0273-2289/87/1502–0131$02.60

Applications of the Air Lift Fermenter

L. A. WOOD* AND P. W. THOMPSON

Celltech Limited, 244-250 Bath Road, Slough SL1 4DY, Berkshire, UK

ABSTRACT

Since the first development of the airlift fermenter by Le Francois in 1955 (*1*), the applications have increased to cover most areas of fermentation. This configuration has likewise been developed into many different forms, including: (a) Concentric tube airlift (*2*); (b) external loop airlift (*3*); (c) jet loop reactor (*4*); and (d) propeller loop reactor (*4*).

The subject for discussion in this paper will be the concentric tube airlift, as shown in Fig. (*1*). This consists of a vertical cylindrical vessel incorporating a concentric draught tube into which is injected a flow of air or other gas mixture suitable for oxygenation. The resulting reduction in bulk density in the central riser causes the contents to move upward thus displacing the contents of the surrounding downcomer, which moves downward, thus inducing circulation of the total contents. This has the advantage of generating liquid mixing and gas transfer without the use of mechanical agitators.

The number of possible applications for the airlift fermenter has increased with the advent of genetic engineering techniques that result in new strains of unicellular organisms capable of producing many novel products. An almost universal requirement is the maintenance of absolute sterility, and the absence of agitation with associated shaft seals may be of benefit here. A typical property of some novel organisms is the sensitivity to shear stress of either the product (often a large, easily denatured protein) or the cells themselves, in which there may be an absence of a thick protective cell wall. The airlift fermenter should therefore be of use in these applications.

*Author to whom all correspondence and reprint requests should be addressed.

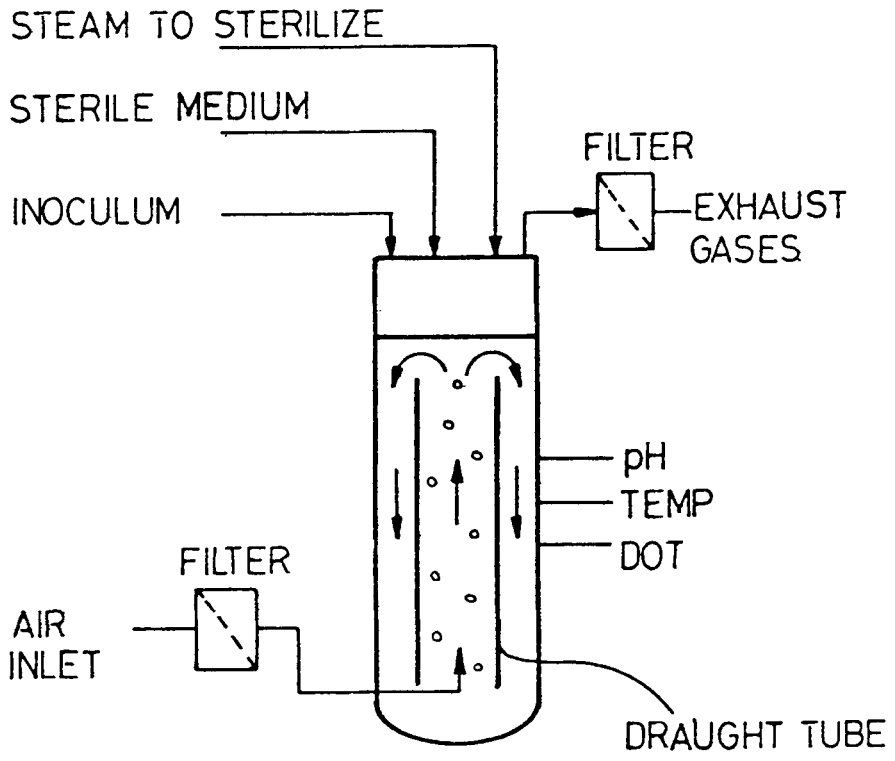

Fig. 1. Principle of an airlift reactor.

CHARACTERISTICS OF THE AIRLIFT FERMENTER

In order to determine the usefulness of the airlift fermenter it is necessary to determine relevant characteristics of importance in fermentation. It is therefore proposed to assess the rate of oxygen transfer and the level of resulting shear stress.

