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Short Communication

RESIDENCE TIME DISTRIBUTION IN THE EXTRA CAPILLARY SPACE OF HOLLOW FIBER BIOREACTORS

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The residence time distribution (RTD) in the extracapillary space (ECS) of hollow fiber bioreactors (HFBRs) has been studied using a high molecular weight protein, bovine serum albumin, as a tracer. The RTD measurements have been carried out at different conditions of flow in the ECS and the intracapillary space (ICS). The RTD results obtained give an indication of the flow patterns existing in the ECS. The implications of these studies on cell cultivation as well as product recovery from HFBRs have been discussed.

KEYWORDS Residence time distribution Hollow fiber bioreactor

INTRODUCTION

Hollow fiber bioreactors (HFBRs) are being increasingly used for mammalian cell culture. Fluid dynamics in the HFBR plays a crucial role in growth factor distribution, cell loading and reactor productivity. Several experimental (Park and Chang, 1986; Drury *et al.*, 1988; Gillies *et al.*, 1989; Pangrle *et al.*, 1989; Piret and Cooney, 1990; Heath *et al.*, 1990; Hammer *et al.*, 1990; Donoghue *et al.*, 1992) and theoretical (Apelblat *et al.*, 1974; Schoenberg and Belfort 1987; Salmon *et al.*, 1988) studies of HFBR fluid dynamics have been reported. However, the extent of macromixing in these reactors has not been characterized. In the present work, residence time distribution (RTD) measurement was used as a tool to investigate the extracapillary space (ECS) flow nonidealities. The results presented here give an indication of the flow patterns existing in the reactor and their probable implications in cell growth and product recovery from HFBRs using mammalian cell cultures.

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The HFBR typically consists of a bundle of hollow semipermeable fibers housed in a cylindrical casing. The reactor is separated into two compartments: the intracapillary space (ICS), through which the medium is perfused and the ECS, where the cells are entrapped. HFBRs thus mimic the vascular system which provides nutrients to cells and tissues through the capillary network. The product secreted by the immobilized cells is retained in the ECS by the ultrafiltration fibers and can be harvested in a concentrated form. The HFBRs offer a low shear environment for the mammalian cells, which can grow to packed densities. HFBRs have been used for the production of cancer and viral antigens (David *et al.*, 1974; Hager *et al.*, 1982), hormones (Knazek *et al.*, 1972) and monoclonal antibodies (Altshuler *et al.*, 1986).

The residence time distribution in the HFBR arises due to the specific flow pattern existing in the reactor. It quantifies mixing at macroscopic level and governs the overall extent of the reaction (Levenspiel, 1972; Wen and Fan, 1975). The non-ideal flow can be characterized by means of the exit age distribution function (Danckwerts, 1953), which can be determined by using various experimental techniques. In these stimulus response techniques, the system is disturbed by a stimulus and the response is analyzed. In our current study, the stimulus was a protein tracer input into the fluid entering the ECS of the HFBR and the response was a time record of the protein concentration at the exit of the reactor. The tracer can be any material that can be detected and does not disturb the flow pattern in the reactor. As it would have been difficult to satisfy these conditions in the presence of cells in the reactor (due to possible adsorption and metabolism of the tracer by the cells), the tracer experiments have been carried out in the absence of cells in the reactor. They are relevant in the initial stages of (approximately two weeks) when the volume by the cells is not significant (less than 1% for a cell density of 10^6–10^7 cells/ml). The effect of cells on the fluid mechanics can be assumed to be negligible especially for cells growing in suspension. However, during this period the distribution of high molecular proteins such as serum and growth factors affects the subsequent distribution and productivity of the cells. Besides, this also ensured that the flow patterns under different experimental conditions did not vary due to possible variations in the ECS cell distribution.

