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

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Abstract

Hydrodynamic information of the flow occurring as a bubble breaks at a gas liquid interface has being 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 through 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 and 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:

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

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):

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

where c is given in feet per second and T is in R. If a 1.7 mm diameter bubble is sea water at 65 F is considered, it can be calculated using Eq. 2 that the pressure wave will propagate 2 bubble diameters in 9.9×10^{-6} sec. As it will be shown later, the collapse of this bubble occurs in about 2.3×10^{-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 θ 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-Newtonain 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 asses 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, τ_{res} , defined by

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

where τ_{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 asses 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 asses 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 (2×10^{-3} sec) for the 35x55 and 70x110 grids. Cells with resultant stresses less than 50 dyne/cm^2 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^2. The frequencies decrease rapidly as the range of stress increased. In both cases only few cells presented resultant stresses larger than 500 dyne/cm^2. 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 ($2x10^{-3}$ 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 dyne/cm^2 were not considered. From Table 2 we see that the largest frequencies are localized in the range of 50 to 100 dyne/cm^2 and then decrease for higher stress ranges. In each case only a few cells show values greater than 650 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 δr to δz 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

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

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,

however, one can calculate every component of the stress tensor for every fluid element. Comparison of this complex flow stress tensor with the shear stress from viscometric studies would require the transformation of the complex flow stress tensor to one of the following forms:

$$\boldsymbol{\tau}^* = \begin{bmatrix} 0 & \tau_{12}^* & 0 \\ \tau_{21}^* & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \text{ or } \begin{bmatrix} 0 & 0 & \tau_{13}^* \\ 0 & 0 & 0 \\ \tau_{31}^* & 0 & 0 \end{bmatrix} \text{ or } \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & \tau_{23}^* \\ 0 & \tau_{32}^* & 0 \end{bmatrix} \qquad (5)$$

for some e_1^*, e_2^*, e_3^*.

In general, if T_{ij} are the Cartesian components of a tensor **T**, then $T_{ii}=T_{11}+T_{22}+T_{33}$ is a scalar invariant to all orthogonal transformations. A condition to transform the complex flow stress tensor, to one of the forms in Eq. 5, upon rotation of coordinates is that $\tau_{ii}=\tau_{11}+\tau_{22}+\tau_{33}=0$. Clearly, this condition does not exist simultaneously for every fluid element in a complex flow such as that occurring when a bubble breaks. We conclude that shear stress by itself should not be considered as the appropriate quantity to analyze our data.

Following the idea that a single, suitable design parameter should be useful to analyze different and independent experiments the following alternatives are proposed.

In trying to find a suitable parameter, it is necessary to consider some quantity which is uniquely defined in space and time and is related to cell damage. If it is assumed that cell damage results from the rate of deformation of the fluid in general (and not from shear stress only as the literature suggests), one is tempted to consider the rate of deformation itself or other quantity related to it such as stress (not shear stress only), which for Newtonian fluids is

linearly related to the rate of deformation. Since the rate of deformation and stress at a specific time and location are characterized by the rate of deformation tensor and the stress tensor respectively, and both are symmetric second order tensors, quantities which are uniquely defined to these tensors could be considered as design parameters. If the stress tensor is considered, for example, possibilities include its second or third invariants (first invariant is zero for incompressible fluids). Another parameter which is relatively easy to understand, that is uniquely defined at a given time and position, and that can be calculated from the rate of deformation is the rate at which work is done on an element of fluid as it moves and deforms. In this approach, as in that involving invariants, no consideration is made on the resultant forces acting on the cell surface since the cell may rotate or deform as a response to the rate of deformation of the fluid, but instead the functionality:

$$\text{Cell damage} = f\,(\text{rate of work per unit volume})$$

is based on the rate of deformation of the continuum (the fluid phase). In addition, the implication is made that cells are contained within the fluid element being considered.

For an incompressible Newtonian fluid the dissipation function representing the rate at which work is converted into heat is given by:

$$\Phi = \mu\,[(\nabla \boldsymbol{v} + (\nabla \boldsymbol{v})^T] : (\nabla \boldsymbol{v})\,. \qquad (6)$$

For example, a value of $\Phi = 2.5\text{x}10^8$ dyne-cm sec^{-1} cm^{-3} means that in one second per cubic centimeter of fluid the energy dissipated is $2.5\text{x}10^8$ dyne-cm. Thus, given a velocity field obtained from the numerical solution of a complex flow such as that resulting when a bubble breaks or from experimental data from agitated containers (using techniques from which local

information of the flow can be obtained) or from well defined flow devices (where the rate of deformation is fixed) the dissipation function can be calculated and compared. In this work the dissipation function is used to analyze the local hydrodynamic information obtained from the computer solution.

