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THE FOAM SEPARATION OF ESCHERICHIA COLI WITH ETHYLHEXADECYLDIMETHYLAMMONIUM BROMIDE

by

Nasrin Sadidi

A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirement for the degree of Master of Applied Science in the Department of Chemical Engineering, University of Ottawa.

Ottawa, Canada
1975

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ABSTRACT

The foam separation of E. Coli strain B from distilled water suspension using a cationic surfactant, ethylhexadecyldimethylammonium bromide (EHDA-Br), was investigated. The experimental results were evaluated in terms of total cell count, using a membrane filtration technique. Throughout the series of experiments, the initial cell concentration was held constant at 2.6×10^7 cells/ml., and the air superficial velocity was 164 cm/min. At these conditions, the effects of EHDA-Br concentration and foaming time were studied. EHDA-Br concentrations in the initial cell suspensions were varied from 0.020 to 0.040 mg/ml., and the foaming times were varied from 2 to 10 min. The best performance corresponded to 99.9% removal at EHDA-Br concentration of 0.030 mg/ml and a 10 min. foaming time. The cell enrichment ratio was a power function of the foaming time. At an initial cell concentration of 2.6×10^7 cells/ml, air superficial velocity of 164 cm/min., EHDA-Br concentration of 0.030 mg/ml., and foaming time of 4 min., the effect of presence of Na Cl, $NaNO_3$ Na_2CO_3, and Na_2SO_4 was investigated. The addition of from 0.17 to 8.56 μ eq./ml of salt decreased the total cell removal.

ACKNOWLEDGEMENT

The author wishes to express her sincere appreciation and thanks to Dr. F.D.F. Talbot, the director of this thesis, for his encouragement and assistance throughout the course of this work.

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INTRODUCTION

The adsorptive bubble separation processes is the generic name given to all separation methods involving the use of selective adsorption at gas-liquid interfaces, the interfaces being generated by gas bubbles in liquid media. Figure 1 is the schematic representation of subdivisions of adsorptive bubble separation processes[1]. The initial division is made by considering the method in which the enriched gas-liquid interfaces are removed from the bulk media. If a foam is involved in the process, then the term foam separation is used, if the separation is carried out without the use of a foam, i. e., with only the passage of gas bubbles through the bulk medium, then the term nonfoaming adsorptive bubble separation is applied.

Foam separationis further subdivided in terms of the nature of the species being separated. If the species being separated are part of a homogeneous solution, then the term foam fractionation is applied. For example, in the removal of surface-active agents, foam fractionation would be the proper term to use.

If the species being separated are insoluble particulates, then the term froth flotation or simply flotation is applied. This in turn is subdivided into seven parts: ore flotation, macroflotation,

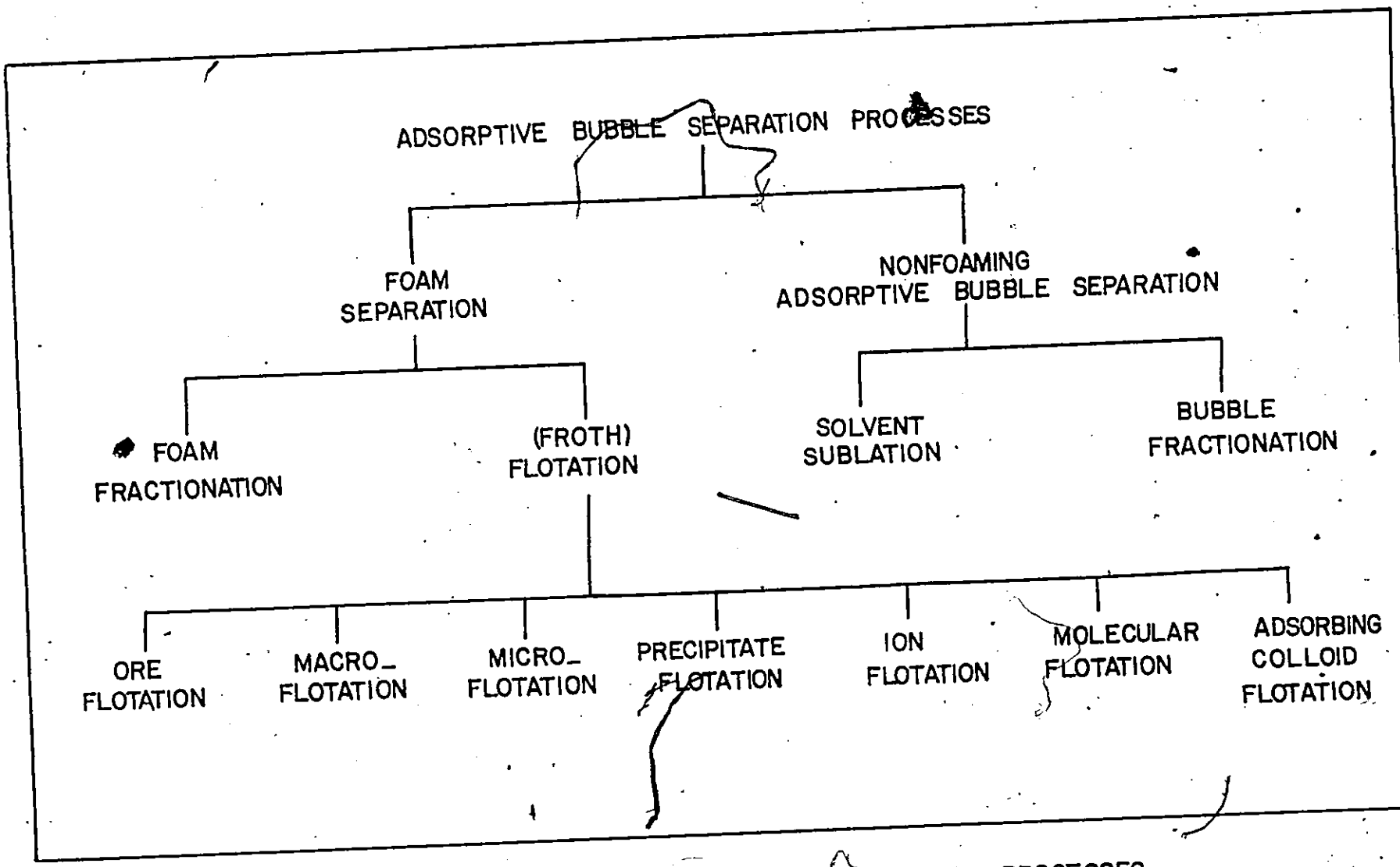

FIGURE 1 – THE SUBDIVISIONS OF ADSORPTIVE BUBBLE SEPARATION PROCESSES

microflotation, precipitate flotation, ion flotation, molecular flotation, and adsorptive colloid flotation.

In the area of nonfoaming adsorptive bubble-separation there are only two categories: solvent sublation and bubble fractionation.

Karger and deVivo[1], and Lemlich[2] have provided a good deal of detail on each of the above-named processes. The interested reader may refer to them. The object in reviewing this classification has been to place microflotation in proper perspective with regard to other adsorptive bubble separation processes.

As the name implies, microflotation involves the separation of microscopic particles by foaming. This process deals with the flotation of microorganisms and colloids (i.e. colloid flotation). Amongst the microorganisms used in flotation studies, bacteria have been the most popular. The fact that bacterial cultures in aqueous medium at neutral pH, behave as colloids, carrying a negative charge[3], has permitted the workers to separate or remove bacteria by the foam separation process, using a cationic surfactant as collector.

Scientists and engineers have been interested in the flotation of microorganisms for the following reasons: First, using high gas flow rates, removal rates are much higher than the conventional separation processes. Second, foaming can provide a means of concentrating cells

for more accurate cell count analysis. Third, the foaming process can be valuable in the water-treatment plants, since the removal of bacteria from dilute suspensions will allow a cutback in disinfectant dosages. Fourth, foaming can be used as a selective method of removing and/or concentrating bacterial spores and vegetative cells from the culture growth medium. Finally, an understanding of the removal mechanism should provide some fundamental information on the surface phenomena of the microorganisms. With these views in mind, it becomes evident that the flotation of microorganisms is well worth studying.

It is the potential use of microflotation in sewage and water-treatment plants which has prompted this work. The organism chosen for the study is Escherichia Coli. There are three reasons for choosing this bacteria. First, because of the universal presence of E. Coli in the human intestinal tract, and because of the ease with which it can be identified and counted in a water sample, the presence of this organism in a water sample is usually used as an index of fecal pollution of the water. Second, compared to other pathogenic bacteria of interest to the sanitary engineer, E. Coli is relatively safe to handle in foaming experiments. Finally, having been investigated by several workers (4, 5, 6, 7, 8), the related literature available could serve as a guideline for the novice.

This work is based on the experiments conducted by R. B. Grieves

et al[4, 5]. The equipment used is similar but not identical to theirs. The object of the work is to study the foam separation of E. Coli with ethylhexadecyldimethylammonium bromide (EHDA-Br) and determine the effects of foaming time, surfactant concentration, and the presence of four inorganic sodium salts on the separation process. Two of the salts used in this study have not been investigated by Grieves and his team.

LITERATURE SURVEY

Microflotation is an application of adsorptive bubble separation processes which is relatively old. In 1941, Dognon[9, 10] bubbled air through a suspension of staphylococci containing a small number of BCG strain of tubercle bacilli and found that the BCG cells collected at the top of the froth. Knowing that most of the organism's remained in the liquid when a clinical specimen was centrifuged for laboratory analysis, he suggested that flotation be used in the diagnostic laboratories to concentrate tubercle bacilli.

Shortly after, Hansen and Gotaas[11] used laurylamine hydrochloride to remove bacteria from sewage. They reported high removal rates for unidentified bacteria in 15-20 min., over 99% being removed by 80-100 mg./l. of the surfactant. The pH range of 6-8 was found to give the best flotation results.

In 1958, Boyles and Lincoln collected Bacillus anthracis spores essentially free of vegetative remains. In studying B. subtilis variety niger spores, they found that the spores were not attached to the foam bubbles until a component of the culture medium which prevented adsorption was removed in a primary frothing. They suggested that flotation could be of value in studies on spore physiology and antigenicity, phagocytosis, and similar problems in which cell preparations free of cellular

remains are desired.

Levin et al[13] reported the development of a flotation procedure for harvesting algae from dilute suspensions. Several species of Chlamydomonas and one specie of Stichococcus and of Chlorella were floated using a surfactant naturally produced by the organisms. Harvesting was carried out in a long cylindrical culture vessel which was aerated from below. In a typical operation, 88% of the cells in 1,200 ml. of feed culture were harvested in 18 minutes.

In 1962, Gaudin et al[6] tried to float various strains of E. Coli from the culture medium and from water suspension without adding a surfactant. Efforts to recover the cells were unsuccessful until sodium chloride was added to the initial suspension. Na Cl acted as a flotation reagent; and although strains responded differently to a given Na Cl concentration, about 85% of the cells were concentrated into one-sixth the original volume when the initial suspension contained 2% Na Cl. In a second study[7], they used other salts as flotation reagents. Although good results were obtained with carbonates and phosphates, the use of bicarbonate, sulfate, nitrate, bromide, and iodide did not help the flotation of E. Coli.

