Bioreactor Design and Analysis
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Lecture 01
Introduction to the course - Part 1

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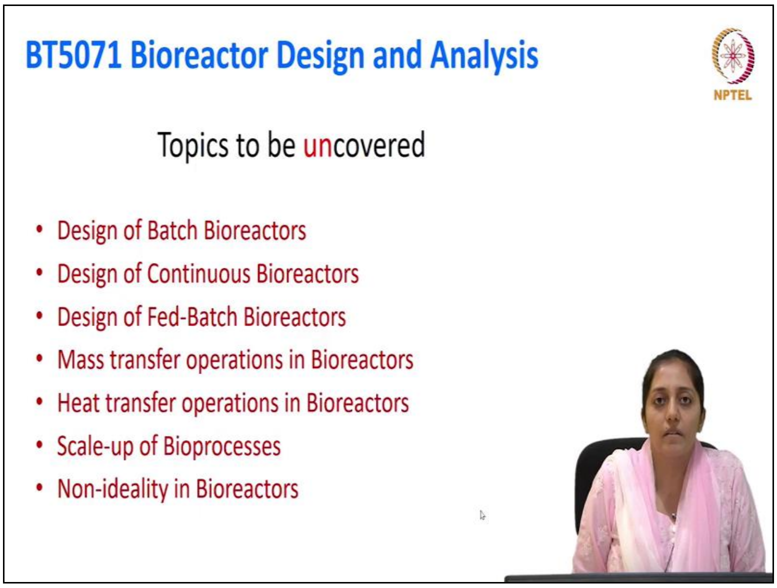

Hello students welcome to the course on bioreactor design and analysis. So I will be giving you an introduction about the course today. We will be talking about what will be the things which we will be uncovering in this course. So the topics which we are going to cover in this course include design of patch reactors, then continuous reactors design of fed patch reactors and mass transfer operations which take place in bioreactors.

Then we will go on to study the heat transfer operations in bioreactors, scale up of bioprocesses and then we will end up with the some introduction on the non-ideality of bioreactors and how to handle it in practical situations.

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These are some textbooks for your reference you can go through it after the lecture.

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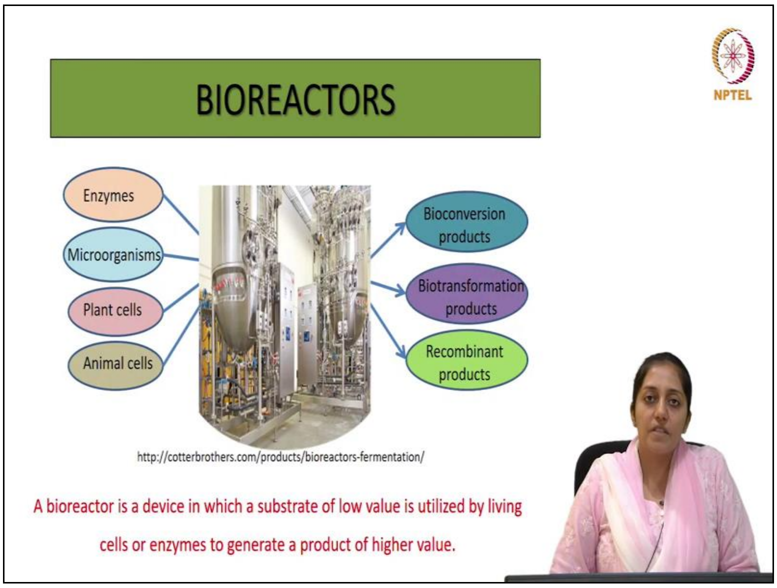

So as you can see on the slides the title of the slide is bioreactors it is important to know what is a bioreactor. So in fermentation industry or biotechnology based industries bioreactors play a crucial role I would rather say they are a foundation stone for these industries. Now what are bioreactors you can call them as devices in which a substrate which is of low value is utilized by living cells or the cellular components which we call as biocatalyst named as enzymes to generate a product of higher value.

So inside this device which we call as bioreactors there are different kinds of production platforms which are used to convert a low cost substrate to a high cost product. Now the different production platforms which are used in industry they vary greatly you will hear about plant cells being used. You will hear about microorganisms then there are animal cells which are used in industry and also rather than using the whole cells industries are also using cellular components or to say bio catalyst which are like chemical enzymes responsible for carrying out desirable bioreactions.

