Post-Harvest Operations and Processing of Fruits, Vegetables, Spices and Plantation Crop Products

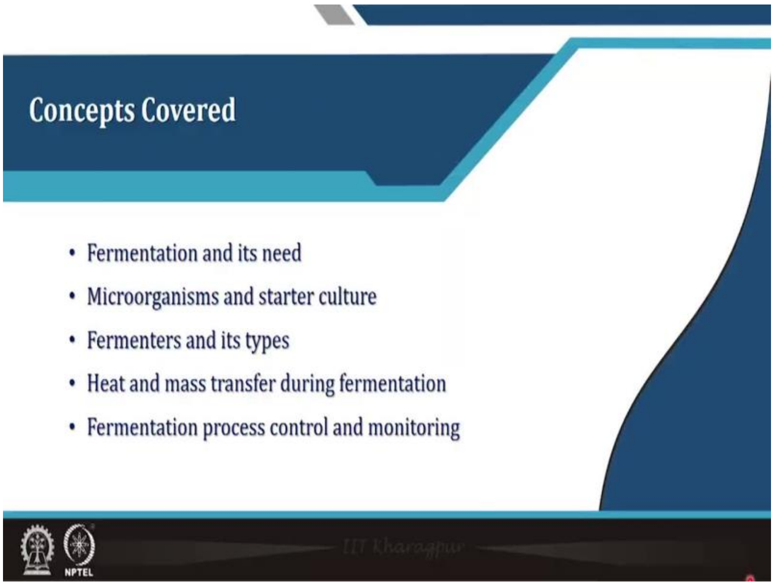
Professor H N Mishra
Department of Agriculture and Food Engineering
Indian Institute of Technology, Kharagpur

Lecture 46

Fermentation Technology

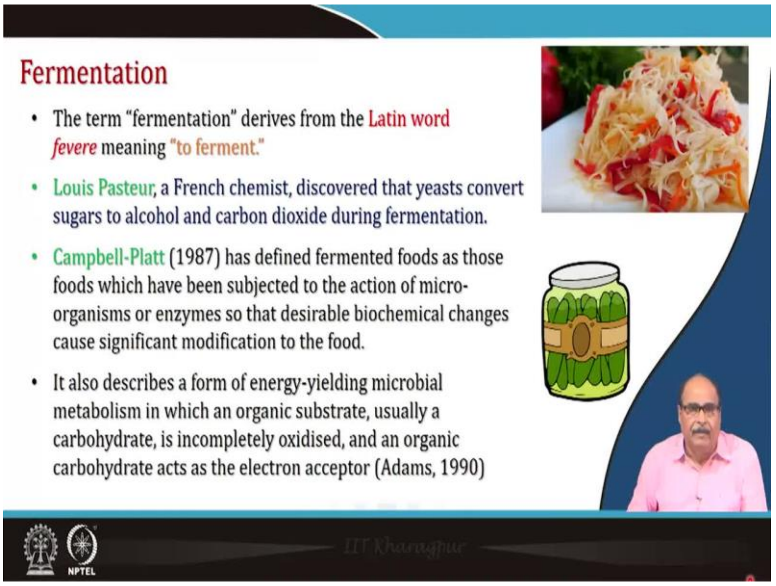
Hello everybody, namaskar, now, we are in the 10th module of the course, and 5 lecture of this 10th module will be devoted on various aspects of plant based fermented foods and beverage. In today's lecture, that is lecture 26, we will discuss the concept, and process basics, and fundamentals of fermentation technology.

Concepts covered



The various aspects of fermentation technology that will be covered in today's lecture: what is fermentation; why it is needed; what are the different microorganisms and the starter cultures used in the fermentation process; fermenters and various types of fermenters. Then, we will also discuss something about heat and mass transfer during fermentation process. And finally, fermentation process control and monitoring.

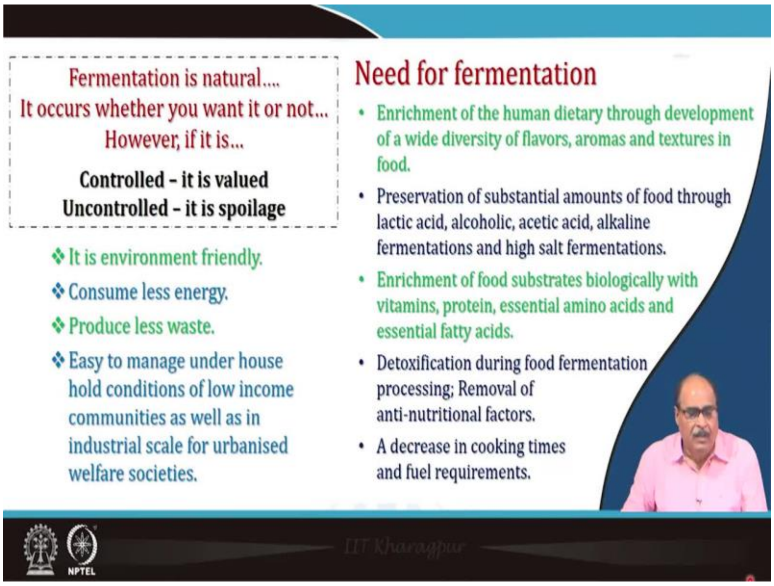
Fermentation



Let us see, what the fermentation is. The term fermentation derives from the Latin word meaning 'fevere'. It means, 'to ferment'. Louis Pasteur, who was the French chemist discovered that yeast converts sugar to alcohol and carbon dioxide during fermentation process.

Campbell Platt in 1987 defined fermented foods as: those foods which have been subjected to the action of microorganisms or enzymes so that the desirable biochemical changes cause significant modification to the food. It is also described as a form of energy yielding microbial metabolism, that is, fermentation is a form of energy yielding microbial metabolism in which an organic substrate, usually a carbohydrate, is incompletely oxidized and an organic carbohydrate acts as the electron acceptor.

Need for fermentation

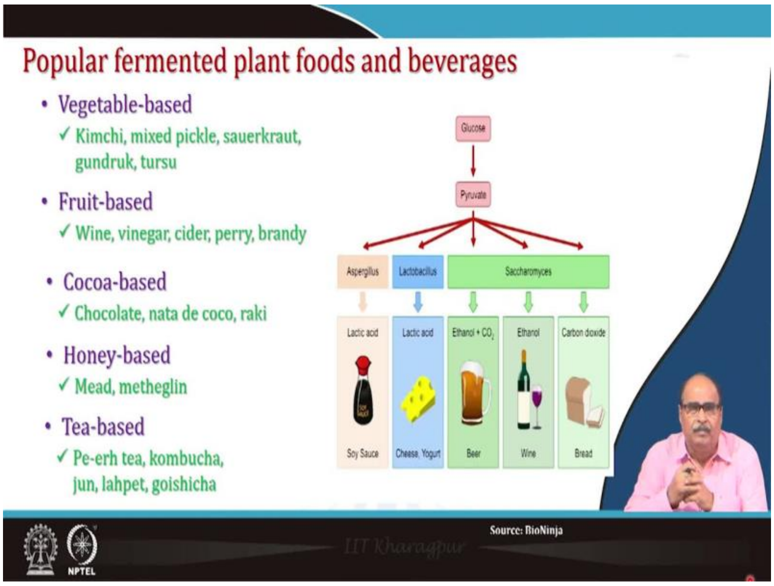


The fermentation is natural; it occurs whether you want it or do not want it. However, if it is controlled, it is a very valuable process. It adds value to the product, but if it is uncontrolled, it will spoil the product. So the fermentation process is environment friendly, it consumes less energy, produces less waste, and it is easy to manage under household conditions of low income community as well as in the industrial scale for urbanized welfare societies.

Fermentation is needed to enrich the human dietary through development of a wide diversity of flavors, aroma and textures in foods. Fermentation is a preservation technology. It preserves the food through lactic acid, alcoholic acetic acid, alkaline fermentation processes and even high salt fermentations.

Also there is enrichment of the food substrates biologically with the vitamins, protein, essential amino acid, salt, etc. These are all many such compounds are produced or generated synthesized by the microbial acts and enzymes in the food. Fermentation also lead to some sort of detoxification of the food, there is a removal of anti-nutritional factors in the fermentation process. There is a decrease in the cooking times and fuel requirements after (fermentation) in the fermented foods.