In 1966, the foam separation of E. Coli from distilled water suspension using ethylhexadecyldimethylammonium bromide (EHDA-Br) as collector was reported by Grieves and Wang[4, 5]. The effect of the

variables: foaming time, initial cell concentration, initial surfactant concentration, gas flow rate, foam port height, added inorganic salts, and multiple additions of surfactant on the removal of E. Coli was studied. Grieves and Wang[14] have further investigated the relative effectiveness with which several species of bacteria can be foam separated from pure suspension. Using similar feed suspensions, the same surfactant (EHDA-Br) at the same concentration, and identical operating conditions, E. Coli, Serratia marcescens, Proteus vulgaris, Pseudomonas fluorescens, Bacillus cereus, and Bacillus subtilis var niger were studied individually. The "best" separation was obtained with Bacillus subtilis var niger, while the "worst" separation was achieved with E. Coli.

Rubin et al[8] reported the developement of a new technique for the foam separation of microorganisms at low gas-flow rates. A stable surface phase was produced by adding an insoluble surfactant such as a long-chain fatty acid or amine. Due to the formation of this insoluble surface phase, low rates of gas flow could be used. The use of flotation aids such as frothers and flocculents improved the efficiency of the technique. The applications discussed were the removal of E. Coli, using lauric acid and alum, and two species of algae, using stearylamine without alum. The frother used was ethanol.

A review published by Dobias and Vinter[15] provides many more examples of applications of foam separation to microorganisms.

The Role of the Cationic Surfactant in the Microflotation Process

It is a well known fact that bacterial cultures in aqueous media behave in many respects as hydrophilic colloids, with the bacteria carrying a net negative charge at neutral pH[3,16]. The existence of this charge on the surface of bacteria has permitted many workers to separate or remove bacteria by the foam separation process, using a cationic surfactant as collector.

The surfactant added as collector-frother in the flotation process serves three functions. First, the adsorption of the surfactant on the surfaces of the particles may cause destabilization of the colloid and makes the particles suitable for bubble attachment. Second, the interaction between the particles plus their adsorbed surfactant and "free" surfactant adsorbed at the gas-liquid interfaces may produce bubble attachment of the particles. Finally, the free surfactant molecules act as a frother producing a stable foam. This is essentially what takes place in most flotation processes. However, in the case of microflotation, the picture is more complicated.

The complication arises from the fact that in microflotation one is dealing with living organisms which are capable of undergoing intricate interactions with surface-active agents. The surfactant ions may be adsorbed at oppositely charged surface groups or within the cytoplasm, may partly dissolve in the surface, or may react chemically

with surface molecules. An extensive amount of research has been done on the adsorption of surfactants on bacteria[17-20], but no single mechanism has been established.

The work of Salton[19, 20] on the adsorption of cetyltrimethyl-ammonium bromide (CTAB) by bacteria is worthy of consideration. In this important work, Salton concluded that the form of the uptake curve of CTAB by Staphylococcus aureus and Escherichia Coli is that of an adsorption isotherm. Also, tests on six different bacteria showed that the maximum amount of CTAB adsorbed varied from one organism to another. Furthermore, electron microscopy of bacteria treated for 5 to 30 min. at 20°C with concentrations of CTAB ranging from 0.045 to 0.900 mg./ml. showed evidence of cytological damage. The damage caused by CTAB to the osmotic barrier (also called permeability barrier) resulting in the leakage of cell solutes, was accompanied by a noticeable contraction of the cytoplasm, which became detached from the cell wall. For E. Coli there was a correlation between the number of cells killed and the proportion of cells in which the cytoplasm had contracted from the wall. Another observation made was that, when treated with large amounts of CTAB (i.e., 0.090 - 0.900 mg. CTAB/ml., for 30 min. at 20°C), the cell was either partly or completely stripped off.

In studying the foam separation of E. Coli from distilled water

suspension using EHDA-Br, Grieves[4] found direct evidence for the 'binding' effect of the cells. Analysis made on the initial suspensions produced EHDA-Br concentrations lower than those which should have existed, based on the quantities of EHDA-Br which had been added. This effect was quite noticeable in the initial suspensions containing 1.0×10^8 cells/ml. Grieves reports that microscopic examination of E. Coli - EHDA-Br suspensions showed no evidence of gross cell damage. However, this has little significance since as Mitchel[21] pointed out the initial breakage of the osmotic barrier is not accompanied by any obvious morphological changes which can be detected in the light microscope.

When one considers that EHDA-Br like CTAB is a cationic surfactant, and more particularly a quaternary ammonium compound, and that the surfactant concentration, contact time, temperature, and most importantly, the organism used in Salton's experiments are similar to those in Grieves' experiments, the work of Salton becomes relevant. The author feels that there is a good chance that there was a certain amount of damage to the osmotic barrier of E. Coli cells in Grives' experiments. This would most definitely cause a leakage of certain cell constitutents[20,21], resulting in the lightening of the cells. This would raise an interesting question. How many cells floated due to buoyancy effects alone?

Based on the foam separation studies carried out on E. Coli and other bacteria, all that can be said is that microflotation is an efficient separation process. However, any effort toward the explanation of the results on the basis of an adsorption or reaction mechanism would be speculative, especially due to the many modes of action that have been suggested for the bactericidal properties of quaternary ammonium salts.

EXPERIMENTAL APPARATUS

Figure 2 shows the foaming apparatus used. The pressure of the oil free compressed air coming from a cylinder was maintained at 6 psig. The air was then filtered on passing through a glass tube 54 cm. long and filled with glass wool. The filtered air was then saturated by a humidifier consisting of a fritted sparger in a flask of distilled water. A calibrated rotameter was used to meter the air. The bubbler in the column was made from a Labpor filter funnel obtained from Canus Equipment Ltd. The funnel sides were cut to leave a linear polyethylene filter disk 8.0 cm. in diameter with 30-40 μ pores. The porous disk was then imbedded in a stainless steel chamber. The cylindrical column had an inside diameter of 8.5 cm., a height of 86.5 cm., and was made of Pyrex. The foam was removed from a port of 1.2 cm. i.d. located 77 cm. above the base of the column and 57 cm. above the initial suspension level. The column had a sidearm 11 cm. above the base, through which the surfactant could be introduced with a syringe.

The bacteria were grown in 50 ml. Corex brand centrifuge tubes with plastic screw caps (Cat. No. 8422-A) containing especially prepared nutrient broth. A constant temperature bath at 37°C was used to incubate the culture tubes. To prepare the initial cell suspension,

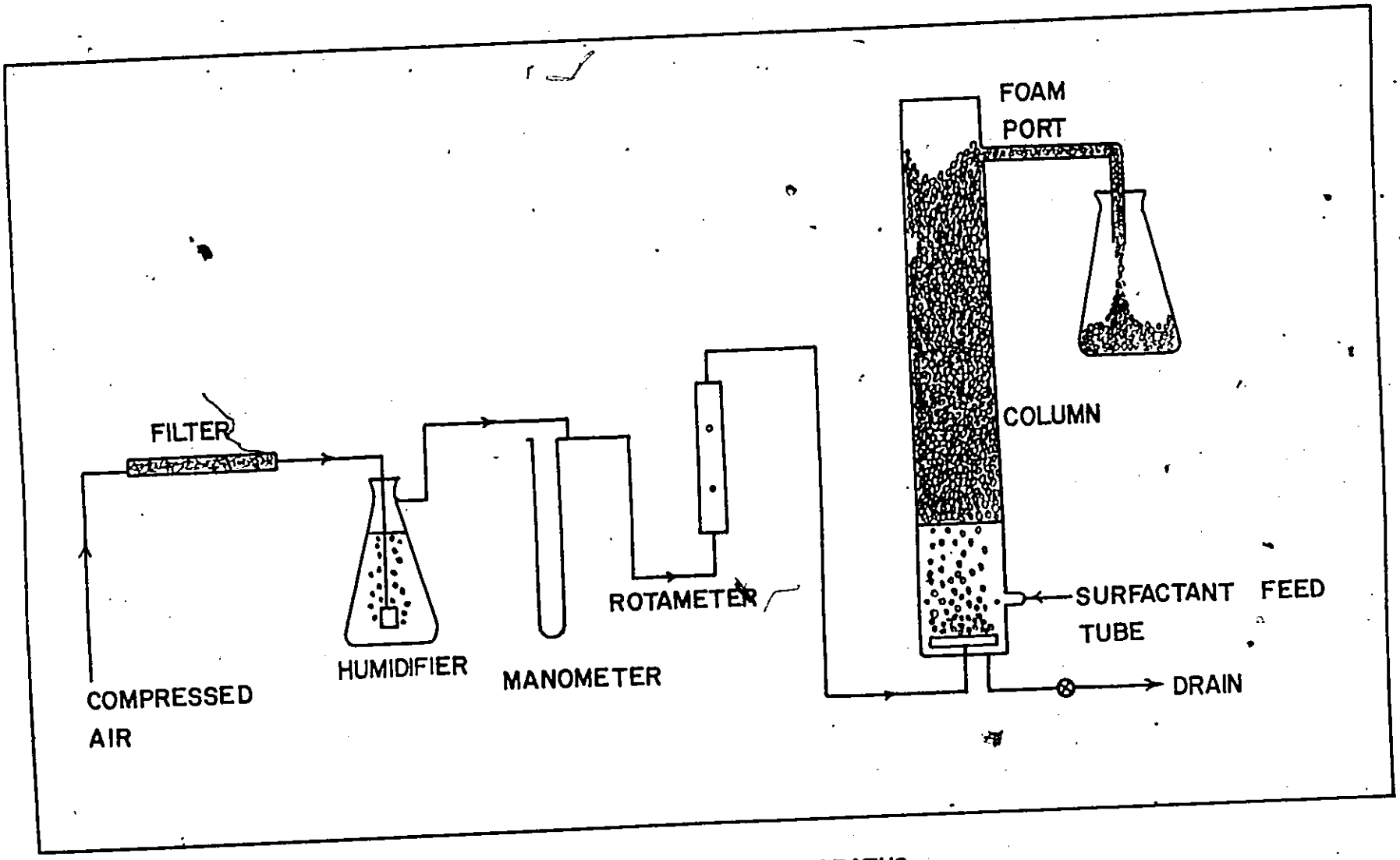

FIGURE 2_ FOAM SEPARATION APPARATUS

the culture tubes were centrifuged by means of an IEC Model HNS centrifuge.

Initial suspension analysis was made with a Bauch & Lomb Precision Spectrophotometer (Cat. No. 33-26-50). Visible light with λ = 620 mμ, a slit width of 0.5 mμ, and cylindrical Spectrosil cells of 50 mm. path length were used.

