Applied Environmental Microbiology

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Lecture – 10
Microbial Energetics IV

Hello students, today in this class we are going to cover the part of microbial energetics that has to deal with glycolysis, which is consumption of glucose. In the previous lecture I talked about how delta G drives any microbial reaction and decides whether a microbial Redox reaction is feasible for microbe or not.

Whether microbe should invest energy in or not, we also talked about certain energy rich molecules within cells such as ADP, ATP, NADH, NAD plus which help, but which help catalyze and which help catalyze these Redox reactions and ensure that the energy produced from this Redox reaction is essentially transferred to the sulphur its essential functions such as cell replication and sustenance.

In this lecture we are going to come back to one particular important part of the previous lecture which is that glucose is the basic substrate of most chemoorganotrophs. So, for most microbes that use organic material as an electron donor, need to break it down into glucose which is a very simple sugar six carbon sugar. And then the glucose can either be fermented or respired; now you must know this from your high school biology that even in human body, we have two forms or respiration one is aerobic and one is anaerobic which happens under extreme conditions.

So, for example, a sprinter is a classical example of an athlete that uses anaerobic respiration to drive their motion. So, when the air is rich with oxygen and there is plenty of oxygen in the blood; most of our respiration and Glycolysis will be governed by aerobic degradation of glucose. But when the air becomes scarce which happens when the body is active very fast or the oxygen in the air is less.

And in case of the sprinter the sprinters body is moving a much faster rate than the oxygen in the blood can cope with; then the body of the athletes which is to an aerobic respiration and lactic acid is produced as a byproduct; which for long time was considered to be the cause for drums which is derived onset muscle soreness. And now

we know that it is not the case, but we still know that after anaerobic respiration our muscles are rich in lactic acid, which give it an uneasy feeling.

So, today we are going to understand what this aerobic respiration and anaerobic fermentation are about, what the pathways are about? Now these pathways are very important to understand because each of these pathways even though in the way we will cover in this lecture is just three steps pathways, but they are in fact, much more complicated.

The web of Glycolysis is so intense and so, diverse also that if we were to draw this on the board behind me, it would be an impossible activity. Definitely impossible to make it legible enough for you to see and I must mention this here there for many decades scientists have been working very hard to uncover the Glycolysis pathways and various enzymes that participate in it.

So, let us start today with exploring fermentation versus respiration.

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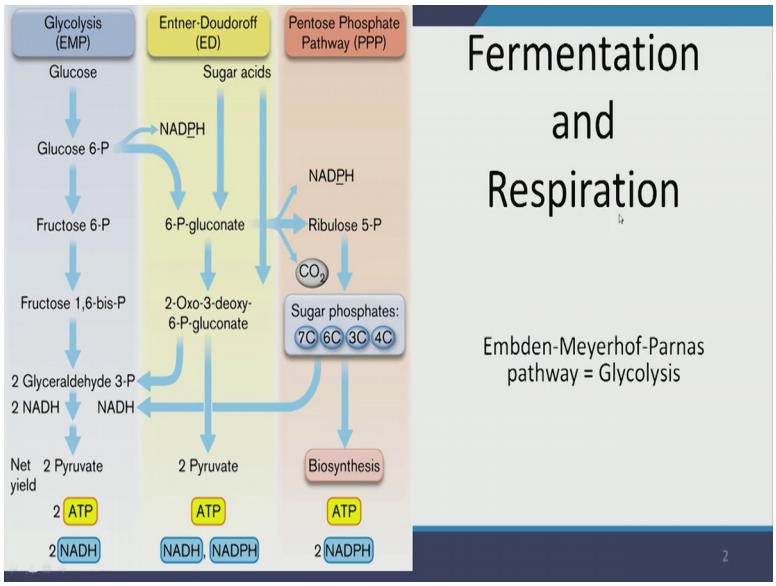

Now, both are methods of Glycolysis which is consumption of glucose; however, fermentation happens under anaerobic condition and respiration under aerobic condition.

