ISBN: 978-93-95847-05-6

EMERGING TRENDS IN BIOCHEMISTRY VOLUME II

Editors:
Dr. Bassa Satyannarayana
Mr. Mukul Machhindra Barwant

BHUMI PUBLISHING
FIRST EDITION: 2024

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FERMENTATION TECHNOLOGY

R. L. KULKARNI[1] AND K. S. SONTAKKE[2]

[1]Department of Botany,
Smt. Radhabai Sarda College, Anjangaon Surji, Amravati -444705, Maharashtra, India.
[2]Department of Botany,
G.S.G. College Umarkhed, Yavatmal - 445206, Maharashtra, India

ABSTRACT

The Latin word fevere, which meaning "to boil, " is where the word "fermentation"originates. Dating back to the Neolithic era (10, 000 years BC), fermentation is one of the oldest methods of food storage and preservation in use today. In the biochemical sense, it is the metabolic process that produces energy (ATP molecules) and breaks down complex organic compounds, especially carbohydrates, into simpler compounds without the need for oxygen (all essentials). The anaerobic breakdown of the sugars in the extracts produces carbon dioxide bubbles, which gives the appearance of boiling (principles). Sugar catabolism is an oxidative process that yields reduced pyridine nucleotides, which need to be reoxidized in order for the process to proceed (principles). For many years, the first industrial method for producing a microbial metabolite was the large-scale production of alcohol through the action of yeast on malt or fruit extracts (principles). Depending on the microbe, fermentation produces different final products(essentials).

KEYWORDS: Fermentation, ATP, Anaerobic, Metabolic.

INTRODUCTION

The Latin word fevere, which meaning "to boil, " is where the word "fermentation"originates. Dating back to the Neolithic era (10, 000 years BC), fermentation is one of the oldest methods of food storage and preservation in use today. In the biochemical sense, it is the metabolic process that produces energy (ATP molecules) and breaks down complex organic compounds, especially carbohydrates, into simpler compounds without the need for oxygen (all essentials). The anaerobic breakdown of the sugars in the extracts produces carbon dioxide bubbles, which gives the appearance of boiling (principles). Sugar catabolism is an oxidative process that yields reduced pyridine nucleotides, which need to be reoxidized in order for the process to proceed (principles). For many years, the first industrial method for producing a microbial metabolite was the large-scale production of alcohol through the action of yeast on malt or fruit extracts (principles). Depending on the microbe, fermentation produces different final products(essentials). With Leeuwenhoek and Hooke's 1665 naming and identification of microorganisms, fermentation's scientific justification was established (essentials).

Five main categories comprise commercially significant fermentations

a) Those who generate microbiological cells.

b) Those who generate microbial enzymes.

c) Individuals who generate microbial metabolites.

d) Individuals who manufacture recombinant products.

e) Individuals who alter the compound added to the fermentation.

TYPES OF FERMENTATIONS

The majority of fermentations that are useful commercially can be categorized as either submerged or solid-state cultures. In solid-state fermentations, capillary water may be present, but the microorganisms grow on a moist solid with little to no "free" water. Examples of fermentations involving solid substrates include the production of cheese, bread, coffee, and compost. A dissolved substrate (such as a sugar solution) or a solid substrate suspended in a lot of water to create a slurry can be used in submerged fermentations. A variety of products, including beer, recombinant insulin, and penicillin, are made by submerged fermentations. Both submerged and solid-state fermentations can be further separated into aerobic processes that need oxygen and anaerobic processes that must be carried out in the absence of oxygen. One example of an aerobic submerged fermentation is production of the antibiotic penicillin by using fungus *Penicillium chrysogenum*.

FERMENTATION PROCESS

Industrial fermentations can be run as continuous cultures, fed-batch operations, or batch wise processes.

