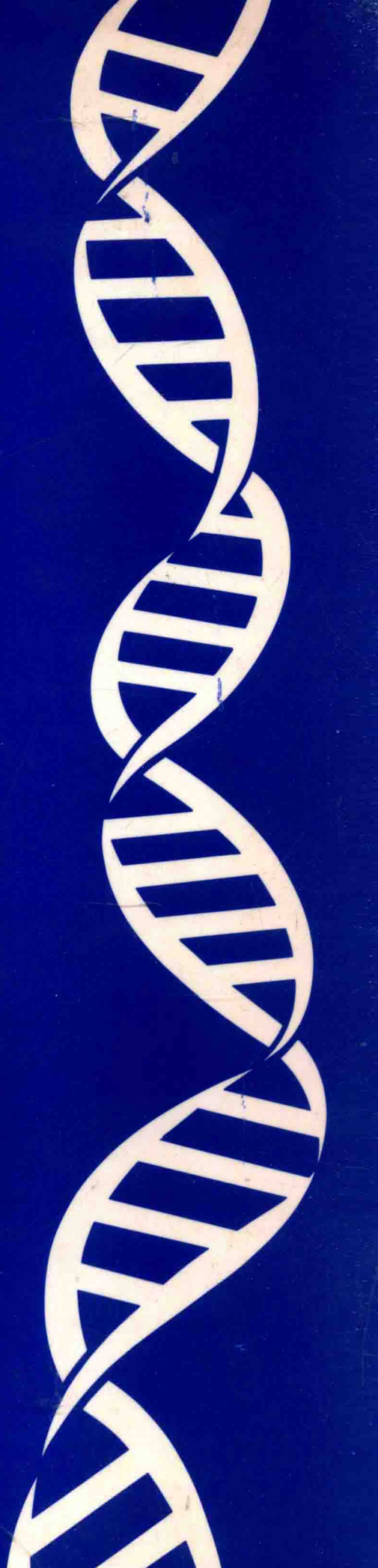
The World Biotech Report 1986

Volume 2: Part 3

BIOPROCESSING



online

Proceedings of the conference held in San Francisco, November 1986

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Introduction

Few new technologies have evoked such a strong and diverse reaction as biotechnology. And fewer still carry with them the burden of so much promise. Since biotechnology emerged as a commercial prospect in the early eighties there has been intense debate among those who see the benefits and those who see the risks of this leading edge technology - one which brings research and commercial application into closer contact than ever before. Yet there can be little doubt that the practical effects of biotechnology are being felt across a widening spectrum of disciplines.

This book focuses on the study of bioprocessing and the application of biotechnology to this field. It contains transcripts of the presentations given during the Bioprocessing Seminar at Biotech San Francisco.

Biotech San Francisco is organized and controlled by Online International Inc. The conference and exhibition continue the highly successful series of events which started with Biotech '83 in London.

Online is one of the world's leading specialists in the design, coordination and management of conferences and exhibitions concerned with the business implications and applications of leading-edge technology. With a schedule spanning some 20 technology areas, many Online events have achieved world forum status. The company was formed in 1971 and now employs more than 100 specialists based in London and New York.

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Considerations in the efficient production of secreted proteins from mammalian cells in large scale culture

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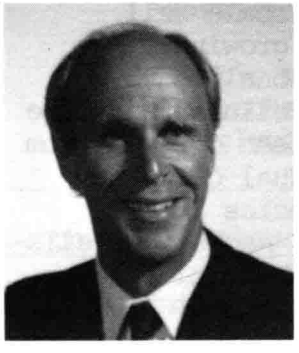
Summary. In order to exploit the wide variety of proteins expressed by mammalian cells, large scale systems must be designed which are both flexible and of sufficient capacity. The Mass Culturing Technique (MCT) is a system that satisfies these requirements.



Biography. Prior to joining Bio-Response as a Senior Scientist in 1983, the author was a postdoctoral fellow in the Department of Biological Sciences at Stanford University in cellular and molecular biology.

