Fermentation

- Fermentation is the process of growing microorganisms in a nutrient media by maintaining physico- chemical conditions and thereby converting feed into a desired end product
- Fermentation technology is the use of organisms to produce food, pharmaceuticals and alcoholic beverages on a large scale industrial basis.

Fermentation

- The basic principle involved in the industrial fermentation technology is that organisms grown under suitable conditions, by provi raw materials meeting all the necessary requirements such as carbon, nitrogen, salts, trace elements and vitamins.
- The end products formed as a result of their metabolism during their life span are released into the media, which are extracted for use by human being and that have a high commercial value.

Major fermentation products

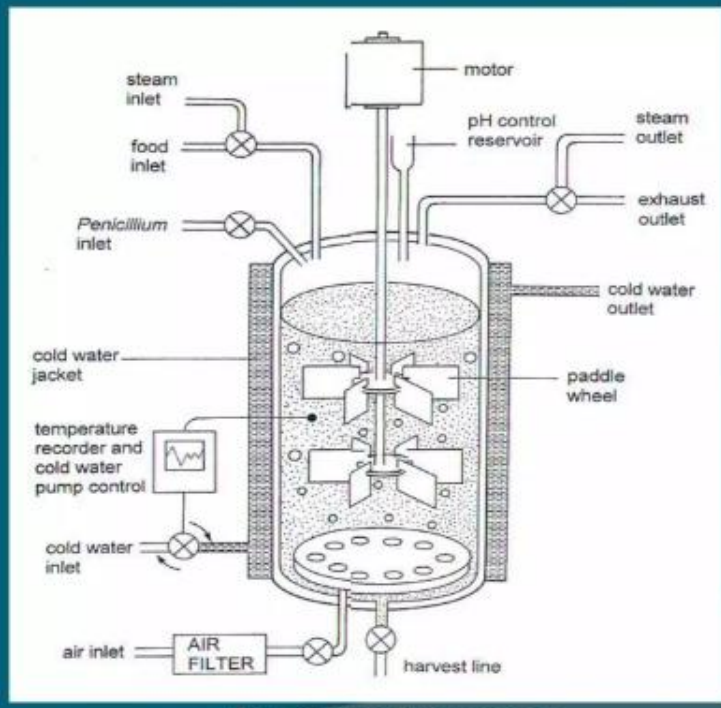
Group	Product	Organism
Industrial chemicals	Ethanol	*Saccharomyces cerev*
	Lactic acid	*Lactobacillus bulgaricus*
Enzymes	α-amylase	*Bacillus subtilis*
	Proteases	*Bacillus species*
	Lipases	*Saccharomyces lipolytica*
Antibiotics	Penicillin	*Penicillium chrysogenum*
	Streptomycin	*Streptomyces griseus*
	Chlorampenicol	*Streptomyces venezuelae*
Vitamins	Riboflavin	*Ashbya gossypi*
	Vitamin B12	*Pseudomonas dentrificians*

DESIGN OF FERMENTER

- A fermentation process requires a fermenter for successful pr
.
- Fermentor is the large vessel containing considerable quantities of nutrient media by maintaining favourable conditions.
- The design and nature of the fermentor varies depending u type of fermentation carried out. Invariably all the fermentors the following facilities for the process such as

  - contamination free environment,
  - specific temperature maintenance,
  - maintenance of agitation and aeration, pH control,
  - monitoring Dissolved Oxygen (DO),
  - ports for nutrient and reagent feeding (antifoam agents, alkali or acid),
  - ports for inoculation and sampling,
  - provide all aseptic conditions at the time of sample withdrawal and addition of innoculum
  - complete removal of broth from the tank and should be easy to clean
  - It should be designed in such away that it consumes less power, have less evaporation, can be used for long periods of operation

DESIGN OF FERMENTER



Figl. An Ideal fermenter

Components of fermenter

- 1. Basic component includes drive motor, heaters, pump, etc.,
- 2. Vessels and accessories
- 3. Peripheral equipment (reagent bottles)
- 4. Instrumentation and sensor

Various components of an ideal fermenter for batch process are:

