Pak. J. Biotechnol. Vol. 7 (1-2) 19-39 (2010) ISSN. 1812-1837

Review Article

THE IMPORTANCE OF FOAMS AND ANTIFOAMING IN BIOPROCESSES

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ABSTRACT

Foams are comprised of thousands of tiny bubbles of mechanical or chemical origin and are generated within a liquid. If these bubbles rise and accumulate at the liquid surface faster than they decay, foaming occurs. Foams are defined as a dispersion of gas in liquid (>95% gas) when the distance between individual bubbles is extremely small and the volume fraction of gas is quite large. The presence of foams in products or processes may or may not be desirable. Foaming occurs during fermentations, which is considered undesirable and is a problem common to many of microbial fermentations, especially where surface active microbial products (bio-surfactants) are involved. Foaming reduces the productive volume, i.e. increasing process costs, and can lead to blockage of the outlets and threat the sterility of a fermenter.

Antifoam action may take the form of addition of antifoam agent, mechanical agitation or ultrasound. The most commonly used method is the addition of chemical antifoams although it can add significantly to process costs and reduce the oxygen transfer rate. That may also exert adverse effects on the cell's physiology.

Conversely, foam separation techniques can be used for the recovery of proteins. In addition, foam fractionation has been successfully applied for the effective separation of surfactants and biological materials such as proteins, microorganisms, suspended solids, aromatic substances and pigments. It is, therefore, important to be able to effectively monitor and control the dynamic formation and collapse of such foam phases. At last, in spite of the important role of foaming in bioprocesses, successful prediction of foaming and defoaming phenomena is not entirely possible at present and further attention and research continues to be needed.

INTRODUCTION

Foams play an important role in several fields of human life including food technology,medicine,cosmetics,oceanography, environmental technology, fire extinguishing, etc. (Schugerl, 2000). The classical definition of foam is that it is a dispersion of gas in liquid, comprised of thousands of tiny bubbles of mechanical or chemical origin with the liquid in the form of thin films separating gas bubbles (Brayant, 1970). If these bubbles rise and accumulate at the liquid surface faster than they decay, foaming occurs. Foams are also defined as a dispersion of gas in liquid (>95% gas) when the distance between individual bubbles is extremely small and the volume fraction of gas is quite large (Vardar-Sukan, 1998; Varley et al., 2004). It means that gas makes up the larger volume fraction of such foam; therefore, the bulk density of the foam approaches that of the gas rather than the liquid. At least five simultaneous processes take place during foam formation/destruction (Vardar-Sukan, 1998):

1. liquid run over from the interfacial films
2. diffusion of gas from smaller bubbles into larger ones
3. redistribution of the liquid along the height of the foam column
4. natural escape of liquid from the foam
5. destruction of inter-bubble films.

If we consider foam bubbles as liquid polyhedral cells encapsulating a gas (see Figure 1) then *Lamellae* is defined as the liquid faces separating two cells, *Plateau Border* is the thicker junction between lamellae and *Vertexes* are the junctions of four Plateau Borders (van Phul and Cummings, 2007).

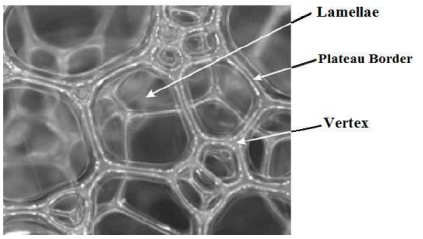

Fig. 1: foam bubbles (van Phul and Cummings, 2007).

Generally two extreme structural situations can be recognized for foams. The first type, *dilute foams*, consists of nearly spherical bubbles separated by rather thick films of somewhat viscous liquid.The other type, *concentrated foams*, are mostly gas phase and consist of polyhedral gas cells separated by thin liquid films (which may develop from more dilute foams as a result of liquid drainage, or directly from a liquid of relatively low viscosity). Similarly, some *solid foams* consist of spherical gas bubbles trapped within a solid network (e.g. foam rubber), whereas others consist of as little as 1 percent solid volume and are composed of polyhedral gas cells separated by very thin solid walls (e.g. expanded polystyrene) (Vardar-Sukan, 1998; van Phul and Cummings, 2007).

The terms *foams* and *froths* are often used interchangeably, but it is more usual to refer to the gas-water macro cluster systems where the broken structure leaves a homogeneous aqueous phase as foam. Froth usually contains dispersed solid particles, so that the broken structure gives a-phase system (aqueous solution and finely divided particles) (Vardar-Sukan, 1998).

Foam stability: Not surprisingly, there is no single theory which can satis-factorily explain the mechanism of foam stability. Only transitory foams can be formed with pure liquids and a third (surface-active) component is necessary to achieve any reasonable degree of stability. In fact, when a gas bubble is introduced below the surface of a pure liquid, it bursts almost immediately as soon as the liquid has drained away. With dilute surfactant solutions, as the air-liquid interface expands and the equilibrium at the inter-face is disturbed then a restoring force is set up which tries to re-establish this equilibrium. This results from the elasticity in the film so that it is less likely to break. There are *two mechanisms* by which this can occur; *the Gibbs* and *the Marangoni* surface elasticity effects. However,since the latter is usually superi-mposed on the former, then their action is usually considered together. If a film of liquid containing a surface-active agent is locally stretched, the surface tension of that part will increase. The stretching leads to a decrease of solute concentration within the film, and therefore, a rise in equilibrium surface tension. This force tends to resist local thinning and to restore the thin area. This is the basis of Gibb's

theory of surface elasticity (Schugerl, 2000; Brayant, 1970). So if a film is subjected to local stretching as a result of some external disturbance, the consequent increase in surface area will be accompanied by a decrease in the surface excess concentration of foaming agent and, therefore, a local increase in surface tension. Marangoni's theory differs from Gibb's only in that it concerns dynamic rather than static systems. According to Marangoni the difference in surface tension caused by thinning is greater in a dynamic system,where equilibrium values are not reached. Since a certain time is required for surfactant molecules to diffuse to this surface region and restore the original surface tension, this increased surface tension may persist for long enough to cause the disturbed film region to recover its original thickness. An absence of the Gibbs-Marangoni effect is the main reason why *pure liquids do not foam* unless a surface active material is present (Brayant, 1970).

Stability of foam depends upon *two principal factors*: (a) The tendency for the liquid films to drain and become thinner and, (b) their tendency to rupture as a result of random disturbances. Owing to their high interfacial area and surface free energy (as in the separated state the gas and liquid have a lower surface energy), all foams are unstable in the thermodynamic sense (Brayant, 1970; Vardar-Sukan, 1998). In kinetic terms, a sharp distinction can be drawn between unstable, *transient foam* with a lifetime of seconds and *metastable or so-called permanent foams* with lifetimes which may be measured in days. It is likely that the surface influences the gas diffusion rate across the lamellae, thus changing the foam structure with time. So based on *characteristics*, foams were classified into various categories (Table 1).