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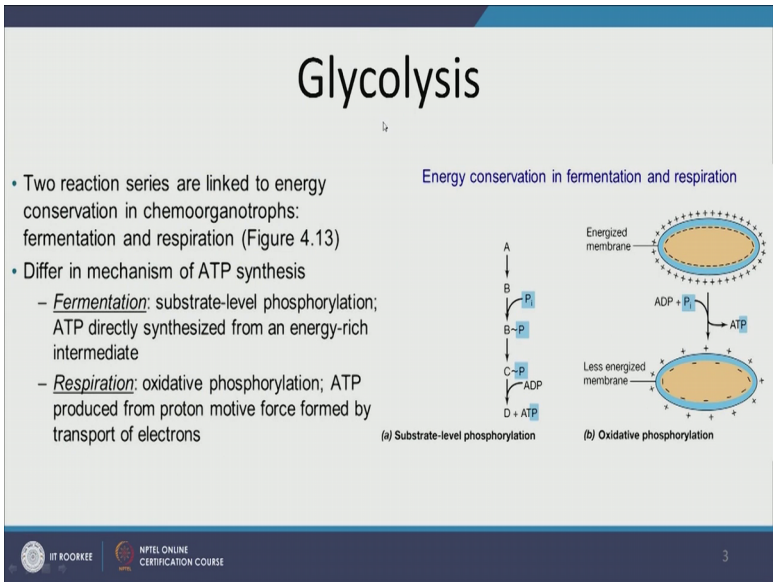

Now, in Glycolysis the cell wants to conserve energy whether it is fermentation or respiration. So, to conserve energy it uses different mechanisms so, producing ATP. In fermentation, it will do what is referred to a substrate level phosphorylation; it will directly phosphorylate the substrate which is glucose. And thus avoid any intermediary and directly get energy from the electron donor which in this case is glucose.

In case of respiration because the oxygen is present; this is oxidative phosphorylation. So, the cell actually uses its proton motive force and this is something we taught you in one of the previous lectures; how naturally the cell membrane behaves likes a battery which carries energy, which stores energy for the cell; the positive charge around it and negative charge inside it. So, in oxidative phosphorylation which happens in aerobic respiration; microbes use this charge to make ATP. So, then they have made ATP charge the voltage around the cellular membrane reduces.

So, in case of Glycolysis there are usually three steps to Glycolysis; in the first step phosphorylation happens by ATP.

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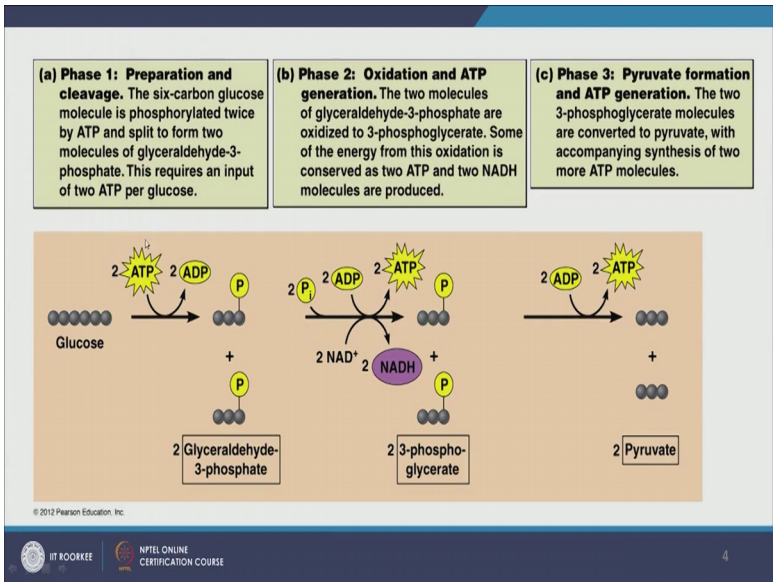

Then it consumes 2 ATP molecules and thus the glucose is broken down into two glyceraldehyde three phosphate molecules. Now these two molecules of glyceraldehyde three phosphate are again process in the second step where ATP is produced. So, in this first step; 2 ATP were produced and 2 ADP were consumed.

So, in this the in the step microbes are actually investing their current stock of ATP into glucose. And in the second stage they oxidize this and they create ATP; so the Glyceraldehyde three phosphate is oxidized into phosphoglycerate and 2 ATP and 2 NADH are produced. And the third step; Pyruvate is formed, so the 2, 3 phosphoglycerate molecules are converted into Pyruvate and 2 ATP are produced and overall microbes produce more ATP than they had spent.