Samples of cell suspension were filtered with a Gelman Borosilicate Filter Funnel (Fisher Cat. No. 9-730-208), a 1l. vacuum flask, and gridded, presterilized Gelman membrane of 0.45 μ pore size. For total cell count analysis, Series 50 American Optical Binocular microscope (Cat. No. L50BUA-QW) was used.

The pH measurements were made with a Ionalyzer model 801/ digital pH meter. Sterilization was done with a AMSCO general purpose autoclave. Throughout the series of runs distilled water was used.

The following is the list of chemicals used:

1. BACTO-AGAR (DIFCO)
   Cat. No. 0140-01-0, Canlab
2. BACTO-Nutrient Borth (DIFCO)
   Cat. No. 0003-0106, Canlab
3. Basic Fuchsin
   Matheson Coleman & Bell

4. Acid Fuchsin
   Cat. No. F-97, Fisher Scientific Co.

5. Methylene Blue
   Cat. No. M-225, Fisher Scientific Co.

6. Concentrated nitric acid

7. Na OH, H Cl research or analytical grade

8. Ethylhexadecyldimethylammonium bromide
   Eastman Organic Chemicals

9. Sodium Chloride (crystal)
   Cat. No. S-271, Fisher Scientific Co.

10. Sodium Nitrate (analytical reagent)
    Analar

11. Sodium Carbonate (anhydrous)
    Cat. No. S-263, Fisher Scientific Co.

12. Sodium Sulfate (anhydrous)
    Cat. No. S-421, Fisher Scientific Co.

13. Prepared Gram stain solutions-obtained from the Department of Biology, University of Ottawa

14. Sodium hypochlorite Solution (12%)
    G. H. Wood & Co. Ltd.

EXPERIMENTAL PROCEDURE

Two specially prepared nutrient broth tubes were inoculated with a loop from a 20-hr E. Coli culture and incubated at 37° C for 20-hr. under static conditions. The tubes were then centrifuged at 3000 g for 20 minutes. The liquid was discarded into a pail containing 10% chlorine solution. The cells were then resuspended in a very small amount of distilled water. A few ml. of this dense suspension were added to distilled water and the volume was made up to 500 ml., to give an 85% transmittance reading at 620 mμ. This suspension was diluted 1:1 to give 1000 ml. of initial cell suspension containing 2.6 x 10^7 cells/ml.

A 2.0 mg./ml. solution of EHDA-Br in distilled water was freshly prepared. The air flow was started and maintained at 9.3 l./min. 1000 ml. of the initial cell suspension were placed in the column. Then the desired amount of EHDA-Br was introduced to the column with a syringe. As soon as the foam appeared in the receiver, the timer was started. Temperature was held at 20 $\pm$ 1°C. At the end of each run, the residual suspension was taken for total cell count analysis. The volume of the residual suspension was carefully measured. After each experiment, the column was washed with EHDA-Br solution and rinsed several times with distlled water.

The membrane-filter technique for total cell count was used. The sample of residual suspension containing 30-300 cells per microscopic field was filtered through a Gelman filter membrane (pore size 0.45 mμ). The filter was then stained and prepared for microscopic examination. For each experiment, sixteen counts (i.e., sixteen microscopic fields) were made.

Details of the experimental procedure will be found in the following sections.

1. Preparation of Culture Media

The test tubes with the screw caps, the borosilicate funnel, and the receiving flask were washed with detergent, rinsed thoroughly with distilled water and then autoclaved. In the meantime, 1l. of nutirent broth solution was prepared by dissolving 8.0 g. of nutrient broth in 1l. of distilled water. The broth was then filtered with the sterile Gelman filtration equipment. If solid medium was required, 1.5% agar was added to nutrient broth. The media were then distributed in the sterile test tubes with the screw caps loosely placed on. The tubes were autoclaved at 15 psi. pressure for 15 min. and were not removed from the autoclave until the temperature had dropped to approximately 50°C. Once taken out, the screw caps were tightened immediately to avoid contamination.

2. Inoculation Procedure and Initial Suspension Preparation

E. Coli strain B was supplied by the Department of Biology, University of Ottawa. The bacteria were subcultured monthly from refrigerated agar slants. Using an inoculating loop, two nutrient broth tubes were inoculated and incubated at 37°C for 20 hr. under static conditions. At the end of the growth period, Gram stains of both culture tubes were made to detect possible contamination. From these tubes, two more nutrient broth tubes were inoculated with a loop and the newly inoculated tubes were refrigerated until such time as to be incubated for the next day's experiment. The 20 hr. culture tubes were then centrifuged at 3000 g for 20 min. The cells were resuspended in a very small amount of distilled water. This dense suspension would soon be diluted to produce the initial cell suspension.

Throughout the series of runs, 1000 ml. of initial cell suspension containing 2.6×10^7 cells/ml. were required. To facilitate the task of preparing this suspension, figure 3 was produced. This figure shows the relationship between the % transmittance reading and the cell concentration of the sample. (The method used for this calibration is described in Appendix A). Since the desired cell concentration of 2.6×10^7 cells/ml. was off the graph, a 500 ml. suspension containing 5.2×10^7 cells/ml. was first prepared. This was done by adding a

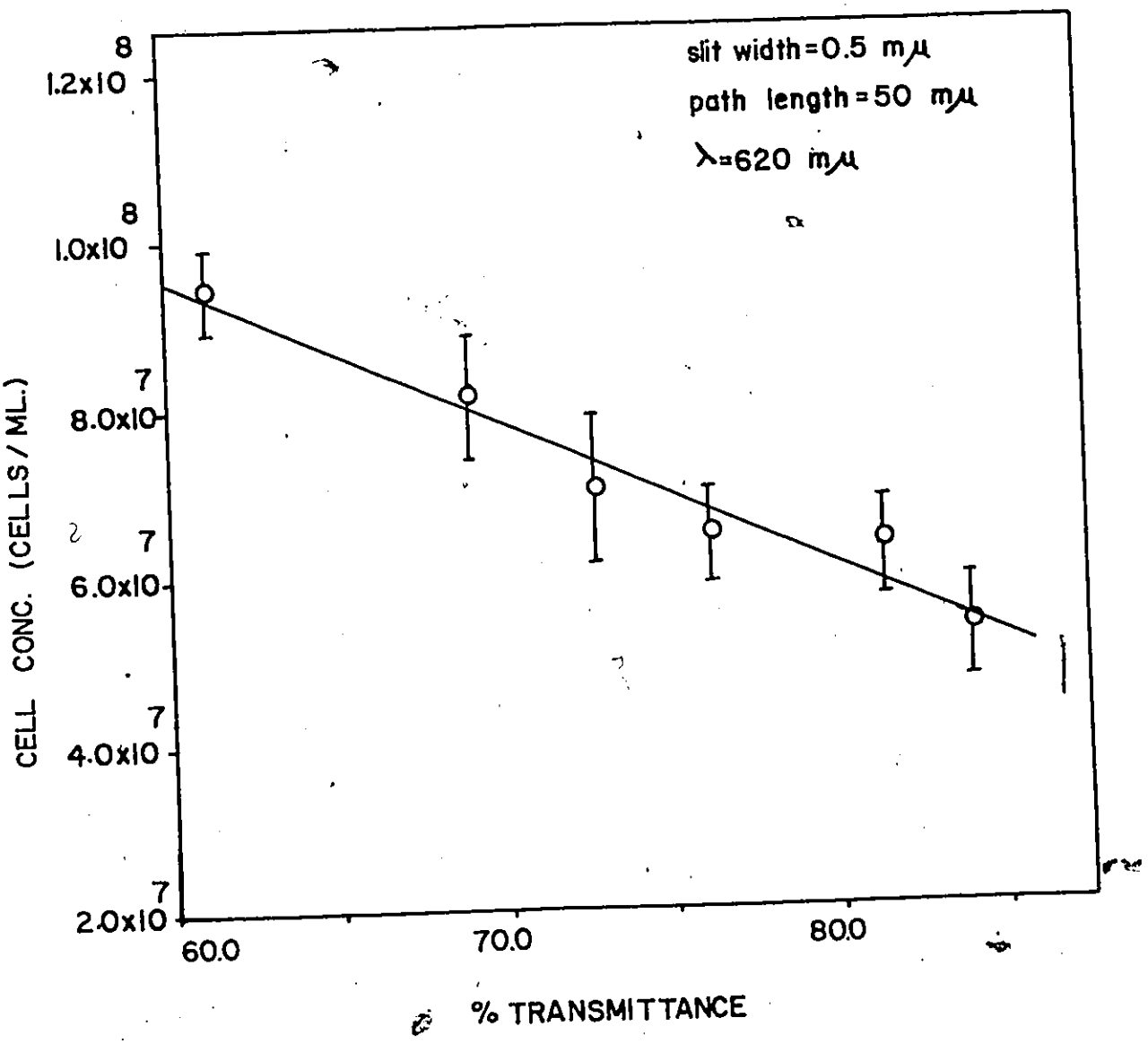

FIGURE 3 - RELATIONSHIP BEETWEEN % TRANSMITTANCE & CELL CONCENTRATION OF THE SAMPLE

few ml. of the dense cell suspension to distilled water and making up the volume to 500 ml. The suspension was then stirred with a magnetic stirrer and a sample was placed in the spectrophotometer cell. If the reading of 85% transmittance was not obtained, the 500 ml. cell suspension was diluted or concentrated accordingly until the desired reading was read out. This suspension was then diluted 1:1 to give 1000 ml. of suspension having a cell concentration of 2.6×10^7 cells/ml.

Throughout the series of runs, the initial cell concentration was held constant at 2.6×10^7 cells/ml. However, with the use of figure 3, it would have been a simple matter to prepare initial cell suspensions of various concentrations.

It would be worthwhile to point out a problem that was encountered in this part of the experiment. The 1% inoculation from a 24hr. culture, advocated by Grieves and Wang[4], was found unsatisfactory. After the 20 hr. incubation period, this method produced aggregates of E. Coli resembling strings under the microscope. Any attempt to break up the aggregates by stirring and/or a pH change of the cell suspension to acid met with failure. The method was abandoned. Instead, the loop inoculation was tried and found very satisfactory.

3. The Foaming Procedure

A 2.0 mg./ml. solution of EHDA-Br in distille water was freshly prepared. The air flow was started and maintained at 9.3 l./min. 1000 ml. of initial cell suspension were poured in the foaming unit through the opening at the top of the column. The opening was then tightened securely. Varying quantities of EHDA-Br solution were injected into the column with a syringe, providing initial concentrations of EHDA-Br ranging from 0.020 to 0.040 mg./ml. As soon as the foam appeared in the receiver, the timer was started. The length of the foam runs was varied from 2 to 10 minutes. Temperature was held at 20 $\pm$ 1°C. At the end of each run, the residual suspension was taken for total cell count analysis and for the measurement of residual cell volume. After each experiment, the column was washed with EHDA-Br solution and rinsed with distilled water. Then, the glass tube containing glass wool was placed in 250°F oven and left there overnight. It would be attached to the foaming unit the following day.

