Dialysis Fermentor Systems for Concentrated Culture of Microorganisms

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ABSTRACT

GALLUP, D. M. (The University of Michigan, Ann Arbor), AND PHILIPP GERHARDT. Dialysis fermentor systems for concentrated culture of microorganisms. Appl. Microbiol. **11**:506–512. 1963.—Cell-mass production of *Serratia marcescens* was studied in dialysis systems in which growth was managed in a fermentor remote from, but connected by conduits and pumps with, a nutrient reservoir. Dialysis was accomplished with membrane tubing in either the fermentor or the reservoir, or best with membrane sheet in a plate-and-frame dialyzer that was remote from but connected with both vessels. Growth trials with these systems demonstrated their ability to produce virtually unlimited population density in a liquid culture, viable counts in excess of 10^{12} cells/ml and partial cell volume of 50% being attained. The system used for growth also may be used, after the growth cycle, to concentrate cells still further by osmotic dehydration with a hydrophilic colloid. The dialyzer-dialysis system that was evolved permits independent control of the component operations and is believed to be adaptable to any desired scale of size.

Existing dialysis culture methods have been limited in scale and control of operation, aeration-agitation, and membrane applicability. Concurrently with the development of a laboratory shaker flask (Gerhardt and Gallup, 1963), we therefore sought to design and test pilot fermentor systems in which the potential advantages offered by semipermeable membranes might be more fully realized. Cell-mass production of a representative aerobic bacterium, *Serratia marcescens* strain 8-UK, was studied.

The key to a successful dialysis culture process was found in the concept of carrying out growth in a fermentor region remote from but integrated with a nutrient-reservoir region; best results were obtained with the dialyzer also in a separate region. This separation of the principal regions makes it possible for each operation to be controlled independently and more efficiently. For example, vigorous agitation may be applied to the culture in the fermentor without affecting the membrane, or nutrient may be supplied to the culture solely by dialysis. Moreover, with the separate dialyzer concept, the process can be adapted to any desired scale of size: the vessels used for the culture or nutrient supply may be of any size or design, provided only that they can be connected with conduits; with a plate-and-frame dialyzer, any dialysis capacity may be attained simply by adjusting the number of chambers used. Furthermore, different types of membranes in sheet form may be employed.

MATERIALS AND METHODS

The media, antifoam, culture procedures, and analyses are described elsewhere (Gerhardt and Gallup, 1963). Cells were grown on either Trypticase Soy Broth (BBL) or a modification of the synthetic medium of Smith and Johnson (1954), with polypropylene glycol (P-2000; Dow Chemical Co., Midland, Mich.) added as an antifoam agent. Inoculations (1% by volume) were made from early stationary-phase, aerated cultures. Samples from the experimental cultures were removed at intervals in the growth cycle and analyzed for the number of cells (both total and viable) and the amount of cell mass [measured as dry weight, deoxyribonucleic acid (DNA), and optical density].

The laboratory fermentors used were of conventional design, with two sets of four-bladed impellors and four vertical baffles. The 5- and 14-liter fermentors were glass (model F-05 and F-14; New Brunswick Scientific Co., New Brunswick, N.J.); the 250-liter fermentor was stainless steel (Access-A-Bilt model; Stainless & Steel Products Co., St. Paul, Minn.). At first, a 20-liter carboy and a 4-gal Pyrex jar were also used, as reservoir vessels. Sterile, metered air was passed into the bottom of the fermentor; temperature was maintained at 30 C by means of a water bath. The circulation of liquid between components of the dialysis system was accomplished through gum-rubber tubing and controlled by peristaltic-action finger pumps (Sigmamotor, Inc., Middleport, N.Y.).

When the dialysis membrane was in the form of tubing, it was spiralled on vertical supports close to the inside diameter of the vessel and held in position by U-shaped retainers. A rubber sleeve device with glass tubing was used to connect the membrane with the rubber tubing. Regular-grade, regenerated cellulose tubing, 0.25 in. in diameter (Visking Co., Chicago, Ill.), was used.

When a dialysis membrane sheet was employed, it was mounted in a plate-and-frame laboratory dialyzer provided with spiral membrane supports (Hi-Sep model; Graver Water Conditioning Co., New York, N.Y.). Alternate

culture and nutrient chambers were connected in parallel, the liquid in both being circulated upwards so as to prevent accumulation of entrained air. Since the dialyzer was made of Lucite, it was pasteurized (at 75 C for 6 hr). The membrane sheets were made by cutting regenerated cellulose tubing, 8.5 in. wide (U-Zephyr; Visking Co.). The comparative properties of this and regular Visking membrane are described elsewhere (Gerhardt and Gallup, 1963). Both types were allowed to become hydrated before use.