These due to a series of reactions so when whole cells are used they undergo a series of reactions which are called as metabolic reactions as a result of which the substrate is converted into product. Mostly these substrates are compounds which are required for the growth and survival of the organism. And due to its metabolic reactions or metabolism to say the end products of this metabolism can be of high value to us which we call them as products.

Then apart from bio conversion reactions other than the metabolic reactions there are also a set of reactions which these bio catalyst or enzymes they carry out like for example detoxification reactions. Reactions which may be needed to convert a toxic metabolite into a non-toxic form. So sometimes such reactions can also be converting a low cost product into a high cost product so they come under bio transformation where the substrate when exposed to either repository of enzymes which we call as whole cells or single enzymes or free enzymes.

Can convert these substrates through a series of reactions by which there is some chemical transformation done in the compound so as to convert it into a desirable form which we call as product. Now there are third set of products which can also be achieved in bioreactors which we call as recombinant products. Now recombinant products they are a result of certain reactions which are not present in the native organism.

So to say which are not inherent to the native organism but the organism has been engineered deliberately to carry out those set of reactions which we call as genetic engineering. So when a substrate is exposed to the organism the organism uses the substrates and the substrates undergoes a series of reactions which are not inherent to the organism's metabolism but is operational due to the genetic intervention done inside the organism and results into a high value products.

So those are called as recombinant products. So now I hope you are able to see the wide array of kind of products which these bioreactors can produce.

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Let us see our day-to-day products in our life which we use let us talk about bread or biscuits which have been fortified to have rich nutrients or personal care products like cream cosmetics or detergents, textile detergents which we use day-to-day to wash our clothes or paper tissue which we frequently use. Then carpets or furniture's polyesters in fact even rubber then bio fuels which I am sure you must have heard of as an alternate to conventional fuel.

Plastics, now plastic has a wide array of applications in our life ranging from food service wear to beverage packaging, food packaging, plastic containers and so on. Now all these products were conventionally produced via chemical reactions but then gradually advancement in the biotechnology industry these products are now being produced as a result of biotechnology driven processes by biotechnology industries.

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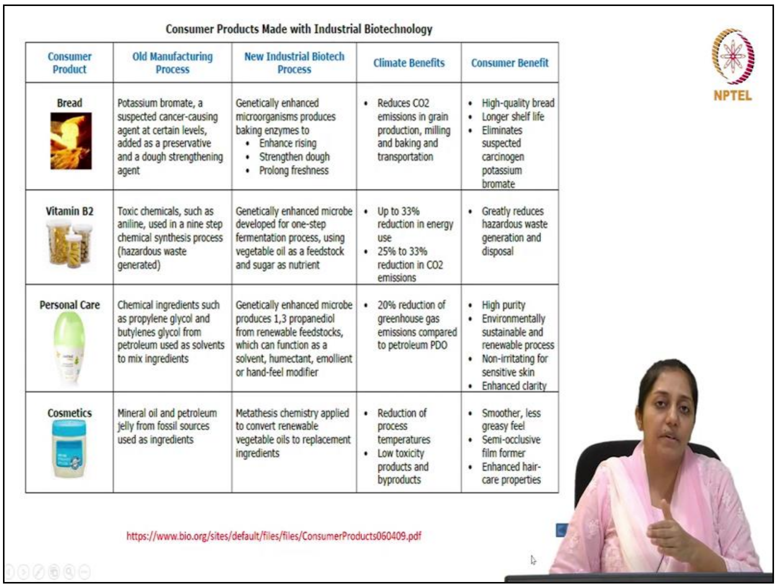

Let us see why this change has happened and these are some of the examples which will tell you how biotechnology intervention has been useful to humankind. Like for example let us take bread now in bread conventionally a chemical which is known to be carcinogen potassium bromate used to be used as a preservative for those strengthening. Now there are some industries which are now using genetically enhanced microorganisms to produce different flavours of breads and with better quality in terms of strengthened dough higher shelf life and high quality.

