Plant Cell Bioprocessing
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Lecture - 27
Bioreactors for Hairy Root cultures

We will be talking about Hairy Root cultures. Apart from plant cell cultivations, hairy root cultures are also being used in the industry commercially for secondary metabolite production.

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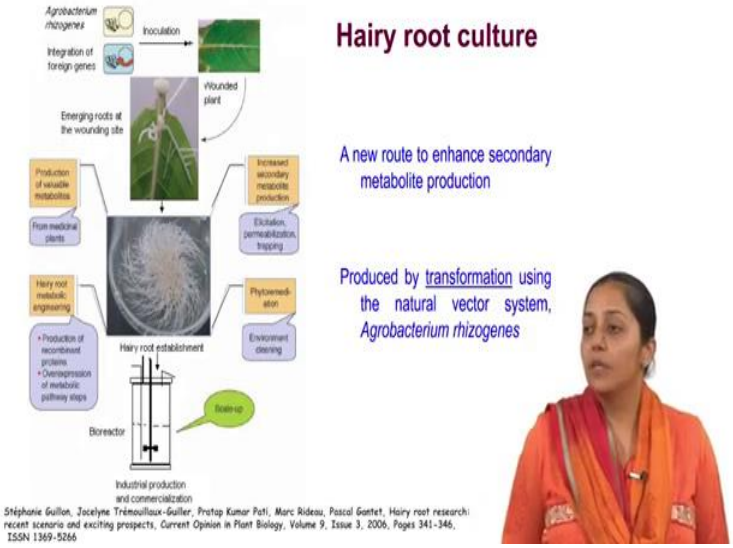

From where do the hairy root cultures come? They are actually a disease in the plant caused by *Agrobacterium rhizogenes*. We have already studied about *Agrobacterium* transformations. If *Agrobacterium tumefaciens* infects the plant cells the plant is affected by crown gall disease, if *Agrobacterium rhizogenes* infects then we get adventitious roots which are called as hairy roots from the point of infection because of the root origin locus rol genes present in the T-DNA.

Now, there are different commercial applications of hairy root cultures, they are being tested for phytoremediation studies, they are used for secondary metabolite production where same strategies can be applied for yield and productivity enhancement. But there are limitations in the scale up of hairy root cultures. This is where bioreactor designing plays an important role.

Applications of hairy roots: Production of high value secondary metabolites from endangered species or medicinal plants. For metabolic engineering, for heterologous expression of proteins

it becomes a more biosynthetically and biochemically stable system because there is a stable T-DNA getting integrated into the plant chromosome.

But, growth rate wise, hairy root cultures being organized structures are slow in comparison to plant cells. This is because plant cells are dispersed and are more exposed to availability of nutrients, hence the specific growth rates are higher. But because biosynthetic capability and stability is more, people prefer hairy root cultures.

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Advantages are that they are fast growing. When we say fast growing it is relative to the natural plant or natural root system because in comparison to the natural root systems there are more number of meristems present. There is extensive branching in hairy roots. Therefore, we see that these specific growth rates are higher than the normal root cultures.

Genetic stability is attained because of the chromosomal integration. Then biochemical stability is attained because it is an organized structure, hence there is a higher secondary metabolite yield. Moreover, they are neoplastic in nature. So, there are no hormones required because auxin synthesizing genes are already present, hence they are cost effective in comparison to plant cell cultures.

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Now, the challenges in bioreactor cultivation or scale up; they grow like a mesh matrix. The problem with hairy roots is that as the working volume increases, even from the day one, depending on the size of the inoculum there can be gradients of nutrient limitation specially oxygen and also nutrients if the size of the matrix is big as it grows.

Hairy roots are self-immobilizing in nature. So, the growth begins, once they come together. As the growth happens they will grow as single matrix and therefore, the inner core is generally found to be devoid of nutrients and gets necrotic.

There is shear sensitivity in hairy roots. It has been observed that if stirred tank reactor configurations are used for hairy root cultures then these roots will break and if the breakage happens the organism takes it as a wound created and there will be callusing happening on the roots, thereby leading to inconsistent product formation.

Then there is a requirement of support matrix because they are self-immobilizing in nature. It has been observed that more dispersed they remain in the liquid media the growth is hampered. So, the growth does not begin. Therefore they are required to come together as it acts like a prerequisite for the growth to begin. Hence there is a requirement for a support matrix. They are highly sensitive to even the material of the support. Generally, stainless steel is the material which is used in the reactors, but it depends on the species. Sometimes it is found that it is very specific and sensitive to the material of the inner parts of the reactors.

