Process scale up steps, laboratory scale, pilot plant and industrial scale

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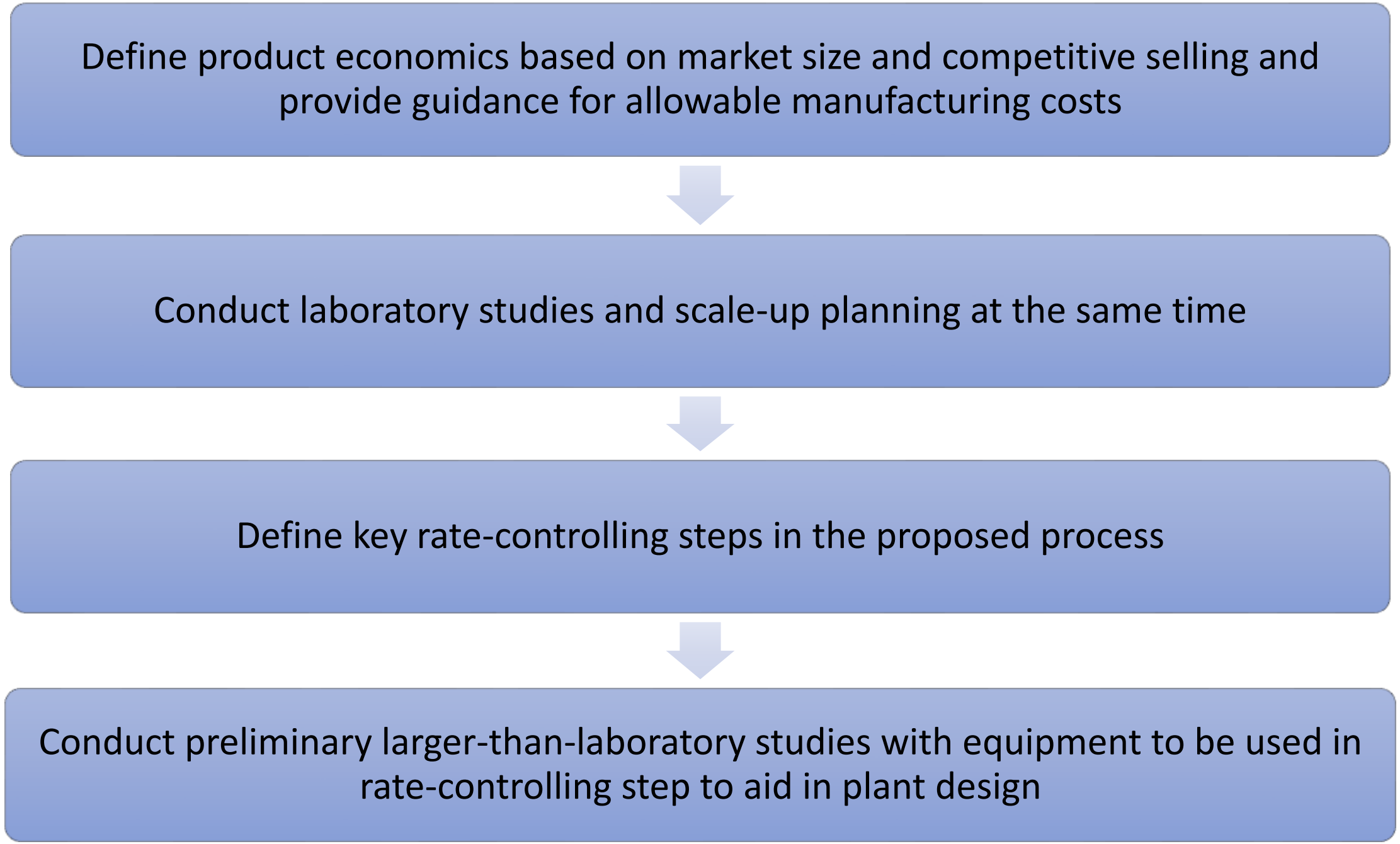
➢ **Definition** :-

- Scale up means the art for designing of large scale apparatus or full size plant (prototype) using the data obtained from the laboratory studies

