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Optimization and Mathematical Modeling of the Transtubular Bioreactor for the Production of Monoclonal Antibodies from a Hybridoma Cell Line

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Abstract This report describes the use of a transtubular bioreactor to study the relative effects of diffusion versus perfusion of medium on antibody production by a hybridoma cell line. The study was performed with a high-density cell culture maintained in a serum-free, low-protein medium for 77 days. It was determined that the reactor possessed a macro-mixing pattern residence time distribution similar to a continuous stirred tank reactor (CSTR). However, due to the arrangement of the medium lines in the reactor, the flow patterns for nutrient distribution consist of largely independent medium path lengths ranging from short to long. When operated with cyclic, reversing, transtubular medium flow, some regions of the reactor (with short residence times) are more accessible to medium than others (with long residence times). From this standpoint, the reactor can be divided into three regions: a captive volume, which consists of medium primarily delivered via diffusion; a lapped volume, which provides nutrients through unilateral convection; and a swept volume, which operates through bilateral convection. The relative sizes of these three volumes were modified experimentally by changing the period over which the direction of medium flow was reversed from 15 min (larger captive volume) to 9 h (larger swept volume). The results suggest that antibody concentration increases as the size of the diffusion-limited (captive) volume is increased to a maximum at around 30 min with a sharp decrease thereafter. As reflected by changes in measured consumption of glucose and production of lactate, no significant difference in cellular metabolism occurred as the reactor was moved between these different states. These results indicate that the mode of operation of the transtubular bioreactor may influence antibody productivity under serum-free, low-protein conditions with minimal effects on cellular metabolism.

Keywords: bioreactor design, antibody production, modeling, perfusion, cell communication

INTRODUCTION

Cell-cell communication is important for protein production by mammalian cells [1-5]. Local cellular communication occurs through various mechanisms including gap junctions and autocrine and paracrine factors. The production of autocrine/paracrine factors from mammalian cells has been well documented [6-12]. These are known not only to be stimulatory for growth, but also for production of proteins [12,13]. In the case of hybridoma cells, various proteins have been proposed that is not only stimulatory [12,13], but also inhibitory [14]. These findings may be due to several reasons including the cell line used, the overall environmental conditions in which the cells are placed and the concentration of these cell-derived factors.

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To examine the influence of the cellular microenvironment for antibody production from a hybridoma cell, a transtubular bioreactor capable of growing and maintaining mammalian cells in a high cell density culture was used. The reactor consists of a set of macroporous Teflon™ tubes for medium exchange and a silicone tube for gas exchange imbedded in a macroporous alginate matrix that is used to entrap, suspend and evenly distribute the cells without agitation. The system can be operated in several different one-pass perfusion flow regimes, which exposes the entrapped cells to environments ranging from a diffusion-limited to a well-perfused regime. Opening and closing the two input and output medium lines controls these various mixing arrays. As has previously been reported [15,16] antibody production from a hybridoma cell entrapped in a transtubular bioreactor may be influenced by both flow rate and the period of line changes. It is not clear why anti-

body production is affected. One possibility is that a long residence time of cell-derived factors may induce higher antibody production. Recently, Roy *et al.* proposed that the Peclet number for media flow to hepatocytes immobilized in a bioreactor influence the behavior of the cell [17]. Hence, the objective of this report is to examine how different flow patterns in a reactor environment might affect antibody production by a hybridoma cell. The period of medium line changes were modified to examine the affects on antibody production and ranged from 15 min (diffusion-limited) to 9 h (well perfused). Experiments reported here suggest that different flow patterns may influence antibody production.

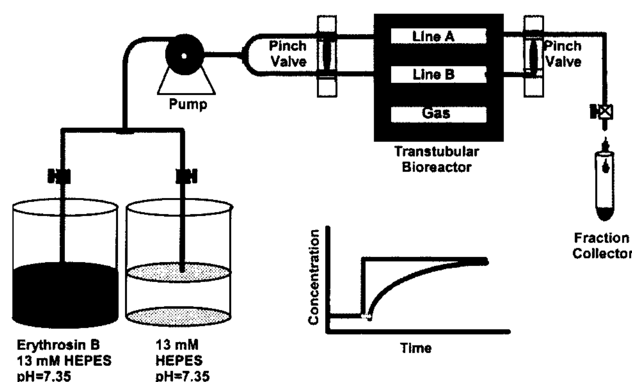

Fig. 1. Schematic diagram of the residence time distribution experimental setup as described in the Materials and Methods section.

