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COLUMN FLOTATION OF BAKERS' YEAST

by

DEVON L. HUSBAND

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Master of Science.**

DEPARTMENT OF CHEMICAL ENGINEERING

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ABSTRACT

The feasibility of column flotation as an alternate method for cell recovery from fermentation broths was investigated using bakers' yeast in the resting state as a model cell type. Flotation experiments were performed on a flotation column with a working height of 1 m and a cross-sectional area of 0.0016 m^2. At a feed concentration and rate of 2.0 g/L and 0.21 cm/s and an air superficial velocity of 0.31 cm/s, continuous operation was successfully maintained over a wash water range of 0-0.063 cm/s. Yeast flotation was aided by an alkylpolyglycoside (APG) surfactant and addition of inorganic salts such as NaCl, KCl, $MgCl_2$, and $CaCl_2$ to the feed in a concentration range of 0-0.5 M. Yeast enrichment in the foam reached values as high as 11-fold when compared with the yeast concentration in the feed, but foam recovery did not exceed 55% of the yeast entering the column in the feed.

The addition of wash water to the foam zone produced two effects: dilution of the foam product (reducing foam enrichment), and yeast detachment from the foam zone (reducing foam recovery). Yeast recovery was virtually eliminated at a wash water rate of 0.031 cm/s. Salt addition to the feed at concentrations of 0.05-0.1 M improved yeast flotation, raising foam enrichment from 7-fold to as high as 11-fold and foam recovery from 25% to as high as 55%. Feed salt concentrations for maximum foam enrichment and

recovery were identical and depended upon the type of salt added to the feed. Yeast zeta potential was identified as a useful indicator of flotation performance. The cation concentration producing a zero yeast zeta potential correlated reasonably well with the feed concentration maximizing foam enrichment and recovery.

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NOMENCLATURE

A	Cross-sectional area of flotation column
Q	Volumetric flowrate
J	Superficial velocity or flux
α	Gas holdup or volumetric gas fraction
ΔP	Pressure difference in bubbly or foam zone
h	Distance between pressure taps
d	Diameter (bubble or particle)
d_{32}	Sauter mean diameter
V_∞	Bubble terminal rise velocity
C	Yeast concentration
C_r	Carrying rate of bubbles
C_a	Carrying capacity of foam
R	Recovery (yeast or surfactant)
E	Enrichment (yeast or surfactant)
ζ	Zeta potential
u	Electrophoretic mobility
ρ	Density
μ	Viscosity
σ	Surface tension

Subscripts

f	Feed
t	Tailings
c	Foam concentrate
l	Liquid
g	Gas
b	Bubble
p	Particle

Chapter 1: INTRODUCTION

In the biotechnology industry, downstream processing represents a large portion of the product cost, as great as 90% for some high-value bioproducts. Progress in biotechnology may now depend as much upon innovations in processing as on advances in the biosciences or developments in bioreactor design and operation, yet research and development in this area are rarely conducted outside industry and consequently access to this information is limited.

Whether a desired bioproduct is extracellular, intracellular or the cells themselves, the removal of biomass is almost invariably the first stage of downstream processing. Harvesting of microbial cells from a fermentation broth is normally accomplished by centrifugation or membrane filtration, but these methods are expensive and prone to certain problems. Centrifugation serves as a cell reduction rather than cell exclusion technique and becomes more difficult as the density difference between cells and supernatant decreases; membrane filtration can ensure complete cell removal but is vulnerable to fouling when subjected to the heavy loading of a fermentation broth. Both methods have difficulty handling foaming broths and are unsatisfactory when non-cell solids are present.

Froth flotation is a potential alternative. A type of foam separation developed in the mineral process industry to handle solids separation, this inexpensive technique is used to recover

valuable minerals from ores. In common with all foam separations, froth flotation employs a sustained foam to concentrate a desired component from a suspension. What distinguishes froth flotation from other foam separations (and makes it suitable for cell recovery) is the insoluble or particulate nature of the component - the feed is a slurry rather than a true solution. Separation relies on the tendency of certain particles to preferentially attach to gas bubbles rising through the liquid into the foam layer, which in turn can be collected and collapsed to yield a concentrate enriched in these particles.

Researchers adapted the technique to cell recovery in the 1950's and 1960's, employing a columnar form of flotation apparatus to survey microbial species for flotability and to identify and study parameters of batch-mode operation. Interest waned over the next decade, and only recently revived with a more intensive exploration of the cellular properties and mechanisms allowing flotation. Despite the advantages conferred by a steady-state system in analyzing the complex factors of cell flotation (and despite the suitability of the columnar flotation apparatus for this mode of operation), very limited data has been collected from continuous operations.

This project was designed to enlarge the body of data available from continuous flotations while probing cell surface properties through the addition of salts to the flotation media. Bakers' yeast in the resting state was chosen as the model cell

type, to be floated from tap water with the aid of a non-ionic carbohydrate surfactant. A variety of inorganic salts were added to give a concentration range of 0-0.5 M. The typical columnar design of the cell flotation apparatus prompted adoption of **column flotation**, the latest innovation in mineral recovery. Column flotation is characterized by the application of a water stream to the foam to improve separation by washing out entrained contaminants. Furthermore, the addition of water tends to stabilize the foam. An evaluation of continuous column flotation as a means of cell recovery and the effects of salt addition on flotation performance will form the focus of this thesis.

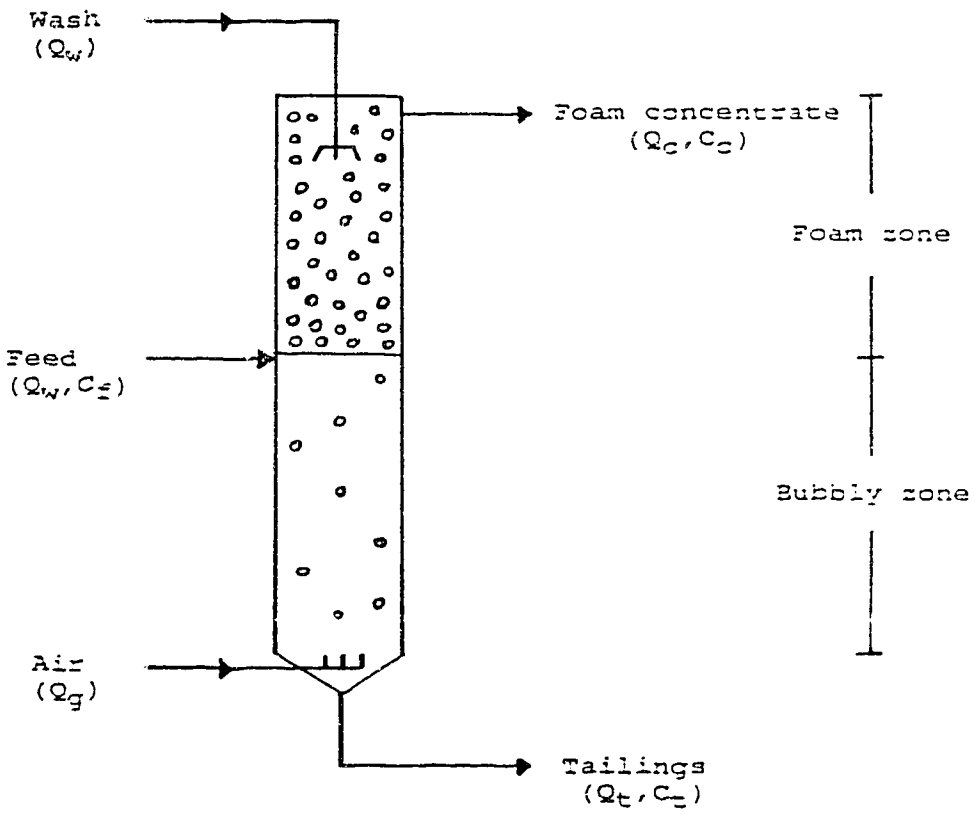
Chapter 2: COLUMN FLOTATION BACKGROUND

2.1 Description

Column flotation is a special application of froth flotation which was conceived in the 1960's and introduced to the mineral process industry in the 1980's. This application is distinguished by several changes to traditional flotation design and practice, including apparatus shape, mode of bubble generation, and use of a wash water stream.

The traditional tank-like flotation equipment is replaced by a flotation column (**Figure 2.1**). Feed is introduced just below the liquid-foam interface separating the bubbly and foam zones, and the level of the interface is maintained by regulating the flow of a tailings stream withdrawn from the bottom of the column. Traditional mechanical agitation and aeration are replaced by internal gas sparging at the base. The large height-to-diameter ratio of the column, commonly at least 10:1, is designed to reduce axial mixing (which would lower recovery and separation). This allows appropriate contact time between gas and suspension during the vertical rise of the bubbles through the bubbly zone. The ascending bubbles effectively "sweep" the suspension for particles before arriving at the zone interface to join the rising foam. A water stream is introduced at the top of the column to stabilize the draining foam and to wash mechanically entrained particles out

Figure 2.1: Schematic Diagram of Flotation Column



of the rising foam, thereby overcoming the requirement of traditional flotation for water from the feed to maintain foam stability. The wash water enters just below the foam overflow to prevent short-circuiting to the foam product. An enriched foam concentrate is obtained after foam breakdown.

The bubbly zone is also known as the collection zone since it is the site of particle adsorption to bubbles. In this region of countercurrent gas/liquid flow, collection depends upon zone retention time, the degree of axial mixing, and particle properties.

The foam zone is also referred to as the cleaning zone since it is the region where entrained contaminants, following in the wake of bubbles, are removed from the foam by the action of the wash water, or more accurately by that portion of the wash water trickling down through the foam to the bubbly zone. The wash water also stabilizes the foam by replacing water draining from between the bubbles, thereby ensuring a greater height of foam zone. A flotation column is normally operated under positive bias, i.e. a downward water flux, such that the tailings flowrate exceeds that of the feed in order to maintain the interface level. The value of the bias can be approximated by the difference in flowrate between wash water and foam concentrate, but this is an underrepresentation ignoring the portion of the flow compensating for solids transfer from the bubbly zone into the foam (**Finch &**

Dobby, 1990). Overall, the column's wash water is able to replace virtually 100% of the carryover water from the bubbly zone.

Foam structure in a flotation column is more open than its traditional counterpart - the thickness of the water film between bubbles is 4- to 5-fold greater than that of a conventional foam, which likely facilitates rejection of entrained particles. Yet the introduction of wash water does not make the foam zone homogeneous. Three separate regions exist in the foam zone: an expanded bubble bed with a high liquid content adjacent to the zone interface, a packed bubble bed with a lower liquid content and a more heterogeneous bubble population located directly above, and a conventional draining foam situated above the wash water inlet (**Finch & Dobby, 1990**). Bubble coalescence, while occurring at all levels in the foam, is particularly vigorous in the expanded bubble bed adjacent to the interface and is the mechanism for cleaning action.

As the foam concentrate (i.e. breakdown product) is normally the product of interest, column performance is judged on the basis of foam recovery and enrichment. Recovery is defined as that proportion of the desired particulate entering in the feed which is recovered in the foam concentrate:

$$R = \frac{Q_c C_c}{Q_f C_f} \qquad (2.1)$$

where Q is the volumetric flowrate, C is the particulate concentration, and the subscripts c and f refer to foam concentrate and feed respectively. Enrichment is a measure of the quality of the foam concentrate and compares the concentration of the desired particulate in the foam concentrate with that in the original feed:

$$E = \frac{C_c}{C_f} \qquad (2.2)$$

2.2 Principles of Separation

Froth flotation relies on the tendency of certain particles in suspension to preferentially attach to gas bubbles rising through the suspension. Particles differ in their degree of attraction to bubbles, which allows selective separation. Those particles with native flotability possess natural hydrophobicity; particles lacking natural hydrophobicity may be made to float by addition of an appropriate collector to promote particle adsorption to bubbles. A collector is a surfactant whose amphipathic nature allows it to adsorb to both bubble and particle - its hydrophobic region is generally a long chain hydrocarbon while its hydrophilic portion may be polar, anionic, cationic or zwitterionic as necessary for adsorption to the particle of

interest. Collector choice depends upon its specificity for the particle and the ionic strength and temperature of the suspending medium.

Although the particle surface must possess a net hydrophobicity in order for the particle to float, the surface may also display a significant number of hydrophilic sites, i.e. hydrophobic particles may also exhibit surface charge. Most particles acquire a surface charge in aqueous suspension due to ionization of surface groups, chemisorption and complex formation between ions and surface species. The surface charge attracts ions of opposite charge from the surrounding media and these counterions, along with complementary co-ions and the charged surface, form an electric double layer (**Barlow, 1970**). The counterbalancing ions ensure overall electroneutrality and form a structure with two regions: the Stern layer of specifically adsorbed ions held tightly to the surface (i.e. fixed), and a diffuse layer of freely-moving counterions and co-ions extending out to the bulk medium. Potential falls sharply from the surface to the outer limit of the Stern layer but declines more slowly in the diffuse layer to a value of zero at its outer edge. The potential change in the Stern layer increases with concentration and valence of counterions, and charge reversal within the layer is possible with polyvalent counterions.

When a charged particle is in tangential motion relative to the aqueous phase, a shear plane develops within the diffuse

region such that not all of the double layer moves as one with the particle. The potential difference at the shear plane, known as the zeta potential, can be determined from electrokinetic measurements and is normally used in place of the surface potential as a measure of the surface charge (**Haydon, 1964**). Zeta potential (ζ) is proportional to the electrophoretic mobility (u) of the migrating particle in the presence of an external applied electric field, and is sensitive to changes in the ionic strength of the suspending medium. One expression relating the zeta potential of a sphere to its electrophoretic mobility is the Henry equation (**Probstein, 1989**):

$$\mathbf{u} = \frac{\mathbf{2}}{\mathbf{3}} \frac{\varepsilon}{\mu} f(\kappa R)\ \zeta \qquad \textbf{(2.3)}$$

where
ε = permittivity
= $\varepsilon_r\ \varepsilon_o$
ε_r = dielectric constant of medium
ε_o = permittivity of vacuum
μ = viscosity of medium
$f(\kappa R)$ = Henry function

Other expressions have been derived from different theoretical treatments and differing methods of electrokinetic measurement.

With small particles (i.e. under 20 μ in diameter) it is reasonable to presume double layer effects are particularly important in flotation, and there is a body of evidence to support

this contention. **Derjaguin & Shukakidse (1961)** demonstrated that the rate of flotation of naturally hydrophobic antimonite dropped sharply as particle zeta potential exceeded a certain value. **Jaycock & Ottewill (1963)** found the rate of flotation of silver iodide particles was maximized when particle zeta potential was zero (although size effects from coagulation could not be ruled out as the cause since the coagulation rate at zero zeta potential would have been high). **Collins & Jameson (1976)** concluded that particle charge had a drastic effect on flotation rate after observing an order of magnitude increase in rate of flotation for polystyrene particles due to a reduction in zeta potential from 60 to 30 mV.

Double layer effects must also include bubbles, even though measurements on small bubbles have been difficult to accomplish. Many studies have shown that gas bubbles acquire a negative charge in pure water and in solutions of simple inorganic electrolytes, and the magnitude of the charge depends upon electrolyte concentration and duration of contact. **Dibbs et al.(1974)** proposed that upon bubble formation in inorganic electrolyte solutions, the ions in the vicinity of the bubble surface are attracted to the bulk of the solution and the degree of attraction is related to the extent of ion hydration. Since cations generally have larger solvation sheaths than anions, they would be attracted more strongly and would leave an excess of negative charge associated with the bubble surface. Ionic surfactants also

produce changes in the bubble surface dependent on the surfactant concentration and the sign of ionic groups.

Clearly the joint effects of particle and bubble charge on flotation performance must be considered. **Dibbs et al.(1974)** found efficient flotation of quartz particles occurred when particles and bubbles had opposite charges. **Collins & Jameson (1977)** noted that bubbles and polystyrene particles carried positive charges which were approximately the same under the same conditions of electrolyte and cationic surfactant, yet the flotation rate increased rapidly as both zeta potentials were reduced. **Fukui & Yuu (1980)** also found positively charged bubbles in the presence of the electrolyte $AlCl_3$, but polystyrene particles were negatively charged (although the particle charge changed sign as flotation time increased). Flotation rate was maximized when zeta potentials of bubbles and particles were opposite in sign, and the rate declined once the absolute value of the product of these potentials exceeded a certain value. **Okada & Akagi (1987)** developed a more accurate apparatus for measuring the zeta potential of small bubbles and employed it to show that the positive charge of bubbles in the presence of a cationic surfactant and the negative charge in the presence of an anionic surfactant declined with increasing electrolyte concentration, although in the latter case the sign of the bubble charge could be reversed with the addition of $Al_2(SO_4)_3$. These bubble measurements were related to flotation efficiency of oil

flotations in later work (**Okada et al.,1988**). The efficiency was strongly dependent on the zeta potentials of both bubbles and oil droplets and was maximized when the oil droplets were negatively charged, bubbles were positively charged, and a parameter defined as the critical adhesion parameter (proportional to the product of the two poentials) remained under a critical value.

A better understanding of these results can be gained by considering the electrostatic force between the bubble and the particle. A fair approximation of the force between two spheres in close proximity is provided by the expression for two parallel plates immersed in a symmetrical electrolyte solution **(Russel et al.,1989)**:

$$F = \frac{e^2 z^2 n_o}{kT} \left[\frac{2\,\zeta_1\zeta_2\,\cosh(\kappa h) - \zeta_1^2 - \zeta_2^2}{\sinh^2(\kappa h)} \right] \qquad (2.4)$$

where
- e = elementary electric charge
- n_o = bulk concentration of electrolyte solution
- z = valence of electrolyte
- k = Boltzmann constant
- T = absolute temperature
- ζ_i = zeta potential of plate i
- h = distance between plates
- κ = inverse Debye length

The force is proportional to the product $\zeta_1\zeta_2$, and a negative value (brought about by zeta potentials with opposite signs) represents attraction.

When **Kubota et al.(1990)** obtained good flotation of barium sulfate particles in the presence of an anionic surfactant, despite negative charges on both bubble and particle surfaces, they suggested that interactions other than electrostatic must be taken into account. **Okada et al.(1990a,1990b)** demonstrated with latex particles that flotation efficiency reached its highest value when zeta potentials of bubbles and particles attained their lowest absolute values in two surfactant systems (anionic and non-ionic) where both potentials remained negative over a wide range of pH. Since the overlapping of like-charged electrical double layers would lead to electrostatic repulsion, the effects of other forces had to be considered - hydrodynamic interactions, van der Waals attraction and the gravitational force of the particles. Particle adhesion to bubbles was presumed to occur as long as the total interaction force was less than or equal to zero, i.e. a repulsive electrostatic force could be overcome if the net surface force composed of electrostatic and van der Waal forces were attractive.

2.3 Column Flotation Parameters

Efficient particle collection by bubbles in the bubbly zone requires operation within the bubbly flow regime characterized by a homogeneous distribution of bubbles of uniform size rising at a uniform rate. Within this regime, there is an approximately

linear relationship between gas holdup or volumetric fraction (α) and gas superficial velocity (J_g). Above a certain gas velocity the bubbly zone develops rapidly rising large bubbles, which displace water and smaller bubbles downward. Gas holdup becomes unstable, bubbles begin to coalesce into "slugs" of air, and the flow begins transition to the churn-turbulent regime. Operating within the bubbly flow regime imposes limits not only on the superficial gas velocity but also on bubble diameter (d_b), liquid superficial velocity (J_l), and feed solids content.

Small bubbles are more efficient particle collectors due to their larger surface area-to-volume ratio. Bubble diameter (d_b) is a function of gas velocity, as expressed in the empirical relationship of **Dobby & Finch (1986)**:

$$d_b = C J_g^n \qquad 0.2 \leq n \leq 0.4 \qquad (2.5)$$

where C is a constant and J_g is the gas superficial velocity. Lower gas flow produces smaller bubbles, yet the gas sparger places a practical limit on bubble size since very fine bubbles cannot be forced out sufficiently quickly to prevent coalescence at the sparger surface. Consequently there is a lower limit to bubble diameter at a given gas velocity (or correspondingly an upper limit to gas velocity at a given bubble diameter) which allows bubbly flow to be maintained. Increasing the liquid flux

lowers the maximum gas velocity tolerated within the bubbly flow regime.

The solids content of the feed is also important. The gas velocity and the bubble particle loading (particles per unit area of bubble) determine the carrying rate (C_r) of the bubbles, i.e. the mass rate of solids per unit cross-sectional area of column. The maximum carrying rate is obtained with a "saturated" bubble surface (which is not 100%), but this theoretical rate is never achieved. The practical measurement of this rate is the carrying capacity (C_a) which is obtained experimentally from the maximum concentrate solids rate as a function of feed solids rate. It is important not to exceed the carrying capacity of the system since coarse particle drop back (out of the foam into the bubbly zone) appears to give a concentrate composed of only the smaller particles. Heavy bubble loading may also increase gas holdup (due to a slower bubble rise velocity brought on by a greater bubble-particle aggregate density) to a point exceeding that which is compatible with the bubbly flow regime. In addition, particles may retard or promote bubble coalescence on the basis of particle surface properties.

Another parameter is particle size. Commonly there is a peak or plateau in foam recovery as a function of particle diameter. As size increases there is a greater chance of collision with bubbles but attachment may become more difficult and bubble-particle detachment may also increase. A decrease in selectivity

with increasing particle size is also commonly observed and may be due to preferential drop-back of large particles from the foam. At the other end of the scale, flotation of very fine particles (under 5-10 μ diameter) is difficult due to the tendency of these particles to follow streamlines around bubbles and so avoid contact.

Particles can also influence foam stability through their size and hydrophobicity. By reducing the film thickness between adjacent bubbles below that of the critical rupture thickness, attached particles can induce bubble coalescence and foam instability. This is especially true of highly hydrophobic particles and is particularly relevant to column flotation, in which foam rejection of hydrophilic particles is very efficient. In heavily loaded foams (or those containing large particles) the particles may provide a physical barrier to film drainage and coalescence of bubble interfaces, and so extend foam stability.

There is some evidence that the foam may play an additional role beyond that as collector of adsorbed particles. Foam depth may contribute some selectivity between particles differing in hydrophobicity. If particles were to be subjected to repeated detachment and reattachment (possibly as the result of bubble coalescence and subsequent reduction of available surface area), those particles with higher probabilities of bubble collection (i.e. the more hydrophobic particles) would be progressively and selectively separated out. Grade profiles have been observed in

some foams, although not in the presence of mixing, fully-loaded or shallow foams. Foam drop-back to the bubbly zone is not well understood but may play a role in selectivity since it is dependent on particle size and is believed to result in extensive recycling (perhaps over 50%).

Wash water is a major operating parameter in column flotation, stabilizing the foam by replacing water lost through drainage and virtually eliminating mechanical entrainment. While the economic benefits to minimizing water consumption are evident, there are even more important reasons related to column performance. Excessive positive bias (i.e. downward liquid flux) promotes mixing of the foam, reduces concentrate quality through dilution, and decreases the bubbly zone retention time for particle collection by elevating the tailings removal rate.

2.4 Kinetics

The kinetics of the collection process have received a great deal of attention. The collection rate of a mineral in the bubbly zone is usually considered to be first order:

$$C(t) = C_i \exp(-kt) \qquad (2.6)$$

where C(t) and C_i are the mineral concentrations at time t and time t=0. The collection rate constant (k) is given by:

$$k = 1.5 \frac{J_g}{d_b} E_K \qquad (2.7)$$

where J_g is the gas superficial velocity, d_b is the bubble diameter, and E_K is the overall collection efficiency **(Finch & Dobby, 1990)**. The collection efficiency can be considered the product of collision and attachment efficiencies (E_C and E_A):

$$E_K = E_C E_A \qquad (2.8)$$

Weber & Paddock (1983) provide the following expression for the collision efficiency:

$$E_C = 1.5 (d_p/d_b)^2 \left[1 + \frac{3/16 \, Re_a}{1 + 0.25 Re_a^{0.56}}\right] \qquad (2.9)$$

where d_p is the particle diameter and Re_a is the Reynolds number of the bubble. The attachment efficiency is not well understood, but is approximated by:

$$E_A \cong \exp\left[-C \frac{d_p}{d_b}\right] \qquad (2.10)$$

where C is a constant. Clearly, the collection rate constant is a complex function of (d_p/d_b).

2.5 Hydrodynamics

Understanding flotation column flow behaviour is essential for column design and control, yet surprisingly little attention has been directed towards this area. Flow characteristics of a flotation column have been studied by **Pal & Masliyah (1989)** using an air/aqueous surfactant system, and the holdup behaviours of the bubbly and foam zones have been linked using the drift-flux model of **Wallis (1969)**. The experimental results of Pal & Masliyah, together with literature data, have been found to correlate well with the Richardson-Zaki equation.

In this approach the drift-flux is defined as:

$$J_{gl} = (1-\alpha)J_g - \alpha J_l \qquad (2.11)$$

where J_g is the gas flux or superficial velocity, J_l is the liquid superficial velocity, and α is the gas holdup. In gravity-dominated flows with uniform cross-sectional gas holdup and negligible wall effects it can be shown that:

$$\frac{J_{gl}}{v_\infty} = f(\alpha) \qquad (2.12)$$

where V_∞ is the bubble terminal rise velocity. The majority of empirical expressions for $f(\alpha)$ reported in the literature fit a general form:

$$\frac{J_{gl}}{V_\infty} = \alpha(1-\alpha)^n \qquad (2.13)$$

where n is an exponent differing from one correlation to another. According to **Richardson & Zaki (1954)**, n is a function of the particle Reynolds number and is equal to 2.39 at $Re_p > 500$. This expression has been shown by Pal & Masliyah to correlate the drift-flux data of the flotation column for two very different air/surfactant systems.

The drift-flux can be determined experimentally by measurement of J_g, J_l, and α. The gas flux is obtained from flow measurement in the gas supply line, while the gas holdup or volume fraction is determined from pressure measurements in the appropriate zone. The liquid flux is evaluated in different ways for the bubbly and foam zones. In the bubbly zone the liquid flux is calculated from the tailings flowrate:

$$J_l = - \frac{Q_t}{A} \qquad (2.14)$$

where A is the cross-sectional area of the column. In the foam zone there are two components of liquid flow to be taken into

account: downward drainage due to gravity and liquid ascent due to the rising foam bed. The net upward liquid flux is determined from a material balance around the top of the column (see **Figure 2.2**):

$$J_l = \frac{(Q_c - Q_w)}{A} \qquad (2.15)$$

The bubble terminal rise velocity is also evaluated in different ways for the two zones. Visual bubble tracking may be used in the foam zone, but an empirical correlation must be employed in the bubbly zone. One such correlation is that of **Clift et al.(1978)**:

$$V_\infty = \frac{\mu_l}{\rho_l \, d_{32}} M^{-0.149} (J - 0.857) \qquad (2.16)$$

where

$$J = \begin{cases} 0.94 \; H^{0.757} & 2 < H \leq 59.3 \\ 3.42 \; H^{0.441} & 59.3 < H \end{cases}$$

$$H = \frac{4}{3} E_o \, M^{-0.149} \left(\frac{\mu_l}{\mu_w}\right)^{-0.14}$$

$$M = \text{Morton no.} = \frac{g \, \mu_l^4 (\rho_l - \rho_g)}{\rho_l^2 \, \sigma_l^3}$$

E_o = Eotvos no.

$$= \frac{g\ (\rho_l - \rho_g)\ d_{32}^2}{\sigma_l}$$

μ_l = viscosity of liquid

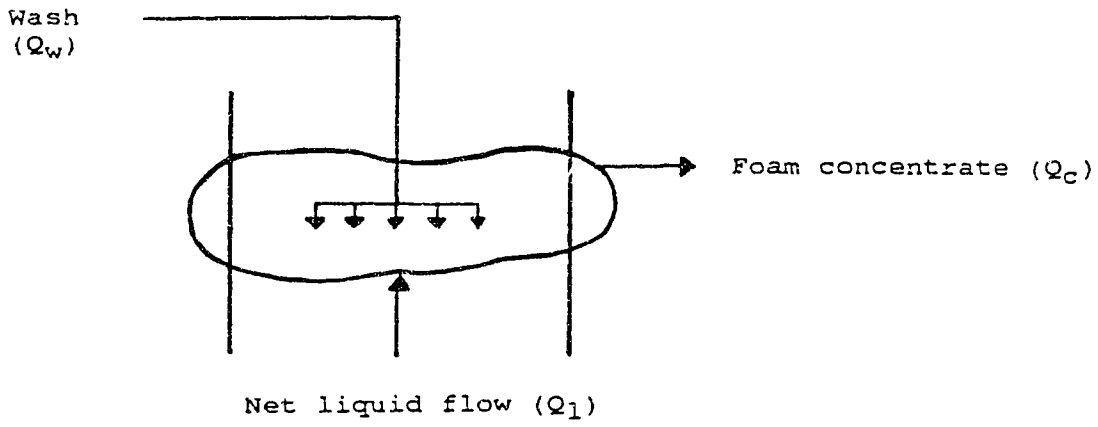
ρ_l = density of liquid

ρ_g = density of gas

σ_l = surface tension of liquid

d_{32} = Sauter mean bubble diameter

Figure 2.2: Material Balance Around Top of Flotation Column



Chapter 3: LITERATURE REVIEW

3.1 Introduction

The impetus to investigate the potential of foam separation, or more accurately froth flotation, for microbial cell concentration and purification came from three sources in the 1940's and 1950's:

1. The requirement for "clean" cell preparations for research
2. The desire for improved water treatment
3. The search for a harvesting method for large-scale algal production

Studies in physiology, immunology, and cytology were hampered by the difficulty in producing purified cell suspensions free of cellular debris. Preparation by centrifugation was an arduous task with frequently low yields, and researchers were devoting considerable effort to developing improvements or alternate methods. Concurrently in the field of fermentation, there was recognition that foaming in aerated fermentors resulted in microbial cell loss. This soon led to the realization that an undesirable process in one field could become a potential solution in another.

Dognon (1941) first noted that *Mycobacterium tuberculosis* cells could be separated from a culture of staphylococcal bacteria by bubbling air through the culture and collecting the froth

above, and proposed using froth flotation for selective recovery of tubercle bacilli in the diagnostic laboratory. When **Boyles & Lincoln (1958)** began growing aerated cultures of *Bacillus anthracis*, they observed material collecting in the foam above the liquid - low culture spore counts resulted when large amounts of the material collected in the foam or after uncontrolled foaming. Microscopic examination of the material revealed essentially clean spores, and Boyles & Lincoln proceeded to experiment with a foam separation process. Independently, **Black et al.(1958)** also concluded that froth flotation had potential as a selective harvesting technique after observing the loss of *Bacillus cereus* spores from aerated cultures after uncontrolled foaming. Both groups were successful in developing laboratory flotation processes to separate spores from vegetative cells in culture.

Water treatment engineers were also becoming interested in flotation as a method of cell removal. Flotation had the potential to concentrate cells from dilute samples and so improve the accuracy of cell count estimations from which disinfectant dosages were calculated. In addition, cell removal prior to treatment could reduce disinfectant requirements, providing economic benefits and eliminating adverse effects of high disinfectant dosages. **Hansen & Gotaas (1943)** and **Gibbs (1950)** investigated flotation for the treatment of sewage and industrial waste. Adopting the use of a collector from the mineral processing tradition, Hansen & Gotaas achieved very high (greater

than 99%) removals of bacteria in only 15 to 20 minutes of batch operation. **Hopper & McCowen (1952)** and **Moore & Bryant (1954)** also made use of foaming aids in their flotation studies - Hopper & McCowen were able to purify surface water of most solid particles, including 99% of bacteria and all cysts of *Endamoeba histolytica*.

At this time serious consideration was being given to large-scale algae production as a potential food source. **Cook (1950)** described a plant design for large-scale continuous culture of *Chlorella* and proposed algal recovery by flotation. The lack of an economical harvesting method for relatively dilute algal suspensions was identified by **Burlew (1953)** as one of the major problems in mass cultivation of unicellular algae for food or other purposes. **Gotaas & Golueky (1957)** carried out extensive investigations of algal flotation using commercial flotants and reported that while flotation could meet the product criterion of 5-8% solids content for economical drying, the flotant requirements would make the process too costly for commercialization.

Work on microbial cell flotation continued through the 1960's, surveying species for flotability, improving equipment and identifying operating parameters. The bulk of our knowledge comes from this period, as interest waned in the following decade and only began to revive in the 1980's with a renewed focus on the effect of cell surface characteristics on flotation.

3.2 Flotation Equipment and Mode of Operation

Comparison of many experimental results from biological flotations is hampered by a lack of documentation on experimental conditions and considerable variation in the flotation equipment. Everything from graduated cylinders (**Hopper & McCowen, 1952**) to Buchner funnels (**Rubin et al.,1966**) have been employed. **Boyles & Lincoln (1958)** used glass chambers with variable foam space. **Gaudin et al.(1960a,1960b)** developed a "subaeration cell" (essentially a malted milk mixer with a sleeve for foam exit), and a pneumatic column (an agitated pulp chamber from which the culture was driven by pressurized air). Often neither flotation chamber dimensions nor flotation volumes were recorded. Gas (air or nitrogen) flowrates were not strictly regulated in early work and aquarium or fritted glass spargers were employed without much concern over the size of bubble generated.

Levin et al.(1962) employed a simple glass column with bottom sparging and sidearm removal of foam at the top of the column, and this apparatus served as the forerunner to a recognizable flotation column; they also specified the rate of aeration and sparger porosity. **Grieves & Wang (1966)** provided the dimensions of their flotation column, introduced a sintered stainless steel sparger, and carefully regulated the gas flowrate. **Viehweg & Schugerl (1983)** were the first to record the relative heights of foam and liquid. With these standardized practices and equipment

it became possible to compare results on a common basis and identify the parameters affecting flotation performance.

Almost all work on cell flotation has been carried out in the batch mode of operation, and the continous change in the liquid and foam composition with time presented difficulties in assessing the effects of various parameters. The liquid in the flotation column was continuously changing not only in cell concentration but also in the concentration of natural surfactants or additives, pH, ionic strength, and liquid level. It is therefore not surprising that the literature yields a wide range of experimental conditions with conflicting results.

When **Boyles & Lincoln (1958)** followed the time course of spore removal from a *Bacillus anthracis* culture over a 95 minute flotation run, they observed a sigmoidal shape to the cumulative spore removal curve with time and a peak in the foam concentrate spore concentration. Other workers observed a somewhat different pattern to the time-dependency of spore removal. **Rubin et al. (1966)** found that batch flotations of *Escherichia coli* and two algae, *Chlorella ellipsoidea* and *Chlamydomonas reinhardtii*, all exhibited an initial phase of rapid cell removal from the feed and a quick leveling out to a plateau concentration. The same pattern was obtained by **Rubin (1968)** with *Enterobacter* (formerly *Aerobacter*) *aerogenes*. In each case the initial slope and the level of the plateau varied with different operating parameters such as gas flowrate or pH. Taking a different approach, **Grieves**

& Wang (1966) followed the time-dependency of *E. coli* cell concentration in the residual feed - both declined exponentially. Grieves & Wang recommended as long as possible a foaming time to achieve the best results, but they may not have had the opportunity to observe a tailing-off in performance since their maximum flotation period was only 20 minutes.

Parthasarathy et al.(1988) went further and developed a simple model for batch flotation to predict the time-dependency of the ratio of cell concentration in the residual to initial feed. The model was based on such assumptions as perfect mixing in the liquid zone, equilibrium between liquid and interface cell concentrations, and constant foam drainage rate. The model successfully predicted a slightly less than linear decline of the cell concentration ratio during flotation of the yeast *Saccharomyces cerevisiae* over a period of 8 minutes, but only when experimentally determined foam concentrate flowrates were employed. The simplicity of the model can be seen in the lack of any parameter incorporating cell characteristics.

Considering the greater ease in studying parameter effects under steady-state conditions, remarkably little data is available on continuous biological flotations. The simple flotation column of **Levin et al.(1962)** was designed to allow continuous as well as batch operation by providing a tailings exit at the bottom of the column; however, it is unclear to what extent the continuous mode may have been employed to obtain experimental results. **Viehweg &**

Schugerl (1983) began their flotation studies on the yeast *Hansenula polymorpha* using the batch mode, but switched to continuous operation once they obtained a 2- to 3-fold improvement in foam cell concentration.

3.3 Operating Parameters

The literature can be broadly divided into the effects of operating parameters such as gas flowrate, bubble size, foam height, feed characteristics, pH, and additives, and parameters reflecting cell characteristics; this section will focus on operating parameters.

3.3.1 Gas Flowrate

Little attention was paid to the accurate measurement of gas flow in early work, let alone its effect on the flotation performance of cells. **Hopper & McCowen (1952)** noted that excessive air flow prevented water drainage from the foam and so produced a large degree of water carryover into the foam. **Boyles & Lincoln (1958)** normally increased the air flowrate with time to offset declining foam height during batch operation and recorded average rather than instantaneous flowrates, but they collected some limited data on the effect of gas flowrate. Foam spore recovery of *Bacillus anthracis* at three air flowrates ("slow",

"medium", and "fast") either did not vary or fluctuated without any clear pattern, but the foam enrichment (i.e. the ratio of spore concentration in the foam to that of the original feed) declined by approximately 50% when the air flowrate was adjusted from "slow" to "fast". Flotation of *Bacillus subtilis var. niger* spores and *Serratia marcescens* cells also required "very slow" and "relatively low" air flowrates, leading to the conclusion that high air flowrates did not produce the best results.

Levin et al.(1962) accurately measured air flowrates to their flotation column, and by providing column dimensions made it possible to transform their data into gas superficial velocities for comparison with later work. It should be noted that even the latest studies have not presented gas flow data in this format. Levin et al. examined the effects of air flow over the superficial velocity range of 0.09-2.39 cm/s on an algal flotation of a *Chlorella* species, and they achieved an 80% increase in foam enrichment (to 45-fold) with a 70% decrease in air superficial velocity. At the low end of the velocity range the improvement was even more dramatic - a further doubling of enrichment (to 90-fold) in the drop from 0.74 to 0.09 cm/s.

Investigations by **Bretz et al.(1966)** and **Grieves & Wang (1966,1967a,1967b)** on a variety of bacteria employed a higher gas superficial velocity range (1.6-3.5 cm/s) with nitrogen rather than air sparging. At first glance their findings appear to contradict those of Levin et al., since elevated gas flows reduced

the cell concentration in the residual feed - they obtained decreases of approximately 75-95%. However, increasing volumes of collapsed foam product with lower cell concentrations indicated that higher gas flows actually increased physical entrainment in the foam; lower gas flows produced more concentrated foams.

The results of **Rubin et al.(1966)** are less easily explained. This group pioneered the use of **microflotation**, a technique inv .ving flotation aids (including an insoluble collector such as a long-chain fatty acid or amine) to form an insoluble surface phase and reduce foaming and therefore gas flow requirements. Unfortunately, insufficient data were provided to calculate gas superficial velocities, so it is difficult to compare their results with those of other workers. Microflotation of *E. coli* using nitrogen sparging revealed that the recovery of cells in the foam increased with gas flowrate but only up to a point, after which the recovery again declined. Rubin et al. attributed the decline at higher gas flowrates to disruption of the insoluble surface layer, causing *E. coli* cells to return to bulk solution. Similar results were obtained with microflotation of the algae *Chlamydomonas reinhardtii*. Yet the conclusion that recovery was proportional to gas flowrate in the lower end of the range appears questionable, since this improvement could only be observed in the first few minutes of flotation. Data on cell concentration in foam or feed residue might have provided an explanation.

Viehweg & Schugerl (1983) explored the effect of gas flowrate on flotation of the yeast *Hansenula polymorpha* using a continuous system, and measured cell concentration rather than recovery in both foam and tailings. Over a much lower superficial gas velocity range (0.060-0.722 cm/s) the tailings cell concentration declined nearly 50% with increasing gas rate before reaching a plateau, while the foam cell concentration remained nearly constant. Viehweg & Schugerl considered this an improvement in flotation performance as separation (i.e. the ratio of cell concentration in foam to tailings), increased linearly by 400%. However, enrichment would have been a better measure of performance - the ratio of cell concentration in foam to initial feed actually fell with increasing gas flow, presumably due to water carryover into the foam.

Parthasarathy et al.(1988) found further evidence for the detrimental effect of increasing gas flowrate during flotation of the yeast *Saccharomyces cerevisiae*. Time plots of the ratio of residual feed to initial feed cell concentration at two gas rates (0.267 and 0.683 cm/s) revealed a faster decline at the higher gas flowrate. Once again, separation rather than enrichment was presented as a function of gas flowrate, and even this ratio declined with gas flowrate.

