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Full Length Research Paper

Simple process capability analysis and quality validation of monoclonal antibody production in bench-top bioreactor

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The production of monoclonal antibody (MAbs) for anti-cancer by fermentation of hybridoma cell in bench-top bioreactor was investigated. The main aim of this study was to increase the percentage of cell viability and to achieve high yield of IgM. Many ways can be applied to improve the process and one of them is by choosing the correct six sigma's design of experiment (DOE). In this study, Taguchi's experimental design was applied to achieve high percentage of cell viability in the fermentation experiment. The process capability of this study was later analyzed by using the MiniTab Software (Ver. 14). Analysis showed that this study fulfilled a minimum target of process improvement as indicated by the Cp and Cpk value of 0.90 and 0.86. This proved that the fermentation experiment has achieved cell viability of more than 80% throughout the study.

Key words: Monoclonal antibody, hybridoma, process capability, Taguchi experimental design.

INTRODUCTION

Since the advent of monoclonal antibodies (MAbs) from hybridoma cells in 1975, hybridoma technology has had a major impact on the scientific and research world. The demand for MAbs has increased considerably in recent years. MAbs are being used in *in vitro* diagnostics, immunoaffinity chromatography, *in vivo* imaging and immunotherapy (Freshney, 2000; Jackson, 1999). While the demand for MAbs will remain primarily stable in other areas, it is projected that human therapy (primarily the treatment of cancer) will require large quantities of highly pure monoclonal antibodies, which can be conservatively estimated at 50 kg/year for each therapeutic MAb. The excessive costs of producing sufficient quantities of MAbs and the small yields from conventional technology are important factors in making research and development of cost effective large scale MAb production essential (Mel and Yumi, 2004; Zola, 2000; Stoll et al., 1996).

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MATERIALS AND METHODOS

Culture medium and cell line

Established RPMI 1640 (Cellgro), supplemented with glucose, L-glutamine and fetal bovine serum, FBS (Cellgro) was used as the culture medium throughout the experiment. The hybridoma cell line C3A8 producing IgM was routinely cultivated in 25 cm^2 culture flask (Nunc) at 37°C, 5% CO_2 in a humidified incubator (Galaxy S, RS Biotech) and subcultivated after 2 or 3 days. For the experiments, cell was taken from the exponential growth phase with the concentration of 1.5 to 1.7 × 10^5 cells/ml as the inoculum.

Cell cultivation in bioreactor

The bioreactor that was used in this study is a 2.5 L bench-top bioreactor with 1 L working volume (Figure 1). The vessel must be thoroughly clean prior to use. The vessel was soaked, rinsed and dried to remove any material that may inhibit cell growth. Later, the bioreactor was assembled with the pH probe, filters and tubes. The pH calibration was done at the Digital Control Unit (DCU) by using two buffer solution having pH 7.0 (green) and 4.21 (red). After filled with about 1 L of distilled water (dH_2O), the bioreactor was autoclaved for 30 minutes at 121°C.

After the sterilization process was completed, the bioreactor was

Figure 1. Benchtop-stirred tank bioreactor for MAb production.

Table 1. Setting parameter for bioreactor operation with Taguchi's design.

Batch	A	B	C	pO_2 (%)	rpm	pH
Run 1	1	1	1	30	50	7.2
Run 2	2	1	2	50	50	7.4
Run 3	1	2	2	30	100	7.4
Run 4	2	2	1	50	100	7.2

reconnected to the DCU. Distilled water was then vacuumed out and was replaced with fresh medium. After that pO_2 probe was calibrated by using nitrogen gas for 0% reading (zero calibration) and air for 100% reading (slope calibration). After the calibration process, the parameters of the bioreactor operation: pH, temperature and impeller speed were set according to desired level. According to Freshney (2000) and Mel et al. (2008), the stirring speed were in the range of 30 and 100 rpm which is sufficient to prevent cell sedimentation and also will not create high shear force that would damage the cells.

Once the parameters have reached the desired values, cells were inoculated into the vessel. In this study, the inoculum size is 10% from the working volume. So, for 1 L working volume, 100 ml of hybridoma cell broth was added to 900 ml culture medium. The inoculum was transferred into the bioreactor vessel via connection tube of the inoculum flask and inoculum pipeline of the bioreactor. The connection tube was clamped-off, and by gravity action, the inoculum inside the inoculum flask flowed through the connection tube into the vessel. After inoculum has been completely transferred into the bioreactor vessel, the connection tube was disconnected aseptically. During the bioreactor operation, 1 M sodium hydroxide (NaOH) solution, 1 M hydrochloric acid (HCl) solutions and CO_2 gas were used to maintain the pH of culture medium at 7.2 ± 0.1. Sampling was done every 6 h to determine the cell growth.