MATERIALS AND METHODS

Cell Line

The cell line used was a murine hybridoma, S3H5/γ2bA2, which produces an anti-idiotypic antibody directed against the antigenic site 38C13 [18]. Both the growth of these cells and their antibody production capabilities have been characterized elsewhere [18,19].

Iscove's Modified Dulbecco's Medium (IMDM) (Gibco Laboratories, Grand Island, NY, USA) was used and either 10% fetal bovine serum (FBS, Hyclone Laboratories, Logan, UT, USA) or 1 mL/L of Insulin-Transferrin-Sodium Selenite Media Supplement (ITS) (5 μg/mL, 5 μg/mL, 5 ng/mL; final concentration) (Sigma Chemical Company, St. Louis, MO, USA) was used as a supplement. To one liter of medium, 10 milliliters of a penicillin, 5,000 units/mL, and streptomycin, 5,000 μg/mL (Sigma) were added. Prior to inoculation into the bioreactor, the cells were grown in T-75 cm^2 plastic cell culture flasks (Bellco Glass, Inc., Vineland, NJ, USA) at 37°C in a humidified CO_2 incubator. The cells were split 1:5 with fresh medium every other day to be maintained in a log phase of growth.

Transtubular Bioreactor Loading Protocol

As previously described [15,16], the transtubular bioreactor used in this study includes two tubes for medium exchange and another for gas exchange (Fig. 1). The tubes are enclosed in a glass housing with a final extratubular volume of 50 mL [15,16]. One line (Silastic™, Dow Chemical, Midland, MI, USA) provides oxygen directly to the contained medium without sparging of gas. Two macroporous Teflon™ (Gore-Tex®, Elkton, MD, USA) tubes with a pore size of 3.5 μm and an overall porosity of 70% are used to deliver medium and remove wastes from the reactor. The cells are suspended evenly in an alginate/gelatin matrix with the maximum hybridoma cell density achieved of 2×10^8 cells/mL [15].

The reactor was loaded initially with a cell density of 3.5×10^6 cells/mL and was operated in a batch recycle mode for 10 d. The reactor was then switched to a cyclic, reversing perfusion (CRP) mode for the rest of the run. Medium enters into one line (A), the outlet of which is blocked, and leaves through the second macroporous tube (B) (Fig. 1). This delivers medium to the cells by forced convection from one medium line to the other. Periodically, at regular intervals, the direction of medium flow was reversed by means of an external solenoid-controlled pinch valve (Bio-Chem Valve Corp., NJ, USA). The pinch valve was controlled using an X-10 controller (X-10 (USA) Inc., Northvale, NJ, USA) which allowed for the inlet line to be alternated between one medium line (A) to the other line (B) at a desired period (here, ranging from 15 to 540 min). The CRP mode was initially introduced to improve the delivery of medium throughout the reactor, but it was subsequently discovered that this mode of operation (depending on the period of line changes) could control the mixing patters in the reactor.

Analytical Methods

Six mL of medium, sampled from the outlet, were taken twice a day and immediately used to measure pH, and, with Yellow Springs Instruments Model 2000 Glucose/Lactate Analyzer (Yellow Springs, OH, USA), glucose and lactate concentrations. The antibody IgG_{2b} concentration in thawed samples was quantified using an enzyme-linked immunosorbent assay (ELISA) [19]. At the termination of the run, the remaining alginate matrix containing the cells was dissolved in isotonic citrate solution (3% aqueous sodium citrate (Sigma) diluted 1:1 in 0.9% NaCl (Sigma) and adjusted to pH 7.4) [20]. The viable cell concentration was determined using trypan blue dye exclusion.