Oxygen Transfer

The mass transfer coefficient for oxygen transfer in the airlift fermenter may be calculated from the following empirical correlation, as derived by Bello et al. (*5*) for external loop airlift fermenters:

$$K_La = b\left[1 + \frac{A_D}{A_R}\right]^{-1.2}\left[\frac{P_G}{V}\right]^{0.8} \quad (1)$$

where

$$\bar{P}_G = nP_1u_1\frac{\gamma}{\gamma - 1}\left[\frac{P_2}{P_1}\frac{\gamma - 1}{\gamma} - 1\right] \quad (2)$$

and

$$P_2 = h\rho g \quad (3)$$

Where K_La is the liquid mass coefficient; L stands for liquid; a and b are constants; A is the cross-sectional area; d stands for downcomer; r for riser; P_G is the pneumatic power input; V is the fermenter volume; n the

number of compressor stages; u is the velocity; γ is the ratio of specific heats at constant pressure and volume density; p stands for pressure; G stands for gas; h is the weight of liquid; g is the gravitational constant; 1 stands for initial, and 2 for final.

This correlation has been tested for accuracy by measuring the K_La in various fermenters at differing air-flow rates. Where low air-flow rates were employed (up to 0.1 vvm), the method of Pirt (*7*) was used in which the rate of change of pO_2 is measured in initially deaerated water at 37°C under constant sparging conditions, where:

$$t = K_La \ln \frac{(C^* - C)}{C} \tag{4}$$

Where t stands for time; C^* is the saturated oxygen concentration; and C is the bulk-phase oxygen concentration.

Where higher air rates were used, the K_La was determined by the method of Cooper et al. (*8*) by measuring the catalyzed rate of air oxidation of aqueous sodium sulfite. From Figs. 2 and 3 it may be seen that

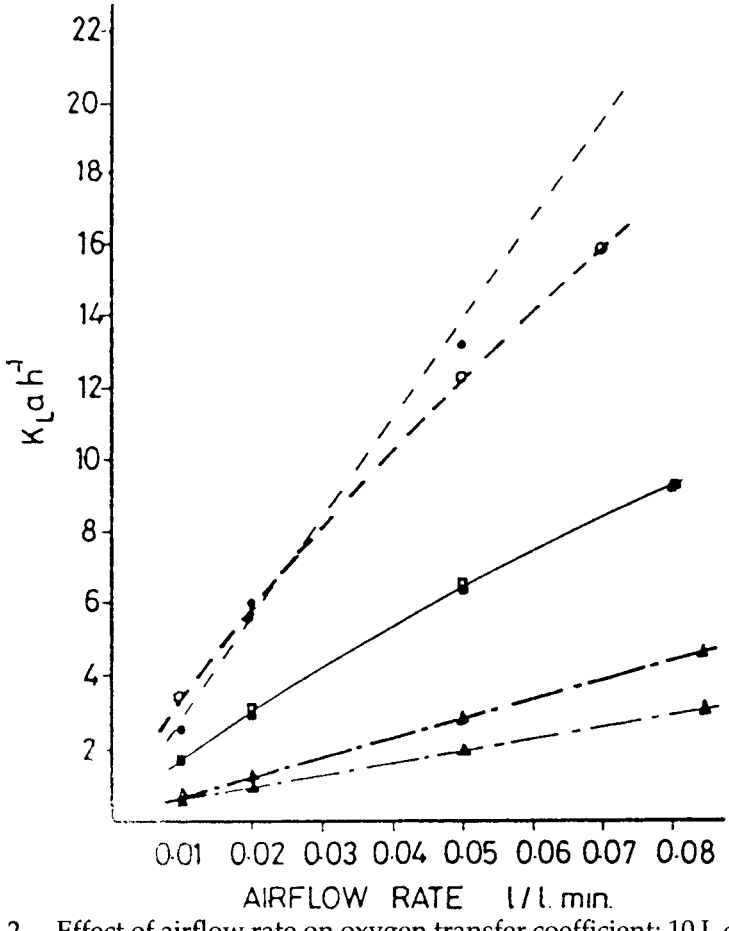

Fig. 2. Effect of airflow rate on oxygen transfer coefficient: 10 L calculated (△); 10 L measured (▲); 100L calculated (□); 100 measured (■); 1000 L calculated (○); 1000 L measured (●).