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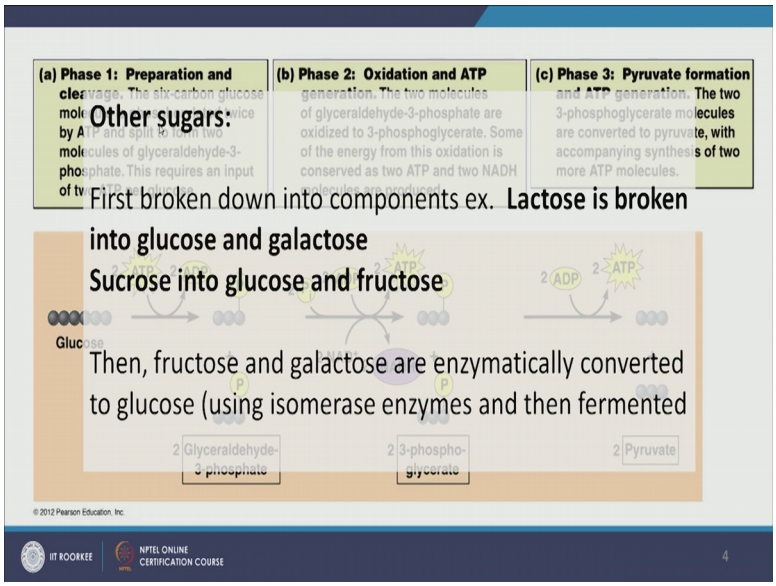

Now, in case of other sugars such as lactose and sucrose; they are first broken down into simpler sugars such as glucose and galactose and sucrose is broken down into glucose and fructose. The glucose will pass through the same three phases that we talked about; first as consumption of ATP and creation of glyceraldehyde phosphate, second step would be oxidation of glyceril oxidation of glyceraldehyde phosphate and generation of ATP and third phase is Pyruvate formation an ATP generation for galactose and fructose the cells would let them go waste.

So, what cell does is it enzyme ethically converts them into glucose using isomerase enzyme and then ferments them.

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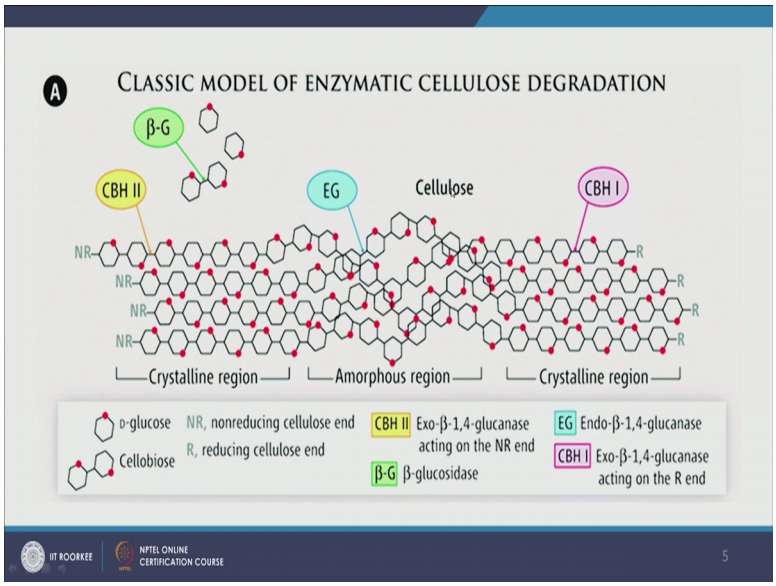

Now, I am here showing you an example of a very complicated organic molecule; a polymer rather. So, we here we were talking about lactose and sucrose which are sugars, but not as simple as glucose. Here we have a polymer of many glucose or cellobiose change; so, cellobiose they look like two glucose molecules that are connected to each other with data 1, 4 glycosidic bond.

So, now this is a very complex molecule of cellulose and you should know that in cellulose because it is a very long polymer and it is perhaps the most abundant naturally formed organic polymer on earth's surface. So, the cellulose in itself in its basic composition has some regions that are crystalline. For example, here it is flanked by crystalline regions on the left side and the right side. And in middle we have amorphous region; so, amorphous region is not crystalline and as you can notice here pretty clearly; now that it two broad categories of enzymes that attack cellulose.