Overall, these experimental results reflect the guidelines established in mineral column flotation, which recognize limitations on the gas flowrate imposed by the need to maintain a

bubbly flow regime and the direct relationship between gas superficial velocity and bubble size (see **Equation 2.5**).

3.3.2 Bubble Size

Bubble size has been rather neglected as a cell flotation parameter. Some initial work did not provide sparger information; later flotation studies specified the average pore size of the sparger but with one exception did not verify actual bubble size, and no workers investigated the effect of flotation aids on bubble size.

Boyles & Lincoln (1958) provided the first evidence for bubble size as a parameter in biological flotation by evaluating disk and tube fritted glass spargers with average porosities of 5, 40, and 160 μ in the flotation of *Bacillus anthracis* spores from culture. Enrichment almost doubled from the 5 μ to the 160 μ sparger, leading Boyles & Lincoln to conclude that larger bubbles improved flotation performance. Yet results from flotation of *Serratia marcescens* appeared to contradict this conclusion - cell removal into the foam proceeded more quickly using the 5 μ sparger than the 40 μ sparger, although the final % recovery and enrichment were similar for the two spargers. The explanation may lie with the air flowrate. The *Bacillus anthracis* flotations were carried out at higher air flowrates, and bubble size tends to increase with gas flowrate (as seen in mineral column flotation).

Moreover, at the higher air flowrates bubble coalescence may have occurred before the bubbles were able to leave the surface of the 5 μ sparger. Also complicating the matter was the practice of ramping the air flowrate to offset declining foam height over time.

Levin et al.(1962) examined the effect of bubble size at constant aeration rate on the algal flotation of a *Chlorella* species. Experimental data were not provided, but an inverse relation between foam enrichment and sparger porosity was described. Levin et al. claimed that enrichment obtained with a 5 μ sparger increased 25% over that obtained with a 65 μ sparger.

Some contradictory data on bubble size effects is available from a continuous mode of operation. **Viehweg & Schugerl (1983)** evaluated spargers having mean pore diameters of 10-500 μ during flotation of the yeast *Hansenula polymorpha*. The cell concentration of both tailings and foam fell with decreasing pore diameter. This result led Viehweg & Schugerl to conclude that water carryover resulted in foam dilution and poor flotation performance as bubble size decreased, in contradiction to the findings of Levin et al. However, cell recoveries in foam and tailings were not calculated, and these may have improved with declining bubble size.

Parthasarathy et al.(1988) attempted to clarify the issue with their simple batch flotation model by comparing flotation performance of yeast with a single capillary for large bubbles and

a sintered disk for small bubbles. The average bubble diameter generated by the single capillary was calculated from a correlation, whereas the average diameter of bubble produced by the sintered disk was estimated photographically, and the two were found to differ by an order of magnitude (i.e. 3.5 mm compared with 0.4 mm). Parthasarathy et al. found that the rate of cell removal from the feed was greater for the smaller bubbles and this was attributed to the much larger interfacial area generated by smaller bubbles at a given gas flowrate. Separation was almost an order of magnitude higher for the larger bubbles, and Parthasarathy et al. concluded that increased water carryover by smaller bubbles contributed to foam dilution. However, once again cell recoveries were not calculated.

At present, the cumulative data suggest that smaller bubbles facilitate more rapid cell removal into the foam during batch operation due to their large interfacial area, and although information is lacking, this effect may lead to greater foam recovery. Contradictory results have been obtained on whether small bubbles enhance foam enrichment or produce a more dilute product due to increased liquid holdup in the foam. In a continuous mode of operation, the importance of establishing a bubbly flow regime would also apply. As demonstrated in mineral column flotation, this flow regime imposes restrictions upon minimum bubble size.

3.3.3 Foam Height

Several investigators have taken an interest in the effect of foam height on the flotation separation of cells. **Boyles & Lincoln (1958)** varied the diameter of the foam chamber above the liquid in order to examine the role of foam drainage. Doubling the foam chamber diameter nearly doubled the concentration of *Bacillus anthracis* spores in the foam concentrate without changing the spore recovery, yet a 50% increase in foam height failed to significantly improve the foam cell concentration. Flotations of *Serratia marcescens* produced the opposite effect - greater foam heights yielded more concentrated foam product while smaller diameter foam chambers outperformed the larger.

Levin et al.(1962) obtained a profile of cell concentration in a standing foam height of 120 cm after discontinuing flotation of an algal *Chlorella* species. Cell concentration increased rapidly with height in the first 15 cm and then remained very constant until another sharp rise in the last 5 cm. The maximum cell concentration achieved was 67% higher than the constant value. Considering the height of the foam layer, foam drainage had a remarkably small effect.

Bretz et al.(1966) attempted to optimize foam height in the floation of *E. coli*, but relied on foam concentrate and feed measurements rather than the cell concentration profile in the foam. They observed that residual cell concentration increased

and collapsed foam volumes decreased with foam height, and the smaller volumes correlated with higher foam cell concentrations. Bretz et al. concluded that extending the foam height might reduce foam recovery but would produce a more concentrated foam product.

The continuous flotation study on the yeast *Hansenula polymorpha* carried out by **Viehweg & Schugerl (1983)** found an almost linear increase in foam cell concentration with increasing foam cross-sectional area, and the improvement was tentatively attributed to a diminishing wall effect (there was no discussion of drainage effects). Viehweg & Schugerl were unique in looking at the influence of liquid height upon flotation performance. Cell concentrations in both the foam and tailings rose with foam height up to 15 cm, beyond which no effect was found. When Bretz et al. (1966) observed a similar response to foam height (although without the cut-off), they accounted for the improved foam cell concentration by enhanced drainage; however, neither group attempted to explain the apparent increase in cell concentration of the tailings or residual feed. Lengthening the liquid zone produced a considerable impact on the tailings - the drop in tailings concentration was again most pronounced with a liquid height up to 15 cm, above which the concentration continued to decline but at a reduced rate. This effect would appear to be due to a longer bubble residence time providing more opportunity for cell collection and removal.

It is unfortunate that only one flotation study considered both foam and liquid heights; of course, this opportunity was only made available by the use of a continuous system. The wide range of initial liquid to foam ratios employed by different workers may have affected their results.

3.3.4 Nature of the Feed

It is important to consider the type of feed employed in biological flotations if the results are to be compared. Approximately equal numbers of studies were performed using whole cultures and washed cells resuspended in water. While the ultimate objective is to carry out flotations with fermentation broths (i.e. whole cultures obtained directly from the fermentor), the poorly characterized nature of culture broths could hamper the identification and investigation of parameters of biological flotation.

Interference may come from media components, cellular debris, and secreted or waste products of the cells. Components from poorly defined media (such as heart infusion broth, yeast extract, trypticase, malt extract, peptone, and casein hydrolysate) may contribute to foaming, as may naturally-produced surfactants. Cellular debris (particularly fragments of cell wall) may act as surfactants whose activity depends upon the degree of lysis within the culture; this may be a factor in the flotation of

sporulating cultures undergoing autolysis. The history of the culture, including cultivation conditions and age, may affect the range of secreted and waste products of the cells. Additives may interact with culture components in unforeseen ways.

For these reasons, the use of washed cells of known history resuspended in water may simplify the identification and investigation of flotation parameters by providing a standard basis for comparison. The variability of feeds in the literature suggests a need for caution in comparing results.

3.3.5 Feed Concentration and Rate

Almost all cell flotation studies have been carried out in batch mode where cell concentration in the residual feed changes continuously with time as cells are removed into the foam. However, this does not trivialize the role of initial feed concentration as a parameter in batch cell flotation, and in the case of continuous operation where steady-state conditions are maintained, the role of feed concentration as a flotation parameter is more easily appreciated.

Feed concentrations in the literature have varied by three to four orders of magnitude and have normally been reported as cell counts (10^5-10^9 cells/mL), although biomass concentrations (2-12 g/L) have been employed in a few yeast studies. These concentrations have been determined from turbidity measurements

(using standard curves), viable counts (plating out samples on growth media to obtain visible colonies from single cells), and direct counts (on membrane filters); the algal study by **Levin et al.(1982)** reported concentrations in terms of the packed cell volume obtained after centrifugation. The different methods provide different measures of cell mass, and the wide concentration range in the literature also reflects the differing culture densities to which microorganisms can be grown depending on the species, growth media and conditions, and culture time. The washed cell preparations used by many workers have the advantage of being adjustable to a standard concentration.

The first indication of the role of cell concentration came from studies conducted by **Boyles & Lincoln (1958)**, who noted that flotation performance of sporulating *Bacillus anthracis* cultures correlated with the degree of autolysis. Spore enrichment in the foam doubled when autolysis (and hence spore release) exceeded 75%. Yet it does not necessarily follow that the increase was due to a greater spore concentration in the culture, since it is known that cell wall components possess some surfactant-like properties.

Levin et al.(1962) specifically addressed the question of feed concentration during flotation of the algae *Chlorella*. Cultures diluted with fresh media provided more enriched foams, and Levin et al. pointed out the suitability of flotation for low-density cultures. **Grieves & Wang (1966)** examined residual feed concentration as a function of initial concentration in the

flotation of washed *E. coli* cells. While the residual concentration could be reduced by using less concentrated feed, a consistent pattern was not observed. Using the yeast *Hansenula polymorpha*, **Desmaison & Schugerl (1980)** demonstrated that flotation performance clearly changed over an initial feed concentration range of 2-24 g/L. Above 10 g/L there was little change in flotation performance and enrichment was marginal; below 10 g/L enrichment increased dramatically, and reached a maximum at 2 g/L.

Viehweg & Schugerl (1983) continued work with *Hansenula polymorpha* in the continuous mode of operation, and obtained further evidence for the inverse relationship between feed concentration and flotation performance. Tailings cell concentration fell sharply and foam cell concentration rose (although less dramatically) as the feed cell concentration was reduced below 10 g/L. The separation ratio also declined. It is unfortunate that the data were not presented as enrichment, since the effect of feed concentration would have been even more impressive.

This last study also provided the only information available on the effect of feed rate on continuous cell flotation. Viehweg & Shugerl varied the feed within a superficial velocity range of 0.016-0.108 cm/s and found that higher feed rates reduced the foam cell concentration and raised the cell concentration of the tailings. They attributed the drop in performance with feed rate

to greater water carryover into the foam and shorter residence times of the liquid in the column.

3.3.6 pH

One parameter which has received widespread attention in biological flotations is pH. Even the earliest studies investigated the effect of pH on flotation performance, and variable responses have been observed depending upon the microorganisms employed.

Hansen & Gotaas (1943) achieved very high removals (greater than 99%) of bacteria from sewage using a cationic surfactant in a pH range of 6-8. Removals did not change significantly at acidic pH, but sharply declined above pH 9. This poor performance at alkaline pH was attributed to the loss of frothing and collecting properties of the surfactant.

A very different response was obtained with spore flotation. **Boyles & Lincoln (1958)** found no significant effect on spore collection of *Bacillus anthracis* over the pH range 6-8, but spore removal from cultures of *Bacillus subtilis var. niger* actually improved in the very alkaline range (i.e. pH 11.5). **Gaudin et al. (1960b)** carried out a more detailed study of pH using washed suspensions of sporulating *Bacillus subtilis var. niger*. As with *Bacillus anthracis*, there was no significant change in the foam spore concentration in the acidic range, although extreme

alkalinity may have provided some assistance. However, pH had a greater impact on recovery - spore recovery in the foam improved as pH moved away from neutral to either acidic or alkaline values, although the greatest improvement was obtained at pH 2. Gaudin et al. could offer no explanation for these confusing findings. Yet while pH manipulation could ensure virtually complete spore recovery, it could not raise the quality of the foam product. The difference in foam recoveries between cellular debris and spores varied by less than 20% throughout most of the pH range 2-12, and actually shrank at low pH.

Much stronger evidence for a pH role in flotation came from algal studies. **Gotaas & Golueke (1957)** observed optimum performance at low pH (under 4). **Levin et al.(1962)** found a dramatic pH effect on foam production of cultures of a high-temperature *Chlorella* species. Relatively little frothing occurred during pH reduction from a culture value of 7.5-8 until pH 4.5, at which point a rigid and much more stable foam appeared. Beyond pH 4 the foam could be broken only after prolonged standing or with the addition of an antifoam. The effect on foam cell concentration was even more pronounced - from an initial pH of 5, cell concentration increased linearly with declining pH until halted by cell decomposition at pH 2.

The same type of pH dependence was not observed in bacterial flotation systems. While more interested in the effects of salts on flotation than pH, **Gaudin et al.(1962a,1962b)** noted that pH did

not appear to be a prominent factor in the flotation of *E. coli*, at least in the range of pH 4.5-6. The presence of flotation aids may have obscured pH effects. For example, the microflotation technique pioneered by **Rubin et al.(1966)** employed flocculents, frothers, and an insoluble collector phase. Flotation experiments with washed *E. coli* cells revealed pH interaction with the flocculent alum (i.e. aluminum sulfate $Al_2(SO_4)_3$). At 100 mg/L alum, cell removal from the feed passed through an optimum in the pH range 5-8; reduction of the alum concentration narrowed the optimum pH range. Flotation could not be achieved at low pH where alum exists as the free Al^{+3} ion, whereas high removals could be obtained at a higher pH where aluminum hydroxide formed and was able to promote flocculation. Consequently, the effective flotation range for *E. coli* corresponded to the effective pH range of the flocculent.

Continuing the investigation of pH / flotation aid interactions, **Rubin (1968)** carried out flotation of *Enterobacter aerogenes* in four experimental systems: anionic collector with and without flocculent (i.e. alum), and cationic collector with and without flocculent. In the absence of flocculent, reduction of pH below 9 brought about an erratic increase in cell removal with anionic collector and a more defined, sharper and sustained increase with cationic collector. These results could be explained on the basis of collector adsorption to microbial cells, which generally carry a net negative charge. At low pH the

cationic collector would possess a positive charge while the anionic collector would be neutral; at higher pH values the cationic collector would be neutral while the anionic collector would become increasingly negative and adsorb poorly to cells. Unlike *E. coli* flotations, flocculent addition to *E. aerogenes* flotations did not introduce an optimum into the pH curve; apparently collector adsorption was not confined to flocculated cells and could therefore overcome the effects of a restricted flocculent pH range.

A pH optimum reappeared in the yeast flotation conducted by **Viehweg & Schugerl (1983).** In their continuous system, the concentration of *Hansenula polymorpha* in the foam product rose approximately 50% from pH 3 to pH 5 before declining once more.

It is difficult to generalize the effect of pH on the basis of these varied systems and microorganisms. Spore flotation may be enhanced under alkaline conditions; considerable evidence suggests that algal flotation should be carried out under strongly acidic conditions. In bacterial flotations there may be an optimum pH range on the slightly acidic side, or no optimum may exist. The influence of pH on flotation aids such as ionic surfactants and especially flocculents (which operate over a restricted pH range) requires careful consideration for each system. What is clear is that different microorganisms have different flotation behaviors along the pH scale, suggesting that pH plays a role in adjusting the ionic state of the cell surface.

Unfortunately, no consideration has been given to the effect of pH on the other surface of interest, namely that of the bubble.

3.3.7 Additives

While investigators such as **Boyles & Lincoln (1958)** and **Levin et al.(1962)** exploited the natural ability of certain microorganisms to undergo flotation, most workers pursued "assisted" flotations as a means of enhancing performance, and separation without additives was seldom practiced. Chemicals have been employed to promote foaming or cell attachment to bubbles; some additives have been selected to perform both roles.

Frothers, Flocculents, and Surfactants

The practice of employing surface-active agents was adopted early in the development of cell flotation and became almost a standard feature regardless of the microorganism to be floated. **Gotaas & Golueke (1957)** used commercial flotants as a matter of course in their quest for an economical harvesting method for large-scale algae production, but concluded that the requirements for algal flotation would be too costly.

The first survey of surfactants for biological flotations was conducted by **Gaudin et al.(1960b)**, who examined some secondary amine and fatty acid compounds for their ability to serve as

bacterial collectors. Preliminary results suggested that cationic surfactants improved foam selectivity for *B. subtilis* spores without toxic effects on spore viability, whereas anionic surfactants were selective for vegetative cells and debris. Gaudin et al. recommended further experimentation with surfactant concentration, hydrocarbon length, and pH to optimize surfactant use for a particular microorganism.

Rubin et al.(1966) introduced a spectrum of flotation aids to the microflotation technique. The method employed an insoluble surfactant such as a long-chain fatty acid or amine to produce a stable surface phase; frothers were used to shrink bubble size and enhance the collector properties of the surfactant while flocculents were added for cell agglomeration and to provide sites for collector adsorption. Flocculent requirements depended on cell concentration in the feed, whereas frother requirements varied inversely with the surfactant concentration. While two algal species (*Chlorella ellipsoidea* and *Chlamydomonas reinhardtii*) were successfully floated with a cationic surfactant, the inability to float *E. coli* led to speculation that charge was not the only consideration in surfactant adsorption to cells. *E. coli* flotation by an anionic surfactant was successful only in the presence of a flocculent, alum. Since aluminum flocs are positively charged, it was postulated that the alum, adsorbed to and bridging flocculated cells, provided positive sites onto which the anionic surfactant adsorbed.

In a later study with *Enterobacter aerogenes*, **Rubin (1968)** found that, unlike *E. coli*, collection by an anionic surfactant was possible in the absence of alum, although removals were generally higher in the presence of the flocculent. However, a cationic surfactant was a more efficient collector.

Grieves & Wang (1966) were able to achieve flotation of *E. coli* with a cationic surfactant by using EHDA-Br (ethyl-hexadecyldimethylammonium bromide) in initial concentrations of 15-40 mg/L. Both cell and surfactant concentration were measured in the residual feed rather than in the foam concentrate, the surfactant by a two-phase titration technique. Interestingly, Grieves & Wang consistently found that the ratio of the concentration of cells to surfactant in the foam was greater than that in the initial feed, i.e. cells were concentrated more efficiently in the foam than the surfactant, but offered no explanation for such behaviour. In a continuation of this work by **Bretz et al.(1966)**, substantial improvement in foam cell concentration was obtained by multiple additions of the surfactant over the flotation period.

Further studies with EHDA-Br conducted by **Grieves & Wang (1967a)** assessed the flotation performance of a variety of bacteria: *Serratia marcescens*, *Proteus vulgaris*, *Pseudomomas fluorescens*, *Bacillus cereus*, and *Bacillus subtilis var. niger*. All species except *B. subtilis* out-performed *E. coli*, and the foam enrichments of *S. marcescens* and *P. vulgaris* were two orders of

magnitude greater. Grieves & Wang attributed the different performances to differing abilities to adsorb the cationic surfactant, which in turn reflected differences in the cell surfaces of the species.

Inorganic salts

Inorganic salts were the first additives to be tested in the quest to achieve flotation of previously non-flotable microorganisms. While **Dognon (1941)** found that salts aided flotation of several microorganisms, **Boyles & Lincoln (1958)** could not improve the collection of *Bacillus anthracis* spores from culture with the addition of 5% NaCl. In somewhat qualitative studies with *E. coli*, **Gaudin et al.(1962a)** observed that the improvement in flotation performance with NaCl addition varied with the strain but was not due to salt-induced flocculation with the exception of one strain. Whereas natural flotation recovered only 15-20% of the cells in the feed, the addition of 3% NaCl recovered improved cell recovery to 98%.

Gaudin et al.(1962b) discovered a wide range of salt efficacy. Noting that the negative surface charge of a microbe could be reduced or even reversed in the presence of cations, they suggested that cation interaction with ionic groups on the cell surface could be a prerequisite to flotation of *E. coli*. While salts with multivalent cations were generally better flotation

promoters than those with univalent cations, Gaudin et al. concluded that the valence and atomic weight of a cation corresponded only roughly to flotation performance. For example, the effectiveness of salt-induced removal of *E. coli* from the feed followed the series $Mg^{2+} \geq Na^{+} > Ca^{2+} > Li^{+}$ and K^{+}; i.e. divalent Ca^{2+} was not more effective than Na^{+} despite its greater valence, while Li^{+} did not outperform Na^{+} despite its greater charge to mass ratio. The choice of anion was also important - the bromide and iodide salts of Na performed poorly compared with the chloride salt, while phosphates were effective but markedly decreased cell viability.

Bretz et al.(1966) also studied the relative effect of Na, Mg, and Ca salts on *E. coli* flotation using the chloride and sulfate series, but concluded on the basis of increased residual feed cell concentrations that salt addition had an adverse effect on flotation. However, the foam data supports a very different picture - the cell concentration of the collapsed foam actually increased after salt addition, demonstrating that the salts improved foam enrichment. The foam data also suggested a different sequence of cation effectiveness: $Na^{+} > Mg^{2+} > Ca^{2+}$. Although based on inconsistencies in the residual feed data, Bretz et al. echoed the conclusion of Gaudin et al.(1962b) that simple valency could not completely account for the observed salt effects, and proposed that site-specific binding of certain ions might play a role in biological flotation. Despite the use of a

cationic surfactant in all flotations, there was no discussion of possible salt interactions.

Expanding the investigation of salt effects to other microorganisms, **Grieves & Wang (1967a)** performed flotations of *Pseudomonas fluorescens* and *Bacillus subtilis var. niger* in the presence of 0.005 N Na, K, Mg, and Ca salts using the chloride and sulfate series. Unfortunately, Grieves & Wang appeared to be more interested in salt effects on the distribution of the cationic surfactant employed in the flotations. As judged by elevated residual feed cell concentration, salt addition was detrimental to flotation of both *B. subtilis* and *P. fluorescens*; Mg produced a disproportional effect compared with Ca, Na, and K. Yet salt addition was not neccessarily detrimental when the more relevant foam cell concentrations were considered - foams of *B. subtilis* were more concentrated after salt addition while *P. fluorescens* foams were more dilute.

Viehweg & Schugerl (1983) included salt effects in their list of flotation parameters under investigation, but furnished few results. Although the surfactant-free flotation of the yeast *Hansenula polymorpha* was well suited to study salt effects, data for only one salt were presented. $NaClO_4$ (described as representative of "structure-breaking" salts) reduced the foaminess of the flotation over the concentration range of 0-0.5 M and elevated the foam cell concentration by approximately 20%.

Unidentified "structure-making" salts apparently produced the opposite effect.

No consistent trend emerges from the limited and confusing information on salt effects in the cell flotation literature. There is some evidence to suggest that cation valency may not be the only consideration, but more data is required on both the individual cation efficacy and the influence of salt concentration.

3.4 Cell Characteristics

The flotation performances of a number of microorganisms, including bacteria and some algae and yeasts, have been evaluated (see **Table 3.1**). While individual responses have varied, so too have the operating conditions under which the data were collected. In particular, additives such as surfactants have been used without necessarily comparing the results to natural flotations. Yet it is still possible to state from the literature data that certain species are suitable for flotation while others are not. For example, **Dognon (1941)** easily recovered *Mycobacterium tuberculosis* in a natural flotation, whereas a number of investigators have failed to recover *E. coli* without recourse to salts or surfactants.

Not only does species-to-species variation exist, but there is evidence for strain and colony differences. **Gaudin et al.(1962a)** found that *E. coli* response to salt addition depended upon the strain - flotation of one strain could be improved by 1-2% NaCl, whereas a second strain could not be properly floated with even 4-6% NaCl and a third strain flocculated after salt addition. **Boyles & Lincoln (1958)** observed distinct differences in the behaviour of colony variants of *Bacillus anthracis*. Compared with a rough variant, a mucoid variant produced a much wetter foam with noticeable amounts of cell debris, and both spore recovery and concentration in the foam were reduced. Colonial differences were also obtained with *Brucella suis*, although in

Table 3.1: Microorganisms Employed in Cell Flotation

	Bacteria
Escherichia coli	Gaudin et al.(1962a,1962b) Grieves & Wang (1966) Bretz et al.(1966) Rubin et al.(1966) Grieves & Wang (1967a)
Serratia marcescens	Boyles & Lincoln (1958) Grieves & Wang (1967a)
Enterobacter aerogenes	Rubin (1968)
Pseudomonas fluorescens	Grieves & Wang (1967a,1967b)
Francisella tularensis	Boyles & Lincoln (1958)
Brucella suis	Boyles & Lincoln (1958)
Bacillus anthracis	Boyles & Lincoln (1958)
Bacillus cereus	Black et al.(1958) Grieves & Wang (1967a)
Bacillus subtilis var. niger	Boyles & Lincoln (1958) Gaudin et al.(1960a,1960b) Grieves & Wang (1967a,1967b)
Mycobacterium tuberculosis	Dognon (1941)
	Yeast
Hansenula polymorpha	Desmaison & Schugerl (1980) Viehweg & Schugerl (1983)
Saccharomyces carlsbergensis	Parthasarathy et al.(1988)
	Algae
Chlorella sp.	Levin et al.(1962)
Chlorella ellipsoidea	Rubin et al.(1966)
Clamydomonas reinhardtii	Rubin et al.(1966)

this case the rough variant performed better than the smooth. Even cell type may affect flotation behaviour. **Gaudin et al.(1960a,1960b)** observed different rates of recovery for dormant spores, germinated spores, and vegetative cells of *Bacillus subtilis var. niger*, and were able to exploit the differences by selecting appropriate collectors. The distinct flotation behaviour may have reflected differences in the outer surfaces of cells not only from strain to strain but between cell types of the same microorganism.

This section will elaborate on parameters from the literature which reflect cellular variation: cell size, cell age, cultivation conditions, and the nature of the microbial cell surface.

3.4.1 Cell Size

Cell size and shape vary widely between microbial species. A bacterial cell may be spherical, rod-shaped or even spiral, and have a typical dimension of 0.5-3 μ; for example, *E. coli* is rod-shaped with approximate dimensions of 2 μ in length and 1 μ in diameter. Yeast cells are spheroid and range 5-30 μ in length and 1-5 μ in diameter, while unicellular green algae may be larger still. Some microorganisms demonstrate a tendency to flocculate under certain conditions, with a resulting increase in particle size.

No researcher has specifically examined whether the size of a microorganism plays a role in its flotation performance. The use of flocculents in the microflotation technique raised this conjecture when **Rubin et al.(1966)** observed differing rates of removal of the algae *Chlorella ellipsoidea* for two different degrees of flocculation with alum, although these results were explained on the basis of a cell-linking alum network providing positive sites for surfactant adsorption. In a later study, **Rubin (1968)** stated without supporting data that the rate of removal was a function of particle size, and suggested that the variable removal of *Enterobacter aerogenes* with pH could reflect a pH-dependent state of aggregation. Unfortunately, no follow-up studies were done, and later investigators did not pursue cell size as a possible parameter of flotation.

3.4.2 Cell Age

Cell size, composition, metabolism and products change during the various phases of culture growth, yet cell age is another potential parameter which has received almost no attention in the flotation literature. **Gaudin et al.(1960b)** included culture harvest age as a variable in the flotation of *Bacillus subtilis var. niger*, and observations on 1-5 day cultures revealed that flotation performance varied with culture age. Spore concentration in the foam reached a maximum at 3 days. Since the

spore concentration in the feed also increased with time, the declining flotation performance after 3 days could not be attributed to fewer spores in the feed. Gaudin et al. suggested the change in flotation performance may have been due to altered proportions of vegetative cells and cellular debris, or the changing nature of the cellular debris itself.

Viehweg & Schugerl (1983) made reference to previous work by Schugerl in which declining foam recovery of *Hansenula polymorpha* correlated with increasing age of the yeast cells, but no further investigations were carried out.

3.4.3 Cultivation Conditions

Since single-celled organisms have few means of controlling their environment, many microorganisms (especially bacteria) exhibit great flexibility in substrate utilization and considerable adaptability in the external barrier erected between the cell and its environment. Depending on conditions (most notably nutrient availability), microorganisms may have the ability to produce an external capsule or slime layer and even adjust to some extent the composition of their outer cell envelope. The array of products excreted by a microorganism may also change with the environment, and these products may possess surface activity. Consequently, microbial cultivation conditions may affect microbial flotation performance.

Recently, **Viehweg & Schugerl (1983)** began to explore the possible effects of cultivation conditions on continuous flotation of the yeast *Hansenula polymorpha*. In addition to standard growth on glucose, cultivation conditions included alternate C sources such as methanol or ethanol and nutrient limitation (P, N or O_2). Yeast grown on methanol produced higher cell concentrations in both foam and tailings, and unfortunately cell recoveries were not provided to clarify the situation. N-limitation adversely affected flotation performance by elevating the tailings cell concentration, and the effect appeared to be more strongly dependent on feed cell concentration than in any other case.

In a continuation of this work by **Bahr et al.(1991)**, *Hansenula polymorpha* culture was fed directly from the fermentor to a continuous flotation column. Flotation performance was affected by a wider range of nutrient limitation - foam cell recovery declined in the sequence P-, N-, C-limitation. Performance was influenced by the degree of culture foaminess, which in turn depended upon extracellular proteins excreted by the yeast during cultivation. Analysis by polyacrylamide gel electrophoresis (PAGE) and gel chromatography determined that the pattern of protein production and distribution in foam and tailings (but not protein concentration) were influenced by the type of growth limitation. Under P-, N-, and C-limitation the molecular weight distributions of the proteins were similar, but the P-limitation proteins possessed a significantly different

electrophoretic behaviour, i.e. charge differences were apparently present. Bahr et al. proposed that the proteins played a dual role as foamer and cell collector. With the exception of a single, strongly acidic protein, all of the extracellular polypeptides were glycoproteins, indicating that they were originally membrane-associated or located in the perisplasmic space of the cells. The latter may very well be the source of these proteins, since the extracellular proteins of *Hansenula polymorpha* were not originally associated with the cytoplasmic membrane.

These results may be repeated in other microorganisms, or at least in other yeast. **Eschenbruch & Rassell (1975)** found that foaming varied with the strain of *Saccharomyces cerevisiae* used in winemaking and foaming correlated with the flotability of the yeast strain. **Clapperton (1971)** reported that foaming could result from the peptides, amino acids and polysaccharides excreted by *Saccharomyces carlsbergensis* during fermentation of wort to produce alcohol. **Molan et al.(1982)** determined that less than 20% of the foaminess of one *S. cerevisiae* strain was due to the cells themselves; the majority of the foaming was produced by three extracellular proteins.

3.4.4 Cell Surface

Even the earliest investigations into cell flotation recognized that the nature of the cell surface played a major role in whether or not a microorganism could be floated. **Boyles & Lincoln (1958)** concluded that cells with a relatively hydrophobic surface were carried by foam, and **Gaudin et al.(1962a)** suggested that salt-induced changes in the bacterial surface configuration exposed more hydrophobic groups. **Gaudin et al.(1960b)** attributed the differences in flotability between *Bacillus subtilis* vegetative cells and spores in the presence of ionic surfactants to polar groups exposed at the cell surface, i.e. both cationic and anionic sites were available, but the relative abundance of sites differed with cell type (as demonstrated by ionic collector preference). **Grieves & Wang (1967a)** considered bacteria to behave as hydrophilic colloids whose surface properties were affected by hydrated water molecules, and proposed that ionic surfactants could adsorb to the cell at oppositely-charged surface groups or even react chemically with surface molecules.

Cell parameters such as age and cultivation conditions may exert part of their influence through the changing nature of the cell surface. For example, **Miyazu & Yano (1974)** found that the surface hydrophobicity of hydrocarbon-grown *Mycotorula japonica* yeast cells was approximately 7-fold greater than that of glucose-grown cells, although the numbers of polar groups differed less

dramatically. Operating parameters such as pH and the use of additives also affect the cell surface. Indeed, **Gaudin et al.(1962b)** deduced from the flotation performance of *E. coli* with various inorganic salts that cation interaction with polar groups on the cell surface might be a prerequisite for flotation.

Clearly a closer look at the microbial cell surface is in order. However, it is very difficult to define the cell surface since the cell wall is freely permeable to water ions and small molecules. The outermost limit at which properties differ from the surrounding environment is the ionic atmosphere held in the neighborhood of the cell by cell surface ionic groups. Within this region are also found nutrients and metabolites diffusing towards the cell and soluble products diffusing away. Further in, bacteria (and some yeasts) may possess a capsule or hydrated slime layer whose thickness varies with growth conditions; most capsules consist of relatively simple polysaccharides containing repeating sequences of two or three sugars and often uronic acids. Next to be encountered is the cell wall, a complex structure of polysaccharides, peptidoglycan, proteins, and lipids whose composition and organization varies greatly between bacteria, yeasts and unicellular algae, and that variation extends to the species and even the strains within each group. The final osmotic barrier is the cytoplasmic membrane, an asymmetrical bilayer of phospholipds, proteins and polysaccharides. Continuous with the membrane are optional appendages such as flagelli (for motility)

and pili (for adherence or genetic exchange). Yeasts and some bacteria possess an additional outer membrane in close association with the inner cell wall, and the two membranes enclose the periplasmic space in which potentially damaging products and enzymes are stored.

The study of hydrolysed cell envelope fractions can provide information on the basal components of the cell wall and membrane for a variety of cell types. Degraded cellular material, however, can provide little information on the nature and quantities of the native molecular species at the cell surface which may be involved in flotation. **Ouichi et al.(1973)** found marked differences between sake yeast and non-foaming mutants in various physico-chemical properties, including agglutination (by *Lactobacillus* cells) and flotability. However, there was no correspondingly significant difference in the yeast cell wall composition (although no lipid determinations were performed), and Ouchi et al. concluded that the physico-chemical changes were likely due to structural changes on the outermost layer of the cell wall.

Electrokinetic techniques based on electrical phenomena at interfaces can provide information on cell surface charge. The region under study (which depends upon the ionic strength of the suspension media) extends 100-200 nm into the liquid phase and is considered to begin at the outer portion of the cell membrane where the space not occupied by macromolecular structures is accessible to ions and small molecules. The presence of ionogenic

groups can be established by electrophoretic studies of cell migration in suspensions of known pH and ionic strength under an applied electric field; similarly, the presence of neutral surface lipid can be established by studies on suspensions containing surfactants. Even though large differences in cell size and charge-determining groups exist, the electrophoretic mobilities of different cells all fall within the same order of magnitude. Consequently, while cell mobility is reproducible under controlled growth conditions, a particular strain cannot be uniquely identified by a single mobility value at a given pH and ionic strength. Since the charge carried by cells in suspension (and hence their mobility) depends upon not only the nature and number of ionogenic cell surface groups but also on the nature of the suspension, it is possible to use the pattern of cell mobility with pH or ionic strength to characterize cell surface groups.

The variation of cell mobility with ionic strength has been used to identify the carboxyl surface nature of spores of *B. megaterium*, *B. cereus* and *B. subtilis* (**Douglas, 1957**) and several yeasts, the partially phosphatide surface of *Mycobacterium phlei* (**Adams & Rideal, 1959**) and the acidic polysaccaride surface of *E. coli* (**Davies et al.,1956**). The method also illustrates the three-dimensional nature of the surface since charged groups lying deeper in the cell envelope are exposed to contribute to the total cell charge as the ionic strength is reduced. The change of mobility with pH at constant ionic strength has been used to

identify the anionic surface nature of *K. aerogenes* (**Gittens & James, 1963**), *E. coli* (**Davis et al.,1956**), slow-growing strains of *Rhizobium* (**Marshall, 1967**) and many mycobacterial strains typical of carboxyl, sulfate and phosphate groups (where ion adsorption appears to be negligible), while a mixed amino-carboxyl surface is characteristic of *B. cereus*, *B. megaterium* and *B. subtilis* (**Douglas & Shaw, 1958**), *Micrococcus lysodeikticus* (**Few et al.,1960**), and *Streptomyces pyogenes* (**James et al.,1965**). Surface lipid other than phospholipids can be detected by surfactant addition - lipophilic surfaces show a significant increase in negative mobility in the presence of an anionic surfactant, while charge reduction and even charge reversal may occur in the presence of a cationic surfactant.

The surface charge of some species may also vary with cell age and the nature and composition of the growth media. For example, *E. coli* has been shown to possess its minimum negative charge during physiological youth, even though no corresponding changes in biochemical properties have been recorded (**Moyer, 1936**). *K. aerogenes* exhibits a slightly greater negative charge during exponential growth, which has been attributed to changes in the amount of capsular polysaccharide produced (**Plummer & James, 1961**). Variation in the mobility of *B. subtilis* during spore germination was presumed by **Tochikubo et al.(1975)** to be due to differences in the outermost surface layer of the spore coat. The increasingly negative charge gained by *Streptococcus pyogenes*

during growth in liquid media was traced to the accumulation of surface hyaluronic acid in active growth and its removal in the stationary phase, while the increasing negative cell mobility during growth in the presence of glycerol was shown to correspond with an increase in surface phosphatide lipid in active growth **(Hill et al.,1963a,1963b)**. A change in the nature of the cell surface, however, may not always be reflected in a mobility change.

Chapter 4: MATERIALS AND METHODS

4.1 Materials

4.1.1 Yeast

Bakers' yeast (*Saccharomyces cerevisiae*) was purchased in bulk as finely powdered active dry yeast from a local health food store. In order to minimize leaching of solids from the yeast, rehydration was carried out in small amounts of 40°C tap water (approximately 1 L per 40 g) for 5 minutes, as recommended by **Reed (1982)**. The yeast slurry was then available for feed preparation.

4.1.2 Surfactant

Chosen as analogues for natural microbial biosurfactants, two alkylpolyglycoside surfactants were obtained from Henkel Corporation (Ambler PA, USA): APG 600CS and APG 625CS. These non-ionic surfactants are formed by the condensation of a carbohydrate and a fatty alcohol, and have the formula and structure depicted in **Figure 4.1**; reported surface activities are presented in **Table 4.1**.

Stock aqueous solutions (1.0 wt%) were prepared by initial mixing of surfactant with a small amount of isopropanol (BDH analytical reagent grade) to aid in dispersal before addition of

Figure 4.1: Typical Structure of an Alkylpolyglycoside

General formula $C_nH_{2n-1}O(C_6H_{10}O_5)_x$

n=12-14	APG 600CS
n=12-14-16	APG 625CS
$1 < x < 2$	

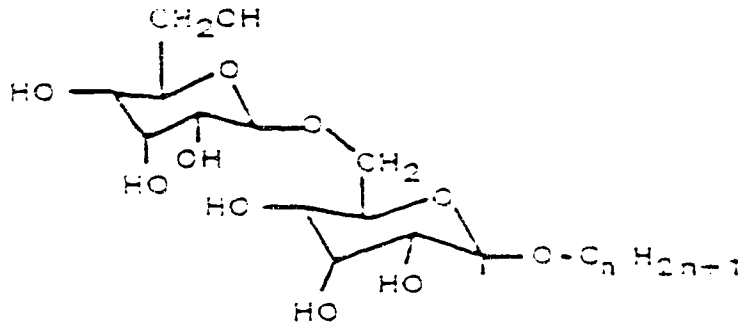


Table 4.1: Surface Activity of Alkylpolyglycosides

Surfactant	Concentration (ppm)	Surface tension at 77°C (N/m)
APG 600CS	100	0.028
	1000	0.026
APG 625CS	100	0.0277
	1000	0.0277

water. The final concentration of isopropanol in the stock solutions was 0.5 vol %.

4.1.3 Inorganic Salts

NaCl, KCl, $MgCl_2 \cdot 6H_2O$, and $CaCl_2 \cdot 2H_2O$ salts (BDH analytical reagent grade) were employed in concentrations of 0.05, 0.1, and 0.5 M during flotation runs.

4.2 Methods

4.2.1 Bubble Photography

Bubble photography was used to determine liquid zone bubble terminal rise velocity (V_t) required in the hydrodynamic characterization of the flotation column. Photographs were taken with a Nikon camera at different frontally illuminated locations along the liquid zone during hydrodynamic runs. From the photographs bubble shape and size were digitized using the Sigma-Scan Measurement System and a Jandel Scientific digitizing tablet. Ellipsoidal bubbles (increasingly numerous with increasing gas flowrate) were identified by shape factor:

$$SF = \frac{\sqrt{A_b}}{P_b} \qquad (4.1)$$

where A is the bubble cross-sectional area and P is the bubble perimeter, and the values of their effective diameter (d_e) were calculated from the volume-equivalent sphere:

$$d_e = \sqrt[3]{\frac{6V}{\pi}} \qquad (4.2)$$

where the ellipsoidal bubble volume (V) was estimated from the bubble area and the horizontal diameter.

Depending upon the gas flowrate (which influenced bubble size and hence the number of bubbles observable on each photograph), anywhere from 300 to 1000 bubble diameters were obtained in this manner for each flowrate and were used to calculate the Sauter mean diameter (d_{32}) at each gas flowrate:

$$d_{32} = \frac{\Sigma(n_i d_i^3)}{\Sigma(n_i d_i^2)} \qquad (4.3)$$

where d_i is the individual bubble diameter (spherical or equivalent) and n_i is the number of bubbles having diameter d_i. Using the Sauter mean diameter, the bubble terminal rise velocity (V_∞) at each flowrate was calculated from the empirical correlation of **Clift et al.(1978)** according to the procedure described in Section 2.5.