Table-1: Classification of foams (Ghildyal et al., 1988).

Type	Characteristics
True	Predominantly gaseous dispersion
Fluid (Dawson - 1961)	Predominantly liquid dispersion with enhanced hold up of gas in a large portion of the liquid
Unstable	equilibrium state is continuously approached
Metastable	Progress to the equilibrium state is arrested
Transient	Lifetime of seconds
Persistent	Lifetime of hours or days if undisturbed

In general, *fluid foams* are encountered in submerged processes and these can be unstable, metastable, transient or persistent. *Unstable* foam continuously approaches the equilibrium state; constantly breaks down as the liquid dries between the bubbles. Its lifetime depends on the concentration of the solution. *Metastable* foam is characterized by the fact that drying of the liquid between the bubbles can stop and the foam can persist indefinitely, if absolutely protected from disturbing influences including vibration, draughts, evaporation,radiant heat, temperature differences, dust and other impurities. Metastability may be conferred on the foam by the presence of a solute that is positively adsorbed at the surface and requires work to remove it from there to the bulk. *True foaming* only occurs when the intervening liquid between two bubbles thins down to lamellae, instead of rupturing at the point of closest approach (Schugerl, 2000; Brayant, 1970).

Foam stability is also related to the drainage rate of liquid from lamellae. The drainage rate declines with increasing viscosity of the bulk liquid; however, it is

unclear if the drainage rate is affected by the surface viscosity of the liquid or not.

Foaming in bioprocesses: Foaming is encountered in bioprocessing in aerated and agitated bioreactors. The presence of foams in products or processes may or may not be desirable. The foaming tendency and its stability of a bioprocess depend on the system and the operating variables. The complexities of biosystems make it difficult to relate their foaming characteristics to individual factors and qualitative differences exist between foaming abilities of liquids and types of foam produced (Phianmongkhol and Varley, 1999).

In submerged culture, foaming is associated with hydrodynamic conditions which in turn are affected by the gas flow rate, the nature and composition of the medium (pH, concentration of salts, proteins, and sugars, presence of alcohols, etc.), the presence of growing cells, and the operating conditions (temperature, rheological properties of the broth, conditions of sterilization, and the composition of gas making up the gas bubbles). Some effects of these variables are summarized below (Vardar-Sukan, 1998; Bumbullis et al., 1979; Bumbullis and Schugerl, 1981; Kotsaridu et al., 1983).

Proteins: In biotechnology, protein foams in combination with surfactants play a significant role. The main components of foam formation in cultivation media are proteins. In fact proteins are used as energy sources for the microorganisms and cells. Bio-systems contain many kinds of proteins and surfactants besides several poorly defined components (e.g. solid particles) which influence the formation and properties of foams in (volume-aerated) submerged cultures. However, because protein foams dominate in cultivation media, it is expected that properties of protein foams and cultivation foams will be similar and that results obtained with model protein foams can be applied for foams of cultivation media. Several authors have applied solutions of particular proteins and used them as model media to investigate the behavior of biological foams (Schugerl, 2000; Brayant, 1970; Vardar-Sukan, 1998).

The high foaming capacity of protein solutions is explained by their strong adsorption at the interface (Schugerl, 2000). According to *Cumper* et al. the adsorption process takes place in three main stages: (a) diffusion of the native protein molecules to the interface and their adsorption, (b) uncoiling of the polypeptide chains at the interface (surface denaturation), and (c) aggregation of the surface denaturated proteins into coagulum largely devoid of surface activity (coagulation) (Cumper and Alexander, 1950).

The foaming capacity of the surfactant or protein solutions is characterized by the foaminess. Foaminess and foam stability were concluded to be complementary properties. The foaminess $\sum$ is defined as:

$$\sum = \frac{Vs}{Vtg}$$

Where Vs is the equilibrium volume of the foam above the liquid layer and Vtg is the volumetric gas flow rate [1]. A comparison of foams formed by various proteins indicates that the foaminess is related to the rate of decrease in the surface tension of the air/water interface by protein molecules whereas the foam stability is related to the structure of the adsorbed protein films (Phianmongkhol and Varley, 1999). Thus flexible protein molecules, which can rapidly reduce the surface tension of the air/water interface, give good foaminess whereas highly ordered global molecules with slow surface

denaturation rates give poor foaminess (Schugerl, 2000).

Rapid build-up of film pressure by proteins tends to lead to formation of coarse foam (with large bubbles) whereas slow increase favors small air bubbles, i.e. creamy foam. Globular protein foams are more stable than foams prepared with proteins of flexible structure. Therefore,the foaminess of proteins with more-or-less random coil molecules (e.g. *b*-casein) differs from that of globular proteins (e.g. BSA).The solubility of proteins as well as the protein type influences the foaminess. The solubility of proteins is lowest at their isoelectric point (IEP) and, therefore, their foaminess is the highest at their IEP, if the proteins do not precipitate (Schugerl, 2000).

Salts: It is well known that inorganic salts influence protein solubility (Hofmeister, 1888).The foaminess of solutions increased in the presence of salts, but the relative foam stability diminished. This influence of salts on the foam formation in protein solutions could be explained by the changes in the protein solubility that accompanied changes in the water structure caused by the salts. The solubility of proteins in water was higher at low salt concentrations and, correspondingly, the foam stability was greater. The converse occurred at higher salt concentrations. When pure salts were added to pure water, no foaming occurred, but the bubble stability increased. The influence of salts on the foaminess is mainly due to their effect on the structure of water. The influence of organic solvents on the foaminess is more complex. They control not only the water structure, and by that the protein solubility, but also the protein structure (Schugerl, 2000; Brayant, 1970; Vardar-Sukan, 1998).

Temperature: Foaminess is inversely proportional to temperature. This may be due to increased liquid drainage because viscosity declines with increasing temperature. Elevated temperature may also enhance evaporation of volatile surface active components. At higher temperatures, the denaturation of proteins increases the foaminess of biomedia. Complex media have a particularly high foaming tendency that can be increased considerably during sterilization. Heat seems to affect mainly the nitrogen sources in biomedia. Nitrogen sources are partly hydrolyzed or otherwise degraded by heat to produce substances that combine with reducing sugars, amino acids, proteins and peptides via the Maillard reaction. Consequently, foam formation is enhanced (Schugerl, 2000; Vardar-Sukan, 1998).

pH: The dependence of foaminess on pH is more complex and the foam formation capacity is significantly influenced by the pH of the medium. Why the foaminess exhibits a minimum at pH 3 and below 3 increases again is not yet clear. The effectiveness of antifoam agents may also depend on pH (Kotsaridu et al., 1983; Tanford et al., 1955).