S.No.	Part	Purpose
1	Top plate	cover (made of steel)
2	Clamp	top plate compressed onto vessel using clamp
3	Seal	separates top plate from vessel (glass) to prevent air leakag
4	Vessel	glass, jacketed, steel with ports for various outputs, input etc
5	Drive motor	used to drive mixing shaft
6	Drive shaft	mixes the medium evenly with its impeller
7	Marine impeller	for plant tissue culture
8	Baffles	prevent sedimentation on sides and proper mixing
9	Sparger	air supplier / after filtration via membranes – ensures efficient dispersal – by attached to impeller
10	Exit gas cooler	like condenser remove as much moisture as possible from exhaust
11	Inoculation needle	port to add inoculum
12	Feed pumps	regulates the flow rates of additives (medium, nutrients) variable speed
13	Peristaltic pumps	fixed speed pumps – used for continuous sampling
14	Syringe pump	using a syringe – mostly used in batch
15	Exit gas analysis	CO_2 analyzer, O_2 analyzer, mass spectrometer
16	Sample pipe	through which samples are drawn
17	3 way inlet	to insert different probes

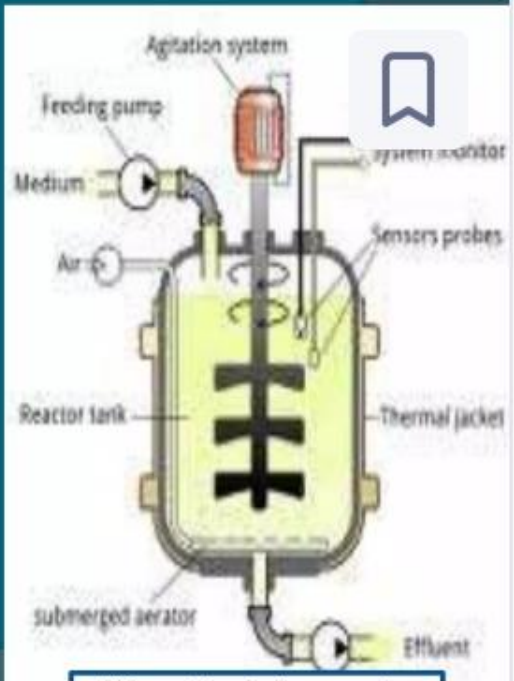
Monitoring and controlling parts of fermenter are:

S.No	Part	Use
1	Pt100	temperature sensor (platinum resistance electrode)
2	Foam probe	kept above the medium level to sense foam formation
3	pH electrode	senses pH
4	O_2 sensor	Monitors dissolved oxygen level
5	Heater pad	directly heats the medium
6	Cold finger	after direct heating – used to cool the vessel contents (closed coil/pipe to pass cool water)
7	Rotameter	variable air flow meter – indicates rate of air flow into vessel – attached to air sparger
8	Pressure valve	attached to rotameter for safer operation
9	Air pump	supply of air
10	Peristaltic pump	to pump in medium, acids, bases, antifoam

Stirred tank fermenter (STF

stirred tank fermenter

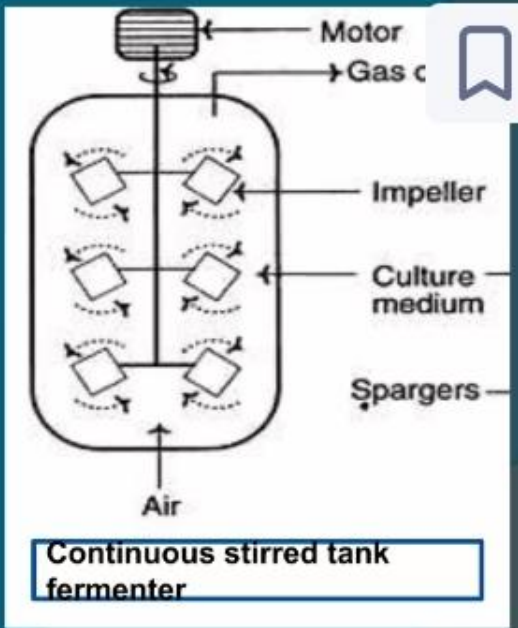
- batch operated fermenter
- agitators consists of one or more impellers mounted on the shaft
- It is rotates with the help of electric motor
- Advantage of this fermenter flexibility in design
- Used in the range of 1-100 ton capacity sizes



Stirred tank fermenter

Continuous stirred tank fermenter (CSTF)

- A continuous stirred tank fermenter consists of a cylindrical vessel with motor driven central shaft that supports one or more agitators (impellers).
- The shaft is fitted at the top of the bioreactor (ref. fig.). The number of impellers is variable and depends on the size of the fermenter