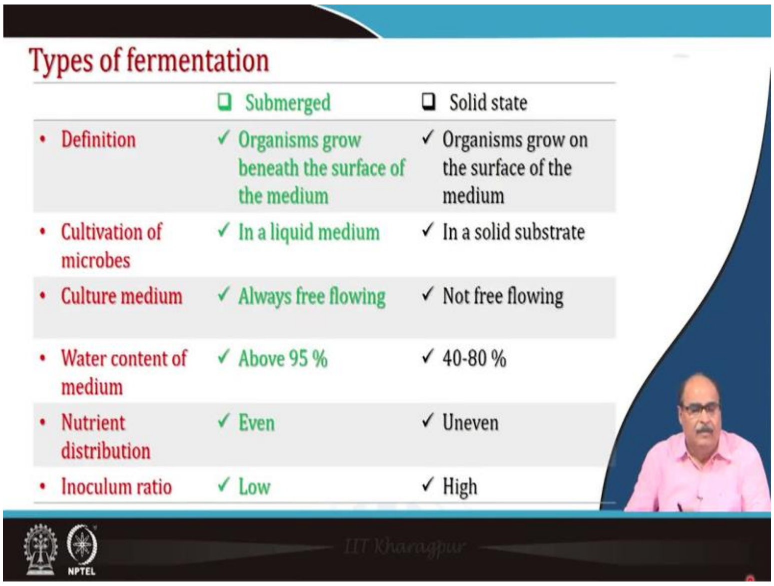
Popular fermented plant foods and beverages



The popular plant based fermented foods and beverages are considered vegetable based like kimchi, mixed pickle, sauerkraut, gundruk. Fruit based product may be wine, vinegar, cider, perry, brandy, etc. Chocolate Nata de coco, raki: these are cocoa based fermented products; even mead and metheglin are honey based or even tea based fermented product include pe-erh tea, kombucha, jun tea, tahpet, goishicha and so on. So the fermentation process as I discussed earlier in the definition that is glucose is converted actually into pyruvate.

Then this pyruvate it may go that is fermented oxidized further by the Aspergillus into lactic acid in the happens in the soya sauce or lactobacillus group of bacteria, it converts pyruvate into lactic acid, this is found in the cheese or yoghurt and it may be yeast fermentation like *Saccharomyces cerevisiae* where it may result in the beer ethanol and carbon dioxide, in the wine, fruit wines etc. This pyruvate is converted into ethyl alcohol or in some other product like bakery products etc. same yeast enzyme is converts it into carbon dioxide the major product.

Types of fermentation

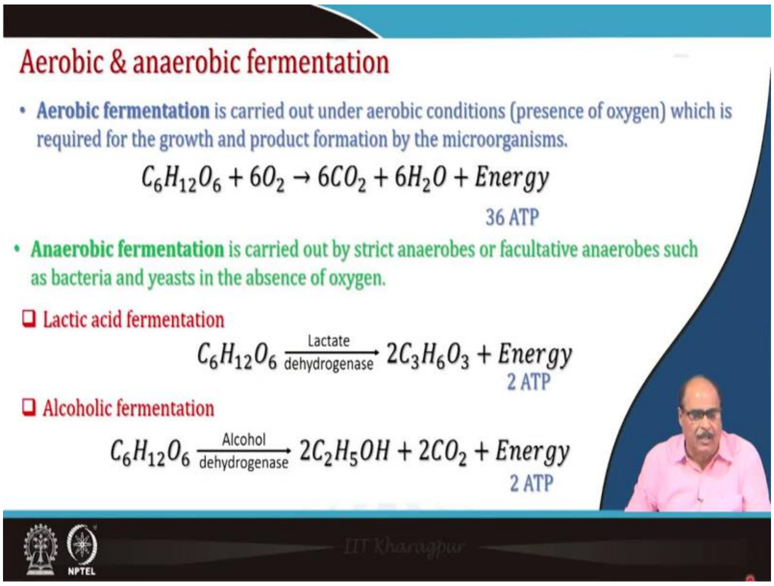


So the fermentation types. Mainly two types of fermentation are there: either submerged fermentation or solid state fermentation. So in the submerged fermentation organism grow beneath the surface of the medium, whereas, in the solid state fermentation organism grow on the surface of the medium.

In the solid submerged it is a liquid medium is generally used, whereas, in the solid state fermentation a solid medium, solid substrate is used. The culture medium is always free flowing in submerged fermentation, whereas, in the solid state fermentation culture medium does not flow freely.

Water content of the medium is about 95 percent or above in the submerged fermentation, about 40 to 80 percent is the solid state fermentation. Nutrient distribution is even in the submerged fermentation but in the solid state fermentation, it is not even maybe uneven. Inoculum ratio is generally low in the submerged fermentation and high in the solid state fermentation.

Aerobic & Anaerobic fermentation

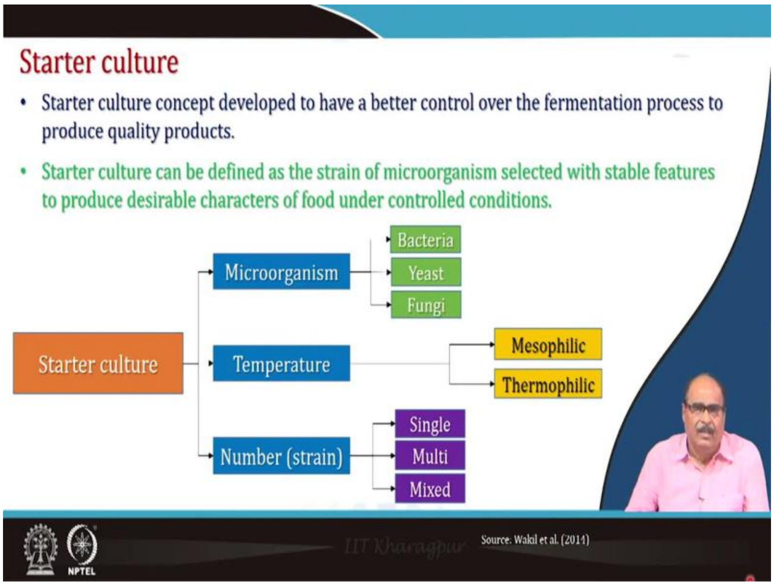


So, the fermentation oxygen consumption depending upon the oxygen availability and its utilization, it may be anaerobic or aerobic fermentation. Aerobic fermentation is carried out under aerobic environment that is the presence of oxygen and here glucose is completely oxidized giving carbon dioxide and water and 36 molecules of ATPs are generated from one glucose molecule.

In the anaerobic fermentation obviously, there is strict anaerobic conditions there even facultative anaerobes also some time they may manage to perform anaerobic fermentation and most of the bacterial or yeast fermentation which occur in absence of oxygen.

This anaerobic fermentation may be lactic acid fermentation, where lactic dehydrogenase enzymes convert glucose into lactic acid and that two moles of ATP generated. In the case of alcoholic fermentation, that is alcohol dehydrogenase, yeast it converts glucose into this ethyl alcohol plus carbon dioxide and also energy in the form of ATP is released.

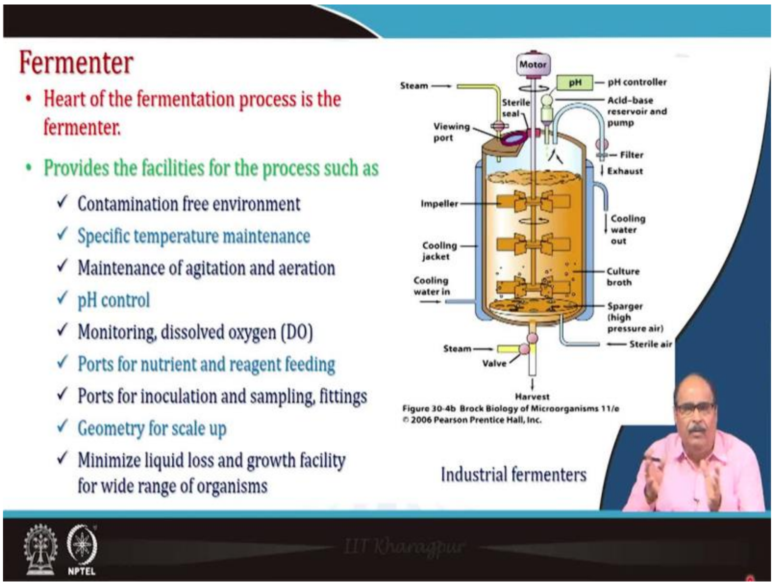
Starter culture



As far as the starter culture concept is concerned, the starter culture concept developed to have a better control over the fermentation process to produce desired quality and good quality product. The starter culture is defined as the strains of microorganism which are selected with stable features to produce desirable characters in the product under controlled conditions.