Now in terms of environment sustainability the process has become more environment friendly because a large reduction in the release of greenhouse gas. And the biotechnological process is of advantage because it is able to avoid the use of the carcinogen potassium bromated. Let us talk about the cosmetics a personal care products where generally like your petroleum jelly where generally products based on petroleum distillates they are used in the market.

Now this process itself leads to a large amount of release of greenhouse gases. So biotechnologically driven processes like for example genetically modified microbes they produce 1 3 propane diol from renewable feedstocks which can then be used as humectants or emiliant or hand field modifier. Now these processes in general they lead to a very high reduction in greenhouse gas emissions up to 50% in some cases.

And it also leads to reduction of harsh conditions or moderate conditions are used in comparison to chemical processes in terms of the requirements of ph temperature.

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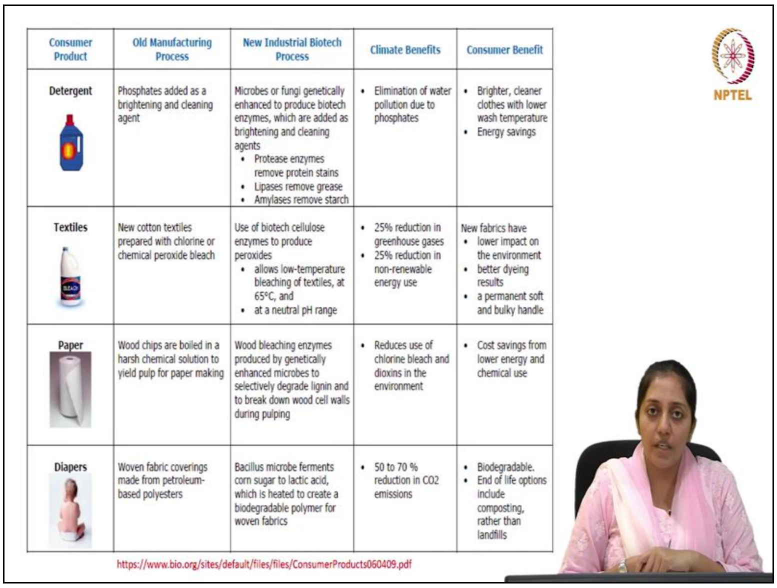

Some more examples like for example your detergent textile or papers where bleaching agents are used like hydrogen peroxide chlorine which can cause harm to the environment when released or sometimes in pulp industry acid is used harsh chemicals are used. So now with biotechnology intervention there are certain microbes which can produce a set of enzymes ranging from being proteases, lipases amylases which have the capability of degrading different types of strains or celluloses which have the capability of degrading the cellulose and making it easier to for processing of pulp in the paper industry.

Now again the result is that there is a large decrease in the release of greenhouse gases you can avoid the use of bleaching agents chemical bleaches less harsh conditions are used in the to carry out these processes and the product quality is enhanced.

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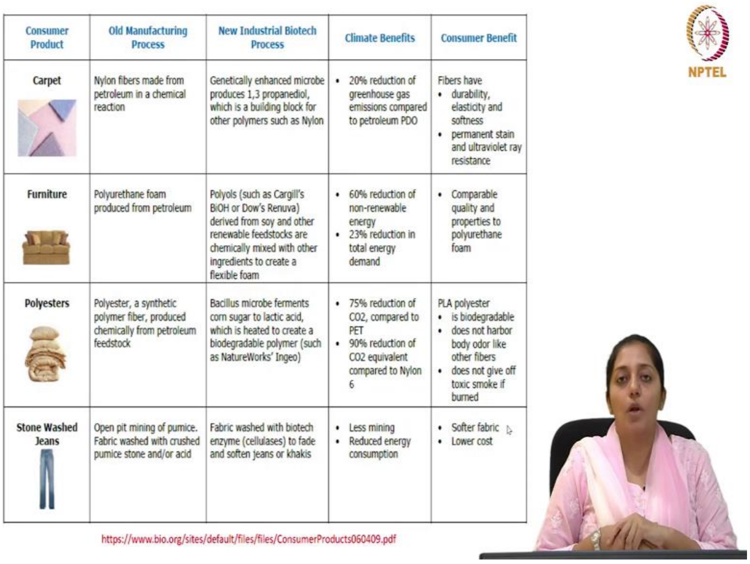

