Foam Control:

Foam is produced during most microbial fermentations. Foaming may occur either due to a medium component, e.g., protein present in the medium, or due to some compound produced by the microorganism. Proteins are present in corn-steep liquor, pharma media, peanut meal, soybean meal, etc.

These proteins may denature at the air-broth interface and form a protein film that does not rupture readily. Foaming can cause removal of cells from the medium; such cell wills undergo autolysis and release more proteins into the medium. This, in turn, will further stabilize the foam. Five different patterns of foaming are recognized; these are listed below.

1. Foaming remains at a constant level throughout the fermentation. Initial foaming is due to the medium, but later microbial activity contributes to it.

2. Foaming declines steadily in the initial stages, but remains constant thereafter. This type of foaming is due to the medium.

3. The foaming increases after a slight initial fall', in this case, microbial activity is the major cause of foaming.

4. The foaming level increases with fermentation duration; such foaming pattern is solely due to microbial activity.

5. A complex foaming pattern that combines features of two or more of the above patterns.

Foaming may lead to several physical and biological problems. Some examples of physical problems are as follows:

(1) The working volume of the fermenter may decrease due to a circulation of oxygen-depleted gas bubbles in the system.

(2) The bubble size may also decrease, and

(3) The heat and mass transfer rates may also decline.

(4) Foaming may interfere with the functioning of sensing electrodes resulting in invalid process data, and incorrect monitoring and control of pH, temperature, etc. The biological problems of foaming include (1) deposition of cells in the upper parts of the fermenter, (2 problems of sterile operation as the air filter exits of the fermenter become wet, and (3) increased risk of contamination. In addition, (4) there may be product loss due to siphoning of the culture broth.

Whenever excessive foaming occurs, the following approaches may be used to resolve the problem:

(1) A defined medium may be used to avoid foam formation. This may be combined with modifications in physical parameters like pH, temperature, aeration and agitation. This approach will be successful in such cases where medium is the main culprit, but will fail whenever microbial activity is the main contributor.

(2) Often the foam may be unavoidable; in such case, antifoam should be used. This is the most standard approach to combat foaming.

(3) A mechanical foam breaker may also be used. Antifoams are surface active agents; they reduce surface tension in the foams and destabilize protein film by the following effects: (a) hydrophobic bridges between two surfaces, (b) displacement of the absorbed protein, and (c) rapid spreading of the surface film. Ideal antifoam should have the following properties.

1. It should disperse rapidly and act fast on existing foam.

2. It should be used at a low concentration.

3. It should prevent new foam formation for a long time.

4. It should not be used up or degraded by the microorganism.

5. It should be nontoxic (to the microorganism as well as animals, including humans).

6. It should not interfere with downstream processing.

7. It should not cause problems in effluent treatment.

8. It should be safe to handle.

9. It should be cheap.

10. It should not affect oxygen transfer.

11. It should be heat stable for heat sterilization.

Several compounds meet most of these requirements, and have been found to be suitable for different fermentation processes; these compounds are as follows: alcohols (stearyl and octyl decanol), esters, fatty acids and their derivatives (especially, triglycerides like cottonseed oil, linseed oil, soybean oil, sunflower oil, etc.), silicones, sulphonates, and miscellaneous compounds like oxaline, Alkaterge C, and polypropylene glycol.

Many of the antifoams are of low solubility; therefore, they are added with a carrier like lard oil, liquid paraffin and castor oil. There carriers, however, may be metabolized, and they may affect the fermentation process. Further, many antifoams would reduce oxygen transfer by up to 50% when used at effective concentrations.

Antifoams are generally added when foaming occurs during fermentation. But foam control in fermentation industry is still an empirical art. Therefore, the best method of foam control for a particular process in one factory is not necessarily the best for the same process in other factories. Further, the design and operating parameters of the fermenters may affect the properties and the foams produced during the fermentation process.

Types of Fermenters:

A variety of fermenters have been described in the literature, but few of them have proved satisfactory for large scale aerobic fermentations. The most commonly used fermenters are based on a stirred upright cylinder with sparger aeration (Fig. 14.3).

