DIALYSIS FLASK FOR CONCENTRATED CULTURE OF MICROORGANISMS

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Received for publication 27 May 1963

ABSTRACT

GERHARDT, PHILIPP (The University of Michigan, Ann Arbor), AND D. M. GALLUP. Dialysis flask for concentrated culture of microorganisms. J. Bacteriol **86**:919–929. 1963.—A twin-chambered dialysis flask was designed with a supported membrane clamped between a reservoir of medium in the bottom and a small volume of culture above, the unit being mounted on a shaking machine to provide aeration and agitation. The performance of different dialysis membranes and membrane filters was compared in glucose-diffusion and bacterial-culture tests. Some of the variables in dialysis culture were assessed and the growth response was characterized, with *Serratia marcescens* as the test organism. The general usefulness and concentrating effect of dialysis culture were demonstrated in trials with 16 representative types of microorganisms. Dialysis culture was shown to be especially suitable for producing dense populations of cells or their macromolecular products in an environment free from complex medium constituents, for removing toxic products that limit growth or fermentation, and for supplying oxygen by diffusion without the damage from usual aeration procedures.

Metchnikoff, Roux, and Salimbeni (1896) pioneered the use of semipermeable membranes for growing microorganisms when they implanted collodion sacs containing cholera vibrios in the peritoneal cavity of animals to establish the existence of a soluble toxin, to enhance virulence, and to study antitoxic serum therapy. Other in vivo applications of dialysis culture followed (e.g., Nocard and Roux, 1898; Nocard, 1898; Novy, 1899; Olitsky and McCartney, 1923; Ogg et al., 1958).

Extension of dialysis culture to in vitro uses was reported independently from three laboratories. Carnot and Fournier (1900) employed a collodion culture sac suspended in a flask containing ordinary medium, so as to enhance virulence and obtain toxin from pneumococci. Ruffer and Crendiropoulo (1900) described a similar arrangement and mentioned possible uses. Frost (1904) exploited the technique to study the antagonism between typhoid bacilli and soil or water organisms, observing what now would be called antibiotic effects.

Although membranes were thereafter employed in various ways (M'Ewen, 1926; Lewis and Lucas, 1945; Harmsen and Kolff, 1947; Wentzel and Sterne, 1949; Gorelick, Mead, and Kelly, 1951), essentially a small sac or tube of culture was suspended in a large reservoir of medium, with diffusion bringing nutrients to the culture and simultaneously carrying away metabolic products. Besides occurring through a membrane, dialysis also takes place when a solute is diffused across an interface. Applying this principle to microbial propagation, Tyrrell, MacDonald, and Gerhardt (1958) devised a simple biphasic system which consisted of a shaker flask containing a base of agar-solidified medium overlaid with a small volume of broth culture.

In addition to the aforesaid uses, the principal result of membrane or interface dialysis culture is a concentration of the cell population during growth, extraordinary densities becoming obtainable. With the agar-liquid system, furthermore, increases in the total yield of cells may occur (Tyrrell et al., 1958). Macromolecular products also accumulate, such as an extracellular enzyme (Hestrin, Avineri-Shapiro, and Aschner, 1943; Rogers, 1948) or toxin (Fredette and Vinet, 1952). Moreover, if the constituents of a complex medium are incorporated into the reservoir of a membrane system or into the agar base of a biphasic flask, water or a simple solution may be used in the culture zone. Thus, the medium immediately supporting growth can be markedly simplified, and clean cells or macromolecular products can be obtained. The principle has been applied, for example, to the production of fastidious bacteria (M'Ewen, 1926; Gerhardt and

Hedén, 1960), mammalian-cell suspensions (Eagle, 1960), bacterial exotoxins (Polson and Sterne, 1946), and spores (Tyrrell, 1962; Schneider, Grecz, and Anellis, 1963). Dialysis culture also has been used for symbiotic propagation (Ritter, 1949; Nurmikko, 1957). Another feature of dialysis culture is the ability to provide air indirectly by allowing it to diffuse through the membrane, without the danger of denaturation by gassing or the toxic effects of antifoam agents (Gladstone, 1948). Still another result is a sparing effect during the terminal phases of the growth cycle: the period of active multiplication is extended (Tyrrell et al., 1958), viability is sustained both in the culture and when cells are washed and stored (Tyrrell, 1962), autolysis is allayed (Gladstone, 1948; Barron and Reed, 1954; Tyrrell, 1962), and spore formation may be prevented (M'Ewen, 1926; Gladstone, 1948).