Operating Conditions: Operating conditions of the reactor such as air flow rate and agitation also influence the foaming. As the gas flow rate increases, the height of the foam layer increases, because more bubbles reach the surface and are converted into foam (Yeh et al., 2006). In some cases, the thickness of the foam layer may decrease with increasing gas flow rate after a maximum thickness has been reached. The reasons for this are not clear.

Cells: Little is known about the direct influence of cells on foaming because cells are always accompanied by proteins and other solutes and also viscosity changes are proportional to cell concentrations. In general, a very dense suspension of cells shows less foaming activity than a very dilute suspension, but this may well be a

viscosity effect. Generally the presence of solids tends to stabilize liquid films if the solids are wetted,as in the case with micro-organisms. One explanation for this is that surface active materials are also adsorbed onto the solid particles, with non-polar ends oriented towards the water phase. This imparts a hydrophobic character to the particles, so that air bubbles adhere to them, resulting in a stabilization of the bubble and longer bubble survival time (Schugerl, 2000; Brayant, 1970; Vardar-Sukan, 1998).

Surface active materials: Antifoam agents are surface active substances and for destabilizing the foam in bioprocesses may be made of oils, fatty acids, esters, polyglycols and siloxanes,alcohols, sulfites and sulfonates.The interaction of different surface active agents and different interfaces may produce a variety of surfactant functions such as emulsification, deemulsification, foaming, defoaming, and spreading (Vardar-Sukan,1998). Table-2 lists examples of surfactants and their reported applications in the petroleum industry (van Phul and Cummings, 2007).

Table-2: Examples of Oil Field Surfactants (van Phul and Cummings, 2007).

Surfactant category	Type	Used in products of type*
Alkyl aryl sulfonates	Anionic	EB, CI
Alkyl sulfates	Anionic	AF
Alkyl ethoxylate sulfates	Anionic	AF
Phosphate esters	Anionic	CI
Quaternary ammonium compounds	Cationic	CI, BC
Fatty amine salts	Cationic	CI
Fatty acid amides	Cationic	EB
Imidazolines	Cationic	CI
Alkyl phenol ethoxylates	Non-ionic	CI, BC, EB
Alkyl poly glycosides	Non-ionic	CI
Ethoxylate-propoxylate polymers	Non-ionic	EB
Fatty alcohol ethoxylates	Non-ionic	BC, CI, EB
Betaines	Amphoteric	CI

*KEY: AF (antifoam); BC (biocide); CI (corrosion inhibitor); EB (emulsion breaker).

Surface-active molecules produced by bacteria, yeasts, and fungi are known as biosurfactants, which are characteristic of high surface activity, low toxicity, high biodegradability and ecological acceptability (Yeh et al., 2006; Mulligan, 2005; Desai and Banat, 1997). Studies of the surface activity and emulsification properties of biosurfactants show that their properties are comparable to those of synthetic surfactants but unlike most synthetic surfactants they are biode-gradable and non-toxic to the environment (Rosenberg and Ron, 1999; Banat et al., 2000). These favorable features make biosurfactants potential alternatives of chemically synthesized surfactants in a variety of applications. In fact, bio-surfactants have been widely utilized in industries like cosmetics, specialty chemicals, food, pharmaceutics, agriculture, cleansers, enhanced oil recovery, and bioremediation of oil-contaminated sites (Desai and Banat,1997; Banat et al., 2000). However, the high production cost of biosurfactants has been the major obstacle for commercial applications (Yeh et al., 2006). Fermentation broths typically contain numerous surface active substances that form adsorption layers and films around interfaces (Vardar-Sukan, 1998). Surface activity in a bioprocess arises through: (a) the normal metabolic activities of exponentially growing cells; (b) enzyme catalyzed degradation during autolysis and release of biosur-

factants during stationary and endogenous metabolism phases of older cultures; (c) physical processes, including the shearing of the cell wall, that result because of culture agitation; (d) uptake and metabolic degradation of previously released surfacetants; and (e) the highly hydrophobic nature of certain microbial cells (e.g., N. amarae) (Vardar-Sukan, 1998).

Beneficial points of foams in bioprocesses: Foams can be utilized in bioprocesses and the role of biofoams in industrial processes is crystal clear. Foam separation and foam fractionation techniques has been successfully applied for the effective separation of surfactants and biological materials such as proteins, microorganisms, suspended solids, aromatic substances, and pigments (Du et al.,2000; Linke et al., 2007; Stevenson et al., 2008; Davis et al., 2001; Nam and Park, 1999). The separation process is mainly dominated by: (a) adsorption of objective substances onto the bubble surface within the bubble dispersed bed and (b) drainage within the foam bed. On the other hand, bio-surfactants have gained importance in the recent years because of the broad range of potential applications in different fields, such as controlled drug delivery, enhanced oil recovery, hydrocarbon bioremediation in soil and water, paint industry, agriculture, etc. (Desai and Banat, 1997; Stevenson et al., 2008; Nam and Park, 1999; Suzuki et al., 2008; Quek et al., 2006).

Polyurethane foam (PUF) is also a polymer which was used as inert support for the growth production (extracellular enzymes), pre-concentration, separation and determination of phenols and other pollutants in water and air, sorbing organic compounds by solvent extraction mechanism, immobilizing hydrocarbon-degrading microorganism in the bioremediation of petroleum hydrocarbons and degrading various petroleum products (Marin-Cervantes et al., 2008; El-Shahawi and Aldhaheri, 1996; El-Shahawi et al., 1994; El-Shahawi and Nassif, 2003).

For more instances, bacteria removal from rearing water and washing water in aquaculture systems, aquariums and fishing port facilities via foaming is the most important means of diminishing the risk of fish diseases, improving public health and ensuring high food quality (Suzuki et al., 2008). Foams are consumed in the form of bread, cake and confectionery and drink products including beer (Phianmongkhol and Varley, 1999). Bioactive and bioresorbable polymers were also developed based on three-dimensional, macro porous foams in tissue engineering for the repair of a damaged tissue, avoiding the need for a permanent implant made of an engineered material. Moreover, the biomass and support are easily separated by PUF into the enzymatic extract with few impurities, which facilitates further purification. For more investigation, here, some titled examples of foaming applications joined by bioprocesses are reviewed.

