

1. E. B. CHAIN and G. GUALANDI. — Aeration Studies II.

Summary. — 1) An amperometric method for continuous measurements of oxygen concentration in aqueous agitated solutions containing the usual ingredients for cultivation of microorganisms was developed. Agitated flasks of various shapes and dimensions and stirred 10, 50, 150, 3,000 and 12,000-litre fermenters with different mechanical conditions of agitation were used. Measurements were made in presence and absence of various microorganisms, dead and living.

2) Respiration rates determined amperometrically for various microorganisms are in satisfactory agreement with the conventional manometric methods.

3) Oxygen diffusion rates in open flasks, on a reciprocating shaker, depend on their shape and the amount of solution. Optimal aeration (about 130 ml O_2 /ml liq./hour) was found in 1-litre Erlenmeyer flasks. On a rotary shaker the oxygen diffusion rates are almost independent of their shape and inversely proportional to the amount of solution. Optimal aerations are somewhat lower than those obtainable with the reciprocating shaker, particularly with volumes of solution exceeding 100 ml. About 10 times higher aeration rates can be obtained employing shake flasks fitted with a baffle plate and agitated on a rotary shaker. In flasks closed with the usual cotton-wool plugs or metal caps, oxygen diffusion rate is very low (about 0.5 ml O_2 /100 ml liq./hour) and, with intensively respiring microorganisms quasi-anaerobic conditions are rapidly established, whatever the method of agitation employed.

4) Oxygen diffusion rate determinations in 10-litre stirred fermenters, confirm previous results obtained by sulphite measurements. In the vortex system of aeration, a linear relationship exists between the number of revolutions of the propeller and the rate of oxygen diffusion, while in the fully baffled system the rate of oxygen diffusion is a function of a power of the r.p.m.

5) Oxygen diffusion rates and power absorption measurements in 50, 150 and 200-litre fermenters stirred with both free and fully baffled systems of agitation have been carried out with the sulphite method, to provide values for the scaling up of the data for aeration efficiency.

A preliminary account of the material published in this paper was given at the 2nd International Congress of Biochemistry held in Paris from July 21 to 27, 1952. The first paper in this series was published in the Bulletin of the World Health Organization (1952) 6, 73.

6) Aeration and power absorption measurements were made in a 3,000-litre tank. With ring spargers the relative diameter dimensions of sparger and propeller blades were shown to be an important factor in determining the rate of oxygen diffusion. Oxygen diffusion rates were higher when the ring sparger was replaced by an open tube placed centrally under the propeller blades. The efficiency ratio (defined as $E_r = 1 \text{ O}_2 \text{ diffused/watt hour consumed}$) was determined for various systems of agitation such as the vortex system, the fully baffled system, and the system of free agitation in the presence of an air sparger. The E_r lies in most cases between 0.25 and 0.50. For different aeration systems utilizing an air sparger, the efficiency ratio does not vary more than a factor of 2. With none of the systems investigated did the efficiency factor differ by as much as a factor of 5.

7) A few values of oxygen diffusion rates were determined in a 12,000 litre fermenter under different conditions of agitation. There again E_r was found to be between 0.30 and 0.45.

8) The introduction of air into the stirred liquids decreases to a varying degree the power required for agitation depending on the rate of air flow and the propeller speed.

9) Power absorption increases by about 30% in the presence of 2% mycelium of *Penicillium chrysogenum*.

10) Oxygen diffusion rates decrease to a varying degree in the presence of different amounts of mycelium of *Penicillium chrysogenum*.

1) The aeration efficiencies of various systems of aeration in reactors of different sizes are compared. While the aeration efficiency E_r is largely independent on the fermenter size (from 10 litre to 12,000 litre capacity) the ratio power absorbed for agitation/power absorbed for air compression decreases markedly with increasing fermenter size. Therefore in scaling up from laboratory to industrial dimensions the aeration rate may be maintained for the same total power expenditure, but the agitation intensity proper is reduced. With certain micro-organisms this may have important physiological effects.

Riassunto. — 1) E' stato sviluppato un metodo amperometrico per misure continue della concentrazione dell'ossigeno disciolto in soluzioni acquose agitate, contenenti gli ingredienti usuali per la cultura di micro-organismi. Sono stati usati palloni agitati di varie forme e dimensioni e fermentatori della capacità di 10, 50, 150, 3.000 e 12.000 litri provvisti

di differenti sistemi di agitazione meccanica. Sono state fatte misure in presenza e in assenza di vari microorganismi, morti e vivi.

2) Le velocità di respirazione di vari microorganismi determinate amperometricamente sono in soddisfacente concordanza con quelle determinate con i comuni metodi manometrici.

3) Le velocità di diffusione dell'ossigeno in palloni aperti, su un agitatore alternativo, dipendono dalla forma dei recipienti e dalla quantità della soluzione. La più elevata aerazione (circa 130 ml O_2 /100 ml liq./ora). è stata osservata in Erlenmeyer da 1 litro, contenenti 100 ml di liquido. In palloni aperti su agitatore rotatorio le velocità di diffusione dell'ossigeno sono quasi indipendenti dalla forma del recipiente e inversamente proporzionali alla quantità della soluzione. Le aerazioni più elevate sono un po' inferiori a quelle ottenibili con un agitatore alternativo particolarmente con volumi di soluzione superiori a 100 ml. Velocità di aerazione circa 10 volte maggiori possono essere ottenute impiegando palloni ad agitazione provvisti di una piastra antivortice, agitati su un agitatore rotatorio. In palloni chiusi con tappi di cotone o con capsule di metallo la velocità di diffusione dell'ossigeno è molto bassa (circa 0.5 ml O_2 /100 ml liq./ora) e quindi con microorganismi a intensa respirazione si stabiliscono rapidamente condizioni di quasi-anaerobiosi indipendentemente dal metodo di agitazione impiegato.

4) Determinazioni di velocità di diffusione dell'ossigeno fatte in fermentatori agitati di 10 litri confermano precedenti risultati ottenuti mediante misure col metodo del solfito. Nel sistema di aerazione a vortice esiste una relazione lineare fra il numero di giri dell'elica e la velocità della diffusione dell'ossigeno, mentre nel sistema ad antivortici la velocità di diffusione dell'ossigeno è funzione di una potenza del numero di giri al minuto dell'elica.

5) Sono state compiute misure di potenza assorbita e di velocità di diffusione dell'ossigeno in fermentatori di 50, 130 e 200 litri agitati sia con il sistema libero che con quello ad antivortici.

6) Sono state compiute misure di aerazione e di assorbimento di potenza in un fermentatore di 3.000 litri. Si è osservato che con diffusori ad anello le dimensioni relative dei diametri del diffusore e delle pale dell'elica sono un fattore importante nel determinare la velocità di diffusione dell'ossigeno. Le velocità di diffusione dell'ossigeno risultarono inferiori quando il diametro del diffusore era alquanto maggiore del diametro dell'elica. Più efficiente aerazione si è ottenuta sostituendo al dif-

fusore ad anello un tubo, ad un solo foro, situato al centro del fermentatore sotto la pale dell'elica. E' stato determinato il rapporto di efficienza (definito come $E_r = 1 \text{ O}_2 \text{ diffusi/watt-ora consumati}$) per vari sistemi di agitazione, a vortice, ad antivortice e a libera agitazione con diffusore d'aria. L' E_r è compreso nella maggioranza dei casi fra 0,25 e 0,50. Nei vari metodi di aerazione che utilizzano un diffusore d'aria il rapporto d'efficienza non differisce più di due volte fra un sistema e l'altro. In nessun caso il rapporto d'efficienza variava più di 5 volte da un sistema all'altro.

7) Sono stati determinati alcuni valori della velocità di diffusione dell'ossigeno in un fermentatore di 12.000 litri con diverse condizioni di agitazione. Anche in questo caso è stato osservato un E_r compreso fra 0,30 e 0,45.

8) L'introduzione d'aria nei liquidi agitati diminuisce la potenza richiesta per l'agitazione, in varia misura a seconda del flusso d'aria e della velocità dell'elica.

9) L'assorbimento di potenza aumenta di circa il 30% in presenza del 2% di micelio di *Penicillium chrysogenum*.

10) Le velocità di diffusione dell'ossigeno diminuiscono in varia misura in presenza di diverse quantità di micelio di *Penicillium chrysogenum*.

11) Sono stati messi a confronto fra loro i rapporti di efficienza per vari sistemi di aerazione in fermentatori di diverse grandezze. Mentre l'efficienza di aerazione E_r è in larga misura indipendente dalla grandezza del fermentatore (dalla capacità di 10 litri fino a quella di 12.000 litri), il rapporto fra la potenza assorbita per l'agitazione e quella assorbita per la compressione d'aria diminuisce notevolmente al crescere delle dimensioni dei fermentatori. Pertanto nel passare di scala dalle dimensioni di laboratorio a quelle industriali può essere mantenuta, con lo stesso totale consumo di potenza, la stessa aerazione, ma l'intensità dell'agitazione meccanica viene ridotta. Questo fatto, con certi microorganismi, può avere importanti effetti fisiologici.

Résumé. — 1) Les Auteurs ont mis au point une méthode ampérométrique du contrôle continu de la concentration en oxygène dans les solutions aqueuses en conditions agitées et contenant les ingrédients usuels pour la culture des micro-organismes. On s'est servi de ballons agités, des diverses formes et de dimensions variables ainsi que de cuves de fermentation de 10, 15, 150, 3.000 et 12.000 litres de capacité soumises à divers systèmes d'agitation mécanique. Les mesures ont été faites

en présence et en absence d'espèces diverses de micro-organismes, morts et vivants.

2) Le taux respiratoire des différents micro-organismes, déterminé par la méthode ampérométrique, concorde d'une façon satisfaisante avec la détermination obtenue par les méthodes manométriques ordinaires.

3) La diffusion de l'oxygène dans les ballons ouverts sur agitateurs à mouvement réciproque dépend de la forme des récipients et de la quantité de la solution. L'optimum d'aération (environ 130 ml. O_2 /100 ml. liq./heure) s'avère dans des Erlenmeyers de 1 litre, contenant 100 ml. de liquide. En ce qui concerne les ballons ouverts sur agitateur rotatoire, la diffusion de l'oxygène est quasi indépendante de la forme du récipient et inversement proportionnelle à la quantité de la solution. Le plus haut degré d'aération est quelque peu inférieur à celui obtenu avec un agitateur réciproque particulièrement si les volumes de solution sont supérieurs à 100 ml. Le taux d'aération peut être dix fois plus élevé si les ballons, placés sur l'agitateur rotatoire, sont munis d'un panneau anti-turbulent.

Dans les ballons fermés par des bouchons de coton ou des capsules de métal, le taux de diffusion de l'oxygène est très lent (env. 0,5 ml. O_2 /100 ml. liq./heure) et, avec des micro-organismes à respiration intense, il s'établit rapidement des conditions quasi anaérobiques, quel que soit le système d'agitation.

4) Les déterminations de la vitesse de diffusion de l'oxygène faites dans des cuves de fermentation de 10 litres, en conditions d'agitation, ont confirmé les résultats précédemment obtenus par la méthode du sulfite. Tandis que dans le système d'aération à vortex, on constate un rapport linéaire entre le nombre de révolutions de l'hélice et la vitesse de diffusion de l'oxygène est fonction d'une puissance du nombre des r.p.m.

5) On a mesuré les vitesses de diffusion de l'oxygène et la puissance absorbée dans des cuves de fermentation de 50, 130 et 200 litres soumises aussi bien à l'agitation libre qu'au système complètement turbulent en se servant de la méthode au sulfite, afin de fournir les valeurs nécessaires à l'établissement d'une échelle pour la mesure de l'efficacité de l'aération.

6) On a mesuré l'aération et la puissance absorbée dans un tank de 3.000 litres. On a observé, avec les disperseurs à anneau, que les dimensions relatives des pales de l'hélice et des diamètres du disperseur sont un facteur important dans la détermination du taux de diffusion de l'oxygène. Les vitesses de diffusion de l'oxygène sont inférieures quand le diamètre du disperseur est de beaucoup plus grand que celui des pales

de l'hélice. On a obtenu une aération plus efficace en remplaçant le disperseur à anneau par un tube ouvert placé au centre sous les pales de l'hélice. Le coefficient d'efficacité (défini comme $E_r = 1 \text{ O}_2 \text{ diffusé/watt-heure consommé}$) a été déterminé pour différents systèmes d'agitation, à vortex, turbulent et à agitation libre avec un disperseur d'air. Dans la plupart des cas l' E_r se situe entre 0,25 et 0,50. Pour les différents systèmes d'aération utilisant un disperseur d'air, le rapport d'efficacité ne varie pas au delà d'un facteur de 2. Dans aucun des systèmes étudiés, le facteur d'efficacité n'a varié jusqu'à un facteur de 5.

7) On a déterminé certaines valeurs de diffusion de l'oxygène dans un tank de fermentation de 12.000 litres, dans différentes conditions d'agitation. Ici encore les valeurs de E_r sont comprises entre 0,30 et 0,45.

8) L'introduction d'air dans les liquides agités diminue plus ou moins la puissance nécessaire pour l'agitation et ceci selon l'importance de la quantité du flux d'air et de la vitesse de rotation de l'hélice.

9) La puissance absorbée augmente d'environ 30% en présence de 2% de mycélium de *Penicillium chrysogenum*.

10) La vitesse de diffusion de l'oxygène diminue plus ou moins en présence de concentrations différentes de mycélium de *Penicillium chrysogenum*.

11) On a comparé entre eux les coefficients d'efficacité de différents systèmes d'aération dans des cuves de fermentation de grandeurs différentes. Tandis que l'efficacité d'aération E_r est presque indépendante de la grandeur de la cuve de fermentation (de 10 à 12.000 litres), le rapport: puissance absorbée pour l'agitation/puissance absorbée pour la compression de l'air, diminue considérablement avec l'augmentation des dimensions des cuves; ainsi, en passant de l'échelle du laboratoire à l'échelle industrielle, si le degré d'aération peut être maintenu pour la même dépense de puissance totale, l'intensité réelle d'agitation est diminuée. Les conséquences physiologiques de cette constatation peuvent être importantes selon les micro-organismes employés.

Zusammenfassung. — 1) Eine amperometrische Methode für kontinuierliche Messungen der Sauerstoffkonzentration in gerührten wässrigen Lösungen mit den üblichen Bestandteilen für die Kultur von Mikroorganismen wurde entwickelt. Hierbei gelangten Schüttelkolben verschiedener Formen und Grössen sowie Fermenter von 10, 50, 150, 3000 und 12000 Litern Inhalt mit Rührvorrichtungen verschiedener Typen zur Verwen-

dung. Die Messungen wurden in Gegenwart sowie in Abwesenheit verschiedener toter und lebender Mikroorganismen durchgeführt.

2) Der amperometrisch festgestellte Sauerstoffverbrauch verschiedener Mikroorganismen stimmte mit den üblichen manometrischen Messungen zufriedenstellend überein.

3) Die Sauerstoffdiffusionsgeschwindigkeit in offenen Gefäßen auf einer Schüttelmaschine mit reciproker Bewegung hängt von der Form der Gefäße sowie von der Menge der vorhandenen Lösung ab. Optimale Sauerstoffdiffusionsgeschwindigkeiten (rund 130 ml O₂/100 ml Flüssigkeit pro Stunde) wurden bei Verwendung von Erlenmeyer-Kolben von 1 Liter Inhalt mit 100 ml Flüssigkeit erreicht. Bei offenen Gefäßen auf einer Schüttelmaschine mit Rotationsbewegung ist die Sauerstoffdiffusionsgeschwindigkeit ziemlich unabhängig von der Form der Schüttelgefäße und umgekehrt proportional zu der Menge der vorhandenen Lösung. In diesem Fall ist die optimale Durchlüftung etwas niedriger als die optimalen Sauerstoffdiffusionsgeschwindigkeiten, die mit einer Schüttelmaschine mit reciproker Bewegung erhalten werden können, besonders wenn Lösungsmengen von mehr als 100 ml verwendet werden. Zehmal höhere Durchlüftungswerte können durch Verwendung von mit Prallplatten versehenen Gefäßen auf einer Schüttelmaschine mit Rotationsbewegung erzielt werden. Bei mit dem üblichen Wattebausch oder mit Metallkappen geschlossenen Gefäßen ist die Sauerstoffdiffusion sehr gering (etwa 0,5 ml O₂/100 ml Flüssigkeit/Stunde), so dass bei intensiv atmenden Mikroorganismen sehr schnell praktisch anaerobe Bedingungen eintreten, gleichgültig welche Schüttelbewegung zur Anwendung gelangt.

4) Die Bestimmung der Sauerstoffdiffusionsgeschwindigkeit in mit mechanischer Rührung versehenen Fermentern von 10 Litern Inhalt bestätigt die früheren, mit Sulfit-Messungen erhaltenen, Ergebnisse. Bei Durchlüftung mittels des « Vortex » systems besteht eine geradlinige Beziehung zwischen der Tourenzahl des Rührers und der Sauerstoffdiffusionsgeschwindigkeit, während bei Rührung in Gegenwart von Prallplatten die Sauerstoffdiffusionsgeschwindigkeit eine Funktion einer Potenz der Tourenzahl des Rührers ist.

5) Messungen der Sauerstoffdiffusionsgeschwindigkeit als Funktion des Kraftverbrauches wurden in mit mechanischer Rührung versehenen Fermentern von 50, 190 und 300 l Inhalt unter Verwendung verschiedener Belüftungssysteme (Vortexsystem und Rührung in Gegenwart von Prallplatten) mit der Sulfit-Methode durchgeführt, um Vergleichswerte für Belüftungsleistungen bei Aufwertung von Laboratoriums- zu Semiindustrialskala zu erhalten.

6) Messungen der Sauerstoffdiffusionsgeschwindigkeit wurden in einem Fermenter von 3000 l Inhalt durchgeführt. Es wurde gezeigt, dass bei Verwendung von ringförmigen Luftverteilern die Sauerstoffdiffusionsgeschwindigkeit weitgehend von dem Verhältnis Durchmesser des Luftverteilers/Durchmesser der Rührflügel abhängig ist. Wenn der Durchmesser des Luftverteilers erheblich grösser als der Durchmesser des Rührflügels ist, sinkt die Sauerstoffdiffusionsgeschwindigkeit erheblich ab. Der Belüftung-Leistungskoeffizient E_r (Diffundierter O_2 (in l)/Wattstunde) wurde für verschiedene Belüftungsverfahren bestimmt und zwar für das Vortexsystem, und Verfahren, die auf Luftverteilung eines durch die Lösung geblasenen Luftstromes in Gegenwart und Abwesenheit von Prallplatten beruhen. In den meisten Fällen liegt der E_r -Wert zwischen 0,25 und 0,50. In Belüftungssystemen in denen ein durch die Lösung geblasener Luftstrom verteilt wird, ändert sich der Belüftungskoeffizient um höchstens einen Faktor von 2. Bei keinem der untersuchten Belüftungssysteme ändert sich der Leistungskoeffizient mehr als zu einem Faktor von 5.

7) Einige Messungen der Sauerstoffdiffusionsgeschwindigkeit wurden in einem Fermenter von 12000 l Inhalt unter verschiedenen Rührbedingungen durchgeführt. Auch hier wurden E_r -Werte zwischen 0,30 und 0,45 gefunden.

