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A Review Scale Up Fermentation Procedure

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ABSTRACT

Due to the larger size and scale of industrial fermentation setups it is vital to succeed beforehand studies of possible complications, due to increase in scale, and to sort out ways to tackle them. Scale up studies are executed at the laboratory or pilot plant scale to generate data that can be used to anticipate and construct the large scale industrial fermenters with ample confidence that it would function according to all its expected behaviors. Hence, scale up studies not only help understanding the technical components of a large-scale fermentation setup but also reduce the economic risk involved in the case of direct investment on large scale production.

Keywords : Fermentation, Scale Up, Pilot Plant Scale, Scale Down

I. INTRODUCTION

Industrial fermentations use the process of fermentation to produce the desire product from the microorganism by using their living cells [13]. To increase product yield and to ensure consistent product quality it is vital to maintain optimum and homogenous reaction conditions, minimizing microbial stress exposure and stabilizing their metabolic activity [18]. The process of fermentation is first designed at laboratory level but the actual industrial scale production means quantitative increase of several thousand liters as compare to laboratory fermentation. So, it is necessary to perform scale up experiments before proceeding to full scale plant installation. However, this increase take place in different phases i.e. laboratory scale experiments lead to pilot scale experiments that in turn leads to industrial production which is called scale up [8]. Fermentation techniques are formulated using flasks and small lab scale fermenters. However, there use to be a significant difference between designs and efficiency of small and large-scale fermenters which calls for determination of proper incubation conditions that are needed to employed at large scale production tanks as based on information obtained from experiments done with various small fermenters. The fermentation is started from laboratory scale where pure and analytic grade chemicals are used, conditions are controlled carefully and fermentation parameters are maintained appropriately. However, at the industrial level managing all these expects effectively becomes nearly impossible which calls for fine tuning of these parameters. A direct changeover from a laboratory scale experiment to industrial production scale may give rise to results against the expectation which means a huge financial loss. For instance, most of industries use media that is crude and unprocessed with variable chemical composition so production flops if fermenting organism fails to use such media and grow. Scale up means to increase the volume. This procedure aims to increase the scale of fermentation (volume) without compromising yield or if reduction in yield occurs then fermentation technologist should able to identify the factors that contribute to decrease and to rectify it [20].

II. SCALE UP CONSIDERATIONS

The speculations that must be considered while developing the process of higher fermentation productions are discussed below;

A. Inoculum development

An inoculum used for fermentation should be in its active state so that length of lag phase remains short in the subsequent fermentations. Inoculums must have been available in sufficiently large volume within its suitable morphological form. It must be contamination free and with product forming capabilities retained. The process that can be adopted to produce such inoculums, that meets forth mentioned qualities, is known as inoculum development. Inoculum development is equally important regardless of its scale as shown by a quotation of Hockenhulll "Once fermentation has been started it can be made worse but not better" [3]. At industrial level relatively, large inoculum volume is used to keep length of lag phase short so that maximum biomass can be produced in shorter time period. So, it means that inoculum has to be developed in a number of stages where two or three steps are performed in flasks then next two or three in seeding fermenters before inoculum is finally added to pilot fermenter or industrial fermenter. Number of stages or steps in between depends on size of fermenter. However, the more the number of stages between master culture and production, fermenter will involve the greater risk of contamination and stain degeneration will be persisting [21].

B. Sterilization

Sterilization in a process of fermentation means using contamination free inoculum, sterilization of media, vessels and materials used in process and maintaining aseptic conditions during fermentation process. However, at large scale achieving sterilization becomes difficult due to larger size of vessels and bulk amount of media. It becomes important to select suitable technique for process within economical limits. For example, for media sterilization there are different treatments available like heat treatment, Ultrasonic treatment or chemical treatment etc. However, besides use of steam, as it is used for sterilization of most of the media, filtration is used for sterilization of media for animal cell cultures and other heat labile media. Filtration adds extra expenses in the overall cost of production that must kept in mind while using filters at higher scales [21].

C. Cleaning

The cleaning of fermentation vessels is important for prevention of blockage and continuous functioning of fermenter which becomes more extensive due to increase in size of fermenter. Mostly the distilled water is used to remove loose residual cultures and other remains. After washing vessels are immediately dried. However, during cleaning examination of fermenters can be done by checking presence of chips, cracks, rust or any other damage [1].

D. Environmental parameters

The surrounding environment of an organism may change with the change in scale of production. Significant gradients of different parameters such as dissolved oxygen, pH etc. can be observed in number of industrial scale fermentation setups [9]. It means that the cells that are fluctuating in a large volume reactor may therefore experience noticeable changes in micro-environment especially in the case of aerobic fermentation [2]. The parameters which may bring such changes are summarized as follows:

- ✓ Nutrient availability
- ✓ pH
- ✓ Temperature
- ✓ Dissolved oxygen concentration
- ✓ Dissolved carbon dioxide concentration
- ✓ Shear conditions
- ✓ Foam production

There are two basic factors that affect all parameters mentioned. These two parameters are agitation (in term of bulk mixing) and aeration (in term of oxygen provision). The parameters such as Nutrient availability, pH, Temperature and shear conditions are dependent on bulk mixing (agitation) while parameters such as dissolved oxygen concentration, dissolved carbon dioxide concentration and foam production depends on air flow or oxygen transfer rate (aeration). It means that agitation and aeration tend to be dominating in discussions about scale up procedures. However, decrease in yield due to difficulties in sterilization and inoculums development can also not be ignored [21].