Immobilization: One application of foams in bioprocesses is to make an immobilized bed to have better culture for a certain purpose. Immobilization is an important strategy for the removal of shear stress (Honda et al., 2001). Poly-urethane foam (PUF) can be prepared for immobilization as an immobilization support in the culture space. It also presents excellent characteristics such as high porosity, low density, and relatively high water absorption. PUF has an adequate pore size which provides a satisfactory environment for fungal growth.

In almost all cases, effective production of biological materials by the immobilized cells has been reported via avoiding

damages due to the hydrodynamic stress. For example, *Honda* et al. developed an immobilized cell system for cultivation of embryogenic rice callus using PUF as porous supports for the immobilization of mycelia cells and plant cells. They found that the immobilized callus maintained high regeneration ability because shear stress and hydrodynamic damage were avoided. On the other hand, this procedure was convenient because their subjects were the calli immobilized in foam, not fragile clumps, and the foams exit in the liquid medium and thus are easy to transport (Honda et al., 2001).

Bioremediation: During bioremediation in marine environment, nutrients and hydrocarbon degraders are often added to increase the rate of degradation. Oil adsorbents can adsorb and concentrate floating petroleum and prevent its migration to shorelines and beaches. If oil adsorbents were immobilized with hydrocarbon degraders, bioremediation may occur in-situ or ex-situ. Of great interest to bioremediation is the potential of immobilizing microorganisms onto polyurethane foams (PUFs), alginate and other matrices to degrade hydrocarbons and toxic wastes. As an example, *Quek* et al. reported the immobilization and performance of a hydrocarbon-degrading microorganism on polyurethane foam (PUF) in the bioremediation of petroleum hydrocarbons. The results suggest the potential of using PUF-immobilized Rhodococcus sp. (designated as F92) to bioremediate petroleum hydrocarbons in an open marine environment (Quek et al., 2006). F92 was efficiently immobilized onto PUF and the immobilized cells were able to degrade a variety of petroleum products such as ALC, ASC, diesel and oil slops (Quek et al., 2006).

Preconcentration and Sorption: The membrane-like structure of the foams with the efficient sorption properties offers many advantages over other solid collectors with other solid materials (El-Shahawi and Nassif, 2003). Solid-phase extraction (SPE) is a very important preconcentration technique in trace metal determination (de Jesus et al., 1998). The importance of PUF in separation and preconcentration of metals has increased in the last few years. PUF has been used as a solid sorbent to separate and preconcentrate a wide variety of inorganic and organic compounds from different media. PUF can be directly used without previous pretreatment. Several investigations were carried out to remove toxic heavy metal ions from waste water by biosorption. Microbial cells loaded with heavy metals were recovered by flotation. PUFs have been proposed for the pre-concentration, separation and determination of phenols and other pollutants in water and air (El-Shahawi and Nassif, 2003). For example, *El-Shahawi* et al., (1994) worked on the sorption mechanism of phenols and other organic contaminates from aqueous media by PUFs and showed that the foams are capable of sorbing organic compounds by solvent extraction mechanism. The PUF sorbent offers unique advantages in rapid separation of complex species from fluid water samples (El-Shahawi and Aldhaheri, 1996;El-Shahawi et al.,1994). Preconcentration of aerosols onto porous PUF plugs has been also reported by other researchers.

Seawater bacterial purification: The foam separation using dispersed bubbles and surface-active substances is a feasible convenient technology for seawater purification as a treatment prior to membrane filtration or ultraviolet irradiation (Suzuki et al., 2008). The foam separation examined has been one of the solid-liquid separa-

tion methods for the removal of bacteria as suspended substances from seawater; its largest advantage is the utilization of protein or natural surface-active substances as a chemical reagent for processing (Du et al., 2000). Therefore, this method is quite different from conventional foam separation. The foam separation unit maintains an oxygen-saturated condition in the rearing water. Furthermore, suspended substances and bacteria absorb onto the stable foam formed from fish mucus (surface-active substance) and are removed from the rearing water with the foam. To develop seawater purification technology for bacterial removal, researchers examined the removal efficiency for several groups of bacteria that are frequently detected in coastal seawater by foam separation using dispersed bubbles and surface-active substances (Suzuki et al., 2008). A schematic diagram (not to scale) of foam separation unit for both batch and continuous-flow system is prepared (Figs. 2 and 3). *Suzuki* et al. (2008) showed that by using batch equipment or the continuous-flow unit of foam separation, major groups of bacteria such as viable bacteria, enterococci, Vibrio, and Salmonella-like bacteria were removed from coastal seawater with foam generation.

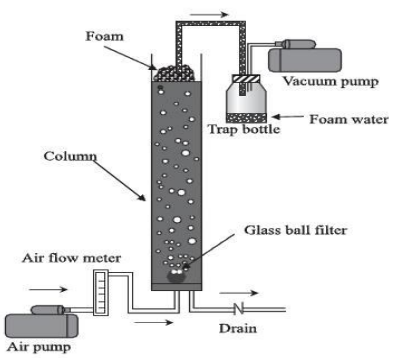

Fig. 2: Schematic diagram of foam separation equipment for batch system (Suzuki et al., 2008).

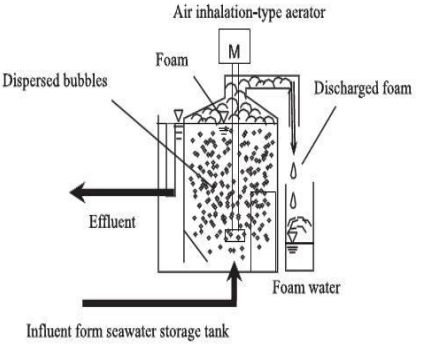

Fig. 3: Schematic diagram of foam separation unit for continuous-flow system (Suzuki et al., 2008).

Flotation: Flotation has been used for centuries in the mining industry for the dressing and concentration of mineral ores and in wastewater engineering. Foams can be used for the recovery of proteins and microorganisms from the cultivation medium by flotation. The recovery of proteins from cultivation medium is usually performed by precipitation, adsorption, flocculation, extraction and ultrafiltration. Foam flotation is especially suitable for protein recovery from aqueous solutions at low protein concentrations (Schugerl, 2000; Vardar-Sukan, 1998).

Cross-flow membrane separation is often used in the lab scale for retention of the cells. Some microorganisms and cells are enriched in the foam; therefore, flotation is suited for the recovery of particular microbial cells from cultivation medium. It was reported that cell separation can be increased by reducing the feed rate and aeration rate and increasing the aerated liquid layer and the foam layer height, as long as the foam remains stable.

It is also increased with a larger column diameter (Schugerl, 2000).

