Studies on the Aerobic Propagation of *Serratia marcescens*[1]

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In the course of our fermentation studies, it became important to investigate the optimal conditions for producing dense populations of bacteria. *Serratia marcescens* was selected as the organism to be studied to facilitate the ease and accuracy of the many counts which would be required. There were two objectives. The first was to devise a medium and to select the other fermentation conditions which would support unusual numbers of highly pigmented viable cells. The second objective was to find means of consistently reproducing the optimal conditions and the high bacterial counts so that ample quantities of physiologically uniform cells would be available for evaluation of stabilizers, protectants, and other pertinent factors in an investigation of freeze-drying. Smith and Johnson (1954) employed a synthetic medium in producing *S. marcescens* cells and showed the importance of aeration in cell production.

Materials and Methods

The stock strain of *Serratia marcescens* employed in the present study was obtained from the Army Chemical Corps, Fort Detrick, as their strain 8 UK, and is hereinafter designated as NRRL B-1481. Subcultures were stabilized essentially as recommended by that agency, but some modifications were made. The final technique employed was as follows: A freeze-dried culture was rehydrated with diluent (formula 1) and streaked on agar plates (formula 2). After incubation at 28 C for 24 hr, 25 red colonies were picked from one plate and each colony was transferred to 25 ml of broth (formula 3) in a wide mouth 250-ml Erlenmeyer flask. The flasks were incubated on a reciprocal shaker for 18 hr at 28 to 29 C. A small portion of the contents of each flask served as inoculum for each of a second series of 25 flasks, and so on through five series. All flasks were held at 8 C until the fifth serial transfer series had been incubated. Cells from flasks of the first series, whose progeny in the fifth series were stable with respect to pigmentation and viable counts, were concentrated and then freeze-dried in tubes. Each tube contained a loopful of cell concentrate in 0.1 to 0.15 ml of sterile bovine serum and served as the source of a single lyophil pellet. The tubes were stored in the cold as stable stock cultures for the preparation of inocula.

Formula	Ingredients	Per Cent
1	NaCl	0.5
	Tryptone (Difco)[5]	0.1
2[6]	Peptone (Difco)	0.5
	Glycerol	1.0
	Edamine (Sheffield)[7]	0.1
	NaCl	1.0
	Agar	2.5
	(Adjusted to pH 6.8–7.0)	
3	Tryptose (Difco)	2.0
	NaCl	0.5
	Glucose (commercial)	0.5

Originally, aliquots of a whole culture, grown in formula 3 medium and refrigerated at 4 C, served as inoculum, but losses of viable cells with time limited their use to 30 days. Further studies revealed that whole cultures of strain B-1481 could be quickly frozen at −60 to −70 C and then held at −25 C for periods up to 3 months with no significant loss in viable count. Frozen whole cultures, held for 10 months and thawed, lost only 18 per cent of the original viable cell population. Consequently, inocula were prepared as follows: A lyophil pellet was used to inoculate 300 ml of the chosen production medium in 2.8-L 16-dented Fernbach flasks, Corman *et al.* (1957). The flasks were agitated on a reciprocal shaker for 20 hr at 28 C. If a culture was normal with respect to terminal pH, pigmentation, and microscopic appearance, 5-ml aliquots were quickly frozen in sterile tubes and stored at −25 C in screw capped metal cans. When new cultures were needed, the tubes were rapidly thawed and served as an excellent source of inoculum at the

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[5] The mention of names of trade products does not imply endorsement of these by the Department of Agriculture over others of equal quality.

[6] Formula 2 was far superior to any other plating medium tested in producing excellent pigmentation and rapid growth of *S. marcescens* B-1481.

[7] Sheffield Farms Co., Inc., Norwich, New York.

rate of 1 ml per 500 ml of medium. Fermentations in flasks were conducted, at the least, in duplicate.

Cell counts of propagations in liquid media were made as follows: A culture was diluted appropriately in saline tryptose broth by standard bacteriological techniques so that 0.3 ml of the final dilution contained between 30 and 300 viable cells. A 0.3-ml aliquot of the final dilution of the sample was pipetted onto each of nine "tempered" (dry surface) agar plates and immediately spread over the entire surface with a glass streaking tool. After incubation at 18 to 20 hr at 29 C, the red colonies were of sufficient size to be counted at twofold magnification with background illumination.