Results and Discussion

Fermentor-dialysis system. The first design of a dialysis system, shown schematically in Fig. 1, consisted of a 5-liter conventional fermentor arranged with a 30-ft length of 0.25-in. dialysis membrane tubing coiled around the periphery of the baffles. The ends of this tubing were connected to rubber conduits leading to a 20-liter carboy which served as the medium reservoir. A 12-liter amount of Trypticase Soy medium was charged into the reservoir, and 3 liters were charged into the fermentor. After sterilization, the system was assembled, allowed to equilibrate for 2 hr, inoculated, and put into operation. The reservoir contents were continuously circulated through the tubing in the fermentor at a rate of 130 ml/min, air was supplied to the fermentor at 9 liters/min, and the fermentor agitator was driven at 275 rev/min. In the control situation, the fermentor was managed in the same way but without the dialysis apparatus.

The results are shown as viable-count growth curves in Fig. 2A, and the different analyses made at 48 hr are included in Table 1. In this and subsequent experiments, complete growth curves were analyzed usually by four different methods, but only representative results are presented here. The concentrating effect of dialysis culture was at once evident from the results, and its relationship to the phase of growth was seen to be the same as that observed in a biphasic (Tyrrell, MacDonald, and Gerhardt, 1958) or dialysis flask (Gerhardt and Gallup, 1963).

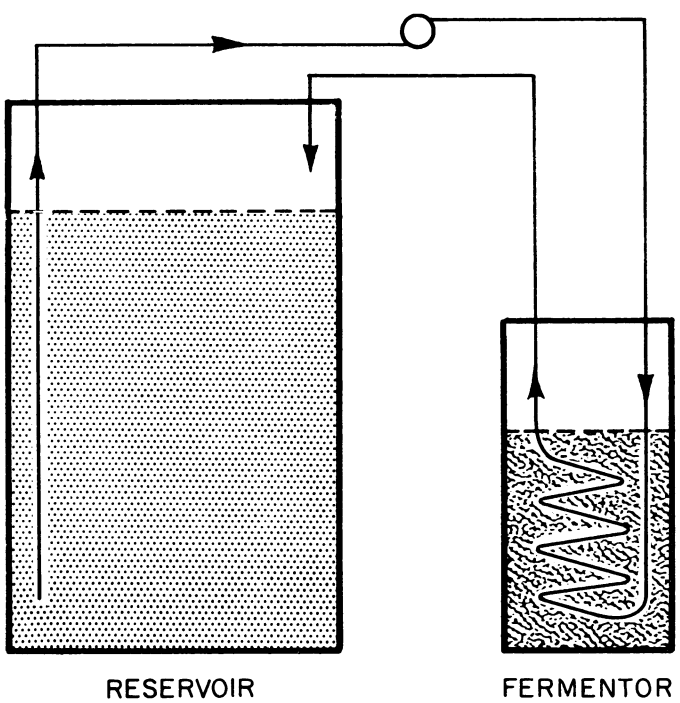

FIG. 1. *Schematic diagram of fermentor-dialysis system.*

In an otherwise similar experiment, Trypticase Soy medium was restricted to the reservoir, only water being put in the fermentor. After overnight equilibration, the system was operated as above. The results (Table 1) show that growth and concentration in a water diffusate and in a complex medium are essentially equal, as shown also in a dialysis flask system (Gerhardt and Gallup, 1963).

When Smith-Johnson synthetic medium was used instead of Trypticase Soy, in both the fermentor and reservoir, the initial pattern of growth remained the same. After a growth peak was reached, however, viability of the control culture decreased, whereas that of the dialysis culture was sustained (Fig. 2B and Table 1).

The equal rates of multiplication in the dialysis and control situations indicated that the dialysis exchange-diffusion rate of nutrients and products was not limiting. A series of experiments was conducted in which the area of membrane, usually 1830 cm^2, was progressively reduced. Otherwise the conditions were as described above, except that the synthetic medium was used. From the results shown in Fig. 3, it was concluded that dialysis did not appreciably restrict the extent of growth until the membrane surface was reduced by two-thirds. Even then the rate remained unaffected, and the terminal loss in viability observed in control cultures was prevented.

The initial series of experiments with the fermentor-dialysis system thus demonstrated the capability of dialysis culture to effect concentration by extending active growth and to allow simplification of the medium immediately supporting growth. The amount of surface needed with a moderately efficient membrane seemed readily attainable. A limitation of this system, however, became evident when attempts to use greater agitation resulted in breaking of the membrane.

Reservoir-dialysis system. To remove the membrane from the fermentor, another design, shown schematically in Fig. 4, was tested. The 5-liter fermentor was used without any modification. The nutrient reservoir vessel consisted of a 4-gal cylindrical jar fitted with a stainless-steel cover, to which was attached a cylindrical support. Around this support, and held in place by retainers, was a spiral of membrane tubing, through which was circulated culture from the fermentor. The contents of the reservoir were vortex-mixed by means of a magnetic stirrer. Otherwise, conditions were essentially as described above.

This reservoir-dialysis system was employed initially to study the response of dialysis culture to increased agitation. The results (Table 2) showed that the final cell density with dialysis, but not in the control, increased

markedly as agitation (and therefore aeration efficiency) was increased.