So, why do they need to attack? Because first of all we need to break down this complex molecule through cellulose into simpler glucose structures; such as the ones represented here that can be either fermented or aerobic re respired by the microorganisms. So, microorganisms they produce a suite of hundreds and hundreds of enzymes that act in synergy to degrade the cellulose.

So, notice here some of the cellulose are at free end; so, these are non reducing cellulose and some of them are connected further they are reducing end.

Most of the cello bios and glucose molecules are in middle; now we need different kinds of enzymes to attack different kinds of molecules fact in this structure. So, for the ones that are at the corner we use exo glu kinase; so, these are exo cellulase that attack cellulose monomers from the end. Then we have end of beta 1, 4 glue kinase which attack cellulose cellobiose monomers which are in the middle and mostly in the amorphous region.

So, here CBH II which is actually carbohydrate binding molecule they are shown here attacking it from the one of the ends. And on the non reducing in that too whereas, CBH I is attacking at it the reduced end. And the endo glue kinase is attacking it from the middle; now when these molecules act when these enzymes break down the cellulose now remember enzymes do not undergo transformation themselves as they carry on the reaction. And that is we need very little amount of these three the types of enzymes to break down cellulose into cellobiose monomers.

Now, look here this is a cellobiose monomer, now this is a sparing of cellobiose by the way. Now there are attached to each other of a beta 1, 4 glycosidic bond. Now this bond is a very strong bond and this is a very stable compound definitely does not want to degrade. In fact, I want to mention here that cellulose is also one of the most stable sequestered form of carbon and beta 1, 4 glycosidic bonds of its cellobiose may give it immense stability.

So, this table bound requires us again a suit of complex enzymes that work together to break it down. And one of the rate limit this is actually the rate limiting step of cellulose degradation. So, for this for breaking down this rate limiting very strong bond between these are two glucose monomers which form together cellobiose, there is a special suit of enzymes and then these enzymes they break down this beta 1, 4 glycosidic bond.

Now, this is very important; this bond is specific to cellulose and different kinds of celluloses. And thus whenever we can find this enzyme we know that cellulose degradation is happening and we will see this later in our future lectures how we use these very specific enzymes as biomarkers for telling us; how much of cellulose degradation is happening? And what kind of cellulose degradation is happening?

So, when these big cellulose polymers have been broken down into cellular bios and then using beta glucoside is enzymes; they have been broken down into glucose monomers

then they can either go fermentation and go into the fermentation pathway or they can go into the aerobic respiration pathway. Now because I give you the example of cellulose here I would like to take a moment to explain to you that the cellulose degradation in itself is a very very complicated procedure phenomena, which involves hundreds of different kinds of enzymes and they are usually very sensitive to the environmental condition.

So, for example I have cellulose degradation happening inside a rumen of a cow; now you know cow eats cellulose rich food and it has the ability to degrade it; unlike human beings we do not have the ability to degrade cellulose in our intestines, but cows do. So, in the rumen they have cellulose degradation going on and the enzymes they use; the hundreds of enzymes that come together for cellulose to degrade in rumen of very different and distinct from the enzymes that a wetland utilizes for degrading cellulose.

And a simple question might be why is this cellulose degradation or any such degradation of organic compounds; so important for us to study; for our studies to happen? And I would like to mention that this is a hint for you to understand how organic contaminants degrade in environment whether I am talking about PHS; which are Poly Aromatic Hydrocarbons or general hydrocarbons; they all use a suite of enzymes to degrade them; break them down into simpler sugars or simpler compounds that can eventually form glucose or other sugars that can be fermented or respired by microorganisms.

One of the chief and highly promoted mechanisms for attenuating contaminants in environment is bioremediation and natural attenuation and both require microorganisms. So, it is very important for you to get an idea of what it takes to degrade a major contaminant. Now some of the contaminants; now that we are talking about contaminants some of the contaminants serve as electron donors and carbon source. However, interestingly there are also contaminants at serviced electron acceptors and donors of carbon. In some cases, there will be only will electron acceptors and you need to get rid of them.