4. Total Cell Count Analysis

To find out the residual cell concentration, the membrane-filter technique for total cell count was used. A sterile Gelman filter of 0.45 mμ pore size was placed asceptically (grid side up) in the autoclaved filter holder. The sample of residual suspension containing 30-300 cells per microscopic field was poured into the funnel and vacuum was applied to start filtration. The funnel walls were rinsed with 20 ml. of fresh distilled water. The funnel was then removed and the filter was transferred to a clean Petric dish. Prior to staining, the filter had to be dried in a preheated 100°F oven. By placing the lid of the Petri dish in a slanted position, the drying period could be reduced to approximately 30 min. The filter was then cut into two sections. One section was kept intact for possible future reference but the other section was stained in the following manner. The filter section was placed on a pad saturated with distilled water for a few seconds so that the water diffused to the entire area. The bottom of the membrane filter was then blotted to remove excess water. The filter was then placed for 2 min. on a pad saturated with 10%, W/V, nitric acid in distilled water, 4 min. on a pad saturated with 0.1%, W/V, acid fuchsin of pH 6.5, and 90 seconds on a pad saturated with 0.02%, W/V, methylene blue of pH 6.4, in this order. After each treatment, the bottom of the filter section was blotted to remove excess solution. The filter was

then placed in a Petri dish and ovendried at 100° F for 30 minutes. For microscopic examination, the prepared filter section was placed on top of a drop of immersion oil on a microscopic slide; another drop of oil was put on the surface of the filter, the whole was covered with a cover slide, and a final drop of oil was put on the slide. The slide was ready for examination when the filter became transparent.

Examination was made with an immersion objective x 100 and ocular x 15 using a micrometer disk in one of the oculars ruled with 100 sections. The bacteria appeared dark red against a faint blue background. For each experiment sixteen counts (sexteen microscopic fields) were made. However, if there were less than 30 bacteria per gridded surface, more fields were counted for higher accuracy.

The staining procedure described above is the author's modification of an improved technique for staining bacteria on membrane filters[22]. Grieves and Wang[4] followed a different staining procedure which involved the use of 0.06% basic fuchsin solution and filtering of solution downward through the filter. With their method, the bacteria appeared dark red against a red background, making the task of enumerating tedious and unreliable.

One of the problems that were encountered in total cell count analysis involved the use of distilled water that had been stored in polyethylene containers for two days or longer. When this water was

used to dilute the cell suspensions or to rinse the filtration funnel, the bacteria could not be enumerated properly. Extraneous material interferred with observation of bacteria. Upon investigation, it was concluded that this material had leached from the polyethylene containers. Thereafter, fresh distilled water and glass containers were used.

5. Sterilization and Disinfection

The use of an autoclave in sterilizing the growth media, old culture tubes, filtration equipment, etc., was indispensable. However, many of the contaminated beakers, graduated cylinders, and flasks were easily disinfected by being left overnight in a 1:10 dilution of 12% sodium hypochlorite solution. The following day, they were washed with detergent and rinsed with distilled water.

RESULTS AND DISCUSSION

The following material balances can be written for the column[4]:

$$V_i = V_r + V_f \tag{1}$$

$$Z_i V_i = Z_r V_r + Z_f V_f \tag{2}$$

where the subscripts i, r, and f refer to the initial, residual, and collapsed foam suspensions, respectively. The volume in ml. is designated by V, and the cell concentration, in number of cells per ml., is designated by Z. Equations (1) and (2) can be used to calculate the concentration of cells in the collapsed foam, provided V_r and Z_r have been measured. As emphasized by Grieves and Wang[4], the quantity $Z_f V_f$ represents the total cells carried from the column in the foam phase as well as the cells that adhere to the glass walls of the column above the liquid level. Thus, $Z_f V_f$ represents the quantity of cells removed from the initial suspension by foam separation.

In describing the efficiency of a foam separation process, the enrichment ratio, Z_f/Z_r, i.e. the concentration of cells in the total

collapsed foam suspension divided by that in the residual suspension becomes a useful parameter.

I. Effect of Foaming Time and Surfactant Concentration

Three sets of experiments were conducted using initial cell suspension of 2.6×10^7 cells/ml., air rate of 9.3 l./min., and surfactant concentrations of 0.020, 0.030, and 0.040 mg./ml. Foaming times were varied from 2 to 10 minutes.

Figure 4 shows the relationship between residual cell concentration and foaming time with parameters of initial surfactant concentration. Each point on the graph is the result of a separate experiment.

As might have been expected, the residual cell concentration decreased as the foaming time was prolonged. The greatest decrease in the residual cell concentration appeared to take place within the first 2 to 3 minutes of foaming. Also, at a constant value of foaming time, lower residual cell concentrations were obtained with lower initial surfactant concentrations and this effect became more pronounced as θ increased. For example, at θ equal to 10 min., the residual cell concentration had dropped to 2.6×10^4 cells/ml. (99.9% removal) for the run with the initial surfactant concentration of 0.030 mg./ml. and 1.45×10^5 cells/ml. (99.4% removal) for that with initial surfactant concentration of 0.040 mg./ml. The experiments with the initial surfactant concentration of 0.020 mg./ml. could not be continued for more than 4 minutes. Even though this surfactant concentration gave the best results for short periods of foaming, after 4 minutes the foam became unstable.

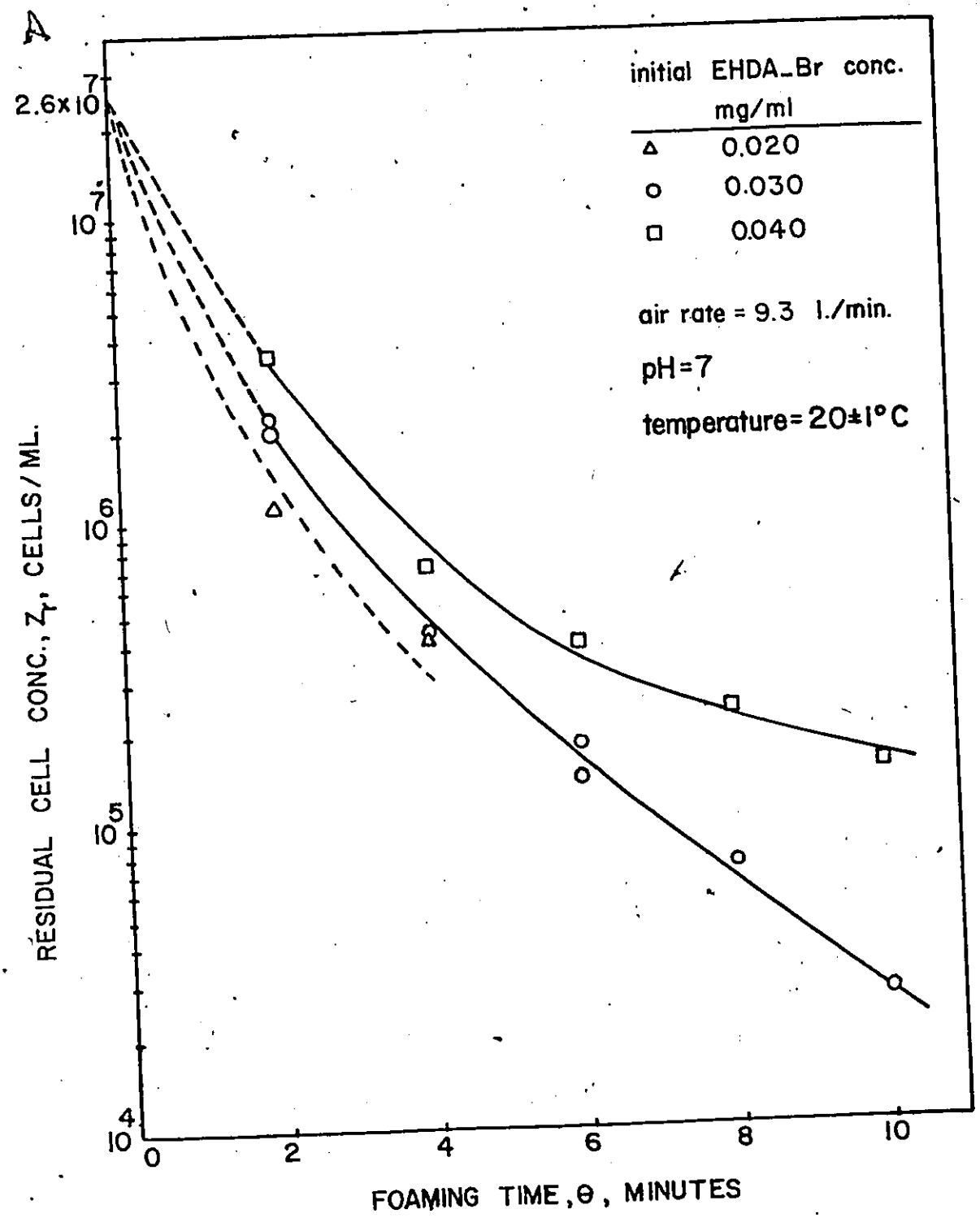

FIGURE 4 - RELATIONSHIP BETWEEN RESIDUAL CELL CONC. & FOAMING TIME WITH PARAMETERS OF INITIAL SURFACTANT CONCENTRATION

There was a minimum limit of bulk entrainment in the apparatus used. This was governed by air rate, surfactant concentration, height of foam column, bubble size, and viscosity of the liquid phase. It looks as if the lower limit was approached at surfactant concentration of 0.030 mg./ml. Below this value, one cannot obtain a stable foam for a reasonable amount of time.

Table 1 presents the values of collapsed foam volumes that were calculated for the three sets of experiments discussed here. At any given foaming time, the amount of entrained bulk suspension in the foam was highest for the experiment with the greatest initial surfactant concentration. Of course the process should be conducted in such a way that a product of least possible volume and the highest possible cell concentration would be finally obtained. This would be equivalent to a very dry foam. The experiments conducted with surfactant concentration of 0.030 mg./ml. came closest to yielding such a product.

The table shows that as θ increased, the foam became drier. An obvious reason was that the surfactant concentration in the column had decreased. Another contributing factor was that after the initial burst of foam, the liquid level in the column had dropped considerably and the distance that the foam had to rise before leaving the foam port had increased, thus allowing the entrained liquid to drain back into the column.

TABLE 1

The collapsed foam volumes for the three sets of runs investigating the effects of foaming time and initial surfactant concentration

	EHDA-Br conc. = 0.020 $\frac{mg}{ml}$		EHDA-Br conc. = 0.030 $\frac{mg}{ml}$		EHDA-Br conc. = 0.040 $\frac{mg}{ml}$	
θ, (min)	V_f, (ml)	R*, (ml/min)	V_f, (ml)	R, (ml/min)	V_f, (ml)	R, (ml/min)
2	125	62.5	275+	137+	337	189
4	85	21.2	281	70.2	491	123
6	-	-	338+	56.3+	394	65.6
8	-	-	248	31.0	427	53.4
10	-	-	362	36.2	449	44.9

* Rate of entrained bulk liquid removal in ml./min.

