FERMENTORS AND DOWNSTREAM PROCESSING:

A PRELIMINARY MARKET AND TECHNICAL OPPORTUNITY ANALYSIS

FOR CANADIAN EQUIPMENT MANUFACTURERS

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I. EXECUTIVE SUMMARY

This report is not intended to be an exhaustive study of the fermentation equipment market. It was produced with both time and dollar constraints. As such, the reader will most certainly encounter inaccuracies, anomalies and deficiencies. Specific deficiencies identified to date, include enzyme reactors and lab/scale fermentors/bioreactors.

The Canadian biotechnology industry, as in the rest of the world, is rapidly moving from research to commercialization. As this process gathers momentum, new opportunity potentials are developing for biotechnology equipment manufacturers.

The primary and secondary research conducted for this report indicates that the Canadian market per se, while healthy, is too small to support a significant manufacturing capability. The sales volume of biotechnology equipment in Canada will unlikely exceed several million dollars annually in the next five years.

There will be limited but steady demand for fermentation vessels at lab (50 - 100L) and pilot (200L +) scales. This segment of the industry is characterized by established firms with established technologies. As the industry scales up to commercial production levels, there will be steady, high growth for "production scale" fermentors; however, these will be predominantly for high value products, and systems will be relatively small. Competitive advantage is and will be defined in terms of technological improvement, proven product reliability, cost, and replacement parts service.

Opportunities in the fermentation industry will arise predominantly from technological improvements that offer greater process control, decreased labour or energy inputs, and/or increased throughput. In this regard, opportunities for developers of biosensors and microcomputer-based control systems are forecast.

In the area of downstream processing, the next five years will see a greater use of membrane technology applications. Ultrafiltration appears to offer particularly significant opportunities.

A. Conclusions

Biotechnology is entering a new, highly competitive phase. Canadian Manufacturers of biotechnology equipment are not proactive in assessing their own strengths and weaknesses in the context of international competition.

Canadian companies are likely to find the greatest opportunities for growth in the markets in the United States. While there is a growing demand for

biotechnology equipment in Canada, the market is too small to sustain a manufacturing industry.

Canadian companies must face the multinational goliaths of the industry with a strategy that maximizes maneuverability and builds upon current strengths. It is therefore imperative that equipment development be targeted and sold to very specific market niches that offer the greatest profit potential, least risk and least competitive activity.

Canadian companies are not gathering enough market intelligence to safely guide development and manufacture.It is imperative that Canadian companies who want to compete within the international framework begin to accumulate very specific information regarding target niche markets.

B. Recommendations

1. Canadian manufacturers must focus their competitive strengths on carefully selected market niches that are appearing in the industry. There are potential opportunities in membrane technology, biosensors and computer-based control systems.

2. Manufacturers should develop a closer working relationship with equipment users, particularly at the process development stage. A significant amount of this effort should be focussed on equipment users in the United States.

3. Manufacturers must establish a marketing presence in the United States. This will entail building an effective distribution channel and appropriate promotional programs. The creation of a market intelligence gathering network is also necessary.

4. There is a need for a coherent and detailed industrial strategy for biotechnology as it moves from research into commercialization on a large scale. A continuing dialogue must be nurtured between researchers, producers and marketers that centres on developing internationally competitive biotechnology products and processes.

5. A market driven approach to the business of biotechnology needs to be fostered at the research level, as it is with equipment users and manufacturers. An emphasis on products and processes with high commercial potential should be encouraged by government and research institutions.

It is recommended that firms and or the industry concentrate on identifying potential market niches that offer a strategic fit with their manufacturing, distribution and financial capabilities. Exploiting profitable market segments will require a well-researched assessment of the potential for specific technologies and an accurate analysis of competitor strengths and weaknesses.

Success for the Canadian equipment manufacturer will depend on how well foreign markets and particularly the U.S., are penetrated.

The success of this penetration is highly dependant on a broader understanding of the World Biotechnology market direction as a whole. It is the authors contention that such information is crucial to successful strategic competition within the whole Biotechnology industry in Canada.

Canadian companies are not going to succeed manufacturing low value equipment. High value niche products, designed and produced as a result of accurate market intelligence will offer Canadian companies the most likely route for market penetration, profit and long term product acceptance.

Canadian companies must become more proactive in defining their development directions. They must begin to define markets first, prior to embarking on expensive research and development programs.

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II. INTRODUCTION

This study has been commissioned by the Ministry of State for Science and Technology in conjunction with the Industry Development Office of the National Research Council in an effort to better define the direction and opportunity for biotechnology equipment manufacturers. The industry sector specifically under consideration is the biotechnological fermentation industry and the equipment manufacturers in the areas of:

A. Mammalian Cell Culture
B. Plant Cell Culture
C. Fungal Fermentation
D. Large Scale Fermentation
E. Membrane Systems
F. Downstream Processing Techniques

This report does not include an analysis of enzyme reactors. The reviewers had exhausted both time and money resources.

The market for biotechnology equipment has to be considered as an ancillary market to the production of actual biotechnology products. It is generally understood that biotechnology has not "taken-off" as some had expected. This is due to a variety of factors including the long lag times in bringing products to market; a result of regulatory requirements and the product development process itself.

Consequently the market for process equipment has also lagged. The performance of process equipment suppliers in biotechnology activities has not been as successful as expected.

It is significant that while in the United States there has been a lot of interest in market surveys and analyses for biotechnology products, there has been no great attention paid to process equipment for biotechnology applications.

One important technical problem in the Canadian context of equipment and process development has been the lack of companies who could be described as process integrators, that is, companies whose business mission is specifically to develop technologies, put pieces together and sell technology or process packages. Various companies have stepped into this void on occasion, but there are not companies who do this in a regular basis. The filling of this void is a crucial consideration for the industry. Both the Canadian firms who currently manufacture and market fermentor equipment are drawn from the ranks of Canadian agents to foreign firms. They also have some fabrication capability of their own.

Various engineering companies have developed process technologies, although most have traditionally remained in the

role of service organizations and have not actively pursued the marketing role. The one group of companies conspicuously absent, are users of equipment who have developed their own process technologies or equipment. For this group, the marketing of technology is the notable exception rather than the rule, due primarily to the possibility that selling technology could jeopardize the competitive position of their own production efforts.

One possible solution, is to encourage user-manufacturer collaborations for the development of technology. While an excellent idea in principle, this concept has pitfalls. Such collaborative arrangements can help solve difficult process problems for users, but they are often viewed with suspicion by the users, because they might benefit the manufacturers at the expense of users.

III. SCOPE OF REVIEW

A. Statement of Purpose

As the number of Canadian companies involved in biotechnology grows, it is becoming apparent that there is a developing demand for fermentation and downstream processing equipment. Some Canadian companies are already aware of these needs and have begun to address the growing market opportunities. However, the industry is fragmented with user companies, not always being aware of the equipment and services that could be provided by Canadian manufacturers. In addition, it is evident that Canadian manufacturers are not aware of the users' requirements.

This study was commissioned to provide information that would assist Canadian companies and researchers in developing and marketing products and processes in this expanding area of commercial biotechnology. It is designed to act as an indicator of specific areas of opportunity in fermentation and downstream processing and as a contact source book for both equipment users and equipment manufacturers.

The specific areas identified as potential opportunities for this study are:

1. Production scale mammalian/plant cell cultures
2. Fungal fermentations for high value biochemical and pharmaceutical products
3. Large scale industrial fermentors for ethanol production or other organic chemicals
4. Membrane bioprocessing
5. New techniques or new processes for downstream processing.

B. Research Method

1. Survey Method

The information provided in this document has been gathered from a number of sources (see Appendix B) in the research and development, commercialization and equipment manufacturing segments of the biotechnology industry. Time and financial constraints precluded the polling of the entire industry. As a result, there are likely sizeable projects scheduled for the near future of which we are unaware.

