

OXYGEN TRANSFER AND AGITATION

In Submerged Fermentations

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A major difficulty in production of antibiotics is the prediction of results in factory scale equipment and the scale-up of results found in laboratory-size fermentors to factory scale fermentors. The present work was undertaken in an endeavor to find a workable method for scale-up. It has been found that the primary scale-up factor between geometrically similar, fully baffled 5-liter, 200-gallon, 10,000-gallon, and 15,000-gallon aerobic fermentors is the rate at which oxygen is supplied from the gas to the liquid phase. The factor is defined as $k_{d_w}P$, where k_{d_w} is the specific oxygen absorption coefficient as determined in a copper-catalyzed sulfite-water system, gram moles of oxygen per milliliter of solution per hour per atmosphere, and P is the average pressure in the fermentor, atmospheres. The primary locus of oxygen transfer has been found to be at the sparged impeller, and a design method is outlined, employing the data of Cooper, Fernstrom, and Miller, for translating data obtained in laboratory fermentors to plant scale fermentors.

FERMENTATION STUDIES in laboratory submerged fermentors were undertaken to provide a background for engineering design and biological understanding and control. Experimental studies and theoretical analyses of oxygen absorption and agitation in aerobic fermentations using strains of *Penicillium chrysogenum* and *Streptomyces griseus* (2, 3), and details of laboratory fermentor construction and operation (1) have been discussed. The present paper shows how the established principles may be used to compare and translate fermentation information from one scale of operation to another. Comparisons are made of streptomycin and penicillin production in 5-liter laboratory fermentors, 200-gallon pilot plant fermentors, and 10,000- and 15,000-gallon factory fermentors.

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Fermentors

Within any fermentation the following variables were maintained constant: type, amount, and age of inoculum, composition of medium, temperature, total pressure and oxygen partial pressure, mechanical agitation, type of sparger, and rate of air flow. Dependent variables measured at time intervals were: antibiotic activity, mycelial weight, residual sugar content, pH, and dissolved and saturated oxygen concentrations. Among the latter variables, antibiotic activity is pertinent to the present paper. Although fermentation characteristics were followed throughout the normal time cycle, harvest times were selected after antibiotic yields substantially ceased to increase.

Pilot plant fermentors were of 200-gallon and factory fermentors of 10,000- and 15,000-gallon total capacity. Both types of units were fully baffled and provided with mechanical agitation.

Generally, the larger units were similar in geometrical proportions to the laboratory fermentors as regards liquid depth to tank diameter, baffles, etc. The agitators were of a radial flow type but unlike in specific detail. The linear velocity of the air through the sparger orifices was of the same order of magnitude for all scales at their normal operating conditions. Figure 1 illustrates schematically the design of all fermentors employed in this study.

The biological systems were those used industrially and they were the same in the comparative factory, pilot plant, and laboratory fermentor studies. Fermentation cycle times were the same in pilot plant and factory as for the laboratory units.

Results

Information obtained in the 5-liter laboratory fermentors (3) has led to the following conclusions.