4.2.2 Tensiometry

Surface tension measurements were used to provide parameter values for hydrodynamic characterization of the flotation column and to determine surfactant concentration in flotation samples. These measurements were performed on a Cahn Dynamic Contact Angle Analyzer DCA-312 (courtesy of the Alberta Research Council) using the Wilhelmy plate method. Standard curves at 21°C were generated for aqueous solutions of surfactants APG 600CS and APG 625CS in the concentration range 5-100 ppm (complete data are provided in **Appendix A**).

4.2.3 Spectrophotometry

Absorption spectroscopy was employed to determine yeast concentration in flotation systems according to turbidity. Absorption measurements at 600 nm were performed on a Shimodzu UV-VIS UV-160 spectrophotometer. A biomass standard curve at 21°C was generated for bakers' yeast suspensions in the concentration range 1-600 mg/L, and is provided in **Appendix D.**

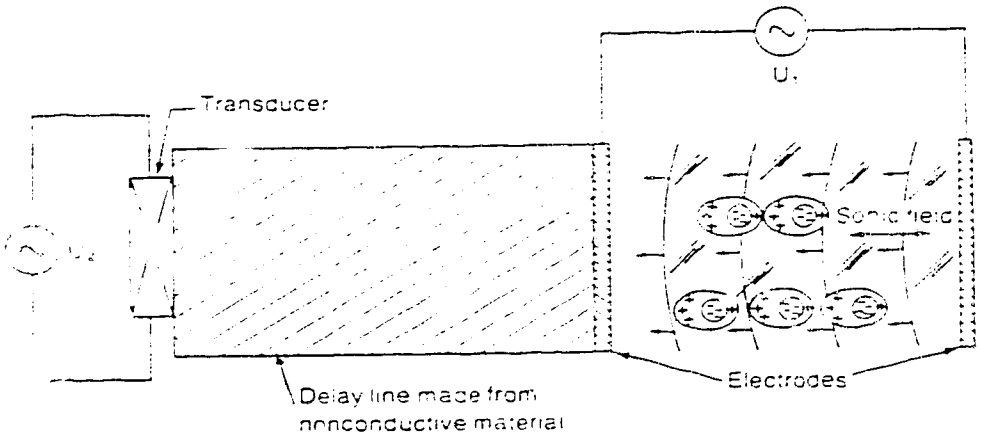
4.2.4 Electrokinetic Sonic Amplitude (ESA)

Traditional electrokinetic techniques for characterizing particle surface charge are optical methods subject to

restrictions on the optical properties, size and dilution of the particulate suspension. A new electroacoustic technique described by **Babchin et al.(1989)** requires a density difference between the continuous and disperse (i.e. particulate) phases, but expands the range of suspensions which can be studied (including opaque suspensions) and is also capable of monitoring coagulation effects. This electroacoustic technique, operated in the ESA mode, was employed to characterize the surface charge associated with bakers' yeast cells during titration with 2.0 M solutions of various inorganic salts (i.e. NaCl, KCl, $MgCl_2$ and $CaCl_2$). The analysis was performed at 21°C on 10 wt % suspensions of yeast in the presence of surfactant at the same concentration as in the flotation experiments, using a MATEC SSP-1 ESA sample cell assembly.

The electroacoustic system depicted in **Figure 4.2** permits two modes of measurement: ultrasound vibrational potential (UVP) and electrokinetic sonic amplitude (ESA). In the first mode a high frequency voltage (U_2) applied to the transducer circuit causes the propagation of a sound wave of the same frequency through the delay line into the particulate suspension, where the resulting relative motion between the particles and their double layers is detected as a variable voltage (U_1). In the ESA mode an alternating electric field applied to the suspension through the high frequency potential at the electrodes (U_1) generates a sound wave of the same frequency, and the acoustic pressure is detected

Figure 4.2: Principle of the Electroacoustic Technique



as a voltage in the transducer circuit (U_2).

Either mode requires a density difference to exist between the particulate and continuous phases of the suspension in order to generate relative motion between the particles and their double layers, since the complementary ionic atmospheres of the particles belong to the liquid phase. If the particle density exceeds that of the continuous phase, the motion of the particles lags behind the motion of the liquid, whereas the particle motion preceeds the liquid motion if the liquid density is the greater.

In the more accessible ESA mode of analysis the acoustic pressure (P_O) and hence the ESA signal are proportional to the frequency-dependent electrophoretic mobility of the particles (u):

$$\mathbf{ESA}(\omega) \quad = \quad \frac{P_O}{E_O} \quad = \quad \phi \, \Delta\rho \, c \, G_f \, u(\omega) \qquad (4.4)$$

where E_O = amplitude of the alternating electric field strength
ω = frequency of the particle oscillation
ϕ = particulate phase volume concentration
$\Delta\rho$ = density difference between the continuous and particulate phases
c = velocity of sound in the suspension
G_f = correction factor for the ... electrode geometry

In turn, the dynamic electrophoretic mobility (u) is proportional to the zeta potential of the particles (ζ):

$$u(\omega) = \frac{|U_o|}{E_o} = \frac{4\pi\varepsilon\zeta f(\kappa R)}{\sqrt{(6\pi\mu\underline{R})^2 + (\frac{4}{3}\omega\rho_{eff}\ R^2)^2}} \qquad (4.5)$$

where $U_o(\omega)$ = magnitude of the particle velocity

ε = permittivity of the continuous phase

μ = dynamic viscosity of the continuous phase

κ = inverse Debye length

R = particle radius (assuming a spherical shape)

$\underline{R}$ = effective particle radius R_{eff} normalized to the radius R

R_{eff} = $R(1 + \kappa/\delta)$

δ = depth of penetration (a measure of the region surrounding the particle in which viscosity has an important influence on the flow, increasing with viscosity and decreasing with oscillation frequency)

ρ_{eff} = effective particle density in oscillatory motion

$$= \rho_o + \frac{g}{4R}\sqrt{\frac{2\mu\rho}{\omega}}\left(1 + \frac{2R}{2\delta}\right)$$

ρ_o = particle density

ρ = density of continous phase

In the limit $\omega \rightarrow 0$, Equation 4.5 reduces to the Henry equation for the static electrophoretic mobility of non-conducting spherical particles (**Equation 2.3**).

Chapter 5: EQUIPMENT AND OPERATING PROCEDURES

5.1 Equipment

A schematic diagram of the flotation apparatus used in this study is shown in **Figure 5.1.** The acrylic flotation column was designed with a working height of 1 m and a diameter of 0.045 m ID, giving a cross-sectional area of 0.0016 m^2 and a height-to-diameter ratio of approximately 20:1. The column was equipped with air sparging at the base, feed entry at mid-column, and wash water delivery at the top of the column (just below the foam exit). Tailings were withdrawn by gravity drainage from the bottom of the column; the flowrate was manually adjusted by a valve to maintain a constant liquid-foam interface level just above the feed entry. Foam exited freely from a side chute at the top of the column, and was collected in a holding vessel to allow complete collapse. The column was also equipped with two diferential pressure cells of the diaphragm type, one for the liquid zone (maximum 1.0 psi span) and one for the foam zone (maximum 0.1 psi span). Exact locations of these features have been provided in **Table 5.1.**

Feed was stored in an impeller-stirred 20 L holding tank and delivered to the column at a preset flowrate by a programmable peristaltic pump. Air for sparging was diverted from a high-pressure line and regulated to 90 psig; the flowrate was

Figure 5.1: Schematic Diagram of Apparatus

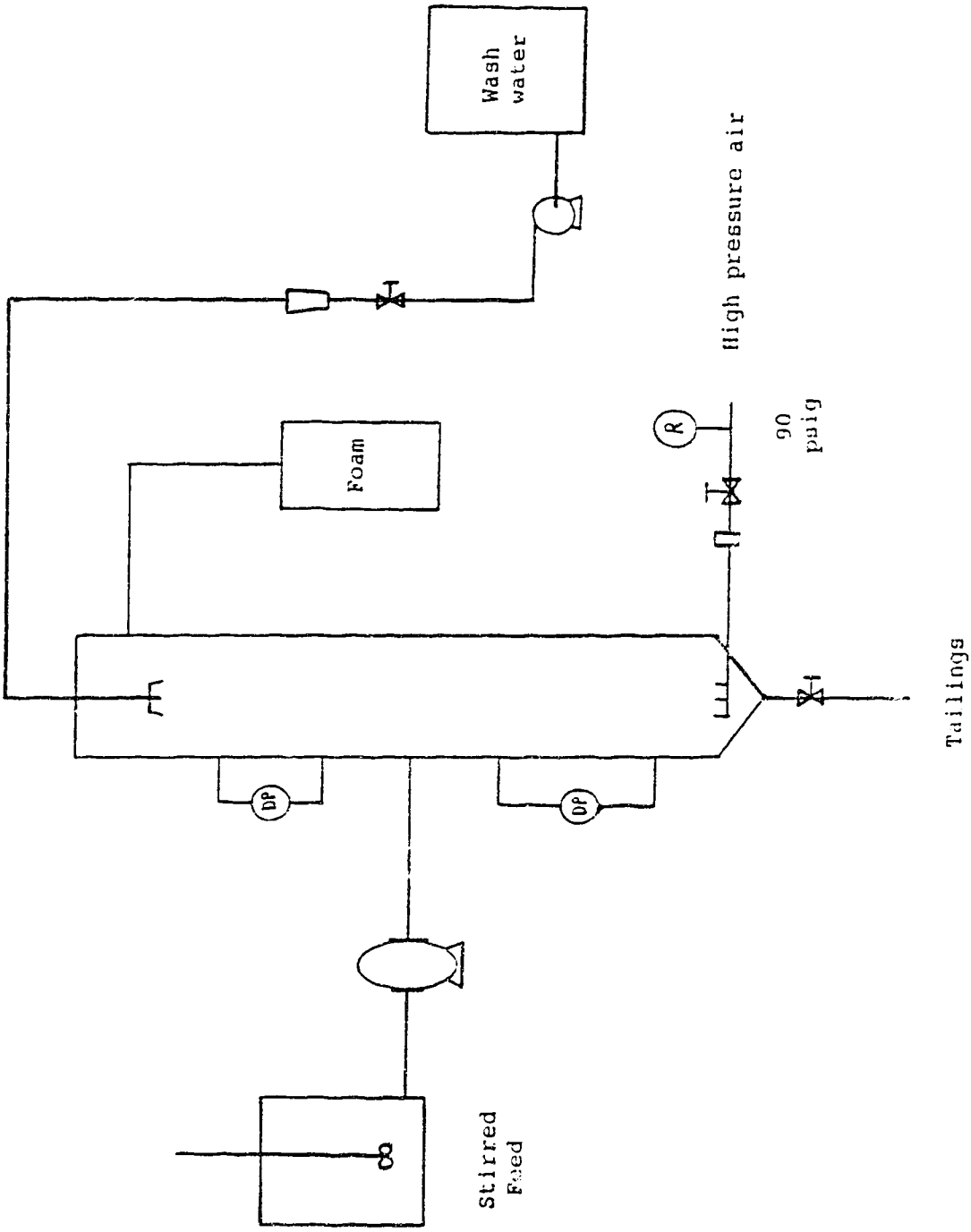


Table 5.1 Flotation Column Features

Port / Feature	Location above sparger (m)	Monitoring distance (m)
Foam exit	1.000	
Wash water distributor	0.980	
Foam zone DP cell		0.150
upper tap	0.837	
lower tap	0.687	
Foam-liquid interface	0.600	
Feed slurry	0.588	
Liquid zone DP cell		0.203
upper tap	0.490	
lower tap	0.288	

controlled by a rotameter equipped with a high-accuracy needle valve. A sintered stainless steel filter element with a 15 μ nominal pore size served as the sparger in the bottom of the column. Wash water was stored in a 20 L holding tank and delivered to the top of the column by a centrifugal pump; the flowrate was also controlled by a rotameter equipped with a needle valve. A small, common plastic nozzle (screwdown adjustable) served as the wash water distributor and provided a cone-shaped spray at all but the lowest flowrate.

5.2 Operation

5.2.1 Experimental Parameters

The feed flowrate to the flotation column was chosen by scale-down from an existing flotation column (**Pal & Masliyah, 1989**) based on superficial velocity. Since unaided yeast flotation was very poor, a non-ionic alkylpolyglycoside surfactant was introduced to the feed to act as a collector; this surfactant also played a secondary role as frother, reducing bubble size and helping to sustain the foam in the column. The surfactant concentration in the feed was selected as the lowest concentration producing good foaming characteristics in the column for both surfactants APG 600CS and APG 625CS. A yeast concentration of 2 g/L was chosen from the lower end of the flotation range from the

literature on the basis that low concentrations produced more enriched foams. Feed pH was adjusted in the presence of yeast to attain the midpoint of the normal fermentation range for bakers' yeast (i.e. pH 4-6). The column was operated at the largest foam to bubbly zone height ratio which could be consistently maintained by the foaming character of the feed, and the upper limit to wash water rate was imposed by the resiliency of the foam.

5.2.2 Hydrodynamic Characterization of Flotation Column

A hydrodynamic characterization of the flotation column was undertaken in order to validate its operation. This characterization was performed on two aqueous surfactant systems (APG 600CS and APG 625CS) in the absence of bakers' yeast, and employed the drift-flux model discussed in Section 2.5.

Feed Preparation

Prior to the start of a hydrodynamic experiment, surfactant feed was prepared in the feed holding tank by diluting 1.0 wt% stock solution of the chosen surfactant (APG 600CS or APG 625CS) in tap water to give a final feed concentration of 40 ppm. Following an initial 5 minute stirring period, feed homogeneity was maintained by continuous agitation throughout the experiment.

Start-up

At the start of each experiment the feed pump drive was set to deliver a constant flowrate of 200 mL/min, the flotation column was charged with feed to a level just above the feed entry port, and feed delivery was started. Air flow to the sparger was initiated at a chosen rate within the range 5-25 cm^3/s (21°C), and the tailings valve was opened and manually adjusted to bring the foam-liquid interface to a constant level at 0.600 m above the sparger. Once the rising foam had reached the level of the foam overflow, wash water delivery to the top of the column was initiated at 1.0 mL/s. The system was allowed to come to steady-state (requiring approximately 10 minutes), with tailings rate adjustment as necessary to ensure a constant interface level.

Run-time

Once steady-state at the chosen air flowrate had been achieved, a series of measurements was initiated; due to the limited capacity of the feed holding tank, multiple runs were normally required to complete the measurements. Tailings and foam concentrate rates were measured by the stopwatch-and-collection method, and the volume of liquid in a foam concentrate sample was determined only once the foam had completely collapsed.

Gas holdup (α) in each zone was obtained from the measured pressure difference (ΔP) according to:

$$\alpha = 1 - \frac{\Delta P}{\rho g h} \qquad (5.1)$$

where ρ is the liquid density between pressure taps and h is the distance between pressure taps. The liquid density was adequately approximated by the density of water at 21°C. Multiple pressure measurements were required for the foam zone to smooth out greater fluctuations caused by foam movement.

Bubble velocity in the foam zone was measured by timed tracking of a selected bubble, and once again multiple measurements were required due to fluctuations in foam movement. Photographs were taken at various heights along the bubbly zone in order to determine bubble velocity in the liquid (see Section 4.2.1).

Once the series of measurements had been completed for the set air flowrate (or once the feed had been exhausted), the flotation apparatus was shut down and cleaned in preparation for the next hydrodynamic run.

Table 5.2 Parameters and Variables of Hydrodynamic Characterization

Parameters

Feed:	Superficial velocity	0.210 cm/s
	Surfactant concentration	40 ppm
Wash water superficial velocity		0.0629 cm/s
Bubbly zone height		0.600 m
Foam zone height		0.400 m

Variables

Air superficial velocity	0.314-3.144 cm/s
Surfactant	APG 600CS APG 625CS

Table 5.3 **List of Hydrodynamic Characterization Experiments**

Surfactant	J_g(cm/s)	Experiments
APG 600CS	0.314	A600-G1-1, A600-G1-2
	0.629	A600-G2-1, A600-G2-2
	0.943	A600-G3-1, A600-G3-2
	1.258	A600-G4-1, A600-G4-2
	1.886	A600-G5-1, A600-G5-2
	2.515	A600-G6-1, A600-G6-2
	2.830	A600-G7-1, A600-G7-2
	3.144	A600-G8-1, A600-G8-2
APG 625CS	0.314	A625-G1-1, A625-G1-2
	0.629	A625-G2-1, A625-G2-2
	0.943	A625-G3-1, A625-G3-2
	1.258	A625-G4-1, A625-G4-2
	1.886	A625-G5-1, A625-G5-2
	2.515	A625-G6-1, A625-G6-2
	2.830	A625-G7-1, A625-G7-2
	3.144	A625-G8-1, A625-G8-2

5.2.3 Yeast Separation Experiments

Separation experiments were performed on rehydrated bakers' yeast suspensions in the presence of the surfactant APG 625CS under conditions of varying salt concentration and wash water rate. Initially, a salt concentration of 0.5 M was chosen on the basis of similar studies in the literature; the concentration was then reduced in subsequent experiments. The separation achieved by the flotation column was assessed in terms of the two performance parameters, enrichment and recovery.

Feed Preparation

Prior to the start of a separation experiment, a 2.0 g/L bakers' yeast feed was prepared in the feed holding tank. The appropriate amount of rehydrated yeast (see Section 4.1.1) was diluted in tap water or previously prepared salt solution and brought to near final volume before undergoing an initial 10 minute stirring period. Salt solutions of NaCl, KCl, $MgCl_2$, and $CaCl_2$ were employed at final concentrations of 0.5 M, 0.1 M, and 0.05 M. Once the yeast suspension was well-mixed, the appropriate volume of 1.0 wt% stock solution of APG 625CS was added to give a final surfactant concentration of 40 ppm, and an additional 5 minute stirring period followed. Feed pH was adjusted with dilute HCl to pH 5, and feed samples were withdrawn for

spectrophotometric analysis. Feed homogeneity was maintained by continuous agitation throught the experiment.

Start-up

The same procedure was followed as in start-up of a hydrodynamic run, with the exception that air flow to the flotation column was held constant at 5 cm^3/s (21°C) corresponding to a superficial velocity of 0.314 cm/s, and wash water flow was initiated at a chosen rate of 0, 0.25, 0.5, 0.75, or 1.0 mL/s (corresponding to superficial velocities of 0, 0.0157, 0.0314, 0.0472, and 0.0629 cm/s). The system normally required a longer time to come to steady state (approximately 15 minutes, but as long as 20 minutes), and more frequent tailings rate adjustments were necessary to ensure a constant interface level.

Run-time

Once steady-state at the chosen wash water rate had been achieved, tailings and foam concentrate samples were collected for spectrophotometric analysis. Tailings and foam concentrate rates were measured by the stopwatch-and-collection method. Gas holdup in the bubbly zone was determined from pressure difference measurements. After concluding the series of measurements at one wash water rate, the wash water rate was adjusted and the

procedure was repeated as soon as a new steady-state had been achieved.

Upon completion of measurements for the entire range of wash water rate (or once the feed had been exhausted), the flotation apparatus was shut down and cleaned in preparation for the next separation experiment.

Table 5.4 **Parameters and Variables of Separation Experiments**

Parameters

Feed:	Superficial velocity	0.210 cm/s
	Yeast concentration	2 g/L
	Surfactant	40 ppm APG 625CS
	pH	5
Air superficial velocity		0.314 cm/s
Bubbly zone height		0.600 m
Foam zone height		0.400 m

Variables

Feed salt concentration	0 - 0.5 M
Wash water superficial velocity	0 - 0.0629 cm/s

Table 5.5 List of Separation Experiments

Description	Salt		Experiments
APS 6[illegible]	No salt		A1, A2
Yeast	No salt		W1, W2, W3
	0.5 M:	NaCl	S5-N1, S5-N2
		KCl	S5-K1, S5-K2, S5-K3
		$MgCl_2$	S5-M1, S5-M2, S5-M3
		$CaCl_2$	S5-C1, S5-C2
	0.1 M:	NaCl	S1-N1, S1-N2
		KCl	S1-K1, S1-K2
		$MgCl_2$	S1-M1, S1-M2
		$CaCl_2$	S1-C1, S1-C2
	0.05 M:	NaCl	S05-N1, S05-N2
		KCl	S05-K1, S05-K2
		$MgCl_2$	S05-M1, S05-M2
		$CaCl_2$	S05-C1, S05-C2

Chapter 6: RESULTS AND DISCUSSION

6.1 Hydrodynamic Characterization of the Flotation Column

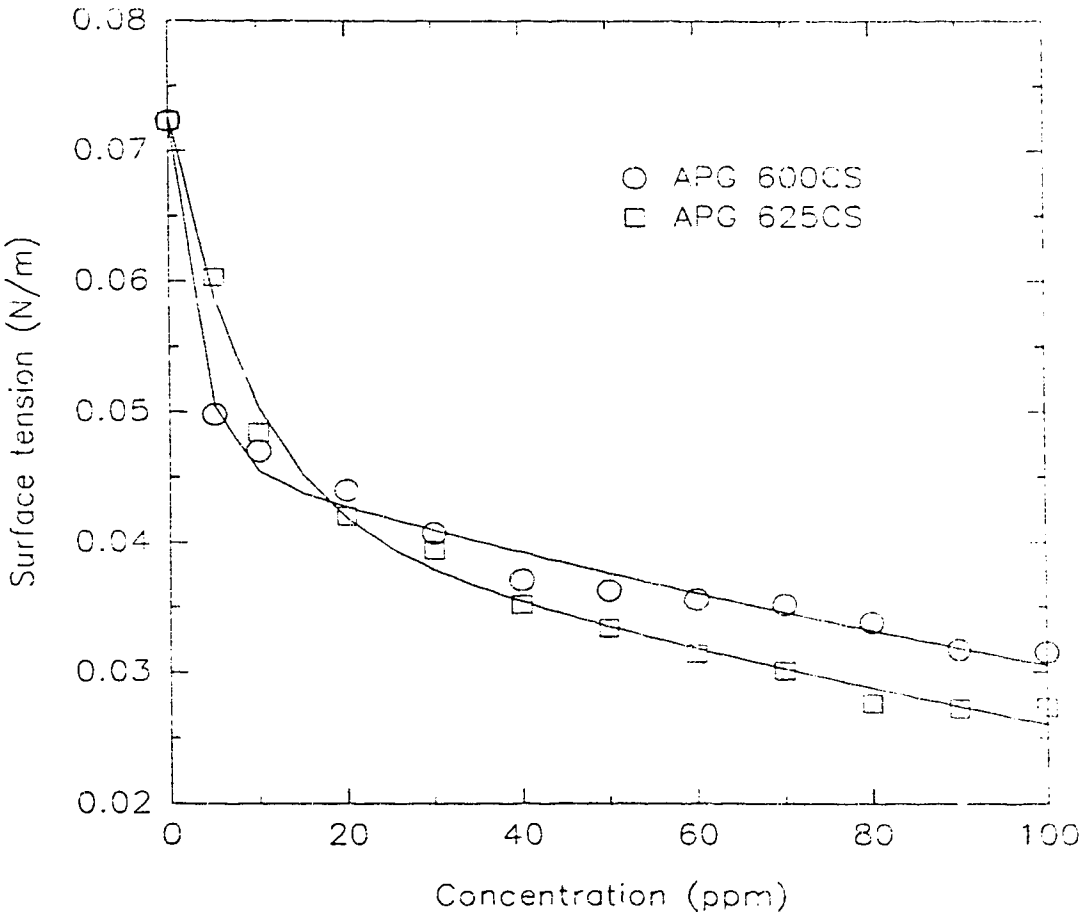
A hydrodynamic characterization of the flotation column was undertaken in order to validate its operation. This characterization was performed on the two aqueous alkylpolyglycoside (APG) surfactant solutions in the absence of bakers' yeast. Experimental parameters were as described in **Table 5.2.**

6.1.1 Surfactant Concentration

The concentration of APG surfactants employed in the hydrodynamic characterization was chosen on the basis of surface tension reduction and foam stability.

The variation of surface tension with surfactant concentration in tap water is presented in **Figure 6.1** (complete data are provided in **Appendix A**). Of the two surfactants, APG 625CS provided slightly superior reduction of surface tension with concentration. Most of the benefit in surface tension reduction was achieved below a concentration of 40 ppm for both surfactants, and flotation tests at this concentration yielded stable and sustained foam production. Consequently, a surfactant concentration of 40 ppm was chosen for conducting the hydrodynamic characterization of the column. The surface tensions generated by

Figure 6.1: Surface Tension of Tap Water with APG Surfactants at 21° C



the two surfactants at this concentration were 0.0393 N/m for APG 600CS and 0.0355 N/m for APG 625CS.

6.1.2 Bubble Size and Velocity

Within the bubbly zone, sparging produced a high frequency of ellipsoidal bubbles at even the lowest gas velocity employed. As shown in **Figure 6.2**, the frequency of ellipsoidal bubbles averaged around 70% over the entire gas velocity range, fluctuating in an apparently random manner rather than increasing as expected with increasing gas rate. The distribution of bubble diameter was generally sigmoidal, and a typical example is provided in **Figure 6.3** (complete distribution data are provided in **Appendix B**). Interestingly, a bimodal distribution appeared at high gas velocities (i.e. $J_g \geq 2.52$ cm/s) when surfactant APG 625CS was employed, but not with APG 600CS; this pattern is illustrated in **Figure 6.4**. Approximately 20-40% of all bubbles in the bimodal distribution had diameters under 3 mm; the remaining larger bubbles were undoubtedly the result of coalescence at the high volumetric gas flowrate. As expected, the mean bubble diameter increased with gas velocity at virtually the same rate for both surfactants, as shown in **Figure 6.5**; the relationship is suitably described by **Equation 2.5**. The corresponding bubble velocity calculated for a given gas velocity using the correlation of **Clift et al.(1978)** as discussed in **Section 2.5** is presented in

Figure 6.2: Bubble Shape in the Bubbly Zone

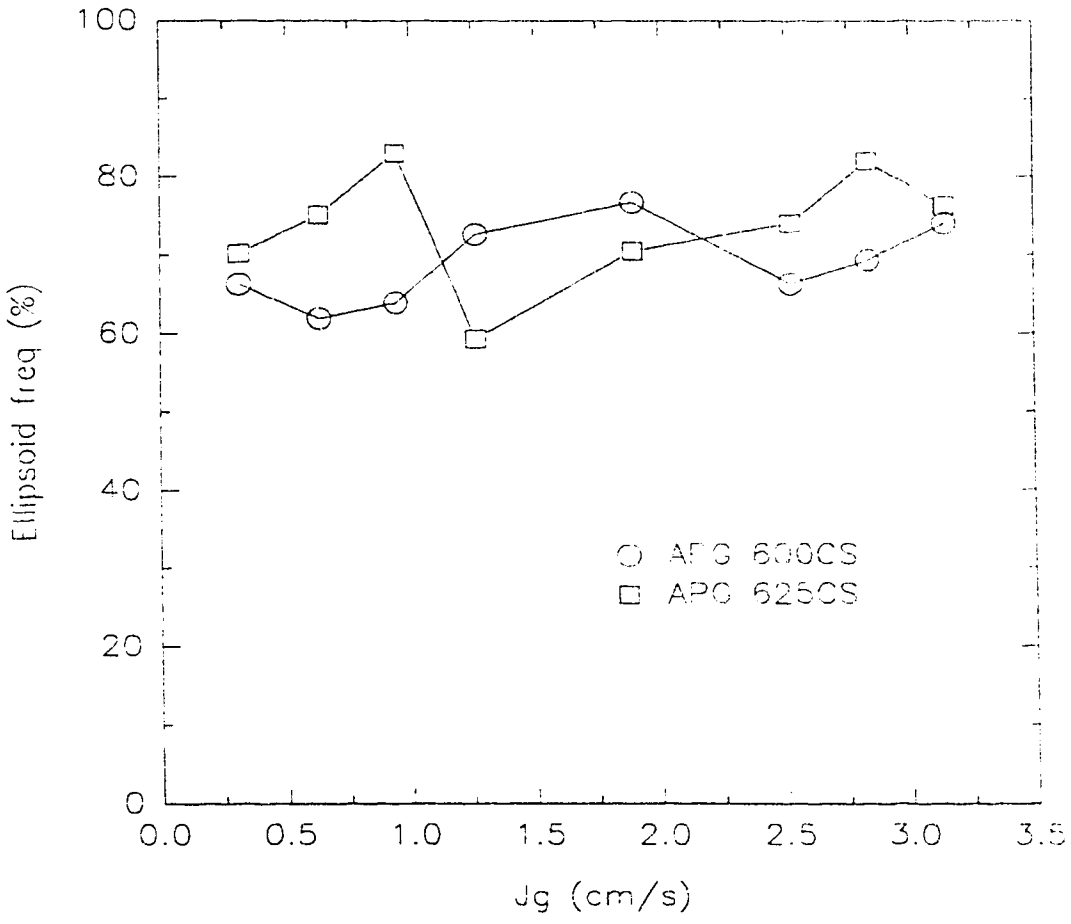


Figure 6.3: Bubble Diameter Distribution with APG 625CS

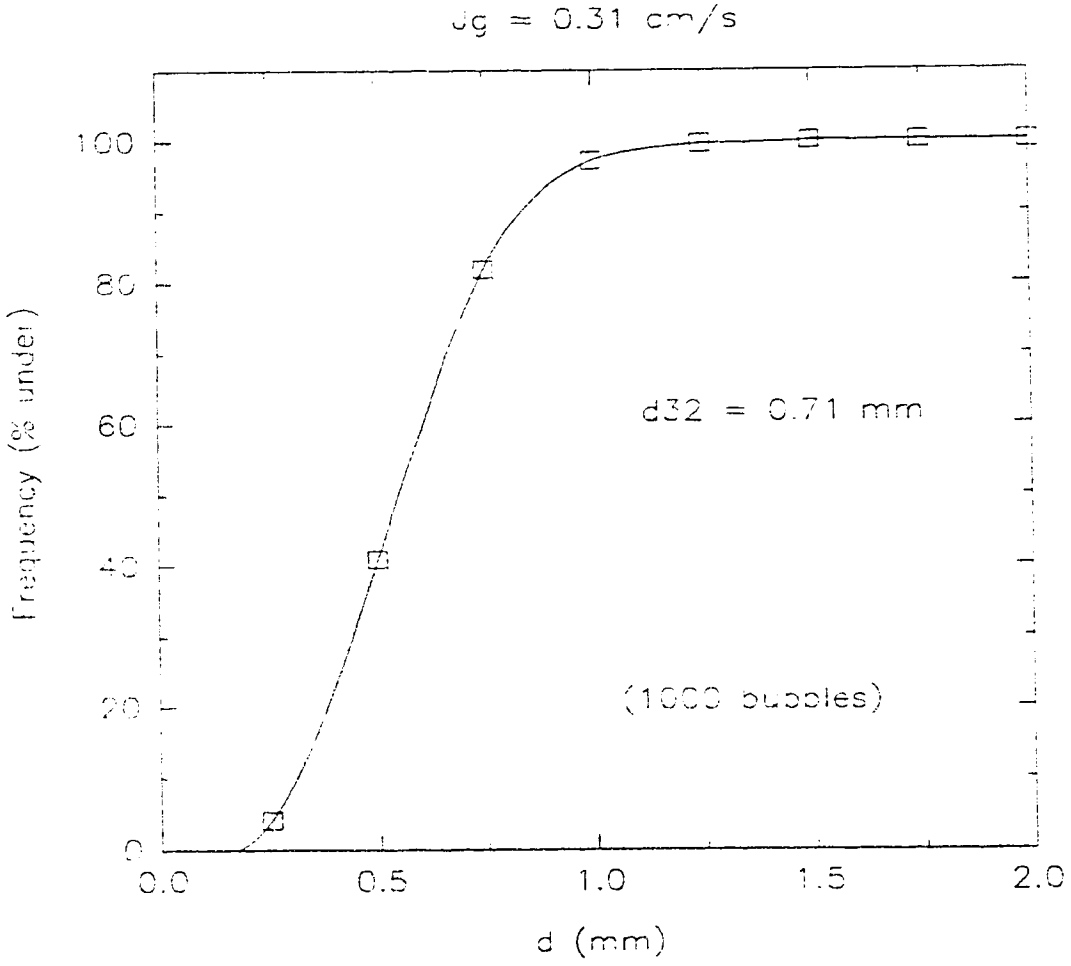


Figure 6.4: Bubble Diameter Distribution with APG 625OS

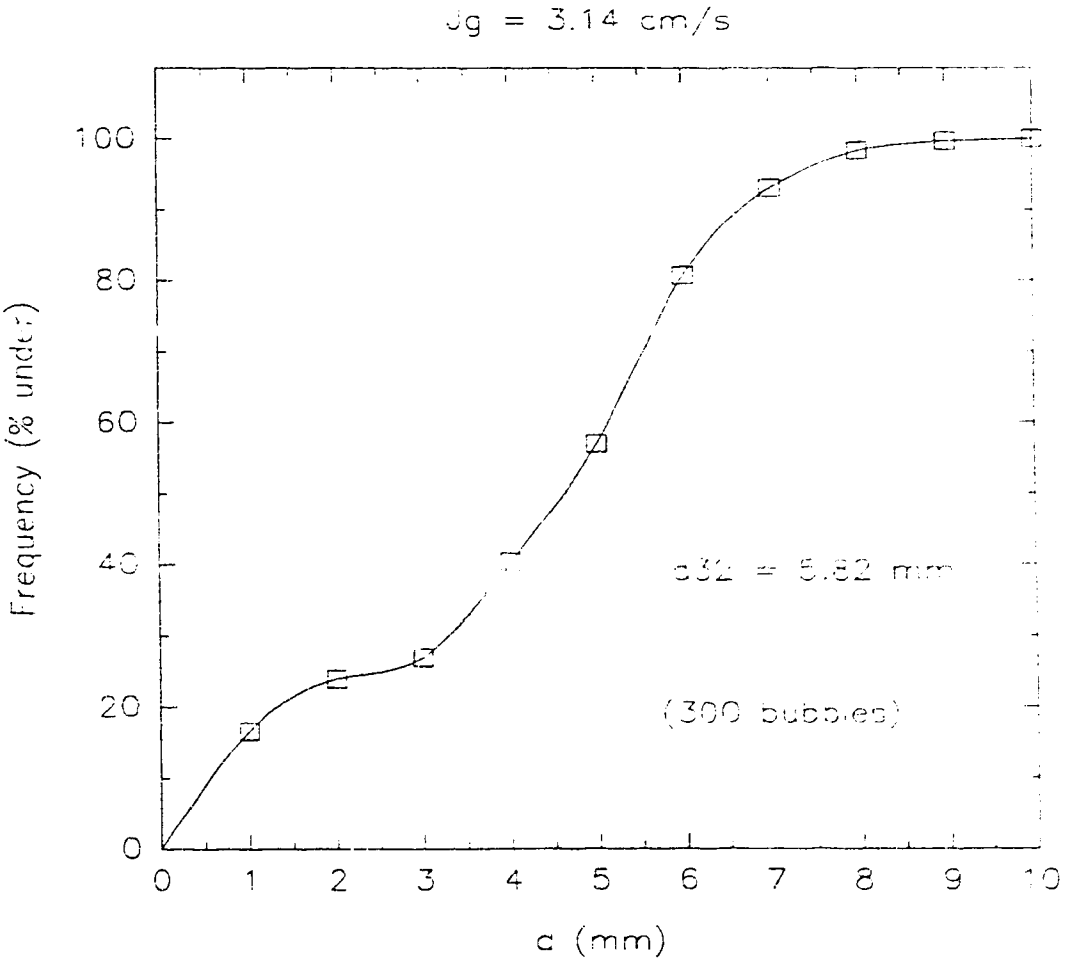


Figure 6.5: Mean Bubble Diameter in the Bubbly Zone

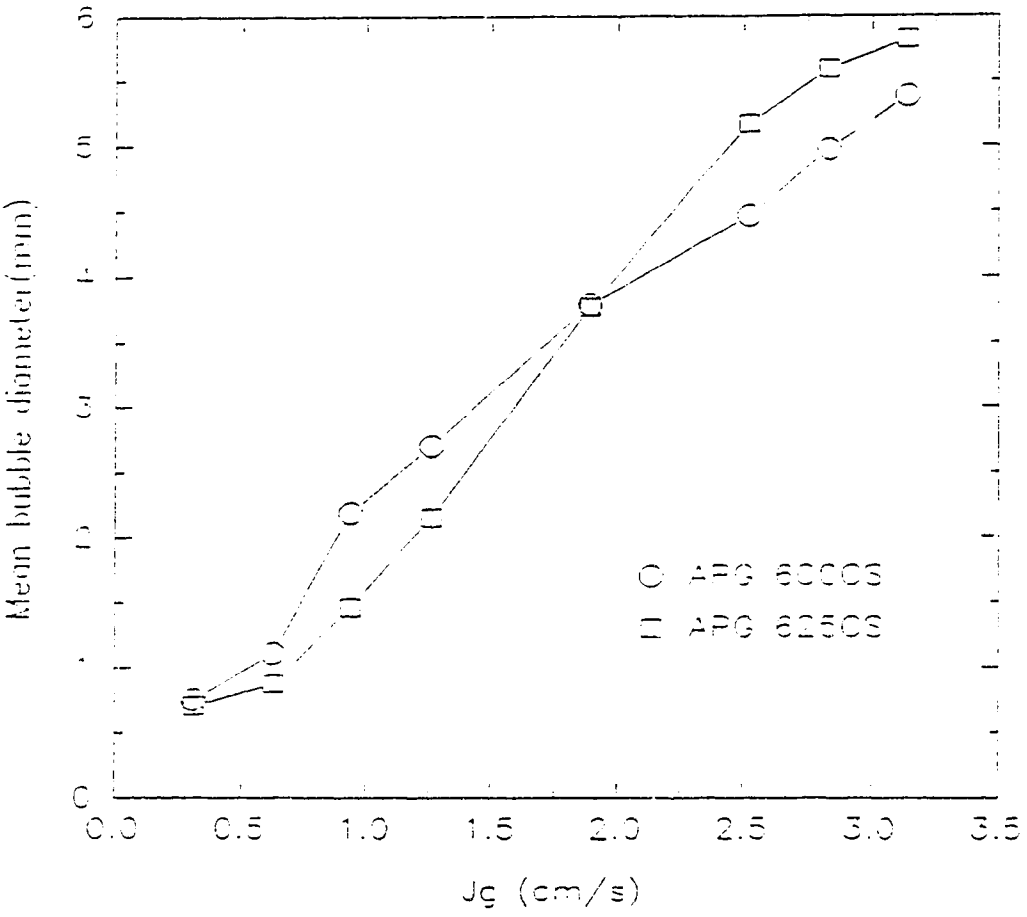


Figure 6.6. Bubble velocity appeared to increase at a slightly greater rate with gas superficial velocity for surfactant APG 600CS, but for both surfactants the bubble velocity leveled out to approximately 21 cm/s at a gas velocity of around 1.9 cm/s. Complete bubble data for the bubbly zone are presented in **Appendix B.**

Within the foam zone, bubble velocity was determined by visual tracking. In contrast to the bubbly zone, bubble velocity appeared to increase at a slightly greater rate with gas velocity for surfactant APG 625CS, at least at the upper limit of gas velocity (as demonstrated in **Figure 6.7**). It should be noted that the accuracy of this method of velocity estimation undoubtedly suffered from the short observation times imposed by the limited height of the foam zone. Complete bubble velocity data for the foam zone can be found in **Appendix C.**