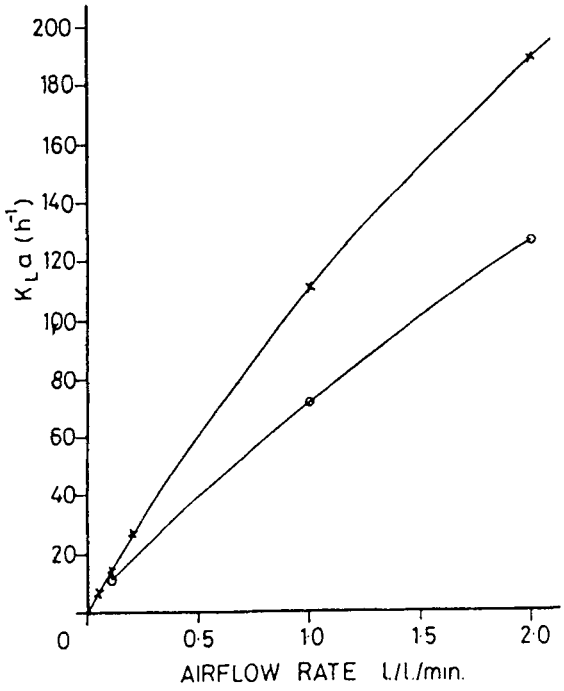

Fig. 3. Effect of airflow rate on oxygen transfer coefficient in the 30-L airlift fermenter: measured (X); calculated (O).

there is a reasonably close correlation, with a maximum variance of about 20%. The large variance in high air-flow rates in the 30-L airlift may be because of inaccuracies in the sulfite oxidation method of measurement. It is recognized that in a real system containing dissolved and suspended solids and antifoam the results may be somewhat different. However, for comparative and scale-up purposes this model is still useful. The aspect ratio of the fermenters used were 5 to 1 (10 L), 12 to 1 (30 L), 6.5 to 1 (100 L), and 8.5 to 1 (1000 L).

Shear Stress

The liquid circulation velocity may be predicted from a correlation also derived by Bello et al. (*5*), as follows:

$$u_{Lr} = aA_d/A_L^{0.7} \quad u_{Gr}^{0.32} \tag{5}$$

This correlation has also been tested experimentally by measuring the time taken for an injected acid sample to pass between two pH probes, again for a range of fermenters at varying flow rates. The accuracy of prediction also falls within a 20% variance, as shown in Figs. 4 and 5.

These liquid velocity results enable us to calculate the maximum shear stress at the vessel wall from the Blasius equation, where:

$$R/\rho u^2 = 0.0396\ R_e^{(-0.25)} \tag{6}$$

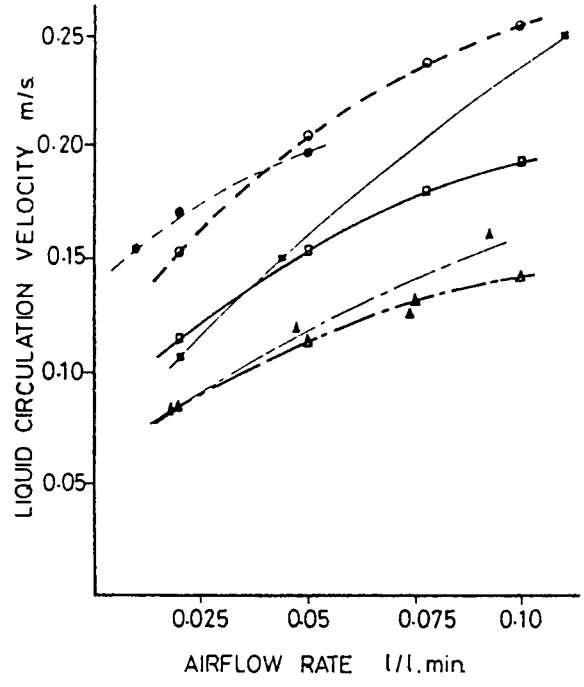

Fig. 4. Effect of airflow rate on liquid circulation velocity: 10 L calculated (△); 10 L measured (▲); 100 L calculated (□); 100L measured (■); 1000L calculated (○); 1000L measured (●).