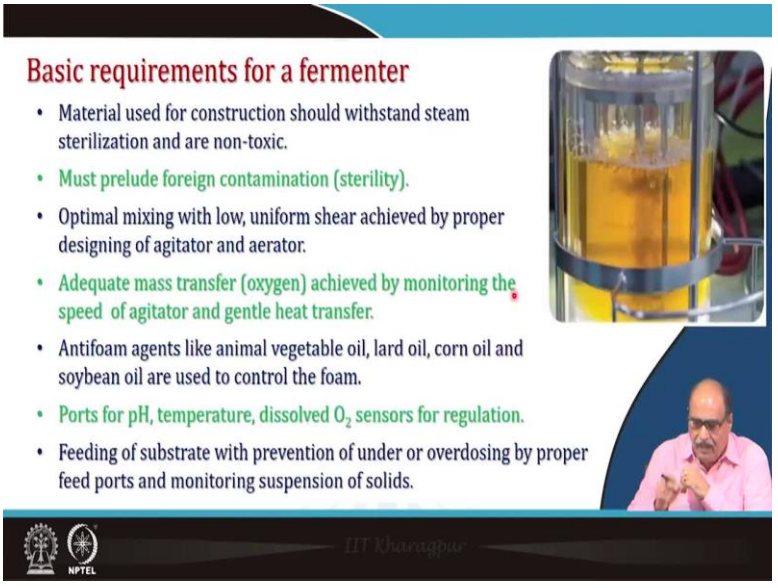
There may be that the microorganisms like bacteria, yeast and fungi are the starter culture and then proper temperature that is all these microorganisms they have some optimum temperature requirement for their growth so that proper temperature should be maintained that is like mesophilic or thermophilic. And then a number of the strain is also important, whether it is the single bacteria or multi bacteria, the mixed mode and so on.

Fermenter



Then let us discuss about the fermenter. It is the heart of the fermentation process. The fermenter is basically a bioreactor where all the control operations, etc. are provided and it facilitates the process like this it gives a contamination free environment. You will be able to control the specific temperature (maintenance and control), maintenance of agitation and aeration, pH control, monitoring of the dissolved oxygen, ports for nutrient and reagent feeding, ports for inoculation and sampling, fitting etc. Geometry for scale up and minimize liquid loss and growth resulting from the wide range of organisms. So all these elements are provided in the fermenter of bioreactor you can see here in the figure.

Basic requirements for a fermenter

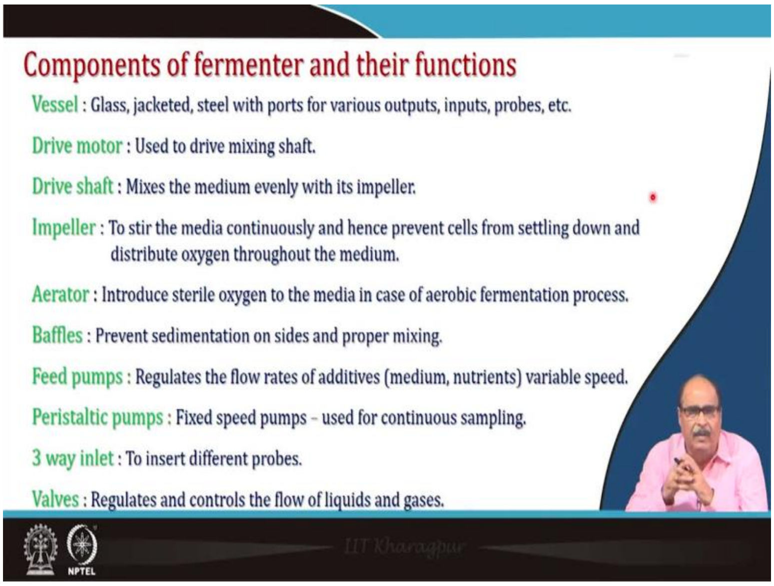


Then the basic requirements of a fermenter: what is a material that is used for the construction so that it withstands the steam sterilization. It (the material) should be nontoxic, non-corrosive. It must prelude foreign contamination, etc. if there's any that it is to be able to be sterilized. Optimal mixing with low uniform shear is achieved by proper designing of the agitator and aerator.

Adequate mass transfer that is of oxygen is achieved by monitoring the speed of the agitator and gentle heat transfer. Antifoam agents like animal vegetable oil, lard oil, corn oil, soybean oil, etc. are used in controlling the foam because many times when you agitate some foam is generated. So anti foaming agents may be added so there should be arrangement for that.

There should be ports for pH, temperature, dissolved O_2, sensors for regulations, etc. Feeding for substrate with preventions of under or overdosing by proper feed ports and monitoring suspension of solids etc. These are the basic requirements of any fermenter.

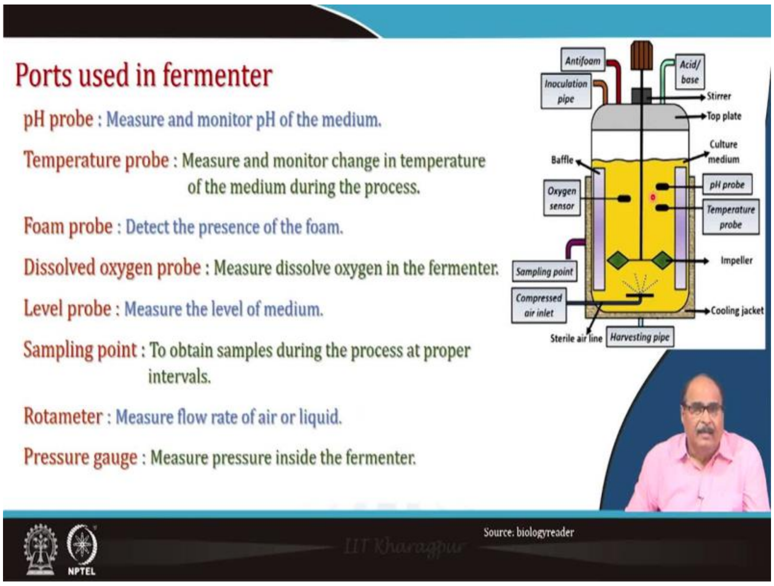
Components of fermenter and their functions



So normally the components of the fermenter and their functions which are there these are given in this table. The major component is the vessel; it may be glass, jacketed, steel vessel with ports, probes. Then drive motor, drive shaft, impeller, aerator, baffles. Baffles prevent sedimentation on sides and ensure proper mixing.

Then feed pumps which regulate the flow rate of additives. Peristaltic pumps are fixed speed pump which is used for continuous sampling. 3 way inlet to insert different probes or valves that regulate and control the flow of the liquid and gases etc., so all these are the components of the fermenter.

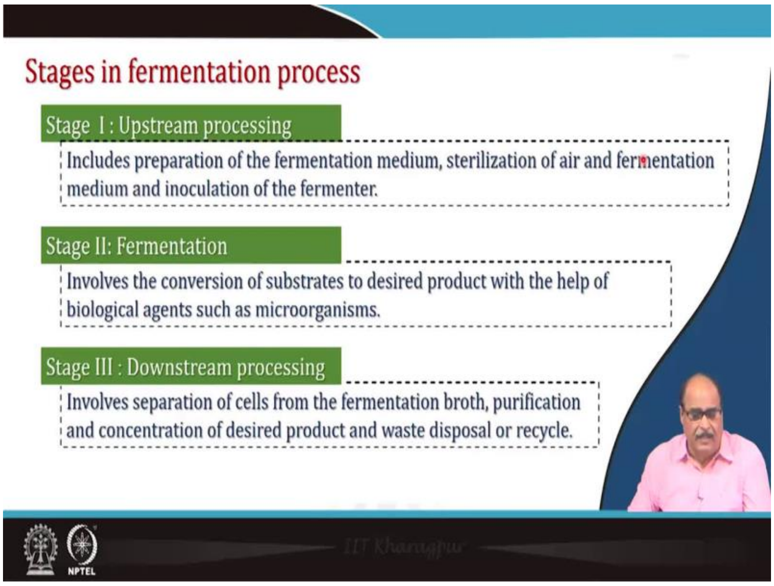
Ports used in fermenter



Then the various ports which are used in the fermenter you can see here in this figure, like pH probe for measuring and monitoring the pH of the medium, temperature probe, foam probe, dissolved oxygen probe, or even level probe that is which will indicate level of the medium inside the reaction vessel.

Then sampling port to obtain samples during the process at proper intervals so as to check the fermentation process and level of alcohol or acetic acid that is produced which facilitate the stoppage of the fermentation process. Rotameter measures the flow rate of the air and the liquid. Pressure gauge measures pressure inside the fermenter. So, all these ports, etc. are provided in the modern fermenters.

