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論　文　内　容　要　旨

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学位論文題目	Studies of large scale culture method of mammalian cell focusing on dissolved carbon dioxide concentration （溶存二酸化炭素の除去能力に着目した動物細胞の大規模培養方法に関する研究）		

内容要旨

Biopharmaceuticals are now become one of main approach to therapy of cancer, rheumatism and other various therapeutic area. More than half of top ten sale pharmaceutical drug are biopharmaceuticals produced in bioprocess using genetically modified cells or microorganism. There are two strategies to obtain production capability, one is scale-out, and another is scale-up strategy. Bioprocess scale-up of biopharmaceuticals production is important to produce drug substance keeping productivity, quality and reducing cost of goods. The monoclonal antibody (Mab) is one of protein drug, which has the largest number of launches and developments in the market. Mabs have often been produced by batch culture or fed-batch culture process of CHO cells. In the fed-batch culture production process, it was reported that Mab concentration was reached to 10 g/L in harvest. This shows that productivity is increased more than 10 times at least in 30 years. Improvements of cell culture and cell line developments are recognized as the great contribution for these progresses. Also, accompanying improvement of productivity, i.e., quality assurance, is required with increase of productivity. In addition, production scales are expected to be increased with the development stage of pharmaceutical pipeline progresses and the manufacturing site changes. There are several stages of pharmaceutical development, including toxicity studies in animals, clinical trials for human use, and commercialization. Quality equivalence should be kept during these development stage and scale-up process to ensure efficacy and toxicity consistency. Various cell culture parameters, such as pH and temperature are known to affect product quality such a glycan profile, aggregation content, and optimal culture conditions have been investigated using various cell lines and medium. It is also well known that the dissolved carbon dioxide concentration (dCO_2) also affects the product quality. However, since CO_2 was released from cells by metabolism of TCA cycle, it is difficult to exactly control the dCO_2 during cell culture and/or scale up processes. The trend of dCO_2 during the cell culture process is related to the parameter $k_La(CO_2)$ which is dependent on shape of the bioreactor and medium composition. It is also known that $k_La(CO_2)$ varies depending on the scale of the bioreactor, agitation and the aeration conditions.

In this thesis, the scale up factors obtained by calculation and by experiment were compared. Power per unit volume (P/V) value for a scale-up factor obtained from the calculation (Chapter 2), and mixing time and k_La for a scale-up factor obtained from the experimental data (Chapter 4) were focused. Mixing time and the k_La ratio calculated by $k_La(O_2)$ and $k_La(CO_2)$ was evaluated for the scale up of bioreactor (Chapter 3). $k_La(O_2)$ was related to aeration rate to control dissolved oxygen concentration (DO). Stripping of CO_2 from medium is related to $k_La(CO_2)$ and aeration rate. If the k_La ratio is kept constant, the time course of dCO_2 during cultivation could be expected to be similar. P/V was adopted for the scale-up factor of a SS bioreactor with similar shape. The agitation condition was determined by P/V, and the other scale-dependent condition, i.e., feed ratio of feed medium, medium volume which was determined by the scale ratio. These other conditions were determined based on the previous experiments. Recently, single-use reactors are commonly used for middle-scale (from several hundreds to thousands liter scale) production of therapeutic antibodies. Various types of single-use bioreactors are provided by various companies and these designs are not the same. However, the calculation of P/V of single-use bioreactor for scale-up is not easy, because the detail design and size of impeller and vessel are not open for users. Therefore, scale-up methodology using scale-up factor based on experimental data, such as k_La ratio, was necessary for commercial-based production. In this thesis, the agitation and the aeration conditions were determined on the basis of calculated k_La ratio. The scale-up methodology using both calculation–based and experimental data-based method from 200 L-scale to 2000L-scale was achieved, and the advantage and disadvantage of both methods were discussed.