Al-Farabi Kazakh National University

Lecture 3
Inoculum Development

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The preparation of a population of microorganisms from a dormant stock culture to a state useful for inoculating a final productive stage is called inoculum development.

Preparation may range in scale and purpose from a small inoculum for a bioassay to 1m3 for the production of a vitamin or antibiotic in a 200 m3 fermenter.

Inoculum aims to

(i) minimize the loss of viable microorganisms during the recovery from dormancy,

(ii) obtain a genotypically identical copy of the population that was stored,

(iii) increase biomass, and

(iv) develop the culture to a physiological state suitable for the performance in the final production stage

It is essential that the culture used to inoculate a fermentation satisfy the following criteria:

1. **It must be in a healthy, active state thus minimizing the length of the lag phase in the subsequent fermentation.**

2. **It must be available in sufficiently large volumes to provide an inoculum of optimum size.**

3. **It must be in a suitable morphological form.**

4. **It must be free of contamination**

5. **It must retain its product-forming capabilities.** The process adopted to produce an inoculum meeting these criteria is called **inoculum development.**

The inoculum development, which invariably involves both laboratory-based and plant-based steps, usually begins with the transfer of cells from an agar slant to a shaken flask by means of an inoculation wire fixed to a metal holder or a glass rod.

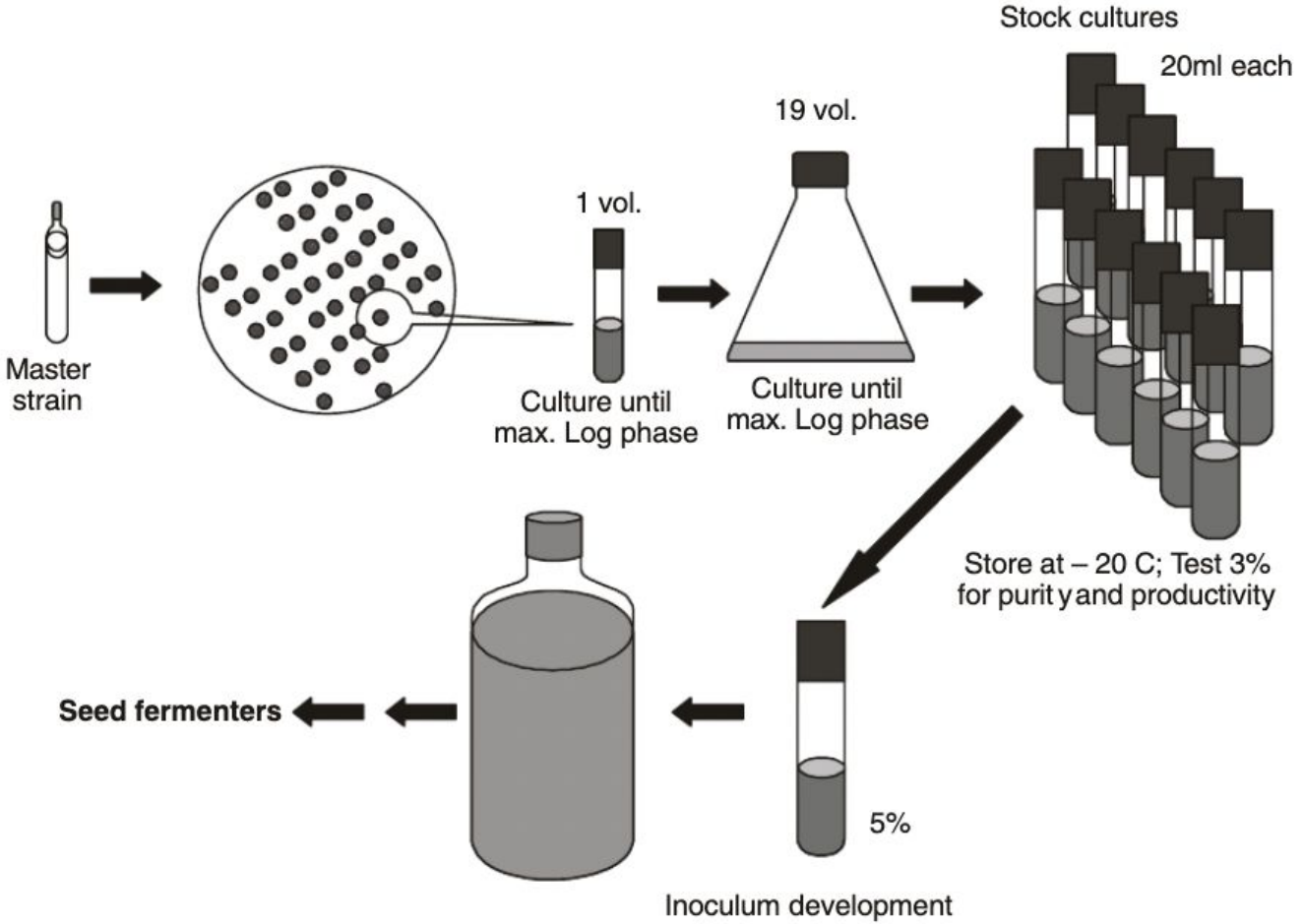
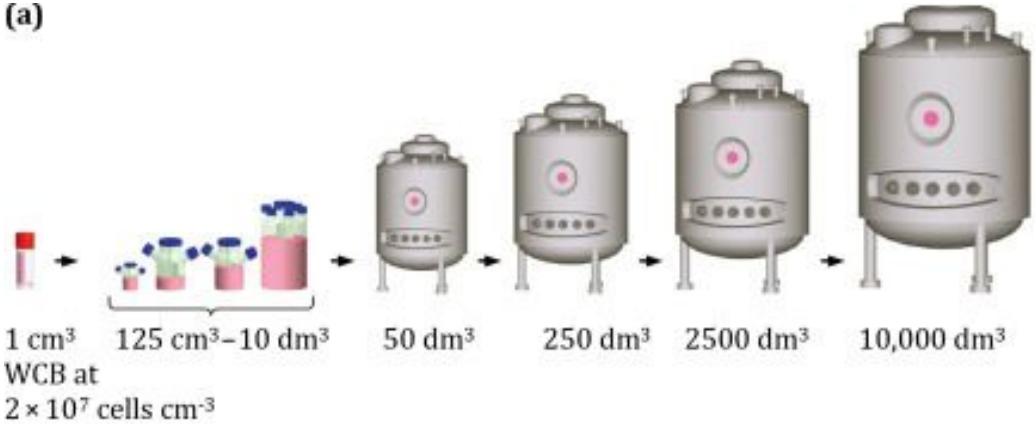