E. Scale up of aeration and agitation regimes in stirred tank reactors

Fox (1978) explained aeration and agitation problem during scale up in a detailed manner. He purposed "Scale up window" to illustrate issues of aeration and agitation. The Scale up window can be used to represent respective boundaries that are imposed by the environmental parameters and cost of the aeration, agitation regime as shown in figure A. A range of aeration and agitation combinations can be used to obtain suitable conditions for mixing and oxygen transfer. The two axes shown in the figure are agitation (y-axis) and aeration (x-axis) while the zone lying within hexagon represents acceptable aeration and agitation regimes [21].

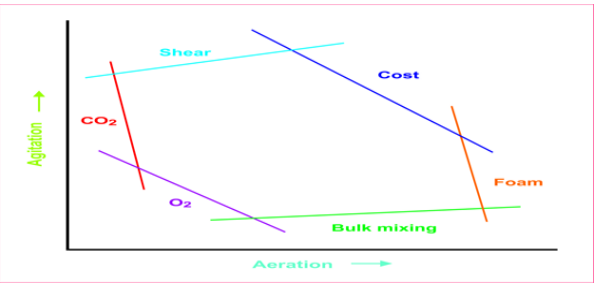

Figure 1. Scale up window [5][11]

The boundaries of hexagon define limits of oxygen supply, carbon dioxide accumulation, and damage of cells due to shear forces, foam formation, cost and bulk mixing. The rate of agitation and aeration should lie within its maximum and minimum values. For instance, the lower limits of aeration are determined by oxygen limitation and carbon dioxide accumulation while upper by foam formation which may become hard and block fermentation vessels in later stages of fermentation. Due to transport limitations in large scale fermenters the oxygen consumption rate may exceed its transportation rate, which might result in depleted oxygen supply. Such issues are most common in fed batch cultivations during aerobic fermentation [12]. Some bacteria may recognize such gradients within few seconds only [19] For example; *E.coli* recognizes oxygen depletion in the time period as short as 13 seconds and start responding by producing undesirable by products [21]. Effect of verified stirrer speed, time of cultivation and type of organism on oxygen gradient was demonstrated by Larsson *et al.* in 1996. The pH gradient may also lead to reduced cell viability. For example, the pH gradients may cause the fermentation acids produced to be driven back in to the microbial cells. Inside cells these acids produce protons in the intracellular expanse so that it hinders normal cellular functions hence disturb viability of microbial cells [4].

III. SCALE DOWN

As the conditions achievable at the laboratory level are impractical at large scale that's why scale down operations, which means are laboratory or pilot scale experiments conducted under conditions, that virtually mimic the conditions occurring at industrial level, are used to study yield objectives at industrial level. It means that scale down operation is actually the part of scale up studies. This approach not only helps development of a new product but also to improve existing fermentation product at its full scale. The procedure of scale down operation is well reviewed by Jem [7].

Aspects that are contemplated while designing laboratory or pilot plant experiments in the context of scale down operations are discussed as follows:

A. Media design

The media used in experiment should be relevant to media suitable for industrial level. However most of the time media from cheaper recourses are preferred to keep cost low. However, proper optimizations of such media are compulsory before application at large scale levels. Different fermentation media have different objective for example if our desire product is primary metabolite or biomass then target is maximum growth of microorganism but in case of secondary metabolite is not related to growth. Industrial fermentation media generally require a carbon source, nitrogen, phosphorus, sulphur and vitamins [17].

B. Media sterilization

Considering bulk amount of media that have to be sterilized at large scale demands for longer exposure time and higher temperature as compare to lab scale specially for batch sterilization. It means that time for sterilization at smaller scale must have to be increased to mimic requirements of industrial scale. Otherwise, media taken from industrial unit which are already functioning can be used for scale down studies. This approach can further add an advantage of highlighting situation under continues sterilization where loss of media quality could occur. However, continuous sterilization can itself be used for laboratory or pilot scale experiments.

C. Inoculation procedure

Inoculate of every fermentation is not necessary be under optimum conditions due to range of possible circumstances. That's why scale down operations can help to predict respective consciences of such events by mimicking them i.e. by using stored inoculums or inoculum of old ages.

D. Number of generations

Higher number of generations is required for completion of fermentation process at industrial level which may rises question on stability of strains used as inoculum. To mimic industrial conditions at down scale operation serial sub cultures can be used to ensure that strain remains suitably stable or not. It becomes more important when dealing with recombinant strains.