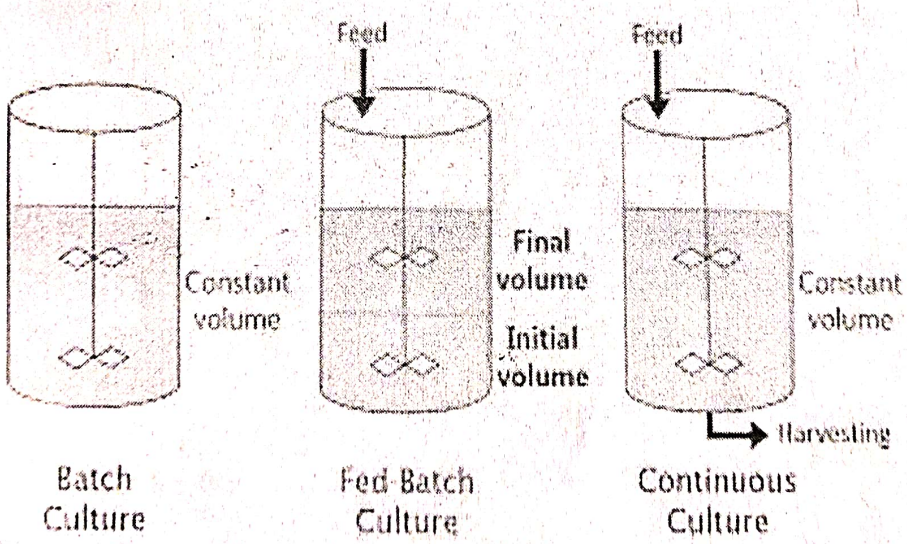

Fig. 1: a) Batch Culture b) Fed Batch Culture c) Continuous Culture

In batch processing, a microbial culture known as the starter culture is added to a batch of culture medium in the fermenter. While most batch fermentations take four to five days, some traditional food fermentations can go on for several months. In fed-batch fermentations, the inoculated fermentation batch receives periodic or continuous additions of sterile culture medium. Continuous fermentations maintain an unchanged fermentation volume by

continuously feeding sterile medium into a fermenter and continuously withdrawing the fermented product.

INOCULUM GENERATION

Generally, sterile Petri dishes or liquid medium in shaker barrels are inoculated with a pure starter culture (or seed), which is kept under strictly regulated conditions. The preculture is used to inoculate the "seed" fermenter once it has grown sufficiently. Owing to the relatively large size of industrial trial fermenters, the inoculum is gradually increased to 5–10% of the production fermenter's working volume through multiple stages. This tactic reduces the batch time in the production fermenter, ensuring maximum utilization of this vessel. Costs go up and productivity (i.e., the amount of product formed per unit time per unit volume of fermenter) decreases with an unreasonably long fermentation time (or batch time).

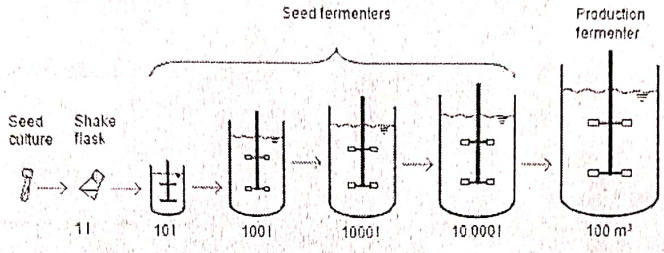

Fig. 2: Generation of inoculum for a large production fermenter

FERMENTATION MEDIUM DESIGN

All the chemical components needed to produce a specific amount of biomass and metabolites must be present in a well-designed fermentation medium, which should also be affordable, easily accessible, and of constant quality. The media frequently has an overabundance of nutrients. Cells and microorganisms generally have the formula $CH_xN_yO_z$. As a result, a fermentation medium needs sources of carbon, nitrogen, and oxygen in order to sustain growth and product formation. Usually, the fermentation broth is continuously aerated to provide oxygen. Usually, carbohydrates or starches are used to supply carbon. Protein hydrolysate is one example of an organic compound that contains nitrogen; other forms of nitrogen supply include inorganic salts. Micronutrients like vitamins might be needed by a medium. trace amounts of certain elements, including phosphorus, iron, zinc and sulphur.