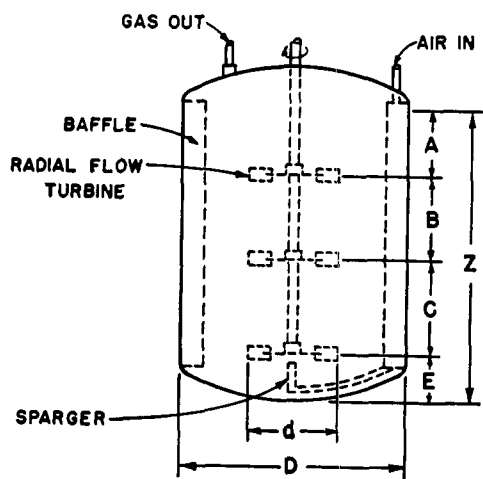


Figure 1. Schematic fermentor

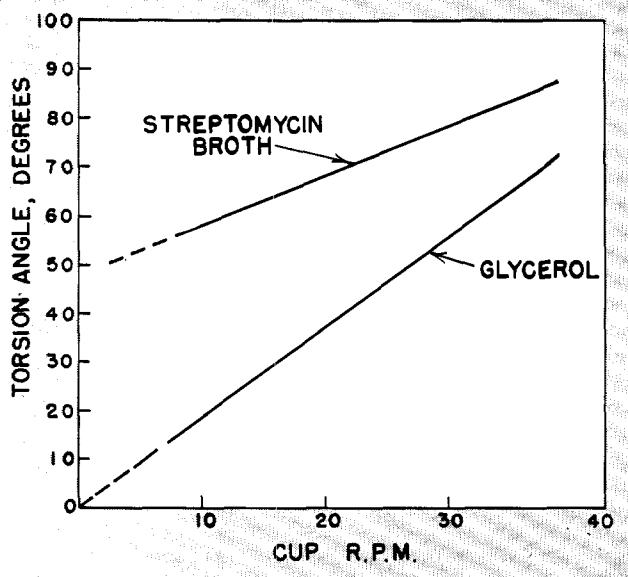
Fermentor Characteristics

Z/D: 1.2 to 1.6 B/d: 1.0 to 1.5
 d/D: 0.34 to 0.5 C/d: 1.0 to 1.2
 A/d: 1.0 to 1.2 E/d: 0.5 to 1.3
 Superficial air velocity: 6 to 175 ft. per hour
 Mechanical power input to liquid: 0.0009 to 0.015 hp. per gal.

Cellular respiration, hence antibiotic production, depends upon the maintenance of an oxygen concentration at the cell wall such that the respiration rate is independent of the oxygen concentration—that is, a zero-order rate. At times oxygen becomes difficultly available to the cells as these become effectively hidden from the oxygen source within aggregates or clumps of cells. That such clumps exist in mycelial-type broths is shown by their non-Newtonian behavior. Figure 2, a plot of torque *vs.* revolutions per minute for streptomycin broth, illustrates the characteristic behavior of this type of broth.

The maintenance of a critical oxygen concentration at the cells depends upon a balance of two rates: the rate of oxygen absorption by the cell and the rate of oxygen transfer to the region of

Figure 2. Non-Newtonian behavior of mycelial-type broth
 MacMichael viscometer, 100-ml. sample, No. 26 wire



the cell walls. Antibiotic yield thus ultimately depends upon the rate of oxygen transfer to the cells.

Oxygen mass-transfer rates (and thus yield) depend upon scale and intensity of turbulence, which are a function of power absorbed from mechanical impellers and the flowing air.

Oxygen transfer and agitation rates are interdependent variables.

The oxygen transfer rate is expressed by means of an absorption coefficient, k_a , defined through the following diffusion rate equation:

$$r_a = k_a H(C - C^*) = k_a' H(C^* - C_c) \quad (1)$$

where

r_a = differential rate of oxygen absorption, gram moles of O_2 /(ml. of solution)(hour)

k_a = diffusion rate coefficient over concentration gradient ($C - C^*$), gram moles of O_2 /(ml. of solution)(hour)(atm.)

k_a' = diffusion rate coefficient over concentration gradient ($C^* - C_c$), gram moles of O_2 /(ml. of solution)(hour)(atm.)

H = Henry's law constant, (atm.)(ml. of solution)/gram moles of O_2

C = aqueous oxygen concentration in equilibrium with oxygen in gas contacting the liquid, gram moles of O_2 /ml. of solution

C^* = oxygen concentration in solution at any time, gram moles of O_2 /ml. of solution

C_c = oxygen concentration at the cell surface, gram moles of O_2 /ml. of solution

Equation 1 may be rewritten to express the oxygen absorption capacity of a fermenting system:

$$r_a = k_a P(y - y^*) = k_a' P(y^* - y_c) \quad (2)$$

where

P = total pressure, atmospheres

y = oxygen mole fraction in inlet gas stream

y^* = oxygen mole fraction in gas in equilibrium with oxygen concentration in main body of solution, C^*

y_c = oxygen mole fraction in gas in equilibrium with oxygen in solution at the cell surface

It will be recognized that k_a together with $(y - y^*)$ represents the oxygen transfer process from bubble surfaces to the main body of solution and k_a' with $(y^* - y_c)$ represents oxygen transfer from solution sites essentially near bubbles to cell surfaces in regions within denser cell clumps. In conventional tanks having pipe spargers and radial flow turbines operating under baffled conditions, the combination of mechanical power input and air flow rates to give a satisfactory k_a value normally lends also to satisfactory turbulence around the cell clumps—i.e., large k_a' .