Frosted glass plates were substituted in place of clear glass plates in the Quebec colony counters. The use of frosted glass serves to eliminate glare. The change is recommended to anyone who counts pigmented colonies of microorganisms. The average number of colonies and 95 per cent confidence limits for each series of nine plates were determined and, after correction for extent of dilution, the viable cell count was expressed in billions of cells per ml. The precision of the counting method was good because the average of all the 95 per cent confidence limits was ±7.7 per cent.

The reciprocal shaker, previously mentioned, made 88 complete 3⅜-in. strokes per min. A rotary shaker, having an eccentric radius of 2¼ in. and normally operating at 200 rpm, was used in some experiments. Investigations were also conducted with 10-L cultures in the 20-L fermentors described by Dworschack *et al.* (1954). The Fernbach flasks with 16 indentations were employed on the rotary shaker to produce highly aerated fermentation conditions. The closure for these flasks was made of three milk-filter discs wrapped in cheese cloth and tied over the flask mouth as described previously by Corman *et al.* (1957).

The methods for carbohydrate and nitrogen analyses were as follows: glucose by the method of Somogyi (1945), total nitrogen by the micro-Kjeldahl procedure of Clark (1943), and ammonia nitrogen by the method of Nichols and Foote (1931). Oxygen absorption rate (OAR) was determined by a modification (Corman *et al.*, 1957) of the procedure of Cooper *et al.* (1944). OAR is defined as mM of oxygen absorbed per L of sulfite solution per minute.

Results

Propagation in Shaken Flasks

Cells propagated in the medium of Bunting *et al.* (1949), containing 2 per cent peptone and 1 per cent glycerol, were deeply pigmented but the count never exceeded 6 billion cells per ml. A combination of 1 per cent glucose and 2 per cent tryptose increased the count to 18.5 billion cells per ml as shown in table 1. The further benefits of phosphate buffer and skim milk powder are also evident from the data presented.

Table 2 records the effects of other variations in the propagation medium under conditions favoring high OAR. At an OAR of 3, a medium containing 2 per cent glucose, 2 per cent tryptose, and 5 per cent skim milk powder produced 179 billion cells; whereas, at an OAR of 0.7, the same medium gave only 90 billion cells per ml (table 1). The ultimate choice was a medium composed of 5 per cent skim milk powder, 3 per cent protopeptone[8] (No. 343), and 2 per cent glucose, herein-

[8] Wilson and Co., Chicago, Illinois.

TABLE 1. *Value of combinations of glucose, tryptose, phosphate and skim milk powder in promoting the growth of Serratia marcescens NRRL B-1481**

Glucose	Tryptose	Phosphate buffer	Skim Milk Powder	pH at 20 hr	Viable Cell Count and 95 Per Cent Confidence Limits
%	%	*M*	%		*billion/ml*
1	2			7.35	18.5 ± 1.2
1	2	0.03		8.3	60 ± 3.8
1		0.03	5	7.4	77 ± 2.9
2	2	0.03	5	7.6	79 ± 1.7
2	2		5	7.85	90 ± 4.3
2			5	6.2	109 ± 7.6
			5	8.2	43 ± 1.6

* Incubated in 300 ml of medium at 28 C and oxygen absorption rate (OAR) of 0.7 in plain Fernbach flasks on reciprocal shaker and sampled after 20 hr. Initial pH, 7.3.

TABLE 2. *Effect of glucose and various nitrogen sources on growth of Serratia marcescens NRRL B-1481**

Glucose	Tryptose	Protopeptone No. 343	Skim Milk Powder	pH at 20 hr	Viable Cell Count and 95 Per Cent Confidence Limits
%	%	%	%		*billion/ml*
1	1		5	7.6	109 ± 17
1	2		5	7.9	122 ± 13
2	1		5	7.2	156 ± 15
2	2		5	7.65	179 ± 19
2		1	5	5.9	173 ± 15
2		2	5	6.35	218 ± 14
2		3	5	7.0	233 ± 15

* Incubated in 300 ml of medium at 28 C and oxygen absorption rate (OAR) of 3.0 in Fernbach flasks with 16 indentations. Sampled after 20 hr on a rotary shaker operated at 200 rpm.

