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Engineering challenges in high density cell culture systems

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Abstract

High density cell culture systems offer the advantage of production of bio-pharmaceuticals in compact bioreactors with high volumetric production rates; however, these systems are difficult to design and operate. First of all, the cells have to be retained in the bioreactor by physical means during perfusion. The design of the cell retention is the key to performance of high density cell culture systems. Oxygenation and media design are also important for maximizing the cell number. In high density perfusion reactors, variable cell density, and hence the metabolic demand, require constant adjustment of perfusion rates. The use of cell specific perfusion rate (CSPR) control provides a constant environment to the cells resulting in consistent production. On-line measurement of cell density and metabolic activities can be used for the estimation of cell densities and the control of CSPR. Issues related to mass transfer and mixing become more important at high cell densities. Due to the difference in mass transfer coefficients for oxygen and CO₂, a significant accumulation of dissolved CO₂ is experienced with silicone tubing aeration. Also, mixing is observed to decrease at high densities. Base addition, if not properly done, could result in localized cell lysis and poor culture performance. Non-uniform mixing in reactors promotes the heterogeneity of the culture. Cell aggregation results in segregation of the cells within different mixing zones. This paper discusses these issues and makes recommendations for further development of high density cell culture bioreactors.

Introduction

Cell culture fermentation is used for the production of several important biologicals including interferons, growth factors, vaccines, hormones, and monoclonal antibodies (Lubiniecki, 1990). The process efficiency of cell culture fermentation should be increased in most cases for economically viable production. Selection of high production clones, minimizing the cost of media preparation, bioreactor design and optimization, and streamlining the product recovery, are all important research areas for cost effective production.

In the area of bioreactor design and optimization, extensive research has been directed towards the development of high density cultures (Griffiths, 1990; Ozturk, 1994). High density culture systems are desirable for the cost effective large scale production of pharmaceuticals, mainly because of their high volumetric production rates (Ozturk, 1994). For a given production capacity, high density reactors are more compact in size and allow stable long term continuous cultivation.

Several high density reactor systems have been developed for high density cultures in the last decade. This paper will discuss the state-of-the-art of these systems and point out some of the areas that need further development. First, we discuss the limitations for increasing cell density and therefore, the reactor performance. Space, operation, aeration, and media formulation can limit the attainable number of the cells in the reactor. We then discuss some of the interesting aspects of high density cultures with regard to process control, mixing, base addition, and heterogeneity.
Limitations for cell density

Although the distinction is relative, high density cultures refer to cell densities in the order of $10^7$ cells per ml (Griffiths, 1990). Although reactor systems can be optimized to achieve higher cell densities, several factors can impose limitations. Physical space, reactor design and operational issues, media formulation, and aeration capacity can determine the maximum number of cells maintained in the reactor.

Space limitations

The theoretical maximum cell density for mammalian cell culture is about $10^9$ cells/ml which can be calculated from the closely packed tissue-like geometries. At cell densities of $10^7$ and $10^8$ cells/ml (typical values reported in the literature), cells occupy only 1 to 10% of the volume, indicating the room for improvement in terms of space limitations. By comparison, in microbial and yeast systems, cell densities can be increased to up to 40% of the reactor volume (Bailey and Ollis, 1986).

Reactor design and operation limitations

In order to increase the cell density, the reactors should be designed to allow the retention of cells in the reactor. The design and operation of the cell retention system determines the performance and applicability of a given high density cell culture system.

Over the last decade, several reactor systems have been developed to accommodate high densities. Although they can achieve very high local densities ($10^9$ cells/ml), membrane systems (hollow fibers and flat sheets) are not practical for large scale production. Still these systems find application for small quantity production of antibodies for diagnostic purposes (Griffiths, 1990). Cell immobilization or encapsulation have also been very attractive for achieving high densities in the order of $10^8$ cell/ml (Tyler, 1990). However, as time progressed, these systems were proven not to be suitable for commercial systems because of intensive labor and the cost involved in cell immobilization. Cell attachment systems such as fiber beds (Chiou et al., 1991), ceramic cartridges (Berg and Biedeker, 1988) and microporous carriers (Karkare et al., 1989) have been more viable for commercial production with cell densities in the range of $10^8$ cells/ml; however, the complexity in their operation limited their acceptance. These systems are more attractive for attachment dependent cell lines.

Except some primary cells used for vaccine production, most cell lines can be adapted to grow in suspension either as single cell or aggregates (Fenge et al., 1992; Moreira et al., 1994b). In the suspension-type retention systems, cells are retained by internal and external cell separation devices. Cell retention is achieved by centrifugation (Tokashiki, 1990; Wie et al., 1991), internal (Avgerinos et al., 1990) and external spin filters (Fenge et al., 1993), and membrane and gravitational separation devices (Hulscher et al., 1992; Hansen et al., 1993). These systems eliminate the need for a solid support and have been used successfully for both native and adapted suspension cells in large scale high density cultures. The cell densities in these systems are in the order of $10^7$ cells/ml. The limitation on cell density is related to the loss in the efficiency of the cell retention system at high densities. The spin filter and membrane systems, for instance, are more susceptible to clogging at higher densities. Another problem is related to the volumetric flow rates allowable for the cell retention system. At high cell densities, the medium exchange rate should be increased to maintain the cells under good growth conditions. As a result, the centrifugal and gravitational cell separation systems lose cell separation efficiency at high throughputs before the reactor reaches higher cell densities.

It is clear that more engineering expertise is needed for the design and optimization of cell retention systems. The reactor and the cell retention system should be integrated optimally. The system should be easy to operate, allow high volumetric flow rates, and should be operable for the long time required for high density systems.

Media formulation

The number of cells attainable in the reactor is also dependent on the nutritional value of the medium used. In batch systems, for instance, only 1–5 million cells/ml can be obtained. Medium exchange is essential for sustaining a higher cell density, and thus achieving higher reactor performance (Figure 2). Most of the current medium formulations can support about $10^7$ cells/ml (calculated based on cell yields on different nutrients) at growth conditions with an exchange rate of one volume per day (Figure 2). To maintain a cell density of $10^8$ cells/ml, the media exchange rate should be increased to 10 volumes per day change. The use of high perfusion rates, however, decreases the efficien-