The above ideas may be expressed analytically in the following equation, which is Equation 2 with y^* eliminated between the two expressions there represented:

$$r_a = \frac{y - y_c}{\frac{1}{k_a P} + \frac{1}{k_a' P}} \quad (3)$$

When agitation is adequate, $k_a' P \gg k_a P$ and we get

$$r_a = k_a P(y - y_c) \quad (4)$$

Thus, in so far as oxygen diffusion rate processes are concerned, the oxygen concentration, y_c , at the cell depend upon the specific rate constant, $k_a P$

Figure 3. k_{a_w} vs. shaft horsepower for 5-liter fermentor
 Z/D = 1.3, impeller diameter = D/2, fully baffled, 27° C., atmospheric pressure

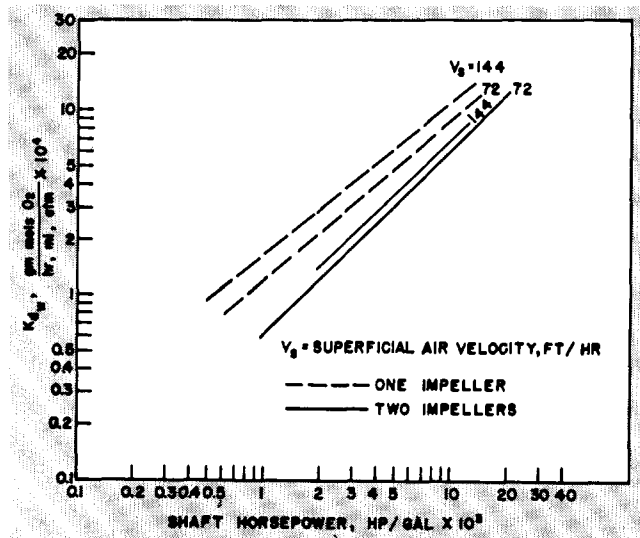
One-impeller system. One 4-bladed radial type turbine located 1.5 inches from bottom of tank

Two-impeller system. Two 4-bladed radial type turbines located 1.5 and 4.5 inches from bottom of tank

Z. Liquid depth

D. Tank diameter

V_s . Superficial air velocity at sparger, feet per hour



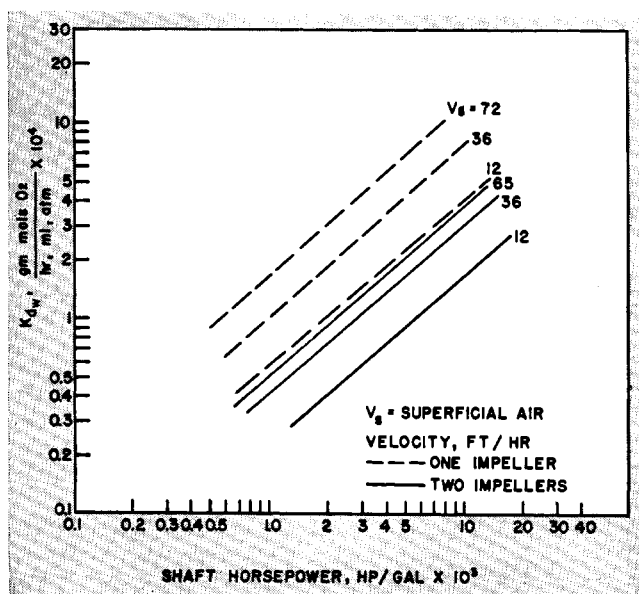


Figure 4. k_{d_w} vs. shaft horsepower for 30-liter fermentor $Z/D = 1.15$, impeller diameter = $D/2$, fully baffled, 27°C ., atmospheric pressure

