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# Kinetic Analysis of the Effect of Cell Density on Hybridoma Cell Growth in Batch Culture

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**Abstract** The effect of cell density on cell growth was investigated in a suspension batch culture of hybridoma cells. The specific growth rate was found to increase with increasing initial cell density and then to decrease with further increases in initial cell density. In order to quantitatively describe the dependence of specific growth rate on cell density, a kinetic model is proposed, which satisfactorily represents the experimental data.

*Keywords*: cell density, growth rate, hybridoma, kinetic model

In recent years, *in vitro* cultivation of hybridoma cells has been increasingly used to produce monoclonal antibodies for research diagnostics and therapeutic purposes [1,2].

Anchorage-dependant mammalian cells, which grow as surface monolayers, are growth-limited by cell density. This growth limitation is due not only to nutritional limitations and toxic metabolites but also to cell contact inhibition. A phenomenological model that describes the dependence of specific growth rate on cell density has been proposed [3]. In the case of hybridoma cells, which can grow anchorage-independently, the specific growth rate is increased upon increasing the initial cell density up to a certain cell density; further increases in initial cell density result in reduced specific growth in batch culture. However, no kinetic study has quantitatively analyzed cell density effects on anchorage-independent cell growth. The determination of the cell growth rates of hybridoma cells is a prerequisite for the development of a bioreactor system for the commercial production of monoclonal antibodies, and cell density effects should be considered to determine the cell growth rate. The objectives of this work were to analyze the effect of cell density on hybridoma cell growth and to propose a cell density inhibition model describing this growth phenomenon to predict growth rates in batch and fed-batch culture.

The hybridoma KA112 secreting IgM type antibody against *Chlamidia trachomatis* L2 type was used in this study. The cell culture medium used was a low serum medium (DMEM supplemented with 1% (v/v) FBS, 2 g/L kanamycin, 2 g/L sodium carbonate, 150 mg/L oxaloacetate, 0.01% (w/v) pluronic F-18, and 75.5 μg/L insulin) [4]. Cells were maintained in tissue culture dishes in a $CO_2$ incubator.

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**Table 1.** The effect of initial cell density on specific growth rate

| Initial cell density ($\times 10^4$ cells/mL) | Specific growth rate ($h^{-1}$) |
|---|---|
| 12 | 0.0230 |
| 17 | 0.0235 |
| 25 | 0.0289 |
| 37 | 0.0302 |
| 47 | 0.0331 |
| 54 | 0.0361 |
| 66 | 0.0325 |
| 76 | 0.0303 |
| 98 | 0.0203 |
| 123 | 0.0198 |
| 157 | 0.0120 |

The specific growth rates were determined by linear regression of the early exponential growth phase data. Each value represents the average of three cultures.

Cells were cultivated for three days in 60 mL of low-serum medium in a spinner flask (Bellco Glass Inc., USA) to measure the specific growth rates at various initial cell densities. The agitation speed used was 40 rpm and all the spinner cultures were performed in a humidified $CO_2$ incubator. A celligen bioreactor (NBS inc., USA) was used for the batch cultivation of hybridoma cells. During batch cultures, DO and pH were controlled at prescribed levels.

The number of cells was determined using a hemocytometer. Viable cells were distinguished by the trypan blue dye exclusion method. Cell viability was found to be above 94% during exponential growth. Glucose concentration was determined by the DNS method.

As shown in Table 1, initial cell density affected the specific growth rate, and the specific growth rate was found to increase with increasing initial cell density up to a certain cell density level (about $54 \times 10^4$ cells/mL). Further increases in cell density resulted in a reduced

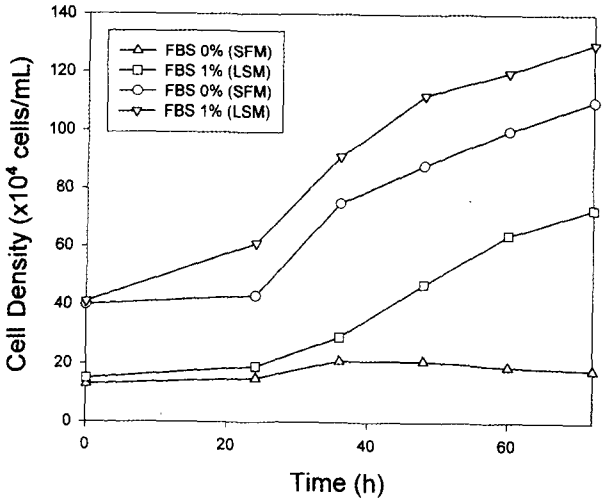


**Fig. 1.** Effect of initial cell density on cell growth at various serum concentrations (FBS, fetal bovine serum; SFM, serum free medium; LSM, low serum medium).

specific growth rate in suspension batch culture. Similar results have been previously reported and it was suggested that the amount of serum and the nutritional requirements of cells are lower at higher cell density [5].