Design of the experiment

Optimization of MAb production in bioreactor was carried out according to Taguchi Robust design experiments (orthogonal arrays). Three selected parameters: Partial O_2 pressure, stirrer speed and pH were varied in this experiment to investigate their optimum value in response to maximum MAb concentration produced. The design consists of 4 runs with each parameter having 3 levels (Mel et al., 2006a; Mel and Lubis, 2006b; Rowlands et al., 2000; Taguchi et al., 2005). The layout of the design is shown in Table 1.

Sample analysis

Five milliliters sample from the vessel was collected into the sampling tube of the bioreactor. Then, the sample was transferred into a centrifuge tube. 10 µl of sample in the centrifuge tube was later taken out to count the cell number and its viability by using the

Table 2. Log viable cell number (VCN) for each bioreactor run.

Time (h)	Log VCN			
	Run 1	Run 2	Run 3	Run 4
0	5.83251	5.89934	5.91291	5.9325
6	5.97559	5.94036	5.97482	5.8404
12	5.98808	5.98081	5.99209	5.9808
18	6.09395	6.03952	5.9345	6.0194
24	6.19638	6.29638	6.29964	6.1964
30	6.04139	6.07918	6.23045	6.1461
36	6.18556	6.12878	6.129	6.2945
42	6.36736	6.27107	6.26922	6.2748
48	6.56075	6.46096	6.36128	6.2634
54	6.36263	6.36263	6.16263	6.1626
60	6.35684	6.36693	6.36839	6.2683
66	6.26628	6.26721	6.26532	6.1672
72	6.26232	6.26335	6.26335	6.1644
78	6.29106	6.46021	6.16128	6.2591
84	6.25563	6.15682	6.25441	6.1544
90	6.35289	6.45276	6.25289	6.5289
96	6.15502	6.15514	6.55023	6.5563
102	6.24314	6.23136	6.24472	6.2431
108	6.14613	6.15534	6.14613	6.1492

trypan blue dye exclusion test. Number of cell counted on the hemacytometer counting chamber was recorded.

RESULTS AND DISCUSSION

Viable cell number (VCN) readings for each bioreactor run were converted into log numbers as shown in Table 2. Figure 2 shows the means plot for the bioreactor experiment. The plot indicates the effect of each parameter on log VCN. From the figure, it can be observed that the pH is the most significant parameter with regards to log VCN. Lower pH yielded greater log VCN of hybridoma cell culture in the bioreactor. Following the pH is the pO_2 which also has significant effect on log VCN in which lower pO_2 increased the log VCN. The third parameter, that is, the stirrer speed has the least effect on log VCN. Nevertheless, it was analyzed that higher stirrer speed would increase the log VCN.

Six Sigma is a condition of the generalised formula m for process capability, which is defined as the ability of a process to turn out a good product (Shina, 2002). It is relationship of product specification to manufacturing variability, measured in terms of *Cp* or *Cpk*, or expressed as a numerical index. *Cp* index is defined as the ratio of specification width over the process spread, while *Cpk* index is defined as a measure of how close a process is running to its specification limits, relative to the natural variability of the process. Six Sigma is achieved when the product specification are at ±6σ (σ is the symbol for standard deviation) of the manufacturing process. In the design community, *Cp* = 1 is also called 3σ design (natural tolerance) and *Cp* = 1.33 is called 4σ sigma design. Both indexes can be calculated by using the following formula:

$$C_p = \left(\frac{USL - LSL}{6\sigma}\right) \quad (1)$$

$$C_{pk} = \min\left(\frac{\mu - USL}{3\sigma}, \frac{USL - \mu}{3\sigma}\right) \quad (2)$$

Where, USL, LSL and σ denote the upper specification limit, lower specification limit and process standard deviation associated with the measurements, respectively.