Determination of the Residence Time Distribution

The mean residence time in the reactor was determined using the following experimental setup: A 50-mL transtubular bioreactor with an immobilized alginate/gelatin matrix was connected to two bottles of media (Fig. 1). After overnight equilibration in a 37°C incubator with a 13 mM HEPES (Sigma, St. Louis, MO, USA),

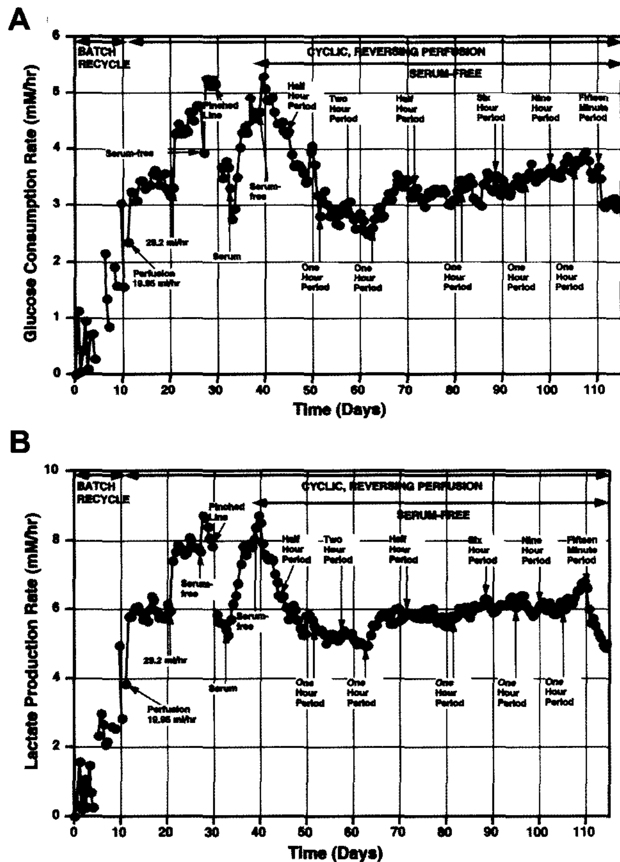

Fig. 2. (A) Glucose consumption rate (mM/h) versus time (days). (B) Lactate production rate (mM/h) versus time (days).

pH 7.35, a step input of a solution of Erythrosin B (Sigma) dissolved in the 13 mM HEPES solution was added to the reactor. Two-minute fractions were collected over a six-hour period using a Foxy (ISCO, Lincoln, NE, USA) fraction collector. The flow rate was kept constant at 40.0 mL/h and samples were measured at a wavelength of 540 nm using a μmax Kinetic Microplate Reader (Molecular Devices, Palo Alto, CA, USA).

RESULTS AND DISCUSSION

Transtubular Bioreactor Run

The transtubular bioreactor was operated for 115 days, the last 77 days of which used a serum-free, low-protein medium. Fig. 2(A) is a graph of glucose consumption rate (mM/h) versus time (d). The reactor was initially operated for 10 days using a batch recycle mode at 40 mL/h and was then switched to a cyclic, reversing perfusion mode on day 11 at a flow rate of 19.95 mL/h (Fig. 2(A)). On day 21 the reactor was switched to 29.2 mL/h and kept at this flow rate for the rest of the run. On day 28, the reactor was switched from a medium containing 10% serum to a serum-free, low-protein medium that contained only insulin, transferrin and selenium. Inadvertently, on day 30 of the run, the inlet tube to the reactor became pinched and the reactor did not receive fresh medium for approximately 8 h. To recover the reactor, the system was shifted back to a medium containing 10% serum. On day 38 of the run, the reactor was returned to a serum-free medium for the duration of the run.