Continuous stirred tank fermenter

Continuous stirred tank fermenter

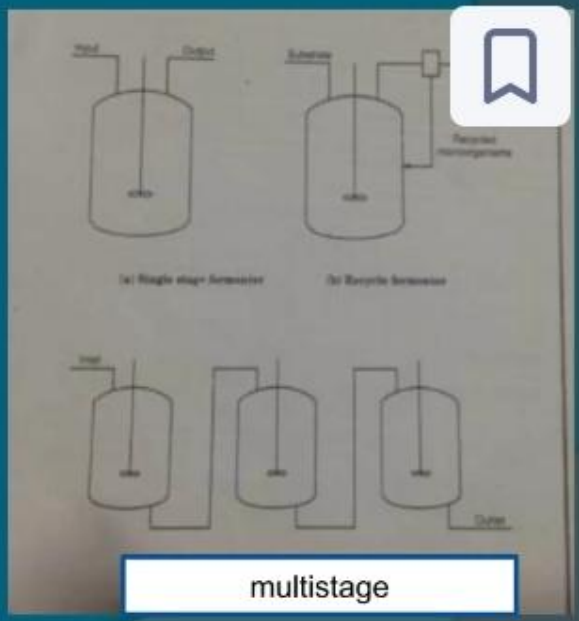
- In this fresh medium is added continuously in the fermenter vessel
- On the other end the medium is withdrawn for the recovery of fermentation products
- As it is a continuous fermenter the Steady state conditions can be achieved by either **Chemostatic** or **Turbidostatic** principles.

Continuous stirred tank fermenter(CSTF)

- Different types of continuous fermer are
  a. Single stage: single fermenter is inoculated and kept in continuous operation by balancing the input and output culture media
  b. Recycle continuous fermentation: a portion of the withdrawn culture or residual unused substrate plus the withdrawn culture is recycled

CSTF

c. Multistage continuous operation: involves two or more stages with the fermenter being operated in sequence



multistage

STF

Advantages of batch operated

- Less risk of contamination because of short growth period
- Process is more economical and simple
- Raw material conversion level is high

Disadvantages:

- Low productivity due to time required fro the sterilizing, filling, cooling, emptying and cleaning
- More expenses are required for subcultures for inoculation, labor and process control

STF

Advantages of continuous operated

- Less labor expenses due to automation of fermentation process
- Less toxicity risk to operator by toxins producing microorganisms
- High yield and good quality product due invariable operating parameters and automation of the process
- Less stress on the fermenter as sterilization is not frequent

Disadvantages:

- Higher investment costs in control and automation equipment
- More risk of contamination and cell mutation

- **There are three different process of fermentation viz.:**
- (1) Batch fermentation
- (2) Feb-batch fermentation and
- (3) Continuous culture.

Batch fermentation:

- Nutrients are added in the fermentation for the single time only and growth continues until the particular nutrients are exhausted

- In the batch process when the microorganism is added into a medium which supports its grov the culture passes through number of stages known as 'growth curve'

A typical growth curve consists of following stages

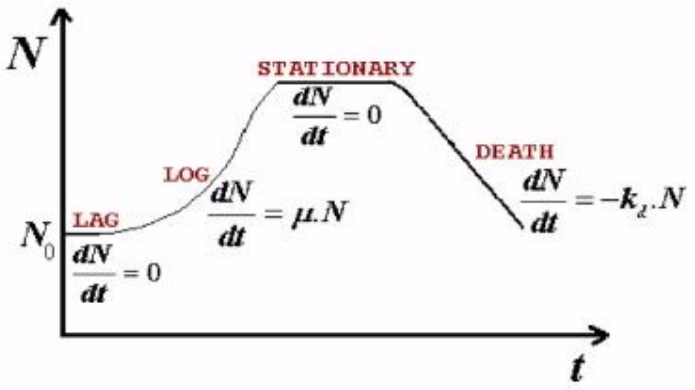
a) Lag phase
b) Acceleration phase
c) Log or exponential phase
d) Deceleration phase
e) Stationary phase
f) Death phase

- **(a) Lag phase:**
- Immediately after inoculation, there is no increase in the numbers of the microbial cells for some time and this period is called lag phase. In this is phase the organisms adjust to the new environment in which it is inoculated into.
- **(b) Acceleration phase:**
- The period when the cells just start increasing in numbers is known as acceleration phase.
- **(c) Log phase:**
- This is the time period when the cell numbers steadily increase.
- **(d) Deceleration phase:**
- The duration when the steady growth declines.

- **(e) Stationary phase:**
- The period where there is no change in the microbial cell number is the stationary phase. This phase is attained due to depletion of carbon sources or accumulation of the end products.
- **(f) Death phase:**
- The period in which the cell numbers decrease steadily is the death phase. This is due to death of the cells because of cessation of metabolic activity and depletion of energy resources.
- Depending upon the product required the different phases of the cell growth are maintained. For microbial mass the log phase is preferred. For production of secondary metabolites i.e. antibiotics, the stationary phase is preferred.

