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Oxygen Absorption Rates in Laboratory and Pilot Plant Equipment[1]

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Submerged, aerobic fermentation is used to produce a variety of substances, including antibiotics, enzymes, vitamins, and organic acids. In laboratory studies of these processes, aeration rates are often indirectly stated as the speed and stroke of a reciprocal shaker or the speed and radius of a rotary shaker. The extent of aeration in vat fermentors is sometimes specified in terms of volume of air supplied per volume of medium. A more quantitative measurement of aeration rate has been provided by the sulfite oxidation procedure of Cooper *et al.* (1944). This method with certain modifications was employed in the present study of factors influencing the oxygen absorption rate in shaken flasks and 20-L fermentors. Oxygen absorption rate (OAR) is defined as millimoles of oxygen absorbed per liter of the solution per minute.

Materials and Methods

In laboratory investigations, both regular and indented Erlenmeyer and Fernbach flasks were used. The indented flasks are described in the section dealing with effects of such modifications on OAR. Two reciprocal shakers were used, one making 88 complete 3⅜ in. strokes per min; the other 2¼ in. strokes with speed varied as desired. In addition, two variable speed rotary shakers were used. One was a Gump[5] sieve shaker that had been converted to a shaker for culture flasks and which moved the flasks in a circle of 2¼ in. radius. The second was a New Brunswick[6] shaker which moved the flasks in a circle of 1 in. radius.

OAR investigations were made also with the 20-L stainless steel fermentors recently described by Dworschack *et al.* (1954). These have an interior diameter of 8.75 in. Four 24-gauge stainless steel baffles 1.57 in. wide were spaced evenly on the inside of each fermentor. The four baffles, which extended the full height of the fermentor, were riveted to two 24-gauge stainless steel hoops so that the entire assembly could be easily inserted or removed as desired. Two types of air spargers were used in this study: (a) an Aloxite[7] porous stone sparger in an unbaffled fermentor, and (b) a stainless steel pipe with six holes each $\frac{5}{64}$ in. in diameter in a baffled unit.

To determine OAR, the method of Cooper *et al.* (1944) was modified as follows: duplicate 5-ml samples of sodium sulfite solution, containing 0.001 M copper sulfate as catalyst, are pipetted from the shaken flasks into 1 by 10 in. test tubes containing a small pellet of dry ice. The CO_2 blankets the sample and thus prevents further oxidation. It also acts as an automatic stirrer during titration. Just prior to titration with iodine, two drops of starch indicator and another piece of dry ice are added. The pellet of dry ice added initially should be small so as to avoid partial freezing of the sample, which causes a fleeting end point in the titration. However, if partial freezing does occur, warming the test tube in the palm of the hand melts the sample and the

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[4] One of the divisions of the Agricultural Research Service, United States Department of Agriculture.

[5] The mention of names of trade products does not imply that they are endorsed by the Department of Agriculture over other products of similar quality. B. F. Gump Co., Chicago, Illinois.

[6] New Brunswick Scientific Co., New Brunswick, New Jersey.

[7] Carborundum Co., Niagara Falls, New York.

titration can be completed to the permanent blue starch-iodine end point.

Although Cooper *et al.* (1944) varied the size of samples from 5 to 100 ml "depending on the size of the tank" and also used approximately 1 N sulfite, we have found it advantageous to titrate a standard volume of sample, and to vary the concentrations of both the sulfite and iodine solutions with the aeration conditions being investigated. The iodine solution is standardized daily against 0.1 N sodium thiosulfate. The initial normality of the sulfite solution should be between 4 and 5 times that of the iodine solution; that is, 20 to 24 ml of iodine should be required to titrate 5-ml sample of sulfite. Thus, by using a 25-ml burette, the oxygen absorption of a number of samples taken at convenient time invervals can be determined before the sulfite in the flask or vat is completely oxidized.

The oxygen absorption rate (OAR), expressed as mM oxygen absorbed per L per min, can be calculated from the following equation:

$$\text{OAR} = \frac{\text{ml titration difference} \times \text{normality iodine soln}}{4} \times \frac{1{,}000}{5} \times \frac{1}{\text{min}}$$

Significant titration differences between 5-ml samples taken at convenient time intervals are obtained by use of iodine solution of appropriate normality. If a 4-ml titration difference is desired for 5-ml samples taken at 10-min intervals while investigating an OAR of approximately 3.0, substitution of these values in the equation indicates that 0.15 N iodine should be used. However, if aeration conditions are such that an OAR of 0.3 is being investigated, 0.015 N iodine solution is more appropriate. The initial concentration of catalyzed sodium sulfite in either case should be adjusted so that 5 ml requires 20 to 24 ml of iodine for titration as previously mentioned.