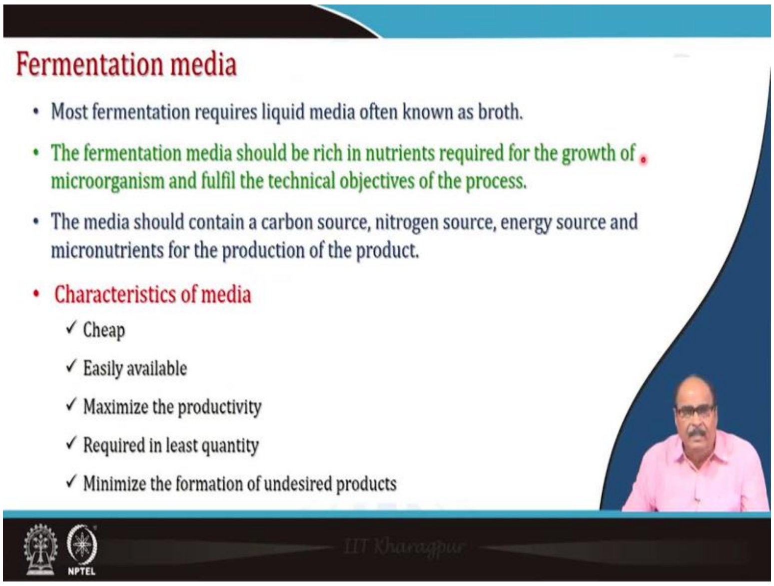
Stages in fermentation process



Stages in the fermentation process, that is there are 3 stages: stage 1, stage 2, and stage 3. Stage 1 is basically upstream processing, which includes preparation of the fermentation medium, sterilization of air and the fermentation medium, and finally inoculation of the fermenter, so these are the upstream processing.

Then the fermentation: actual fermentation process which involves the conversion of the substrate to desired product with the help of biological agents like microorganism etc. So there is a bioreactor where the reaction normally takes place that is actual fermentation process. And then finally the downstream processing: it involves separation of cells from the fermentation growth medium, purification and concentration of the desired product and the waste disposal or recycling.

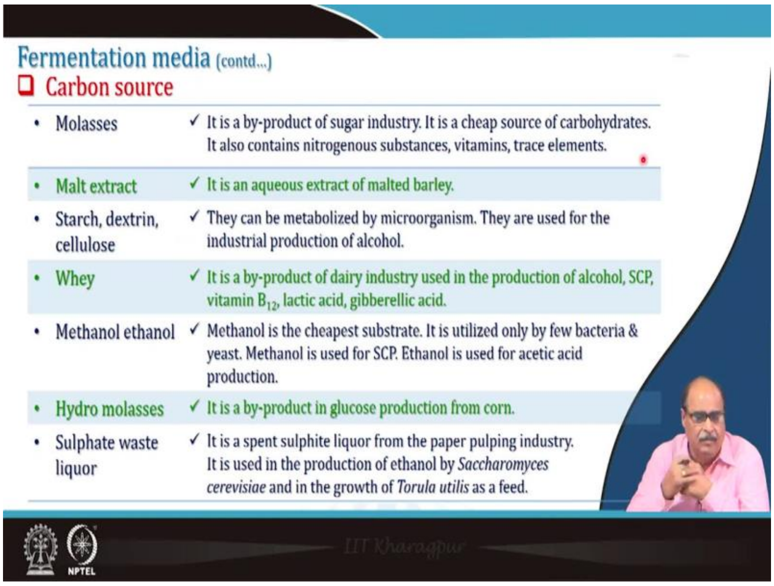
Fermentation media



The fermentation media is the main component. Most fermentation requires the liquid media and it is often known as broth. The fermentation media should to be rich in the nutrients required for the growth of the microorganism and it should fulfil the technical objectives of the process.

The media should contain a carbon source, a nitrogen source, energy source, and the desired micro nutrient required for the utilization and growth of the microorganism and then finally for the production of the products. So the characteristics of a good media includes: it should be cheap, should be easily available, it should maximize the productivity, so that it is required in least quantity, and should minimize the formation of undesirable products.

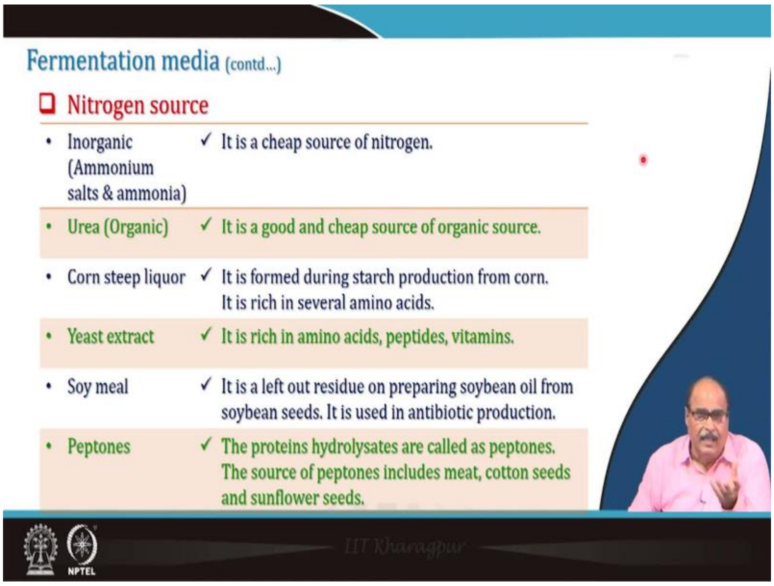
Fermentation media – Carbon source



Generally the carbon sources in the fermentation media includes molasses, malt extract, starch, dextrin, cellulose, whey, methanol or ethanol, hydro molasses or sulphate waste of the liquor, etc. So either biomass or from various organic sources all these. Then like for example, molasses is a byproduct of the sugar industry, so it is a cheap source of the carbohydrate, it contains also nitrogenous substances, vitamins, trace elements, etc.

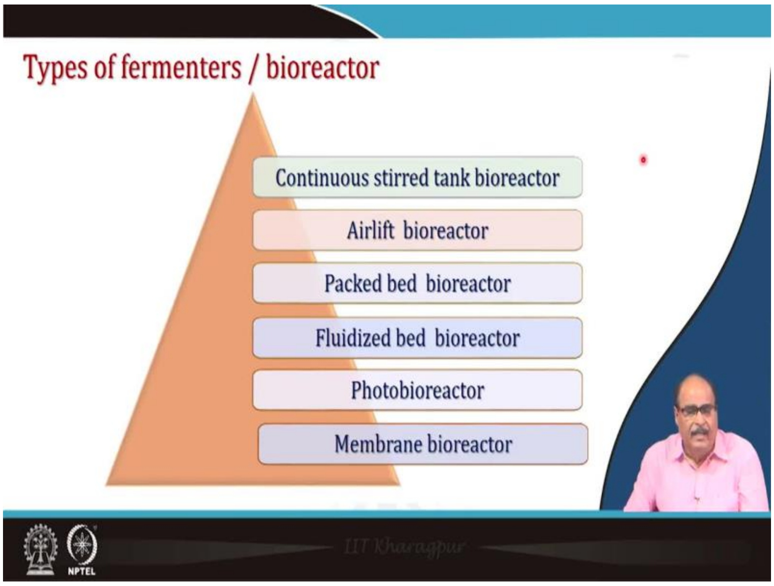
Sulphite wastes: it is spent sulphite liquor from the paper pulp industry, which is used in the production of ethanol by *Saccharomyces cerevisiae* and the growth of *Torula utilis* as a feed, so these are the various carbon sources.

Fermentation media – Nitrogen source



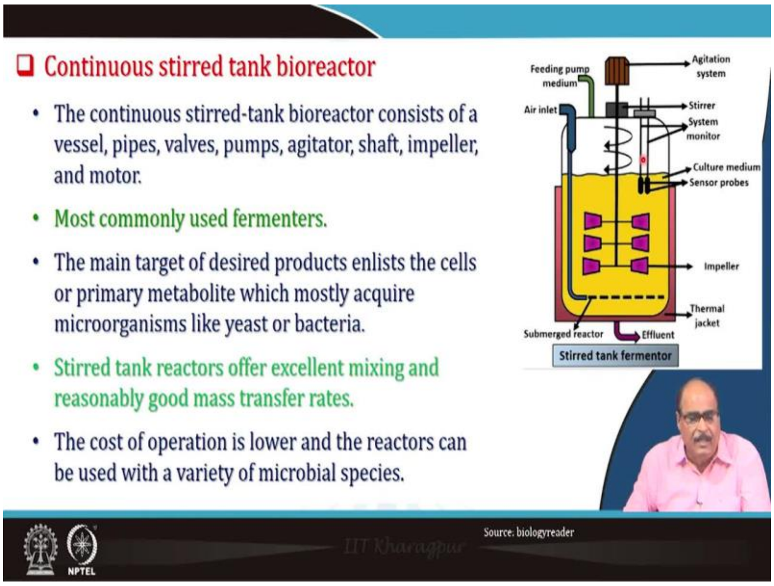
The various nitrogen sources may be inorganic form of nitrogen like ammonium salts or ammonia, etc. Then urea: it is an organic source of nitrogen; then there is corn steep liquor, yeast extract, soy meal, peptones, etc. So a suitable source of the nitrogen, carbon or nutrients etc. energy source will be provided in the microbial media.