➢ **Objectives :-**

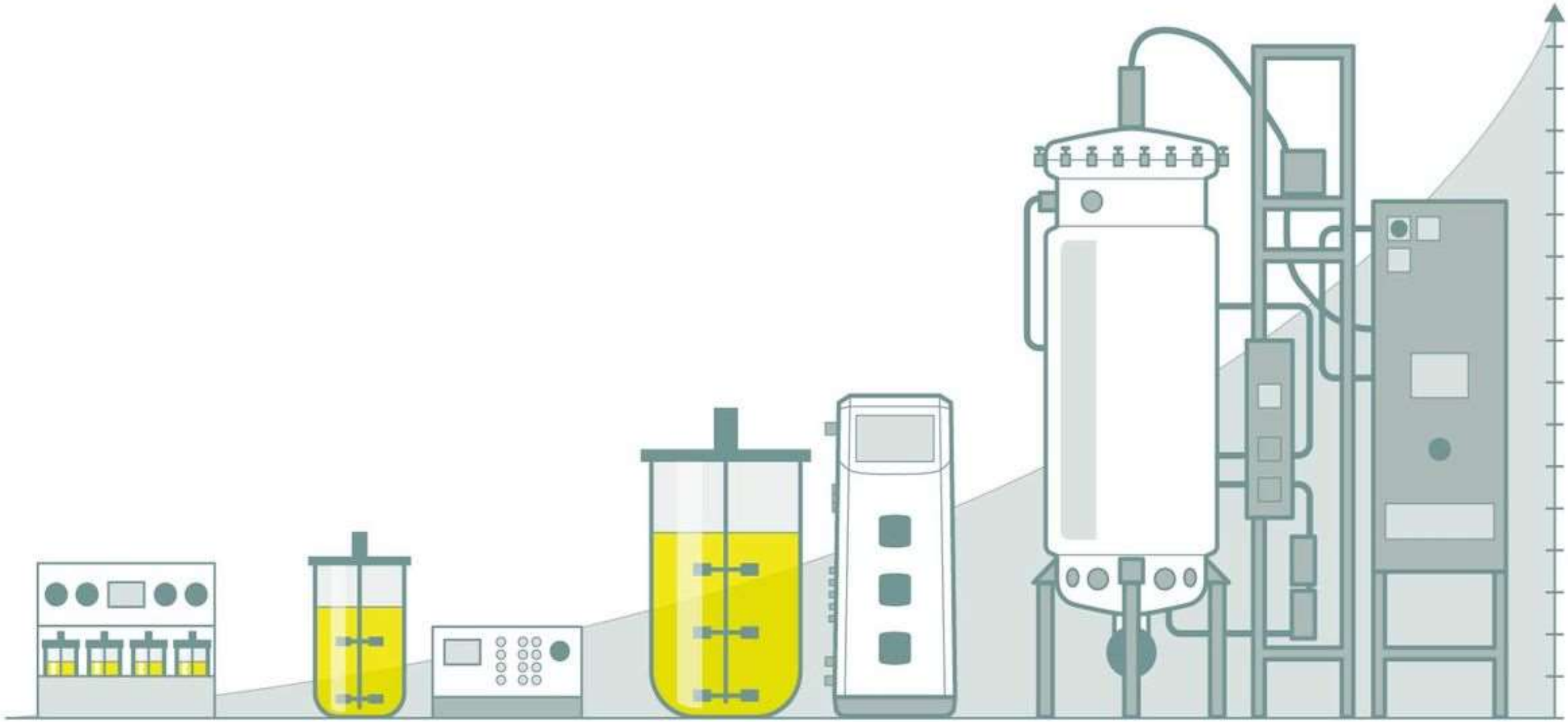
- To provide master manufacturing formula
- To identify the critical features of the process
- Evaluation, Validation and Finalization of process
- Guidelines for production and process control
- Review of the processing equipment
- To produce physically and chemically stable products


Define product economics based on market size and competitive selling and provide guidance for allowable manufacturing costs
Conduct laboratory studies and scale-up planning at the same time
Define key rate-controlling steps in the proposed process
Conduct preliminary larger-than-laboratory studies with equipment to be used in rate-controlling step to aid in plant design