Samples taken at varying time intervals indicated that initial OAR values were different from those obtained later. The variation may be caused by the time required for the system to attain equilibrium. Therefore, a sufficient number of samples must be taken over a period of time for a determination of oxygen absorption rate. A plot of cumulative mM oxygen absorbed per L (as ordinate) *versus* time in min (as abscissa) results in points on a straight line whose slope is the OAR for the conditions under investigation (figure 1).

The advantages of the above direct titration procedure over the original method are: (a) reduction of possible manipulative errors, (b) prevention by CO_2 of further oxidation of the sample, (c) automatic stirring by CO_2 during titration, (d) elimination of back titration and (e) increased sensitivity of end point by titration with iodine rather than by titration of excess iodine with thiosulfate (Blasdale, 1928).

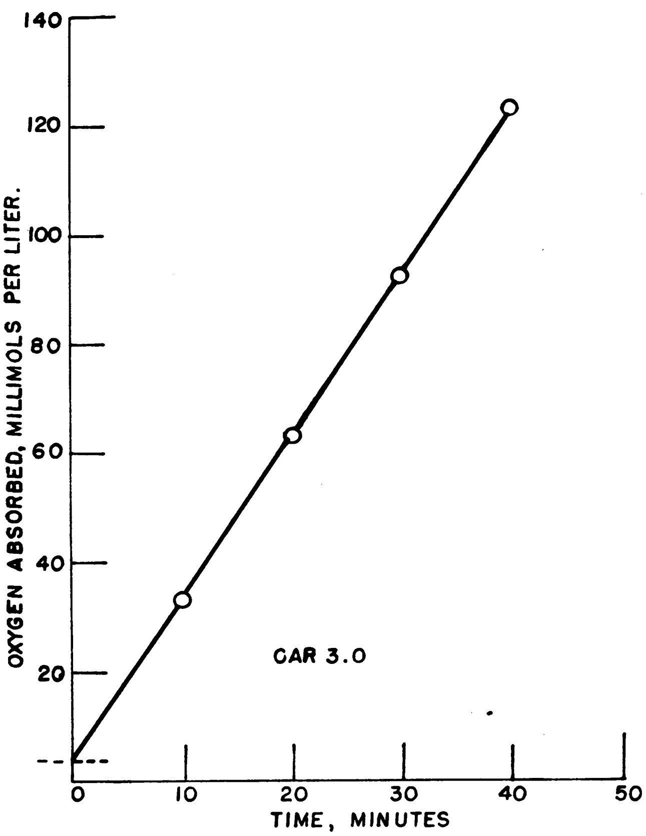

FIG. 1. Plot of oxygen absorption in an indented Fernbach flask. See table 3.

RESULTS AND DISCUSSION

OAR in Laboratory Equipment

Effect of flask size on OAR. The effect of flask size on the OAR of 100 ml of sulfite solution shaken on the Gump shaker at 200 rpm and on the reciprocal shaker was investigated with 300-, 500- and 1,000-ml Erlenmeyer flasks stoppered with cotton plugs. The data in table 1 show that the slight but steady increase in OAR, from 0.15 to 0.25 mM of oxygen absorbed per L per min, is due to increase in size of the Erlenmeyer flask. Agitation on the reciprocal shaker making 88 complete 3⅜-in. strokes per min causes the solution in a 1-L Erlenmeyer flask to attain 3 times the OAR value of that

TABLE 1. *Effect of size of Erlenmeyer flask on on oxygen absorption rate (OAR)*

Capacity of Flask	Volume of Solution	Oxygen Absorption Rate*	
		Gump shaker at 200 rpm	Reciprocal shaker† at 88 rpm
ml	*ml*		
300	100	0.15	0.16
500	100	0.20	0.18
1000	100	0.25	0.49

* mM Oxygen absorbed/L/min.
† Stroke of 3⅜ in.

noted for the solution in a 300-ml Erlenmeyer flask. The increased OAR from 0.16 to 0.49 is due to greater turbulence of liquid shaken in the larger flask on the reciprocal shaker.