Some results were reported to explain the specific growth rate variation with cell density [6-12]. Three reasons have been proposed to explain why the specific growth rate increases with increased initial cell density up to a certain level. First, cells can secrete growth factors that stimulate cell growth during the initial lag phase. This assumption is based on experimental results, which show that high initial cell density does not require serum to provide various types of growth factors. As shown in Fig. 1, cells at low initial cell density ($10 \times 10^4$ cells/mL) do not grow well in serum-free medium, whereas cells at a high initial cell density ($40 \times 10^4$ cells/mL) grow well. Second, cells possess detoxification ability in their environment. Table 2 shows the effect of toxic metabolites, such as the ammonium ion and lactate, on specific growth rate and maximum cell density in batch culture. Cells cannot grow on a medium with 5 mM or 10 mM of ammonium ion at low initial cell density ($19 \times 10^4$ cells/mL), whereas cells at high initial cell density ($40 \times 10^4$ cells/mL) grow well (Fig. 2). Third, there is a specific cell-cell interaction. Many mammalian cells including hybridoma cells stimulate cell growth *via* intercellular contact [3].

Further increases in initial cell density result in a reduced specific growth rate. It can be inferred that high maintenance energy and the accumulation of toxic metabolites at high initial cell density reduce specific growth rate. Since cellular viability at high initial cell density remains good, a high maintenance energy requirement might be the main reason for this specific growth rate reduction. Hybridoma normally requires 60% of total energy as maintenance energy and KA112 needs up to 81% of total energy as maintenance energy,

**Table 2.** The effects of initial glucose, glutamine, lactate, and ammonia concentrations on specific growth rates

| | Concentration (mM) | Specific growth rate ($h^{-1}$) |
|---|---|---|
| Glucose | 2.7 | 0.030 |
| | 5.4 | 0.033 |
| | 16 | 0.032 |
| | 24 | 0.034 |
| Glutamine | 4 | 0.033 |
| | 6 | 0.034 |
| | 8 | 0.035 |
| | 12 | 0.033 |
| Lactate | 0 | 0.034 |
| | 10 | 0.035 |
| | 20 | 0.028 |
| | 30 | 0.021 |
| Ammonia | 0 | 0.035 |
| | 1 | 0.032 |
| | 5 | 0.027 |
| | 10 | 0.020 |
| | 15 | 0.009 |

The specific growth rates were determined by linear regression of the early exponential growth phase data. Each value represents the average of three cultures.

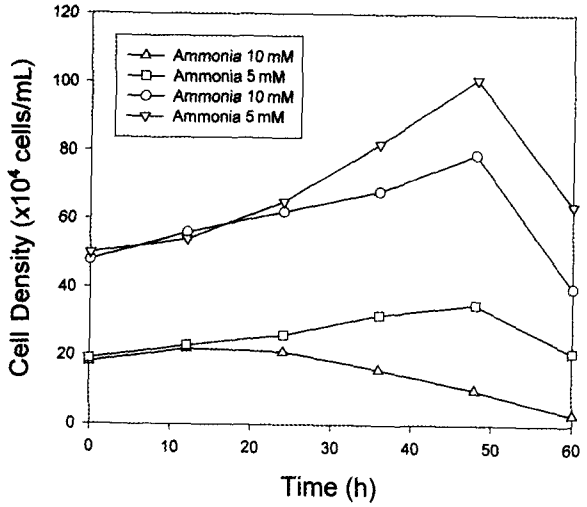


**Fig. 2.** Effect of initial cell density on cell growth at various ammonia concentrations.

from kinetic analyses of batch cultures.

