CHAPTER 2

LITERATURE REVIEWS

Currie et al (1972) investigated the release of protein from disrupted baker's yeast (*Saccharomyces cerevisiae*) using a 4.2 liter vertical agitator mill. Rate of protein released from disruption in the mill followed the first-order law. The rate constant was influenced by six operating parameters as temperature (5-42^{o}C), agitator speed (500-1800 rpm), bead size (0.5, 0.8, 1.1, 2.0, and 2.8 mm), weight of beads (3-9 kg), feed rate of yeast suspension (1-8 l/min), and yeast concentration (0.3-0.75 g packed yeast/ml). It was found that the disruption efficiency increased with the agitator speeds, loading of beads, while it decreased when the feed rate of yeast suspension was increased. Operating temperature showed insignificant effect on cell disruption.

Kunas and Papoutsakis (1990) showed that when freely suspended hybridoma cells were cultured in an agitated bioreactor, two fluid-mechanical mechanisms could cause cell damage and growth retardation. The former was presented when there existed gas phase dispersion associated with vortex formation and accompanied by bubble entrainment and breakup. The latter occurred with the absence of a vortex and bubble entrainment. Cells could be damaged only at very high agitation rates, above approximately 700 rpm, by stresses in the bulk turbulent liquid. However, the entrainment and motion of very fine bubbles cause no growth retardation even at agitation rate as high as 600 rpm.

Bavarian et al (1991) studied the interaction of two suspended insect cell with air and oxygen bubbles by microscopic, high-speed video technology. Based on the observation, cell-bubble adhesion and cell-bubble collision were the main cause of cell damage. Bubble rising could form high shear in the suspension. Thus, cell would deform by

the fluid flow around the bubbles. It was reasonable to assume that the cell would experience a surface shear stress.

Papoutsakis (1991) was interested in damage of animal cells in bioreactors incorporated with agitation and/or aeration. With microcarrier (polymeric or glass beads, typically 120-400 μm in diameter), cells were damaged due to forces generated by the interaction of microcarrier beads with each other and also with small turbulent eddies. There was a strong experimental evidence that bead-to-fluid interactions were detrimental to cell disruption. Additionally, the effects of the bead-to-bead interactions were also believed as the main source of cell damage in microcarrier bioreactors. However, for freely suspended cells grown in mixed bioreactors, cell damage was most frequently due to bubble breakup or fast-draining liquid films around rearraging gas-liquid interfaces.

Wang et al (1994) studied a modeling framework that was proposed to assess the detrimental effects of air sparging and agitating on damage of suspended cells in an aerated, agitated bioreactor. It was assumed that cells might be rendered nonviable by bubble breakup/ coalescence within the medium, by bubble formation at the sparger, or by bubble bursting at the free surface. Some conjectural mechanisms were perused from the dominant parameters, such as the cell-bubble encounter rate and the bubble breakup/ bursting rate. Finally, the specific cell death rates were shown to be linearly proportional to the specific bubble interfacial area (total bubble surface area per unit volume of media).

Chalmers (1994) studied various parameters which could provide effect on the cells growth e.g. In his study bubbles were the cause of cell detriment that could be classified into three distinct regions in a bioreactor: (i) bubble generation region (ii) bubble rising region (iii) bubble disengagement region. The hypothetical killing volume correlation demonstrates that an increase in the height to diameter ratio resulted in the decreased cell damage. From his experimental results, rupturing bubbles at gas-liquid interfaces with attached cells was believed to result in cell death. On the other hand, additives in the media which prevented cell attachment to gas-liquid interfaces could protect cells from damage.

Carlson et al (1995) evaluated five different mechanical cell disruption methods to extract plasmids from bacterial cells. The methods used were sonication, nebulization, homogenization, microfluidization and bead milling. The recovery yields of intact plasmids from the various methods were measured by quantitative gel electrophoresis. Although cell disruption was obtained in all the methods tested, only two methods, microfluidization and bead milling, resulted in high recovery of intact plasmid with total intact recovered of over 90% and around 50%, respectively. Other methods resulted in substantial plasmid degradation, with recoveries no greater than 20% of the total intact plasmid.