E. Mixing

Degree of mixing decreases with the increase in scale. Laboratory experiments can be modified by using pulse medium feeds or fluctuating process conditions such as oxygen concentration, pH and temperature which may allow predicting suitability of new strains for industrial exploitation.

F. Oxygen transfer rate

High oxygen transfer rates at laboratory level is easy to maintain as compare to industrial level that's why a scale down operation should reflect oxygen transfer rates achievable at full scale fermentation. In suspension of aerobic microorganisms, the concentration of dissolved oxygen depends on the rate of oxygen transfer from gas to liquid phase [6]. It is necessary as it will be unrealistic if fermentation is designed at high oxygen transfer rate that is difficult to achieve at industrial scale [21].

IV. PILOT PLANT STUDIES

A pilot plant can be referred as a model that mimics its commercial prototype fermenter. There are number of definitions suggested for pilot plants. Ogorzaly [14] defined pilot plant as "An assembly of equipment devoted to studying the critical features of process operation". An even general definition of pilot plant was given by Paluzzi [15] "A pilot plant can be referred as a tool that intends to allow investigation of a process or process related problems on a manageable scale in a more realistic manner and within manage able time. It is also defined as a part of the

pharmaceutical industry where it is used to produce viable product from a lab process [16].

The definitions clearly demonstrate that the pilot plant is not the end itself; however, it is a way to achieve a goal of installing full scale, commercial and productive fermentation unit. Furthermore, a manageable scale means limited time utilization, less risk of capital loss and security of other resources.

A. Important aspects of pilot plants

1. Size

Pilot plants can be divided into different classes on the bases of their size as demonstrated below.

1a. Bench tip pilot plants (micro units)

It includes pilot plants that may fit to bench top or inside a small laboratory. Generally, volume of fermentation under study use to be smaller than 1 L in a total ground area of 0.5 to 1.0 m^2.

1b. Research scale pilot plants

Such plants seem as the workhouse of the industry. However, the research plants may vary in size from several frames to a unit that may occupy the small size building. The tanks used in plants have volume around 4 m^2 occupying area of 2-15 m^2.

1c. Prototype units

These plants work at the scale close to actual industrial unit having very large tanks of 4 to 40 m^3 occupying area as much as 900 m^2.

2. Costs

The pilot plant uses to be generally expensive as same number of instruments is required for pilot plant as for a full scale industrial unit. However, for full scale industrial process some extra instruments and process controllers have to be installed. By using reduced scale pilot plants i.e. mini units the cost of a pilot plant can be cut off up to 40%. An important expects to be considered using reduced scale is the fact that the stability of process decreases due to difficulty in designing it by mean of process control and management. For instance, certain materials use to start accumulating in recycling stream, where such accumulations are not easily detectable at bench scale, which decreases quality of study done.

3. Time scheduling

The stage of industrial plan at which the pilot plant should be built remains an important question. Due to pressure of commercial competition the industrialists might look to reach production phase as soon as possible even sometimes by jumping to full scale and skipping pilot plant. A possible solution for this problem is to start pilot and full-scale plant at same time where pilot plant can be constructed in shorter time period so that the process can be studied before completing of full scale unit. However, it involves risk that if pilot plant tests prove selection of instruments, that would have been installed in full scale production plant also, wrong then a huge economical loss must be expected. The equipment with short installation and adjustment time may also help to start production in manageable time after testing pilot plant.

4. Flexibility

The pilot plant must be designed along with suitable flexibility. It means that there must be enough possibilities to take frequent samples and its analysis without disturbing basic process. Furthermore, a continuous supply of raw material and easy collection of products should be possible.

5. Recycle streams

To reduce the amount of waste and cost of a process the recycle stream should be part of design. However, recycling may have some adverse effects on process, i.e. fouling of equipment's, which means it should be intelligently managed.

6. Safety

Possible risks should be mapped before starting any pilot plants that are dealing with any harmful raw materials or products. An effective safety plan should available while working on such pilot plants.

7. Use of computers

There is an essential role played by computers in the operation of pilot plant. Most of the process controlling, online analysis and data processing use to b computerized. For example, use of computers to

control feed stream increase the speed of process so it makes feed stream more productive besides better control and safety. Computers can also be used to stimulate process by using model study. It means that if certain data relative to process is already available then it can be further used to make simple experiment sufficient enough to obtain the data on required parameters. It decreases the cost of product due to decrease in labour which may add a further advantage to list. However, a good computer model is that which provides a quick and simple stimulation of a process within least number of required experiments. Somehow, risk of premature and over enthusiastic use of models for scaling up can lead to wrong assessment of certain parameter. For example, complex process of large scale aerobic fermentation cannot be simply scaled up by computers directly [15].

V. CONCLUSION

Scale up studies must execute before constructing a large-scale fermentation unit to understand the technical components of a large-scale fermentation setup and to reduce the economic risks. To keep scale up studies within defined economical limits scale down operations are important as they mimic the challenges of large scale production displayed at the scale that is reduced and easier to manage. However, conducting such studies use to be a challenge over their own.

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