\+ Average of two experiments

The relationship between cell enrichment ratio and foaming time with parameters of initial surfactant concentration is presented in figure 5. Upon examination of this figure, once again it becomes evident that the best performance is obtained with the smallest initial concentration of surfactant with as long a foaming as is possible.

Using a log-log curve fit program, the equations of lines passing through the points were found to be

$$Z_f/Z_r = 7.06\ \theta^{2.50} \quad \text{for initial EHDA-Br conc. of 0.030 mg./ml.}$$

$$Z_f/Z_r = 4.86\ \theta^{1.93} \quad \text{for initial EHDA-Br conc. of 0.040 mg./ml.}$$

which indicate that the cell enrichment ratio is a power function of the foaming time.

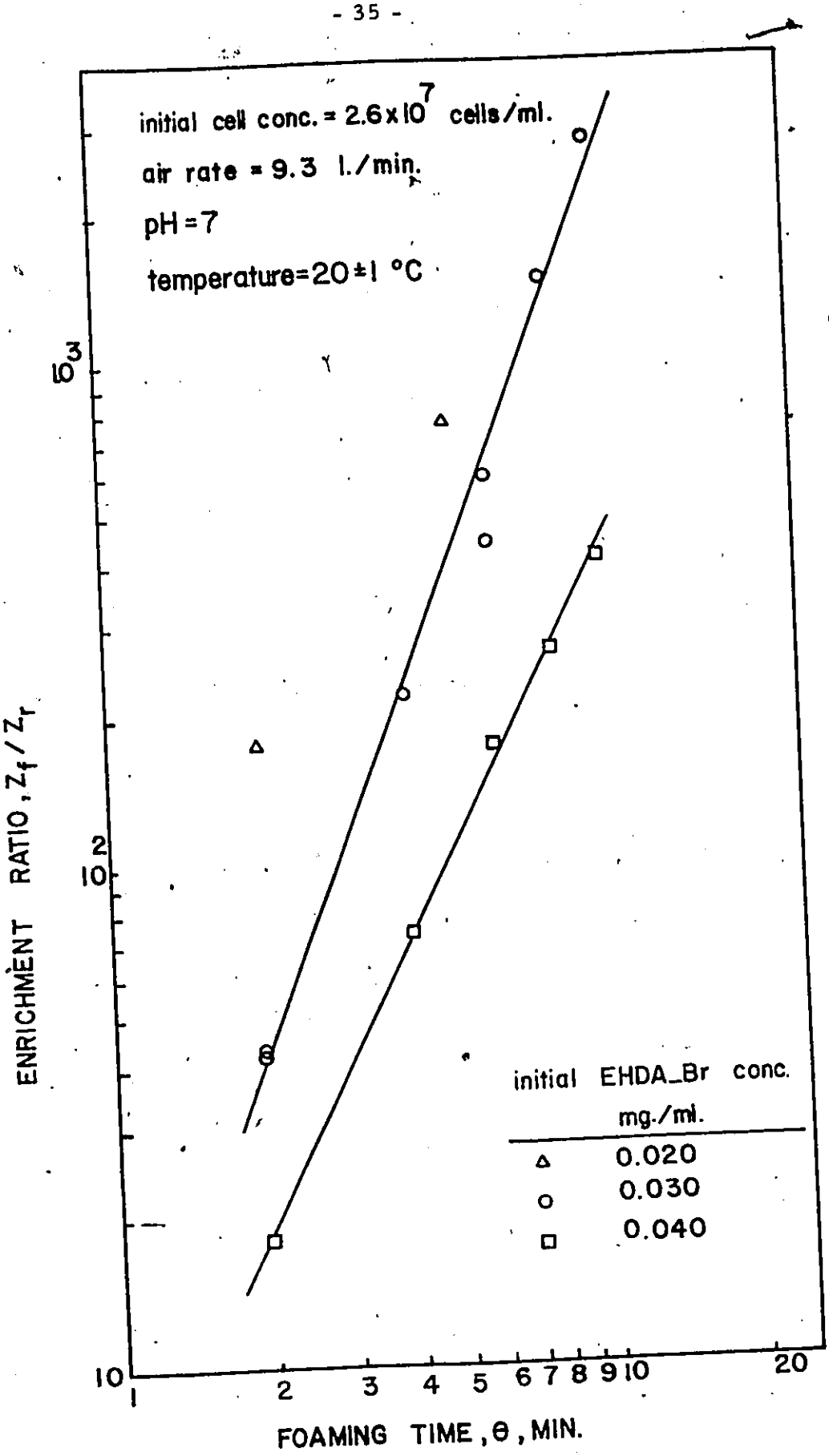

FIGURE 5_ ENRICHMENT RATIO VS. FOAMING TIME

II. Effect of the Presence of Inorganic Sodium Salts

To investigate the effect of the presence of inorganic salts on the foam separation of E. Coli, a second series of experiments was conducted with an initial cell concentration of 2.6×10^7 cells/ml., air rate of 9.3 l./min., initial surfactant concentration of 0.030 mg./ml., and a foaming time of 4 minutes. In each experiment, 0.17, 0.856, 1.71, 3.42, or 8.56 μ equivalent/ml. of NaCl, $NaNO_3$, Na_2CO_3, or Na_2SO_4 were added to the initial suspensions before the addition of the surfactant. The results were evaluated in terms of the ratio of the residual cell concentration with salt (Z_{rs}) to the control without salt (Z_r) and related to the salt concentration in the initial suspension.

By using salts of the same cation, i.e., sodium, one might investigate the relative effects of the radicals Cl^-, NO_3^-, $CO_3^=$, and $SO_4^=$ on the foam separation of E. Coli. Competition between the cells and Cl^-, NO_3^-, $CO_3^=$, and $SO_4^=$ radicals for $EHDA^+$ ions was expected. Also it seemed reasonable to assume that the competition shown by $CO_3^=$ and $SO_4^=$ would be roughly twice that shown by Cl^- and NO_3^-. To evaluate the effect of the radicals on the same basis (i.e., valency), Z_{rs}/Z_r ratios were plotted against salt normalities instead of salt molarities. Consequently, it was expected that at each normality reading, Z_{rs}/Z_r ratios would be similar for all the salts.

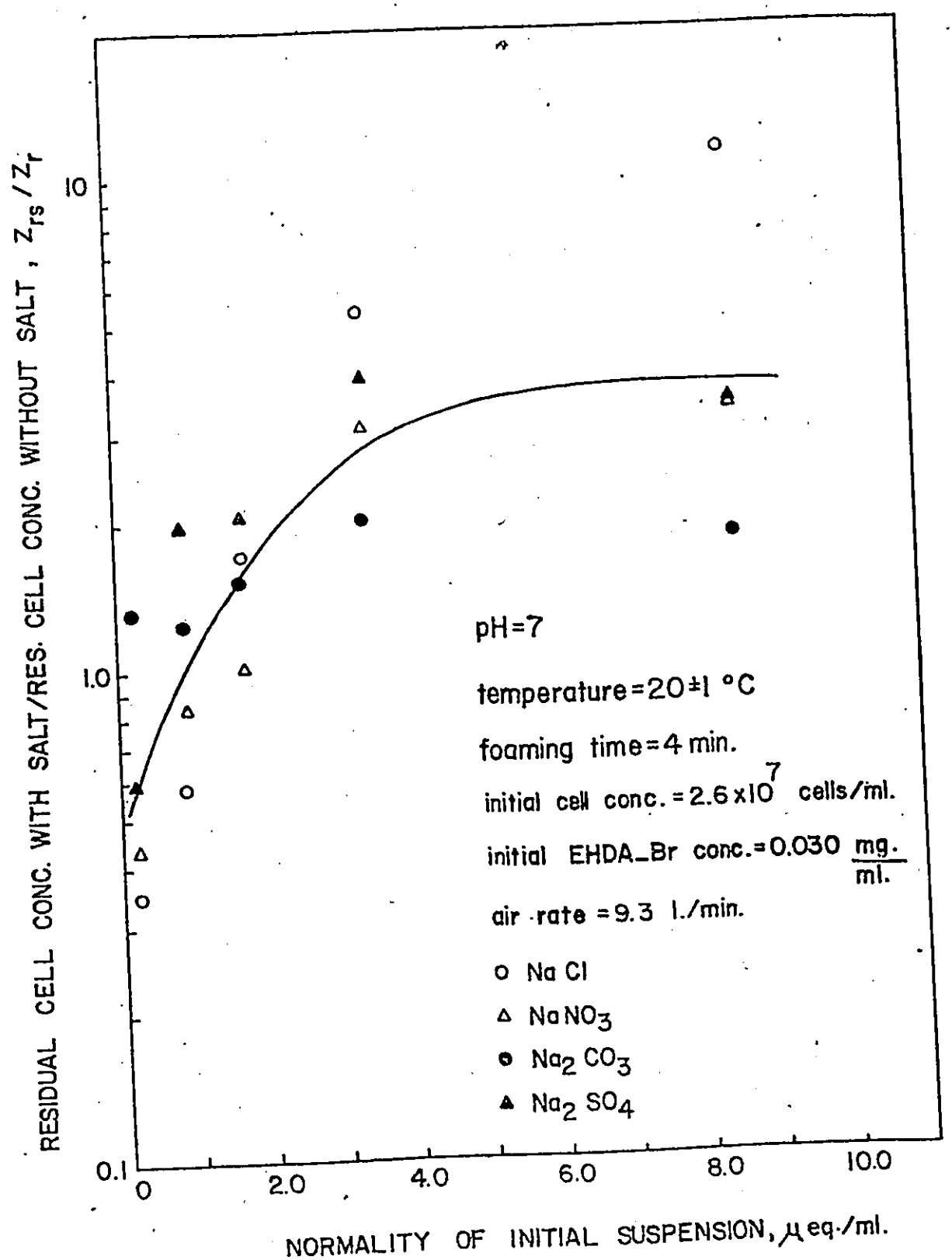

FIGURE 6 _RATIOS OF RESIDUAL CELL CONCENTRATIONS FROM SUSPENSIONS CONTAINING SALTS OF SPECIFIED NORMALITIES TO RESIDUAL CELL CONC. WITHOUT SALT

Figure 6 shows the results. With the exception of Na_2CO_3, the addition of 0.17 μ eq./ml. of any salt decreased the residual cell concentration. However, as the salt concentration in the initial suspension increased, the residual cell concentration increased significantly and with the exception of NaCl, the Z_{rs}/Z_r ratio began to level off at salt concentrations greater than 3.5 μ eq./ml. In general, it may be stated that all the salts used in this study had an adverse effect on the foam separation of E. Coli.