Restriction of nutrient or oxygen delivery to the central mass of the tissue happens, then resistance to flow takes place due to the interlock matrix. Now, the size of the biomass itself is like an immobilized system and is quite big. So, there will be thicker boundary layers in comparison to your gas liquid boundary layer.

The mass transfer limitation in these systems is much more severe than when you are working with plant cells or microbial cells. In the case of microbial cells, the cell size is small, so the solid liquid boundary layer thickness is smaller. But in hairy roots, the solid liquid boundary layer limitation is higher in comparison to liquid gas. It also depends on the species on which we are working and the root itself can be very thick.

Generally, if we work with herbs then we will have very fine roots, but if we are working with tree species then you may have very brittle and thick morphology, like when we were working with *Azadirachta indica*. Initially, when hairy roots were induced, it was like a tap root system. There was one single root and then branch; the branching was also less.

So, it was very difficult to do liquid culture of those roots as growth rates were very slow, so we had to optimize the media and then we applied gibberellic acid as one of the hormones and we could clearly see the morphology completely changed. We had seen a complete difference in the morphology because by adding gibberellic acid what we observed were ‘n’ number of meristems which came out. So, gibberellic acid is known to increase branching in root systems.

There was heavy branching because of which the morphology became thin and it became easier to scale up the system and mass transfer limitations were also reduced. It has to be a balancing act between the biological needs of the tissue without inducing an additional biological response for example, wounding which will include callusing in the roots.

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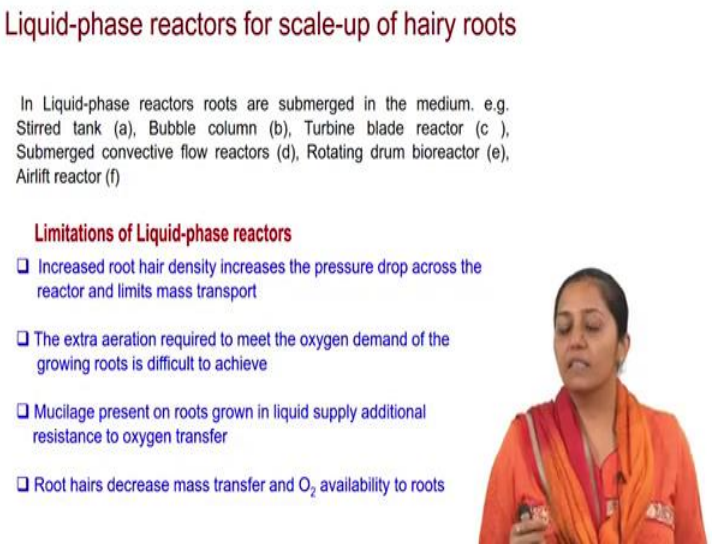

The reactors for hairy root cultures are divided into two types, liquid phase reactors and gas phase reactors. Liquid phase reactors are generally the kind of reactors which we work with in the microbial fermentation or plant cell fermentations. How are the two different? In liquid phase reactors, the gas is the dispersed medium in the liquid. In gas phase reactors, the liquid is the dispersed medium in the gaseous environment.

Let’s talk about the liquid phase reactors. In liquid phase reactors, mixing is done by stirring the tank and there is bubble column, where convective flow carries out mixing. Rotating drum and airlift reactors are some other examples.

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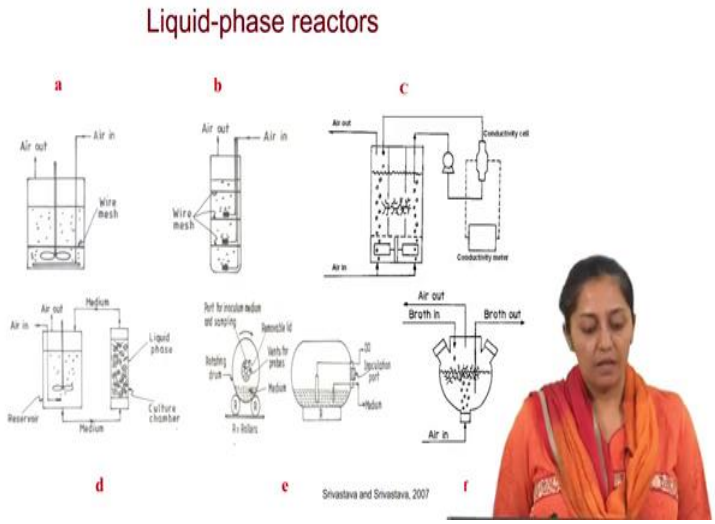

Since root cultures are self-immobilizing they need a support. So, even in these kind of liquid phase reactors a support is provided such that it can facilitate self-immobilization of the hairy roots. In picture ‘a’ we can see a stirred tank reactor. There is a mesh provided on to which inoculation is done and the impeller is kept below the mesh such that it cannot generate large shear forces on the inoculated roots.

