Plant Cell Bioprocessing
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Lecture - 26
Plant Cell Bioreactors - Part 2

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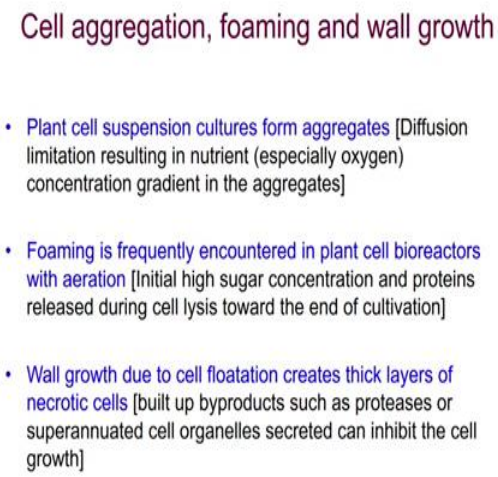

Last class, we discussed about the characteristics of plant cells, how they are different from microbial fermentations and what impact a bioreactor's different operating parameters would have on plant cell fermentations. So, we spoke about cell aggregation which is peculiar to plant cell fermentation. These appear more frequently in plant cell fermentations. We also saw about foaming and wall growth.

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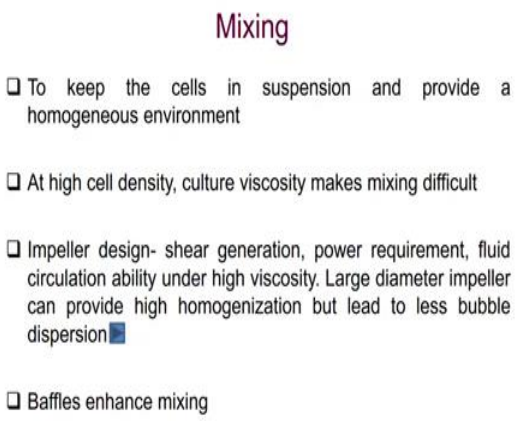

Then we spoke about mixing where we need radial as well as axial mixing. We also saw that in plant cell fermentation, since they are heavy and dense as the cell density increases, suspension efficiency has to be enough and is an important aspect which has to be taken care apart from dispersion of the gas bubbles or homogeneity inside the reactor. For this the impeller design is crucial.

Generally, for plant cell fermentations, high width to diameter ratios are preferred. The distance between the sparger and the impeller is important. The kind of spargers, for example, point spargers when used, will have more instability as there is higher chance and lesser dispersion possible.

Helical ribbon or setric impellers and centrifugal impellers are specific impeller designs which are frequently used for plant cell fermentations because they can create both axial and radial movements, have less shear forces on the cells and better kLa in comparison to impellers like radial impellers for microbial fermentations.

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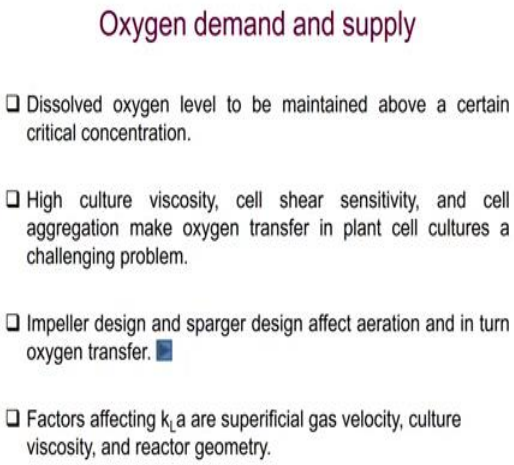

Mixing in pneumatically agitated reactors is a limitation because there are no moving parts, but shear rates are less.

What is pneumatically agitated?

Pneumatically agitated means air driven. There are no moving parts involved. Whatever mixing is being done is provided by the air flow rates by sparging. Dissolved oxygen levels should be maintained above the critical oxygen concentration. High culture viscosity, cell shear sensitivity and cell aggregation; all these phenomena make oxygen transfer in plant cell cultures a challenging problem. So, the impeller design and sparger design will help in overcoming these problems.