Figure 6.6: Bubble Velocity in the Bubbly Zone

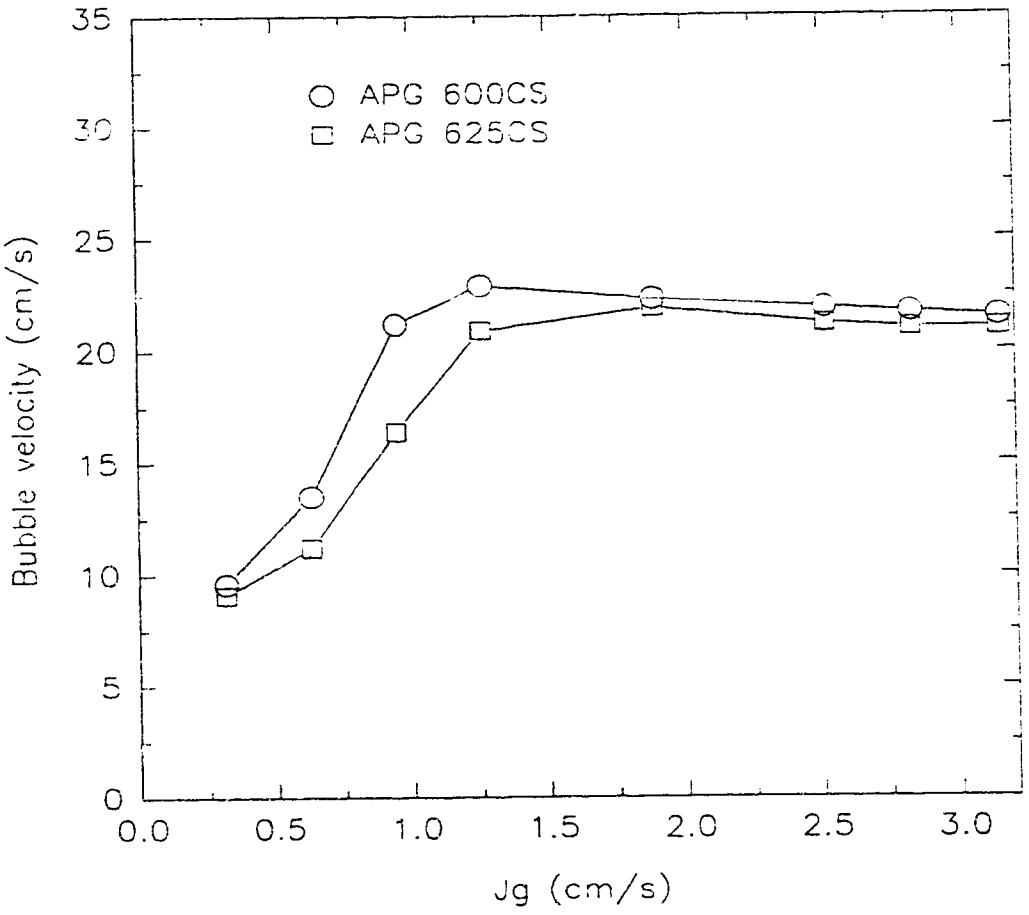
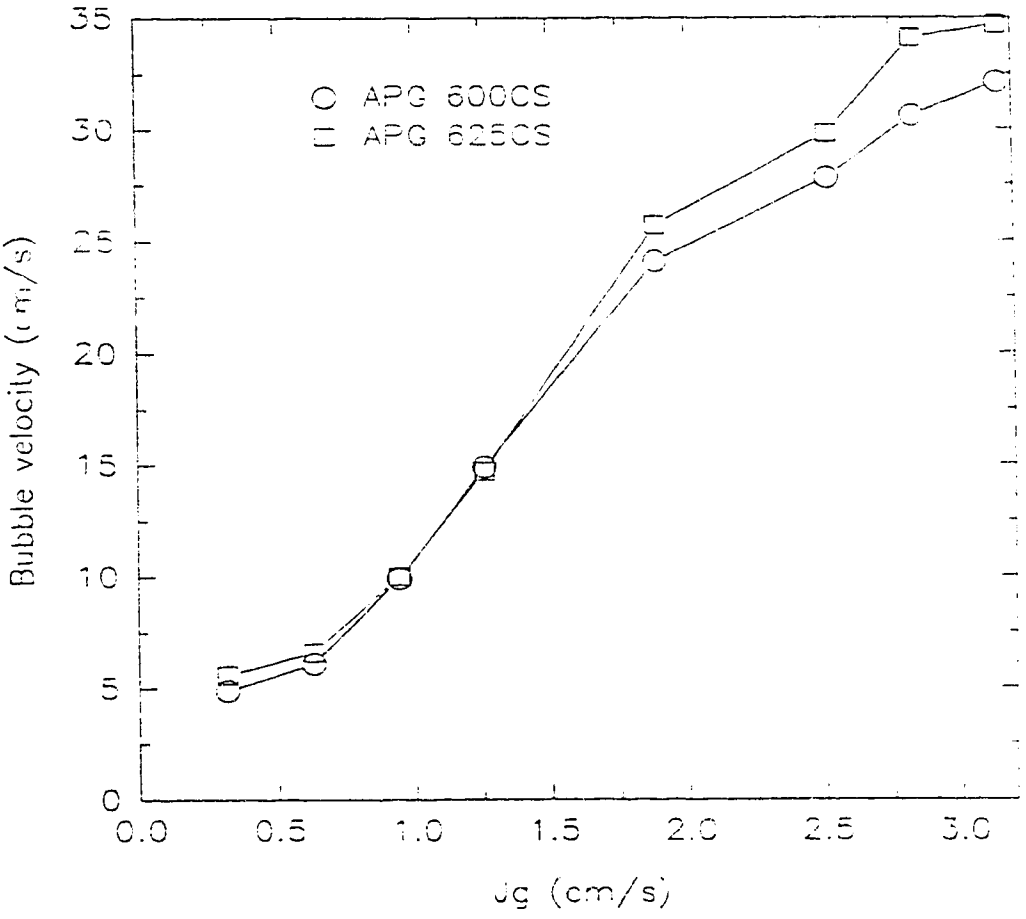


Figure 6.7: Bubble Velocity in the Foam Zone



6.1.3 Bubbly Flow Regime Verification

Since the efficient performance of the flotation column required operation within the bubbly flow regime, the variation of gas holdup in the bubbly zone with gas velocity was examined during the hydrodynamic experiments. As shown in **Figure 6.8**, gas holdup varied in an approximately linear manner with gas velocity, a characteristic feature of bubbly flow. Complete data are presented in **Appendix C.**

6.1.4 Drift-flux Model Fit of Hydrodynamic Data

In addition to gas holdup and bubble velocity, hydrodynamic data collected from the bubbly and foam zones of the flotation column at a variety of sparging rates included liquid and gas fluxes. Since the liquid flux was estimated from tailings, foam concentrate and wash water flowrates (depending upon the zone), a liquid volumetric balance was performed on the flotation column for each run. Total inlet and outlet liquid flowrates differed by only 5% on average, and the worst case did not exceed 9% (complete data may be found in **Appendix C**).

Applying the drift-flux model to the hydrodynamic data revealed a good fit to the Richardson-Zaki correlation (**Equation 2.13**) within the bubbly zone, but a poorer fit within the foam zone. Data collected with surfactant APG 600CS (presented in

Figure 6.8: Bubbly Flow Regime Verification

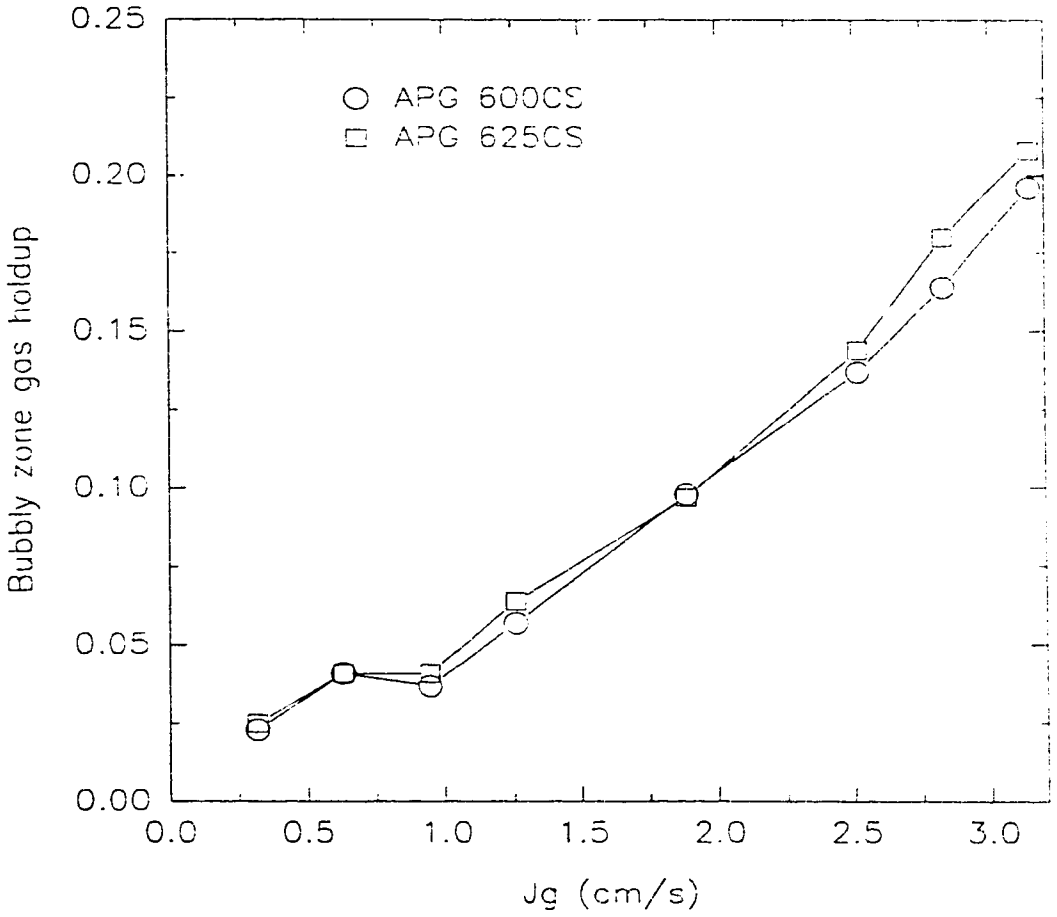


Figure 6.9), and with surfactant APG 625CS (presented in **Figure 6.10**) exhibit very similar patterns in both zones, as expected from the similar effects of the surfactants on surface tension (already seen in **Figure 6.1**). While the data display considerable divergence from the correlation within the foam zone, it must be noted that the methods and instrumentation for data collection within the foam zone in particular were hardly sophisticated, especially considering the rapidly fluctuating pressure difference measurements encountered within this region. Furthermore, the literature data presented in **Figure 6.11** also display considerable divergence from the correlation in the foam zone at gas holdups in excess of $\alpha = 0.75$. The experimental data lie within literature values for this range of gas holdup. Consequently, the hydrodynamic characterization of the flotation column was considered to have successfully validated its operation.

Figure 6.9: Drift-flux Model Fit of APG 600CS Hydrodynamic Data

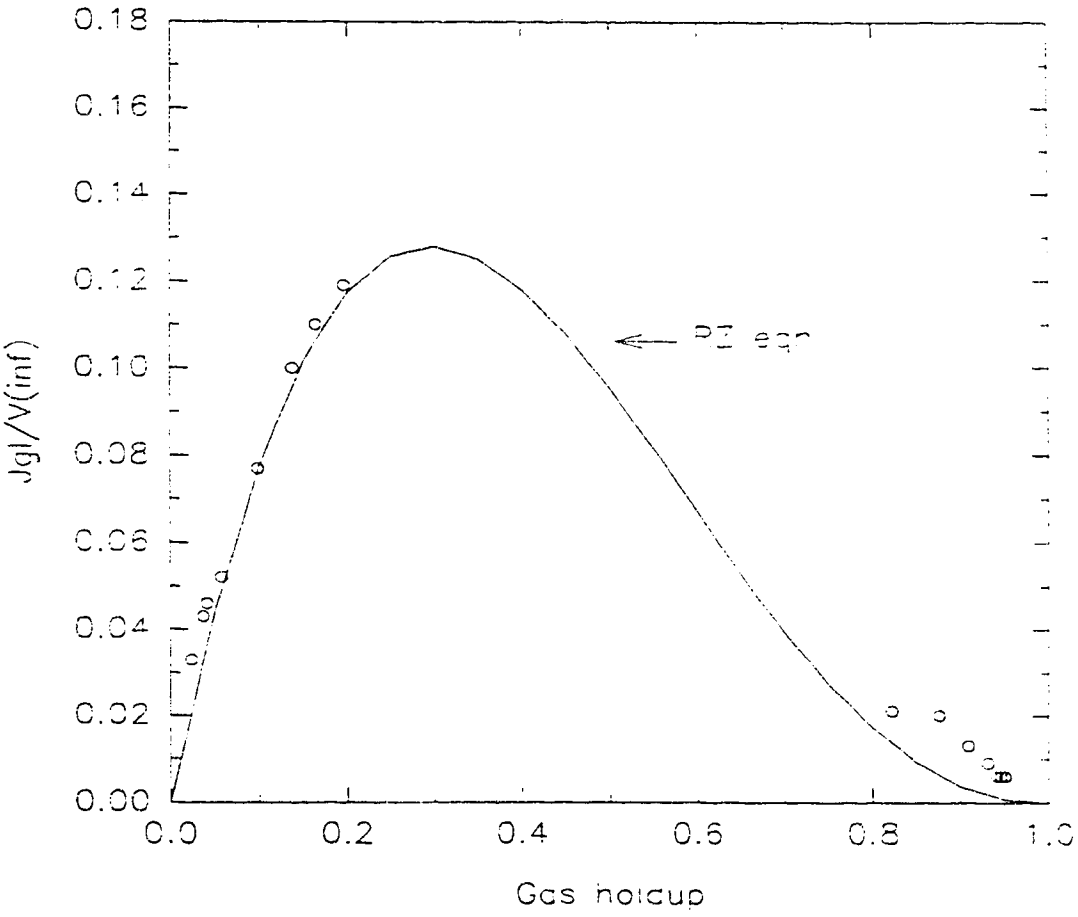


Figure 6.10: Drift–flux Model Fit of APG 625CS Hydrodynamic Data

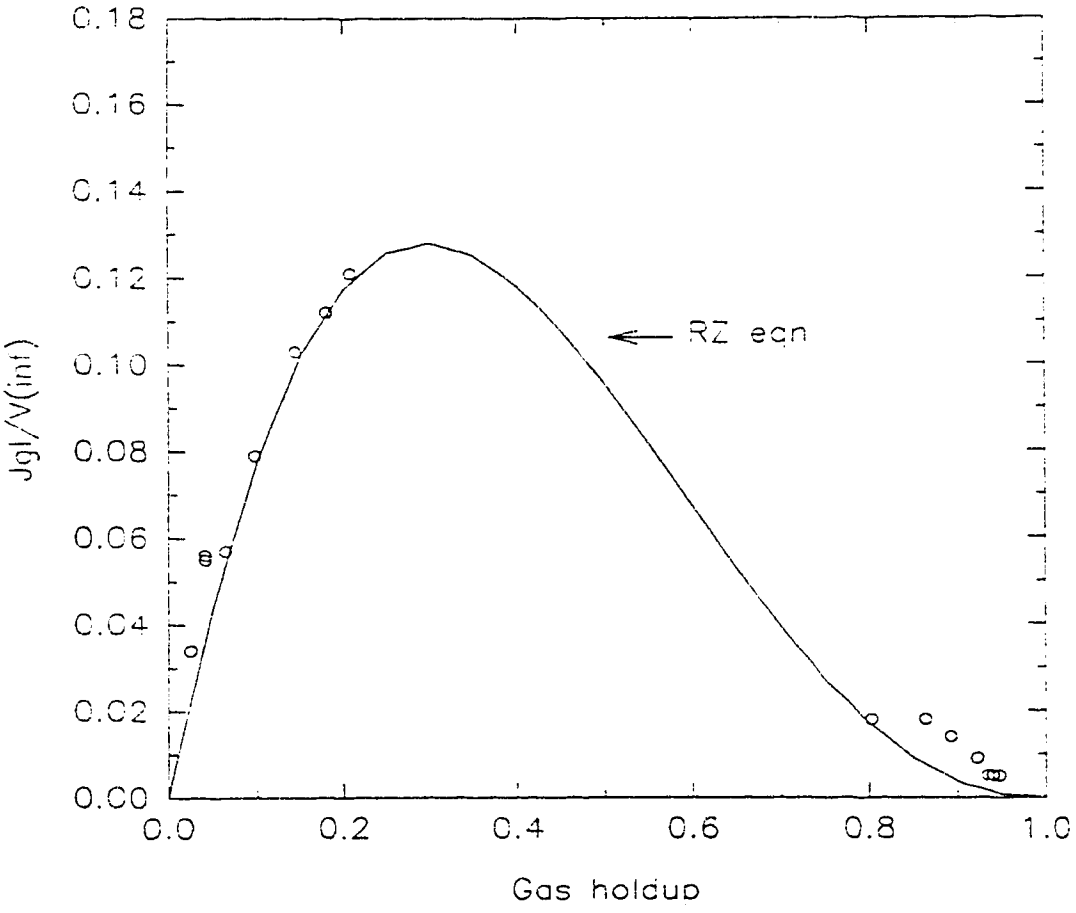
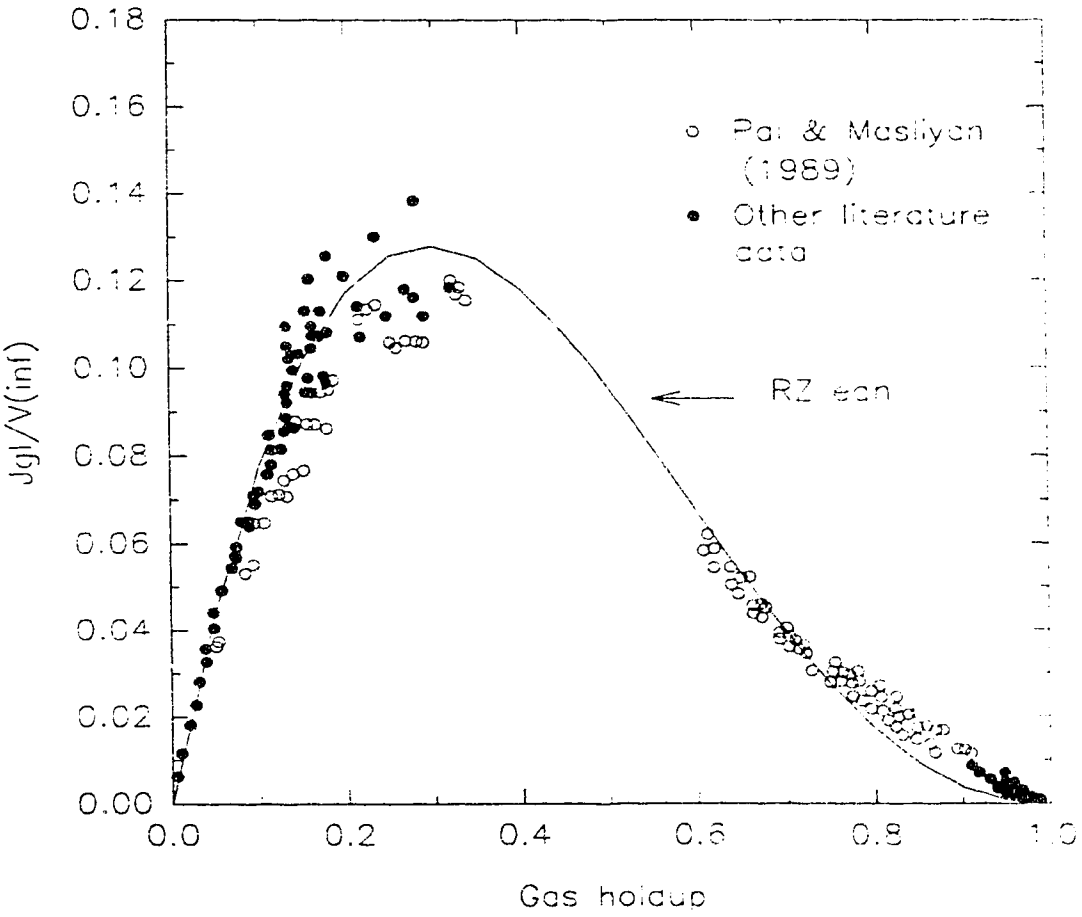


Figure 6.11: Drift-flux Model Fit of Literature Hydrodynamic Data



6.2 Yeast Separation Experiments

Yeast separation by the flotation column was examined under conditions of varying wash water rate and salt concentration in the feed, using a variety of inorganic salts (i.e. NaCl, KCl, $MgCl_2$ and $CaCl_2$) to investigate the effects of cell surface charge. Experimental parameters were as described in **Table 5.4;** bubble diameter was assumed to be unchanged from that obtained at the same gas flowrate in the surfactant-only system, i.e. d_b = 0.71 nm.

6.2.1 Surfactant Selection and Distribution

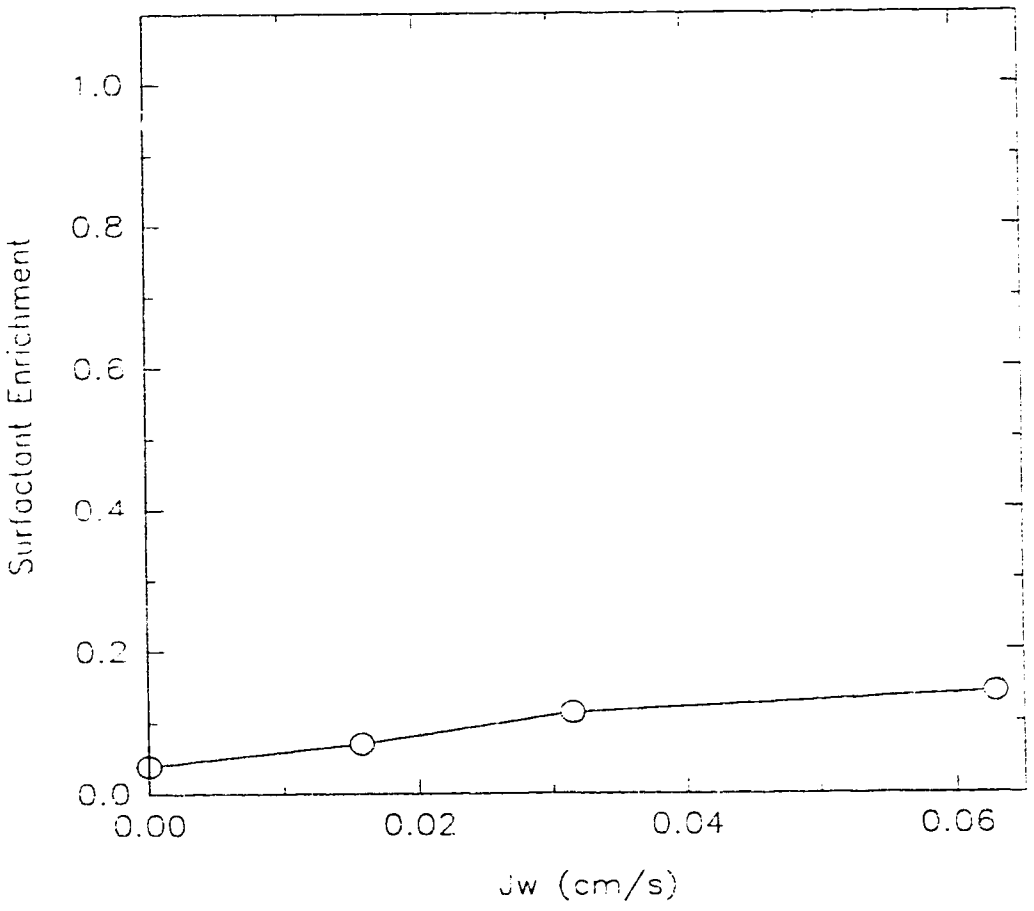
Of the two surfactants employed in the initial hydrodynamic characterization of the flotation column, APG 625CS was selected for further use in yeast separation experiments. This was primarily on the basis of its slightly superior surface tension reduction, although an additional factor in this decision was the surfactant's greater ease in handling.

The distribution of surfactant between foam and tailings product streams was checked during surfactant flotations in the absence of yeast. Surface tension measurements were used to detect low surfactant concentrations in the tailings, but this technique was not suitable for the high surfactant levels expected in the foam concentrate. Since there was no chemical assay

specific for alkylpolyglycoside surfactants, the distribution of surfactant was estimated solely on the basis of surfactant reduction in the tailings.

The suitability of column flotation for surfactant recovery was reflected in the low residual surfactant concentration in the tailings compared with the feed. In the absence of wash water, the tailings surfactant concentration was less than 4% of the feed. Wash water influence on the tailings surfactant enrichment, i.e. the ratio of surfactant concentration in the tailings to that in the feed, is shown in **Figure 6.12** (complete data are given in **Appendix A**). The addition of a wash water stream to the flotation column resulted in some surfactant detachment from the foam, as demonstrated by the rising tailings surfactant enrichment with increasing wash water rate. However, this effect was relatively minor over the range of wash water rates employed, since the residual surfactant concentration in the tailings was less than 15% of the feed concentration even at the upper wash water limit of 0.063 cm/s.

Figure 6.12: Surfactant Enrichment in Tailings



6.2.2 Water Quality

Tap water was employed for all yeast separation experiments. An analysis of the water quality performed by the local water treatment plant is provided in **Table 6.1.** The naturally occurring concentrations of cations in the feed water were clearly sufficiently low when compared with the salt additions as to be neglected. However, it is not known whether these naturally occurring concentrations in the wash water may have had any significant effect on yeast attachment in the foam zone.

Table 6.1: Concentration of Cations Occurring Naturally in Water Used for Yeast Separation Experiments

Cation	Concentration
Na	1.65×10^{-4} M
K	1.71×10^{-5} M
Mg	4.56×10^{-4} M
Ca	6.29×10^{-4} M

6.2.3 Wash Water Effects

The effects of wash water on yeast separation were examined over the wash water range of J_w = 0-0.063 cm/s. The upper limit of the wash water rate, which was imposed by the loss of foam stability beyond this point, varied with salt concentration in the feed. Feeds with a salt concentration of 0.05-0.1 M could not support foaming beyond a wash water rate of 0.047 cm/s, whereas feeds with either a high salt concentration (i.e. 0.5 M) or completely lacking in salt were able to support wash water rates up to 0.063 cm/s. Foam appearance changed dramatically with addition of wash water, expanding with the increased water content and displaying larger bubbles.

Bias rates for the different salt cations at each feed salt concentration are compared with the salt-free bias rate, with typical results presented in **Figure 6.13**. Similar results were obtained at all three feed salt concentrations (complete data available in **Appendix F**). While these rates are overestimates which do not take into account yeast transfer into the foam, the error should be minor at the low yeast concentration employed in the feed (i.e. 2 g/L). Negative bias values occurred only in the absence of wash water. Consequently, column flotation was always performed under positive bias (i.e. downward liquid flux) to ensure proper cleaning action of the wash water in the foam zone, as anticipated for the low air flowrate employed. This

requirement would be important for selective recovery from a multicomponent feed such as a fermentation broth, but in this case the positive bias served only to wash salt from the foam (although this was not experimentally verified by measuring salt concentration). The crossover from negative to positive bias was interpolated to occur at a wash water rate of 0.005-0.015 cm/s, depending upon the concentration (and type) of salt added to the feed. All bias rates were low, never exceeding 0.061 cm/s. In order to maximize retention time in the bubbly or collection zone and to minimize water consumption, bias rates should be as small as possible. The experimental results fall within the range of 0.02-0.1 cm/s recommended by **Finch & Dobby (1990)**.

Yeast separation by the flotation column was evaluated in terms of yeast enrichment and recovery in the foam concentrate and tailings product streams, as defined in **Equations 2.1** and **2.2**. Addition of wash water to the flotation column produced similar trends in yeast enrichment and recovery regardless of the salt concentration in the feed; only the magnitude of the responses differed.

The variation of foam enrichment in yeast with wash water rate is presented in **Figures 6.14-6.16**. Foam yeast enrichment was maximized in the absence of wash water, reaching 3.5- to 11-fold improvement over the feed yeast concentration (depending upon the concentration of salt in the feed). The introduction of wash water to the column was not beneficial to foam yeast enrichment -

even the lowest wash water rate (J_w = 0.016 cm/s, corresponding to a bias rate of under 0.01 cm/s) brought about a significant reduction for all salts and even for the salt-free case. Foam yeast enrichment declined with increasing wash water rate up to 0.031 cm/s, beyond which no further reduction was observed. Yet even at this wash water rate, a 1.5- to 3-fold improvement over the yeast concentration in the feed was obtained, proving that selective adsorption was taking place. If cell flotation were only by mechanical entrainment, enrichment could not exceed a value of 1. An apparent minimum in foam yeast enrichment at a lower wash water rate (i.e. J_w = 0.016 cm/s) for the divalent salt cations Mg^{+2} and Ca^{+2} at a concentration of 0.5 M in the feed was not observed at lower salt concentrations. This most probably reflected difficulties in maintaining steady-state operation at the lower limit of wash water rate and the upper limit of salt concentration (rather than being a divalent charge effect).

Conventional foam drainage in the region above the wash water entry leading to the foam overflow does not appear to have been sufficient to cope with the increased water content of the foam zone. Consequently, the addition of wash water led to dilution of the foam concentrate.

However, there is some evidence in the variation of tailings yeast enrichment with wash water rate (presented in **Figures 6.17-6.19**) for a detachment mechanism, whereby the wash water removed yeast from the foam zone by detachment from the rising bubbles.

If foam dilution were the sole wash water effect, then an increase in wash water rate would be expected to reduce the yeast concentration in the foam product while having no effect on the tailings yeast concentration. The data show that the tailings yeast concentration remained very close to that of the feed regardless of the wash water rate, but a small increase was observed over the range of wash water in which foam yeast enrichment was reduced (i.e. J_W = 0-0.031 cm/s).

More conclusive evidence for the detachment mechanism is provided in the variation of foam yeast recovery with wash water rate, presented in **Figures 6.20-6.22.** The highest foam yeast recoveries were obtained in the absence of wash water, ranging from 10-55% of the yeast entering the column in the feed (depending upon the concentration of salt in the feed). Yet even these modest recoveries declined upon wash water addition up to a wash water rate of 0.31 cm/s, until stabilizing around 1-15%. Clearly, yeast detachment from the bubbles in the foam zone caused significant losses at even the lowest wash water rate, and resulted in almost complete removal of the yeast from the foam by a wash water rate of 0.031 cm/s, a trend seen for all salts and even in the absence of salt in the feed.

This effect can be viewed in the corresponding variation of tailings recovery with wash water rate, measured independently of foam recovery (complete data are presented in **Appendix F**). Initial tailings recoveries of 40-85% of the yeast entering in the

feed in the absence of wash water (depending upon the feed salt concentration) rose to in excess of 90% at a wash water rate of 0.031 cm/s. Another apparent maximum in tailings recovery at this wash water rate (reaching in excess of 100% for several cations at all three concentrations of salt in the feed) corresponds with the greatest deviations in yeast balances performed on the flotation column. While standard deviations in yeast balances over the column were consistently higher than the norm of 5% in volumetric liquid balances, occasional values as high as 20% were obtained at the higher wash water rates.

These results establish the importance of yeast detachment in the foam zone of the flotation column when employing wash water. Clearly, the adhesion of the cells to the bubbles is weak and easily disrupted by wash water. The difficulty in maintaining foam stability and a steady flow of foam product beyond what is actually a fairly low wash water rate also poses a problem. In a multicomponent feed such as a fermentation broth, these disadvantages to the use of wash water would have to be balanced against the potential gain in the purity of the foam product achieved through removal of non-cell solids (assuming these contaminants were hydrophilic). However, it should be noted that even a slightly positive bias is sufficient to provide this cleaning action.

The loss of yeast from the foam at the higher wash water rates cannot be attributed to significant washout of surfactant

from the foam zone. In **Section 6.2.1** it was observed that while surfactant washout increased with increasing wash water rate, the effect was relatively minor even at the highest wash water rate employed (J_w = 0.063 cm/s). Although these results were obtained from yeast-free flotations, visual observation of the foaming properties of tailings from both surfactant-only and yeast-with-surfactant flotations suggests that there is no reason to suspect significantly greater surfactant washout in the latter case.

