Biotechnology and Bioprocess Engineering **2005**, 10: 564-570 ©KSBB

Protective Effect of Bcl-2 in NS0 Myeloma Cell Culture is Greater in More Stressful Environments

B. T. Tey[1]* and M. Al-Rubeai[2]

[1] Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[2] Department of Chemical and Biochemical Engineering, University College Dublin, Belfield, Dublin 4, Ireland

Abstract In the present study, the protective effects of Bcl-2 over-expression in a suspension culture (without any adaptation) and spent medium (low nutrient and high toxic metabolite conditions) were investigated. In the suspension culture without prior adaptation, the viability of the control cell line fall to 0% by day 7, whereas the Bcl-2 cell line had a viability of 65%. The difference in the viability and viable cell density between the Bcl-2 and control cell lines was more apparent in the suspension culture than the static culture, and became even more apparent on day 6. Fluorescence microscopic counting revealed that the major mechanism of cell death in the control cell line in both the static and suspension cultures was apoptosis. For the Bcl-2 cell lines, necrosis was the major mode of cell death in the static culture, but apoptosis became equally important in the suspension culture. When the NS0 6A1 cell line was cultured in spent medium taken from a 14 day batch culture, the control cell line almost completely lost its viability by day 5, whereas, the Bcl-2 still had a viability of 73%. The viable cell density and viability of the Bcl-2 cell line cultivated in fresh medium were 2.2 and 2.7 fold higher, respectively, than those of the control cultures. However, the viable cell density and viability of the Bcl-2 cultivated in the spent medium were 8.7 and 7.8 fold higher, respectively, than those of the control cultures. Most of the dead cells in the control cell line were apoptotic; whereas, the major cell death mechanisms in the Bcl-2 cell line were necrotic.

Keywords: apoptosis, *bcl-2,* spent medium, NS0 myeloma cells, necrosis

INTRODUCTION

Mammalian cells are commonly grown in stirred tank bioreactor due to its simplicity, ease of process control and scale up. However, the relative fragility of animal cells compared to bacteria and fungi increases their susceptibility to fluid-mechanical shear stress in suspension cultures. Based on experimental results, two types of fluid-mechanical stresses causing cell death have been proposed: bubble disengagement, which is caused by direct sparging or gas entrainment and liquid flow [1-3], and turbulence, which is caused by the impeller blade [4,5]. Initially, cell death caused by fluid-mechanical stresses was generally assumed to be via necrosis. However, a report by Al-Rubeai *et al.* [6] suggested that apoptosis, as well as necrosis, could be induced by hydrodynamic stresses in suspension cultures. Shive *et al.* [7] also reported that a low level of shear stress (0.002 N m^{-2}) induced apoptotic death in neutrophils. In contrast, a shear stress level of 0.045 N m^{-2} has been shown to suppress the endothelial cell apoptosis induced by the presence of TNF or following growth factor withdrawal [8]. Interestingly, Hu *et al.* [9] reported that exposure of bovine aortic endothelial cells to laminar shear stress (0.012 N m^{-2}) caused a transient (less than 2 h) activation of c-Jun NH2-terminal kinase (JNK), but there was no induction of apoptosis. In the same study, colchicine treatment induced a sustained activation of JNK that lasted for 12 h and apoptosis was induced. These results suggested that sustained activation of JNK is a requirement for the induction of apoptosis. Therefore, apoptosis in cells cultivated in a bioreactor is probably due to a sustained activation of JNK induced by constant subjection to shear stress.

***Corresponding author**
Tel: +60-3-8946-6289 Fax: +60-3-8656-7120
e-mail: btey@eng.upm.edu.my

Reusing spent medium as a strategy for reducing production costs has been proposed and demonstrated by several authors [10-12]. Dutton *et al.* [13] demonstrated the use of spent medium to reduce the lag phase and increase the average viable cell density. Indeed, in the 1960's, Rubin and co-workers reported that spent medium could be used to enhance the growth of small numbers of chick embryo cells. They suggested that the cells had conditioned the spent medium, with what they described as 'stimulatory substances' [14,15]. Spent medium has also been considered as being rich in protective substances and other expensive proteins of serum and

their substitutes [10,12]; hence, can be used to reduce the concentration of serum required for proliferation and maintenance of cell viability [16-18]. Conversely, spent medium is not only low in nutrients, but has also been reported to contain toxic metabolites, such as methylglyoxal [19], formate [20] ammonia and lactic acid [21-24], as well as unknown low molecular weight auto-inhibitors [25,26]. Due to the presence of these toxic metabolites and auto-inhibitors, cells cultured in undiluted spent medium may stop growing, even if sufficient nutrients are supplied [25]. The limited nutrients and presence of toxic metabolite in the culture medium have been previously been shown to be potent apoptotic stimuli for most industrially important cell lines [27-31]. Therefore, the cells cultivated in the spent medium containing these two stimuli are expected to be more susceptible to apoptotic cell death.