Types of fermenters / bioreactor



Then, we will discuss types of the fermenters or bioreactors; they are of different type like continuous stirred tank bioreactor, airlift bioreactor, packed bed bioreactor, fluidized bed bioreactor, photobioreactor or a membrane bioreactor.

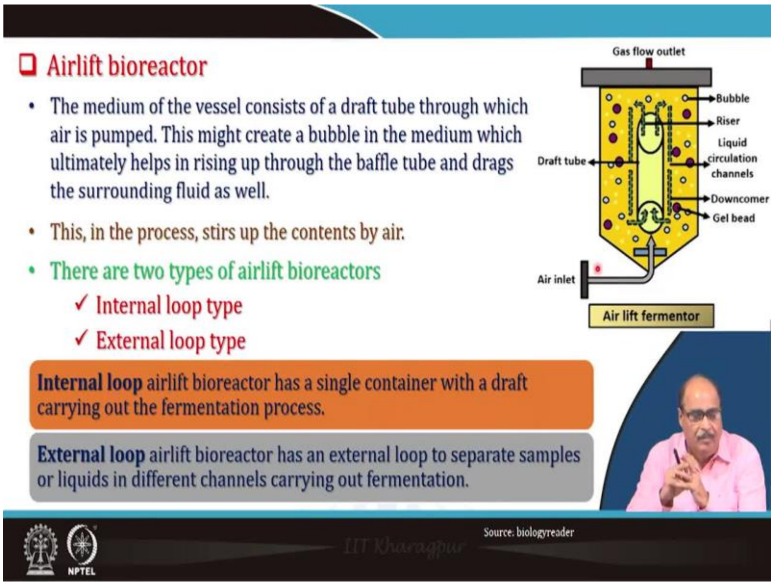
Continuous stirred tank bioreactor



The continuous stirred tank bioreactor, as you can see here in the figure, it consists of a vessel, pipes, agitator, shafts, impellers and motors. It is most commonly used fermenter. The main target of the desired product enlists the cell or primary metabolites, which mostly acquires microorganisms like yeast or bacteria.

The stirred tank reactors offer excellent mixing and reasonably good transport, mass transfer rate. The cost of operation is lower and the reactors can be used with a variety of microbial species.

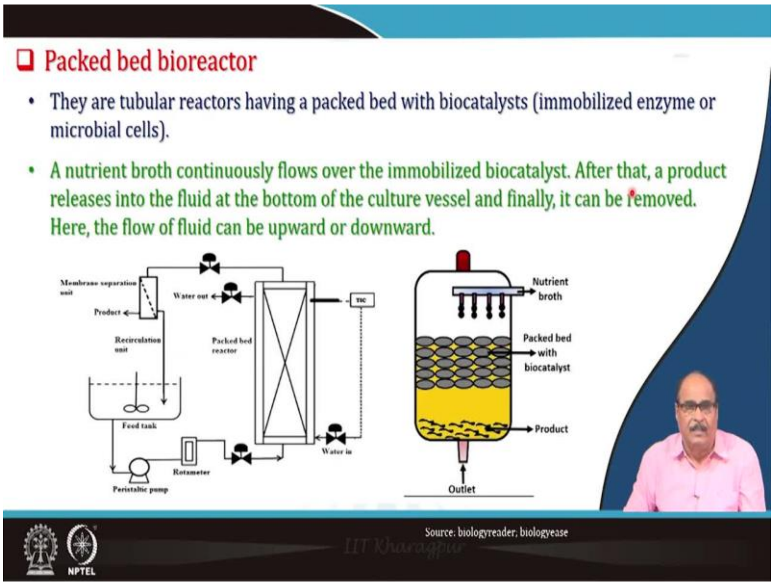
Airlift bioreactor



Then airlift bioreactor. You can see here: the medium of the vessel consists of a draft tube through which the air is pumped inside. So here the air is pumped, this might create a bubble in the medium which ultimately helps in rising up through the draft tube and drags the surrounding fluid as well. This, in the process, stirs up the the content by air.

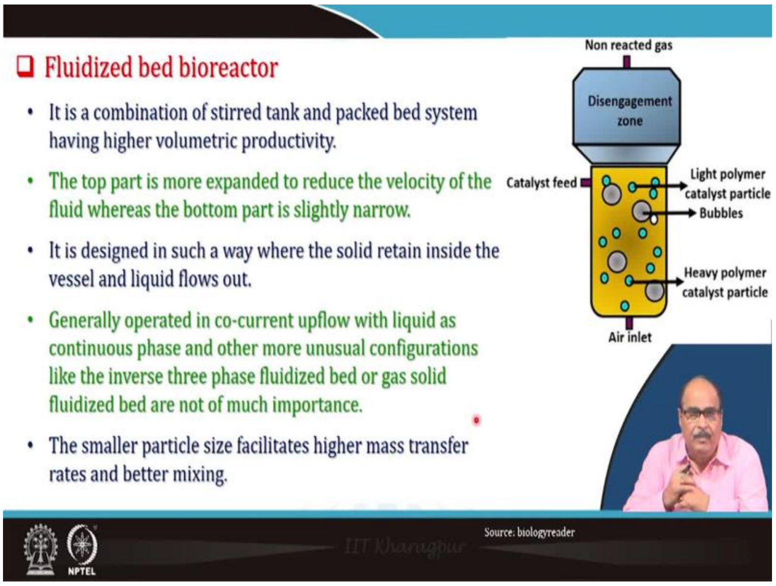
There are 2 types of airlift bioreactors: one is the internal loop type and the external loop type. Internal loop airlift bioreactor has a single container with a draft carrying out the fermentation process, whereas, the external loop airlift bioreactor has an external loop as the name itself indicates to separate samples or liquid in different channels carrying out the fermentation process.

Packed bed bioreactor



The packed bed bioreactors are tubular reactors having a packed bed with biocatalyst that is immobilized enzymes or microbial cell. You see here packed bed with the biocatalyst in the reactor. So a nutrient broth continuously flows over the immobilized biocatalyst. After that a product releases into the fluid at the bottom of the container, you can see here in the figure, and finally it can be removed. Here the flow of the liquid can be either upward or downward as the case may be.

Fluidized bed bioreactor



Fluidized bed bioreactor is a combination of stirred tank and packed bed system having higher volumetric capacity. The top port is more expanded to reduce the velocity of the fluid, whereas the bottom port is slightly narrow. You can see here in the figure. It is designed in such a way where the solid retain inside the vessel and the liquid flows out.

Generally operated in co-current up-flow with liquid as continuous phase and other more unusual configuration like the inverse three phase fluidized bed or gas solid fluidized bed are not of much importance. The smaller particle size facilitates higher mass transfer rates and better mixing. You see here that is material they are kept in the bubble or fluidized and it gives intimate mixing so the reaction is better.