STERILIZATION OF AIR AND FERMENTATION MEDIUM

To avoid contamination with undesirable microorganisms, the medium and any air used to supply oxygen in the majority of industrial fermentations must be sterilized. Every time, air is filtered through hydrophobic membrane filters to sterilize it. There are membrane filters that can capture nearly all microorganisms, with particles as small as 0.1 μm. The culture medium and fermenter can be sterilized simultaneously, or a separately sterilized batch of medium can be put into a fermenter that has already been presterilized. Sterilisers and media are typically sterilized by heating to 121 °C and remaining there for 20 to 30 minutes. Sterilization of heat-sensitive media without suspended solids is achieved by filtering through hydrophilic membrane filters with an absolute rating. Membrane microfiltration is a commonly used

method of sterilizing media used for animal cell cultivation. In certain microbial fermentations, heat may be used to sterilize a portion of the medium, and the heat-sensitive components are subsequently filtered out separately. Microfiltration is a low-cost method of sterilizing salt and water solutions. The slow heating and cooling processes in the fermenter take time to sterilize a large volume of medium in one batch. Continuous sterilization is often used as a workaround for this. The raw medium is heated from room temperature to a higher temperature prior to continuous sterilization. After passing through a heater, the preheated medium's temperature is increased to about 150 °C. After that, the heated medium passes through a "holding coil, " which maintains the temperature at 150 °C for a brief period of time (about 2-4 s). Heat from the hot sterilized medium is transferred to the incoming raw medium in the preheater through a metal wall as it leaves the holding coil. The heated medium is immediately poured into the presterilized, empty fermenter after being further cooled to fermentation temperature.

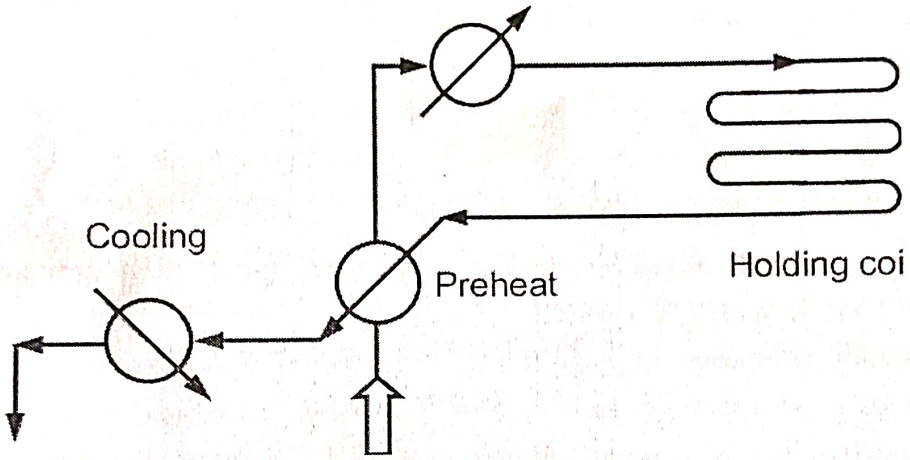

Fig. 3: Fermentation medium (continuous sterilization)

ENVIRONMENTAL FACTORS

Temperature, pH, ionic strength, medium type and composition, dissolved oxygen, dissolved carbon dioxide, and shear rates in the fermenter are just a few of the variables that can affect a fermentation. Fermentations are also influenced by the mode of operation (e.g., batch, fed-batch, continuous, precursor feeding) and mixing (cycling through different environments). Numerous industrial fermentations are conducted in extremely controlled environments with respect to temperature, pH, dissolved oxygen, and other variables. On the other hand, traditional food fermentations are frequently carried out with little oversight. The type of microorganism, the type of substrate, and the qualities of the intended product determine the particular conditions needed for a fermentation. The kind of substrate, the concentration of cells, and the microbial species all affect how much oxygen a fermentation needs. Fermentation will be oxygen limited if the oxygen supply does not meet the oxygen demand. Meeting the oxygen demand in viscous fermentation broths and broths with a high concentration of oxygen-consuming cells can be particularly challenging. Generally speaking, a fermenter's capacity to supply oxygen is determined by the rate of aeration, the degree of agitation, and the characteristics of the culture broth. Oxygen transfer in large fermenters becomes challenging

