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）

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Chapter 1. Introduction

1.1. Production of biopharmaceuticals

Biopharmaceuticals are now become one of main approach to therapy of cancer, rheumatism and other various therapeutic area (1). More than half of top ten sale pharmaceutical drug are biopharmaceuticals produced in bioprocess using genetically modified cells or microorganism (2). There are two strategies to obtain production capability, one is scale-out, and another is scale-up strategy. Scale-out is a method of increasing production capacity by increasing the number of production equipment. Scale-up is a method of increasing the volume of production in a single manufacturing opportunity by increasing the capacity and scale of production equipment. Each method has its advantages, but in many cases, a combination of both methods seems to have effectiveness and flexibility. Thus, bioprocess scale-up of biopharmaceuticals production is important to produce drug substance keeping productivity, quality and reducing cost of goods. The types of biopharmaceuticals include protein drugs, antibody-drug conjugate (ADC), Chimeric Antigen Receptor-T Cell Therapy (CAR-T), and cell therapies. Table 1 shows example of biopharmaceuticals.

Table 1 Example of Modality and indication of biopharmaceuticals*1

Modality	Product name	Indication	Launch
Mab	HUMIRA ®	rheumatoid arthritis	2008

	OPDIVO®	Cancer	2014
	KEYTRUDA®	Cancer	2016
ADC	ADCETRIS®	Cancer	2011
	KADCYLA®	Cancer	2013
	ENHERTU®	Cancer	2020
CAR-T	KYMRIAH®	ALL*2	2017
	YESCARTA®	DLBCL*3	2017
Gene therapy	ZOLGENSMA®	Spinal muscular atrophy	2019

*1 This table was created by the author based on the Nikkei Bio yearbook.

*2 ALL: Acute Lymphoblastic Leukemia

*3 DLBCL: Diffuse Large B-Cell Lymphoma

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 (3, 4, 5, 6, 7).

This shows that productivity is increased more than 10 times at least in 30 years (8). In addition, the perfusion culture process, which has been attracting attention as one of the new type production processes, has been developed in industry. In this perfusion process, the concentration of cells in the process is reported to be 10 times higher than that in fed-batch culture, and the concentration of antibodies is reported to be 10 times higher.

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 (9).

1.2. Scale-up method of bioreactor for CHO cell

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. Table 2 summarized pharmaceutical product development stage. Quality equivalence should be kept during these development stage and scale-up process to ensure efficacy and toxicity consistency.

Table 2 Pharmaceutical product development stage

Stage	Pre-clinical	Clinical trial Phase I	Clinical trial Phase II	Clinical trial Phase III	Commercial
Target	Animal	Human	Human	Human	Human
Purpose	Toxicology, Efficacy	Safety	Safety, Efficacy	Safety, Efficacy	Medical treatment

Scale	Minimum	Small	Medium	Large	-

In order to ensure such quality equivalence, additives to various culture media have been reported (10, 11). Although these methods control the physical properties of biopharmaceuticals themselves, there are various studies for cell culture parameters to control the quality of protein drugs. Various cell culture parameters, such as pH and temperature are known to affect product quality such a glycan profile, aggregation content (9, 12), 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 (13). 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. Figure 1 describes partial diagram of dCO_2 behavior in the bioreactor.

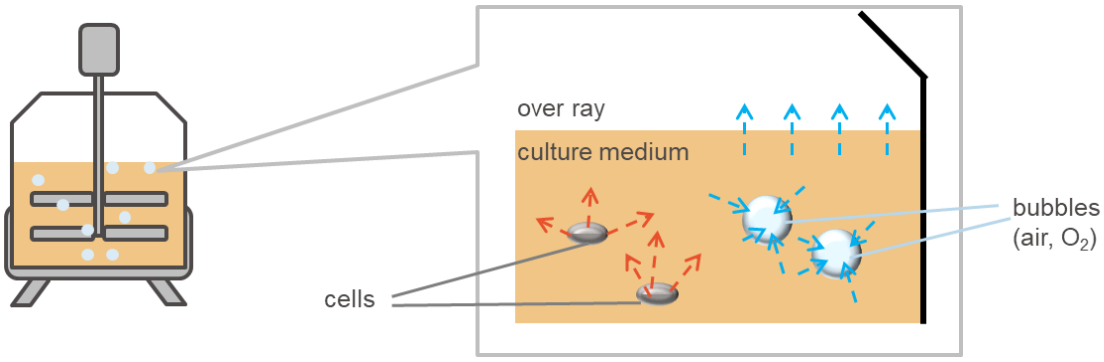

Figure 1 Diagram of the behavior of carbon dioxide in a bioreactor. Red arrow shows carbon dioxide release from cells. Blue arrow shows carbon dioxide stripping by bubble and release to over ray in the bioreactor.

On the other hands, Feng Li also reported the importance of the construction of proper scale-down model (14). To solve the scale -up problem, it is important to construct the simulated scale-down model because to it is expensive and time-consuming to use large-scale culture for investigation. 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 (15, 16).

1.3. Summary of my research

In this thesis, I compare the scale up factors obtained by calculation and by experiment. I focused on 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). 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. I adopted P/V 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 (Figure 2). I achieved the scale-up methodology using both calculation–based and experimental data-based method from 200 L-scale to 2000L-scale, and discussed the advantage and disadvantage of both methods.

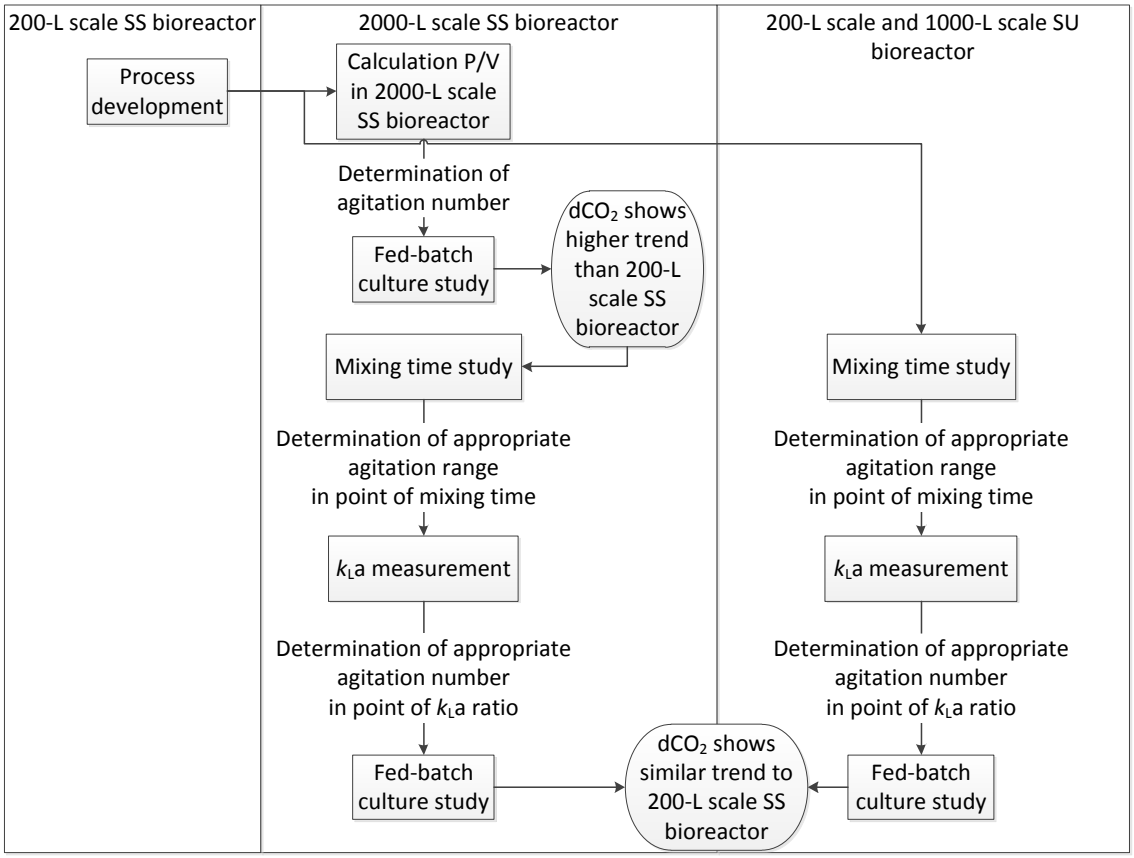

Figure 2 Experimental strategy for this thesis. Developed process in 200-L scale SS bioreactor was scaled-up to 2000-L scale SS bioreactor and single-use bioreactors (200-L and 1000-L scale).

