CELL SUSPENSION CUTURE

How single cells are produced?

Callus cultures fall into two types viz., compact and friable. In compact callus the cells are compactly and densely arranged and in friable type the callus cells are loosely arranged and they are soft and breaks apart easily. Friable callus provides inoculum for cell suspension culture when friable callus is placed in liquid medim and agitated single cells are released in the medium.

What is a cell suspension culture?

It a type of culture in which single cells or small aggregates of cells multiply while suspended in agitated liquid medium . It is also referred to cell culture or cell suspension culture

What is plant cell culture?

 It is an *in vitro culture* of single or relatively small groups of plant cells i.e., the callus culture

Why plant cell suspension culture?

 Establishment of single cell cultures provides and excellent opportunity to investigate the properties and potentialities of plant cells and for mutation induction.

Spatial heterogeneity

 The individual cells within a population of cultured cells invariably show cytogenetical and metabolic variations depending on the stage and growth cycle and culture conditions, such variables is termed as spatial heterogenity.

Critical Initial Density (CID)

The particular initial cell density that is able to grow in liquid medium is called critical initial density.

Different categories of suspension culture

 Broadly speaking there are three types of suspension cultures

1. batch culture
2. continuous culture
3. immobilized cell cultures

Batch culture

 It is a suspension culture where the cell cultures are maintained in a definite volume of agitaged liquid with repeated subculturing of a small aliquot of cell culture to a fresh medium at regular intervals. Generally cell suspensions are grown in flasks (100 – 250 ml) containing 20 –75 mil of the culture medium incubated on orbital platform shakers at the speed of 8- 120 rpm..

After subculture the cells divide and the biomass of the culture increases in a characteristic fashion, until nutrients in the medium are exhausted and/or toxic by - products build up. . The cell number of a batch culture exhibits a typical sigmoidal curve containing

1. a lag phase ( during which period the cell or biomass remains unchanged
2. a log phase , an exponential phase which includes rapid increase in cell numberdue to active cell division
3. stationary period during which period the cell number does not changedue to the depletion of nutrients in the media and or by toxic substances

stationary

Exponential stage (log phase)

 Cell number

Lag phase

 time

figure:. Model curve showing different growth phases in batch culture

Batch cultures are characterized by a constant change in the pattern of cell growth and metabolism.

Uses of batch culture

1. for cloning
2. for cell suspension

Continuous culture / mass culture

 In continuous culture, the cell population is maintained in a steady state by regularly replacing a portion of the used or spent medium by fresh medium

There are two types of continuous culture types

1. closed continuous culture
2. open continous culture

closed continuous culture

cells are separated from the used medium taken out for replacement and added back to the culture so that the cell biomass keeps on increasing

open continuous cultures

both cells and the used medium are taken out from open continuous cultures are replaced by equal volume of fresh medium . Here, the steady state of suspension culture is maintained

 immobilized cell cultures

plant cells and groups may be encapsulated in a suitable material or entrapped in membranes or stainless screens. To provide the nutrients to the cells, liquid medium is continuously run through the column where the immobilized cells are packed and sufficient aeration is also provided.

Assessment of cells in suspension

1. cell count

cell count ( at the beginning of culture and after certain days of incubation) , is a relatively more accurate measure adopted to determine the growth and cultures Increase in cell number depends on Mitotic Index (MI) of cells in suspension cultures

 Mitotic Index refers to the percentage of cells undergoing mitosis.

 total number of divided cells

 MI = \_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_ X 100

 total observed cells

1. packed cell volume (PCV) = (Biomass volume) ml / g of culture
2. cell fresh weight (gm / ml)
3. cell dry weight(gm / ml)
4. by measuring the optical density of the liquid medium the turbidity level could be estimated which is directly proportional to cell number

Test for viability of cell

 using Fluoresein Di Acetate (FDA), Evans blue stains the viable cell count could be made

applications of cell culture

1. it helps in understanding of an organ formation or embryoid formation starting from single cell or small cell aggregate
2. suspension culture derived from medicinally important plants can be studied for the production of secondary metabolites such as alkaloid
3. it helps in mutant selection in relation to crop improvement
4. to produce valuable products including secondary metbolites through bio transformation a technique utilized with the help of microbes

(eg) Datura cell culture possess ability to convert hydroquinone into arbutin

1. single cell cultures are also being used for production of Single Cell Proteins (SCP)

SCP are any microbial biomass from both uni and multicellular bacteria, yeasts, filamentous fungi or algae which can be used as food or feed additives.

 (eg) Spirulina

comparison of callus and cell suspension cultures

|  |  |  |
| --- | --- | --- |
| Parameters | Callus | Cell suspension |
| Growth | Slow  | Fast |
| Cell or cell content  | Cells in contact | Dissociated |
| Medium | Only lower layer is in contact with the medium | All cells are in direct contact with the medium |
| Precursors | Not available to all cells | Available to cells  |
| Subculture period |  Long, 4-8 weeks | Short , 7-21 days |
| Accumulation of metabolites | Higher than cell suspension | Lower than callus culture |
| Scale up in bioreactor | Not possible | Cell suspension ar grown in bioreactor |

 Applications of cell culture

 cell culture offers enormous opportunities in the study of single cells and group oc cells

 in the isolation of protoplasts

 in cell cloning by the plating technique with or without specific treatment like mutagens,

 development of cell lines for various types of resistance like salt or drought tolerance, toxin resistant lines

 in scale up technology using bioreactors of various types