FACULTY OF ENGINEERING & TECHNOLOGY

LT.10. Scaling up of cell culture

Outline

1. Scaling up definition
2. Methods of scaling up
    a. Scale-up in suspension
    b. Scale-up in monolayer
    c. Immobilized cell cultures
    d. Microcarrier culture
3. Test your understanding

Definition: Scaling up

•Scaling up refers to increase in size of cell culture

•Scale-up involves the development of culture systems in stages from (small scale) laboratory to (large scale) industry

•The methodology adopted to increase the scale of a culture depends on the proliferation of cells

Methods of Scaling Up of cell culture

a. Scale-up in suspension
b. Scale-up in monolayer
c. Immobilized cell cultures
d. Microcarrier culture

Scale up in suspension culture

Scale-up in suspension is the preferred method as it is simpler. Scale-up of suspension culture primarily involves an increase in the volume of the culture. Small scale generally means the culture capacity less than 2 litres volume (or sometimes 5 litres).

Stirred suspension cultures:

It is usually necessary to maintain cell strains in stirred suspension cultures, by agitation (or stirring) of the medium. The stirring of the culture medium is achieved by a magnet encased in a glass pendulum or by a large surface area paddle. The stirring is usually done at a speed of 30-100 rpm. This is sufficient to prevent sedimentation of cells without creating shear forces that would damage cells.

Static suspension cultures:

Some cells can grow in suspension cultures, without stirring or agitation of the medium, and form monolayer cells. However, static suspension cultures are unsuitable for scale-up.

•Scaling up in suspension culture involves following elements:

(i) An increase in the volume of the culture medium

(ii) Agitation is done when the depth exceeds 5mm and above 5-10 cm.

(iii) Sparging with CO_2 is done to maintain adequate gas exchange.

(iv) Stirring should be between 30-100rpm as:-

(v) Stirring at higher speed creates shear forces that would damage the cells.

(vi) It should be sufficient enough to prevent cell sedimentation.

(vii) Antifoam or Pluronic F68 is added at conc. 0.01-0.1% when serum conc. is 2%.

(viii) Carboxymethylcellulose(1-2%) is added to inc. the viscosity of the medium.

Reactors used for large scale suspension culture

•Stirred Bioreactors

•Continuous flow reactors

•Air lift fermentors

Stirred Tank Bioreactors : These are glass (smaller vessels) or stainless steel (larger volumes) vessels. These are closed systems with fixed volumes and are usually agitated with motor-driven stirrers with considerable variations in design details

Continuous-Flow Cultures

•The medium enters from inlet side and the cells along with product exit from outlet side.

•Continuous cycle is maintained.

•It can operate at chemostat (constant chemical constituent in reactor) or turbidostat mode (constant cell density) in reactor.

Airlift Fermenters Cultures in such vessels are both aerated and agitated by air (5% CO_2 in air) bubbles introduced at the bottom of vessels. The vessel has an inner draft tube through which the air bubbles and the aerated medium rise.

Monolayer Culture This type of culture is essential for anchorage dependent cells. Scaling up of such cultures is based on increasing the surface area of the substrate in proportion to the number of cells and the volume of medium and therefore tends to be more complex than suspension cultures. The available surface area can be increased by using plates, spirals, ceramics and micro carriers.

Following strategies are used for scaling up of cell culture as monolayer

a. Roux bottle
b. Plastic film
c. Roller bottle
d. Helicell vessels
e. Multitray unit
f. Bead bed reactor
g. Synthetic hollow fibre cartridge
h. Heterogenous reactors
i. Opticell culture system

Roux Bottle It is commonly used in laboratory and is kept stationary so that only a portion of its internal surface is available for cell anchorage. Each bottle provides Ca. 175- 200 cm2 surface area for cell attachment and occupies 750-1000 cm3 space.

Roller Bottle This vessel permits a limited scale up as it is rocked or preferably rolled so that its entire internal surface is available for anchorage. Several modifications of roller bottle further enhance the available surface,

Roller bottle


Roux bottle

https://www.sciencedirect.com/topics/engineering/roller-bottle
https://www.thomassci.com/Laboratory-Supplies/Culture-Bottles/_/ROUX-CULTURE-BOTTLE

Multitray Unit

A standard unit has 10 chambers stacked on each other, which have interconnecting channels; this enables the various operations to be carried out in one go for all the chambers.

Each chamber has a surface area of 600 cm^2.

This polystyrene unit is disposable and gives good results similar to plastic flasks.

NuNc Cell factories

Scale up as Immobilized culture

By immobilizing the cell in cultures, there stability and specificity increases. Two basic mechanisms used for immobilization of animal cells are; Immurement culture methods and Entrapment culture methods

Immurement culture– In this type of culture, cells are encapsulated in a polymeric matrix by adsorption. Matrix used for immobilization are gelatin, polylysine, alginate and agarose.

Entrapment culture– In this type of culture, the cells are held within an open matrix through which the medium flows freely. The cells may be entrapped within the porous ceramic walls of the unit or cells also can be enmeshed in cellulose fibers

Scale up as microcarrier culture

Monolayers can be grown on small spherical carriers or micro-beads (80-300 pm diameter) referred to as micro-carriers. These systems use 90-300 pm dia particles as substrate for cell attachment. Initially, Dextran beads (Sephadex A-50) were used by Van Wezel in 1967; these were not entirely satisfactory due to the unsuitable charge of beads and possibly due to toxic effects. The micro-carriers are made up of any one the following materials (trade names given in brackets).

i. Plastic (acrobeads, bioplas).

ii. Glass (bioglass, ventreglas).

iii. Gelatin (ventregel, cytodex-3).

iv. Collagen (biospex, biospheres)

v. Cellulose (DE-52/53).

vi. DEAE Dextran (cytodex I, dormacell).

Test your understanding

For adherent culture scale up can be done as

a. Monolayer
b. Suspension
c. Immobilized
d. None of the above

For transformed cells can be scaled up as

a. Monolayer
b. Suspension
c. Immobilized
d. None of the above

What do you understand by microcarrier culture scale up

a. Scaling up in suspension
b. Scaling up as monomalyer
c. Scale up using Dextran or glass bead
d. None of the above

NUNC cell factories is used for scale up of

a. Adherent cells
b. Non-adherent cells
c. Transformed cells
d. Lymphoblast cells

Static suspension culture uses magnetic stirrer for stirring (True/ False)

Suggested reading

Further reading

1. Watson, J.D., Gilman, M., Witowski J.and Zoller, M. Recombinant DNA, 2nd ed., Scientific American Books, 1983
2. Glick, B.R. and Pasternack, J.J. Molecular Biotechnology, 3rd ed., ASM Press, 2003
3. Davis J.M. Basic Cell Culture: A Practical Approach, IRL Press, 1998
4. Freshney R.I. Animal Cell Culture a practical approach, 1987