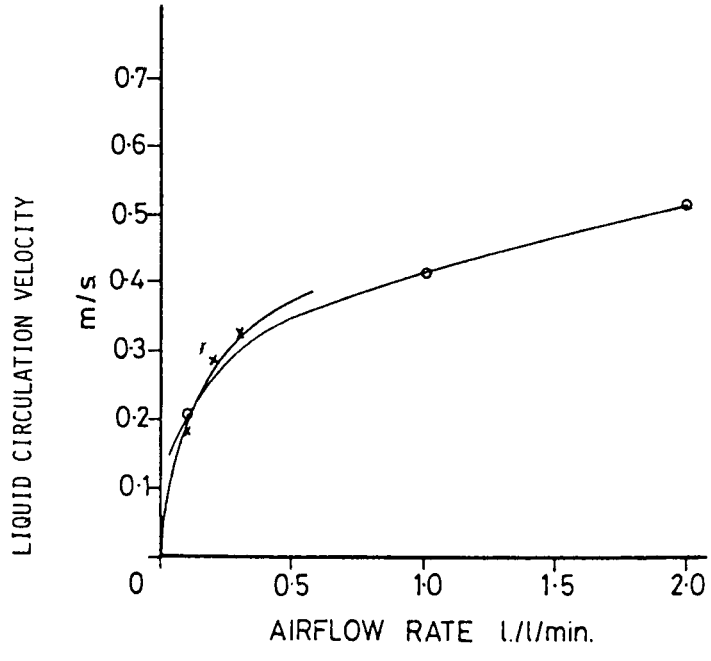

Fig. 5. Effect of airflow rate on liquid circulation velocity in the 30-L airlift fermenter: measured (X); calculated (O).

where

$$2500 < R_e < 100{,}000 \tag{7}$$

where R stands for shear stress; and R_e for Reynolds number.

This correlation has not been tested.

Figures 6 and 7 illustrate how the maximum shear stress is affected by achieving a particular oxygen transfer rate (OTR), where OTR is calculated by the equation:

$$\text{OTR} = K_L a\ (C^* - C) \tag{8}$$

It is therefore possible to predict the maximum achievable OTR if the maximum permissible shear stress is known. It is interesting to note that to achieve the same OTR at a larger scale, the air-flow rate required generated a lower, maximum shear stress.

EXAMPLES OF HYBRIDOMA CULTURE IN AIRLIFT FERMENTERS

The growth of hybridoma cells in tissue culture for the production of monoclonal antibodies has increased greatly since their original use by Kohler and Milstein in 1975 (*10*). Hybridoma culture was originally

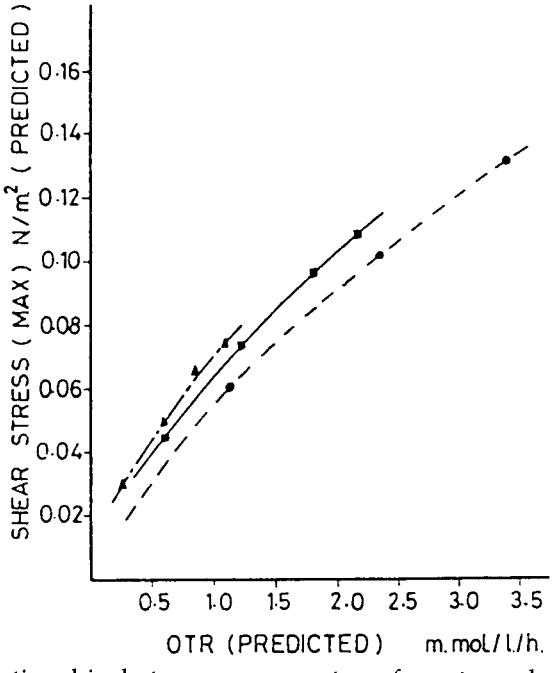

Fig. 6. Relationship between oxygen transfer rate and maximum shear stress both as derived from predictive correlations: 10 L (▲); 100 L (□); 1000 L (●).

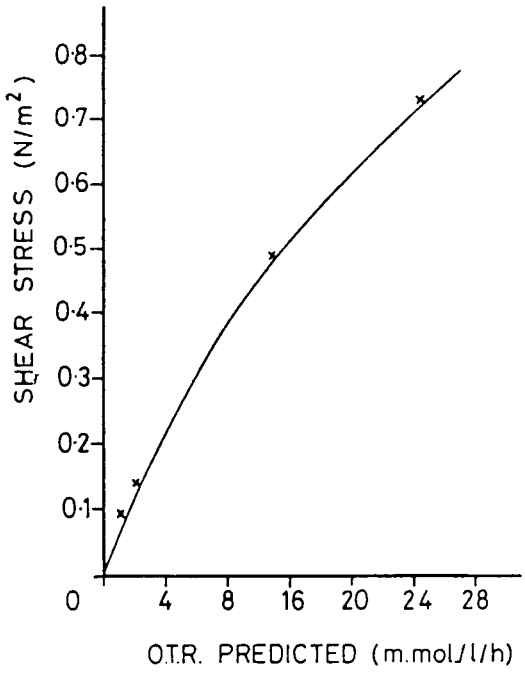

Fig. 7. Relationship between oxygen transfer rate and maximum shear stress both as derived from predictive correlations for 30-L airlift fermenter.

carried out in vivo using mice. However, the requirement for larger quantities of monoclonal antibodies in such applications as tumor imaging, immunopurification, and in vivo therapy (*11–13*) in multikilogram quantities means that very large numbers of mice would be used for production in a potentially cumbersome and costly process.