The volumes ranging from 1 l to several thousand I. A general description of the following types of fermenter is given in the following sections: (1) stirred tank reactor, (2) airlift fermenter, (3) tower fermenter and (4) bubble up fermenter.

Stirred Tank Fermenter:

These are glass (smaller vessels) or stainless steel (larger volumes) vessels of 1-1,000 1 or even 8,000 1 (Namalva cells grown for interferon; but in practice their maximum size is 20 1 since larger vessels are difficult to handle, autoclave and to agitate the culture effectively).

These are closed systems with fixed volumes and are usually agitated with motor-driven stirrers with considerable variation in design details, e.g., water jacket in place of heater type temperature control, curved bottom for better mixing at low speeds, mirror internal finishes to reduce cell damage, etc. Many heteroploid cell lines can be grown in such vessels.

The needs for research bio-chemicals from cells are met from 2-50 1 reactors, while large scale reactors are mainly used for growing hybridoma cells for the production of monoclonal antibodies although their yields from cultured cells is only 1-2% of those obtained by passaging the cells through peritoneal cavity of mice.

Continuous-Flow culture systems, a type of stirred tank reactors, are either of chemostat or turbidostat type. In both the types, cultures begin as a batch culture. In a chemostat type, inoculated cells grow to the maximum density when some nutrient, e.g., a vitamin, becomes growth limiting.

Fresh medium is added after 24-48 hours of growth, at a constant rate (usually lower than the maximum growth rate of culture) and at an equal rate the culture is withdrawn. When the rate of growth equals the rate of cell withdrawal, the cultures are in a 'steady state', and both the cell density and medium composition remain constant. One of the constituents of the medium is used at a lower concentration to make it growth-limiting. However, chemostat is the least efficient or controllable at the cell's maximum growth rate hence the steady-state growth rates in them are much lower than the maximum.

In contrast, in a turbidostat cells grow to achieve a pre-decided density (measured as turbidity using a photoelectric cell). At this point, a fixed volume of culture is withdrawn and the same volume of fresh normal (not having a growth-limiting factor) medium is added; this lowers the cell density or turbidity of the culture.

Cells keep growing, and once the culture reaches the preset density the fixed volume of culture is replaced by fresh medium. This system works really well when the growth rate of the culture is close to the maximum for the cell line.

The continuous-flow cultures provide a continuous source of cells, and are suitable for product generation, e.g., for the production of viruses and interferons. It is often necessary to use a two-stage system in which the first stage supports cell growth, while the second stage promotes product generation.

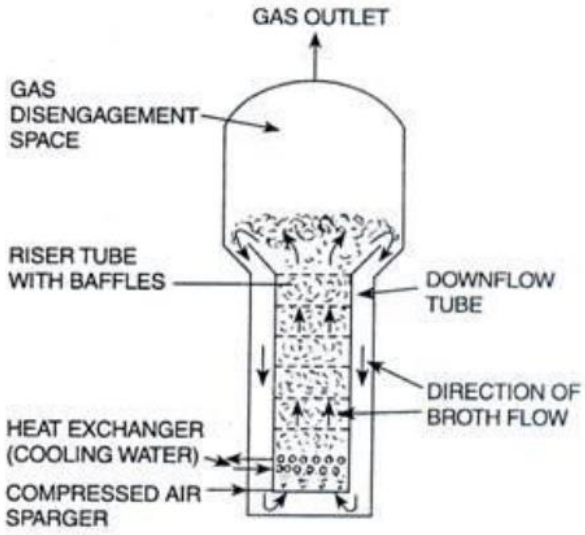

FIG. 14.4. Air-lift fermenter with externally placed riser tube.

Airlift Fermenter:

An airlift fermenter consists of a gas light baffled riser tube or draught tube (broth rises through this tube) connected to a down-comer tube (broth flows down through this tube). The riser tube may be placed within the down-comer tube as shown in Fig. 14.4, or it may be externally located and connected to the latter (Fig. 14.5). Air/gas mixture is introduced into the base of the riser tube by a sparger.