Zheng et al. (1998) showed the application of foam flotation for the recovery of enzymes which are often impaired by their denaturation and activity loss. By using nitrogen or carbon dioxide as sparging gas, respectively, instead of air, low volumetric flow rates (0.79 cm s^{-1}) and operating at 16°C and pH 3, denaturation can be suppressed. A schematic picture of applied flotation column is shown in Figure 4.

Fractionation: Foam fractionation is a gentle, environmentally compatible, inexpensive and selective method for the effective separation of surface-active compounds from diluted aqueous solutions (Linke et al., 2007). This process was first patented in 1920 and received renewed interest in separation of biological materials such as proteins, microorganisms, suspended solids, aromatic substances, and pigments in recent years (Linke et al.,2007; Stevenson et al., 2008). However, continuous multistage operation has the highest performance (Gehle and Schugerl, 1984); the most common mode of operation of foam fractionation employed by various investigators is the single stage semi-batch unit. The protein solution was used in batch mode and aerated continuously (Uraizee and Narsimhan, 1990). Surface-active molecules adsorb to the surfaces of bubbles in rising foam; when this foam is broken into the so-called 'foamate' stream, the solution is seen to be enriched in surfactant concentration. This foam has a relatively high surface area for a specific volume of interstitial liquid (Stevenson et al., 2008).

Handling morphology to enhance application: *Nam and Park* showed successful preparing of biodegradable polymeric microcellular foams by modified thermally induced phase separation method (TIPS).

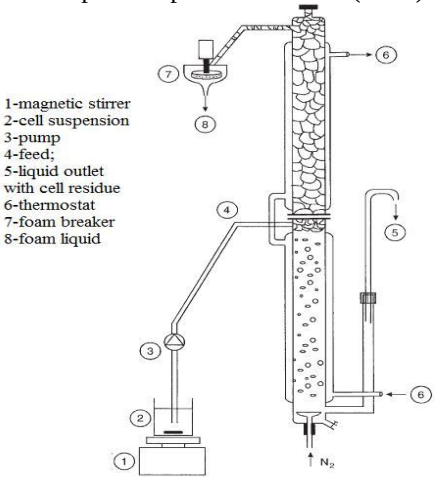

Fig. 4: Continuous cell flotation column (Viehweg and Schugerl, 1983)

It has been shown that various microcellular and porous foam morphologies could be obtained by adjusting the TIPS parameters. A slight changes in the parameters, such as types of polymer, polymer concentration,solvent/ nonsolvent ratio, and the most importantly, thermal quenching strategy, significantly affect the resultant foam morphology. In particular, the addition of polymeric surfactant in the TIPS formulation enhances the size of pores and improves their inter-connectivity. The prepared foams can find applications in a number of fields such as controlled drug delivery and in joining by other methods, such as immobilization or fractionation, to enhance them (Nam and Park, 1999).

Drawbacks of foams in bioprocesses: From another point of view, the interest in foam which is formed during fermentation is purely negative and is a problem common to many of microbial fermentations, especially where surface active

microbial products (bio-surfactants) are involved (Davis et al., 2001).

Generally the problems created by excessive foaming fall into two classes: those that are caused by its appearance within the reactor, and those which are caused by its escape if some control is not exercised (Vardar-Sukan, 1998). Foaming leads to a loss of culture liquid and micro-organisms through air exhaust and reduces the productive volume (increasing process costs) and can lead to blockage of the outlets and threat to the sterility of a fermenter (Varley et al., 2004; Boon et al., 2002). Materials carried into the foam often deposit on the fermenter walls or lid, where they are no longer useful, and interfere with process measurements and sampling. Enzymes, microorganisms and animal cells are carried into the foam layer by froth flotation (Chisti, 1993). In continuous culture, because of the outflow of foam, the effluent from the fermenter may not be representative of the bulk contents. Sometimes, foam generation is autocatalytic, i.e. small amounts of foam can create conditions that promote lysis of some cells and this in turn leads to greater production of foam. Detrimental effects are observed also in the mass and heat transfer patterns of foaming processes. The enhanced gas holdup, made by foaming, decreases the apparent viscosity of the liquid, resulting in a decrease in power dissipation and circulation rate. The presence of tiny bubbles within the liquid also affects the transport properties. These limitations create additional hetero-geneities within the reactor, interfering with process monitoring and control of both on-line and off-line parameters. The denaturation of proteins or enzymes due to the stresses associated with bubble formation may also be a serious problem.

Furthermore,whenever a surface is created in a solution containing surface active materials, the surface active molecules diffusing to the surface tend to remain there, increasing their concentration near the surface. An instance is the occurrence of thick and stable foams on activated sludge plants which is a world- wide phenomenon and a significant problem. Their presence not only reduces the effluent quality of the plant but can also pose public health problems, as some bacteria present are pathogenic (Carr et al., 2005).

Even if the foam does not expand to such an extent as to lead a loss from the fermenter, its presence is still undesirable. The reasons are that the conditions of aeration and agitation will in general then be different from those in a non-foaming system (Brayant, 1970). Care is required in selecting an appropriate culture medium to minimize the unwanted-foaming tendency without affecting the qualitative characteristics of the process.

To sum up disadvantages, a classified list of problems created by foaming in bioprocesses is prepared in the next (Vardar-Sukan, 1998).

Physical Effects

- Increased heterogeneity of broth
- Enhancement of gas-liquid oxygen transfer
- Increased effective reactor volume
- Reduction in the working volume
- Enhanced gas holdup
- Changes in air bubble size and composition
- Decreased power dissipation
- Changed pattern of dissolved gases due to heterogeneous dispersion
- Reduction in apparent viscosity
- Lower mass and heat transfer rates
- Invalid process data due to interference at the electrodes

- Decreased circulation rate
- Incorrect monitoring and control
- Reduction in aeration and mixing
- Blockage of inlet and exit gas filters

Biological Effects

- Enrichment of cells in the stagnant liquid film around the air bubbles
- Deposition of cells on upper parts of the bioreactor
- Loss of culture fluid from exit lines causing product and biocatalyst loss
- Microbial lysis
- Changes in microbial metabolism due to nutrient limitations
- Froth flotation and foam separation causing preferential removal of surface active agents
- Protein denaturation in the foam layer
- Problems in sterile operation
- Risk of environmental contamination due to aerosol formation

Since unwanted foams are wasteful and the uncontrolled foams are often hazardous, elimination of foaming is imperative in bioprocesses. As mentioned, however, sometimes foaming is unavoidable and foam breaking methods must be used; the preferred practice is to prevent foaming rather than destroy the foam that has already formed (Vardar-Sukan, 1998; Varley et al., 2004).