Apoptosis is an active and highly regulated process, in which the cell itself activates and executes a cascade of death events, which are controlled by a group of endogenous proteins in response to environmental stress and stimuli. An early event is the increase of the ratio of pro-apoptotic to anti-apoptotic proteins of the Bcl-2 family involved in the regulation of apoptosis. This may be followed by a dissipation of the mitochondrial inner transmembrane potential ($\Delta\Psi m$), with the release of mitochondrial inter-membrane proteins, such as cytochrome-c. The release of cytochrome-c results in the activation of the protease caspase (cysteine aspartase) cascade. Active caspase cleaves a wide range of cellular protein targets, which are responsible for the morphological and biochemical changes accompanying apoptosis [32]. Apoptotic cell death is an irreversible process, which can limit the success of the cell culture process. Given the highly regulated nature of apoptosis, it should; therefore, be possible to delay this form of cell death by genetic manipulation of the apoptotic pathway. In the present study, the protective effects of Bcl-2 protein were shown to be higher under more stressful culture environments, such as cultivation of cells in suspension culture prior to any adaptation (compare to static culture) and spent medium (compared to fresh medium).

MATERIAL AND METHODS

Cell Lines

The parent cell line, NS0 6A1, was kindly supplied by LONZA Biologics (Slough, UK), and had previously been transfected with the glutamine synthetase (GS) expression system carrying a gene for a human-mouse chimeric antibody (cB72.3). In the present study, the cells were further transfected with the expression and control vectors *pEFbcl-2* and *pEFneo*, respectively, as previously described [30].

Culture Medium

The culture medium used for the maintenance of the NS0 6A1 cells was GMEM medium (Gibco, Paisely, UK), supplemented with 5% foetal calf serum (FCS) (Gibco), MEM non-essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), 500 μM glutamic acid, 500 μM asparagine, 30 μM adenosine, 30 μM guanosine, 30 μM cytidine, 30 μM uridine, 10 μM thymidine, 2.7 g/L sodium bicarbonate and 100 μM methylionine sulphoximine (MSX) (all chemicals from Sigma, Poole, UK).

Cell Density and Viability

The cell density and viability were determined using a haemocytometer, *via* the trypan blue exclusion method, as previously described [33].

Apoptosis and Necrosis Levels

The apoptosis and necrosis levels were measured by fluorescence microscopic analysis of the nuclear morphology and plasma membrane integrity by double staining with acridine orange and propidium iodide, as previously described [33].

Batch Culture in Spent Medium

At the mid-exponential phase of the batch culture, the cells were harvested by centrifugation, resuspended at 2×10^5 cells/mL in spent medium, and then transferred into triplicate 25 cm^2 T-flasks. The cultures were incubated at 37°C. The spent medium was from a 15 day old NS0 Bcl-2 batch culture, which had been centrifuged twice at 2,000 rpm for 10 min to remove all cell debris. Samples were taken daily to assess the viable cell density, viability and levels of apoptosis, as described above.

Comparison of Suspension and Static Culture

At the mid-exponential phase of the batch culture, the cells were harvested by centrifugation, resuspended at 2×10^5 cells/mL in fresh medium, and then transferred into triplicate 25 cm^2 T-flasks and 100 mL spinner flasks. The cultures were incubated at 37°C. Samples were taken daily to assess the viable cell density, viability and levels of apoptosis, as described above.