Smith and Johnson (1954) showed that media thought to be optimal at limiting oxygen levels are actually suboptimal when the oxygen demand is met. Analysis after the above dialysis experiments with a high agitation rate revealed that the carbon supply had become exhausted. In an effort to extend the concentration attainable with dialysis culture, supplemental feeding was employed together with agitation at 875 rev/min and aeration at a rate of 9 liters/min in the fermentor. In addition to the usual synthetic medium, a concentrate equal to 0.25% complete medium was added to the reservoir at 3, 6, 9, and 12 hr. At 14 hr, an additional concentrate equivalent to 1% glucose was added. Similar additions and conditions were made in a control experiment without dialysis.

The results (Table 3) show that, with dialysis culture, this type of nutrient manipulation was not only feasible but also productive of an extremely high population density, which was unobtainable in the usual method of culture. The viable-cell count, for example, reached 2.5×10^{12}/ml in the dialysis culture. Figure 5 illustrates the terminal results even more strikingly: 50% partial cell volume, and turbidity visible even after diluting the suspension 10,000 times. From the 3 liters of final culture, 1.4 kg of cells could have been harvested by centrifugation.

The degree of culture concentration also can be regulated by the ratio established between the volume of nutrient in the reservoir and the volume of culture in the fermentor, as demonstrated by the results summarized in Table 4. A ratio of 1:10, for example, produced a population density comparable with that described above, together with a somewhat greater substrate-conversion efficiency than was obtained with lower ratios. The rate of multiplication, as

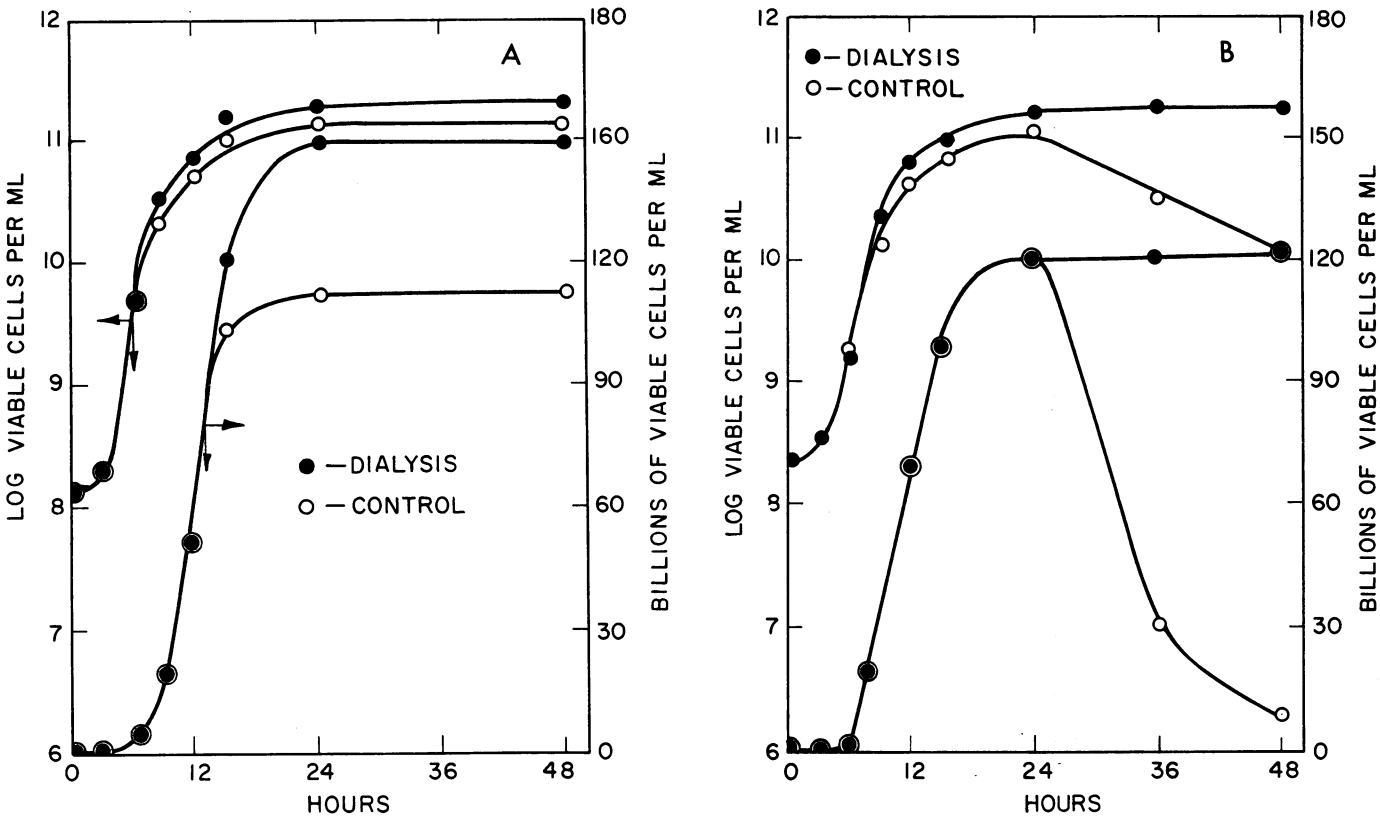

FIG. 2. *Viable-count growth curves for fermentor-dialysis and control cultures in Trypticase Soy (A) and synthetic medium (B). The arrows in this and subsequent graphs indicate which ordinates are applicable to the respective curves.*