Prevention,circumventing and breaking foams: A chemical way to prevent foam formation is adding antifoams as an inhibitor to the medium. This will be explained in the following section but the best way to avoid foaming is to choose cultivation conditions which circumvent foam formation. Foam formation may be minimized by using lower rates of aeration and agitation and higher oxygen content in the gas inlet. Foaming tendency may be reduced also by employing shorter periods of sterilization. Another solution may be the utilization of especial mutants and tailored biomedia that prevent the formation of foam. Use of mixed microbial cultures is another promising alternative for foam control.

Ghildyal et al. (1988) diminished foam formation by reducing the temperature from 32 to 28 °C. This control was more effective than the use of chemical agents; however, it is often not possible to change the cultivation temperature without reducing the growth and production rate considerably. *Chisti* showed by the use of spargers with large holes, which produce large bubbles, unstable foam was formed and the flotation of hybridoma cells was reduced (Chisti, 1993). However, especially in the case of animal cells, large bubbles can impair the viability of cells.

Foams can often be broken by spraying with small quantities of substances such as ethers and n-octanol. As a result of their high surface activity, these foam breakers raise the surface pressure over small regions of the liquid films and spread from these regions, displacing the foaming agent and carrying with them some of the underlying liquid.

Antifoams: Anything that has destabilizing effect on the foam is antifoam. The main concern is to discover how to prevent its appearance, or, if this is impossible or impracticable, how to destroy it (Brayant, 1970). Antifoam action may take the form of addition of antifoam reagent (as foam inhibitor or foam breaker), mechanical foam breaking or physical methods. The most commonly used method is chemical antifoam agents (AFAs). Although it can add significantly to process costs and reduce the rate of oxygen transfer, or affect the downstream operations and the quality of the final product, and may exert adverse effects on the cell's physiology (Varley et al., 2004).

Microscopic examination of the fresh foam is often the best way to determine which, and thus what remedial action is necessary. Antifoaming agents act against the various factors which promote foam stability and, therefore, a number of mechanisms may be operative. Here all form of antifoam actions are summarized (Carr et al., 2005).

Mechanical foam breaking is largely based on subjecting the foam lamellae to shear stress. Various methods to achieve mechanical foam control have been developed including:

- Injectors. ejectors, and orifices where an occasional sudden pressure drop causes the bubbles to burst
- Revolving disks, impellers, and stirrers where the shear stress is increased by rapidly alternating pressure fields
- Centrifuges and cyclones where the rotational force is superimposed on the centrifugal force and the especial design features enhance the twisting of foam strands

The disadvantages of mechanical foam breakers include high operating costs, complicated designs, possible shear damage to the product or microorganisms, risk of disturbances to the unit operations, and their limited effectiveness (light foam, limited foaming). However, mechanical foam breakers are preferred to overcome the disadvantages associated with the chemical antifoam agents (e.g., reduced mass transfer rate, reaction inhibition, toxicity, adverse effects on downstream processing). Although Mechanical foam control in stirred tank fermenters substantially reduces the agitation power demand by increasing gas holdup (Varley et al., 2004; Boon et al., 2002; Chisti, 1993; Carr et al., 2005; Gogate et al., 2000).

Boon et al. (2002) tested the ability of a variety of radial and axial pumping impellers to disrupt foams. It was shown that, for all impellers, the predominant mechanism behind foam disruption is foam entrainment, as for all impellers the gas hold-up increases sharply when the impeller starts to operate as a defoaming device (Boon et al., 2002; Gogate et al., 2000).

Physical methods for foam control include the use of *ultrasound*, and *thermal* or *electrical treatments*. These methods are not widely used because micro-organisms are quite sensitive to such physical factors.

- The destruction of foam by sonic defoamers is attributed to acoustic pressure, undirected radiation pressure, induced resonant vibrations in the bubbles, high internal pressure in foam bubbles as compared to that in surrounding particles, vacuum caused by sonic energy, and turbulence produced by sonic waves.
- Collapse of the foam by the thermal method is based on the expansion of the bubbles, evaporation of moisture and solvent causing foam, decrease in surface viscosity, thermal degradation of the foam producing material, freezing, and reduction in surface tension.
- Electrical foam breakers are based on passing an electric discharge through the foamy region to break up the foam. The exact mechanism of foam breakage by this method is not known, the effect is probably based on the appearance of forces which act differently on the liquid and the gas.

In industrial production, with a few exceptions, mechanical foam breakers (e.g. steroid biotransformation) are not used because of their high power input demand, which is often higher than the power input by the stirrer. Physical

methods are not used either, because ultrasound, heat or electric treatment can impair the viability of the microorganisms. Mechanical devices action is enhanced by simultaneously using chemical antifoams at the lowest possible concentration. Mechanical devices destroy foam only after it has been formed whereas chemical antifoam agents can prevent foam formation as well as destroy the existing foam (Schugerl, 2000; Vardar-Sukan, 1998). Therefore, a single method may not be effective enough to eliminate the foam problem and the combined action of more than one method may have to be employed.

Chemical Foam Control: Chemical Foam Control is another way which antifoams action. There are thousands of different chemical antifoams.As mentioned previously, chemical foam control substances are often added to the aqueous phase, *prior to foam formation*, and act as foam inhibitors or antifoamers to prevent or inhibit foam formation from within the aqueous phase, and often are used as defoamers or foam breakers, added to eliminate an *existing foam* and usually act on the outer surface of the foam (A foam is a closed system and the defoamer can only reach the outer surfaces). Frequently, the separation into roles is confusing but often the mechanisms are different; for example, alcohols such as octanol are effective defoamers but ineffective as antifoamers. According to *Ross and Robinson and Woods*, AFAs may affect foam in two different ways: (a) the antifoam agent is dispersed into very small droplets which penetrate into the foam lamellae and form a duplex film. This film spreads on the lamellae. It bursts because of the strain caused by the extension of the duplex film. (b) The antifoam agent penetrates into the lamellae and forms a mixed monolayer on the lamellae which has less cohesion than the lamellae-stabilizing protein film in the absence of antifoam (Ross, 1950).

Although the use of chemical antifoam agents offers advantages such as simplicity, ease of operation and acceptable economics, in most cases its disadvantages are sometimes serious. Liquid foams in many industrial plants and sites (distillation columns, paints, foods, oil recovery, water discharges, etc.) can reduce the process efficiency and cause environmental problems in waste discharge. In addition, the use of breaking chemicals or inhibitors may contaminate the product and cause additional pollution (Vardar-Sukan, 1998; Varley et al., 2004).