The sample we have interviewed, while selective, offers an opportunity to gauge the size of the market and its direction. In some instances, interviewees have been reluctant to disclose the precise nature of their future process equipment needs. This, in itself, is a significant characteristic of the industry. We have respected the respondents' right to privacy in each instance. In other cases, interviewees have been forthright in discussing their plans and prospects. In general, the individuals we contacted have been most cooperative.

The questionnaire in its skeletal form is included in Appendix C. In practice, we sought to allow the conversations to some extent to follow their own course. Beyond the acquisition of the hard data of specific equipment purchasing plans, we have attempted to determine the wishes, expectations and predictions prevalent in these areas of biotechnology. Consequently, we have received opinions on a wide range of topics. Where we were able to identify common themes, we have commented in the report.

Biotechnology is a global market. It became quickly apparent that success for Canadian equipment manufacturers would be predicated on their ability to compete internationally. However, resources have constrained our investigation of world markets to a preliminary secondary data search.

2. Additional Considerations

In the determination of potential opportunities for manufacturers, a number of basic considerations, aside from individual market sizes, were identified as potentially important in increasing the possibility of a successful manufacturing venture in this sector. These factors are listed as follows:

a. Potential for crossover applications of the technology into other non-biotechnology areas.

The biotechnology market for process equipment in Canada is by itself too small to support manufacturing. The size of this market in Canada is estimated to be only several millions of dollars per year and larger projects, like many biotech products, are high value/low volume with only a few major opportunities available in a five year span. For some types of equipment, even the total world market may not be very large. Biotechnology processes and equipment must therefore be considered more in the context of "generic enabling technologies," that is, technologies with other applications. The more crossover to a variety of

other applications, the higher the probability of commercial viability. For example, the closest parallel activity to bioprocessing is food processing and an exploration of this is most important.

b. Scope for technical improvements in the processes or equipment.

Concentration on rapidly evolving areas rather than areas of matured technologies.

Concentration in areas where there is less technical expertise.

Concentration in areas where there is a competent interdisciplinary scientific research base available, such as NRC or university laboratories, from which to base new improvements or processes.

IV. TECHNICAL REVIEW

A. Mammalian Cell Culture

In recent years, the majority of attention in biotechnology has been focused on microbial fermentation systems and in particular on the use of recombinant DNA modified bacterial systems to produce desired chemicals and proteins. Increasing attention is now focusing on the culturing of isolated cells from higher plants and animals in a controlled environment. The greatest activity has been in the culturing of mammalian cells, however, many of the techniques and approaches employed have application to other mammalian and even plant cells. At present, the major production uses of mammalian cell cultures are for viral vaccines and monoclonal antibodies, using hybridoma cells, for use as diagnostics, purifying and therapeutic agents. Increasingly however, mammalian cells are being viewed as a viable alternative for the direct production of many complex biological compounds in the category of high value therapeutic agents that are of mammalian and particularly human origin. By far, the greatest attention is on human blood products and especially the group of enzymes called Plasminogen Activators (PA) used in treatment of blood clots in heart attack victims. Other prominent products are the hormone Erythropoietin (EPO) for stimulation of blood cell growth and Factor VIII for blood clotting. A more complete summary of the kinds of products that can be produced from mammalian cell culture is provided in Mizrahi (1986).

While bacteria and other microbes in general are robust, culture easily, reproduce and grow rapidly, and have fairly predictable behaviour, they are limited in the kind, size and complexity of proteins they can produce as surrogates through recombinant DNA techniques. The constraints which apply to bacteria and to a certain extent to lower eucaryotes such as yeast and fungi, are recognized as follows:

(1) Limited genetic machinery available.
(2) Lack of or inappropriate glycolysation (sugar attachment).
(3) Lack of or inappropriate three dimensional protein configuring (folding and bridging).
(4) Lack of product secretion.
(5) Presence of pyrogenic impurities.

While mammalian cells are capable of producing and excreting even the most complex biological chemical in a fully active form, they are extremely difficult to grow in culture. Important characteristics of mammalian cells that impact on bioreactor design can be listed as follows:

(1) Delicate and susceptible to damage.

(2) Grow and reproduce very slowly.
(3) Highly specialized nature with diverse behaviour and needs.

The diversity of mammalian cells is an especially important consideration affecting bioreactor design. Specialization, which is essential in the functioning of a total organism, causes problems in cell culture. A complex and carefully balanced environment of hormones, gases, nutrients, wastes and growth additives must be maintained, that can be highly specific and even detrimental to other cell lines. One major distinction can be made in the way cells grow, that effects reactor design as follows:

(1) Some mammalian cell lines tend to grow better in free suspended culture and are termed Suspension, Anchorage Independent or Non-adherent cells.

(2) The majority of mammalian cell lines, grow better attached to a solid surface and are termed Anchorage Dependent or Adherent cells.

Due to mammalian cell diversity and specialization, bioreactor development has been fairly empirical according to the specific needs of the cells and a wide assortment of configurations and approaches have been employed ranging from simpler tanks to elaborate circulatory mimicking systems. Currently, there is no single optimum solution for growing and producing products from mammalian cells.

Another important aspect of mammalian cell culture, compared to other conventional fermentations, is the small production volumes of the complex and expensive products involved. In the production of monoclonal antibodies for instance, gram quantity lots are considered production scale and very large production operations produce only several kilograms per year. The corresponding size of bioreactors is therefore smaller than those normally associated with alternate fermentation processes.

1. Available Commercial Technologies

The different types of systems available were grouped into the following general categories beginning with simpler approaches and moving toward those that are more complex. Because of the wide array of alternatives, which can be quite unique, any such systematic categorization of mammalian cell systems is unfortunately somewhat complicated and arbitrary.

a. In-Vivo Production

In-vivo techniques, such as the use of mice and other animals for monoclonal antibodies, are still major and important production technologies. While such methods are beyond the scope of consideration here, they must definitely be considered as possible alternatives to process equipment systems.

b. Batch Tank Suspension Culture

Suspension type cells which prefer to grow in free submerged culture, are suitable for use in modified versions of standard tank fermentors. The emphasis in such systems has been the modification of the aeration/agitation modules to reduce turbulence and shearing. Two main configurations are employed, agitated and airlift types. The reduction and/or elimination of internal protrusions such as coils and baffles which cause shear damage,is another major modification.

In agitated systems, which include simple spinner flasks, the flat beaded turbine impellers associated with microbial systems are replaced by low shear mixer devices. Most common are marine impellers, rotating flexible sheets or sails and vibratory mixers. There is also a wide variety of more specialized mixing devices such as gas-exchange impellers and floating impellers, designed to remain at the air/liquid interface. The cell bioreactor development pursued by Pegasus Industrial Specialties of Toronto, in collaboration with NRC, involved a specialized mixing device of this type.

Impeller rotational speeds are kept low in these mammalian cell systems. In smaller versions, there is often provision for controlled acceleration on start-up and magnetic couplings are usually employed. In larger systems, impellers are either top or bottom driven with double mechanical seals on the drive shaft to ensure containment.

The oxygen requirements of mammalian cells are much lower than microbial systems. Good oxygen transfer is still extremely important. As a result, the use of vigorous direct sparging for aeration in vessels is either replaced by: fine direct bubbling, sparging behind fine mesh protective screens to prevent direct contact, or eliminated by the use of surface diffusion or supply through membrane diffusers.

Agitated tank bioreactor systems, specially modified to accommodate mammalian cells are available from most major fermentor suppliers.

In airlift systems, a gentle infusion of gas bubbles is employed to provide both aeration and agitation. This eliminates the need for mechanical seals. In all other aspects this system is similar to standard tank systems. The British firm, Celltech, has been prominent in the development of large airlift technology (ca 1000L) for mammalian cell growth. It is licensed under the trade name Cytair. Airlifts suitable for mammalian cells are also available from many other major fermentor suppliers.

The major advantages of agitated and airlift tank systems, when they can be employed, are their proven reliability, operation simplicity and scaleability. Cell concentrations in submerged cultures tend to be low, no more than about 2x10e6/mL and correspondingly, larger sized vessels are required to achieve desired product yields.

