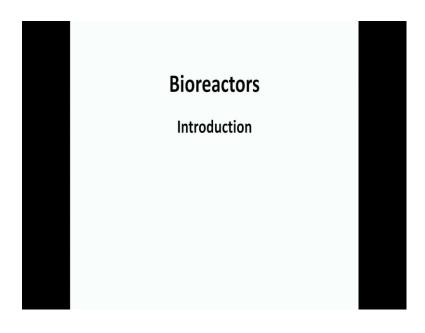
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Lecture – 01 Introduction

Welcome to this mooc on bioreactors. This is going to be a 10 lecture mooc spread over about 4 weeks. This mooc will have these lectures and there will be some assignments that come once a week or so. In addition, there will be some numerical problems or thought based problems that we will discuss in class and probably assign some problems you would have time to do it and we will check up with solutions in the next class.

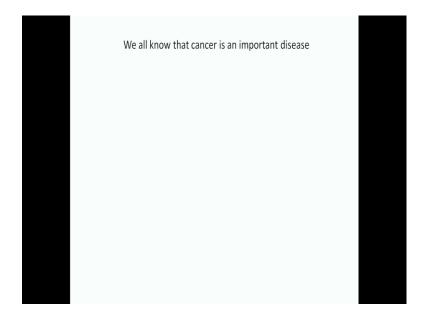
The organization of this course probably you may not find in text books in a complete way. However, two books may be useful. You can consider them as reference books. The first one is Shuler and Kargi. I will give you the details in one of the files that you can download from the course site, that is a nice book. In addition, there is an older book, very old '86, it is by Bailey and Ollis. That is also a good book but it is somewhat dated. In going forward, this would be an introduction, the first lecture is going to be an introduction.

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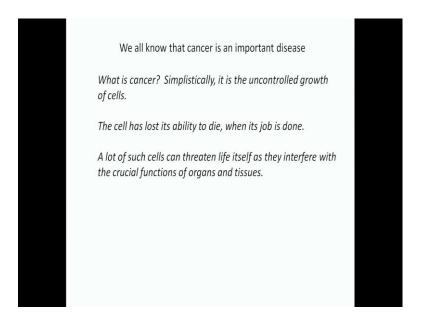
Let us think about Cancer; let us start with Cancer. We all know that cancer is an important disease nowadays.

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It has been an important disease for a very long time, decades maybe and still it has not been completely conquered. There is lot of progress that has been made in treating cancer, in diagnosing cancer, but we have still not completely overcome it and it is still one of the important diseases of today.

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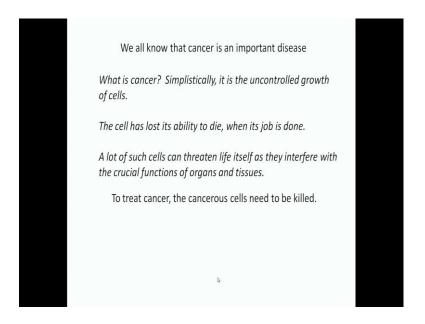


What is cancer? Very simplistically speaking, it is the uncontrolled growth of cells. We all know this. The cell has lost it is ability to die when its job is done and when that happens, cancer happens. Or, that is one of the ways cancer happens. For example, this is a nice example to give you. If you look at our hand and if you look at how this hand started out in a fetus, it would have been a completely filled kind of a mass. Then the cells here started dying away and that is what gave rise to the digits. Therefore, in other words, the cells here actually committed suicide and that is what gave us these digits, which are functional.

So, cell death is a necessary part of the developmental process, the regeneration process and so on in the body. And if that goes haywire and the cell continues to live, then it results in cancer. Why is cancer dangerous? It is just cell that is there. Cells that have lost their ability to die. So, why should it bother us? It bothers us because, a lot of such cells in the body can threaten life itself as they interfere with the crucial functions of organs and tissues. For example, let us take the lung. If the lung cells become cancerous, they start growing uncontrolled and they interfere with the gas exchange that happens in the lungs, which is vital to keep up life and so on and so forth. You can talk of different organs in the body - maybe liver, maybe kidney. And, if there are cells that continue to grow and refuse to die, then it interferes with the normal function of those organs and

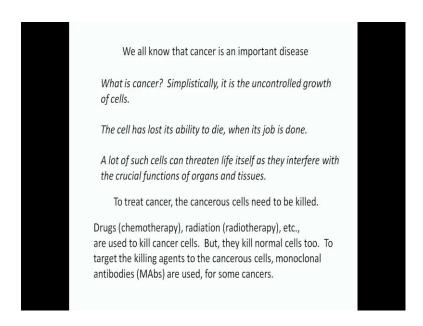
that ultimately leads to death in a simplistic sense. There are other complications also there, but this is one of the things that leads to death.

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Therefore, again, in a straightforward thinking fashion. To treat cancer, the cancerous cells need to be killed. How do you kill it?