Consumer Product	Old Manufacturing Process	New Industrial Biotech Process	Climate Benefits	Consumer Benefit
Carpet	Nylon fibers made from petroleum in a chemical reaction	Genetically enhanced microbe produces 1,3 propanediol, which is a building block for other polymers such as Nylon	• 20% reduction of greenhouse gas emissions compared to petroleum PDO	Fibers have • durability, elasticity and softness • permanent stain and ultraviolet ray resistance
Furniture	Polyurethane foam produced from petroleum	Polyols (such as Cargill's BiOH or Dow's Renuva) derived from soy and other renewable feedstocks are chemically mixed with other ingredients to create a flexible foam	• 60% reduction of non-renewable energy • 23% reduction in total energy demand	• Comparable quality and properties to polyurethane foam
Polyesters	Polyester, a synthetic polymer fiber, produced chemically from petroleum feedstock	Bacillus microbe ferments corn sugar to lactic acid, which is heated to create a biodegradable polymer (such as NatureWorks' Ingeo)	• 75% reduction of CO2, compared to PET • 90% reduction of CO2 equivalent compared to Nylon 6	PLA polyester • is biodegradable • does not harbor body odor like other fibers • does not give off toxic smoke if burned
Stone Washed Jeans	Open pit mining of pumice. Fabric washed with crushed pumice stone and/or acid	Fabric washed with biotech enzyme (cellulases) to fade and soften jeans or khakis	• Less mining • Reduced energy consumption	• Softer fabric • Lower cost

https://www.bio.org/sites/default/files/files/ConsumerProducts060409.pdf

Now talking about the use of polymers now nylon which is a well known polymer and has a very frequent use in our lives can also be produced by our genetically modified organisms which produce one three propane diol which forms the building block of nylon. Then polyols which can replace polyurethane forms. Now they can be produced using renewable feedstocks from certain microorganisms which can then be chemically mixed with other ingredients to create flexible forms for use.

All these processes again lead to up to 60% reduction in the greenhouse gas emissions or the use of renewable energy and the durability the environmental friendliness like the biodegradability is an added advantage. The well-known biofuels where ethanol is conventionally produced by acid hydrolysis of starch can now be produced by a biotechnology using enzymatic hydrolysis of starch and cellulose.

Thereby reducing the use of chemicals and energy or toxic by-products release and also fewer release of greenhouse gases.

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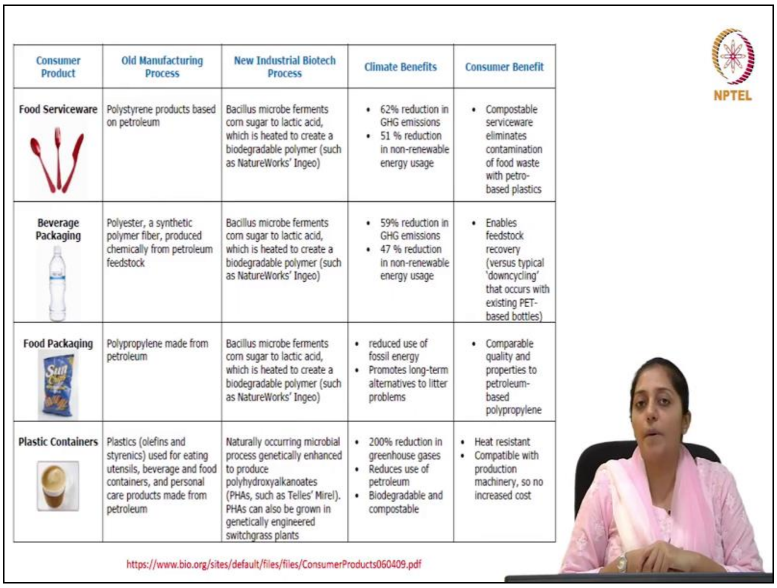