The demonstrated and potential usefulness of dialysis culture is limited in existing systems, however, by restrictions on their capability for expansion in size and on the aeration-agitation necessary to support extremely dense populations. Accordingly, we sought to design, construct, and test new systems to implement these practical needs and to provide ready means for examining principles. Described below is a membrane-dialysis flask suitable for laboratory investigations and relatable to the pilot fermentor systems developed concurrently (Gallup and Gerhardt, 1963). The flask was used to test membranes, study variables and growth curves, compare the growth of various microorganisms, and demonstrate some of the unique results obtainable with dialysis culture.

MATERIALS AND METHODS

Figure 1 shows the dialysis flask assembled and disassembled. It was designed for mounting on a shaking machine and with provision for clamping a supported membrane between its two glass chambers. In use, the lower reservoir is filled with medium which is stirred by the rotation of a steel ball and is accessible through a rubber diaphragm. The upper chamber contains a small volume of culture, aerated and agitated by baffled swirling, and accessible through a top opening.

The chambers consist of two stock fittings of Pyrex pipe 4 in. in diameter, clamped together by standard metal flanges. Other sizes of glass pipe could also be used. The upper chamber at first was made from a pipe cap with an opening in which a cotton plug could be inserted. The design later was improved by using a 4-in. × 1.5-in. pipe reducer (Fig. 1), the neck of which prevents splashing on the closure and makes possible the use of cotton-gauze pads for better aeration. A 5-in. circle of membrane is positioned between the chambers and supported above and below by 4.5-in. circular plates, made from a perforated stainless-steel sheet ($\frac{1}{32}$ in. thick, $\frac{3}{32}$-in. staggered holes on $\frac{5}{32}$-in. centers, 32% open). Silicone- or gum-rubber gaskets, $\frac{1}{32}$ in. thick, seal adjacent components and also space the membrane apart from the supporting plates, so as to prevent perforating the membrane and to permit bathing it completely with liquid. On the support plate in the upper chamber are mounted baffles to produce turbulence. It was discovered that perforations in the baffles enhance their effectiveness. Initially four baffles were used, but experimentation proved that best results occurred with a single baffle positioned at an angle of 40° to the tangent of the wall circumference.

The lower chamber has a drilled 0.5-in. hole in which is fitted a flanged vaccine-bottle stopper, which allows access by a hypodermic needle. A 1-in. stainless-steel ball rotates freely in the bottom, or can be suspended from the lower support plate, to provide mixing of the reservoir contents. This feature permits use of a deep reservoir of medium without limiting the rate of diffusion, which is not possible in the biphasic flask system (Tyrrell et al., 1958). The assembled flask is fixed to an angle-iron carriage, and the unit is bolted on a rotary shaking machine, operated at 200 rev/min through a 1-in. stroke.

To use the flask, the bottom chamber is charged with medium constituents made up to 950 ml, just less than capacity. With some media or organisms, it is necessary to add an antifoam agent such as polypropylene glycol (P-2,000, Dow Chemical Co.) at a final concentration of 50 μg/ml. The flask components are then assembled and carefully positioned in ascending order, but the metal flanges are not tightened. Dialysis membranes must be hydrated and then kept moist. If charged with hot liquid, the flask can be sterilized in 20 min at 120 C. The flanges are then drawn up evenly. Afterward, separately sterilized medium or water is added by syringe to fill the bottom chamber except for an expansion bubble