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You use drugs - cancer drugs and such a treatment is called chemotherapy. You could use radiation, such a treatment is called radiotherapy and so on to kill cancer cells. However, these therapies, you know you've taken drugs to kill the cancer cells, it would also kill the other normal cells in the body. And that is what leads to the undesirable side effects such as the hair falling out and various other side effects of chemotherapy and radiotherapy, which is not desirable. So, it becomes helpful to target the killing agents - the drugs that kill the cancer cells, directly to the cancerous cells so that the effect on the normal cells is minimal. To target these drugs to the cancer cells, nowadays something called monoclonal antibodies or MAbs are used, at least for some cancers. For some cancers, it has been very effective. These ideas started decades - probably a decade and a half ago, or more ago and then they have come to fruition probably a few years ago. Now they are effective against some types of cancer.

Monoclonal antibodies are made by cells called hybridomas. Hybridoma, as the name suggests, hybrid - oma, it is a fusion or a mixture of a cell that can produce one type of antibody, and therefore you call them monoclonal antibodies and another type which does not die - which is a cancerous cell. So, if you fuse them together, you have a cell that produces one type of antibodies - monoclonal antibodies, and can live forever - which becomes useful outside the body when you trying to produce these monoclonal antibodies that are used to target the drugs to cancerous cells. The hybridomas themselves were developed in the mid 70's by Kohler and Milstein and it's potential was realized quite soon - as soon as it was developed and monoclonal antibodies became a rage even in the early 80's. Some of my work, doctoral work, was related to monoclonal antibody production in a bioreactor.

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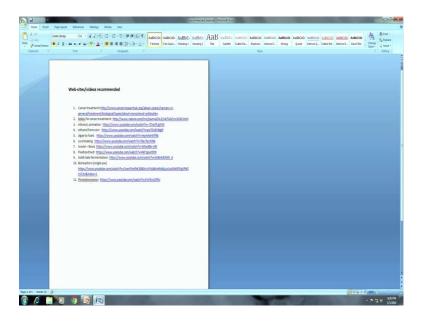
These are some of the monoclonal antibodies that are being used nowadays to treat cancer. Rituximab, Trastuzumab, Bevacizumab, Cetuximab, Panitumumab, Ipilimumab and so on and so forth. If you see here, it ends in MAb which stands for monoclonal antibody and each one is effective against certain kinds of cancers.

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For more information, you could look at this website. I am going to list all these websites in a separate file and put it up on your course page, so that it is easier for you to reach these websites. We will have some of these websites throughout the course, more so in the introductory module, so that you can go and read about them, get in depth of those aspects. Because we will cover whatever is necessary for us in this particular course, but they by themselves could be very interesting to some of you. So, you could go and read about them and dig further about them based on your interest.

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The file is going to be something like this. The URL that I gave you just now, is the first URL - cancer treatment here. This one says websites/ videos recommended. There could be some videos also along with this. This is a website cancer treatment and this was the address that was given here. You could download this file and click on the appropriate link. I guess you need to use a control or something like that before you click here and then you would access the website and see through it. This is a nice website for cancer treatment. I will highlight as we go through the lecture, the various websites.

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There is another website that is given there. I think which is number 2 on that list. We just saw that list. And let us now come back from cancer to our bioreactors course. Now what is the relevance here?

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The relevant question here is that - how are monoclonal antibodies or MAbs made in

large quantities that are needed for therapy? The amounts would be very small if you grow hybridomas in small vessels as they were done initially. But the demand for MAbs is huge now a days.

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So, how do you produce the large quantities of MAb that are needed for therapy? The answer is through something called a bioprocess. Let me leave it there for the time being and we will keep visiting this bioprocess and then I will tell you what that is.

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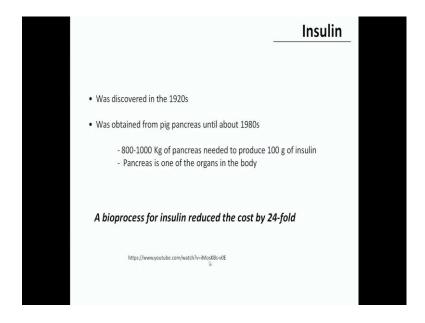
Let us (Refer Time: 10:57) switch gears. The alternative liquid fuels were in great demand or were kind of hot a few years ago. They are still of great interest. They are fuels that would replace the petroleum or diesel that comes from crude oil. The justification was, the need was - the crude oil sources are running out and therefore, we need alternative liquid fuels. It is rather difficult to replace liquid fuels. It is rather difficult to imagine how a plane would fly without a liquid fuel, at least for some time to come, although there are other alternatives available now on an experimental basis. Still, probably we need a lot of liquid fuels. Two examples of liquid fuel are bio-ethanol and bio-diesel. Ethanol, you know C2H5OH, that is found in a lot of these intoxicating drinks also. But in a purer form, if it is mixed with some amount of petrol and or gas -as it is called in some countries, and used in cars, then, that is a very good fuel. But how do you get bio-ethanol? That is, the source of bio-ethanol is corn or other plant sources that ultimately give us the ethanol. How do you go from corn, for example, to ethanol? Because corn contains various other things, it does not contain ethanol. So, how do you contain whatever corn has to the ethanol that is useful as a bio-fuel. That is a long process. It is also through a bioprocess.

