Scale up of Fermentation Processes

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Introduction

- Two of the most common phrases one often met in fermentation technology research is 'Scaling up' and 'Scaling down' studies.
- However, the phrase 'scaling up' is more commonly understood and practiced during the designing of industrial scale fermentors.
- Where as 'scaling down' studies are rarely heard that frequent.
- In reality many fermentation technologists are not aware that during most times of their work they are doing 'scale down studies‘.
- Maybe the phrase 'scale up' has more impact factor than 'scale down' studies.

Why Scale Up/Down?

- The process of generating product in increased quantities can take the form of setting many replicates of the cultivation system originally used for detection or induction.
- Conversely it could require establishing an efficient process in a larger volume vessel optimized for cultivation in larger volumes, e.g. a stirred tank reactor.
- In contrast, once a product has been commercialized, there may be a need to investigate changes observed or that require introduction at the large scale, and for economic, strategic or environmental constraints unable to be studied or investigated at this scale.
- A scaled-down model of the production process is an extremely cost effective, environmentally aware, option for exploring potential change options in a production operation.
- The intermediary activities translating a successful bench product to production scale, or alternatively a system for translating a production process back to bench scale, is the concept of scale up/scale down, tending to involve microtitre plates, shake flasks, laboratory, pilot and production scale equipment.

Scale Up

- Cambridge Dictionary defines scale-up as increasing something in size, amount, or production.
- Microbial processes involve cultivation of microbes in bioreactors (also referred to as fermentors) to produce a product, as well as the subsequent recovery and purification of the product and disposal of associated wastes.
- Scale-up of microbial processes is undertaken typically for a commercial purpose, specifically to provide product benefits to customers and to generate a financial return for investors.
- A process developed in a laboratory (e.g. 0.5–10 L fermentors) must be translated into a full manufacturing scale process (e.g. 20 000–2000 000 L fermentors), with scale factors ranging anywhere from thousands to millions.

INITIAL SCALE UP STUDIES

- Most scale up studies are usually carried at different phases involving different scales of fermentors.
- Preliminary work are carried out at the level of petri dishes and small scale laboratory fermentors to establish whether the process is:
- **Technically viable**, meaning it is possible to produce such fermentation process and the products on the small scale.
- Additional parameters not provided by petri dishes studies and for more confidence are obtained by carrying further studies using submerged liquid fermentation using various sizes laboratory scale fermentors and even a pilot plant fermentor.
- **There are a few rules of the thumb followed when doing scale up studies such as:**
- Similarity in the geometry and configuration of fermentors used in scaling up.
- A minimum of three or four stages of increment in the scaling up of the volume of fermentation studies.
- Each jump in scale should be by a magnitude or power increase and not an increase of a few litres capacity.
- Slight increase in the working volume would not yield significant data for scale up operation.

… INITIAL SCALE UP STUDIES

- The exercise in scaling up involved a number of programmed research or steps that has to be established so as to predict the final behaviour of the final large scale production fermentor.
- Studies carried out during scale up include:
  - Inoculum development
  - Sterilization establishing the correct sterilization cycle at larger loads
  - Environmental parameters such as nutrient availability, pH, temperature, dissolved oxygen, dissolved carbon dioxide
  - Shear conditions, foam production

Variables to be Considered when Changing Fermentation Scale

Aeration and Agitation

- Aeration provides two related functions in an aerobic fermentation process: (i) to provide mixing, and (ii) to supply oxygen.
- ***Microtitre Plates and Shake Flasks***
- In these cultivation systems oxygen is present in the gaseous phase above the liquid surface, and transfer is by diffusion through the gaseous phase, through the gas/liquid interface and into solution.
- The demand for oxygen by respiring organisms provides the continuous diffusion gradient maintaining supply by this method, and agitating the surface helps to prevent stationary boundaries developing and promotes diffusion and mixing in both gaseous and liquid phases.
- ***Stirred Tank Reactors***
- In stirred tank reactors, supply of air is optimized to provide maximum gas transfer by the use of shear, power and turbulence, to maximize the gaseous surface area to liquid volume ratio and gas velocity.