Now, factors that affect the kLa which is mass transfer efficiency in the reactors includes superficial gas velocity, culture viscosity and reactor geometry. We see that these factors will be affecting the kLa, then we will be able to find an empirical co-relationship between mass transfer, characteristics and how they are related to various operating parameters of the reactors like impeller width, impeller diameter, superficial gas velocity, gas flow velocity and power input to the impellers. These are some of the parameters in the reactors which have been found to be related with the mass transfer coefficient of the reactor vessel.

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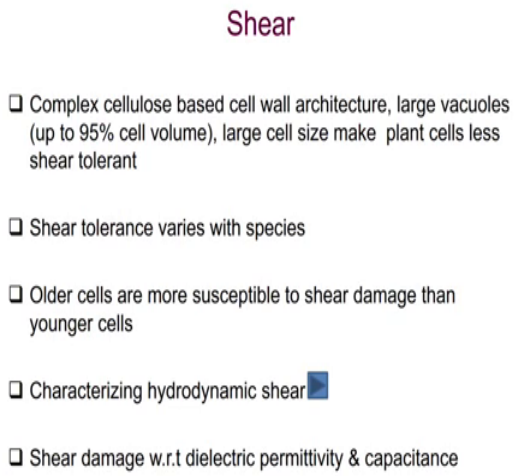

Why are plant cells less shear tolerant when compared to microbial fermentations?

This is because there is an inverse relationship that when the plant cells are in the early log phase, rapidly multiplying, they are more flexible. But as the cell density increases they become less flexible to shear forces.

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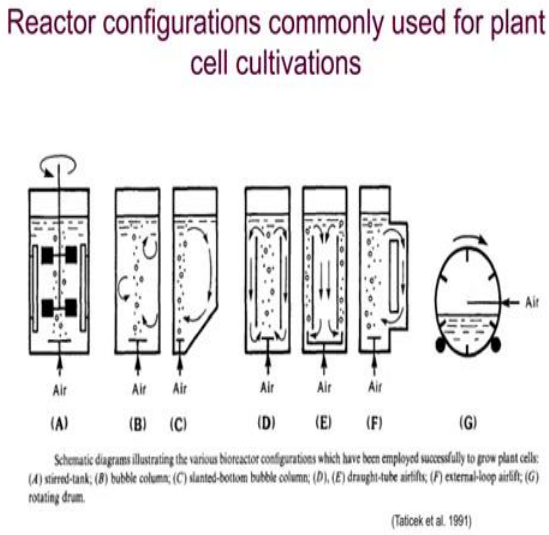

So, reactor configurations, the first one is a stirred tank reactor. The number of impellers can also be one of the factors for mixing and the amount of mixing required which is quantified in terms of mixing time.

In the pneumatically driven reactors you will find, there are tapered bubble column reactors, then there will be airlift reactors, external internal loop reactors, etc. Now that we know these are pneumatically driven reactors, what is the limitation of such kind of reactors?

Mixing is the limitation. So, any changes like making an external loop to it or making it tapered in the design improves the mixing time, trying to overcome the limitation of the pneumatically driven reactors. The position of the sparger i.e, where the sparger should be placed in an airlift of a bubble column, will also determine whether dead zones can be available at the bottom or not. Refer to the diagram.

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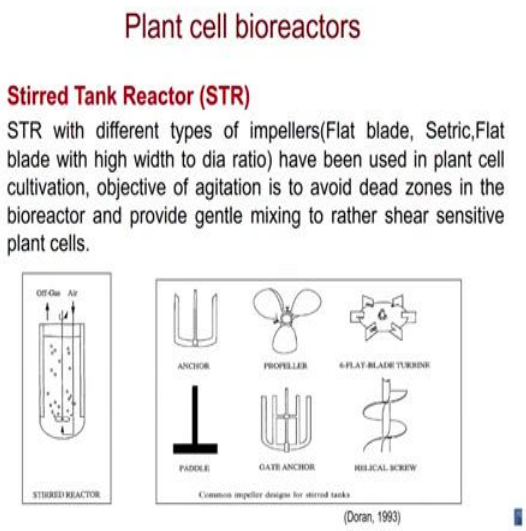

In a study, the position of the sparger was changed and they could find that in an inner loop reactor when the position of the sparger is well above the draft tube, then the circulation currents were better because the bubbles could drive the currents from inside to outside. But, if the sparger was placed well below the draft tube, then there is a chance that dead zones will be created as the bubbles will get dispersed at the bottom, rather than flowing through that channel, creating density gradient and circulation currents.