While tank type systems can be considered by themselves for simple suspension cultures, they can also be used in association with immobilization techniques, such as microcarriers and continuous operation techniques to expand their scope of application. These aspects are discussed in subsequent sections.

c. Anchorage Dependent Culture

A wide variety of techniques have been employed in order to cultivate Anchorage Dependent cells. All share the characteristic of providing an appropriate surface on which cells can attach, grow and produce desired products.

Among the simplest techniques, are roller bottles (and modified roller bottles). They are still used today in large quantities for production runs. Cells anchor to the rotating bottle surface from which they are bathed in nutrient and exposed to air for oxygenation. While such bottles are simple, several disadvantages exist. They have a small capacity relative to their size, a relatively low cell concentration (<10e6/mL) and the method tends to be labour intensive.

The use of immobilization techniques, especially microcarriers and microporous matrices, is a common means of providing attachment sites for Anchorage

dependent cells. Since immobilization techniques can be applied to both anchorage and non-anchorage dependent cells, such methods are described together in the following section.

d. Immobilization

Immobilization techniques are attractive for the growth of animal cells, especially mammalian. The main benefit of immobilization is the increased cell density that can be obtained. This translates to higher productivity which in turn means that smaller bioreactor volumes are required. Immobilization also allows for an easier separation of media from cells, which is more important in continuous systems. The methods of immobilization fall under two categories, those which provide only surface attachment, including microcarriers, porous matrices and solid surface matrices; and those which provide some measure of isolation of the cells from their physical environment, such as gel entrapment, encapsulation, and membranes. Individual methods are discussed as follows:

(1) Microcarriers

A widespread technique to provide attachment sites for Anchorage Dependent cells has been the use of microcarriers. Microcarriers are small bead particles of up to several hundred microns in diameter made out of various materials including polysaccharides such as dextran, gelatin, or collagen; synthetic polymers such as polystyrene or polyacrylamide and inorganics such as diatomaceous earth, ceramics or glass. Initially, microcarriers were solid in nature and only allowed attachment and growth on the exterior surface. This lead to a trade off in size since increasingly smaller beads provided more surface area, while good cell growth demanded minimum cell numbers per bead. The trend, however, has been towards the development of porous microcarriers with large internal voids, which allow for interior growth of cells. Such beads create a pseudo-entrapment, as cells on the interior are provided with some measure of protection from the external environment.

Microcarriers allow for the growth of Anchorage Dependent cells in most standard agitated tank bioreactor systems available, as discussed

previously and depending on the density of the carriers, they can also be employed in fluidized bed type systems.

(2) Entrapment

A method of immobilization generally applied to suspension type cells but also applicable to anchorage types is entrapment, wherein cells are inoculated and allowed to grow within a protective matrix. Such techniques include gel entrapment within beads of polysaccharide, such as alginate or agarose and encapsulation. Entrapped cells can, as in the case of microcarriers, be used in agitated tank and fluidized bed systems.

The most prominent method of encapsulation is the Encapsel process developed by Damon Biotech. This process involves the staged entrapment of inoculum cells and the formation of a spherical polyaminoacid microcapsule membrane. Encapsulated cells are grown in simple 40L vessels which, because cells are protected by the capsules, can be aerated by conventional sparging. While nutrient media is continuously added over the culturing period of several weeks, the process is effectively batch since the permeability of the capsule membranes is adjusted in order to retain and accumulate the desired protein product. Cell concentrations within the capsules ultimately reach high values (ca 10e8/mL of capsules) as do product concentrations. Perhaps the most unique advantage of this system is the effective preconcentration of protein afforded by capsule membranes. These concentrations are such that very high crude protein product purities can be achieved (45% to 75%), thereby reducing downstream processing requirements.

(3) Solid/Porous Matrices

Solid and porous matrix materials can serve as sites for the attachment of anchorage dependent cells. Since these systems are generally used in a continuous fashion they are discussed in more detail in a subsequent section.

(4) Membranes

Asymmetric semipermeable membranes can act as sites for cell attachment and retention, both within the porous structure of the membrane or

more frequently, on one side of the membrane. The pore structure of the membrane must be such that it is semipermeable, (cells cannot pass through but nutrients, wastes and end products can).

Membrane systems lend themselves to more continuous operations. Specific membrane systems are discussed in the following section.

e. Continuous Systems

Because of the inherent traits of long reproductive times and product secretion, the design of cell-culture systems has tended toward increasing the cell densities and more continuous production operations with long term production runs.

Long reproductive times mean that cells can be maintained in culture for extended time periods. Product secretion means the necessity of batch harvesting of cells and the subsequent re-seeding is eliminated. While the methods of cell retention vary, so-called perfusion systems share the following traits. Nutrients and gases are continually added, while wastes and products are continuously removed.

Continuous operation has been observed to have other specific advantages. Higher specific productivities have been observed and such systems have been found to require much less serum in the nutrient media once high cell densities have been achieved, further reducing downstream processing requirements.

(1) Perfusion Vessel Reactors

Continuous perfusion has been conducted in vessels with both suspension and anchorage type cells. These are virtually the same, but for the addition of microcarriers. Simpler tank bioreactor systems are often employed for more continuous perfusion operations with the addition of external media, waste and/or recirculation reservoir vessels.

A more sophisticated perfusion system has been developed by Invitron. This perfusion chemostat system consists of a main growth vessel to which fresh media is supplied at a constant rate. A satellite filter vessel connected to the main growth vessel, allows for removal of used media and products while returning cells to the culture vessel. Once the cell density in the growth vessel

has reached an optimum level, unseparated culture media and cells are continuously removed to a third harvest vessel. Cell densities of 10 to 30 times those in conventional bioreactors are claimed. Four size ranges have been developed ranging from 4 to 200L, as well as a version adapted for the use of microcarriers.

(2) Fixed Matrix Bed

A variety of matrix materials have been used to make packed beds which can be operated in a continuous manner. Bio-Response, a major US firm, has developed a bed of immobilized glass beads to provide a gentle growth environment for the production of hormones from cell cultures. Agitation arises only from fluid percolation. Hoffeman-LaRoche has developed a stainless steel coil matrix to pack an air agitated fermentor which could produce a continuous stream of desired protein product for over one year. Available surface area of about 4 m2 was obtained in a 3L reactor.

A surface attachment porous ceramic matrix serves as the central feature in the continuous Opticell system from Charles River Biotechnical Services. The ceramic core contains a large number of parallel channels through which media is continually perfused. While this system has a similar appearance to a hollow fiber membrane cartridge, cells are instead immobilized on surfaces in direct contact with the media flow. Anchorage cells attach directly to smooth ceramic surfaces, while a rougher ceramic version is employed for suspension cells so that they can become trapped in small convolutions. The ceramic cores have a high surface to volume ratio and are available in sizes from .45 m2 to 12 m2. Cell densities are high with values for some cell lines claimed to be as high as 3x10e9/mL.

A matrix core is also a central feature in the Invitron Static Maintenance Reactor, which is discussed in a subsequent section.

(3) Fluidized Beds

Another continuous production approach, possible with the use of cells immobilized on carriers and in beds, is the fluidized bed. Two approaches to agitation have been used. The Verax fluidized bed

system employs two phases (solid and media) with only the flow of nutrient media used to achieve any agitation. In this case, aeration of the media is performed externally in a membrane gas exchanger. This system is available in 1.6 and 20L sizes and claims high cell densities are achieved using a collagen based macroporous mirocarrier.

The second approach, is three phase fluidization (solid, liquid and gas) where the gas flow is used primarily to agitate the system. This is the approach used in the Hoffmann-LaRoche/Bellco fluidized bed reactor, which features a taper bottom design with a 3L volume and gentle air agitation at rates of less than 50 mL/min. High cell densities approaching 10e8/mL are claimed.

Development of a three phase fluidized bed bioreactor system for mammalian cells is presently being pursued by ChemBioMed of Edmonton in concert with researchers from the University of Calgary.