Antifoam agents are surface active substances which destroy the surface elasticity and surface viscosity of the foaming system and prevent meta-stable foam formation. The antifoam agent must have, therefore, low surface tension to spread on the foam lamellae. An AFA for bioprocesses should be suitable for use with a living system, and it should not interfere with analytical devices such as pH probes or dissolved oxygen electrodes. Because AFAs must be sterilized, they should not deteriorate under sterilization conditions or promote the formation of corrosive products. AFAs are typically added on demand when the reactor contents are foaming vigorously; thus rapid antifoamming action is wanted to prevent overdosing. The antifoaming agent must be supplied in sufficient quantity to maintain a high surface concentration even under the dynamic conditions found in a reactor; therefore, a low solubility is advantageous also the antifoam should have low intermolecular cohesive forces so that it does not itself contribute to surface viscosity or rigidity (Ross, 1950).

In sum up, some factors that affect antifoam performance include (van Phul and Cummings, 2007):

1. **Solubility,** most antifoams exhibit extremely low solubility in aqueous solution.
2. **Droplet size,** the entry force required to allow the antifoam droplet to enter the bubble wall generally increases as antifoam droplets become smaller.
3. **Presence of hydrophobic solids,** liquid-solid mixtures are usually more effective than either component used alone.
4. **Environmental shear,** some antifoams are inactivated by too much shear
5. **Repeated exposure to foaming,** often, repeated exposure to foaming eventually exhausts the antifoam's ability to inhibit formation.
6. **Competing chemical constituents,** other surface active chemical constituents have been found to occupy interfacial area and reduce the effects of antifoams.
7. **Surfactant concentration,** higher surfactant concentrations tend to reduce antifoam effectiveness by increasing the entry force necessary to bridge the interfacial film.
8. **Dissolved salt species and concentration,** the presence of high valence metal ions reduces antifoam effecttiveness.

AFAs are added to nearly all submerged fermentations. The mode of action of a chemical antifoam agent depends on the nature of the compound, the type of the foam, and the nature of the substances causing foaming. AFAs belonging to different groups of surface active agents may affect the process differently. Here, some effects are summarized (Schugerl, 2000; Brayant, 1970; Vardar-Sukan, 1998).

Effects on Microbial Metabolism: Certain types of antifoam chemicals are toxic to microorganisms, while some others may favorably affect growth or product formation. Therefore, this has been reported to increase or to decrease microbial growth, product formation and substrate utilization. Microbial enzyme systems may be damaged by some of the oils used as carriers, causing rates of sugar utilization to decrease and production of desired metabolites such as antibiotics to be inhibited. Therefore, for enzyme production, inert antifoam agents that cannot be metabolized by the microorganisms are preferred.

The antifoam may also have a physiological effect by being metabolized. For example, the pH of the medium may be affected when fatty acids are released into the culture medium through hydrolytic action of lipases and the resulting free fatty acids are utilized as carbon sources to have a marked effect on the overall metabolism (Schugerl, 2000; Vardar-Sukan, 1998).

Effects on Mass Transfer: Addition of antifoam to fermentation broth often affects the oxygen transfer performance. The precise effect is the interactive outcome of many variables including aeration and agitation,turbulence, viscosity, oxygen gradient, concentration and morphology of the microorganisms, contact time and surface parameters of the system. Addition of antifoaming agents alters the surface tension, surface viscosity and ionic strength, thus affecting the surface area, coalescence behavior, and rigidity of the bubbles (Yeh et al., 2006).

Generally the surface active antifoam agents may enhance or reduce the mass transfer. Some reports decline that the values of volumetric mass transfer coefficient (kLa) in the presence of antifoams

were larger than the values in the absence of the antifoam at high driving forces; however, the observed kLa values were lower in presence of the antifoaming agent at low driving forces. Therefore, up to an extent a foaming liquid may have a higher kLa. However, the net effect of adding an antifoam agent is difficult to predict because the effect depends on the limiting stage in the oxygen supply process (Vardar-Sukan, 1998; Varley et al., 2004).

For more investigation, we know that kLa is strongly enhanced by increasing the aeration rate. At low superficial gas velocities (<2.5 $cm.s^{-1}$),the bubble coalescence can be neglected. It was assumed that below this gas velocity the difference between kLa values in distilled water, in cultivation media in the presence and absence of AFA is caused by only kL and that this difference in kL holds true for higher gas velocities as well. Above this critical superficial gas velocity the volumetric mass transfer coefficient due to the specific interfacial area 'a' is enhanced, but the bubble coalescence is also increased, which reduces 'a'.

After addition of an antifoam agent to the cultivation medium, the balance between oxygen uptake rate (OUR) and oxygen transfer rate (OTR) is disturbed. The increase in the dissolved oxygen concentration (DOC) is probably caused by the stronger reduction of OUR of the fungus (due to its diminished respiration of the fungus) than OTR. The decrease in DOC above this value is due to the stronger reduction of the OTR than the OUR (Schugerl, 2000; Brayant, 1970).

Effects on Process and Unit Operation: Antifoam agents may induce considerable changes in the physical properties of the culture broth, leading to possible deterioration of bioreactor performance parameters. Products and installations contaminated with highly surface active agents may sometimes be forced into shutdown.

Excessive foaming can adversely affect the unit operations in product recovery, separation and isolation. Foam can foul ultrafiltration and microfiltration membranes and reduce permeate flux. This fouling effect results from several factors including the molecular mass of solutes, the type and the material of membranes and antifoaming agents. Other negative effects of antifoams have been reported in unit processes such as adsorption, extraction, electrophoresis and crystallization.

Each emulsion, according to its structure, process and plants which is used in, has an optimal concentration at which they are efficient and in another situations are not. For instance, pure silicone and polymer AFAs are not suited for foam suppression in large reactors, because of their inadequate distribution in the medium. In addition, these AFAs quickly lose their effectiveness, because they are deposited on the reactor wall. These optima are at very low concentrations. At these low concentrations, the AFAs are not toxic for the microorganisms and they do not impair their growth and product formation. However, they often reduce the OTR and by that they can cause oxygen limitation. Even if the antifoam does not interfere with downstream operations, its presence in the final product may create serious problems with respect to product quality and toxicology.

Schugerl by adding an AFA to the cultivation medium, showed the mean bubble velocity instantaneously increased by a factor of about two in the airlift tower loop reactor during the cultivation of *E. coli* (Schugerl, 1996). After about half an hour, the bubble velocity dropped to the original value, which indicates that the

antifoam had disappeared from the cultivation medium. However, after several antifoam additions, the base line and the maxima of the bubble velocity gradually increased. The cultivation medium became more and more coalescence promoting. Monitoring the intensity of the reflected ultrasound allowed the specific gas/liquid interfacial area to be measured in situ. The specific interfacial area 'a' instantaneously reacted to the addition of an AFA (SE9) to the medium (Koch et al., 1995).