Let us talk about the different configurations. Stirred tank reactor is a well-known configuration which is very well used for microbial fermentations. The difference which you will find in plant cell fermentation is with respect to the sparger design, impeller design, absence and presence of baffles.

Now, even people are working with the aspect ratio. The kind of reactors, for example disposable bioreactors or wave bioreactors will have more surface to volume ratio. The kind of impellers, like anchor impeller, propeller six and flat blade turbine are well used for microbial fermentations.

Now, why these designs?

This is because these designs impact flooding and loading. Now, this impacts the dispersion of the gas bubbles in the reactors. More the dispersion of the gas bubbles better is the mixing. But there is a limitation of creating suspension of the cells. As far as the sparger is concerned, using a point sparger or a ring sparger depends on the reactor geometry. So, all these aspects have to be taken into account when you are designing reactors.

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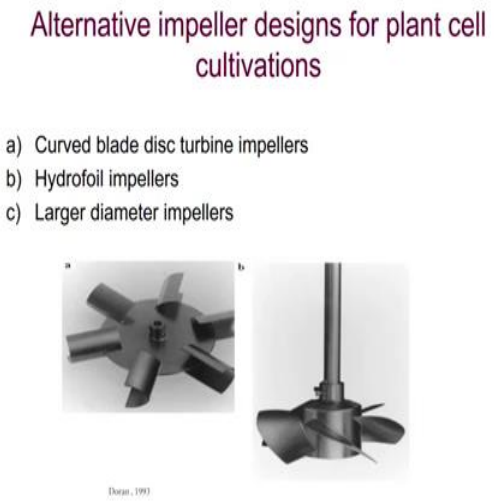

Some alternative designs include disc turbine curved blade reactors and large diameter impellers.

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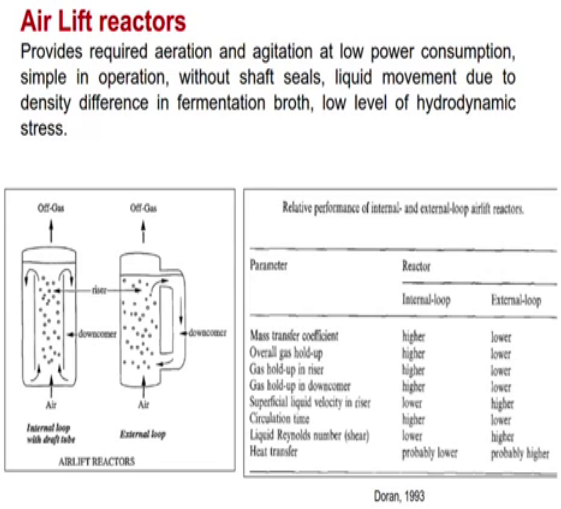

Airlift reactors: So, the first picture is the inner loop reactor, the second is the outer loop reactor. Outer loop reactor will improve mass transfer. Why? It is said that the outer loop improves mass transfer of oxygen and gas liquid mass transfer was improved.

In both the cases air is being sparged. But how does the external loop work? It is a simple enclosure which is in continuity with the inner reactor.

So, what is increasing? Is there an extra path? Yes, Surface area. So, what? You are giving more time for gas hold up. More time is given for exchange to happen. So, more circulation time.

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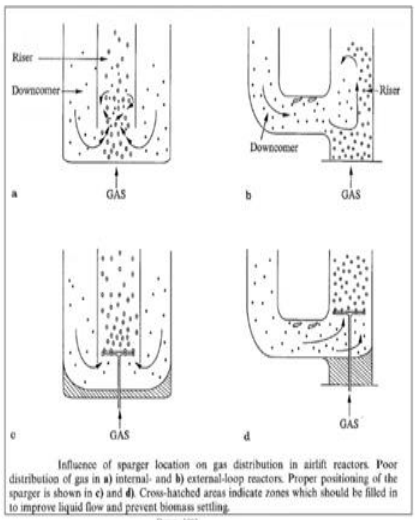

Influence of sparger location on gas distribution in airlift reactors. Poor distribution of gas in **a)** internal- and **b)** external-loop reactors. Proper positioning of the sparger is shown in **c)** and **d)**. Cross-hatched areas indicate zones which should be filled in to improve liquid flow and prevent biomass settling.