(4) Membrane Systems

Membranes are excellent immobilization matrices applicable to both anchorage dependent and suspension cell devices. They have been used in a variety of configurations, especially hollow fibers. Such systems have been employed to mimic the circulatory system with media flowing down the centre of the lumen fiber. Usually the membrane is an ultrafiltration membrane. Varying molecular weights of membranes can be employed so that large products can either be retained or allowed to pass through. Two sets of membranes are also employed where one set allows nutrient and waste to pass, while the other is more open and allows product to pass.

The Amicon Vitafiber system was one of the first such systems developed. It employs a bundle of hollow fiber membranes of polysulfone or acrylic copolymer in a disposable cartridge with cells occupying the extracapillary space between lumen fibers. Cartridge units of 2.5, 25 and 250 mL extracapillary chamber volumes are available with corresponding available surface areas of 60, 1000 and 100,000 cm^2. Concentration levels near 10e8/mL are achieved. The hollow fibers are Asymmetric ultrafiltration membranes with the skin layer on the inside lumen surface. Single units or several cartridges in parallel can be used, with nutrient

media recirculated from an external reservoir through the interior of the lumen, perfusing through the membrane to the cells. Media composition and pH readjustment are all done in the external reservoir. Oxygen transfer to the media is also accomplished externally through surface adsorption. The hollow fiber membranes are available in 10,000, 50,000 and 100,000 nominal molecular weight cut offs and the unit is most effectively employed when products are retained in the extracapillary space. By periodically opening a permeate port to allow flow out, product uncontaminated by media serum is obtained.

Membrane perfusion systems are available from other companies, including the Dynacell system form Millipore, the Membroferm system from Sulzer and the Tricentric polypropylene fiber within fiber system from Separation Equipment Technologies.

More elaborate production systems have been developed based on membranes by Endotronics and Bioresponce, two firms also involved as contract product manufacturers.

The Endotronics' Acusyst system employs hollow fibers to mimic the circulatory system. This system usually is comprised of several bioreactors in tandem, each consisting of looped hollow fibers. Cells grow on the outside of the fibers. They secrete product internally through pores on the fibre from which they are collected.

Careful computer control is incorporated to monitor pressure differences between hollow fibers and an external chamber connected to the extracapillary space and to prevent the formation of lethal stagnant regions. Cell densities reach high values of 10e8/mL and several reactors in tandem can be employed in order to increase protein production.

Bio-Response has developed a series of bioreactors called Mass Culturing Technique (MCT) which consist of cells sandwiched between two semipermeable membranes, one sufficiently open to allow products through and the other closed enough to allow only nutrients and wastes to pass. This system also mimics the circulatory system with very high cell density claims of 10e9/mL. With provision for continuous flow of nutrients wastes

and products, this reactor system can function for virtually unlimited periods of time and cells can be maintained in a steady state by altering the composition of the nutrients.

f. Non-Growth Systems

The growth of a cell culture is not necessarily desirable for the production of certain products, with an inverse relationship often existing between growth and cell specific product formation and secretion. Thus certain bioreactors are being developed specifically to maintain cells in a steady non-proliferative state. The best example is the much publicized Invitron SMR. The SMR or Static Maintenance Reactor was designed to maintain cells, grown in a separate perfusion system, at very dense concentrations (ca 10e8/mL) for extended periods of time, minimizing division and growth.

This reactor consists of a semirigid matrix with two sets of tubing running through it. One set is porous to allow constant perfusion of nutrients and waste removal, while the other is gas permeable to deliver gases. The reactor is claimed to have extremely high productivity.

While the SMR is the only reactor specifically designed to operate under non-growth conditions, other reactor systems such as the Bioresponse can be made to run in a similar manner, making it more conducive to high protein output.

2. Future Developments

Despite the diversity of systems, three technical trends are apparent in the culturing of animal and particularly, mammalian cells.

Firstly, is the movement toward increasing cell densities, with the motivation to increase yields and reduce capital, labour and space requirements. Particularly important in this respect, is the development of improved immobilization and microcarrier materials and technologies.

Secondly, is the movement toward continuous operation and particularly toward non or low growth protein production, again with the motivation of increasing product yields.

Finally, there is a trend toward complete automation of fermentation processes. Mammalian cell systems require the maintenance of a precise and delicately balanced environment

where there is a high degree of interrelation between control of pH, aeration and nutrients. These requirements coupled with production movements to higher cell densities and continuous operations, are putting severe strains on the process controls. Automation is becoming a necessity.

B. Plant Cell Culture

Higher plants represent a potential source of a wide variety of valuable chemical products, including pesticides, pharmaceuticals, food and cosmetic colours, flavours and fragrances. The production of such naturally occurring substances by the use of plant cell culture, which is termed phytoproduction, offers new alternatives to many existing production methods by chemical synthesis or extraction from whole plant material, as well as providing an opportunity to develop completely novel substances.

The use of plant cell cultures, however, lags far behind the explosion of activity associated with animal and particularly mammalian cell culture. This may be due in part to a lack of the same kind of strong market pull generated by very high value products, such as monoclonal antibodies or plasminogen activator enzymes, that are essentially unavailable by other means, but also because of the well developed and entrenched nature of competing conventional production technologies. There are also unattractive long batch cycle times and low product concentrations associated with plant cultures, as well as a general lack of understanding of plant systems in culture, especially with regard to nutrition. Thus, while plant tissue culture has been recognized as having potential, it is still expensive and uncertain.

The first and still the only chemical product to be made via plant tissue culture on a large scale is shikonin, the red dye and anti-inflammatory produced by Mitsui Petrochemicals primarily for use in cosmetics. While this product is fairly valuable (ca $4500/kg), an important motivating factor in its production via cell culture was the rarity of the plant that it was normally extracted from. While there has also been some other smaller activities in Japan and Germany, the commercialization of plant tissue production systems has been very slow and there are at present, only a handful of products which could be derived economically via plant tissue culture.

Like mammalian cells, plant cells are very fragile and sensitive to shear damage. In suspension culture they must be gently agitated. Their metabolic processes are much slower than microbes and so correspondingly, their requirements for oxygen are much lower. A notable difference with mammalian cells is that plant

cells generally tend to accumulate products within vacuoles and must be induced to secrete or leak products. Another unique requirement for some plant cell cultures is the need for a light source, with luminous intensity becoming a limiting factor, in some instances. The biochemistry of plants cells in culture is poorly understood, so that media development is highly empirical.

Product formation rates in plant cultures are low. While this can be improved by selection techniques, its combination with slow growth rates makes plant cell cultures susceptible to replacement by other microbial producers.

1. Available Commercial Technologies

Plant cells can be grown in suspension cultures with modifications to the process operation parameters, specifically the reduction of agitation and aeration. The production of shikonin is conducted in fairly standard large batch reactors with conventional mixers for agitation.

A less detrimental method of suspension culture is the use of airlifts, however, there are problems in mixing if viscosities become too high and with potential overaeration.

Immobilization techniques have been applied to plant cultures, including entrapment in hollow fibers membranes and gels and on inert surfaces. Immobilization is advantageous since it provides contact between cells which can be important in growth.

There is, at present, a lack of developed technologies specifically for plant cultures, however, given the similarities, much of the technology presently developed for mammalian cultures may be transferable wholesale to the application of plant tissue culture.

2. Future Developments

As in the case of mammalian cell cultures, the long growth and reproduction times associated with plant tissue culture, as well as the inverse relation between growth and production of certain secondary metabolites, make continuous operating systems an attractive objective. By accomplishing this through immobilization a number of advantages, such as cell to cell contact and enhanced productivity due to cell densities, are achieved. As suggested in Sahai and Knuth (1985), the trend toward immobilization and continuous operation, coupled with the inducement of product leakage could potentially enable production of plant derived products at much lower costs.