Calculation of Specific Growth Rate

In a batch culture,

$$\frac{d(x_v)}{dt} = \mu . x_v - k_d . x_v \qquad (1)$$

During the exponential phase, the death rate constant, $k_d=0$ and; therefore, the rate of accumulation of dead cells was negligible, and this expression can be reduced to:

$$\mu \approx \frac{1}{x_v} . \frac{dx_v}{dt} \ \ (\text{day}^{-1}) \qquad (2)$$

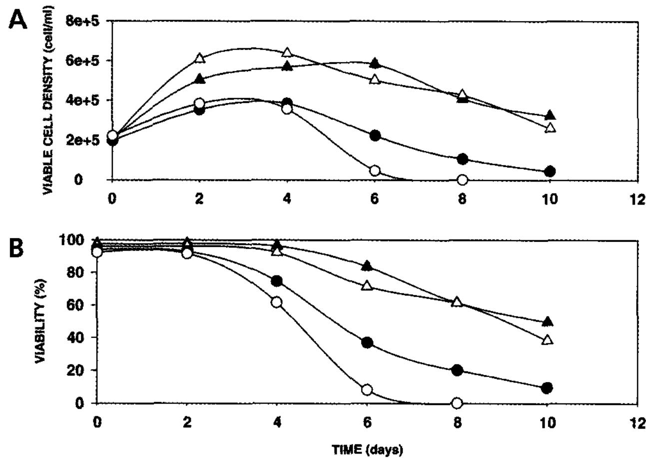

Fig. 1. (A) Viable cell density and (B) viability of the NS0 6A1 batch culture (Keys: ▲, Bcl-2 static culture; △, Bcl-2 suspension culture; ●, control static culture; ○, control suspension culture).

or, by integration, $\mu = \dfrac{\ln x_{v2} - \ln x_{v1}}{t_2 - t_1}$ (3)

where x_{v2} and x_{v1} are the density of viable cells at time t_2 and t_1, respectively. The error in measuring $\mu(\Delta\mu)$ between these two points can be calculated from the error combination formulae [34]. The final equation is as follows:

$$\left|\frac{\Delta\mu}{\mu}\right| = \left|\frac{\Delta x_{v2}/x_{v2}}{\ln\frac{x_{v2}}{x_{v1}}}\right| + \left|\frac{\Delta x_{v1}/x_{v1}}{\ln\frac{x_{v2}}{x_{v1}}}\right| \quad (4)$$

where Δx_{v1} and Δx_{v2} are the standard error of x_{v1} and x_{v2}, respectively.

RESULTS AND DISCUSSION

Suspension vs Static Cultures - Fluid-Mechanical Stress in Suspension Culture

Fig. 1 shows the viable cell density and viability of the NS0 6A1 cells in the suspension culture prior to any adaptation compared to the static culture. In the suspension culture, the viability of the control cell line fall to 0% by day 7, whereas that of the Bcl-2 cell line was 65%. The difference in the viabilities and viable cell densities between the Bcl-2 and control cell lines in both the static and suspension cultures became apparent, even on day 6. In the static culture, the viable cell density and viability of the Bcl-2 cultures were 2.3 and 2.6 fold higher, respectively, than those of the control cultures. However, the viable cell density and viability of the Bcl-2 suspension cultures were 8.8 and 11 fold higher, respectively, than those of the control cultures. This indicates that Bcl-2 has greater protective effects under more damaging culture conditions with a suspension culture.