Since hybridomas are the fusion product of a myeloma cell that confers immortality and a lymphocyte that produces the antibody, they arewell suited to growth in tissue culture by fermentation.

From the limited information available it appears that animal cellshave a widely differing tolerance to shear stress. Augenstein et al. (*14*)suggest that 500 N/m^2 causes significant disruption of HeLa S3 andmouse L929 cells in a capillary tube, whereas Midlar and Finn (*15*) quote about 4 N/m^2 for the protozoa *Tetrahymena*. Fazekas de St. Groth (*16*), using a stirred fermenter, suggests that an impeller rip speed of 0.19 m/s equivalent to 0.05 N/m^2 is the maximum permissible for the cultivation of B-cell hybridomas. According to Fig. 6, 0.05 N/m^2 would enable an OTR of between 0.6 and 1.0 mmol/L/h to be achieved in the airlift fermenter.

Results obtained by Boraston et al. (*17*) indicate that an OTR of up to 0.56 mmol/L/h is required by NB1 hybridomas to support growth of the cells up to a density of 3×10^6 cells/mL, which is typically the maximum viable cell density.

METHODS

The following example refers to the culture of mouse NB1 cells that produces a monoclonal antibody against human blood group B antigen (*17*). A 30-L airlift fermenter was sterilized empty with steam via the air inlet line and left to cool and to let the sensors stabilize. The medium was then filter sterilized directly into the vessel, and after sensor calibration and medium equilibration the inoculum of mouse NB1 cells was added.

The medium used in the process was proprietary, but Dulbecco's modified Eagles medium is an example of a culture medium that can be used in this type of fermenter. The medium was usually supplemented with heat-inactivated fetal calf serum at a level to suit the particular cells. The culture was sparged with a mixture of gases. Carbon dioxide was added as and when required for pH control and air was added to maintain a suitable dissolved oxygen tension in the medium. The inlet gas mixture was filter sterilized prior to sparging into the vessel. The exhaust gas passed through a condenser to remove water droplets and vapor before final filtration to prevent back contamination. The pH was controlled by the addition of carbon dioxide or a sodium hydroxide solution. Temperature was controlled by the circulation of water through the vessel jacket with water being heated or cooled as required. Cells were counted using a hemocytometer. Viable cells were distinguished by their ability to exclude trypan blue stain. Antibody titer was measured by the level of agglutination of type B red blood cells calibrated against a standard as used by Sacks and Lennox (*18*).

RESULTS

Cells were inoculated into the fermenter at 1.42×10^5 cells/mL and after a minimal lag phase grew exponentially to reach a maximum cell

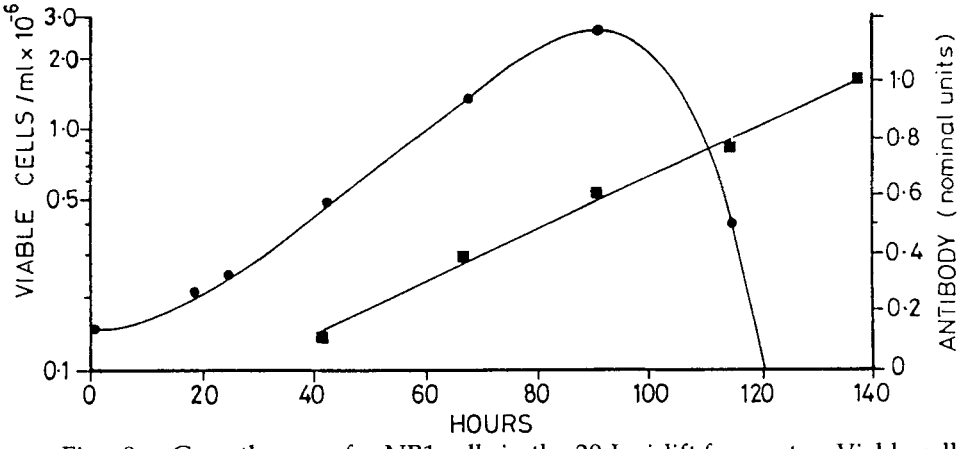

Fig. 8. Growth curve for NB1 cells in the 30-L airlift fermenter. Viable cell population (●); antibody titer (■).