C. Microbial Fermentation

The major workhorses of production by fermentative means are microbes, including bacteria, yeast and fungi. While microbial fermentation per say is an ancient technology with roots extending far back into prehistory, sophisticated fermentors are manufactured for more modern biotechnology applications, especially for the use of recombinant DNA organisms. It is important to consider the technology available for microbial fermentation, first, because of its dominance and secondly, as there is substantial overlap into other areas.

1. Available Commercial Technology

For biotechnical applications employing microbes, the dominant technology is the use of batch stirred tank fermentation vessels.

The batch mode of operation is dominant for microbial fermentations. Despite the conceptual productivity gains associated with continuous operation, it remains, even for large industrial applications, very much the exception. While the maintenance of asepsis for long periods of time is often mentioned as a problem, the major concern with continuous processing has been the potential adverse mutation of the organism. The use of feed-batch systems for increasing productivity has gained more attention.

The configuration of microbial fermentors has not altered radically in recent years, with the exception of control and instrumentation. The basic features of fermentors can be summarized as follows:

a. Containment Vessel - Usually of stainless steel (and/or glass in laboratory scale versions for visualization), however, other material can be used.

b. Agitator - Usually flat blade turbine impeller, often magnetically coupled in small versions and direct top or bottom drive with double mechanical seals in larger units. A variety of more sophisticated mixing devices including airlifts are also available.

c. Aeration Mechanism - Usually a sparger. Sterilization filters on inlet and exhaust gases are also of importance.

d. Heating and Cooling Systems - External jacketing, internal coils, or heat exchangers.

e. Sterilization Capability - Either through direct heating or steam injection.

f. Inoculation and Sampling Ports

g. Foam Control (usually optional)

h. Monitoring Probes

i. Control System

The worldwide market for standard microbial fermentors, which are sold as prepackaged, instrumented systems and rarely as simple components, is dominated by Swiss manufacturers who include Chemap (part of the Alfa-Laval group), Bioengineering and MBR (part of the Sulzer group). There are other less dominant international supply companies including New Brunswick Scientific (US), LH Engineering (UK), Biolafitte (Fr) and Braun (FRG). A brief listing of most of the available manufacturers' is provided in Van Brunt (1987). All the major international suppliers have established technical performance reputations and perhaps more importantly, have well established worldwide marketing networks, including Canada, to sell their products.

Fungal fermentations were included in this section along with other microbes because these organisms can be cultured in virtually the same systems. Fungi are well established in antibiotic manufacturing and have been utilized as enzyme sources, as well as surrogates for recombinant DNA production techniques. There are certain unique problems associated with these organisms, such as the formation of mycelial mats, high viscosities, aeration differences and high fragility. Such problems can be overcome in conventional fermentor systems. The biggest problem for fungi is not the production of fermentors. It is a lack of basic understanding of the physiology associated with these organisms within fermentors.

The biggest improvements, particularly in newer fermentor models have been in controls, especially in the computer supervision of control loops and data logging. The trend toward increased process automation is not unique, but is important to all types of fermentation and cell culturing.

The major limitation in fungal fermentor control is in the area of on-line sensoring, where only a relatively few parameters can be measured using in-situ on-line probes. Those available currently include pH, temperature, dissolved oxygen, dissolved carbon dioxide and to a certain extent cell concentration (i.e. via turbidity or fluorescence). The biggest constraint affecting the development of probes

appears to be the ability to maintain a sterile instrument.

2. Future Developments

The more widespread application of computer automation systems will in the near term have a great impact in improving microbial fermentor productivity, probably more than can be achieved by the wholesale development of new process concepts and/or configurations. While most fermentor manufacturers have developed unique automation systems, there has been a fairly high turn over of systems, even those applied by individual manufacturers. Most are tending toward the use of smaller and more powerful microcomputers (i.e. PC-AT equivalents).

Another major priority appears to be in the development of new and improved probes and sensors. These include, but are not limited to, the so called "biosensor". Most effort seems to have been directed toward the monitoring of substrates, and sterilizable probes have been developed for glucose. However, in order to monitor and more effectively control the progress of complex biological processes involved in fermentation, especially in the production of secondary metabolites, it is desirable to have direct on-line measurements of many additional parameters including the concentrations of desired end products, intermediates and important inhibitors or contaminants.

D. Large Scale Industrial Fermentation (low-value, high volume)

Fermentation has been long recognized as a possible means to produce a wide array of organic chemicals including ethanol, butanol, acetone, isopropanol, glycerol and acetic acid. Of these major use bulk chemicals, only ethanol is produced in any significant quantities via fermentation. Industrial ethanol has a wide range of uses as a solvent, disinfectant, feedstock precursor, component in pharmaceutical preparations and most importantly, from a potential volume standpoint as a motor fuel component. Although initially, fermentation ethanol was touted more as a renewable fuel extender, a recognition of its superior octane properties has emerged, such that its major role is now viewed as a potential octane enhancer, especially in the replacement of lead additives.

The economic viability of producing low-value bulk chemicals such as ethanol via fermentation, depends primarily on the cost of substrate raw material and the price of competing commodities made from other feedstocks. Even though the prices of grains and other potential substrates have, in general, been depressed in

recent years, markets for chemicals from fermentation, have been stagnant because of low oil prices.

1. Available Commercial Technology

Large scale ethanol production systems can be divided into three sections, namely preparation, fermentation and product recovery. It should be noted that there is substantial overlap in the systems.

A large component of a fermentation process is substrate preparation. In the case of ethanol, a wide variety of low cost substrates have or could potentially be employed. These can be divided into three categories of increasingly complex pretreatments, namely simple sugars, such as molasses; starches, such as grains or corn; and lignocellulosics. Starches and cellulosics require saccharification prior to use. On a world wide basis, most ethanol is derived from sugar cane, while in North America corn is the major substrate.

In terms of the fermentation component itself, by far the vast majority of production is via conventional batch fermentation processing. While other organisms have been proposed, the yeast Saccharomyces cerevisiae is almost always used for the fermentative conversion process.

Although a wide array of more advanced process concepts have been developed, relatively few have been employed on a large scale. Most notable, is the elegant Biostil process developed by Alfa-Laval. The key areas of development for improving productivity have been as follows:

a. Continuous Processing - to reduce batch down time.
b. Cell Density - to increase production rates.
c. Product Removal - to decrease product poisoning of the yeast.

While continuous cascade systems have been used on a large scale, the use of cell retention or recycle methods is a more attractive means of achieving a continuous operation. Cell recycle by use of membranes or centrifuges, or cell retention by surface or entrapment immobilization provide the opportunity to retain a very high cell population density with correspondingly much higher rates of conversion within the fermentor. Although a 20,000L column reactor of alginate immobilized yeast has successfully been operated by Kyowa Hakko Kogyo Co., such immobilization technologies are still relatively expensive and tedious at production scale, as well as imposing severe limitations on the tolerance of particles in the feed in order to avoid plugging.

Ethanol is a classic inhibitory product and various schemes have been proposed for simultaneous removal of this product during fermentation such that conversion rates can remain at higher levels. These include the following:

Vacuum Stripping - less practical from energy considerations.

Gas Stripping

Selective R/O Membranes - pass product but retain substrate.

Solvent Extraction - forms two liquid phases in fermentor.

Of these, the latter, which exploits chemical interaction differences, offers the best potential for full scale application.

The Biostil process is the most successful advanced ethanol production system, with nine full scale plants now operating worldwide using sugar substrates. A small production scale pilot plant was also built and run using grain substrates. Key features of this process, include continuous operation with a flow portion continually removed from the fermentor and return after ethanol stripping by conventional distillation; high cell densities maintained by cell recycle with centrifuges; and an ability to handle very concentrated substrates. In addition to the productivity advantages of continuous, high density fermentation, the process also offered enhanced energy efficiency, because less water had to be added. The process is a non-aseptic operation, relying instead on a dense, vigorous population to out-compete contaminants. This is particularly well suited to operations in tropical regions.

