
470	1.16	10.7×10^{-3}	3.75×10^{-3}	9.3
370	1.16	7.4 "	3.75 "	4.9
470	0.31	12.3 "	4.0 "	5.8
470	1.81	10.5 "	4.0 "	9.5
470	0.73	11.8 "	4.75 "	4.9
100	1.16	2.2 "	2.0 "	1.5
270	1.16	4.4 "	3.5 "	2.6
370	1.16	7.4 "	4.25 "	3.5
270	1.81	4.3 "	3.75 "	3.0
270	0.31	4.6 "	3.0 "	2.5
100	1.81	2.2 "	1.75 "	1.5
100	0.73	2.2 "	1.62 "	1.3
270	1.81	4.4 "	3.0 "	4.8
270	0.73	4.5 "	3.12 "	2.4

The pre-exponential coefficients were determined by the least square method. The equations are also graphically presented in Figures 14, 15, 16, 17.

The effect of cell concentration on the rate of oxygen transfer was investigated. The experimental methods have been described previously. The cell concentration effect was studied at two different levels of agitator speeds and air flow rates. The results of these experiments are given in Tables IV (A) and IV (B) and presented in Figure 18. From Figure 18, it was found that a linear proportionality exists between the cell concentration and the rate of oxygen transfer.

C. Penicillium chrysogenum Respiration

Penicillium broths prepared on a rotary shaker were placed in the five liter fermentor and studied with respect to the effects of agitation and air flow on the respiration rate. The exhaust gas from the fermentor was analyzed to determine its carbon dioxide content; from this the rate of oxygen transfer to the culture was calculated. The results obtained are given in Table V.

It has been previously described in the sulfite oxidation section that Equation (III-7a) suggests linear correlations when suitable groups of variables are plotted on logarithmic coordinates. Following the same graphical trial and error procedure, an empirical equation was obtained:

$$KA = 5.55 \times 10^{-7} N^{1.6} G^{0.2} E^{-0.1} \qquad \text{(IV-7)}$$

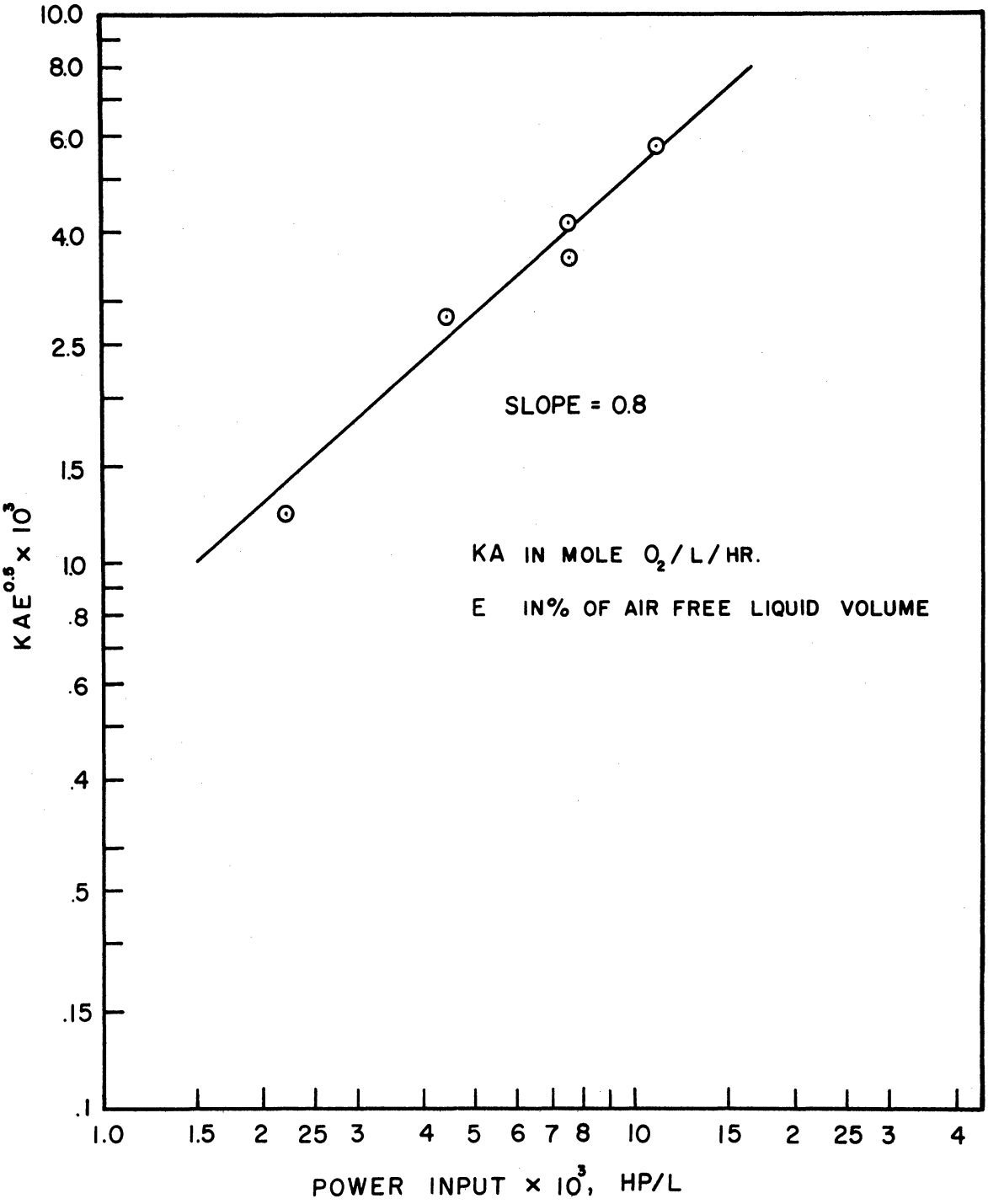


Fig. 14 Effect of Power Input on the Rate of Pseudomonas ovalis Fermentation.

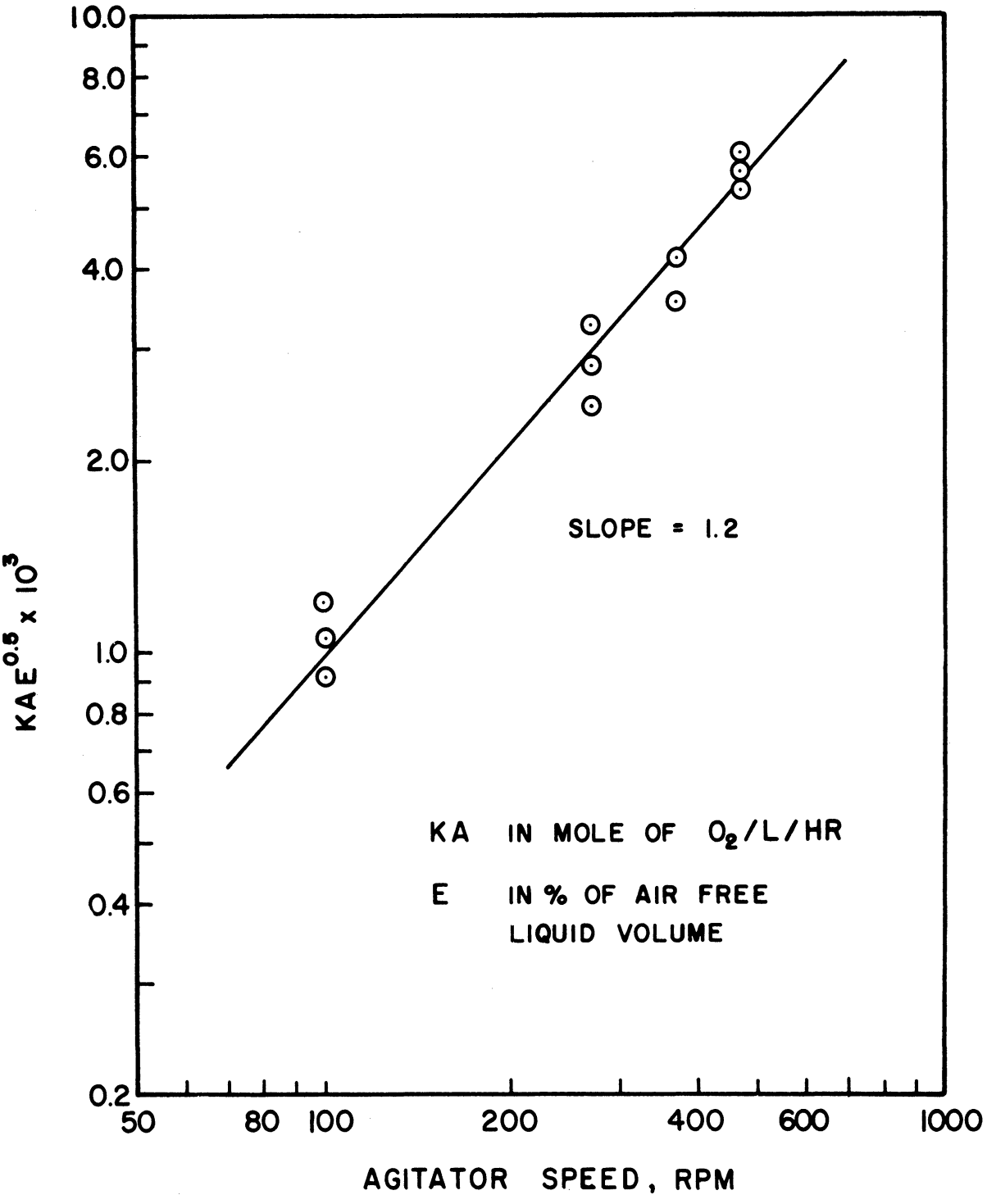


Figure 15. Effect of Agitation on the Rate of Pseudomonas ovalis Fermentation.