Similarly, bio-diesel. Bio-diesel is being used in our country, from plant sources, to run railways and things like that to a reasonable extent. You could also use something called

microalgae to produce bio-diesel. Because microalgae accumulates what is called bio-oil and that bio-oil can be converted, it is somewhat equivalent of the crude oil, not exactly but somewhat equivalent of the crude oil and that bio-oil can be processed to produce bio-diesel. So, if you are using microalgae, which is a higher generation bio-fuel compared to the plant based bio-fuel, bio-diesel, then, you need to use a bioprocess that converts the raw materials. Sometimes, it could just be the carbon dioxide in the air and in the presence of sunlight and so on through photosynthesis to yield bio-oil, which can then be converted to bio-diesel. So this process also is a bioprocess. So, bioprocesses are required for production of certain alternative liquid fuels such as bio-ethanol and bio-diesel. I would like you to look at this animation which gives you details about ethanol making. It gives you, in a nice way, how ethanol is made from corn or some other source here. Similarly, this is ethanol from corn, this is ethanol making in general and animation and then this is algae to fuels.

If you are unable to read it here or use it directly from this presentation, please look at, I think it is 4, 5. Ethanol animation is 3, ethanol from corn is 4 and algae to fuels is number 5 on this list. You could go through these sites, which are commonly available videos and get some information in a nice way, in a non-boring way and that might be useful. Let us get back to our presentation here. So the production of bio-ethanol and bio-diesel are also done through bioprocesses. Another switch here.

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You have all heard of insulin. What is insulin? It is a drug that is used to treat diabetes. A widely used drug that is used to treat diabetes. If you look at what kind of a molecule it is, it is something called a protein. I hope you have some background in biomolecules and so on. So, it is one kind of a biomolecule, amino acid and a polymer of amino acid is a protein. So insulin is a really small protein.

It was discovered in the 1920s, a long time ago and soon people found that you could use it to treat diabetes. And the initial source of insulin was pig pancreas. Probably a few years after this, till about 1980s, they were obtained from the pancreas of pigs. The numbers are quite staggering. You need about 800 to 1000 kg of pancreas to produce 100 grams of insulin, which is a very, very small amount. Compared to the worldwide demand for insulin to treat diabetes, it is a very, very small amount. Not just that, pancreas is only one of the organs of the body. So, you can imagine what needed to be done to get insulin when no other method was known.

However, when a bioprocess for insulin became available, because of the ease of getting insulin, this immediately reduced the cost of insulin by 24-fold and this is also a bioprocess.

This is the next video there on insulin that is given in your list. You can go and look at this, to get some idea of the relevance of insulin, the production of insulin through a bioprocess and so on. Ok!.

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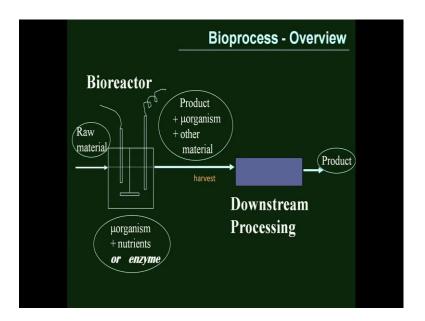
Now let us come closer to home. How do you think curd or yoghurt is made? You take milk, you heat it to certain warmth in a vessel and then you add some old curd to this slightly warmish milk and set it aside. There are also curd makers that maintain this vessel at 37-degree C just by some electrical means. And when this is done in a curd maker, curd forms in about 2 hours or so. If you live it overnight at home, assuming that the outside temperature is reasonable, then curd forms overnight very nicely.

What is happening here? Let me introduce some terms that we will be using frequently in this course. The milk that you initially take - we can call it the medium. Right? The nutrients, a nutrient broth. Using the nutrients in the nutrient broth, the inoculum - the small amount of curd that you added that contains organisms, the organisms utilize the nutrients in the medium and grow. When these organisms grow, these particular type of organisms - they are called *Lactobacillus*, when they grow they produce something called lactic acid. You all know what an acid does. It reduces the pH. And because of the controlled production of acid in an appropriate fashion, the pH gets reduced in an

appropriate way along with other things happening in the milk. And a protein in the milk called casein curdles and that is how we get curd. Appropriately, you know, even the taste is made appropriate in the process because of the way in which it gets done and then you could eat curd.