The aerated medium/broth of the riser tube has a lower density, while that in the down-flow tube it is relatively much less aerated and, as a consequence, has a higher density. This density difference drives the circulation of broth.

The lighter medium in the rise tube flows upward till it reaches the gas disengagement space of the fermenter. The O_2 is continuously consumed by the cells and CO_2 is generated by respiration.

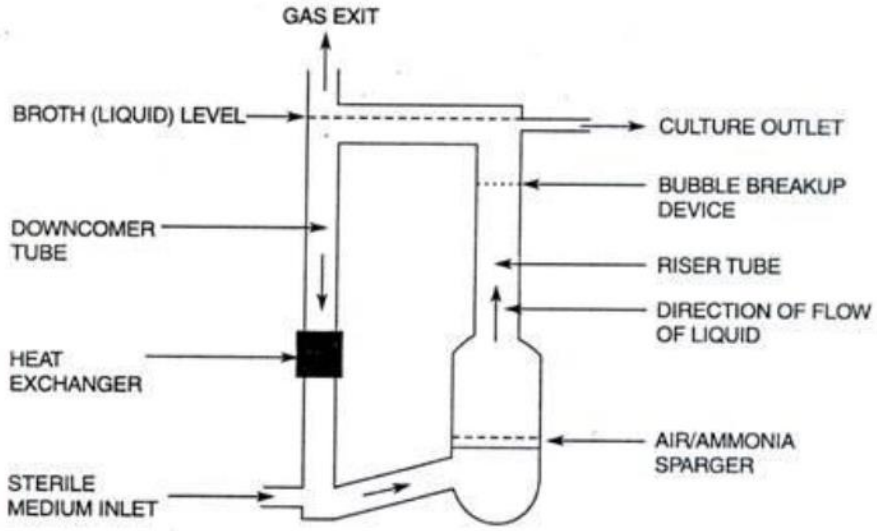

FIG. 14.5. Air-lift fermenter with inner loop.

The bulk of CO_2 and other gases move out of the medium broth into the gas phase, and the un-aerated medium flows down through the down-flow tube. Circulation times in loops of 45 m height may be 120 seconds.

Single cell protein (SCP) production by Marlow Foods, U.K uses an air-lift fermenter in which the riser tube is externally placed (Fig. 14.6). Air and gaseous ammonia are introduced at the base of riser tube, while sterilized glucose, biotin and mineral salts are pumped into the fermenter at the base of the down-flow tube.

An internal heat exchanger coil is located at the bottom loop connecting the riser and down-flow tubes; it maintains the temperature at 30°C. The upper loop connecting the riser and down-flow tubes acts as an air outlet assembly through which CO_2 is continuously extracted. The removal of

CO_2 and continuous consumption of O_2 dissolved in the medium increases the density of the culture broth, which causes it to settle down through the down-flow tube.

SCP is harvested through a port at the base of riser tube, which leads into an RNA reduction vessel; steam is injected into the vessel to raise the temperature to 60°C, which reduces RNA content of SCP. After RNA reduction, SCP is harvested and processed.