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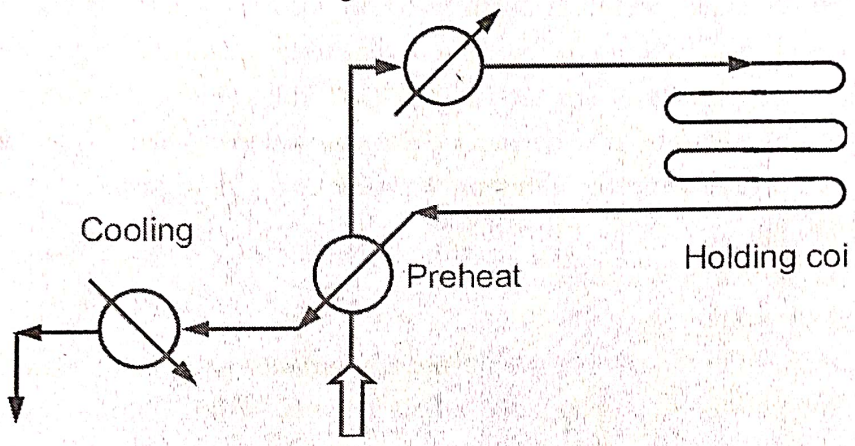

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when demand rises above 4-5 kg/m3 per hour. Every fermentation produces heat. Microbial activity usually accounts for 3–15 kW/m3 of the heat output in submerged cultures. Furthermore, when the broth is mechanically stirred, up to 15 kW/m3 are produced. As a result, cooling a fermenter is necessary to avoid temperature rise and culture damage. Since the cooling water's temperature is usually only a few degrees lower than the fermentation broth's, heat removal is typically difficult. Heat transfer capacity is often the limiting factor in industrial fermentations. The surface area available for heat exchange, the temperature differential between the coolant and the broth, the characteristics of the coolant and the broth, and the turbulence in these fluids all affect how much heat can be removed. The fermenter's geometry determines the available area for heat exchange. Solid state fermentations have lower biomass levels (10–30 kg/m3) than submerged culture. However, compared to submerged cultures, solid state fermentations typically generate a lot more heat per unit fermenting mass due to the low water content. Because there isn't much water to absorb the heat, the temperature can rise quickly. It has been reported that the cumulative metabolic heat generation in koji fermentations for a range of products is 419–2387 kJ/kg solids. Solid-state fermentations of steamed grain or soybeans are known as koji fermentations. Asian fermented foods like saké and soy sauce are made mostly with these fermentations. During composting, higher values—up to 13 398 kJ/kg—have been recorded.

FERMENTATION EQUIPMENT

Submerged fermenter

Steam under pressure can sterilize submerged fermentation equipment, which is typically made for monoseptic cultures. Stirred tank, bubble column, airlift, fluidized-bed, and trickle-bed fermenters are the main varieties of submerged fermenters.

Stirred Tank Fermenter

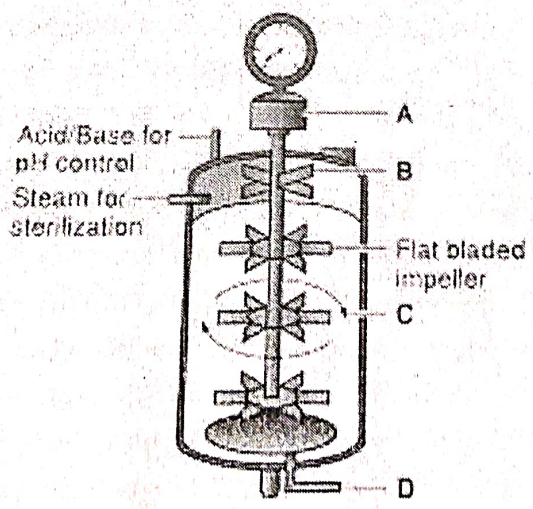

Fig. 4: Stirred Tank Fermenter

Due to its versatility, stirred tank fermenters are among the most widely used varieties. Comprising a cylindrical vessel with an aspect ratio (working height-to-diameter ratio) of 3.4, it