  ***However, as small bubbles have less buoyancy than large bubbles, they tend to rise more slowly having a negative impact on gas transfer by reducing gas velocity, but also a positive impact on gas transfer as surface area/volume ratio is increased.**

... Variables to be Considered when Changing Fermentation Scale

- Some production operations employ bubble columns at the production scale.
- These fermenters are extremely energy efficient, low shear vessels, but are typically not directly scaleable from stirred tank reactors, as shear is significantly reduced and superficial gas velocity plays a much greater role in gaseous transfer processes.
- In spite of some scaling limitations, however, it is typical to use a standard scaling route using stirred tank reactors at the laboratory and pilot scale, merely adjusting to equipment constraints when the process reaches production scale.
- **Nutrient transfer and product transfer** would be expected to follow similar principles to oxygen delivery.
- Therefore, if oxygen transfer is used as a focus for optimizing equipment, it would be expected that other transfer steps could be similarly modelled on the same principles.

Sterilization of Fermentation Media and System

- Batch thermal sterilization processes tend to be used for liquid and equipment in fermentation systems, as these tend to be reliable and cost-effective options for both small and large scale systems, and there is a degree of confidence in being able to assure sterility in a batch operation.
- Continuous sterilization of medium can be used for fermentation systems, but it tends to be the result of specific process benefit.
- Example, reduction in utilities or specific nutrient characteristics, which are provided by continuously sterilised media.

Small Scale Fermentation Systems

- Typically sterilization methods for small scale cultivation systems (equipment and/or media) rely on the use of autoclaves.
- Chemical degradation can be limited by sterilizing vulnerable components separately (e.g., glucose sterilized separately from amino nitrogen sources can limit degradation and the generation of Maillard compounds – to which some fermentation processes can be extremely sensitive).
- Chemical degradation during sterilization may lead to significant pH changes, which may require adjustment after sterilization to bring the pH into a more useful range.

... Sterilization of Fermentation Media and System

- High value or sensitive products (e.g., antibiotics required for retention of a plasmid) may usefully be filter sterilized rather than exposing them to heat and potential degradation.

Pilot and Production Scale Fermentation Processes

- Fermenters greater than 5 lits in volume are sterilized in situ using live steam injection.
- Often, as processes move from laboratory autoclaves to pilot vessels, differences in process performance are observed, due to chemical changes affecting medium components during the sterilization process.
- If the differences are found to be significant, then often the best way of offsetting these changes is to record each sterilization process and adjust to longer or shorter cycles (if possible) and observe the impact.
- There are also useful scaling parameters that can be directly applied if computer data logging is available to generate sterilization curves; a particularly useful set of parameters to use for scaling sterilization is the concept of *Fo/Ro.*
- *Fo is the integral of sterilization* from 90 °C where it is considered that significant heat degradation of components starts to occur, and *Ro is the integral of sterilization from 120 °C, where it is considered that* significant biological 'kill' starts to take place.
- For a process susceptible to heat degradation of media, a low *Fo/Ro would be preferred, whereas for a process that benefits from* some heat degradation of media, a longer *Fo/Ro would be required.*

Inoculum Development and Culture Expansion

- In inoculum generation it may be relevant to explore any of the variables listed below for optimizing biomass yield and generating biomass suitable for producing product.
  - Inoculum type (e.g., vegetative cells, spores, pregerminated spores). Extremely different results can be generated depending on type and number of inoculum points)
  - Cultivation temperature.
  - Agitation/mixing rates
  - Medium development.
- Once an inoculum process permitting productivity has been developed, there may be a need to expand it – introducing another transfer step to a culture expansion or 'seed' vessel, with the aim of increasing biomass in the vegetative phase to sufficiently larger volumes to use a sufficient volume of mature inoculum usefully to inoculate a production vessel.
- Traditionally it has been recommended that attempts be made to get as close as possible to transfer of 10% by volume of inoculum into a final stage production scale, so maximizing equipment utilization at the largest scale of operation.