TABLE 3. *Effect of oxygen absorption rate (OAR) on the population of Serratia marcescens NRRL B-1481 propagated in SPG medium**

Fernbach Flask Type	Volume Culture/flask	Type Shaker	OAR†	pH at 20 hr	Viable Cell Count and 95 Per Cent Confidence Limits
	ml				*billion/ml*
Plain........	500	Rotary	0.2	6.85	42 ± 2.9
Plain........	500	Reciprocal	0.7	7.5	125 ± 4.3
Modified.....	300	Rotary	3.0	7.0	212 ± 13

* SPG medium: skim milk solids, 5 per cent; protopeptone, 3 per cent; and glucose, 2 per cent. Temperature, 28 C; initial pH, 7.0; and fermentation time 20 hr.

† mM of oxygen absorbed/L of sulfite solution/min.

after referred to as SPG medium. As further shown in table 3, the full potentialities of this medium could not have been realized without accelerated aeration and a greatly increased supply of oxygen for the rapidly multiplying cells.

Unusually high counts of strain B-1481 were obtained consistently with SPG medium in 16-dented Fernbach flasks on the rotary shaker. The average count of viable cells in one series of 14 trials, using as inocula four different lots of frozen whole culture liquor, was 194 billion per ml. The lowest count was 172 billion per ml and the highest was 217 billion per ml. The changing pH in the fermentation cycle was reproducible, starting at pH 6.8 to 7.0, dropping to 5.2 to 5.5 at 6 hr with rapid utilization of the glucose present, and thereafter rising gradually to 7.0 to 7.5 at 20 to 22 hr. Some idea of the various phases of the fermentation, obtained during an early stage of the medium development studies, can be obtained from figure 1.

Smith and Johnson (1954) noted a marked difference in the sizes of pigmented *versus* nonpigmented cells of *S. marcescens*. Our carefully stabilized stock cultures grown in SPG medium contained only highly pigmented cells. The average size of these pigmented cells decreased as the OAR was increased up to 3.0, the highest OAR tested. The results are shown in table 4. Stained smears prepared from surface colonies on agar plates showed that cells of strain B-1481 are rod-shaped, averaging 1.5 to 2.0 μ in length by 1.0 μ in width. Cultivation in liquid medium produces coccobacilli at low and medium OAR and coccus forms, occurring mainly as diplococci, at the highest OAR. Some growth trials on the synthetic medium of Smith and Johnson were made, employing 300 ml of medium in 16-dented flasks. When cells previously grown in synthetic medium were used as inocula, the size of the inoculum was not critical. However, when the cells were grown in SPG medium, the size of inoculum had to be decreased markedly to obtain comparable counts of viable cells. The results are shown in table 5.

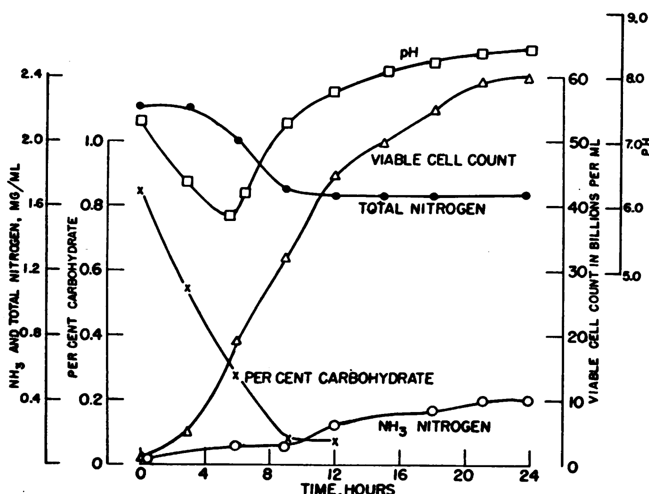

FIG. 1. Progressive changes in fermentation of *Serratia marcescens*. Five hundred ml of medium containing 1 per cent glucose, 2 per cent tryptose, and phosphate buffer (0.03 M) was agitated in 2.8 L plain Fernbach flasks on the reciprocal shaker at an OAR (oxygen absorption rate) of 0.7.

