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Separation and Concentration of Bacterial Spores and Vegetative Cells by Foam Flotation

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Selective methods for removing and/or concentrating bacterial spores and vegetative cells from the growth medium are essential in studies on spore physiology and antigenicity, phagocytosis, and similar problems in which cell preparations free of cellular debris are desired. Development of a method to obtain "clean" preparations followed the observation that masses of material collected above the liquid level in the head of foam when *Bacillus anthracis* was grown in aerated deep cultures. When this material was smeared and stained, microscopic observation indicated that the material was composed of essentially clean spores. Frequently, on those occasions when masses of the material collected or when foam was incompletely controlled and lost through the air vent, the spore count of the culture was low. These observations led to the conclusion that a collection process could be developed that would separate *B. anthracis* spores from vegetative cells and cellular debris contained in the culture medium. Such a method of purification by flotation was developed and is reported in this paper. While this manuscript was being edited, it was learned that similar independent observations had been made and the same conclusions

drawn by Black *et al.* (1958) who observed the loss of *Bacillus cereus* spores from deep culture fermentations after uncontrolled foaming.

In the present study, simple glass cylinders equipped with spargers or diffusers were used as foaming chambers. *Serratia marcescens* cells were first successfully separated and collected and, with the development of equipment and a working procedure, cells of *Brucella suis* and *Pasteurella (Bacterium) tularensis* and spores of *B. anthracis* and *Bacillus subtilis* var. *niger* (*B. globigii*) were processed. Although major emphasis has been placed on the results obtained with *B. anthracis*, data on several other species are given to supply additional information on the flotation of cells or spores.

THEORY AND BIOLOGICAL APPLICATION OF FOAM PRODUCTION

Production of foam requires a lowering of the surface tension and a certain degree of heterogeneity of molecules at the gas-liquid interface. High viscosity and presence of long-chain molecules aid foam formation.

Foam stability depends upon concentration of foam-producing agents, whereas the amount of foam is a function of the method by which it is formed and the

¹ Deceased.

amount of surface-active agent present. Maximum stability of foam occurs when (a) the concentration of surface-active agent is within certain limits of low concentration, (b) viscosity is increased, (c) long-chain molecules are present, (d) adsorbed solid particles are present on foam bubbles, and (e) bubble size is small.

Froth flotation is used for certain separations of ores. Usually mechanical agitation is employed to produce bubbles, and three classes of chemicals may be added to improve the separation and collection of the desired mineral. Collectors, such as the xanthates, give the mineral particles a hydrophobic coating, thereby increasing the angle of contact between air bubble and particle. Frothers, of which cresols or terpeneol are representative, lower surface tension and increase surface heterogeneity, thereby making foam production easier. Modifiers, such as sodium cyanide or copper sulfate, either depress the flotability of the undesired particles or increase the coating by the collector of the particle desired in the froth.

Biological industries make less use of flotation than does the mining industry. Dognon (1941) used flotation to separate bacterial cells from salt solutions. By a foam process, Hopper and McCowen (1952) purified surface water of most solid particles, including 99 per cent of the bacteria and all cysts of *Endamoeba histolytica*. In the large scale culturing of algae, flotation is used to concentrate the mature culture (Cook, 1950). Flotation has been used for treatment of sewage and industrial waste (Hansen and Gotaas, 1943; Gibbs, 1950), for separation (a) of corn gluten from the starchy material of the corn grain (Boie, 1938; Smith, 1950), (b) of catalase from a solution of lysed erythrocytes (Dognon and Gougerot, 1949), (c) of oleic acid from the exhausted solution containing free NaOH (Raison, 1949), (d) of ripe grain from ergot (Plante and Sutherland, 1944), and (e) for certain other separations (Gaudin, 1957; Roe, 1948). The book by Gaudin (1957) provides a general reference, whereas the paper of Gaden and Kevorkian (1956) represents an applied treatment of the subject.

MATERIALS AND METHODS

Cognizance of the potential hazards of handling pathogens required that all work be conducted in accordance with the general philosophy of laboratory operations outlined by Reitman and Wedum (1956).