Design and construct a pilot plant including provisions for process and environmental controls, cleaning and sanitizing systems, packaging and waste handling systems, and meeting regulatory agency requirements

Evaluate pilot plant results (product and process) including process

Economics to make any corrections and a decision on whether or not to proceed with a full scale plant development



Laboratory scale

- To demonstrate process feasibility or generate design data for a process, then a mini plant may be more appropriate than a pilot plant.
- Includes all recycle streams and can be extrapolated easily
- Uses same components as the lab testing (pumps, etc.), which is often standardized and can be used in many other mini plants
- Operated continuously for weeks or months so some automation is required.
- Is used in combination with process modeling and simulation of the industrial scale process.

Pilot scale

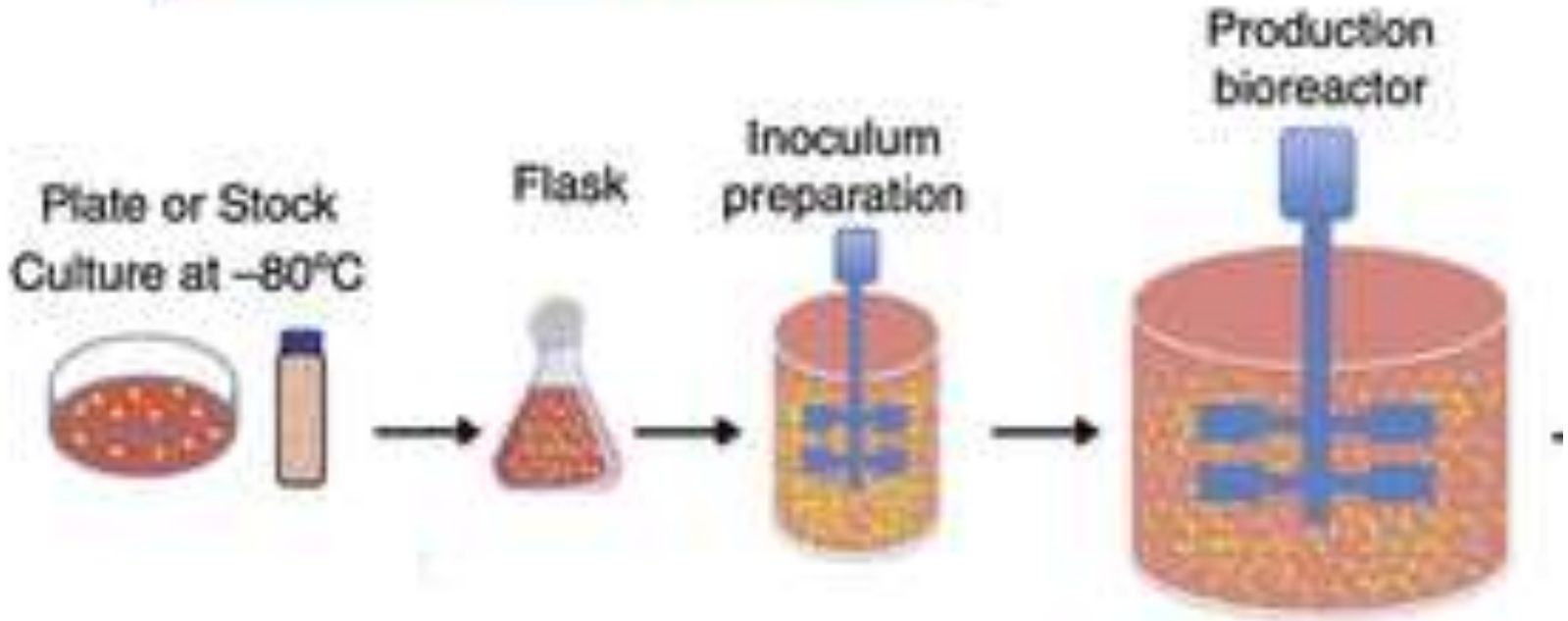
- Pilot scale means an experimental system that represents the part it corresponds to in an industrial unit.
- A pilot plant allows investigation of a product and process on an intermediate scale before large amounts of money are committed to full-scale production
- It is not possible to design a large complex food processing plant from laboratory data alone with any degree of success