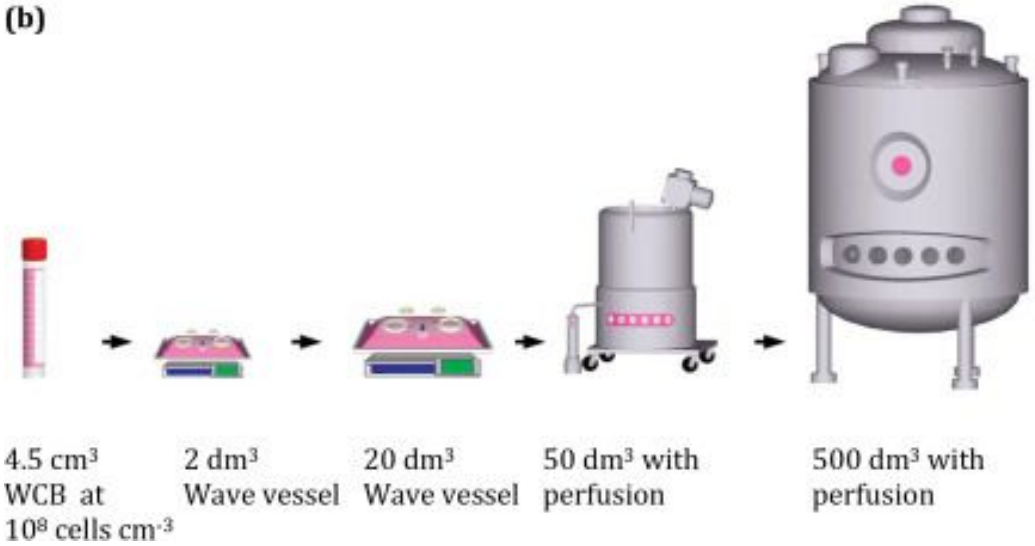
Fig. 7.4. Classical steps in inoculum development.

INOCULUM DEVELOPMENT FOR BACTERIAL CULTURE

- For bacterial fermentation the size of inoculum is optimized at 3% to 10% of the total volume of production fermentation medium
- Time to transfer of this inoculum also determine the total time of fermentation time
- Usually the inoculum at its log phase growth is transferred aseptically to production fermentation vessel
- Composition of inoculum medium medium and production medium is usually kept identical to minimize the lag period of the inoculum culture in the fermentation process

Alternative Inoculum Development Programs for a CHO Fermentation





*working stock culture (WSC)

(a) Inoculum program initiated with a 125 cm3 spinner flask inoculated with a standard WCB vial (1 cm3 at 2.5 × 107 cells cm−3) leading to batch reactors.