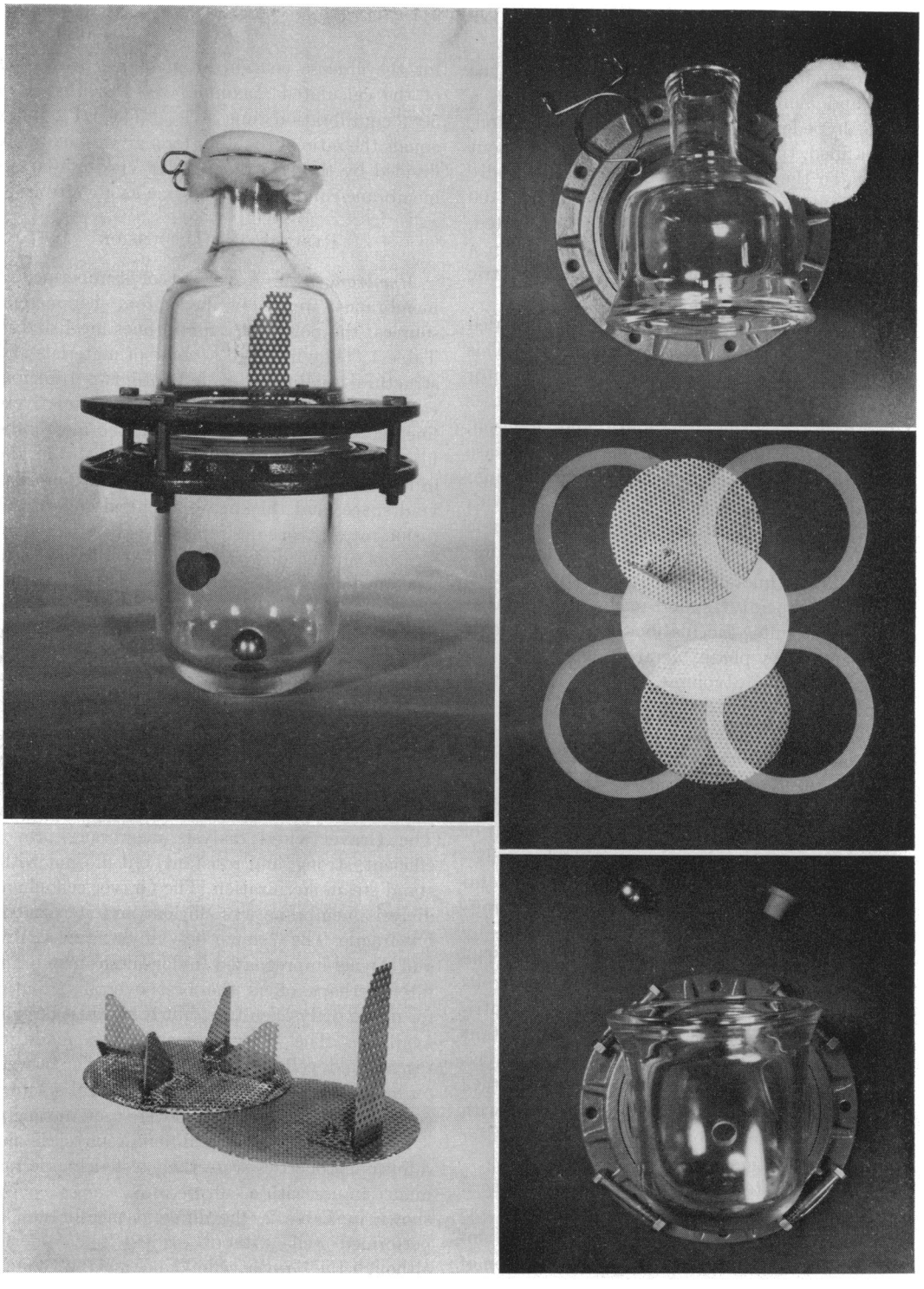

FIG. 1. *Dialysis flask. Top left: complete assembly. Bottom left: two types of baffled support plate used in top chamber. Right: disassembled parts (bottom to top)—bottom chamber and flange with agitator ball and sampling diaphragm, bottom support plate and gaskets, membrane, top baffled support plate and gaskets, and top chamber and flange with cotton closure and spring retainer.*

of a few milliliters accomplished by tipping the flask and venting the displaced air through a sterile hypodermic needle plugged with cotton. When filled, the bottom chamber contains about 1 liter. To the top chamber is added aseptically the desired amount of sterile liquid, usually 100 ml of water. When the contents have equilibrated, ordinarily overnight, the flask is mounted on a shaker, the steel ball is rocked to an eccentric orbit, and the unit is set in motion.

Culture tests in the flask usually were carried out with *Serratia marcescens* strain 8UK. This strain was used in our related dialysis fermentor work, and its nutrition and aeration responses have been characterized (Smith and Johnson, 1954). Cells were grown either on Trypticase Soy Broth (BBL) or on the synthetic medium formulated by Smith and Johnson (1954), with dibasic ammonium citrate substituted for citric acid in the latter medium. Cultures were incubated in a humidified room at 30 C. Other bacteria, temperatures, and media are indicated below in Results. Inoculations were made from early stationary-phase, aerated cultures in an amount of 1% by volume, equal to a starting population of about 500 million viable cells per ml. Control flasks had a sheet of gum rubber in place of the membrane, and the same volume of medium in the upper chamber. Samples were removed at intervals and assayed for the number of cells, both total and viable, and the amount of cell matter, measured as dry weight, deoxyribonucleic acid (DNA), and optical density. Total counts were made conventionally with a Petroff-Hausser slide, and viable counts were made by streak-plating on Trypticase Soy Agar. The amount of cell matter was measured with cells washed one time with distilled water in a centrifuge, either by oven-drying at 105 C to constant weight or by analyzing DNA by the diphenylamine method of Seibert (1940). The optical density of cell suspensions was determined with a Klett colorimeter, by use of a 420-mμ filter and a 1:10 dilution of the culture.