As shown in Figure 3, the value of *Cp* and *Cpk* indexes obtained in this study was 0.90 and 0.86, respectively. The process mean falls short of the target and the process distribution mean lies on the left of the target. Also, the left tail of the distribution falls outside the lower specification limit. Therefore, some log VCN will not meet the lower specification of 5.9. The capability analysis command also produced a table of statistics. The *Cpk* index indicates whether the process will produce units within the tolerance limits. A *Cpk* index of 1 means that a process is exactly capable of meeting specifications; while less than 1 means that the process did not meet the specification limits (Rezaie et al., 2006; Shina, 2002). The *Cpk* index for MAb production is only 0.86, which indicates

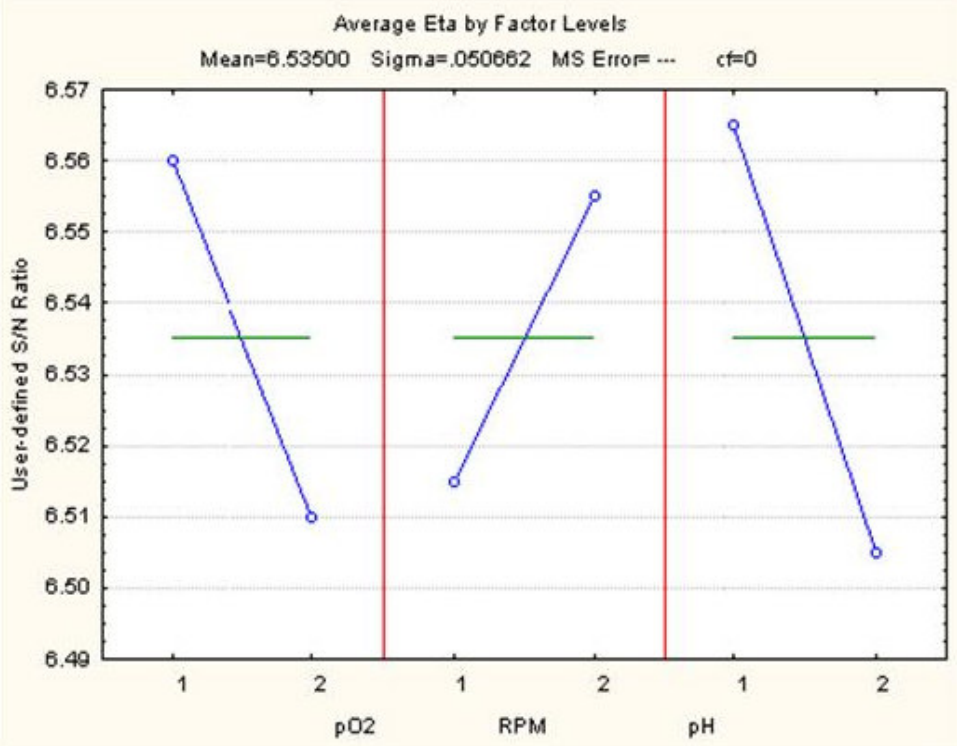

Figure 2. Means plot for Log VCN.