8) Die Einführung von Luft in Flüssigkeiten während des Rührens vermindert in verschiedenem Ausmass den für das Rühren erforderlichen Kraftverbrauch je nach der Stärke des Luftstroms und der Tourenzahl der Rührers.

9) Der Kraftverbrauch erhöht sich um etwa 30% in Gegenwart von 2% Mycelium.

10) Die Sauerstoffdiffusionsgeschwindigkeit vermindert sich in verschiedenem Ausmass in Gegenwart verschiedener Mengen von Mycelium.

11) Die Leistungsfähigkeit von Belüftungssystemen in Reaktionsgefässen verschiedener Grösse wurde verglichen. Während der Leistungskoeffizient E_r weitgehend von der Grösse des Fermenters (von 10 l bis zu 12000 l Fassungsvermögen) unabhängig ist, vermindert sich das Verhältnis Kraftverbrauch für Rührung zu Kraftverbrauch für Luftkompression sehr stark mit der Zunahme der Grösse des Fermenters. Daher bleibt der Gesamtkraftverbrauch (Rührung plus Luftkompression) für eine bestimmte Sauerstoffdiffusion beim Aufwerten von Laboratoriums- zu Industrieskala konstant, jedoch sinkt der Kraftverbrauch für die Rührung. Dieser Faktor kann für einige Mikroorganismen wichtige Änderungen im Stoffwechsel hervorrufen.

INTRODUCTION

One of the factors determining the course of metabolism of aerobic micro-organisms in submerged culture is the amount of oxygen available to them. The oxygen demand of micro-organisms differs with their age, their respiration rates being greatest when they are young and actively multiplying. If the oxygen demand required to maintain their respiration rates at maximum level is higher than the oxygen supply the micro-organisms will adapt themselves to the conditions of oxygen deficiency with more or less profound changes in their metabolism, according to the degree of oxygen deficiency. The amount of the oxygen available to the micro-organisms depends on the rate with which the oxygen diffuses into the culture fluid; only oxygen dissolved in the culture fluid can be utilized; there is no evidence that micro-organisms can absorb oxygen directly from the gaseous phase.

In order to maintain aerobic micro-organisms in the desired state of metabolism it is necessary to maintain suitable oxygen diffusion rates at a constant level. The availability of methods for the determination of rates of oxygen diffusion into the culture media is therefore of considerable importance for the evaluation of the effectiveness of the aeration methods employed.

Oxygen diffusion rates into water can be measured efficiently by the sulphite method of COOPER, FERNSTROM and MILLER (1944) in which the catalytic oxidation by air of sulphite to sulphate ions in the presence of copper ions is followed iodometrically.

In this method the rates of oxidation of sulphite to sulphate ions is equal to the rate of oxygen diffusion into the sulphite solution at an oxygen back pressure of zero.

Extensive use of this method has been made in the first paper of this series (CHAIN, PALADINO, CALLOW, UGOLINI and VAN DER SLUIS, 1952) in which a systematic study was undertaken of the mechanical factors on which aeration efficiency depends, such as agitation in the presence or absence of baffles, air flow, sparger types, etc. While the sulphite method is an excellent means of great simplicity for comparing the efficiency of different aeration devices in different types of fermentation vessels it is obviously not applicable to biological systems.

An alternative method for determining oxygen diffusion rates is the polarographic method of measuring dissolved oxygen (for literature see KOLTHOFF and LINGANE, 1952). This method which has been ap-

plied successfully to biological systems, e.g. for measuring the respiration rates of different micro-organisms(BAKER & BAUMBERGER, 1940; BAUMBERGER, 1938; BAUMBERGER, 1938; BAUMBERGER, 1939; BAUMBERGER LEONG and BARDWELL, 1948; DIRSCHLER and BERGMAYER, 1950; DU BUY and OLSON, 1940; HIXSON and GADEN, 1950; OLSON, BRACKETT and CHRICKARD, 1949; PETERING, DUGGAR and DANIELS, 1939; SKERMAN and MILLIS, 1949; WINZLER, 1949; ZIMMERMANN and BERRY, 1949) can also be used for the determination of aeration rates in culture media in the presence of micro-organisms.

The efficiency of an aeration device can be measured quantitatively by determining the rate with which oxygen diffuses into aqueous solutions previously freed from oxygen by gassing out with nitrogen. The concentration of the oxygen dissolved in the aqueous solutions may be determined polarographically at different time intervals until saturation is reached. A typical logarithmic diffusion curve is thus obtained from which the rate of diffusion of oxygen into the aqueous solution can be determined for any desired concentration of oxygen. Several polarographic methods of determining oxygen diffusion rates have been published, all involving the use of the dropping mercury electrode. One of the main technical difficulties encountered in this type of work arises from the fact that all aeration solutions are turbulent, the degree of aeration efficiency being dependent on the degree of turbulence of the water-air mixture; the turbulence is created by different agitation and (or) sparger devices (CHAIN *et al.*, 1952).

Turbulence affects the size and dropping time of the mercury drops and hence the accuracy and reproducibility of the polarographic measurements. It is therefore essential to take precautions to ensure that the polarographic determinations of dissolved oxygen by means of the dropping mercury electrode are made under conditions in which interference with the formation of the mercury drops is reduced to a minimum. Three methods for the polarographic determination of the oxygen diffusion rates in aerated and agitated solutions have been published in which the circumvention of the above difficulty has been attempted in different ways. Two of these are discontinuous (HIXSON & GADEN, 1950; WISE, 1951); they are based on removing samples at different time intervals from the turbulent solutions and transferring them to a conventional dropping mercury electrode. These methods have several disadvantages. 1) It is impossible to measure accurately high aeration rates because of the difficulty of withdrawing samples sufficiently rapidly. 2) In the presence of living micro-organisms it is necessary

to kill these practically instantaneously while taking the sample, in order to avoid that the micro-organisms should continue absorbing oxygen from the solutions. In practice this is a very difficult condition to satisfy. 3) It is not easy to avoid the entry of small amounts of air during the transfer of the sample from the agitated vessel to the dropping mercury electrode.

The other method for measuring aeration rates is continuous (BARTHOLOMEW, KAROW, SFAT and WILHELM, 1950). The dropping mercury electrode was placed in the aerated and agitated culture liquid, but shielded from the turbulence by means of a wire gauze. Measurements of the concentration of dissolved oxygen were made in the presence or absence of micro-organisms. The disadvantage of this method is that it is difficult to find wire gauze of just the right mesh size, sufficiently small to exert an efficient shielding action against the turbulence of the fluid, but not too small to interfere with the diffusion of the liquid. This factor is of particular importance when micro-organisms such as filamentous fungi, are present which may block the wire gauze to an unknown and unreproducible degree.

A second continuous method available for the measurement of the concentration of dissolved oxygen utilizes a mechanically « shocked » mercury electrode (BERMAN, SAUNDERS and WINZLER, 1951). This method, which appeared while the work reported in this paper was in progress, has been applied to the determination of respiration rates of micro-organisms in liquids agitated by a stream of gas and it may be possible to adapt it for determining oxygen diffusion rates in aerated and agitated reactors. It is, however, mechanically rather complex and cannot be adapted to some of the conditions of aeration, such as aeration in shake flasks, with which this paper is concerned.

Dissolved oxygen can also be determined polarographically by means of the rotating platinum electrode (BRONK, 1946; GIGUERE and LAUZIER, 1945; KOLTHOFF and LAITINEN, 1950; LAITINEN and KOLTHOFF, 1941; MADER and FREDIANI, 1951). In the following paper the authors have adapted the rotating platinum electrode for the continuous amperometric determination of oxygen diffusion rates in reaction vessels commonly used for carrying out aerobic fermentations, such as shake flasks of different shapes and dimensions and stirred 10 l containers under different conditions of agitation and aeration.

They have also determined oxygen diffusion rates in larger ferment-

ters of 3,000 and 12,000 l capacity and for this purpose have adopted the vibrating platinum electrode (HARRIS and LINDSEY, 1948; HARRIS, 1951).

METHODS AND MATERIAL

Arrangement for the continuous amperometric measurement, by means of the rotating or vibrating Pt electrode, of oxygen diffusion rates into aqueous solutions in shake flasks, a 10 l stirred reaction vessel and agitated reactors of 3,000 and 12,000 l capacity.

Reaction vessels.

Aeration rates were measured a) in Erlenmeyer and round bottom pyrex flasks of different sizes agitated either on a reciprocating or a rotary shaker, b) in a cylindrical reaction vessel of perspex of 10 l total capacity containing 5 l of solution which could be subjected to mechanical agitation of different intensity and, c) in stainless steel reactors of 3,000 and 12,000 l capacity. Aeration measurements were also made in stainless steel fermenters of 50, 130 and 200 l capacity, but in these cases the sulphite method was used.

Shakers.

Aeration measurements in shake flasks were made using the reciprocating and rotary types of shaker.

a) Reciprocating shaker.

The same reciprocating shaker of special construction was used which had been employed for the previously reported aeration studies (CHAIN *et al.*, 1952). It had a stroke of 6 cm and a frequency of 95 oscillations per minute. Its shaking action was intensified by a system of springs which gave the liquid in the shake flasks an extra accelerating motion at the end of each stroke. This action of the springs helped to increase the turbulence of the fluid in the shake flasks and, in consequence, the aeration rate, but it had the disadvantage of making the shaking motion dependent on the total load and the position of the flask, thus resulting in a poor reproducibility of the aeration rates.

b) Rotary shaker.

The model of the rotary shakers used for the present study was designed by PALDINO (1954). It is characterised by simplicity of construction; essentially it consists of a board made to move on four metal spheres by mean of an eccentric drive. It has been in continuous use for periods of many months and has given excellent results with regard to mechanical stability and reproducibility of results.

Rotating Platinum electrode.

The electrode consisted of a short glass tube (length 80 mm, diam. 4 mm; these dimensions are not critical) into which a platinum wire was introduced through a small hole about midway along its length. The end of the platinum wire was melted in the oxygen flame to a small sphere (diam. about 1.5 mm) and one end of the glass tube sealed around the platinum wire in such a way that only the platinum sphere protruded. The electrode was pushed through the centre of a rubber cap of a penicillin bottle and by means of a small metal ring, was held in position so that it could not slide along the tube (fig. 1).

The shake flasks used for the aeration measurements had a short tube sealed centrally into their bases through which the rotating electrode was inserted by means of the rubber cap. In the perspex

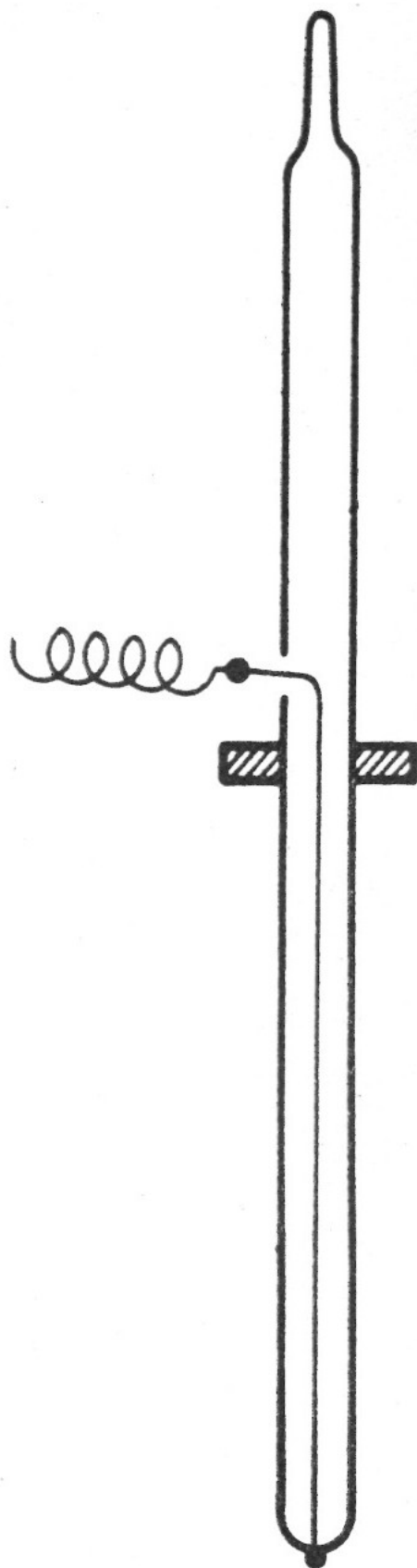


Fig. 1. - Rotating platinum microelectrode.

vessel the electrode was inserted laterally through a hole cut into the perspex. The general experimental arrangement is illustrated in fig. 2.

After insertion into the reaction vessels through the appropriate openings it was driven by a bicycle dynamo acting as a constant speed

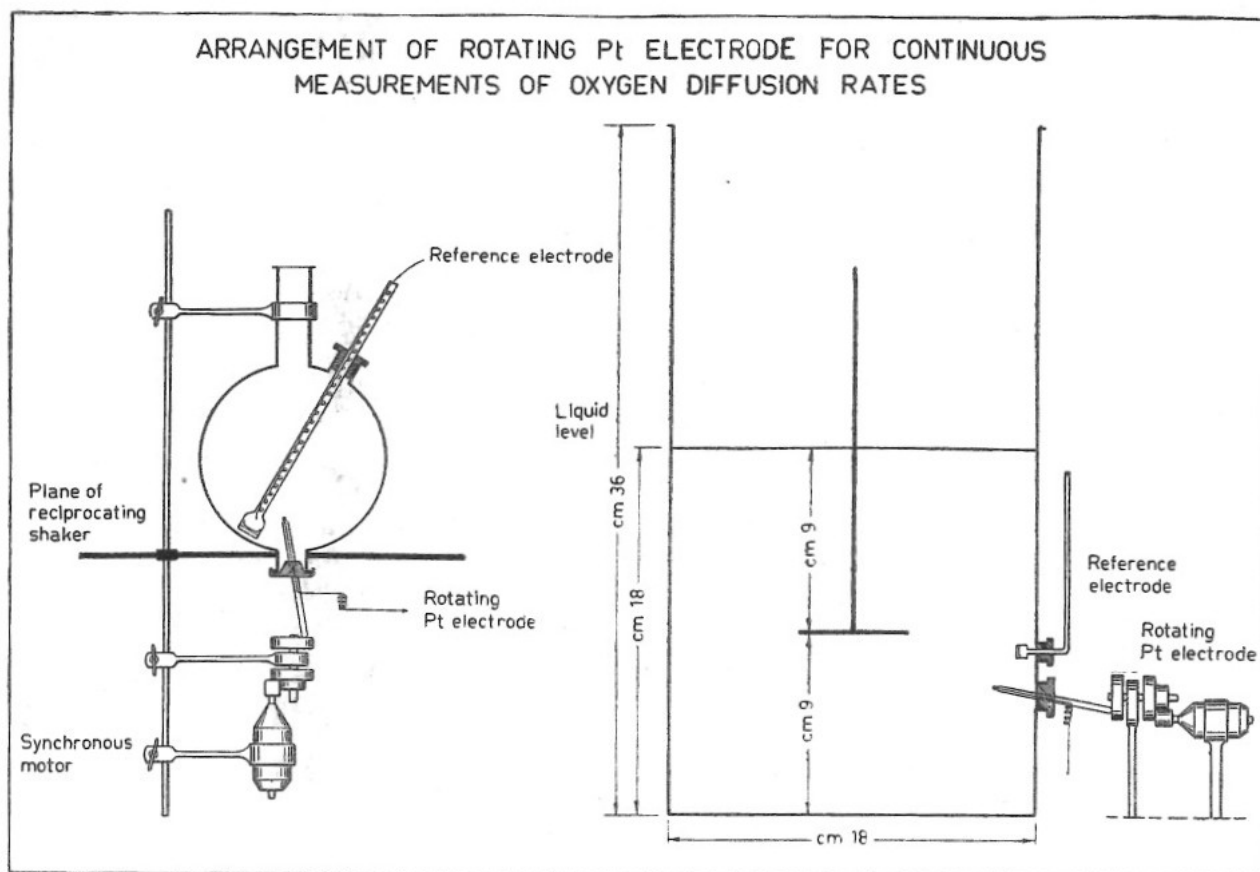


Fig. 2. - Arrangement of rotating Pt electrode for continuous measurements of oxygen diffusion rates.

synchronous motor (1.5 watts; 6 volts; speed 1,500 r.p.m.); transmission was effected through a step pulley by which speeds of 600 to 1,500 r.p.m. could be obtained, according to the degree of sensitivity required. The electrode tube inserted eccentrically into the pulley wheel was made to describe a double conical movement so that the electrode point made a circular movement inside the reaction vessel.

By introducing the electrode through a flexible rubber cap it was made to rotate inside the reaction vessel without having to use a stuffing box, liquid seal or similar devices.

Vibrating Platinum electrode.

For measurement of oxygen diffusion rates in aqueous solutions contained in fermenters of 3,000 and 12,000 l capacity a vibrating platinum

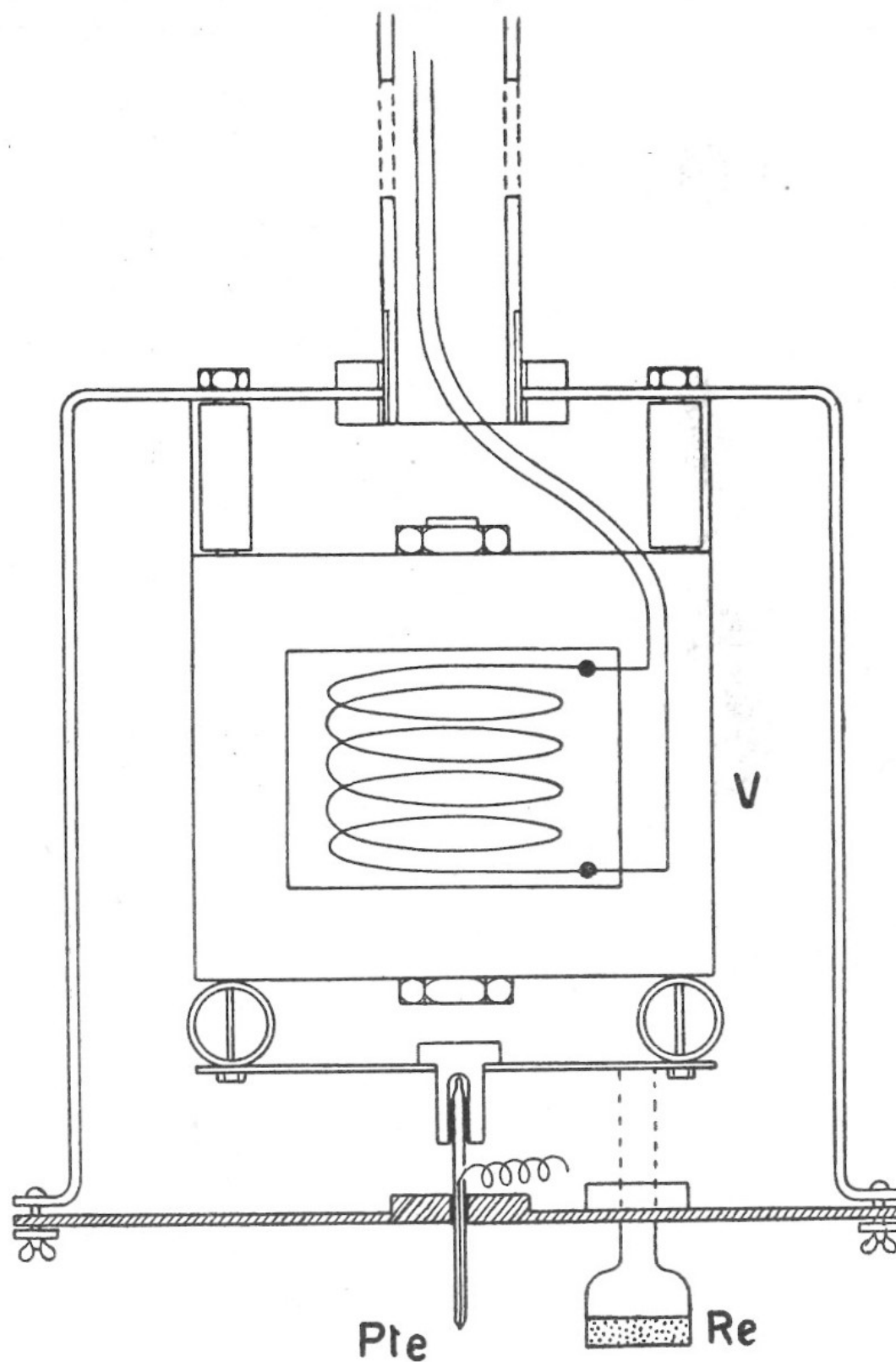


Fig. 3. - Vibrating platinum microelectrode.

