



Figure 12.4. Time trajectories of hybridoma growth and monoclonal antibody production at different dissolved-oxygen concentrations in a 3-l fermenter. (With permission, from S. Rueuveay, D. Velez, J. D. Macmillian, and L. Miller, *J. Immunol. Methods* 86:53–59, 1986.)

Animal cells require 0.06 to 0.2×10^{-12} mol $O_2/h \cdot$ cell, which is about five times less than the oxygen requirements of many plant cells and much lower than for microbial cells. Typical animal cell concentration in suspension culture media is 0.1 to 1 g/l (5×10^5 to 5×10^6 cells/ml) for suspension cultures and 10 to 50 times higher than this in immobilized/surface culture systems. For a cell culture density of 10^6 cells/ml, the oxygen requirement is on the order of 0.1 to 0.6 mmol $O_2/l \cdot h$. For shallow culture depths of 2 to 5 cm, oxygen supplied by air in the head space of the vessel should meet the oxygen requirements of cells. Forced aeration is required for submerged cultivation of denser cultures. However, animal cells are very shear sensitive, and rising air bubbles may cause shear damage to cells, particularly at the point of bubble rupture. Very small bubbles are less damaging. For this reason, special aeration and agitation systems are designed for animal cell cultures. Techniques that decouple agitation (and shear) from oxygenation are particularly attractive. Chemicals (e.g., Pluronic® F-68) can be added to provide shear protection. Since the oxygen requirements of animal cells are relatively low, the oxygen transfer coefficient need not be as high as for bacterial fermentations. Typical values of $k_L a$ for suspension cultures (10^6 cells/ml) are 5 to 25 h^{-1} . Animal cells are usually cultivated in spinner flasks of 0.5 to 10 l in the laboratory. Spinner flasks contain a magnetically driven impeller or spoonlike agitators that operate at 10 to 60 rpm. Aeration is usually by surface aeration using 5% CO_2 -enriched and filtered air for mammalian cell lines. Spinner flasks are set on a magnetic stirrer plate in a CO_2 incubator.