TABLE 1. *Effect of differences in medium on cell production in fermentor-dialysis (D) and control (C) cultures (results after 48 hr)*

Medium in fermentor	Viable cells (billions/ml)			Cell mass (mg/ml)					
				Dry wt			DNA		
	D	C	D/C	D	C	D/C	D	C	D/C
Trypticase Soy Broth	340	120	2.8	34.2	12.7	2.7	2.7	0.80	3.8
Water diffusate of Trypticase Soy Broth	290	120	2.4	29.8	10.5	2.8	2.6	0.80	3.7
Smith-Johnson synthetic medium	170	120	1.4	32.6	7.6	4.3	2.9	0.85	3.5

evidenced in the growth curves (not shown), also was affected, being greatest with the 1:10 ratio.

It has become evident (Kohn, 1959; Flodin, Gelotte, and Porath, 1960) that a protein solution within a sac of dialysis membrane can be osmotically dehydrated if the sac is placed in contact with a hydrophilic colloid such as polyethylene glycol or dextran. In preliminary tests, the principle also was found applicable to bacterial suspensions. Consequently, after the conclusion of the growth cycle in the reservoir-dialysis system, the spent medium was withdrawn from the reservoir and replaced with a thick slurry of polyethylene glycol (molecular weight, 20,000; Union Carbide Chemicals Co., New York, N.Y.). As the cell suspension continued to be circulated, it lost water and became greatly concentrated, to a point that pumping was no longer possible. Although slow, such osmotic dehydration provides a useful and safe method for terminally concentrating microorganisms without the need for centrifugation and additional equipment.

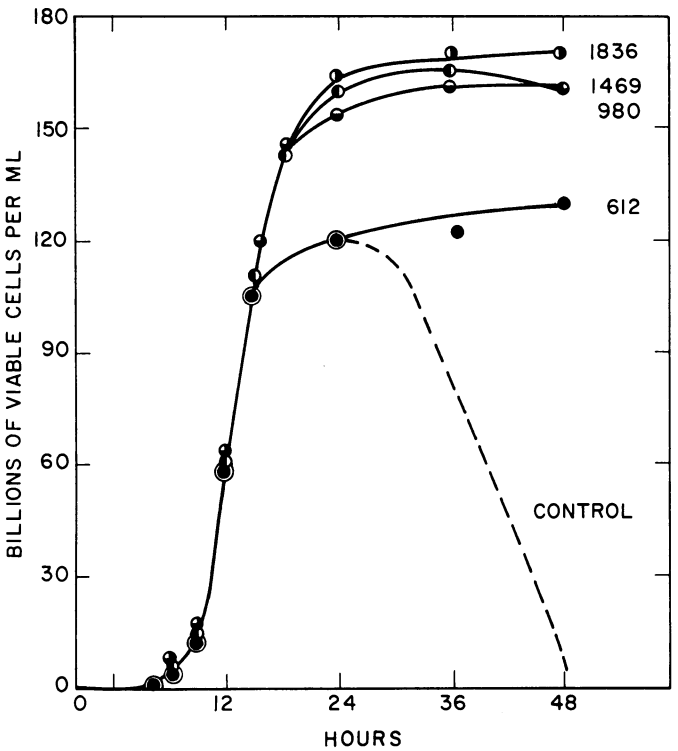

FIG. 3. *Viable-count growth curves for fermentor-dialysis cultures with varying area of membrane. Numbers indicate cm² of exposed surface. The broken line is an extrapolation based on other results.*