One-impeller system. One 4-bladed radical type turbine located 3 inches from bottom of tank

Two-impeller system. Two 4-bladed radical type turbines located 3 and 9 inches from bottom of tank

Z. Liquid depth

D. Tank diameter

V_s . Superficial air velocity at sparger, feet per hour

and this constant is therefore suggested as a correlating variable for antibiotic production. Growth and antibiotic production are constant above a minimal value of y_e (3). The cell oxygen concentration, y_e , also depends upon the concentration of cells and the specific uptake rate of oxygen by the cells in accord with the following equation:

$$\tau_r = \tau_d = k_r c_m \quad (5)$$

where

τ_r = differential rate of oxygen absorption, gram moles of O_2 /(ml. of solution)(hour)

k_r = specific uptake rate of oxygen by the organism, gram moles of O_2 /(grams of mycelium)(hour)

c_m = concentration of mycelium at any time, grams of mycelium per ml. of solution

As different fermentation batches

were harvested at approximately the same cell concentrations and antibiotic activities in the different scales of experimentation, the contribution of oxygen demand by the cells upon y_e may be considered constant between experiments, and y_e therefore remains dependent primarily upon $k_d P$.

All the above mass-transfer expressions are written for a point condition; hence, in a large fermentor the product $k_d P$ will not be constant for all parts of the tank, but will vary as $P^{1/3}$. This is shown by the following:

For a given volumetric air flow and a given tank, at any point in the liquid,

$$k_d = ka$$

where

k = oxygen absorption coefficient, gram moles of O_2 /sq. cm., (hour) (atm.)

a = interfacial contact area between air and liquid, sq. cm./(ml. of solution)

The bubble size will vary with the pressure according to the relationship, $a \propto P^{-2/3}$ and thus, $k_d P a \propto P^{1/3}$

Since the dependency of $k_d P$ on P is small, it can ordinarily be assumed constant throughout the fermentor.

As k_d values are available only for 5-liter fermentors (2), recourse is made to the specific oxygen-absorption coefficient, k_{d_w} , which is defined as that coefficient obtained from measurements of a copper-catalyzed sodium sulfite-water system [gram-moles of oxygen/(milliliters of solution)(hour)(atmosphere)]. This is the system employed by Cooper, Fernstrom, and Miller (4) in their study of stirred gas-liquid con-

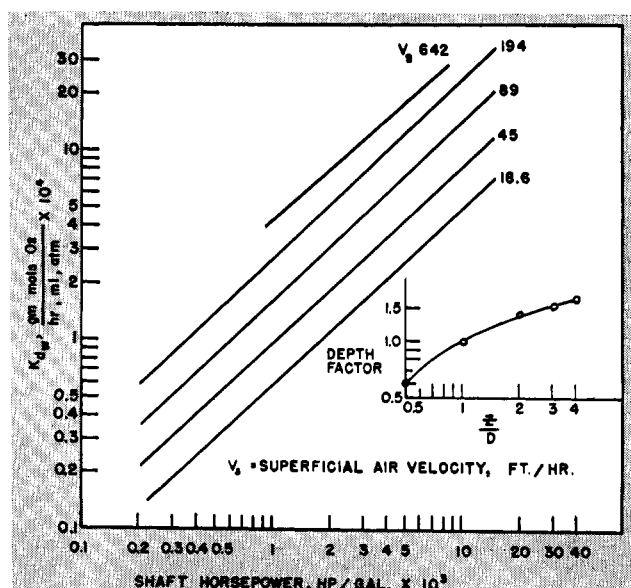


Figure 5. k_{d_w} vs. shaft horsepower replotted from data of Cooper *et al.* (4)