Grieves and Wang[5] have studied the effect of presence of NaCl Na_2SO_4, $MgCl_2$, $MgSO_4$, $CaCl_2$, and $CaSO_4$ on the foam separation of E. Coli, using EHDA-Br as surfactant. It was found that with the exception of very low concentrations of $CaCl_2$, or $CaSO_4$, the addition of any of the above named salts increased significantly the residual cell concentration. This effect was much more pronounced when $MgSO_4$ was used. The fact that low concentrations of $CaSO_4$ did not increase Z_{rs}/Z_r in a manner similar to $MgSO_4$ indicated that simple valency was not the only salt effect on the foam separation of bacteria, and that the nature of the cation also played a role.

Similarly, from figure 6, one can see that NaCl does not increase Z_{rs}/Z_r in a manner similar to $NaNO_3$. This indicates that the intrinsic nature of the radical also plays a role.

Actually, these results should not be surprising. Besides an obvious element of competition that exists between the cells and the radical ions for $EHDA^+$ adsorption, the presence of electrolytes affects the magnitude of the negative charge at the cell surface. It has been reported[3] that the bacterial surface acts as a biological interface interposed between solid and liquid phases and that at the site of this interface, ions are positioned in such a way that the solid phase (i.e., the cell) maintains a net negative charge balanced by the net positive charge of the liquid phase. At neutral pH, the magnitude of this charge depends (i) on structural features of the cell surface, such as the dissociation of carboxyl and amino groups, and (ii) the nature and number of ions or ionic groups available for adsorption, i.e., ionic strength. It is possible that the salts that were added to the initial suspensions reduced (each to a certain extent) the bacterial surface charge, thereby rendering the cells unsuitable for flotation.

The collapsed foam volumes were higher for the experiments with salts than for the control without salt. The combination of high collapsed foam volumes and high residual cell concentrations makes the presence of salts undesirable.

CONCLUSIONS

Based on the studies carried out, the following conclusions can be drawn regarding the foam separation of E. Coli with EHDA-Br:

1. In the case of this study, the best performance (99.9% removal) is obtained with an initial cell concentration of 2.6×10^7 cells/ml., air superficial velocity of 164 cm./min., pH of 7 , temperature of 20 °C, EHDA-Br concentration of 0.030 mg./ml., and a 10 min. foaming time.

2. The presence of NaCl, $NaNO_3$, Na_2CO_3 , and Na_2SO_4 in the initial suspension had an adverse effect on the foam separation of E. Coli with EHDA-Br.

3. The high efficiency of the separation process and the simple equipment set-up, make microflotation a valid means of separating cells from aqueous medium.

RECOMMENDATIONS FOR FUTURE WORK

The experience gained from the foam separation of E. Coli with EHDA-Br, and a study of the available literature on microflotation, has convinced the author that there is a great need for understanding the removal mechanism. For as long as this remains a mystery, no scientific explanation can be furnished for the results.

Taking the case of E. Coli as a model, a plan of research would include the following elements:

1. An attempt would be made to see if the uptake curve of EHDA-Br by E. Coli is that of an adsorption isotherm.

2. Electron microscopy of E. Coli - EHDA-Br suspensions would be made in order to determine if, at the same conditions as for microflotation (i.e., minimum surfactant concentration giving a stable foam, specific pH, contact or foaming time, temperature, etc.), the osmotic barrier or any other part of the cells have been damaged.

3. The effect of various cations and anions on the foam separation of E. Coli would be investigated. An attempt would be made to see if the results can be explained on the basis of the double layer theory.

Considering the practical aspect of E. Coli flotation:

1. Foam separation experiments would be conducted with initial suspensions containing salts in concentrations comparable to those found in the water of a sewage treatment plant. The presence of salts at such concentrations might reduce the removal rate of the cells to such a level that the use of microflotation in a sewage treatment plant may be questioned.

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APPENDIX A

How to relate the % transmittance reading to cell concentration of the sample

METHOD

A 20 hr. culture tube of E. Coli was centrifuged and the cells were resuspended in approximately 125 ml. of fresh distilled water (see section 2 of experimental procedure). A clean Spectrosil cell of 50 mm. path length was filled with this initial cell sample, and at λ = 620 mμ, slit width of 0.5 mμ, the % transmittance (%T) reading was taken. A 1.0 ml. sample of this suspension was pipetted into a 100 ml. graduated flask and the glass was filled up to the mark with distilled water. From this second dilution, a sample giving between 30 and 300 cells per microscopic field was filtered, and the cells on the filter were enumerated (see section 4 of experimental procedure).

For each filter, sixteen counts were made. However, if there were counts where the number of bacteria per gridded surface was less than 30, more fields were counted for higher accuracy. The following equation was used to calculate C, the number of cells per ml. in the initial cell sample,

$$C = \frac{S.q.\ 10^6}{s.V} \quad \text{cells/ml} \qquad (3)$$

where S is equal to the "working" surface of the filter (mm^2), s is the area of the entire gridded surface on the micrometer disk (measured in μ^2 with a stage micrometer at the same magnification), q

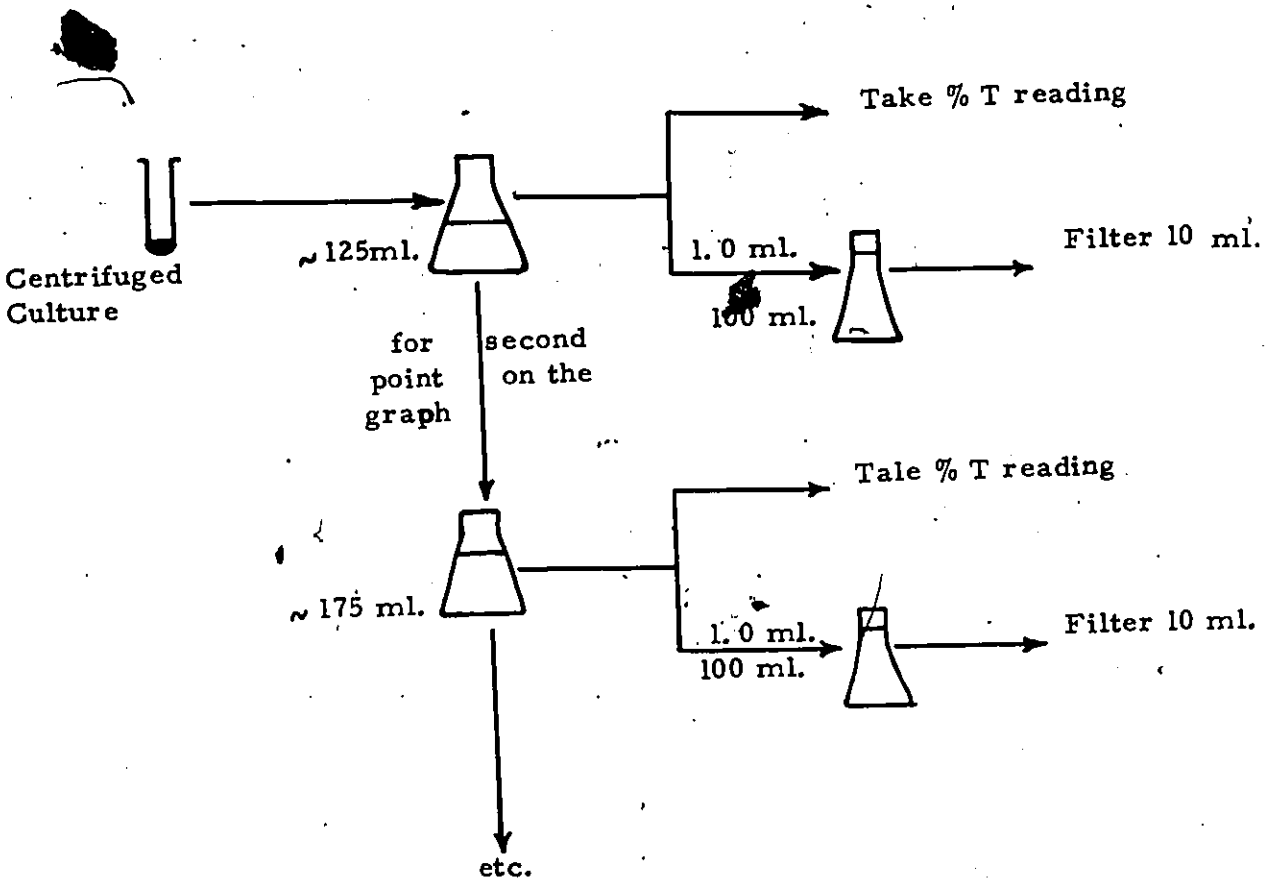
is the average number of bacteria per gridded surface, and V is the volume (ml.) of the initial cell sample which was filtered.

To calculate S, the inside diameter of the borosilicate funnel resting on the filter was measured,

$$S = \frac{\pi D^2}{4} = \frac{3.14\,(35.5)^2}{4} = 989.8 \text{ mm}^2$$

and s was found to be .5625 μ^2. With these constants evaluated, one only had to determine q and V each time that C would be related to a specific %T reading.

The procedure described above is for obtaining one point on the cell concentration versus %T graph. However, the same batch of bacteria could be used to obtain another or indeed more points on the graph. The only consideration would be not to keep the cell suspension overnight (to avoid settling, clustering, etc.). To take more readings, one had to further dilute the original suspension, take another %T reading, rinse the filtration funnel thorougly with distilled water, and filter a sample of the suspension. The procedure can be shown graphically,



It is impossible to give exact dilution instructions as this will depend on the number of cells present in the 20 hr. culture tube. However, if the tube has been inoculated with a loop, the above diagram can serve as a guide.

Experimental and calculated data for plotting C vs. %T

Example and sample calculation: The centrifuged cells were re-suspended in approximately 125 ml. of distilled water and the%T reading of the suspension was found to be 61.3. 1.0 ml. of this suspension was pipetted into a 100 ml. flask and the flask was filled up to the mark with fresh distilled water. From this second dilution, 10.0 ml. were filtered; this represented 0.1ml. of the initial suspension, i.e., V = 0.1 ml. The filter was then prepared for microspic examination and sixteen counts were made,

$$\bar{q} = \frac{q_1 + q_2 + \ldots + q_{16}}{16}$$

$$= (53 + 63 + 64 + 55 + 52 + 50 + 44 + 53 + 49 + 51 + 59 + 48 + 47 + 56 + 59) / 16$$

$$= 53.6$$

Using equation (3), the cell concentration of the initial suspension was calculated,

$$C = \frac{989.8 \times 53.6 \times 10^6}{5625 \times 0.1} = 9.44 \times 10^7 \text{ cells/ml.}$$

Actually, 9.44×10^7 cells/ml. is the mean ($\bar{x}$) of a sample of sixteen counts. To determine the "confidence limits" within which

the population mean ($\bar{x}_p$) might fall, one must consider that since the number of items (i.e., counts) in the sample is small, the sampling distribution follows the t distribution[23]. In other words,

$$\frac{\bar{x} - \bar{x}_p}{\sigma_{\bar{x}}} = t \tag{4}$$

is distributed according to the t distribution. In equation (4), $\sigma_{\bar{x}}$ is the standard error of means drawn from samples (the value which would be obtained if one computed the standard deviation of means of all possible samples of N items). $\sigma_{\bar{x}}$ can, in turn, be evaluated from the following expression,

$$\sigma_{\bar{x}} = \frac{\bar{\sigma}}{N}$$

$$= \frac{\frac{\sum x^2}{N - 1}}{N} \tag{5}$$

where $\bar{\sigma}$ is the standard deviation of the population as estimated from the sample;

$\sum x^2$ is the sum of the squared deviations of each item in the sample from the sample mean;

N is the number of items in the sample.