density of 2.6×10^6 cells/mL after 90 h, maintaining a specific growth rate of 0.035/h (Fig. 8). Oxygen was not limited. Cell death ensued rapidly, culminating in zero viability after 137 h. Antibody titer increased mainly during cell growth and continued to increase during the stationary and death phases of the culture. From this it may be presumed that antibody production is not totally growth dependent. Cell viability remained in excess of 90% up to the point at which the maximum population was obtained, indicating that conditions for growth remained favorable during that time. The oxygen-transfer rate was therefore sufficient and the shear forces low enough to avoid cell damage, as had been predicted.

EFFECT OF COMBINATION OF HYDRODYNAMIC FORCES AND MEDIUM COMPONENTS ON CELL VIABILITY

The apparently disparate values for critical shear stress quoted by Augenstein et al. (*14*), Midler and Finn (*15*), and Fazekas de St. Groth (*16*) above may possibly be explained by the associated phenomenon of surface denaturation of proteins at the gas–liquid interface of bubbles in a sparged fermenter. Donaldson et al. (*19*) have reported significant protein denaturation at the gas–liquid interfaces, which is probably related to a combination of surface oxidation and surface tension forces. Virkar et al. (*20*) also have indicated that although shear stress alone has little effect on proteins, shear stress in the presence of entrained gas bubbles causes significant denaturation.

It is possible that the effect of shear forces on protein denaturation is masked because of the protective action of high levels of serum proteins. It might therefore be expected that the culture and productivity of cells in airlift fermenters may suffer if a serum-free medium were to be used.

Figure 9 shows growth and antibody synthesis for NB1 hybridomas growing in a 5-L airlift fermenter (*21*) in serum-free medium containing about 1 g protein/L. The antibody titer from the same cell line in medium containing 5% serum (2–3 g protein/L medium) is typically 100 mg/L, as measured by enzyme-linked immunosorbent assay (ELISA), which is comparable to that seen in Fig. 9. The presence of serum proteins in the medium appears, therefore, to have little effect on the susceptibility of cells to shear forces.

SCALE-UP OF AIRLIFT FERMENTERS

Although the 30-L fermenter is suitable for producing several grams per batch, larger quantities require larger working volumes. Figure 6 indicates that a 1000-L fermenter should achieve suitable OTR at an acceptable shear stress. However, the increased height of a 1000-L vessel (about

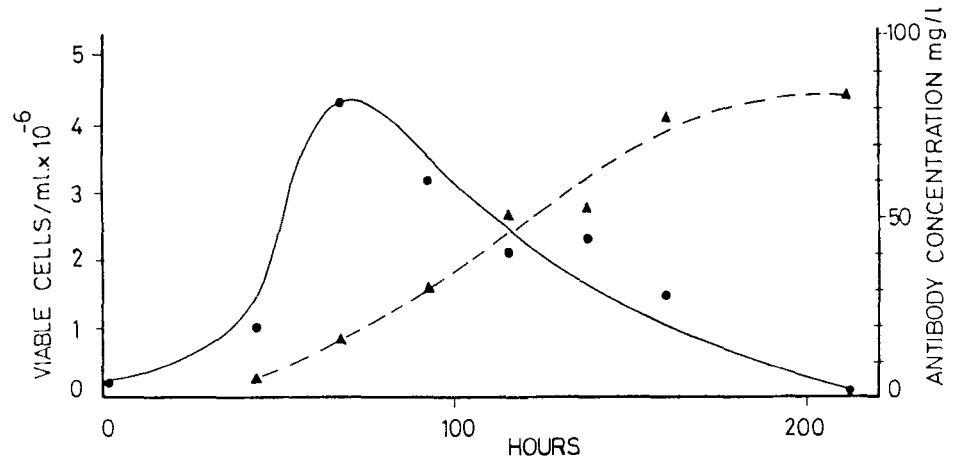

Fig. 9. Batch culture of a mouse hybridoma cell line, NB1, growing in serum-free medium in an airlift fermenter: Viable cells/mL × 10 (●); antibody concentration^{-6} mg/L (▲).

5.0 m) means that cells in suspension will be subject to pressure cycling of increased magnitude, the effects of which are unknown.

Preliminary tests on cell suspension were carried out by subjecting a culture of NB1 cells to pressure variations in a pressure vessel. No harmful effects on the cell viability were detected (Fig. 10).