Dissipation of energy in the fluid has been used in the past to correlate cell damage on microcarriers (Croughan *et al.*, 1987). It has also been used as a design parameter using a turbulent eddy model (Croughan *et al.*, 1987; Cherry and Papoutsakis, 1986). This model, assumes that cell damage will occur if the Kolmogoroff eddy microscale calculated from

$$\eta = \left(\frac{\nu^3}{\epsilon}\right)^{\frac{1}{4}} \qquad (7)$$

is of comparable size to the microcarrier size, where ε is the average specific rate of energy dissipation. The turbulent eddy model does not take into account local values of the flow. Events at the liquid interface where, as it will be shown, highly localized energy dissipation takes place as sparged or entrained bubbles burst are not consider in this turbulent eddy model.

Figures 8, 9, and 10 show a sequence of free surface plots (left) and dissipation function distribution (right) for bubbles with diameters 0.77, 1.7, and 6.32 mm, respectively. The dissipation function is shown using a gray scale that spans from white to black. For each bubble size, the corresponding range of these gray scales is presented in the second column of Table 3. Values greater than the upper limit where normalized to be shown by the maximum gray level, (i.e. black). This saturation value (the upper limit of the range) was

used because only a small number of cells presented dissipation function values near the maximum value. Had we selected saturation values equal to the maximum, only very tiny black regions would have been observed in the graphs of the dissipation function distribution at times before and after the maximum was calculated. The corresponding time for every frame is indicated in the legend of each figure.

As it can be seen in Figures 8, 9, and 10, a region of highly localized energy dissipation starts to develop in the initial stages of the bubble collapse. It is located at the edge of the bubble cavity where the fluid starts to move at high speeds due to high pressure differences located in this region. In subsequent frames the region of localized energy dissipation grows and moves towards the axis of symmetry and down into the cavity wall. When the flow converges at the axis of symmetry a maximum for the dissipation function is calculated. These maximum values and the time at which they are detected are shown on the third and fourth columns of Table 3, respectively. After the convergence of the flow upward and downward jets are observed and the dissipation function losses its intensity. The total elapsed time is shown on the fifth column of Table 3.

It is important to point out that even though similar characteristics are observed for the three bubble sizes considered, the calculated dissipation function decreases as the bubble size increases. For example, the maximum, calculated values for the dissipation function decrease 3 orders of magnitude when the 6.32mm bubble diameter is compared to the 0.77 mm bubble. This decreasing relationship is nearly semi-logarithmic

Figure 11 shows plots of the dissipation function corresponding to the time when the maximum is calculated. In this Figure the range of numerical values for the gray scale is

from 0 to the corresponding maximum value of the dissipation function for each bubble size.

Region of maximum values for the dissipation function.

Even though the maximum values of the dissipation function are concentrated in a small region, its importance becomes evident if we consider the hydrodynamics of the rupture process and animal cells-bubble interactions.

MacIntyre (1972, 1968) has described the bubble rupture process as a boundary layer "microtome". Using high-speed cinematography and dyed bubbles he observed that a very thin liquid layer next to the bubble boundary is transported towards the axis of symmetry and eventually ejected in the upward and downward jets. This is schematically shown on Figure 12. In visualization studies, Chalmers and Bavarian (1991) and Bavarian *et al.* (1991), have reported that some cell lines attach to rising bubbles. Garcia-Briones and Chalmers (1991), collected a liquid sample from the upward jet produced as a bubble ruptured in a suspension of insect cells. In this liquid sample they found that more than 95% of the cells were dead as compared with less than 10% in the bulk suspension.

Given the above observations, the argument can be made that even though the energy dissipation is maximum in a small region (See Figure 11), cells attached or closed to the bubble cavity will be eventually transported to the region where high localized energy dissipation (high rates of deformation) occurs.

Comparison with data from the literature

In this section a comparison of our results with data from the literature is made.