Figure 6.13: Bias Rates with 0.05 M Salt in Feed

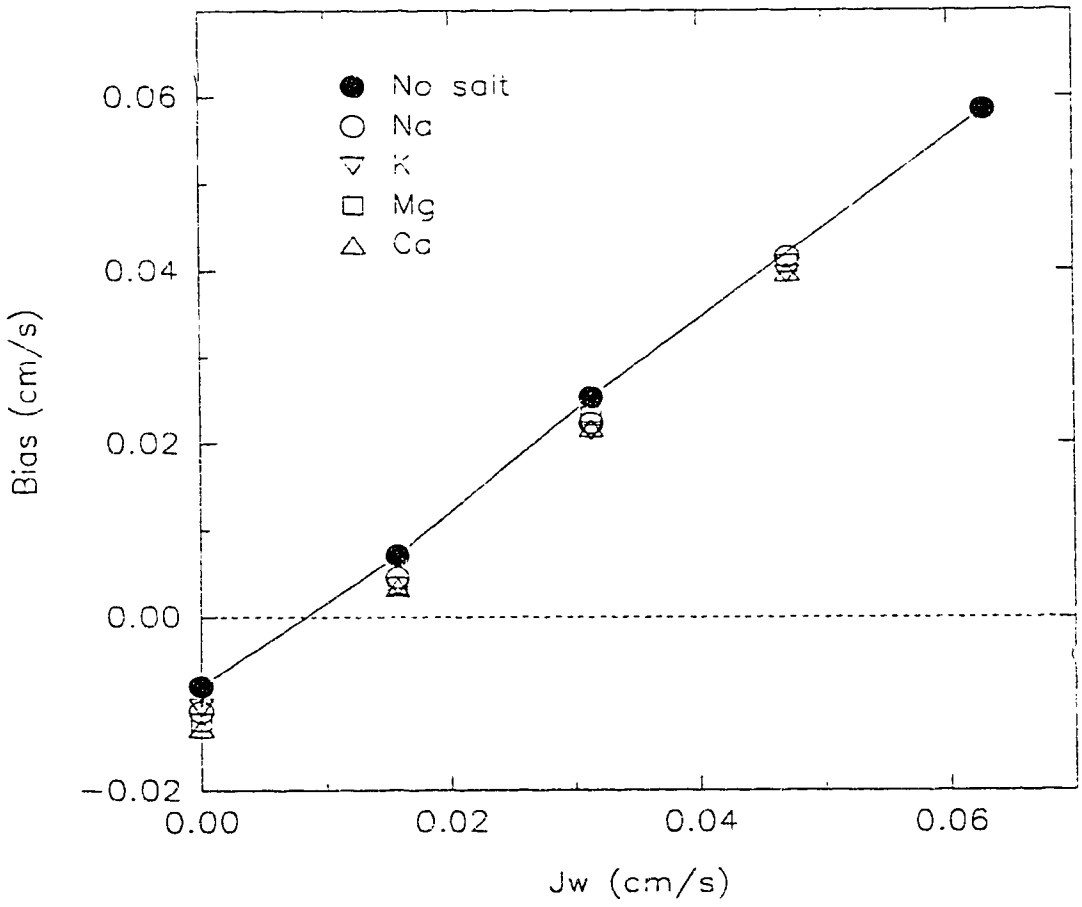


Figure 6.14: Wash Water Effect on Foam Yeast Enrichment

0.5 M Salt in Feed

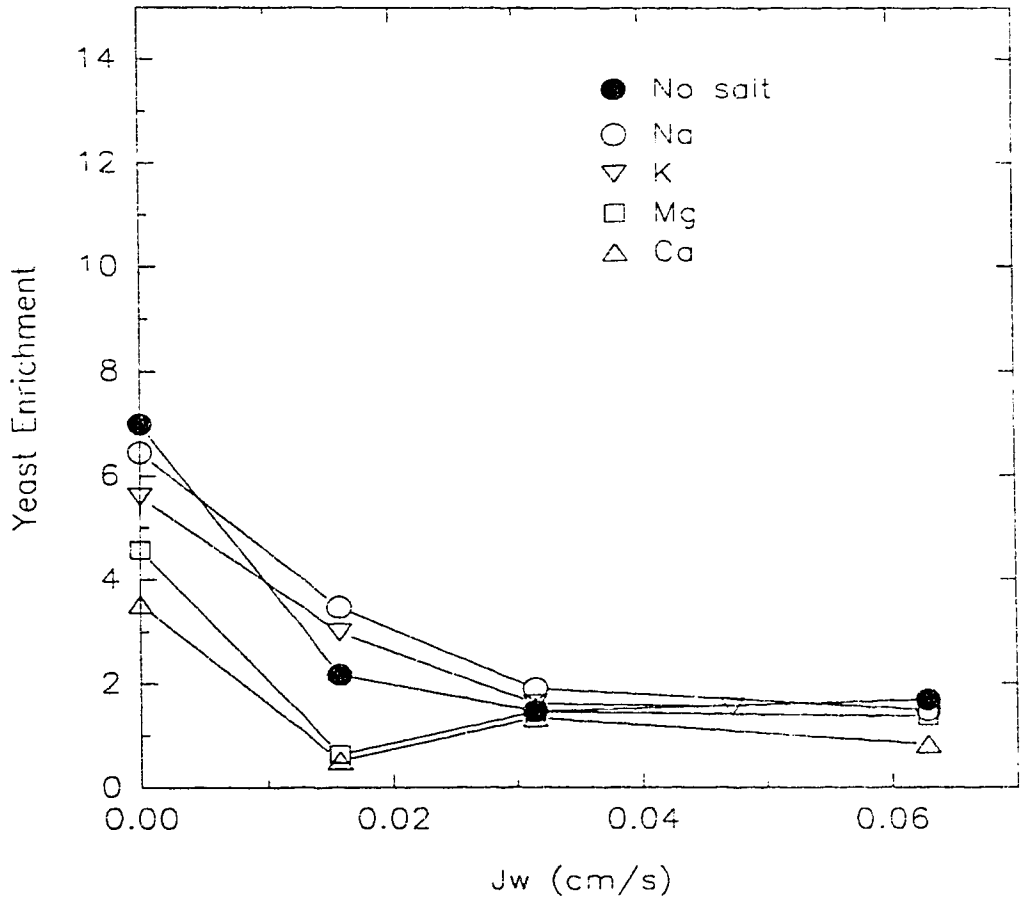


Figure 6.15: Wash Water Effect on Foam Yeast Enrichment

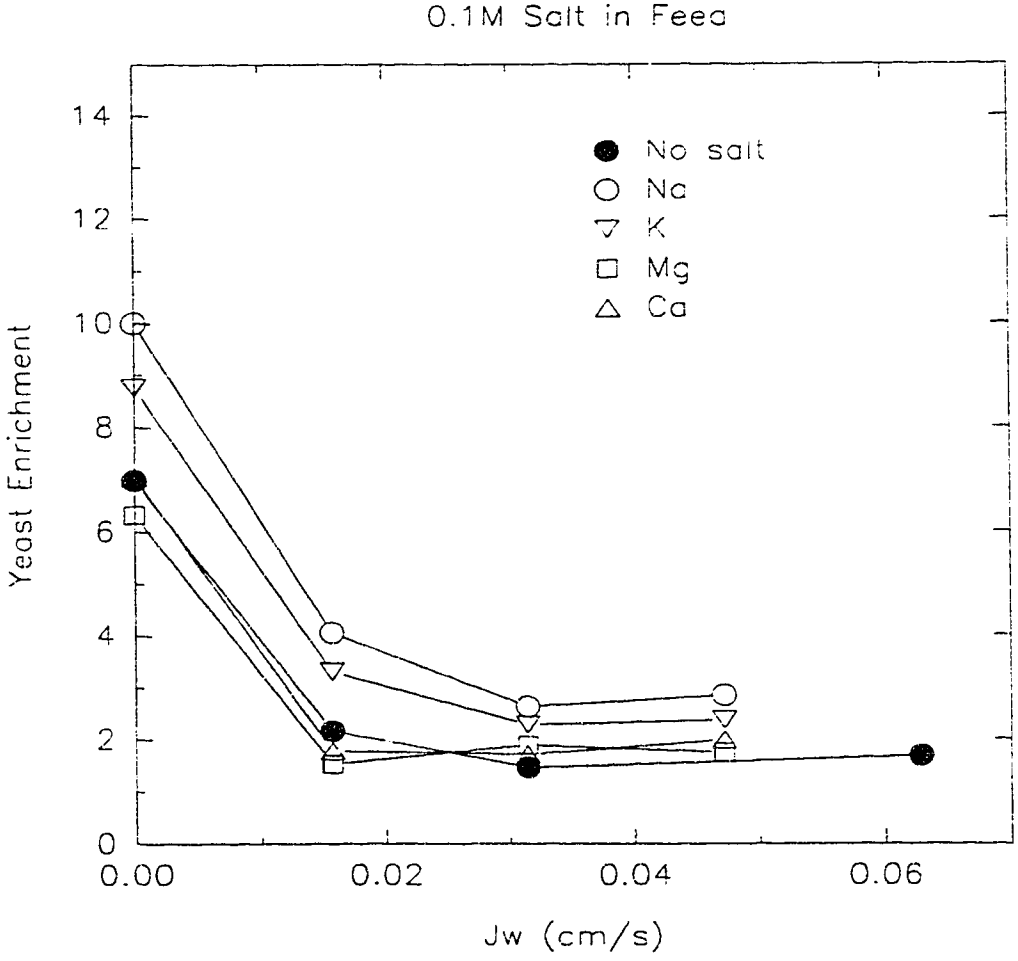


Figure 6.16: Wash Water Effect on Foam Yeast Enrichment

0.05 M Salt in Feed

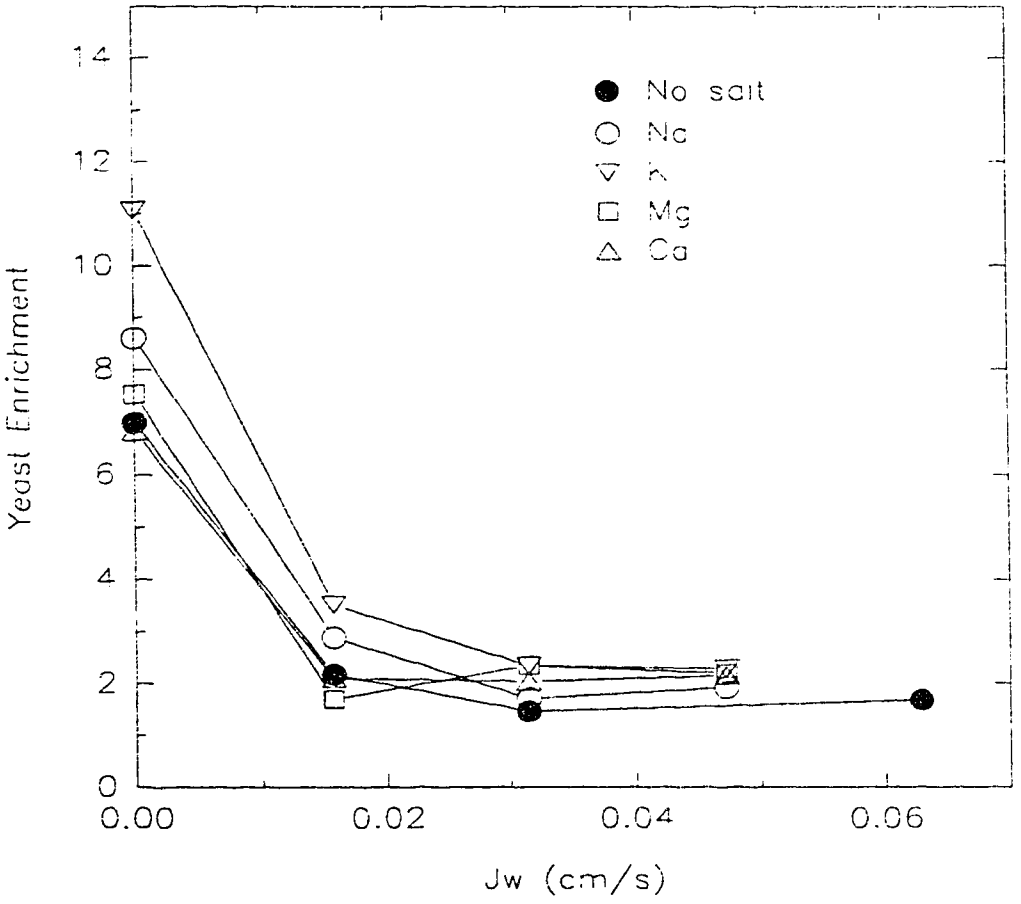


Figure 6.17: Wash Water Effect on Tailings Yeast Enrichment

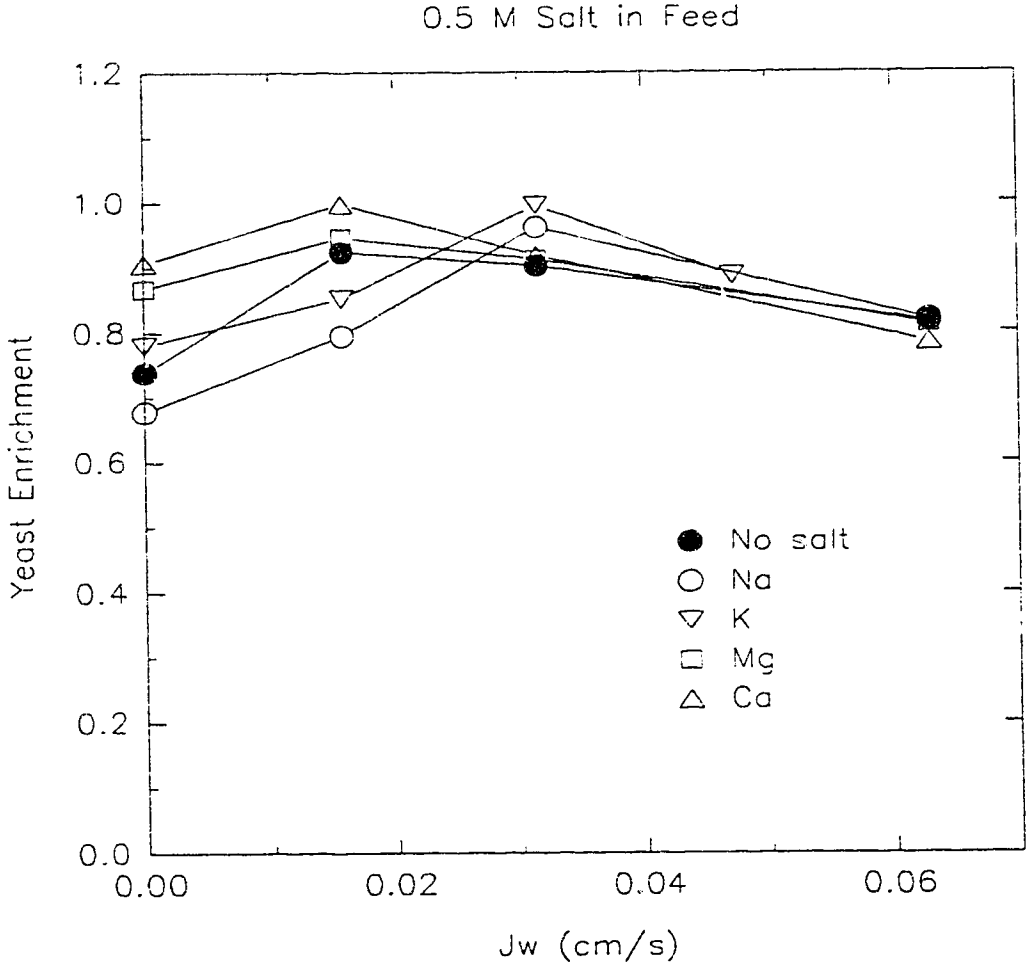


Figure 6.18: Wash Water Effect on Tailings Yeast Enrichment

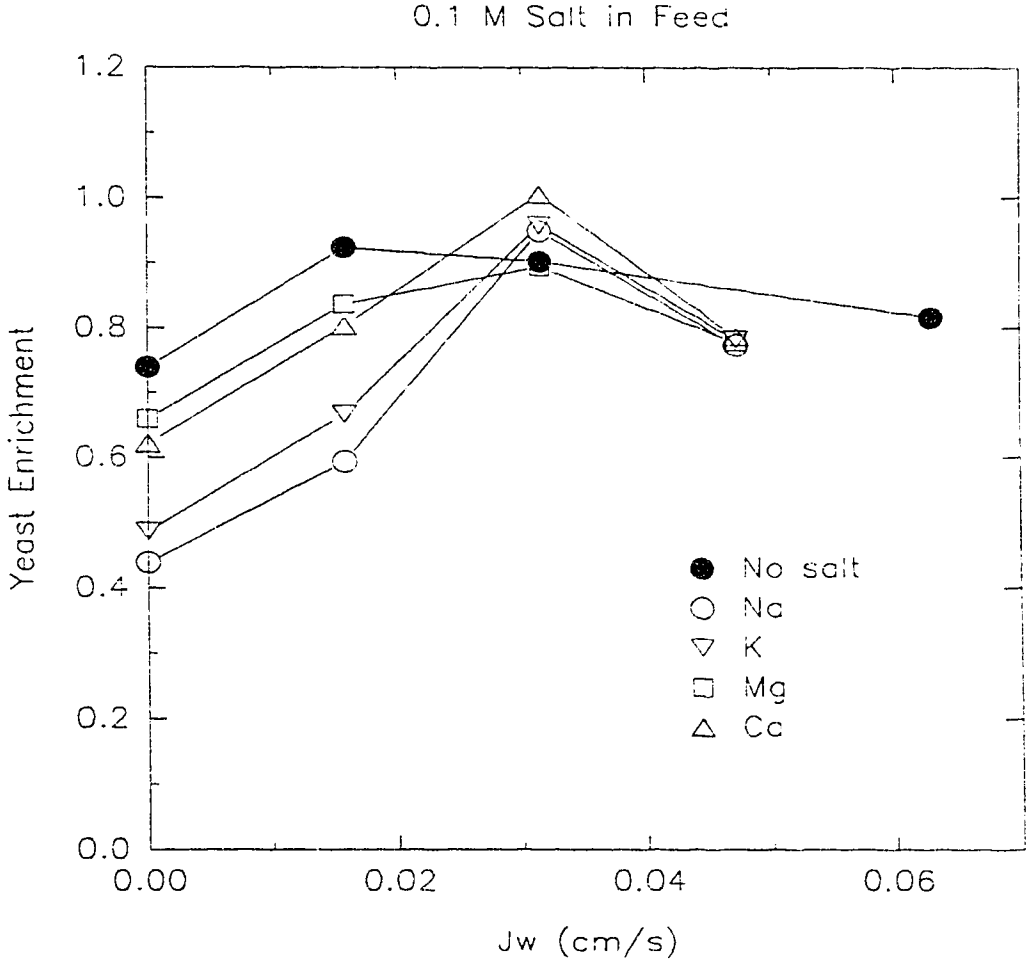


Figure 6.19: Wash Water Effect on Tailings Yeast Enrichment

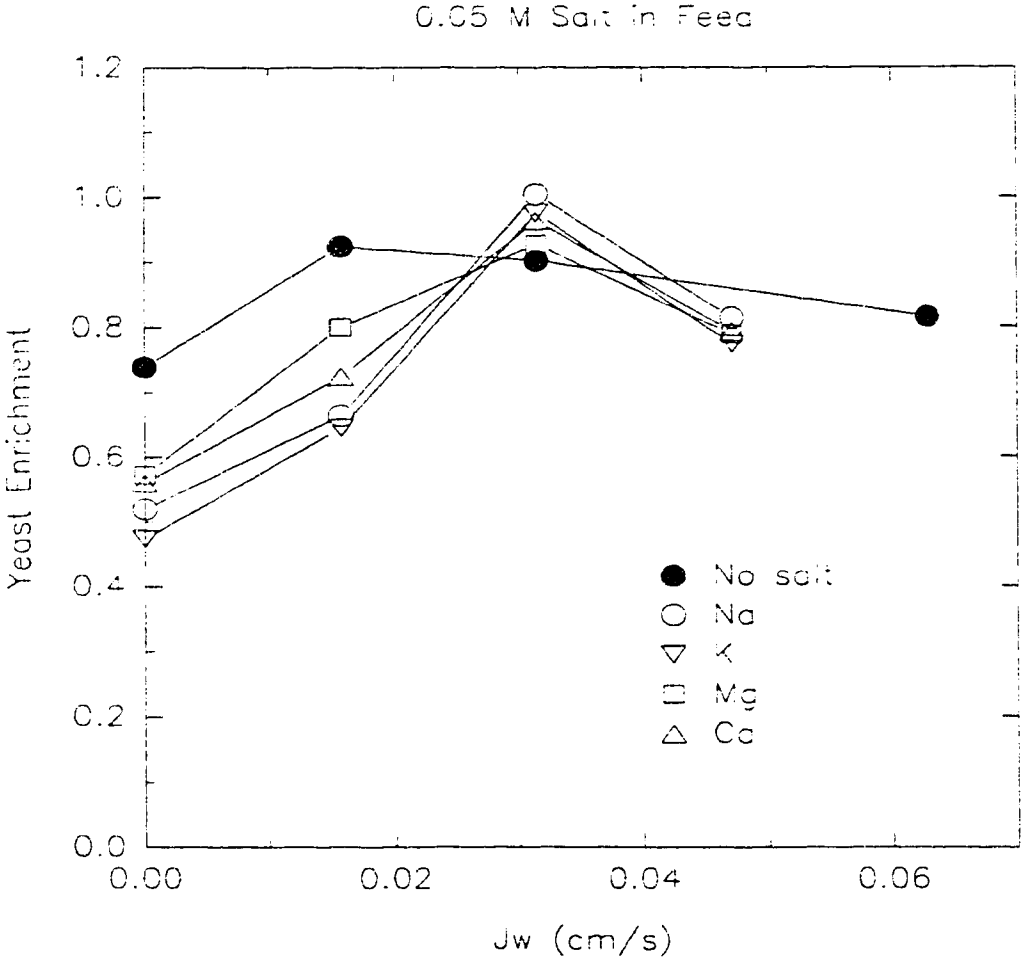


Figure 6.20: Wash Water Effect on Foam Yeast Recovery

0.5 M Salt in Feed

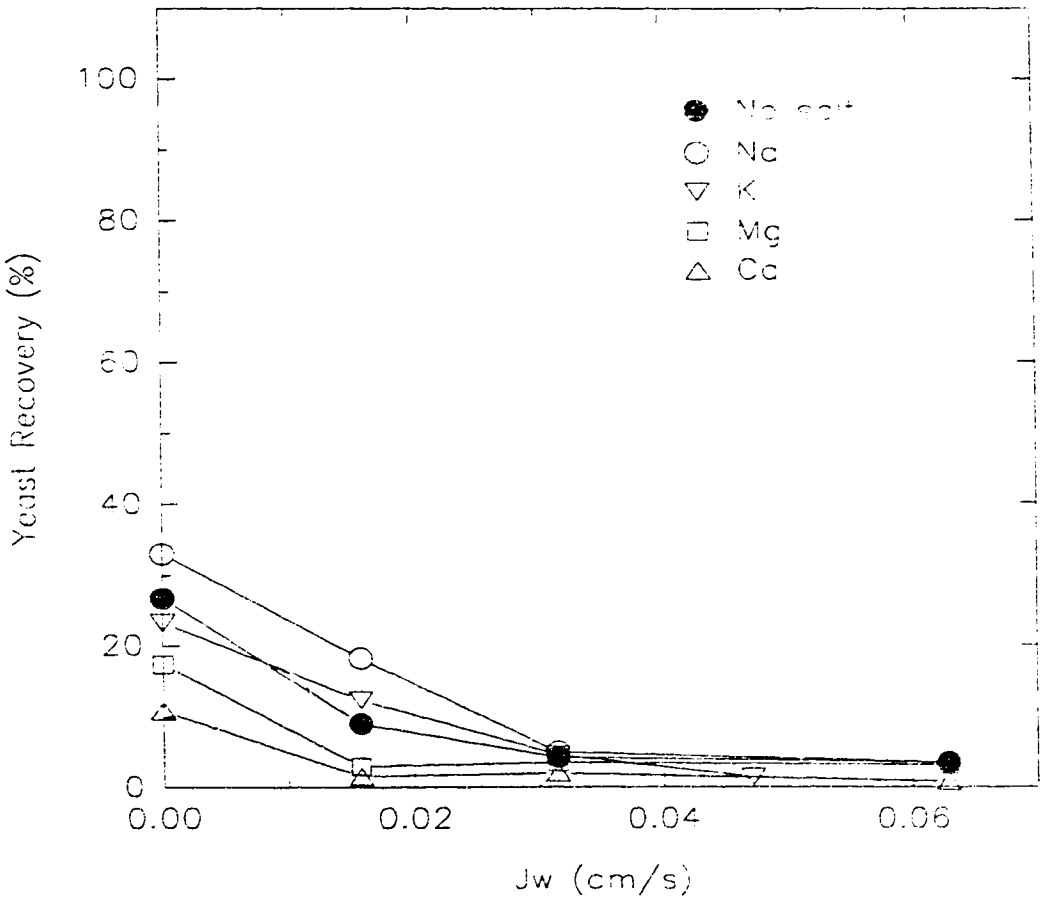


Figure 6.21: Wash Water Effect on Foam Yeast Recovery

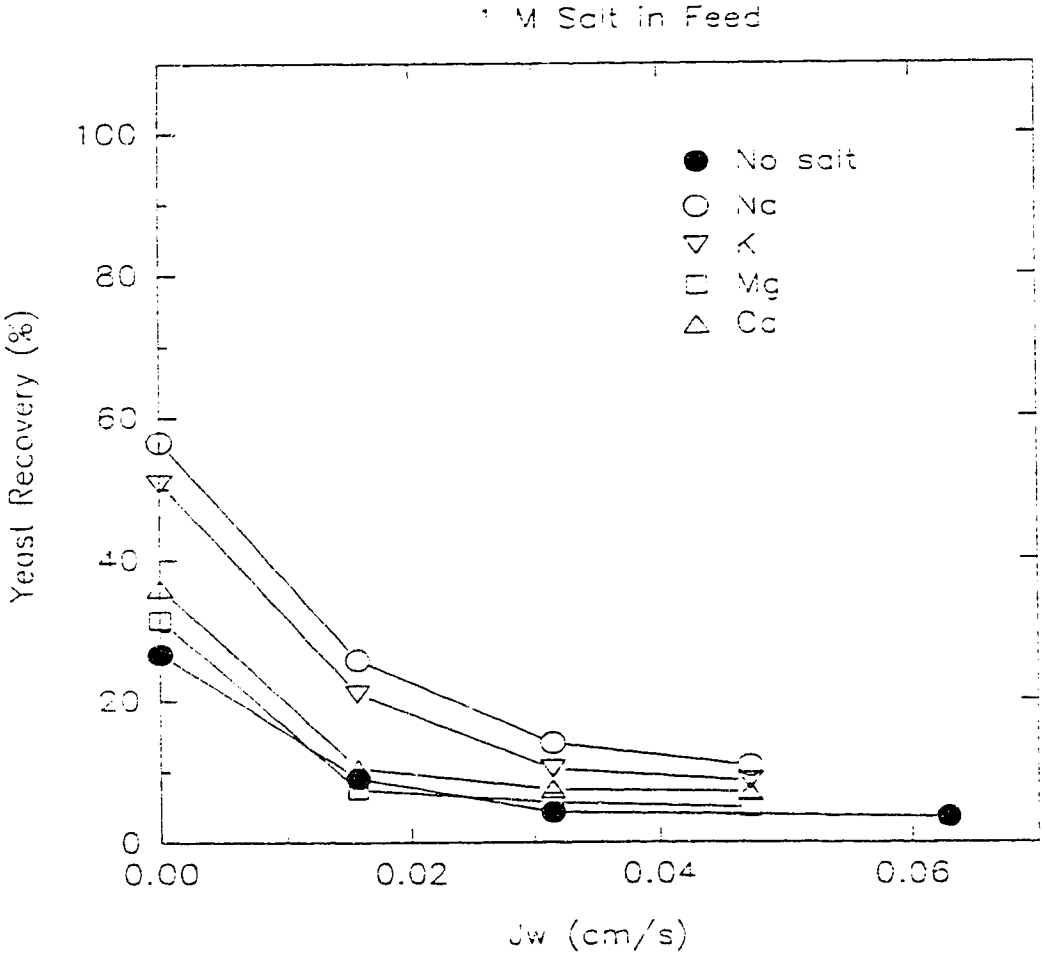
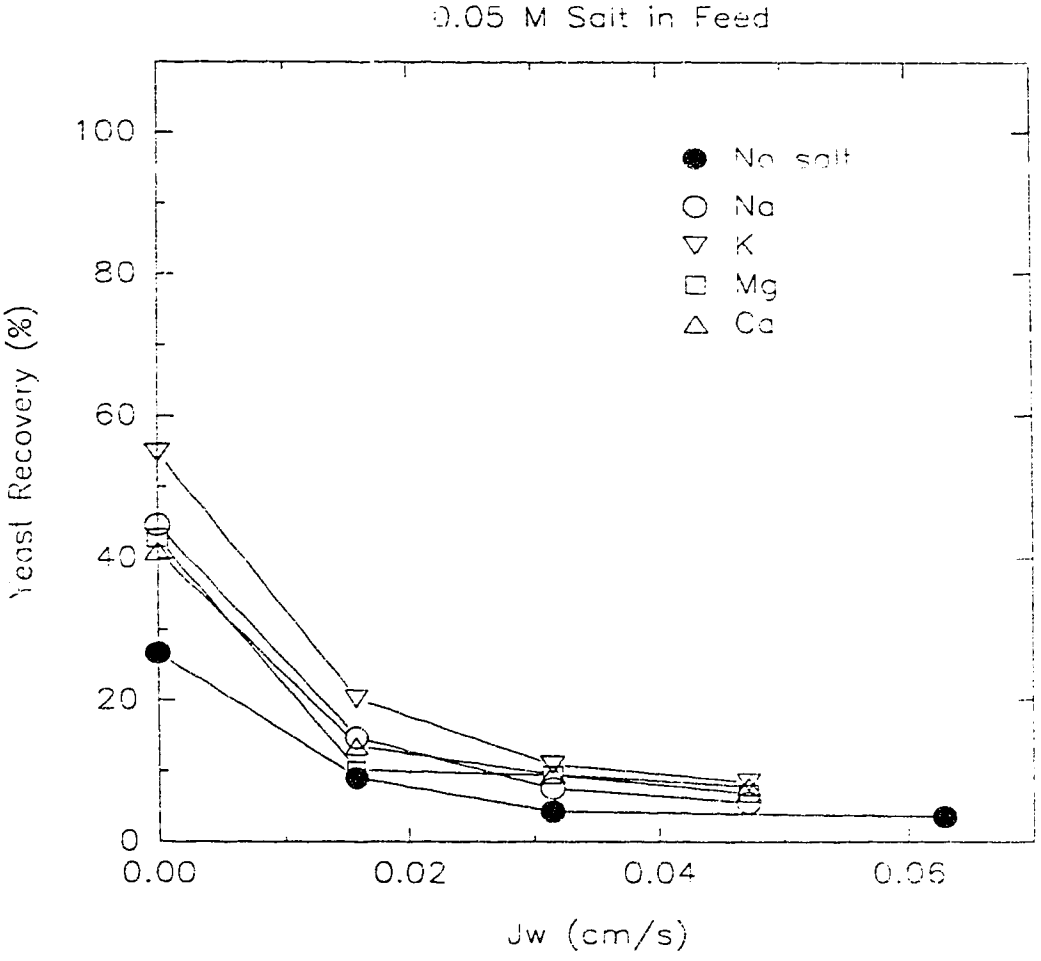


Figure 6.22: Wash Water Effect on Foam Yeast Recovery



6.2.4 Salt Effects

The effects of salt addition to the feed on yeast separation by the flotation column were explored in terms of salt concentration, cation charge and size, using the chloride series NaCl, KCl, $MgCl_2$, and $CaCl_2$ over a concentration range of 0-0.5 M in the feed. In the following data presentation, salt and wash water effects have been separated by choosing three wash water rates (J_w = 0, 0.016, and 0.031 cm/s) at which to examine the variation of each flotation performance parameter with feed salt concentration.

It has already been shown that wash water addition has a detrimental effect on foam yeast enrichment and recovery, slight influence on tailings yeast enrichment, and a beneficial effect on tailings yeast recovery (the latter through yeast detachment from the foam zone). However, a comparison of these wash water effects in the absence and presence of salt in the feed reveals that salt addition most often played a mitigating role - there was a smaller decline in foam yeast enrichment and recovery at a given wash water rate when salt was present in the feed, and a smaller improvement in tailings yeast recovery.

The variation of foam yeast enrichment with feed salt concentration at the three chosen wash water rates is presented in **Figures 6.23-6.25.** As previously observed, the magnitude of the variation was greatest when wash water was not employed (**Figure**

6.23). In the absence of salt in the feed, a 7-fold enrichment of yeast in the foam concentrate over the feed yeast concentration was obtained. With salt addition to the feed, an initial increase to as high as 11-fold foam yeast enrichment was observed within the feed salt concentration range of 0-0.1 M (depending upon the cation). Beyond this region, foam enrichment declined with increasing feed salt concentration, and the beneficial salt effect vanished at high salt concentration (i.e. 0.5 M). All cations with the exception of Ca^{2+} generated this type of response, and the only difference with Ca^{2+} was a plateau in foam yeast enrichment in lieu of the initial increase over the no-salt case. All cations did not generate the same magnitude of response - monovalent cations were effective in improving foam yeast enrichment by 40-60% over the no-salt case within a low salt concentration range (under 0.1 M), and did not have a significant detrimental effect even at a concentration of 0.5 M. On the other hand, divalent cations generated little or no improvement in foam yeast enrichment within this feed salt concentration range, and constituted a liability at a high concentration (0.5 M) where foam yeast enrichment dropped to well below the no-salt level. The data support the existence within the low concentration range of 0-0.1 M of an optimum feed salt concentration, whose value depends upon the cation employed. Optimum feed concentrations could be readily assigned for the monovalent cations at 0.1 M for Na^{+} and 0.05 M for K^{+}. With the divalent cations the situation was much

less clear, since little or no improvement in foam enrichment was observed within this concentration region, although higher concentrations definitely reduced foam enrichment. An optimum feed concentration of 0.05 M could be assigned to Mg^{+2}, but there was uncertainty over the existence of an optimum for Ca^{+2}.

At any feed salt concentration, foam yeast enrichment declined in the presence of wash water due to yeast detachment from the foam zone. More importantly, the patterns of foam yeast enrichment with feed salt concentration for individual salt cations changed when wash water was employed. While monovalent cations continued to outperform divalent salt cations at a wash water rate of 0.016 cm/s (see **Figure 6.24**), no maximum in foam yeast enrichment was observed as a function of K^{+} concentration, and the foam enrichments of both divalent salt cations in this region, while remaining below the no-salt level, increased with declining feed salt concentration. While these results may simply reflect an overall reduction in foam yeast enrichment, they could also appear to suggest an apparent shift in optimum feed salt concentration to lower values upon wash water addition.

Detachment of yeast from the foam zone at a wash water rate of 0.031 cm/s (see **Figure 6.25**) virtually eliminated any improvement in foam yeast enrichment by either monovalent or divalent cations over the no-salt level. However, it is possible to discern a small maximum in foam yeast enrichment with divalent cations at low feed salt concentration. Furthermore, foam yeast

enrichments with divalent cations in this region exceeded the no-salt level. Once again, these results could suggest an apparent downward shift in optimum feed salt concentration for the divalent cations with wash water addition.

Very similar results were obtained for the variation of foam yeast recovery with feed salt concentration, presented in **Figures 6.26-6.28.** Once more, foam yeast recovery was greatest when wash water was not employed (**Figure 6.26**). In the absence of salt in the feed, approximately 25% of the feed yeast was recovered in the foam concentrate. With salt addition to the feed, an initial increase in foam yeast recovery to as high as 55% was observed within the concentration range of 0-0.1 M salt in the feed (depending upon the salt cation). Beyond this point, yeast recovery in the foam declined with increasing feed salt concentration, to a level of 10-35% at a feed salt concentration of 0.5 M. Unlike foam yeast enrichment, foam yeast recovery continued to experience some beneficial effect of salt addition to the feed even at high concentrations, at least in the case of the monovalent salt cations. Overall, the variation of foam yeast recovery with feed salt concentration was very similar to that of foam yeast enrichment, with the exception that an optimal concentration of Ca^{2+} was clearly visible at low feed salt concentration (i.e. 0.05 M) even in the absence of wash water. The remaining salt cations exhibited optimal feed salt

concentrations for foam yeast recovery identical to those for foam yeast enrichment.

Foam yeast recovery upon wash water addition closely paralleled the response of foam yeast enrichment. No sharp maximum in yeast recovery was observed as a function of K^+ concentration at a wash water rate of 0.016 cm/s (see **Figure 6.27**). Stronger evidence for an apparent downward shift in the optimal feed salt concentration with wash water addition was not obtained, since a clearly visible Ca^{2+} optimum concentration of 0.05 M was observed in the absence of wash water and retained at all rates of wash water employed.

The variation of tailings yeast recovery with feed salt concentration, determined independently of foam yeast recovery and presented in **Appendix F**, was almost the reverse of foam yeast recovery, as expected. Some inconsistencies with the corresponding foam yeast recovery data were observed - for example, at a wash water rate of 0.031 cm/s, the relative order of tailings yeast recovery for the salt cations was not the reverse of foam yeast recovery, although the values were within standard deviation. These discrepancies were due to larger deviations in yeast balances than the norm of around 5% observed in volumetric liquid balances; yeast balance deviations reached as high as 20% at the higher wash water rates.

Clearly, yeast enrichment and recovery in the foam concentrate benefitted from low concentrations of salt in the

feed, i.e. 0.1 M or under. The optimal concentration depended upon both the salt cation and the rate of wash water employed. Monovalent cations outperformed divalent cations at all but the highest wash water rates. Different enrichments and recoveries were obtained between individual cations of the same valence. Wash water at sufficiently high rates negated the benefits of salt addition to the feed by detaching yeast from the foam zone.

If yeast flotation is dependent only on the yeast surface charge, and this is affected by simple valency of the salt cation, then a divalent salt cation should have an optimum feed salt concentration at half the value of a monovalent salt cation. The optimal concentration of a divalent salt cation may very well fall below the employed feed salt concentration, which could account for any apparent downward shift in the optimal concentration observed with wash water addition. In the absence of wash water, the salt concentration in the bubbly zone is comparable to the feed salt concentration; with wash water addition, the positive bias (i.e. downward liquid flux) in the foam zone increases with wash water rate and dilutes the salt concentration in the bubbly zone. Consequently, a more accurate picture of the salt concentration dependency of yeast flotation may be given by the salt concentration in the bubbly zone.

In order to investigate this explanation for the apparent downward shift in feed salt concentration optima, the surface charge associated with bakers' yeast was characterized by an

electrokinetic technique during titration with 2.0 M solutions of the various inorganic salts (complete data are provided in **Appendix G**). The variation of the yeast ESA signal (which is proportional to the yeast zeta potential) with salt concentration for each cation has been superimposed on the corresponding yeast enrichment and recovery for that salt cation in **Figures 6.29-6.36.** If yeast separation is dependent solely on yeast surface charge, then foam yeast enrichment and recovery should be maximized at a salt concentration resulting in no net surface charge on the yeast cell, i.e. a zero ESA signal.

While the zero point of the ESA signal with NaCl was in the neighborhood of the feed salt concentration which maximized foam yeast enrichment and recovery (see **Figures 6.29-6.30**), the correspondence was not exact. For the case of **Figure 6.29**, electrokinetic measurements suggested that a net zero yeast surface charge was obtained at a concentration of 0.049 M NaCl, whereas optimum yeast separation occurred at approximately 0.1 M NaCl. The concentrations of KCl, $MgCl_2$, and $CaCl_2$ producing a net zero yeast surface charge were also all below the feed concentrations of these salts for optimum yeast separation (see **Table 6.2**). Although the correspondence was not exact, the relative order of salt cation concentrations for production of zero yeast surface charge corresponds with the relative order of cation concentrations to maximize foam yeast enrichment and recovery.

It is particularly interesting that cations of the same valency were not equivalent. Although Na^+ generally produced greater foam yeast enrichment and recovery than K^+, this cannot be explained on the basis of charge density since Mg^{2+} often did not equal the performance of Ca^{2+} in these results. In fact, the monovalent cation K^+ was equivalent to the divalent cation Mg^{2+} in terms of the salt concentration producing a net zero yeast surface charge and the feed salt concentration for optimum yeast separation. Such results are possible because changes in the thickness of the electric double layer and the zeta potential of both particles and bubbles depend upon the chemistry of the surfaces and the mode of gaining surface charge. Unless a good surface ionization model is available, it is difficult to predict changes in the zeta potential with electrolyte addition. A rule of thumb is that a 10-fold change in concentration will bring about a 2-fold change in zeta potential.