Photo bioreactor

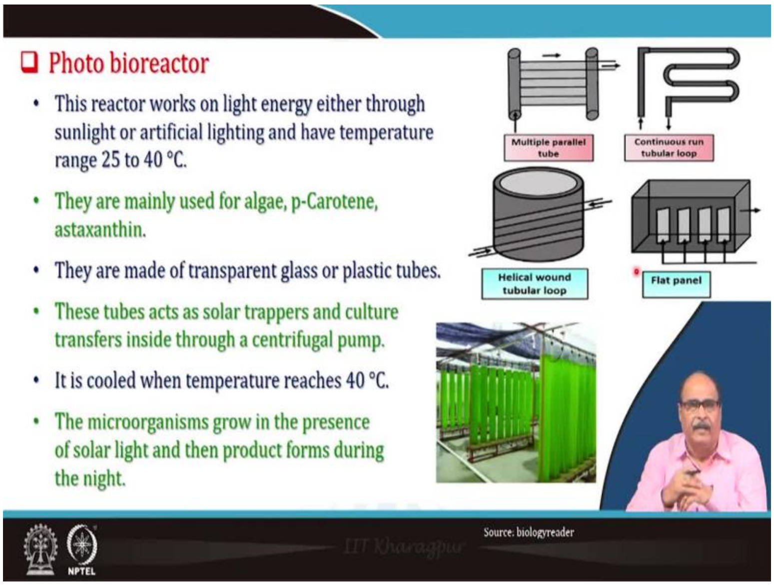
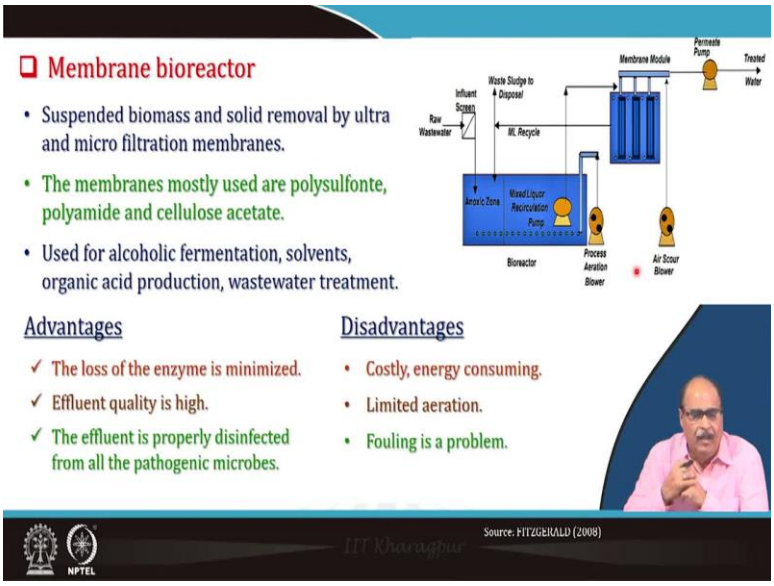


Photo bioreactor work on light energy either through the sunlight or artificial light and have a temperature of around 25 to 40 degrees Celsius in the reaction medium. They are mainly used for algae production, Carotene production, astaxanthin production, etc.

They are made of transparent glass or plastic tubes. These tubes act as solar tappers and culture transfer inside through a centrifugal pump. It is cooled as and when the temperature reaches about 40 degrees Celsius. The microorganisms grow in the presence of the solar light and then product forms during the night.

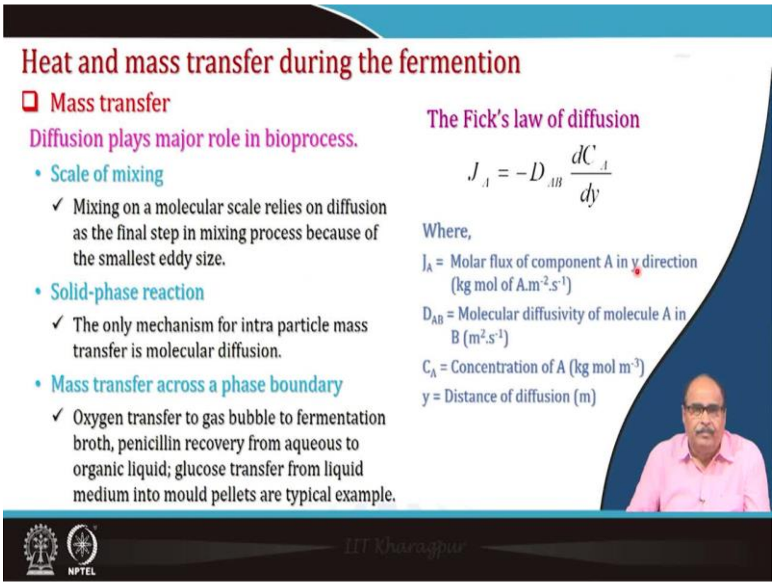
Membrane bioreactor



Membrane bioreactor: here the suspended biomass and solid removal is done by ultra and micro filtration membranes. The membranes mostly used are polysulfonate, polyamide or cellulose acetate membrane. They are used for alcoholic fermentation, solvents, organic acid production, wastewater treatment, etc.

The advantages of the membrane bioreactors include that the loss of the enzyme is minimum. Effluent quality is high and the effluent is properly disinfected from all the pathogenic microorganisms. However, these are costly, it consumes more energy, it has limited aeration capacity, and the fouling of the membrane is a major disadvantage.

Heat and mass transfer during the fermentation



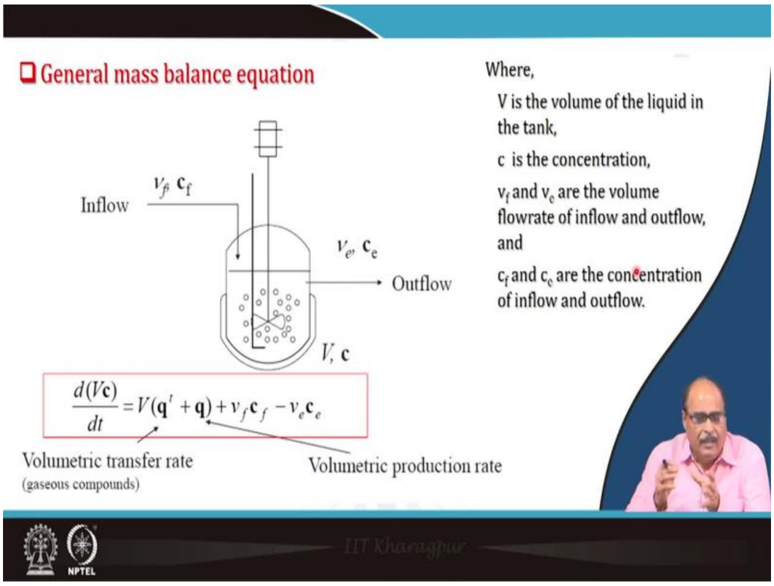
Now, let us see some heat and mass transfer phenomena during the fermentation. Mass transfer: here the diffusion plays a major role in the bio process and the Fick's law of diffusion is:

$$J_A = -D_{AB}\frac{dC_A}{dy}$$

where J_A the molar flux component of A in y direction, D_{AB} is the molecular diffusivity of molecule, C_A is the concentration of A and y is the distance of the diffusion.

So this Fick's law of diffusion holds good here. So it is that scale of mixing, solid phase reaction, and mass transfer across a boundary membrane. Then the oxygen transfer to the gas bubble to the fermentation broth, penicillin recovery from the aqueous to the organic liquid or glucose transfer from liquid medium to the mole pellets are typically examples of the mass transfer across a phase boundary.

General mass balance equation

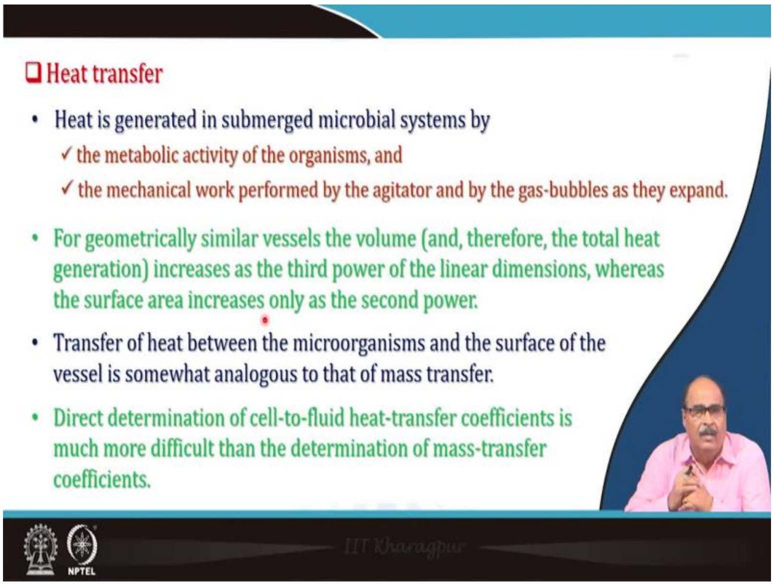


The general mass balance equation:

$$\frac{d(Vc)}{dx} = V(q' + q) + v_f c_f - v_e c_e$$

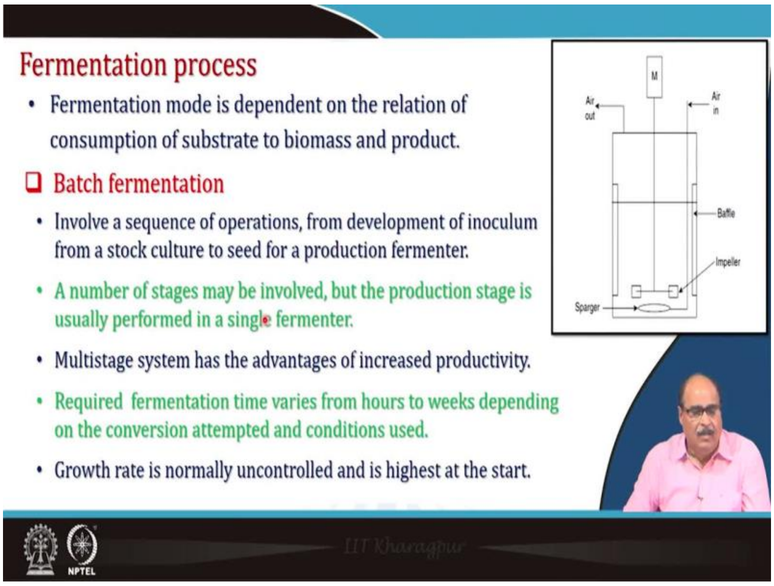
where q is the volumetric transfer rate, q-dash is the volumetric transfer rate and q is the volumetric production rate. V_f is the flow and concentration of the flowing substrate is the c_f. It is outflow is the v_e and c_e and v and c are the volume and the concentration of liquid inside the reaction medium. So this general mass balance equation is all good and this can be calculated.