Pte = Platinum electrode
Re = Reference electrode.
V = Vibrator

electrode was used because of its greater ease of manipulation under these conditions. The Pt electrode used in conjunction with the vibrator and the reference electrode was the same as that described above. A small electromagnetic vibrator absorbing 20 watts and specially constructed for the purpose was used. The amplitude of the vibrations was 0.1 to 1.5 mm and the frequency 50 cycles per second. The general arrangement is shown in fig. 3.

The vibrating and the reference electrodes were encased in a brass cylinder closed at one end by a rubber sheet through the middle of which

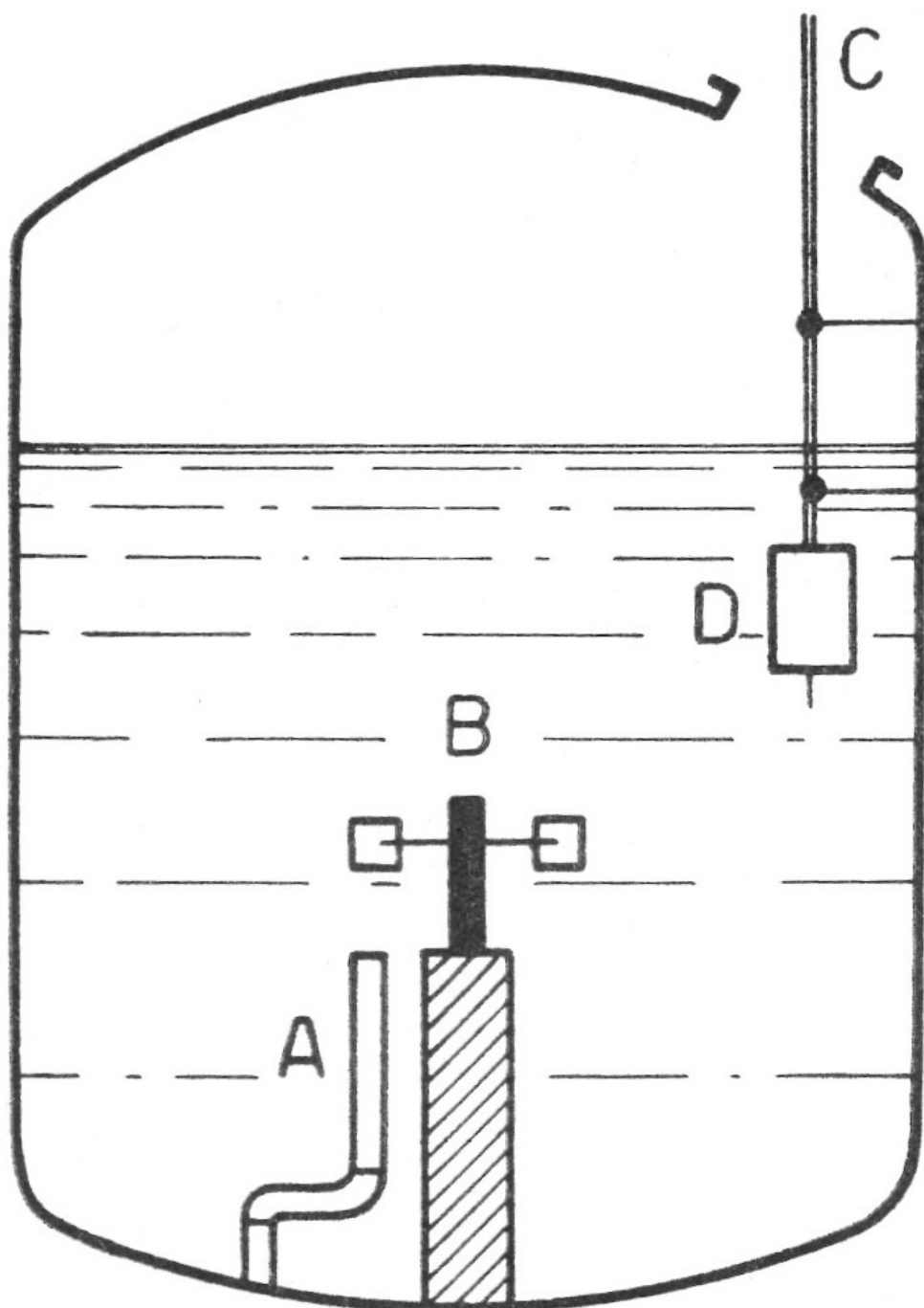


Fig. 4. - Arrangement of vibrating Pt electrode for measurement of oxygen diffusion rates in industrial fermenters.

A = air sparger
B = propeller

C = manhole
D = vibrating electrode

the electrodes protruded. The rubber sheet was reinforced by layers of thin rubber at the point through which the vibrating electrode protruded from its protecting case. The case containing the electrodes was connected to a long metal tube which carried the wire connections to the measuring instruments and served to insert the electrode case into the fermenter through the manhole at the fermenter head so that it was completely immersed in the liquid (fig. 4).

Reference electrode.

An Ag/AgCl/sat.KCl electrode was used as reference electrode. Its construction was similar to that of BROWN (1934), but it contained sat. KCl instead of HCl. This electrode provided a sufficiently large anodic surface and was not sensitive to shocks and vibration, unlike the usual mercury-calomel half cell.

Polarographs.

Two Sargent polarographs were employed for the work reported in this paper, Model XII and the self-recording Model XXI. All amperometric measurements were made at a constant applied potential of -0.9 volt and at a temperature of $25^{\circ} \pm 1^{\circ}$ C.

Measurement of oxygen diffusion rates.

a) *In liquids contained in shake flasks.* — The shake flasks containing the desired amount of liquid (0.1 N KCl) were placed on the shaker which was then put in motion. With the electrodes operating, the dissolved oxygen in the liquid was removed by gassing out with commercial nitrogen through a sintered glass sparger until its concentration, as recorded on the polarograph, had reached its minimum value. The shaker was then stopped, the sparger tube removed, and air gently sucked over the surface of the liquid to replace the nitrogen. This is important; considerable errors in measuring the diffusion rates occur if this precaution is omitted. The shaker was then started again and the oxygen concentrations measured amperometrically were recorded continuously until saturation was reached.

b) *In mechanically stirred liquids in a 10 l perspex reaction vessel.* — The liquid volume used in all experiments was 5 litres

(0.1 N KCl solution). All measurements were made in the above mentioned 10 l cylindrical perspex reactor provided with mechanical agitation. Aeration rates were determined for two aeration methods involving mechanical, agitation 1) the vortex system in which the air is sucked into the liquid from the atmosphere above by a vortex formed by a fast rotating propeller and is dispersed by the propeller blade at the apex of the vortex (see CHAIN *et al.*, 1952) and 2) the fully baffled system in which the air enters the liquid through a sparger tube and is dispersed by the combined stirring action of propeller blades and baffles. Stainless steel propellers of the disk-turbine type, as previously described (CHAIN, *et al.*, 1952) with eight blades (height 15 mm; for illustration see fig. 12) and three diameter sizes, (55 mm, 65 mm, and 75 mm) were used for the agitation. The propellers were made to rotate at half height of the liquid level. Four metal strips (width 1/10 of the diameter of the vessel) placed equidistantly in the liquid acted as baffles, as previously described (CHAIN *et al.*, 1952).

Gassing out for aeration measurements using the vortex system was done by introducing the nitrogen through a sintered glass sparger placed at the bottom of the vessel as near as possible to the propeller blade, with the electrode operating and the propeller in motion; during the gassing out the vessel was covered with a suitably cut perspex top to avoid back diffusion of air into the liquid. When the polarograph indicated that the oxygen concentration in the liquid had reached its minimum, the propeller was stopped, the sparger tube withdrawn, a stream of air blown over the surface of the liquid and finally the perspex top lifted. The propeller was set in motion at the desired speed and the rate of oxygen diffusion measured amperometrically and recorded continuously until saturation was reached. For measurements of aeration rates in the fully baffled system nitrogen was passed into the solution through a constricted one hole sparger as previously described (CHAIN *et al.*, 1952) with electrodes and propeller operating. When the oxygen had been completely replaced, air instead of nitrogen was passed through the sparger without interrupting the agitation and the oxygen diffusion rates were measured and recorded as above.

c) *In liquids contained in 3,000 and 12,000 l reactors provided with mechanical agitation.* — The gassing out technique was similar to that used for the corresponding experiments on the 10 l scale. All measurements were carried out at $20^{\circ} \pm 1^{\circ}$ C. The liquid volumes were 2,250 and 10,000 l respectively. Stainless steel eight-bladed propellers of the disk-turbine type were used (for diameter sizes see below).

Amperometric determination of respiration rates of micro-organisms.

For the amperometric determination of respiration rates of micro-organisms by means of the rotating platinum electrode a reaction vessel specially designed for the purpose was used (fig. 5).

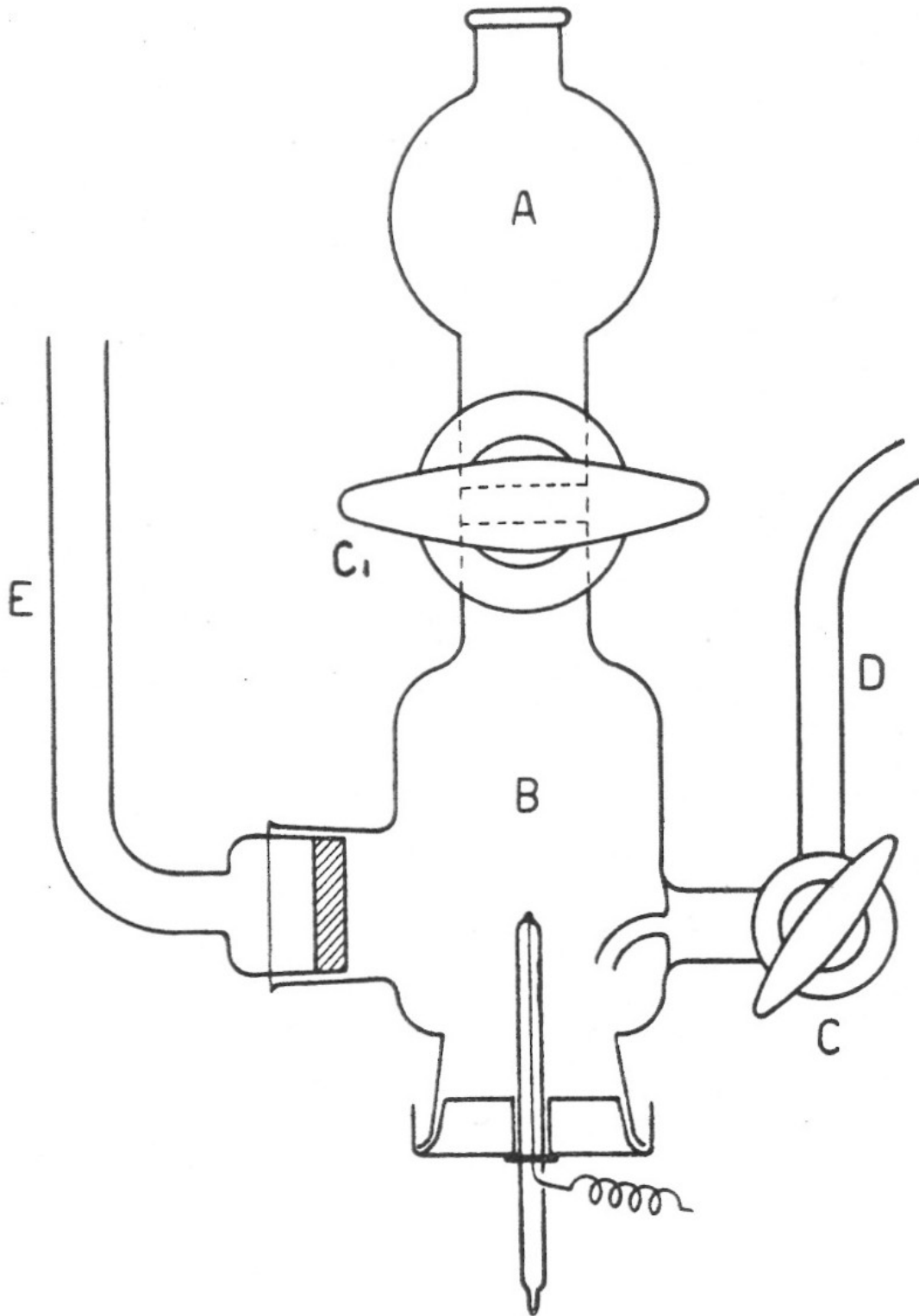


Fig. 5. - Cell for measurements of oxygen uptake in biological systems

- | | |
|----------------------|----------------------------------|
| A = bubbler | C and C ₁ = stopcocks |
| B = reaction chamber | b = gas inlet |
| | E = reference electrode |

It consisted of a reaction chamber B (capacity 25 ml) provided with a side tube D through which oxygen or air could be passed, a short side arm to receive the reference electrode E, and the bulb A

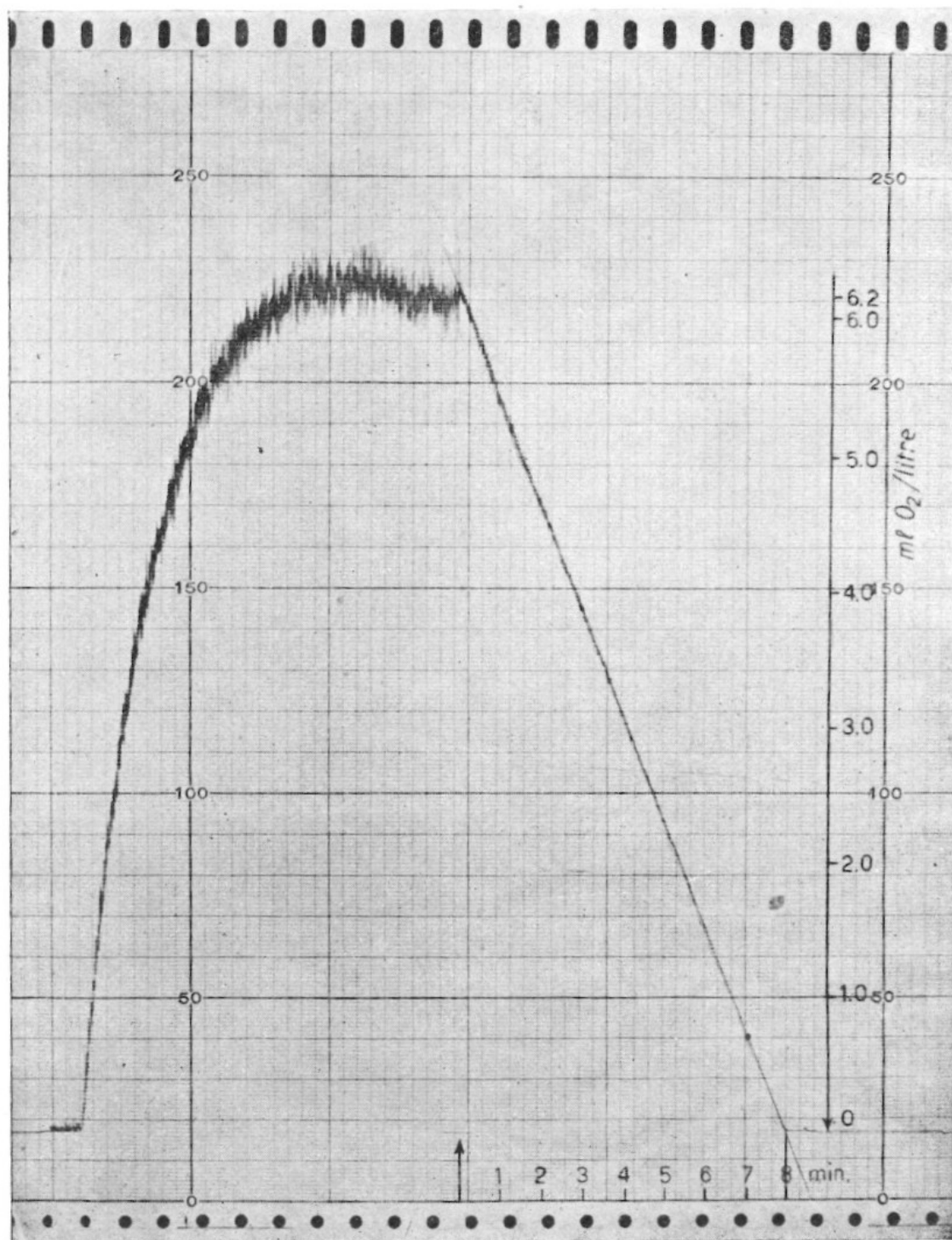


Fig. 6. - Typical oxygen absorption curve followed by amperometric measurement.

serving as bubbler (capacity 10 ml). The rotating platinum electrode was inserted through the rubber cap and driven as described above. The reaction chamber was filled with culture medium until the bulb A was half full. A very small amount (0.1 to 1.0 ml) of the suspen-

sion of micro-organisms to be analyzed was introduced into the reaction chamber through the bulb A by means of a Pasteur pipette and a stream of oxygen bubbled through the tube D through the solution in B and A with the electrodes operating, until the oxygen concentration in the solution had reached saturation level. The rotating Pt electrode acted as a good mixer in this system. When the amperometric measurements had shown that the solution was saturated with oxygen the gas flow was stopped, stopcocks C and C₁ closed and the decrease of oxygen concentration due to the respiration of the micro-organisms measured continuously and recorded. The amount of micro-organism introduced in the reaction chamber must be small enough for the reaction to proceed at a sufficiently low rate to make accurate reading possible. The slope of the oxygen absorption curve indicates the rate of respiration. Fig. 6 shows a characteristic absorption curve of a suspension of *Penicillium chrysogenum*.