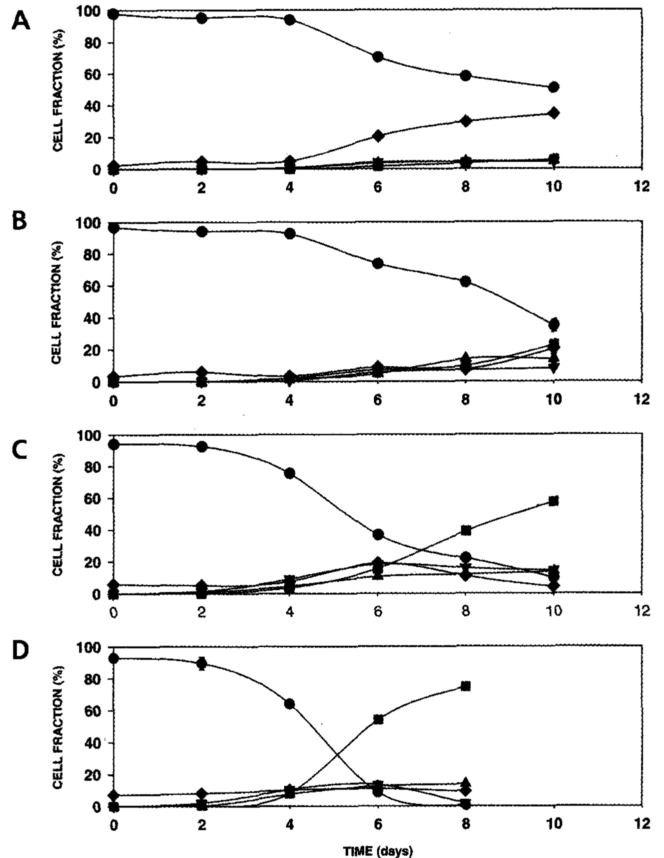

Fig. 2. Dual staining with acridine and propidium iodide of NS0 6A1 (A) Bcl-2 static culture, (B) Bcl-2 suspension culture, (C) control static culture, and (D) control suspension culture. (Keys: ●, viable cells; ▼, early apoptotic; ■, late apoptotic; ◆, necrotic; ▲, ghosts).

Fluorescence microscopic counting revealed that the major mechanism of cell death in the control cell line in both static and suspension cultures was apoptosis (Fig. 2). However, for the Bcl-2 cell lines, necrosis was the major mode of cell death in the static culture, but apoptosis became equally important in the suspension culture. Clearly, the over-expression of Bcl-2 in the NS0 cells provided protection from the apoptosis induced due to the suspension culture conditions. Similarly, Singh *et al.* [35] has reported that a plasmacytoma cell line underwent apoptotic cell death when first transferred to a suspension culture, but this was prevented by Bcl-2 over-expression. The over-expression of Bcl-2 also enabled hybridoma cells to be immediately cultivated under suspension culture conditions without the induction of significant levels of cell death [28]. Furthermore, studies by Perani *et al.* [36] clearly demonstrated the ability of Bcl-2 over-expression to suppress fluid-mechanical stress induced apoptosis and promoted cell growth of a hybridoma cell line.

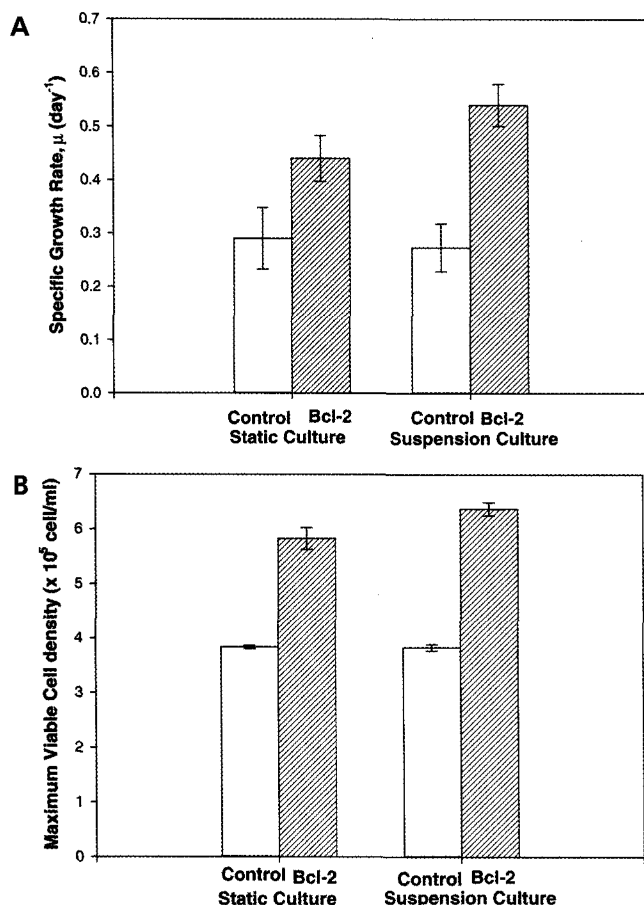

Fig. 3. (A) Specific growth rate and (B) maximum viable cell number in the static and suspension batch cultures of NS0 6A1 cells. (Keys: □, control; ▨, Bcl-2).