Chapter 2. Scale up based on Power per unit volume

2.1. Introduction

In the microbial fermentation for food, drink, and pharmaceutical production, scale-up has been studied in various ways. P/V, Reynolds number, and agitator tip speed were used as the scale-up indicator for these studies. The tip speed of the impellor is known to be associated with shear stress to the cells. Although this is a considerable point when scaling up, in this thesis, it was not treated as a direct scale up factor. The Reynolds number is an index of the flow condition of the liquid phase. In the previous study, in case the Reynolds number was more than 10000 in the agitation tank, the flow is considered to be turbulent. It was confirmed that the flow was turbulent in all the agitation conditions of the bioreactor evaluated in this thesis (data not shown). P/V represents the amount of energy consumption per unit volume and has been calculated using an equation that is estimated from electricity consumption data. In this thesis I focused on the mixing and gas exchange conditions in the bioreactor. As an evaluation of mixing condition, I attempted to scale up the process of CHO cells culture using the P/V formula. In this chapter, scale-up study on the basis of P/V for 2000-L scale bioreactor was reported.

2.2. Materials and Methods

2.2.1. Power per unit volume calculation

Power per unit volume (P/V) was calculated for 2000-L scale bioreactor and 200-L scale stainless steel (SS) bioreactor for scale-up.

$$P/V = \frac{Np \times \rho \times N^3 \times Di^5}{V} \quad (1)$$

where Np (-) is power number explained by Nagata (17), ρ (kg m^{-3}) is degree of density of water, N (rotation per seconds) is agitation speed, Di (m) is impeller diameter, V (m^3) is estimated maximum volume in fed-batch culture process. During fed-batch culture, culture volume continuously increases by the addition of feed medium. In this P/V calculation, I set V to maximum volume at the end of cultivation which is calculated from the time course of our previous 2-L scale fed-batch culture using glass vessel.

2.2.2. Cell line and medium

Cell lines A expressing Mabs A was established using CHOK1SV cells as the host cell and the GS Gene Expression System provided by Lonza Biologics (Slough, Berkshire, UK). This cell line was selected in CD CHO medium (Life Technologies, Carlsbad, CA, USA) containing methionine sulfoximine. Chemically defined basal medium was developed for the fed-batch process by modifying the CD CHO medium with in-house proprietary supplements. This protein-free, chemically defined feed media developed in-

house were used for the fed-batch culture for this thesis.

2.2.3. Fed-batch culture

Fed-batch cultures were performed using IgG-producing CHO cell line A. In-house serum-free basal and feed medium were used in this thesis. Agitation was kept at speed which was determined to show the expected power per unit volume. The temperature was kept at 37°C. The pH was controlled by pure CO_2 sparging and by the addition of 1.0M sodium carbonate. DO was controlled at 50% air saturation by aeration to headspace and, oxygen and air mixed gas aeration from sparger. Small amount of sample was taken from reactor and DO, dCO_2 and pH were measured by a blood gas analyzer (Rapidlab 348 blood gas analyzer (Bayer Corp., MA, USA)). Cell viability and viable cell density were measured by Vi-CELL XR (Beckman Coulter, CA, USA). Glucose and lactate concentrations in the supernatant were determined with a BF-5 bioanalyzer (Oji Scientific Instruments, Hyogo, Japan). After centrifugation, Mab concentration was measured using an HPLC system (Waters/mS, USA) with a UV detector and a POROS PA affinity column (Applied Biosystems/mA, USA). Specific growth and production rates were calculated according to previous report (19).

2.3. Results

2.3.1. Evaluation of power per unit volume calculation of Stainless Steel (SS) bioreactor

In order to scale up from 200-L bioreactor to 2000-L bioreactor, P/V of each bioreactor was calculated. For calculation, estimated maximum volume V in fed-batch culture process was used in each scale. The agitation speed in the 200-L bioreactor was set for 1 m/sec tip speed. For 2000-L SS bioreactor, the control agitation speed was determined by the condition showing similar P/V value to 200-L SS bioreactor. P/V in control condition of 200-L was 82 W/m^3, and P/V in control condition of 2000-L was 84 W/m^3, respectively.

2.3.2. Fed-batch culture result comparing SS 200-L to 2000-L scaled up based on power per unit volume calculation

In order to evaluate the scale-up process by agitation condition determined by P/V, fed-batch cultures of CHO cell line A using 200-L and 2000-L scale SS bioreactor were carried out. Fed-batch culture was continued to 16 days. Sampling was taken daily during the culture period to measure viable cell density, viability, glucose and lactate concentrations (data not shown), and Mab concentration. Figure 3 shows time course of Mab concentration, viable cell density, viability, and dCO_2. Mab concentration, viable cell density, viability showed the similar trend between 200-L and 2000-L cultivations. The maximum viable cell density was about 20 x 10^6 cells/mL on both scales. The

viability gradually decreased after the 12th day of culture. The Mab concentration reached about 6 g/L at the time of harvest. dCO_2 shifted around 60 mmHg in the 200-L scale throughout the culture period, but it was kept at around 80 mmHg on the 2000-L scale. dCO_2 tended to be higher on the 2000-L than that on 200-L cultivation. Specific Mab production rate was 33 ± 0.6 pg $cell^{-1}$ day^{-1} in 200-L scale fed-batch culture, and 33 ± 0.2 pg $cell^{-1}$ day^{-1} in 1000-L scale fed-batch culture, respectively. Specific growth rate during day 1 to day 7 were 0.021 ±0.003 h^{-1} in 200-L scale fed-batch culture, and 0.023 ±0.001 h^{-1} in 2000-L scale fed-batch culture, respectively. Even the different dCO_2 during cultivation, cell growth and the productivity were similar between 200-L and 2000-L cultivations.