Inasmuch as *S. marcescens* readily utilizes a number of carbon sources besides glucose for growth, experiments were conducted with several compounds, mainly carbohydrates, at the same level employed for the glucose controls. The results are shown in table 6. Viable cell counts obtained with sucrose, maltose, mannitol, and glycerol were quite comparable to those obtained with glucose. There was little or no utilization of the added lactose, xylose, and soluble starch.

Prior to making trials in the 20-L vat fermentors, a wide variety of antifoam agents were tested, alone, and

TABLE 4. *Effect of the oxygen absorption rate (OAR) on cell size of Serratia marcescens NRRL B-1481 propagated in SPG medium for 18 hr at 29 C*

OAR	Viable Cell Count and 95 Per Cent Confidence Limits	Packed Cell Vol/Ml*	Calculated Count/Ml Packed Cells	Morphology and Approximate Size of Cells by Microscopic Observation†
	billion/ml	*ml*	*billion/ml*	
0.13–0.20	27.1 ± 3	—‡	—	Coccobacilli, 0.5–1.5 μ, averaging 1.0 μ
0.70	89.3 ± 7	0.140	630	Coccobacilli, averaging 0.85 μ
3.0	150 ± 12	0.164	910	Coccus forms, 0.4–0.7 μ, averaging 0.6 μ

* 1.0 ml cell suspension spun down at 2,700 rpm for 30 min.

† Average size of 50 cells measured with calibrated ocular micrometer with oil immersion, × 970. Negative staining with 2% aqueous solution of Congo Red.

‡ Could not be accurately determined because milk solids were not completely utilized during growth and could not be separated from the cells.

TABLE 5. *Effect of source and size of inoculum on counts of viable cells of Serratia marcescens NRRL B-1481 produced in synthetic medium of Smith and Johnson**

Source of Inoculum	No. of Viable Cells in Inoculum used/Ml Medium	Viable Cell Count in Synthetic Medium after 18 hr at 28 C
	billion/ml	*billion/ml*
Synthetic medium†	0.2 –1.5	77–86
SPG medium‡	0.05–0.4	78–94
SPG medium	1.0	55–61
SPG medium	2.0	37–42

* Three hundred-ml lots of medium were shaken for 20 hr in 16-dented Fernbach flasks on the rotary shaker at an OAR of 3.0.

† Medium composed of $(NH_4)_2SO_4$, 2 per cent; glucose, 2 per cent; sodium citrate, 2 per cent; and KH_2PO_4, 2 per cent.

‡ Medium contains skim milk solids, 5 per cent; protopeptone, 3 per cent; and glucose, 2 per cent.

in combination with Hodag KG-1,[9] employing the 16-dented flasks at an OAR of 3. The most interesting results from the standpoint of high counts of viable cells were obtained with Hodag KG-1 and tributyl phosphate (TBP) as shown in table 7. Although 0.25 per cent TBP was toxic to the growth of B-1481, the addition of 0.75 per cent Hodag KG-1 seemed to counteract the toxicity of the former and gave excellent counts of viable cells. Tributyl phosphate alone, however, could not be successfully employed in vat fermentors; levels approaching those which caused toxicity in flasks yielded orange-colored growths.

The addition of Hodag KG-1 appeared to stimulate the growth of the test organism in shaken flasks, and a combination of 1 part of the former to 9 parts soybean oil was not toxic at the highest level tested, that is, 5 per cent. Ordinarily not over one per cent was used.

[9] Hodag Co., Chicago, Illinois.

TABLE 6. *Viable cell counts of Serratia marcescens NRRL B-1481 grown in a medium containing 5 per cent skim milk solids and 3 per cent protopeptone plus 2 per cent of various carbon compounds**

Carbon Source	Final pH after 20 Hr	Viable Cell Count and 95% Confidence Limits
		billion/ml
Glucose	6.9	197 ± 20
Sucrose	6.25	194 ± 11
Maltose†	5.7	176 ± 16
Mannitol	5.95	199 ± 15
Glycerol	6.4	195 ± 22
Lactose	7.1	89 ± 3.1
Xylose	4.2	92 ± 2.5
Soluble starch	8.0	88 ± 3.2
Control: without accessory carbon source	8.2	87 ± 3.0

* Three hundred-ml amounts of medium were shaken in indented Fernbach flasks on the rotary shaker for 20 hr at 28 C and OAR of 3.

