

Quadrant II – Transcript and Related Materials

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Unit: 02 Types of fermentation processes, bioreactors and measurement of fermentation parameters

Module Name: Solid-state and liquid-state (stationary and submerged) fermentations

Module No: 13

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Notes

We all know by now that fermentation is a process whereby there is biological conversion of complex substrates into simple compounds by various microorganisms such as bacteria, yeast and fungi. These compounds are also called 'bioactive compounds' since they possess biological activity. During the course of the breakdown, fermentation products called Primary metabolites are formed during the growth phase (trophophase).

Additional compounds called secondary metabolites may also be formed during the stationary phase (idiophase). Primary metabolites are those which are required by the microorganisms for their growth and maintenance, while secondary metabolites are the end products of the primary metabolism and mostly have an ecological function. Trophophase and idiophase may sometimes overlap, depending on the nutritional environment presented to the culture.

Further, in the previous class we have seen that Fermentation is classified based on the type of substrate used. If the substrate used is in the form of a solid we say it is Solid State Fermentation. And when we use a liquid substrate it is called Liquid State Fermentation.

Fermentation may be carried out as Surface or Submerged.

Surface Fermentation

In Surface fermentation the organism is grown on the surface of a liquid or solid substrate as a batch culture, without any agitation. For example in Citric acid fermentation by surface fermentation. *A. niger* grows as a mycelium layer on the

surface of the liquid substrate in trays. These trays are stacked in fermentation rooms supplied with filtered air which serves both as a source of oxygen, as well as to control the temperature of fermentation. Surface fermentation is easy to control and economical. It needs no agitation device. Temperature and humidity are the only two parameters that need proper controlling. Most surface fermentations however are done using a solid substrate. Because surface fermentation has a greater surface area exposed to the environment, the fermentation broth evaporates faster and gets concentrated during fermentation. Therefore, recovery of product and its purification are more economical.

Disadvantages:

- 1) Building investment costs are high.
- 2) Personnel expenses are high in developed industrial countries with extremely high wages.
- 3) Fermentation time is long and therefore productivity is low.

Submerged fermentation

In submerged fermentation the organism is grown within the media with vigorous agitation. Mostly submerged type of fermentors are preferred in industries, because they require less space for setting up, they have a better design, and overall control is easier. Liquid state Fermentation is not always submerged but may also sometimes be used as a surface and fermentation.

Submerged fermentations can be carried out either as a batch, fed batch or continuous fermentation.

Batch fermentation:

Batch fermentation is the type of fermentation wherein the substrate and microorganisms are added all at once and the fermenter is not emptied until fermentation is completed. During the course of fermentation, the microorganisms go through 6 phases of growth. A visible change is seen in the culture media, the number of microorganisms and the amount of the product formed (i.e. the metabolite). The six phases of the microbial growth are:

(a) Lag phase:

This phase occurs immediately after inoculation. This is a period when the organisms gets acclimatized to the new environment into which it is inoculated. There is no significant increase in cell numbers at this stage and so it is called the lag phase.

(b) Acceleration phase:

The period when the cells just start increasing in numbers is called the acceleration phase.

(c) Log phase:

The log phase (sometimes called the logarithmic phase or the exponential phase) is a period when the cell numbers steadily increase i.e. there is cell doubling. If substrate limitation is not there, growth will continue, and the cell population will double at a constant rate with time.

(d) Deceleration phase:

Is the phase where there is a steady decline in growth.

(e) Stationary phase:

Stationary phase is the period where there is no change in the microbial cell number as the rate of formation of new cells is equal to the death rate of the old cells. This happens due to depletion of nutrients or accumulation of toxic products of metabolism. In this stage growth ceases but cells remain metabolically active.

(f) Death phase:

The period in which the number of living cells decreases exponentially and the population growth experiences a sharp decline. This is due to death of the cells because metabolic activity ceases due to depletion of energy resources. Eventually the dead cells will also lyse and the cell debris will be used as nutrients by the surviving bacteria.