Introduction

As little as five years ago interest in the large scale growth of mammalian cells in culture was largely confined to producers of viral vaccines and interferons. With the evolution of hybridoma technology and the explosive growth of commercial concerns focusing on the life sciences, methods and applications of large scale mammalian culture have become of paramount importance in bridging the gap between research and the marketplace for certain key products. These products include but are not restricted to monoclonal antibodies of mouse and human cell origin, plasminogen activators, erythropoietin, Factor VIII, GM-CSF, protein C, and subunit viral vaccines. While it was apparent from the outset that monoclonal antibodies would most likely be produced from mammalian cells, it has become clear within the past few years that using recombinant DNA techniques some large and complex human proteins can only be produced in biologically active form in mammalian cells. The reasons for this stem from the inherent inability of bacteria and yeast, the favored organisms for large scale fermentation, to properly fold and post translationally modify the engineered mammalian proteins.

To those unfamiliar with mammalian cell culture, the plethora of approaches to large scale culture must seem bewildering and unnecessarily complicated when compared to techniques of classical fermentation. The simple observations driving this diversification of techniques are, however, that mammalian cells are much more difficult to grow than microorganisms, are more diverse in growth requirements, and that there exists a paucity of fundamental biology and experience with large scale cell culture in general. The increased difficulty of growing a diversity of mammalian cells is perhaps best rationalized by recalling that microorganisms are primitive, unicellular and free living systems whereas all mammalian cells have been derived from complex, multicellular organisms in which individual cells have developed specialized functions and interdependencies at the expense of autonomous growth. More specifically, mammalian cells in culture require many of the environmental and nutritional states found *in vivo*: constant temperature, oxygenation, approximately neutral pH, complex nutrient melieu, protection from mechanical damage, and isolation from pathogenic microorganism. Duplicating or at least partially substituting for these conditions, therefore, is the challenge of any cell culture system and, as with most scaled up processes, these challenges become more severe in large scale culture systems.

Approaches to Large Scale Mammalian Cell Culture

It would be beyond the scope of this article to exhaustively evaluate the various cell culture systems employed by those in the

field. However, a summary of approaches currently used is included in Table 1. An important point that does emerge, however, is that each mammalian cell bioreactor may have a particular advantage for a given cell type or product. For example, a deep tank system may be well suited for the active growth of cells in suspension such as hybridomas but may not be the system of choice for an anchorage dependent cell line which simply will not grow or secrete its relevant protein under these growth conditions.

Table 1 - Large scale mammalian cell culture	reference
1. Suspension adapted cell lines only	
-deep tank systems: stirred or air-lift	4,5
-encapsulation: agarose	Bellco, Karyon
2. Anchorage-dependent cell lines only	
-microcarriers, solid	Pharmacia, Solohill
polysaccharide, glass, gelatin	Ventrex, KC
-immobilized support matrix	
glass bead, ceramic A	4,3
3. Dual Capability (e.g. 1 and 2)	
-hollow fibers	4
-encapsulation: alginate ± collagen	4, Damon
-immobilized support matrix,	
Ceramic B	4
-parallel flat membranes	Bio-Response, Millipore, MBR
-static maintenance reactor	4
-microcarrier, porous: collagen ±	
glycosaminoglycan	4, F. Cahn

Batch vs. continuous perfusion

In addition to the actual design and routine process control of the bioreactor itself, the operating mode of the system plays an important role in determining the overall advantages of any cell culture system. Specifically,certain systems such as the deep tank systems rely heavily on a batch or fed-batch process whereas other systems such as the hollow fiber bioreactors are ideally suited for a continuous perfusion mode of operation. For biological processes that are by their nature time limited such as virus or interferon production the batch mode is appropriate; for processes that are not time limited such as monoclonal antibody production continuous perfusion may be more desirable from a number of viewpoints.