MATERIALS AND METHODS

HFBR Cartridge

The Acusyst-R HFBR (Endotronics Inc., USA) used in this study essentially consisted of a bundle of hollow fibers potted at the ends with epoxy resin and enclosed in a transparent polycarbonate casing (23 cm × 2 cm dia). The fibers are made of regenerated cellulose and the effective volume of the ECS was 110 ml. Figure 1 shows a schematic diagram of the reactor. The geometric construction of the reactor and the flow distributor at the inlet and outlet of the ECS were examined by making transverse sections of the cartridge. The dimensions of the individual wetted hollow fibers were measured using a micrometer slide under an optical microscope (Axiophot, Carl Zeiss, Germany).

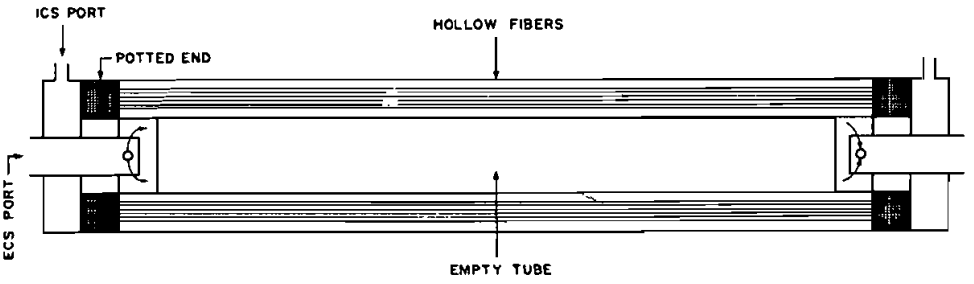

FIGURE 1 A schematic representation of the geometric construction of the HFBR.

Tracer Studies

The HFBR was equilibrated with a 0.85% NaCl solution. A bovine serum albumin (Sigma Chemical Co., USA) solution was stained with Coomassie Brilliant Blue G-250 (Sigma Chemical Co., USA). The solution was dialyzed extensively against 0.85% NaCl to remove unbound stain molecules. A 60 g/l stock solution of the dye-protein complex in 0.85% NaCl was used as a tracer. The staining of the tracer helped its visual observation. A small aliquot (about 200 µl) of the stock solution was injected into the ECS feed stream at the entry point of the HFBR (Fig. 1) using a hypodermic syringe. The exit concentration of the tracer was determined by monitoring the absorbance (at 220 nm) of the effluent on-line using a spectrophotometer equipped with a flow cell (Pharmacia, Sweden). The spectrophotometer was connected to the exit of the ECS using a thin tubing (1 mm, id, with a volume of 2 ml). All the exit fluid was collected and the protein concentration present in the total effluent was measured by measuring the absorbance at 280 nm. Equal flow rates at the ECS inlet and outlet were ensured by the use of peristaltic pumps at both the end.

The fiber membrane permeability was measured according to the procedure described by Patkar *et al.* (1993). The secondary Starling flow in the ECS was approximately calculated using this measured permeability by assuming linear axial pressure drop in the ICS (Tharakan and Chau, 1986; Heath *et al.*, 1990) and ECS. The expected tracer residence time was calculated from the ECS volumetric flow rate and the ECS volume. For calculating the average residence time and variance from RTD data, the tail of the curve was approximated as an exponential decay (Fogler, 1986).

RESULTS AND DISCUSSION

The tracer studies were performed under different flow conditions in the ICS and ECS.The physical parameters of the reactor and the experimental conditions used are summarized in Tables I and II. In the absence of ICS flow the tracer concentration profiles were essentially symmetrical Gaussian RTD curves with an extended tail

TABLE I

Physical Properties of the HFBR System Used

Inner radius of fiber	1.05×10^{-4} m
Outer radius of fiber	1.26×10^{-4}
Krogh radius	2.07×10^{-4} m
Length of the fiber	0.23 cm
Number of fibers	5070
Area of the ECS	0.92 m^2
Membrane permeability	4.94×10^{-10} m/Pa s
Fluid viscosity	1×10^{-3} kg/m s

TABLE II

ECS Mean Residence Time and Starling Flows Under the Experimental Conditions Used