Biological Material.

For comparing the amperometric with the manometric method of determining oxygen absorption rates, the following oxygen absorbing systems were used:

a) washed suspensions of brewers' yeast in M/15 phosphate containing 20 mg/ml of glucose; b) a washed suspension of *Penicillium chrysogenum*, strain Q 176 in the synthetic medium of JARVIS and JOHNSON (1947); c) a solution of d-amino acid oxidase prepared according to KREBS (1935) containing 1.0 M dl-alanine.

The *Penicillium chrysogenum*, used for all experiments was grown in agitated stainless steel 10 l fermenters described above. In order to study quantitatively the effect of mycelium on aeration rates different amounts of mycelium killed by heating at 60° for 15-30 minutes or by adding 1% of phenol and washed on a buchner funnel, were added to the culture fluid and the aeration rates determined amperometrically.

Calculations.

The oxygen diffusion rate is defined as ml O₂ diffused per hour per 100 ml of solution at a concentration of gaseous oxygen of 0.21 atm. corresponding to the oxygen partial pressure of air under standard conditions.

The diffusion rate of oxygen into a liquid, a first order process, is expressed by the well known equation:

$$(1) \quad \frac{dc}{dt} = \phi (c_{\text{sat}} - c_i)$$

where

$\frac{dc}{dt}$ = rate of variation of oxygen concentration in ml per 100 ml solution per hour.

c_{sat} = concentration of oxygen at saturation level, in 0.1 N KCl = 0.56 ml/100 ml sol. at 25° C and 0.21 atm. partial pressure (SEIDELL, 1940).

c_i = concentration of oxygen in ml in 100 ml solution at a given time t_i .

ϕ = Kinetic constant (hour⁻¹)

The oxygen diffusion rate is, by definition,

$$\phi \times c_{\text{sat}} \text{ ml O}_2/100 \text{ ml solution/hour}$$

Integration of equation (1) followed by a simple rearrangement gives

$$(2) \quad 2.303 \log c_{\text{sat}} - 2.303 \log (c_{\text{sat}} - c_i) = \phi \Delta t$$

If R_{sat} and R_i are the galvanometer or penrecorder deflections corresponding to the concentrations c_{sat} and c_i , equation (2) can obviously be expressed as

$$(3) \quad 2.303 \log R_{\text{sat}} - 2.303 \log (R_{\text{sat}} - R_i) = \phi \Delta t$$

When $\log (R_{\text{sat}} - R_i)$ is plotted against time a straight line results with the slope = $- 2.303/\phi$, hence

$$(4) \quad \phi = - 2.303/\text{slope}$$

The oxygen diffusion rate, as defined above, can be calculated if ϕ is known. Fig. 7a shows a typical oxygen diffusion curve; in fig. 7b

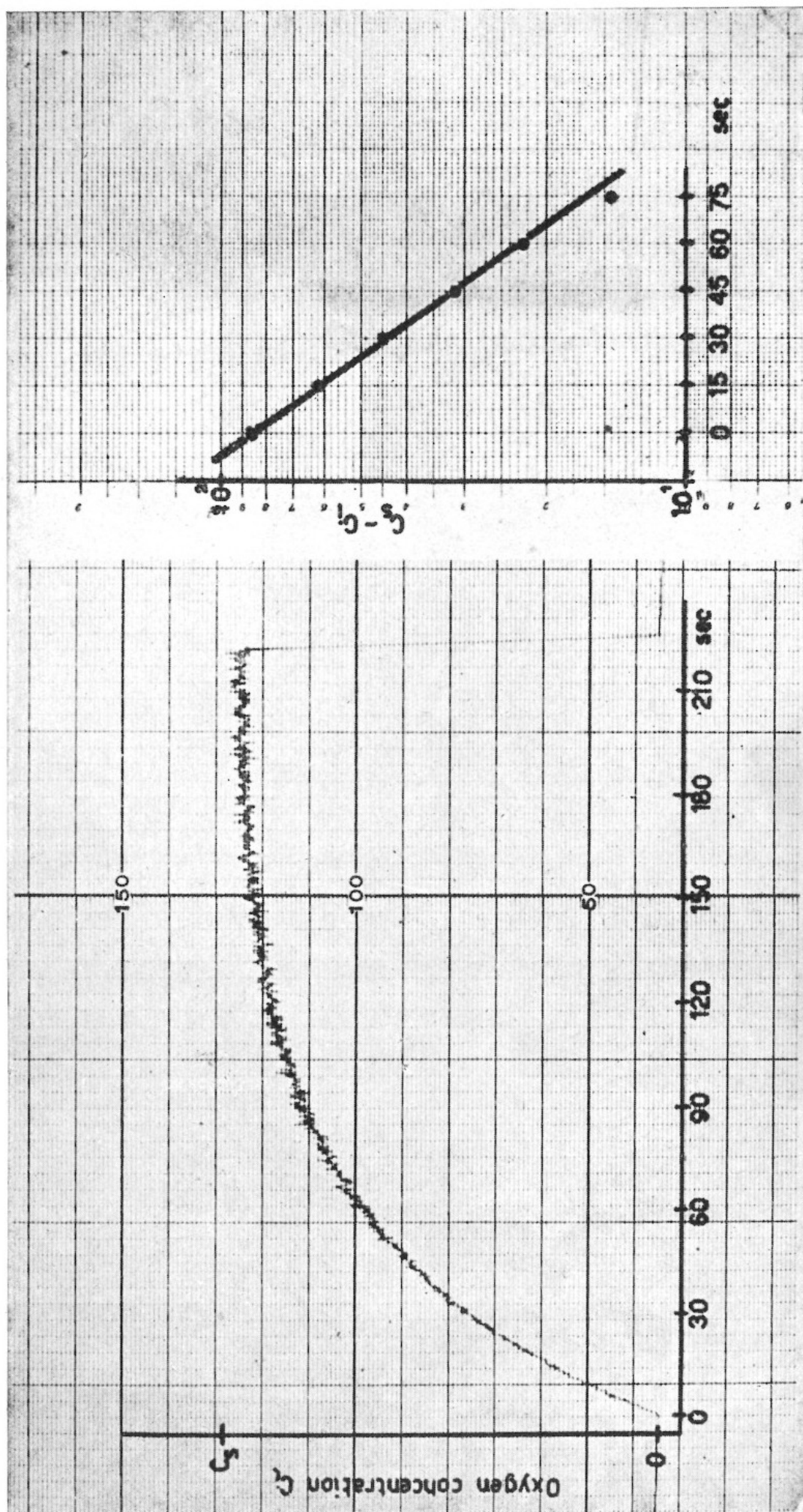


Fig. 7. - Typical oxygen saturation curve followed by amperometric measurement.

the values for $\log (c_{\text{sat}} - c_i)$, calculated from this curve, are plotted against time.

Power measurements.

The measurements of the power required by the various systems of agitation were carried out with a three phase kilowatt-hour-meter inserted on the power input of the motor. When necessary, suitable shunts were used. The measurements were made by counting the number of seconds required for the disk to make a given number of revolutions (usually 10).

The power necessary for the air compressor, was evaluated from the mean power absorption of various compressors of both reciprocating and turbine type. This power was calculated as 3.0 watt/litre for an air flow of 1.0 vol. air/vol. liq./min. Pressure at the compressor: 2.0 atm.

RESULTS.

Relation between height of polarographic wave and concentration of dissolved oxygen.

Curve A in fig. 8 represents a polarogram of a 0.1 N KCl solution saturated with air; curve B shows the course of the displacement of oxygen by commercial nitrogen measured amperometrically at -0.9 volt constant applied potential, while curve C is the polarogram of the 0.1 N KCl solution saturated with commercial nitrogen.

As can be seen from curve C, the commercial nitrogen used contained a small amount of oxygen which gave a small residual oxygen wave; this had to be taken into account in the calculation of absolute values in oxygen concentrations while it did not matter in the calculations of the oxygen diffusion rates.

The above measurements were performed in the 10 l perspex vessel containing 5 l of solution, with a propeller of a diameter of 65 mm rotating at a speed of 1,100 r.p.m.

To determine the relationship between oxygen concentration and

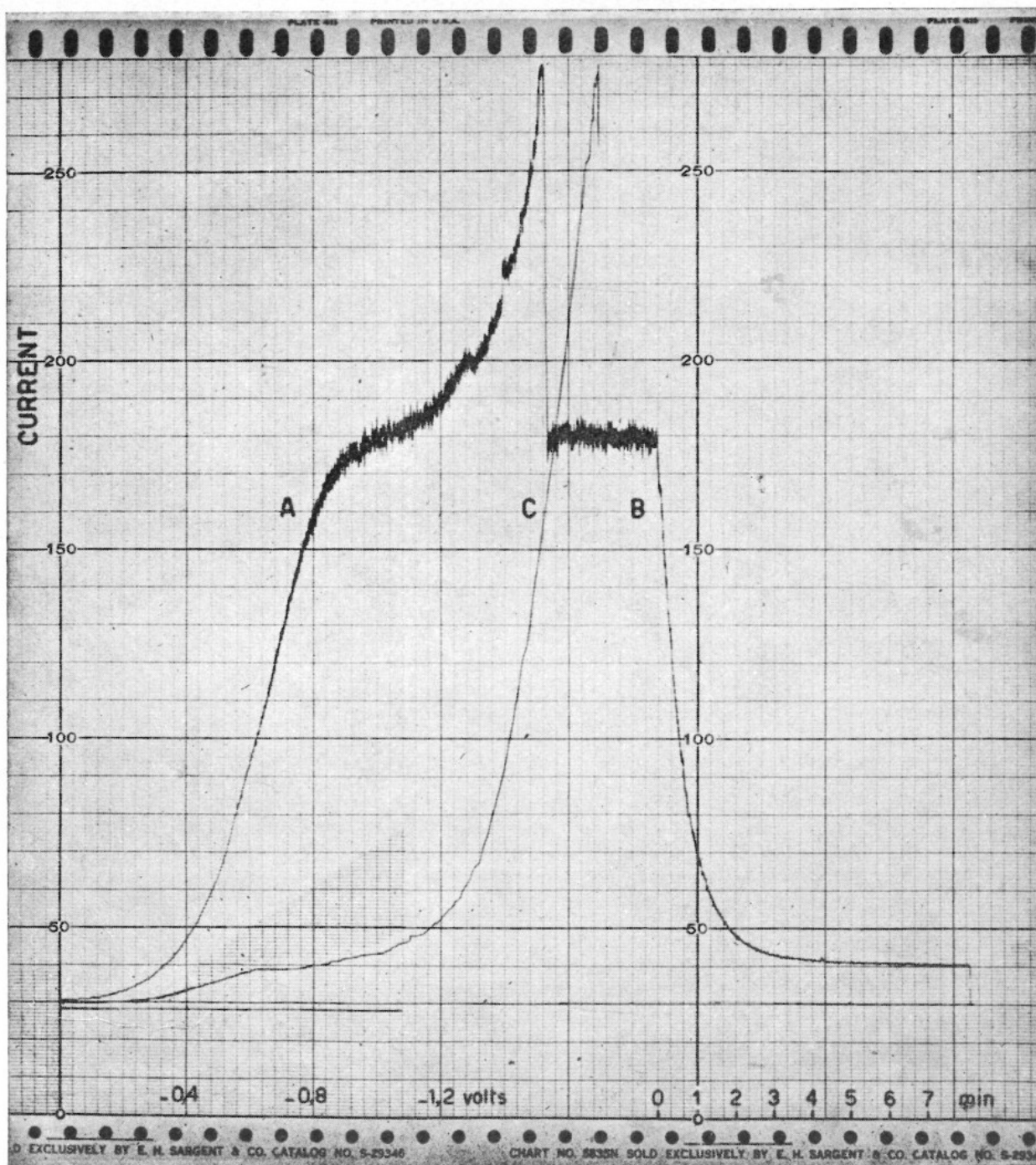


Fig. 8. - Typical polarogram obtained with rotating platinum electrode in a stirred solution.

A and C = polarographic curves
B = amperometric curve

polarographic wave height, nitrogen-oxygen mixture containing different percentages of oxygen (determined manometrically) were bubbled through a solution of 0.1 N KCl in the cell used for respiration measurements, as described above, until saturation was reached, and polarograms of the solution were made.

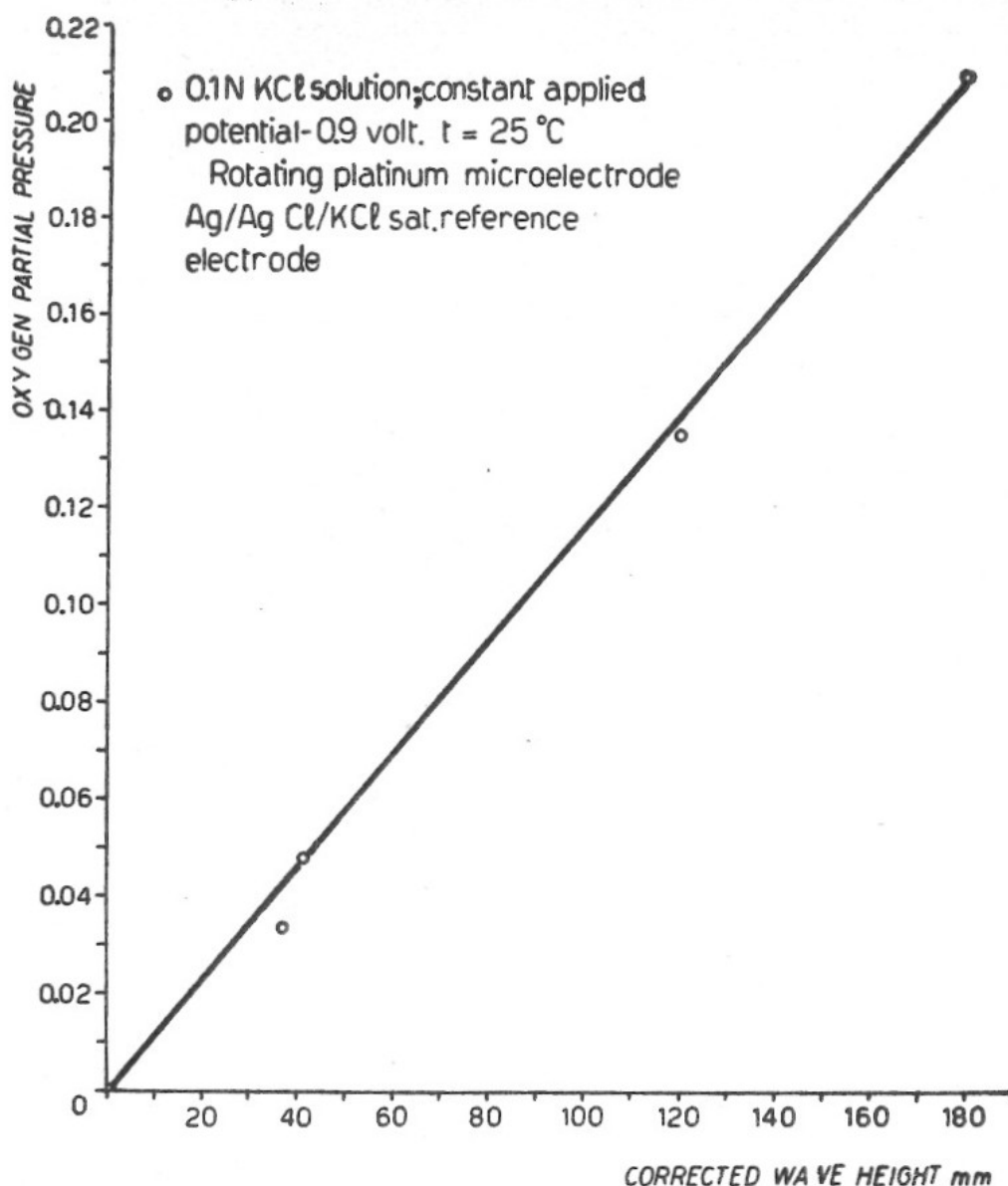


Fig. 9. - Relation between oxygen partial pressure and polarographic oxygen wave height.

Fig. 9 shows linearity between oxygen concentration and the height of the polarographic oxygen wave. The same result was obtained when culture media, such as peptone-glucose and cornsteep-lactose, were used instead of 0.1 N KCl solution. The saturation levels in the culture media were about the same as in 0.1 N KCl solution.

COMPARISON OF MANOMETRIC AND AMPEROMETRIC DETERMINATIONS OF OXYGEN ABSORPTION RATES.

The results of a comparison between manometric and amperometric determinations of different oxygen absorbing systems are given in table 1.

COMPARISON OF MANOMETRIC AND AMPEROMETRIC DETERMINATION OF OXYGEN ABSORPTION

Oxygen consuming system	Oxygen absorption	
	manometric method $\mu\text{L}/\text{mL}$ of solution/hour	amperometric method
yeast suspension n° 1	88.9	92.4
" " " 2	80.7	95.5
" " " 3	63.8	71.5
" " " 4	93.1	90.6
" " " 5	71.7	72
" " " 6	68.1	68.8
" " " 7	50.6	59
suspension of <i>Penicillium Chrysogenum</i> n° 1	102	115
n° 2	45	49
solution of D-amino acid oxidase	94.9	92

Tab. 1. - Comparison of manometric and amperometric determination of oxygen absorption.

The manometric measurements were carried out in Warburg vessels, the amperometric measurements in special cells as described above. The agreement between the two methods was found to be reasonably good. Fig. 6 represents a typical oxygen absorption curve for a suspension of *Penicillium chrysogenum*. It is evident that the rate of oxygen absorption is constant down to a very small partial pressure of oxygen. The same independence of the rate of oxygen absorption of the partial pressure of oxygen has also been observed for yeast suspensions and *B. coli*. This confirms the observations of previous authors (WARBURG, 1929; HIXSON and GADEN, 1950; WISE, 1951), that the respiration of many micro-organisms is independent of the oxygen pressure down to very small oxygen concentrations.

AERATION IN SHAKE FLASKS.

a) On a reciprocating shakers.

The results of amperometric measurements of oxygen diffusion rates in open shake flasks of different sizes and shapes, and containing different volumes of liquid are represented in table 2. The polarographic measurements of the aeration rates were checked by sulphite oxidation measurements, and good agreement between the two methods was obtained. Aeration efficiency was found to be dependent on the ratio of the volume of liquid to the total volume of the flasks. Erlenmeyer flasks gave more aeration than round bottom flasks; in the latter the movement of the fluid was less turbulent than in the former. With 1 l Erlenmeyer flasks containing 100 ml of liquid fairly high oxygen diffusion rates were obtained, up to 130 ml/100 ml solution/hour.

b) On a rotary shaker.

For the determination of aeration rates in shake flasks on a rotary shaker, the sulphite methods was used throughout because of its simplicity and its good agreement with the polarographic method. The results are given in table 2.

which also gives comparative values for aeration rates of shake flasks of the same volume and shape on the reciprocating and rotary shaker. In the rotary shaker the fluids in the shake flasks are subjected to a regular centrifugal swirling motion and the liquid distributes itself along the walls of the flasks in such a way that centrifugal and gravity forces are balanced.

The rates of aeration in shake flasks on a rotary shaker depend on the surface area of the solutions exposed to the air, there being no mixing of the gas and liquid and air dispersion into fine bubbles as occurs on the reciprocating shaker.