As described in the Materials and Methods section, the delivery of medium can be controlled using external solenoid pinch valves. Hence, the convective delivery of medium to the reactor can be switched from line A to line B and from line B to line A at various periods, using a controller timer. These changes are represented in Fig. 2(A) as "Half Hour Period", etc. Initially the reactor was operated with a one-hour period. On day 45 of the run, the reactor was switched to a half-hour period and the glucose consumption rate decreased to a value of approximately 3.0 mM/h. The reactor system was shifted to a one-hour period and the consumption rate leveled out to just under 3.0 mM/h. On day 57 of the run, the reactor system was switched to a two-hour period. This change caused a decrease in the glucose consumption rate to a value of approximately 2.5 mM/h. The reactor was then shifted back to a one-hour period whereupon the glucose consumption rate increased to a value of about 3.3 mM/h. From day 70 to day 95, including periods of a half-hour (day 72), one hour (day 82), and six hours (day 88) the glucose consumption rate remained around the value of 3.3 mM/h. From day 95 to 107 the glucose consumption rate increased to approximately 3.8 mM/h. This included the periods of one-hour (day 95), nine-hour (day 100) and one-hour (day 105). On day 110, the period was switched to a 15-minute period and the glucose consumption rate dropped from 3.5 to 3.0 mM/h. On day 113, an electrical storm eliminated power to the reactor system for approximately 8 h. This caused a decrease in the glucose consumption rate to below 3.0 mM/h.

Fig. 2(B) shows the lactate production rate (mM/h) versus time (days). The values ranged between 0 to 4.0 mM/hr for the batch recycle mode and reached as high as 8.5 mM/h during the initial change to a serum-free, low-protein medium. From day 45 to day 52 (half-hour period), the lactate production rate decreased from 6.2 to 5.5 mM/h. From day 52 to 63, (one-hour and two-hour periods) the lactate production decreased from 5.5 mM/h to 5.0 mM/h. From day 63 to 68 (one-hour period), the lactate production rate increased from 5.0 mM/h to 5.8 mM/h. From day 63 to 82 (one hour and half-hour periods), the lactate production remained constant at a value of approximately 5.8 mM/h. On day 82, the lactate production increased to a value of 6.2 mM/h and fluctuated around this point until day 105 (one hour, six-hour, one-hour, and nine-hour periods). On day 105 (one-hour period), the lactate production rose to a value of 7.0 mM/h. On day 110 (fifteen-minute period), the lactate production rate decreased to 5.8 mM/h. On day 113, the lactate production rate dropped to below 5.0 mM/h.

Fig. 3 is a graph of antibody (μg/mL) and total anti-

Fig. 3. Antibody (μg/mL) and total antibody produced (μg) versus time (days).

body produced (μg) versus time (days). The antibody concentrations ranged between 33 μg/mL while operating with 10% serum-containing medium, to 4.5 μg/mL when the reactor was operated in a serum-free, low-protein medium with a 15-min period. A total of 0.96 gram antibody was produced during this run.

As shown in Fig. 3, on day 38, when the reactor was shifted from a 10% serum medium to a serum-free, low-protein medium the antibody titer decreased from an average value of 23 μg/mL to approximately 15 μg/mL (day 44). The reactor was then shifted from a one-hour to a half-hour period. The antibody titer appeared to reach steady state at a concentration of approximately 13.6 μg/mL. On day 51 of the run the reactor was switched back to a one-hour period. This was accompanied by an immediate drop in the antibody titer from an average concentration of 13.6 μg/mL to an average value of 10.0 μg/mL. On day 57, the reactor was switched to a two-hour period. As seen in Fig. 4, the average antibody concentration was 9.3 μg/mL. On day 62 of the run, the reactor was switched back to a one-hour period and the average antibody concentration was 9.9 μg/mL. On day 72 of the run, the reactor was switched to a half-hour period and the average antibody concentration increased to 12.6 μg/mL. On day 78, while the reactor was still operating with a half-hour period, the antibody titer decreased to 10.0 μg/mL. This brought the final average antibody titer during the half-hour period to 10.9 μg/mL. On day 82, the reactor was switched to a one-hour line change and the antibody concentration dropped to an average concentration of 9.5 μg/mL. On day 88 of the run, the reactor was switched to a six-hour period. The antibody concentration immediately dropped to an average antibody titer of 7.7 μg/mL. On day 95 of the run, the reactor was switched back to the one-h period mode with an average antibody concentration of 8.8 μg/mL. On day 100 of the run, the reactor was shifted to a nine-h period. The average antibody concentration dropped to 7.9 μg/mL. On day 105 of the run, the reactor was changed to a one-h period with an increase to an average antibody titer of 9.4 μg/mL. On day 110 of the run, the reactor was switched to a fifteen-min period and the antibody titer immediately dropped to 5.7 μg/mL. The reactor was shut down on day 115 and the alginate from the entire reactor volume (as described in the Materials and Methods section) was dissolved for a viable cell count. Unfortunately, presumably due to the loss of power to the reactor system on day 113 for approximately 8 hours, the percent viability was extremely low (5-10%).