The expression N-1 represents the degrees of freedom (n) present.

In the case of the cell suspension having %T reading of 61.3 ,

$$\bar{x} = 9.44 \times 10^7 \text{ cells/ml.}$$

$$\bar{\sigma} = \frac{989.8 \times 10^6}{5625 \times 0.1} \times \sqrt{\frac{475.75}{15}} = 9.910 \times 10^6 \text{ cells/ml.}$$

and $$\sigma_{\bar{x}} = \frac{9.910 \times 10^6}{16} = 2.48 \times 10^6 \text{ cells/ml.}$$

By considering equation (4) and referring to the t table[23], opposite n = 15, one would observe that the sample means would vary $\pm 2.131 \sigma_{\bar{x}}$ or more from the population mean in about 5 samples out of 100. Assuming that the 0.05 level of significance is satisfactory as a criterion, one finds that, if the observed mean is below the population mean, the population mean might be $9.44 \times 10^7 + (2.131)(2.48 \times 10^6) = 9.96 \times 10^7$ cells/ml.; while if the observed mean is above the population mean, the population mean might be $9.44 \times 10^7 - (2.131)(2.48 \times 10^6) = 8.91 \times 10^7$ cells/ml. It can be concluded, then, that it is likely that the population mean falls between 9.96×10^7 cells/ml. and 8.91×10^7 cells/ml., since the fiducial probability is 95 out of 100 that the population mean falls between these limits.

A program was written to perform the above calculations for each %T reading. The mean cell concentration values (and their 95% confidence limits) were plotted against the corresponding %T readings. Using a second program, the line of best fit was drawn through the points (fig. 3, page 21).

```
6  REM   PROGRAM TO CALCULATE MEAN CELL CONC.& 95% CONFIDENCE LIMITS
7  PRINT
10  DIM X[20],Y[20]
15  READ T,V1,N,T1
20  LET S1= 0
25  LET S2= 0
27  FOR I=1 TO N
28    READ X[I]
30    LET S1=S1+X[I]
32  NEXT I
35  LET X1=S1/N
40  LET N1=989.8*X1*1E+6/(5625*V1)
44  PRINT "%T",T
45  PRINT
50  PRINT "CELL CONC.",N1
51  PRINT "(CELLS/ML)"
52  PRINT
53  PRINT "MEAN",N1
55  FOR I=1 TO N
60    LET A=X[I]-X1
65    LET S2=S2+A*A
67  NEXT I
70  LET S=S2/N
71  LET S3=S2/(N-1)
80  LET N2=989.8* SQR (S)*1E+6/(5625*V1)
81  LET N3=989.8* SQR (S3)*1E+6/(5625*V1)
82  LET N4=N3/ SQR (N)
85  PRINT "STD  DEV",N2
87  PRINT "STD DEV POP",N3
89  PRINT "STD ERR MEAN",N4
90  PRINT
92  LET N5=N1+(N4)*(T1)
93  LET N6=N1-(N4)*(T1)
94  PRINT "95%CONF.LIMITS ARE",N5,N6
95  PRINT
100  DATA  61.3, .1, 16, 2.131
110  DATA  53, 63, 64, 55, 55, 52, 50, 44
120  DATA  53, 49, 51, 59, 48, 47, 56, 59
999  END

RUN

%T              61.3

CELL CONC.      9.43609E+7
(CELLS/ML)

MEAN            9.43609E+7
STD  DEV        9.5952E+6
STD DEV POP     9.90989E+6
STD ERR MEAN    2.47747E+6

95%CONF.LIMITS ARE          9.96404E+7     8.90814E+7

*READY
```

```
100  DATA  69.1,.1,16,2.131
110  DATA  45,48,41,60,50,43,56,51
120  DATA  47,47,33,38,50,32,57,43
RUN

%T              69.1

CELL CONC.      8.14935E+7
(CELLS/ML)

MEAN            8.14935E+7
STD  DEV        1.35692E+7
STD DEV POP     1.40142E+7
STD ERR MEAN    3.50356E+6

95%CONF.LIMITS ARE          8.89596E+7     7.40274E+7


*READY

100  DATA  72.7,.1,19,2.101
110  DATA  45,27,35,21,40,53,35,51,18,58
120  DATA  46,40,45,41,35,37,32,55,45
RUN

%T              72.7

CELL CONC.      7.02932E+7
(CELLS/ML)

MEAN            7.02932E+7
STD  DEV        3.99179E+7
STD DEV POP     1.90648E+7
STD ERR MEAN    4.37377E+6

95%CONF.LIMITS ARE          7.94824E+7     6.11039E+7


*READY

100  DATA  76.2,.1,18,2.110
110  DATA  42,32,32,27,40,34,23,36
120  DATA  51,31,33,36,37,37,37,44,49,37
RUN

%T              76.2

CELL CONC.      6.43248E+7
(CELLS/ML)

MEAN            6.43248E+7
STD  DEV        1.19377E+7
STD DEV POP     1.22838E+7
STD ERR MEAN    2.89531E+6

95%CONF.LIMITS ARE          7.04339E+7     5.82157E+7
```

```
*READY

100  DATA  81.4,.1,20,2.093
110  DATA  40,37,39,43,25,40,34,25,34,26
120  DATA  32,28,53,35,39,33,44,39,40,30
RUN

%T              81.4

CELL CONC.      6.29952E+7
(CELLS/ML)

MEAN            6.29952E+7
STD  DEV        1.21479E+7
STD DEV POP     1.24635E+7
STD ERR MEAN    2.78692E+6

95%CONF.LIMITS ARE          6.88283E+7     5.71622E+7


*READY


100  DATA  84.0,.2,16,2.131
110  DATA  41,79,51,66,45,55,56,87
120  DATA  54,53,62,66,55,84,64,47
RUN

%T              84

CELL CONC.      5.30643E+7
(CELLS/ML)

MEAN            5.30643E+7
STD  DEV        1.15208E+7
STD DEV POP     1.18986E+7
STD ERR MEAN    2.97466E+6

95%CONF.LIMITS ARE          5.94033E+7     4.67253E+7


*READY
```

```
LIST
 5  REM    PROGRAM FOR LINE OF BESTFIT
 6  REM     Y=A+BX
 8  DIM X[6],Y[6]
 9  READ N
 10  LET A= 0
 11  LET B= 0
 12  LET C= 0
 14  LET D= 0
 15  FOR I=1 TO N
 20    READ X[I],Y[I]
 25    LET A=A+X[I]
 26    LET B=B+X[I]*X[I]
 27    LET C=C+Y[I]
 28    LET D=D+X[I]*Y[I]
 30  NEXT I
 31  PRINT
 32  PRINT
 40  LET Z=N*B-A*A
 41  LET I=(C*B-D*A)/Z
 42  LET S=(N*D-A*C)/Z
 44  PRINT "Y-INT",I
 45  PRINT "SLOPE",S
 46  PRINT
 47  PRINT "XEXPT","YEXPT","YCALC","% ERROR"
 50  FOR H=1 TO N
 55    LET Q=I+S*X[H]
 60    LET E=(Q-Y[H])*100/Y[H]
 65    PRINT X[H],Y[H],Q,E
 70  NEXT H
 80  GOTO  10
 100  DATA  6
 110  DATA  61.3, 9.43E+7
 120  DATA  69.1, 8.15E+7
 130  DATA  72.7, 7.03E+7
 140  DATA  76.2, 6.43E+7
 150  DATA  81.4, 6.3E+7
 160  DATA  84, 5.31E+7
 999  END
 RUN

 Y-INT          1.993E+8
 SLOPE         -1.72993E+6

 XEXPT          YEXPT          YCALC          % ERROR
  61.3           9.43E+7        9.32556E+7    -1.10753
  69.1           8.15E+7        7.97622E+7    -2.1323
  72.7           7.03E+7        7.35344E+7     4.6009
  76.2           6.43E+7        6.74797E+7     4.94507
  81.4           6.3E+7         5.84841E+7    -7.16815
  84             5.31E+7        5.39862E+7     1.669
```

APPENDIX B

Tables of Experimental Data

In conducting the experiments to determine the effects of foaming time and initial surfactant concentration, the following parameters were held constant:

temperature	20 ± 1°C
air rate	9.3 l./min.
initial cell conc.	2.6×10^7 cells/ml.
initial volume	1.0 l.

Table 2 - Experimental data for the runs with initial EHDA-Br concentration of 0.020 mg./ml.

Run	foaming time θ (min.)	residual vol. Vr (ml.)	vol. of residual suspension filtered V (ml.)	microscopic counts q i
1	2.0	875	10.0	64, 90, 66, 40, 85, 70, 71, 58, 59, 83, 77, 51, 36, 57, 60, 73
2	4.0	915	25.0	59, 88, 91, 44, 46, 43, 52, 45, 46, 48, 30, 63, 65, 45, 53, 91

Table 3 - Experimental data for the runs with initial EHDA-Br concentration of 0.030 mg./ml.

Run	foaming time θ (ml.)	residual vol. Vr (ml.)	vol. of residual suspension filtered V (ml.)	microscopic counts q i
3	2.0	740	5.0	53, 38, 97, 68, 38, 63, 72, 103, 70, 81, 82, 58, 69, 56, 56, 40
4	2.0	711	5.0	53, 47, 65, 58, 49, 18, 41, 54, 56, 76, 38, 66, 56, 65, 63, 88
5	4.0	719	25.0	82, 55, 57, 58, 70, 53, 82, 74, 61, 41, 61, 60, 43, 60, 55, 50
6	6.0	570	70.0	50, 52, 52, 53, 62, 56, 50, 56, 70, 45, 35, 76, 53, 65, 56, 40
7	6.0	754	73.0	66, 54, 66, 44, 72, 117, 50, 135, 74, 75, 62, 55, 64, 61, 57, 120
8	8.0	752	202	107, 94, 97, 92, 79, 54, 88, 83, 65, 93, 79, 78, 53, 104, 60, 83
9	10.0	638	306	52, 57, 31, 47, 48, 37, 37, 38, 50, 48, 51, 52, 38, 44, 38, 60

Table 4 - Experimental data for the runs with initial EHDA-Br concentration of 0.040 mg./ml.