Because a wider range of OAR values was found for the reciprocal shaker than for the rotary shaker, a more detailed investigation was conducted of the OAR of sulfite solution in flasks on a reciprocal shaker. A reciprocal shaker with a 2¼ in. stroke and equipped with a variable speed drive was used to study the effect of shaker speed, flask size, and liquid volume on OAR. The closures for all flasks consisted of three Rapid-flo[8] (fiber bonded) discs, 6½ in. in diameter. Wrapping the discs in a single thickness of cheese cloth, before tying them over the mouth of the flask, produces a uniform closure that can be autoclaved several times. Although this closure offers less resistance to air flow than conventional cotton plugs, it effectively prevented contamination of cultures incubated in shaken flasks for as long as 168 hr.

The OAR values of varied volumes of sulfite solution in Erlenmeyer and Fernbach flasks, shaken at 80 and 100 complete strokes per min, are shown in table 2. Variation in solution volumes in Erlenmeyer flasks significantly influences the OAR values. On the other hand, variation of the liquid volume in Fernbach flasks exerts very little effect. The data also indicate that the shaking speed has a marked effect upon OAR values in Erlenmeyer flasks but only a minor effect in Fernbach flasks. This difference in effective turbulence is likely due to relationship of diameter of shaken flasks to extent of displacement, or stroke, of the reciprocal shaker. Data, presented below, show that an analogous situation exists with rotary shakers where the relationship between flask diameter and eccentricity greatly influences OAR values. The type of data presented in table 2 has been used for scale-up of laboratory experiments. For example, an OAR of 1.0 is obtained with 25, 50, and 100 ml of solution contained in 300-, 500- and 1000-ml Erlenmeyer flasks, respectively, all shaken at 100 strokes per min on this particular shaker.

[8] Johnson and Johnson Company, New Brunswick, New Jersey.

TABLE 2. *Oxygen absorption rate (OAR) with reciprocal shaker* at 25 C*

Liquid Volume	Size and Type Flask Used at 80 and 100 Complete Strokes/Min							
	2,800-ml Fernbach		1,000-ml Erlenmeyer		500-ml Erlenmeyer		300-ml Erlenmeyer	
	80	100	80	100	80	100	80	100
ml								
25							0.48	1.03
50			0.78	1.50	0.42	0.96	0.25	0.58
100	0.67	0.67	0.51	1.00	0.16	0.53	0.14	0.23
200	0.62	0.63	0.22	0.78	0.08	0.19†		
300	0.62	0.69	0.10	0.57†	0.01			
500	0.58	0.72	0.06					
700	0.50	0.56						

* Stroke of 2¼ in.

† Flask closures became wet.

The OAR under two "still culture" conditions was determined for comparative purposes. When the flasks were allowed to stand undisturbed, the OAR of 100 ml of sulfite solution in Fernbach flasks was 0.21 while the OAR of 50 ml of sulfite solution in 500-ml Erlenmeyer flasks was 0.04.

Flask modification. A 500-ml bottle with a square base was fitted with a cotton plug and used as a shaken flask in an effort to increase turbulence in solutions agitated on the Gump shaker set at 200 rpm. Under the conditions, the OAR of 100 ml of sulfite solution was 0.45 while the OAR for 50 ml was 0.79. These values, substantially higher than those reported in table 1, indicated that some modification in the shape of the shaken flask was desirable for attainment of high OAR on a rotary shaker.

Dale *et al.* (1953) increased the OAR of media in 1-L Florence flasks, agitated on a rotary shaker, by means of numerous indentations in the flasks. The data in table 3 show that the addition at random of 72 indentations in the base of a Fernbach flask accounted for the increase in OAR from 0.2 to 3.5. Both the plain and the indented flasks were equipped with the filter disc closure.

Although highly aerobic conditions were obtained by use of this indented Fernbach flask on the Gump shaker, the flask was difficult to make. Observation of the action of the liquid, when the flask was shaken on the Gump shaker, indicated that the peripheral indentations near the base of the flask were primarily responsible for turbulence. Another Fernbach flask was then modified by means of 16 vertical indentations 1½ in. high and ½ in. deep, evenly spaced about the base (figure 2). As noted in table 3, an OAR of 3.0 was attained with 300 ml of sulfite solution in this flask. Consequently, a number of the Fernbach flasks with 16 indentations were made and are used routinely. Chain and Gualandi (1954) also increased the OAR of liquids in round bottom as well as Erlenmeyer flasks, shaken on a rotary shaker, by fusing a baffle inside the flask.

