Effects of the Na^+/K^+ ratios on hybridoma cell growth

박준영, 박홍우
한양대학교 화학공학과 생물공학연구실
전화 (02) 2290-0487, FAX (02) 2299-9496

Abstract

In hybridoma cell culture, NH_4^+ is the most important toxic byproduct so far identified. It has been postulated that NH_4^+, which is similar to K^+ in size, is taken up non-specifically by the cells through a potassium transport system, and that the addition of K^+ to the culture medium may have a detoxifying effect of NH_4^+. Thus, in this article the effects of high K^+ concentrations in the range of 10 mM to 60mM on hybridoma cell growth and metabolism were investigated. No significant differences in growth were found for K^+ concentrations up to 40 mM, but cell death in the death phase was slightly delayed in the cultures with K^+ addition. At 60mM, growth was initially poor but the cells could be adapted after approximately 13 passages. With similar growth levels for high K^+ concentrations having been identified in batch cultivations using basal medium, we are currently investigating how such high levels of K^+ will affect cell growth in fortified batch cultures where the accumulation of NH_4^+ is more problematical.

Introduction

Batch cultivation is the preferred mode in many industrial applications of monoclonal antibody production because its process simplicity and flexibility. The cell growth in batch mode, however, is limited by the depletion of essential nutrients and the accumulation of toxic byproducts such as lactate and ammonia(1).

NH_4^+, which is an important toxic byproduct in animal cell culture, is similar in size to K^+ and is taken up non-specifically by the cells through a potassium transport system. It has been also suggested that the addition of K^+ to the culture medium may have a detoxifying effect of NH_4^+. Detoxification by K^+ addition has been studied in T-flask cultivations for relatively low levels of K^+ less than 10mM(2).

The purpose of this study is to investigate the effects of high concentrations of

K^+ in the culture medium for potential application in large-scale, fortified batch or fed-batch culture.

Materials and Methods

The cell line used in this study is the hybridoma 5F12AD3 (ATCC HB 8209) secreting IgG_1 against erythropoietin. IMDM(GibcoBRL) with 5% FBS was used as the basal medium, with modifications of KCl and NaCl concentrations according to the specific experiments. IMDM media with K^+ concentrations of 10mM, 40mM and 60mM were manufactured (Table 1). The NaCl concentration was adjusted to offset the osmolality increase which resulted from the addition of K^+.

The experiments were carried out in 500㎖ spinner flasks (working volume 200 ㎖), at 40rpm, 37℃ and 5% CO_2. The pH was controlled at 7.0~7.2 by the addition of 0.5M sodium bicarbonate. The inoculation density was 2×10^5 cells/㎖. The cells used for inoculation were previously grown in each medium for two passages to adapt the cells to the modified media. The viability of the cells used for inoculation was more than 90%.

The viable and total cell densities were determined by microscopic counting with a hemocytometer and trypan blue exclusion.

Glucose and lactate concentrations were determined with HPLC (Waters) using a Aminex HPX-87H column(Bio-Rad Co.). Amino acid concentrations were determined by the Waters Pico-tag method.

	NaCl	KCl
control (IMDM)	77.59	4.4
IMDM with 10 mM K^+	74	10
IMDM with 40 mM K^+	44	40
IMDM with 60 mM K^+	24	60

Table 1. Concentrations of K+ and NaCl in the culture media (mM)

Results and Discussions

• Cell growth & death

The cells were grown in each medium prior to inoculation to adapt the cells to the modified media including 10mM, 40mM or 60mM potassium. Cell growth in

medium containing 60mM potassium was poor and was therefore excluded from the spinner flask experiments. In separate adaptation experiments, the cells could be adapted to the medium containing 60mM potassium after cultivation for 13 passages.

There was no significant difference in cell growth in the various media, with the maximum viable cell density being highest with 11.6×10^5cells/㎖ in the medium containing 10mM potassium.

In the declining phase after approximately 60 hours, the rate of cell death was slightly slower in IMDM containing 40mM potassium than in IMDM(Figure 1).