Z. Liquid depth

D. Tank diameter

V_s . Superficial air velocity at sparger, feet per hour

Table I. Streptomycin Productivity as a Function of Oxygen Transfer

Fermentor	No. of Batches	Superficial Air Velocity V_s , Ft./Hr.	Total Shaft Horsepower, Hp./Gal.	$k_{d_w}^a$, G. Moles O_2 / Mi., Atm., Hr. $\times 10^4$	Av. Pressure, P, Atm.	$k_{d_w} P$, G. Moles O_2 / Mi., Hr. $\times 10^4$	Relative Streptomycin Conc.
5-liter (2 impellers)	6	72	0.0009	0.6	1	0.6	0.2
	11	72	0.0019	1.2	1	1.2	1.0
	9	72	0.0062	3.8	1	3.8	1.0
	19	36	0.0092	4.9	1	4.9	0.96
	6	36	0.0028	1.4	1	1.4	0.5
	4	24	0.0030	1.2	1	1.2	0.09
	5	12	0.0036	1.0	1	1.0	0.06
	4	6	0.0120	2.4	1	2.4	0.53
	4	12	0.0114	3.4	1	3.4	0.85
	4	72	0.0150	9.0	1	9.0	0.82
	15,000-gal.	27	174	0.0007	1.0	1.6	1.6
19		174	0.0013	1.0	1.6	1.6	0.99
15		174	0.0041	5.1	1.6	8.1	0.96
16		174	0.0019	3.4	1.6	5.4	0.96
11		114	0.0023	3.4	1.6	5.4	0.94

^a k_{d_w} values for 5-liter fermentors determined experimentally, for 15,000-gallon fermentors calculated from data of Cooper *et al.* (4).