In addition, fermenters at both the 100- and 1000-L scales have been operated under up to 10 psig top pressure, with no detectable deleterious effects on cell growth or product formation.

A full-scale experiment was carried out (*21*), and Fig. 11 shows growth and antibody production by a mouse hybridoma producing an IgG antibody in a 1000-L production fermenter. In this experiment the fermenter was inoculated from a 100-L fermenter to provide an initial cell

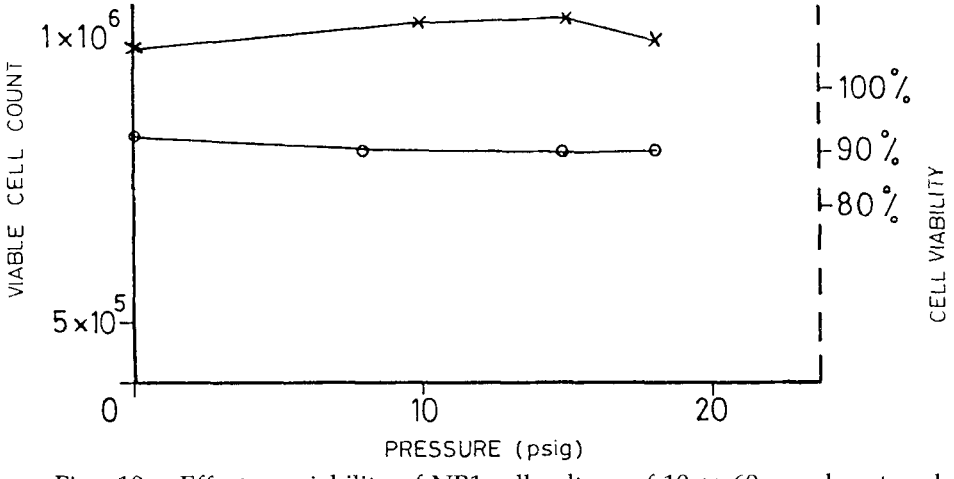

Fig. 10. Effect on viability of NB1 cell culture of 10 × 60 s cycles at each pressure: Viable cell count (X); cell viability (O).

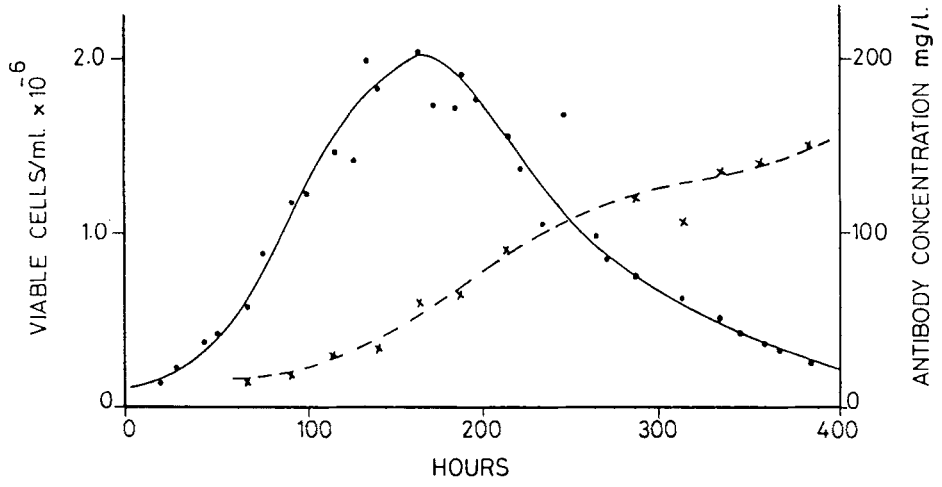

Fig. 11. Batch culture of a mouse hybridoma cell in a 1000-L fermen ter: Viable cells/mL × 10 (●); antibody concentration^{-6} (X).

density of 1.0×10^5 cells/mL. The cells grew exponentially, with a specific growth rate of 0.05/h to a maximum cell density of 2×10^6 cells/mL. Synthesis of antibody occurred during the growth and stationary phases to a maximum of 150 mg antibody/L, which was equivalent to that seen at the 100-L scale. Adequate oxygen transfer was achieved even at maximum cell densities, with the maximum air rate not exceeding 0.05 L/L/min, thus confirming the expectations previously outlined. Visual observation of the cells during the growth phase indicated no damage related to mechanical disruption, viabilities remained in excess of 90% right up to maximum cell density, after which time viability naturally decreased, as seen in roller bottles or static flasks. No direct measurement of the denaturation of proteins was made, but it can be assumed to be negligible in the light of the cell-growth rate and specific antibody production rate, which was comparable or greater than that in static or roller culture. From these results it may be concluded that hybridoma culture may be successfully scaled up at least to the 1000-L scale.