Well defined flow devices. Table 4 shows the dissipation function for 4 different experiments performed in 4 different viscometers. To illustrate how the dissipation function

was calculated, let us take the plate and cone viscometer. Considering spherical coordinates, the tensor $\nabla \mathbf{U}$ for the cone and plate viscometer is given by:

$$\nabla \boldsymbol{U} = \begin{bmatrix} 0 & 0 & \frac{\partial U_\phi}{\partial r} \\ 0 & 0 & \frac{1}{r}\frac{\partial U_\phi}{\partial \theta} \\ -\frac{U_\phi}{r} & -\frac{U_\phi}{r}\cot\theta & 0 \end{bmatrix} \tag{8}$$

where it has been considered that $U_r = U_\theta = 0$ and no change in the ϕ direction exists, thus $\partial U_\phi / \partial \phi = 0$. The product $\Phi = \mu[(\nabla \mathbf{U}) + (\nabla \mathbf{U})^T] : (\nabla \mathbf{U})$ is then

$$\Phi = \mu\left[\frac{\sin\theta}{r}\frac{\partial}{\partial\theta}\left(\frac{U_\phi}{\sin\theta}\right)\right]^2. \tag{9}$$

We also notice that the only non-zero components of the stress tensor for this flow become

$$\tau_{\theta\phi} = \tau_{\phi\theta} = -\mu\left[\frac{\sin\theta}{r}\frac{\partial}{\partial\theta}\left(\frac{U_\phi}{\sin\theta}\right)\right]. \tag{10}$$

For a properly design plate and cone viscometer where the angle of the cone is small we have

$$\tau_{\theta\phi} = \tau_{\phi\theta} = \tau_{plate} = \mu \frac{\Omega}{\theta_o}. \qquad (11)$$

Comparison of Eq. 9, 10 and 11 indicates that the dissipation function can be calculated by

$$\Phi = \mu (\dot{\gamma})^2 \qquad (12)$$

where

$$\dot{\gamma} = \frac{\Omega}{\theta_o}. \qquad (13)$$

It can be shown that the dissipation function can be calculated with an equation of the same form as Eq. 12 for concentric cylinders and double cup and bob viscometers.

Mixed bioreactor experiments. Even though no local values of the dissipation function are available in the literature for mixed bioreactor studies, a comparison can be made with the reported average values of the specific turbulent power dissipation rate calculated from

$$\epsilon = \frac{N_p n^3 d_i^5}{V_d}. \qquad (14)$$

In this case an average dissipation function can be calculated from

$$\Phi_{ave} = \epsilon \rho \qquad (15)$$

where in the absence of more information, the density can be taken to be equal to the density of water.

Table 5 shows the average dissipation function for experiments in mixed bioreactors.

Discussion of results

Looking at the data presented on Table 4 we see that when the dissipation function was 5.81×10^3 erg cm^{-3} no appreciable reduction in cell viability was observed. In contrast, for the experiment for which the dissipation function was 4.8×10^8 erg cm^{-3} a reduction of 84.5% in viable cells was observed in 0.3 sec. This order of magnitude for the dissipation function is comparable to the maximum values calculated for the 0.77 and 1.7 mm diameter bubbles shown on Table 3.

In well defined flow devises, time is always involved. The question arises as to what is more detrimental to cells, a low rate of energy dissipation applied for a long time or the combination of a high rate of energy dissipation applied for a very short period of time. The data in Table 3 suggests that at a sufficiently high rate of energy dissipation cell damage becomes independent of time for the time scales of hydrodynamic events common in bioreactors. This may very well be what happens when small bubbles break. The rate of energy dissipation is sufficiently high ($\sim 1 \times 10^9$ for 0.77 mm diameter bubbles) that the very short time of action is not important regarding its effect in cell viability. This can actually be experimentally tested using a viscometer, and subjecting the cells to high rates of deformation for very short periods of time.

Data shown on Table 5 shows that when the average dissipation function was in the order of $2.99x10^4$ to $2.90x10^5$ the growth rate was seen to decrease significantly. On the other hand for an average dissipation function in the range of $2.07x10^1$ to $2.97x10^4$ no effect on cell growth and physiology was observed. The range shown on Table 5 for the dissipation function where a reduction in the growth rate was observed ($2.99x10^4$ to $2.90x10^5$) may seem to be low, however, these values are average values for the dissipation function. Higher values than the average most probably exist in the trailing vortices behind the impeller (Placek *et al.*, 1986). These local values of the dissipation function can only be calculated if local information of the flow is known.

The magnitude of the calculated maximum value for the dissipation function decreased from $9.52x10^8$ to $9.4x10^5$ as the bubble diameter was increased from 0.77 to 6.32 mm. The effects of sparging reported by Handa *et al.* (1987) show a reduction of cell viability as the bubble size was decreased. Oh *et al.* (1992) found that bubbles larger than 5 mm are much less detrimental than small bubbles. Clearly the energy dissipation function helps us to understand why small bubbles are more detrimental to cells than larger ones.

In this paper, only the energy released in the boundary of the bubble cavity has been presented. It is our belief that the rate of energy released as the bubble film retracts can be even greater than the calculated maximum values shown here.