has a central shaft supporting 3.4 impellers spaced approximately 1 impeller-diameter apart. It is possible to use a variety of impeller types that direct the flow radially (outward from the shaft) or axially (parallel to the shaft). On the same shaft, axial and radial flow impellers are occasionally used. Four vertical baffles that are evenly spaced and extend from the vicinity of the walls into the vessel are provided. The breadth of the braid usually makes up 8–10% of the vessel diameter.

Bubble Column Fermenter

This is made up of a cylindrical vessel that has a 4–6 working aspect ratio. Compressed gas agitates it as it is sparged at the bottom. Despite its simplicity, its low performance in comparison to other systems prevents it from being widely used. Large amounts of solids or very viscous broths are not appropriate for it.

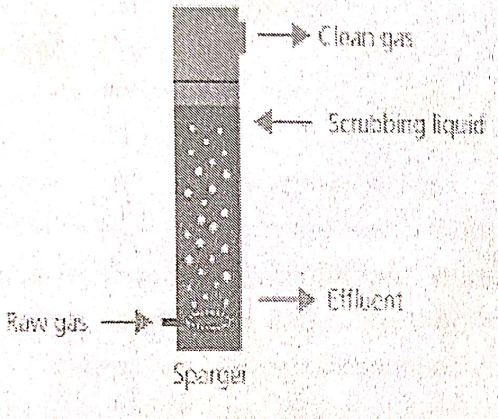

Fig. 5: Bubble Column Fermenter

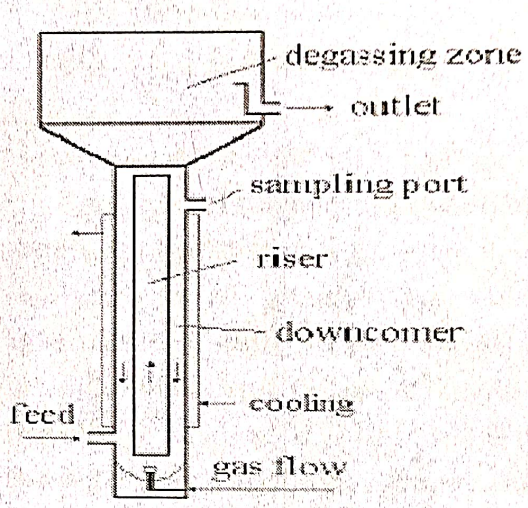

Fig. 6: Airlift Fermenter

Airlift Fermenters

These are available in designs with internal and external loops. The aerated riser and the unaerated descender in the internal-loop configuration are housed in the same shell. The riser and the down comer are independent tubes that are connected near the top and bottom in the external-loop configuration. Between the riser (up flow) and the down comer (down flow), liquid flows. Airlift fermenters have a working aspect ratio of six or higher. These are excellent fermenters overall, with the exception of the thickest broths. They are good at transferring heat, oxygen, and suspended solids. There is little hydrodynamic shear. In the industrial sector, the external-loop design is comparatively uncommon.

Fluidized Bed Fermenter

These resemble bubble columns in that the top of the column has an enlarged cross-section. Continuous pumping of fresh or recirculated liquid at a speed sufficient to fluidize the solids or keep them suspended occurs into the bottom of the vessel. Fluidized beds require a separate pump. In order to prevent the solids from being washed out of the bioreactor, the expanded top section slows the local velocity of the upward flow.

