Note

High Cell Density Culture of a Hybridoma Using Perfluorocarbon to Supply Oxygen

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In order to culture animal cells in suspension at high density, the supply of an ample amount of oxygen is an important requisite. An insufficient oxygen supply to a culture results in poor growth of the cells or a poor yield of the products. Aeration with gaseous oxygen is the most widely used method for supplying oxygen to a suspension cell culture system, but cells suffer some damage due to the aeration, especially in a high cell density culture. Although for bacterial fermentations various methods have been reported,^{1~4)} little work has been done to develop new methods for supplying oxygen to a high cell density culture system.

The purpose of this investigation was to develop a novel oxygen supply system. In the system developed, oxygenized liquid perfluorocarbon is fed into the culture vessel as an oxygen carrier, oxygen being supplied to the cells by means of cell-liquid contact. After the release of oxygen, the liquid perfluorocarbon sediments to the cone-shaped bottom of the vessel, because of its high specific gravity and insolubility in the medium. The perfluorocarbon is then conducted to an separate vessel to be oxygenized again with gaseous oxygen. Cells are supplied with oxygen through the oxygenation-deoxygenation cycle of perfluorocarbon. Gaseous oxygen is highly soluble in perfluorocarbon, the solubility being about ten times that in water, and perfluorocarbon has actually been studied as artificial blood, both in animals and humans. The specific gravity of the liquid ranges from 1.6 to 1.9, which makes it easy for the liquid to be separated from the culture medium.

The perfluorocarbon employed in the present study was Fluorinert FC-40 (3-M, St. Paul, MN, U.S.A.). Some properties of FC-40 are as follows: boiling point, 155°C; specific gravity, 1.85; solubility of gaseous oxygen (1atm, 25°C), 37 ml/100 ml FC-40.

Figure 1 shows a scheme of the system used. This system comprised a culture vessel, a new medium flask, apparatus for solubilizing oxygen in the perfluorocarbon and three peristaltic pumps.

The culture vessel (165 mm × 80 mm) and aerator (500 mm × 50 mm) were made of glass and all the tubing was composed of silicone. The culture vessel was equipped with a dissolved oxygen (DO) sensor (Oriental Yeast Co., Ltd.) and a spinner filter made of sintered stainless steel (pore size, 10 microns; area, 95 cm²). 225 ml of RPMI 1640 medium containing 10% fetal calf serum (FCS) was charged into the culture vessel and then mouse-mouse hybridoma 4C10B6 (parent: P3/x63-Ag·U1) cells that secreted IgG_{2b} were inoculated at density of 1.5×10^5 cells/ml. Air containing 5% CO₂ was passed over the surface of the culture medium. The cells were cultured at 37°C. The filter was rotated by a motor on the top of the culture vessel and the medium was stirred at 150 rpm. Living cell numbers were determined with a hemacytometer after trypan blue staining, and the concentration of IgG in the medium was determined by an enzyme-linked immunosorbent assay (ELISA).

The growth of hybridoma 4C10B6 cells, and the concentration and productivity of IgG are collectively shown in Fig. 2. On the 4th day the concentration of living cells was found to be 9.2×10^5 cells/ml and fresh medium was continuously fed at a rate of 225 ml/day from line 1 in Fig. 1. Simultaneously, spent medium was continuously separated from cells by the rotating filter and conducted out of the system through line 2 by a peristaltic pump so that the volume of culture liquid in the vessel remained constant at 225 ml. The amount of the medium fed into the culture vessel was increased to 700 ml/day as the cell density increased. On the 7th day, the supply of air containing 5% CO₂ was stopped and the oxygen supply using perfluorocarbon was started. The oxygenized perfluorocarbon was added to the aqueous medium dropwise from the top of the culture vessel. The perfluorocarbon coalesced in the FC-40 phase (maintained at about 3 cm height) was

FIG. 1. Scheme of the Culture System for High Density Culture of 4C10B6 Mouse-Mouse Hybridoma Cells.

New medium was fed into the culture vessel *via* line 1 and spent medium was removed *via* line 2.

FIG. 2. Growth and IgG Production in a High Density Culture of Hybridoma 4C10B6 Cells.

Cells were cultured in 10% FCS-RPMI1640 medium in the system shown in Fig. 1. The numbers of total (○) and viable (●) cells were determined with a hemacytometer. The concentration of IgG (△) was determined by ELISA and the IgG productivity (▲) of the hybridoma cells was calculated from the total cell number and the concentration of IgG. After 7-day culture, oxygen was supplied to the system using perfluorocarbon as an oxygen carrier (indicated by an arrow).

continuously removed from the culture vessel, re-aerated with oxygen gas and repeatedly used. The culture vessel contained 100 ml of FC-40, and the total volume of the

perfluorocarbon in the system was 300 ml. The concentration of dissolved oxygen was monitored with DO sensor and maintained at 3 ppm by controlling the flow of perfluorocarbon with a peristaltic pump. Sufficient oxygen was supplied using perfluorocarbon as an oxygen carrier for the hybridoma cells to grow, because the hybridoma cells had proliferated up to the maximum cell density of 9.8×10^6 cells/ml on the 9th day. The viability of the hybridoma cells did not change (>95%), and the concentration and productivity of IgG remained almost constant throughout the culture.

In this experiment it was demonstrated that perfluorocarbon was capable of supplying so much oxygen that cells could attain to such a high density as 9.8×10^6 cells/ml. Mouse-mouse hybridoma 4C10B6 cells are rather strong as to aeration, so the results above did not show that the use of perfluorocarbon as an oxygen carrier was decidedly superior to aeration. But this method will make it possible to culture feeble cells that suffer serious damage due to aeration.

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