Consumer Product	Old Manufacturing Process	New Industrial Biotech Process	Climate Benefits	Consumer Benefit
Food Serviceware	Polystyrene products based on petroleum	Bacillus microbe ferments corn sugar to lactic acid, which is heated to create a biodegradable polymer (such as NatureWorks' Ingeo)	• 62% reduction in GHG emissions • 51 % reduction in non-renewable energy usage	• Compostable serviceware eliminates contamination of food waste with petro-based plastics
Beverage Packaging	Polyester, a synthetic polymer fiber, produced chemically from petroleum feedstock	Bacillus microbe ferments corn sugar to lactic acid, which is heated to create a biodegradable polymer (such as NatureWorks' Ingeo)	• 59% reduction in GHG emissions • 47 % reduction in non-renewable energy usage	• Enables feedstock recovery (versus typical 'downcycling' that occurs with existing PET-based bottles)
Food Packaging	Polypropylene made from petroleum	Bacillus microbe ferments corn sugar to lactic acid, which is heated to create a biodegradable polymer (such as NatureWorks' Ingeo)	• reduced use of fossil energy • Promotes long-term alternatives to litter problems	• Comparable quality and properties to petroleum-based polypropylene
Plastic Containers	Plastics (olefins and styrenics) used for eating utensils, beverage and food containers, and personal care products made from petroleum	Naturally occurring microbial process genetically enhanced to produce polyhydroxyalkanoates (PHAs, such as Telles' Mirel). PHAs can also be grown in genetically engineered switchgrass plants	• 200% reduction in greenhouse gases • Reduces use of petroleum • Biodegradable and compostable	• Heat resistant • Compatible with production machinery, so no increased cost

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Talking about the use of plastics now plastics as we know it has become a menace in the world. Now people have been trying to invent bioplastics to replace the chemically originated plastics. Now these bio plastics are nothing but polymers produced by the micro organisms as a result of their metabolism. So, bacillus for example a micro which can ferment corn sugar to lactic acid which is heated to create the biodegradable polymer.

So like polylactic acid is one of the key polymers which is used in packaging industry has a use in food service where making or food packaging. So it can replace the conventional plastic or poly hydroxy alkanoid which are produced by certain bacteria as carbon reserves these polymers can in turn be used to replace the conventional plastics. The advantage being that bioplastics are completely biodegradable in nature so they are environment friendly substitute to plastics there is lesser greenhouse gas emissions up to 50% reduction is observed and less energy requirements in the process.

Because of moderate conditions under which biochemical reactions take place. So these were some of the examples where biotechnology has played a key role in substituting certain harmful chemical reactions for commercial use.

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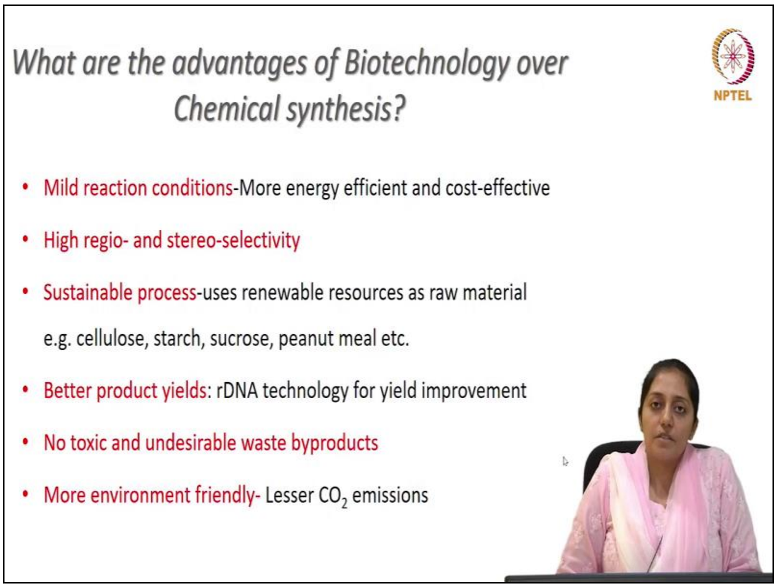

Now let us see what are the advantages after looking at these examples which I talked about I am sure you must have got an idea of the various advantages which biotechnology or biochemical reactions have over the chemical reactions. Now how biotechnology can be better than chemical synthesis as you can see on the slide. Biochemical reactions they can be carried out under milder process conditions.