Doran, 1993

For effective mass transfer to happen in pneumatically driven reactors, mixing of suspension should happen. So, what is causing this suspension and mixing? It is the circulation currents.

What is causing the circulation currents? The density gradient.

What is the cost for the density gradient? The gas sparging.

So, it is very well demonstrated that if you change the position of the sparger, a little up in the draft tube then the gas bubbles would drive in the direction in which it is desired, thereby creating the right circulation currents and improving mixing. So, lesser is the chance for dead zones at the bottom of the reactor.

The other thing is that if the lower edges can be filled rather than keeping it very erect at the bottom and they have been made circular thereby filling it with glass then this will improve the circulation rates of the medium, avoiding the dead zones in at the bottom of the reactor.

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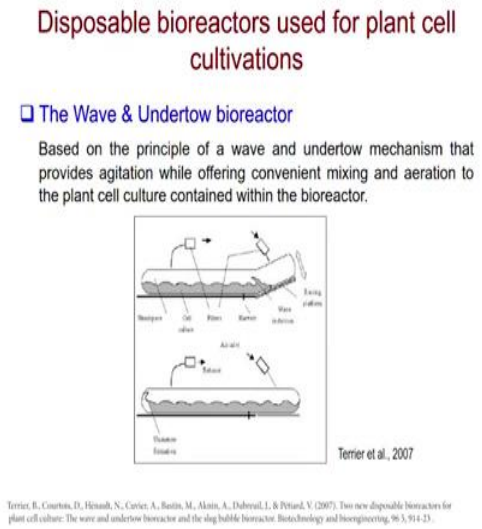

The disposable bioreactor is a well-known bioreactor used for animal cells, but people have started using it for plant cell fermentations. It works on the principle of wave and undertow. So, why this mechanism has been spoken about? There are no moving parts.

What causes mixing? The rocking motion.

What will these rocking motion create? Ripples, currents; it will create a wave at the surface. This is the same principle which works at the beach, when the wave comes to the beach then there is a large mass flux of the liquid towards the beach, but there is an equivalent mass flux against the beach which is beneath the turf. That is undertow. Those currents flow back beneath the wave. They work in the opposite directions. So, this is what will cause mixing to happen when this wave gets created, this is what is called as wave and undertow mechanism.

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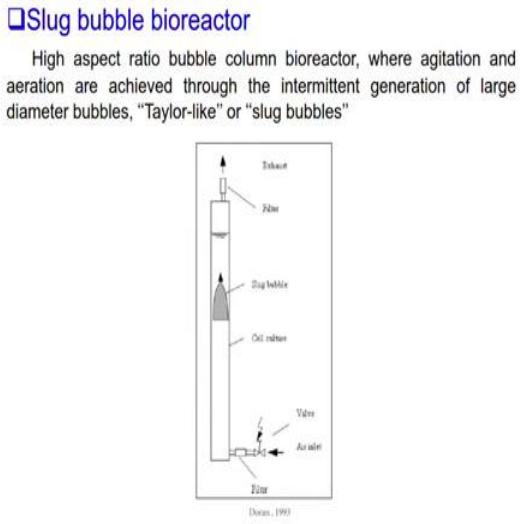

Slug bubble reactors have very high aspect ratio reactors. High aspect ratio means the height to diameter ratio is large. A single bubble is created which can span the entire column, entire reactor volume as it move slowly and the mass transfer takes place, as it would move on the edges of the bubble, it is said that it will create a plug flow.

On the edges of the bubbles there is a very thin film of liquid which is adjacent to the glass column or the reactor wall. The transfer between the liquid and the gas takes place through that thin film which is adjacent to the bubble and the wall at the top of the bubble.

You can see on the sides of that bubble which is shown in "filled grey color". So, these are called slug bubbles because they will create slug flow. You can assume it is closer to plug flow with no boundary layers.

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Apart from choosing the right kind of configuration, we want to improve the productivity. Now, we have spoken about different strategies which you can use to improve the yield of the product or the productivity of the product. Then, under those conditions you choose the right kind of bioreactor, you apply those conditions and get to some x productivity.