For diffusion tests, 250 ml of water were placed in the unbaffled upper chamber, and glucose was added to the filled lower chamber of an unsterilized flask to give a starting concentration of 0.15%. The flask then was shaken at 30 C and diffusate samples were removed from the upper chamber at intervals for analysis by the conventional anthrone reaction. The time required for the glucose concentration to reach one-half of the calculated maximum was recorded as a 50% equilibration time (ET_{50}). The ET_{50} Index equals the ratio of the ET_{50} for a given membrane divided by the ET_{50} for regular Visking dialysis membrane run at the same time as a control.

RESULTS AND DISCUSSION

Membrane tests. A variety of semipermeable membranes were obtained from commercial sources; the potentially useful ones are listed in Table 1. They included a range of materials and structural qualities but fell into two principal categories: dialysis membranes with a relatively fine porosity in the order of 3 mμ, and membrane filters with a relatively coarse porosity in the order of 300 mμ. Although membrane porosity, void space, and thickness in general govern diffusion rates, it was desirable to make direct comparisons in the dialysis flask with glucose as a test substance.

The results of the diffusion tests demonstrated a wide variation in rates (Table 1). Among membranes of a given type, such as the ultrafine cellulose series, the rate of glucose diffusion corresponded to porosity. Certain of the bacteria-withholding membrane filters provided rapid diffusion; their fragility and cost, however, may limit their use. All of the cellulosic membranes potentially are susceptible to bacterial digestion. The Graver vinyl dialysis membrane proved efficient, strong, and resistant, but did not withstand steam sterilization. The Graver cellophane dialysis membrane was efficient and sterilizable, but fragile. The Gelman polyvinylacetate (PVA) and Dynel-impregnated nylon-mesh membrane filter perhaps offers the best overall potential for use in dialysis culture, but it had just become available. The dialysis membranes made of regenerated cellulose (Visking "regular") were on hand from the start, embodied a satisfactory combination of qualities, and were used routinely.

Membranes possessing different physical and diffusion properties were then tested for performance in use with a culture of *S. marcescens*. As shown in Table 2, the different membranes all performed well, without evident toxic effects, although the Graver cellophane and the Gelman PVA-Dynel-nylon membranes supported the highest yields. These experiments also demonstrated that concentration during growth does in fact take place in a dialysis flask and that a

TABLE 1. *Glucose diffusion through different membranes tested in the dialysis flask*

Material	Distributor	Trade designation	Pore diam*	ET_{50}	ET_{50} index
Regenerated cellulose	Visking Co., Chicago, Ill.	Regular	<0.003	125–150	1.00
		U-Zephyr	<0.003	143	1.1
		Exptl N or C	<0.003	90	0.60
Cellulose-nitrate	Schleicher & Schuell Co., Keene, N.H.	Membrane Coarse	3.0–0.75	35	0.23
		Membrane Medium	0.75–0.5	180	1.20
		Membrane Dense	0.5–0.2	240	1.60
Cellulose-acetate	Schleicher & Schuell Co.	UF Coarse	0.2–0.08	40	0.27
		UF Medium	0.08–0.05	100	0.67
		UF Dense	0.05–0.01	165	1.10
		UF Very Dense	0.01–0.005	195	1.30
		UF Super Dense	<0.005	270	1.80
Cellophane	Graver Water Conditioning Co., New York 11, N.Y.	Hi-Sep	~0.003	83	0.67
Vinyl	Graver Water Conditioning Co.	Hi-Sep-70	0.007	90	0.60
Cellulose-nitrate	Gelman Instrument Co., Ann Arbor, Mich.	Membrane AM-5	0.7	15	0.10
		Membrane AM-7	0.3	62	0.50
		Membrane AM-8	0.2	45	0.30
Cellulose-acetate-nitrate	Gelman Instrument Co.	Membrane GM-8	0.2	>360	>2.9
Cellulose-paper	Gelman Instrument Co.	Process 1119	0.4	>360	>2.4
		Process 1121	0.2	>360	>2.4
Polyvinyl alcohol-Dynel-nylon	Gelman Instrument Co.	Acropor 6408	0.2	71	0.57
Dynel-nylon	Gelman Instrument Co.	Acropor 6427	~0.1	86	0.69
Resin-nylon	Gelman Instrument Co.	Acropor WA 6402	0.1	65	0.43
		Acropor WB 6403	0.1	200	1.33
Teflon-glass	American Machine & Foundry Co., Springdale, Conn.	AMFAB TX-40-H-70	1–70	>360	>2.4
Plastic	S. C. Johnson & Son, Inc., Racine, Wis.	Porelon	>0.3	>360	>2.4
Asbestos	F. R. Hormann Co., Inc., Newark 4, N.J.	D6	0.50	>360	>2.4
		D7	0.10	>360	>2.4
		D8	0.05	>360	>2.4
Unglazed porcelain	Selas Corp. of America, Dresher, Pa.	No. 015	<1.4	45	0.30
		No. 03	<0.6	>360	>2.4
Porous stainless steel	Micro Metallic Corp., Glen Cove, N.Y.	Grade H, 1/16 in.	5	>360	>2.4
Sintered glass	Corning Glass Works, Corning, N.Y.	Medium, 5/16 in.	10	10	0.07
		Fine, 5/16 in.	15	15	0.10
Agar-impregnated sintered glass	Corning Glass Works	Medium, 5/16 in.	~0.001	>360	>2.4