Method of producing foam. Foam has been produced by passing compressed air through fritted glass of a specified average pore size into the culture containing bacterial cells. Disc and tube types of fritted glass spargers were used. Air was sterilized by passage through layers of Chemical Corps no. 6 paper,² and regulated

² M. S. A. Ultra-Aire Space Filter Paper E6, Mine Safety Appliance Company, Pittsburgh, Pennsylvania.

by rotometers constructed to meter air at rates between 0 and 15,000 ml per min.

Foam chambers and flotation procedure. Foam chambers 35, 51, or 70 mm in outside diameter and 8, 12, or 16 in. in height were fused to the disc or tube type spargers. Chambers with disc or tube type spargers, a modified type used for head-space experiments, and the arrangement of spargers and collection flasks for both a one- and two-step foam process are illustrated in figure 1. If foam persisted in the collection flask, caprylic alcohol was added. The concentration factor of the spores or cells is expressed as the ratio of

$$\frac{\text{Cells per ml in volume collected}}{\text{Cells per ml in original culture}}$$

Growth medium. Except as specifically indicated, cells were produced in a medium composed as follows: Casein hydrolyzate, 1.2 mg Kjeldahl nitrogen per ml; autolyzed yeast solids, 0.5 per cent; glucose, 0.5 per cent; K_2HPO_4 , 0.5 per cent; $MgSO_4 \cdot 7H_2O$, 0.005 per cent; $MnSO_4 \cdot 4H_2O$, 0.003 per cent; $FeSO_4 \cdot 7H_2O$, 0.001 per cent; tap water to volume; pH 7.0 ± 0.1 adjusted with NaOH or H_2SO_4 .

Casein was hydrolyzed as follows: 10 g of casein, 40 ml of water, 30 ml of 2 M H_2SO_4 , and 2 ml of 2 M H_3PO_4 were autoclaved at 20 lb pressure for 4 hr. After

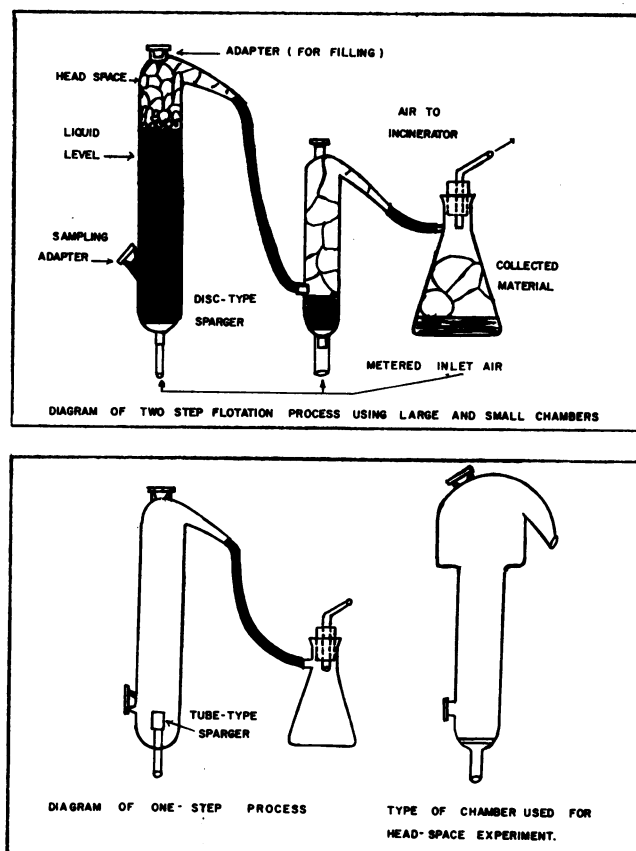


Figure 1. Diagram of foam chambers and flotation process

cooling, the hydrolyzate was neutralized to pH 7.5 with 10 N NaOH. Volume was increased to 100 ml with water, 1 g of Celite³ was added, and the hydrolyzate was clarified by filtration through filter paper.

Cultural conditions. All cultures were incubated in Erlenmeyer flasks on a reciprocating shaker furnishing 99 cycles of 3-in. amplitude per min.