Fig. 4. Expanded view of antibody (μg/mL) versus time (days) from day 51 to 115.

Residence Time Distribution

Why does the antibody production rate depend upon the period of switching medium between the two lines? In order to answer this question, a better understanding is needed of how these periods of switching affect the overall mixing inside the reactor. A wide variety of flow paths are contained within the reactor due to the pseudo-random packing of the tubes. Initially, an empirical mathematical model was developed (not shown) in an attempt to understand the flow distribution of medium down the length of the tube. This mathematical model is based on solving four differential equations simultaneously and observing how different percent transtubular flow rates will affect the distance that the fluid will leave or enter a tube in the reactor. The boundary conditions used for this model were the following: pressures in both medium lines were set equal to 0 at the outlet of the reactor and the reactor operated under a constant pressure. This model does not take into account the use of an alginate matrix. According to this mathematical model, under conditions of 100% unidirectional transtubular flow, medium will leave the tube throughout its entire length with the greatest medium exchange near the inlet of the reactor. However, the model describes a system that contains a constant pressure throughout the reactor (unlike a reactor which contains an uneven distribution of matrix) and does not take into account how cyclic, reversing perfusion can effect the flow down the length of the tubes. To incor

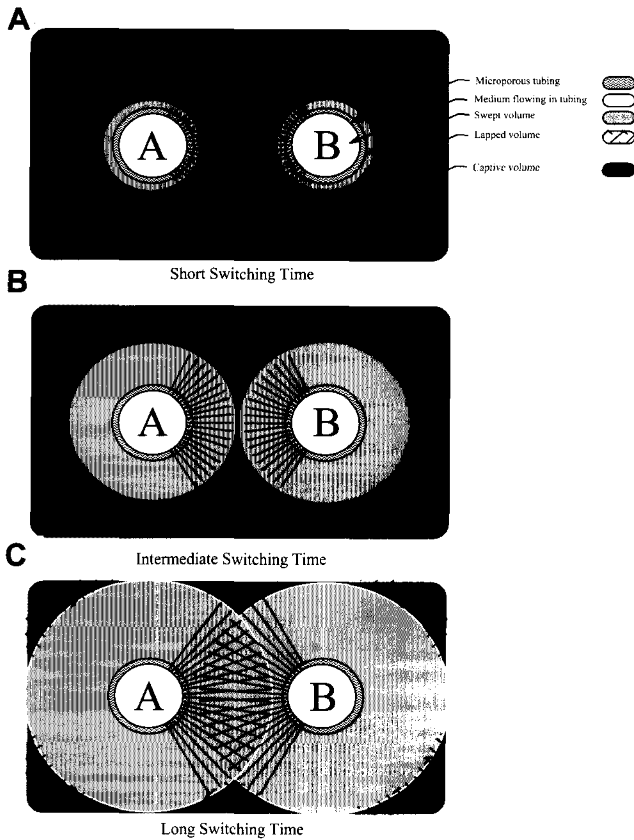

Fig. 5. Hypothetical mixing regimes in relationship to the period of switching medium lines as described in the materials and methods section. (A) Short period of switching lines causes the majority of the reactor to reside in the captive volume. (B) Intermediate switching time places part of the reactor in a diffusional limited state and part of the reactor in a convective regime. (C) Long switching time allows the majority of the cells to see medium via a well-perfused regime.