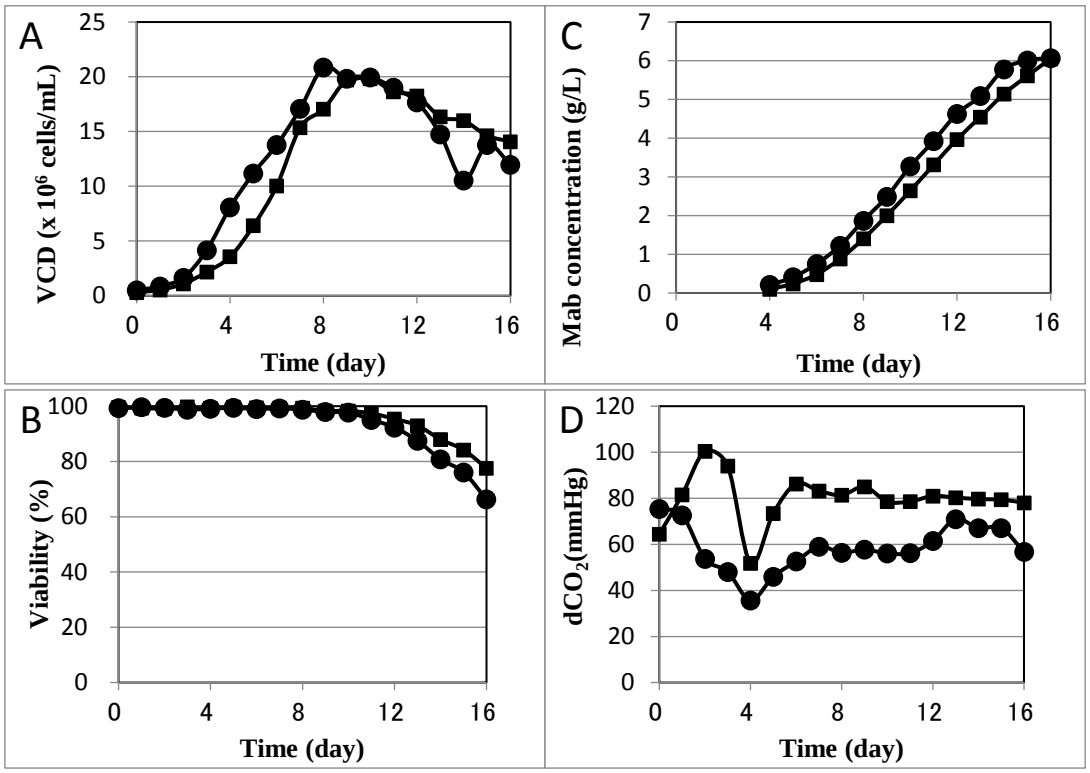

Figure 3 Time course of cell line A cultivation in 2000-L scale SS bioreactor with P/V based agitation condition. A: Mab concentration, B: Viable cells density, C: Viability, D: dCO_2. Symbols are 200-L scale (closed circle) and 2000-L scale (closed square).

2.4. Discussion

In the similarly-shaped 200-L and 2000-L scale bioreactor, agitation conditions were determined, that showed P/V of 82 W/m^3 and 84 W/m^3, respectively. The stainless steel (SS) bioreactor was easy to calculate the P/V value, because bioreactor was designed by ourselves. This is an advantage of the stainless steel (SS) bioreactor. In the 2000-L scale, the concentration of dissolved CO_2 tended to be higher than those in 200-L scale after day 1 during the fed-batch culture. One considerable reason is that there was a difference in

the amount of aeration to remove dissolved carbon dioxide from the culture medium between 200-L and 200-L aerations. The aeration rate per unit volume of culture medium was higher on the 200-L scale (Data not shown). The cell growth and viability of cells that were affecting by dCO_2 during cell culture process were similar in both scales in this thesis. However, in the larger bioreactor than 2000-L scale, any further dCO_2 accumulation may has negative impact to cell growth. The tip speed was higher on the 200-L scale than that on the 2000-L scale. The shear stress on agitation speed in this 200-L scale had no effect on cell growth and antibody productivity. For the further analysis of shear stress effect in the bioreactor, computational fluid dynamics (CFD) appear to be effective.

Chapter 3. Mixing time and k_La evaluation for bioreactors

3.1. Introduction

In this chapter, for the performance evaluation of bioreactors mixing time study and k_La study were reported. In mixing time study, various agitation conditions were evaluated in view of mixing performance. In k_La study, k_La ratio was evaluated in various agitation conditions between appropriate agitation range for SS 2000-L scale bioreactor and single use bioreactors in view of gas exchange conditions.

The mixing time is the time it takes for the bioreactor to become from non-uniform to 95% uniform (18). In a scale of less than 2,000 L cultivation for CHO cells, the mixing time was reported to be within approximately one minutes. A formula for estimating the mixing time in a tank with the following conditions is also reported(16, 17, 19).

1. Liquid height and diameter are 1:1

2 There are four baffle plates, and the ratio of plate width and tank diameter range are from 1:10 to 1:12.

3 Impellor blades of 1/3 to 1/2 of the tank diameter

4 The position of the impellor blade is 1/3 to 1/4 of the height from the bottom of the tank.

In this thesis, it was thought that all of the above conditions may not be suitable for SS bioreactors. For single use bioreactor, at least one of condition is not suitable. Then, I decided to perform measurements of the mixing time.

In the field of chemical engineering, k_La is an index that comprehensively expresses the gas exchange capacity of between the gas and liquid phases in a vessel. In bioprocess for CHO culture, pure oxygen is supplied in the bioreactor for supplying oxygen to the cells. At the same time, the bubbles remove the carbon dioxide from the culture medium. Therefore, in the higher k_La (O_2) condition, the less oxygen is supplied, and the less carbon dioxide is removed. As the k_La (O_2) increases, the k_La (CO_2) also increases. However, the sensitivity of the change of k_La to the airflow rate change is different. Consequently, I considered that it important to keep the ratio of k_La (O_2) to k_La (CO_2) below a certain level in order to remove dissolved carbon dioxide. Therefore, the k_la ratio of oxygen to carbon dioxide was evaluated.

3.2. Materials and Methods

3.2.1. Mixing time

Mixing time in the bioreactor was evaluated by pH measurement. A calibrated pH sensor (Mettler toledo, OH, USA) was installed in the bioreactor, and purified water was filled into a bioreactor at room temperature and agitated under various bioreactor scale and agitation speed. After confirming the uniform mixing in the bioreactor by observation, 5 M NaOH (Merck KGaA, Darmstadt, Germany) was shortly added from the top of the bioreactor. The time until pH was stable was evaluated as the mixing time.

3.2.2. k_La measurement and evaluation

Both *k*La (O_2) and *k*La (CO_2) were measured by dynamic gassing-out method (20) using phosphate buffered saline (PBS) instead of serum-free medium. In brief, PBS (T900, Takara bio, Shiga, Japan) diluted in purified water to 50% was filled and kept at 37 ºC in each scale bioreactors. In k_La(O_2) study, DO sensor (METTLER TOLEDO, OH, USA) was used for DO measurement. DO sensor was calibrated in 100% on air saturation condition. Nitrogen gas was sparged into the bioreactor until DO was decreased and became stable, and then calibrated to 0%. After 0% calibration, Nitrogen gas in head space of bioreactor was replaced to air. Agitation and air-sparge were started and then DO was recorded until saturated by air. The k_La (O_2) was calculated according to below equation (2).

$$k_L a(O_2) = \frac{\ln([DO]_s - [DO]_0) - \ln([DO]_s - [DO]_t)}{t} \qquad (2)$$

where $[DO]_s$ is saturated value (% air saturated), $[DO]_0$ is value in t=0 (min), and $[DO]_t$ is value at elapsed time t. k_La was calculated as a slope of the linear plot of $\ln([DO]_s-[DO]_0)-\ln([DO]_s-[DO]_t)$ against t.

In k_La(CO_2) study, dCO_2 sensor (METTLER TOLEDO, OH, USA) was used for dCO_2 measurement. Pure CO_2 gas was sparged into the bioreactor with PBS until dCO_2 value

became stable, and then dCO_2 sensor (METTLER TOLEDO, OH, USA) was calibrated to 100%. After 100% calibration, CO_2 gas in head space of bioreactor was replaced to air. Agitation and air sparge were started and then dCO_2 was recorded until fully dropped and became stable. From recorded data, $k_La(CO_2)$ was according to below equation (3).

$$k_La(CO_2) = \frac{\ln([dCO_2]s-[dCO_2]0)-\ln([dCO_2]s-[dCO_2]t)}{t} \quad (3)$$

where $[dCO_2]_s$ is saturated value, $[dCO_2]_0$ is value in t=0, and $[dCO_2]_t$ is value at elapsed time t. k_La was calculated as a slope of the linear plot of $\ln([dCO_2]_s-[dCO_2]_0)-\ln([dCO_2]_s-[dCO_2]_t)$ against t.