Expt. Number	ECS Flow rate (ml/min)	ICS Flow rate (ml/min)	Flow Direction*	Calculated Residence time (h)	Exptl. Residence time (h)	Starling Flow (ml/min)
1	0.17	0	NA	11	10.66	0
2	1.67	0	NA	1.1	1.31	0
3	1.67	100	+	1.1	0.75	5.3
4	1.67	200	+	1.1	0.51	10.7
5	1.67	100	−	1.1	0.80	5.5
6	1.67	200	−	1.1	0.86	10.9

*+ Cocurrent to ECS flow
− Countercurrent to ECS flow

portion (Fig. 2A, B). The Gaussian portions of the curves indicate significant deviation from plug flow in the HFBR ECS. This dispersion may occur due to nonhomogeneous fiber packing and nonuniform ECS flow distribution. The extended tail could possibly be due to stagnant zones in the reactor and/or slow desorption of protein adsorbed on the fibers. The adsorption of the protein was found to be negligible as the losses in recovery of protein in the effluent were less than 1%. The ECS feed enters a small cylindrical region from where it is distributed to the ECS (Fig. 1). The reverse process occurs at the ECS outlet. Stagnant regions could be present in both these distribution regions resulting in a long tail in the RTD. An increase in the ECS flow rate resulted in a decrease in the mean residence time. The mean residence time and the expected residence time calculated from the flow rate and the ECS volume were in good agreement in experiments performed at zero ICS flow.

Highly asymmetric protein concentration profiles, with long tails, were obtained in the presence of ICS flow, either cocurrent or countercurrent to the ECS flow (Fig. 3A, B). This asymmetry indicates a significant increase in dispersion (Levenspiel, 1972). With cocurrent ICS flows, the observed residence times were substantially less than the calculated residence times (Table II). The mean residence times were lower even when the ICS flow was countercurrent, although the differences were less than that in the cocurrent case.