RESULTS

Collection of spores of Bacillus anthracis. (1) Relation of autolysis of cultures and collection of spores: In preliminary studies designed primarily to demonstrate that spores could be separated from a variety of media, concentration factors varied from 1.4 to 16.8. The relatively inconsistent results seemed to be correlated with the degree of sporulation and autolysis of the cultures. Collection of spores was inefficient in cultures less than 75 per cent sporulated, and a greater degree of sporulation resulted in a corresponding increase in efficiency of

³ Johns-Mansville Co., New York, New York.

TABLE 1
Effect of sparger pore size on collection and concentration of spores

Sparger Pore Size	No. of Replicates	Collected Spores, 10 ⁹ /ml	Conc Factor
Fine.....	5	8.0	8.8
Medium.....	8	15.0	12.6
Coarse.....	8	20.9	16.8

the process. Certain data have been summarized as follows:

	Conc factor
Autolysis >75% complete: Single foam process	9.0
Double foam process	19.7
Autolysis <75% complete: Single foam process	4.4
Double foam process	10.9

(2) Importance of pore size of sparger: Spargers with average porosities of 5, 40, and 160 μ , hereafter referred to in the text as fine, medium, and coarse, respectively, were evaluated for their effect on both the collection and the concentration of spores. The results of these experiments are given in table 1. As sparger pore size increased, the number of spores collected and the number of spores per ml, with a corresponding increase in the concentration factor, increased. Coarse spargers produce a greater proportion of large, thin-walled, dry bubbles than do spargers with small pore size, thereby entraining less water and increasing efficiency.

(3) Collection of spores at various rates of air flow: Experience showed it was necessary to use relatively low rates of air flow at the beginning of the process and to increase the rate when visual observations indicated the foam volumes to be decreasing. The concentration factor and the volume of liquid collected show that maximum concentrations were not obtained with fast rates of air flow. Certain data supporting this conclusion are presented in table 2. The coarse sparger is more efficient as regards spores collected than the medium

TABLE 2
Efficiency of flotation chambers operated at different air flow rates in collection of *Bacillus anthracis* spores

	Rate of Air Flow											
	40 μ Sparger pore size operated at indicated air flow rate			160 μ Sparger pore size operated at indicated air flow rate								
	Fast	Medium	Slow	Fast	Medium	Slow						
Original spore count (10 ⁸ /ml) ...	1.2	1.6	1.4	1.2	1.6	1.4						
Collected spores (10 ⁹ /ml).....	8.8	17.9	20.0	9.9	19.8	27.0						
Spores collected, %.....	100.9	74.0	83.7	79.3	78.9	78.2						
Concentration factor*.....	7.3	11.1	14.3	8.2	12.4	19.3						
	Volume of air used during indicated successive time periods (in min) of the collection process											
	min	ml/min	min	ml/min	min	ml/min	min	ml/min	min	ml/min	min	ml/min
Successive time periods of process of froth flotation process	0-8	312	0-41	75	0-39	25	0-5	325	0-20	65	0-12	25
	8-14	475	41-48	100	39-51	75	5-9	575	20-34	425	12-22	50
	14-18	582	48-53	400	51-56	125	9-14	750	34-46	537	22-37	125
	18-22	682	53-86	512	56-75	487	14-20	825	46-76	487	37-56	155
	22-32	882			75-90	250	20-30	1150	76-87	812	56-90	271
	32-35	1075			90-128	337	30-35	1350	87-89	1120	90-128	437
				128-144	550	35-37	1675			128-147	1032	
Total time for process, min ...	35		86		144		37		89		147	
Total air used (min \times ml/min), ml.....		22,447		22,671		31,181		34,225		42,426		50,963

* Concentration factor = (spores/ml collected)/(spores/ml in original culture).

sparger but less efficient when volume of air used for the process is considered.

(4) Influence of head space on collection: The space between the surface of the liquid being foamed and top of the foam chamber, referred to as "head space," provides room in which the foam drains and dries as excess liquid drains while the foam is being lifted to the outlet of the foam chamber. Head space may be varied by changing the diameter, the height, or both height and diameter of the flotation chamber. Two series of experiments were completed in which head space was varied by changing the diameter of the chamber above the liquid level so that foam was produced in a 35 mm chamber but aged in a chamber of 35, 51, or 70 mm in diameter. All chambers were equipped with a coarse sparger. These data are summarized in table 3. The evaluation of the variable of head space was confounded. The size of the bubbles was directly related to head-space volume, with larger bubbles produced in the chambers with enlarged head space. Comparatively speaking, the 70 mm diameter chamber gave a higher concentration of spores than did the 35 mm chamber. Efficiency of collection and time required to complete a flotation run was not entirely dependent on chamber diameter, as each increase in chamber diameter increased the amount of air necessary to complete the flotation.