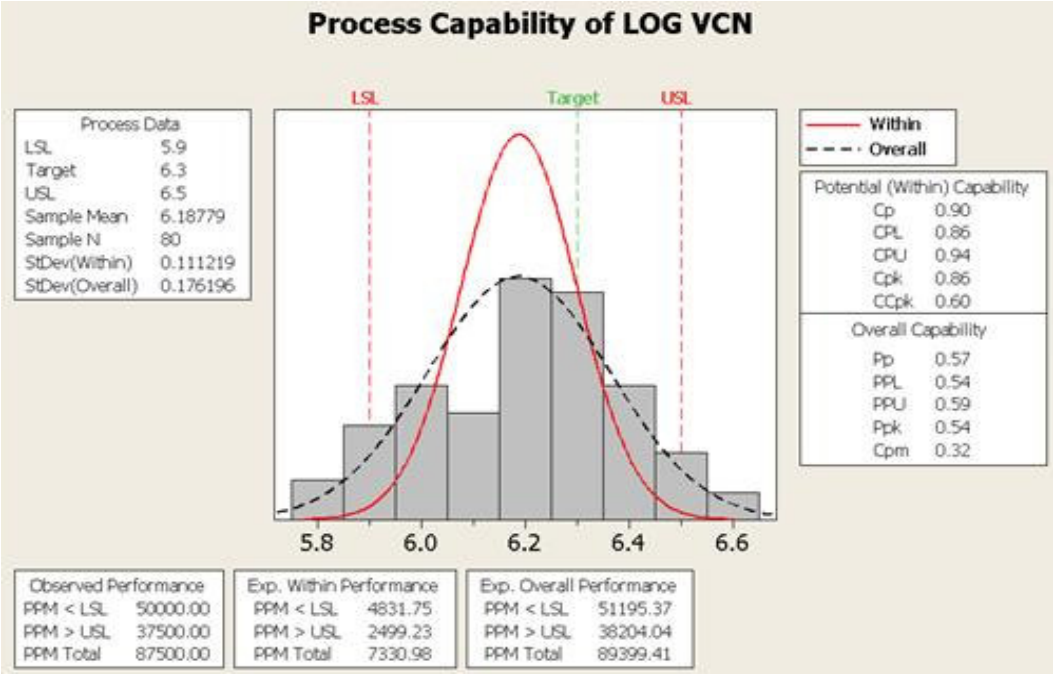

Figure 3. Process capability of Log VCN.

cates that improvements must be done to increase the process capability, for example, by reducing the variability or by cantering the process on the target.

Control chart have been traditionally used as a method to determine the performance of manufacturing process over time by statistical characterization of a measured

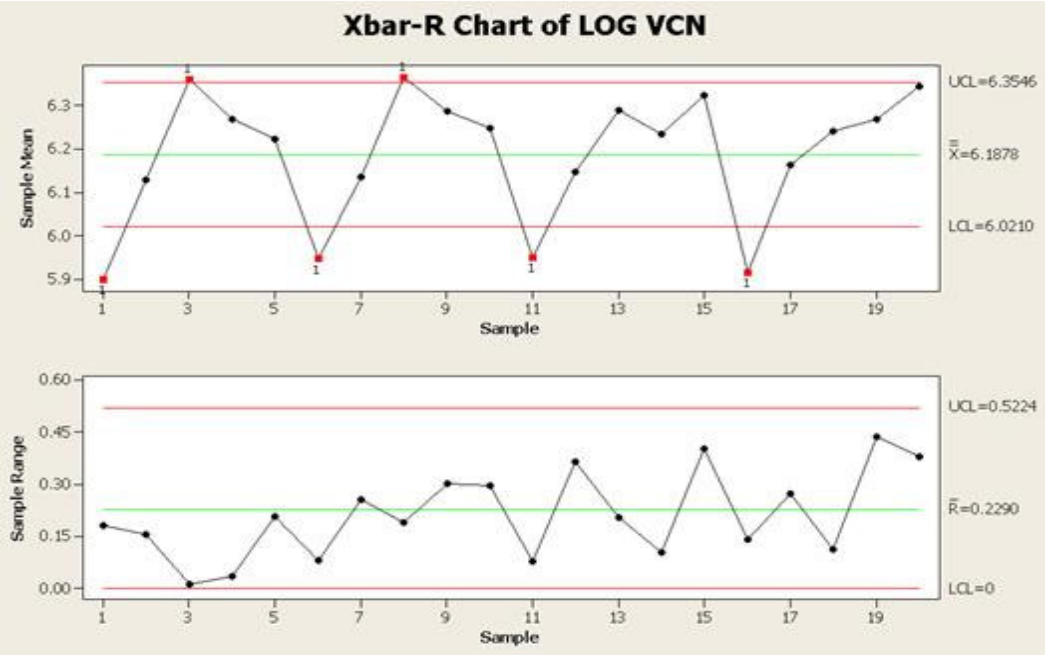

Figure 4. Xbar-R chart for Log VCN.

parameter which is dependent on the process. They have been used effectively to determine whether the manufacturing process is in statistical control (Montgomery, 2005; Rezaie et al., 2006). There are two major types of control charts: A variable chart which plot continuous data from the observed parameters, and attribute charts, which are discrete and plot accept/reject data. Variable chart are also known as Xbar, R chart for high volume and moving range charts for low volume. Xbar and R control charts are used to monitor the average and the standard deviation of production. The Xbar chart shows whether the manufacturing process is centred around or shifted from the historical average. The R charts shows the uniformity or consistency of the manufacturing process.

According to the Xbar and R charts illustrated in Figure 4, both means and ranges for log VCN appear to be in control, although, it can be observed that the mean is 6.18, and not 6.38. The average range for log VCN is 0.2290. This means that there is a slight uniform effect on the process of bioreactor operation.

Conclusion

Process capability index has been the most popular index used in the manufacturing industry as a process performance indicator. Since *Cpk* is indirectly related to the percentage of non conforming products, it may be used as an estimation of the process capability to produce products that are confirmed to be specification in terms of the specific process characteristics. The presented *Cpk* index shows that this bioreactor process needs to be improved further.

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