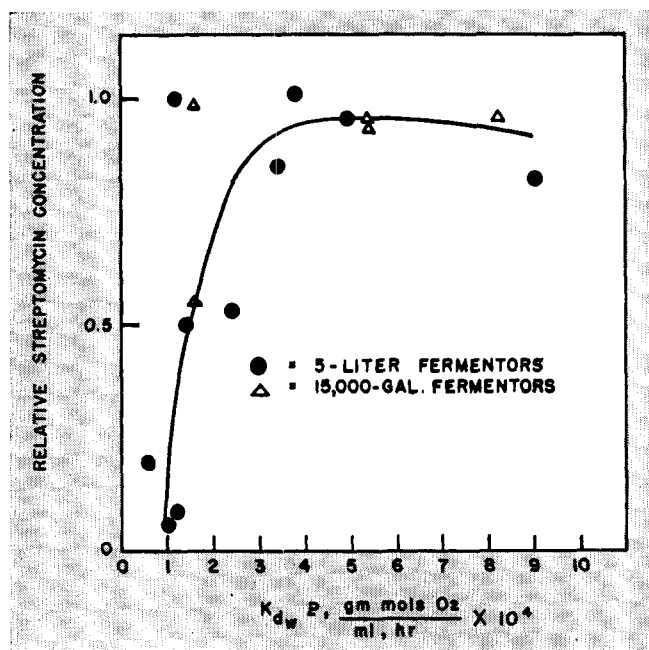


Figure 6. Streptomycin productivity vs. $k_{d_w}P$

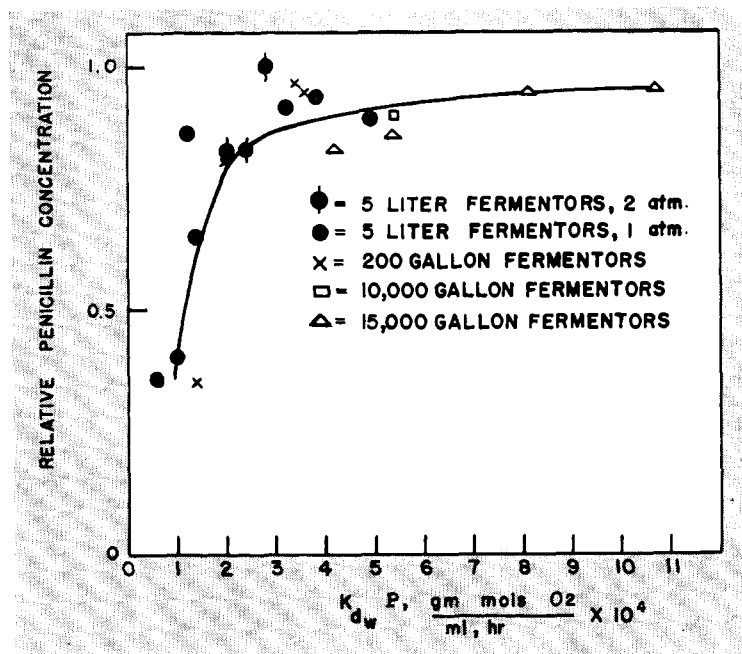


Figure 7. Penicillin productivity vs. $k_{d_w}P$

factors. This coefficient is related to k_d , because both are affected proportionally by the same variables—i.e., air flow and agitation—and for convenience is used in place of k_d .

Cooper, Fernstrom, and Miller have reported k_{d_w} data for several tank sizes and have correlated the oxygen absorption characteristics with shaft horsepower and superficial linear air velocity, V_s . Their data are restricted to a system containing but one impeller. Data obtained in this laboratory in 5- and 30-liter units employing one and two radial type turbines show that, although the addition of the second impeller increases the shaft horsepower, the oxygen ab-

sorption coefficient is not increased proportionally. From this it may be inferred that for the physical arrangement of the impellers employed, the sparged impeller contributes most to the absorption of oxygen. This is illustrated in Figures 3 and 4. Thus, k_{d_w} values for plant scale fermentors were obtained by considering that only the bottom sparged impeller was contributing to gas absorption, and these values were picked from a replot of Cooper, Fernstrom, and Miller data (Figure 5) by using the shaft horsepower contributed by the gassed impeller at the known air velocity. It is recognized that this procedure is not exact, but, in

lieu of other information, it yields usable data and allows for practical design.

In Tables I and II are tabulated antibiotic activities and fermentor oxygen absorption characteristics for the streptomycin and penicillin fermentations. The k_{d_w} values included in these two tables were determined experimentally in the case of the 5-liter and 200-gallon fermentors. In the case of the 10,000- and 15,000-gallon fermentors, the k_{d_w} values were obtained from the data of Cooper, Fernstrom, and Miller. In Figures 6 and 7 are plots of $k_{d_w}P$ vs. antibiotic activity for streptomycin and penicillin, respectively. Considering the

Table II. Penicillin Productivity as a Function of Oxygen Transfer

Fermentor	No. of Batches	Superficial Air Velocity, V_s , Ft./Hr.	Total Shaft Horsepower, Hp./Gal.	$k_{d_w}^a$, G. Moles O_2 / Ml., Atm., Hr. $\times 10^4$	Av. Pressure, P, Atm.	$k_{d_w}P$, G. Moles O_2 / Ml., Hr. $\times 10^4$	Relative Penicillin Concn.
5-liter (2 impellers)	3	72	0.0009	0.6	1	0.6	0.33
	5	12	0.0036	1.0	1	1.0	0.40
	5	36	0.0028	1.4	1	1.4	0.65
	6	72	0.0019	1.2	1	1.2	0.86
	4	12	0.0114	3.4	1	3.4	0.92
	8	36	0.0095	4.9	1	4.9	0.89
	10	72	0.0062	3.8	1	3.8	0.94
	1	12	0.0036	1.0	2	2.0	0.82
	2	36	0.0028	1.4	2	2.8	1.0
	2	72	0.0019	1.2	2	2.4	0.83
200 gal.	1	15	0.0036	1.0	1.4	1.4	0.35
	2	29	0.0034	1.4	1.4	2.0	0.81
	2	87	0.0026	2.4	1.4	3.4	0.98
10,000 gal.	1	142	0.0021	2.6	1.4	3.6	0.95
15,000 gal.	22	152	0.0023	3.6	1.5	5.4	0.89
	1	87	0.0025	2.6	1.6	4.2	0.84
	12	174	0.0019	3.4	1.6	5.4	0.86
	2	87	0.0050	5.1	1.6	8.1	0.95
	22	174	0.0041	6.7	1.6	10.7	0.95

^a k_{d_w} values for 5-liter and 200-gallon fermentors experimentally determined, for 10,000- and 15,000-gallon fermentors calculated from data of Cooper *et al.* (4).

fact that the k_{aw} values for the factory scale tanks are not precise, the correlation for the several scales of operation is excellent. This is in agreement with the recently published data of Wise (6).