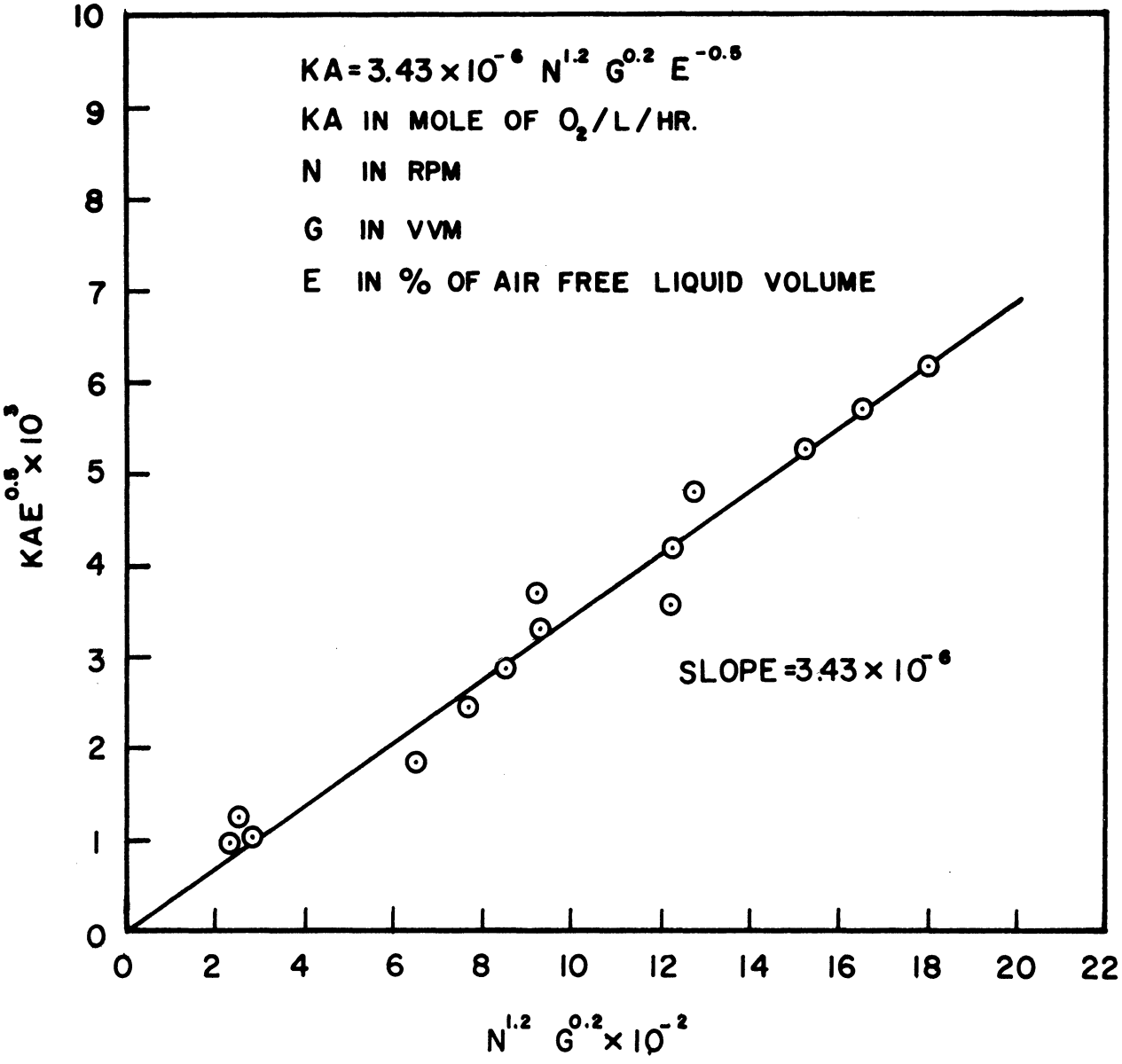


Figure 16. Combined Effect of Agitation and Air Flow on the Rate of Pseudomonas ovalis Fermentation.

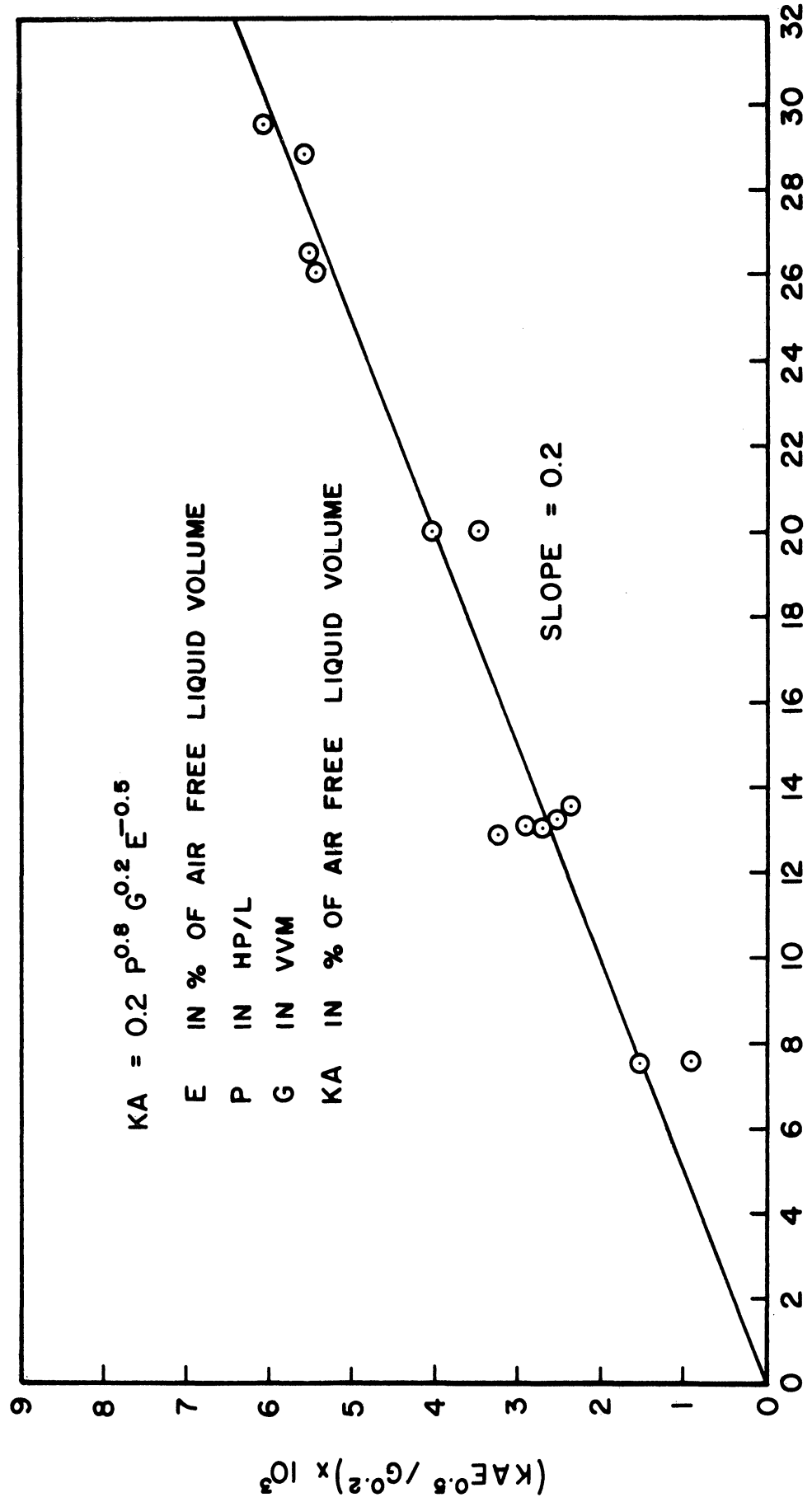


Figure 17. Combined Effect of Power Input and Air Flow on the Rate of *Pseudomonas ovalis* Fermentation.

TABLE IV-A

THE EFFECT OF CELL CONCENTRATION ON THE RATE OF GLUCONIC ACID PRODUCTION* BY RESTING CELLS OF Pseudomonas ovalis NRRL-B-8

Cell concentration of the Cell Suspension	Rate of Acid Production	Turbidity** of the Cell Suspension	Volume*** of 24 Hour Broth Used
cells/ml	mole/l/hr		ml
0.88×10^{10}	2.25×10^{-3}	22	440
0.88 "	1.50 "	--	440
1.32 "	3.24 "	42	660
1.76 "	4.0 "	57	880
1.76 "	3.75 "	--	880
1.76 "	3.50 "	55	880
2.64 "	6.6 "	86	1320
3.52 "	7.0 "	101	1760

* At an agitator speed of 470 rpm and an air flow rate of 1.16 vvm.

** Using a brown filter no. 66 with a photoelectric colorimeter, model 800-3, manufactured by Klett-Summerson Company.

***Volume of the 48 culture broth from which the resting cells of P. ovalis were prepared. (The broth contained 4×10^{10} cells per ml. (average of 65 plate counts)).

TABLE IV-B

EFFECT OF CELL CONCENTRATION ON THE RATE OF GLUCONIC ACID PRODUCTION* BY RESTING CELLS OF Pseudomonas ovalis NRRL-B-8

Cell Concentration of the Cell Suspension	Rate of Acid Production	Volume** of 24 Hour Broth Used
cells/ml	mole/l/hr	ml
0.44×10^{10}	0.4×10^{-3}	220
0.88 "	1.1 "	440
1.32 "	0.8 "	660
1.76 "	1.4 "	880
1.76 "	2.0 "	880
2.64 "	1.5 "	1100
3.52 "	2.5 "	1320

* At an agitator speed of 300 rpm and an air flow rate of 0.73 vvm.

**Volume of the 24 hour culture broth from which the resting cells of P. ovalis were prepared. (The broth contained 4×10^{10} cells per ml. (average of 65 plate counts)).