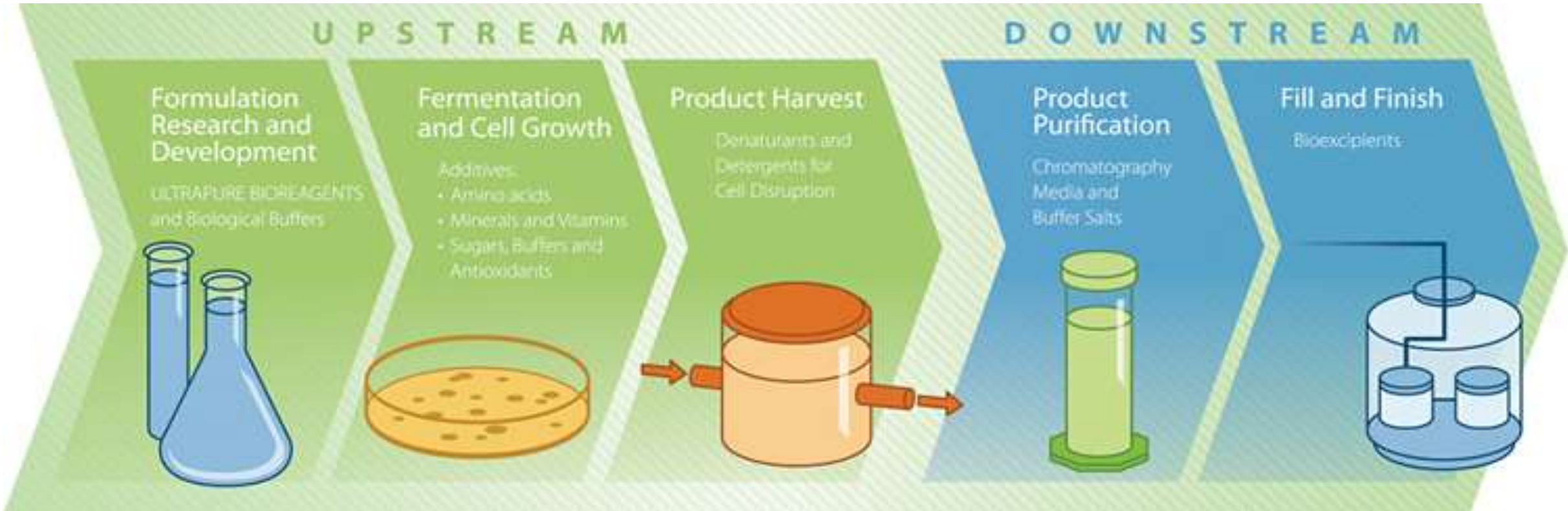
- Producing small quantities of product for sensory, chemical, microbiological evaluations, limited market testing or furnishing samples to potential customers, shelf-live and storage stability studies
- Determining possible salable by-products or waste stream requiring treatment before discharge
- Providing data that can be used in making a decision on whether or not to proceed to a full-scale production process and in the case of a positive decision, designing and constructing a full-size plant or modifying an existing plant

Requirements for scale up process

- Personnel Requirements
- Space Requirements
- Administration and Information Process
- Physical Testing Area
- Standard Pilot-plant Equipment Floor Space
- Storage Area
- Review of Formula
- Raw materials
- Equipment
- Production rates
- Process Evaluation
- Preparation of Master Manufacturing Procedures
- Product Stability and Uniformity

UPSTREAM PROCESS




UPSTREAM
DOWNSTREAM
Formulation Research and Development
ULTRAPURE BIOREAGENTS and Biological Buffers
Fermentation and Cell Growth
Additives:
• Amino acids
• Minerals and Vitamins
• Sugars, Buffers and Antioxidants
Product Harvest
Denaturants and Detergents for Cell Disruption
Product Purification
Chromatography Media and Buffer Salts
Fill and Finish
Bioexcipients

Bioreactor Scale-Up

- Bioreactors play an important role in many industries, including fermentation, food, pharmaceuticals, and wastewater treatment.
- Recently, the major challenge of bioreactor scale-up is how to translate the laboratory-scale product designs into large-scale production. During the scale-up of cell culture processes, reduced productivity has often been reported, which can be attributed to any of a couple of factors, including shear stress, oxygen supply, and gas composition.