Very simplistically speaking, curd making is also a bioprocess. You have a vessel, you have a medium/ which contains a medium and then you add curd to it. So you have added inoculum to it. The cells in the inoculum multiply and make more cells, make a product lactic acid and the lactic acid does something to the milk to give you curd. So this, simplistically speaking, is actually a bioprocess. Therefore, curd making is also a bioprocess. So, let us look at what actually a bioprocess is.

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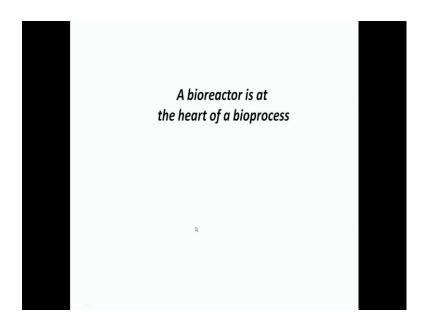


This is an overview of the bioprocess. The bioprocess has two major aspects. One is the bioreactor aspect - this is also called the upstream aspect and a huge aspect here called the downstream processing aspect. The bioreactor is represented here by this, we will see this in detail later. The raw material, for example it could be the milk in the case of curd, this enters the bioreactor. The bioreactor contains the microorganism of interest along with the nutrients as we saw in the case of curd making, the nutrients that are getting converted to curd as well as the lactobacillus that converts it into curd. Or, it could even

have just an enzyme which does some enzymatic conversion .So, after this process is done - there are various ways in which the process is done, we will look at the details later.

After the process is done, what comes out of the bioreactor, what is harvested from the bioreactor contains the product of interest, the microorganism and probably other material. We all are interested only in the product. Therefore, we need to separate and purify the product from these other things that are there and that is what is done through what is called downstream processing. There could be very many steps in downstream processing. In fact, the number of steps in downstream processing could constitute about 95 percent of the steps in a bioprocess. So large number of steps in downstream processing. And finally, at the end of it all, we have the final purified product. So, this is a bioprocess. This course will focus on the upstream side or the bioreactor side. Downstream processing of course is very important. But the focus in this particular course, because we need to focus on something to get something useful out of whatever we are doing and we are going to focus on the bioreactor aspect or the upstream aspect. Typically, the bioreactor aspect and that is why this mooc is called a Bioreactors.

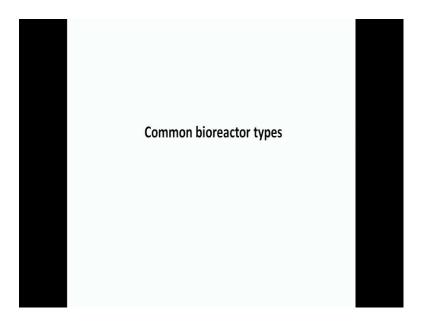
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As was clear from the previous slide, the bioreactor is at the heart of a bioprocess that is

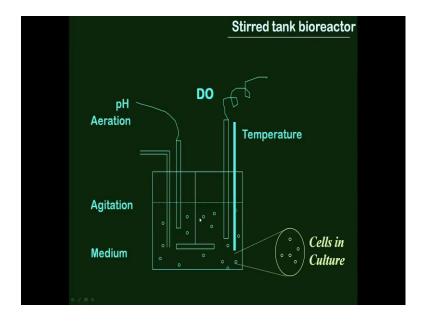
necessary for a bioprocess to exist.

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Now let us look at some of the common bioreactor types. Some of the kinds of bioreactors that are used commonly.

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The first one that we are going to look at, is called the stirred tank bioreactor. In fact, this is pretty much the work horse of the bio industry. Many industries use this stirred tank bioreactor. As the name suggests, it is a tank, which is represented by this rectangle here. It is actually cylindrical in reality. It could be as small as something that sits on this table maybe a 1- 2 liter reactor or maybe something that sits on a lab bench, may be about 15 liter reactor, to something that occupies huge spaces in the industry - maybe 1 Lakh liters, few Lakh liters and so on so forth that are required in some industries. Stirred tank, all these are effectively the same except their sizes are so different. All these stirred tank reactors will have a stirrer which stirs the contents of the bioreactor and that is why it is called a stirred tank bioreactor.

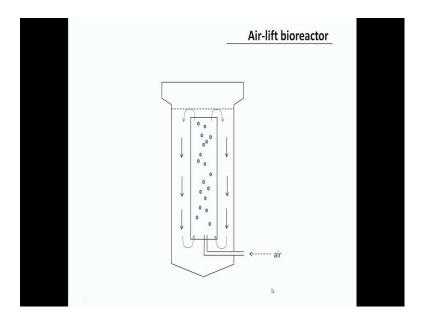
It is, any bioreactor for that matter, is an instrumented, there are various instruments here and highly controlled kind of an environment here. The typical instruments that are used are those that are needed to measure temperature, there is a thermometer or an RTD device that measures the temperature of the bioreactor contents. There is something called DO. This is not do. This is DO which stands for Dissolved Oxygen, which happens to be an important parameter. Especially, when we use certain kinds of microorganisms in the bioreactor. pH is an important parameter that is measured controlled. Many bioprocesses or bioreactions take place in a narrow range of pH. The aeration happens here, air is used here. Agitation of course we talked about and the medium we already talked about.