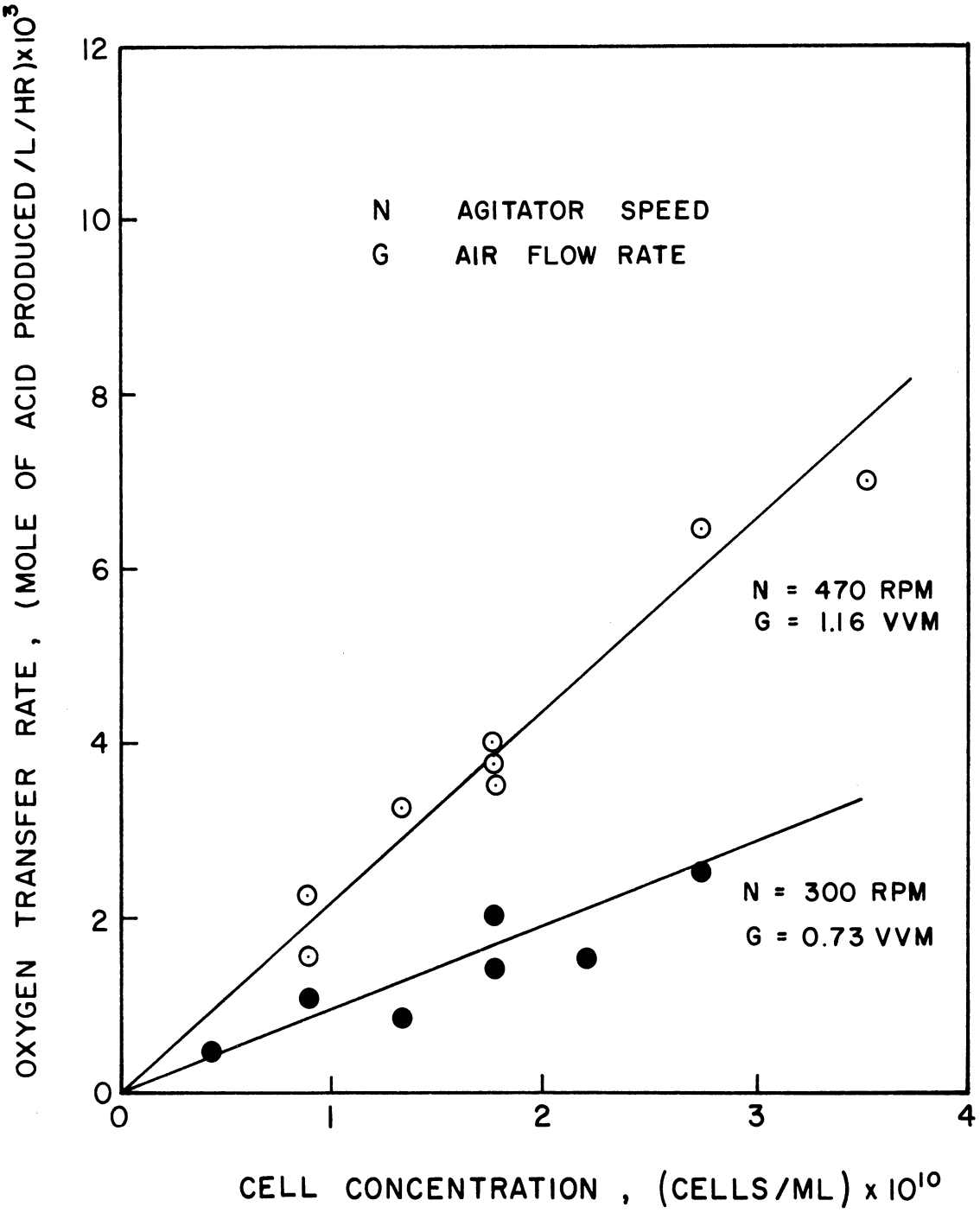


Figure 18. Effect of Cell Concentration on the Rate of Oxygen Uptake by Resting Cells of Pseudomonas ovalis (expressed as the rate of gluconic acid production).

TABLE V

EFFECTS OF POWER INPUT, AGITATION AND AIR FLOW ON THE RATE OF CARBON DIOXIDE PRODUCTION BY Penicillium chrysogenum N329

Agitator Speed	Air Flow Rate	Power Input	Rate of CO_2 Production	Liquid Volume Expansion
N rpm	G vvm	P hp/l/hr	mole/l/hr	E % by vol
470	1.16	4.93×10^{-3}	9.62×10^{-3}	7.3
470	0.73	5.15 "	8.41 "	7.05
470	0.31	5.48 "	10.01 "	6.8
470	1.60	4.82 "	9.00 "	7.5
470	0.52	5.7 "	6.4 "	6.95
470	1.37	4.82 "	7.35 "	7.4
370	1.16	2.63 "	8.39 "	4.5
370	0.31	2.96 "	6.06 "	4.0
370	0.73	2.85 "	5.68 "	4.2
370	1.60	2.63 "	5.57 "	4.8
205	1.16	0.88 "	2.78 "	1.5
205	0.31	0.88 "	1.98 "	1.1
205	0.73	0.88 "	2.92 "	1.3
205	0.52	--	2.09 "	1.2
370	1.16	2.63 "	6.54 "	4.5
305	0.73	1.71 "	5.46 "	2.8
225	0.73	1.01 "	4.32 "	2.5
225	1.16	1.05 "	3.27 "	3.0
225	0.73	1.01 "	2.74 "	2.5
280	0.73	1.47 "	3.91 "	3.5

By replacing N with P, the following equation was obtained by the graphical trial and error method.

$$KA = 7.57\ P^{0.8}\ G^{0.2}\ E^{-0.1} \qquad \text{(IV-8)}$$

The pre-exponential coefficients were both determined by the least square method. The equations are also graphically presented in Figures 19, 20, 21, 22.

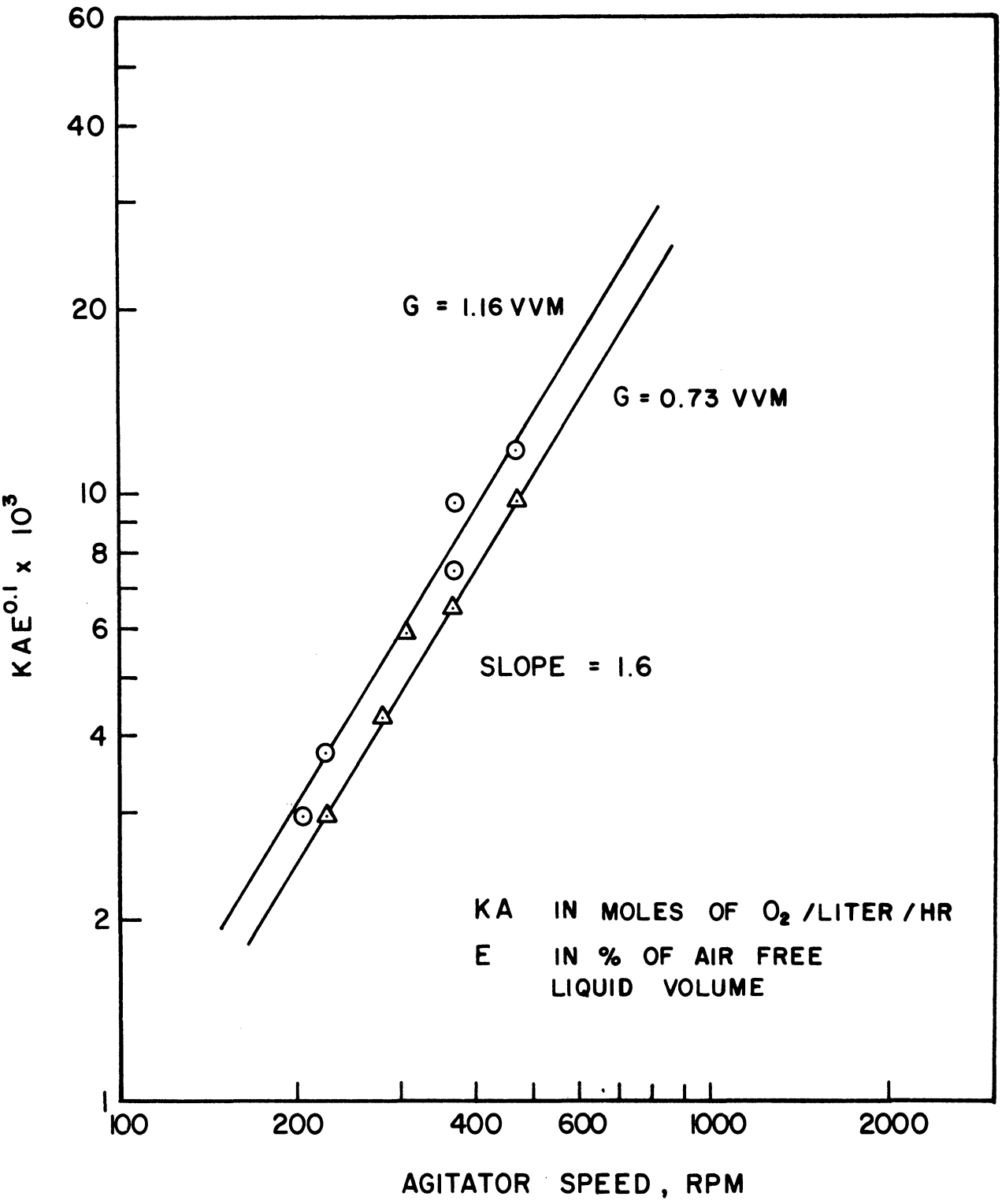


Figure 19. Effect of Agitation on the Rate of Respiration of Penicillium chrysogenum.

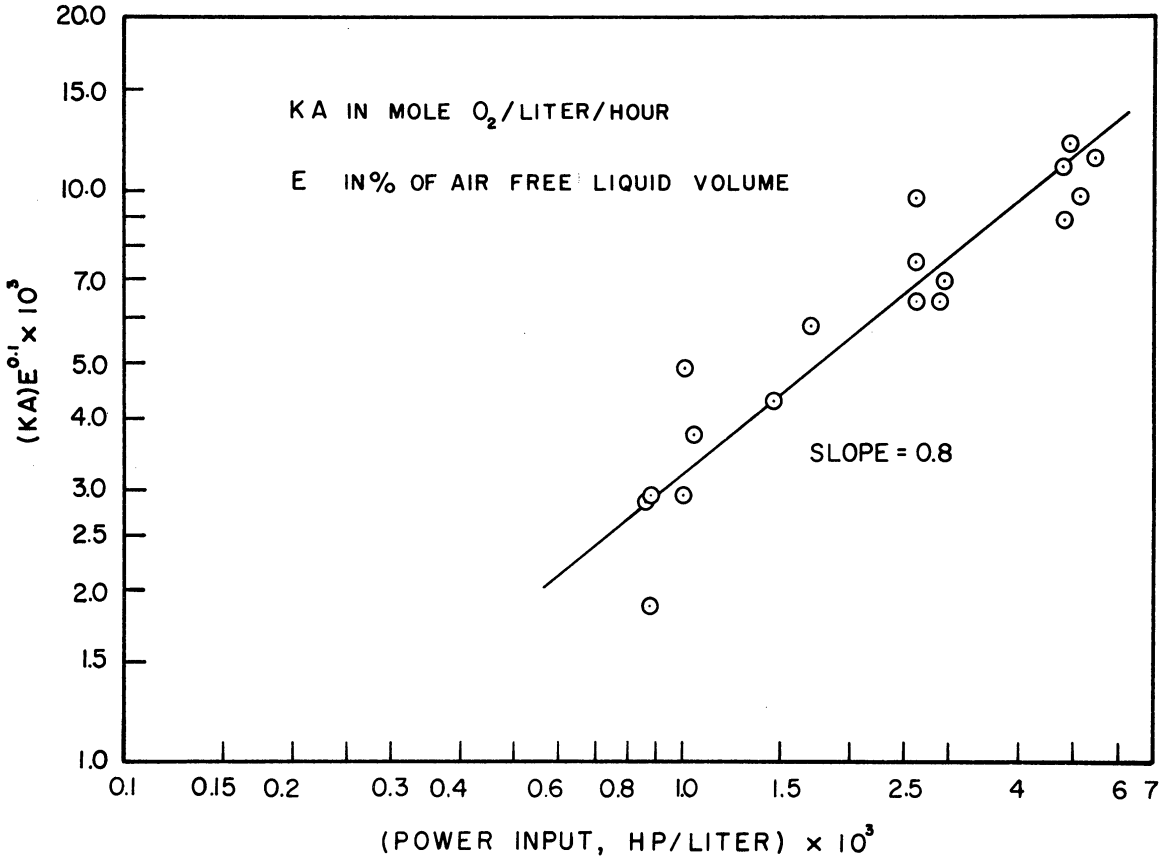


Fig. 20 Effect of Power Input on the Rate of Respiration of Penicillium chrysogenum.