- **Analysis**

The glutamine consumption of the cells grown in IMDM decreased in the death phase almost to zero, whereas there was a significant consumption of glutamine in the cultivation using IMDM including 40mM potassium as the medium. This may indicate a higher cellular activity of these cells in the death phase, as compared to cells grown in IMDM(Figure 2).

Analysis of glucose and lactate concentrations with HPLC after 60 hours showed that the glucose consumption was similar with 3g/ℓ in all three cultivations, whereas there was a slightly lower production of lactate in the potassium-containing media(Figure 3, 4).

- **Discussions**

In conclusion, a similar growth and possibly an improved cellular activity was observed for hybridoma cells grown in IMDM containing high concentrations of potassium as compared to basal IMDM. We are currently investigating the effects of K^+ on ammonium toxicity, and whether cultivation in K^+- containing medium offers advantages in high density systems such as fortified batch and fed-batch culture.

References

1) Bibila et al., "Monoclonal antibody process development using medium concentrates"(1994), Biotechnol. Prog., 10, 87-96
2) Martinelle K. and Haggstrom L., "Effect of NH_4^+ and K^+ on the energy metabolism in Sp2/0-Ag14 myeloma cells"(1999), Cytotech., 29, 45-53
3) Mirabet M., Navarro A., Lopez A., Canela E. I., Mallol J., Lluis C. and Franco R., "Ammonium toxicity in different cell lines"(1997), Biotech. Bioeng., 6, 530-537

4) Lim Dongjin, "A study on the ammonium toxicity for high-density hybridoma cell culture"(1999), Hanyang University

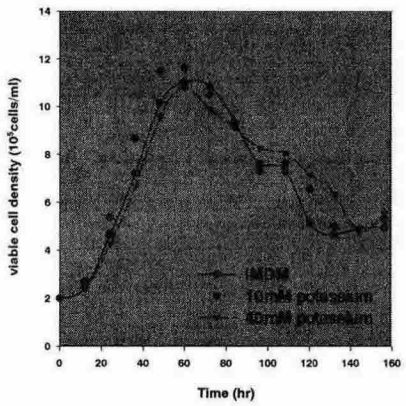

Figure 1. viable cell density on variable potassium conc.

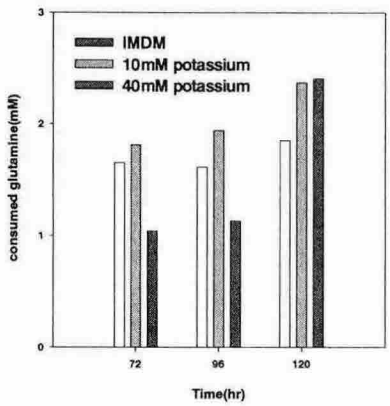

Figure 2. Glutamine consumption in different potassium concentration

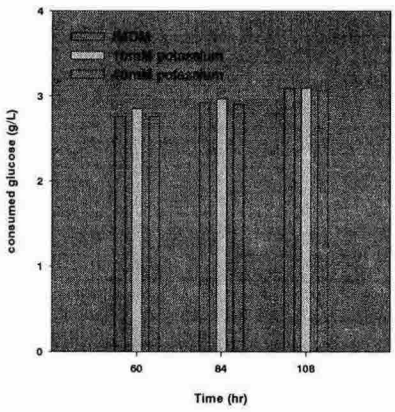

Figure 3. The consumed glucose concentratins(g/L)

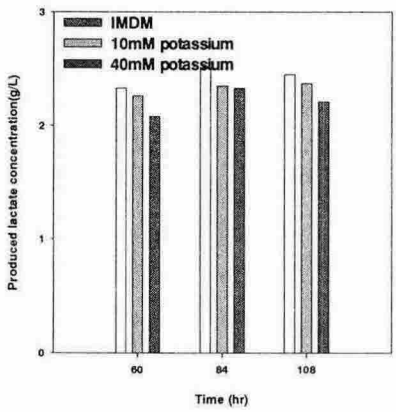

Figure 4. Production of lactate in potassium-added growth media