Schugerl (1996) presented key parameters for recombinant *E. coli* batch cultivation in a 60L working volume airlift tower loop reactor at constant aeration rate up to 16 h, whereupon the temperature was increased from 30 to 42°C and gene expression was induced. At the same time, concentrated Luria-Bertani (LB) medium was added to the reactor. To avoid oxygen limitation, the aeration rate was increased. At 12 h the foaming increased and SE9 was added to the medium. The bubble velocities and the specific gas/liquid interfacial area quickly increased and passed a narrow maximum, but kLa dropped and the OTR was not influenced. After the induction of the gene expression by a temperature increase and medium supplement the dissolved oxygen concentration with respect to the saturation increased due to the elevation of the aeration rate; the mean bubble velocity and specific interfacial area decreased, OTR increased and kLa remained at low values. The mass transfer coefficient with respect to the liquid phase kL dropped from about 1.67 to 0.67 ms^{-1} after the addition of SE9 to the medium (Koch et al., 1995).

In Fig. 12 the variations in the specific growth rates of recombinant *E. coli* during cultivation in a 2.5L stirred tank reactor at different SE9 concentrations are shown. AFAs in large reactors only slightly influence the cell concentration and product formation. SE9, a silicone oil emulsion, caused a significant increase in the concentration of *E. coli* and intracellular product and did not impair the CFU (colony forming units) and plasmid stability. The effect of AFAs on the OTR and kLa is significant at the beginning of the cultivation. Later, this effect is gradually decreased.

Monitoring and Measurement: Foaming importance is clear now and, therefore, it is important to be able to effectively monitor and control the dynamic formation and collapse of such foam phases. Foam sensors are the major components of any foam control system and are used to detect foam so that a control mechanism can be activated. Foam sensors can be classified into two main types: *contact sensors* and *contact-less sensors*. Contact sensors function either as capacitance sensors or as conductivity sensors. Contact sensors have a number of disadvantages such as microbial overgrowth, electrode polarization and erosion. Contactless detectors overcome the shortcomings of the former group of sensors. Contactless devices based on ultrasound, photo-detection or detection of head pressure variations, are available.

Protein foams play an important role in both food and biotechnological processes. A sound understanding of foaming properties of proteins relevant to such processes is useful (Phianmongkhol and Varley, 1999). In general, measurements of changes in foam volume (volumetric method) are used for foam characterization. However, recently there has been increased interest in the use of measurement methods based on conductivity and capacitance. Foams are currently monitored by means of

single or dual conductivity probes, which provide an indication of the presence or absence of foam at a particular height in a vessel. If unwanted foam is present at the location of one of these probes, anti-foam may then be added. This method of measurement takes little account of the physical nature or dynamics of the foam, e.g. anti-foam would be added regardless of whether the foam detected was stable or about to collapse naturally (Varley et al., 2004). It is developed and tested for some time a multi-segment conductivity probe, which allows measurement of the conductivity across a foam phase at a range of heights. The change in conductivity can be monitored as a function of time thus giving an indication of the rate of foam formation and collapse processes. In general there is good agreement between volumetric and conductimetric measures however the conductivity measurements appear more sensitive to both protein concentration and operation parameters as compared to the commonly used volumetric method. This type of conductimetric techniques could be used for foam characterrization in systems in which the foam cannot easily be visualized, e.g. fermentations.

Varley et al. (2004) reported a dynamic multi-point measurements of conductance across a foam phase formed during a continuous *Pseudomonas* sp. fermentation for a range of process regimes in which the only variables were gas flow rate, gas composition and impeller speed. On the basis of dynamic multi-point measurement of foam behavior, the dependence of foaming frequency on some key process control variables has been identified. The data obtained is potentially useful for defining fermentation foam control strategies.

Pugh reported several techniques to study the structure and the stability of froths and foams. Image analysis also proved useful for detecting structure changes in 2-D foams and has enabled the drainage process and the gradients in bubble size distribution to be determined. However, studies on 3-D foams require more complex techniques such as Mutiple-Light Scattering methods, microphones and optical tomography (Pugh, 2005). Under dynamic foaming conditions, the Foam Scan Column enables the water content of foams to be determined by conductivity analysis.

Consider that the best control method established in one plant for a particular process is not necessarily suitable for a similar process elsewhere. This is because complex natural products are generally used in preparing the culture media and properties of those natural components are not particularly well defined. At last, it is important to be able to effectively monitor and control the dynamic formation and collapse of foam phases. Despite numerous advances, successful prediction of foaming and defoaming phenomena in bioprocesses is not entirely possible at present and further research continues to be needed.

CONCLUSIONS

Foaming is encountered in bioreactors. Protein foams play an important role in both food and biotechnological processes. The foaming tendency and its stability of a bioprocess depend on the system and the operating variables. The complexities of bio systems make it difficult to relate their foaming characteristics to individual factors. Foaming is widely used in applications such as furniture industry,packing,coatings, decorating,building construction,insulation, shoe industry, transportation and etc. Foam

separation is an efficient method for the objective recovery of proteins, surface-active products, enzymes, microorganisms etc. On the other hand, the interest in the foam formed during fermentation is purely negative and is a problem common to much microbial fermentation, especially where surface active microbial products are involved. Care is required in selecting an appropriate culture medium to minimize the unwanted-foaming tendency without affecting the qualitative characteristics of the process. The main concern is to discover how to prevent its appearance, or, if this is impossible or impracticable, how to destroy it. The best way to avoid foaming is to choose cultivation conditions which circumvent foam formation. Foam formation may be minimized by using lower rates of aeration, higher oxygen content in the gas inlet, employing shorter periods of sterilization and etc. Besides, antifoam action may take the form of addition of antifoam agent (as foam inhibitor or foam breaker), mechanical foam breaking or physical methods to preventing and breaking foaming. The most commonly used method is the addition of chemical anti-foam agents. However, it would have significant undesirable effects. Other methods have their disadvantages too. Therefore, a single method may not be effective enough to eliminate the foam problem and the combined action of more than one method may have to be employed. It is also important to be able to effectively monitor the dynamic formation and collapse of such foam phases. Foam sensors are the major components of any foam control system and are used to detect foaming. Recently there has been increased interest in the use of measurement methods based on conductivity and capacitance.

In spite of the important role of foaming in bioprocesses, successful prediction of foaming and defoaming phenomena is not entirely possible at present and further attention and research continues to be needed while foam control remains an empirical art.

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