Now, this is also a good opportunity for me to explain that most of these mechanisms as I mentioned just a while ago are very sensitive to the environmental condition. So, if we change environmental condition; the mechanism of cellulose degradation would change.

The mechanism of glucose utilization would change, now a simple question you might ask well what is there to change in glucose degradation.

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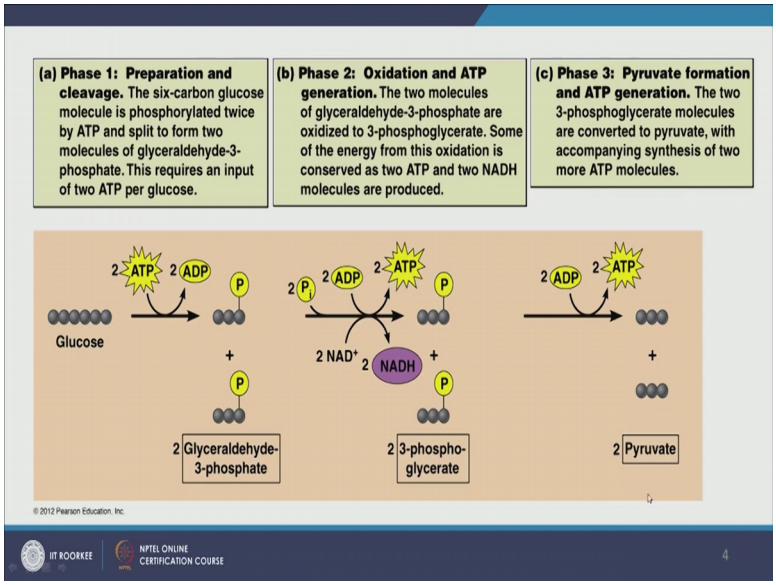

Its fermentative pathway is so, well laid out; by the way after pyruvate, Pyruvate is very easy to degrade some microbes either further reduce it oxidize it; reduce it into methane oxidize it into carbon dioxide and produce more energy. And there is a very nice, very beautiful cycle that happens for Pyruvate degradation, but I do not think you need to know this for this particular course.

So, now simple question that arises here is that everything about degradation of glucose is known. So, we already know the biochemistry of life, we already know how things are broken down. How they are consumed by microbes? But the interesting part is that is not correct. Almost every year scientists across the world are surprised by simple diversity in microbial degradation of these organic compounds; not only are we finding new enzymes, but also new pathways.

So, every year we add on more information into the metabolism of microbes; to summarize there are two forms of respiration aerobic and anaerobic.

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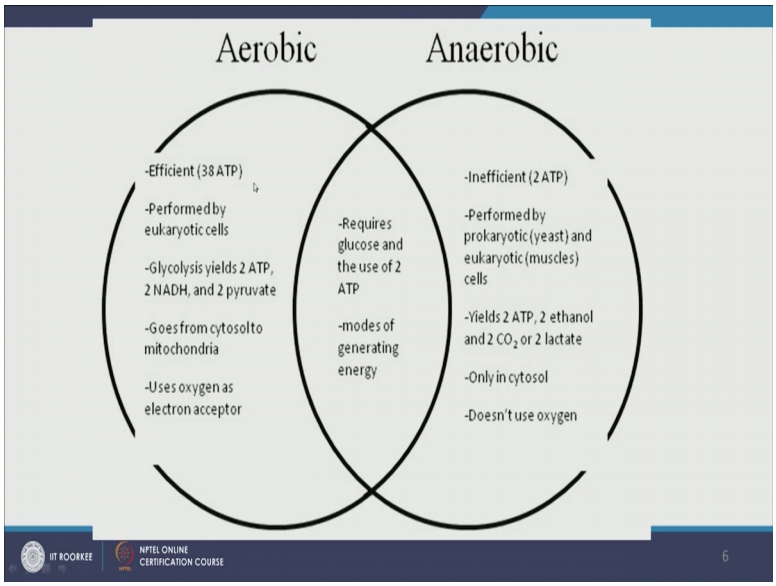

Aerobic is more efficient it produces 38 ATP for every glucose molecule that it respires. Usually performed by eukaryotic cell it actually is also performed by many prokaryotic cells; so, do not worry about that. The Glycolysis itself will lead to ATP to NADH and to pyruvate; the Pyruvate can be broken down further. In case of eukaryotic cell, it goes from cytosol to mitochondria and they use; obviously, oxygen as the electron acceptor because oxygen is available.