Heat transfer



Then heat transfer: the heat generated in submerged microbial system by the metabolic activities of the microorganism and the mechanical work performed by the agitator and by the gas bubbles as they expand. So for geometrically similar vessels the volume increases as the third power of the linear dimension, whereas, the surface area increases only as the second power. Transfer of heat between the microorganism and the surface of the vessel is somewhat analogous to the mass transfer and direct determination of cell to fluid heat transfer coefficient is much more difficult than the determination of mass transfer coefficient.

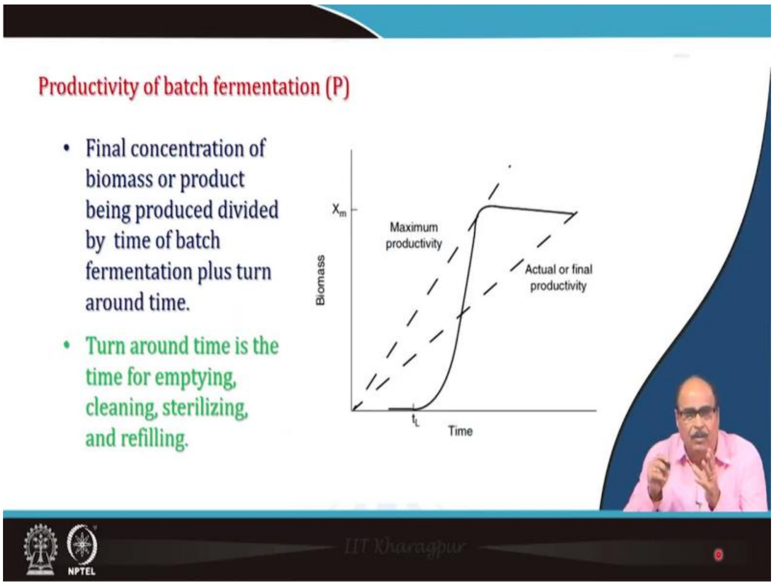
Fermentation process



The fermentation process: the mode of the fermentation is dependent on the relation of the consumption of substrate to the biomass and the product, it may be batch fermentation, it may be continuous fermentation. A batch fermentation involves a sequence of operations from development of inoculum from a stock culture to seed for a production of a fermentation.

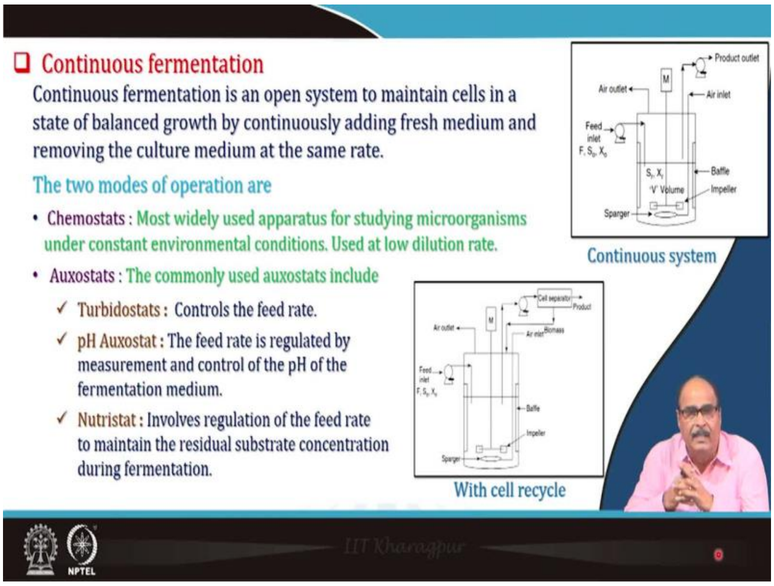
So a number of stages may be involved, but the production stage is usually performed in a single fermenter. Multistage system has the advantage of increased productivity, that is required fermentation time varies with the hours to week, depending on the convergent attempted and the conditions that are used. Growth rate is normally uncontrolled and it is highest at the start of the fermentation.

Productivity of batch fermentation (P)



So the productivity in the batch fermentation process that is the final concentration of the product or biomass that is for P is divided by the time of batch fermentation plus turnaround time, so this gives the productivity of batch fermentation. The turnaround time is the time for empyting, cleaning and sterilizing and finally, refilling the reactor.

Continuous fermentation

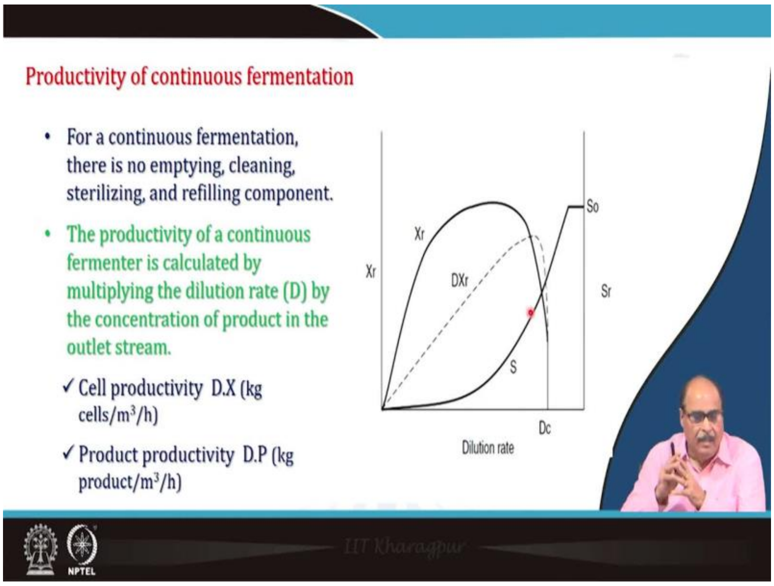


The continuous fermentation is open system to maintain cells in a state of balanced growth by continuously adding the fresh medium and removing the culture medium at the same time. You can see there is a continuous system where continuously the feed is added and a particular concentration is maintained and then continuously the product is formed.

So the rate of the product goes out at the rate of the feed added so balance is maintained. And there is other system, the cell recycle system, it is also that in the continuous but in this the product goes out there is some underdeveloped cells etc., they are separated and they are recirculated, so this is a cell recycle system.

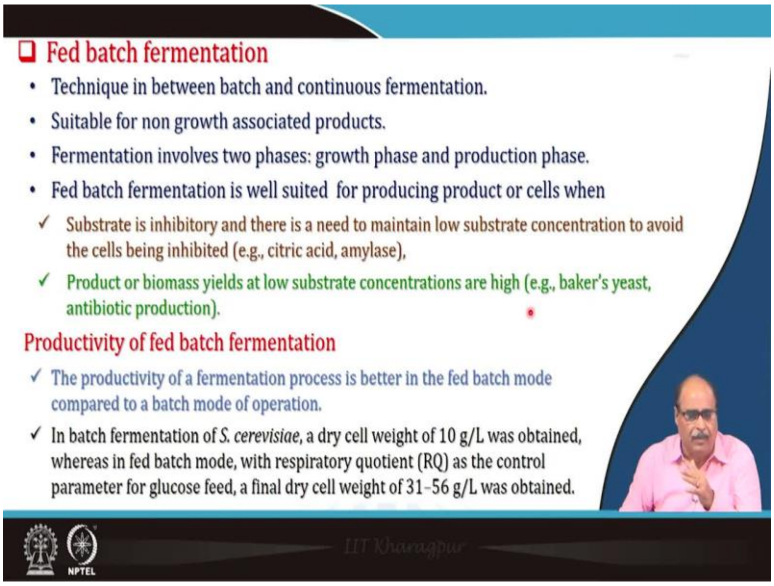
So 2 modes: there are 2 modes of operation like chemostats were most widely used apparatus for studying microorganism under constant environment conditions, it uses low dilution rate. And Auxostats are the Turbidostats, which controls the feed rate, pH state. The feed rate is regulated by measurement control of the pH of the fermentation medium. And Nutristat involves regulation of the feed rate to maintain the residual substrate concentration during the fermentation process.