Raw Materials and Nutrient Availability

- Media invariably change during scaling.
- The constraints of microtitre and shake flask cultivation systems introduce compromises that can change once the process is operating in stirred tanks.
- For example, particulate containing media can be used and pH control becomes possible in stirred tanks.
- In addition, in making changes of scale there may be changes due to chemical change during sterilization and the opportunity to intensify the process by feeding concentrated nutrients to sustain phases of productivity.
- A medium that is useful for producing product in small vessels may not be viable from a cost basis at the large scale.
- A cheaper raw material options supplying a similar mixture of vitamins, minerals and cofactors may require evaluation at pilot tank scale; e.g. testing corn steep liquor, dried yeast, or mixed bulk protein sources as a replacement of yeast extract.
- It becomes possible to start feeding nutrients to a stirred tank reactor and prolong and intensify the productivity of the process, as final yields in excess of twice that achievable typically from batched materials becomes possible.
- This is one of the huge benefits of scale up: that productivity increases can be achieved by focusing on the biochemistry and induction kinetics of the organism of interest.

pH

- pH tends to be a sensitive fermentation output variable and potentially a controlling parameter, so it is therefore valuable to track pH at all stages of a scaling operation as it can be a route for optimization.
- Consideration should be given as to whether an inorganic buffer (e.g., phosphate) could be useful, or whether it may interact with an inducible process, particularly relevant for secondary metabolite processes that may rely on nitrogenor phosphate derepression for biosynthesis.
- pH changes can be the result of degradation or uptake processes, e.g. proteolytic breakdown can result in release of ammonia, causing pH increases.
- Carbon source supplied in excess in some fermentations can lead to
- the conversion of the carbon source into organic acids using the TCA cycle, with the organic acids being excreted into the growth medium. When carbon sources become limiting, the organic acids are then consumed, altering pH

Shear

- Shear tends to be low in shake flask cultivation systems, increasing dramatically when a process is scaled to stirred tank reactors, as these are designed to be high shear mixing systems for optimal gas transfer.
- Shear rates can also change during a fermentation, if the organism produces a polymeric product such as, for example, *Xanthamonas campestris, producing xanthan gum, which* shows pseudoplastic tendencies, or if it produces enzymes which can degrade viscous substrates e.g. pectinases from some filamentous fungi such as *Cochliobolus sativus.*
- For unicells such as *E. coli, fermentation processes tend to be described as shear insensitive,* whereas shear sensitivity may be experienced in cultivation of eukaryotes and filamentousorganisms.
- In the worst case, cells are friable and lyse in a high shear environment.
- Shear requirements and responses can therefore generally be considered culture and cultivation system specific.

Temperature Maintenance

- At a small scale venue, biomass quantities are relatively low and heating can be supplied relatively easily to an incubator or stirred tank reactor.
- However, as reactor volume increases, so significant heat is generated during aeration and agitation.
- Processes are intensified, often biomass increases are achieved, and although the specific heat output may stay the same, the overall cooling requirement would increase due to an increase in biomass density.
- Cooling capacity can be delivered to a large fermenter by using a jacketed vessel supplied with cooling or chilled water, or the vessel may have internal cooling coils.
- Or the vessels can be sited in the open air and be sprayed with water to cause cooling by evaporative loss.
- In addition, if cooling capacity is limited, then it is valuable to introduce temperature as a variable in a scale-up process or to get some estimations of cooling requirements for changes introduced at the small scale.
- It has been proposed that the use of thermotolerant organisms for production processes could alleviate this constraint.

