Module-24: Aeration and Agitation

Introduction

The main function of aeration is to supply enough oxygen to the microbes in submerge culture technique for proper metabolism, while agitation provides proper mixing of the nutrient so that each and every organisms get proper nutrients.

Each fermentation process requires unique type of aeration and agitation system.

The parts of the fermenter involved in aeration and agitation are:

(a) The agitator (impeller).

(b) The aeration system (sparger).

The agitator (impeller)

The main aim of the agitator is to provide homogenous environment all over the fermenter. It is also used for mixing of different phases, oxygen and heat transport.

The aeration system (sparger)

A sparger is a tool used for introducing air into the fermentation medium.

Three basic types of sparger

1. The porous sparger,
2. The orifice sparger (a perforated pipe) and
3. The nozzle sparger (an open or partially closed pipe).

Porous Sparger

The porous sparger is mainly used for laboratory scale non agitated fermenter. It is made up of sintered glass, ceramics or metal.

Orifice Sparger

In small stirred fermenters the perforated pipes were arranged below the impeller in the form of crosses or rings (ring sparger), approximately three-quarters of the impeller diameter.

Nozzle Sparger

Most modern mechanically stirred fermenter designs from laboratory to industrial scale have a single open or partially closed pipe as a sparger to provide the stream of air bubbles. Ideally the pipe should be positioned centrally below the impeller and as far away as possible from it to ensure that the impeller is not flooded by the air stream (Finn, 1954). The single-nozzle sparger causes a lower pressure loss than any other sparger and normally does not get blocked.
**Need For Aeration and Agitation**

The majorities of fermentation processes are aerobic and, therefore, require the provision of oxygen. If the stoichiometry of respiration is considered, then the oxidation of glucose may be represented as:

\[ C_6H_{12}O_6 + 6O_2 = 6H_2O + 6CO_2 \]

Thus, 192 grams of oxygen are required for the complete oxidation of 180 grams of glucose. However, both components must be in solution before they are available to a microorganism and oxygen is approximately 6000 times less soluble in water than is glucose (a fermentation medium saturated with oxygen contains approximately 7.6 mg dm\(^{-3}\) of oxygen at 30°C). Thus, it is not possible to provide a microbial culture with all the oxygen it will need for the complete oxidation of the glucose (or any other carbon source) in one addition.

Therefore, a microbial culture must be supplied with oxygen during growth at a rate sufficient to satisfy the organisms' demand.

The aeration and agitation of the fermentation medium, provides necessary oxygen to the industrial fermentation process. However, the productivity of many fermentations is limited by oxygen availability and, therefore, it is important to consider the factors which affect a fermenter's efficiency in supplying microbial cells with oxygen.

**The Oxygen Requirements of Industrial Fermentations**

Although a consideration of the stoichiometry of respiration gives an appreciation of the problem of oxygen supply, it gives no indication of an organism's true oxygen demand as it does not take into account the carbon that is converted into biomass and products.

It has been studied that a culture's demand for oxygen is very much dependent on the source of carbon in the medium. Thus, the more reduced the carbon source the greater will be the oxygen demand.

However, it is inadequate to base the provision of oxygen for fermentation simply on an estimation of overall demand, because the metabolism of the culture is affected by the concentration of dissolved oxygen in the broth.

It may be seen that the specific oxygen uptake rate increases with increase in the dissolved oxygen concentration up to a certain point (referred to as \( C_{crit} \)) above which no further increase in oxygen uptake rate occurs.

Thus, maximum biomass production may be achieved by satisfying the organism's maximum specific oxygen demand by maintaining the dissolved oxygen concentration greater than the critical level. If the dissolved oxygen concentration were to fall below the critical level then the cells may be metabolically disturbed.
However, it must be remembered that it is frequently the objective of the fermentation technologist to produce a product of the micro-organism rather than the organism itself and that metabolic disturbance of the cell by oxygen starvation may be advantageous to the formation of certain products.

Equally, provision of a dissolved oxygen concentration greater than the critical level may have no influence on biomass production, but may stimulate product formation. Thus, the aeration conditions necessary for the optimum production of a product may be different from those favoring biomass productions.

The oxygen demand of fermentation largely depends on the concentration of the biomass and its respiratory activity, which is related to the growth rate.

By limiting the initial concentration of the medium, the biomass in the vessel may be kept at a reasonable level and by supplying some nutrient component as a feed, the rate of growth and hence the respiratory rate, may be controlled.

**Oxygen Supply**

Oxygen is normally supplied to microbial culture in the form of air, this being the cheapest available source of the gas.

The method for provision of a culture with a supply of air varies with the scale of the process:

1. **Laboratory scale**

   Cultures may be aerated by means of the shake-flask method. Flask are shaken on a platform contained in a controlled environment chamber

2. **Pilot and Industrial Scale**

   Air is provided to the cultures by specific types of fermenter (Bubble fermenter)

Bartholomew et al. (1950) represented the transfer of oxygen from air to the cell, during fermentation, as occurring in a number of steps:

(i) The transfer of oxygen from an air bubble into solution.

(ii) The transfer of the dissolved oxygen through the fermentation medium to the microbial cell.

(iii) The uptake of the dissolved oxygen by the cell.

The rate of oxygen transfer from air bubble to the liquid phase may be given by the equation:

\[
dC_L / dt = K_La (C^* - C_t)
\]

Where \( CL \) is the concentration of dissolved oxygen in the fermentation broth (mmole dm\(^{-3}\)),

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t is time (hours),

dC_L/dt is the change in oxygen concentration over a time period, i.e. the oxygen transfer rate (mmole O_2 dm^{-3} h^{-1}),

KL is the mass transfer coefficient (cm h^{-1}),

a is the gas/liquid interface area per liquid volume (cm^2 cm^{-3}),

C^* is the saturated dissolved oxygen concentration (mmoles dm^{-3}).