Experience shows that start up efforts for any bioreactor system far outweigh long term maintenance efforts of a properly established continuous perfusion system. These start-up expenses therefore may be amortized by a continuous as opposed to batch process. Because systems designed for continuous perfusion generally are more modest in size than batch systems of comparable capacity, capital expenditures for continuous systems may be less. Finally, from a process control standpoint, extensive monitoring of operating parameters such as oxygen and pH are not as crucial once continuous perfusion systems have reached their maintenance phase in which most processes achieve virtual equilibrium. As a result, properly characterized and managed continuous perfusion systems may require minimal instrumentation and operator intervention short of uninterrupted media and gassing supplies.

Apart from economics, continuous systems have the advantage that secreted products are removed throughout the production run and residence time within the bioreactor may be minimized. This is particularly important for zymogens such as plasminogen activators (tPA and KPA), Factor VIII, or Protein C which may be prematurely cleaved to their active form by extended exposure to cell culture medium containing released cellular proteases. Finally, certain secretory systems such as endogenous plasminogen activator secretion from human fibrablasts may be subject to negative feedback control which could, in theory, be broken through continuous removal of product as opposed to accumulation in batch culture (1).

The problems associated with continuous perfusion are generally a result of increased complexity of these systems. Since higher cell densities are usually achieved, mass transfer becomes more problematical than in batch systems. Also, from a quality control standpoint meaningful in-process quality control steps should be implemented which monitor consistency of the product of interest throughout a continuous run. Finally, little is known about the nutritional requirements of mammalian cells in continuous perfusion versus static (i.e., batch) culture. For example, glucose in conventional culture medium is routinely established at 2 -- 4.5 grams/L whereas in continuous perfusions, adequate nutrition is achievable at glucostat concentration at least 10-fold lower (data not shown). As a result, initial optimization studies for continuous processes should be done in more complicated perfusion systems rather than more simplistic static culture conditions found in all cell culture laboratories.

The Mass Culturing Technique (MCT) System

The MCT system is a continuous perfusion cell culture system that has the flexibility to successfully support the growth of a wide variety of anchorage dependent and suspension cell lines. A

schematic of the basic MCT system is outlined in figure 1. Incoming medium supplements a loop of conditioned medium which is continuously pumped by specially designed, long wearing peristaltic pumps. Upstream of the bioreactor, gasses (O_2 and CO_2) are exchanged across silicone rubber based membranes in the oxygenator. Nutrient and oxygen-rich medium enters the bioreactor, is metabolized and exits enriched in secreted product and largely depleted of these nutrients. A portion of the effluent stream is removed at a rate equal to the input of fresh medium to the loop. The volume of the circulating loop is kept to an absolute minimum in order to minimize the dilution of input medium. The rate of circulation is varied according to, in part, oxygen demand and bioreactor design. Further downstream an optional dialysis circuit may be employed which continuously exchanges low molecular weight metabolites such as ammonia and lactic acid for additional amino acids and vitamins from fresh medium. Using this circuit may help conserve costly protein supplements and facilitate pH control. The entire system is operated within a 37°C chamber and no extra heat exchangers have been required. Sample ports are placed both before and after the bioreactor and, in the case of hollow fiber bioreactors, in the reactor itself. Through these ports samples are sterilely removed and rapidly analyzed off-line for the variety parameters listed in table 2 and appropriate adjustments effected.