What I would like you to bear in mind is that the stirred tank is a vessel - that is a bioreactor. Whereas the actual reactors, the bioreactors or the factories that are producing the products are - the cells in the bioreactor that make these products. It could be the ethanol that we talked about, the lactic acid that we talked about, the MAbs that we talked about, the insulin that we talked about or the bio-oil that we talked about. All these are made by these microscopic cells. The size of these cells are anywhere between, let's say, 2 microns to 10 microns typically. They could be larger too. And therefore, these cells in so called culture are the actual factories that produce the product.

We need to keep this in mind whenever we look at bioreactors. In the initial part of the course, we will look at the bioreactor as this vessel and do things necessary for this

vessel and this will be in the background. And later, towards the later part of the course, we will focus some attention on how to operate or come up with strategies based on how these cells actually function. That is what makes more sense because, these cells are the actual factories that produce products. And therefore, we need to have an idea of as to how they are doing for the bioreactor to be effective. Looking at it as some sort of a black box may not be very helpful in many situations. So, that is a stirred tank bioreactor.

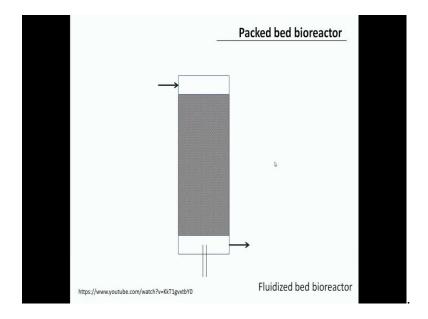
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The second type, second common type that is used is what is called an air-lift bioreactor. This schematic shows some important features of an air-lift bioreactor. The main difference that you would immediately notice between a stirred tank bioreactor and an air-lift bioreactor is that there is no stirrer here. However, you need to keep the bioreactor broth in agitation. Only then will the bioreactor function properly. The agitation - the mixed nature, is brought about by air which is pumped in here somewhere. The air rises through this, it takes in, this is a cylinder inside the reactor, the air rises through this and when it rises through this it carries the cells along with it and then it kind of circulates. This is a cylinder. I hope you are able to visualize this. This is one cylinder. This is the outer cylinder and therefore, air rises through the center comes like this - quite vigorously and because of that, it is able to keep the cells in suspension and thereby the reactor operates. This is the liquid level that is shown as the dotted line here.

So, this is an air lift bioreactor. This has some advantages over the stirred tank bioreactors and some disadvantages. We would probably look at some of them in the passing, after we know something more about bioreactors.

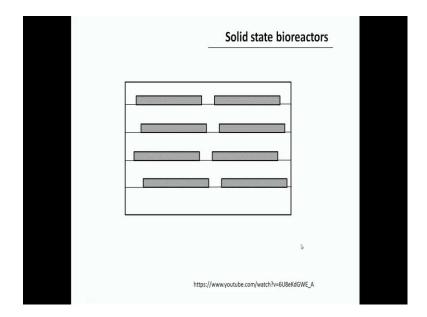
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The third kind, that I would like to present to you, is something called a packed bed bioreactor. You have a bed here which is packed. There is an inlet here, there is an outlet here. It could be continuous. Typically these packed bed bioreactors are operated in a continuous way. And this is where the bioreactions take place to convert the reactant into the desired product. This is a video that you may want to watch. I think it is, let me go back to - the packed bed, fluidized bed (Refer Time: 30:13). That is the fluidized bed - I will come to that next. The Packed bed is just a stationary bed, which contains these organisms which convert the reactants to the products. The fluidized bed, which is a variant of the packed bed, would have this part fluidized. Fluidized in the sense fluid like so that it will be, the bed would not be stationary. It will be jiggling around, jumping around and so on so forth. That brings in certain desired aspects in the operation of bioreactors and fluidized bed bioreactor are used in some situations.

A packed bed bioreactor is used commonly in water treatment and so on so forth. And this could just be a sand bed through which the water to be treated flows and what flows out of here is cleaner water.

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Another type of bioreactor is called the solid-state bioreactors. As the name indicates, there is typically no liquid here. It is all solid. And this can be used for producing useful substances – say, enzymes, so on and so forth, from natural substances which are kind of solids. They could be cut down, chopped down, so on and so forth and then placed in these trays in probably a large enclosure and appropriate conditions maintained here and the organisms added to the appropriate substrate as a solid. The organisms convert the substrate into the desired product. So, such a reactor is called a solid state bioreactor. They are used in the industry, they were heavily used in the industry at one point in time to produce very useful products such as enzyme. They could still be used.