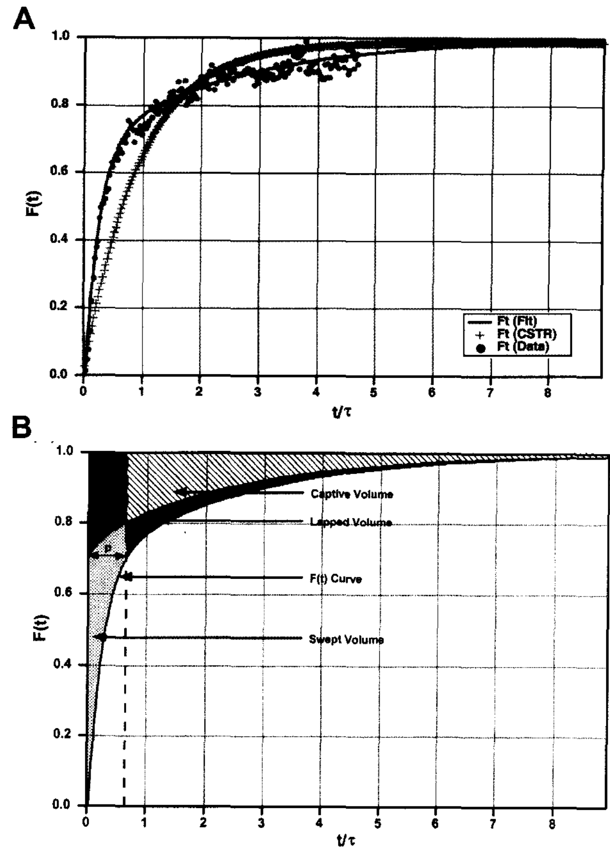

Fig. 6. (A) Cumulative residence time distribution ($F(t)$) versus t/τ. The data points were generated by introducing a step function of dye into the reactor at a flow rate of 40.0 mL/h. A non-linear curve fit was used to derive the three exponential curve (Equation 1.0). The CSTR curve was generated using the definition of the $F(t)$ for a CSTR. (B) Hypothetical volumes associated with the $F(t)$ curve.

porate the CRP, one needs to examine the distribution of tubes in the reactor and how they will effect medium flow. Short path lengths will exist where an inlet tube is adjacent to an outlet tube and long path lengths where the medium leaving one tube resides in the reactor for a period of time before leaving through the outlet medium line.

Fig. 5 is a schematic drawing illustrating how the switching time of the medium lines can effect these various path lengths for delivery of medium to the cells. Three volumes are shown in this figure. *Captive Volume*: Except for micromixing (diffusion), this is the fraction of reactor volume that never receives new medium when the reactor is operated in a cyclic, reversing medium mode. *Swept Volume*: Except for micromixing (diffusion), this is the fraction of reactor volume that receives fresh medium during every period, alternating from medium tubes A and B. *Lapped Volume*: Except for micromixing (diffusion), this is the fraction of reactor volume that receives fresh medium only during alternate periods, and only from either medium tube A or B. Fig. 5(A) depicts a short switching time. As shown in this Figure, a large percentage of the reactor volume will be diffusion limited (Captive Volume), with unidirectional convective flow (Lapped Volume) being the other influential medium volume. Fig. 5(C) depicts the situation of long switching time between medium lines. As depicted in this figure, the majority of the cells will see fresh medium through bilateral convective flow (Swept Volume) with a very small percentage of the reactor volume being diffusion limited (Captive Volume). As mentioned above, due to the placement of the tubes in the reactor, short and long path lengths exist. These various path lengths are randomly dispersed throughout the reactor and hence, instead of creating large regions in the reactor, small volume pockets are created.

To address the issue of these various volumes in the reactor, it is necessary to consider the residence time structure of the reactor. As described in the Materials and Methods section, a residence time distribution experiment was performed using a step input of erythro-

sin B with two-minute samples collected for a 6-h period. A flow rate of 40.0 mL/h was used for this study. Fig. 6(A) is a graph of F(t), the cumulative residence time distribution[21], versus time (min). The dots are data and the line is a fitted triple exponential curve, given by

$$Fit = 1 - 0.332Exp(-0.005t) + 0.115Exp(-0.614t) - 0.783Exp(-0.054t). \quad (1)$$

Also shown in Fig. 6(A), for comparison, is a second line generated for a continuous stirred tank reactor (CSTR) [21].