The surface area of the solutions can be increased by either increasing the eccentric throw or the rotary speed of the shaker, the former being less effective than the latter. Values for aeration rates in 500 ml Erlenmeyer flasks with two different volumes of solutions and two eccentrics of different throw are reported in fig. 10.

The curves in fig. 10 show that there exists practically a linear relationship between aeration rates and speeds of the rotary shaker.

AERATION RATES IN SHAKE FLASKS ON RECIPROCATING AND ROTARY SHAKER

type and volume of flasks ml	volume of liquid ml	Oxygen diffusion rate ml/100 ml of liquid/hour		
		reciprocating shaker *		rotary shaker**
		amperom. method	sulphite method	
 250	50	—	—	56
 500	50	18 -	24.5	99
	100	11.5	18 -	59
	200	14 -	21 -	—
 1000	100	40.5	44.5	108
	200	38.5	33.5	62
	300	28 -	28.5	—
 1500	200	32.5	28.6	—
	300	38 -	34 -	—
	400	34.5	32 -	—
 250	50	—	—	59
 500	50	92 -	102.5	101
	100	52.5	57.5	64
	200	36 -	35.5	—
 1000	100	129	112 -	103
	200	96	80 -	56
	300	48	44.5	—

Tab. 2. - Aeration rates in shake flasks on reciprocating and rotary shaker.

* Stroke 60 mm — Oscillations per minute 95.

** Eccentric throw 50 mm — Revolutions per minute 285.

As is to be expected the aeration rates on the rotary shaker are inversely proportional to the volumes of the solutions.

Another striking difference between aeration conditions of the

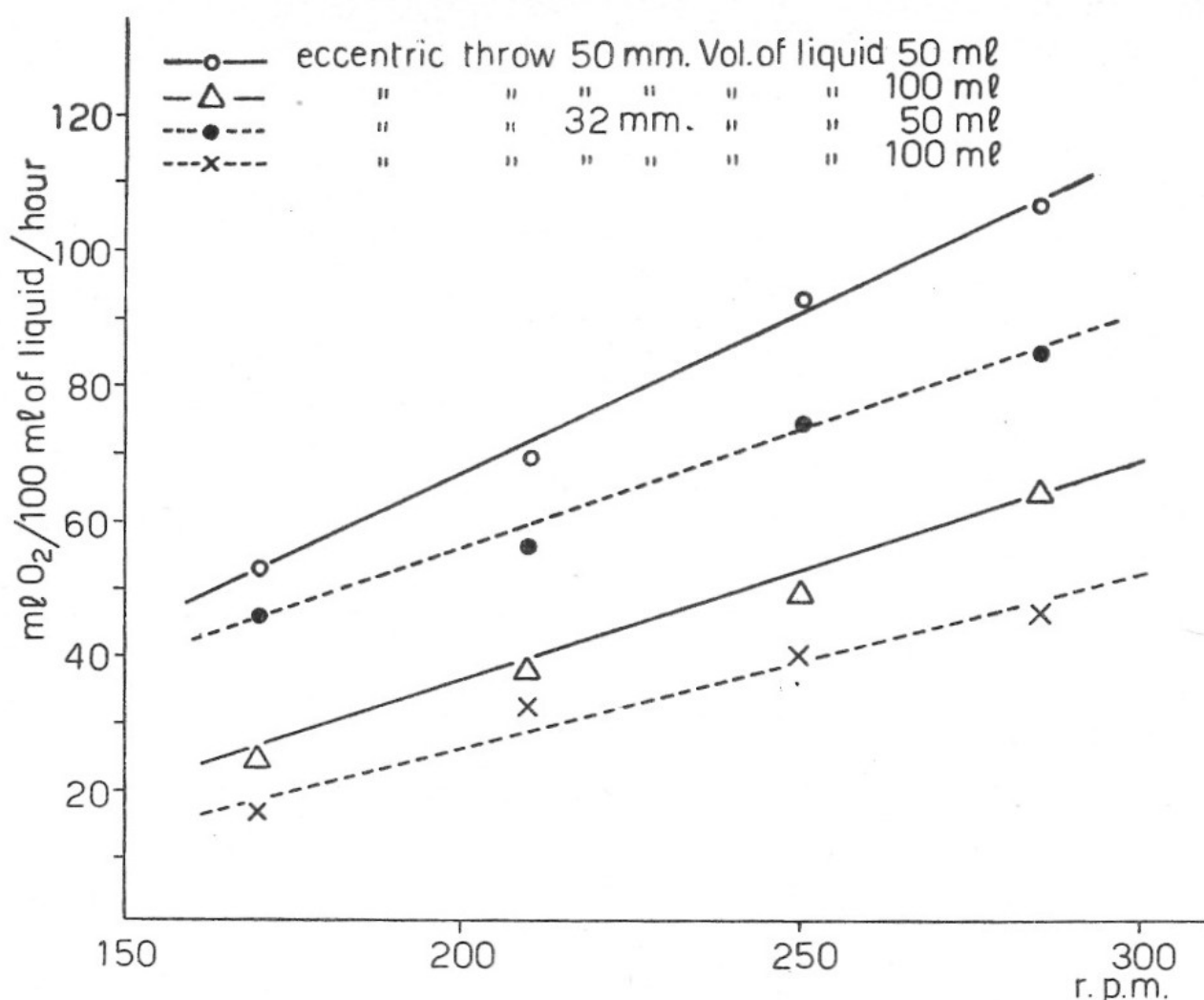


Fig. 10. - Aeration rates in 500 ml Erlenmeyer shake flasks on rotary shaker with different volumes, throws and speeds

rotary and reciprocating shaker is that the aeration rates on the rotary shaker are independent of the geometrical shape of the shake flasks while they are very markedly influenced by this factor on the reciprocating shaker (table 2).

From the point of view of the mechanical stability of the shaker and the ease of its construction it is more convenient to have a higher number of revolutions with a smaller eccentric. Experiments with eccentrics of different throw have shown that satisfactory aeration rates were obtained with an eccentric of a throw of 50 mm; increasing the throw beyond this value did not appreciably increase the aeration efficiency of the rotary shaker while the mechanical strain was considerably augmented. In view of this result the rotary shaker in use in this laboratory was constructed with a throw of 50 mm and a normal working speed of 285 rev./min.

A comparison of aeration rates obtained on the rotary and the reciprocating shaker is given in table 2.

The highest aeration values obtainable on the reciprocating shaker are somewhat higher than those obtainable on the rotary shaker. This applies particularly to volumes of solution exceeding 100 ml. For volumes of solution of 50 and 100 ml, which are most commonly used in series experiments with shake flasks, there is little difference between the efficiencies of rotary and reciprocating shakers with Erlenmeyer flasks; with round bottom flasks the aeration rates on the rotary shaker (which, as stated above, are similar to those obtained with Erlenmeyer flasks) are higher than those obtained on the reciprocating shaker.

The chief advantage of the rotary shaker over the reciprocating shakers is the higher degree of reproducibility of the conditions of agitation, a factor of particular importance in metabolic studies of micro-organisms. There is much less deposit of spores and mould mycelium on the walls, and hence less surface growth, in shake flasks in the rotary than in the reciprocating shaker.

In the presence of mycelium of *Penicillium chrysogenum* in the filamentous form the aeration rates were considerably reduced, depending on the amount of mycelium present, as is shown in table 3.

REDUCTION OF AERATION RATES IN SHAKE FLASKS IN PRESENCE OF DIFFERENT AMOUNTS OF DEAD MYCELIUM OF *PENICILLIUM CHRYSOGENUM*

Type and volume of vessel ml	Volume of suspension ml	Oxygen diffusion rate ml O ₂ /100 ml of liquid/hour			
		no mycelium	0.5 % dead myc.	1.0 % dead myc.	2.0 % dead myc.
 1000	200	38.5	31	20	—
 1000	200	96	80	48	11

Tab. 3. - Reduction of aeration rates in shake flasks in presence of different amounts of dead mycelium of *Penicillium chrysogenum*.

Turbulence and aeration rates in shake flasks may be considerably increased by inserting a baffle (see DALE, AMSZ, PING SHU, PEPPLER and RUDERT, 1953). This may be done by fusing a pyrex plate into Erlenmeyer or round bottom flasks. Such baffled shake flasks are illustrated in fig. 11.

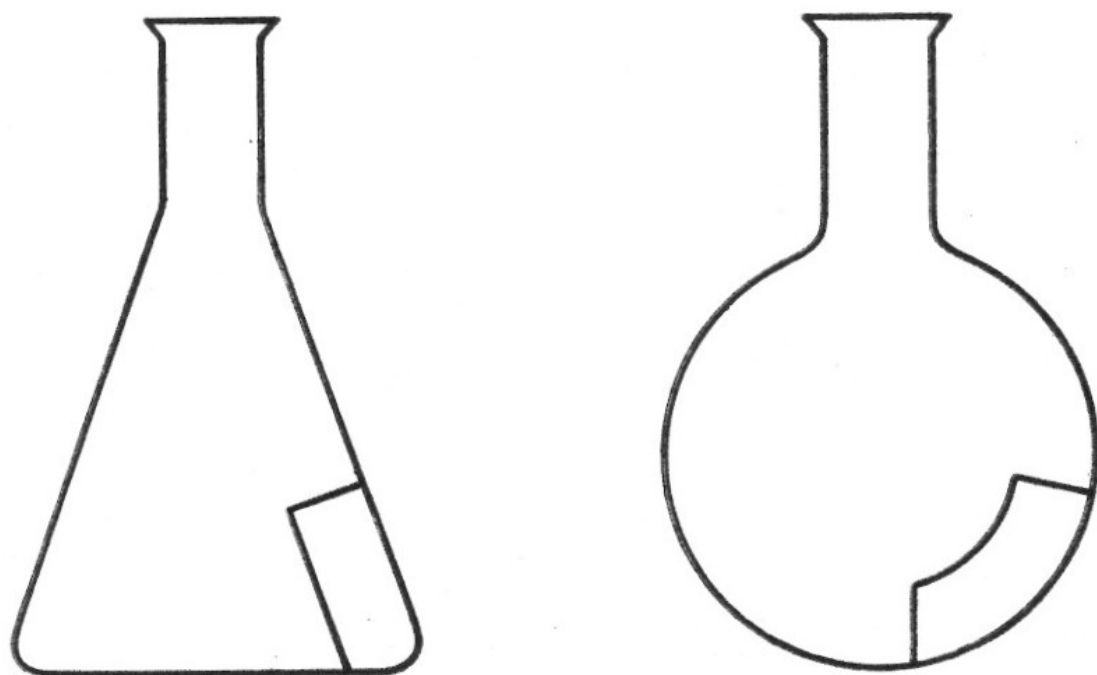


Fig. 11. - Types of baffled shake flasks.

(Dimension of baffles: length 60 mm, width 22.5 mm, thickness 2 mm). If well annealed they were quite resistant to breakage and withstood repeated sterilization. Aeration rates in baffled shake flasks, measured by the sulphite method, are given in table 4.

It is evident that the insertion of one baffle increased the aeration rates up to twenty times. The insertion of a second baffle was without much effect on the aeration rate; this is analogous to the observation reported previously (CHAIN *et al.*, 1952) that the aeration rates in stirred fermenters are not appreciably increased by fitting a second or third propeller on the shaft. Evidently the main air dispersion takes place when the air stream meets the first obstacle.

All the above reported aeration rates have been measured in open flasks. In stoppered shake flasks filled with nitrogen the diffusion of air through the stopper into the liquid was slow, irrespective of whether cotton wool plugs or aluminium caps were used. Cotton wool plugs were found to allow a somewhat higher diffusion rate than aluminium caps (table 5).

TYPE AND VOLUME OF FLASKS ml	VOLUME OF LIQUID ml	NUMBER OF BAFFLES	REVOLUTIONS PER MINUTE		
			145	180	220
OXYGEN DIFFUSION RATE ml/100 ml of liquid/hour					
 1000	100	0	—	—	61
		1	395	515	640
	200	0	—	—	34
		1	251	414	595
	400	0	10	12	18
		1	141	274	470
 500	50	0	47	58	69
		1	324	509	873
		2	453	682	875
	100	0	18	28	37
		1	234	425	716
		2	341	515	795
	200	0	9	10	13
		1	118	234	369
		2	151	290	428

Tab. 4. - Aeration rates in baffled shake flasks on rotary shaker.

In aerobic fermentations carried out in plugged or capped shake flasks the oxygen concentration in the atmosphere continuously diminishes because the oxygen is replaced by air and the rate of equilibration of the atmosphere above the liquid inside the flasks and the outside air through the plugs is low; sooner or later, depending on the rate of respiration of the micro-organisms quasi anaerobic conditions are established. The efficiency of the shaker has only a very limited effect on the air diffusion rate through the plugs of the shake flasks. Consequently, when the point is reached where the oxygen originally present in the shake flasks has largely been used up the respiring organisms and the rate of diffusion of oxygen into the liquid is mainly determined by the rate of diffusion of air through the plugs, the type of the shaker used, rotary or reciprocating, has obviously little influence on the aeration rate of the culture fluid.

It is clear that in the ordinary capped shake flasks the conditions of air supply are completely different from those in stirred fermenters where the air is continuously renewed and thus the oxygen concentration in the atmosphere is kept constant during the course of the experiment. The usefulness of shake flasks for metabolic studies is therefore limited to those reactions which do not require continuously

Type of vessel total volume 1000ml volume of solution 200 ml	Open	With cotton wool plug	With aluminium cap
	ml O ₂ /100	ml of solution / hour	
	38.5	0.25	0.15
	96.0	0.6	0.21

Tab. 5. - Rate of oxygen diffusion in stoppered shake flasks.

high aeration rates. In view of the different environmental conditions it is difficult to draw conclusion as to the metabolic behaviour of micro-organisms in stirred fermenters from results obtained in capped shake flasks. It is of course possible to maintain constant the gas atmosphere in shake flasks by blowing air continuously over the surface of the culture fluid. In this way the conditions in the shake flasks approach those in stirred fermenters, but however simple the arrangement for maintaining a continuous gas stream may be, a good deal of the simplicity of design of the ordinary shake flasks is lost, which is their main attraction and their main advantage over the stirred fermenters.

AERATION IN MECHANICAL STIRRED AQUEOUS SOLUTION CONTAINED IN A 10 l REACTION VESSEL

a) *Vortex aeration.*

In confirmation of previous results obtained with the sulphite method (CHAIN *et al.*, 1952) it was found that for the vortex system of aeration the rate of oxygen diffusion is a function of the diameter of the propeller blade and a linear function of the propeller speed. The

results of the measurements with the propeller blades of three diameter sizes are given in fig. 12.

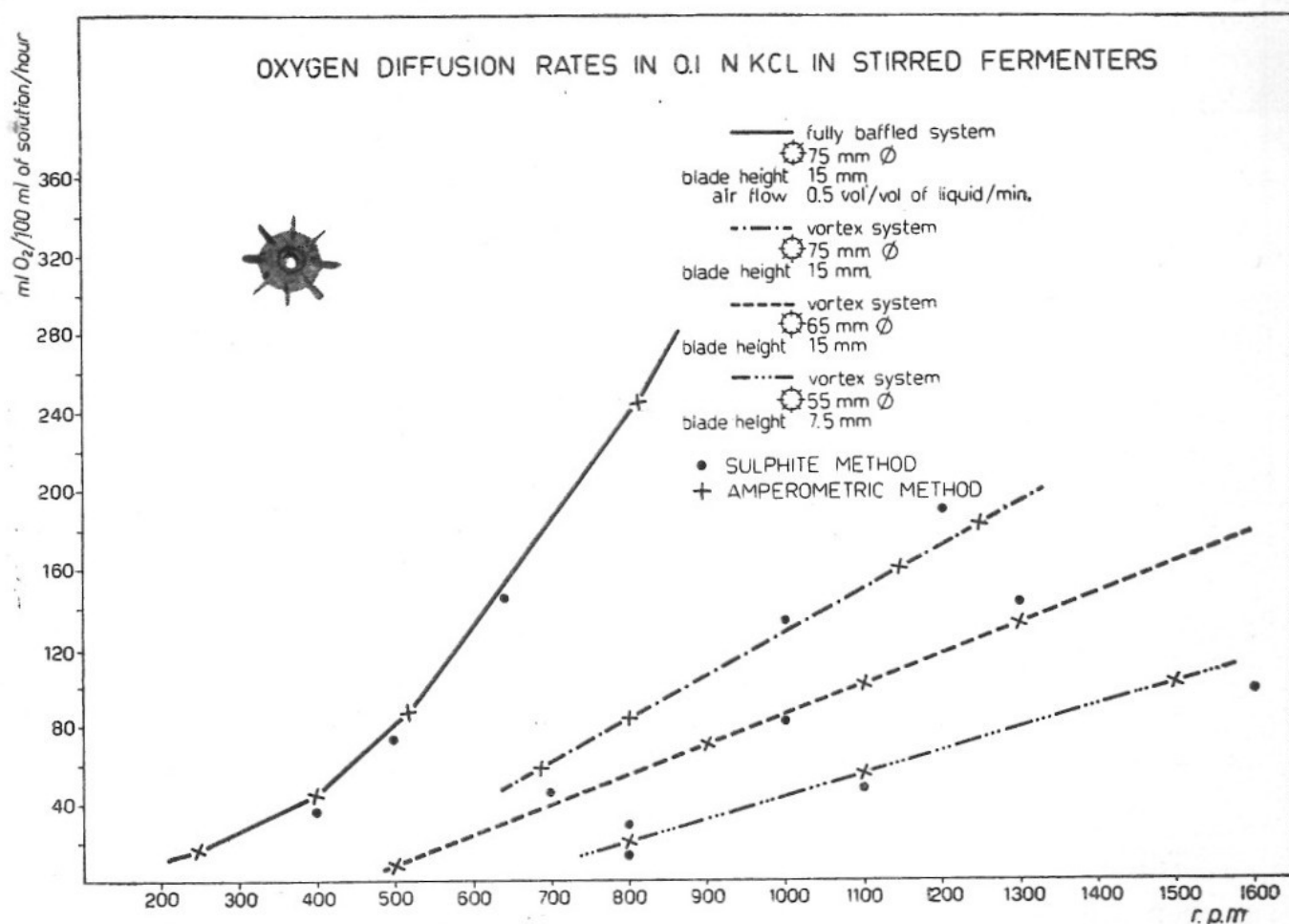


Fig. 12. - Oxygen diffusion rates in 0.1 N KCl in stirred fermenters.

It was found possible to calculate satisfactorily the respiration rate of *Penicillium chrysogenum* from the amperometric values for oxygen diffusion rates. These experiments, made to check the accuracy of the amperometric method, were carried out as follows: A suspension of *Penicillium chrysogenum* of a known dry weight was suspended in 5 l of culture fluid and the propeller speed was so adjusted that the oxygen demand of the suspension just equalled that of the supply, i.e. that there was no excess of oxygen in the culture fluid. The oxygen diffusion rate was determined at this speed in presence of the same amount of mycelium killed by the addition of phenol and from this value the Q_{O_2} was calculated.