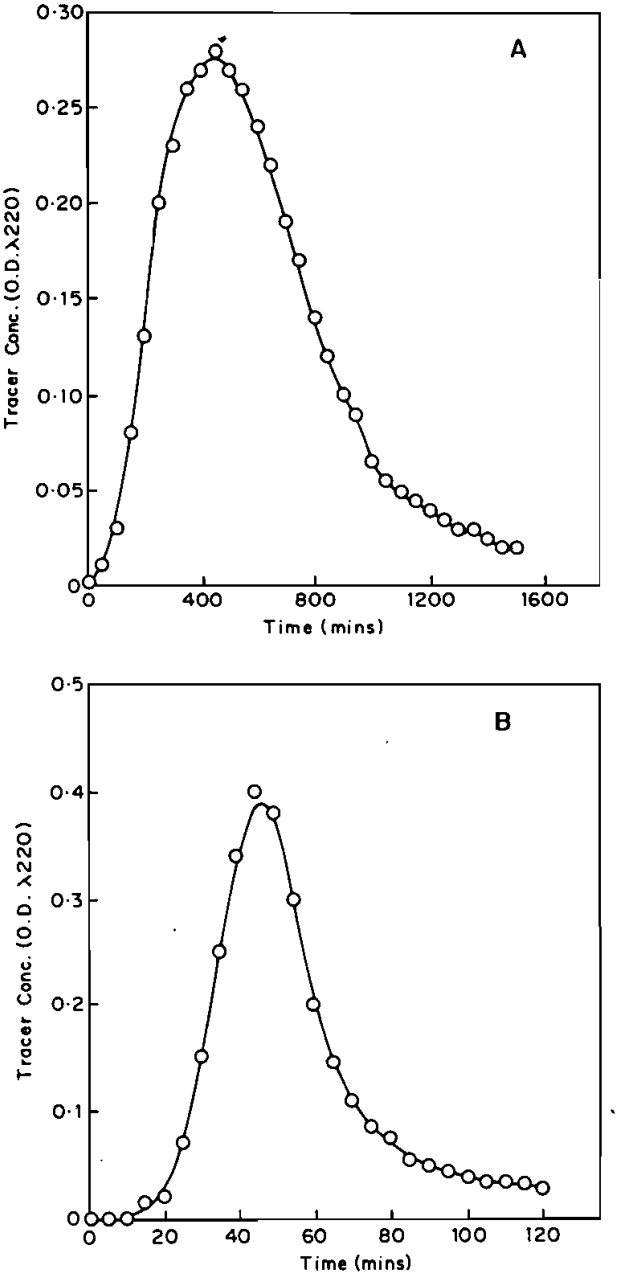

FIGURE 2 The exit tracer concentration profile when the flow is only in the ECS: The protein tracer concentration was monitored at the exit of the ECS at (A) a flow rate of 10 ml/h, and (B) a flow rate of 100 ml/h.

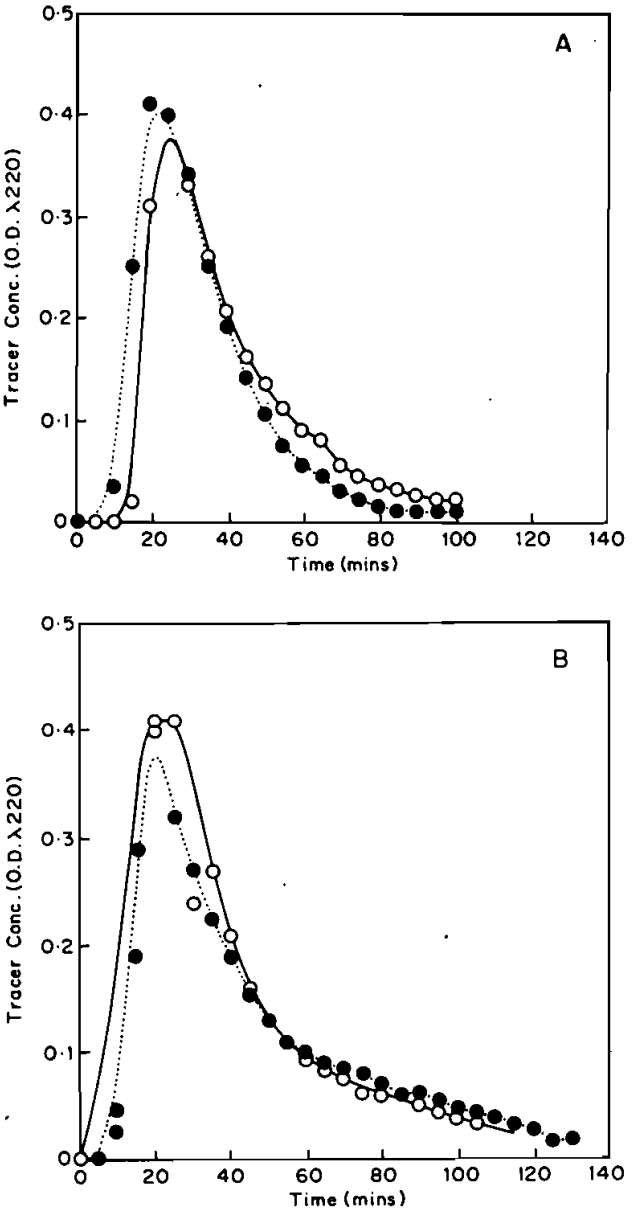

FIGURE 3 Theh exit tracer concentration profile when there is flow in the ICS and ECS: The protein tracer concentration was monitored at the exit of the ECS at a flow rate of 100 ml/h and the ICS flow rate was 100 ml/h (-○-) and 200 ml/h (-●-). (A) The direction of ICS flow was cocurrent to the ECS flow, and (B) The ICS flow was countercurrent to the ECS flow.

The results could be qualitatively explained. The pressure gradients in the ICS and the ECS cause a secondary convective flow in the ECS (Apelblat, 1974). The ICS fluid enters the ECS through the fiber membrane near the reactor entrance and reenters the ICS near the reactor exit. A simple calculation based on ICS and ECS pressure

gradients and membrane permeability was used to approximate the magnitude of average Starling flow in the ECS (Table II).