Growth kinetics of batch culture

The number of living cells (population of growth rate dN/dt)varies with time in a batch system as shown below:



where;

N = number of bacteria at any time t in the reactor

t = time

N_0 = initial number of bacteria in the reactor after inoculation

LAG Phase:

Number of bacteria does not change with time in lag phase

$$\frac{dN}{dt} = 0$$

LOG Phase:

Number of bacteria increases exponentially in log phase.

$$\frac{dN}{dt} = \mu.N$$

where;

μ = specific growth rate

During log phase the number of organisms in the reactor at any time t can be calculated, by using rate equation shown below:

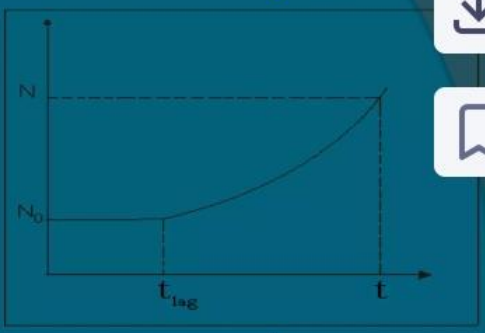
$$\frac{dN}{dt} = \mu . N$$

This rate equation can be integrated:

$$\int_{N_0}^{N} \frac{dN}{N} = \int_{t_{lag}}^{t} \mu . dt$$

$$\ln \frac{N}{N_0} = \mu . (t - t_{lag})$$

$$N = N_0 . e^{\mu . (t - t_{lag})}$$



where;

N_0 : initial number of bacteria at t_0 (starting time)

t_{lag} : time where lag phase ends

According to last equation, number of bacteria in the reactor at any time t during log phase can be calculated, as it is seen in the graph.

STATIONARY Phase:

There is no net change in number of bacteria wi e in stationary phase. Bacteria divide but also die at equal rate. Most of the important biological proc (especially secondary metabolites like antibiotic biomass are produced during this phase.

The biomass concentration at stationary phase is determined by following equation

$$X = Y . S_R$$

X=cell concentration

Y= yield factor for limiting nutrient

S_R = original nutrient concentration in the medium

- Y' measures the efficiency of a cell in converting nutrients into biomass
- So the biomass at a particular time in the during the fermentation is given by the following equation.

$$X = Y (S_R - s)$$

S= nutrient concentration at particular time

thus 'Y' is represented by the following equation

$$Y = X/ (S_R - s)$$

- **Feb-batch fermentation:**
- In this type of fermentation, freshly prepar culture media is added at regular intervals without removing the culture fluid. This increases the volume of the fermentation culture. This type of fermentation is used for production of proteins from recombinant microorganisms.
- The total amount of the biomass in the vessel increases but biomass concentration is maintained constant

Continuous operations

Continuous fermentation:

- The growth rate and physiological conditions microorganisms can be maintained by using process of continuous culture (chemostat)
- In this the products are removed continuously along with the cells and the same is replenished with the cell girth and addition of fresh culture media. This results in a steady or constant volume of the contents of the fermenter. This type of fermentation is used for the production of single cell protein (S.S.P), antibiotics and organic solvents.

CONTINUOUS fermentation process

- The dilution rate is the ratio of inflowing amount of medium to the volume of the culture.
- Thus
- $D = F / V$

D= dilution rate (h^{-1})

F= flow rate (dm^3 /h)

V= volume (dm^3)

- The change in cell concentration of cells at perticular time period is expressed by the following equation

  dx/dt= growth rate – output

  Or $dx/dt = \mu x - Dx$

In the process of continuous culture technique the output is balanced by growth hence,

$\mu x = Dx$

$\mu - D$

$Dx / dt = D$

- The biomass concentration in the chemostat is determined by the following equation

$X = Y(S_R - s)$

X= steady state concentration

Or $dx/dt = \mu x - Dx$

In the process of continuous culture technique the output is balanced by growth hence,

$\mu x = Dx$

$\mu - D$

$Dx / dt = D$

- The biomass concentration in the chemostat is determined by the following equation

$X = Y(S_R - s)$

X= steady state concentration

S= steady state residual concentration in the medium

Advantages and disadvantages of batch an continuous operations

BATCH SYSTEMS

- easy to operate and control
- genetic stability of organism could be controlled if it is genetically engineered biocatalyst.
- lower contamination risk
- non-productive down time is a disadvantage
- batch to batch variability is problem
- accumulation of inhibitory products is problem

CONTINUOUS SYS

- degeneration of biocatalyst
- higher contamination risk is a disadvantage
- efficient, higher productivity
- product is obtained with uniform characteristics; quality of the product is almost same from time to time
- no accumulation of