(b) Inoculum program initiated with a 2 dm3 Wave reactor inoculated with a high cell density WCB (4.5 cm3 at 108 cells cm−3) leading to perfusion reactors.

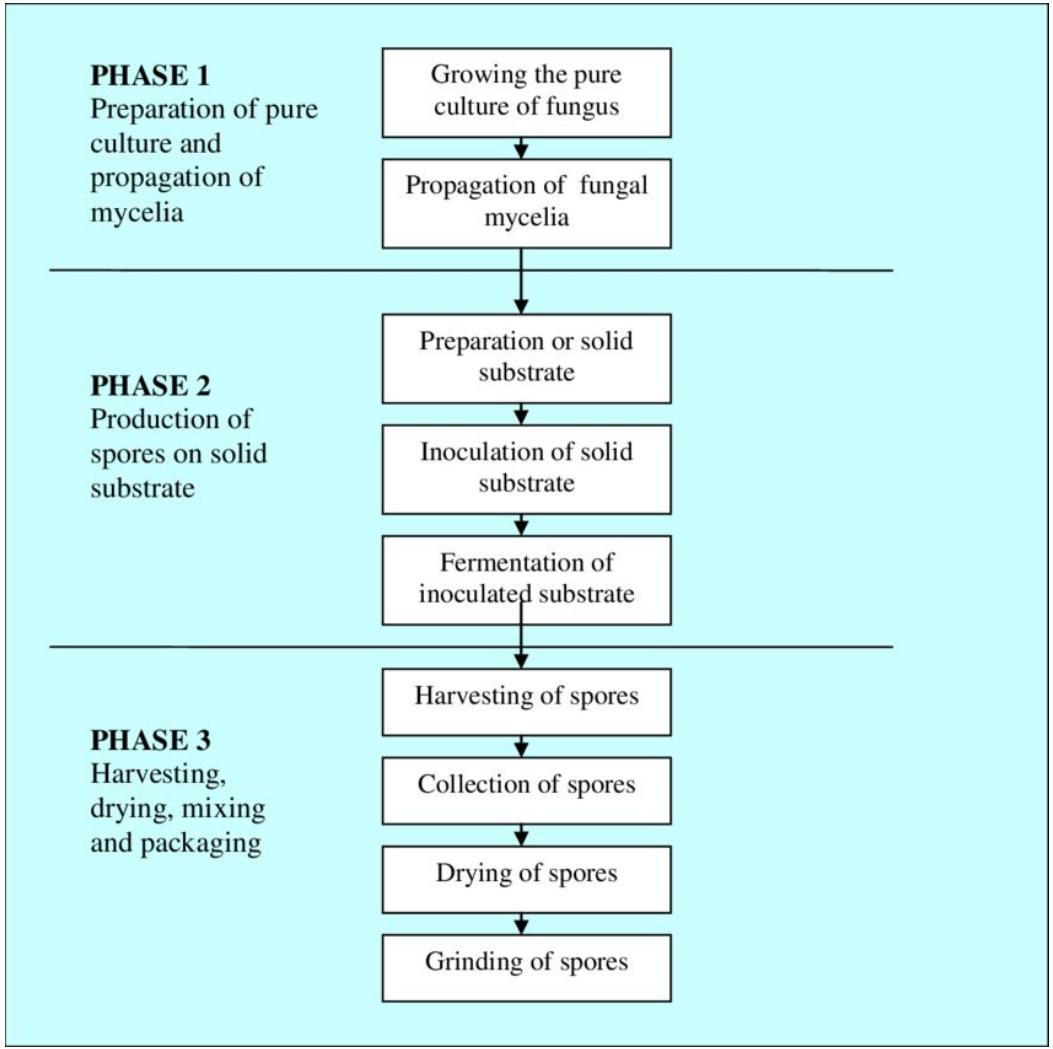
INOCULUM DEVELOPMENT MYCELIAL CULTURE

- Since most of mycelial fungi and streptomycetes are used to prepare inoculum culture in fermentation involving these kind of microorganisms.

- Therefore spores are used to prepare inoculum culture in fermentation involving these kind of microorganism.

- Thus the first stage in preparation of inoculum of spore suspension is production of large amount on spore on a suitable medium in lab.

Production of entomopathogenic fungus, *Metarhizium anisopliae*



https://www.researchgate.net/figure/Chart-showing-the-flow-of-the-process-involved-to-produce-the-spores-of-Metarhizium_fig1_236010090