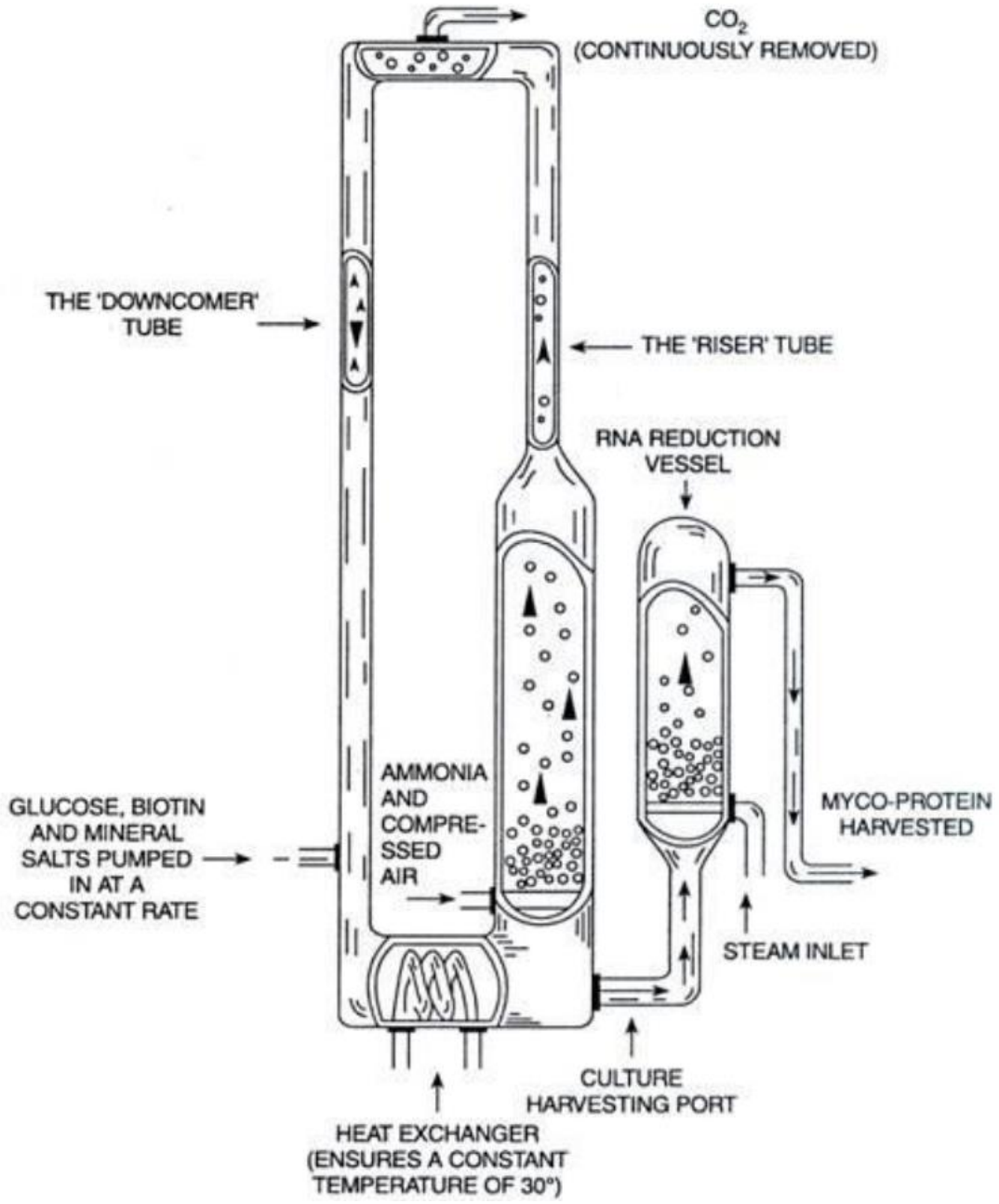

FIG. 14.6. A schematic diagram of the air-lift fermenter used by Marlow Foods, U.K. for the production of myco – protein Quorn in continuous flow culture.

This air-lift fermenter of 43 m^3 volume is used in a continuous mode for the production of mycoprotein Quorn from Fusarium gaminareum grown on wheat starch-based medium. It allows production of long hyphae due to low shear, which is the preferred form of the product.

However, it gives lower biomass yields (only 20 g l^{-1}) due to lower oxygen transfer rates in the high viscosity broth resulting from fungal hyphae. This fermenter is a modification of that designed by ICI pic, U.K. for SCP production using methanol as substrate.
The fermenter was developed to reduce production costs by minimising cooling costs since agitated vessels would generate additional heat. ICI pic used it in a continuous process to produce SCP for animal feed, but the process had to be discontinued because of high methanol cost and competition from animal feeds based on protein-rich crop produce. The mycoprotein production is, however, primarily for animal food.

Modifications of airlift fermenters include various modifications of draught (riser) tubes and multiple air-lift fermenters.