What are the limitations? These are reactors which have already been used for hairy root cultures (refer picture). There are reactors where there will be a support system and an impeller for mixing. We may also find that there can be two separate compartments, one can be culture compartment, the other can be your medium reservoir. So, if you want to avoid your shear forces on to the biomass then the reservoir is aerated and mixed separately and then circulated through the culture chamber, like you can see in the picture ‘d’.

The configuration remains the same, be it bubble column, airlift reactors, but the geometry is differed, so as to work around with the mass transfer limitations for the nutrients or for oxygen. And because support is needed they are self-immobilizing, so some structures inside the same reactor configurations are provided to balance out the mass transfer and the shear forces.

The limitations which have been found with liquid phase is the increase in the pressure drop across the reactor and mass transport limitation. For example, if you are using a packed bed system, the biomass will start growing there will be increased pressure drop with the increased root density. Then there will be mass transfer limitations.

Similarly, if we use configurations where they have used small baskets, polypropylene mesh, and they inoculate the roots onto those mesh and then they drop the entire mesh with the inoculated roots inside the liquid medium. Now, as the roots would grow around the mesh the branching would happen then you can imagine that the inner core of the roots which is there in the mesh will not have the same oxygen availability that the outer surface would have.

These are severe mass transfer challenges in hairy root cultures. What can be a way to overcome this problem? You need support, the roots are self-immobilizing, hence you cannot disperse them. How will you improve? What strategy would you use to improve mass transfer? What can you do? The following are the kind of reactors which are generally used.

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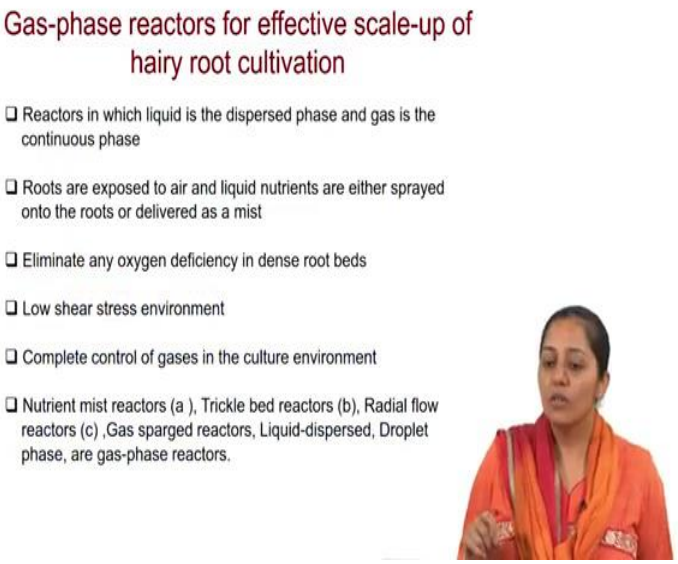

Gas phase reactors. In these reactors a liquid is dispersed into the gaseous medium. Liquid being dispersed into the gaseous medium, for example, trickle bed reactors or mist bioreactors, where the media is provided in the form of cloud and mist on to the roots and the roots were kept on the support system. This results in a significant improvement in the growth of the roots.

Why do you think it might be helping? There is a limitation of scale up, but this was working and hydrodynamic stress could be avoided; In liquid phase reactors, roots when completely submerged in the liquid medium are observed to have a lot of mucilage which was getting collected on to the root surfaces which was inhibiting the growth of the roots. This phenomenon is called hyperhydricity. Lot of phenolics and mucilage can get collected onto the root surface.

When we read the literature, we found that they are sensitive to hyperhydricity i.e, too much of water retention on to the roots. How to avoid that? By using mist bioreactors. Now we can even use trickle bed system, but there the droplet size would be greater but if we use mist the droplet size will be reduced.

Even in mist bioreactors the cycle of the mist and the size of the droplet can be optimized for best results. This is one kind of gas phase reactor system. Roots are exposed to air and liquid nutrients which are sprayed on to the roots and delivered as a mist.

Coming back to the spray bioreactors - we had also got custom made small setups where we were using a nozzle and this nozzle could create a spray of the liquid medium and we put the roots on to the support and beneath the support we had that spray. Directly it does not hit the roots. It used to go up to the head plate and from there it used to fall down the spray. It did not give us good results. We saw that there was lot of water retention on to the roots and the roots became necrotic by the end of 25 days.