Consequently, the k_La ratio [k_La (CO_2) / k_La (O_2)] was evaluated from below equation (4).

$$k_La \text{ ratio} = \frac{k_La\ (CO_2)}{k_La\ (O_2)} k_La \text{ ratio} = \frac{k_La\ (CO_2)}{k_La\ (O_2)} \quad (4)$$

3.3. Results

3.3.1. Mixing time study

The characteristics of the bioreactor were also evaluated by experimental data for scale-up. The mixing time was analyzed using pH measurement. Figure 4 summarized the results of mixing time study. The mixing time of the 200-L scale SS bioreactor was 21 seconds under the control agitation condition. In the 2000-L SS bioreactor, mixing time was 40 seconds under the agitation condition which was determined based on P/V. At this

agitation condition of the 2000-L bioreactor, the cell growth, specific Mab production rate was similar to those of the 200-L scale. Specific Mab production rate were 33 ± 0.6 pg $cell^{-1}$ day^{-1} in 200-L scale fed-batch culture and 33 ± 0.2 pg $cell^{-1}$ day^{-1} in 2000-L scale fed-batch culture. And Specific growth rate during day 1 to day 7 were 0.021 ±0.003 h-1 in 200-L scale fed-batch culture and 0.023 ±0.001 h^{-1} in 2000-L scale fed-batch culture. It was presumed that mixing time did not affect the cell growth and Mab productivity. The mixing time of single-use bioreactors from vendor A, B, and C were also evaluated. Vendor A provided 200-L scale and 1000-L scale, vendors B and C provided the 200-L scale. On the 200-L scale, the mixing time for vendor A reactor was about 30 seconds between 45 rpm and 100 rpm, the time for vendor B reactor was about 35 seconds at more than 100 rpm, and the time for vendor C reactor was less than 35 seconds at more than 210 rpm. From the results of mixing time and comparison with a SS bioreactor, it was estimated that the appropriate agitation range for 200-L single-use reactor was 45 rpm or more for vendor A, 100 rpm for vendor B and 210 rpm or more for vender C, respectively. For the vendor A 1000-L scale single-use bioreactor, the mixing time was around 60 seconds under between 25 and 50 rpm agitation condition, but it was less than 30 seconds under more than 65 rpm. Based on this result, it seems that appropriate agitation condition is more than 65 rpm in this 1000-L scale bioreactor from viewpoint of mixing time. From

the result of scale-up in SS bioreactor based on P/V, under 40 second mixing time may not affect the cell growth and productivity of 200-L and 2000-L scale fed-batch culture using cell line A. It's supposed that the suitable agitation condition (40 second mixing time) should exist between 50 rpm and 65 rpm.

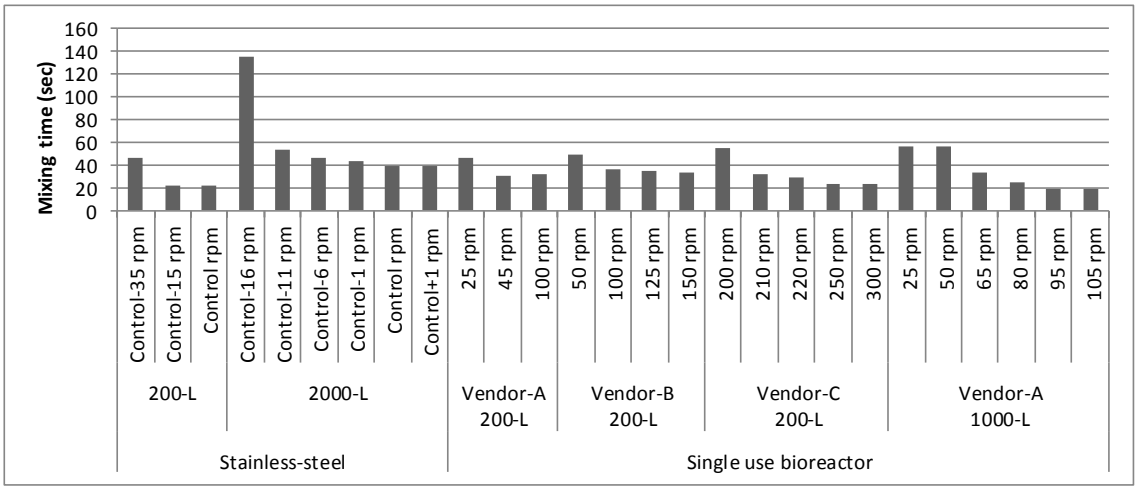

Figure 4 Mixing time in various bioreactor. Mixing time was evaluated under various agitation conditions at SS bioreactors and single-use bioreactors.

3.3.2. k_La study in SS bioreactor

The appropriate agitation condition was determined from the investigation of mixing time. Agitation speed and aeration condition which showed the similar k_La ratio as that of 200-L scale SS bioreactor were used for study. Determined conditions are, with the 0.012 vvm head-space aeration, and either 0.0085 vvm or 0.035 vvm the sparge rate in a 200-L scale bioreactor. For 2000-L scale, the head-space aeration is 0.012 vvm and the sparge rate is 0.0085 vvm or 0.015 vvm.

In 2000-L scale SS bioreactor, $k_La(O_2)$ and $k_La(CO_2)$ were measured at agitation speed determined based on P/V result. As shown in Table 3, the k_La ratio in 2000-L bioreactor was lower than that in 200-L bioreactor at the agitation speed which was previously determined condition showing the similar P/V. $k_La(O_2)$ of 2000-L scale bioreactor was 37% higher than k_La (O_2) of 200-L scale bioreactor under control agitation condition. $k_La(CO_2)$ of 2000-L scale bioreactor was 6% lower than $k_La(CO_2)$ of 200-L scale bioreactor under control agitation condition. It was suggested that CO_2 tends to accumulate in the 2000-L scale bioreactor medium under control agitation condition. k_La changes with depending on agitation condition. In order to evaluate the effect of agitation condition, the trend of k_La ratio when changing the agitation speed on the 2000-L scale was evaluated. Agitation condition presumed to be similar k_La of dCO_2 to the 200-L scale was determined as a result of evaluation by sensitivity study of agitation speed. Agitation speed was changed from minus 6 rpm of control to plus 1 rpm of control condition. As a result, k_La ratio was decreased with the increase of agitation speed of 2000-L scale. The reason of this was that $k_La(O_2)$ is more sensitive than $k_La(CO_2)$ by change of agitation speed in 2000-L scale. Matsunaga also reported about the relationship that of culture volume and k_La sensitivity (15, 16).

Table 3 $k_La(O_2)$, $k_La(CO_2)$ and k_La ratio in SS bioreactors.

$k_La(O_2)$ (h^{-1})

	Agitation condition	Air sparge 0.0085 (vvm)	Air sparge 0.015 (vvm)
SS 200-L	Control*	1.57	-
SS 2000-L	Control-6 (rpm)	1.37	-
	Control-5 (rpm)	1.44	-
	Control-4 (rpm)	1.54	2.78
	Control-3 (rpm)	1.68	-
	Control-2 (rpm)	1.84	-
	Control-1 (rpm)	2.09	-
	Control*	2.15	4.05
	Control+1 (rpm)	2.80	-

$k_La(CO_2)$ (h^{-1})

	Agitation condition	Air sparge 0.0085 (vvm)	Air sparge 0.015 (vvm)
SS 200-L	Control*	0.83	-
SS 2000-L	Control-6 (rpm)	0.67	-
	Control-5 (rpm)	0.69	-
	Control-4 (rpm)	0.70	1.12
	Control-3 (rpm)	0.72	-
	Control-2 (rpm)	0.77	-
	Control-1 (rpm)	0.81	-
	Control*	0.78	1.24
	Control+1 (rpm)	0.83	-

k_La ratio

	Agitation condition	Air sparge 0.0085 (vvm)	Air sparge 0.015 (vvm)
SS 200-L	Control*	0.53	-
SS 2000-L	Control-6 (rpm)	0.49	-
	Control-5 (rpm)	0.48	-
	Control-4 (rpm)	0.46	0.40
	Control-3 (rpm)	0.43	-
	Control-2 (rpm)	0.42	-
	Control-1 (rpm)	0.39	-
	Control*	0.36	0.31
	Control+1 (rpm)	0.30	-

*Agitation speed at SS 2000-L and 200-L were not shown because of company's confidential information.

3.3.3. k_La study in single-use bioreactor

In the single-use bioreactor, the $k_L a(O_2)$, $k_L a(CO_2)$ and k_La ratios were similarly evaluated within the range determined by previous mixing time study. As shown in Table 4, under the condition of 100 rpm, the 200-L scale of vendor A with 0.0085 vvm (0.56) and 0.035 vvm (0.46) air sparging and the 200-L scale of vendor B with 0.035 vvm (0.42) air sparging showed the same k_La ratio as that of SS 200-L bioreactor (0.53 in 0.0085 vvm and 0.43 in 0.035 vvm) .