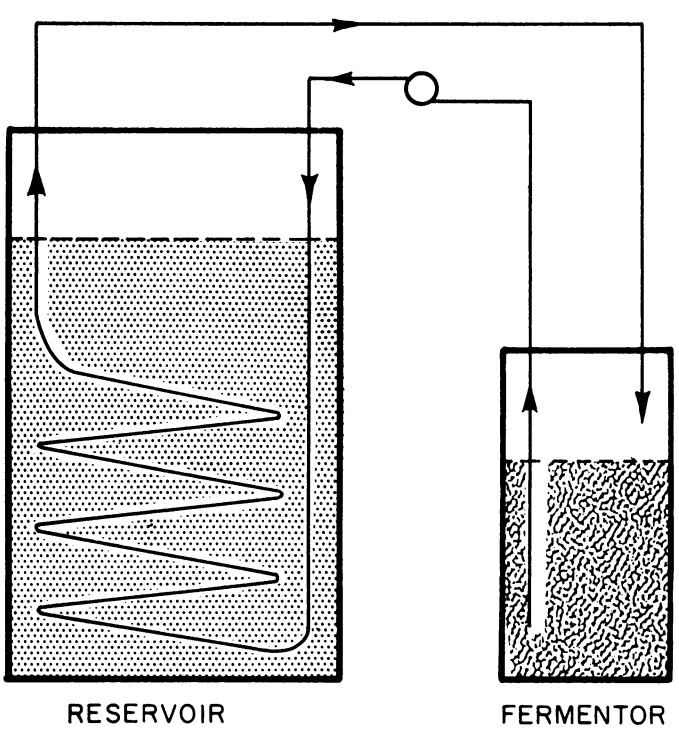

FIG. 4. *Schematic diagram of reservoir-dialysis system.*

This series of experiments with the reservoir-dialysis system thus further demonstrated the capability of dialysis culture to produce an extremely high population density by manipulation of the nutrient and oxygen supply during the growth cycle. Afterward, if needed, the system can be used to concentrate the cells still further by means of osmotic dehydration with a hydrophilic colloid.

Still, both of the above systems had inherent limitations in the control of each component operation, the use of the process in any desired scale of size, and the application of different types of membranes.

Dialyzer-dialysis system. To overcome these limitations, all three regions—fermentor, reservoir, and dialyzer—were separated. The design that evolved is diagrammed in Fig. 6. In this system, the fermentor (5 liters) and the

TABLE 2. *Effect of fermentor agitation rate on cell production in reservoir-dialysis (D) and control (C) cultures (results after 48 hr)*

Agitator speed (rev/min)	Viable cells (billions/ml)			Cell mass (mg/ml)					
				Dry wt			DNA		
	D	C	D/C	D	C	D/C	D	C	D/C
170	170	120	1.4	32.6	7.6	4.3	2.95	0.85	3.5
550	240	140	1.7	32.0	8.3	3.8	3.00	0.90	3.3
875	350	140	2.5	63.3	13.4	7.1	6.10	1.25	4.9

TABLE 3. *Effect of supplemental feeding on cell production in reservoir-dialysis (D) and control (C) cultures*

Age of culture	No. of cells (billions/ml)				Cell mass (mg/ml)			
	Viable		Total		Dry wt		DNA	
	D	C	D	C	D	C	D	C
hr								
0	0.35	0.36	0.68	0.80				
3	5.5	4.2	10	10				
6	50	15	95	31				
9	100	45	160	80	18.2	7.0	1.75	.55
12	250	80	400	170	23.6	8.0	2.5	.75
24	1300	190	2100	300	83.1	15.8	7.9	1.60
48	2500	50	5600	260	148	14.7	13.1	1.43