Then we tried to put the nozzle on top and spray the roots with smaller droplet size, but all these configurations did not work and the reason which we could decipher was probably the amount of water retention which was happening on the roots. Even though we can change the support, the support did not have any holes initially. We then created holes onto the support, so that the water could drain down and go back to the reservoir.

That did not help a lot because we could see lot of water retention on to the roots. Then the mist design came up which worked, but then scale up is an issue there. Things can work at small scale, but imagine the production scales. It is quite costly.

Roots are exposed to air and liquid nutrients. They are either sprayed on to the roots or delivered as a mist. These are mist bioreactor designs. What can they do? They can eliminate oxygen deficiency in the dense root beds and we could see that very clearly. Once we harvested the reactors and we opened up the root mesh and till the inner core we could see small branches of fresh roots coming out. In total the biomass was one of the best in comparison to all the reactor designs which we saw.

There is a lower shear environment because there are no moving parts. There is complete control of gases in the gaseous environment. It can travel till the inner core and hyperhydricity is also removed. One of the best advantages was that there was good gas transfer happening.

Why do you think that happens? Better mass transfer takes place in oxygen than in the liquid, which could have been the reason for improvement. Oxygen gets to the roots through the mist.

Now the gas transfer is happening directly from the air to the root. Oxygen is sparingly soluble in the liquid which is not the case in air. So, there is better availability of oxygen for gas transfer to happen which could have been the reason to get significant change.

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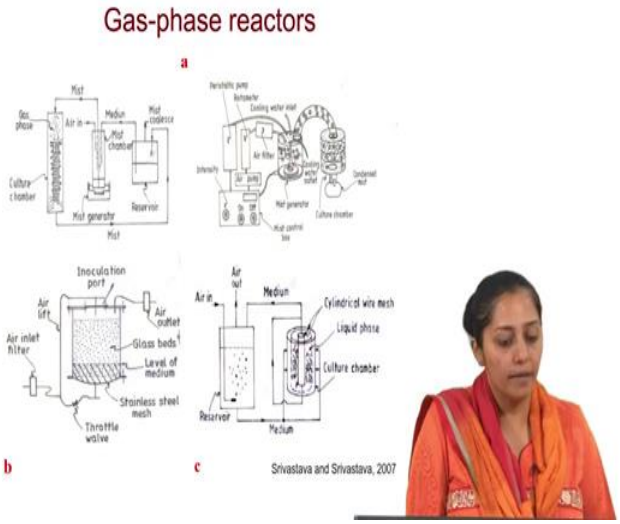

In the centre there was a mist chamber in which you can use ultrasonic transducers, where the mist was created. It then goes to the culture chamber where we have inoculated our roots. Then whatever condensate is obtained can then be recycled back to the reservoir.

Moreover, the quality of support or the kind of support you use also matters. We tried glass beads, we tried polypropylene basket or polypropylene mesh, we tried polyurethane foam and we tried stainless steel mesh as support and all these different kinds of supports gave us different results. So, the kind of material you use as a support can also become a critical factor for results.

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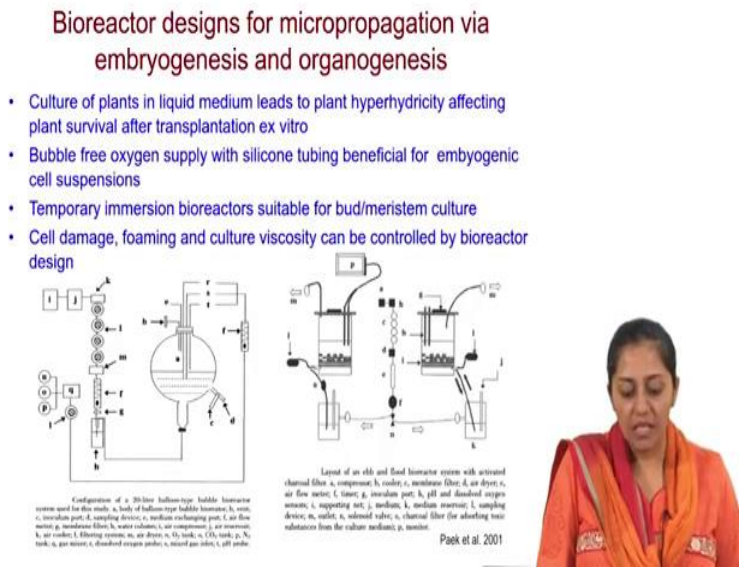

These are some of the other reactors which have been used in literature for other organ cultures for shoot multiplication or somatic embryos. For somatic embryo scale up people have used balloon kind of bioreactors. Generally you will observe either they are used as fill and draw kind of a system to remove the phenomena of hyperhydricity and the entire drum would rotate like in rotating drum bioreactor.