DETERMINATION OF THE Q_{O_2} FROM OXYGEN DIFFUSION RATES

Age of Mycelium (hours)	dry weight %	r.p.m.	rate of oxygen diffusion ml O_2 /100ml hour	Q_{O_2}	
				calculated from oxygen diff. rates	directly determined
36	0.60	630	27.5	45	40
60	0.67	560	17.5	27	25
84	0.85	575	19.0	22	23

Tab. 6. - Determination of the Q_{O_2} from oxygen diffusion rates.

Table 6 shows a comparison of Q_{O_2} values obtained indirectly from oxygen diffusion rates and those obtained by direct manometric determinations in Warburg flasks. The agreement between the two methods was fairly satisfactory and proves that the accuracy of the amperometric determination of oxygen diffusion rates is fairly high.

Effect of presence of mycelium on aeration rates.

In the presence of dead mycelium of *Penicillium chrysogenum* the rates of oxygen diffusion were reduced. (Fig. 13).

Effect of foam formation on aeration rates.

The main advantage of the vortex system of aeration over systems in which an air sparger is employed is that foam formation presents no mechanical problem. However, though foam does not rise out of

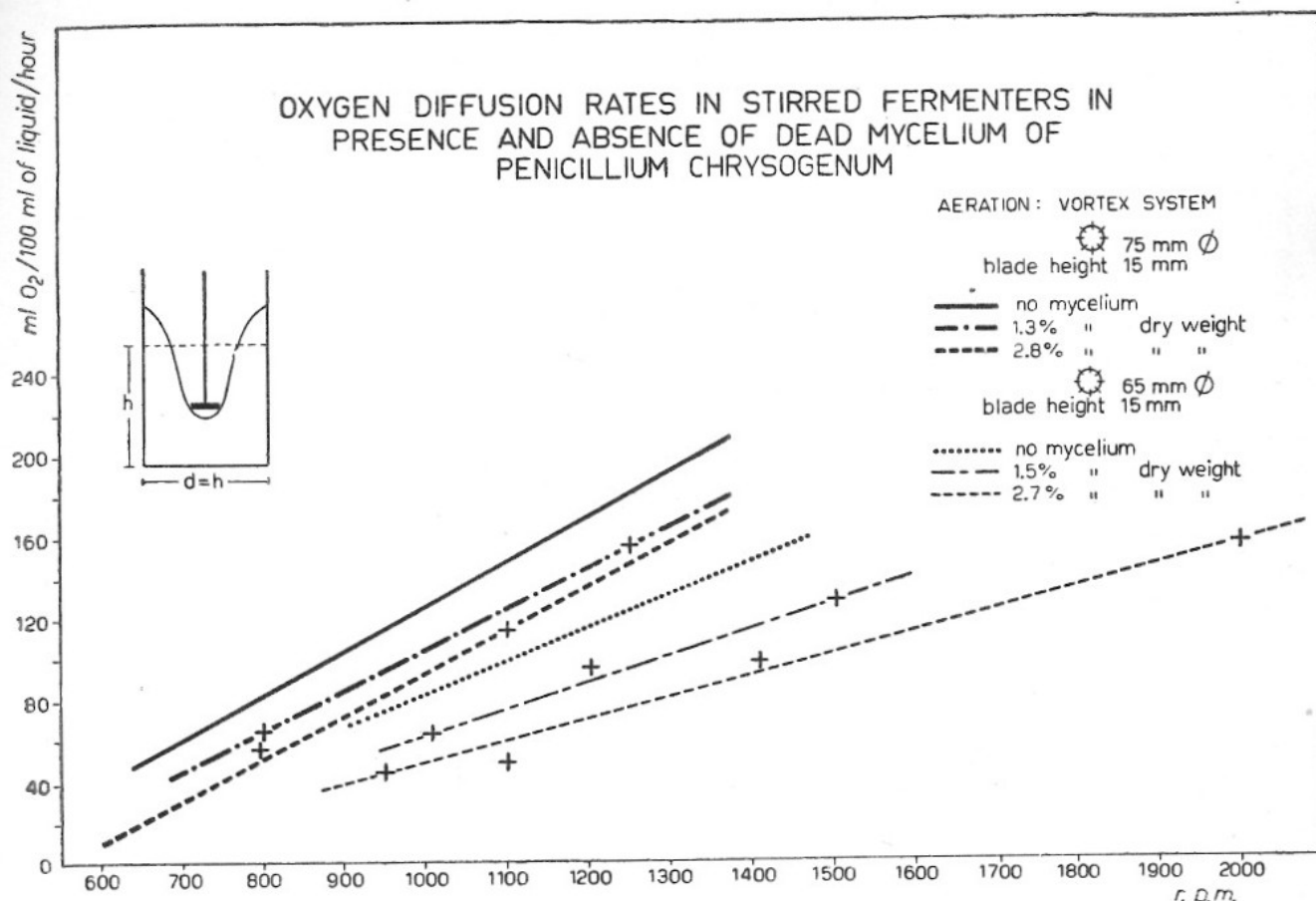


Fig. 13. - Oxygen diffusion rates in stirred fermenters in presence and in absence of dead mycelium of *Penicillium chrysogenum*. Fully baffled system of agitation.

the liquid and the fermentation vessel when vortex aeration is used, it is nevertheless formed by the fast agitation and manifests its presence by an increase in the liquid volume. In the presence of living micro-organisms the foam is recycled by the vortex movement of the liquid and a stable emulsion results, formed of nitrogen, culture fluid, and micro-organisms, which prevents the access of oxygen from the air to the cells and thus interferes with aeration.

The effect of the foam formation and of the addition of antifoam agent to foaming solution on oxygen diffusion rates into culture medium is demonstrated in fig. 14.

In the experiment recorded in this figure 5 l of a culture medium containing 3 per cent lactose and 3 per cent cornsteep solids was agitated for 15 minutes by means of a propeller blade of 65 mm diameter rotating at 1,200 r.p.m. Under these conditions a strong vortex was formed.

Foam formation became visible through an increase of the liquid

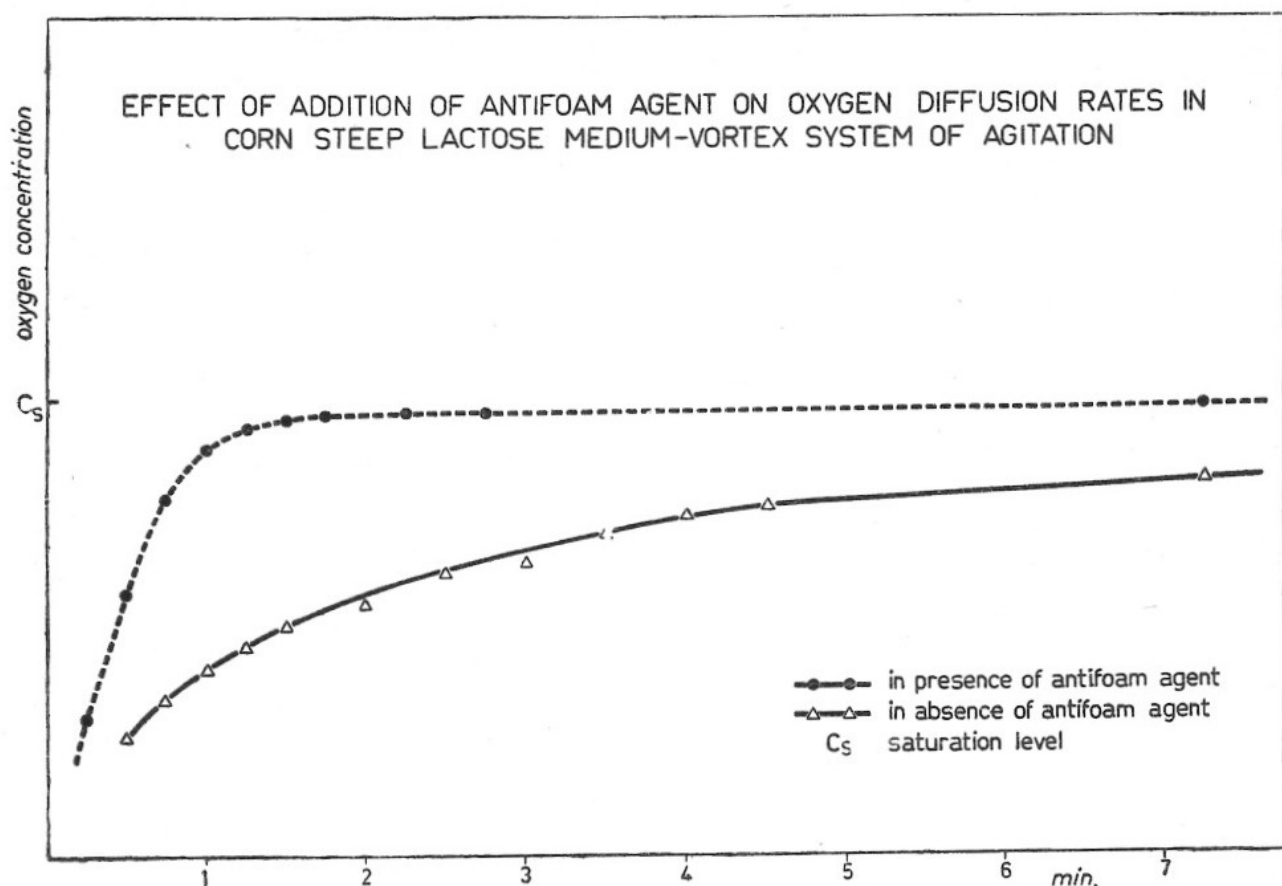


Fig. 14. - Effect of addition of antifoam agent on oxygen diffusion rates in corn-steep lactose medium. Vortex system of agitation.

volume amounting to about 20 per cent. The foam containing solution was gassed out with nitrogen in the usual manner and it was noticed that removal of the oxygen by the gassing out process took much longer than it did normally. Subsequent determinations of the oxygen diffusion rates showed that it was considerably lower than the normal rate (curve marked ▲—▲). After the termination of the amperometric measurements 1 ml of an antifoam agent (grape seed oil containing 3 per cent of octadecanol) was added to the solution; this led to an almost instantaneous destruction of the foam, as could be seen by the immediate fall of the liquid level. The oxygen diffusion rate, determined after gassing out with nitrogen in the usual manner, was found to be considerably increased by a factor 10 (curve marked ●---●). The reason of this effect is probably the formation of a stable nitrogen-culture fluid emulsion into which the oxygen from the air diffuses more slowly than into an emulsion-free homogeneous aqueous solution.

b) Sparger aeration in presence of baffle plates.

Fig. 15 shows aeration rates in fully baffled agitated 0.1 N KCl solution. As has been shown previously the oxygen diffusion rate is a function of a power of the number of the propeller blades. In order to

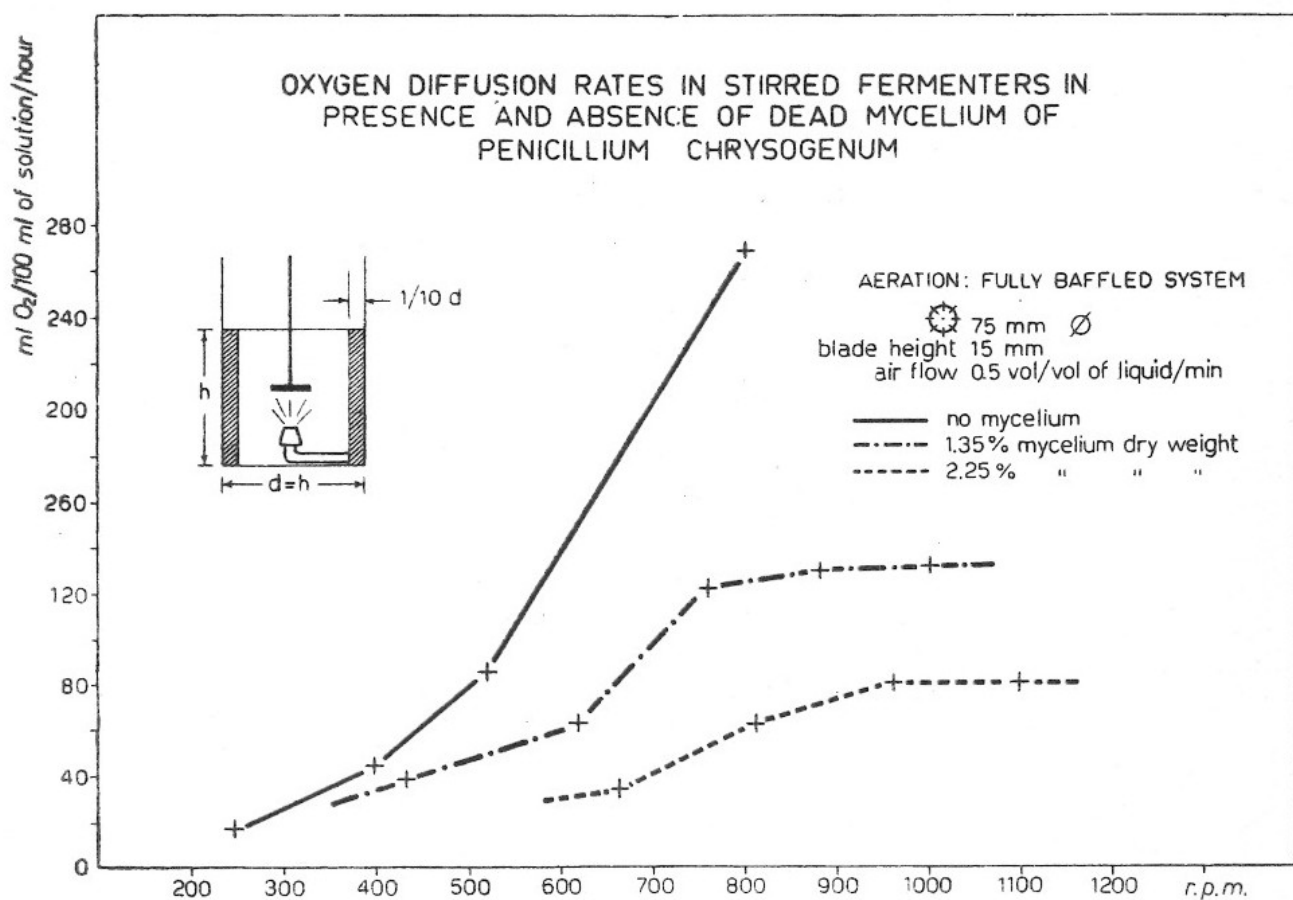


Fig. 15. - Oxygen diffusion rates in stirred fermenters in presence and absence of dead mycelium of *Penicillium chrysogenum*. Vortex system of agitation.

compare the efficiencies of the sulphite and the amperometric methods for the determination of aeration rates, measurements of oxygen diffusion rate were carried out under identical conditions by the two methods. As can be seen from fig. 12 very good agreement between the sulphite method and the amperometric method was obtained.

Addition of mycelium to the liquid decreased the aeration rate, the reduction being the greater the more mycelium was added. The effect was more pronounced than that obtained with the vortex system of aeration. It was interesting to note that the increase of the propeller speed above a certain value, depending on the amount of

mycelium present, did not lead to higher aeration rates. The power above this critical value is not utilized for increasing the water-air turbulence and thus enhancing aeration, but dispersed as heat.

AERATION MEASUREMENTS IN FERMENTERS OF 50, 150 AND 200 l CAPACITY.

Some aeration measurements using both the vortex system of aeration and the baffled system with an air sparger were carried out in pilot plant fermenters of the type described in a previous communication. (PALADINO, UGOLINI and CHAIN, 1954).

The sulphite method was used for these measurements, the results of which are presented in figs. 16 and 17.

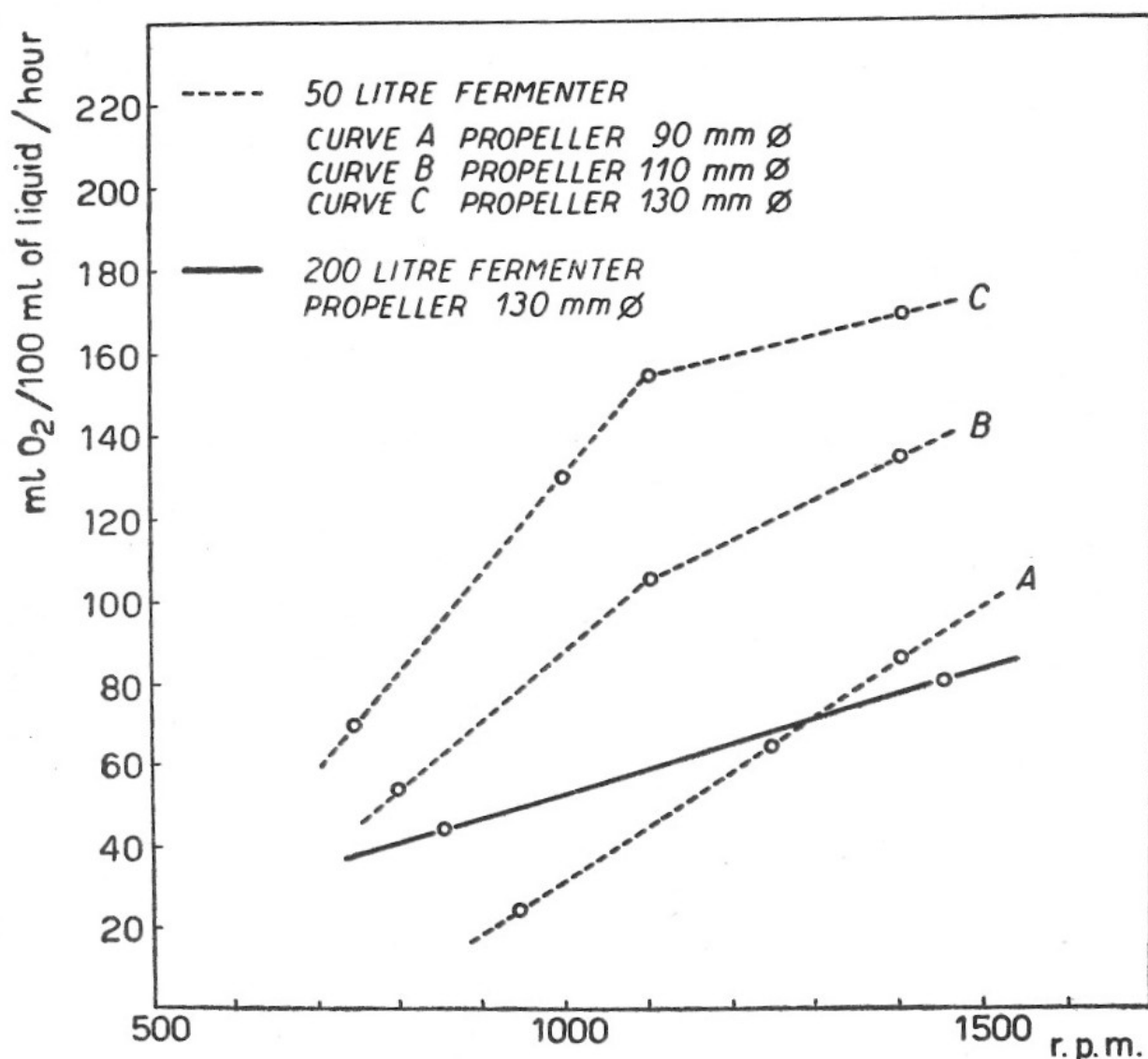


Fig. 16. - Oxygen diffusion rates in 50 and 200 litre fermenters. Vortex system of agitation.