* Provided from distributors' data.

bacterium can readily be grown in an equilibrium diffusate of a complex medium. Consequently, it became routine practice to use only water in the culture chamber and to restrict medium to the reservoir.

Variables and growth response. The importance of adequate aeration and agitation was evidenced from changes in design of the flask and the resulting increases in the growth response of *S. marcescens*. Best results were attained by using a wide-mouth top chamber, a cotton-pad closure, and a single perforated baffle positioned at an acute angle to flow. This arrangement resulted in an oxygen-transfer rate (Cooper, Fernstrom, and Miller, 1944) of about 1 mmole of O_2 per liter per hr and concentrations (Table 2) up to 1.66×10^{11} viable cells per ml, depending on the membrane and other conditions employed.

At this level of cell density and oxygen supply, the usual provision of nutrient became limiting. If the strength of Trypticase Soy or synthetic medium in the reservoir was doubled, still greater cell concentration was achieved, up to 3.3×10^{11} viable cells per ml after 48 hr of incubation. Relatively little further increase in concentration was attained, however, even with a combination of measures, including the use of double-strength Trypticase Soy medium, humidified and sterilized air bled directly into the medium, shaking at a rate of 240 rev/min, a 1:10 culture-reservoir ratio, and an efficient PVA-Dynel-nylon membrane filter. The apparently maximal results after 48 hr of incubation in the dialysis flask (compared with control results in parentheses) were 4.0×10^{11} (1.4×10^{10}) viable cells per ml, 4.1×10^{11} (6.2×10^{10}) total cells per ml, 33.8 (4.9) mg (dry weight)/ml, and 0.88 (0.57) mg of DNA per ml.

Possible limitation of growth by the rate of nutrient diffusion was studied in a series of experiments in which the exposed area of Visking regular dialysis membrane was masked (Table 3). Under such ordinary conditions, the rate and extent of growth became restricted only when the effective membrane area was reduced by more than 30%. With extraordinary conditions and high cell concentrations, however, the need for the entire surface and also a more efficient membrane became evident, as shown above.

The degree of concentration of a culture in the dialysis flask could be regulated simply by restricting the volume in the top chamber, as shown by the results in Table 4. The amount of nutrient in each instance was maintained constant, since only water was added in the culture chamber. A relationship between culture concentration and the ratio of culture-reservoir volume was evident.

TABLE 2. *Performance of selected membranes with concentrated culture of Serratia marcescens in the dialysis flask (results after 48 hr)*

Series	Membrane	No. of cells (billions/ml)		Cell mass (mg/ml)	
		Viable	Total	Dry wt	DNA
1*	Visking cellulose, regular	90	125	9.5	1.10
	Gelman cellulose ester, AM-8	80	120	9.8	0.98
	Gelman cellulose ester, process 1119	95	98	8.7	0.80
	Gelman resin-nylon, WA 6402	90	90	8.5	0.80
	Graver vinyl, Hi-Flex	80	85	9.1	0.85
	Rubber blank (250-ml control)	20	28	1.9	0.18
	No membrane (1250-ml control)	10	25	1.9	0.15
2†	Visking cellulose, regular	100	150	14.1	1.24
	Graver cellophane	145	390	20.9	1.40
3†	Visking cellulose, regular	72	108	9.2	—
	Gelman PVA-Dynel-nylon, 6408	166	170	23.2	—
	Gelman Dynel-nylon, 6427	61	98	21.1	—

* In the first test series, the culture chamber contained 250 ml of water and the reservoir contained Smith-Johnson medium. A narrow-mouth flask top with an aeration-restricting cotton plug was used.

† In the second and third test series, the culture chamber contained 100 ml of water and the reservoir contained Trypticase Soy medium. A wide-mouth flask top was used and covered with a cotton pad, providing better aeration.

Above a 1:10 ratio, however, concentration was achieved at the expense of yield, and this ratio was adopted for routine use.