Preparation of the Inoculum

- The microbial inoculum has to be prepared from the preservation culture so that it can be used for the fermentation. The process involves multiple steps to ensure maximum yield.
- First-generation culture is prepared from the preservation culture on agar slants, which is than subcultured to prepare working culture. At this stage, microorganisms start growing. In small fermentation processes, working culture is used as an inoculum, but for large-scale fermentation, inoculum preparation involves additional steps. Sterile saline water or liquid nutrient medium containing glass beads is added to the agar slant and shaken so that a microbial suspension is obtained.

- This suspension is transferred to a flat-bed bottle, which contains sterile agar medium. The microorganisms are allowed to grow by incubating the bottle. Then, the microbial cells from flat-bed bottles are transferred to a shake flask containing sterile liquid nutrient medium, which is placed on a rotary shaker bed in an incubator.
- The aeration helps microorganisms to grow at a rapid rate. The purpose of this step is to increase the microbial biomass, which influences the final yield of the fermentation process because yield is defined as a ratio of biomass to mass of substrate.

Inoculum Development Process for Fermentation

➢ **Strain Improvement :-**

- The yield of products will be much less when naturally available microorganisms are used. Providing optimum growth conditions increases the yield marginally. Therefore, to increase the productivity of microorganisms, it is necessary to modify their genetic structure.
- Change in genetic structure also influences the culture medium and nutritional requirements. Genetic changes in microorganisms can be induced by various methods such as improvement of a classical strain by mutation and selection or by the use of recombinant DNA technology.

➢ Mutation :-

- Every time a microbial cell undergoes division, there is a little probability that the strain may undergo mutation, which alters the genetic makeup of the wild-type strain making it mutant. The probability of mutation can be increased by exposing the wild-type strain to mutagenic agents such as nonionizing radiations (ultraviolet (UV) rays), ionizing radiations, and various chemical agents such as nitrous acid etc.
- However, the exposure of the microbial population to the mutagen dosage results in death of a large number of cells. The survivors may still include some mutants that produce low levels of the industrially desired product. Out of the entire microbial population, there may be a very small population of survivors that are improved with respect to the production of the desired metabolite after the exposure to mutagenic agent.

➢ Recombinant DNA technology :-

- Recombination is a process, which aids in the generation of new combinations of genes that were originally present in two different organisms, done either in vitro or in vivo.
- Protoplast fusion, which is a type of recombination done in vivo, has been achieved in filamentous fungi, yeast, and bacteria, which has greatly enhanced the properties of the resultant recombinant. In vitro recombinant DNA technology has been applied to organisms such as *Streptomycetes* and filamentous fungi for the improved yield of the final fermentation product. In vitro DNA recombination has contributed to the development of improved strains for the production of important antibiotics in the field of medicine.

➢ Selecting natural variant :-

- Microorganisms undergo a slight genetic change with every cell division. After several divisions, the culture medium includes microbes with a wide range of genetic structures. From these varieties, maximum yielding strains can be selected for fermentation.

Monitoring Inoculum Development

- Standardization of culture conditions and the monitoring system is required for determining the optimum transfer time, to maintain proper physiological conditions, and the optimized production process.
- Biomass is a key factor in the fermentation process, directly influencing the performance of the fermentation system as well as the quality and yield of the product.
- Biomass levels can be measured by monitoring parameters such as packed cell volume, dry weight, wet weight, turbidity, respiration, residual nutrient concentration, and morphology.
- A new generation of highly specific biosensors has been developed by interfacing the immobilized enzymes with electrochemical sensors, that is, glucose and sensitive alcohol electrodes. For example, a glucose sensor determines concentrations based on the glucose oxidase enzyme.