When the height of the head space above the liquid in the 51 mm flotation chamber was varied from 3.0 to 4.5 in., there was no significant difference in the concentration factor, per cent recovery, or volume of air used for a collection.

Further experimentation on optimum size and shape of the sparging chamber to accomplish maximum concentration needs to be done as it is expected that, if the height of the foam chamber were increased, a drier foam and a greater concentration factor would result.

(5) Effect of pH, NaCl, and genetic colonial type on collection of spores: Addition of 5 per cent NaCl or adjustment of the pH of the cultures from the normal pH range of about 7.5 to a pH of 6.0, 7.0, or 8.0 had no significant effect on the collection of spores.

A typical colony of the V1b strain of *B. anthracis* ap-

pears raised, rough, and rhizoid and was described earlier by Zelle *et al.* (1946) as colonial type RSI. The colony appears smooth when grown on bicarbonate agar. The collection of colonial variants was inefficient, both with respect to per cent of spores collected and concentration achieved. With mucoid variants, the foam appeared wetter than normal, and the collected product contained noticeable amounts of debris.

(6) Concentration achieved by the foam process: Six trials were made to determine the concentration of spores obtainable when a two-step foam process was employed. In these experiments, the foam from a 16 in. chamber was collected in an 8 in. chamber in which the rate of air flow was maintained at a minimum until collection of spores from the first chamber was complete. The rate of air flow in the second chamber then was adjusted to force foam into the collection flask. Both chambers were 51 mm in diameter and contained medium spargers. Data obtained for this process, given in table 4, show that collection was independent of the initial count of the culture and that the efficiency of the collection is high.

After these experiments were completed, coarse spargers became available. The results of seven runs made by a one-step process with coarse spargers and a foam chamber of 51 mm in diameter and 16 in. high are presented in table 5.

An average concentration of 40-fold was achieved

TABLE 4

Values for certain operational variables in separating spores of *Bacillus anthracis* by a two-step foam process*

	Rate of Air Flow					
	Fast		Medium to slow		Slow	
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
Spores in culture, 10 ⁹ /ml.	1.1	1.3	1.9	0.7	0.9	1.3
Spores remaining in 16 in. chamber, %	18.3	19.6	9.7	19.7	18.2	10.5
Spores remaining in 8 in. chamber, %	4.2	2.0	1.9	2.1	2.7	2.9
Spores collected by process, %	90.4	69.6	70.0	86.1	75.5	96.5
Spores in concentrate, 10 ⁹ /ml.	35.5	43.5	75.0	36.0	45.0	70.0
Concentration factor†	32.5	34.8	35.5	55.3	52.2	54.2
Total air used 16 in. chamber, L.	20.2	34.3	44.1	82.5	76.1	73.5
Total air used 8 in. chamber, L.	8.3	11.5	13.1	20.0	33.6	22.0
Operation time 16 in. chamber, min.	56.0	55.0	91.0	77.0	176.0	192.0
Additional time required for 8 in. chamber	18.0	25.0	24.0	15.0	43.0	17.0

* Chambers were 51 mm in diameter; spargers had a 40 μ pore size.

† Concentration factor = (spores/ml collected)/(spores/ml in culture).

TABLE 3

Ratio of results obtained using chambers of indicated diameter

	Diameter of Chamber, mm		
	35*	51*	70†
Relative concentration	1.0	1.71	1.88
Relative recovery (efficiency)	1.0	1.08	0.97
Relative volume of air used	1.0	2.33	3.95
Relative time of operation	1.0	1.08	1.29

* Three replications.