The specific growth rates for control cultures are very similar in both static and suspension cultures. However, there was about a 25% increase in the specific growth rate in the Bcl-2 suspension culture compared to the static culture (Fig. 3). Agitation in suspension cultures has been shown to enhance the mass transfer rate of oxygen and nutrients, which in turn increases the dissolved oxygen and nutrient levels in a bioreactor. Indeed, the dissolved oxygen level has always been a limiting factor in the intensive culture of animal cells, such as in a perfusion culture [37]. The increase of the dissolved oxygen and nutrient levels in the culture medium increases their concentration gradients, which are the driving forces of the passive diffusion involved in the uptake of oxygen and nutrient by cells. Increases in the nutrient and oxygen uptake rates; in turn, improve the cellular enzymatic reaction rates and; hence, the growth rate. However, in a control suspension culture, the enhanced mass transfer rate does not show improvement in maximum viable cell density. This may be due to the apoptotic cell death induced by the fluid-mechanical stress caused by agitation in a suspension culture (Fig. 2D). Therefore, the higher apoptotic death rate counters the positive effect of the higher mass transfer rate in the control suspension culture. Conversely, a higher growth rate resulted from an enhanced mass transfer rate in the suspension culture, and a lower apoptotic death rate as a result of the protective effects of Bcl-2; the maximum viable cell density was increased about 10% in the Bcl-2 suspension culture compared to the static culture. The ability of the Bcl-2 protein to protect cells from hydrodynamic stress induced apoptosis has a very important implication for large-scale bioprocesses, since suspension cultures are more easily scaled-up and controlled. Furthermore, the shorter suspension adaptation time of the Bcl-2 cells can expedite the cell line development process.

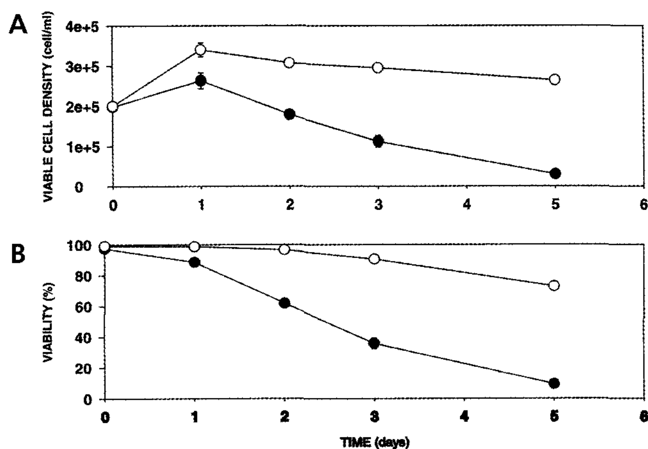

Fig. 4. Batch culture of NS0 6A1 Bcl-2 (○) and control (●) cells in spent medium from a 15 day old batch culture: (A) viable cell density and (B) viability.

Spent Medium

Spent medium contains very low levels of nutrients and high levels of toxic metabolites, such as methylglyoxal [19], formate [20] ammonia and lactic acid [21-24], as well as unknown low molecular weight auto-inhibitors [25,26]. Both nutrient limitation and excess levels of ammonia are potent inducers of apoptosis, as shown in previous experiments [30,31]. When the NS0 6A1 cell line was cultured in spent medium taken from a 14 day batch culture, the control cell line almost completely lost viability by day 5; whereas, the Bcl-2 cell line still had a viability of 73% (Fig. 4). The viable cell density and viability of the Bcl-2 cell line cultivated in fresh medium were 2.2 and 2.7 fold higher, respectively, than those of the control cultures. However, the viable cell density and viability of Bcl-2 cultivated in the spent medium were 8.7 and 7.8 fold higher, respectively, than those of the control cultures. Clearly, the results of this experiment showed that the Bcl-2 protein has greater protective effects under the more stressful culture conditions introduced by the spent medium. Fluorescence microscopy revealed that most of the dead cells in the control cell line were apoptotic. By contrast, almost all the dead cells of the Bcl-2 cell line cultures were necrotic (Fig. 5). The

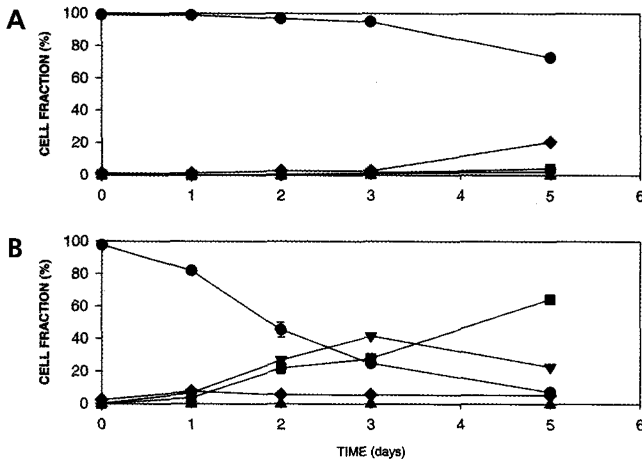

Fig. 5. Dual staining with acridine and propidium iodide of NS0 6A1 (A) Bcl-2 and (B) control cells in spent medium batch cultures. (Keys: ●, viable cells; ▼, early apoptotic; ■, late apoptotic; ◆, necrotic; ▲, ghosts).