Interior of a large scale production vessel illustrating the use of internal cooling coils for providing cooling capability

Partial Pressures

- In larger fermenters, back pressure tends to be used to help protect the sterile envelope of the fermenter.
- In addition, in production scale venues of 100 000 litres plus, hydrostatic pressure at the base of the fermenter can be significant.
- Both of these pressures would tend to influence gaseous partial pressures in the liquid medium, potentially changing gaseous gradients.
- For oxygen, typically slight increases in vessel overpressure would not be expected to have a significant effect on metabolism, but if the organism is sensitive to a gaseous product that can dissolve in broth, then unexpected events can be observed.
- Typically carbon dioxide is the molecule most often observed as having an adverse impact on processes.

Shake Flask Scale Process Stage (50–500 mL)

- The shake flask scale is ideally suited to evaluating:

  (i) Temperature ranges (temperature of incubator)

  (ii) Responses to high and low agitation conditions (speed of shaking)

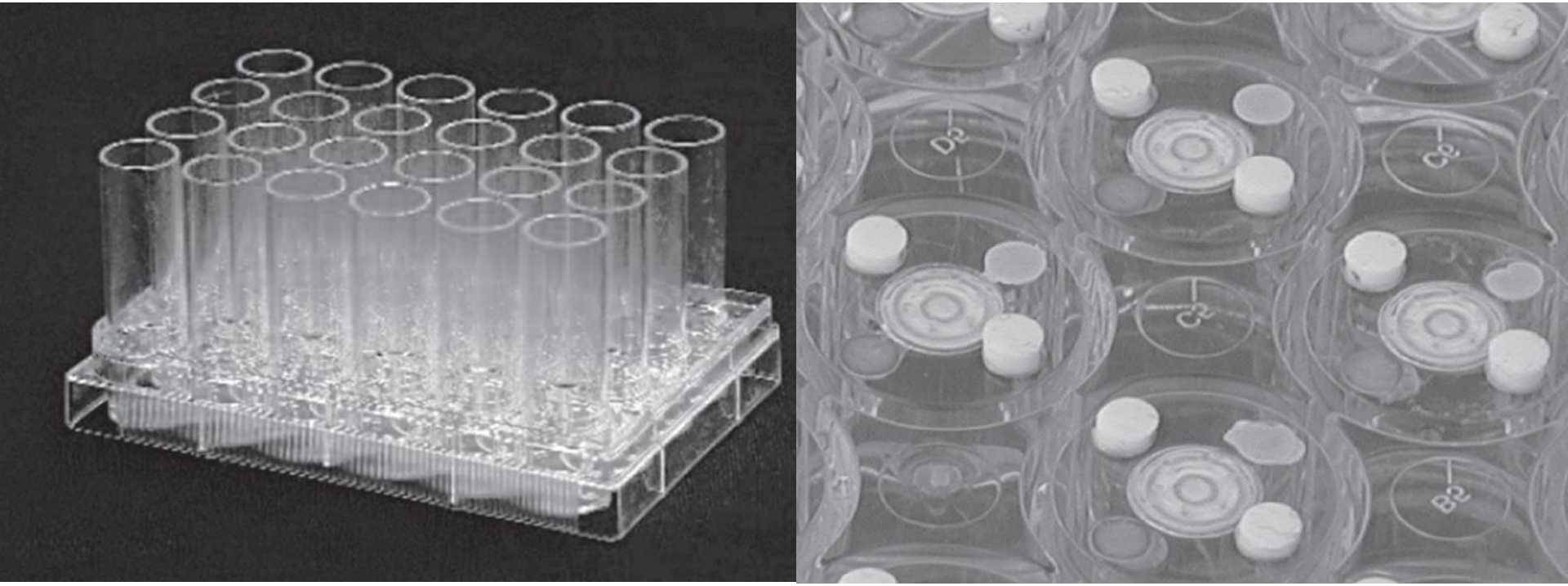
  (iii) pH

  (iv) Range of nutritional responses on a media using either inorganic, organic or mixed sources of carbon and nitrogen.