Conclusions

It has been shown that as a bubble collapses at a gas-liquid interface a significant amount of energy is released in a very small region of liquid. The calculated rate of energy dissipation for a 0.77 mm diameter bubble can be as high as $\sim 1x10^9$ erg cm^{-3}. As the bubble

size increases the rate of energy dissipation decreases. It has also been shown that the concept of dissipation function is a general concept which is meaningful and provides a logical framework to understand animal cell damage in the region of bubble disengagement. In addition, it has the potential to be used as a criterion for the design of animal cell bioreactors. The only assumption behind this parameter is that cell damage is a function of the rate of deformation of the fluid in general and not shear stress only. If the dissipation function is to be applied in bioreactor design, details of the flow at all relevant areas in the bioreactor are needed in order to calculate local values of the dissipation function. One could then modify the reactor design (or operation) with the purpose of having a dissipation function less than a threshold value corresponding to the limit that a particular cell line can withstand. This limit can be determined experimentally using well defined flow devises.

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References

Augenstein, D.C., Sinskey, A.J. and Wang, D.I.C., 1971, Effect of shear on the death of two strains of mammalian tissue cells. *Biotech. Bioeng.* 13, 409-418.

Bavarian, F., Fan, L. S. and Chalmers, J. J., 1991, Microscopic visualization of insect cell-bubble interactions. I: rising bubbles, air-medium interface, and the foam layer. *Biotechnol. Prog.* **7,** 140-150.

Blanchard, D. C. and Syzdek, L. D., 1978, Seven problems in bubble and jet drop researches. *Limnol. Oceanogr.* **23,** 389-400.

Brodkey, R. S., The Phenomena of Fluid Motions, Addison-Wesley, Reading, MA, 1967.

Chalmers, J., Bavarian, F., Tsutsumi, A. and Fan, L. S., 1990, Microscopic visualizations of cell-bubble interactions. Paper No. 103C, Annual AIChE Meeting, Chicago, IL, Nov. 12-15.

Chalmers, J. J., Bavarian, F., 1991, Microscopic visualization of insect cell-bubble interactions. II: the bubble film and bubble rupture. *Biotechnol. Prog.* **7,** 151-158.

Cherry, R.S., Papoutsakis, E.T., 1986, Hydrodynamic effects on cells in agitated tissue culture reactors. *Bioproc. Eng.* **1,** 29-41.

Croughan, M.S., Hamel, J.F., Wang, D.I.C., 1987, Hydrodynamic effects in animal cells grown in microcarrier cultures. *Biotechnol. Bioeng.* **29,** 130-141.

Garcia-Briones, M. and Chalmers, J. J., 1992, Cell-bubble interactions: Mechanisms of suspended cell damage. *Ann. N.Y. Acad. Sci.* **665,** 219-229.

Goldblum, S., Bae, Y., Hink, W. F. and Chalmers, J. J., 1990, Protective effect of methylcellulose and other polymers on insect cells subjected to laminar shear stress. *Biotechnol. Prog.* **6,** 383-390.

Handa, A., Emery A. N., and Spier R. E., 1987, On the evaluation of gas-liquid interfacial effects on hybridoma viability in bubble column bioreactors. *Develop. Biol. Standard.* **66,** 241-253.

Handa-Corrigan, A., Emery, A. N. and Spier, R. E., 1989, Effect of gas-liquid interfaces on the growth of suspended mammalian cells: mechanism of cell damage by bubbles. *Enz. Microb. Technol.* **11**, 230-235.

Hirt, C.W. and Nichols, B.D., 1981, Volume of fluid method for the dynamics of free boundaries. *J. Computational Physics* **39,** 201-225.

Jobses, I., Martens, D. and Tramper, J., 1991, Lethal events during gas sparging in animal cell culture. *Biotech. Bioeng.* **37,** 484-490.

Kunas., K. T., and Papoutsakis, E. T., 1990, Damage mechanisms of suspended animal cells in agitated bioreactors with and without bubble entrainment. *Biotech. Bioeng.* **36,** 476-483.

MacIntyre, F., 1972, Flow patterns in breaking bubbles. *J. Goephys. Res.* **77,** 5211-5228.

MacIntyre, F., 1968, Bubbles: a boundary-layer "microtome" for micron-thick samples of liquid surface. *The Journal of Physical Chemistry* **72,** 589-592.

Medrow, R. A., 1968, Floating bubble configurations and charges of jet drops produced by bursting bubbles. Ph. D. Thesis, University of Illinois, Urbana, Illinois.

Oh, S. K. W., Nienow, A. W., Al-Rubeai, M., and Emery, A. N., 1989, The effects of agitation intensity with and without continuous sparging on the growth and antibody production of hybridoma cells. *J. Biotech.* **12,** 45-62.