The recovery of product ethanol is an area of intense development given the large energy requirements associated with conventional distillation methods. Vapour recompression has been employed as a means to boost the efficiency of distillation methods. Amongst other technologies considered, are the use of sorbants and selective molecular sieves, solvent extraction, supercritical fluid extraction and membrane processes including R/O, UF, Pervaporation and Vapour Separation.

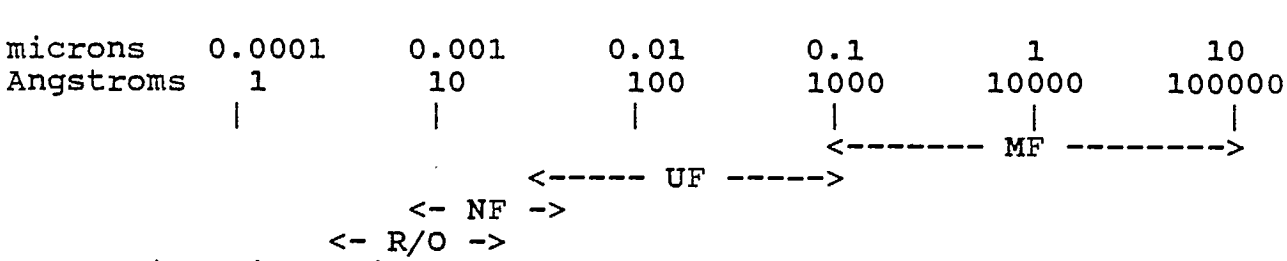
2. Future Developments

Future developments for this technology are considered nominal for Canadian Equipment Manufacturers. Discussions

regarding this topic are carried out in Section V: Market Analyses.

E. Membrane Systems

Membrane filtration describes generically the separation by selective permeation of molecules (solute) through a membrane material. While membrane separations are often characterized as sieving processes relying only on the relative size of solutes and "pores" in the membrane, membrane-solute chemical interactions are very important. Different names have been applied to separations as the membrane "pore" size is reduced approximately as follows:



MF - Microfiltration
UF - Ultrafiltration
NF - Nanofiltrataion
R/O - Reverse Osmosis, sometimes referred to as Hyper Filtration (HF) where virtually only the solvent (i.e. water) passes through the membrane

1. Available Commercial Technology

Membranes are commercially available in all the size ranges shown above. Most available membranes are made of organic polymers although inorganic ceramic membranes are available in MF and upper range UF applications.

In MF applications membranes may be asymmetric or uniform in nature. They are used in either a tangential (across surface) or depth (through membrane) flow configuration. The UF and smaller membranes are made in an asymmetric nature, with very thin active surface layers to perform the separation and a much more porous substructure. This allows for the maintenance of high separation and also high flux. Polymeric UF and R/O membranes are usually made of cellulosic derivatives, polysulfones and related polymers, or composites, called TFC's (or Thin Film Composites), with added selective surface layers. UF and smaller pore membranes are almost always run with a tangential flow configuration across the membrane surface. Available tangential flow membrane configurations are: Flat, Spiral, Tubular and Hollow Fiber. A fairly comprehensive listing of membrane manufacturers is provided in Cheryan (1986).

In reverse osmosis (R/O), extremely small pore sizes are employed such that only the solvent (i.e. water) passes through the membrane. In order to overcome the osmotic pressures involved, high pressures must be used. The application of R/O is most important for water purification and in this use, membranes are a well established technology. This application is important to biotechnology processes but more as a utility. R/O membranes have also been applied in bioprocessing streams for dewatering. Most R/O systems are flat plates or spirals, with the spirals being much cheaper, but less tolerant of particulates.

Microfiltration has been applied in whole cell and large particle separations and for fluid sterilization. Tangential flow systems tend to be used for product separations, such as whole cell separations, while depth flow configurations are usually employed for sterile filtration.

Ultrafiltration has also been applied to whole cell separations and extensively for pyrogen removal from water streams, but is of particular interest in bioprocessing applications requiring the separation of macromolecules. Because of the small pore sizes involved, ultrafiltration membranes are usually rated by molecular weight cut-off (MWCO) and are available in the listed ranges of from about 1000 to 500,000 Daltons. Ultrafiltration systems are usually run in batch with the recirculation of retained flow back to a feed vessel. The term diafiltration is applied when solvent water is added to the recycled feed in order to progressively dilute and wash out impurities. Ultrafiltration systems can also be run in a continuous manner, either with a single stage or with sequential multiple stages. The dominant company in MF or UF downstream processing membrane applications in Canada is Millipore, the majority of systems being small sized flat plate units.

The potential of fractionating proteins and other macromolecules by ultrafiltration has remained elusive in all but a few practical applications. In order to reliably separate molecules, there must be an order of magnitude difference in size. Presently in Canada, UF membranes are used more for crude, but often critical, separations, such as cell harvesting, broth clarification, product dewatering, and concentration. In a few applications, they have been used successfully to separate widely different sized proteins, however, the method of choice in fractionating macromolecules remains chromatography.

The major problems with membranes include: the decline of permeate flux, due to the build up of non-permeating species against the membrane, termed concentration polarization, as well as irreversible fouling. A second problem, is the lack

of reproducibility of permeate selectivity from membrane to membrane and particularly from manufacturer to manufacturer. Unfortunately, membrane manufacturing is still more art than science with membrane performance at best a statistical function of the pore size distribution. While membranes are marketed on the basis of specified nominal MWCO's, such values are definitely not hard and fast. Actual performance is extremely application dependent and this situation is vividly illustrated by a case study presented in McGregor (1986). In general, hollow fiber membranes have less reliable molecular weight cutoff values than flat plate membranes. Hollow fibers bound into cartridges, however, are more robust and unlike the usual case for flat plate membranes, can be easily repaired by blocking damaged fibers.

The uncertainty of actual membrane performance is enhanced by a lack of good communication between users and manufacturers. Users tend to be confronted by the confounding situation of having to test a wide variety of available modules by trial and error until a satisfactory solution is found. Similarly, manufacturers are usually supplied minimal information about the desired products to be separated from a complex fermentation-derived soup, usually because of their expensive and proprietary nature and are asked to supply a solution to a problem they do not fully understand.

An important aspect of a membrane module, is the design of the peripheral flow and control systems, which can account for as much as 80% of the cost of a total system. The most important component of a membrane system from a cost point of view, is usually the pump. While a variety of pumps are employed in small scale designs, in larger scale systems only two tend to be used, namely centrifugal and rotary lobe positive displacement pumps. Centrifugal pumps are least expensive and are preferred if the product can tolerate the potential shearing from the pump. For more sensitive products, rotary lobe pumps are employed, often oversized to reduce the rotational speed. It should be noted that a move from centrifugal to positive displacement pump can more than double the cost of a membrane system.

Aside from the more conventional discrete separation processes described above, membranes have a unique potential to be used to exploit other separation techniques in combination and also to be used directly with fermentation or bioconversion steps to form combined conversion and separation processes.

An already well established combined separation technique is electrodialysis, which is used primarily for desalting.

Electodialysis employs membranes, which incorporate ion exchange groups and are operated under the influence of a direct electric current, to achieve selective ionic migration and separation. The more recently commercialized use of centrifugal effects and the concepts of combining extraction and affinity phenomena with membranes are discussed in the next sections.

Of particular interest to biotechnology applications, is the use of membrane bioreactors. Membrane modules can be employed for continuous cell or enzyme recycle in conjunction with more conventional fermentors and such complete systems are available. Membrane modules themselves have also been applied as reactors with biocatalyst materials (either whole cells or enzymes) immobilized on the feed or permeate side of the membrane or within the membrane itself. Through immobilization or retention, such systems can achieve very high densities of cells or enzymes, with consequent high volumetric productivities. At the same time, the presence of the membrane allows the retention of, or alternately the passage of certain molecules, thus providing for at least partial purification of products.