This is a video that would talk something about these solid-state bioreactors. You might want to watch that.

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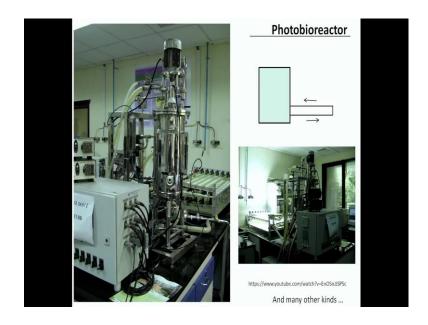


What has become popular nowadays, in some industrial applications and also in some lab applications, more so in industrial applications, is the use of single use bioreactors. Use it, throw out. This has significant advantages in the process of validation. Because a process of validation is rather stringent and people need to establish various different aspects in reproducible fashion, when approvals are given for the production and approvals are given after inspections and so on so forth. Such operations become significantly simplified if you can use and throw the bioreactor. And therefore, the single use bioreactors are quite popular. The same stirred tank could be made of a disposable plastic vessel and that becomes a disposable reactor. Everything else remains the same. Even the sizes could be reasonably large if you use disposable plastic vessels. Therefore, instead of the stainless steel or appropriate material that is used for bioreactor construction which is a permanent there, you could use disposable plastic vessels.

There are advantages as we talked about earlier, but there are disadvantages too. There are some disadvantages that this video lists. You can go and take a look at that. And more recently or I think about the same time, bioreactors called wave bioreactors came into being. This is also given, I think in the same video. You can take a look at what wave bioreactors are. They are nothing but plastic bags. They could be large plastic bags with huge capacity of 25 liters or so, which can be used for the production of low volume high

value products. So, the disposable aspect helps in the validation, maintenance and so on so forth and they work. They are essentially plastic bags that are kept on rockers as the video would demonstrate and the production happens in such a condition.

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The next kind of bioreactor that I would like to talk about is what is called a photobioreactor. A photobioreactor means that it employs light. When do we need light? - whenever an organism needs light. And something that requires light is called a photosynthetic organisms, when you use them, you need to use something called a photobioreactor. Remember we talked about microalgae for the production of bio-diesel? Microalgae happen to be photosynthetic organisms. They convert the carbon dioxide in the air to bio-fuel and that is why, it is also considered a carbon neutral kind of a situation. That is, it does not take any carbon from the earth. It just takes the carbon from the air, converts it into bio-fuel and even if it gets back into air, it is still a neutral kind of a situation, there is no addition of carbon dioxide from the earth into the atmosphere.

So, when a microalgae is grown to produce bio-oil, you use something called a photobioreactor. The process is rather straightforward and simple. Everything is known about the process. This is the vessel here which contains the broth and this vessel has an

outlet which leads to this photo section. I think the next picture would show the photo section a little better. You know this is the photo section lighted up. This is from this angle and the second photograph is from this angle. I hope you are able to understand that. It is the same reactor. This is taken from our lab. We use a photobioreactor. We did some work on microalgae. So this vessel, there is an outlet which goes into the photo section this (Refer Time: 37:14). The organisms receive light here when they are in the photo section which are made up of glass tubes and then they return to the vessel and that happens continuously. So, this is a photobioreactor. Coming back to the process of biodiesel or bio-oil production using microalgae, the process is rather simple. You grow microalgae. The microalgae convert the carbon dioxide in the air and in that process they accumulate lipids or bio-oil. The lipids can be gotten out of the microalgae after production by just pressing them using a press. The oil flows out and then you can use the oil for whatever you need.

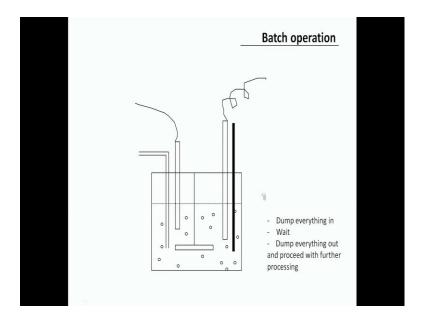
The process is very simple. A lot is known about the process, but the economic production of bio-oil from this route is still not happening. And that is the reason why this process is still not available commercially anywhere in the world. Except on an experimental basis may be, but it is not really commercially viable. It has lot of promise, a lot of challenges - some of which we will talk about in this course. If those challenges are overcome, then it could be an economic reality and this could be a very good alternative source for bio-oil and bio-diesel and many other products. Even jet fuel can be made from this process. This is the schematic of the vessel here and the photo section here. This way you might be able to understand things a little better here. This contains the microalgae. They are let out here. There is a pump that takes it through the glass tubes in the photo section. They are exposed to light as is shown here and they come back and they photo synthesize to accumulate bio-oil. This is a video that you could watch from that list that is taken there. I think it is number 11 here, before the bioreactor. That would give you an idea as to how it is possible to produce bio-oil from microalgae, through a bioprocess that involves a bioreactor. And there are very many kinds of bioreactors. Its not possible to go through all of them. These are the common types that are used for producing useful products through the biological route and you have seen some of them.