A wide mouth, 500-ml Erlenmeyer flask was similarly modified but with only 6 vertical indentations (figure

TABLE 3. *Effect of modification of flask on oxygen absorption rate (OAR)*

Type of Flask	Volume of Solution	OAR with Gump Shaker at 200 Rpm
	ml	
Nonmodified Fernbach......	300	0.2
Fernbach with 16 indentations	300	3.0
Fernbach with 72 indentations	300	3.5

2). When only 25 ml of liquid was used in this flask on the Gump shaker at 200 rpm, turbulence was so violent that the closure became wet. The excessive turbulence was due primarily, as will be discussed later, to use of the modified flask of small diameter in conjunction with a shaker having relatively large eccentricity.

Flask closure. When highly aerobic conditions were first attained on the Gump shaker by use of an open Fernbach flask with 72 indentations, it was thought that the type of closure might be limiting the air supply. Verification of this suspicion is shown in table 4 in which the OAR value for the flask with the filter disc closure is compared to that of the flask without any closure. However, it will be noted that the use of the filter disc closure results in an OAR that is more than twice the value obtained with the cotton plug closure. This advantage, in addition to those previously mentioned, has made the three-filter disc closure most desirable, especially for the attainment of highly aerobic conditions in shaken flasks.

Relationship of flask size and eccentricity of shaker. As noted above, agitation of 25 ml of solution in the modified 500-ml Erlenmeyer flask on the Gump shaker at 200 rpm resulted in wetting of the closure due to excessive turbulence. However, this wetting does not occur on the New Brunswick shaker at 200 rpm, which suggested that under comparable conditions OAR values achieved with the Gump shaker might be higher than those obtained with the New Brunswick shaker. Accordingly, comparison was made of OAR values obtained with two flasks of different size on the same shaker, and also, the same sized flasks on the two different shakers operated at the same rpm. Wide mouth 500-ml Erlenmeyer flasks with 6 indentations and Fernbach flasks with 16 indentations were both employed with the New Brunswick shaker. Other variables, including liquid volume, filter disc closure, rpm, and eccentricity of shaker, were kept constant. The increase in flask size decreased the OAR from 7.7 to 1.3 (table 5). Modified Fernbach flasks were then tried on both the New Brunswick and Gump shakers to demonstrate the effect of eccentricity on OAR. Other variables were once more held constant so that the increase in OAR from 1.3 to 5.2 is due to difference in eccentricity of the shakers (table 5).

The data in table 5 thus indicate that highly aerobic conditions can be attained with a rotary shaker by taking advantage of the eccentricity of shaker and radius of modified shaken flask, as well as shaker speed. Turbulence obviously depends on the ratio of the eccentricity of the shaker to the radius of the shaken flask:

$$\frac{\text{Eccentricity, (E)}}{\text{Flask Radius, (FR)}},$$

which is analogous to the term, "percentage baffling" encountered in using stationary fermentors. Because the diameter of the base of a Fernbach flask is 8 in., the ratio, E:FR is 1:4 or 0.25 when the New Brunswick shaker is used, and 2.25:4 or 0.56 when the Gump shaker is employed. This substantial increase in ratio, because of increased eccentricity, accounts for the significantly greater turbulence and the resultant fourfold increase in OAR from 1.3 to 5.2. When a modified, wide mouth, 500-ml Erlenmeyer flask having a 3½ in. diameter base is used in conjunction with the New Brunswick shaker, the E:FR ratio is 1:1.75, or 0.57. Thus a high OAR should be expected, and a value of 7.7 was attained. The excessive turbulence of liquid

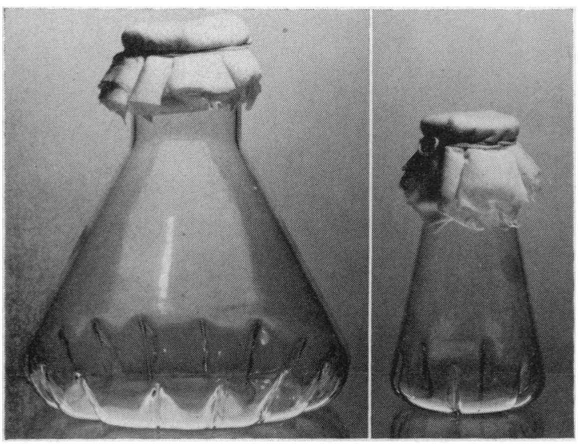

FIG. 2. Fernbach flask with 16 indentations and 500-ml Erlenmeyer flask with 6 indentations.