† Cell color, en masse, is extremely dark-purplish red when grown in maltose medium.

TABLE 7. *Effect of Hodag KG-1 and tributyl phosphate (TBP), alone or in combination, on the growth of Serratia marcescens in SPG medium**

Per Cent Antifoam Added	Final pH after 20 Hr	Viable Cell Count and 95 Per Cent Confidence Limits
		billion/ml
0.75 Hodag KG-1	6.3	226 ± 14
0.94 Hodag KG-1	6.2	197 ± 23
0.985 Hodag KG-1	5.9	208 ± 9.0
0.015 TBP	7.25	198 ± 11
0.06 TBP	7.1	225 ± 20
0.25 TBP	7.05	74 ± 7.6
0.25 TBP 0.75 Hodag KG-1	6.4	253 ± 21
0.06 TBP 0.94 Hodag KG-1	5.9	262 ± 16
0.015 TBP 0.985 Hodag KG-1	6.15	249 ± 8.0

* Three hundred ml of medium was fermented for 20 hr at 28 C and OAR of 3. Indented Fernbach flasks and the rotary shaker were used.

Propagation in 20-L Fermentors

The effect of OAR on the propagation of *S. marcescens* in 20-L fermentors at 28 C is illustrated in table 8. Counts of viable cells were made at five other time in-

TABLE 8. *Effect of oxygen absorption rate (OAR) on the viable cell count of Serratia marcescens NRRL B-1481 propagated in 20-L vat fermentors*

Fermentation Time	OAR 0.5	OAR 1.0	OAR 2.0	OAR 4.0
	VVM* 0.1	VVM* 0.2	VVM* 0.5	VVM* 1.0
	Viable cell count, billion/ml			
hr				
6	28.9 ± 3.0	38.1 ± 3.3	41.9 ± 3.3†	40.1 ± 2.3
15	89 ± 5.6	126 ± 16	142 ± 22†	174 ± 22
22	126 ± 10	184 ± 20	161 ± 25†	159 ± 18
30	165 ± 14	214 ± 2.3	189 ± 17†	154 ± 15

* VVM designates volume of air/volume of medium/minute.

† Average counts of duplicate trials; other values are the means of triplicate vat runs.

Conditions in common:

Ten L of SPG medium were fermented in each vat with Hodag KG-1 and soybean oil (1:9) as antifoam agents.

A 5 per cent inoculum of 22-hr culture grown at OAR of 0.7 in a plain Fernbach flask on the reciprocal shaker was used and the 0 hr counts in the vats averaged 3.9 billion/ml.

The agitation was 350 rpm in the fermentors.

TABLE 9. *Effect of antifoam on counts of viable cells of Serratia marcescens propagated at OAR 1.0 in 20-L fermentors*

	Fermentor A	Fermentor B	Fermentor C
Initial antifoam mixture*	100 ml	50 ml	25 ml
Hodag KG-1 added drop wise during fermentation	0 ml	26 ml	40 ml
Sample time	Viable cell counts		
hr		*billion/ml*	
0	4.4 ± 0.3	4.3 ± 0.3	4.0 ± 0.3
6	17.7 ± 2.5	23.9 ± 4.5	31.0 ± 2.3
9	57 ± 2.4	55 ± 2.5	76 ± 2.8
12	87 ± 6.8	105 ± 6.9	121 ± 8.0
15	118 ± 8.8	140 ± 3.8	159 ± 12
23	144 ± 14	169 ± 10	166 ± 18

* This mixture was made by adding 2 ml Hodag KG-1 per 1 g Dow-Corning Antifoam A. (Dow-Corning Corp., Midland, Michigan).

Conditions in common:

Ten L of SPG medium were fermented in each vat.

A 5 per cent inoculum of 22-hr culture grown at OAR of 0.7 in a plain Fernbach flask on the reciprocal shaker was used and the 0 hr counts in the vats averaged 3.9 billion/ml.