Run	foaming time θ (min.)	residual vol. Vr (ml.)	vol. of residual suspension filtered V (ml.)	microscopic counts q i
10	2.0	623	5.0	107, 62, 83, 117, 97, 143, 82, 85, 115, 106, 84, 79, 103, 137, 99, 109
11	4.0	509	24.8	85, 132, 117, 98, 104, 102, 95, 87, 85, 116, 79, 97, 115, 109, 86, 101
12	6.0	606	50.0	114, 177, 107, 93, 111, 121, 98, 109, 91, 98, 114, 100, 122, 70, 98, 117
13	8.0	573	82.0	112, 147, 110, 116, 131, 104, 88, 118, 93, 111, 80, 101, 97, 114, 68, 120
14	10.0	551	72.0	36, 43, 59, 62, 57, 66, 72, 67, 54, 37, 58, 66, 69, 59, 61, 84

In conducting the experiments to determine the effect of presence of inorganic sodium salts, the following parameters were held constant:

temperature	20 ± 1°C
air rate	9.3 l./min.
initial cell conc.	2.6×10^7 cells/ml.
initial volume	1.0 l.
initial EHDA-Br. conc.	0.030 mg./ml.
foaming time	4.0 min.

Table 5 - Experimental data for the runs with Na Cl

Run	Na Cl added (g./l.)	residual vol. Vr (ml.)	vol. of residual suspension filtered V (ml.)	microscopic counts q i
15	0.0100	596	50.5	45, 44, 31, 57, 52, 30, 51, 37, 43, 38, 46, 56, 53, 44, 41, 45
16	0.0502	574	77.0	88, 111, 98, 113, 80, 109, 68, 131, 127, 93, 130, 110, 109, 123, 89, 105
17	0.0999	542	21.0	99, 116, 88, 97, 75, 76, 78, 105, 83, 75, 97, 86, 66, 72, 73, 71
18	0.1997	645	19.6	169, 293, 260, 281, 188 195, 306, 231, 244, 276 207, 296, 246, 264, 234 289
19	0.5002	-	2.0	45, 43, 54, 62, 80, 44, 60, 51, 85, 62, 70, 42, 50, 52, 25, 35, 37

Table 6.- Experimental data for the runs with Na NO_3

Run	Na NO_3 added (g./l.)	residual vol. Vr (ml.)	vol. of residual suspension filtered V (ml.)	microscopic counts q i
20	0.0146	626	58.0	77, 66, 95, 47, 36, 42 69, 56, 65, 59, 47, 5 65, 55, 51, 64
21	0.0736	596	47.5	119, 79, 79, 109, 120, 93 69, 79, 104, 77, 70, 12 86, 114, 91, 107
22	0.1450	582	23.8	52, 79, 74, 51, 63, 46 41, 91, 63, 34, 37, 55 49, 71, 62, 45
23	0.2906	567	9.1	69, 90, 74, 58, 80, 69 71, 68, 44, 52, 69, 61 71, 46, 82, 62
24	0.7279	693	5.8	58, 55, 34, 60, 59, 50 50, 39, 54, 48, 45, 30 44, 44, 36, 31

Table 7 - Experimental data for the runs with $Na_2 CO_3$

Run	$Na_2 CO_3$ added (g./l.)	residual vol. Vr (ml.)	vol. of residual suspension filtered V (ml.)	microscopic counts q i
25	.0091	500	15.3	44, 34, 44, 52, 56, 45, 52, 36, 66, 51, 48, 61, 53, 45, 34, 49
26	.0455	509	16.1	39, 49, 61, 55, 69, 49, 44, 48, 57, 34, 34, 61, 44, 44, 35, 38
27	0.0910	614	15.0	43, 44, 66, 71, 59, 52, 71, 62, 48, 53, 78, 47, 36, 38, 45, 53
28	0.1810	642	15.1	54, 80, 65, 67, 45, 93, 77, 65, 63, 75, 89, 87, 77, 50, 109, 60
29	0.4533	663	12.8	47, 54, 57, 60, 69, 40, 70, 50, 79, 41, 43, 50, 57, 57, 54, 62

Table 8 - Experimental data for the runs with $Na_2 SO_4$

Run	$Na_2 SO_4$ added (g./l.)	residual vol. Vr (ml.)	vol. of residual suspension filtered V (ml.)	microscopic counts q i
30	0.0122	578	30.0	47, 38, 41, 32, 52, 73, 33, 32, 45, 43, 43, 32, 35, 57, 34, 32
31	0.0610	555	17.6	99, 83, 107, 95, 79, 85, 47, 82, 90, 85, 68, 87, 70, 79, 94, 68
32	0.1214	570	10.0	59, 54, 51, 51, 33, 37, 26, 52, 87, 56, 47, 56, 49, 58, 26, 49, 34, 56
33	0.2428	601	5.0	56, 45, 53, 40, 50, 38, 66, 35, 74, 43, 40, 47, 53, 38, 27, 48, 31
34	0.6086	545	5.0	68, 30, 43, 38, 50, 41, 47, 25, 59, 18, 36, 42, 47, 41, 38, 35, 52, 33

APPENDIX C

Sample Calculation and Tables of Calculated Data

Calculations for run 1

The residual cell concentration, Z_r, was calculated from equation (3) in Appendix A.

$$Z_r = \frac{S.q.\ 10^6}{s.V}$$

For run 1, $q = (64 + 90 + 66 + 40 + 85 + 70 + 71 + 58 + 49 + 83 + 77 + 51 + 36 + 57 + 60 + 73)/16$

$= 65$

and $V = 10.0$ ml.

Therefore, $Z_r = \frac{989.8 \times 65 \times 10^6}{5625 \times 10.0} = 1.14 \times 10^6$ cells/ml.

To calculate the enrichment ratio, Z_f/Z_r:

$$V_i = V_r + V_f \quad (1)$$

$$Z_i\ V_i = Z_r\ V_r + Z_f\ V_f \quad (2)$$

From (1) and (2) $\frac{Z_f}{Z_r} = \frac{Z_i\ V_i - Z_r\ V_r}{Z_r\ (V_i - V_r)}$

Substituting the values for run 1

$$\frac{Z_f}{Z_r} = \frac{2.6 \times 10^7\ (1000) - 1.14 \times 10^6\ (875)}{1.14 \times 10^6\ (1000 - 875)}$$

$= 175$

Calculations for run 15

First, the residual cell concentration for the experiment with salt is calculated,

$$Z_{rs} = \frac{s.q.\ 10^6}{s.\ V}$$

For run 15 $q = (45 + 44 + 31 + 57 + 52 + 30 + 51 + 37 + 43 + 38 + 46 + 56 + 53 + 44 + 41 + 45)/16$

$= 44.6$

and $V = 50.5$ ml.

Therefore, $Z_{rs} = \frac{989.8 \times 44.6 \times 10^6}{5625 \times 50.5} = 1.55 \times 10^5$ cells/ml.

The control experiment without salt is run 5, for which $Z_r = 4.2 \times 10^5$ cells/ml. Then, the ratio of the residual cell concentration for the experiment with salt to the residual cell concentration of the control without salt is,

$$Z_{rs}/Z_r = \frac{1.55 \times 10^5}{4.2 \times 10^5} = 0.37$$

Table 9 - Calculated data for runs 1 to 14

Run	Z_r (cells/ml.)	Z_f/Z_r
1	1.14×10^6	175
2	4.0×10^5	754
3	2.2×10^5	42.6
4	2.0×10^6	43.5
5	4.2×10^5	2 18
6	1.4×10^5	438
7	1.8×10^5	584
8	7.1×10^4	1470
9	2.6×10^4	2760
10	3.5×10^6	17.8
11	7.1×10^5	73.2
12	3.8×10^5	172
13	2.3×10^5	263
14	1.4×10^5	398

For the log-log plot of enrichment ratio versus foaming time, with parameters of initial EHDA-Br concentration, the following program was used to find the lines of best fit through the sets of points:

```
6  REM   LOG-LOG CURVE FIT
7  REM
8  DIM X[20],Y[20],W[20],Z[20]
9  READ N
10  LET A= 0
11  LET B= 0
12  LET C= 0
14  LET D= 0
15  FOR I=1 TO N
20    READ W[I],Z[I]
21    LET X[I]= LOG (W[I])
22    LET Y[I]= LOG (Z[I])
25    LET A=A+X[I]
26    LET B=B+X[I]*X[I]
27    LET C=C+Y[I]
28    LET D=D+X[I]*Y[I]
30  NEXT I
31  PRINT
32  PRINT
40  LET Z=N*B-A*A
41  LET I=(C*B-D*A)/Z
42  LET S=(N*D-A*C)/Z
44  LET I= EXP (I)
45  PRINT "Y-INT",I," SLOPE",S
46  PRINT
47  PRINT "XEXPT","YEXPT","YCALC","% ERROR"
49  LET A=I
50  FOR I=1 TO N
55    LET Q=A*W[I]↑S
60    LET E=(Q-Z[I])*100/Z[I]
65    PRINT W[I],Z[I],Q,E
70  NEXT I
100  DATA  7
110  DATA  2, 42.6
120  DATA  2, 43.5
130  DATA  4, 218
140  DATA  6, 584
150  DATA  6, 436
160  DATA  8, 1470
170  DATA  10, 2760
999  END
RUN

Y-INT          7.06168        SLOPE          2.50415

XEXPT          YEXPT          YCALC          % ERROR
 2              42.6           40.0619        -5.95788
 2              43.5           40.0619        -7.90358
 4              218            227.277         4.25541
 6              584            627.354         7.42357
 6              436            627.354         43.8884
 8              1470           1289.37        -12.2877
 10             2760           2254.52        -18.3145

*READY
```

```
*READY

100  DATA  5
110  DATA  2, 17.8
120  DATA  4, 73.2
130  DATA  6, 172
140  DATA  8, 263
150  DATA  10, 398
RUN

Y-INT          4.85923        SLOPE          1.93549

XEXPT          YEXPT          YCALC          % ERROR
 2              17.8           18.587          4.42134
 4              73.2           71.0968        -2.8732
 6              172            155.838        -9.39664
 8              263            271.951         3.40349
 10             398            418.851         5.23884

*READY
```

Table 10 - Calculated data for runs 15 to 34

Run	Salt conc. (μ eq./ml.)	Z_{rs} (cells/ml.)	Z_{rs}/Z_r
15	0.171	1.55×10^5	0.37
16	0.856	2.40×10^5	0.57
17	1.71	7.11×10^5	1.7
18	3.42	2.23×10^6	5.3
19	8.56	4.65×10^6	11.1
20	0.171	1.81×10^5	0.43
21	0.856	3.51×10^5	0.84
22	1.71	4.22×10^5	1.0
23	3.42	1.29×10^6	3.1
24	8.56	1.40×10^6	3.3
25	0.171	5.53×10^5	1.3
26	0.856	5.20×10^5	1.2
27	1.71	6.35×10^5	1.5
28	3.42	8.42×10^5	2.0
29	8.56	7.65×10^5	1.8
30	0.171	2.45×10^5	0.58
31	0.856	8.23×10^5	2.0
32	1.71	8.61×10^5	2.0
33	3.42	1.62×10^6	3.9
34	8.56	1.45×10^6	3.4