Figure 6.23: Salt Concentration Effect on Foam Yeast Enrichment

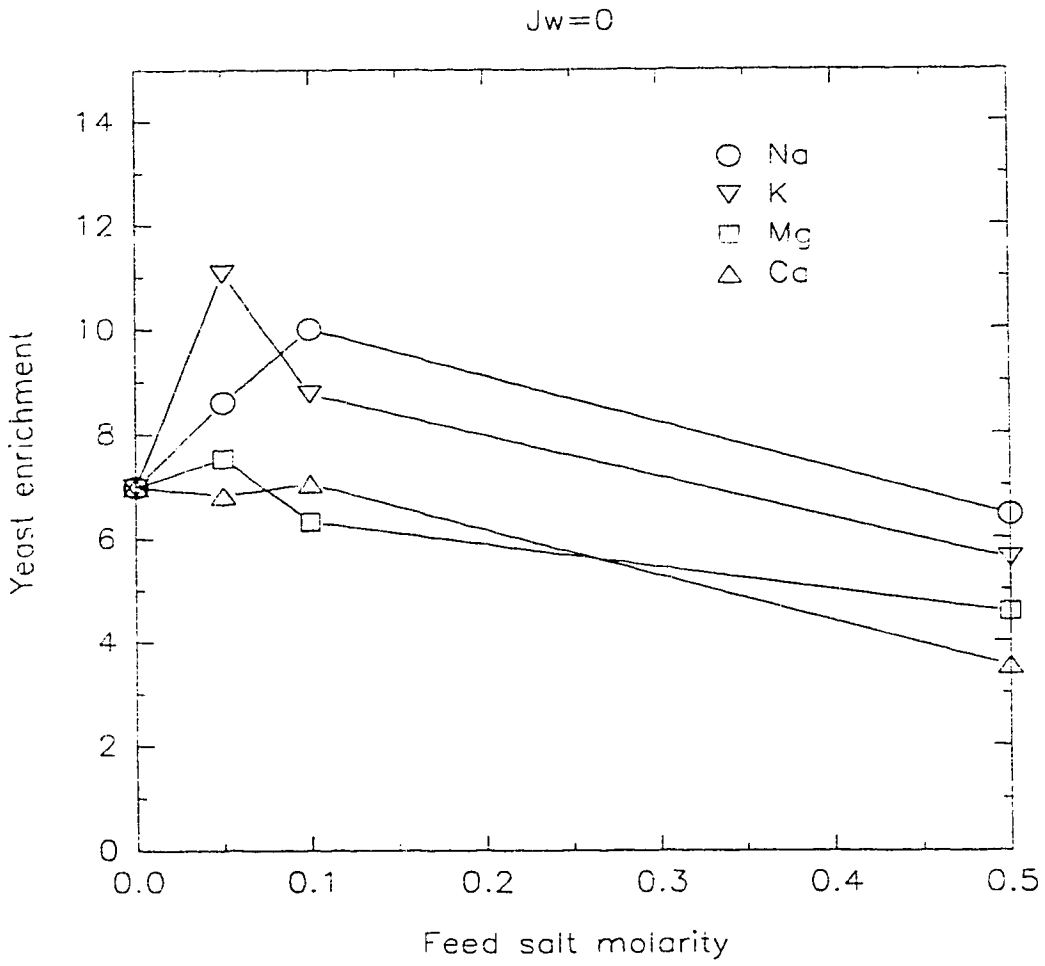


Figure 6.24: Salt Concentration Effect on Foam Yeast Enrichment

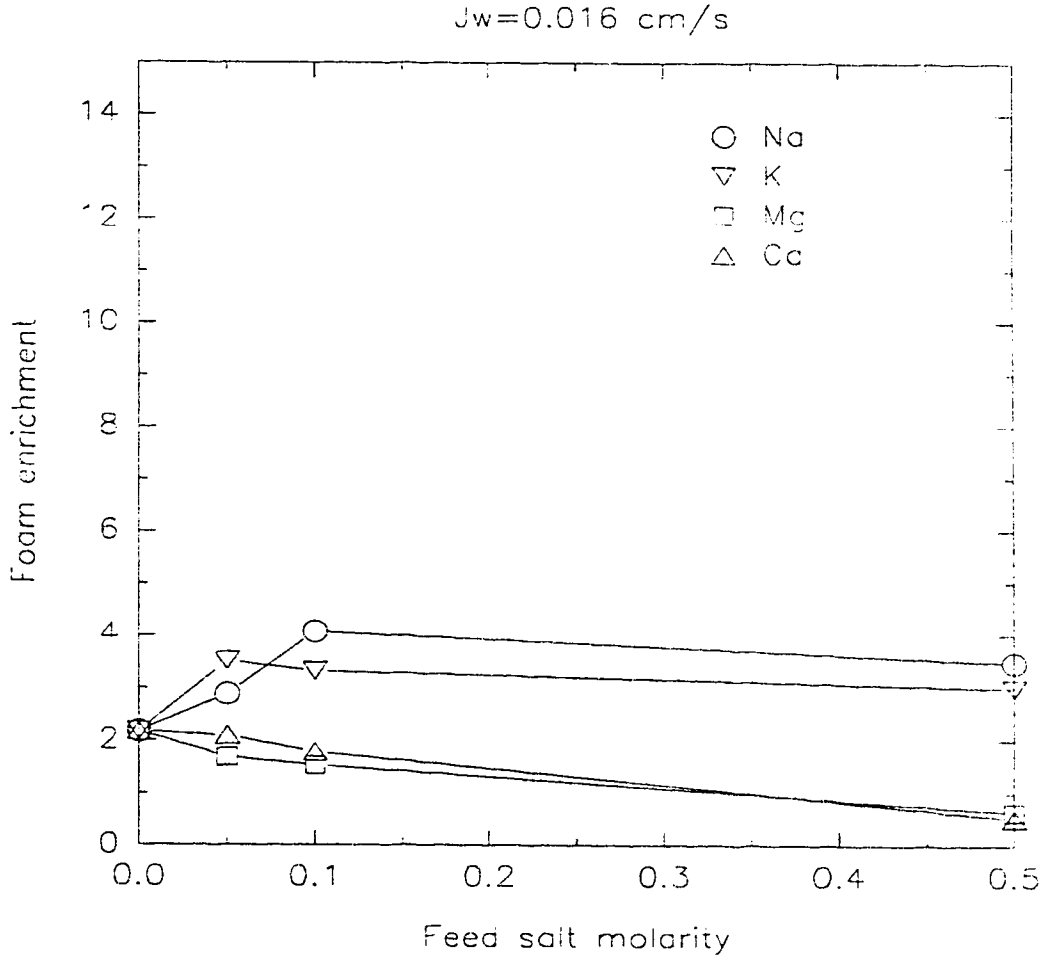


Figure 6.25: Salt Concentration Effect on Foam Yeast Enrichment

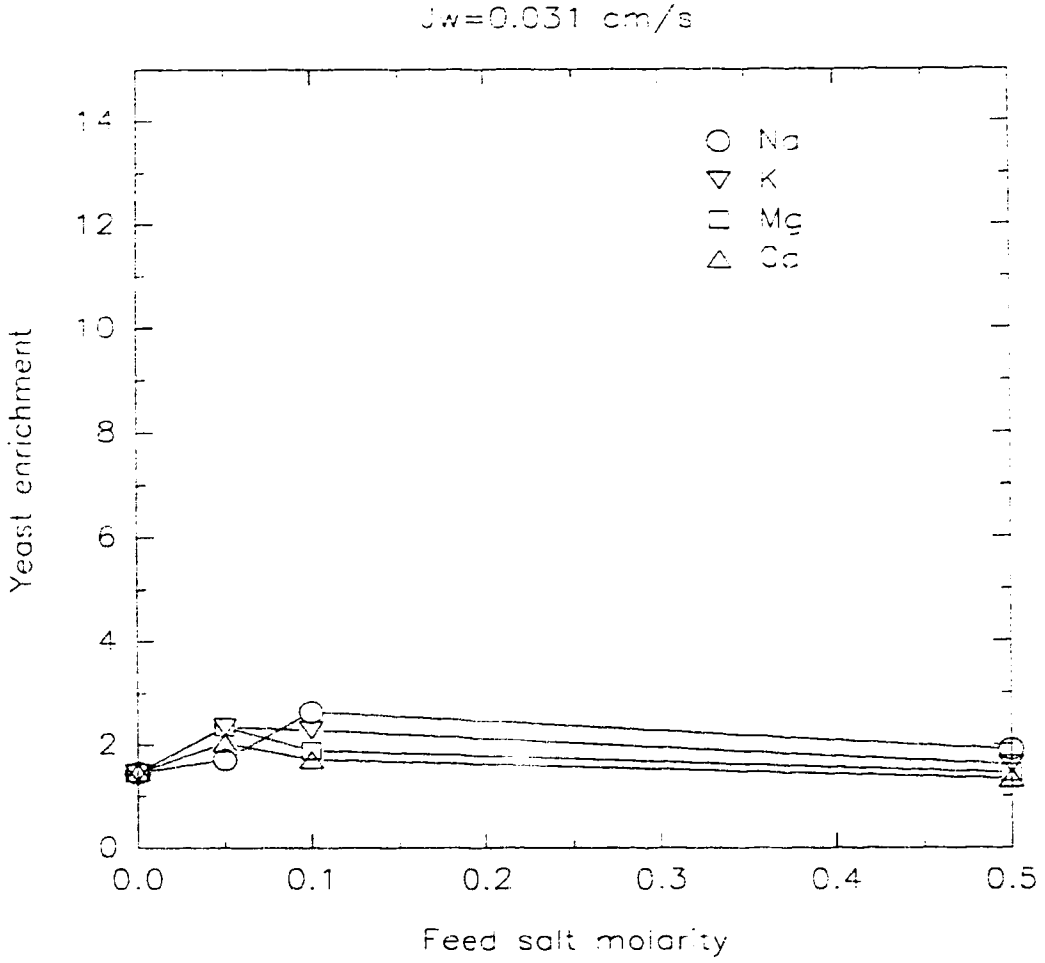


Figure 6.26: Salt Concentration Effect on Foam Yeast Recovery

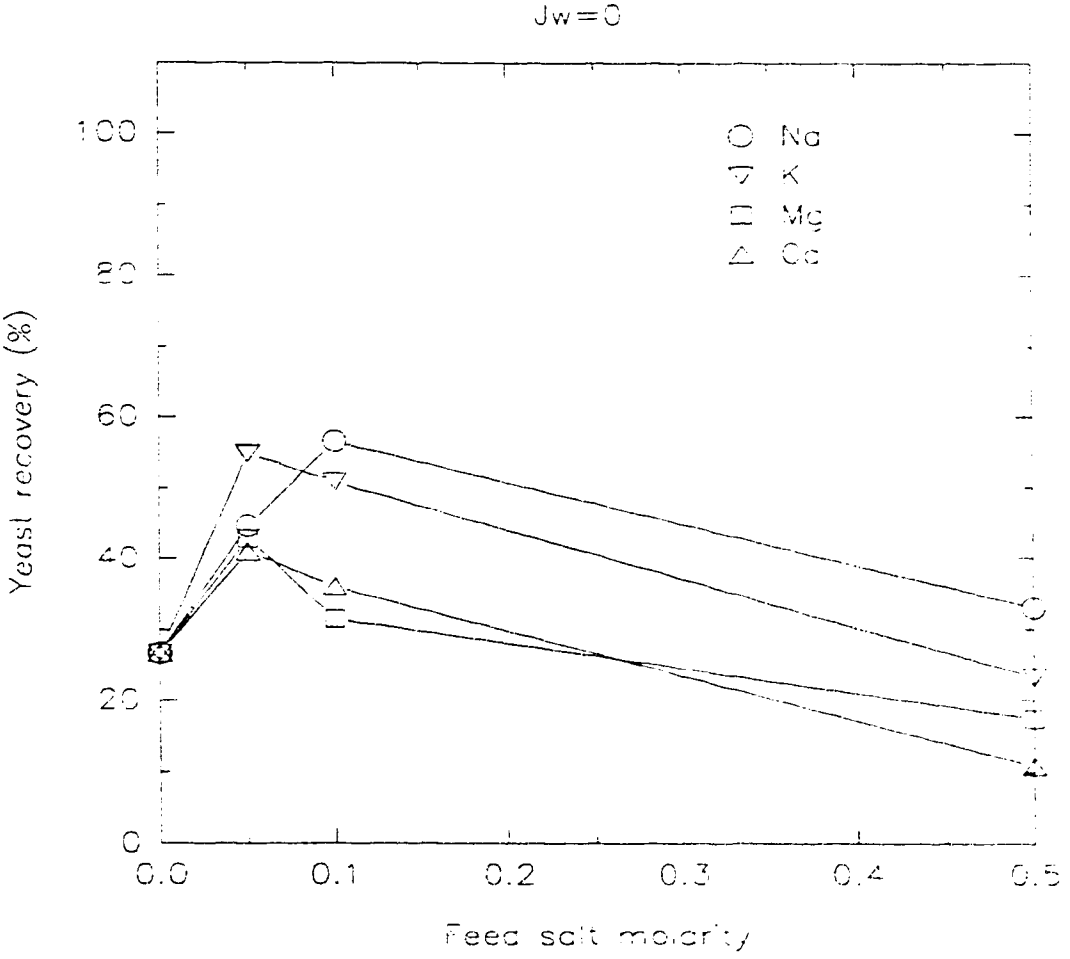


Figure 6.27: Salt Concentration Effect on Foam Yeast Recovery

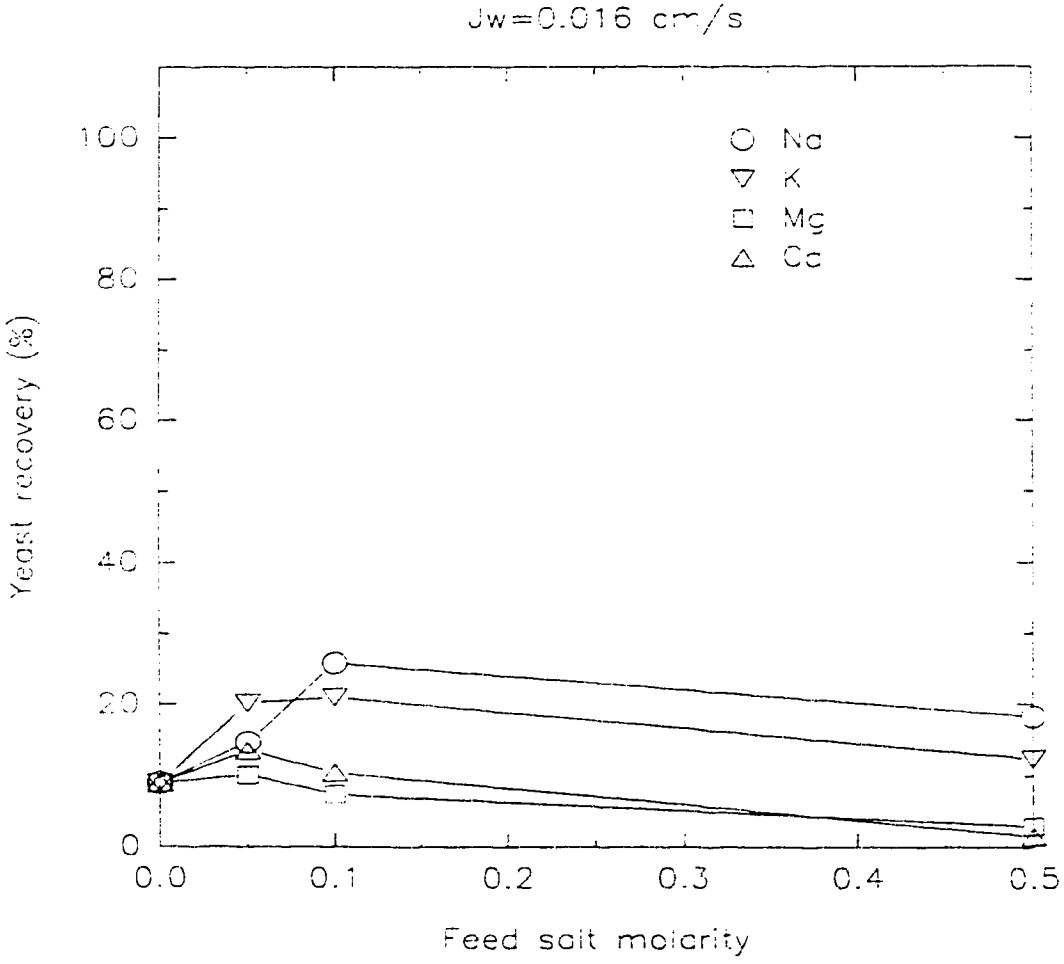


Figure 6.28: Salt Concentration Effect on Foam Yeast Recovery

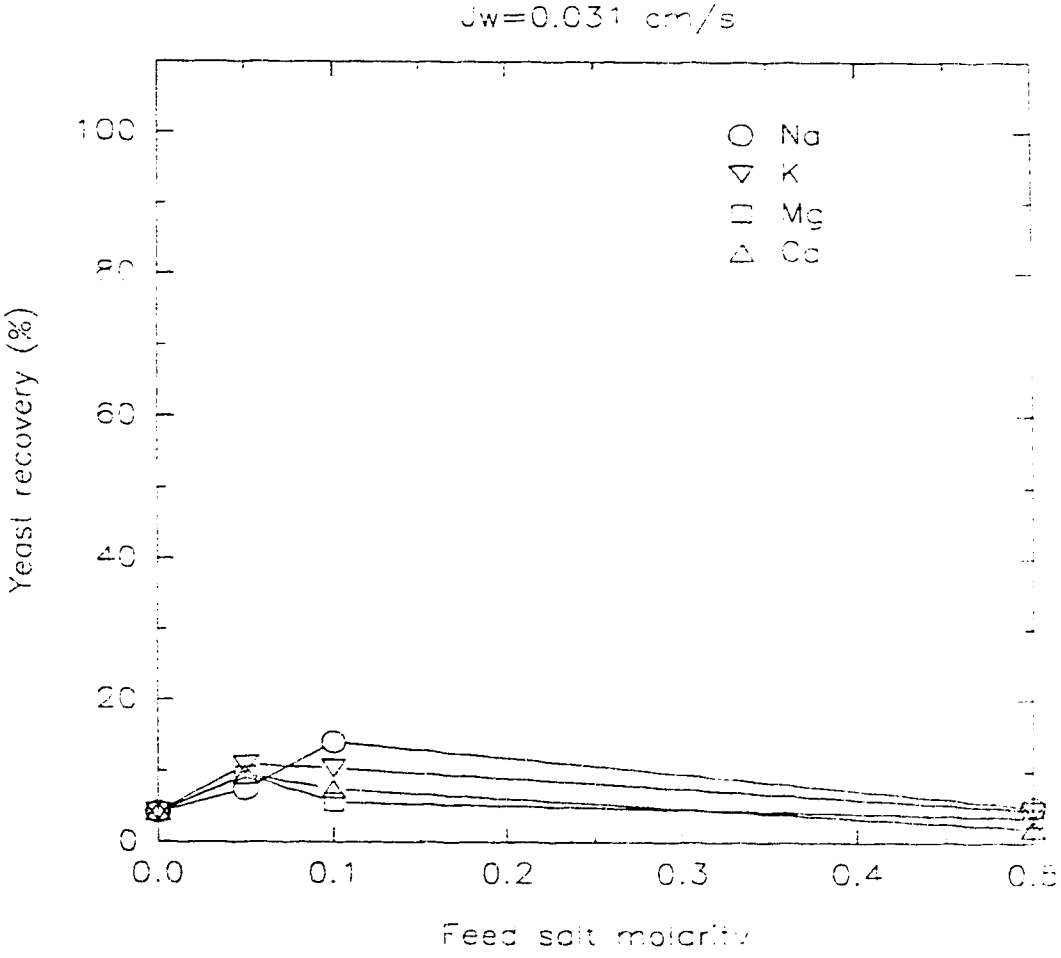


Figure 6.29: Salt Concentration Effect on Yeast ESA and Foam Yeast Enrichment Using NaCl

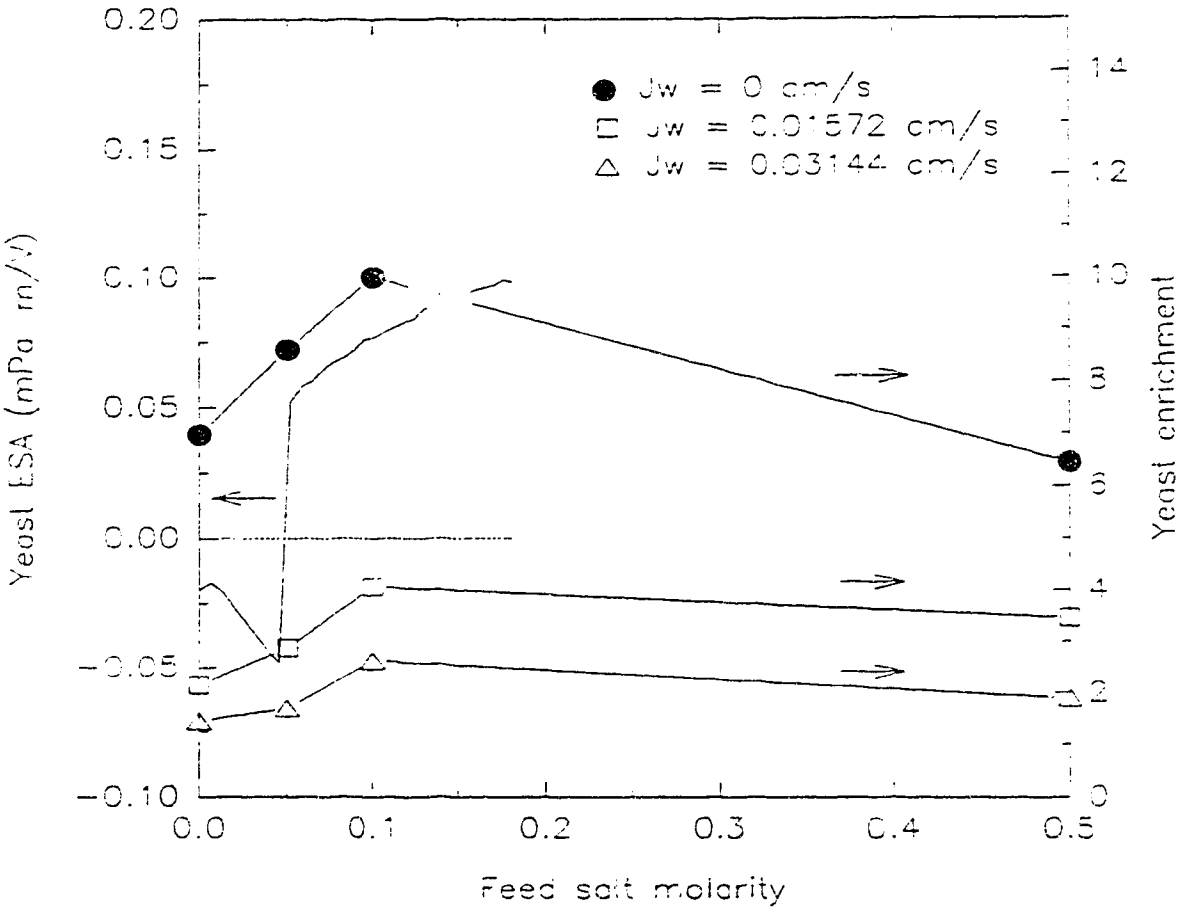


Figure 6.30: Salt Concentration Effect on Yeast ESA and Foam Yeast Recovery Using NaCl

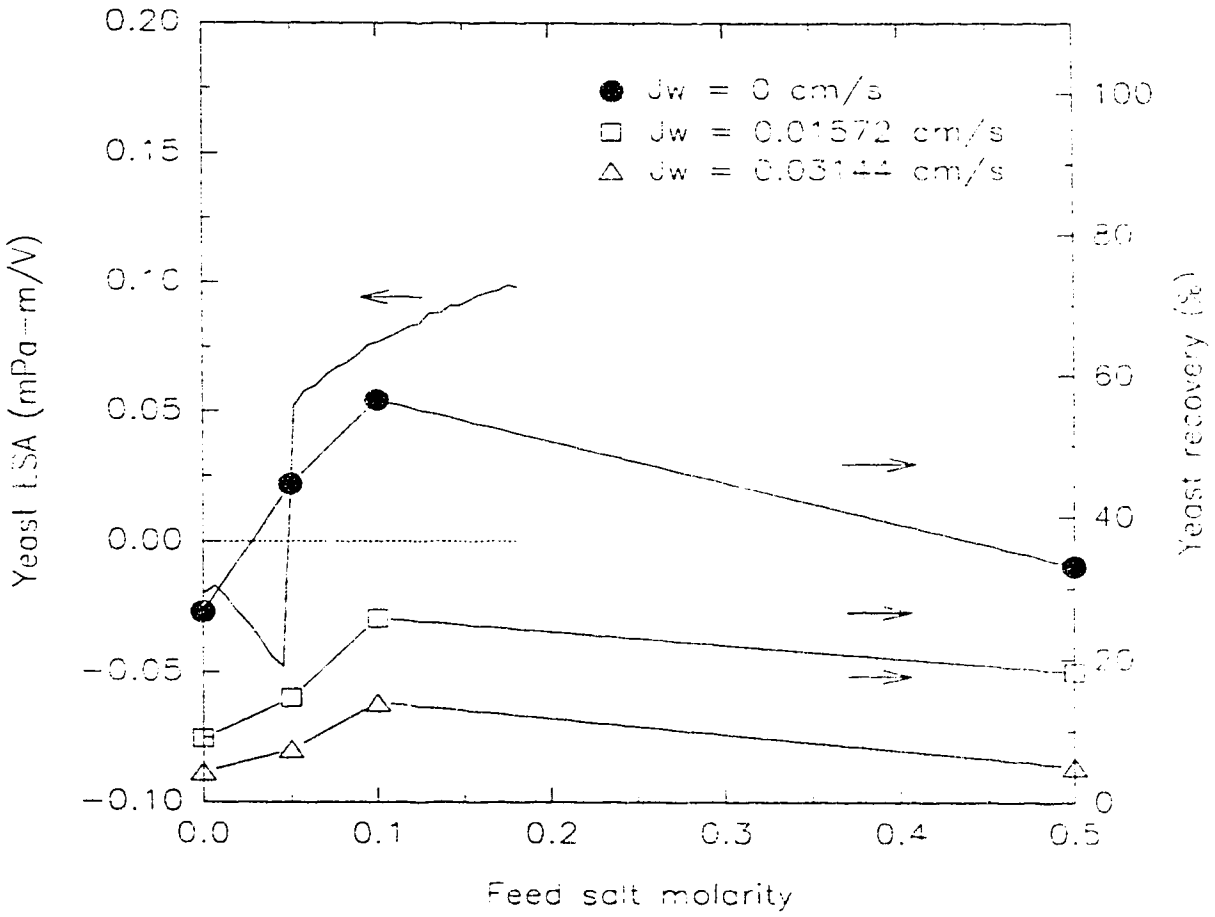


Figure 6.31: Salt Concentration Effect on Yeast ESA and Foam Yeast Enrichment Using KCl

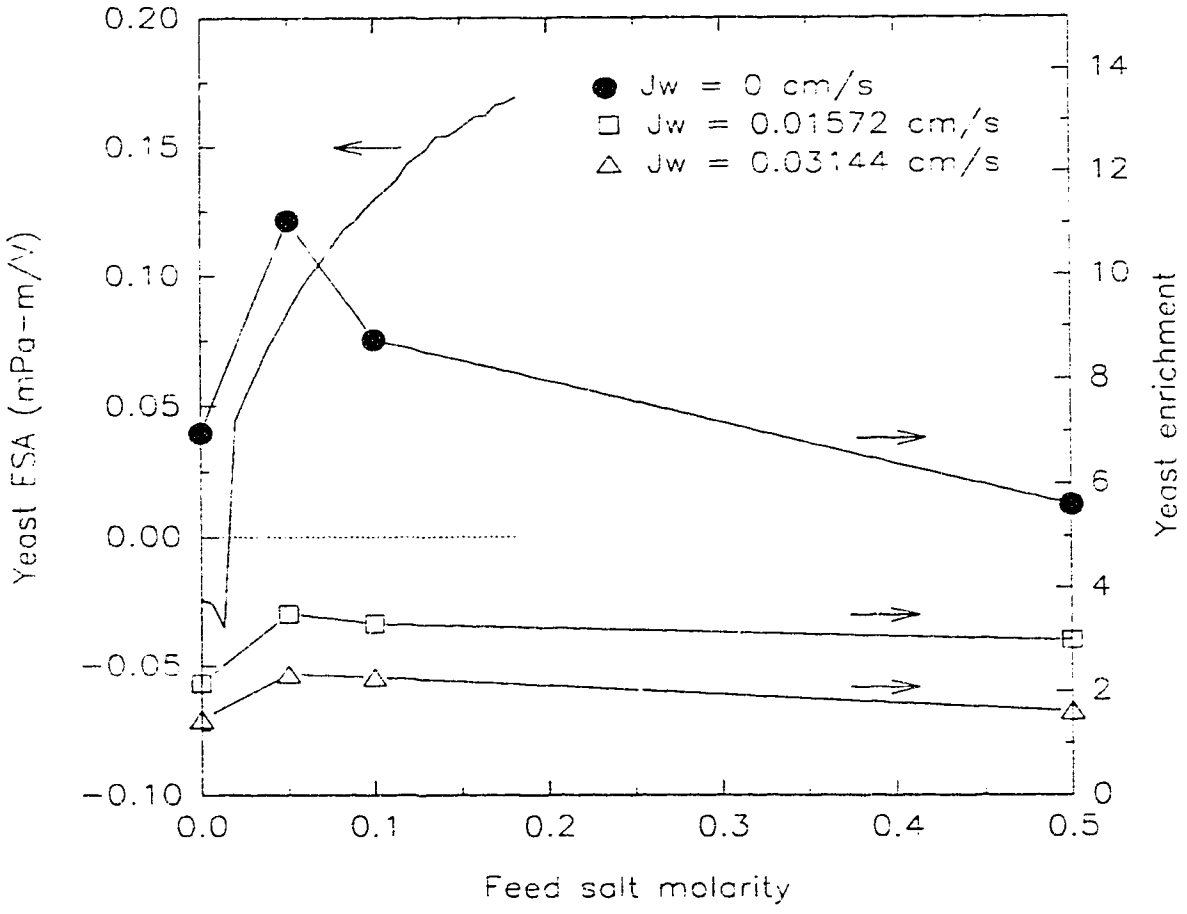


Figure 6.32: Salt Concentration Effect on Yeast ESA and Foam Yeast Recovery Using KCl

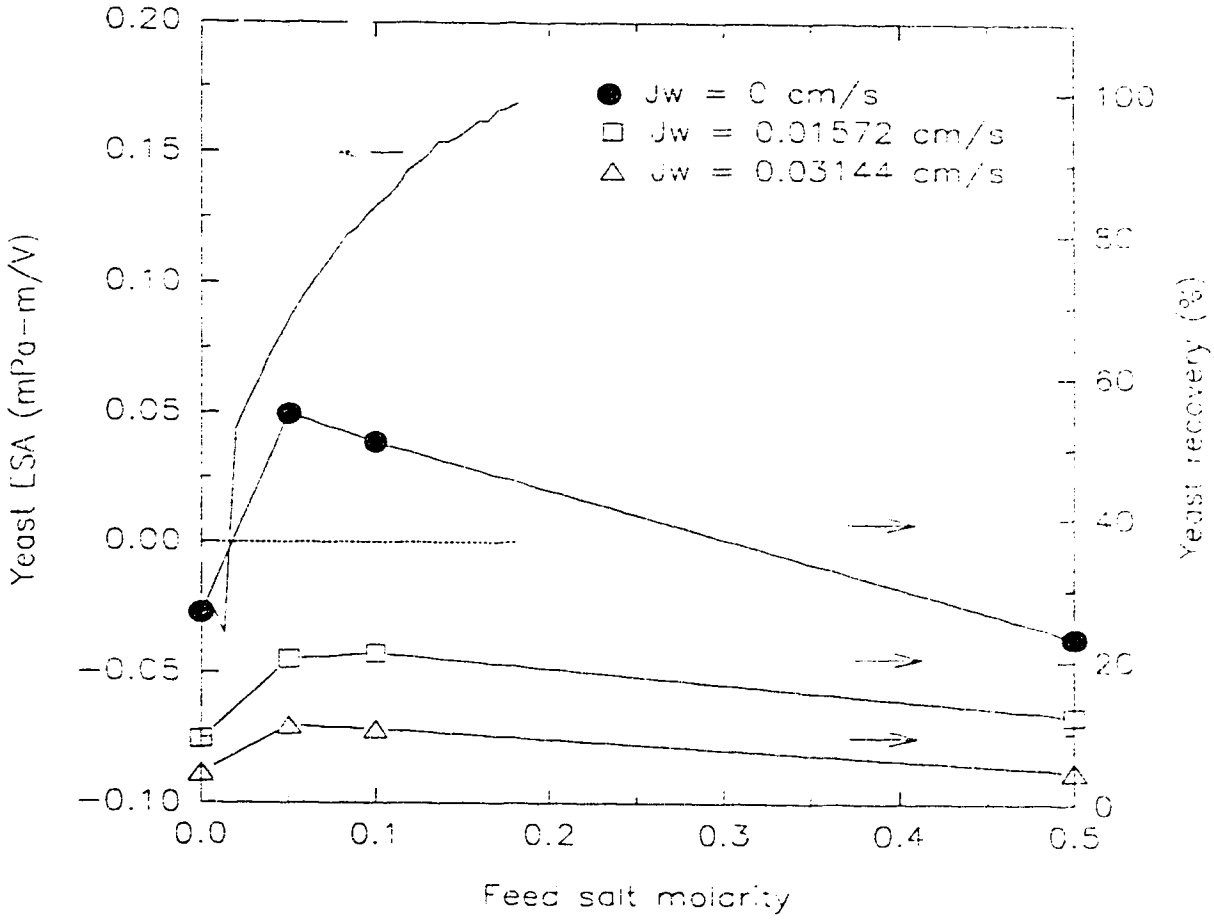


Figure 6.33: Salt Concentration Effect on Yeast ESA and Foam Yeast Enrichment Using MgCl2

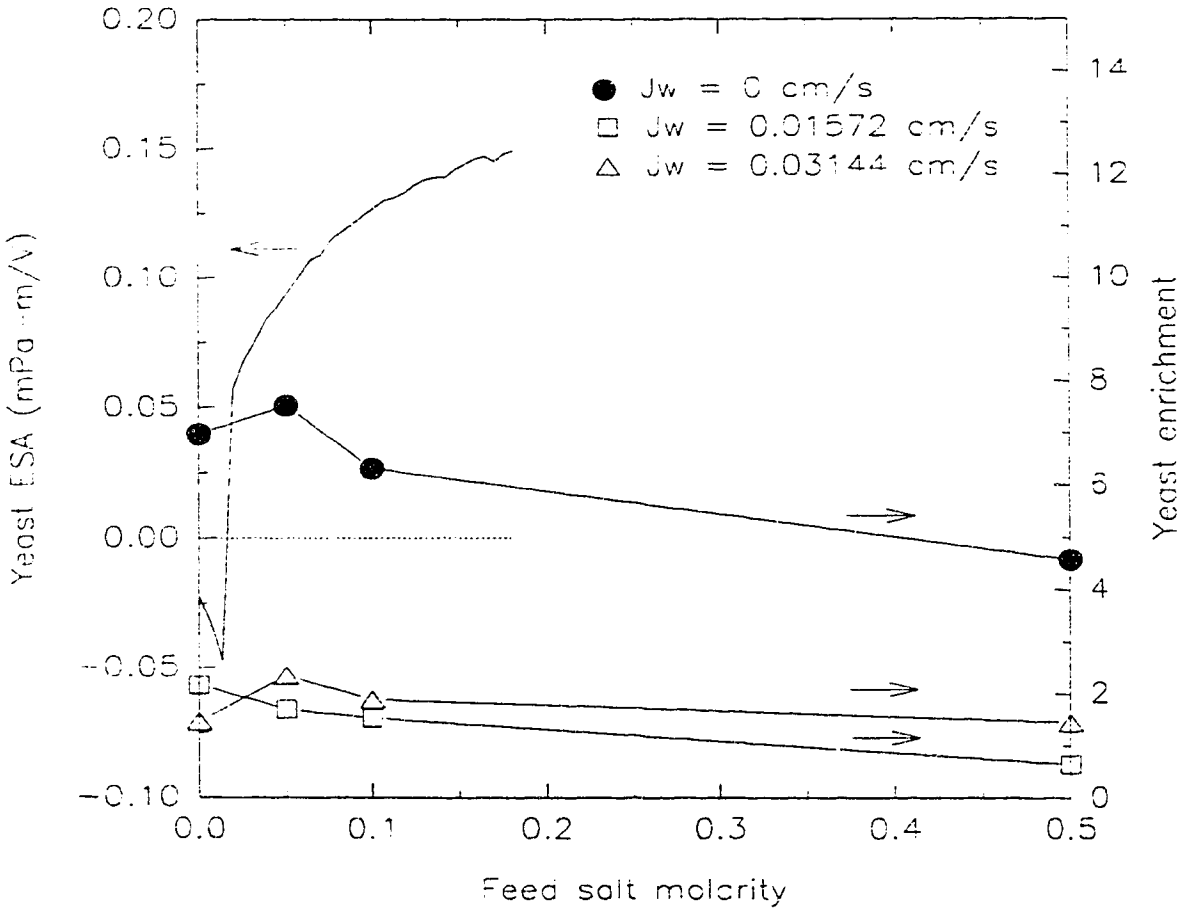


Figure 6.34: Salt Concentration Effect on Yeast ESA and Foam Yeast Recovery Using MgCl2

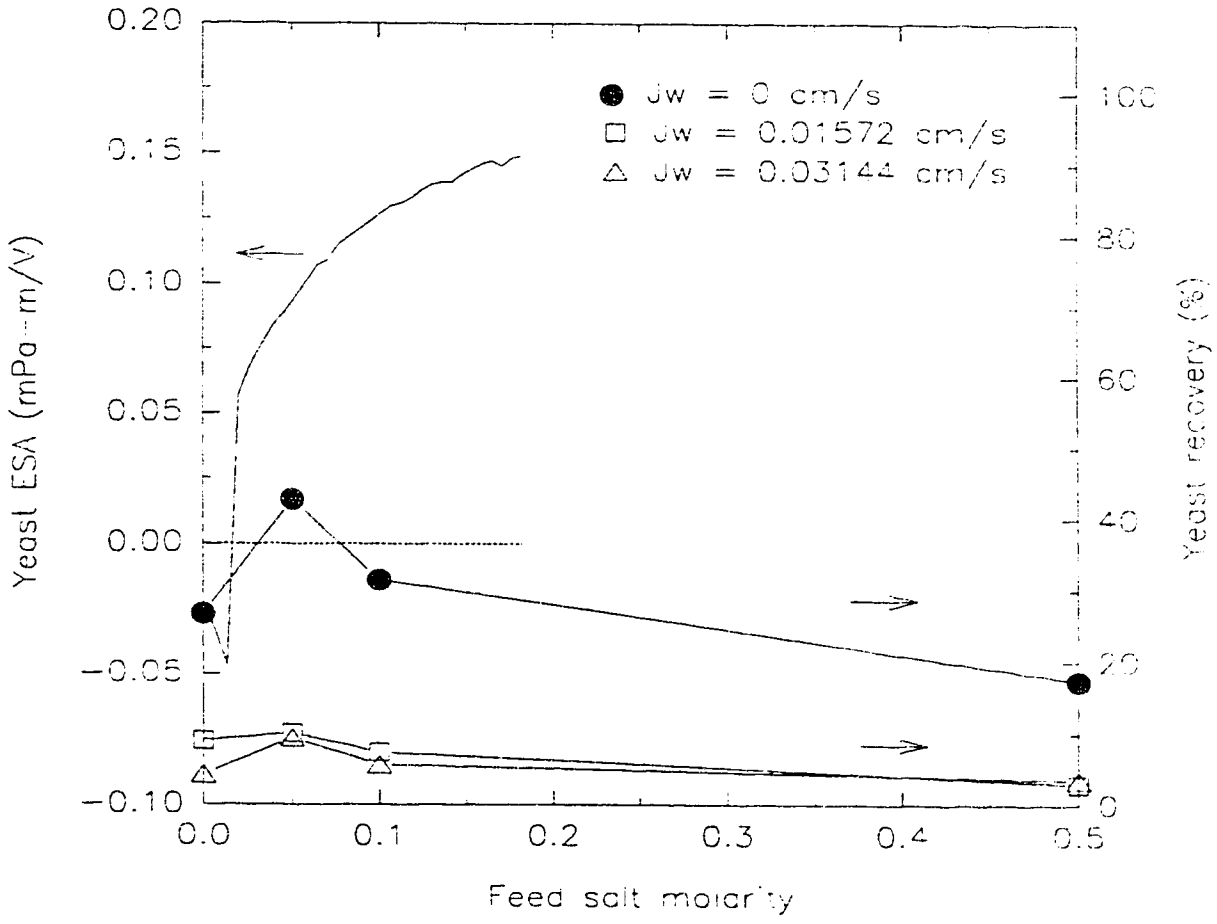


Figure 6.35: Salt Concentration Effect on Yeast ESA and Foam Yeast Enrichment Using CaCl2

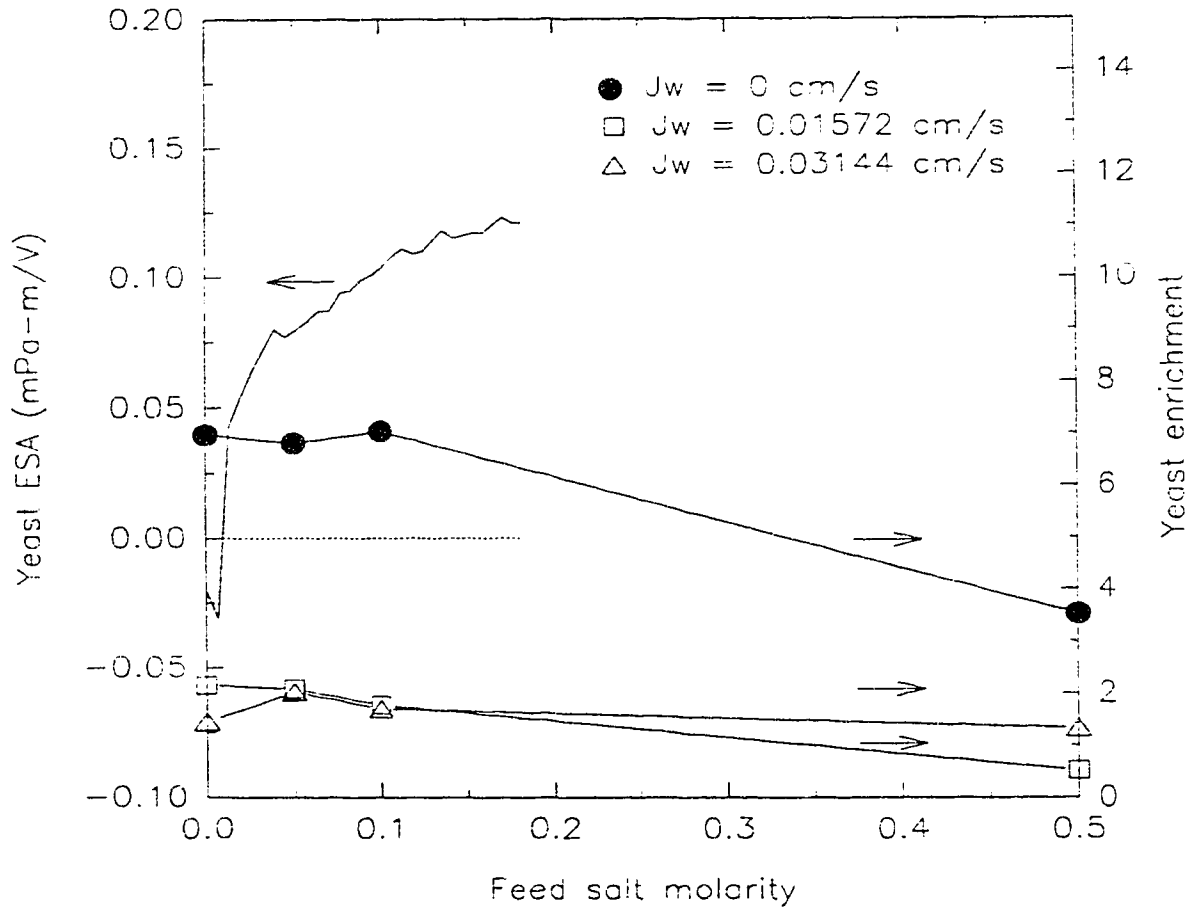


Figure 6.36: Salt Concentration Effect on Yeast ESA and Foam Yeast Recovery Using CaCl2

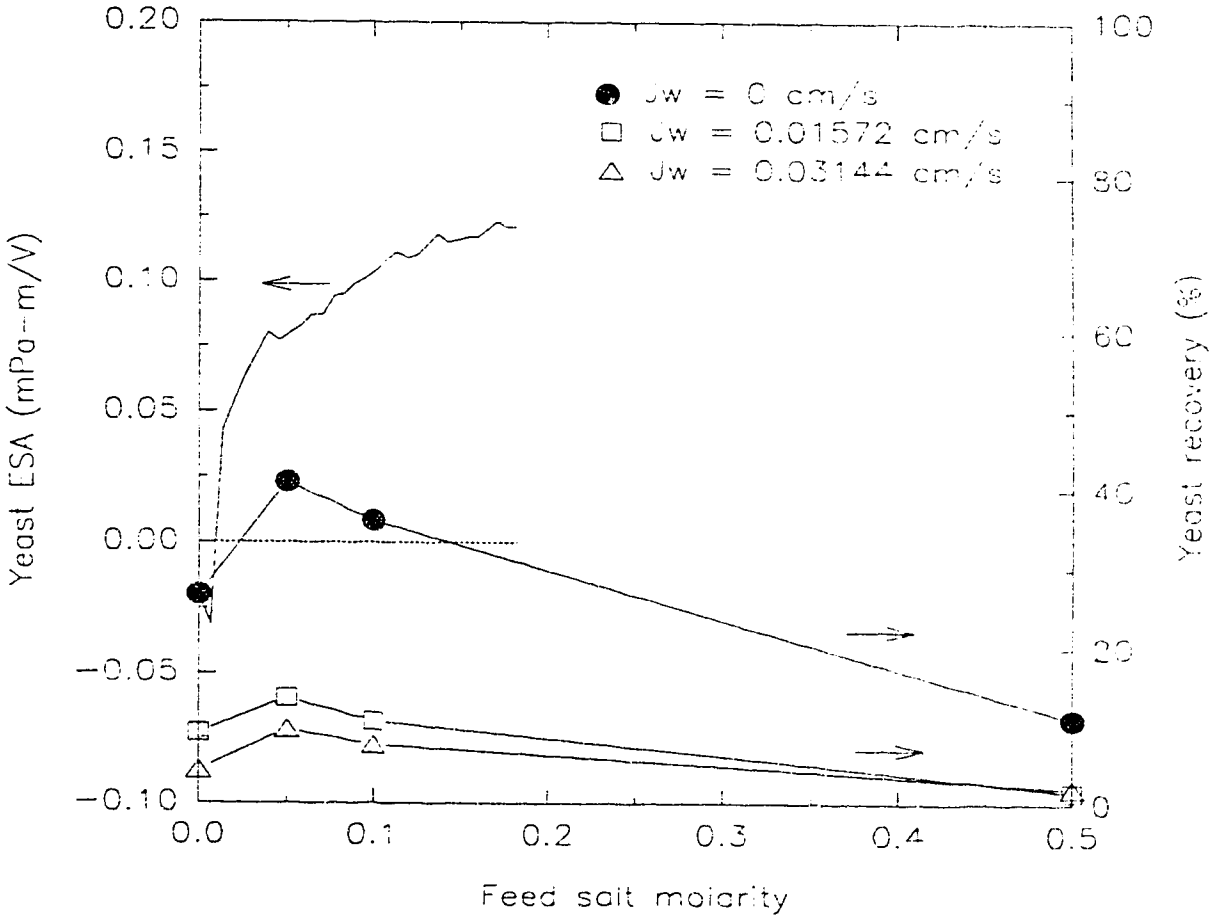


Table 6.2: Salt Concentrations for Zero Point of ESA Signal and Maximum Foam Yeast Enrichment and Recovery

Salt	Salt concentration for ESA=0	Salt concentration for max. foam yeast enrichment and recovery
NaCl	0.049 M	0.1 M
KCl	0.016 M	0.05 M
$MgCl_2$	0.016 M	0.05 M
$CaCl_2$	0.009 M	< 0.05 M

Chapter 7: CONCLUSIONS

The operation of the flotation column was validated by the drift-flux model using two aqueous alkylpolyglycoside surfactant solutions. At a concentration of 40 ppm in tap water, APG 600CS and APG 625CS generated surface tensions of 0.039 N/m and 0.036 N/m respectively. The bubble diameter distribution was typically sigmoidal, although APG 625CS produced a bimodal distribution at high gas superficial velocities (J_g > 2.52 cm/s). A bubbly flow regime was maintained with each surfactant throughout the range of gas superficial velocities employed (J_g = 0.31-3.14 cm/s). This flow regime was essential for efficient column operation, and was demonstrated by an approximately linear variation of gas holdup in the bubbly zone with gas superficial velocity. The drift-flux model provided a good fit to the bubbly zone hydrodynamic data (encompassing a gas holdup range of α = 0.02-0.21) for both surfactants. The fit to the foam zone data (encompassing a gas holdup range of α = 0.80-0.95) was not as good, although still within range of literature values. It should be noted that the drift-flux approach is less successful at modeling the literature hydrodynamic data within this region of high gas holdup.