Villermaux presented a model we adapted for describing the flow regimes in the reactor [22]. The model depicts a segregated reactor with a series of parallel tubes with fluid leaving and entering a system at different time points. This was developed to model micromixing in reactors. The model we developed is shown diagrammatically in Fig. 6(B). The following integrals were used to calculate the volumes associated with Fig. 6(B):

Captive Volume (γ_C) is the area lying above the $F(t+p)$ curve and bounded by $F(t) = 1$ and the period of line change, as shown in Fig. 6 (B) and is described by:

$$\gamma_C = 1/\tau \int_p [1 - F(t+p)]dt \quad (2)$$

where τ is the mean residence time 80.72 min for the data shown in Fig. 6(A)), $F(t)$ is the cumulative residence time distribution, and p is the switching period. Simplifying the above equation leads to:

$$\gamma_C = 1/\tau \left[\int_{2p}^{\infty} tE(t)dt - 2p(1 - F(2p)) \right] \quad (3)$$

Where $E(t)$ is the residence time distribution function.

For the fitted $F(t)$,

$$\gamma_C = 0.8226Exp(-0.01p) - 0.0023Exp(-1.228p) + 0.18Exp(-0.108p) \quad (4)$$

If $F(t)$ had been that for a CSTR, one would obtain,

$$\gamma_C = Exp(-2p/\tau) \quad (5)$$

Swept Volume (Fig. 6(B)) is the area lying between the $F(t)$ curve and the $F(t+p)$ curve bounded by the period of line change and can be described by:

$$\gamma_S = \int_0^p -[1 - F(t+p) - (1 - F(2p))]dt \quad (6)$$

simplifying:

$$\gamma_S = 1/\tau \left[2p[F(2p) - F(p) + \int_0^p tE(t)dt - \int_p^{2p} tE(t)dt \right] \quad (7)$$

For the fitted $F(t)$,

$$\gamma_S = -1.645Exp(-0.005p) + 0.823Exp(-0.01p) + 0.0046Exp(-0.614p) - 0.00232Exp(-1.228p) - 0.36Exp(-0.054p) + 0.18Exp(-0.108p) + 1 \quad (8)$$

If $F(t)$ had been that for a CSTR, one would obtain,

$$\gamma_S = (-2Exp(-p/\tau) + Exp(-2p/\tau) + 1 \quad (9)$$

Lapped Volume γl is described in Fig. 6(B) and is determined by the following:

$$\gamma l = 1 - \gamma_C - \gamma_S \quad (10)$$

For the fitted $F(t)$,

$$\gamma l = -1.645Exp(-0.005p) + 0.00464Exp(-0.614p) - 0.36Exp(-0.054p) \quad (11)$$

If $F(t)$ had been that for a CSTR, one would obtain,

$$\gamma l = 2Exp(-p/\tau) - 2Exp(-2p/\tau) \quad (12)$$

This segregated model does not include the effects of cell metabolism, growth, and dissolution of alginate, which can change the flow patterns and micromixing in the reactor.

The purpose of the experiment described in this report was to determine if the period of cyclic, reversing fresh medium flow might influence the production of antibody from hybridoma cells growing in a serum-free, low-protein medium. The experiment was conducted over a period of 115 days. Two conclusions can be drawn from this run: 1) A high cell density culture can survive and produce adequate amounts of antibody in a serum-free, low-protein medium for an extended period of time (77 days). As previously discussed[15], this provides a benefit for economic production of protein products for both pharmaceuticals and diagnostics. 2) Medium mixing patterns in the reactor may influence antibody titer with little affect on cellular metabolism.

The effects of the switching rate on antibody concentration is shown in Fig. 7. This figure depicts the percent volumes (Captive, Swept and Lapped) and average antibody concentration versus switching time between line changes divided by the mean residence time for the measured RTD. The antibody concentrations are those reported for the reactor run described above. Fig. 7 shows that the highest antibody titer was obtained with a half-h-switching period, whereas, with the exception of the 15-min switching period, the lowest antibody titer was observed at the six- and nine-hour periods. These values correspond to high and low captive volumes respectively. It should be noted that, except for the 15-min period, no significant change in glucose consumption or lactate production occurred during these various changes. In addition, as seen in Fig. 4, the antibody concentrations increased to approximately the same value when the period of line switching was changed from a six-h to a one-hour period and from a nine-hour period to a one-h period. The changes seen for the 15-min period may be due to the majority of the reactor being placed in the Captive Volume regime, which