† Six replications.

with coarse spargers with cultures averaging 1.1 billion spores per ml. Three runs made at this time using medium spargers effected only a 20-fold concentration. Approximately the same concentration factor was achieved by the one-step process with coarse spargers or the two-step process with medium spargers; however, the former required a longer operation time and more air. The greater efficiency of the coarse spargers

TABLE 5

Values for certain operational variables and concentration of *Bacillus anthracis* spores achieved using 160 μ spargers and slow air flow rate

	Trials							Avg
	1	2	3	4	5	6	7	
Spores in culture, 10 ⁹ /ml...	1.1	0.9	1.4	0.9	1.6	0.7	1.2	1.1
Spores in concentrate 10 ⁹ /ml.....	43.5	50.5	36.0	31.0	40.5	35.1	60.1	42.4
Concentration factor*	38.0	59.4	26.4	35.0	25.4	48.7	48.8	40.2
Time, min.....	363.0	398.0	383.0	197.0	147.0	446.0	240.0	312.0
L of air/min—minimum.....	0.2	0.5	0.3	0.1	0.1	0.8	0.1	0.3
L of air/min—maximum.....	1.6	0.8	1.0	0.8	2.0	2.0	3.2	1.6
Total volume of air used, L...	67.9	85.8	90.1	68.5	59.4	122.5	91.0	83.6

* Concentration factor = (spores/ml collected)/(spores/ml in original culture).

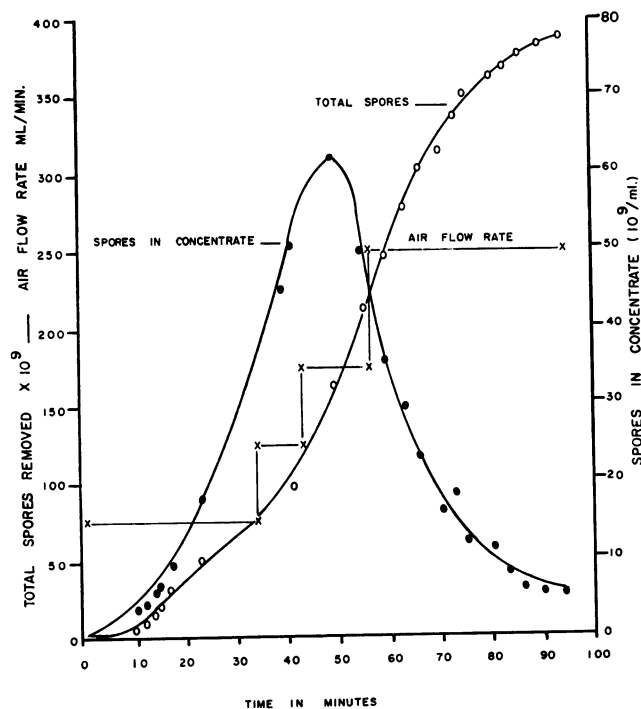


Figure 2. Removal of spores of *Bacillus anthracis* from culture medium by foam produced with a sparger having a pore size of 40 μ in diameter. Concentration factor obtained, 15.8.

is attributable to production of a drier foam, especially at the start of the process.

(7) Progressive removal of the spores by foam: The progress of spore removal was studied in 16 in. foam chambers of 51 mm diameter, equipped with medium or coarser spargers. The results obtained with the medium sparger are plotted in figure 2. Spores per ml increased progressively up to 48 min; the gradual decrease thereafter occurs independently of the rate of air flow. When a coarse sparger was used (figure 3) the initial rate of air flow was increased too rapidly at 64 min and again at the 97 min period, resulting in a wet-appearing foam of poor carrying capacity. Consistent with previous observations, the medium sparger used only half as much air as the coarse sparger.

(8) Description of product: Spores collected by foam flotation are individual and free of vegetative parts of the cell, and little debris is collected by the foam. The concentrate is cream colored, flows freely, and can be easily pipetted or diluted. In this respect, it contrasts markedly with centrifuged concentrates. Microscopic examination of the material remaining after spore collection shows vegetative cells singly or in chains, spores, and debris produced by cell autolysis or spore germination.