  (v) Inoculum preparation

Minifermenters

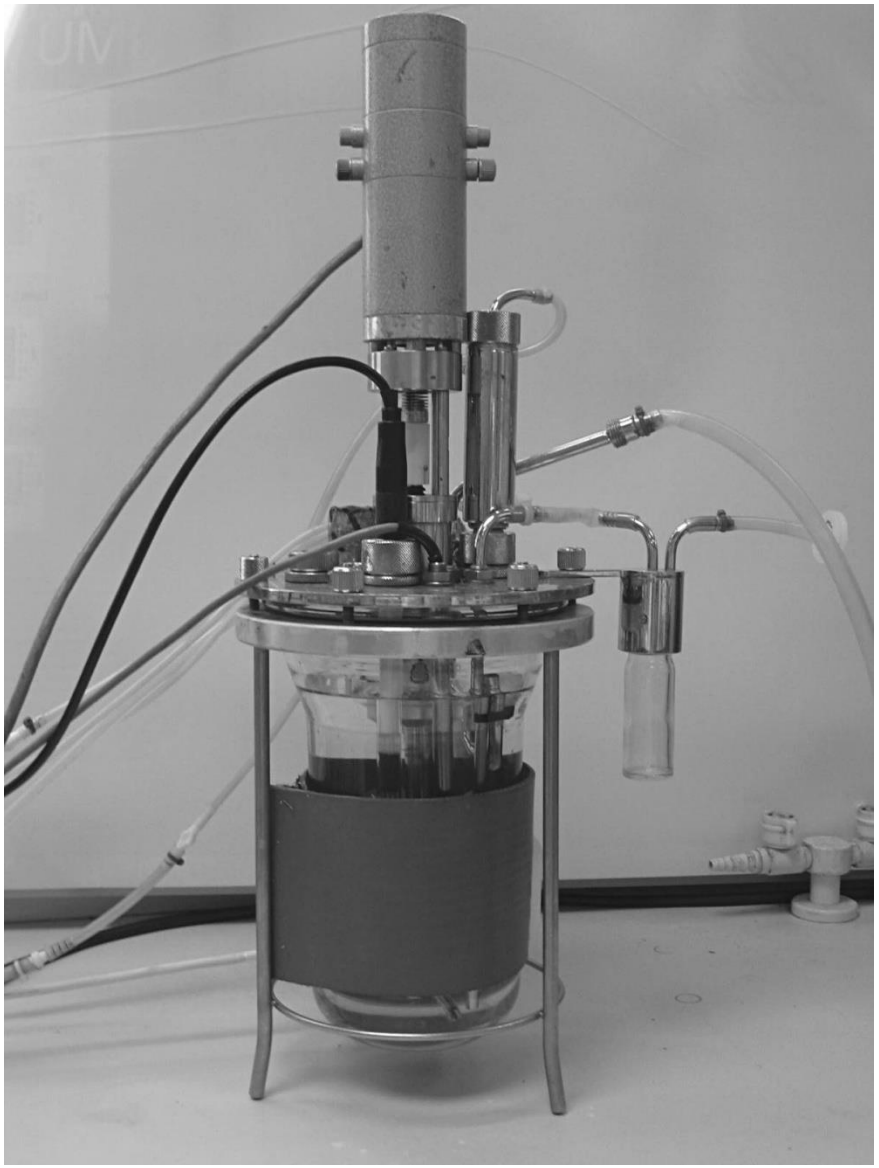
- This is a technology untested by the author, but showing promise as an investigational and scaling venue.
- It has some of the benefi ts of microtitre plates, shake flasks and laboratory fermenters combined into an array of 5 × 10 minifermentation units of 10 ml volume capable of running with stirred agitation, independent temperature and pH control.
- It is showing promise as a venue for exploring a range of variables rapidly, and with minimal equipment and medium components prior to using stirred tanks for further evaluation.