FUTURE DEVELOPMENTS

The use of the simple mathematical model above indicates that a 10,000-L vessel of about 6–8 m liquid depth would generate a maximum shear stress of 0.03 N/m^2 at the wall in order to satisfy an oxygen requirement of 0.56 mmol/L/h, which is well within the limits so far determined. At a depth of 8 m the head of liquid would create a pressure in the region of 12 psig, this has been shown to be insufficient to effect cell viability. From the results obtained to date further scale-up to a 10,000-L airlift fermenter may be confidently predicted.

CONCLUSIONS

Theoretical and experimental results obtained to date, both by hydrodynamic prediction and experimental culture, have shown that for cell lines with a low oxygen transfer requirement, the airlift fermenter provides a suitable environment for growth and product formation up to 1000 L scale and very likely beyond.

ACKNOWLEDGMENT

Acknowledgment for reproduction of this paper is gratefully given to both the authors and the British Hydromechanics Research Association, London. This paper was presented at a meeting held at Cambridge University. Organized and sponsored by BHRA, The Fluid Engineering Centre, with the support of The Institution of Chemical Engineers and the Society of Chemical Industry.

REFERENCES

1. Le Francois, L. Mariller, L. G., and Mejane, J. V. (1955), French Patent no. 1, 102–200.
2. Sinclair, C. G., and Ryder, D. N. (1975), *Biotechnol. Bioeng.* **17,** 375.
3. Lin, C. H., Fang, B. S., Wu, C. S., Fang, U. Y., Kuo, T. F., and Hu, K. C. Y. (1976) *Biotechnol. Bioeng.* **18,** 1557.
4. Blenke, H. (1979) *Advances in Biochemical Engineering* vol. 13. (Ghore, T. K., Fiechter, A., and Blakeborough, N., ed.), Springer Verlag, Berlin.
5. Bello, R. A., Robinson, C. W., and Moo-Young, M. (1984), in *Advances in Biotechnology,* vol. I, p. 547.
6. Coulson, J. M., and Richardson, J. F. (1965), in *Chemical Engineering,* vol. I, Pergamon, London, p. 1970.
7. Pirt, S. J. (1975), *Principles of Microbe and Cell Cultivation,* Blackwell Scientific, London.
8. Cooper, C. M., Fernstrom, G. A., and Miller, S. A. (1944), *Ind. Eng. Chem.* **36,** 504.
9. Slater, L. E. (1974), *Food Eng.* July, 68.
10. Kohler, G., and Milstein, C. (1975), *Nature* (London) **256,** 495.
11. Mach, J-P., Bucheggar, F., Farni, M., Ritschard, J., Berche, C., Lumbroso, J-D., Schreyer, M., Giradet, C., Accola, R., and Carrel, S. (1981), *Immunol. Today,* December, 239.
12. Hill, C. R., Birch, J. R., and Benton, C. (1984) in *Bioactive Microbial Products III—Downstream Processing Special Publication of the Society for General Microbiology.* Academic, London.
13. Marx, J. L. (1982), *Science,* **216.**
14. Augenstein, D. C., Sinskey, A. J., and Wang, D. C. (1971), *Biotech. Bioeng.* **13,** 409.
15. Midler, M., and Finn, R. K. (1966) *Biotech. Bioeng.* **8,** 71.
16. Fazekas de St. Groth, S. (1983), *J. Immunol. Methods* **57,** 121.

17. Boraston, R., Thompson, P. W., Garland, S., and Birch, J. R. (1983) *Dev. Biol. Stand.* **55,** 103.
18. Sacks, S. H., and Lennox, E. S. (1981), *Vox Sanguinic* **40,** 99.
19. Donaldson, T. L., Boonstra, E. F., and Hammond, J. M. (1980), *J. Colloid. Interface. Sci.* **74,** 2, 441.
20. Virkar, P. D., Narendranatham, T. J., Hoare, M., and Dunmill, P. (1981), *Biotech. Bioeng.* **23,** 425.
21. Birch, J. R., Lambert, K., Boraston, R., Thompson, P. W., Garland, S., and Kenney, A. (1985), *Proceedings of the Nato Advanced Studies Institute.* Plenum, In press.