So they become more energy efficient and more cost effective being driven by biocatalysts called enzymes they have higher regio and stereo selectivity so that is a very big advantage. Like for example Will give you an example of alpha tocopherol alpha tocopherol which is the most bioactive component of vitamin E can be extracted from plant tissues it is chemically also produced however chemically produced has a recimic mixture results in a recimic mixture.

Where in nature due to the metabolism in the plant the one particular isomeric form is the most bioactive and is the one which is preferentially absorbed by the human body and is useful to us. So that is one example where if a natural reaction or a biological reaction is carried out it can result in desirably producing the right isomer of the product. However in chemical reactions there is less control over the kind of isomer which can be obtained.

Now biotechnology driven processes are more sustainable in nature because they mostly use renewable resources as raw materials or as substrates like for example cellulose starch sucrose, peanut meal and many others. Biochemical reactions they are also amenable to optimizations which means there is a scope to improve the product yield while changing the process conditions and being controlled reactions in biologically driven reactions there are less toxic byproducts released in comparison to chemically driven reactions.

And they are found to be more environmentally friendly as I told you during giving the examples where up to 50 to 60% reduction has been observed in the greenhouse gas emissions when chemically driven processes were replaced by the biologically driven processes.

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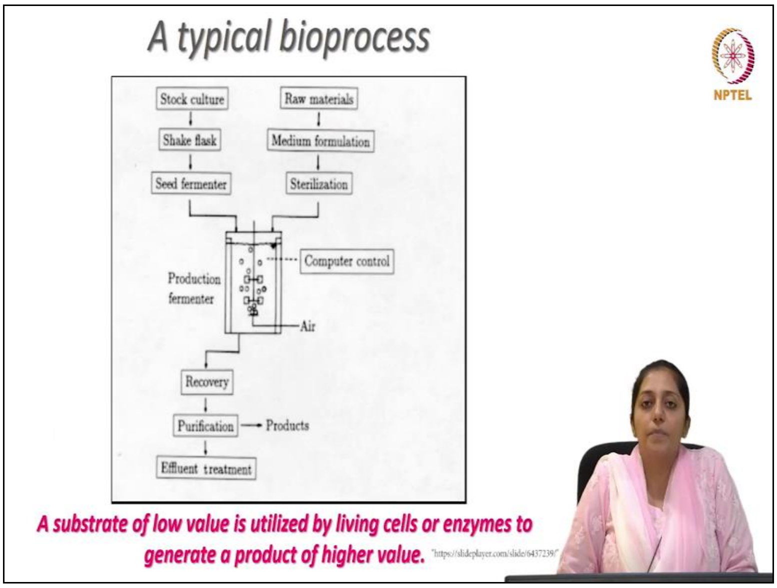

Let us see what is a typical bio process now this is a schematic which talks about what are the different steps involved to convert a low cost substrate to a high cost product. So in a typical bio process if you see on the left hand side top corner is the stock culture stock culture means your master cell bank which can be cryopreserved or maintained for its continuous use in the production. So that stock culture is brought to a working culture state where it is called as working cell bank by exposing it to favourable nutrients or rich nutrients at a very small scale which is generally shake flask level.

Now the shake flask level you can optimize the medium composition to find out the right medium nutrients for maximum product formation. And shake flask studies are also used for screening of cell lines or finding the highest yielding cell line. So from the stock culture you make it into a working stock by bringing it to a shake flask level. Now from the shake flask you bring it to you prepare the inoculum which is called as the starter culture for the production scale.

Now this is called as the seed fermenter this is a small size reactor where you will must multiply the starter culture to bring it at a stage where it is ready to be inoculated in the production fermenter. Then on the right hand side top corner there is raw material which is your substrate sometimes the raw material is required to be pre-treated or processed before it can be used in the reactor.

So that raw material is processed then medium formulation is done where the media which involves macronutrients, micronutrient and depending on the production platforms there might be a need for other compounds like hormones or growth promoting substances for the organism. Now the key is that bioreactor is a controlled system in which only monoseptic operations can be done.

So in order to enable monoseptic culture the media with the substrate has to be sterilized which means it has to get rid of any contaminant or any other microorganism which can contaminate the production fermenter. So there are different ways in which sterilization can be done so this sterilization is also one of the key steps in fermentation technology. After the sterilization is completed the sterile medium with the substrate is then fed into the production fermenter and the production fermenter is inoculated with the starter culture from the seed fermenter.