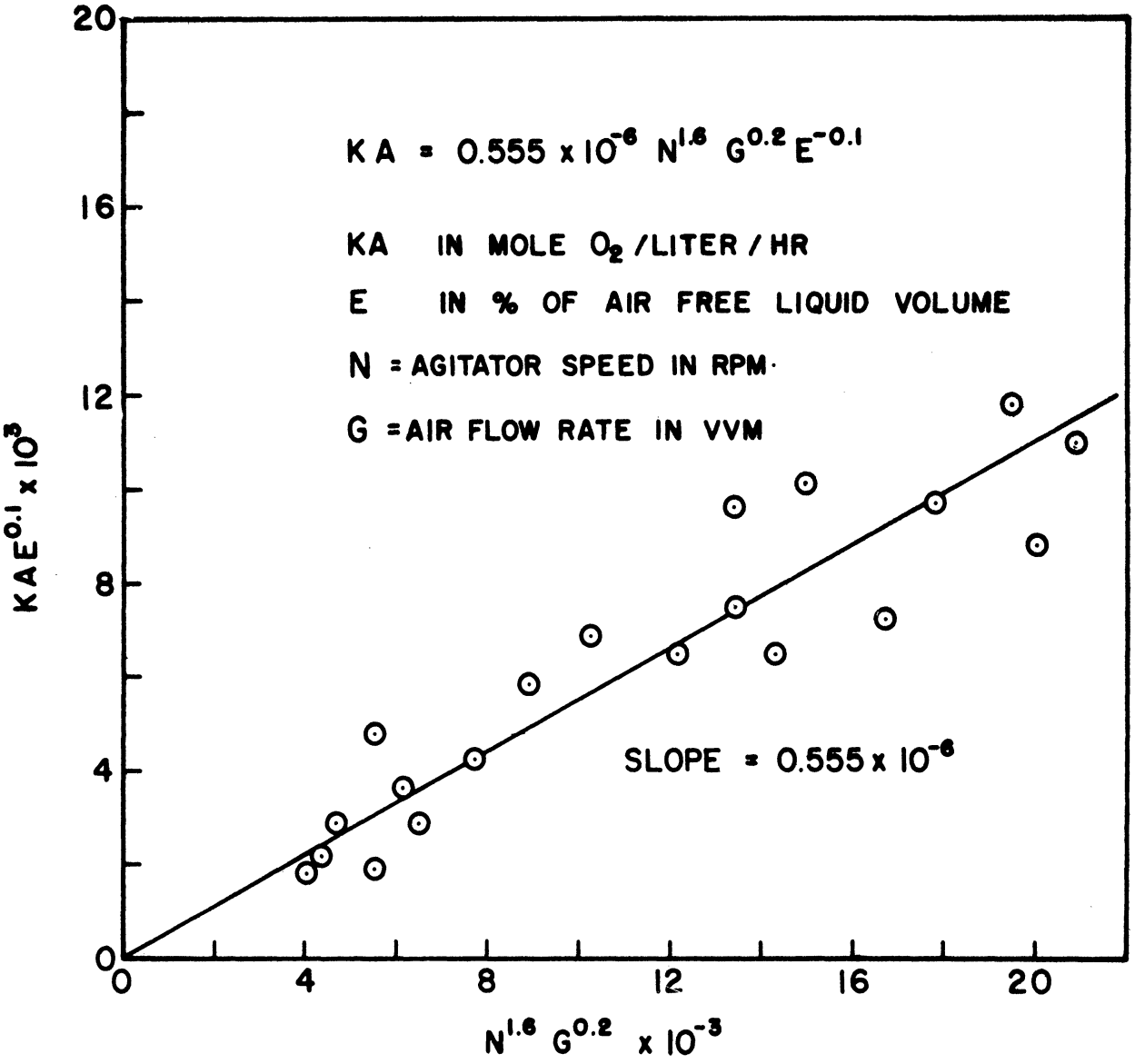


Figure 21. Combined Effect of Agitation and Air Flow on the Rate of Respiration of Penicillium chrysogenum.

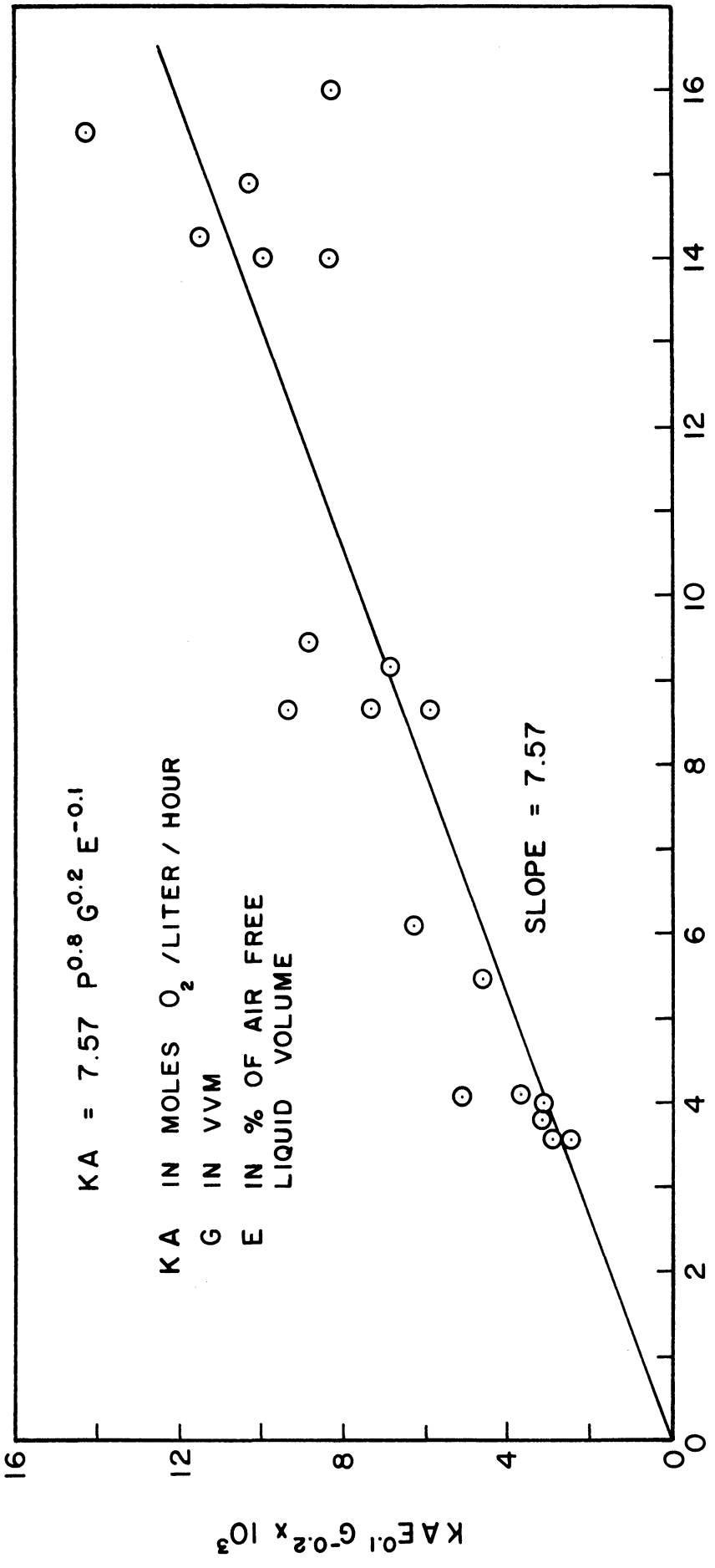


Figure 22. Combined Effect of Power Input and Air Flow on the Rate of Respiration of Penicillium chrysogenum.

CHAPTER V

DISCUSSION

Due to the complex nature of biological phenomena, knowledge of fermentation processes is still very limited. It is common practice among scientists working with a complicated system, to first fractionate the system into its basic elements and then study these elements systematically. The accumulated knowledge helps to build an integrated picture of the system. This general procedure is not unfamiliar to chemical engineers. The studies of unit operations and unit processes are based upon this concept. A large industrial chemical plant, to chemical engineers, is simply a combination and permutation of unit operations and unit processes.

Aerobic fermentation is a process in which oxygen is required to support micrbial activities. Therefore, oxygen transfer can be considered as a unit operation of the aerobic fermentation process; a thorough understanding of this operation is essential for an adequate understanding of such fermentations.

The overall process of oxygen transfer in aerobic fermentation broths can be visualized as consisting of several individual steps. First of all, oxygen contained in gas bubbles must dissolve into the liquid; this establishes a gas-liquid interface. Then, the dissolved oxygen molecules must migrate to the vicinity of the microbial cells where oxygen becomes available for microbiological use only after crossing the boundary between the liquid and the cell wall. An ordinary aerobic fermentor is provided with mechanical agitation. The turbulence caused by the agitator

and by air passing through the broth has sufficient mixing effect to deliver the dissolved oxygen molecules quickly to the neighborhood of the microbial cells. Therefore, the most influential factors controlling the rate of overall oxygen transfer are the resistances at the boundaries between the gas and the liquid and between the liquid and the cell wall. Based upon the master reaction concept, which has been discussed by Hinshelwood[19] and others, the research work of the present program is essentially a special problem of interfacial mass transfer.