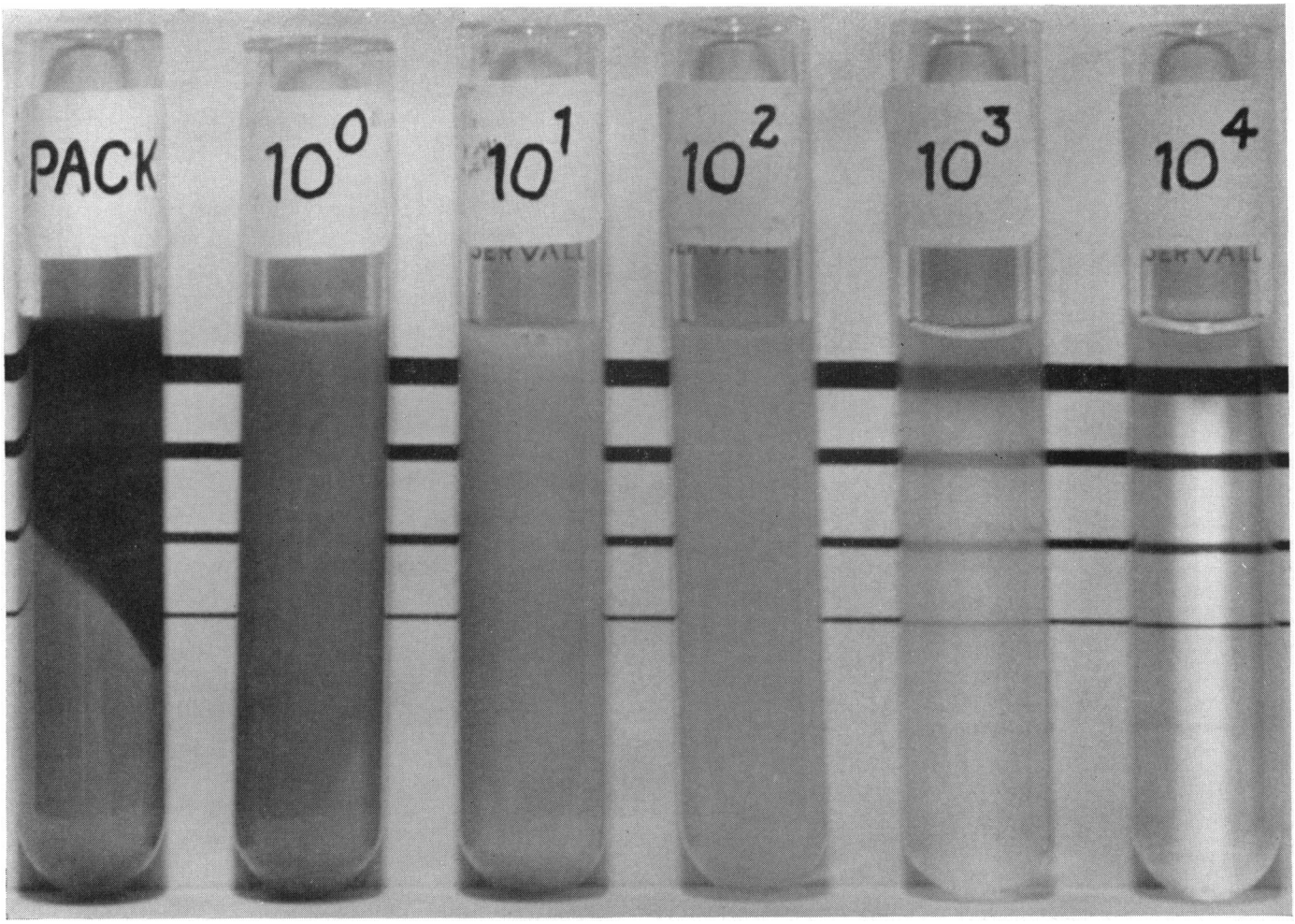

FIG. 5. *Terminal results of reservoir-dialysis culture with supplemental feeding. Left tube: centrifuged sample, showing 50% partial cell volume. Right five tubes: dilution series of sample, showing turbidity through 1:10,000 dilution.*

TABLE 4. *Effect of fermentor-reservoir volume ratio on reservoir-dialysis culture (results after 30 hr)*

Fermentor-reservoir ratio	Volume (liters)		No. of cells (billions/ml)		Cell mass (mg/ml)		Substrate conversion*
	Fermentor	Reservoir	Viable	Total	Dry wt	DNA	
1:0 (control)	3	0	90	240	8.3	0.76	18.6
1:2	3	6	190	340	18.2	1.90	14.7
1:4	3	12	350	525	32.0	3.00	15.2
1:6	2	12	650	1290	53.5	5.25	18.0
1:8	2	16	700	1390	68.3	6.55	18.3
1:10	1.5	15	1340	2100	91.9	8.95	20.0

* Substrate conversion = mg (dry weight) of cells per 100 g of total substrate supplied.

nutrient reservoir (14 liters) both were of conventional fermentor design without any special modification. Remote from these vessels but connected with them by conduit and pumps was a plate-and-frame dialyzer containing two chambers for sterile medium and one for culture, and

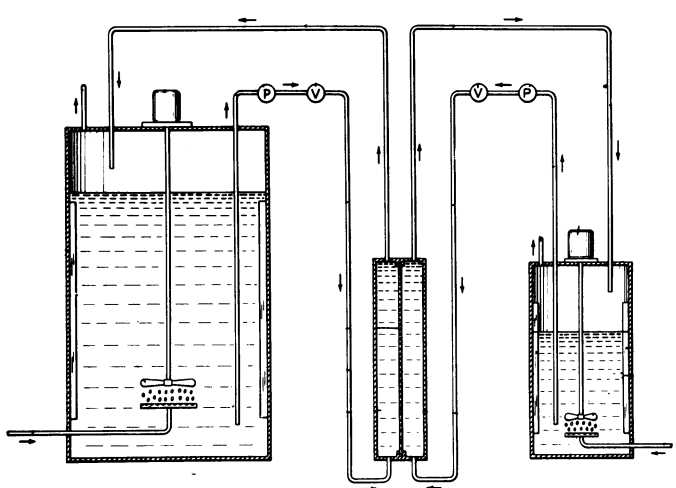

FIG. 6. *Diagram of dialyzer-dialysis system. The nutrient reservoir is on the left, the dialyzer in the center, and the culture fermentor on the right. P designates a pump, and V designates a valve. The arrows show the direction of circulation of medium and culture through the connecting conduits.*

