Difference between solid state fermentation and submerged fermentation:

Solid state fermentation	Submerged fermentation
1. Utilizes solid substrates like bran, bagasse and paper pulp.	1. Utilizes free flowing liquid substrates, such as molasses and broths.
2. Substrates are utilized very slowly, need not be replaced.	2. Substrates are utilized quite rapidly, need to be replaced constantly.
3. Best suited for fungi that require less moisture content.	3. Best suited for bacteria that require high moisture content.
4. Culture system involves three phases – solid, liquid and gaseous.	4. Culture system involves two phases – liquid and gaseous.
5. Inoculum ratio is always larger.	5. Inoculum ratio is always small.
6. System may or may not involve agitation.	6. System often needs agitation.

The stages in the chronological development of the fermentation industry:

Stage	Main products	Process	Culture Method	Strain Selection
Stage 1: Pre – 1900	Alcohol Vinegar	Use of Thermometer, hydrometer, heat exchanger	Batch Batch	Pure yeast, Fermentation inoculated with good vinegar
Stage 2: 1900 – 1940	Glycerol, Citric acid, lactic acid, acetone/ butanol	pH electrodes, Temperature control	Batch and fed culture	Pure culture used
Stage 3: 1940 – 1963/date	Penicillin, streptomycin, antibiotics, amino acids	Sterilizable pH and Oxygen electrodes	Batch Fed common continuous	Mutation/ Selection
Stage 4: 1964-1978/date	Single cell protein (SCP)	Computer Linked control loops	Continuous culture	Genetic engineering
Stage5: 1979-date	Heterologous protein, monoclonal antibody	System using control and sensor	Batch, fed, continuous	Foreign gene r-DNA

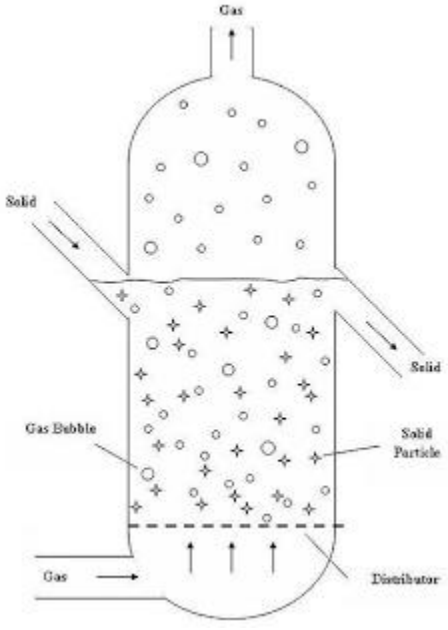

Fig: Fluidized bed bioreactor

4. **Packed bed bioreactor:** A bed of solid particles usually with confiding walls constitutes a packed bed.

