

**Computer Simulation of the Rupture of a Gas Bubble at a Gas-Liquid Interface and its Implications in Animal Cell Damage.**

M. A. Garcia-Briones<sup>1</sup>, R. S. Brodkey<sup>1</sup>, and J. J. Chalmers<sup>1,2</sup>

<sup>1</sup> Department of Chemical Engineering, Ohio State University, 140 W. 19th Ave, Columbus, OH 43210.

<sup>2</sup> Corresponding Author

**Abstract**

Hydrodynamic information of the flow occurring as a bubble breaks at a gas liquid interface has been obtained from computer simulations. The transient rate of energy dissipation per unit volume in the region close to the bubble interface has been calculated. It was found that when a bubble collapses, there is a very high, localized energy dissipation which can be used to explain animal cell damage in sparged suspended animal cell cultures. The rate of energy dissipation was found to be a strong function of bubble size, increasing rapidly as the bubble size decreases.

**Key words**

Bubble breakup, animal cells, stress, energy dissipation

## Introduction

The number of products and product applications derived from the *in vitro* culture of animal cells has increased in the last few years. As a result of this expansion the need of systematic methods to scale up production processes are considered a major priority. The traditional system used to grow animal cells in suspended cultures at small scales is the agitated bioreactor. Some of the advantages associated with this system are homogeneous conditions and simple control of operation parameters such as temperature, pH, oxygen and nutrient concentration. As the bioreactors are scaled up, the supply of oxygen by simple diffusion through the gas head space is insufficient; consequently, oxygen transfer is enhanced by injection of air or oxygen into the liquid phase. This operation, however, has been observed to result in cell damage. Experimental observations reported by different research groups have suggested that cell damage occurs at the region of bubble disengagement (Handa *et al.*, 1987; Handa-Corrigan *et al.*, 1989; Tramper *et al.*, 1986; Tramper *et al.*, 1987; Jobses *et al.*, 1991; Kunas and Papoutsakis, 1990; Oh *et al.*, 1989; Oh *et al.*, 1992; Garcia-Briones and Chalmers, 1992; Chalmers *et al.*, 1990; Chalmers and Bavarian, 1991; Bavarian and Chalmers, 1991). A thorough understanding of the events at the liquid interface is needed if a systematic scale-up method is to be developed. In this report, hydrodynamic information obtained from the computer solution of the flow occurring as a bubble breaks has been used to calculate the rate of energy dissipated in the liquid forming the vicinity of the bubble. These results are compared with data from experiments in well defined flow devices and mixed bioreactors reported in the literature. Three bubble sizes are used (0.77, 1.7, and 6.32 mm in diameter) to determine the dependency of the rate of energy dissipation with bubble

size.

### **Software and computer system**

The numerical simulations were performed using the computer program FLOW-3D (Flow Science, Inc., Los Alamos, NM). This code uses a control volume method to produce a transient solution of the fluid conservation laws of mass, momentum and energy. Particular capabilities of FLOW-3D essential for our application are its general free surface tracking method and its incorporation of surface tension forces.

Numerical solutions are obtained using a grid of rectangular control elements of variable size. Location of fluid within this grid is accomplished with the Volume-of-Fluid (VOF) method, which allows for the coalescence and breakup of fluid masses. The basic solution algorithms used in FLOW-3D for incompressible flows are a direct descendent of the Marker-and-Cell (MAC) method developed at the Los Alamos National Laboratory in the early 1960's. The original (VOF) publication (Hirt and Nichols, 1981) offers an excellent overview of the solution methodology used in FLOW-3D. This commercial code has been extensively refined and validated over the past 10 years for a variety of applications. In this report we have explored its ability in solving small scale flows where surface tension forces dominate.

The code was executed on a Cray Y-MP 8/864 supercomputer at the Ohio Supercomputer Center (OSC). The numerical results were plotted using apE. This is a special tool developed at OSC, designed for visualization of scientific and engineering data.

## **Initial conditions**

The initial state of the fluid was specified as the shape of the bubble resting at the liquid interface without the bubble film. The force driving the motion of the liquid close to the cavity wall is dictated by the Laplace equation:

$$\Delta P = \gamma (1/R_1 + 1/R_2) \quad (1)$$

where  $R_1$  and  $R_2$  are the principal radii of curvature. According to Eq. 1 no realistic results can be expected unless an accurate bubble profile is determined.