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Next, let us look at the bioreactor operation modes. Many of those reactors - stirred tank, air-lift and the other kinds of reactors that we saw, the packed bed, fluidized bed and so on. They can be operated in one of three modes, three main modes.

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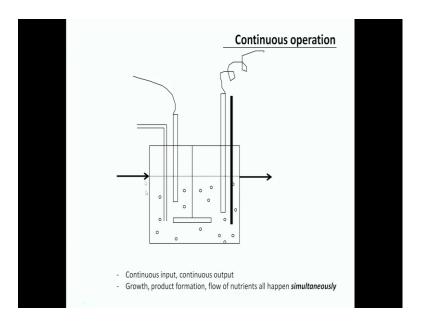


The first one is a batch operation. This is the same stirred tank that we considered earlier

and this is a stirrer here, which stirs and keeps the cells in suspension. When the stirred tank is operated in a batch mode, what is done is, we dump everything in that is - the medium, the cells, the inoculum and so on so forth. And then the reaction takes place. Therefore, we wait for the bio products to be made and at the end of the time we dump everything out and proceed with further processing, the downstream processing steps to separate and purify the product from the other things.

So, this kind of an operation where you dump everything in, wait for the process to complete and dump everything out, that kind of a process is called a batch process or a batch operation of a bioreactor. And many processes in the industry are batch. It is very simple, comparatively speaking, to operate batch bioreactors compared to other types of bioreactors, other kinds of operation.

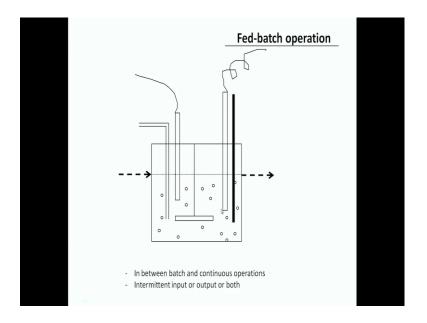
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The next one is a continuous operation of a bioreactor. The same stirred tank here, you could have a continuous input of the substrate as it is called, the nutrient broth as it is called. This need not even contain any cells. The cells could be here and there is a continuous output from the bioreactor with the product made. The times are adjusted so that the product is made appropriately and such an operation is called continuous operation.

What is important to note here is that during this process, there is growth of cells, the product formation and the flow of nutrients - all happen simultaneously. That is important to internalize. In a continuous operation, all these happen simultaneously and that internalization is required to appropriately look at it, analyse it and then use it. And probably design something of interest to each one of you separately.

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Between these two, the batch and the continuous you have something called a fed-batch operation. It is in between the two and you could guess what that is. There could be intermittent input, there could be intermittent output and or both. You could have some input and some output. What I mean by that - you start with probably a batch and then for some reason, well known reasons, you add some substrate maybe, in intervals. Not continuously, in intervals. That is a fed-batch. You may not even remove anything. Or you could remove some part of the broth, the reacting broth, intermittently. That could happen. Or you could do both - you could add intermittently and remove intermittently and any of these kind of operations is called a fed-batch operation. And there are various reasons. For example, some products that are made by the cells could be toxic to the cells themselves. Simple thing is ethanol, that is made so much in so many industries that produce ethanol. And ethanol above some 10, some 16 percent or 18 percent is actually toxic to the cells. So it cannot be allowed to build up here. There are other products that

could be toxic at much lower concentrations and to handle the toxicity, a part of the broth is removed and fresh broth is added so that the concentration of the toxic product remains in check.

Now, those were the types of bioreactors that we saw, the common types and the modes of operation of those types of bioreactors. Some bioreactors lend themselves to all kinds of operation modes or all the three operation modes - batch, continuous and fed-batch. Whereas maybe a packed bed bioreactor or a fluidized bed bioreactor, it may not be very easy to operate them on maybe a batch basis. A continuous basis would be easier to operate. Typically, a continuous operation is a lot more difficult. Many times more effort are required to run a continuous operation, continuous batch, continuous bioreactor compared to the batch operation.

Now let us look at some other aspect. We are using organisms, micro organisms, to produce the product of our interest. Therefore, only the organisms of interest need to be present in that space that produces the product. However, organisms are present everywhere. In this room around us, in the space where this bioreactor is used, in hospital rooms and so on and so forth, there are organisms present in the air in reasonably large amounts and that is not good. For example, you don't want infectious organism that is present in the air to get into your body. The body has certain defense mechanisms that minimize the effects, but still, it could lead to complications. That is why many of us get these infectious diseases when the body's defense mechanism is not able to cope. More critically when a person is operated upon, when a surgery is done, the person's internals are exposed and there is no skin or the skin is compromised for a certain purpose - for the purpose of the surgery.