Collection of spores of *Bacillus subtilis* var. *niger* by flotation. There seems to be a factor in culture liquors of *B. subtilis* var. *niger* which initially prevents adsorption of spores by foam bubbles. During the first stages of foaming this factor is depleted and removal of spores

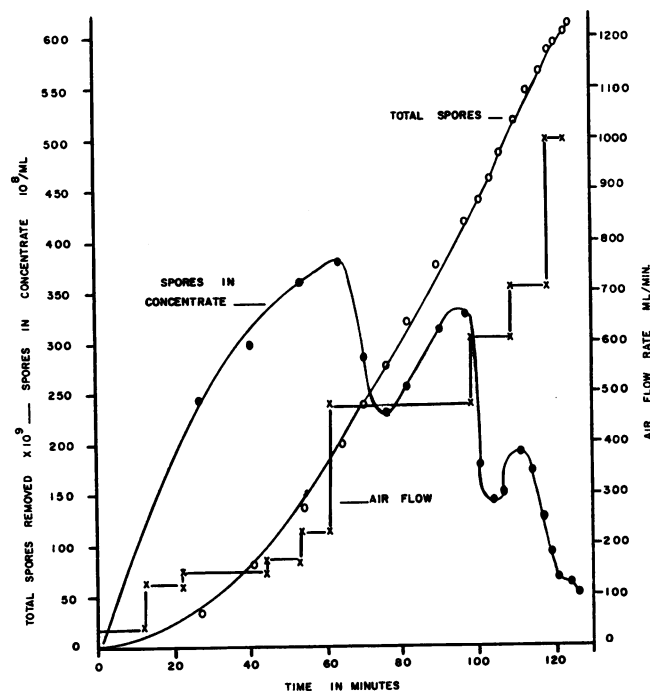


Figure 3. Removal of spores of *Bacillus anthracis* from culture medium by foam produced with a sparger having a pore size of 160 μ in diameter. Concentration factor obtained, 11.8.

is more efficient. Water dilution or adjustment of the liquor to pH 11.5 increased removal. Six trials on liquors diluted with two parts of water, adjusted to pH 11.5, and processed at a very slow rate of air flow yield a concentration factor of 8.6. As with *B. anthracis*, autolysis of the culture was essential before attempting foam separation, but, in contrast, foam produced by fine spargers carried more spores than did foam produced by medium spargers.

When spores of *B. subtilis* var. *niger* were suspended in acetone, dried, and resuspended in casein hydrolyzate medium, their separation and concentration by foam was easily accomplished.

Collection of cells of Serratia marcescens by flotation. Cells of *S. marcescens* were collected employing both fine and medium spargers. The concentration of cells and data on the variables of eight runs are given in table 6.

If the processed medium contained 2.0 per cent casein hydrolyzate the persistent foam initially produced was followed by a thinner, nonpersistent type that removed cells more efficiently toward the end of the process. Initial dilution of the medium with three parts of water yielded a cell-carrying foam equivalent to the nonpersistent foam mentioned above. These results probably are related to molecular heterogeneity of the surface layer, with the diluted broth providing more homogeneity of the surface layer in respect to water than does the 2.0 per cent broth, the latter provides homogeneity of the surface layer in respect to the protein-like constituents.

When 16 and 27 in. chambers of 35 mm diameter were used with equal volumes of culture, the one providing the higher head space between the liquid and the outlet was more efficient both in concentration attained and percentage of cells collected. When the

TABLE 6
Concentration of cells of *Serratia marcescens* achieved by froth flotation

Expt No.	Height of Foam Chamber	Casein Hydrolyzate	Original Viable Cell Count	5 μ Pore Size Sparger				40 μ Pore Size Sparger			
				Volume collected	Cells collected	Time	Total air used	Volume collected	Cells collected	Time	Total air used
4	8	2.0	42.6	35	50.7	45	1.24	69	54.2	60	7.92
5	8	2.0	31.8	38	57.0	40	1.68	77	76.5	50	8.87
6	8	1.6	30.5	41	69.3	34	1.77	68	71.8	60	7.79
7	8	1.3	30.8	53	51.3	40	1.10	113	87.3	56	9.99
9	8	1.0	46.2	46	61.6	40	1.25	73	72.5	53	9.23
15	8	0.5	39.5	33	79.1	66	1.78	44	80.2	120	6.29
17	16	2.0	42.0	18	68.7	50	1.00	47	73.1	110	9.89
19	16	2.0	51.5	19	70.4	131	3.03	46	84.0	149	21.70

height of head space above the liquid and volume of foamed liquid was held constant, then a more efficient removal of cells occurred in the 35 mm diameter chambers as compared with the larger 51 or 70 mm sizes.