Productivity of continuous fermentation



Then productivity of a continuous fermentation process is calculated by multiplying the dilution rate D by the concentration of the product in the outer outlet stream. That is the cell productivity is D.X in terms of kg cells per cubic meter per hour or product productivity is the D multiplied by P that is the kg of the product per cubic meter per hour. So the productivity in the continuous fermentation process, it can be either the terms of cell or product whatever you say. There is no turnaround time because in the continuous fermentation, inputting, cleaning, sterilizing, refilling, etcetera are not a problem.

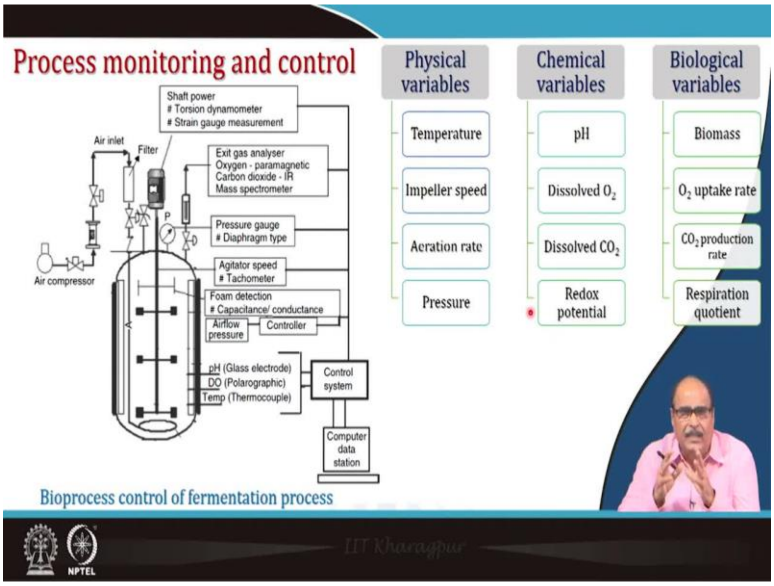
Fed batch fermentation



Fed batch fermentation is a technique in between the batch and continuous fermentation. It is suitable for non-growth associated products. Fermentation involves two phases, growth phase and production phase. So fed batch fermentation is well suited for producing the product or cells when the substrate is inhibitory, and there is a need to maintain low substrate concentration to avoid the cell being inhibited or when the product or biomass yield at low substrate concentrations are high as in the case of baker's yeast, antibiotic production, etc.

The productivity in the fed batch fermentation process: it is better than compared to the batch mode of operation. In batch fermentation of *Saccharomyces cerevisiae* a dry cell weight of 10 gram per liter was obtained, whereas, in the fed batch mode, the respiratory quotient RQ as the control parameter for glucose feed, a dry cell weight of around 31 to 56 gram per liter was obtained. So the productivity in the fed batch fermentation medium is better than that of the batch medium.

Process monitoring and control

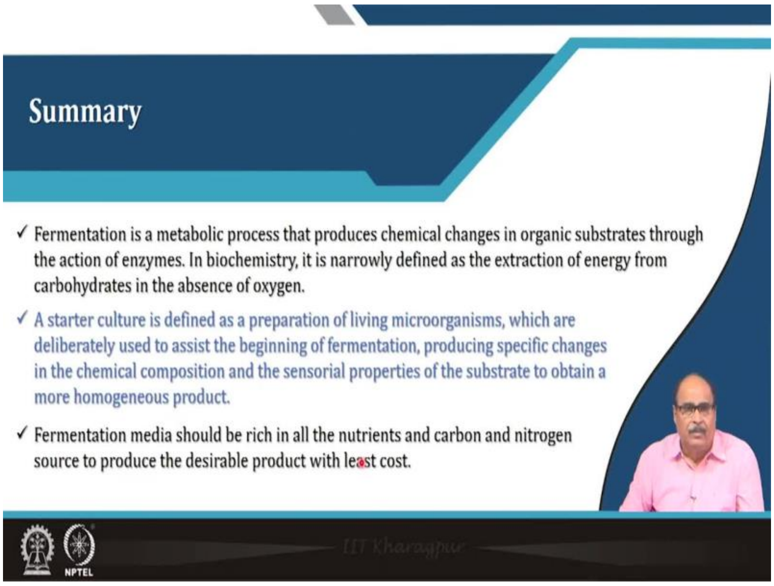


So the process monitoring and control is very-very important in the fermentation process, because, if this is not maintained (for example: if the temperature is not maintained or if in case of aerobic fermentation, oxygen level is not maintained and particularly in the larger reactors, throughout in the liquid the oxygen level is to be maintained or in case of the anaerobic fermentation oxygen should not be completely at all; even the pH and all (is to be maintained)), so most of the modern fermenters they are provided with all a shaft power for the torsion dynamometer, strain gauge measurement, there are filter assembly provided, a compressor, there is exit gas cylinders like oxygen paramagnetic, carbon dioxide, regulators, mass spectrophotometer, or presser gauge, agitator speed, foam detection meters, etc.

Then gauge electrode, pH, temperature thermocouple control, and even the most of the modern fermenters they are properly controlled and even a computer controlled their computer data and station are provided with the computer system. So mostly the physical variable like temperature, impeller speed, aeration rate, pressure, chemical variable like pH, dissolved oxygen, dissolved carbon dioxide, redox potential and biomass, oxygen uptake rate, carbon dioxide production rate, respiration quotient, and all these things are properly monitored and controlled and the modern aerator should be provided with the necessary arrangement to maintain these parameters etc.

In order to get the product desired or to control the process in such a way that the desirable microorganism are only growing and the desired product is produced with the desired characteristics and undesirable or wild growth of the microorganisms are in control so that is very important otherwise, if it is a fermentation processes is not controlled properly, then wild microorganism, spoilage microorganism or wild yeast etc. they may grow and they may spoil the product.

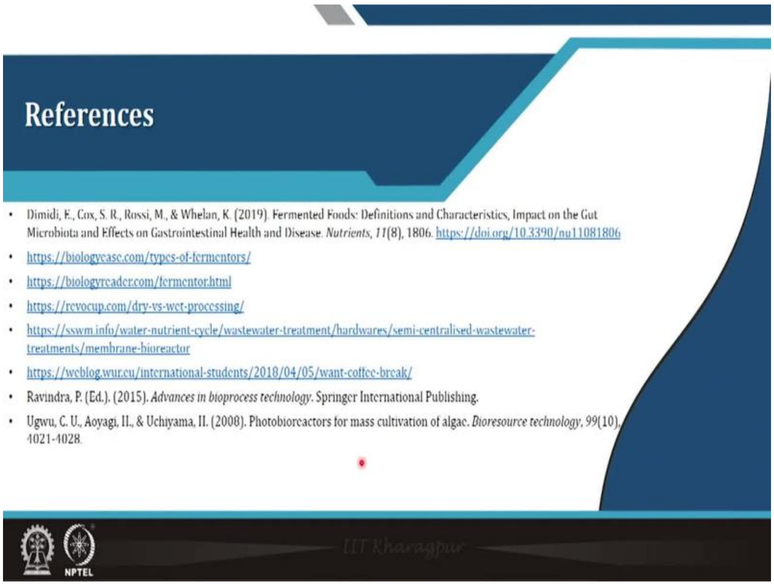
Summary



So I will summarize this lecture by saying that yes there are two-three important aspects in the fermentation process: there should be a proper well-defined substrate and a proper bacterial or microbial or yeast or mold proper culture and this selection of the culture and the substrate is proper like we take the juice maybe grape juice and then *Saccharomyces cerevisiae* yeast in proper form and then before adding the yeast this grape juice is calculated for its sugar content, pH, etc.

So proper condition or the proper characteristics is ensured in the substrate, proper quantity of ingredients are there and then you provide the desired conditions for the bacteria or yeast or other microorganism to grow and at optimum requirement of pH, temperature, other things should be maintained so that you get a proper label of the product with desired characteristics. Fermentation media should be rich in all the nutrients and carbon and nitrogen source and the fermenter should be properly designed and controlled to give the desired product.

References



So these are the references used in this lecture. Finally thank you very much for your patience. Thank you.