When the skin is compromised, the organisms that are present in the air, if they are present, can enter and cause infection and that should not happen and therefore, the surroundings of the surgery must be kept clean of organisms. Similarly, the surroundings of a bioreactor or the bioreactor itself must be clean with no organisms. And then, the organism of interest alone is added to the bioreactor and that is made to multiply and produce a product of interest. Therefore, there is a huge need for a clean slate.

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You might be surprised. You have organisms to the concentration of about 10 power 3 cells or 10 power 4 cells per ml in the air around us. There could be many types of organisms and you don't want them to be present either in your body or in the bioreactor which is designed to produce a particular type of product.

What happens is, if the other organisms come, maybe those organisms can grow much faster than the organism of interest and that will compete with the organism for the food that is available and the bioreactor will be a failure. That is called contamination of the bioreactor. You don't want that. So, to avoid contamination we need a clean slate. How do we achieve this clean slate is rather interesting. Let us discuss this and we will close the introduction lecture. We have maybe about 9 minutes left. We'll close the introductory lecture with this discussion. It is very interesting. How do you sterilize a hospital room? How do you remove all the organisms from a bioreactor? Organisms can be present anywhere (Refer Time: 48:50), any nook and corner of the bioreactor. There are 3 major ways in which this is done. One is, we use very high temperatures, maybe a 120, 121 degree C for about 15 minutes under wet conditions, typically used. A high temperature can be used and that achieves a clean slate. This has been shown repeatedly. It works very well.

You could use chemicals - liquids or vapors and that is what is typically done to sterilize spaces, such as the operation rooms or labs. For example, labs when we get contamination, then we want to remove all the organisms that are contaminating our work related organisms. And therefore, what we do is, we use formalin solution. Add potassium permanganate to it to cause an exothermic reaction and the formaldehyde actually, formaldehyde vapors that come out of it, kills all the cells in that space. It is rather dangerous and that is why when a lab is sterilized, there are notices put up everywhere. The doors are sealed with sealing tape. The windows are sealed completely and the person who places these aluminum boats with this formalin solution at strategic places in the lab and works out a way by which a person can get out and seal the last door. The other doors are already sealed, the other windows are already sealed. And the person keeps dropping these potassium permanganate pellets into these aluminum boats as the person walks out quickly, comes out of the lab, seals the door and the vapors kill all the organisms. No vapor is allowed to escape outside the lab and the lab is kept sealed for a couple of days. And then you enter, you can actually smell the cleanliness or the lack of organisms in the lab along with the chemical smell and so on so forth.

So, this is a very common procedure for using chemicals, in this case it was formalin. You could use 70 percent ethanol to kill the organisms on your hands, on your gloves and so on so forth, while working with organisms, while transferring organisms from one part to another in a lab. Also, you could use radiation such as UV radiation, gamma radiation, which are high energy radiations that can cause changes in the DNA and so on so forth of the organisms and kill the organisms. When you cannot use high temperature, for example, when working with temperature sensitive plastics maybe, syringes and so on so forth, you cannot use this high temperature process. The high temperature process is called autoclaving and that is used for substances made of metal such as bioreactors made of metal. If it is a small bioreactor, you can place the whole bioreactor inside what is called an autoclave, that helps reach the conditions that we mentioned earlier - 121 degree C, slightly elevated pressure, steam. Because steam exists under only those conditions, if you remember the thermodynamics. And if those conditions are maintained for about 15 minutes to half an hour, then you could get a sterile space, then you take that space and make sure that the sterility is maintained and then at the right time, you add the organisms of interest. That is how the autoclaving is done.

If the bioreactors are large, we talked of bioreactors that have capacities of may be lakh few lakh liters, then in situ sterilization is done. You use similar kind of mechanisms - high temperature caused in place because, you cannot move those reactors very easily and a clean slate is achieved. Radiation such as UV and gamma rises we mentioned are used to treat or used to kill the organisms in places where we probably cannot use high temperature to kill or chemicals to kill. So these are some of the common sterilization methods, that are used to achieve the clean slate for a bioprocess to begin. I think we will stop here for this lecture. I think we are almost out of time. I briefly introduced you to this mooc, it is a 10-hour mooc on bioreactors. I mentioned a couple of books that you could look at, but only very loosely, this organization is very different. However, I will also give you the corresponding chapters as we go through the various material. Some of the material may not be there, but whatever is there I will give you the corresponding chapters in the books so that you can go on read the book.

At the end of every week, we will have some assignment to submit, mostly through multiple choice questions. However, it will be good for you to develop the skills of problem solving, at least closed ended problem solving, where you know everything. You will have to analyze, apply and get a useful result and for that I will probably pose the problem in one class, give you enough time to solve it after the lecture and then show you the solution in the next class. Let us meet again in the next class.