Now, in an aerobic environment; the anaerobic degradation of glucose is relatively inefficient. And its performed by many prokaryotic organisms and many eukaryotic mussels. As I mentioned earlier that even human body will undergo anaerobic respiration under (Refer Time: 15:29) it is less than amount of ATP some ethanol, some lactate or carbon dioxide.

It does not happen in mitochondria in case of eukaryotic cell; happens only in cytosol, it is not use oxygen; obviously, both of them require glucose both of them initially use two ATP's and both of them generate energy and are sufficient for life to move on and sustain.

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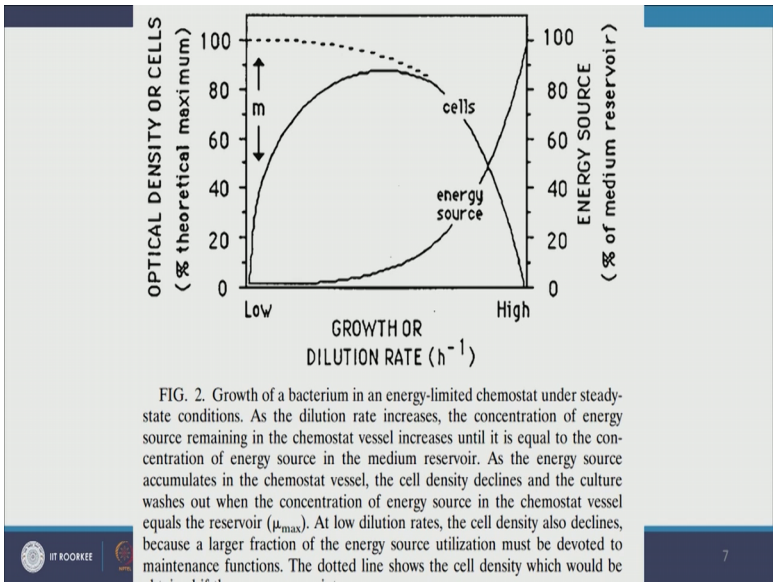

FIG. 2. Growth of a bacterium in an energy-limited chemostat under steady-state conditions. As the dilution rate increases, the concentration of energy source remaining in the chemostat vessel increases until it is equal to the concentration of energy source in the medium reservoir. As the energy source accumulates in the chemostat vessel, the cell density declines and the culture washes out when the concentration of energy source in the chemostat vessel equals the reservoir (μ_{max}). At low dilution rates, the cell density also declines, because a larger fraction of the energy source utilization must be devoted to maintenance functions. The dotted line shows the cell density which would be

Now, before I go any further; I would like to take few moments to explain to you what a chemostat is and how it works and how we can use a chemostat to optimize cell yield to make best use of whatever microbial energetics we understand. In previous lectures, I mentioned to you about batch reactors in which we bring our microbes into a particular medium that has enough food for them, enough nutrition for them to grow to there through the lock phase, through the exponential phase, their stationary phase and their death phase.

Now, the reason why they die is because they are not of nutrients and also they produce dot of products that are likely to be toxic to the microbial community. And as such scientists few decades ago came up with the idea of chemostat. So, chemostat is basically a set up where there is a continual input of nutrients and an continual output of my biomass, consume products and waste material; so a chemostat might look like this.