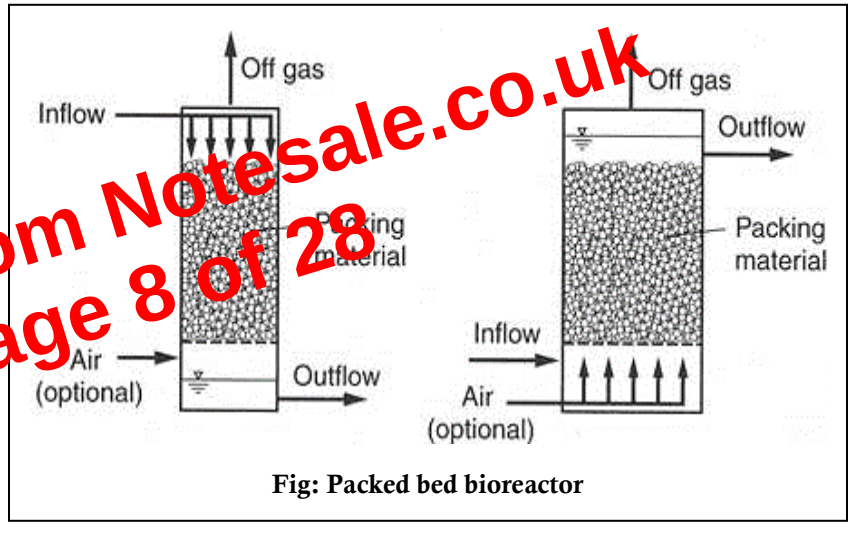

Fig: Packed bed bioreactor

Design and working: It is a static bed of solid inert particles e.g. glass beads. The biocatalyst is supported on or within the matrix of solids. The depth of the bed is by several factors including the density and the compressibility of the solids, the need to maintain the certain level of critical nutrient e.g. oxygen through the entire depth, flow rate that is needed for a given pressure drop.
The medium is recirculated through packed bed by pump and oxygenated by input of air in a secondary vessel. A fluid containing nutrients flow continuously through the bed to provide the needs of the immobilized catalysts. Metabolites and products are released into the fluid and removed in the outflow. The flow may be upward or downward but downflow under the gravity is normal. beads with greater void volume permit greater flow velocities through them but the concentration of the biocatalyst in a given bed volume deceases as the voidage increases.
Problem faced: The environment of a packed bed is non-homogenous as proper mixing is not done. Maintenance of pH becomes difficult due to non-homogenous environment.

5. **Photobioreactor:** Some Cyanobacteria and Micro-algae require sunlight or artificial illumination which is important for their growth and proper production of the product. In that case, photobioreactors are preferred.
Design and working: Photobioreactors are of two types:
   - **Outdoor or open photobioreactors:** They are promising for large-scale production. Open ponds or raceways are often used to culture micro-algae.
   - **Closed photobioreactors:** Closed photobioreactors for monoculture consist of arrays of transparent tubes that may be made of glass or more commonly a clear plastic.

Methods of batch sterilization

The batch sterilization of the medium for a fermentation may be achieved either in the fermentation vessel or in a separate mash cooker. Richards (1966) considered the relative merits of in situ medium sterilization and the use of a special vessel.

Advantages of a separate medium sterilization vessel:

1. One cooker may be used to serve several fermenters and the medium may be sterilized as the fermenters are being cleaned and prepared for the next fermentation, thus saving time between fermentations.
2. The medium may be sterilized in a cooker in a more concentrated form than would be used in the fermentation and then diluted in the fermenter with sterile water prior to inoculation. This would allow the construction of smaller cookers.
3. In some fermentations, the medium is at its most viscous during sterilization and the power requirement for agitation is not alleviated by aeration as it would be during the fermentation proper. Thus, if the requirement for the agitation during in situ sterilization were removed, the fermenter could be equipped with a less powerful motor. Obviously, the sterilization kettle would have to be equipped with a powerful motor, but this would provide sterile medium for several fermenters.
4. The fermenter would be spread the corrosion which may occur with medium at high temperature.

Disadvantages of a separate medium sterilization:

1. The cost of construction a batch medium sterilizer is much the same as that for the fermenter.
2. If a cooker serves a large number of fermenters complex pipework would be necessary to transport the sterile medium, with the inherent dangers of contamination.
3. Mechanical failure in a cooker supplying medium to several fermenters would render all the fermenters temporarily redundant. The provision of contingency equipment may be prohibitively costly.

Overall, the pressure to decrease the 'down time' between fermentations has tended to outweigh the perceived disadvantages of using separate sterilization vessels. Thus, sterilization in dedicated vessels is the method of choice for batch sterilization. The capital cost of a separate batch sterilizer is similar to that of a continuous one and the problems of transfer of sterile media are then the same for both batch and continuous sterilization. Thus, two of the major objections to continuous systems (capital cost and aseptic transfer) may be considered as no longer relevant.