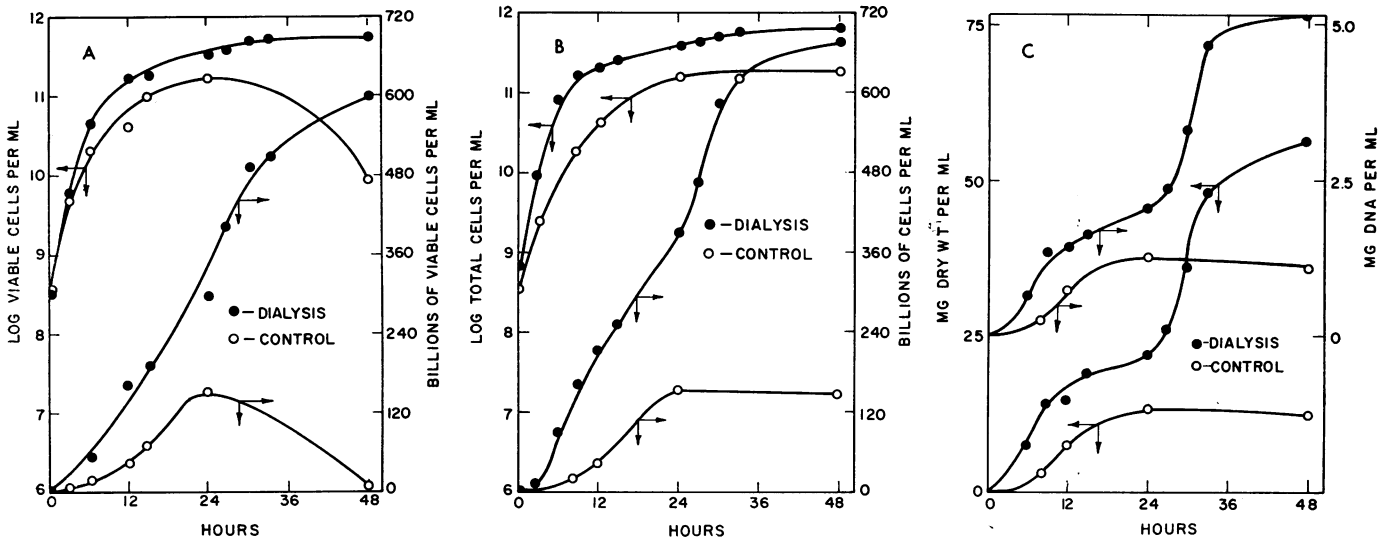

FIG. 7. *Growth curves for dialyzer-dialysis and control cultures in 3-liter fermentor with 12-liter reservoir, as measured by viable cell count (A), total cell count (B), and dry matter and DNA content (C).*