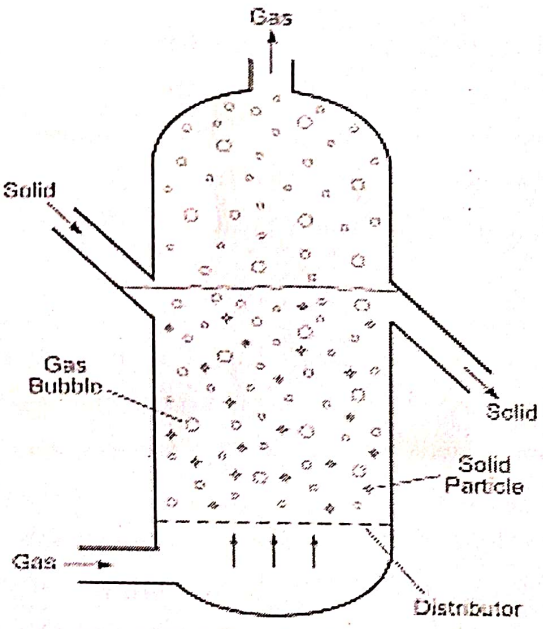

Fig. 7: Fluidized Bed Fermenter

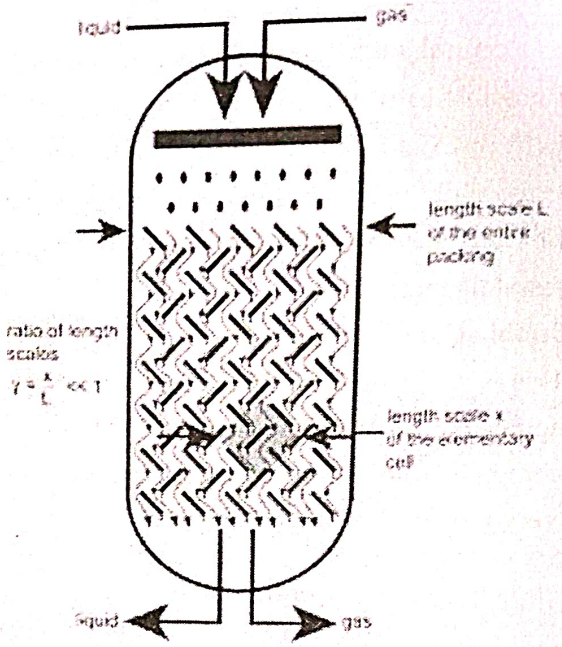

Fig. 8: Trickle Bed Fermenter

Trickle Bed Fermenter

This is made up of a cylindrical vessel filled with filler material (like plastic structures, rocks, or wood chips). There are lots of open areas on the support where gas and liquid can move. The microbes develop in attachment to the sturdy support. A nutrient-rich liquid broth is sprayed on top and then slowly trickles down the bed. Air may flow up the bed in the opposite direction of liquid flow. These fermenters are employed in various processes, including the manufacturing of vinegar. They work well with low viscosity liquids and those that have few suspended solids.

SOLID STATE FERMENTER

The technological sophistication of solid-state fermentation devices ranges from extremely basic methods like wrapping banana leaves, placing bamboo baskets inside, and piling substrate to highly automated systems primarily utilized in Japan. In large-scale processing, some "less sophisticated" fermentation techniques, like fermenting cocoa beans in heaps, work quite well.

Tray Fermenter

These are easy to use and frequently found in Asia's small- and medium-sized koji operations. Wood, metal, or plastic trays are common, and for better aeration, they frequently have a wire mesh or perforated bottom. The substrate ferments in shallow layers, about 0.15 metres deep. Cheese cloth can be used to cover trays to prevent cross-contamination. There is non-sterile processing. Trays can be placed singly or in stacks, in areas with ventilation or in chambers with controlled humidity and temperature. Manual methods are used for mixing and inoculation. Automated handling, filling, emptying, and washing of trays is occasionally possible. Tray fermenters need a lot of space and labour, even with some automation.