Toba (1959) solved for the equations defining the shape of floating bubbles. His model is not restricted to gas-liquid interfaces so it applies to any two immiscible systems which may have similar densities. The solution reported by Toba (1959), however, involves semi-graphical methods. Medrow (1968) solved the governing equations proposed by Toba (1959) using a different approach. He developed an analytic solution for the shape of small bubbles and a combination of numerical and trial and error procedures for big bubbles. These solutions were experimentally verified for a broad range of bubble sizes. For details in the derivation of the model used to find the bubble shape as well as the solution methodology the reader is referred to the work reported by Toba (1959) and Medrow (1968). Figure 1 shows the bubble profiles for the bubble sizes used in this work.

## **Problem description**

When a bubble breaks, its film retracts collecting the liquid forming the film so that a toroidal rim develops. The initial condition used in this work is the instant at which the expanding toroidal rim reaches the edge of the cavity wall. At this instant the velocities of

every element of fluid were taken to be zero. This also implies that the upward drift velocity of the bubble (produced by buoyancy forces) was regarded to be zero -i.e. the bubble was at rest at the interface before it ruptured. If we consider the small mass of the bubble film, and the fact that the collected liquid from the film may break into small droplets, the assumption of zero velocity at the edge of the bubble cavity seems to be a reasonable approximation. Because of this initial condition, no information will be provided about the hydrodynamics of the film rupture. Currently there is no experimental evidence that would indicate the individual contributions of the film retraction and bubble cavity collapse to cell damage.

In defining the problem, a uniform pressure above the liquid phase was specified. This condition is justified if one considers that once the bubble film breaks, pressure equilibrium inside the bubble cavity is reached in a few microseconds. The propagation of a pressure wave in air (velocity of sound) can be estimated by the following formula (Brodkey, 1967):

$$c = 49.02 \sqrt{T} \quad (2)$$

where  $c$  is given in feet per second and  $T$  is in  $R$ . If a 1.7 mm diameter bubble in sea water at 65  $F$  is considered, it can be calculated using Eq. 2 that the pressure wave will propagate 2 bubble diameters in  $9.9 \times 10^{-6}$  sec. As it will be shown later, the collapse of this bubble occurs in about  $2.3 \times 10^{-4}$  sec. Then, it can be safely assumed that the propagation of the pressure wave will not have any effect on the evolution of the bubble cavity collapse and that uniform pressure above the liquid phase can be specified.

## Numerical description.

A type of solids modeling capability is used in FLOW 3-D to define the initial fluid region. Regions are defined in terms of general quadratic shapes (e.g., spheres, cones, cylinders, rectangular blocks, etc.) that may be added or subtracted from one another. The intersection of these shapes with the computational grid is computed to determine the initial fractional volume of fluid in each grid cell. The fractional volume of fluid (or fluid fraction) is one for liquid-phase cells and zero for gas-phase cells. Intermediate values between one and zero are assigned for cells at the interface. The quadratic polynomial provided by the code to define general quadratic shapes was extended to include terms of higher order which were required to accurately define the bubble profile.

The problem was solved using cylindrical coordinates assuming symmetry in the  $\theta$  direction. Incompressible Newtonian fluid under isothermal conditions was also assumed. It has been shown that the growth medium used for insect and animal cells is Newtonian and that the cell density normally obtained is sufficiently low to prevent non-Newtonian effects (Goldblum *et al.*, 1990). The outer and bottom boundaries were set to about two times the bubble diameter. For one bubble size (1.7mm diameter bubble) the outer and bottom boundaries were also set at 50 and 100 bubble diameters to assess the influence of the proximity of these boundaries. In all cases the outer and bottom boundaries were specified as fixed hydrostatic pressure boundaries. The top boundary was defined as an output boundary, through which the fluid could freely flow.

In order to improve the numerical results, the mesh was concentrated at the center of the bubble. In this region the greatest rates of change in the flow variables were expected.

Figure 2 shows the grid used for the 1.7 mm diameter bubble where the outer and bottom boundaries were set at 2 bubble diameters apart. The actual azimuthal segment used in the computation is shown in Figure 3.

### **Comparison of the computer solution with actual photographs.**

A natural way to know how close the computer solution resembles the actual process is to compare free surface plots with pictures of the bubble breakup at the same elapsed time. Figure 4 shows a comparison of the computer solution and photographs reported by Woodcock *et al.* (1953). Several factors may contribute to the differences observed in Figure 4 and are discussed below.

**Erratic behavior of the bubble rupture process.** Blanchard and Syzdek (1978) have reported on the erratic nature of the bubble rupture process in regard to drop ejection heights. They indicated that drop ejection heights often vary in the same experiment from one bubble to the next in distilled water where bubbles burst instantly. A possible explanation suggested by these authors is that this erratic behavior may be associated with the exact position of the bubble at the interface when the bursting occurs. The bubble of the photographic experiment shown in Figure 4 was assumed to have come to static equilibrium (zero upward velocity) before rupture. Evidence supporting or disproving this assumption is not available in the reference from which these results were taken. Because of this erratic behavior in drop ejection heights it is plausible to expect an erratic behavior in the stages previous to the jet formation. In other words, the time sequence pictures of two rupturing bubbles of the same size may not be exactly equal. This points out the difficulty in arriving at an accurate judgment on the extent by which the computer simulation results presented here deviate from

the experimental photographs.