Column flotation of bakers' yeast was performed on low concentration suspensions (2.0 g/L) at a feed rate of J_f = 0.21 cm/s and a gas superficial velocity of J_g = 0.31 cm/s. Continuous operation was successfully maintained over a wash water

range of J_w = 0-0.063 cm/s. Bias rates in the foam zone were low (under 0.06 cm/s) and always positive when operating with wash water, as expected for the low gas superficial velocity and as required for proper cleaning action in the foam zone.

Foam enrichment in yeast significantly above that of the feed concentration was achieved, although yeast recovery in the foam concentrate was quite poor. In the absence of wash water, foam enrichments of up to 11-fold and recoveries of up to 55% were obtained. Wash water addition was not beneficial to yeast enrichment or recovery in the foam. Two wash water effects were observed: foam dilution and yeast detachment. Conventional foam drainage in the region above the wash water distributor was insufficient to handle the increased water content of the foam zone, with resultant dilution of the foam concentrate. Wash water detachment of yeast from bubbles in the foam zone also reduced foam recovery. At a wash water rate of J_w = 0.031 cm/s, foam enrichment in yeast dropped to 1.5- to 3-fold and yeast recovery in the foam was virtually eliminated. The latter effect could not be attributed to surfactant washout from the foam, since the surfactant residual in the tailings (only 4% in the absence of wash water) increased to only 15% at the highest wash water rate.

Salt addition to the feed mitigated these wash water effects to a certain extent. In the absence of salt, a maximum 7-fold yeast enrichment in the foam was obtained, while yeast recovery did not exceed 25%. At feed salt concentrations of 0.05-0.1 M,

foam enrichment increased to as high as 11-fold, and recovery improved to 55%. However, these benefits were lost at wash water rates in excess of J_W = 0.031 cm/s. Optimum feed salt concentrations for yeast enrichment and yeast recovery in the foam were identical and depended on the salt cation: 0.1 M for NaCl, 0.05 M for KCl and $MgCl_2$, and under 0.05 M for $CaCl_2$. Cations of the same valency were not equivalent in yeast flotation performance.

Yeast zeta potential upon electrolyte addition (as determined by the ESA signal of a 10% yeast suspension) was identified as a useful indicator of flotation performance. Although the salt concentration producing the zero point of the yeast ESA signal did not exactly correspond with the optimum feed concentration of that salt for foam enrichment and recovery, the cation order was identical: 0.049 M for NaCl, 0.016 M for KCl and $MgCl_2$, and 0.009 M for $CaCl_2$. Cations of the same valency were not equivalent in generating the zero point of the yeast ESA signal. This is a reflection of the zeta potential dependency upon surface chemistry and the mode of gaining surface charge. Without a good surface ionization model, it is difficult to predict changes in zeta potential with electrolyte addition.

These results demonstrate that improvements in cell flotation are possible by manipulation of the cell surface charge through salt addition. However, the weakness of the cell adsorption to the bubbles, taken with the natural surfactant distribution,

iggest an alternate application for the flotation column - surfactant separation. Column flotation would be well suited to remove cells in the tailings and recover surfactant in the foam. Wash water effects could be supplemented by manipulation of the cell surface charge through adjustments to the electrolyte concentration.

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APPENDIX A: Surface Tension Data

Table A.1: Surface Tension of Tap Water with Surfactants APG 600CS and APG 625CS at 21°C

Conc (ppm)	Surface tension (N/m)							
	APG 600				APG 625			
			avg	sdev			avg	sdev
0	0.0723	0.0723	0.0723	0.0000	0.0723	0.0723	0.0723	0.0000
5	0.0491	0.0504	0.0498	0.0009	0.0611	0.0595	0.0603	0.0011
10	0.0471	0.0468	0.0470	0.0002	0.0498	0.0470	0.0484	0.0020
20	0.0441	0.0439	0.0440	0.0001	0.0401	0.0438	0.0420	0.0026
30	0.0390	0.0424	0.0407	0.0024	0.0393	0.0395	0.0394	0.0001
40	0.0349	0.0393	0.0371	0.0031	0.0332	0.0372	0.0352	0.0028
50	0.0361	0.0364	0.0363	0.0002	0.0340	0.0327	0.0334	0.0009
60	0.0360	0.0351	0.0356	0.0006	0.0307	0.0320	0.0314	0.0009
70	0.0352	0.0351	0.0352	0.0001	0.0300	0.0302	0.0301	0.0001
80	0.0331	0.0344	0.0338	0.0009	0.0280	0.0272	0.0276	0.0006
90	0.0324	0.0310	0.0317	0.0010	0.0273	0.0270	0.0272	0.0002
100	0.0321	0.0308	0.0315	0.0009	0.0275	0.0271	0.0273	0.0003

Surface tension as a function of concentration:

ST = 0.02581*exp(-0.33001C) + 0.04643*exp(-0.00420C APG 600CS
ST = 0.02972*exp(-0.11420C) + 0.04294*exp(-0.00501C APG 625CS

Table A.2: Surfactant Enrichment in Tailings

Jw (cm/s)	Run	Feed		Tailings		
		Surface tension (N/m)	Surfactant (ppm)	Surface tension (N/m)	Surfactant (ppm)	Enrichment
0	A1	0.0366	35.0	0.0683	1.3	0.038
		0.0359	37.9	0.0677	1.5	
			36.5		1.4	
	A2	0.0350	42.2	0.0693	0.8	0.040
		0.0356	39.3	0.0650	2.4	
			40.8		1.6	
						avg 0.039
						sdev 0.001
0.0157	A1	0.0366	35.0	0.0662	2.0	0.070
		0.0359	37.9	0.0631	3.1	
			36.5		2.6	
	A2	0.0350	42.2	0.0635	3.0	0.070
		0.0356	39.3	0.0641	2.7	
			40.8		2.9	
						avg 0.070
						sdev 0.000
0.0314	A1	0.0366	35.0	0.0574	5.6	0.126
		0.0359	37.9	0.0619	3.6	
			36.5		4.6	
	A2	0.0350	42.2	0.0619	3.6	0.101
		0.0356	39.3	0.0595	4.6	
			40.8		4.1	
						avg 0.113
						sdev 0.018
0.0629	A1	0.0366	35.0	0.0606	4.1	0.118
		0.0359	37.9	0.0599	4.4	
			36.5		4.3	
	A2	0.0350	42.2	0.0531	8.0	0.165
		0.0356	39.3	0.0578	5.4	
			40.8		6.7	
						avg 0.141
						sdev 0.034

APPENDIX B: Bubble Data for Surfactant Flotations

Table B.1: Bubbly Zone Bubble Shape, Mean Diameter, and Velocity

Surfactant	Jg (cm/s)	Bubble Count	% Ellipsoid	d32 (mm)	V(inf) (cm/s)
APG 600	0.31	1000	66.3	0.76	9.6
	0.63	1000	61.9	1.11	13.5
	0.94	1000	63.9	2.19	21.2
	1.26	800	72.6	2.70	22.9
	1.89	600	76.7	3.79	22.3
	2.52	300	66.3	4.46	21.9
	2.83	300	69.3	4.97	21.7
	3.14	300	74.0	5.38	21.5
APG 625	0.31	1000	70.2	0.71	9.1
	0.63	1000	75.1	0.88	11.2
	0.94	1000	83.0	1.46	16.4
	1.26	1000	59.3	2.15	20.9
	1.89	799	70.5	3.77	21.9
	2.52	600	74.0	5.17	21.2
	2.83	300	82.0	5.59	21.0
	3.14	300	76.3	5.82	21.0

Figure B.1.1. Bubble Diameter Distribution with APG 6000S

Jg = 0.31 cm/s

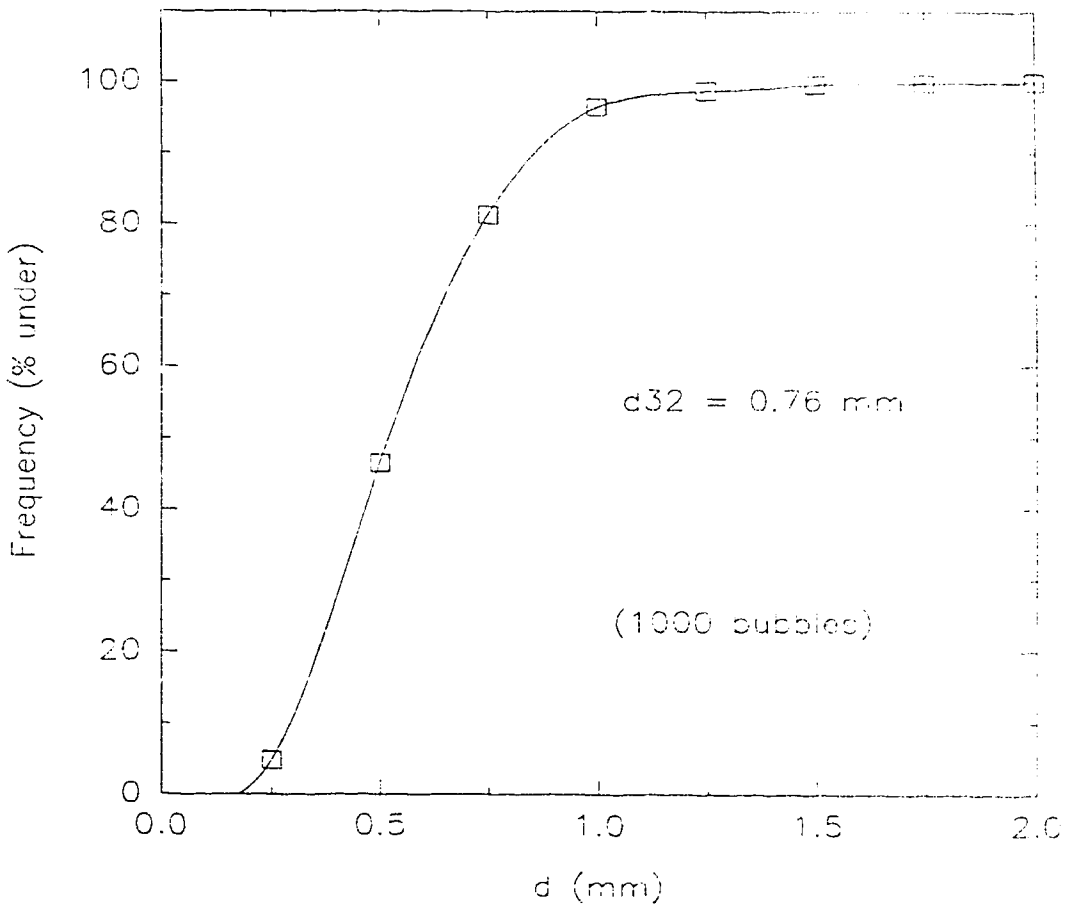


Figure B.1.2: Bubble Diameter Distribution with APG 600CS

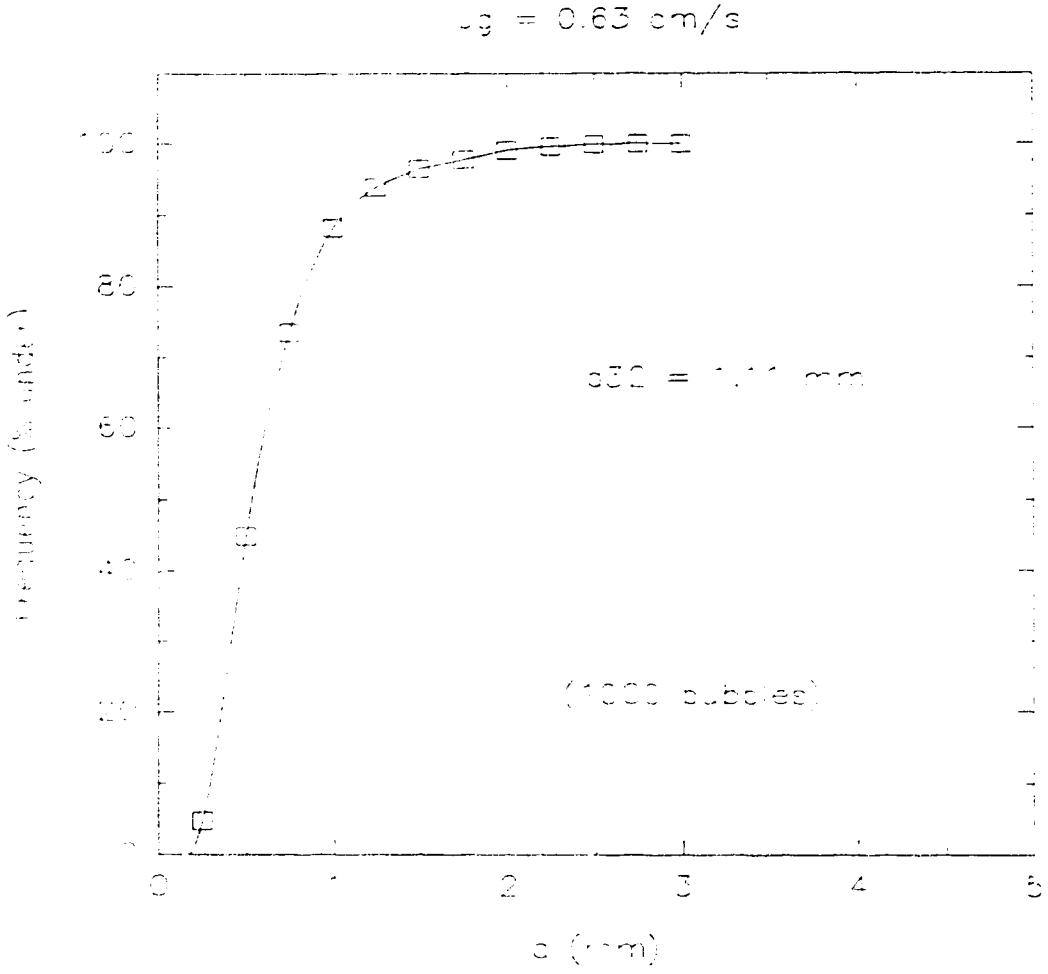


Figure B.1.3: Bubble Diameter Distribution with APG 6000S

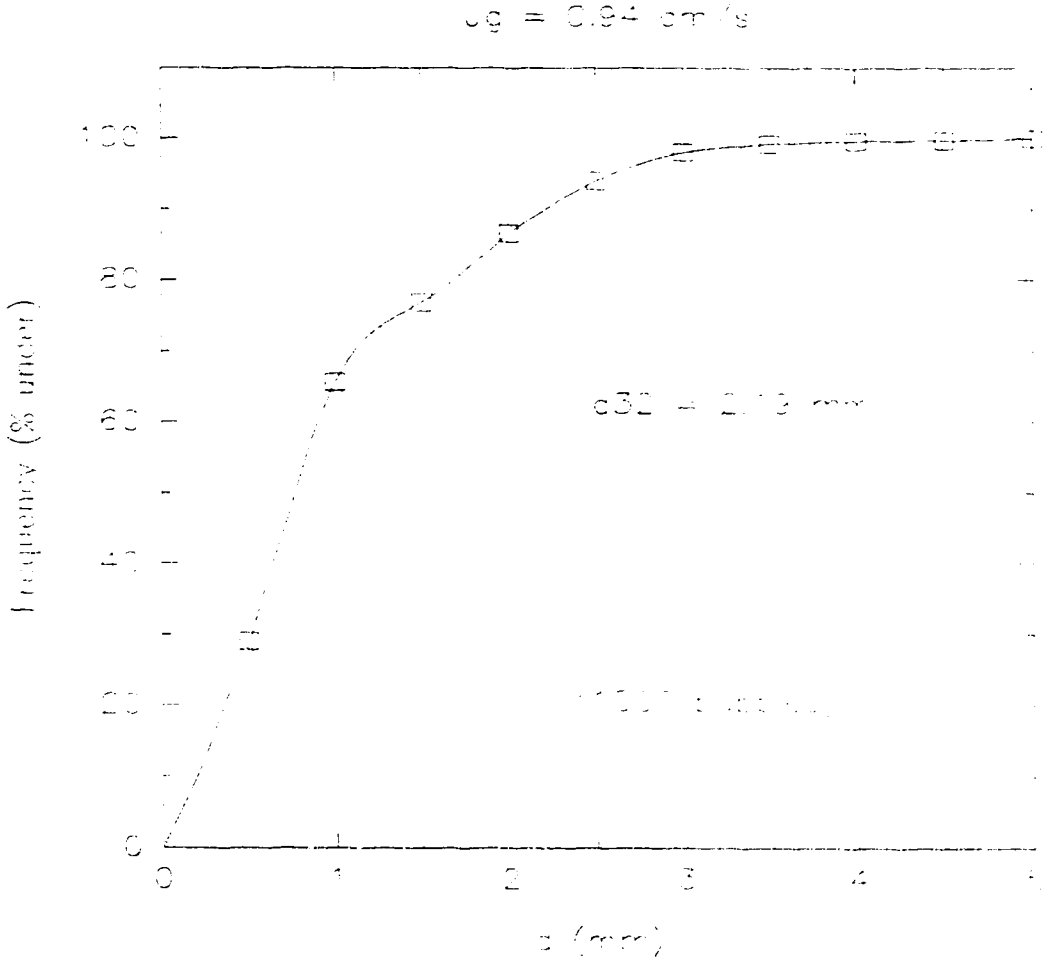


Figure B.14: Bubble Diameter Distribution with APG 600CS

Jg = 1.28 cm/s

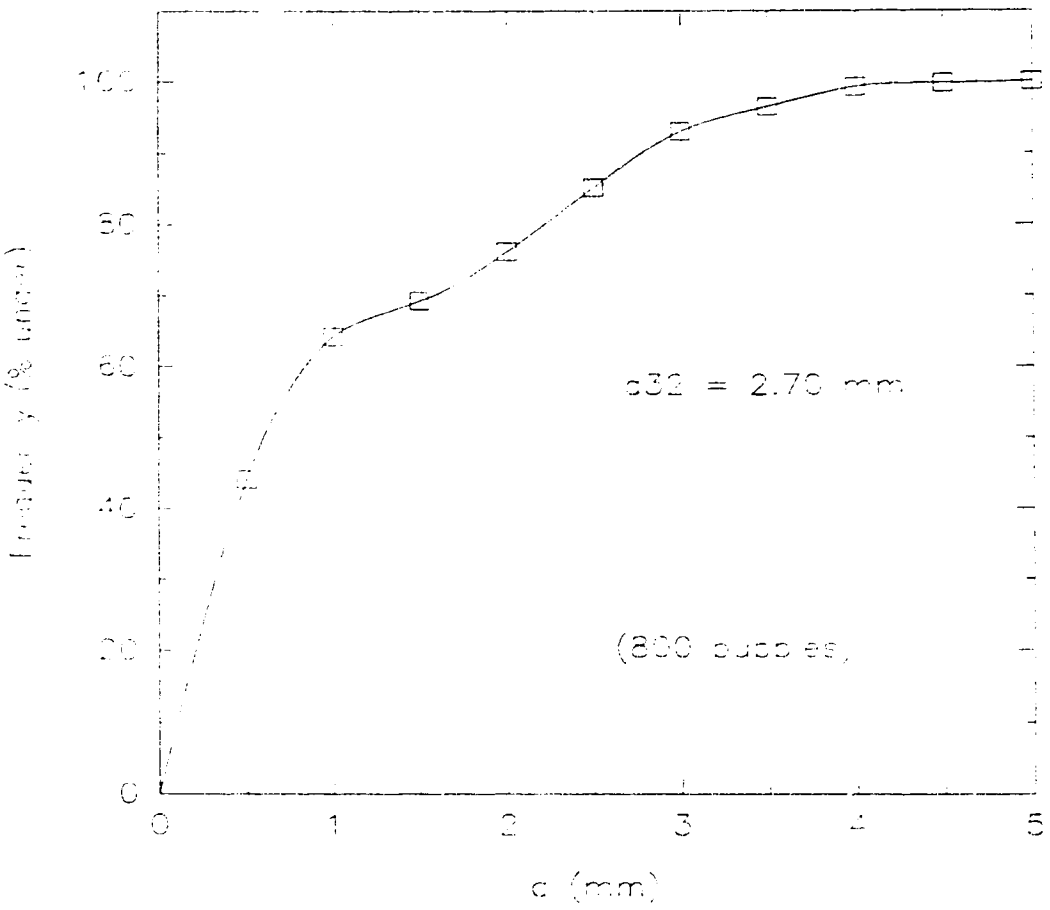


Figure B.1.5: Bubble Diameter Distribution with AFS 80305

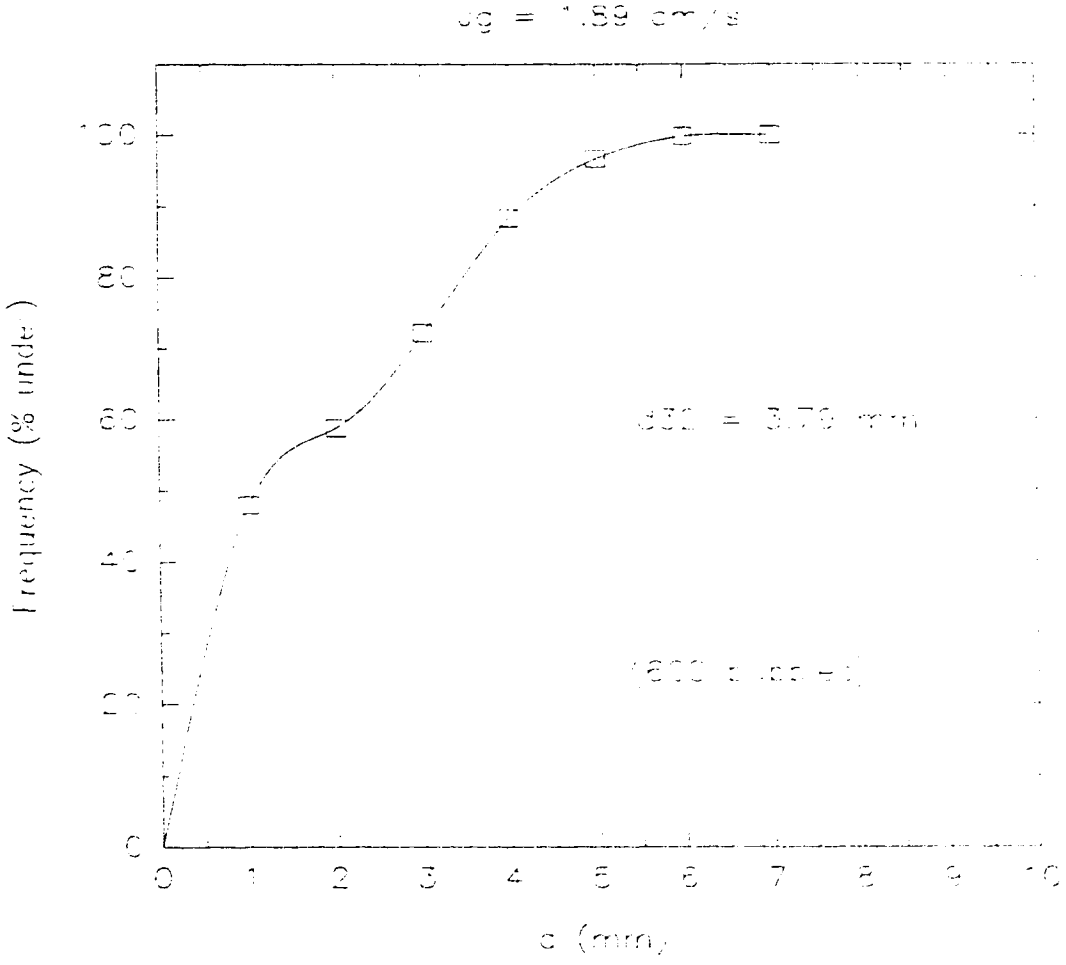


Figure B.1.6: Bubble Diameter Distribution with APG 600CS

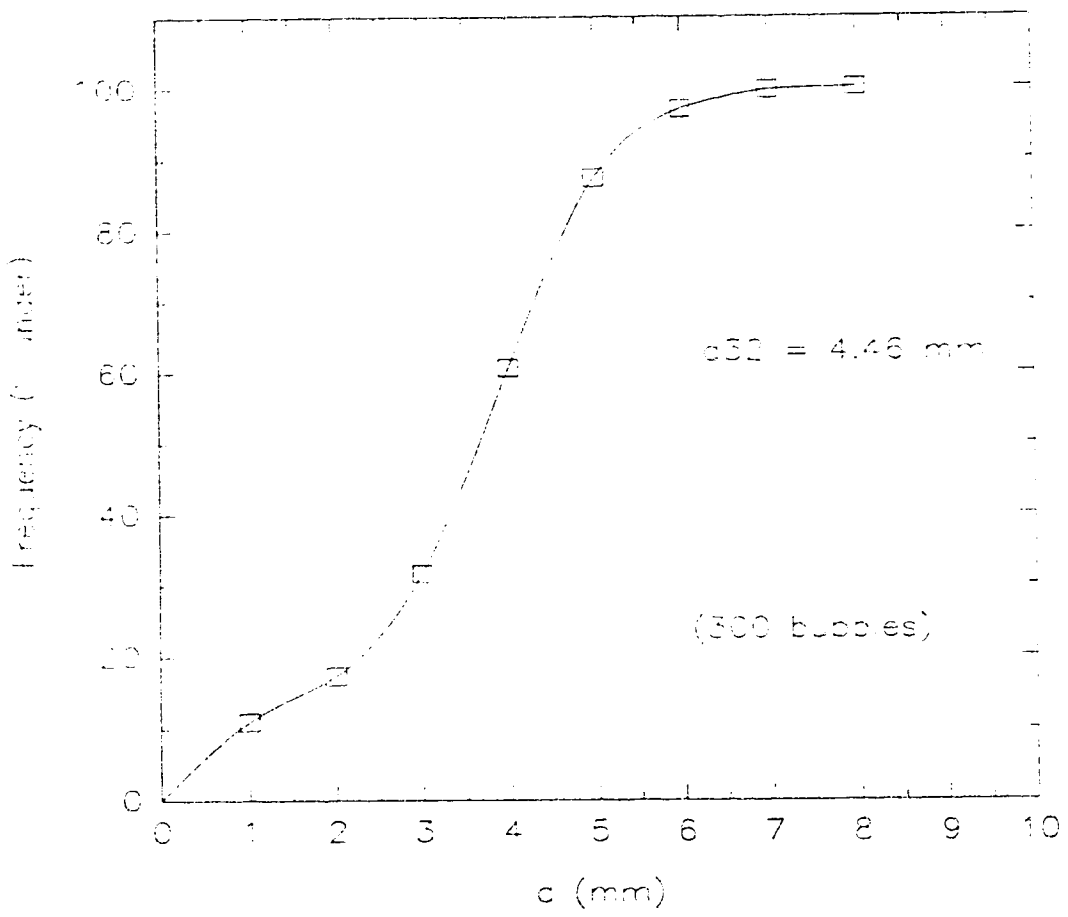


Figure B.1.7: Bubble Diameter Distribution with APG 60003

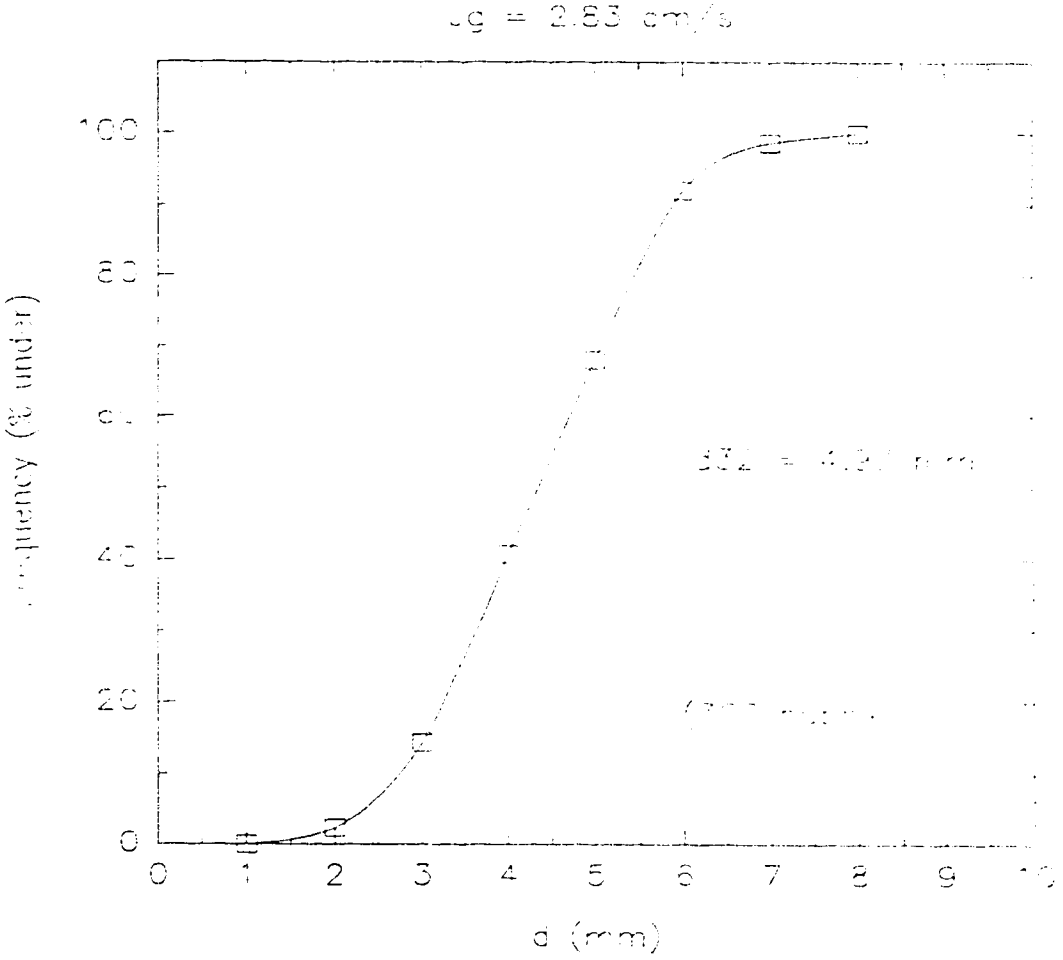


Figure 8.1.8: Bubble Diameter Distribution with APG 6000S

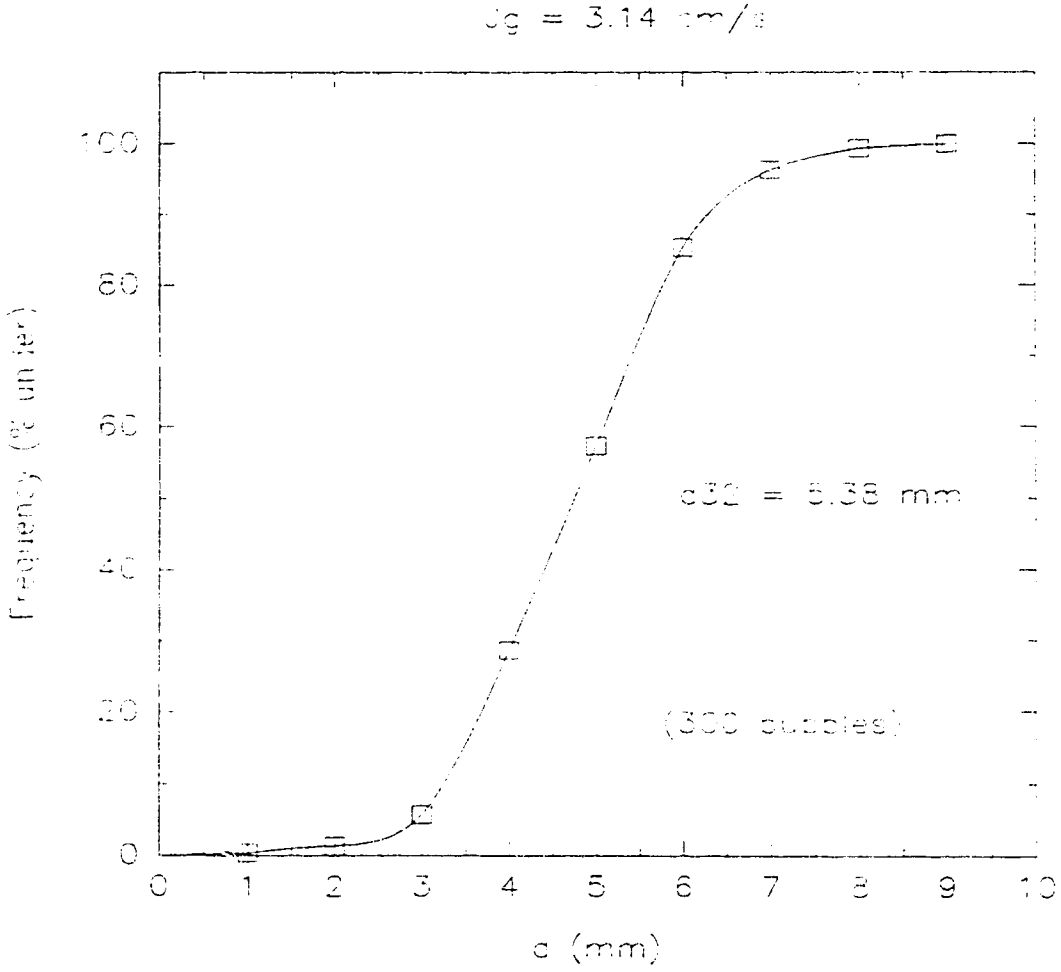


Figure B.2.1: Bubble Diameter Distribution with AR3 [illegible]