Figure 1. Schematic of the MCT system

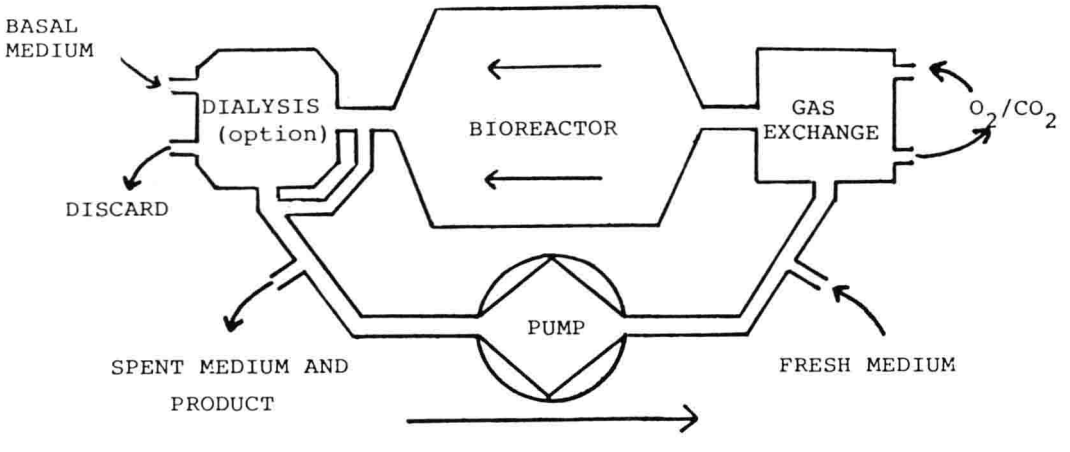


Table 2 MCT Process Control

parameter group	response
1. O_2, CO_2, pH	alter gas composition, circulation rate, medium throughput
2. glucose, ammonia, lactic acid	alter medium throughput
3. lactate dehydrogenase, relevant product, viable cells*	alter medium throughput, remove excess cells*

*hollow fiber bioreactors

Perhaps the most unique aspect of the MCT system and the key to the flexibility of the system is the choice of both bioreactor type and size. For the growth of suspension cell lines hollow fiber bioreactors have proven to be both productive and relatively inexpensive to operate. Alternatively, the packed glass bead bioreactor has worked extremely well for the growth of anchorage dependent cells. With either configuration, high density, long term perfusion culture has been routinely achievable. Specific examples will be discussed below.

The MCT System: hollow fiber bioreactor

We have learned that optimal long term culture of a wide variety of hybridoma cell lines is best achieved using membranes that allow for the greatest permeation of liquids. While ultrafiltration (UF) hollow fiber membranes were utilized in the original work of Knazek (2) we have been more successful with the larger and more open pore distribution of microporous (M.P.) hollow fibers (i.e., 0.2 micron) presumably because these structures have greatly reduced resistance to liquid flow compared to UF membranes. Overall flux rate is vitally important because all mass transfer occurs across these membranes, from the lumen to the extracapillary volume where the cells reside. Conversely, metabolic wastes are removed by flow in the opposite direction. While UF membranes can deliver relatively high concentrations of secreted products selectively excluded from the membranes, (4) this benefit is minimal compared with the total amount of product delivered over time from MP based bioreactors that we have developed.

An example of mouse monoclonal antibody production from one of these hollow fiber based systems is shown in figure 2. Over the 5 months that this system was maintained, approximately 60 grams of antibody was collected at a concentration of about 100

ug/ml. Furthermore, upwards of 400 grams of this antibody in total have been produced to date in additional hollow fiber bioreactors. Since this cell line grew well in specially formulated serum-free medium, initial antibody purity was in excess of 50 percent. This high initial purity and low total protein content (approx. 200 ug/ml) enabled us to concentrate the preparation a minimum of 50-fold using commercially available membrane-based concentrators prior to purification. Periodic viable cell counts revealed cell densities approaching 1 x 10^8 cells/ml within the extracapillary volume of approximately 800 ml. It should also be noted that this was not a particularly prolific hybridoma inasmuch as antibody production in conventional culture was in the order of 20 ug/10^6 cells/24 hours. Finally, the production of consistently high quality product was monitored throughout the production run by comparisons of total immunoglobulin content with functional titer. Thus a kind of specific activity measurement was obtained and compared with that of a standard preparation.

In addition to the use of MP membranes, novel operating strategies contribute to long term cultivation of cells in hollow fiber bioreactors. In particular, cell packing is discouraged through periodic removal of excess cells, physical agitation of the entire module, and secondary flow patterns induced within the extracapillary volume.

Figure 2. Mouse monoclonal antibody production