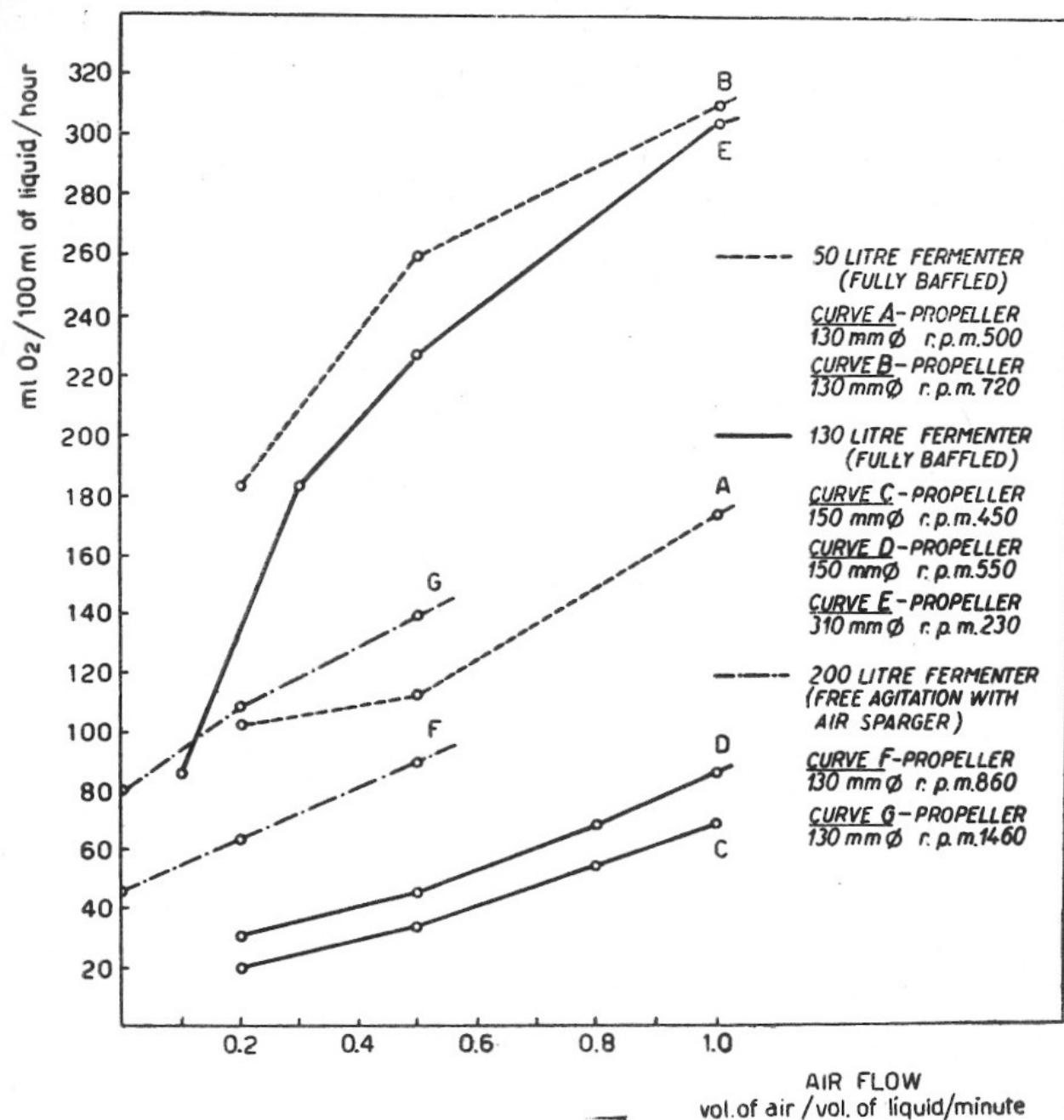


Fig. 17. - Oxygen diffusion rates in 50, 130 and 200 litre fermenters. Free agitation with air sparger and fully baffled agitation.

AERATION IN MECHANICAL STIRRED AQUEOUS SOLUTIONS CONTAINED IN A FERMENTER OF 3.000 l CAPACITY.

Oxygen diffusion rates in 1% NaCl solutions were studied with three methods of air dispersion; a) the vortex system, b) agitation in the absence of baffle plates and c) agitation in presence of baffle plates. In the vortex system the air was passed over the surface of the liquid through tubes in the fermenter head; it was sucked into the liquid by the vortex created by the rotating propeller and dispersed by the propeller.

blades. For the other two aeration systems the air was introduced by a sparger placed at the bottom of the fermenter below the propeller. Stainless steel propellers of the disk turbine type with eight blades (for illustration see fig. 18) of two diameter sizes (300 mm and 360 mm) were used for the agitation. The propellers were made to rotate at about half the liquid height. Four metal plates (width $1/10$ the diameter of the fermenter) placed equidistantly in the liquid acted as baffles.

Effect of position of air sparger in relation to propeller blades.

Two types of spargers, both located at the bottom of the fermenter, were used: 1) a one hole sparger tube (diam. 40 mm) placed centrally under the propeller; 2) a ring sparger with 90 holes (diam. of each hole 3 mm) of a diameter approaching the diameter of the fermenter, i.e. placed at the periphery of the fermenter as far as possible away from the propeller.

The centrally placed one hole sparger was found to be much more effective than the peripherally located 90 hole ring sparger, particularly with high aeration rates (Table 7).

TYPE OF SPARGER *	AIR FLOW vol. of air/vol. of liquid/min oxygen diffusion rate : ml O ₂ /100 ml/hour			
	0.1	0.3	0.5	1.0
RING SPARGER WITH 90 HOLES (3 mm ϕ) ABOUT FOUR TIMES THE DIAMETER OF THE PROPELLER	42	54	80	—
ONE HOLE SPARGER TUBE (40 mm ϕ) PLACED CENTRALLY UNDER THE PROPELLER	60	95	112	163

Tab. 7. - Effect of position of air sparger on aeration efficiency.

* Propeller diameter 360 mm; propeller speed 380 r.p.m.; volume of liquid (1.0% NaCl solution) 2,250 l.

This confirms similar observations made in a 10 l fermenter (CHAIN *et al.*, 1952). The reason for the lower efficiency of the peripherally located ring sparger is that most of the air leaving the holes escapes along the walls of the fermenter without coming into the sphere of the dispersing action of the propeller blades.

The curves in fig. 18. show the relationship between the total power requirement (i.e. power for agitation plus power for air compressor) and the oxygen diffusion rate in various systems of agitation. To compare the aeration efficiency of various systems of agitation it was found useful to introduce the « efficiency ratio » E_r which is defined as follows:

$$E_r = \frac{1 \text{ O}_2 \text{ diffused litre of liquid/hr}}{\text{watt litre of liquid}} = \frac{1 \text{ O}_2 \text{ diffused}}{\text{watt hour consumed}}$$

Table 11 contains the values of E_r for various aeration systems.

a) *Vortex system.*

The curve marked —.—.— on the graph 18 relates to the vortex system of agitation. Two propellers (360 and 300 mm) were used and air blown over the surface of the liquid at a rate of 0.1 vol. air/vol.liq./min. The values of the oxygen diffusion rates were low, particularly with the lower stirring speeds. For this system the values for the efficiency ratio ranged between 0.06 for the lower agitation speed of 350 r.p.m. and a propeller diameter of 360 mm, to 0.15 for the higher agitation speed of 720 r.p.m. and propeller diameter of 300 mm.

b) *Free agitation with air sparger.*

The curves D, E and F marked ————— on the graph 18 relate to the free agitation system, in which the air enters the solution through a sparger and is dispersed by the rotating propeller blades in the absence of baffle plates. Two propellers (diameter 360 and 300 mm) and various rates of air flow (from 0.1 to 1.0 vol. air/vol.liq./min.) were used, the air being introduced through a one hole sparger, as described above. With a propeller of 360 mm diameter (corresponding to about 1/4 of the fermenter diameter) rotating at 380 r.p.m. good efficiency ratios of about 0.45 were obtained with air flows between 0.3 and 1.0 vol. air/vol.liq./min. (curve F in the graph 18). Under these conditions it is not possible to increase the oxygen diffusion rate by introducing more air than 1.0 vol. air/vol.liq./min. Most of the power requirement is

due to the air compressor and only 0.75 watts/litre is absorbed by the agitation (table 8). If the air flow were further increased beyond the value of 1.0 vol. air/vol.liq./min., the propeller would revolve almost

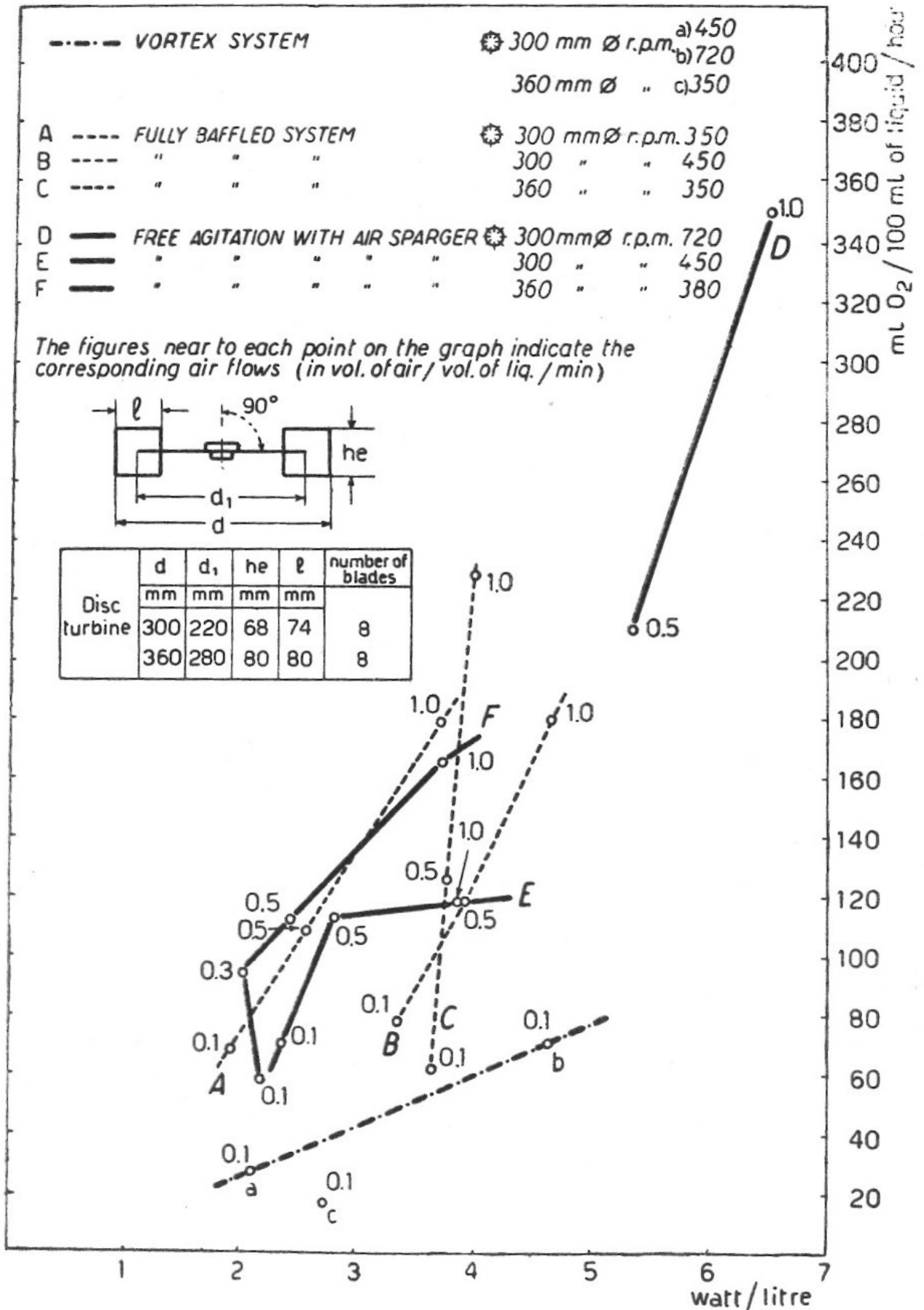


Fig. 18. - Oxygen diffusion rates and power requirements in 3,000 litre fermenter.

completely in air alone so that its dispersing capability would be negligible.

Good efficiency ratios were obtained with a propeller of 300 mm diameter ($1/5$ of the tank diameter) rotating at the high speed of 720 r.p.m. E_r reaches a value of 0.50 for an air flow rate of 1.0 vol. air/vol.liq./min. and falls to 0.40 when the air flow is reduced by half (curve D in graph 18). With the lower speed of 450 r.p.m. and the same propeller the best value for E_r (0.40) was found with 0.5 vol. air/vol.liq./min.

For 1.0 vol. air/vol.liq./min. the curve flattens and the efficiency ratio decreases to 0.30 (curve E in the graph 18). In this case the amount of air introduced exceeded the diffusing capacity of the propeller.

c) Fully baffled system.

The curves marked — — — on the graph 18 relate to the fully baffled system of agitation.

With a propeller of 300 mm (about $1/5$ the diameter of the fermenter) rotating at 450 r.p.m. the efficiency ratio lies between 0.23 and 0.38 with rates of air flow between 0.1 and 1.0 vol. air/vol.liq./min. (curve C, fig. 18). With the same propeller and the lower agitation speed of 350 r.p.m., for the same amount of air introduced (from 0.1 to 1.0 vol. air/vol.liq./min.) the E_r is higher by about 25% than the corresponding values at the speed of 450 r.p.m. It lies between 0.36 and 0.47 (curve B, fig. 18).

With the larger propeller used, of diameter 360 mm, (corresponding to about $1/4$ the diameter of the fermenter) rotating at 350 r.p.m. the efficiency ratio is very low for low rates of air flow (0.1 vol. air/vol. liq./min.; $E_r = 0.16$); with larger volumes of air E_r increases to 0.32 for 0.5 and 0.54 for 1.0 vol. air/vol.liq./min. (curve A fig. 18). In this case the amount of air flow is the limiting factor for the oxygen diffusion rate, because the volume of air supplied is much less than that which the propeller is able to diffuse. This effect may be considered to be the reverse of that shown in curve E of figure 18 and previously discussed. In the figure 18 it is seen that within the limits of efficiency of the various systems, the oxygen diffusion rates are about the same for the same amount of air introduced. This means that, when the power supply is no problem, suitable oxygen diffusion rates can be obtained by the introduction of adequate amounts of air.

From the graph. 18 it may also be observed that in none of the systems investigated did the efficiency values differ by as much as a factor of 5.

With aeration systems utilizing an air sparger all the plots of diffusion rates against power absorbed lie on a rather narrow band; for a given oxygen diffusion rate the efficiency E_r does not change by a factor larger than 2. Hence the question of power absorption is not the most important factor determining the choice of any particular aeration system, but other criteria, such as the biological effect and mechanical factors such as simplicity of construction and running efficiency, will also have to be taken into consideration.

Effect of air introduction on power absorption.

The introduction of air into agitated liquids decreases their specific gravity and viscosity and therefore the power required for agitation. This effect has already been observed by FOUST, MACK and RUSHTON (1944) and COOPER, FERNSTROM and MILLER (1944).

In table 8 the values of the power absorption in watts per litre,

TYPE OF AGITATION	DIAMETER OF THE PROPELLER mm	r. p. m.	Net Power requirement for agitation watt/liter *			
			NO AIR	0.1 vol.air / vol. liq./ min	0.5 vol.air/vol. liq./ min	1.0 vol.air/ vol. liq./ min
FREE	360	380	2.53	1.91	0.95	0.75
	300	450	1.78	1.51	1.32	0.98
	300	720	4.34	—	3.95	3.60
FULLY BAFFLED	360	350	4.07	3.39	2.30	1.14
	300	350	2.05	1.63	1.12	0.77
	300	450	4.38	3.14	2.53	1.67

Tab. 8. - Effect of air flow on power requirement for agitation in 3,000 litre fermenter.

* Not including power requirements for the air compressor.



corrected for the power absorbed by the transmission system and the stuffing box for the agitation alone of 2,250 litres of water contained in the 3,000 l fermenter, are reported for the three aeration system studied, i.e. the vortex system, the system of free agitation and the fully baffled system, in the absence of air and with different rates of air flow.

The reduction of power absorption was particularly marked in the fully baffled system under all conditions tested, amounting to 60-70%. In the absence of baffle plates the reduction of the power absorption was most pronounced with the lowest speeds of agitation and became smaller with increasing propeller speed.

A few measurements (executed by prof. S. PALADINO to whom the authors wish to express their thanks) were made on a fully baffled fermenter containing 50,000 l of culture fluid.

Three eight bladed propellers of the curved paddle type were mounted on the propeller shaft. Rotating at 90 r.p.m. their power absorption was 1.10 watts per litre; in the absence of air; this was reduced to 0.90 watts per litre with an air flow of 0.5 vol. air/vol.liq./min. and to 0.70 watts per litre with an air flow of 1.0 vol. air/vol.liq./min. corresponding to a power reduction of about 20% and 28% respectively. This reduction is less pronounced than in the 3,000 l fermenter. This

r. p. m. *	volume of air/volume of liquid/min	OXYGEN DIFFUSION RATE ml/100 ml/ hour		
		no mycelium	1.2 % dead myc.	2.1 % dead myc.
450	0.5	112	67	45
	1.0	117	67	50
720	0.5	209	85	70
	1.0	~ 350	140	110

Tab. 9. - Reduction of aeration rates in 3,000 litre fermenter in presence of different amounts of dead mycelium of *Penicillium chrysogenum*.

* Free agitation with air sparger. Diameter of the propeller 300 mm.

is most probably caused by the fact that in the latter case one single propeller was present, while in the larger fermenter three were mounted on the shaft. The air flow passing through one single sparger mounted at the bottom of the fermenter affected significantly only the power absorption of the propeller with which the air came first into contact, i.e. the lowest of the three.

Effect of presence of mycelium on power absorption.

The presence of 2% of heat killed mycelium increased the power required for agitation by about 30% under all conditions tested.

*Effect of mycelium of *Penicillium chrysogenum* on aeration rates.*

In presence of dead mycelium of *Penicillium chrysogenum* the oxygen diffusion rates were reduced by varying degrees, depending on the amount of mycelium. In the presence of about 2% of mycelium this reduction amounted to 50-70%, (table 9).

SYSTEM OF AGITATION	AIR FLOW vol./vol. of liq./min	OXYGEN DIFFUSION RATES ml O ₂ /100 ml of liquid / hour	
		amperometric method	sulphite method
FULLY BAFFLED *	0.5	125	100
	1.0	200	225
FREE * *	0.25	88	—
	0.5	116	—
	1.0	165	—

Tab. 10. - Oxygen diffusion rates in 12,000 litre fermenter.

Diameter of the fermenter 2,600 mm. Propeller speed 160 r.p.m.

* Two propellers diameter 600 mm placed respectively at 1/3 and 2/3 of the height of the liquid.

** One propeller at half the liquid height; diameter 800 mm.

AERATION MEASUREMENTS IN A FERMENTER OF 12,000 l CAPACITY.