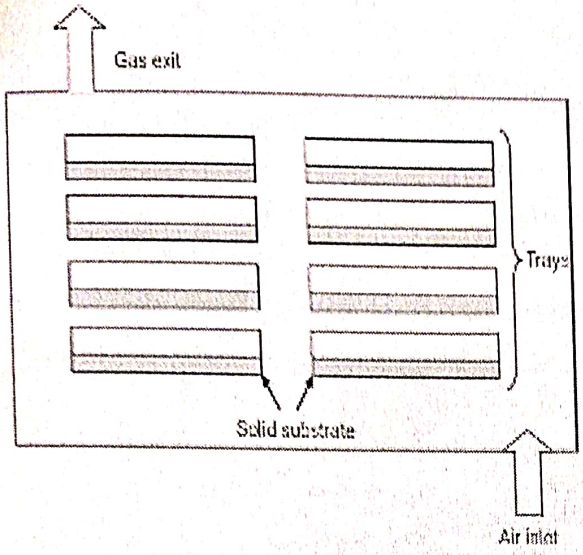

Fig. 9: Tray Fermenter

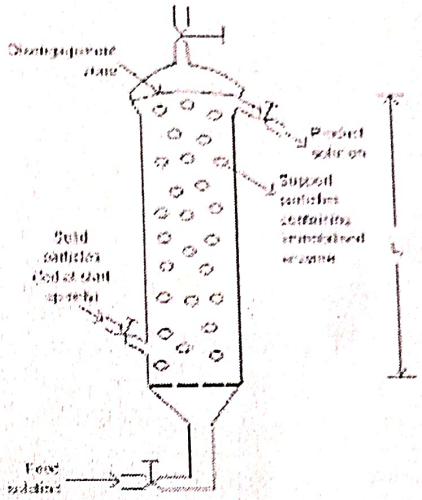

Fig. 10: Static Bed Fermenter

Static Bed Fermenter

This is a tray fermenter modified to fit this purpose. It makes use of one static substrate bed that is deeper, bigger, and housed in an insulated chamber. Through forced aeration through the substrate bed, oxygen is supplied.

Tunnel Fermenter

This is a static bed device modified for use. The solid bed is usually quite long and does not typically go below 0.5 m. With mechanisms for mixing, inoculation, continuous feeding, and substrate harvesting, tunnel fermenters can be highly automated.

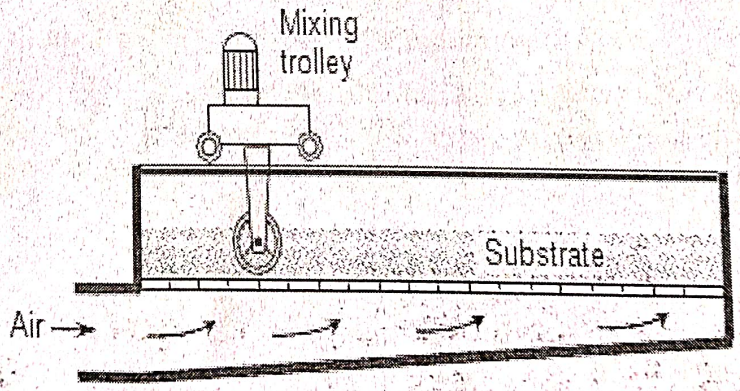

Fig. 11: Tunnel Fermenter

Rotary Disk Fermenter

The upper and lower chambers of the rotary disc fermenter are each equipped with a circular perforated disc to support the substrate bed. The discs are rotated by a single central shaft. The upper chamber is filled with the inoculated substrate, which is then gradually transferred to the transfer screw. The partially fermented solids are transferred by the upper screw to the lower chamber, where additional fermentation takes place, via a mixer. The lower transfer screw is utilized to harvest the fermented substrate. Air that has been temperature-regulated and humidified is used to aerate both chambers. In Japan, koji are made on a large scale using rotary disc fermenters.