The Starling flows were much greater (3–6 times at ICS flow rate of 100 and 200ml/min respectively) than the superimposed ECS flow. The Starling flow increases with an increase in the ICS flow rate. The magnitude of the Starling flow is about 5% of the bulk lumen flow. When the ICS flow is cocurrent the tracer is pushed towards the exit of the reactor at a faster rate due to the Starling flow. At the end of the reactor the fluid reenters the ICS and since the tracer cannot enter the ICS, it leaves the reactor at the exit. This results in channeling or bypassing, where the tracer protein is not distributed throughout the hollow fiber bundle and passes through only a small center portion of the entire bundle. This process is shown schematically in Figure 4A. In the presence of countercurrent flow a similar mechanism can be visualized, where the tracer protein now travels towards the outer radius of the hollow fiber bundle and bypasses the central region of the bundle (Fig. 4B). These channeling and bypassing phenomena may cause a reduction in the observed residence times.

These results indicate that the flow in the ECS deviates significantly from plug flow. The Starling flow was shown to have a significant effect on the RTD in the reactor. This has significant implications in the use of HFBRs for cell cultivation for the production of secretory products. During the operation of HFBRs the cells are supplied with high molecular weight nutrients by passing serum through the ECS. Distribution of proteins in the ECS affects the distribution and amount of cell growth in HFBRs. Piret and Cooney (1990), demonstrated that downstream polarization of growth factors (induced

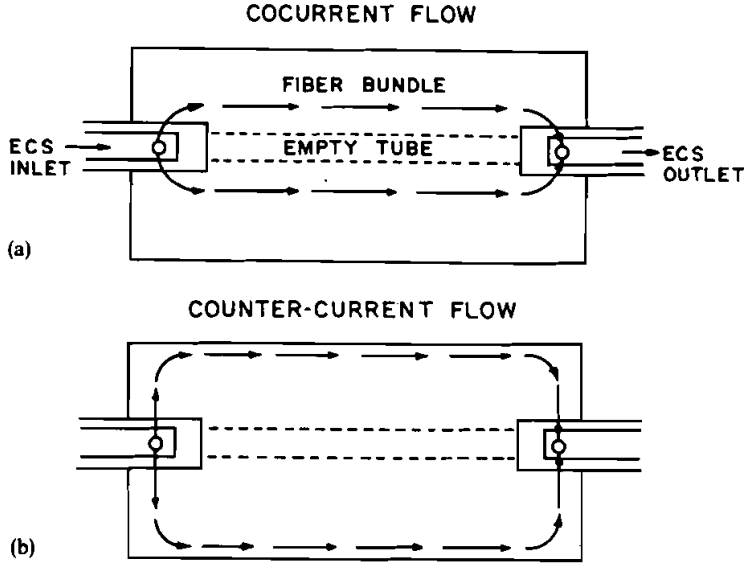

FIGURE 4 A schematic of the fluid flow pattern in the ECS of the HFBR: The fluid flow pattern existing in the ECS is described schematically when (A) The ECS and ICS flows were cocurrent, and (B) The ECS and ICS flows were countercurrent.

by Starling flow) resulted in downstream cell growth, under utilization of reactor volume and corresponding lower reactor productivity.

These phenomena may also play a major role in product recovery from HFBRs. This is normally done by adding medium (containing a small amount of serum) through the ECS inlet and collecting the product in the ECS outlet stream. Assuming that the product formed is uniformly distributed throughout the ECS volume, a reduction in mean residence time by 30–50% (when the ICS flow is cocurrent) implies that the effective ECS volume is correspondingly reduced and hence only 30–50% of the product is recovered. Considering that animal cells secrete products at very low concentrations, this loss is quite significant. The product recovery may be improved by increasing the volume of the medium passed through the ECS for harvesting the secreted protein. Alternative designs for creating the right hydrodynamic conditions, which will avoid these problems, need to be urgently sought.

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