Bioreactors can also incorporate so called "membrane sandwich" arrangements with more than one type of selective membrane, some for instance, allowing passage of nutrients, while others allowing passage of larger or smaller products. While membrane bioreactors have been investigated extensively in the laboratory, the important application on a commercial basis has been for mammalian cells, as discussed in a previous portion of the report.

2. Future Developments

Recent developments in commercial membranes have tended toward increasing flux performance and improving durability. Membranes are now becoming available with increased pH tolerance as well as resistance to cleaning agents. The reduction of fouling has been a major imperative to increasing flux performance. The approaches more generally employed, have been to use membranes with surface charges or chemically modified surfaces to reduce the adhesion of foulants. Surface channelling to increase turbulence at the membrane surface has also been attempted.

Inorganic ceramic membranes have been gaining increasing attention. They offer interesting possibilities for bioprocessing because of their inertness, which makes them less prone to fouling and their stability, which makes them more tolerant of extreme conditions and more readily sterilizable. The major constraint with such materials has been the limit of pore size.

There has also been a movement from reverse osmosis membranes to slightly more open membranes in the so called nanofiltration range with less NaCl separation but higher flux and lower operating pressure requirements. Such membranes are useful for the removal of salts and solvent water, while retaining sugars and small polypeptides.

Systems have been recently commercialized, such as the dynamic pressure filter developed by Sulzer, which employ axial rotation of the filter to aid in separation. The resistance effects of non-permeating species building up against a membrane, are usually reduced by increasing the tangential velocities at the membrane surface, which is achieved by increasing the throughput flow past the membrane, but at the cost of increasing pressure drop. The dynamic filtration technique instead relies on secondary flows created by the axial rotation of the filter element to reduce the buildup of a resistance layer. The result is a higher comparable flux performance and lower pressure drop.

A newer membrane process more recently commercialized is pervaporation, or transmembrane permeation-evaporation. This process has liquid flowing on the feed side of the membrane and vapour, usually created by vacuum, on the downstream side of the membrane. Pervaporation is suitable for the selective removal of either volatile products, or water, as in the case of commercial ethanol dehydration systems marketed by Vogelbush.

3. Future Developments

The development of improved membrane materials including many new and modified polymers, especially of hydrophilic nature, is a major trend. Of particular interest to biotechnology applications is the development of inorganic ceramic membranes with small pore sizes, potentially into the reverse osmosis range.

Gas separation membrane systems have not yet been applied extensively to fermentation operations, but offer potential for the enrichment of oxygen in aeration streams.

Extensive work has been conducted on combining membranes with affinity processes. Approaches include the direct attachment of ligands to a flow-through membrane, in which case, the membrane acts more as a solid support and the retention of large or macrosolute bound ligands by ultrafiltration membranes. In this latter case, the target solute is selectively bound by a ligand so that it is retained and concentrated on the feed side of the membrane. Alteration of the process conditions allow the solute to

then be eluted and separated from the ligand itself by permeating through the membrane. This latter concept is being actively pursued by a variety of researchers including groups in Canada.

Membranes also have potential application in conjunction with extraction processes. In this case, a membrane can be used at the phase interface as a means of phase separation, reducing entrainment of surface active agents and solids into a non-aqueous extractant, or the extractant itself can be incorporated into the capillary spaces of a membrane, thereby forming in effect a "liquid membrane". This latter concept is especially useful for expensive extractants.

One final area of further development is in membrane bioreactors, where the application of more specific and selective membranes, as well as new membrane configurations are of interest.

Ultrafiltration membranes would benefit from the development of performance standards to better reflect the needs of biotechnology uses, as well as more consistent product standards on the part of manufacturers.

F. Downstream Processing

Downstream (and upstream) processing is important for the preparation, separation and purification of products from fermentation and may, in some cases account for upwards of 80% of total processing costs. Because of the wide extent of equipment and processes that could be possibly employed, discussion of downstream processing activities was limited to the following major unit operations:

Sterilization
Centrifugation
Cell Disruption
Extraction
Chromatography

Each of these operations is discussed in more detail.

1. Sterilization

The sterilization of media is important in ensuring the purity of desired cultures by eliminating contaminant organisms. Sterilization can be accomplished by filtration with exclusion on the basis of size or, much more commonly, through thermal means.

a. Available Commercial Technology

Filter sterilization employs flow through a filter material or membrane with usual maximum size openings in the range of 0.2 to 0.45 micron depending on requirements. Filter sterilization is especially important for heat labile components and as such, is most employed in cell culture processes. Due to the greater expense and complexity, this method is usually a second choice to thermal techniques.

The exploitation of high temperatures to achieve thermal death of organisms has been long exploited. The most usual method employed is batch sterilization, either in-situ in the fermentor vessel or done separately using an autoclave. The temperature of the media is elevated, usually to around 120 C for around 20 minutes and then cooled down.

Due to time, energy and media quality considerations, the more recent trend has been toward UHTST (ultra high temperature, short time) continuous processing, where a continuous flow of media is heated up to very high temperatures in the range of 140 C, held for a very short time of no more than a few minutes in a holding

tube and subsequently cooled. Such heating operations are sometimes staged and usually employ several heat exchangers, which could be tubular, plate or spiral, in order to recoup energy from the exiting flow stream. These techniques are taken almost directly from food processing and packaged systems are offered by major food processing suppliers including Alfa-Laval and APV.

St. Lawrence Reactors of Mississauga developed a tubular starch hydrolysis reactor system which during its operation also provides continuous sterilization of the processed media. One such small scale system, was supplied by them to the University of Waterloo specifically for the purpose of sterilization.

b. Future Developments

Continuous media sterilization systems specific to biotechnology applications have only been available for a very few years. As the technology is new, there may be potential for the technical improvement and development of new systems.

2. Centrifugation

Centrifugation exploits density differences, magnified by the application of centrifugal force many times that exerted by simple gravity settling through rapid rotation, in order to obtain solid/liquid and liquid/liquid separations.

a. Available Commercial Technology

Centrifuges are mechanically sophisticated process machines and are available in a variety of configurations. Those most commonly applied to pilot and full scale bioprocessing are horizontally rotating decanters and vertically rotating tubular bowl and disk-stack machines, all of which continuously process feed. Decanters are the simplest in terms of operation, but are limited primarily by the "g" force which can be generated. Tubular bowl machines have much simpler configurations and are most often applied to small scale operations. They can generate very high "g" forces, however, they suffer from a limitation in solids holding capacity and more importantly, a lack of containment.

Disk-stack centrifuges are available in a variety of bowl configurations including solid bowl, which retains

solids and is intended for very low solids loadings; nozzle bowl, which allows for continuous discharge of solids; and solids-ejecting bowl, which intermittently discharges solids. Such machines have been used in a wide variety of industrial and food applications, including yeast separation in breweries and cream separation in dairies. While the capacities of such individual units can be very large, upwards of 150,000 L/h, they have in the past been limited in the "g" force which could be developed.

More recently, specialized high-speed, solids-ejecting, disk-stack machines, as described in Aronsson (1987), have been specifically modified for application to biotechnology uses. Such units develop high "g" forces and are capable of bacterial and even cell debris separations. A major feature of such units is that total containment of the bowl and discharge reception areas around the bowl are achieved through the use of special axial mechanical seals, sterile vents for pressure equalization on discharge, etc. Thus aerosols and potentially dangerous organisms are totally isolated within the bowl hood, while potential contaminants are prevented from entering the unit and contacting the process stream. Such machines also can be cleaned and sterilized in place (CIPable and SIPable). There are two major suppliers of this type of advanced equipment, namely Alfa-Laval and Westfalia, who are both European.

b. Future Developments

No major developments in this industry are considered pertinent to this study or to the directions anticipated for Canadian Equipment manufacturers.

3. Cell Disruption

Cell lysis or disruption is important for the recovery of intracellular proteins. This operation can be accomplished by a variety of chemical, enzymatic and mechanical means, however, at production scale, mechanical methods now dominate.

a. Available Commercial Technology

On a production scale, the preferred methods of mechanical cell disruption have been high pressure homogenization and bead milling, both of which are continuous operations. Bead milling involves essentially grinding by glass beads in a rotating chamber.