Now this fermenter will have different key parts where there is if it is an aerobic fermentation which means the organism if it needs oxygen for its growth then there are parts in the reactors which will facilitate oxygen transfer to these organisms. This will include sparging device there will be a mixing device which we call as impellers to create a homogeneous environment inside the reactor.

So this is the portion where various heat transfer operations mass transfer operations are carried out in order to enhance your biological rate of reactions or to optimize the biological rate of reaction. Then this reactor is also under computer aided control of a bio controller where there is an automated control of the process conditions. Now because these are biologically driven reactions they work best at certain set of conditions can be physical and chemical.

So in order to maintain those required set of conditions at their optimum values to maximize the productivity or the production rates there is an automated control which can help in maintaining the culture conditions of this controlled system environment at its optimum. Generally you will find there is pH dissolved oxygen temperature these are the key parameters which can be controlled in a reactor.

There is a sparging device through which air can be continuously sparsed in inside the reactor to make oxygen available for growth of the organism. Then below the reactor the steps involved they come under downstream processing which involve the recovery of the product which may involve separation of the product from the biomass. Now if the product is extracellular then the cell need not be disrupted and the product has to be separated from the broth.

Otherwise if it is intracellular then first the biomass has to be harvested which means separated from the liquid broth spent medium and then the biomass has to be separately disrupted to bring out the product which comes under product recovery. Then comes product purification which also involves concentration of the product to have higher concentrations of the product. So; where you remove the other by-products or undesired products from the liquid broth.

Then whatever is the spent medium remaining cannot be released in the environment as such so it is treated and brought under the safe limits before the release into the environment as base streams. So this entire process comes under effluent treatment which is a must for most of the process industries. So this entire schematic is responsible while converting a substrate of low value getting utilized by the living cells to produce a product of high value.

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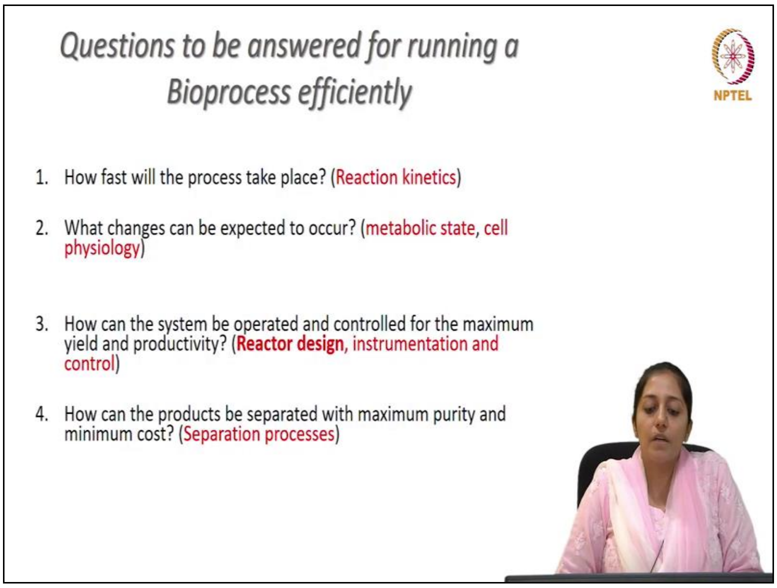

Now the questions which are required to be answered for running a bio process efficiently involve questions like how fast will the process take place which includes investigating the reaction kinetics. Then what changes can one expect to occur during this process which includes investigating the metabolic state or the physiological state of the cell which is being cultivated inside the reactor.

One should know how can this system be operated and controlled for the maximum yield and productivity be it in terms of biomass or in terms of product. So this step is where reactor design plays a crucial role this also involves instrumentation and control but here we are going to focus in this course on the reactor design aspect. What do we mean by reactor design when we need to ensure that the system is operated and controlled to achieve maximum productivity.

And then there is one more question which is how can the product be then separated once achieved for maximum purity in minimum cost. So this is a different set of study which comes under downstream processing which is another course in itself where different ways in which the product is separated and purified are discussed.