TABLE 4. *Effect of type of closure on oxygen absorption rate (OAR) using Fernbach flask with 72 indentations*

Type Flask	Volume of Solution	Type Closure	OAR with Gump Shaker at 200 Rpm
	ml		
Fernbach with 72 indentations	300	Open	5.1
Fernbach with 72 indentations	300	3 filter discs	3.5
Fernbach with 72 indentations	300	Cotton plug	1.6

TABLE 5. *Effect of size of modified flask and of eccentric throw on oxygen absorption rate (OAR)*

Volume of Sulfite Solution	Type Flask*	Eccentric Throw	Rpm	OAR
ml				
25	ME	1 in. (New Brunswick shaker)	292	7.7
25	MF	1 in. (New Brunswick shaker)	292	1.3
25	MF	2¼ in. (Gump shaker)	292	5.2

* ME = modified 500-ml Erlenmeyer flask; MF = modified 2,800-ml Fernbach flask.

which caused the closure of the modified, wide mouth, 500-ml Erlenmeyer flask to become wet, when the flask was agitated on the Gump shaker at 200 rpm, was likewise caused by the large E:FR ratio.

OAR in Pilot Plant Equipment

Introduction of fine air bubbles versus air dispersion with baffles. The OAR of media in fermentors is dependent on several factors including the size of the air bubbles entering the liquid, that is, on the dispersion of air in the liquid. The two different systems of aeration with agitation described previously were compared. Ten L of sodium sulfite having appropriate normality and containing 0.001 M copper sulfate was used in each test run.

Inspection of the data in table 6, obtained from experiments on 20-L fermentors equipped with two types of spargers, indicates that increased agitator speed causes an increased OAR in both systems at all air flow rates investigated. It should be noted, however, that the relative rate of increase of OAR, with increased agitation, is much greater for the pipe sparger with baffles than for the porous stone without baffles. When agitator speed was held at 300 rpm, and aeration rates were varied between 0.25 to 1.0 volume of air per volume of medium per min (VVM), OAR values of the baffled system exceeded those of the porous stone s arger system without baffles. The advantage of the pipe sparger and baffle system over the stone sparger alone, which requires higher air pressure to overcome the greater resistance to air flow, is obvious.

Assuming there is 20 per cent oxygen in air, then at 25 C and 760 mm pressure, 8.18 mM O_2 is supplied per L of solution per min when the air flow rate is 1 L of air per L of liquid per min. On this basis, the OAR values in table 6 have been recalculated and expressed in table 7 as percentage of oxygen utilized in each case. Percentage utilization of oxygen supplied by the stone sparger system without baffles is practically independent of the air flow rate at each rpm investigated. For this reason, the per cent oxygen utilization at all four air flow rates has been averaged at each rpm for the stone sparger system without baffles. The data in table 7, which are graphically depicted in figure 3, readily show the greater increase in efficiency of the pipe sparger and baffle system with increased agitator speed. By use of baffles, excellent air dispersion is attained at the admittedly high speed of 550 rpm. Thus, at air supply rates of 1.0, 0.5, and 0.25 VVM, 65, 86, and 98 per cent,

TABLE 6. *Oxygen absorption rate (OAR) in 20-L fermentors*

Air Flow VVM*	Agitator Speed, Rpm									
	0		100		200		300		550	
	S†	P‡	S	P	S	P	S	P	S	P
0.25	0.08	—	0.11	0.03	0.15	0.41	0.39	1.1	—	2.0
0.5	0.14	—	0.23	0.06	0.40	0.65	0.82	1.6	—	3.5
1.0	0.34	—	0.54	0.10	0.95	0.70	1.40	1.8	—	5.3
2.0	0.89	—	1.19	0.14	1.9C	0.83	2.70	2.5	—	7.2

* Volume of air/volume of medium/min.
† Stone sparger without baffles.
‡ Pipe sparger plus baffles.

TABLE 7. *Per cent utilization of oxygen supplied in 20-L fermentors*

Air Flow	Oxygen Supplied	Agitator, Rpm							
		0	100		200		300		550
		Type of Sparger*							
		S	S	P	S	P	S	P	P
VVM†	*mM/L/min*	%	%	%	%	%	%	%	%
0.25	2.04	4	5	1	7	20	19	54	98
0.5	4.09	3	6	1	10	16	20	39	86
1.0	8.18	4	7	1	12	9	17	22	65
2.0	16.36	5	7	1	12	5	17	15	44

* S = Stone sparger without baffles; P = pipe sparger plus baffles.
† Volume of air/volume of medium/min.