A few measurements of aeration rates were carried out in a fully baffled fermenter of 12,000 l capacity, containing 10,000 l of 1% NaCl solution. Both the sulphite and the amperometric method (with the vibrating Pt electrode, as for 3,000 l fermenter) were used. The results are presented in table 10. As in the case of the aeration measurement in the fermenter of smaller size, the agreement between the two methods was fairly satisfactory. The values for the power consumption for aeration and agitation are given in table 11.

Table 10 contains also measurements of aeration rates with the free system of aeration.

COMPARISON OF AERATION EFFICIENCY OF DIFFERENT AERATION SYSTEMS IN REACTORS OF DIFFERENT SIZE.

Table 11, contains values of oxygen diffusion rates and E_r for different aeration systems in fermenters of different sizes.

As can be expected, in the vortex system, with propellers of the eight bladed disk-turbine type used throughout this work, and with the peripheral speed of the propeller blades maintained at an approximately constant value, the aeration efficiency decreases with increasing fermenter volumes. E_r decreases from 0.60 to 0.06 when the fermenter volume is increased from 5 to 3,000 l. The vortex system of aeration is therefore most economical for fermenters of smaller capacities. In fact, in the author's opinion, its mechanical simplicity and its advantages with regard to foam control make it the system of choice for laboratory and pilot fermenters.

With the fully baffled system under conditions where the rate of air flow and the propeller dimensions are so adjusted in relation to each other that the air dispersing capacity of the propeller is fully utilized, the aeration efficiency does not change very much with increasing fermenter volume within the limits investigated, i.e. from 5 l up to 12,000 l capacity, the E_r lying in most cases between 0.35 and 0.55. For fermenters up to 12,000 l propeller dimensions of $1/5$ to $1/2$ the diameter of the fermenter are suitable for efficient air dispersion with rates of air flow from $1/5$ up to one volume of air per volume of liquid per minute.

System of Agitation	Vol. of Liquid litres	Propell. Diamet. mm	r.p.m.	Air flow vol. air / vol liq. / min	Oxygen Diff. Rate ml/100ml/hour	Power Absorption			Er
						for agitator	for air	total watt/litre	
Vortex	5	90	725	0.1 *	115	1.56	0.3	1.86	0.62
	50	90	1320	0.1 *	75	2.34	0.3	2.64	0.28
	200	150	1470	0.1 *	80	1.34	0.3	1.64	0.49
	2250	200	1470	0.1 *	81	9.05	0.3	9.35	0.09
		300	720	0.1 *	70	4.34	0.3	4.64	0.15
		300	450	0.1 *	27	1.80	0.3	2.10	0.13
		360	350	0.1 *	17	2.52	0.3	2.82	0.06
Fully baffled	5	75	650	0.5	175	3.30	1.5	4.80	0.39
	50	130	720	0.2	185	4.90	0.6	5.50	0.34
				0.5	260	4.30	1.5	5.80	0.45
				1.0	310	3.40	3.0	6.40	0.49
	130	300	230	0.3	184	4.20	0.9	5.10	0.36
				0.5	223	4.00	1.5	5.50	0.40
				1.0	307	3.32	3.0	6.30	0.49
		150	450	0.3	23	1.07	0.9	1.97	0.11
				0.5	36	1.05	1.5	2.54	0.14
				1.0	70	1.00	3.0	4.00	0.17
		150	550	0.3	35	2.75	0.9	3.65	0.10
				0.5	47	2.70	1.5	4.20	0.11
				1.0	85	2.40	3.0	5.40	0.16
	2250	300	350	0.1	69	1.63	0.3	1.93	0.35
				0.5	108	1.12	1.5	2.62	0.41
				1.0	178	0.77	3.0	3.77	0.47
		300	450	0.1	77	3.15	0.3	3.34	0.23
				0.5	117	2.53	1.5	4.03	0.29
				1.0	178	1.67	3.0	4.67	0.38
		360	350	0.1	60	3.39	0.3	3.69	0.16
				0.5	124	2.30	1.5	3.80	0.32
				1.0	225	1.14	3.0	4.14	0.54
	10000	600	160	0.5	125	2.10	1.5	3.60	0.33
				1.0	200	1.50	3.0	4.50	0.44
Free with air sparger	2250	300	450	0.3	70	1.50	0.9	2.40	0.29
				0.5	112	1.32	1.5	2.82	0.39
				1.0	117	0.97	3.0	3.97	0.29
		300	720	0.5	209	3.90	1.5	5.40	0.38
				1.0	350	3.60	3.0	6.60	0.53
		360	380	0.1	58	1.88	0.3	2.28	0.26
				0.3	95	1.15	0.9	2.05	0.46
				0.5	110	1.00	1.5	2.50	0.44
				1.0	163	0.75	3.0	3.75	0.43
	11000	800	160	0.25	88	1.55	0.75	2.30	0.38
				0.5	115	1.30	1.5	2.80	0.41
				1.0	165	1.00	3.0	4.00	0.41

Tab. 11. - Comparison of the efficiency of various systems of agitation in fermenters of different size.

* Air blown on the surface of the liquid.

The formula proposed by Rushton for the calculation of the power absorption of rotary propellers acting in a fully baffled reactor in a liquid having a Reynolds number $> 3 \times 10^3$ is

$$P = \Phi \rho N^3 D^5 \times 10^{-10} (*)$$

The authors have checked the applicability of this formula for conditions in one of their agitation systems in which the 3,000 l reactor was employed. The characteristics of this system were:

Total capacity of reactor = 3,000 l

Volume of liquid agitated = 2,250 l of water

$D = 30$ cm

$\rho = 1$ g/cm³

$N = 6$ revolutions per second

$\mu = 0.01$ poise

The Reynolds number, R_n , for this system, determined by the formula

$$R_n = \frac{D^2 N \rho}{\mu}$$

was found to be 5.4×10^5 , hence the Rushton formula was applicable. The value for Φ was taken from the data of RUSHTON (1950) as 6.9.

From these figures the power consumption for a six blade propeller of the above dimensions is calculated as 3.54 Kws and for an eight bladed propeller $3.54 \times 5/4 = 4.45$ Kws. The experimentally found value was 4.62 Kws, which is in good agreement with the calculated value.

For the calculation of the oxygen diffusion rate from the power absorption values it must be borne in mind that the power absorption for the agitation decreases in the presence of an air current passing through the liquid. For a rate of air flow of 0.5 vol. air/vol.liq./min.,

(*) *Explanation of symbols.*

P = power absorption, in Kws.

Φ = a constant characteristic for the system used and depending on Reynolds number R_n .

ρ = density, in gms/cm³.

N = number of revolutions per second.

D = diameter of propeller, in cm.

μ = viscosity, in poise.

the decrease is about 40% and for 1.0 vol. air/vol.liq./min., it is about 60% for propellers of the dimension used. The power absorption for agitation alone in the absence of air is $4,620 \text{ watts}/2,500 \text{ l} = 2.05 \text{ watts/l}$. In the presence of an air current of 0.5 vol. air/vol.liq./min., it is reduced to 1.12 watts/l; in the presence of an air current of 1.0 vol. air./vol.liq./min., to 0.77 watts/l.

To these values must be added the power absorption of the air compressor, which is approximately 1.5 watts per litre for an air flow of 0.5 vol. air/vol.liq./min. and 3.0 watts per litre for an air flow of 1.0 vol. air/vol.liq./min. The total power absorption is therefore $1.12 + 1.5 = 2.62 \text{ watts/l}$ and $3.0 + 0.77 = 3.77 \text{ watts/l}$ for the two rates of air flow respectively. Assuming an E_r of 0.40 to 0.50, the oxygen diffusion rate corresponding to the above values of power absorption is between 100 and 130 ml O_2 /100 ml liquid/hr for an air flow of 0.5 vol. air/vol.liq./min. and between 150 and 190 ml O_2 /100 ml liquid/hr for an air flow of 1.0 vol. air/vol.liq./min.

The found values were 108 and 178 ml O_2 /100 ml liquid/hr respectively.

For scaling-up effects with regard to power absorption for agitation and aeration in penicillin fermentations in fermenters of different size see WEGRICH and SHURTER (1953) and CHAIN, PALADINO, UGOLINI, CALLOW, TONOLO, KAUSHAL and CARILLI (1954).

Some figures for power absorption, air flow and oxygen diffusion rates have been reported by KAROW, BARTHOLOMEW and SFAT (1953) on the bases of which E_r values can be calculated. In the following table 12 the figures of KAROW *et al.* have been recalculated in units used by the authors in the present paper. KAROW *et al.* measured the oxygen diffusion rates by the sulphite method in the case of fermenters of 5 and 750 l capacity while for the larger fermenters they extrapolated from the figures of COOPER *et al.* (1944) which had been obtained by sulphite measurements of oxygen diffusion rates in reaction vessels from 3 l to 8,500 l capacity.

The E_r calculated from the figures of KAROW *et al.* are of the same order of magnitude as those found by the authors in the cases where the oxygen diffusion rates were determined experimentally by KAROW *et al.*

In contradistinction, the E_r values derived by extrapolation of the figures of COOPER *et al.*, are much higher than those experimentally found by the present authors. The reason for this, in the opinion of the authors, is that the figures of COOPER *et al.* do not lend them-

Fermenter capacity ℓ	Air flow vol./vol. of liq./min.	Oxygen diffusion rate ml O ₂ /100 ml liq./h.	Power for agitation w/ℓ	Power for air compressor w/ℓ	Total power absorption w/ℓ	Er
5	1.5	27	0.18	4.50	4.68	0.06
	0.22	45	0.71	0.66	1.37	0.33
	0.7	63	0.56	2.10	2.66	0.23
	1.5	54	0.38	4.50	4.88	0.11
	0.22	153	2.25	0.66	2.91	0.52
	0.7	220	1.87	2.10	3.98	0.56
	1.5	170	1.22	4.50	5.72	0.30
750	0.06	45	0.71	0.18	0.89	0.50
	0.12	63	0.70	0.36	1.06	0.59
	0.36	108	0.50	1.10	1.60	0.67
	0.60	117	0.42	1.80	2.22	0.53
37 500	0.17	162	0.45	0.51	0.96	1.69
56 500	0.09	117	0.50	0.27	0.77	1.52
	0.18	153	0.38	0.54	0.92	1.66
	0.09	227	1.00	0.27	1.27	1.79
	1.18	300	0.82	0.54	1.36	2.20

Tab. 12. - Power absorption and oxygen diffusion rates data recalculated from Karow *et al.* (1953).

selves to extrapolation because in their experiments the superficial air velocity (m/hr), but not the volumetric air velocity (m³/hr) is kept constant; if this is done, the quantity of air per unit volume of liquid increases according to square function with increasing fermenter capacity, while the increase of fermenter capacity follows a cubic function. Consequently, the quantity of air passing per unit volume of liquid decreases with increasing fermenter size and may become insufficient for effective dispersion by the propeller blades.

Further more, the oxygen diffusion values of COOPER *et al.* which were used by KAROW *et al.* for extrapolation were obtained with a vaned disk propeller whereas the fermenters used by KAROW *et al.* were equipped with flat turbine propellers.

**EFFECT OF SCALING-UP ON THE RATIO: POWER ABSORBED FOR AGITATION/POWER
ABSORBED FOR AIR COMPRESSION.**

While the aeration efficiency E_r in fully baffled fermenters is largely independent of the fermenter size i.e. the total power absorption for agitation and air compression remains constant irrespective of the fermenter capacity from 5 to 12,000 l, the ratio: power absorbed for agitation/power absorbed for air compression decreases considerably with increasing fermenter size (table 13). That is to say with increas-

Volume of liquid litres	Diameter of the Propeller mm	R. P. M. *	Air flow vol. air / vol. liq. / min	Power Requirement w/ℓ			E_r	Power for agitation / Power for air
				for the air compressor	for agitation	total		
5	75	650	0.5	1.5	3.3	4.8	0.39	2.20
50	130	720	0.5	1.5	4.3	5.8	0.45	2.85
			1.0	3.0	3.4	6.4	0.49	1.14
130	300	230	0.5	1.5	4.0	5.5	0.40	2.65
			1.0	3.0	3.3	6.3	0.49	1.10
2250	360	350	0.5	1.5	2.3	3.8	0.33	1.55
			1.0	3.0	1.1	4.1	0.54	0.40
10 000	600	160	0.5	1.5	2.1	3.6	0.33	1.40
			1.0	3.0	1.5	4.5	0.44	0.50

Tab. 13. - Effect of scaling - up on the ratio power absorbed for agitation/power absorbed for air compression.

* Fully baffled system of agitation.

ing fermenter size the mechanical dispersion of air by the propeller becomes more efficient and the shearing forces per unit volume exerted by the propeller become smaller. Effects observed in scaling-up fermentations from laboratory to industrial scale may have their explana-

tion in this phenomenon in cases where the micro-organisms concerned are sensitive to mechanical damage. (See DION, CARILLI, SERMONTI and CHAIN, 1954).

It has been shown (HIXSON and BAUM, 1942; RUSHTON, COSTIC and EVERETT, 1950; RUSHTON, 1951; RUSHTON, 1952; RUSHTON and OLDSHUE, 1953) that, in contrast to the fully baffled system, scaling-up calculations for the power absorption of agitators from laboratory to industrial scale in nonbaffled systems i.e. in the presence of a vortex, are difficult, if not impossible, so that in such systems the geometrical dimensions and hydrodynamic properties of the agitated fluids do not maintain similitude with increasing volume.

In any case, in the authors' opinion the free system of agitation for aeration in fermenters of industrial size has no significant advantages over the fully baffled system, neither in regard to the total power absorption, nor in regard to the ratio: power absorbed for agitation/ /power absorbed for air compression.

ACKNOWLEDGMENTS.

The authors wish to express their thanks to ing. prof. S. PALADINO and ing. F. UGOLINI for their valuable help in the construction of the propeller blades, stuffing box and bearing unit of the fermenter of 3,000 l capacity and the numerous general discussions, to Dr. O. E. VIRTANEN, Dr. G. MILLER, Dr. A. VIRGILIO who have carried out many sulphite measurements and to Mr. D. S. CALLOW for the Warburg experiments.

Roma — Istituto Superiore di Sanità - International Research Centre for Chemical Microbiology.

REFERENCES

- BAKER E. G. S. and BAUMBERGER J. P. (1940) *J. Cell. & Comp. Physiol.*, 17, 285.
BAUMBERGER J. P. (1938) Abstr. from Kongress Bericht II, XVI Int. Physiol. Congress, Zürich.
BAUMBERGER J. P. (1938) *Am. J. Physiol.*, 123, 10.
BAUMBERGER J. P. (1939) Cold Spring Harbor Symposia Quant. Biol., 7, 195.
BAUMBERGER J. P., LEONG G. F. and BARDWELL K. (1948) *Federation Proc.*, 7, 6.
BARTHOLOMEW W. H., KAROW E. O., SFAT M. R. and WILHELM R. H. (1950) *Ind. Eng. Chem.*, 42, 1801.
BERMAN D. A., SAUNDERS P. R. and WINZLER R. J. (1951) *Anal. Chem.*, 23, 1040.
BRONK A. J. (1946) *American Scientist*, 34, 55.
BROWN A. S. (1934) *J. Am. Chem. Soc.*, 56, 646.

- CHAIN E. B., PALADINO S., CALLOW D. S., UGOLINI F. and VAN DER SLUIS J. (1952) Bull. World Hlth Org., 6, 73.
- CHAIN E. B., PALADINO S., UGOLINI F., CALLOW D. S., TONOLO A., KAUSHAL R., with a note by CARILLI A. (1954) To be published.
- COOPER C. M., FERNSTROM G. A. and MILLER S. A. (1944) Ind. Eng. Chem., 36, 504.
- DALE R. F., AMSZ J. Jr., PING SHU, PEPPLER H. J. and RUDERT F. J. (1953) J. Appl. Microbiol. 1, 68.
- DION W., CARILLI A., SERMONTI G. and CHAIN E. B. (1954) Rend. Ist. Sup. Sanità (1954) 17, English Ed. p. 187.
- DIRSCHLER W. and BERGMAYER H. U. (1950) Biochem. Z., 321, 68.
- DU BUY H. G. and OLSON R. A. (1940) Am. J. of Botany, 27, 401.
- FOUST M. C., MACK D. E. and RUSHTON J. H. (1944) Ind. Eng. Chem., 36, 517.
- GIGUERE P. A. and LAUZIER L. (1943) Can. J. Research, 23B, 76.
- HARRIS E. D. (1951) Analyst, 76, 647.
- HARRIS E. D. and LINDSEY A. J. (1948) Nature, 162, 413.
- HIXSON A. W. and BAUM S. J. (1942) Ind. Eng. Chem. 34, 120.
- HIXSON A. W. and GADEN E. L. (1950) Ind. Eng. Chem. 42, 1792.
- JARVIS G. and JOHNSON M. J. (1947) J. Am. Chem. Soc., 69, 3010.
- KAROW E. O., BARTHOLOMEW W. H. and SFAT M. R. (1953) J. Agric. & Food. Chem., 1, 302.
- KOLTHOFF J. M. and LAITINEN H. A. (1950) Science, 92, 152.
- KOLTHOFF J. M. and LINGANE J. J. - « Polarography », 2nd Ed., Vol. II (1952) Interscience Publishers, New York.
- KREBS H. A. (1953) Biochem. J., 29, 1620.
- LAITINEN H. A. and KOLTHOFF J. M. (1941) J. Phys. Chem., 45, 1079.
- MADER W. J. and FREDIANI H. A. (1951) J. Am. Pharm. Ass., 49, 24.
- MARSH G. A. (1951) Anal. Chem., 23, 1427.
- OLSON R. A., BRACKETT F. S. and CHRICKARD R. G. (1949) J. Gen. Physiol. 32, 681.
- PALADINO S. (1954) Rend. Ist. Sup. Sanità (1954) 17, English Ed. p. 145.
- PALADINO S., UGOLINI F. and CHAIN E. B. (1954) Rend. Ist. Sup. Sanità (1954) 17, English Ed. p. 87.
- PETERING H. G., DUGGAR B. M. and DANIELS F. J. (1939) J. Am. Chem. Soc., 61, 3525.
- RUSHTON J. H. (1951) Chem. Eng. Prog., 47, 485.
- RUSHTON J. H. (1952) Chem. Eng. Prog., 48, 33 and 95.
- RUSHTON J. H. COSTIC E. W. and EVERETT H. J. (1950) Chem. Eng. Prog., 46, 395 and 463.
- RUSHTON J. H. and OLDSHUE J. Y. (1953) Chem. Eng. Prog., 49, 161.
- SEIDELL A. « Solubilities of Inorganic and Metal-Organic Compounds », 3rd Ed., Vol. I (1940) D. Van Nostrand Co. Inc., New York.
- SKERMAN V. B. D. and MILLIS E. B. (1949) Australian J. Expl. Biol. Med. Sci., 27, 183.
- WARBURG O. and KUBOWITZ F. (1929) Biochem. Z., 214, 5.
- WEGRICH O. G. and SHURTER R. A. Jr. (1953) Ind. Eng. Chem., 45, 1153.
- WINZLER R. J. (1940) J. Cell. and Comp. Physiol., 17, 263.
- WISE W. S. (1951) J. gen. Microbiol., 1, 167.
- ZIMMERMANN J. F. and BERRY L. J. (1949) Biochim. et Biophys. Act., 3, 615.
-