Vendor B single-use bioreactor have two types of spargers, i.e., sinter pipe and drilled hole. In the case of sparge with sinter pipe at 0.035 vvm and 150 rpm, $k_L a(O_2)$ increased 1.5 times (8.30 h^{-1} on drilled hole sparger and 12.02 h^{-1}on sinter pipe sparger) comparing with drilled hole sparger under the same sparging condition (0.035vvm and 150rpm), but $k_L a(CO_2)$ with sinter pipe sparger (2.45 h^{-1}) was almost the same with drilled hole sparger (2.35 h^{-1}). Therefore, the k_La ratio with sinter pipe sparger become lower than that in drilled hole sparger.

The agitation condition for vendor C single-use bioreactor was determined for 210 rpm or more based on the previous mixing time study. However, at more than 210 rpm condition, I could not find the agitation condition showing the similar k_La ratio of 200L SS bioreactor (between 0.0085 vvm to 0.0035 vvm). To seek the condition showing similar k_La ratio to 200-L SS bioreactor, $k_L a(O_2)$ and $k_L a(CO_2)$ were measured under the

condition of additional pure nitrogen gas sparging in 0.001, 0.002 and 0.005 vvm at 210 rpm, and the k_La ratio was evaluated. Nitrogen gas sparging decreased $k_L a(O_2)$, because O_2 concentration in supplied gas to bioreactor was decreased by nitrogen gas sparging, and increased $k_L a(CO_2)$ because total amount of supplied gas was increased. When nitrogen gas sparging rate was 0.001 vvm, it showed the same k_La ratio as 200 L SS bioreactor in conditions of both 0.0085 vvm air sparging and 0.035 vvm air sparging. It was concluded that 210 rpm agitation and nitrogen aeration 0.001 vvm nitrogen aeration are recommended for the fed batch cultivation using vendor C single-use bioreactor.

Table 4 k_La and k_La ratio in single-use bioreactors.

$k_L a(O_2)$ (h^{-1})			
	Agitation condition /nitrogen gas sparge	Air sparge 0.0085 (vvm)	0.035 (vvm)
SS 200-L	Control* / NA	1.57	4.06
Vendor-A 200-L (Open pipe sparger)	25 (rpm) / NA	0.29	0.83
	45 (rpm) / NA	0.37	0.92
	100 (rpm) / NA	1.45	3.69
Vendor-A 1000-L (Open pipe sparger)	50 (rpm) / NA	-	5.21
	60 (rpm) / NA	2.35	7.85
	70 (rpm) / NA	3.95	11.09
	80 (rpm) / NA	6.45	13.50
Vendor-B 200-L (Drilled hole sparger)	100 (rpm) / NA	-	4.18
	125 (rpm) / NA	-	6.73
	150 (rpm) / NA	2.33	8.30
Vendor-B 200-L (sinter pipe sparger)	150 (rpm) / NA	-	12.02
Vendor-C 200-L (Drilled hole sparger)	210 (rpm) / NA	2.97	10.65
	220 (rpm) / NA	3.28	10.95
	250 (rpm) / NA	4.64	12.12

	210 (rpm)	/ 0.001 vvm	2.80	10.65
	210 (rpm)	/ 0.002 vvm	2.50	10.48
	210 (rpm)	/ 0.005 vvm	1.79	9.96

$k_La(CO_2)$ (h^{-1})

	Agitation condition /nitrogen gas sparge		Air sparge 0.0085 (vvm)	Air sparge 0.035 (vvm)
SS 200-L	Control*	/ NA	0.83	1.76
Vendor-A 200-L (Open pipe sparger)	25 (rpm)	/ NA	-	-
	45 (rpm)	/ NA	0.27	0.76
	100 (rpm)	/ NA	0.81	1.69
Vendor-A 1000-L (Open pipe sparger)	50 (rpm)	/ NA	-	2.40
	60 (rpm)	/ NA	0.85	2.80
	70 (rpm)	/ NA	0.97	3.31
	80 (rpm)	/ NA	1.04	3.78
Vendor-B 200-L (Drilled hole sparger)	100 (rpm)	/ NA	-	1.77
	125 (rpm)	/ NA	-	1.99
	150 (rpm)	/ NA	-	2.35
Vendor-B 200-L (sinter pipe sparger)	150 (rpm)	/ NA	-	2.45
Vendor-C 200-L (Drilled hole sparger)	210 (rpm)	/ NA	1.23	4.26
	220 (rpm)	/ NA	1.32	4.38
	250 (rpm)	/ NA	0.87	2.52
	210 (rpm)	/ 0.001 vvm	1.39	4.40
	210 (rpm)	/ 0.002 vvm	1.52	4.53
	210 (rpm)	/ 0.005 vvm	1.83	4.79

k_La ratio

	Agitation condition /nitrogen gas sparge		Air sparge 0.0085 (vvm)	Air sparge 0.035 (vvm)
SS 200-L	Control*	/ NA	0.53	0.43
Vendor-A 200-L (Open pipe sparger)	25 (rpm)	/ NA	-	-
	45 (rpm)	/ NA	0.72	0.83
	100 (rpm)	/ NA	0.56	0.46
	50 (rpm)	/ NA	-	0.46

Vendor-A 1000-L (Open pipe sparger)	60 (rpm)	/ NA	0.36	0.36
	70 (rpm)	/ NA	0.24	0.30
	80 (rpm)	/ NA	0.16	0.28
Vendor-B 200-L (Drilled hole sparger)	100 (rpm)	/ NA	-	0.42
	125 (rpm)	/ NA	-	0.30
	150 (rpm)	/ NA	-	0.28
Vendor-B 200-L (sinter pipe sparger)	150 (rpm)	/ NA	-	0.20
Vendor-C 200-L (Drilled hole sparger)	210 (rpm)	/ NA	0.41	0.40
	220 (rpm)	/ NA	0.40	0.40
	250 (rpm)	/ NA	0.19	0.21
	210 (rpm)	/ 0.001 vvm	0.50	0.41
	210 (rpm)	/ 0.002 vvm	0.61	0.43
	210 (rpm)	/ 0.005 vvm	1.02	0.48

*Agitation speed at SS 200-L was not be shown because of company's confidential information.

3.3.4. Discussion

The mixing time tended to be shortened with increase of the agitation speed in all the bioreactor. However, there was no linear relationship between mixing time and agitation speed. A linear relationship equation was not reported in the previous study. As a mixing time is complicatedly affected by the shape of the bioreactor, the shape and number of impellors, and other factors, it is difficult to establish an exact prediction formula. Experiments in a 2000 L scale SS bioreactor showed that the mixing time was extremely long under the agitation conditions of Control-16. It was visually confirmed that the movement of the liquid phase was slow and insufficient for mixing during measurement. It was suggested that the performance of the bioreactor could be extremely decreased

without a certain number of agitations condition.

In all bioreactors, k_La increases in both oxygen and carbon dioxide with increase of the agitation speed. From the comparison between Vendor-B sinter pipe and drilled hole sparger, it was shown that the both of k_La increased by the small size of the bubbles. If bubbles with diameters of 20 μm and 2 mm, respectively, are supplied at the same airflow rate, the total contact surface area of 20 μm bubbles between the bubbles and the liquid is 100 times larger than that of 2 mm. However, the k_La(O_2) and k_La(CO_2) was only 1.4 and 1.1 times larger, respectively. The results suggest that factors other than surface area may be largely responsible for the increase of k_La. The sensitivity of k_La(O_2) and k_La(CO_2) to the change in the agitation speed are different. Therefore, the k_La ratio was not constant.

Chapter 4. Scale up based on k_La ratio

4.1. Introduction

In this chapter, fed-batch culture results of the process based on kLa ratio were reported.

In Chapter 3, appropriate cell culture conditions in k_La ratio was found in SS bioreactor and SUBs. Summary of these cell culture conditions was shown in Table 5.