The removal of cells and volume of air used in processing a culture is presented in table 7. Separation of cells from the liquor was rapid, and the cell removal efficiency during each period was more or less constant throughout the process; however, during the final periods, the foam was thin and easily broken, requiring longer times to collect a unit number of cells.

In contrast to the results with *B. anthracis*, cultures of *S. marcescens* were found to collect more efficiently in foam produced by fine spargers than with medium spargers. The same percentage of cells eventually was collected by either sparger. The process with *S. marcescens* was characterized by use of a relatively low rate of air flow and rapid collection of the cells into a small volume.

Concentration of cells of Brucella suis by flotation. Attempts to remove cells of the smooth strain of *B. suis* from casein hydrolyzate medium were unsuccessful. The thin, unstable form produced had the appearance of

TABLE 7
Cell removal and volume of air used in concentrating *Serratia marcescens* cultures by foam*

Sparger with Pores of 5 μ				Sparger with Pores of 40 μ			
Time interval	Air flow	Cumulative total of cells removed	Efficiency of removal†	Time interval	Air flow	Cumulative total of cells removed	Efficiency of removal†
min	L	10 ⁹ /ml	%	min	L	10 ⁹ /ml	%
0-5	0.17	7.3	17.2	0-5	0.24	4.5	10.6
5-10	0.17	9.5	6.3	5-15	0.48	4.9	12.8
10-15	0.30	21.7	40.0	15-25	1.96	4.8	14.5
15-20	0.31	29.4	37.0	25-35	2.30	2.9	10.2
20-25	0.18	31.5	16.0	35-45	2.64	12.8	11.4
25-40	0.56	33.8	20.9	45-60	1.35	11.2	57.1
Cells remaining uncollected, 10 ⁹ /ml.				Cells remaining uncollected, 10 ⁹ /ml.			
8.7				6.4			
Concentration factor 7.9				Concentration factor. . . 8.5			

* Original culture contained 42.5 × 10⁹ cells/ml.
† Per cent removal during the indicated period of cells present at the start of that period.

TABLE 8
Results of collecting *Bacillus suis* by foam with respect to capsular material

	Smooth	Mucoid	Rough
Original culture, 10 ⁹ /ml	40.5	16.5	30.5
Remaining cells, 10 ⁹ /ml	34.0	3.5	12.5
Collected cells, 10 ⁹ /ml	27.2	62.0	158.6
Concentration factor	0.67	3.75	5.1
Time of run, min.	270	76	300

one formed in the presence of a strong antifoaming agent. Collection of cells was not affected by water dilution or the addition of approximately 1.0 to 1.5 per cent of methycellulose, casein, Tween 80,⁴ Triton,⁵ Roccal,⁶ sodium lauryl sulfate, tannic acid, formaldehyde, gum tragacanth, pectin, Cerophyl,⁷ lecithin, rosin, or N-dodecyl- β -alanine. Some of these materials gave bulk to the foam produced but did not result in the removal of cells in the foaming process.

When the culture of *B. suis* was autoclaved at 15 psig for 15 min before processing, microscopic observation confirmed that dead cells were readily transported by the foam.

Smooth, rough, or mucoid types of *B. suis* differ in respect to the capsular material which surround the cells. The rough type generally is easily agglutinated by agents other than antibodies and is believed to be more hydrophobic than the smooth type. The results of collecting these types by foam is presented in table 8.

Cells of the smooth strain were not collected by the foam, whereas cells of the mucoid or rough type were collected with fair efficiency.

Concentration of Pasteurella tularensis by flotation. Our efforts failed to concentrate viable cells of *P. tularensis* by froth flotation of cultures produced in casein hydrolyzate medium. Several trials were made at air flow rates of either 100 or 1200 ml per min and continued for as long as 350 min. An average of only 20 per cent of the cells remained viable at the conclusion of the foaming, and the concentration factor of cells in the collected material averaged 1.87.