Fed-batch fermentation:

In Fed batch fermentation, substrate is added at regular intervals but the product is only removed at the end of the fermentation. This increases the total volume of the fermentation vessel. Fed batch fermentation is of two types: Fixed volume and variable volume.

Continuous fermentation:

In this type of fermentation the products are removed continuously along with the cells and an equal amount of fresh culture media is added so as to maintain the growth of microbial cells in a steady state. Therefore, the volume of the contents of the fermentor remains fixed.

- Submerged Fermentation (SmF)/Liquid Fermentation (LF) SmF takes place in large vessels (fermenter) with volumes of up to 1,000 cubic meters. The fermentation media used needs to be sterilized depending on its composition, for example whether it is a crude raw material like maize, sugars, and soya or refined media. SmF utilizes substrate which are in the free flowing liquid state. Fermentation techniques have to be optimized for each substrate, because each

organism reacts differently to each substrate, based on which productivity will vary. Aqueous substrates are used in submerged fermentation such as molasses, soluble sugars, vegetable and fruit juices, and sewage or waste water. These are utilized rapidly by the fermenting microorganisms, hence need to be constantly replenished. Measurement and control of fermentation parameters like pH, temperature, dissolved oxygen and exhaust gases needs to be optimized.

- The resultant bioactive compounds are secreted into the fermentation broth. The fermentation technique is best suited for microorganisms such as bacteria with a high moisture requirement.
- Examples of products made by submerged fermentation are as enzymes and organic acids like citric acid and lactic acid.
- Several types of submerged type of fermentors are known and they may be grouped in several ways: shape or configuration, whether aerated or anaerobic and whether they are batch or continuous.
- The most commonly used submerged fermentor is the Constantly Stirred Tank Reactor (CSTR)

How does SSF differ from SmF?

SOLID STATE FERMENTATION –

- Substrates: Wheat bran, Rice and wheat straw, Fruit and vegetable waste, Paper pulp, Bagasse, Coconut coir.
- Batch fermentation
- Fermentation in large trays /vessels
- Inoculum in solid or liquid form usually added on surface
- Not stirred
- Aeration is by passing sterile air
- Secondary metabolites not produced
- There is wastage of fermentation media
- If a tray is contaminated, there is loss of a single tray and not the entire batch

LIQUID STATE FERMENTATION-

- Substrates: molasses, soluble sugars, wheat or rice bran, vegetable and fruit peels or juices, and sewage or waste water
- Batch or continuous Fermentation in large fermenters
- Inoculum is usually in liquid phase, may be added to surface or submerged within
- Agitation necessary but sometimes stationary
- Aeration by sparger and impeller
- Can benefit the production by yielding many secondary metabolites
- Entire media is used by microbes for growth and product formation, so no wastage
- If a batch gets contaminated there is loss of the entire batch

ADVANTAGES

- 1) Best suited for bacterial fermentations which need high water content.
- 2) Easier to control fermentation parameters like pH and temperature.
- 3) Since inoculum is usually in liquid form, lesser quantities required.
- 4) Aeration and agitation ensures even distribution of inoculum throughout the medium.
- 5) Shorter fermentation time.
- 6) Lesser space required.
- 7) Purification of products is easier.
- 8) Automation is easier.
- 9) An additional advantage of this technique is that the purification of products is easier.
- 10) Bacterial and yeast cells are evenly distributed throughout the medium.
- 11) Lower total investment costs
- 12) Improved process control
- 13) Lower labor costs
- 14) Simpler operations
- 15) Easier maintenance of aseptic conditions on an industrial scale.

DISADVANTAGES:

- High costs due to the expensive media.
- Expenses for equipment are higher
- Consumption of electrical energy is higher
- The process is very sensitive
- Agitation is often essential
- Chance of contamination
- More effluent generation

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