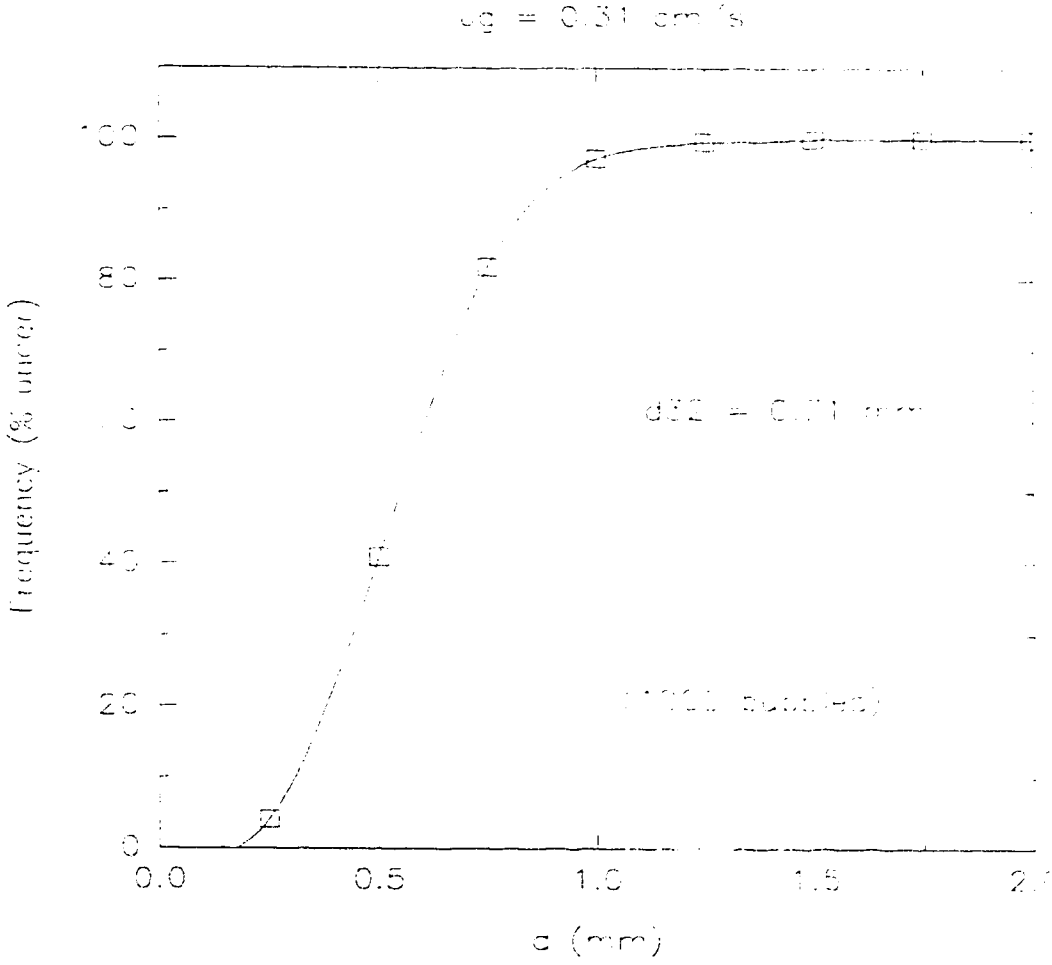


Figure B.2.2: Bubble Diameter Distribution with APG 625CS

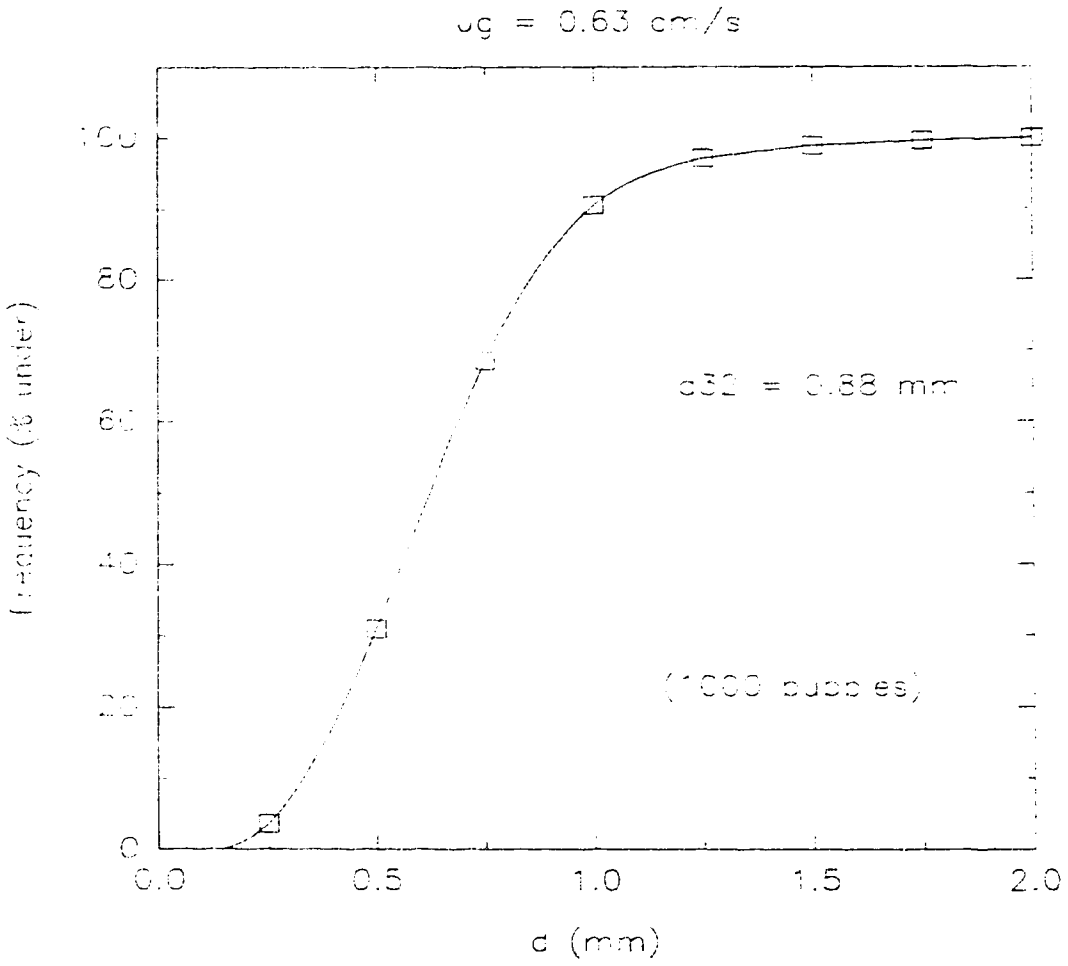


Figure B.2.3: Bubble Diameter Distribution with APG 8250S

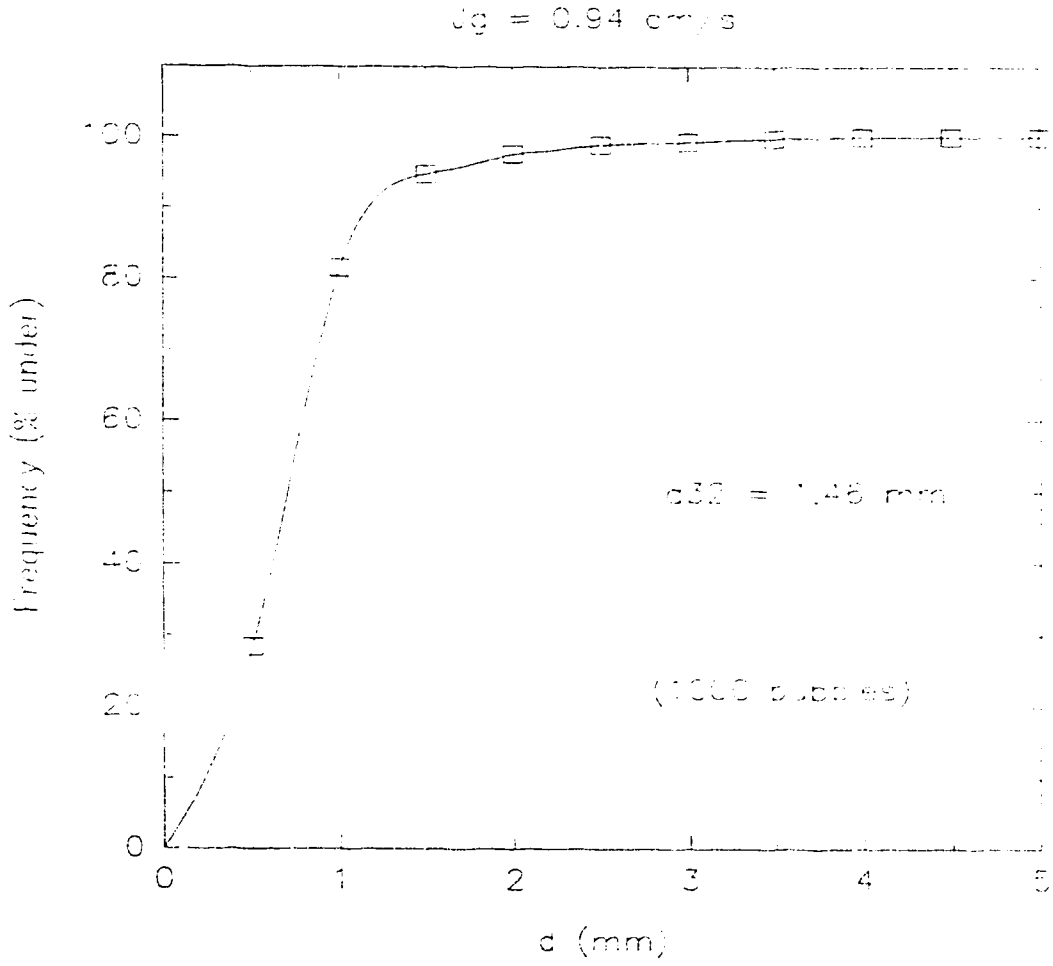


Figure B.2.4: Bubble Diameter Distribution with APG 625CS

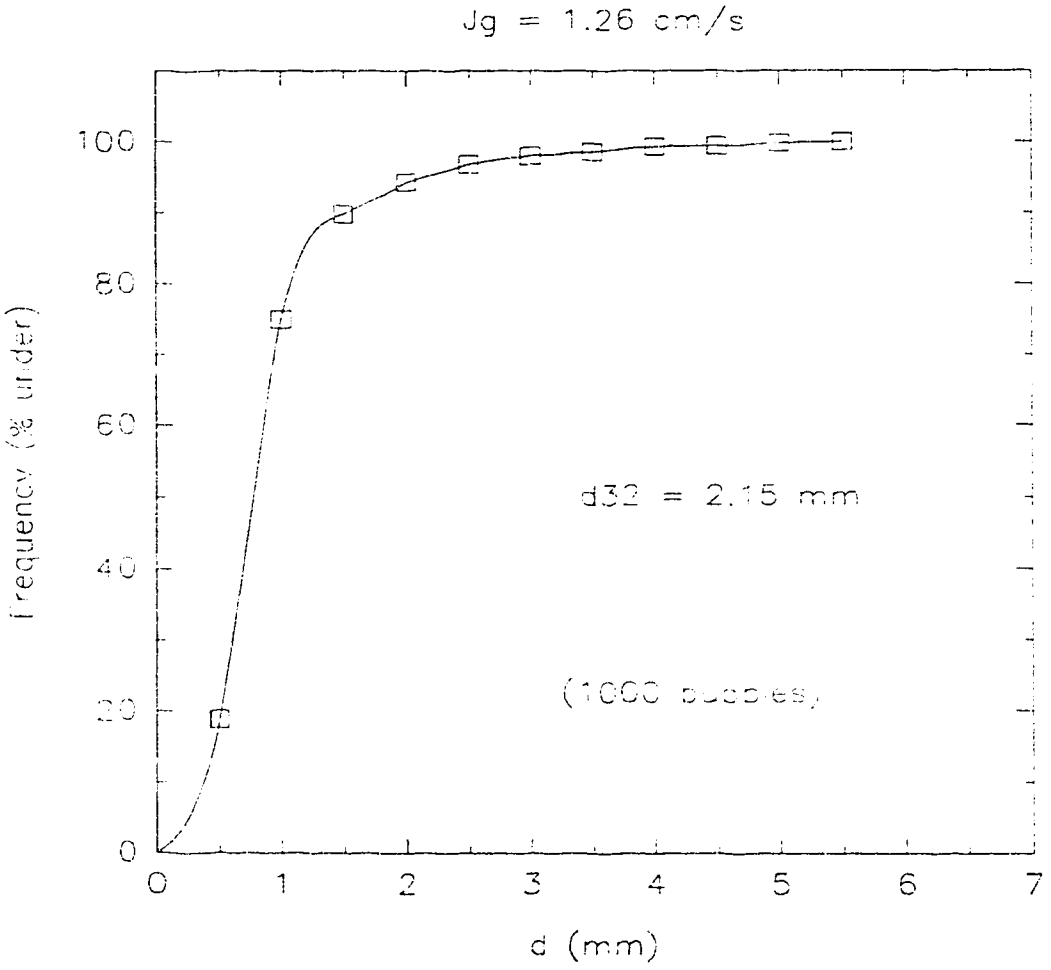


Figure B.2.5: Bubble Diameter Distribution with APG 625CS

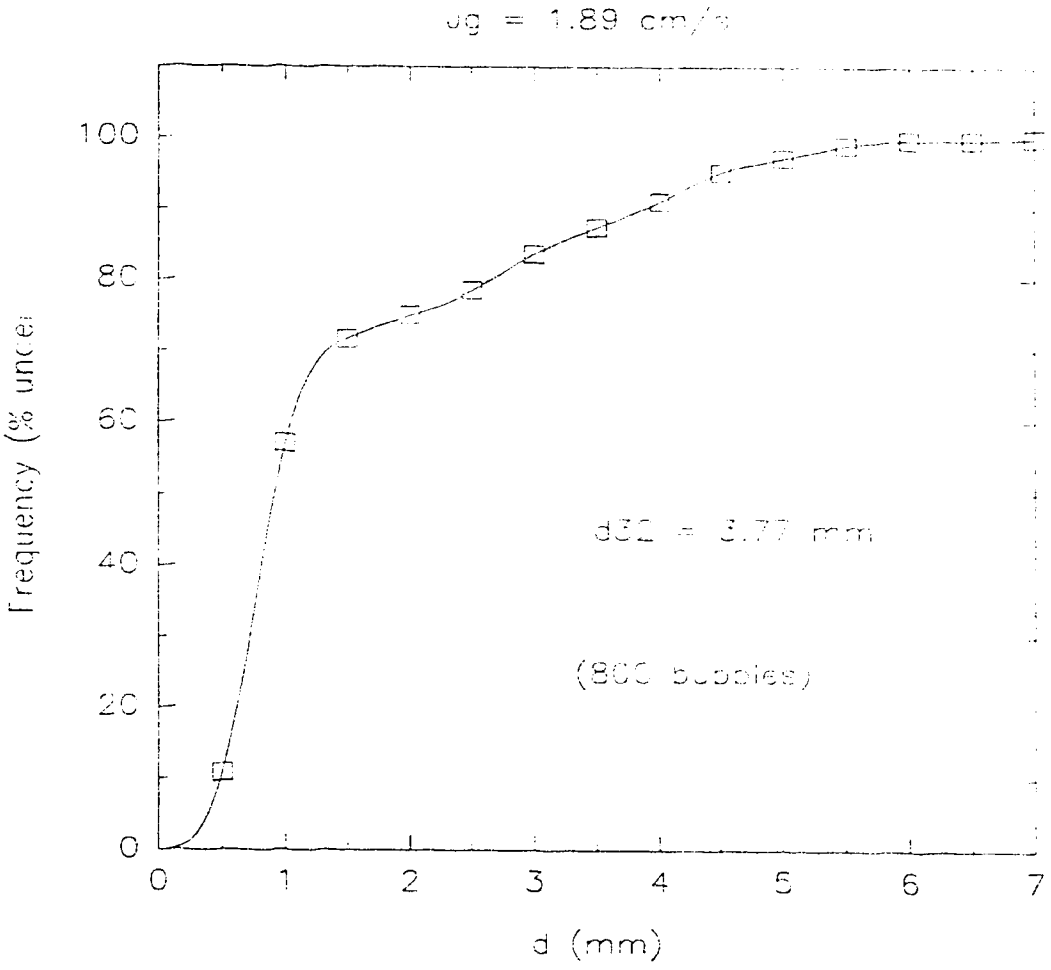


Figure B.2.6: Bubble Diameter Distribution with APC 625CS

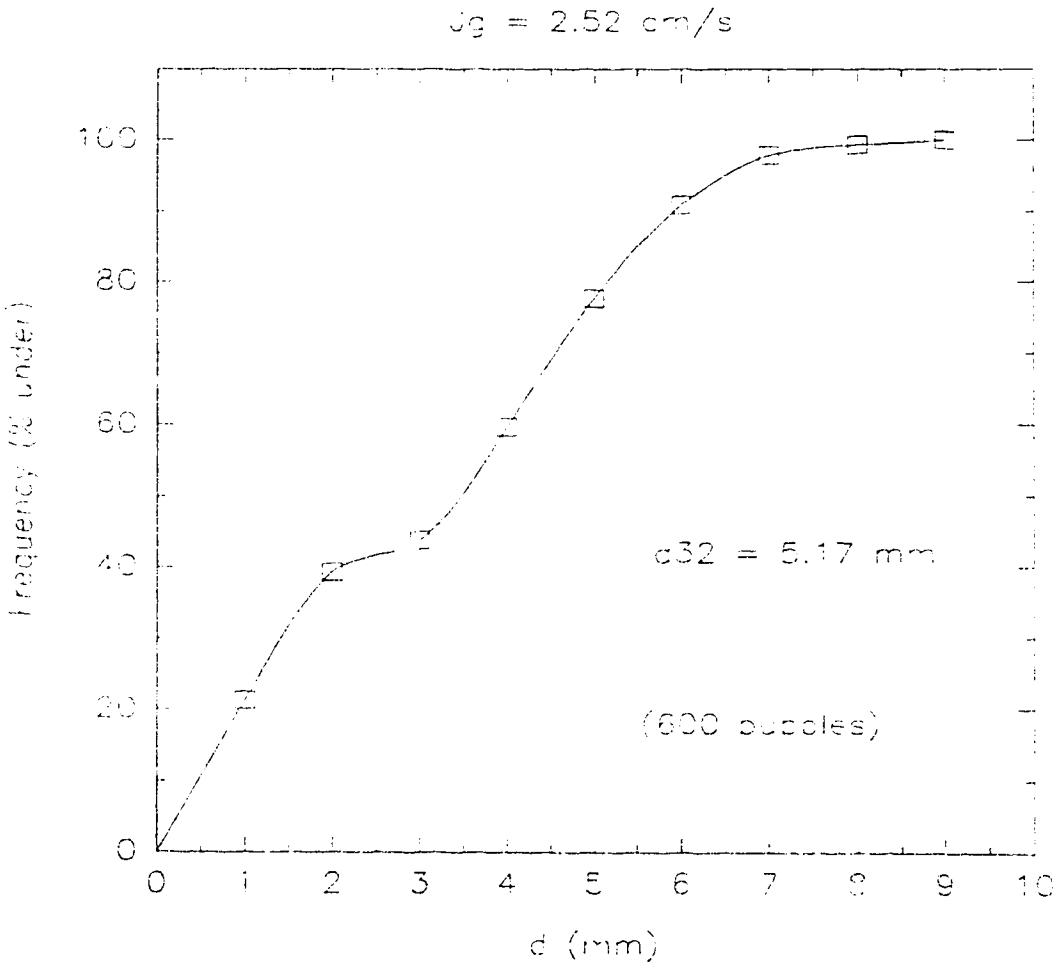


Figure B.2.7: Bubble Diameter Distribution with APG 6250S

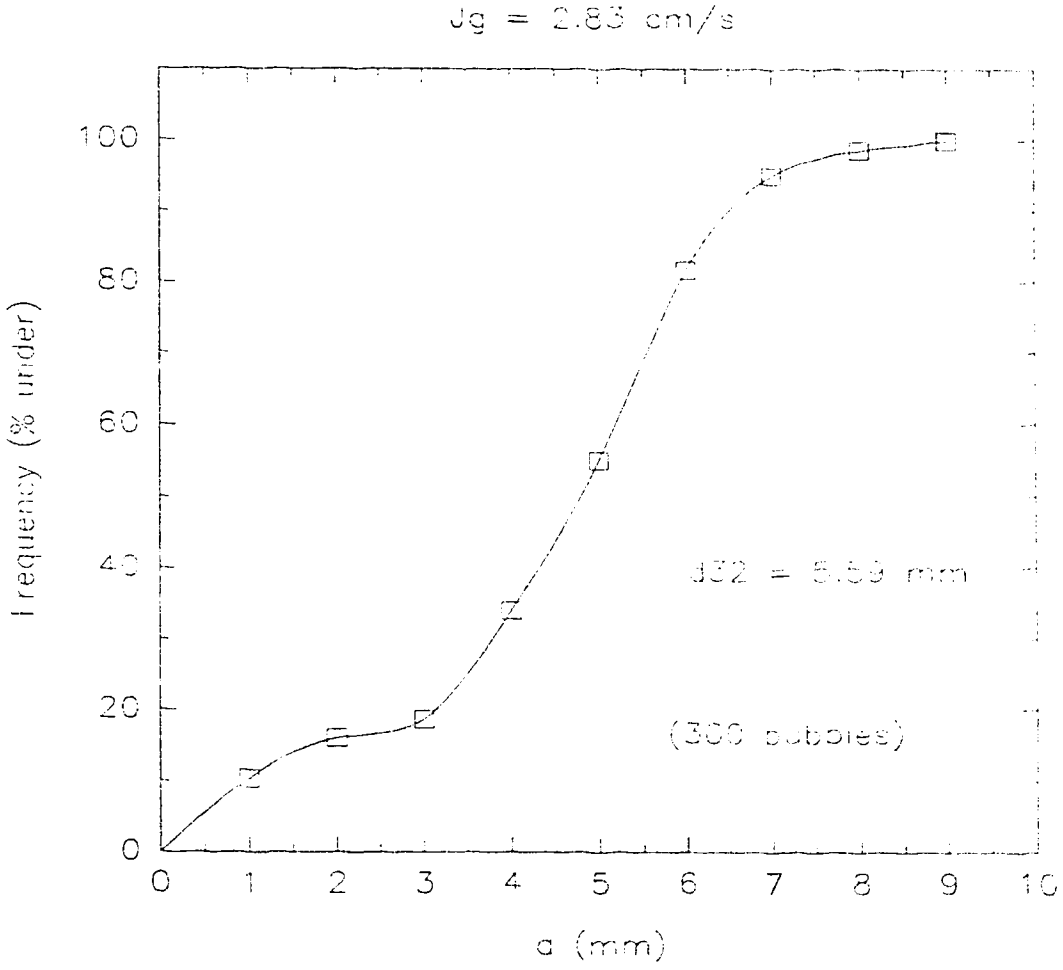
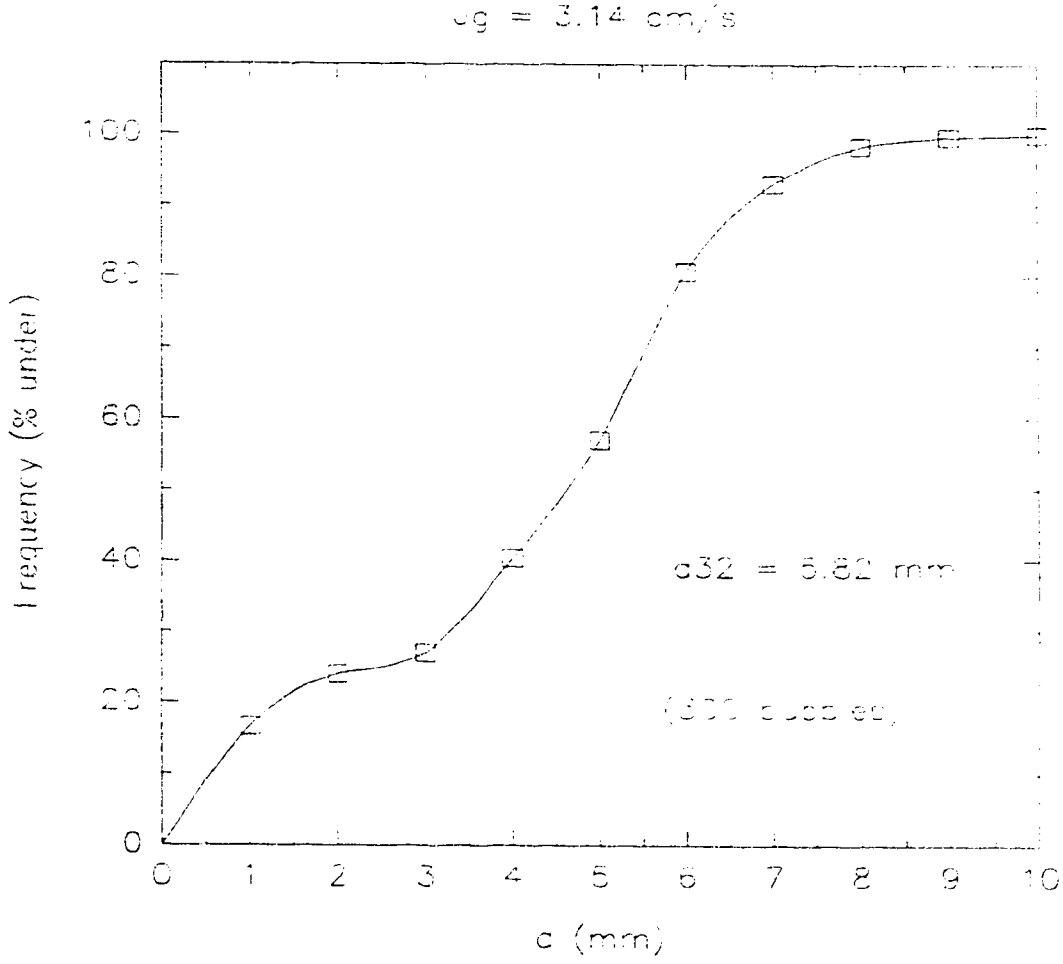


Figure B.2.8: Bubble Diameter Distribution with APG 625CS



APPENDIX C: Hydrodynamic Data for Surfactant Flotations

Table C.1: Bubbly Zone Liquid Flux and Gas Holdup

Surfactant APG 600 CS

Run	Jg (cm/s)	Jw (cm/s)	Vt (mL)	Tt (s)	Qt (mL/s)	Jl (cm/s)		dP (Pa)	gas holdup	
A600-G1-1	0.31	0.063	1000	239.38	4.177		-0.263	1931		0.025
A600-G1-2	0.31	0.063	1000	239.45	4.176		-0.263	1937		0.022
						avg	-0.263		avg	0.023
						sdev	0.000		sdev	0.002
A600-G2-1	0.63	0.063	1000	245.49	4.073		-0.256	1903		0.039
A600-G2-2	0.63	0.063	1000	245.26	4.077		-0.256	1896		0.043
						avg	-0.256		avg	0.041
						sdev	0.000		sdev	0.002
A600-G3-1	0.94	0.063	1000	253.35	3.947		-0.248	1910		0.036
A600-G3-2	0.94	0.063	1000	251.73	3.973		-0.250	1903		0.039
						avg	-0.249		avg	0.037
						sdev	0.001		sdev	0.002
A600-G4-1	1.26	0.063	1000	260.79	3.835		-0.241	1869		0.057
A600-G4-2	1.26	0.063	1000	261.02	3.831		-0.241	1869		0.057
						avg	-0.241		avg	0.057
						sdev	0.000		sdev	0.000
A600-G5-1	1.89	0.063	1000	268.21	3.728		-0.234	1786		0.098
A600-G5-2	1.89	0.063	1000	275.95	3.624		-0.228	1786		0.098
						avg	-0.231		avg	0.098
						sdev	0.005		sdev	0.000
A600-G6-1	2.52	0.063	1000	278.76	3.587		-0.226	1710		0.137
A600-G6-2	2.52	0.063	1000	280.43	3.566		-0.224	1710		0.137
						avg	-0.225		avg	0.137
						sdev	0.001		sdev	0.000
A600-G7-1	2.83	0.063	1000	291.89	3.426		-0.215	1655		0.164
A600-G7-2	2.83	0.063	1000	295.00	3.390		-0.213	1655		0.164
						avg	-0.214		avg	0.164
						sdev	0.002		sdev	0.000
A600-G8-1	3.14	0.063	1000	308.95	3.237		-0.204	1593		0.196
A600-G8-2	3.14	0.063	1000	300.44	3.328		-0.209	1593		0.196
						avg	-0.206		avg	0.196
						sdev	0.004		sdev	0.000

Surfactant APG 625 CS

Run	Jg (cm/s)	Jw (cm/s)	Vt (mL)	Tt (s)	Qt (mL/s)	Jl (cm/s)		dP (Pa)	gas holdup	
A625-G1-1	0.31	0.063	1000	249.29	4.011		-0.252	1924		0.029
A625-G1-2	0.31	0.063	1000	248.74	4.020		-0.253	1937		0.022
						avg	-0.253		avg	0.025
						sdev	0.000		sdev	0.005
A625-G2-1	0.63	0.063	1000	257.23	3.888		-0.244	1903		0.039
A625-G2-2	0.63	0.063	1000	258.86	3.863		-0.243	1896		0.043
						avg	-0.244		avg	0.041
						sdev	0.001		sdev	0.002
A625-G3-1	0.94	0.063	1000	268.20	3.729		-0.234	1903		0.039
A625-G3-2	0.94	0.063	1000	266.77	3.749		-0.236	1896		0.043
						avg	-0.235		avg	0.041
						sdev	0.001		sdev	0.002
A625-G4-1	1.26	0.063	1000	275.24	3.633		-0.228	1855		0.064
A625-G4-2	1.26	0.063	1000	278.52	3.590		-0.226	1855		0.064
						avg	-0.227		avg	0.064
						sdev	0.002		sdev	0.000
A625-G5-1	1.89	0.063	1000	281.40	3.554		-0.223	1793		0.097
A625-G5-2	1.89	0.063	1000	280.99	3.559		-0.224	1786		0.098
						avg	-0.224		avg	0.097
						sdev	0.000		sdev	0.002
A625-G6-1	2.52	0.063	1000	293.90	3.403		-0.214	1703		0.140
A625-G6-2	2.52	0.063	1000	290.11	3.447		-0.217	1689		0.147
						avg	-0.215		avg	0.144
						sdev	0.002		sdev	0.005
A625-G7-1	2.83	0.063	1000	313.31	3.192		-0.201	1627		0.178
A625-G7-2	2.83	0.063	1000	315.33	3.171		-0.199	1620		0.182
						avg	-0.200		avg	0.180
						sdev	0.001		sdev	0.002
A625-G8-1	3.14	0.063	1000	328.80	3.041		-0.191	1572		0.206
A625-G8-2	3.14	0.063	1000	342.84	2.917		-0.183	1565		0.210
						avg	-0.187		avg	0.208
						sdev	0.006		sdev	0.002

Table C.2: Bubbly Flow Regime Verification

Jg (cm/s)	Bubbly Zone Gas Holdup			
	APG 600CS		APG 625CS	
	avg	sdev	avg	sdev
0.31	0.023	0.002	0.025	0.005
0.63	0.041	0.002	0.041	0.002
0.94	0.037	0.002	0.041	0.002
1.26	0.057	0.000	0.064	0.000
1.89	0.098	0.000	0.097	0.002
2.52	0.137	0.000	0.144	0.005
2.83	0.164	0.000	0.180	0.002
3.14	0.196	0.000	0.208	0.002

Table C.3: Foam Zone Liquid Flux and Gas Holdup

Surfactant APG 600 CS

Run	Jg (cm/s	Jw (cm/s)	Vc (mL)	Tc (s)	Qc (mL/s	Jl (cm/s)		dP (psi)		avg	dP (Pa)	gas holdup	
A600-G1-	0.31	0.063	25.5	212.41	0.120	-0.055		0.0433	0.0435	0.0434	299	0.796	
A600-G1-	0.31	0.063	23.0	201.28	0.114	-0.056		0.0446	0.0439	0.0443	305	0.846	
						avg	-0.056					avg	0.821
						sdev	0.000					sdev	0.035
A600-G2-	0.63	0.063	45.5	209.45	0.217	-0.049		0.0361	0.0355	0.0358	247	0.875	
A600-G2-	0.63	0.063	46.5	223.38	0.208	-0.050		0.0358	0.0359	0.0359	247	0.875	
						avg	-0.050					avg	0.875
						sdev	0.000					sdev	0.000
A600-G3-	0.94	0.063	45.0	206.21	0.218	-0.049		0.0262	0.0263	0.0263	181	0.909	
A600-G3-	0.94	0.063	45.0	203.41	0.221	-0.049		0.0259	0.0261	0.0260	179	0.909	
						avg	-0.049					avg	0.909
						sdev	0.000					sdev	0.000
A600-G4-	1.26	0.063	44.5	186.02	0.239	-0.048		0.0195	0.0200	0.0198	136	0.931	
A600-G4-	1.26	0.063	33.5	149.88	0.224	-0.049		0.0200	0.0197	0.0199	137	0.931	
						avg	-0.048					avg	0.931
						sdev	0.001					sdev	0.000
A600-G5-	1.89	0.063	44.5	184.41	0.241	-0.048		0.0154	0.0155	0.0155	107	0.946	
A600-G5-	1.89	0.063	59.5	176.34	0.337	-0.042		0.0156	0.0158	0.0157	108	0.945	
						avg	-0.045					avg	0.946
						sdev	0.004					sdev	0.001
A600-G6-	2.52	0.063	102.5	210.87	0.486	-0.032		0.0138	0.0142	0.0140	97	0.951	
A600-G6-	2.52	0.063	102.0	212.00	0.481	-0.033		0.0141	0.0145	0.0143	99	0.950	
						avg	-0.032					avg	0.951
						sdev	0.000					sdev	0.001
A600-G7-	2.83	0.063	166.0	254.55	0.652	-0.022		0.0174	0.0169	0.0172	118	0.940	
A600-G7-	2.83	0.063	160.0	250.49	0.639	-0.023		0.0173	0.0166	0.0170	117	0.941	
						avg	-0.022					avg	0.941
						sdev	0.001					sdev	0.001
A600-G8-	3.14	0.063	165.0	206.95	0.797	-0.013		0.0178	0.0178	0.0178	123	0.938	
A600-G8-	3.14	0.063	147.0	214.20	0.686	-0.020		0.0157	0.0164	0.0161	111	0.944	
						avg	-0.016					avg	0.941
						sdev	0.005					sdev	0.004

Surfactant APG 625CS

Run	Jg (cm/s	Jw (cm/s)	Vc (mL)	Tc (s)	Qc (mL/s	Jl (cm/s)		dP (psi)			dP (Pa)	gas holdup	
										avg			
A625-G1-	0.31	0.063	31.0	96.63	0.321		-0.043	0.0477	0.0485	0.0481	332		0.774
A625-G1-	0.31	0.063	25.5	105.06	0.243		-0.048	0.0477	0.0488	0.0483	333		0.832
						avg	-0.045					avg	0.803
						sdev	0.003					sdev	0.041
A625-G2-	0.63	0.063	25.5	72.64	0.351		-0.041	0.0392	0.0389	0.0391	269		0.864
A625-G2-	0.63	0.063	26.0	71.38	0.364		-0.040	0.0388	0.0392	0.0390	269		0.864
						avg	-0.040					avg	0.864
						sdev	0.001					sdev	0.000
A625-G3-	0.94	0.063	19.0	51.47	0.369		-0.040	0.0315	0.0313	0.0314	217		0.891
A625-G3-	0.94	0.063	50.0	135.41	0.369		-0.040	0.0310	0.0308	0.0309	213		0.892
						avg	-0.040					avg	0.892
						sdev	0.000					sdev	0.001
A625-G4-	1.26	0.063	25.0	66.02	0.379		-0.039	0.0223	0.0225	0.0224	154		0.922
A625-G4-	1.26	0.063	21.5	57.56	0.374		-0.039	0.0219	0.0222	0.0221	152		0.923
						avg	-0.039					avg	0.923
						sdev	0.000					sdev	0.001
A625-G5-	1.89	0.063	32.5	54.96	0.591		-0.026	0.0179	0.0177	0.0178	123		0.938
A625-G5-	1.89	0.063	40.0	70.27	0.569		-0.027	0.0165	0.0157	0.0161	111		0.944
						avg	-0.026					avg	0.941
						sdev	0.001					sdev	0.004
A625-G6-	2.52	0.063	35.0	56.51	0.619		-0.024	0.0159	0.0147	0.0153	105		0.947
A625-G6-	2.52	0.063	37.0	54.63	0.677		-0.020	0.0151	0.0148	0.0150	103		0.948
						avg	-0.022					avg	0.948
						sdev	0.003					sdev	0.001
A625-G7-	2.83	0.063	131.5	147.70	0.890		-0.007	0.0168	0.0176	0.0172	119		0.940
A625-G7-	2.83	0.063	49.5	53.84	0.919		-0.005	0.0174	0.0175	0.0175	120		0.939
						avg	-0.006					avg	0.940
						sdev	0.001					sdev	0.001
A625-G8-	3.14	0.063	94.0	60.51	1.553		0.035	0.0191	0.0188	0.0190	131		0.934
A625-G8-	3.14	0.063	78.5	69.38	1.131		0.008	0.0184	0.0186	0.0185	128		0.936
						avg	0.022					avg	0.935
						sdev	0.019					sdev	0.001

Table C.4: Foam Zone Bubble Velocity

Surfactant APG 600 CS

Jg	H	T				V(inf) (cm/s)					
(cm/s)	(cm)	(s)								avg	sdev
0.31	30.0	6.00	6.02	6.29	6.24	5.0	5.0	4.8	4.8	4.9	0.1
0.63	30.0	5.11	4.96	4.97	4.79	5.9	6.0	6.0	6.3	6.1	0.2
0.94	30.0	2.89	3.20	3.02	2.99	10.4	9.4	9.9	10.0	9.9	0.4
1.26	30.0	1.92	1.98	2.07	2.08	15.6	15.2	14.5	14.4	14.9	0.6
1.89	30.0	1.16	1.32	1.28	1.23	25.9	22.7	23.4	24.4	24.1	1.4
2.52	30.0	1.01	1.07	1.08	1.17	29.7	28.0	27.8	25.6	27.8	1.7
2.83	35.0	1.13	1.23	1.10	1.13	31.0	28.5	31.8	31.0	30.6	1.5
3.14	35.0	1.11	1.07	1.07	1.11	31.5	32.7	32.7	31.5	32.1	0.7

Surfactant APG 625 CS

Jg	H	T				V(inf) (cm/s)					
(cm/s)	(cm)	(s)								avg	sdev
0.31	30.0	5.65	5.62	5.24	4.97	5.3	5.3	5.7	6.0	5.6	0.3
0.63	30.0	4.66	4.39	4.68	4.40	6.4	6.8	6.4	6.8	6.6	0.2
0.94	30.0	3.18	3.15	2.76	2.99	9.4	9.5	10.9	10.0	10.0	0.7
1.26	30.0	2.06	2.05	2.05	2.03	14.6	14.6	14.6	14.8	14.7	0.1
1.89	30.0	1.17	1.15	1.18	1.17	25.6	26.1	25.4	25.6	25.7	0.3
2.52	30.0	1.00	1.00	1.04	0.99	30.0	30.0	28.8	30.3	29.8	0.6
2.83	35.0	1.02	1.04	1.01	1.04	34.3	33.7	34.7	33.7	34.1	0.5
3.14	35.0	1.03	1.00	1.00	1.01	34.0	35.0	35.0	34.7	34.7	0.5

Table C.5: Volumetric Balance on Flotation Column for Hydrodynamic Runs

Surfactant APG 600CS

Run	Inlet			Outlet			Diff
	Qf (mL/s)	Qw (mL/s)	Q(in) (mL/s)	Qt (mL/s)	Qc (mL/s)	Q(out) (mL/s)	
A600-G1-1	3.333	1.000	4.333	4.177	0.120	4.297	-0.8%
A600-G1-2	3.333	1.000	4.333	4.176	0.114	4.291	-1.0%
A600-G2-1	3.333	1.000	4.333	4.073	0.217	4.290	-1.0%
A600-G2-2	3.333	1.000	4.333	4.077	0.208	4.286	-1.1%
A600-G3-1	3.333	1.000	4.333	3.947	0.218	4.165	-3.9%
A600-G3-2	3.333	1.000	4.333	3.973	0.221	4.194	-3.2%
A600-G4-1	3.333	1.000	4.333	3.835	0.239	4.074	-6.0%
A600-G4-2	3.333	1.000	4.333	3.831	0.224	4.055	-6.4%
A600-G5-1	3.333	1.000	4.333	3.728	0.241	3.970	-8.4%
A600-G5-2	3.333	1.000	4.333	3.624	0.337	3.961	-8.6%
A600-G6-1	3.333	1.000	4.333	3.587	0.486	4.073	-6.0%
A600-G6-2	3.333	1.000	4.333	3.566	0.481	4.047	-6.6%
A600-G7-1	3.333	1.000	4.333	3.426	0.652	4.078	-5.9%
A600-G7-2	3.333	1.000	4.333	3.390	0.639	4.029	-7.0%
A600-G8-1	3.333	1.000	4.333	3.237	0.797	4.034	-6.9%
A600-G8-2	3.333	1.000	4.333	3.328	0.686	4.014	-7.4%

Surfactant APG 625CS

Run	Inlet			Outlet			Diff
	Qf (mL/s)	Qw (mL/s)	Q(in) (mL/s)	Qt (mL/s)	Qc (mL/s)	Q(out) (mL/s)	
A625-G1-1	3.333	1.000	4.333	4.011	0.321	4.332	0.0%
A625-G1-2	3.333	1.000	4.333	4.020	0.243	4.263	-1.6%
A625-G2-1	3.333	1.000	4.333	3.888	0.351	4.239	-2.2%
A625-G2-2	3.333	1.000	4.333	3.863	0.364	4.227	-2.4%
A625-G3-1	3.333	1.000	4.333	3.729	0.369	4.098	-5.4%
A625-G3-2	3.333	1.000	4.333	3.749	0.369	4.118	-5.0%
A625-G4-1	3.333	1.000	4.333	3.633	0.379	4.012	-7.4%
A625-G4-2	3.333	1.000	4.333	3.590	0.374	3.964	-8.5%
A625-G5-1	3.333	1.000	4.333	3.554	0.591	4.145	-4.3%
A625-G5-2	3.333	1.000	4.333	3.559	0.569	4.128	-4.7%
A625-G6-1	3.333	1.000	4.333	3.403	0.619	4.022	-7.2%
A625-G6-2	3.333	1.000	4.333	3.447	0.677	4.124	-4.8%
A625-G7-1	3.333	1.000	4.333	3.192	0.890	4.082	-5.8%
A625-G7-2	3.333	1.000	4.333	3.171	0.919	4.091	-5.6%
A625-G8-1	3.333	1.000	4.333	3.041	1.553	4.594	6.0%
A625-G8-2	3.333	1.000	4.333	2.917	1.131	4.048	-6.6%

Table C.6: Richardson-Zaki Fit of Hydrodynamic Data

Surfactant APG 600 CS

Zone	Jg (cm/s)	Jl (cm/s)	Jgl (cm/s)	V(inf) (cm/s)	gas holdup	Jgl/V(inf)
Bubbly	0.31	-0.26	0.31	9.6	0.023	0.033
	0.63	-0.26	0.61	13.5	0.041	0.046
	0.94	-0.25	0.92	21.2	0.037	0.043
	1.26	-0.24	1.20	22.9	0.057	0.052
	1.89	-0.23	1.72	22.3	0.098	0.077
	2.52	-0.23	2.20	21.9	0.137	0.100
	2.83	-0.21	2.40	21.7	0.164	0.110
	3.14	-0.21	2.57	21.5	0.196	0.119
Foam	0.31	-0.06	0.10	4.9	0.821	0.021
	0.63	-0.05	0.12	6.1	0.875	0.020
	0.94	-0.05	0.13	9.9	0.909	0.013
	1.26	-0.05	0.13	14.9	0.931	0.009
	1.89	-0.04	0.14	24.1	0.946	0.006
	2.52	-0.03	0.15	27.8	0.951	0.006
	2.83	-0.02	0.19	30.5	0.941	0.006
	3.14	0.02	0.20	32.1	0.941	0.006

Surfactant APG 625 CS

Zone	Jg (cm/s)	Jl (cm/s)	Jgl (cm/s)	V(inf) (cm/s)	gas holdup	Jgl/V(inf)
Bubbly	0.31	-0.25	0.31	9.1	0.025	0.034
	0.63	-0.24	0.61	11.2	0.041	0.055
	0.94	-0.24	0.91	16.4	0.041	0.056
	1.26	-0.23	1.19	20.9	0.064	0.057
	1.89	-0.22	1.73	21.9	0.097	0.079
	2.52	-0.22	2.19	21.2	0.144	0.103
	2.83	-0.20	2.36	21.0	0.180	0.112
	3.14	-0.19	2.53	21.0	0.208	0.121
Foam	0.31	-0.05	0.10	5.6	0.803	0.018
	0.63	-0.04	0.12	6.6	0.864	0.018
	0.94	-0.04	0.14	10.0	0.892	0.014
	1.26	-0.04	0.13	14.7	0.923	0.009
	1.89	-0.03	0.14	25.7	0.941	0.005
	2.52	-0.02	0.15	29.8	0.948	0.005
	2.83	-0.01	0.18	34.1	0.940	0.005
	3.14	0.02	0.18	34.7	0.935	0.005

APPENDIX D: Yeast Standard Curve

Table D.1: Bakers' Yeast Standard Curve Data

Concentration (mg/L)	Absorbance at 600 nm			Fit
25	0.048	0.048	0.052	0.054
50	0.101	0.103	0.103	0.107
75	0.154	0.149	0.152	0.159
100	0.208	0.200	0.205	0.210
150	0.309	0.303	0.309	0.308
200	0.404	0.404	0.403	0.401
250	0.495	0.496	0.491	0.490
300	0.577	0.579	0.578	0.573
350	0.653	0.657	0.655	0.652
400	0.724	0.726	0.720	0.727
450	0.794	0.794	0.795	0.796
500	0.860	0.861	0.858	0.861

Concentration as a function of A(600nm):

$$C = A600 * [\ 171.782\ A600+428.751)\ ]$$

Figure D.1: Bakers' Yeast Standard Curve