- Control of particular parameters involves a sensor, which can measure the property, and a controller, which compares the measurements with a predetermined set point and activates equipments to adjust the property back toward the set-point.
- The adjustment usually involves the modification of a valve opening or pump settings. Sensor may be online, that is, connected to the fermentor installation or in contact with the process stream, or off-line, where a sample is aseptically removed for analysis. Commonly online sensors are used for the physical measurement of temperature, pressure, impeller rpm (rotation per minute), liquid and gas flow rate, and for the physiochemical measurement of pH and gas concentrations in the liquid and gas phases.
- Computers can be used in fermentation processes to log data coming from sensors. They can analyze or process the data, present the analysis on display devices, and store it or use it for process control by activation switches, valves, and pumps. Fully computerized integrated fermentation system requires detailed process models, which can detect and report changes in the culture conditions that may influence cell physiology and productivity.

Transfer of Inoculum to the Fermentor Vessel or Scale-Up Process

- The inoculum is aseptically transferred to the final fermentor. For this, the inoculum (which should normally be no more than 5–10% of the total culture volume) is transferred to a sterile, disposable syringe of suitable size.
- The syringe needle is quickly pushed through the membrane and the inoculum is transferred into the vessel. The vessel is actively aerated to minimize the chance of getting contamination into the vessel. The syringe is quickly withdrawn and the silicon membrane reseals.
- During the scale-up process the when the microorganisms are transferred from small to large system in the subsequent processes, there is a difficulty in maintaining homogeneity in large systems.
- This may be due to the changes in surface-to-volume ratios or alterations in the culture itself as a result of increased culture time. Also in the scale-up process, many factors influence bacterial growth and yield of the fermentation process oxygen supply and heat removal are the key factors.

Effects of Process Parameters on Biological Performances

- The main objective of bioreactor selection, design, and control is to provide the optimal environment for a biological reaction system. The bioreactor should provide optimum conditions (e.g., temperature, pH, oxygen transfer, mixing, and substrate concentration), in addition to its basic function of containment.

➢ Temperature

- Temperature is one of the most critical parameters to be closely controlled in a bioreactor.
- Microorganisms are often classified according to their growth temperature as either thermophiles (growth temperature: >50 °C), mesophiles (growth temperature: from 20 to 50 °C), or psychrophiles (growth temperature: <20 °C) [17].
- Regardless of the microorganism type, microorganisms always have a quite narrow optimal temperature range for growth. If grown at a temperature below the optimum, growth occurs slowly resulting in a reduced rate of cellular production and product synthesis. On the other hand, if the growth temperature is too high, not only will death occur, but protein expression or metabolite synthesis will also be seriously affected, lowering product yield or affecting product quality

➢ pH

- Different biological systems have different optimal pH ranges. Most microorganisms grow best between pH 5 and 7. During fermentation, pH can change. As the cells grow, metabolites are released into the medium; substrate consumption also causes pH change. A number of researchers have investigated the effect of pH on the growth kinetics of microrganisms, enzymatic activities, and product synthesis.
- In animal cell culture processes, culture pH is often controlled by the addition of an alkaline reagent, such as $NaHCO_3$ or NaOH, to neutralize the acidic effects of lactate and CO_2 production during cell growth. Another scheme for pH control in animal cell culture process is CO_2 addition. CO_2 is added to a sodium bicarbonate-containing medium in order to control the pH.

➢ Oxygen Transfer

- Oxygen transfer is always a concern in aerobic biological systems. Most nutrients required for cellular growth and metabolism are highly soluble in water; sufficient and timely supply of these nutrients can be achieved in a well-mixed bioreactor. However, oxygen transfer often becomes a limiting step to the optimal performance of biological systems and also for scale-up because oxygen is only sparingly soluble in aqueous solutions. When the supply of oxygen is limited, both cell growth and product formation can be severely affected

➢ Mixing

- In bioreactors, adequate mixing is essential in order to ensure the adequate supply of nutrients and to prevent the accumulation of toxic metabolites. For a bioreactor designed for a suspension system, mixing time is a critical parameter to be studied and evaluated. The fluid hydrodynamics, fluid rheology, impeller type, power input, and vessel size can all influence the mixing conditions.


THANK
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