The agitation was 350 rpm in the fermentors.

tervals, but were not included in the condensed table. Although there is little difference between the 6-hr counts at the four different rates of oxygen absorption, the differences in the 15-hr counts are quite marked. At the highest aeration level, that is, at an OAR of 4, the cultures had reached their peak in 15 hr as shown by the 22- and 30-hr counts. The highest counts in the fermentors were consistently obtained at an OAR of 1.0.

Addition of antifoam agents to the catalyzed sulfite solutions which are used to determine OAR caused a precipitous drop in the OAR values. It would be of interest to know whether a similar drop in the OAR occurred when antifoam was added in an actual fermentation. Chain *et al.* (1952) reported a benefical effect on the addition of antifoam agent to a culture liquor aerated in their Vortex aeration system. Data showing the effect of antifoam additions on viable cell counts of *S. marcescens* grown in 20-L fermentors are summarized in table 9. The superior counts in fermentors B and C over those in A at 12- and 15-hr emphasize the value of having low antifoam levels at the start, with addition of minimal amounts of antifoam, as required, during the course of the fermentation.

Effect of Automatic pH Control

It was desirable to study the effect of pH control on 22-hr cell counts. Four 20-L stainless steel fermentors each equipped with automatic pH controller and recorder were employed. They were operated at 28 C and an OAR of 1.0 with the SPG medium adjusted initially to pH 7.3 before autoclaving. Propagation was allowed to proceed without pH adjustment in three fermentors until the pH in each fermentor dropped to 4.9, 5.9, and 6.7, respectively, while the pH in the fourth fermentor was adjusted immediately after inoculation to 7.7. Sterile acid or alkali was then added automatically, as required, to maintain the desired pH values $\pm$0.1 pH unit for the remainder of the 22-hr fermentation period. Final cell counts, in billion per ml for the cultures with varying pH were: 4.9, 49.7 $\pm$ 3.0; 5.9, 127 $\pm$ 10; 6.7, 201 $\pm$ 15; 7.7, 189 $\pm$ 22. This indicates that maintenance of cultures between pH 6.7 and 7.7 gave the highest counts of viable organisms.

Discussion

The main objective of the present study has been attained, namely, to develop an improved medium composed of readily available materials together with information concerning fermentation conditions which would give consistently high reproducible counts of *S. marcescens*. For inoculum, the employment of frozen and thawed whole cultures was important in obtaining consistent results. Their use necessitates four serial transfers: from tube of thawed culture to flask, to small-scale fermentor, to seed tank, and to large fermentor. The use of 5 ml samples was sufficient to inoculate four 500-ml flasks and conserve the supply of stabilized, lyophilized stock culture. This process could be advantageous in securing a better product, or more product, in fermentations wherein more than four transfers of the organism results in colonial variation and decreased yield.

The results of these studies offer further evidence of the importance of an adequate supply of oxygen, as well as other required nutrients, to obtain maximal cell yields of aerobic microorganisms. In the case of *S. marcescens* B-1481, the laboratory investigations were readily scaled up to 10-L volumes in 20-L fermentors. The information gained in the present study enabled us to prepare uniform cell concentrates for subsequent studies on a variety of chemicals as potential stabilizers in freeze-drying.

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Summary

A very marked requirement for abundant aeration in the production of maximum counts of viable cells of *Serratia marcescens* was demonstrated.

Counts of *Serratia marcescens* NRRL B-1481 ranging up to 260 billion cells per ml were obtained with a skim milk powder, protopeptone, glucose medium which was highly oxygenated and incubated at 28 C.

Frozen and thawed whole cultures used as inocula gave reproducible fermentations. This technique should be of value in the propagation of other aerobic microorganisms and should be of value, generally, in aerobic fermentations to insure a uniformly high yield of end product.

Sucrose, maltose, mannitol, and glycerol as well as glucose supported good growth of strain *Serratia marcescens* B-1481. Added lactose, xylose, and soluble starch were very poorly used, if at all, for growth.

Maintenance of cultures between pH 6.7 to 7.7 in vat fermentors gave high counts of viable cells.

Studies on the depressing effect of antifoam agents on the oxygen absorption rate indicate that minimal amounts of antifoam should be added to fermentors during fermentation.

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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 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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[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.