Wu et al (1995) investigated the detrimental effect of direct gas sparging on insect cells in bubble columns by varying gas flow rates and bubble sizes. The first-order death rate was shown to be directly proportional to the gas flow rate and inversely proportional to the bubble size. It was found that small bubbles were more detrimental to the cell suspended. The specific killing volume of a bubble (killing volume per unit volume of bubble) was found to have a linear correlation with the specific interfacial area of a bubble. Based on the experimental results and the analysis of bursting bubbles at the liquid surface, it was concluded that the killing volume of a bubble is in the liquid layer surrounding the bubbles before their rupture, and in the liquid layer beneath the bubble cavity. Cell damage in the bubble film cap was relatively insignificant compared to that in the liquid layer underneath the bubble cavity, except for very large bubble (i.e., bubble diameter over 5 mm).

Shimizu et al (1998) discovered another method for cell disruption, Theta-composer, which was equipment developed for preparing composites of various particulate materials. From analysis of cell disruption, it was found that the rate constant of soluble protein released was in proportion to the of yeast cell disruption rate. It was also found that critical point of cell disruption could be obtained at rotor speed of 2500 rpm.

Heim and Solecki (1999) proposed a novel bead mill with a multi-disk impeller for the disintegration of yeast, baker's *Saccharomyces cereviseae*. In their investigation operating variables were the concentration of yeast suspension (0.05-0.20 g dry mass/mL), disk-to-

disk distance (5-40 mm) and rotational speed of the impeller (1000-3500 rpm). With the batch operation, the yeast cell disruption rate followed the first order rate law. They also reported that the dominant destruction mechanisms were due to the interaction of bead element and the neighbouring microorganisms.

Camacho et al (2000) studied the effects of mechanical and hydrodynamic forces acting on cells of microalga *Porphyridium cruentum*, aerated in stirred vessels. They report that shear stress could lead to associated damage of microalgal cells in stirred vessel. Additional mechanisms of cell damage were manipulated by: (i) the agitation rate at a low constant aeration rate; (ii) the air flow rate at low rates of mechanical agitation; (iii) the culture height at constant aeration rate. The specific rate of cell death varied linearly with the specific interfacial area, suggesting the importance of bubble-cell contacting. It was found that low rates of mechanical agitation, bubble break-up at the suspension surface was the predominant cause of damage. In contrasts, at higher agitation intensities, cell damage was caused predominantly by hydrodynamic stress in bulk turbulent flow.

Suksamai et al (2000) investigated the disruption of baker's yeast cells (*Saccharomyces cerevisia*) using a three-phase fluidized bed with agitator. The system consists of yeast suspension, air bubble and glass beads employed as liquid phase, gas phase and solid phase, respectively. The operating parameters were impeller speed, the presence of draft tube in the column, superficial gas velocity, superficial liquid velocity and bead size. Their experimental results showed that an increase in the superficial gas velocity between 10 to 40 cm/min led to a decrease in rate of yeast cell disruption. The circulation of yeast suspension using centrifugal pump between 10 and 40 cm/min provided insignificant effect on the yeast cell disruption. They also reported that at the optimal condition the yeast cell disruption of 90 percent could be achieved.

Camacho et al (2001) examined the effect of direct air sparging on damage of *Phaeodactylum tricornutum* microalgal cultures in bubble columns and air lift photobioreactors. Superficial air velocities and types of spargers were varied in their study. The experiments showed that small bubbles brusting at the surface of the culture were

apparently the main cause of cell damage in batch cultures using laboratory-scale bubble columns. Supplementation of the microalgal culture medium with carboxymethyl cellulose at concentrations of 0.02% or greater could help protect the damages of algal cells due to hydrodynamic stress.