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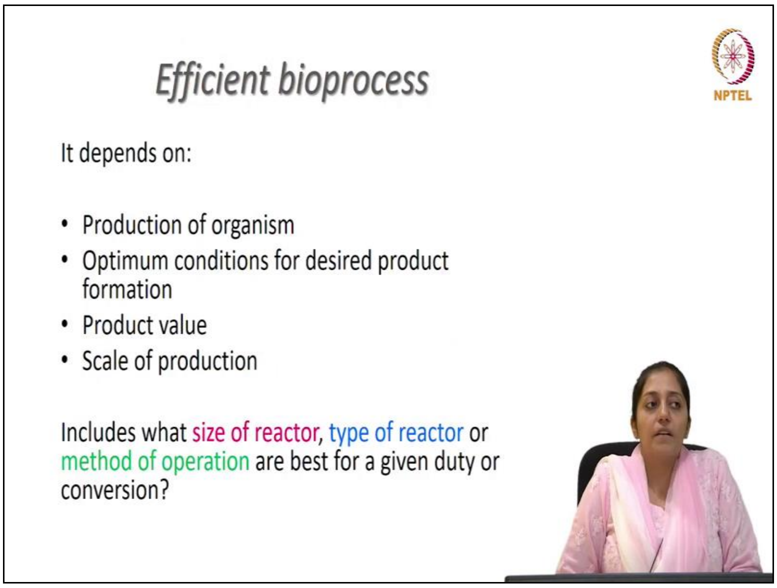

So to say what is an efficient bioprocess it depends on the following things. One on the production of the organism inside the reactor how well the organism is getting mass multiplied which means maximizing the biomass which is achieved. What conditions are required to obtain the desired product formation see being metabolic reactions there can be more than one products getting formed simultaneously.

So what process conditions should be applied so as to drive the carbon flux desirably towards your desired product. Then the value of the product and the scale of operation which means at what scale you should run the process to keep it cost effective and to meet the market demand. So these are some of the questions which are dealt with in bioreactor design and analysis as well. Now in what sense like for example in order to answer these questions you should be able to know what size of reactor is needed to run the operation.

What type of reactor you will be needing and what method of operation will be best for the given substrate duty or conversion.

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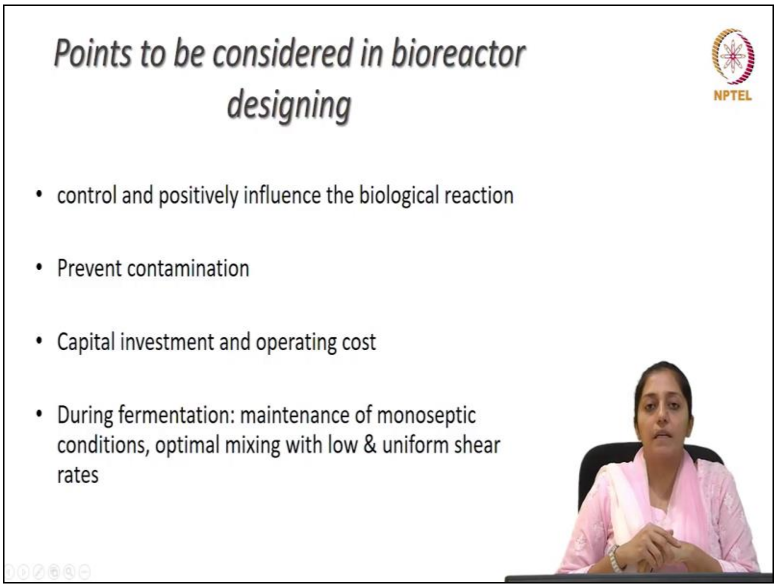

So the points which we will consider in bioreactor design they should be able to control and positively influence the biological reaction that is key. It should be able to prevent contamination the bioreactor design should be such that the capital investment can be kept as low as possible and similarly the operational cost. And one has to take care that in the chosen reactor design it facilitates maintenance of monoseptic conditions during the entire fermentation period.

Depending on the production platforms being used the fermentation time can vary from hours to days and even sometimes to months. So the chosen reactor design should therefore also look into factors like optimal mixing, optimal heat transfer and with low and uniform shear requirements for maximum viability of the culture to achieve maximum productivity.