Table 5 Summary for cell culture condition based on k_La ratio

Type		**Scale**	**Agitation condition**	**Additional sparge**	**Nitrogen**
Stainless-steel		200-L	Control agitation condition	NA	
		2000-L	Control-4 agitation condition	NA	
Single-use bioreactor	Vendor A	200-L	100 rpm	NA	
	Vendor B	200-L	100 rpm	NA	
	Vendor C	200-L	210 rpm	0.001 vvm	
	Vendor A	1000-L	55 rpm	NA	

4.2. Materials and Methods

4.2.1. Cell line and medium

Two recombinant CHO cell lines were used in this thesis. Cell lines A and B expressing Mabs A and B respectively, were established using CHOK1SV cells as the host cell and the GS Gene Expression System provided by Lonza Biologics (Slough, Berkshire, UK). These cell lines were selected in CD CHO medium (Life Technologies, Carlsbad, CA, USA) containing methionine sulfoximine. Chemically defined basal medium was developed for the fed-batch process by modifying the CD CHO medium with in-house proprietary supplements. This protein-free, chemically defined feed media developed in-house were used for the fed-batch culture for this study.

4.2.2. Fed-batch culture

Fed-batch cultures were performed using IgG-producing CHO cell line A and B. In-house serum-free basal and feed medium were used in these studies. Agitation was kept at speed which was determined to show the expected power per unit volume or $k_L a$ ratio. The temperature was kept at 37°C. The pH was controlled by pure CO_2 sparging and by the addition of 1.0M sodium carbonate. DO was controlled at 50% air saturation by aeration to headspace and, oxygen and air mixed gas aeration from sparger. Small amount of sample was taken from reactor and DO, dCO_2 and pH were measured by a blood gas analyzer (Rapidlab 348 blood gas analyzer (Bayer Corp., MA, USA)). Cell viability and

viable cell density were measured by Vi-CELL XR (Beckman Coulter, CA, USA). Glucose and lactate concentrations in the supernatant were determined with a BF-5 bioanalyzer (Oji Scientific Instruments, Hyogo, Japan). After centrifugation, Mab concentration was measured using an HPLC system (Waters/mS, USA) with a UV detector and a POROS PA affinity column (Applied Biosystems/mA, USA). Specific growth and production rates were calculated according to previous report (19).

4.3. Results

4.3.1. Fed-batch culture using SS 200-L and 2000-L based on k_La ratio scale-up

In order to evaluate the agitation condition determined by the k_La ratio, fed-batch culture using 200-L and 2000-L SS bioreactor were carried out and dCO_2 was evaluated during cultivation. Fed-batch cultivation was carried out for 16 days using Cell line B producing Mab-B. As shown in Figure **5**, antibody concentration, viable cell density, and viability in 2000L cultivation showed the same tendency with 200L result. The antibody concentration reached about 5.5 g/L. Specific Mab production rate was 42 ± 1.7 pg $cell^{-1}$ day^{-1} in 200-L scale and 49 ± 0.9 pg $cell^{-1}$ day^{-1} in 2000-L scale. The maximum viable cell density was obtained at 8 days of cultivation for the 2000-L scale, but at 10 days for the 200-L scale. On 200-L scale maximum viable cell density was about 14 x 10^6 cells/mL and on 2000-L scale maximum viable cell density was about 13 x 10^6 cells/mL,

respectively. Specific growth rate during day 1 to day 7 were 0.022 ±0.002 h^{-1} in 200-L scale and 0.022 ±0.002 h^{-1} in 2000-L scale respectively. However, dCO_2 tended to be kept lower at the 2000-L scale and showed similar value at end of cultivation. When Cell line A was cultured based on P/V as shown in Figure 3, the dCO_2 in the 200-L scale was lower throughout the cultivation. However, the agitation speed optimized based on the k_La ratio was set to 2000-L scale bioreactor, dCO_2 showed the similar tendency during cultivation, especially at the end of the cell culture. At the end of the cell culture, dCO_2 was 78 mmHg in 2000-L scale bioreactor process based on P/V and 57 mmHg in 200-L scale, respectively. On the other hands, lower dCO_2 was observed in 2000-L scale bioreactor process optimized by k_La ratio from day 6 to day 16. At the end of the cell culture, dCO_2 was 43 mmHg in 2000-L scale bioreactor process and 48 mmHg in 200-L scale, respectively.

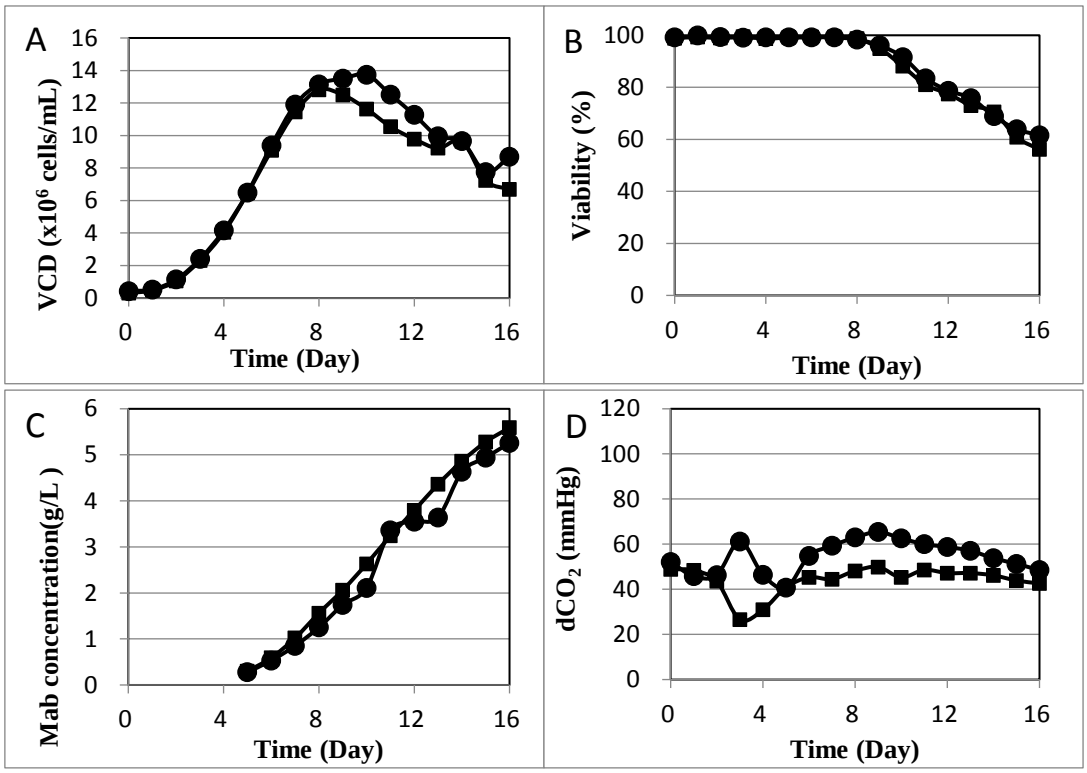

Figure 5 Time course of cell line B cultivation under agitation conditions based on k_La ratio on 2000-L scale SS bioreactor. A: Mab concentration, B: Viable cells density, C: Viability, D: dCO_2. Symbols are 200-L scale (closed circle) and 2000-L scale (closed square).

4.3.2. Fed-batch culture result comparing SS 200-L bioreactor to single-use bioreactors scaled up based on k_La ratio

The fed-batch culture was performed in the single-use bioreactor using the conditions determined by the agitation and aeration conditions determined by k_La ratio. In order to compare various types of single-use bioreactor, the agitation and aeration conditions for fed-batch culture was determined 100 rpm agitation for vendor A, 100 rpm for vendor B and 210 rpm with constant 0.001 vvm aeration of nitrogen for vender C respectively,

according to the result of k_La ratio. Sparger type is open pipe in vendor A and drilled hole sparger for vendor B, and C.