Mass transfer across biphasic boundaries is a process that interests physical as well as biological scientists. However, knowledge of this process has not progressed to the point where details of the transfer phenomena involved can be explained with complete satisfaction. For this reason, it is probable that empirical correlations involving the overall transfer process can be useful. With this viewpoint in mind, oxygen transfer has been treated empirically in this study.

As briefly described in the chapter on historical review, the sulfite oxidation reaction has been used to evaluate the efficiency of fermentors for transferring oxygen from sparged air to fermenting broths. Many workers have studied actual oxygen transfer in fermentations, but due to the complicated systems involved, the data obtained is difficult to interpret. Because of the fact that agitator speed and air flow rate are the two importance factors in fermentation process design, they have been intensively investigated. The results accumulated in the literature have been briefly presented in the section entitled "Historical Review". Correlations of oxygen transfer rates as functions of power input, of agitator speeds at constant rates of air flow and of air flow rates at

constant agitator speeds have been presented in many articles. However, a single correlation combining these factors had not been reported.

Based upon the results obtained in the present study, it appears possible to effectively correlate the data obtained by use of an additional measurement, namely expansion of the liquid volume. Such expansion occurs when sulfite solutions and fermentation broths are mechanically stirred and sparged with air, because some of the air is entrained.

Since the first step in the overall process of oxygen transfer is the dissolution of oxygen molecules from the air into the liquid, the interfacial area between the air bubbles and the surrounding liquid must have a direct effect upon the rate at which oxygen is dissolved. The volume of air retained in the bulk of liquid cannot be directly used to measure the interfacial area because this area is a function of the size and shape of the air bubbles as well as of the volume of air entrained. Nevertheless, expansion of the liquid volume in oxygen transferring systems affects the interfacial area, and, consequently, the rate of oxygen transfer. Therefore, the factor of liquid volume expansion is incorporated in the development of the empirical correlations used in the present work. Several such equations have been developed by the dimensional analysis and graphical trial and error procedures described previously. They are summarized below:

(1) Sulfite solution,

$$KA = 634\ P^{1.1}\ G^{0.4}\ E^{-0.6} \qquad \text{(44.5 liter fermentor)} \qquad \text{(IV-3)}$$

$$KA = 17.8\ P^{1.4}\ G^{0.4}\ E^{0} \qquad \text{(5 liter fermentor)} \qquad \text{(IV-4)}$$

(2) Penicillium broth,

$$KA = 7.57\ P^{0.8}\ G^{0.2}\ E^{-0.1} \qquad \text{(5 liter fermentor)} \qquad \text{(IV-8)}$$

(3) Pseudomonas broth,

$$KA = 0.2\ P^{0.8}\ G^{0.2}\ E^{-0.5} \qquad \text{(5 liter fermentor)} \qquad \text{(IV-6)}$$

and (1a) Sulfite solution,

$$KA = 5.11 \times 10^{-7} N^{2.5}\ G^{0.4}\ E^{-0.6} \qquad \text{(44.5 liter fermentor)} \qquad \text{(IV-1)}$$

$$KA = 0.26 \times 10^{-7} N^{2.5}\ G^{0.4}\ E^{0} \qquad \text{(5 liter fermentor)} \qquad \text{(IV-2)}$$

(2a) Penicillium broth,

$$KA = 0.555 \times 10^{-6}\ N^{1.6}\ G^{0.2}\ E^{-0.1} \qquad \text{(5 liter fermentor)} \qquad \text{(IV-7)}$$

(3a) Pseudomonas broth,

$$KA = 3.43 \times 10^{-6}\ N^{1.2}\ G^{0.6}\ E^{-0.5} \qquad \text{(5 liter fermentor)} \qquad \text{(IV-5)}$$

The above equations correlate oxygen transfer rate, air flow, liquid expansion and power input (or agitaor speed). This kind of correlation has not been previously reported for fermentation systems.

Rushton et al.[13,35,36,37] studied the performance of liquid-gas contacting equipment and developed an equation expressing liquid volume expansion as a function of air flow and power input. Bartholomew et al.[1] investigated the rates of oxygen transfer into a soybean medium and reported data for liquid expansion at different agitator speeds and air flow rates. Otherwise, the importance of liquid volume expansion has not been noted.

For the purpose of further confirmation of the effect of liquid expansion on oxygen transfer, several published articles have been examined

mathematically. The data of Cooper, Fernstrom, and Miller[7] are reproduced in Table VI and Figures 23, 24. These workers attempted to correlate the three variables, transfer coefficient, power input, and air flow rate. As shown in Figure 23, they obtained a curve rather than a straight line; therefore, their results cannot be fitted with an equation of exponential form similar to those of Equations (III-7a) and (III-9a) without including the factor of liquid volume expansion. Because no data concerning liquid volume expansion were reported by Cooper and his co-workers, the expansion results collected from the present work were used as an approximation in an attempt to rationalize their data. By incorporating the transfer coefficient, power input, and air flow rates given by these authors together with the liquid expansion results collected from the experiments described previously, it was possible to plot the line shown in Figure 24. Because the geometrical configurations of the fermentors used by Cooper *et al.* were not the same as the one from which the liquid volume expansion was measured, Figure 24 can only be considered as an approximation. However, the importance of a liquid expansion factor for correlating purposes is demonstrated.

Bartholomew *et al.*[1] studied oxygen transfer in a soybean medium and reported agitator speed, air flow rate, transfer coefficient and liquid volume expansion in several graphs. Their data, which were obtained from the experiments using a constricted pipe to supply air to the fermentor, were also reviewed with the equations developed in the present work. It was found that one single equation could be used to relate all of the four factors together in one expression (Table VII),

$$KA = k\, N^{2.5}\, G^{0.45}\, E^{-0.3} \qquad (V\text{-}1)$$

TABLE VI

EFFECTS OF POWER INPUT AND AIR FLOW ON THE RATE OF SULFITE OXIDATION STUDIED BY COOPER et al. (7)*

Transfer Coefficient K_V lb moles / cuft-hr-atm	Air Flow Rate V_s ft/hr	Power Input P_V ft-lb / min-cuft	Liquid* Volume Expansion E % by vol.	$\frac{K_V}{V_s^{0.67}}$	$\frac{K_V E^{0.6}}{V_s^{0.67}}$	$\frac{1}{k}$
For 9.5 inch diameter tank; $k = 3.99 \times 10^{-6}$						
0.0149	19	946	9.3	0.00209	2050	
0.00436	19	298	6.3	0.000612	415	
0.00128	19	55.2	3.1	0.00018	88.7	
0.0367	19	2870	14.7	0.00515	6460	
0.00206	65	754	10.1	0.00127	1270	
0.00734	65	222	6.9	0.000453	362	
0.00236	93.5	603	10.3	0.00115	1160	
0.00818	93.5	200	7.1	0.000397	323	
0.00367	93.5	62.2	3.8	0.000178	99.4	
0.0715	93.5	2060	15.8	0.00347	4560	
0.0246	149	460	10.3	0.000869	881	
0.0104	149	203	7.1	0.000369	298	
0.00488	149	62.2	3.8	0.000173	96.6	
0.0291	212	427	10.3	0.000813	824	
0.0075	212	63.7	3.8	0.000813	117	
0.130	212	1300	15.8	0.00364	4790	
For 6.0 inch diameter tank; $k = 5.0 \times 10^{-6}$						
0.00694	46.1	202	9.6	0.000538	418	
0.0143	248	156	9.8	0.00362	286	
0.0192	46.1	765	15.7	0.00149	1530	
0.0264	248	429	15.8	0.000668	700	
0.0706	46.1	2740	20.0	0.00543	6520	
0.0702	248	1370	20.0	0.00178	2140	

*The values of liquid volume expansion, E, were from the experimental results of the present research program. Volume expansion of water in the 5-liter fermentor used in the present research program were recorded at different agitator speeds and air flow rates. These data were then plotted in a chart. The expansion values used in the present table were read from the chart at same agitator speeds and air flow rates of those reported by Cooper et al.