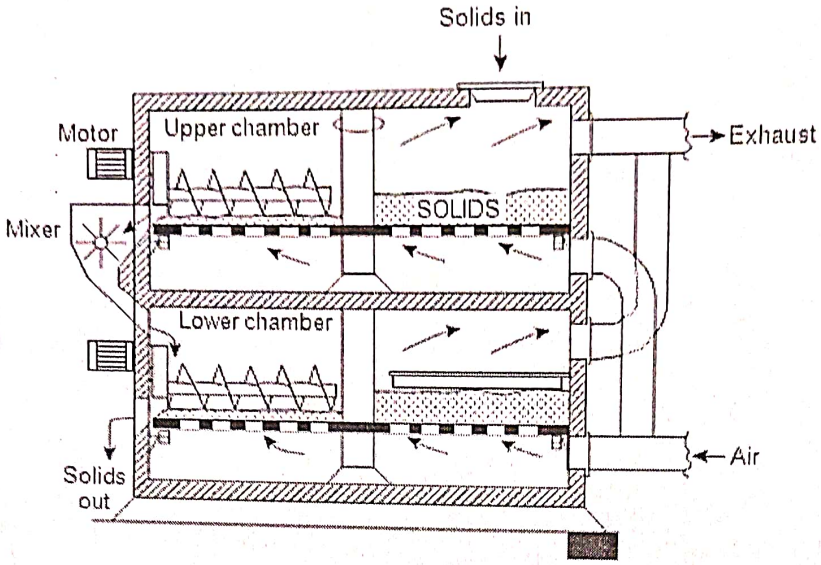

Fig. 12: Rotary Disc Fermenter

Rotary Drum Fermenter

With the help of rollers, the cylindrical drum of the rotary drum fermenter rotates at a speed of 1 to 5 revolutions per minute around its long axis. Depending on the stage of fermentation, rotation may be intermittent and the speed may change. Inside the drum, straight or curved baffles help to turn the substrate, which enhances temperature control and aeration. Occasionally, an inclined drum can cause the substrate to rotate from the higher inlet end to the lower outlet. Coaxial inlet and exhaust nozzles provide aeration.

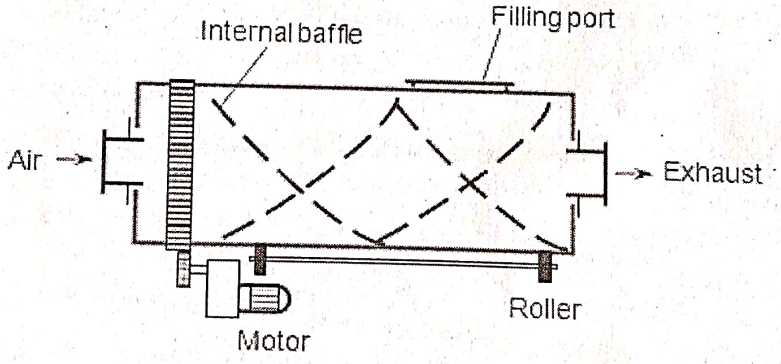

Fig. 13: Rotary Drum Fermenter

HOW TO RECOVER FERMENTATION PRODUCTS?

Fermentation produces crude broth, which needs to be further processed downstream to recover the desired product in a sufficiently pure state. Usually, downstream processing entails a number of stages that come together to produce the product at the required degree of purity. The factors that need to be taken into account when creating an economically feasible scheme for product purification and concentration, based on a limited number of the numerous processing operations that are available, are the only topics covered in this discussion. It is crucial to pay attention to the design of an economical downstream process since product

recovery and purification frequently account for 70–80% of the total cost of product production. Enhancing a product's purity beyond what is necessary for a particular use is costly and wasteful. Reasonable purity requirements that align with the intended application. The physical and chemical differences between the components of a mixture are used by recovery and separation processes to separate them. For instance, the separation achieved by filtration of cells from a broth is facilitated by the disparity in size between the fluid molecules and the cells. In general, the best overall separation result will be obtained if the downstream proce individual steps take advantage of as many physical–chemical differences between mixture's components as they can in order to achieve separation. For instance, gel filtra which separates molecules based on size, and ion exchange chromatography, which sep molecules based on charge differences, may be a better option when two chromatogr processes are to be used in series.

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