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Review Article

Biosimilars: Scale up from R&D to Commercial Stage

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ABSTRACT

The scale up of recombinant proteins involves a multi- dynamic strategic approach to achieve maximum output consistently. Various parameters during the fermentation will limit the synthesis of desired protein, which otherwise can be produced with ease in case of classical fermentation. In order to synthesis the protein, it is very important to understand the process at laboratory scale – both from scale up point of view and productivity improvement at large scale. Some of this parameters and scale up parameters are discussed below in this review.

Keywords: Biosimilars, Research, Recombinant Proteins, Market

INTRODUCTION

Production of recombinant proteins is considered as a growing market worldwide. Recombinant DNA technologies enable the production of a wide range of peptides and proteins with naturally non-producing cells. Examples of commercial recombinant proteins are biopharmaceutical protein products and industrial enzymes. Biopharmaceutical protein products include blood clotting factors, thrombolytic agents, hormones, growth factors, interferons, interleukin-based products, monoclonal antibodies and antibody fragments, vaccines, nucleic-acid based products, and therapeutic enzymes. Industrial enzymes are used for example for the treatment of food, feed, detergents, paper-pulp, or for the manufacturing of biomass based products. In addition to commercial products, production of recombinant proteins enables the wide field of protein research, including protein structural and functional research.

In research laboratories, recombinant proteins are mostly produced in milliliter scale in shake flasks. However, shake flasks are often not sufficient to produce enough recombinant products. Besides small scale cultivation methods, bioreactor cultivations are often applied after initial experiments. However, industrial production is usually done in large scale bioreactors. Therefore, process development in conditions mimicking large scale processes, and scale up of the developed recombinant protein process, are crucial if commercial production is the target.

The goal for optimizing production of recombinant proteins is to produce the highest amount of functional product per unit volume per unit time. For *E. coli*, or any other fermentation system, the level of intracellular accumulation of a recombinant protein is dependent on the final cell density and the specific activity of the protein, or, in other words, the level of accumulation relative to total protein. Four strategies are typically taken for optimizing the production of a recombinant protein. These are: choice of culture medium, mode of cultivation, strain improvement, and

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expression system control. Much of the effort aimed at increasing recombinant protein production in bacterial strains has been directed at maximizing the biomass production and little is known about the effects of media composition on the expression of recombinant proteins. However, it is well known that the production of secondary metabolites in microbial strains can depend on the composition of the medium in which the organism is grown. Despite this, little attention has been paid to the effects of medium formulation on the accumulation of recombinant proteins.

Statistical methods for developing the best medium formulation for maximizing the production of metabolites are a well-established practice. The basic approach used to develop an optimal medium formulation relies on empirical and trial-and-error processes. The use of statistical techniques for experimental design has provided a more elegant means of designing the best medium. Nevertheless, a rigorous and extensive research program is needed to devise the most suitable medium for the production of a given recombinant protein.

The first step in developing the optimum formulation is to screen for the critical components. These are the factors which affect the production of the desired product. There are two experimental designs that can be applied. The first is a Plackett-Burman design. This is a 2-level matrix in which each factor is tested an equal number of times at its high and low values. Further, the design is balanced in that each factor is tested against the high and low of the other factors an equal number of times. This design is good when the number of factors is less than five and is well suited for detecting significant factors. It does not permit a test of interactions. The second type of experimental design used for screening factors is the so called fractional factorial design. This is again a 2-level matrix design which can measure interactions. The factors are set at two levels, a high and a low, and consists of 2^{k-p} trials where k is the number of factors and $(1/2)^p$ is an integer of the fraction of full factorials. The main utility of this technique is that the main effects along with interactions can be examined.

Once the critical factors are determined, optimization can begin. First, curvature of the main effects is assessed by running replicate mid-points of the factor settings. If the mean of the mid-point outcome exceeds the mean of the factorial points the optimum is within the design space. Alternatively, if the mid-point mean is lower than the mean of the factorial points, then the optimum is outside the design space. The non-critical factors are set at their mid-points are best values based on the data. Mathematical models are then employed to describe the optimal process. Statistical software packages are available which greatly simplify this process. Typically, 2 to 5 factors are examined at 3 or 5 factor settings. A response surface modeling design is then used to develop the model. The model developed predicts the optimal values for the factors in question which are tested in subsequent rounds of experimentation[1]

However, as the scale increases important biological, chemical and physical parameters affecting cell growth, as well as protein expression will also change. This makes the scale-up process a challenging task. The common problems associated with scale-up originate from poor mixing, which increases circulation time and creates stagnant regions. This leads to imbalanced and zonal distribution of oxygen, nutrients, pH, heat and metabolites inside the bioreactor Therefore, several strategies have been used as principles to scale up E.coli fermentation to minimize the

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differences between scales by keeping one or more parameters constant from laboratory to pilot and plant scale bioreactors. These parameters include power input per liquid volume (P/V), oxygen transfer rate (OTR), oxygen mass transfer coefficient (kLa), impeller tip speed, mixing time and impeller Reynolds's number (NRe) Traditionally, the constant P/V has been shown to be a successful scale-up criterion for large scale fungal and mammalian cell fermentation, but may be limited to recombinant E.coli culture because of its high energy requirement[2].

The product concentration is among the most important parameters in determination of fermentation successfulness. After a thorough cell line development, the recombinant protein expression with naturally non-producing cells has been shown to be dependent on multiple variables (e.g. specific growth rate, specific production rate, specific substrate uptake rate, dissolved oxygen tension, medium composition, inducer concentration, temperature and pH).
It is known that the agitation rate (mixing time, power input, kLa) plays an important role in product improvement along with dissolved Oxygen in the fermentor broth will having impact of altitude, tank pressure and liquid height. It is very important to maintain the tip speed so as to achieve maximum product output at different log hrs. Cell density wrt nutrient source should be balance in such a way that the cells are able to synthesis the product continuously in the broth.
For commercial production of recombinant proteins, the fermentation usually starts in a laboratory scale bioreactor (e.g., 5–30 l) to identify suitable growth and protein expression conditions. The process then transfers to pilot level (e.g., 40–600 l) to establish optimal operating parameters and finally to manufacturing scale (e.g., over 2,000 l) to reach high productivity. The scale-up process for any recombinant protein should aim for high productivity with consistency in the protein quality and specific yield.

In recent times the secondary role of oxygen on maintenance of cell physiology and quality of the recombinant protein has been a major concern for the high volumetric yield of recombinant protein from E. coli. Fluctuations in oxygen contents during high productive fermentation process can cause oxidative stress within the cells leading to limitation in amino acid production, plasmid instability and more importantly oxidation of proteins These effects altogether may affect the quality of the final product. Oxygen often becomes limiting in HCDCs owing to its low solubility. The saturated dissolved oxygen (DO) concentration in water at 25°C is ~7 mg/l but oxygen supply can be increased by increasing the aeration rate or agitation speed.

The oxygen mass transfer coefficient, kLa plays an important role towards carrying out the design, scaling up and economic of the process. Efforts are to be focused on improving the design and scaling up studies to achieve adequate supply of oxygen at higher scales. By varying the incubation time during overproduction, the protein yield is affected. In this process, the longer the incubation, the higher the yield of the obtained solid phase result that consist of protein (cell pellet)3.

CONCLUSION

The scale up of recombinant proteins is based on each culture used, product and media. Therefore, each scale up strategy for each product will differ and requires extensive studies at laboratory scale.

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