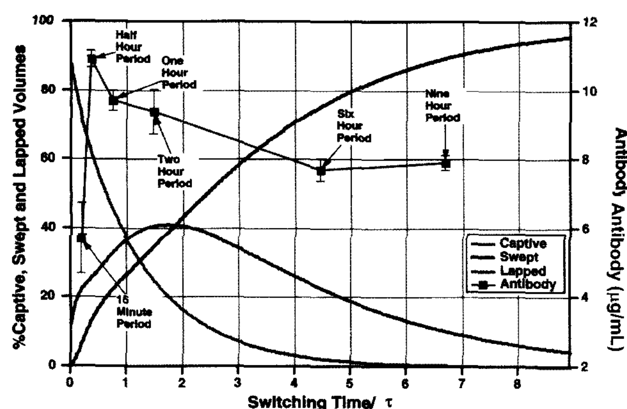

Fig. 7. Percent captive, swept and lapped volumes and average antibody concentrations versus switching time divided by the residence time (τ).

caused a decrease in cell viability due to the insufficient fresh medium being provided to the majority of cells in the reactor. It should be emphasized that the data generated are derived solely from a single reactor run. Whether or not similar findings will be obtained with other reactors with different packing of tubes remains to be determined.

CONCLUSION

When other reactors were operated using either half-hour or six-hour periods, the half-h period reactor runs produced higher titers of antibody (data not shown). Whether these differences were due to the period of line change or to other reasons such as different cell densities remains to be seen. Although not internally controlled, data from the runs with half-h and six-hour switching periods are consistent with the results at the more controlled run reported here. This supports the hypothesis that, when the majority of the reactor is placed into a diffusion-limited environment, the residence time of the cellular autocrine/paracrine factors is increased. This explanation could account for the apparent stimulation of antibody production.

Factors for antibody stimulation from hybridoma cells have been shown to exist. Yamada *et al.* [23] have isolated an immunoglobulin production stimulating factor (IPSF) from the Namalwa myeloma cell line that is used as a fusion partner for the formation of human-to-human hybridoma cells. This cell may produce a similar autocrine factor. In addition, interleukins have been shown to be stimulatory for both growth and differentiation for B cells [7,8,24].

Other explanations could account for the changes in antibody production observed when the reactor is operated in different switching modes. These include the effects of other components of the microenvironments surrounding the cells, the periodic nature of delivery of various factors to the cells, and stress, possibly caused by local pH gradients. Cells placed under stressful conditions often produce stress proteins that are stimulatory to either protein production or protein release [25,26]. Interest in the possible role of periodic delivery of factors derives from knowledge concerning the effects of pulsatile delivery of hormones. In many cases, the frequency and amplitude of these hormone pulses have major effects on the response of the target cells [27-30]. Whether or not periodic reversal of delivery of medium serves to deliver autocrine and paracrine growth factors in variously effective manners similar to those observed for hormone pulses remains to be seen. If none of these mechanisms were operating in the reactor, then one might expect the antibody titer to remain relatively constant independent of mixing patterns in the reactor (except for the extreme case when the reactor is diffusion-limited, *e.g.* a high captive volume).

As described [15,16], the transtubular bioreactor can be used in different modes and possesses a number of advantages over other perfusion bioreactors. These include: the ability to deliver oxygen directly to the cellular environment without causing deleterious shear forces, the long residence time of medium and the ability to operate the reactor in a perfusion mode at relatively slow flow rates. In this report, the ability to control the cellular environment through control of medium flow was shown to confer additional advantages. This phenomenon has been shown to be important in the *in vitro* development of three-dimensional tissues and to foster cell/cell communication [17,31,32]. In an attempt to optimize production from bioreactors, the general trend has been to develop well-mixed homogeneous systems [33,34]. However, this approach may not be the most desirable, perhaps because it does not take into account the advantages conferred by fostering cell-cell communication. The results presented from the reactor run described here suggest that a diffusion-limited region co-mixed with a well-perfused region may provide a more optimal environment for the production of proteins in a serum-free, low-protein medium.

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