Oh, S. K. W., Nienow, A. W., Al-Rubeai, M., and Emery, A. N., 1992, Further studies of the culture of mouse hybridomas in an agitated bioreactor with and without continuous sparging. *J. Biotech.* **22,** 245-270.

Placek, J., Tavlarides, L.L., Smith, G.W., and Fort, I., 1986, Turbulent flow in stirred tanks. Part II: A two-scale model of turbulence. *J. AIChE* **32,** 1771,1786.

Schurch, U., Kramer, H., Einsele, A., Widmer, F. and Eppenberger, H.M., 1988, Experimental evaluation of laminar shear stress on the behaviour of hybridoma mass cell cultures, producing monoclonal antibodies against mitochondrial creatine kinase. *J. Biotech.* **7,** 179-184.

Smith, C.G., Greenfield, P.F. and Randerson, D.H., 1987, A technique for determining the shear sensitivity of mammalian cells in suspension culture. *Biotechnology Techniques* **1,** 39-44.

Tramper, J., Williams, J. B., Joustra, D. and Vlak, J. M., 1986, Shear sensitivity of insect cells in suspension. *Enzyme Microb. Technol.* **8,** 33-36.

Toba, Y., 1959, Drop production by bursting of air bubbles on the sea surface (II) Theoretical study on the shape of floating bubbles. *J. Oc. Soc. of Japan* **15,** 121-130.

Tramper, J., Williams, J. B., Joustra, D. and Vlak, J. M., 1986, Shear sensitivity of insect cells in suspension. *Enzyme Microb. Technol.* **8,** 33-36.

Tramper, J., Joustra, D. and Vlak, J. M., 1987, Bioreactor design for growth of shear-

sensitive insect cells. in *Plant and animal cell cultures: Process possibilities* (Webb, C., Ativuna, F., eds), pp. 125-136, Chichester: Ellis Horwood.

Woodcock, A. H., Kientzler, C. F., Arons, A. B. and Blanchard, D. C., 1953, Giant condensation nuclei from bursting bubbles. *Nature* **172,** 1144-1145.

List of symbols

bd	bubble diameter
c	velocity of sound
d_i	impeller diameter
e	unit vector
N_p	power number
n	agitation rate
R	radius
r	radial direction
T	tensor
T	temperature or component of the stress tensor **T**
U	velocity vector
U	component of the velocity vector
V_d	dissipation volume
V_t	total liquid volume

Greek letters

Δ	difference
γ	surface tension or shear rate
ε	specific turbulent power dissipation rate
η	Kolmogoroff eddy microscale
θ	theta direction in spherical coordinates
μ	dynamic viscosity
ν	kinematic viscosity
ρ	density
τ	stress tensor
τ	component of the stress tensor
ϕ	phi direction in spherical coordinates
Φ	dissipation function
Ω	angular velocity

Operators

∇	nabla operator

Superscripts

*	quantity is associated to the cartesian coordinates $\mathbf{e}^*_1$, $\mathbf{e}^*_2$, $\mathbf{e}^*_3$.
T	transpose

Subscripts

ave	average
i	index
j	index
max	maximum
o	fixed quantity
res	resultant

Figure legends

Figure 1 Bubble shape solution. When properties of water and air are considered the actual diameters (maximum diameters) are 0.77, 1.7 and 6.32 mm (Medrow, 1968).

Figure 2 Computational grid. 35 cells are used in x (radial direction) and 55 in z. The bubble shape at time=0 is shown. The outer and bottom boundaries are set to about 2 bubble diameters from the still liquid level and axis of symmetry (left side) respectively.

Figure 3 Free surface plot at time=0.0 showing the azimutal segment used in the computation. The bubble is 1.7 mm in diameter.

Figure 4 Comparison of high speed photographs (Woodcock et al., 1953) and computer results for the 1.7 mm diameter bubble.

Figure 5 Free surface plots for the 35x55 (left) and 70x110 (right) grids. Time from top to bottom is: 0.0, 8.0×10^{-4}, 1.4×10^{-3}, and 2.0×10^{-3} sec.

Figure 6 As the total number of cells decreases the imposed condition to calculate stresses (fluid fraction equal to 1 for the cell being considered and its surrounding cells) becomes more restrictive. Most of the stresses in the higher range ($>$ 50 dyne/cm^2) are located at cells close to the bubble cavity interface where high rates of deformation ocurr.

Figure 7 Free surface plots for grid arrangements where the outer and bottom boundaries were set at 2 (left), 50 (middle), and 100 (right) bubble diameters (bd) at selected times. The window used is the same to facilitate comparison.