(1) In one modification of draught tube, stainless steel four-mesh tubes were placed at the top and bottom of the tube. This fermenter was used for growing Aspergillus terreus for itaconic acid production. The sieves modulated the fungal morphology so that the biomass was in an intermediate state between pellets and pulp. The type of culture gave double the yields of itaconic acid per unit volume per unit culture time.

(2) The multiple air-lift fermenters has three air-lift fermenters placed in a single vessel. The medium is fed into the central fermenter from where it flows in the middle one and then finally into the outer compartment from where it is eventually discharged.

(3) Another modification of air-lift fermenters is described in Section 14.9.3.4.

Animal cell cultures are also grown in such vessels that are both aerated and agitated by air bubbles introduced at the bottom of vessels (Fig. 14.7). The vessel has an inner draft lube through which the air bubbles and the aerated medium rise since aerated medium is lighter than non-aerated one; this results in mixing of the culture as well as aeration. The air bubbles lift to the top of the medium and the air passes out through an outlet.

The cells and the medium that lift out of the draft tube move down outside the tube and are re-circulated. O_2 supply is quite efficient but scaling up presents certain problems. Fermenters of 2-

90 1 are commercially available but 2,000 1 fermenters are being used for the production of monclonal antibodies.

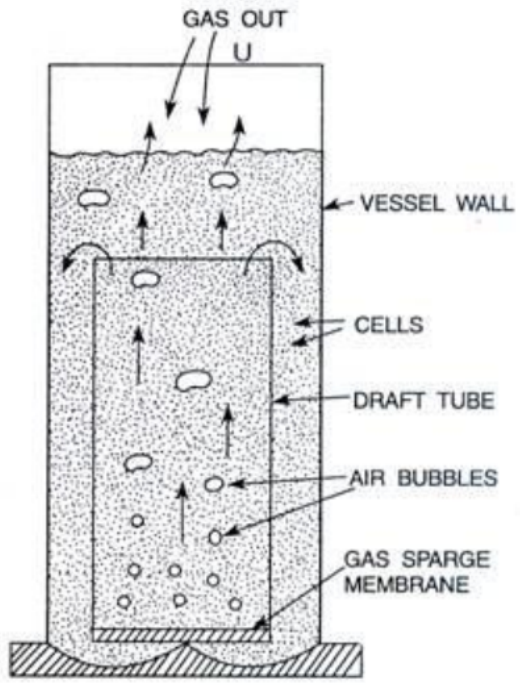

FIG. 14.7. A schematic representation of an airlift fermentor.

Tower Fermenter:

A tower fermenter has been defined by Green-shields and co-workers as an elongated non-mechanically stirred fermenter that has an aspect ratio (height to diameter ratio) of at least 6 : 1 for the tubular section and 10 ; 1 overall, and there is a unidirectional How of gases through the fermenter. There are several different types of tower fermenters, which are grouped as follows on the basis of their design: (1) bubble columns, (2) vertical-tower beer fermenter and (3) multistage fermenter systems.

1. Bubble Column Tower Fermenters:

These are the simplest type of tower fermenters; they consist of glass or metal tubes into which air is introduced at the base. Fermenter volumes from 3 / to up to 950 / have been used, and the aspect ratio may be up to 16 : 1. These tower fermenters have been used for citric acid and tetracycline production, and for a range of other fermentations based on mycelial fungi.

2. Vertical-Tower Beer Fermenters:

These fermenters were designed for beer production and to maximise yeast biomass yields. A series of perforated plates are placed at intervals to maximise yeast yields. It has a settling zone free of gas; in this zone, yeast cells settle down to the bottom and return to the main body of the tower fermenter, and clear beer could be removed from the fermenter. Tower of up to 20,000 / capacity and capable of producing up to 90,000 I beer per day have been installed.

3. Multistage Tower Fermenters:

In these fermenters, a column forms the body of vessel, which is divided into compartments by placing perforated plates across the fermenter. About 10% of the horizontal area of plates is perforated. In a variant of this type of fermenter (down-flow tower fermenter), the substrate is fed in at the top and overflowed through down spouts to the next section, and the air is supplied from the base. These fermenters have been used for continuous culture of E. coli, S. cerevisiae (baker's yeast), and activated sludge.