The agitation condition for 1000-L scale of vendor A are desirable at 65 rpm or more from the result of mixing time, but at 50 rpm from the result of k_La ratio. In previous result as shown in Figure 4, the mixing time greatly changed from 56 seconds to 34 seconds between 50 and 65 rpm. It is estimated that the mixing time of 1000-L scale of vendor A might be the same value as that of 200L SS bioreactor between 50 and 65 rpm conditions. Based on this assumption, we selected agitation speed for 55 rpm in 1000-L scale single use bioreactor of vendor A.

Fed-batch cultures were performed using Cell line A producing Mab-A using 200-L scale stainless steel bioreactor, (control rpm,), 200-L scale single use vendor-A (100 rpm, open pipe type sparger), 200-L scale single use vendor-B (100 rpm, drilled hole type sparger), 200-L scale single use vendor-C (210 rpm, additional constant nitrogen gas sparge at 0.001vvm, drilled hole type sparger), and 1000-L scale single use bioreactor of vendor A(55 rpm, open pipe type sparger). Sixteen days of culture was performed and sampling was carried out daily during the culture period to measure viable cell density, viability, glucose and lactate concentrations (data not shown), and antibody concentration. Figure 6 shows times courses of antibody concentration, viable cell density, viability, and dCO_2.

Antibody concentration, viable cell density, and viability showed the similar trend among bioreactors. The maximum viable cell densities in all bioreactors were between 17 and 21 x 10^6 cells/mL. Compared with cell culture experiment shown in Figure 3, the viability gradually decreased after day 12 of cultivation. Specific growth rate during day 1 to day 7 were 0.021 ±0.003 h^{-1} 200-L scale SS bioreactor, 0.020 ±0.002 h^{-1} in 200-L scale vendor-A 0.020 ±0.002 h^{-1} in vendor-B, 0.021 ±0.001 h^{-1} in 200-L scale vendor-C and 0.019 ±0.002 h^{-1} in 1000-L scale vendor-A. The antibody concentration reached about 6 g/L at the end of cultivation. Specific Mab production rate was 33 ± 1.7 pg $cell^{-1}$ day^{-1} in 200-L scale SS bioreactor, 33 ± 0.5 pg $cell^{-1}$ day^{-1} in 200-L scale vendor-A, 35 ± 05 pg $cell^{-1}$ day^{-1} in vendor-B, 29 ± 0.5 pg $cell^{-1}$ day^{-1} in 200-L scale vendor-C and 35 ± 1.8 pg $cell^{-1}$ day^{-1} in 1000-L scale vendor-A. Among all fed-batch cultivation dCO_2 gradually decreased until the day 4 of the cultivation.

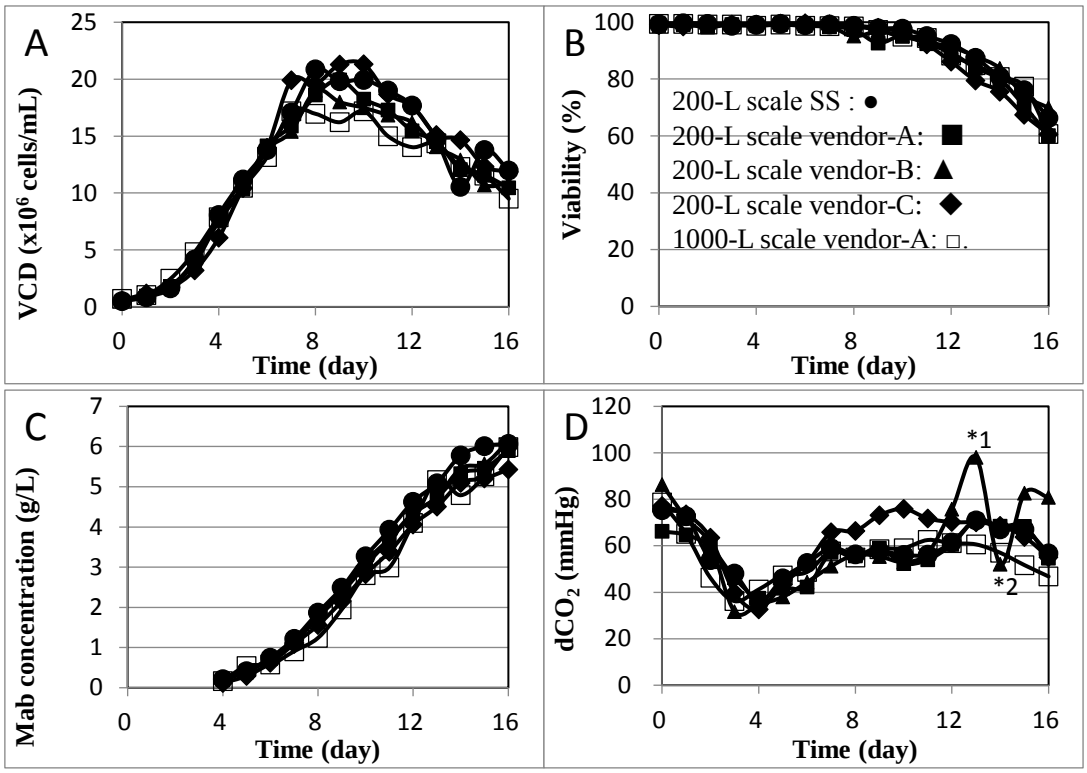

Figure 6 Time course of cell line A cultivation under the agitation conditions based on k_La ratio on single use bioreactors. A: Viable cells density, B: Viability, C: Mab concentration, D: dCO_2. Symbols are 200-L scale stainless steel (closed circle), 200-L scale vendor-A (closed square), 200-L scale vendor-B (closed triangle), 200-L scale vendor-C (closed rhombus), and 1000-L scale vendor-A (open square). Regarding 200-L scale vendor-B, agitation speed was changed from 100 to 90 rpm (*1) and from 90 to 100 rpm again (*2).

The aeration and agitation conditions were 200-L scale stainless steel (Control rpm), 200-L scale vendor-A (100 rpm, open pipe type sparger), 200-L scale vendor-B (100 rpm, drilled hole type sparger), 200-L scale vendor-C (210rpm, additional constant nitrogen gas sparge at 0.001vvm, drilled hole type sparger), and 1000-L scale vendor-A (55 rpm, open pipe type sparger).

4.3.3. Discussion

The accumulation dCO_2 at the end of the cultivation was only observed in the vendor B single-use bioreactor, but the time-course trend of the viable cell density was similar. It was considered that accumulation of foam on the surface of the culture fluid which could

not be depressed by the antifoam agent addition might cause the dCO_2 accumulation. The accumulation of foam seems to be related to the size of the aeration bubble, which is closely related to the shape of the sparger and the aspect ratio of the bioreactor. The dCO_2 accumulation was observed on day 13 of culture using vendor B bioreactor. To decrease dCO_2, the agitation condition of vendor b bioreactor was changed from 100 rpm to 90 rpm. And then, the aeration rate automatically increased in order to keep constant DO and dCO_2 decreased. However, dCO_2 dropped to lower level than those of other bioreactors cultivation at the time of sampling on the day 14 of culture, I changed the agitation rate to100 rpm until harvest time. The results of quality analysis of Mab A at the end of cultivation were shown in Table 6. Charge heterogeneity is important for safety and efficacy of therapeutic antibody (20). Aggregates may induce adverse immune response (21). Glycan profile also affects the ADCC activity, CDC activity and half-life in blood (22). Wang reported that galactosylation ratio in glycan profile was increased in low dCO_2 condition (23). In this fed-batch culture study all these results of quality analysis were similar in all bioreactors as shown in Table 6.

Table 6 Quality analysis for Mab A produced in single-use bioreactor.