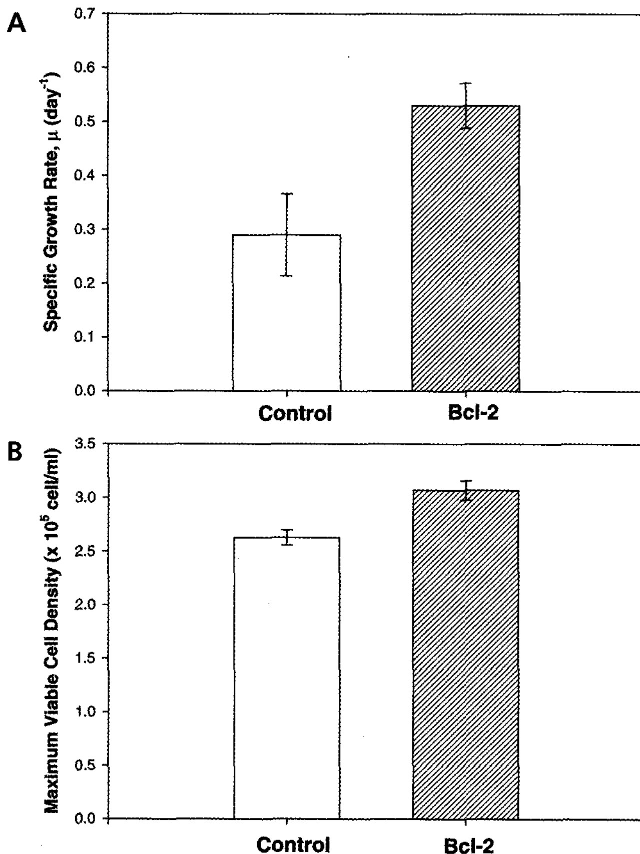

Fig. 6. (A) Specific growth rate and (B) maximum viable cell number of the NS0 6A1 cells a batch culture in spent medium. (Keys: □, control; ▨, Bcl-2).

present study has provided the first demonstration that the over-expression of Bcl-2 can protect NS0 myeloma cells from the death induced under the combination of nutrient exhaustion and the accumulation of toxic metabolites. Indeed, the result of our previous study has shown that Bcl-2 provide better protection to NS0 myeloma cells with a low compared to a high dilution rate in a chemostat culture [38]. Besides, the higher efficiency of nutrient metabolism demonstrated by *bcl-2* transfected cells [33,37,39] has contributed to the lower apoptotic cell death in spent medium. Thus, the suppression of apoptosis should allow the cells to fully benefit from cultivation in spent medium. Such benefit is seen from the 70% increase in the viable cell density and the 80% increase in the specific growth rate without the provision of any additional nutrients or serum (Fig. 6). This will be an important finding for the development of reusing spent medium in large-scale animal cell culture processes for the reduction of production costs [12].

CONCLUSION

The results of the present study have demonstrated that Bcl-2 over-expressed in NS0 cells has greater protective effects in more stressful culture environments. Bcl-2 cell lines have become more robust compared to their control counterparts when cultivated under suspension prior to adaptation rather than in a static culture and under low nutrient and high toxic metabolite conditions in spent rather than fresh medium. This characteristic is very important during the scale-up of a production process, where a stirred tank bioreactor is preferred for the large-scale production of animal cell cultures. Nevertheless, its ability to proliferate in spent medium may lead to the development of reusing spent medium for the reduction of production costs.

Acknowledgements We would like to thank Dr. John Birch (Lonza Biologics plc, U.K.) for the NS0 6A1 cell line used in this study. This work was supported by EC Framework IV and the Malaysia Government; IRPA Grant No. 09-02-04-0454-EA001 to T.B.T.

REFERENCES

[1] Jobses, I., D. Martens, and J. Tramper (1991) Lethal events during gas sparging in animal cell culture. *Biotechnol. Bioeng.* 37: 484-490.