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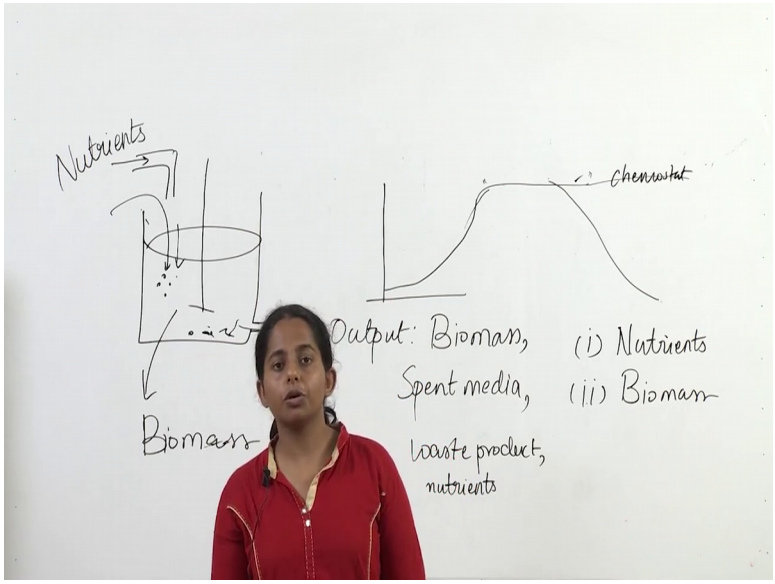

So, in chemostat and I have made the one in which we have the facility to continually stir the media. This is the media and at the beginning again like we did in our simple broth; we inoculated with microbes. So, we add microbes here and microbes find plenty of good food here in this not consuming it.

So, again as usual they go through their lag phase and then hit an exponential increase phase; exponential phase. So, in the exponential growth phase; they use these nutrients and up till here it is very much like what we started earlier, but now we have changed something; we have an continual inflow of nutrients.

So, microbes really never run out of nutrients and because they never run out of nutrients their exponential phase is pretty good; and once they reach their stationary phase, they sustain this stationary phase for very long. Now this is very important once we discovered how to sustain the stationary phase for very long and this might go very long. I give an example for my dissertation; I maintain this stationary phase for cellulose degradation for more than 200 days. Something which can only be maintained for up to a month maximum and I did it for nearly two thirds of a year, which is pretty neat doing it for 8 months.

So, in simple growth culture it might have distorted decaying from here, but in case of chemostat; it will continue in stationary phase for much longer. Because then they never run out of nutrients; now another thing that happens is for example, in case of cellulose

degradation or integration of other organic compounds. Often the daughter products that are formed; so, remember in cell replication we talked about parent cell and daughter cells similarly in case of organic degradation we talked about the parent compound or the original contaminant and then it degrades into daughter compounds.

Now, some of the daughter compounds have a feedback mechanism in which they inhibit the degradation of the parent compounds and in case of cellular cellular degradation that definitely is the case. So, the waste materials that they produce or the cell debris that they produce might inhibit the reaction and push it towards cell decay. So, not only are nutrients being continually added, but also there is a continuous outflow of the mixed media here which definitely includes some of the biomass, some of the spent media, some of the waste products and nutrients.

Now, if we change our nutrients the inflow of nutrients; we can change how the cell yield would be. How much the microbes can be produce? If we change our output and input, we can also change; we can also determine how much microbes will sustain in this single long run or in the stationary phase. Therefore, chemostat gives us the ability to control two things; it allows us to control nutrients in the reactor; in the chemostat, it also allows us to control how many bio how much biomass will be present in the reactor.

So, I can have high nutrient low biomass, I can have low nutrient high biomass, I can have higher both and lower both any of the combinations I can create within a chemostat. So, that flexibility is produced; now why do we talk about chemostat when I am talking about microbial energetic? Because most of the studies in microbial energetic; utilized chemostat.

It was very helpful when we could alter the nutrition lever; in other words we could change glucose levels, we could change levels of cellulose and other organic compounds and see how that impacted the microbial community. You could also give microbes a longer duration of healthy time. Remember we talked about how microbes are healthiest in the exponential growth phase and still second best healthy in the stationary phase.

So, we could prolong these phases the exponential growth phase in and the stationary phase up to sufficient length; that we could study these microbes more deeply. And we could understand what enzymes they are making, we could use some of the wonderful tools of microbiology to study these enzymes individually and understand for cellulose

degradation we have two kinds of bonds that they need to be break in broken are there two different kinds of enzymes that are doing it; one is exo; one is in door. And then we have a cellular wires that are being broken by beta blocker cities and all the study utilize is the chemostat.