DISCUSSION

The spore-carrying capacity of foam seems to be related to type of foam produced. During the initial treatment of a culture, a fairly stable foam composed of fine, small bubbles with a lacelike appearance is produced. As surface active materials are removed, bubble sizes gradually become larger, and the foam may appear milky due to adsorbed cells. A faster rate of air flow than the initial one is required to maintain production. The final stage requires relatively large volumes of air and develops bubbles that rise singly, stretching completely across the broadest chambers employed, and tend to break before leaving the foam chamber. When the rate of air flow is increased too rapidly, a wet-appearing foam composed of smaller bubbles is produced. Those foams which had not broken by the time they entered the collection chamber could be dispersed by a slight shaking of the collection flask. It seems probable that the progressive removal of surface-active materials necessitates an increased rate of air flow to maintain a

constant foam head. A decrease in the relative number of collisions between cells and bubbles as spores or cells are removed also may contribute to decreased collection and stability of the foam. In experiments reported here, processing was conducted as an art, nevertheless, it is believed that by the use of foam probes and conductivity measurements of the foam more exact and predictable control of the process could be readily developed.

Where autolysis of sporogenic strains was more than 75 per cent complete prior to processing, practically all the spores could be accounted for. This was not true for incompletely autolyzed cultures. Of the vegetative species foamed, quantitative recoveries were obtained only with *S. marcescens*. The results obtained with *P. tularensis* wherein only 20 per cent of the cells remained viable suggest that the foam process may inactivate sensitive cells.

Whether or not a cell is carried by foam seems to depend upon the nature of the cell surface. Certain genetic variants and autoclaved cells, or solvent dried spores resuspended in liquor, collect readily in foam, whereas variants of some species and vegetative or unautolyzed cells of *B. anthracis* did not. It is concluded that cells with a relatively hydrophobic surface are carried by foam. Presumably the hydrophobicity of the cell surface can be modified by chemical and physical treatments, but those tried in this work had little effect. The technique of flotation opens another approach to determining the composition, charge, and molecular orientation of cell surfaces.

The selective nature of the flotation process should be emphasized as it appears to furnish much cleaner spore lots from the morphological standpoint than does centrifugation. To obtain clean batches of spores, it is reasonable to presume that flotation, centrifugation and precipitation by gels (Hodge and Metcalfe, 1958) should be combined. Relatively clean spore concentrates should be of interest for studies on physiology and antigenicity, and for the production of vaccines. Other applications of flotation to biology are less obvious, but the technique may have application in the separation of ether-soluble constituents of cultures. The observation of Mudd and Mudd (1924) that certain cells will collect at an oil-water interface might be extended to develop an oil-water-air flotation system with new applications in both types and kinds of products collectable and selectivity of collection.

The flotation phenomena may explain losses of cells or of products due to air flow rate plus inadequate foam control during the final stages of aerobic fermentation. Even if foam is partially controlled, cells in the foam layer may collect on the walls of the vessel, thereby being lost unless the walls are deliberately washed down.

⁴ Atlas Powder Co., Wilmington, Delaware.

⁵ Rohm and Haas Co., Chicago, Illinois.

⁶ Blair and McGuire Corp., Holbrook, Massachusetts.

⁷ Cerophyl Laboratories Inc., Kansas City, Missouri.

SUMMARY

A selective method of removing and/or concentrating bacterial spores and vegetative cells from the culture growth medium by foam flotation is described. Foaming chambers were made of simple glass cylinders equipped with spargers.

In collecting spores of *Bacillus anthracis*, well autolyzed cultures were essential but cultures with high spore counts were not required. Coarse spargers were more effective than fine or medium spargers. Head space within the limits tested was not important, although concentration of spores was directly related to chamber diameter. Concentration of spores to 40 billion per ml was obtained provided a rate of air flow was maintained that kept a dry, carrying-type foam. The volume of air needed depended on the pore size of the sparger and concentration of spores desired.

Spores from autolyzed cultures of *Bacillus subtilis* var. *niger* and cells of *Serratia marcescens* were collected more effectively with fine rather than with coarser spargers, although the same percentage of cells eventually were collected by either sparger. Relatively low rates of air flow were used, and the cells were collected in a small volume.

Cells of a smooth strain of *Brucella suis* were not collected in foam under a variety of conditions, whereas, rough or mucoid type cells could be collected with moderate efficiency.

Cells of *Pasteurella tularensis* did not collect in foam under the conditions of our experiments.

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