Discussion and Conclusions

The data show that the primary scale-up factor for submerged aerobic fermentors of conventional design is the oxygen absorption rate, which is defined as $k_{aw}P$ when air is employed as the source of oxygen. This analysis has been limited to fully baffled vessels provided with agitation, the primary function of which, therefore, is to deliver oxygen from the gas phase to the liquid phase. A secondary function of the agitators is to overcome liquid-mycelia diffusion resistances and to account for satisfactory heat transfer coefficients at the broth-heat transfer surface of the fermentor.

Proper design of large scale units of the type described in this paper requires that a suitable $k_{aw}P$ be experimentally determined through correlation of fermentation yield *vs.* $k_{aw}P$ measured in small scale equipment for any given fer-

mentation, with the proviso that the small scale units be fully baffled and geometrically similar to the contemplated production units (4, 5). From this point the scale-up involves the following steps:

1. Select an operating pressure, thus fixing P .
2. Select an air flow sufficient to supply the peak molar oxygen demand of the broth, but not so high as to cause excessive foaming. A safe starting point would be to set the air flow to correspond to five times the peak molar oxygen demand of the broth. The peak oxygen demand can be determined readily by oxygen-uptake studies as detailed in a previous paper in this series (2).
3. If more than one sparging impeller is employed, multiple sparging is good practice for the most effective use of mixing energy to achieve a given oxygen absorption rate. Then, the tank must be considered as divided into separate units, each provided with one sparged impeller. Thus, the value of V_s for each section will be the volumetric air flow through the sparger of that section divided by the free cross-sectional areas of the section.
4. Determine the shaft horsepower per gallon required from Figure 5. As k_{aw} and V_s have been established, this can be obtained readily. This defines the power

which must be delivered by each sparged impeller at the fixed V_s .

Acknowledgment

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VAPOR CONCENTRATION Control in Closed Spaces

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Maintaining a low but very constant concentration of a given vapor in a space is very difficult in a static system, especially because of adsorption factors. A dynamic method for establishing such a vapor concentration has been developed, in which vapor is introduced into the space by evaporation of a liquid from a container of specific dimensions, while the space air is continuously ventilated or purified. An equilibrium is established, giving a constant vapor concentration. An equation is derived in which the vapor concentration is expressed in terms of the vapor pressure of the liquid, the dimensions of the container from which the liquid evaporates, the volume of the space, the mutual air-vapor diffusion coefficient, the barometric pressure, and the rate of air purification or ventilation. The method may be applied to control of vapor concentrations for olfactory measurements, treatment of perishables with fungicidal vapors, and other uses.

IT IS OFTEN IMPORTANT to maintain a low but constant concentration of a particular vapor in a space—for example, stored fruit may be protected from fungus diseases by a very small concentration of a toxic vapor in the storage atmosphere. When such a method is used, it is essential to prevent the concentration of the toxic vapor from reaching the point where it becomes dangerous for plants or humans. It is therefore necessary to set up a system in which the desired low vapor concentration is reliably maintained.

In studying the migrations of insect

pests it may be revealing to determine how strongly a particular plant odor influences the insects' travels, either as an attractant or as a repellent. If this can be determined experimentally in the laboratory, it affords priceless information which is a valuable aid in the strategy of the fight against the insect pest, and in predicting or controlling its habits. To set up such laboratory tests, however, it is necessary to duplicate and maintain the marginal odors often present in nature. Such odors are produced by extremely low concentrations of vapors (4). This is essentially a study in

insect olfaction. Similar experimental problems are involved in studies of human olfaction.

To set up a low vapor concentration in a space merely by a single "shot" of the required gas is ineffective because the gas quickly dissipates, with nothing to take its place. Even when there is no ventilation, the adsorption of vapors on various solid surfaces causes rapid dissipation when the initial concentration is very low. The problem can be solved by setting up a system in which the required vapor is continuously injected into the space and simultaneously re-