Figure 2 shows representative growth curves of *S. marcescens*, both in dialysis culture and in the control, with changes in cell numbers and mass measured by four different methods. Both exponential and linear plots are shown for the viable- and total-count data. Dialysis culture was observed not to affect the lag period nor the rate of exponential multiplication. The concentrating effect of dialysis culture resulted from a time extension of the exponential phase so that the maximal stationary phase occurred at a higher level. Dialysis culture also prolonged this phase and even effected a gradual rise in viable count. In the control, however, the population viability tended to decline gradually after reaching a peak. These differences illustrated the necessity for complete growth curves in comparing different culture situations.

TABLE 3. *Effect of membrane area on the concentration during growth of Serratia marcescens*

Per cent of membrane area exposed	Per cent of control results at 48 hr*		
	Optical density	Viable count	Dry wt
100 (control)	100	100	100
90	100	96	94
80	97	102	93
70	95	103	90
50	90	60	60
25	60	51	30
13	55	48	25

* Optical density of 1:10 dilution = 2.0. Viable count = 2.45 to 2.66 × 10^{11}/ml. Dry weight = 46.9 to 52.8 mg/ml.

General applicability. The dialysis flask was used in culture tests with a number of microorganisms representative of different types. These results are summarized in Table 5, and instructive viable-count growth curves are shown in Fig. 3. Complete growth curves were analyzed with each organism, however, much as is shown in Fig. 2 for *S. marcescens*.

Altogether, the results demonstrate the general usefulness of dialysis culture as a means of acquiring dense populations in an environment free from complex medium constituents. The densities that were attained varied with different organisms and with the method of measurement. Dry weight gave a fairly comparable parameter, and for most of the organisms averaged about 18 mg/ml in dialysis culture as compared with about 5.6 mg/ml in the control situation. Unlike concentrated culture in an agar-liquid system (Tyrrell et al., 1958), concentration ratios in the dialysis flask never exceeded theoretical values for the culture-reservoir volume ratio used, probably because of the absence of agar-adsorption effects possible in the biphasic flask.

Special applications. The principle of medium simplification was especially well illustrated by the comparatively dense populations of *Haemophilus influenzae* attained simply in water diffusate of the hemoglobin-enriched medium otherwise necessary (Fig. 3a). It was also found that heated whole blood can similarly be employed but not fresh blood, since hemolysis causes diffusion of heme and coloration in the diffusate. The principle would seem to have application in

TABLE 4. *Effect of culture volume in the dialysis flask on the concentration during growth of Serratia marcescens (results after 48 hr)**

Volume of culture	Culture-reservoir ratio	No. of cells (billions/ml)		Cell mass†	Cell yield (trillions/flask)	
		Viable	Total		Viable	Total
ml						
250 (control)	1:0	8.5	16.5	0.22	2.1	4.1
500	1:2	13	25	0.33	6.5	12.5
250	1:4	46	75	0.50	11.5	18.8
100	1:10	200	220	1.20	20.0	22.0
50	1:20	139	220	1.35	7.0	11.0

* Experimental conditions as in first footnote of Table 2. Regular Visking dialysis membrane was employed.

† Optical density of a 1:10 dilution.

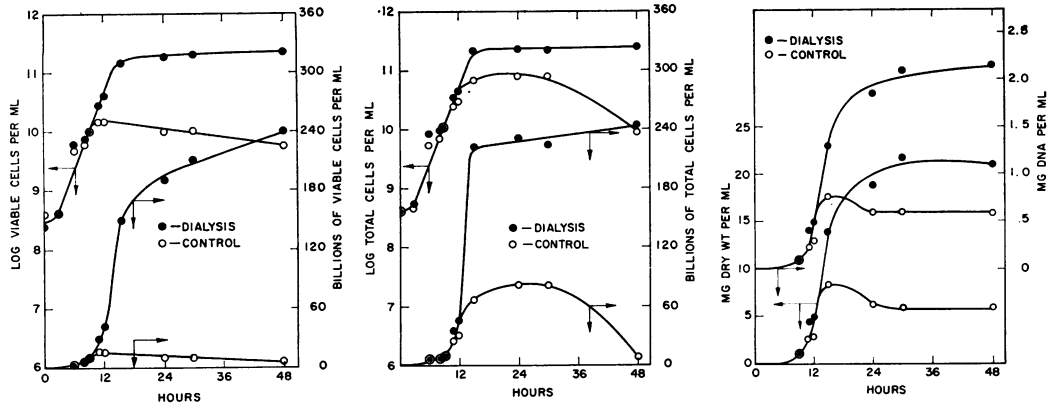

FIG. 2. *Growth curves of Serratia marcescens, in dialysis and control culture, with cell numbers and mass measured as indicated on the ordinates. The arrows on each curve indicate the corresponding axes. Smith-Johnson synthetic medium was used in the lower reservoir and 100 ml of water in the upper culture chamber. The control flask contained 100 ml of medium, restricted to the culture chamber.*