**Proximity of the walls and meniscus.** No information is available about the proximity of the walls in the photographs of Figure 4 nor for the exact shape of the free surface (meniscus) before the bubble was injected. In the calculation presented, infinitely removed boundaries (see problem description) and a horizontal free surface (implied in the derivation of the equations governing the bubble shape at equilibrium) were used.

**Influence of the film rupture.** As indicated in the problem description, the hydrodynamics of the film rupture is not considered. Even though we believed that this simplification will not affect the motion of the bubble cavity its exact influence is unknown.

### **Resultant stresses**

Another important aspect of the computer solution is its numerical stability. In particular, we were interested in the effect of grid refinement and proximity of the boundaries on the rate of deformation of the fluid. The change of deformation rates was assessed by looking at the distribution of resultant stresses,  $\tau_{res}$ , defined by

$$\tau_{res} = \left( \sum \tau_{ij}^2 \right)^{\frac{1}{2}} \quad (3)$$

where  $\tau_{ij}$  represents every component of the stress tensor. The components of the stress tensor were calculated from the velocity field using linear approximations for partial derivatives. The stress tensor can be used since it is linearly related to the rate of deformation for Newtonian fluids. The value of the resultant stress at every fluid element calculated from Eq. 3 is a measure of the components of the stress tensor. This resultant

stress is not invariant to the coordinate system but it can be used to assess the change in deformation rates as long as the same coordinate system is used in all comparisons.

### **Grid refinement.**

The dependency of the solution on grid refinement was determined for the 1.7 mm diameter bubble shown in Figure 2 by increasing the number of cells by a factor of 2 i.e.- twice as many cells were used in both r and z directions. In the following discussion we will refer to the grid shown in Figure 2 as the 35x55 grid and the refined grid as 70x110 grid. The objective of this analysis was to assess the extent by which the numerical solution changes as the grid is refined. This will in turn determine whether further grid refinement is indeed required. Figure 5 shows the time sequence of free surface plots for the 35x55 and 70x110 grids at selected elapsed times. The differences observed result from the fact that the curvature of the free surface is more accurately calculated as the number of cells in the grid increases. The free surface plots shown in Figure 5 show that as the grid is refined the computer results resemble the actual process to the same extent when compared to the photographic results.

The change in deformation rates with grid refinement are assessed by looking at the distribution of resultant stresses. Table 1 shows the frequency of cells with resultant stresses in the indicated range over the time domain of the calculation ( $2 \times 10^{-3}$  sec) for the 35x55 and 70x110 grids. Cells with resultant stresses less than 50 dyne/cm<sup>2</sup> were not considered since we were interested in the range of high resultant stresses. The frequencies of cells with resultant stresses are dependent on the time interval selected to obtain data outputs from the computer program of the hydrodynamic state of the flow. For this grid refinement study 15

data outputs were used. As shown in Table 1, the highest frequencies corresponded to cells with stresses in the range of 50 to 100 dyne/cm<sup>2</sup>. The frequencies decrease rapidly as the range of stress increased. In both cases only few cells presented resultant stresses larger than 500 dyne/cm<sup>2</sup>. The frequency for all intervals is more than twice as high for the 70x110 grid when compared to the 35x55 grid. This is because the condition imposed to calculate the stresses. For any given cell, its associated stresses are calculated only if the fluid fraction for the cell and its surrounding cells is one. This condition becomes more restrictive as the total number of cells used decreases and it is not linear with the refinement factor. This is schematically shown in Figure 6. However, as a percentage of the total number of cells with an associated resultant stress, both the 35x55 and 70x110 grids have a similar distribution of resultant stresses (See Table 1). From the results presented in Figure 6 and Table 1 it can be concluded that further refinement of the 35x55 grid does not produce significant changes in the distribution or the range of the calculated, resultant stresses.