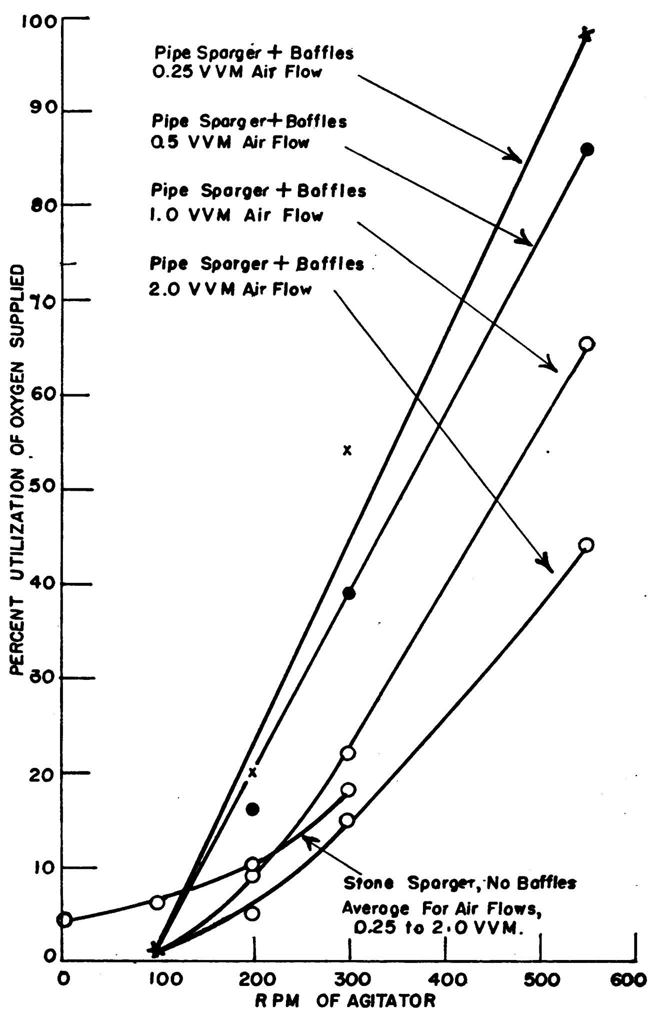

FIG. 3. Efficiency of baffling and of different sparging systems at various air flows.

respectively, of the supplied oxygen are actually used to oxidize the sulfite solution.

Effect of antifoam on OAR. The effect of various antifoams in the fermentors was also investigated. Aeration conditions were adjusted so that an OAR of 3.4 was attained in 10 L of catalyzed sulfite solution in the fermentor. Addition of 2 ml of Hodag KG-1[9] antifoam to the fermentor caused the OAR value to drop to 0.4. Other antifoam agents including octadecanol in alcohol and Dow Corning DCA[10] antifoam had a similar depressing effect. A similar effect has been reported by Deindoerfer and Gaden (1955). They used 3 per cent Alkaterge C[11] in lard oil as the antifoam agent. Such data indicate the desirability of keeping antifoam addition to a minimum, or eliminating the use of antifoam agents by mechanical defoaming devices. Inasmuch as certain media tend to foam more than others, it may be possible to manipulate the composition of the medium to diminish foaming.

ACKNOWLEDGMENT

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SUMMARY

An improved procedure for oxygen absorption rate (OAR) determinations is presented.

A rather wide range of useful OAR values can be attained by judicious selection of fluid volumes and sizes of culture flasks agitated on a reciprocal shaker.

Vertical indentations spaced evenly about the base of culture flasks shaken on rotary shakers increase OAR values.

The relationship between size of modified flasks and eccentricity of a rotary shaker must be considered for achievement of a desired OAR.

The type of flask closure has a significant effect on OAR. A simple, uniform type of closure with less resistance to air flow than offered by a cotton plug is described.

Installation of baffles in a fermentor improves the utilization of the air supplied, especially at low aeration rates.

Antifoam agents markedly depress oxygen absorption by liquids. Therefore, to maintain highly aerobic conditions during a fermentation, a minimum amount of antifoam should be added.

[9] Hodag Co., Chicago, Illinois.

[10] Dow-Corning Corporation, Midland, Michigan.

[11] Commercial Solvents Corporation, Terre Haute, Indiana.

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