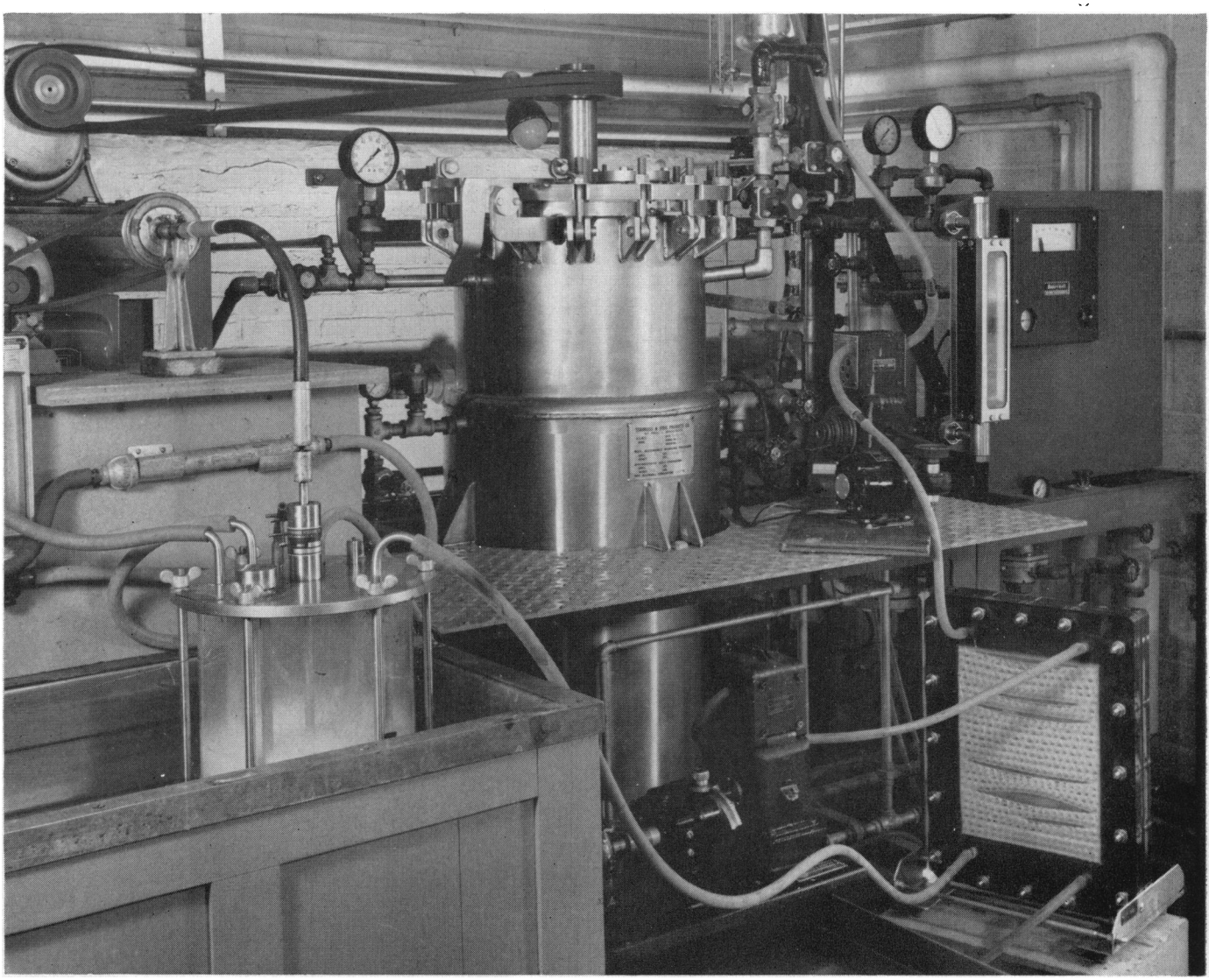

FIG. 8. *Experimental assembly of dialyzer-dialysis system with 10-liter fermentor (left) and 100-liter reservoir (center). The plate-and-frame dialyzer is seen at the right.*

two membranes with an area of about 800 cm² each. Medium and culture were circulated cocurrently upward through the dialyzer at a rate of 130 ml/min. The fermentor agitator was driven at 875 rev/min, and the reservoir agitator at 350 rev/min. Sterile air flowed through the reservoir vessel at a rate of 9 liters/min and, thus humidified, passed on to the fermentor. A 3-liter amount of the synthetic medium was put in the fermentor, and 12 liters were put in the reservoir (a ratio of 1:4).

In Fig. 7 is shown the course of such a dialyzer-dialysis culture of *S. marcescens* over a 48-hr cycle, with growth assayed as the number of cells (both viable and total) and the amount of cell matter (both dry weight and DNA). The results revealed that the growth pattern for both viable cells (Fig. 7A) and total count (Fig. 7B) was much as observed before in dialysis culture. However, a higher level was attained than was expected for this fermentor-reservoir ratio, and the population continued to increase over an extended period of time. Moreover, the rate of growth now was greater than in the control situation. A remarkable, but unexplained, two-stage curve also became evident in the dialysis culture when the amount of cell matter was plotted (Fig. 7C).

The capacity of this dialyzer-dialysis system for independent control of each operation and for use in any desired scale of size was demonstrated in another experiment. The system was adapted to a fixed stainless-steel fermentor with a volume of about 250 liters. Used as the reservoir, this vessel was charged with 100 liters of the synthetic medium, maintained at 25 C, aerated with 50 liters/min of air, and agitated at a rate of 300 rev/min. The fermentor in this system was the 14-liter glass unit charged with 10 liters of medium, for a fermentor-reservoir ratio of 1:10. The fermentor was maintained at 30 C, aerated with 20 liters/min of air, and agitated at a rate of 875 rev/min. The difference in operation of the reservoir and fermentor was deliberate to demonstrate the feasibility of controlling each component independently. Medium from the reservoir and culture from the fermentor were continuously circulated through tubing to the dialyzer, both at a rate of about 500 ml/min. The dialyzer was assembled and left without temperature regulation, as in the preceding experiment. The experimental assembly is pictured in Fig. 8.

The results of this trial generally were similar to and confirmed those obtained with the smaller assembly, although an increase in both the rate and extent of growth was expected because of the 1:10 fermentor-reservoir ratio. The lack of change may reflect limitation in diffusion because of insufficient membrane surface.

The experiments with the dialyzer-dialysis system thus demonstrated the attainment of a process inherently capable of independent control of the component operations, of adaptation to any desired scale of size, and of use of membranes in sheet form. The process also seems capable of achieving the results obtained with the preliminary dialysis fermentor systems and a dialysis flask (Gerhardt and Gallup, 1963).

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LITERATURE CITED

FLODIN, P., B. GELOTTE, AND J. PORATH. 1960. A method for concentrating solutes of high molecular weight. Nature **188:** 493–494.

GERHARDT, P., AND D. M. GALLUP. 1963. Dialysis flask for concentrated culture of microorganisms. J. Bacteriol. **86:**919–929.

KOHN, J. 1959. A simple method for the concentration of fluids containing protein. Nature **183:**1055.

SMITH, C. G., AND M. J. JOHNSON. 1954. Aeration requirements for the growth of aerobic microorganisms. J. Bacteriol. **68:** 346–350.

TYRRELL, E. A., R. E. MACDONALD, AND P. GERHARDT. 1958. Biphasic system for growing bacteria in concentrated culture. J. Bacteriol. **75:**1–4.