TABLE 5. *Concentrated culture of representative microorganisms in the dialysis flask (results after 48 hr)**

Organism	Type of medium	Temp	No. of cells (billions/ml)				Cell mass (mg/ml)				Optical density of 1:10 dilution	
			Viable		Total		Dry wt		DNA			
			Dialysis	Control	Dialysis	Control	Dialysis	Control	Dialysis	Control	Dialysis	Control
		C										
Bacillus cereus terminalis	Glucose-yeast extract-salts	30	7	1.5	115	2.5	15	3.9	1.35	0.26	0.58	0.31
B. megaterium KM	Peptone	30	14	2	18	2.5	26	5.6	1.6	0.40	0.50	0.33
Bacteroides sp.	Thioglycolate	37	12	1.3	15	1.8	6.4	3.8	0.55	0.30	0.63	0.36
Escherichia coli K-12	Trypticase Soy	30	18	4.5	28	6	15	4.1	1.39	0.38	0.84	0.40
Haemophilus influenzae	Proteose-peptone-hemoglobin	37	8.5	1.6	10	1.8	9.6	2.1	0.85	0.18	0.60	—
Lactobacillus acidophilus	Glucose-yeast extract-salts	37	—	—	66	9	14.2	3.4	1.25	0.25	1.18	0.51
Penicillium notatum	Glucose-urea-yeast extract-salts	28	—	—	—	—	19.3	10.1	1.25	0.84	—	—
Pseudomonas ovalis†	Trypticase Soy	30	9.3	0.62	8.7	2.2	7.8	1.2	0.36	0.12	—	—
Saccharomyces cerevisiae	Molasses-urea-salts	28	4.5	1.2	4.8	1.8	35.6	7.7	3.10	0.85	1.60	0.75
Salmonella enteriditis 795†	Trypticase Soy	37	5.4	—	20	—	8.8	—	1.0	—	0.83	—
Serratia marcescens 8UK	Glucose-citrate-salts	30	220	15	235	87	21	5.8	1.95	0.49	1.38	0.48
Staphylococcus aureus†	Trypticase Soy	37	266	22	380	42	52.5	10.2	1.7	0.56	0.73	0.18
Streptomyces griseus	Glucose-urea-yeast extract-salts	28	—	—	—	—	20.0	8.9	1.41	1.00	—	—
Streptococcus lactis	Milk solids-vegetable juice	29	4.1	3.6	4.5	3.8	—	—	—	—	0.05	0.07
Tetrahymena pyriformis	Glucose-peptone-yeast extract-salts	28	0.003	0.001	0.0034	0.0013	—	—	—	—	0.36	0.20
Vibrio comma†	Trypticase Soy	37	10	4.0	12	8.2	8.4	3.0	—	—	0.28	0.16

* The culture chamber contained 100 ml of water and usually was fitted with a cotton plug in a narrow opening.

† In these tests, a wide-mouth flask top was used and covered with a cotton pad, providing better aeration.

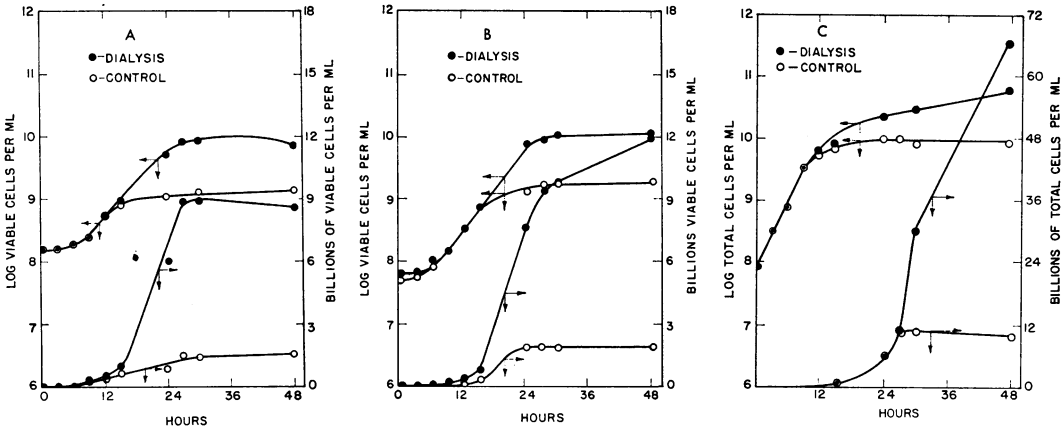

FIG. 3. *Growth curves of representative test organisms in dialysis and control culture, measured as indicated on the ordinates and with the experimental conditions shown in Table 5. (A) Haemophilus influenzae. (B) Bacteroides sp. (C) Lactobacillus acidophilus.*