[2] Handa-Corrigan, A., A. N. Emery, and R. E. Spier (1989) Effect of gas-liquid interfaces on the growth of suspended mammalian-cells-mechanisms of cell-damage by bubbles. *Enzyme Microb. Technol.* 11: 230-235.

[3] Kioukia, N., A. W. Nienow, M. Al-Rubeai, and A. N. Emery (1996) Influence of agitation and sparging on the growth rate and infection of insect cells in bioreactors and a comparison with hybridoma culture. *Biotechnol. Prog.* 12: 779-785.

[4] Kunas, K. T. and E. T. Papoutsakis (1990) Damage mechanisms of suspended animal-cells in agitated bioreactors without bubble entrainment. *Biotechnol. Bioeng.* 36:

476-483.

[5] Papoutsakis, E. T. and K. T. Kunas (1989) Hydrodynamic effects of cultured hybridoma cells CRL 8018 in an agitated bioreactor. pp. 203-211. In: R. E. Spier, J. B. Griffiths, J. Stephenne, and P. J. Crooy, (eds.). *Advances in Animal Cell Biology and Technology for Bioprocesses*. Butterworths, Kent, UK.

[6] Al-Rubeai, M., R. P. Singh, M. H. Goldman, and A. N. Emery (1995) Death mechanisms of animal-cells in conditions of intensive agitation. *Biotechnol. Bioeng.* 45: 463-472.

[7] Shive, M. S., M. L. Salloum, and J. M. Anderson (2000) Shear stress-induced apoptosis of adherent neutrophils: A mechanism for persistence of cardiovascular device infections. *Proc. Nat. Acad. Sci.* 97: 6710-6715.

[8] Dimmeler, S., J. Haendeler, V. Rippmann, M. Nehls, and A. M. Zeiher (1996) Shear stress inhibits apoptosis of human endothelial cells. *FEBS Lett.* 399: 71-74.

[9] Hu, Y.-L., S. Li, J. Y. Shyy, and S. Chien (1999) Sustained JNK activation induces endothelial apoptosis: studies with colchicine and shear stress. *Am. J. Physiol.* 277: 1593-1599.

[10] Kempken, R., H. Buntemeyer, and J. Lehmann (1991) The medium cycle bioreactor (MCB): monoclonal antibody production in a new economic production system. *Cytotechnology* 7: 63-74.

[11] Mizrahi, A. and A. Avihoo (1977) Growth medium utilization and its re-use for animal cell cultures. *J. Biol. Stand.* 5: 31-37.

[12] Riese, U., D. Lutkemeyer, R. Heidermann, H. Buntemeyer, and J. Lehmann (1994) Reuse of spent cell-culture medium in pilot-scale and rapid preparative purification with membrane chromatography. *J. Biotechnol.* 34: 247-257.

[13] Dutton, R. L., J. M. Scharer, and M. Moo-Young (1999) Hybridoma growth and productivity: effects of conditioned medium and of inoculum size. *Cytotechnology* 29: 1-10.

[14] Rein, A. and H. Rubin (1968) Effects of local cell concentrations upon the growth of chick embryo cells in tissue culture. *Exp. Cell Res.* 49: 666-678.

[15] Rubin, H. (1966) A substance in conditioned medium which enhances the growth of small numbers of chick embryo cells. *Exp. Cell Res.* 41: 138-148.

[16] 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: 369-375.

[17] Lee, G. M., B. K. Han, J. H. Kim, and B. O. Palsson (1992) Effect of calcium-chloride treatment on hybridoma cell viability and growth. *Biotechnol. Lett.* 14: 891-896.

[18] Kim, Y. N., H. G. Kim, S. K. Lim, K. Y. Park, J. S. Park, H. Murakami, and H. Y. Lee (1996) The economic production of anti-microbial factor from human promyelocytes in low serum containing medium under chemostat cultivation. *Cytotechnology* 19: 73-78.

[19] Chaplen, F. W. R. (1998) Incidence and potential implications of the toxic metabolite methylglyoxal in cell culture: A review. *Cytotechnology* 26: 173-183.

[20] Ihrig, T. J., B. D. Maulawizada, B. D. Thomas, and F. S. Jacobson (1997) Fomate production by CHO cells: Biosynthetic mechanism and potential cytotoxicity. pp. 193-197. In: M. J. T. Carrondo, B. Griffiths, and J. P. Moreira (eds.). *Animal Cell Technology: From Vaccines to Genetic Medicine*. Kluwer Academic Publishers, Dordrecht, Netherlands.