KL may be considered as the sum of the reciprocals of the resistances to the transfer of oxygen from gas to liquid and (C^*-CL) may be considered as the 'driving force' across the resistances. It is extremely difficult to measure both KL and 'a' in a fermentation and, therefore, the two terms are generally combined in the term KLa, the volumetric mass-transfer coefficient, the units of which are reciprocal time (h^{-1}).

The volumetric mass-transfer coefficient (KLa) is used as a measure of the aeration capacity of a fermenter. The aeration capacity of the system will be more if KLa, is higher.

The KLa value will depend upon the design and operating conditions of the fermenter and will be affected by such variables as aeration rate, agitation rate and impeller design.

These variables affect 'KLa' by reducing the resistances to transfer and affect 'a' by changing the number, size and residence time of air bubbles.

**Determination of KLa Values**

1. **The sulphite oxidation technique**

   \[ Na_2SO_3 + 0.5O_2 = Na_2SO_4 \]

   The rate of reaction is such that as oxygen enters solution it is immediately consumed in the oxidation of sulphite, so that the sulphite oxidation rate is equivalent to the oxygen-transfer rate.

2. **Gassing-out techniques**

   a. **The Static Method of Gassing Out**

   The technique was first described by Wise (1951); the concentration of oxygen in the solution is decreased by passing nitrogen gas into the liquid, this will remove all the oxygen from the solution.

   The aeration and agitation of deoxygenated liquid increase the dissolved oxygen which is monitored using some form of dissolved oxygen probe.

   This technique has the advantage over the sulphite oxidation method in that it is very rapid (normally taking up 15 minutes) and may utilize the fermentation medium, to which may be
added dead cells or mycelium at a concentration equal to that produced during the fermentation.

b. The Dynamic Method of Gassing Out

The procedure involves stopping the supply of air to the fermentation which results in a linear decline in the dissolved oxygen concentration due to the respiration of the culture.

The aeration and agitation of deoxygenated liquid increase the dissolved oxygen which is monitored using some form of dissolved oxygen probe.

The dynamic gassing out method has the advantage over the previous methods of determining the $K_{L}a$ during an actual fermentation and may be used to determine $K_{L}a$ values at different stages in the process. The technique is also rapid and only requires the use of a dissolved oxygen probe, of the membrane type.

3. The oxygen-balance technique

The oxygen balance technique is used for the determination of transportation of amount of oxygen into the fermentation medium in a given period of time. It is also used for the measurement of $K_{L}a$ of a fermenter.

The procedure involves measuring the following parameters:

1. The amount of medium in the fermenter (dm$^3$)
2. The rate of flow of air (incoming and outgoing air), dm$^3$ min$^{-1}$
3. The total pressure at inlet and outlet (atm)
4. The temperature of the gases of the inlet and outlet, (K)
5. The partial pressure of oxygen of the inlet and outlet

The oxygen balance technique appears to be the simplest method for the assessment of $K_{L}a$ and has the benefit of measuring aeration efficiency during fermentation. The sulphite oxidation and static gassing out techniques have the disadvantage of being carried out using either a salt solution or an uninoculated, sterile fermentation medium.

The factors affecting $K_{L}a$ values in fermentation vessels

1. The air flow rate employed
2. The degree of agitation
3. The rheology properties of the medium
4. The presence of antifoam agents

1. The effect of air-flow rate on $K_{L}a$

The rate of air flow in fermentation media in agitated and non-agitated fermenter will be different

Mechanically Agitated Reactors
The air-flow rate of 0.5-1.5 volumes of air per volume of medium per minute is to be maintained constant on scale-up. If the impeller is unable to disperse the incoming air then extremely low oxygen transfer rates may be achieved. Thus proper flow rate should be maintained by agitator.

**Non-Mechanically Agitated Reactors**

Bubble columns and air-lift reactors are not mechanically agitated

**Mixing and aeration is dependent on the air passage**

**Bubble columns**

Bubble column reactor cannot be used for highly viscous medium.

Pattern of gas bubbles in a bubble column reactor is dependent on the gas superficial velocity.

Gas velocity should be 1-4 cm per second for uniform bubbles throughout medium which will provide proper mixing.

If gas velocity is higher or lower than uniform bubbles will not be produced, thus when bubbles coalesce produces differences in fluid density which will disturb air flow rate.

**Air lift reactors**

In this fermenter, medium circulation is also accomplished with bubble formation. KLa obtained in air-lift reactor will be less than bubble fermenter due to shorter contact time between bubble and medium.

2. **The degree of agitation**

- Agitation is playing a vital role in the oxygen transfer rate in agitated fermenter.
- Agitation increases the area available for oxygen transfer by dispersing air into the medium
- It increase the contact time for bubbles in the medium
- It prevents coalesces of air bubbles
- It decreases thickness of liquid film at gas-liquid interface

3. **Medium Rheology (Medium Flow characteristics)**

Mostly the product of fermentation process is not interfering with medium flow rate or viscosity. But certain bacterial strain producing polysaccharide which can increase the viscosity and hence affect the medium rheology. Thus bacterial polysaccharide will decrease the oxygen transfer rate and bulk mixing.

4. **Antifoam agent**

Antifoam agents collapse the foam and thus increase the oxygen transfer rate of the fermentation medium. Thus KLa value decreases due to use of antifoam agent. If inadequate
space is provided above the liquid level for foam control, then abundant amount of antifoam must be used to prevent loss of broth from the vessel. Thus, it is more productive to operate a fermenter at a lower working volume.

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