producing vaccines, toxins, enzymes or other macromolecular products, spores, and cells for other purposes, i.e., in any situation where it is desirable to exclude particles or macromolecules of medium from the immediate growth environment. If it became desirable just to restrict cells to the culture chamber but to allow passage of macromolecules, for example, hydrolytic enzymes, then a more porous membrane could be employed, such as one of the membrane filters.

Dialysis-flask culture of *Bacteroides*, incorporating a closed nitrogen atmosphere, illustrated applicability of the method to a strict anaerobe and also an instance where a gradual increase in the viable population occurred during the plateau phase (Fig. 3B).

An even greater extension of active multiplication was seen in the dialysis culture of *Lactobacillus acidophilus* (Fig. 3C). The result may be explained by the diffusion of lactic acid and its dilution into the reservoir contents, product toxicity thus being allayed. Titration revealed that lactic acid production also was increased by dialysis culture. The principle thus would seem to have practical application in improving lactic acid and also other product-limited fermentations. With another lactic bacterium, *Streptococcus lactis*, the dialysis culture in the water diffusate of a milk medium inexplicably grew scarcely better than in the control, leaving doubt whether the method could be directly used in producing dairy starter cultures.

Still another potential application of dialysis culture was demonstrated by inoculating a facultatively aerobic organism into the bottom chamber of the flask, rather than the top as in the usual procedure. The oxygen demand then was met only by air going into solution and diffusing through the membrane to the culture. This

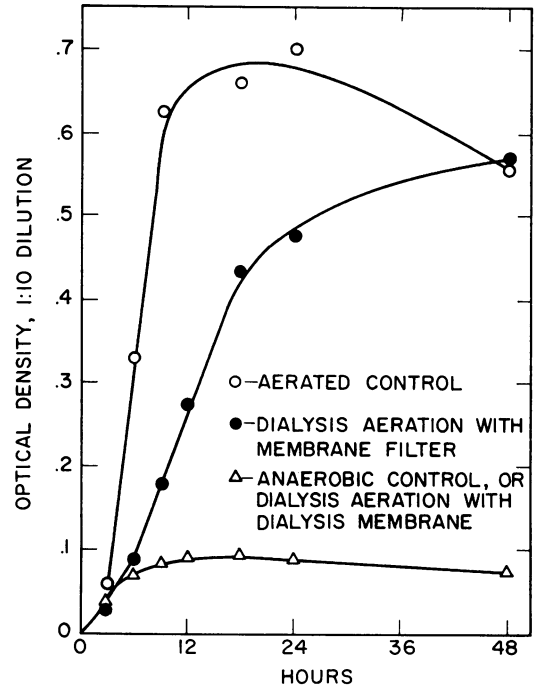

FIG. 4. *Turbidity growth curves of Serratia marcescens grown in the reservoir chamber of the dialysis flask and aerated by diffusion through a membrane filter or a dialysis membrane, as compared with aerobic and anaerobic controls.*

principle of dialysis aeration was examined by inoculating *S. marcescens* into the reservoir chamber filled with Trypticase Soy Broth, the top chamber containing 100 ml of water. A concentrating effect was not, of course, expected from these conditions. The resulting viable-count growth curves are shown in Fig. 4. A slower rate of multiplication and a less dense population were reached in the dialysis-aerated culture, with a Gelman AM-7 membrane filter, than in the aerated control, reflecting a limited oxygen supply. But both the rate and extent of growth in the dialysis-aerated culture were remarkably higher than in the anaerobic control. When dialysis aeration was attempted with a Visking regular dialysis membrane, however, only a small and ineffectual amount of oxygen apparently diffused into the culture, as the resulting growth was virtually the same as that obtained with anaerobic conditions. The principle of dialysis aeration seems especially applicable to the propagation of aerobic but fragile organisms, such as protozoa or mammalian cells that may be injured by direct gassing or the addition of antifoam compounds. It might also be useful for handling microaerophilic organisms. These and other extensions of dialysis culture warrant further study, which we have undertaken.

ACKNOWLEDGMENTS

Capable technical assistance was provided by Glynn E. McArn, J. U. Pedersen, and Edna A. Elias. The research was contract-supported by the Biological Laboratories of the Army Chemical Corps, Fort Detrick, Frederick, Md.

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