Bubble-up Fermenter:

It is a bubble column fermenter that is fitted with an internal cooling coil (Fig. 14.8). Air is introduced from the bottom of the column. In this vessel, the cooling coil effectively separates the column into an inner riser/draught tube and the outer down-flow tube. The cooling coil assembly functions as a leaky draught tube.

The culture broth rises in the compartment enclosed by the cooling coils and it moves down in the compartment outside the coil, although back- mixing also occurs through the coils. The region above the cooling coil shows good mixing, and there were no poorly oxygenated zones in the vessel. It can generate liquid velocities of 1 m sec^{-1}, giving circulation times of 9-12 seconds and mixing times of 14-18 seconds.

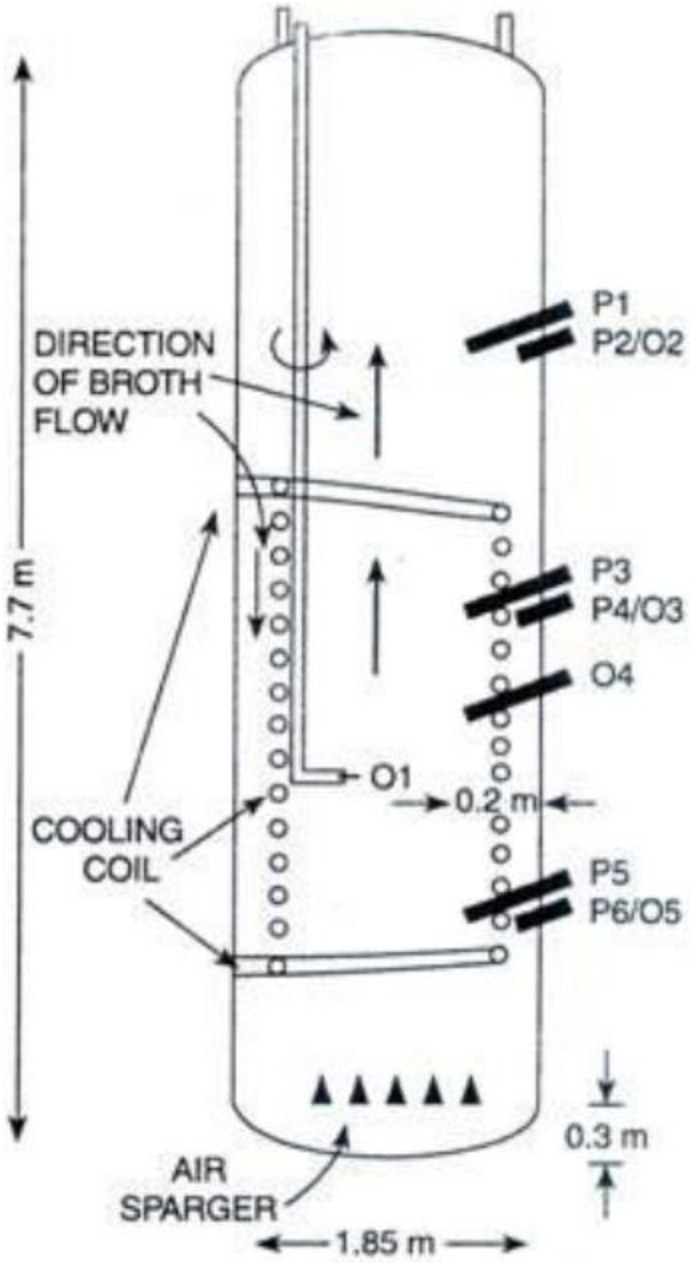

FIG. 14.8. Schematic diagram of a 20 m^3 bubble column fermenter fitted with internal cooling coil. P_1, P_2, etc. indicate location of pH electrodes; and O1, O2, etc. depict the locations of oxygen electrodes to measure the amount of dissolved oxygen.