What is the limitation? We need to improve further, beyond that. Is it possible? Is the bioprocess now optimized? No there is still ample scope for productivity enhancement. There are ‘n’ number of strategies which we have learned that can improve the product yield, secondary metabolite yield and productivity. The limitation in the reactor is the time when we will have to stop the reactor. That time would be invariably when the nutrients are done. Can we do anything to never allow nutrients to be done till whatever point we want?

That can be done when you overcome with the mode of cultivation. We will have to design which nutrient is to feed and at what time and flow rate to feed and how we should be optimizing the nutrient composition. Say, we did statistical optimization and found that carbon is the most critical nutrient for biomass, but phosphate is the highest impacting nutrient for our secondary metabolite.

If we want to improve the productivity which is the sum of the biomass and the product, what should we do? It is better to run in one reactor itself and have a two stage cultivation.

The composition of the feed will also be governed by what we have studied in the batch i.e., whatever optimization was done in the shake flask. So, the concentration of the feed and composition of the feed should be determined at shake flask level before going to the reactor level which can then further improve the productivity. These are the bioreactor operating strategies, where you can even do it as a fed batch or a continuous cultivation in the chosen reactor configuration.

An effective bioreactor operating strategy provides a high productivity and a high product yield. We saw about high product yield with respect to biomass and high product yield with respect to substrate. High product yield with respect to biomass will reduce downstream cost. High product yield with respect to substrate will reduce production cost.

So, that is why these two factors should also be optimized and can be optimized separately. The operating strategy is determined based on the pattern of product formation. To relate the pattern of product synthesis, appropriate index for cell growth is needed.

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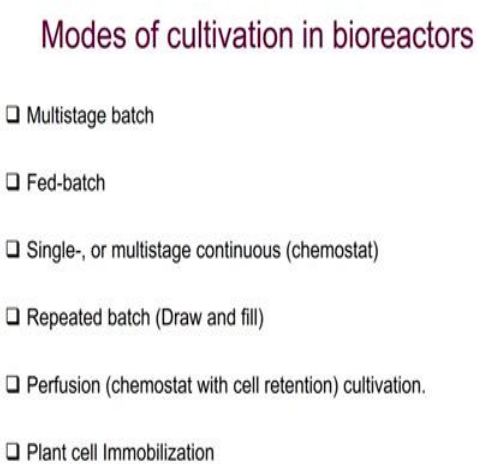

Now, modes of cultivations which you will come across will be multistage, batch, fed batch reactors, single and multi-stage continuous chemostat, then repeated batch which are also called as fill and draw systems.

Perfusion reactors are nothing but cell recycle reactors; the there is chemostat with cell retention and immobilized bioreactors.

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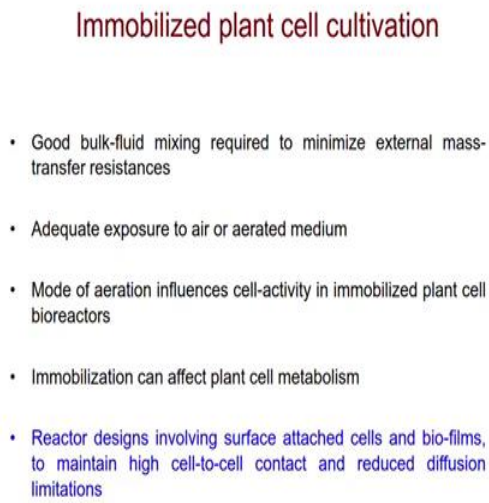

Good bulk mixing is required to minimize external mass transfer limitations so that there are lesser boundary layers around the beads or the stagnated biofilm in immobilized reactors for plant cells.

Adequate exposure to air or the aerated medium is necessary. Sometimes you will see the culture is separate from the medium in the reactor. This is called as a reservoir and the biomass is kept in a separate reactor. If suppose it is being worked as a packed column reactor or packed bed reactor then there is a reservoir from where the media is circulated and the spent media is again recirculated to the reservoir.