Photograph of the minifermentation system

Stirred Tank Reactor Process Stage (1 litre – 500 + litre)

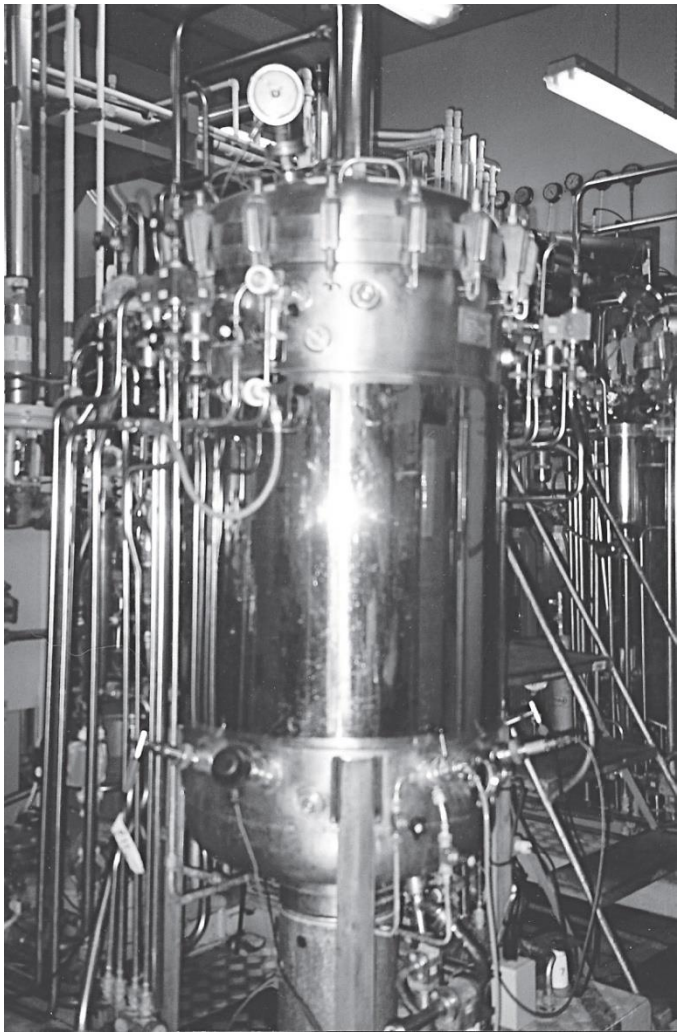
- *Laboratory fermenters Typically these are of the order of 500 ml–5 litre working volume* equipped with agitators, temperature control and are sparged with air.
- Laboratory fermenters are a more sophisticated version of the shake flask system with the following benefits:
- (i) Increased *KLa over shake flask; typically being in the range 20–100 times increased* volume over shake flask, permitting sampling.
- (ii) The option of controlling pH using acid and base.
- (iii) The option of feeding nutrients, typically carbon or nitrogen sources, using continuous or shot-fed/ramped feed designs.
- (iv) The option of using off-gas monitoring.
- (v) The option of computer control and data logging.
- This still differs from a typical production scale system in that medium is sterilized by autoclave rather than the more usual *in-situ live steam injection used on production* scales.



Photograph of laboratory fermenter

Pilot-scale fermenters (in-situ sterilizable)

- Pilot-scale fermenters usually tend to have working volumes of 20, 100, and 1000 litres.
- A pilot scale up stage can be valuable for establishing which, if any, medium components are critical for the fermentation, for identifying, defining and exploring critical process parameters.
- Typical physical and business risk areas can include dimensions of cooling capacity constraints, foaming considerations for optimized media, product chromatographic species distributions for product, and conformation considerations for expressed proteins.
- Risk factors for high value products or biotech products may differ from those of natural products and it could be valuable to share information describing potential process options relatively early in the pilot evaluation, in order to gather suggestions and commitment to problem solving or developing loss prevention strategies.



Pilot-scale fermenter

Photograph of production fermentation facility used for expressing recombinant protein in an *E. coli* expression system