So, now because we started studying chemostat; we started studying these using chemostat we started growing these enzymes at sufficient concentration for us to isolate them to study the genes that actually translate these enzymes. And we found out that it is not just one enzyme that catalyzes step 1 for any chemical reaction or step 2 or step 3 and so on so forth, but there are multiple enzymes.

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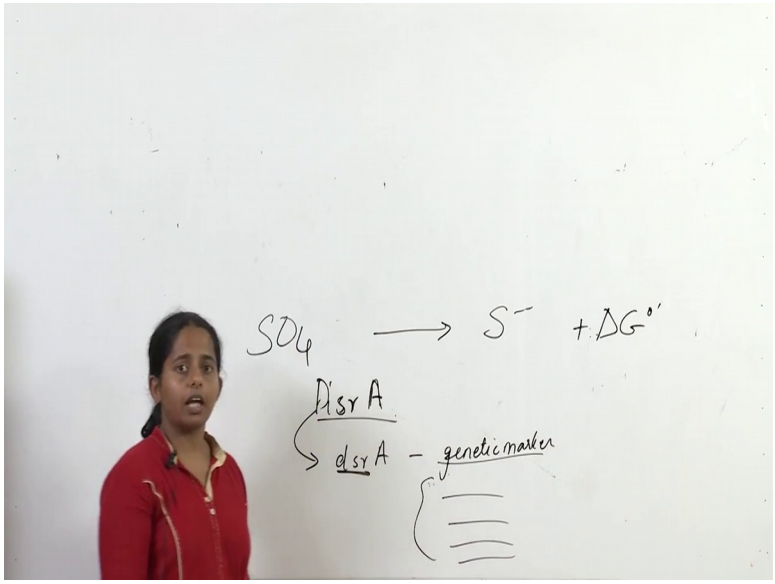

So, for example, we have sulfate and it needs to be reduced to sulfide form and this is sulfate reduction. And this is not one step process as I have drawn here; there are multiple phases the sulphur undergoes to go from its highly oxidized form to the highest reduced form it can go to.

Now in this, we have at every step of sulfate reduction different kinds of enzymes that are working. Now for example, let us talk about dissimilatory; sulfate reductase A; this is one enzyme where it can be summarized as dsr A; dissimilatory sulfate reductase A. This is one of the enzymes that catalyzes reduction of sulfite, which is an intermediary between sulfate and sulfide.

Even though thus this enzyme is very well characterized and the gene that makes it or that codes for it defined as dsr A again; same similar name. Now, this gene is very well known and it is utilized for as a genetic marker. Now let me write the down because this is an important term and we will return to it. We know the sequence of this gene; so, in other words we can imagine that we know this the structure of this enzyme, we know how it works.

But what we are noticing increasingly so, is that dsr is not the only gene that can catalyzes. And we are also noticing that there is a huge diversity within this enzyme. So, for example, the even in the genetic code; there is a diversity, slightly different gene sequences that encode this gene.

And I am sharing this with you to explain to you; a simple reduction of sulfate is not pure chemical reaction. Because if you remember sulfate to sulfide had a plus delta G and thus catalysis is very important and there are different catalysts that act together to carry forward this reaction. And that is where the beauty; the real beauty of bioenergetics comes in.

So, once you know the basic drivers of bioenergetics which we talked in this lecture and the previous lecture, which simply is that delta G should be than 0 for any reaction. And then you know the two formula for calculating delta G, you will be given the tables. And once you understand and grasp this and you know how the; bigger the reduction in delta G you can achieve by any reaction, the more energetically profitable it is.

Once you understand this then we can proceed and look at the microbial aspect or bioenergetics and understand that how microbes actually carry this energy using ATP? How they mediate the Redox reaction is using NADH? And how different enzymes help ATP and to convert into ADPN and revert back to ATP?

Then we move forward and try to understand the diversity of enzymes that carry on these beautiful biochemical reactions. For example, Glycolysis I presented it as a very simple three step chemical reaction, but in your homework for this week you will explore how beautifully complicated and in and diverse this pathways.

So, in the next lecture I am going to talk to you about the diversity within prokaryotes within the microbes. And we will proceed from our current understanding of microbial

energetic; how microbes degrade different electron donors? How they use electron acceptors in environment? And we will see how this diversity is a representation of the eminent diversity within the microbes themselves. So, that is all for today.

Thank you.