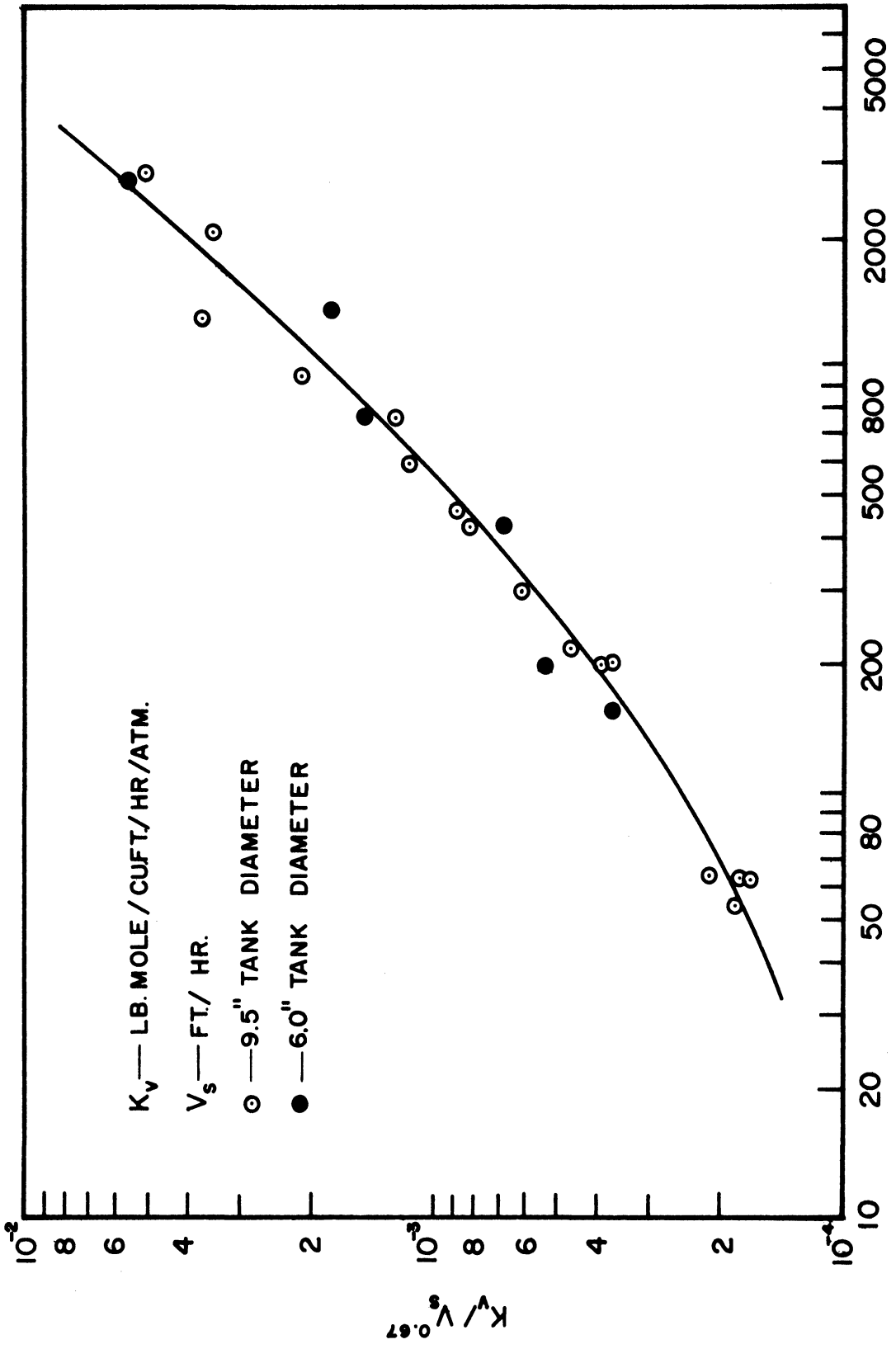


Figure 23. Effects of Power Input and Air Flow on the Rate of Sulfite Oxidation Studied by Cooper et al.(7)

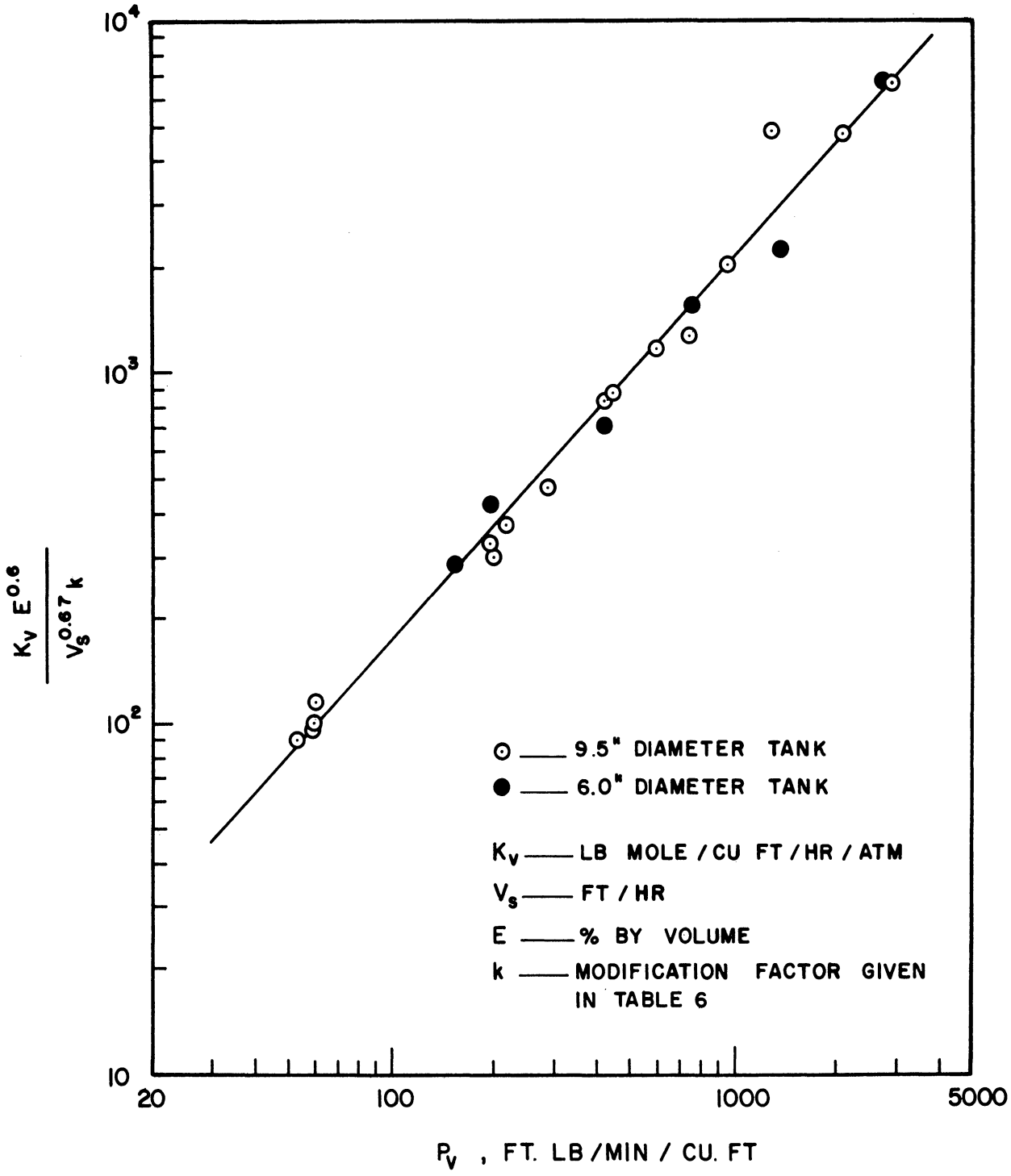


Figure 24. Modified Correlation of the Data Shown in Figure 23 by Incorporating the Factor of Liquid Volume Expansion.

TABLE VII

OXYGEN TRANSFER IN SOY BEAN MEDIUM
STUDIED BY BARTHOLOMEW et al. (1)*

Agitator Speed	Air Flow Rate	Liquid Volume Expansion	Transfer Coefficient		
N	G	E	k_d	$k_d E^{0.3}$	$N^{2.5} G^{0.45}$
rpm	liters/min	% of vol	gm-mole/ml/hr/atm		
375	1	1.5	4	4.5×10^{-4}	2.74×10^{6}
375	2	3.0	6	8.3 "	3.62 "
375	3	4.0	6.5	9.3 "	4.25 "
375	4	4.6	6.5	10.3 "	4.77 "
375	6	5.4	6.5	10.9 "	5.61 "
560	1	3.5	9.5	13.7 "	7.40 "
560	2	5.5	12.5	20.9 "	9.75 "
560	3	6.8	14.0	24.8 "	11.48 "
560	4	7.5	15.0	27.4 "	12.88 "
560	6	8.3	15.5	29.3 "	15.55 "

*Using a constricted pipe as the air supply device.

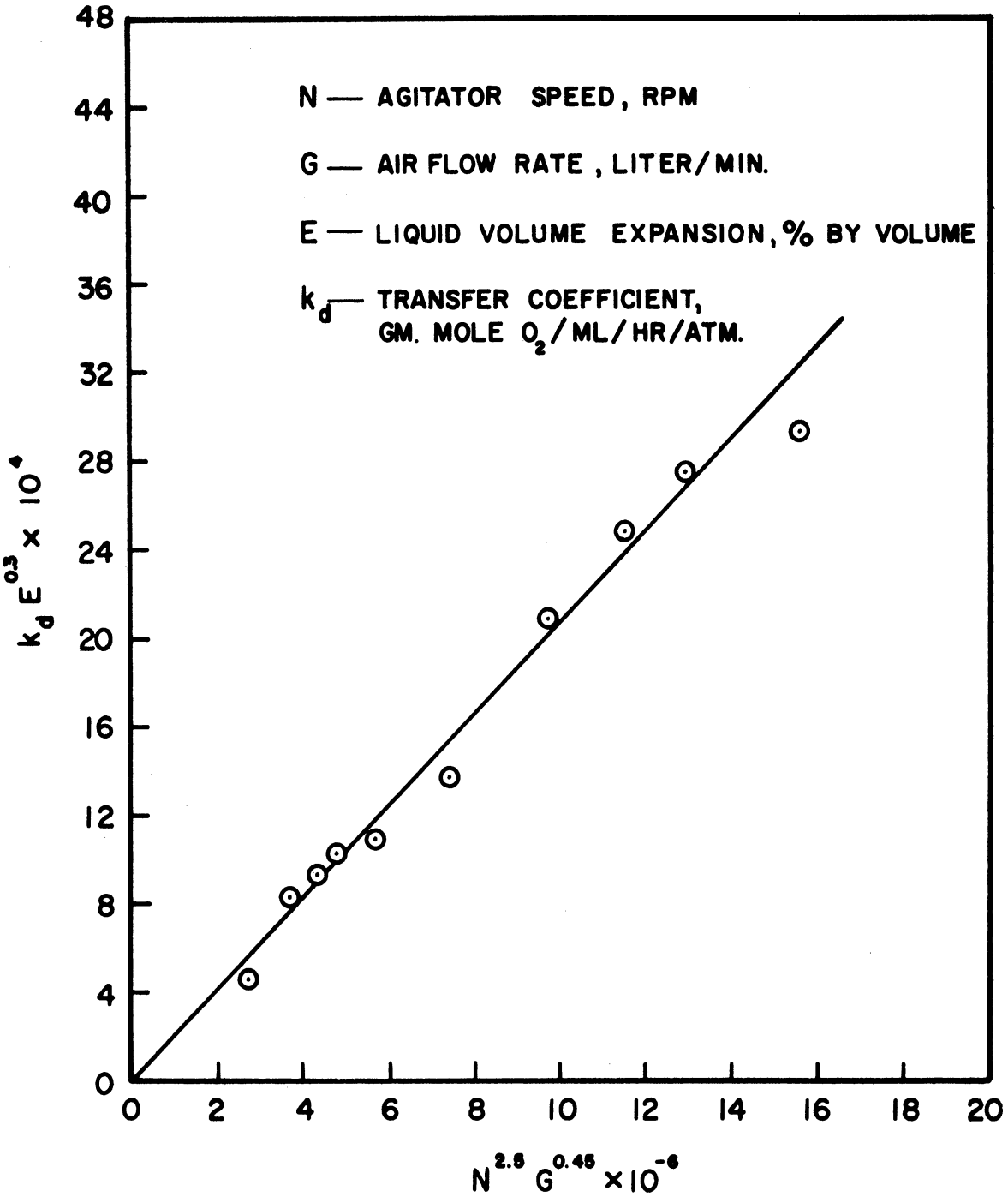


Figure 25. Oxygen Transfer in Soy Bean Medium Studied by Bartholomew et al.(1)

Equation (V-1) is presented graphically in Figure 25.