Test Items		Stainless 200-L scale	Vendor-A 200-L scale	Vendor-B 200-L scale	Vendor-C 200-L scale	Vendor-A 1000-L scale
Imaged Capillary Isoelectric Focusing (icIEF)	pI :	9.49	9.49	9.49	9.50	9.49
	Acidic Species (%) :	42.6	43.17	43.14	41.36	43.69
	Major Isoform (%) :	53.7	53.4	53.4	54.4	52.9
	Basic Species (%) :	3.71	3.44	3.46	4.26	3.4
Size-Exclusion Chromatography (SEC)	% Monomer :	99	99	99	99	99
	% Aggregates :	0.48	0.49	0.46	0.48	0.46
Glycan profile	GN1G0 (%) :	0.4	0.4	0.5	0.5	0.4
	G0 (%) :	4.9	4.9	5.6	5.7	5.1
	GN1G0F (%):	0.1	0.1	0.1	0.1	0.1
	G0F (%) :	88.4	87.7	87.1	87.3	87.7
	Man5 (%) :	1.3	1.3	1.6	1.8	1.3
	G1F (%) :	2.3	2.4	2.0	2.6	2.1

P/V-based scale-up has the advantage of short process design time because the scale up procedure could be attained by calculation on the basis of bioreactor design. On the other hand, the k_La ratio-based scale-up requires experimental data for k_La (O_2) and k_La (CO_2) under various agitation and sparging conditions. However, it was suggested that it may be possible to construct a comparable scale up process with higher probability than P/V. In addition, kLa ratio-based scale-up could be applied for various single-use bioreactor scale-up, even their shape and blade type are not the same. In this research, this scale-up strategy was able to be used in two cell lines. And k_La may be estimated by computational fluid dynamics (24, 25). Therefore, it is considered to be a powerful tool for technology transfer to manufacturing site i.e., contract manufacturing organization (CMO) who are using various types of bioreactor. In early development stage of biopharmaceuticals, a period of process development is very limited. Under this circumstance, scale-up method based on P/V is suitable for quick process development. In contract, for development of late stage programs, it might be better to use k_La ratio scale-up for determination of agitation condition to construct robust process to achieve stable and reproducible dCO_2 trend.

From the view point of performances of the bioreactor, I focused on the k_La ratio and used it for the scale-up factor. Both k_La(O_2) and k_La(CO_2) changed with increases of the

sparging rate and agitation speed. However, the effect of the sparging rate and agitation speed on $k_La(O_2)$ and $k_La(CO_2)$ value are not the same. CO_2 in medium could be removed by oxygen gas sparging which supplied for DO control. The difference in $k_La(O_2)$ under various bioreactor operation causes the difference of aeration rate of oxygen, and also caused the difference of accumulation of CO_2 in each cultivation. It was confirmed that accumulation of dCO_2 did not occur at the determined agitation and aeration condition where the k_La ratio is similar to the bioreactor process in which dCO_2 does not accumulate. In lower agitation condition, k_La ratio was higher as shown in Table 2 and Table 3 (e.g. 0.49 in Conrol-6 agitation condition and 0.30 in Control+1 agitation condition with 0.0085 vvm in SS 2000-L, 0.46 in 50 rpm and 0.28 in 80 rpm with 0.035 vvm in Vendor-A 1000-L). However, in lower agitation condition, the mixing time could be delayed, causing non-uniform mixing in the bioreactor, affecting the cell culture performance. The mixing time and accumulated CO_2 were supposed to be in a trade-off relationship.

Chapter 5. Conclusion

Biopharmaceuticals are now one of the main approaches to the various therapeutic area. More than half of top ten selling pharmaceutical drugs are biopharmaceuticals produced by bioprocess. Scale-up and scale-out method are major strategy to obtain production capability for bioprocess. Bioprocess scale-up in biopharmaceuticals production is important to produce drug substance keeping comparability of productivity and quality and reducing cost of goods.

Several Mabs have been launched in the market and a lot of Mabs are developing now. Mabs have often been produced by batch or fed-batch culture process using CHO cells. Accompanying improvement of productivity, quality assurance, is required. In addition, production scales are expected to be increased with the development stage of pharmaceutical pipeline progresses and the manufacturing site changes. Quality equivalence (comparability) should be kept during these development stage and scale-up process to ensure efficacy and toxicity consistency.

As various cell culture parameters, such as pH and temperature are known to affect a product quality such a glycan profile, and aggregation content, optimal culture conditions have been investigated using various cell lines and cell culture medium. It is also well known that the dCO_2 also affects the product quality. However, since CO_2 was produced from cells by cellular metabolism of TCA cycle, it is difficult to exactly control the dCO_2

during cell culture and/or scale up processes. In this thesis, I compare the scale up factors obtained by calculation and by experiment. I evaluated both factors in view of cell growth, productivity and dCO_2 trend. Advantage and disadvantage were discussed in this thesis.

In Chapter 2, I focused on Power per unit volume (P/V) value for a scale-up factor obtained from the calculation. Fed-batch culture was conducted in 200-L and 2000-L scale SS bioreactor. The maximum viable cell density was about 20 x 10^6 cells/mL in both fed-batch culture. The viability gradually decreased after the 12th day of culture. The Mab concentration reached about 6 g/L at the time of harvest. dCO_2 shifted around 60 mmHg in the 200-L scale throughout the culture period, but it was kept at around 80 mmHg on the 2000-L scale. dCO_2 tended to be higher on the 2000-L than that on 200-L cultivation. Even the different dCO_2 during cultivation, cell growth and the productivity were similar between 200-L and 2000-L cultivations.

In Chapter 3, mixing time and k_La for a scale-up factor obtained from the experimental data. The mixing time was analyzed using pH measurement. The mixing time of the 200-L scale SS bioreactor was 21 seconds under the control agitation condition. In the 2000-L SS bioreactor, mixing time was 40 seconds under the agitation condition which was determined based on P/V. In the 2000-L SS bioreactor, mixing time was similar in the agitation conditions among control to control-6. The mixing time of single-use

bioreactors from vendor A, B, and C were also evaluated. Vendor A provided 200-L scale and 1000-L scale, and vendors B and C provided the 200-L scale. On the 200-L scale, the mixing time for vendor A reactor was about 30 seconds between 45 rpm and 100 rpm. The time for vendor B reactor was about 35 seconds at more than 100 rpm, and the time for vendor C reactor was less than 35 seconds at more than 210 rpm. From the results of mixing time and comparison with a SS bioreactor, it was estimated that the appropriate agitation range for 200-L single-use reactor was 45 rpm or more for vendor A, 100 rpm for vendor B and 210 rpm or more for vender C, respectively.

In Chapter 4, the k_La ratio calculated by $k_La(O_2)$ and $k_La(CO_2)$ was evaluated for the scale-up parameter of bioreactor. In order to evaluate the agitation condition determined by the k_La ratio, fed-batch culture using 200-L and 2000-L SS bioreactor were carried out and dCO_2 was evaluated during cultivation. Mab concentration, viable cell density, and viability in 2000L cultivation showed the same tendency with 200 L result. The antibody concentration reached about 5.5 g/L in both scales. The agitation speed optimized based on the k_La ratio was set to 2000-L scale bioreactor. dCO_2 showed the similar tendency during cultivation, especially at the end of the cell culture. Fed-batch cultures were performed using Cell line A producing Mab-A using 200-L scale stainless steel bioreactor, (control rpm,), 200-L scale single use vendor-A (100 rpm, open pipe type sparger), 200-

L scale single use vendor-B (100 rpm, drilled hole type sparger), 200-L scale single use vendor-C (210 rpm, additional constant nitrogen gas sparge at 0.001vvm, drilled hole type sparger), and 1000-L scale single use bioreactor of vendor A (55 rpm, open pipe type sparger). Mab concentration, viable cell density, and viability showed the similar trend among bioreactors. The antibody concentration reached about 6 g/L at the end in all bioreactor cultivation. The cell culture performance and product quality at the end of the cultivation process were comparable for all tested SUBs.

Through this research, all scale-up studies were achieved successfully. In the scale-up with P/V dCO_2 were slightly higher in large scale. However, this accumulation didn't have impact on productivity. The scale-up method to avoid accumulation of dCO_2 was developed in this research. I concluded that the k_La ratio is a powerful scale-up factor useful to control dCO_2 during fed-batch cultures.

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