In terms of microbial cell growth, the growth patterns of cell are governed by a hyperbolic relationship and cell density is limited. To describe such growth kinetics, it has been proposed the logistic equation:

$$\mu = \mu_m [1 - X/X_m] \quad (1)$$

where $\mu$: specific growth rate
$\mu_m$: maximum specific growth rate

$X$: cell density
$X_m$: maximum cell density

Another modified logistic equation, which includes an index for the inhibitory effect, accounting for the deviation of growth from the exponential relationship was introduced [13].

$$\mu = \mu_m [1 - (X/X_m)^n] \quad (2)$$

where n: index for the inhibitory effect

An empirical model for contact inhibition of anchorage-dependent cell was also proposed [3].

$$\mu = \mu_m [1 - \exp\{(-C)(1-X/X_m)\}] \quad (3)$$

where $C$: index for contact inhibition

However, the above model equations are monotonically decreasing functions of cell density and describe only the inhibitory effect caused by increased cell density in the course of cell growth. A model is needed which describes not only the inhibitory effects but also the stimulatory effect. Hence, we propose the following equation, which takes into account the fact that cells act as activators at low cell density and as inhibitors at high cell density.

$$\mu = \mu_m \frac{X}{K_x + X}[1 - X/X_m]^n \quad (4)$$

where $K_x$: inhibition constant (cells/mL)
$X_m$: maximum cell density which growth is completely inhibited (cells/mL)
n: index for inhibitory effect of cell density

On fitting the proposed model with experimental data, it was assumed that the stimulatory effect or the inhibitory effect can be lumped into a single cell density term, $X$. As it was difficult to obtain the value of $X_m$ by experiment, $X_m$ was obtained by linear regression extrapolation using the data collected in the declining region of specific growth rate curve. The fitting between the proposed equation and the experimental data is shown in Fig. 3, which shows good agreement between the theoretical curve and the experimental data.

To see the effect of various $X_m$ values on specific growth rates and curve patterns, the $X_m$ value was increased from 180($\times 10^4$ cells/mL) to 300. The transition between maximum specific growth rate and zero specific growth rate changes from concavity downward to concavity upward and the value of n increases from 0.746 to 3.374. To remove the dependence of $\mu$ on $X_m$, and to determine the effect of n alone on $\mu$, dimensionless cell density, $\chi$, as $(X/X_m)$, dimensionless growth rate, $\gamma$, as $(\mu/\mu_m)$, and the dimensionless parameter, $\kappa$, as $(K/K_x)$ were defined. The proposed model equation can then be written in dimensionless form.