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

[22] Newland, M., P. F. Greenfield, and S. Reid (1990) Hybridoma growth limitations: the roles of energy metabolism and ammonia production. *Cytotechnology* 3: 215-229.

[23] Ozturk, S. S., M. R. Riley, and B. O. Palsson (1992) Effects of ammonia and lactate on hybridoma growth, metabolism, and antibody production. *Biotechnol. Bioeng.* 39: 418-431.

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

[25] Buntemeyer, H., S. Siwiora, and J. Lehmann (1997) Inhibitors of cell growth: accumulation and Concentration. pp. 651-655. In: M. J. T. Carrondo, B. Griffiths, and J. P. Moreira (eds.). *Animal Cell Technology: From Vaccines to Genetic Medicine*. Kluwer Academic Publishers, Dordrecht, Netherlands.

[26] Lee, Y. K., P. K. Yap, and A. P. Teoh (1995) Correlation between steady-state cell concentration and cell-death of hybridoma cultures in chemostat. *Biotechnol. Bioeng.* 45: 18-26.

[27] Mercille, S. and B. Massie (1994) Induction of apoptosis in nutrient-deprived cultures of hybridoma and myeloma cells. *Biotechnol. Bioeng.* 44: 1140-1154.

[28] Simpson, N. H., A. E. Milner, and M. Al-Rubeai (1997) Prevention of hybridoma cell death by Bcl-2 during suboptimal culture conditions. *Biotechnol. Bioeng.* 54: 1-16.

[29] Singh, R. P., M. Al-Rubeai, C. D. Gregory, and A. N. Emery (1994) Cell death in bioreactors: A role for apoptosis. *Biotechnol. Bioeng.* 44: 720-726.

[30] Tey, B. T., R. P. Singh, L. Piredda, M. Piacentini, and M. Al-Rubeai (2000) Bcl-2 mediated suppression of apoptosis in myeloma NSO cultures. *J. Biotechnol.* 79: 147-159.

[31] Tey, B. T., R. P. Singh, L. Piredda, M. Piacentini, and M. Al-Rubeai (2000) Influence of bcl-2 on cell death during cultivation of a Chinese hamster ovary cell line expressing a chimeric antibody. *Biotechnol. Bioeng.* 68: 31-43.

[32] Tey, B. T., R. P. Singh, and M. Al-Rubeai (2001) Programmed cell death-An overview of apoptosis in cell culture. *Asia Pac. J. Mol. Biol. Biotechnol.* 9: 1-28.

[33] Tey, B. T. and M. Al-Rubeai (2005) Bcl-2 over-expression reduced the serum dependency and improved the nutrient metabolism in a NSO cells culture. *Biotechnol. Bioprocess Eng.* 10: 254-261.

[34] Leelavatcharamas, V. (1997) *Growth, Gamma Interferon Production and Cell Cycle in Batch, Continuous and Perfusion Cultures*. Ph.D. Thesis. The University of Birmingham, Birmingham, UK.

[35] Singh, R. P., A. N. Emery, and M. Al-Rubeai (1996) Enhancement of survivability of mammalian cells by overexpression of the apoptosis-suppressor gene *bcl-2*. *Biotechnol. Bioeng.* 52: 166-175.

[36] Perani, A., R. P. Singh, R. Chauhan, and M. Al-Rubeai (1998) Variable functions of Bcl-2 in mediating bioreactor stress-induced apoptosis in hybridoma cells. *Cytotechnology*

28: 177-188.
[37] Tey, B. T. and M. Al-Rubeai (2004) Suppression of apoptosis in perfusion culture of Myeloma NS0 cells enhances cell growth but reduces antibody productivity. *Apoptosis* 9: 843-852.
[38] Tey, B. T. and M. Al-Rubeai (2005) Effect of Bcl-2 overexpression on cell cycle and antibody productivity in chemostat cultures of myeloma NS0 cells. *J. Biosci. Bioeng.* In press.
[39] Simpson, N. H., R. P. Singh, A. Perani, C. Goldenzon, and M. Al-Rubeai (1998) In hybridoma cultures, deprivation of any single amino acid leads to apoptotic death, which is suppressed by the expression of the *bcl-2* gene. *Biotechnol. Bioeng.* 59: 90-98.

[Received June 15, 2005; accepted September 27, 2005]