The extent of liquid volume expansion is believed to be a function of many factors. These include, among others, the design and location of baffles and impeller, the shape of the vessel, the ratio of height to depth of liquid, etc., as well as properties of the liquid such as viscosity, surface tension, etc.

The equation obtained from the sulfite experiments carried out in the two fermentors used in this work have different exponential coefficients for the term E. In the 44.5 liter fermentor, only one impeller was used and this was fixed on the agitator shaft close to the bottom of the vessel; in the five liter fermentor, two impellers were used. Here, one impeller was placed one inch below the liquid surface and the other near the bottom of the vessel. The top impeller may have sucked air into the bulk of the liquid from the top surface. Therefore, it is likely that the actual, effective value of the air flow rate, G, is larger than the value measured with the rotometer. The under-estimation of G could compensate for the overestimated effectiveness of E. This is shown in Equations (IV-1) and (IV-2), where the exponential coefficient increased from -0.6 to 0 when the number of impellers was changed from one to two.

Development engineers are faced with the problem of scaling up fermentation processes. Unfortunately, information obtained from laboratory apparatus may not be directly applicable to industrial equipment. Also, discrepancies between different production fermentors are very often observed. The disagreement of oxygen transfer rates in different vessels may be caused by many factors. One of these is the difference in geometrical configuration. If the factor E is eventually found to be concerned

with physical factors of the medium and of configuration of the fermentor, it may provide a useful criterion for specifying fermentation systems and for scaling up such processes. Therefore, it is suggested that further study of the meaning of the factor E should be undertaken. It should be valuable to know how this factor will behave if changes are made in other physical factors such as the size of the baffle plates, impeller arrangement, liquid depth, fermentor diameter, etc.

The slow progress being made in understanding the mechanism of oxygen transfer in fermentation systems is principally due to the complex nature of the biological processes involved. However, there are also reasons of technical nature. First among these is the relatively slow and complicated preparatory steps needed for fermentation studies as compared with those required for chemical processes. Also, contamination and mutation of the microbial culture over a prolonged period of operation in a mechanically complicated fermentor can be troublesome. Even the methods available for measurement of oxygen concentration in liquids are not adequate. For example, the polarographic method, which is commonly used, usually requires stripping oxygen from the broths with nitrogen and then reoxygenation of the liquid. The rate at which the oxygen concentration changes during the reoxygenation period is used as the basis for calculating the oxygen transfer coefficient. Therefore, the measurements are actually made during an unsteady reoxygenation period and not during the actual fermentation. Furthermore, if nitrogen affects the microbial cells, the measurements will not reveal the oxygen transfer coefficient in the actual fermenting broths. Oxygen "starvation" during the gassing out period, though lasting only for a short length of time, may severely affect the metabolic pattern of the organisms.

In the present research program, the rate of Penicillium respiration was measured by infra-red analysis of exhaust gases, and Pseudomonas respiration was evaluated by determination of the rate of acid production at controlled pH. The short period culture technique was used with both systems.

A general method for determining the rate of oxygen transfer in actual fermentations has been developed. This is based upon recognition of a specific metabolic product that has a direct relation to the oxygen consumed and which can be conveniently measured. As soon as such a metabolic product is found, the instantaneous rate of oxygen transfer in actual fermentation processes can be determined. The carbon dioxide measurement in the Penicillium respiration experiments and the determination of gluconic acid in the Pseudomonas fermentation are two examples of the general method described above. It should be possible to apply the pH control technique to many acid-producing, aerobic fermentations for determination of the oxygen transfer rate. Also, the infra-red analysis of gaseous products may have relatively broad applications.

The short period technique used in this research has solved the contamination and mutation problems. Because each batch of mash is used for only a short period of time in the fermentors, contamination and mutation cannot be appreciably alter the properties of the mash. In fact, before the effects of contamination and mutation can become important, the oxygen transfer measurements will have been made, the experiment terminated and the mash abandoned. Furthermore, because the broths are prepared in vessels separate from the fermentors used for the oxygen transfer

study, the broths can be prepared uniformly and the variations in many otherwise uncontrolled factors can be minimized. In this manner, the short period method uses a fermentor only to obtain the data needed for the actual oxygen transfer determination. This method has, therefore, increased the utility of laboratory fermentors by reducing the time required for an experimental run to about four hours instead of the several days which would otherwise be required.

The sulfite oxidation method has been extensively used to characterize the oxygen transferring ability of fermentors. However, it has been repeatedly found that sulfite results do not necessarily agree with the amount of oxygen obtained by microorganisms in actual fermentation processes. Philips and Johnson[32] stated that sulfite results indicate only the maximum amount of oxygen available for microorganisms in fermentation systems. The basic reason for this discrepancy is probably concerned with the presence of a solid phase in fermentation systems. In a sulfite solution, there are only gas and liquid phases. In some systems, the major resistance to oxygen transfer occurs at gas-liquid boundary. In actual fermentation broths, three phases are found; these are gas, liquid and microbial cell solids. The rate of oxygen transfer can be limited by either of the two biphasic boundaries, i.e. the gas-liquid or the liquid-cell interfaces. If the liquid-cell boundary is rate controlling, a discrepancy between the sulfite results and the actual fermentation systems is unavoidable.

In sulfite solutions, turbulence caused by the agitator and the sparged air affects the rate of oxygen transfer in two ways. First, the

agitation breaks the air into many fine bubbles. Thus, the intefacial was between the gas and liquid phases in a unit volume of liquid will be larger when the size of each individual air bubble is smaller; increased agitation will increase the magnitude of A in Equation (III-3a).

$$R = K A Y_g \qquad \text{(III-3a)}$$

Second, turbulence will reduce the interfacial resistance by increasing the value of K in Equation (III-3a). Therefore, the effects of agitation and air flow on the rate of oxygen transfer are twofold.

In actual fermentation broths where the liquid-cell boundary is rate limiting, the effects of agitation and air flow will be different. Equation (III-5a) instead of Equation (III-3a) should be considered.

$$R = K A X_\ell \qquad \text{(III-5a)}$$

Here A refers to the area of the liquid-solid boundary, not to that of the gas-liquid interface as previously discussed. In those cases where the liquid-cell boundary is rate limiting, turbulence will not increase the magnitude of A since this area is merely a function of the cell concentration. Agitation and air flow will be concerned only to the extent that they reduce the transferring resistance; this is reflected in the magnitude of the transfer coefficient, K, of Equation (III-5a). Therefore, in those cases where the gas-liquid boundary controls the rate of oxygen transfer, agitation affects both the transferring area, A, and the transfer coefficient, K; in cases where the liquid-cell wall boundary is rate-limiting, agitation affects only the magnitude of transfer coefficient because the transferring area is principally a function of the cell concentration.

Expressions showing the effect of agitation on oxygen transfer were developed from the present research. They are given as Equation (IV-2), (IV-5), and (IV-7), for the sulfite, Pseudomonas and Penicillium systems, respectively. Based upon the foregoing discussion, it is apparent that a relatively greater effect of turbulence should be expected on the oxygen transfer rate in those systems where the gas-liquid boundary is rate limiting than those cases where the liquid-solid boundary controls the rate of oxygen transfer. This is demonstrated by comparing the magnitude of the exponential coefficients of the agitator speed terms in the equations; here it is found that the agitation effects are much greater in the sulfite oxidation than in the Penicillium and Pseudomonas respiration experiments. The exponential coefficient in the equation for the sulfite system is 2.5, while for the Penicillium and Pseudomonas systems, respectively, they are 1.6 and 1.2.

Bartholomew et al.[1] studied oxygen transfer in an uninoculated soybean medium. Their results have been correlated by Equation (V-1) of the present investigation. The exponential coefficient of the term for agitator speed in Equation (V-1) is 2.5 which agrees closely with that of the corresponding term in the sulfite equation, Equation (IV-2). In the work with soybean medium, no microbial culture was present, hence no oxygen transfer at a liquid-cell boundary was involved. The major resistance to the oxygen transfer, therefore, was at the gas-liquid boundary. It has been explained that the rate of oxygen transfer in sulfite solutions is also controlled by this same kind of resistance.

In systems involving gas, liquid and cell-solid phases, the rate of oxygen transfer to the microbial cells can be controlled by the liquid-cell interface. The total surface area of the microbial cells is the sum

of the surface area of all individual cells; provided that the cells are of similar size, their total surface area is directly proportional to the cell concentration. In fermenting mashes, the rate of oxygen transfer may be controlled by a resistance at the cell surface. In this case the rate of oxygen transfer should be linearly proportional to the transferring area and, consequently, also to the cell concentration. This statement may need modification when the cell concentration is so high that the cell solids appreciably alter the hydrodynamic properties of the fermenting mash. In the Penicillium mash, solid contents as high as twenty percent were used. However, in the Pseudomonas system, it is estimated that the cell solid only contributed about 0.2 percent of the total volume of the cell suspensions used. This is due to the extremely small size of the Pseudomonas cells. Therefore, it is probable that even a very large number of these cells does not appreciably change the hydraulic properties of the medium in which they are suspended.

The studies of cell concentration effects on the rate of oxygen transfer in the Pseudomonas systems have been described previously. The results are shown in Figure 18. They confirm the postulated linear proportionality between the rate of oxygen transfer and the cell concentration. This is considered to be a good support for the postulate that the rate of oxygen transfer is controlled by the liquid-cell solid boundary in the Pseudomonas system studied in the present program.

The value of the sulfite oxidation method for expressing the amount of oxygen that can be consumed by microbial cells in actual fermentations has been debated by many workers. A discrepancy between sulfite

results and the actual amount of oxygen received by the microorganism has been repeatedly found. This discrepancy is unavoidable in actual fermentations, if the rate of oxygen transfer is controlled by resistance at the liquid-solid boundary. Consequently, determination of the rate controlling boundary is of basic importance in studies concerned with oxygen transfer in fermentations. The technique used for studying cell concentration effects with the Pseudomonas ovalis cells provides a method whereby this can be determined. However, in fermentations involving mycelious organisms, it is expected that the determination will be more difficult because of changes in the hydrodynamic properties of the fluid due to the presence of large quantities of cell-solids.