#### **Proximity of the outer and bottom boundaries.**

The effect of the proximity of the outer and bottom boundaries was evaluated by specifying these boundaries at 2, 50 and 100 bubble diameters for a 1.7 mm diameter bubble. In expanding the grid to 50 and 100 bubble diameters, the grid of Figure 2 was preserved and additional cells were added in both r and z directions. Beyond the grid presented in Figure 2, the size of individual cells expanded uniformly until the required proximity of the boundaries was achieved. The grid can be expanded in r at a position far from the axis of symmetry (by far we mean beyond 2 bubble diameters) since the motion of the fluid at this distance is very slow as compared with speeds measured close to the bubble interface and particularly at the

axis of symmetry. In a similar way, the grid can be expanded in the z direction at a position far from the still liquid level since the high speed of the downward jet does not propagate more than 2.5 bubble diameters during the calculation time ( $2 \times 10^{-3}$  sec). In addition, this speed slows down as the downward jet propagates due to viscous dissipation. Figure 7 shows selected surface plots for grid arrangements where the outer and bottom boundaries were set at 2, 50 and 100 bubble diameters at selected times. To facilitate the comparison of these results the plots are shown using the same output window. This provides plots of identical physical dimensions. The proximity of the boundaries, however, were set as it was described above. Based upon comparisons of the outputs, extending the distance of the outer and bottom boundaries results in differences in the position of the free surface of at most two cells, and the free surface plots for every case resemble the actual process to the same extent. As with the grid refinement study, the change of deformation rates as the proximity of the boundaries was increased was evaluated. Table 2 shows the distribution of resultant stresses over the time domain. Again for each case, 15 data outputs of the hydrodynamic state were used and cells with stresses less than  $50 \text{ dyne/cm}^2$  were not considered. From Table 2 we see that the largest frequencies are localized in the range of 50 to  $100 \text{ dyne/cm}^2$  and then decrease for higher stress ranges. In each case only a few cells show values greater than  $650 \text{ dyne/cm}^2$ . Figure 7 and Table 2 show that the numerical solution does not change appreciably as the proximity of the outer and bottom boundaries increase beyond 2 bubble diameters. These results are expected when one considers that the driving force is surface curvature and the displacement of the still liquid level (due to the presence of the bubble at the interface) becomes very small at distances longer than two bubble diameters.

## **Discussion of the computer solution.**

The numerical results are sensitive to the initial conditions, boundary conditions, and numerical resolution used in the calculation mesh. However, as it was shown for the 1.7 mm diameter bubble, a grid definition can be found for which the solution no longer changes appreciably. In doing this, one has to be careful in maintaining an appropriate cell aspect ratio (ratio of  $\delta r$  to  $\delta z$  for any cell), specially in those regions where surface curvature is being defined. That is, where there are significant surface tension forces. The evaluation of surface curvatures from data available in the VOF method is a difficult numerical problem. Using large cell aspect ratios exasperates these difficulties because it implies that curvatures can be more accurately calculated in one direction than another and this incorrectly biases the computed force.

During the calculation of the three cases presented, a point is reached at which the convergence of flow at the axis of symmetry (instant at which opposite jets are formed) results in the formation of a very small bubble. That is, as the cavity wall approaches the axis of symmetry it closes slightly above the bottom of the original bubble (data not shown). The development of this small bubble is not an unrealistic result. MacIntyre (1972) have reported a downward ejection of small bubbles upon bubble breakup. In fact, these observations corroborated the formation of the downward jet theoretically predicted. This phenomenon has also been observed in visualization studies in our laboratory. Some of the energy released in the original bubble is conserved as surface energy as smaller bubbles are formed. These smaller bubbles eventually will break and released their energy. It is likely that a minimum bubble size exists at which smaller bubbles are produced.

## Analysis of the hydrodynamic information obtained from the computer solution

One of the goals of this work is to extract a quantity from the numeric solution which can be related to cell damage. Ideally, this parameter should be of general nature, i.e.- it should be useful not only to analyze the results of our computer solution but also results from other independent experiments. If these characteristics are met, this quantity has the potential to be used as a criterion for the design of animal cell bioreactors. Inversely, given a suitable design parameter one should be able to use it to analyze results from any particular experiment.

Shear stress is the parameter that traditionally has been used to correlate cell damage. It has been used for example, in experiments with rotational and capillary viscometers where cells are subjected to deformation of the fluid in which they are suspended.

A comparison of shear stress from two different viscometer designs such as plate and cone and concentric cylinders can be made. This is because at a given position in the flow of any of these viscometers an orientation of a Cartesian coordinate system exists for which normal stress components, as well as two of the three different shear stress components of the stress tensor are equal to zero. For example, for the cone and plate viscometer

$$\boldsymbol{\tau} = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & \tau_{23} \\ 0 & \tau_{32} & 0 \end{bmatrix} \quad (4)$$

for some  $e_1, e_2, e_3$ . These non-zero shear stress components have been associated to changes in cell physiology or death.

When local information of a complex flow such as the velocity field is available,