The design of continuous sterilization process: The design of continuous sterilization cycles may be approached in exactly the same way as for batch sterilization systems. The continuous system includes a time period during which the medium is heated to the sterilization temperature, a holding time at the desired temperature and a cooling period to restore the medium to the fermentation temperature. The temperature of the medium is elevated in a continuous heat exchanger and is then maintained in an insulated serpentine holding coil for the holding period. The length of the holding period is dictated by the length of the coil and the flow rate of the medium. The hot medium is then cooled to the fermentation temperature using two sequential heat exchangers – the first utilizing the incoming medium as the cooling source (thus conserving heat by heating-up the incoming medium) and the second using cooling water. The major advantage of the continuous process is that a much higher temperature may be utilized, thus reducing the holding time and reducing the degree of nutrient degradation. The required Del factor may be achieved by the combination of temperature and holding time which gives an acceptably small degree of nutrient decay.

Types of continuous sterilizer: Two types:

- The indirect heat exchanger and
- The direct heat exchanger (steam injector)

They are discussed below:

- **The indirect heat exchanger:** The most suitable indirect heat exchangers are of the double-spiral type which consists of two sheets of high-grade stainless steel which have been curved around a central axis to form a double spiral. The ends of the spiral are sealed by covers.
  Process:
  To achieve sterilization temperatures, steam is passed through one spiral and medium through the other in countercurrent streams.
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  Spiral heat exchangers are also used to cool the medium after passing through the holding coil.

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Incoming unsterile medium is used as the cooling agent in the first cooler so that the incoming medium is partially heated before it reaches the sterilizer and thus, heat is conserved.

Advantages:

1. The two streams of medium and cooling liquid, or medium and steam, are separated by a continuous stainless-steel barrier with gasket seals being confined to the joints with the end plates. This makes cross contamination between the two streams unlikely.
2. The spiral route traversed by the medium allows sufficient clearances to be incorporated for the system to cope with suspended solids. The exchanger tends to be self-cleaning which reduces the risk of sedimentation, fouling and burning-on.

- **The indirect heat exchanger:** Indirect plate heat exchangers consist of alternating plates through which the countercurrent streams are circulated. The plates are separated by gaskets and failure of these gaskets can cause cross-contamination between the two streams. Also, the clearances between the plates are such that suspended solids in the medium may block the exchanger and thus, they system is only useful in sterilizing completely soluble media. However, the plate exchanger is more adaptable than the spiral system in that extra plates may be added to increase its capacity. The continuous steam injector injects steam directly into the unsterile broth.

Advantages:

1. Very short (almost instantaneous) heating up times.
2. It may be used for media containing suspended solids.
3. Low capital cost.
4. Easy cleaning and maintenance.
5. High steam utilization efficiency.

Disadvantages:

1. Foaming may occur during heating.
2. Direct contact of the medium with steam requires that allowance be made for condense dilution and requires clean steam free from anticorrosion additives.

Sterilization of the fermenter: If the medium is sterilized in a separate batch cooker, or is sterilized separately before the sterile medium is added to it. This is normally achieved by heating the jacket or coils if the fermenter with steam and sparging steam into the vessel through all entries, apart from the air outlet from which steam is allowed to exit slowly. Steam pressure held at 15 psi in the vessel for approximately 20 minutes. It is essential that sterile air is sparged into the fermenter after the cycle is complete and a positive pressure is maintained; otherwise a vacuum may develop and unsterile air be drawn into the vessel.

Sterilization of the feeds: A variety of additives may be administered to a fermentation during the process and it is essential that these materials are sterile. The sterilization method depends on the nature of the additive, and the volume and feed rate at which it is administered. If the additive is fed in large quantities then continuous sterilization may be desirable. Batch sterilization of feed liquids normally involves steam injection into the material held storage vessels. Whatever the sterilization system employed it is essential that all ancillary equipment and feed pipework associated with the additions are sterilizable.

Sterilization of liquid wastes: Process organisms which have been engineered to produce foreign products and therefore contain heterogenous genes are subject to strict containment regulations. Thus, waste biomass of such organisms must be sterilized before disposal.

Process: Sterilization may be achieved by either batch or continuous means, but the whole process must be carrier out under contained conditions.

Batch sterilization involves the sparging of steam into holding tanks, whereas continuous processes would employ the type of heat exchangers.

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