The effects of power input, agitator speed, air flow rate and liquid-volume expansion on the rate of oxygen transfer are shown by the exponential coefficients of these factors in the related equations. However the actual amount of oxygen transferred is determined by the pre-exponential coefficients of the equations as well as by the magnitude of power input and other factors. The importance of the pre-exponential coefficients is evident.

The pre-exponential coefficient may be a function of many factors. Actually, all of the factors held constant in the experiments of the present work affect the pre-exponential coefficient. Variables such as, geometrical configuration of the fermentors, viscosity, surface tension, and other properties of the fermenting broths, etc. are involved. It is considered likely that the pre-exponential coefficient and the exponential coefficient of the term E are related to the physical configuration of the fermentors. Examination of these factors should yield information of value for scaling up pilot plant fermentation data to production processes.

CHAPTER VI

SUMMARY

The rates of oxygen transfer were studied in the systems of sulfite oxidation, respiration of Penicillium chrysogenum and fermentation of Pseudomonas ovalis; the effects of both agitation and volumetric air flow on the rate of oxygen transfer were investigated in detail. The experimental results obtained were correlated with two sets of empirical equations which were developed by a dimensional analysis and a graphical trial and error method. In one set of equations, the oxygen transfer coefficient was correlated in terms of power input, air flow rate and liquid volume expansion; in the other, agitator speed was substituted in place of power. A correlation for oxygen transfer that includes volume expansion has not been previously reported in the literature. The importance of this factor was demonstrated in the equations used for correlating the experimental data. Its value was further tested with data reported from several published articles.

In actual fermentations, the rate of oxygen transfer can be controlled by resistances at either the gas-liquid or the liquid-cell wall boundary. A knowledge of which of these is dominant in a particular situation is of basic importance. This was studied in the Pseudomonas system by using a resting cell technique. A linear proportionality was found between the rate of oxygen transfer and the cell concentration of the broth. This indicated that the overall oxygen transfer in the Pseudomonas system was controlled by a resistance at the liquid-cell wall boundary.

A general method for measuring oxygen transfer in actual fermentation systems was proposed. This method was illustrated by its applications to the Penicillium respiration and the Pseudomonas fermentation experiments conducted in the present work.

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APPENDIX

(I) Dynamometer

A spring type dynamometer was used in the experiments for measuring the power input into the Pseudomonas, Penicillium as well as the sulfite oxidation systems. The dynamometer was attached on the central shaft of the fermentor in order to measure the torque required to drive the central shaft so that the power input to the system could be determined. An enlarged diagram of the dynamometer is shown in Figure 26. On the central shaft, A, a pulley, B, was fixed with set screw. To pulley B, a pulley, C, was linked with a flexible spring. The spring was arranged so that its screw propagation direction was the same as the rotational direction of the shaft, A. Pulley C was also centrally mounted on shaft A but they were not fixed together between D and E, thus they were free to move relatively to each other. Pulley C was driven by a motor using a V-belt. Through spring F, pulley B was driven and consequently the shaft, A. The amount of deformation of spring F due to the applied torque required for driving the central shaft was indicated by the relative displacement of a pointer, G, which was fastened to shaft A. The amount of deformation was determined with a stroboscope while the shaft was rotating. The torque applied could be read directly from a scale on pulley C, which had been previously calibrated with known torques.

(II) Sulfite Analysis by Iodometric Titration

Iodometric titration has been so widely used that it is covered almost in any quantitative analytical chemistry text[46]. The fundamental

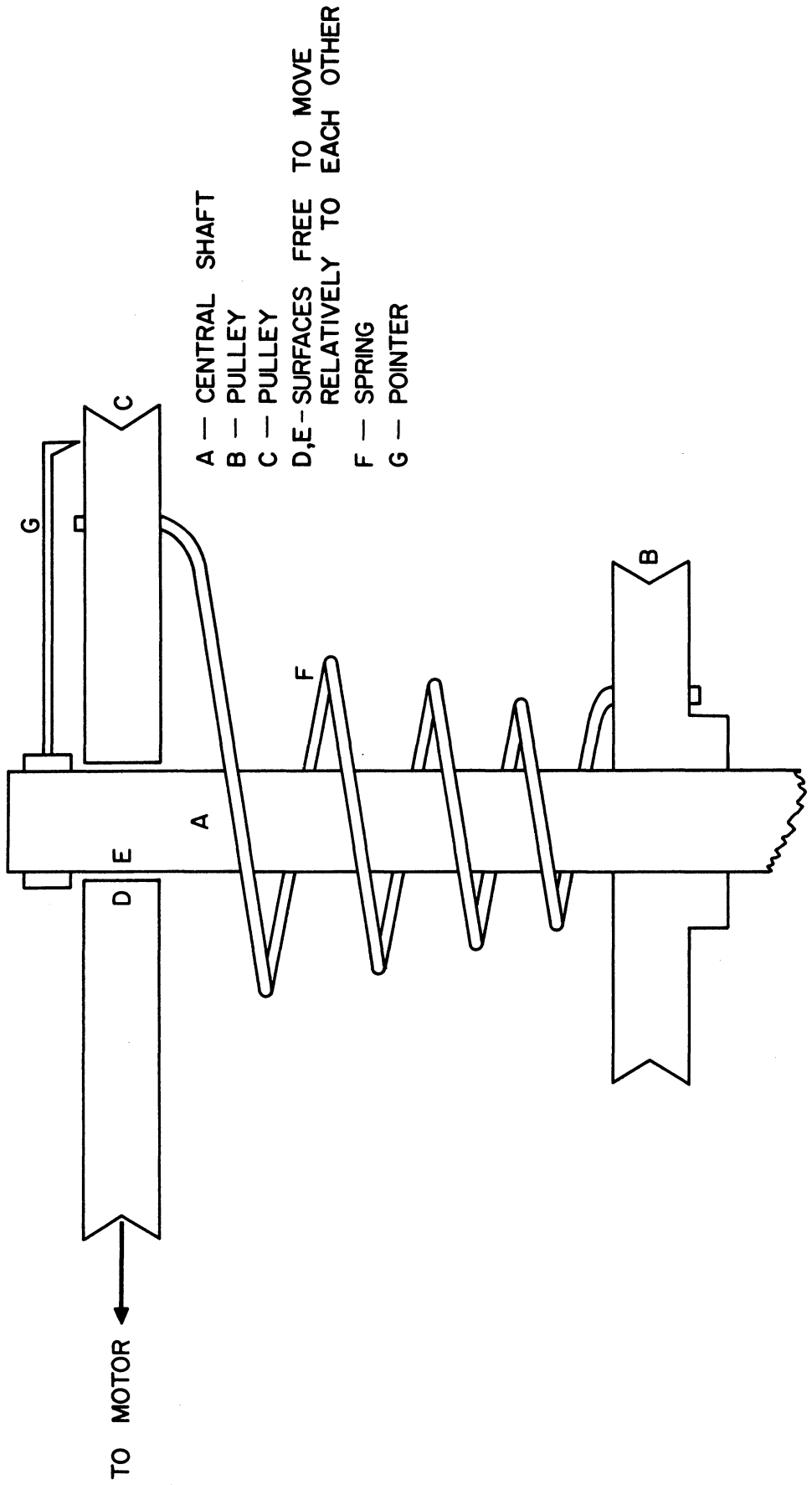

Figure 26. Diagram of the Spring-Type Dynamometer.

reaction can be expressed as below:

$$2\ Na_2\ S_2\ O_3\ +\ I_2 \longrightarrow 2\ NaI\ +\ Na_2S_4\ O_6$$

The method used in the present work involved the addition of the sulfite sample from the sulfite oxidation system into a solution containing an excess amount of iodine. The mixture was then titrated with a standard thiosulfate solution from a burette. The thiosulfate solution was standardized by a potassium dichromate solution.

Potassium dichromate crystals (Reagent grade, B and A, Allied Chemical and Dye Corporation) were heated at 140°C for 48 hours, 4.903 grams of which were dissolved in distilled water and diluted to one liter in a volumetric flask. The solution thus prepared had a concentration of 0.1667 mole of dichromate ions per liter and was used as the primary standard. The thiosulfate solution was made by dissolving 24.8 grams of sodium thiosulfate ($Na_2S_2O_3.5H_2O$) in one liter of distilled water, which gave a concentration of approximately 0.1 mole of thiosulfate per liter. The thiosulfate solution was standardized with the dichromate solution in the following manner[46].

Three grams of potassium iodide was placed in a 500 ml. Eryenmeyer flask. Twenty five milliliters of the standard dichromate solution was added into the flask with a pipette and then 50 ml. of distilled water and 10 ml. of concentrated hydrochloric acid. The mixture reacted in the dark for five minutes and was added dropwise with the thiosulfate solution from a burette until the starch end point.

The iodine solution used in the analysis was prepared by dissolving 166 grams of potassium iodide and 62.5 grams of iodine in distilled water to make one liter of solution which had a concentration of about 0.2 N of iodine.

10 ml. of each sulfite solution sample taken from the sulfite oxidation system was first pipetted into a 250 ml. Eryenmeyer flask. After addition of about 50 ml. of distilled water, the mixture was titrated with the standard thiosulfate solution until the disappearance of the characteristic brown color of iodine.

(III) Preparation of the Slant Culture and the Media Used for the Penicillium Respiration Study

Penicillium chrysogenum N329 was used for the respiration study. The slant culture of this organism was prepared by inoculating the stock culture into a fresh agar medium and the subsequent incubation at 27°C over a period of 8 days. The culture thus obtained was stored in a refrigerator. The agar medium contained the following ingredients:

Glucerol	7.5 gm.
Molasses (Brer Rabbit Green Label)	9.375 ml.
$MgSO_4$	0.05 gm.
KH_2PO_4	0.06 gm.
NaCl	4.0 gm.
$FeSO_4$	0.003 gm.
$CuSO_4$	0.001 gm.
$CaCO_3$	0.25 gm.
Curbery B. G. (U. S. Industrial Chemicals)	2.5 gm.
Peptone	5.0 gm.
Agar	20.0 gm.
Tap Water	1.0 liter

For performing a respiration experiment, the slant culture was first inoculated into 100 ml. liquid medium. The liquid medium used here and in the later part of the experiment which was described in the chapter, "Materials and Methods", had the following composition:

Cerelose	20 gm.
Corn steep solids	10 gm.
$CaCO_3$	5 gm.
$NaNO_3$	1 gm.
K_2HPO_4	0.5 gm.
$ZnSO_4$	0.1 gm.
$MgSO_4$	0.1 gm.
Tap Water	1 liter