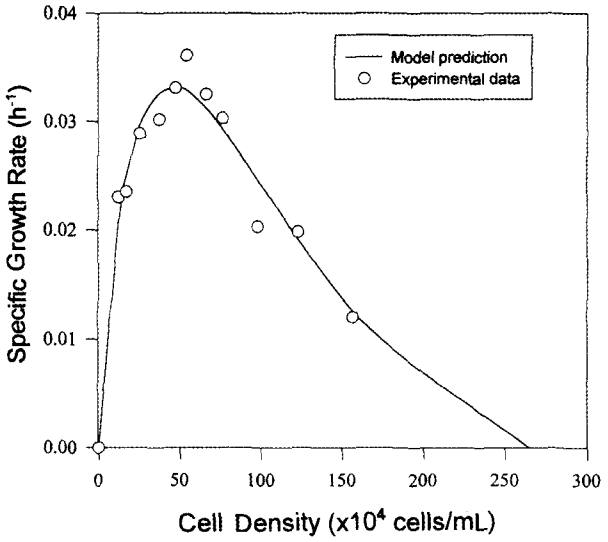


**Fig. 3.** Comparison of model-predicted values of specific growth rate with experimental data.

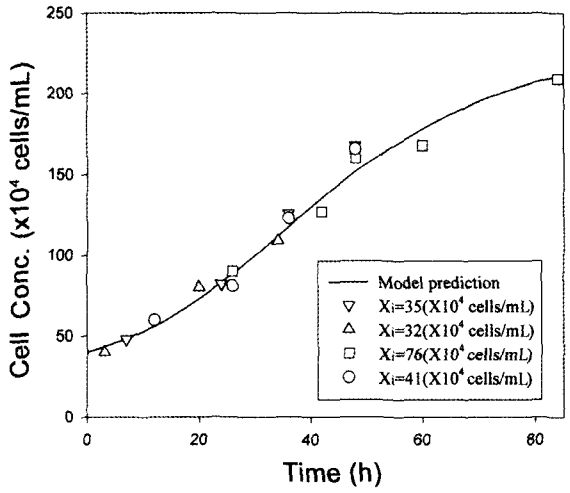


**Fig. 4.** Fitting of the predicted growth curve to the experimental data.

$$\gamma = \frac{\chi}{\kappa + \chi}[1 - \chi]^n \quad (5)$$

where $\gamma$: dimensionless growth rate
$\chi$: dimensionless cell density
$\kappa$: dimensionless inhibition constant

The effect of n on the relation between $\gamma$ and $\chi$ was analyzed. When $n<1$, a slow initial decrease in the specific growth rate is followed by a rapid drop to zero. When n is unity, a linear decrease appears and a rapid initial drop followed by a slow decrease to zero occurs when $n>1$. A similar trend was reported in the case of substrate inhibition [13].

With the proposed model, it was possible to simulate the culture profile of hybridoma satisfactorily. Since lag phase data were not taken into account in the estimation of parameter of the proposed model, the batch growth data without initial lag phase data was used when the proposed model equation was used to simulate experimental data. Simulation results for the batch cultivation of hybridoma cells are shown in Fig. 4, using the proposed model with a n value of 1.330 and a $K_x$ value of 25 ($\times 10^4$ cells/mL). The predicted curve agrees very well with the experimental data. It is evident that the proposed cell density kinetic model adequately describes the effect of cell density on specific growth rate in batch culture. Batch and fed-batch culture processes are more practical than continuous culture, and the prediction of hybridoma growth rate is a prerequisite for the development of batch and fed-batch culture processes. This model is very practical and useful for the prediction of hybridoma growth rate and cell density in batch and fed-batch culture.

## REFERENCES

[1] Stein, K. E. (1997) Overcoming obstacles to monoclonal antibody product development and approval. *Trends Biotechnol.* 15: 88-90

[2] Spier, R. E. (1988), Animal cells in culture: moving into the exponential phase. *Trends Biotechnol.* 6: 2-6.

[3] Frame, K. K. and W. S. Hu (1988) A model for density dependent growth of anchorage-dependent mammalian cells. *Biotechnol. Bioeng.* 32: 1061-1066.

[4] Freshney, R. (1986) *Animal Cell Culture*: A *Practical Approach*. pp. 13-31. IRL press, NY, USA.

[5] Lee, G. M., T. K. Huard, and B. O. Palsson (1989) Effect of serum concentration on hybridoma cell growth and monoclonal antibody production at various initial cell densities. *Hybridoma* 8: 369-376.

[6] Glacken, M. W. (1988) Catabolic control of mammalian cell culture. *Bio/Technology* 6: 1041-1050.

[7] Banik, G. G. and C. A. Heath (1995) Hybridoma growth and antibody production as a function of cell density and specific growth rate in perfusion culture. *Biotechnol. Bioeng.* 48: 289-300.

[8] Sanfeliu, A., J. J. Cairo, C. Casas, C. Sola, and F. Godia (1996) Analysis of nutritional factors and physical conditions affecting growth and monoclonal antibody production of the hybridoma KB265 cell line. *Biotechnol. Prog.* 12: 209-216.

[9] Hornsby, P. J. (1982) The role of vitamin E in cellular energy metabolism in cultured andrenocortical cells. *J. Cell. Physiol.* 112: 207-216.

[10] Schneider, M., I. W. Marison, and U. von Stockar (1996) The importance of ammonia in mammalian cell culture. *J. Biotechnol.* 46: 161-185.

[11] Schneider, M., M. E. Alaoui, U. von Stockar, and I. W. Marison (1997) Batch cultures of a hybridoma cell line performed with in situ ammonia removal. *Enzyme Microb. Technol.* 20: 268-276.

[12] Banik, G. G. and C. A. Heath (1996) High-density hybridoma perfusion culture. *Appl. Biochem. Biotechnol.* 61: 211-229.

[13] Han, K. and O. Levenspiel (1988) Extended Monod kinetics for substrate, product, and cell inhibition. *Biotechnol. Bioeng.* 32: 430-437.

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