So, why do you think this is not being done in one reactor? How will that help? There is a separate reservoir because it will help to aerate the medium. Once it comes out separately from the reservoir, the medium is aerated such that you have enough dissolved oxygen and then it is circulated because mass transfer is a limitation in immobilized

reactor systems. Immobilization can also affect plant cell metabolism. So, cell viability has to be tested.

Surface attached cells as biofilms will facilitate cell to cell contact. We can see that as the cell to cell contacts improve it may impact the secondary metabolite biosynthesis.

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Taticek et al., 1991

Methods employed to immobilize plant cells.

Immobilization method	Species
Ca-alginate entrapment	*P. somniferum* *C. roseus* *M. pruriens*
Reticulate polyurethane matrix	*C. frutescens* *D. deltoidea*
Ceramic hollow fiber	*C. roseus*
Polyester fiber matrix	*C. roseus* *N. tabacum* Soybean
Surface-coated fiberglass	*C. roseus*

These are some of examples in literature where people have used immobilized reactors. We were talking about hollow fibre reactors where the cells can be immobilized either inside the tube or outside the tube. But, again with growth there would be mass transfer limitations, so the flow rates have to be optimized.

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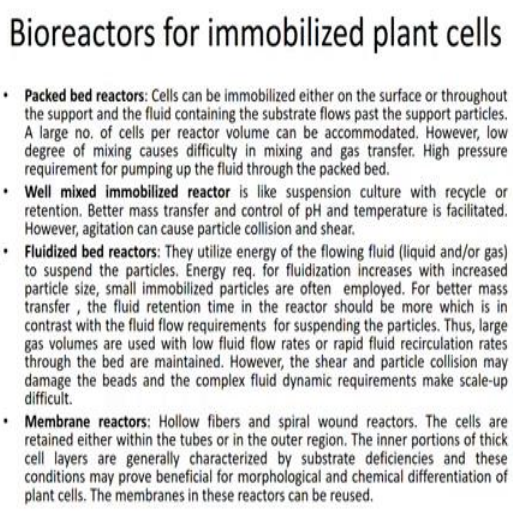

What is the limitation of packed bed reactors? There will be higher pressure drops in packed bed reactors because there is more resistance to flow but that limitation is taken away in a well-mixed immobilized reactor. We use the cells and make them freely suspended in solution. They become like reactors with cell retention. The limitation of external mass transfer can be avoided.

In fluidized bed reactors, there is fluid movement to improve the mass transfer, but cells are kept suspended depending on the flow rate of the gas or the fluid flowing because the fluid flow rate determines the suspension efficiency of the cell mass also.

If we improve the flow rate there will be better suspension, but then there is less time given for the mass transfer to happen. There are circulations developed through matrices such that we drive the liquid currents or the fluid is forced to spend more time with the cells or lots of gas sparging needs to be done.

Now, gas sparging is done in these reactors to improve the bulk mixing and to facilitate mass transfer between the gas bubbles and the liquid medium. That is one way of improving the mass transfer of oxygen in these reactors. But then, the limitation would be that we are having high gas velocities with liquid circulation currents, so there can be high amount of shear which may get generated.

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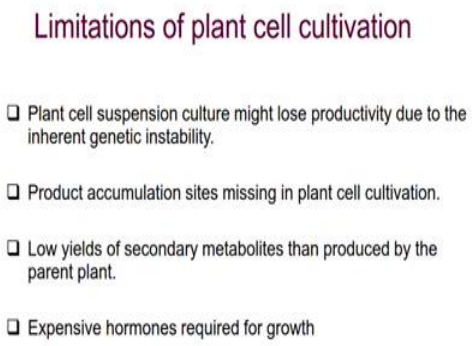

Despite all this, there are known limitations in plant cell fermentations. One thing is that, the minute it is brought to *in vitro* cell lines the yields drop.

Plant cell suspensions are created from callus. Hence, there is somaclonal variation because of which the biosynthetic capacity may vary. In order to avoid this, people exploited the *Agrobacterium* mediated transformed cultures, especially the hairy root cultures. Why? This is because they are organ cultures and heterologous expression can be done which will be more stable because there is genome integration of the T-DNA into the plant chromosome. It is a more stable transformation event. The other limitation in plant cell fermentation is that, we need hormones but in hairy root cultures the cost will come down because they do not need hormones for growth.