Homogenization involves pressurizing a feed stream to high pressure, usually in a three stage high pressure piston pump and then dropping the pressure rapidly down to atmospheric through a special orifice, which creates high shear. Of these two methods, the most utilized is homogenization.

Homogenizer technology has been transferred intact from dairy and food applications and is thus dominated by dairy manufacturers. These include APV-Gaulin, Bran & Leubbe (part of Alfa-Laval group) and Rannie.

b. Future Developments

Homogenization, which dominates cell disruption, is a well developed technology with marginal technical and operational improvements likely. Few of these types of units have been sold here in Canada for bioprocessing uses, a reflection of the relatively small market involved.

Given that mechanical cell disruption by whatever means is energy and capital intensive, with high maintenance requirements and potential damage to products, there is motivation for replacement with enzymatic or other biological techniques. The necessity for any cell disruption technique can be eliminated altogether by using organisms that secrete products or altering organisms to induce secretion. Cell disruption is considered unattractive for exploration by Canadian manufacturers.

4. Extraction

Extraction exploits chemical interactions between component species and different liquid phases to achieve separation.

a. Available Commercial Technology

Liquid/liquid extraction is well established in a wide variety of chemical processes and in the purification and concentration of antibiotics. Such processes rely on the selective equilibrium to draw desired products from one phase (usually aqueous), into another, (usually organic). The solubility of penicillin and other antibiotics, for instance, in organic solvents can be easily manipulated by adjustment of pH conditions. Usually, reducing the pH favours the distribution of the product (partitioning) toward organic solvents, while higher pH conditions favour the aqueous phase and can be used to recover product. When

the acid conditions required are detrimental, a variety of centrifugal contactors have traditionally been employed in order to provide rapid phase contacting.

b. Future Developments

While the application of extraction to antibiotic processes is well established, there are a variety of other potential applications which have been identified.

Extensive work has been directed toward the use of extraction to recover organic chemicals produced via fermentation, in particular ethanol and organic acids. The motivation in these cases has primarily been the high energy requirements to recover products by conventional distillation. This is especially important in the case of organic acids where a fairly dilute stream of non-volatile acid must be concentrated by evaporating all the water.

A wide variety of solvent systems have been investigated for ethanol, including both low boiling point solvents, such as freons and high boiling point solvents, such as long chain, branched acids and alcohols. Of particular interest in the production of ethanol and other inhibitory products, has been the development of extractive fermentation processes in which the product is recovered directly within the fermentor. In this situation, solvent toxicity becomes a critical consideration. Important development work in the area of extractive fermentation has been done in Canada.

Another major motivation for extraction processes is the delicate nature of many products. Aqueous phase liquid fractionation techniques, which exploit the formation of two distinct aqueous phases by the addition of polyethylene glycol and other polymers or salts, have been used, although infrequently, to extract biologically active enzymes that are heat and solvent sensitive. Another such separation technique that could be classified as extraction, is foam separation. While such methods exploit surface interactions rather than true liquid/liquid phase partitioning, they can produce a selective separation with proteins tending toward the foam phase.

Another recent technology that is attractive for heat and solvent sensitive products is supercritical extraction. This technology, which is already well established in caffeine removal from coffee, operates

at temperatures and pressures such that the solvent is in a fluid state, above its critical point. Usually fairly moderate temperatures are employed in conjunction with high pressures. Under these supercritical conditions small changes in operating conditions can cause large changes in solubility, such that equilibrium distribution of desired product between aqueous and solvent phases can be manipulated. The solvents often considered include carbon dioxide, freon, ammonia, sulfur dioxide, ethane and other light hydrocarbons. The supercritical extraction technique is of interest not just for bioprocessing operations, but has potential application in food processing.

Among the newer developments in extraction processes, have been the application of membranes, as mentioned in the membrane portion of the report, where the membranes are used either to provide an interface between phases, or actually incorporate an extractant in effect to form a liquid membrane.

While some aspects of extractive technology are quite old, there is good scope for improved techniques and new applications. This area may also offer applications in other industries such as food processing, particulary in the case of supercritical extraction.

5. Chromatography

Chromatography provides what is termed high resolution separation of desired protein products. It exploits interactional differences between solutes and packing materials to achieve a separation.

a. Available Commercial Technology

Chromatographic techniques, in general, involve aqueous flow through a column of specific packing material. Interactional differences between the various species in solution and the packing are exploited to achieve selective adsorption and separation, with elution or desorption achieved by a step change or gradual change in the buffer solvent properties. The packing media is the central feature of such processes. The type of separation is determined by the interactions that are afforded by the media. While a very wide array of interactions are exploited for laboratory or analytical purposes, fewer are actually applied to production processes. The major techniques employed at production scale are identified as follows and each is discussed in more detail:

(1) Gel Filtration - molecular size
(2) Ion Exchange - net ionic charge
(3) Affinity - specific chemical/biological interaction

The leading supplier of chromatographic process systems is the Swedish firm Pharmacia, with various other international firms, such as Amicon and Millipore, extensively involved. There is a wide number of companies world wide, making separation media, especially for affinity purposes.

In the separation and purification of protein products, one single chromatographic step is rarely sufficient. In general, two to four steps are required and the order in which different chromatographic techniques are employed is usually as follows: Ion Exchange, then Affinity and finally Gel Filtration. The determination of an effective separation strategy is thus an important activity.

(1) Gel Filtration

Gel filtration is a chromatographic technique used to separate molecules on the basis of size by molecular sieving. Its description as filtration, rather than implying a mechanism, is a misnomer based on tradition. As solutes flow through the matrix bed, smaller molecules will tend to more easily enter pores in the matrix and thus will be retained longer. The packing matrix is hydrophilic and quite inert in order to avoid other interactions and for the separation of proteins it contains large pores. The most common packing materials employed include dextrans, modified dextrans, polyacrylamide and agarose in the form of microspheres in the range of 50 to 200 micron diameter.

While this technique is very well established, it suffers from a variety of limitations. Most important are the very low throughputs and the poor mechanical strength of the deformable gels usually employed. The process is very slow, especially if proteins of similar size are to be separated, when very large bed volumes are necessary to process even small amounts of feed material. The large elution volumes of buffers required also tend to dilute products. Resolution is dependent on the relative rates of migration of solutes and on column height, which is limited by

the mechanical strength of the gels, although this has been overcome to a certain extent by stacking a number of staged columns in series.

Since this technique is slow and expensive it tends to be used as the very last step in a separation sequence, where there are fewer contaminants and smaller volumes to be processed.

(2) Ion Exchange

Ion exchange exploits differences in ionic charge to achieve separation of proteins and is the most widely employed chromatographic method on a process scale. Hydrophilic matrix packings can be modified to possess either positive or negative charges, that can also be either weak or strong in nature. When a feed stream is passed through the matrix column, proteins with opposite charge will adhere, while those that are neutral or similarly charged, pass through. By subsequently altering the pH or ionic conditions of washing buffers, the adhered species can be eluted from the column.

This technique is fairly well developed with resolution dependent on the elution strategy, rather than column length. Durable and inexpensive packing media are also available with high protein loading capability. Due to the high absorptive capacity and stable and inexpensive nature of the media, ion exchange tends to be employed as a first step for sample volume reduction in a separation sequence.

(3) Affinity

The most exciting developments in chromatography for biotechnology applications are the so called affinity techniques, which exploit highly specific binding interactions between proteins and packing materials. Such techniques make it possible at least in theory to separate and purify a single component of a complex mixture in a single step. While this sort of separation is rarely if ever achieved, purification using affinity is nevertheless an order of magnitude better than that achieved by other methods.

The technique currently employs an inert hydrophilic support matrix to which a ligand, or specific binding site molecule, is coupled. While the ligand is tailored for each individual case,

the support matrices usually consist of compressible gel materials such as agarose, polyacrylamide and cellulose, or more rigid packings such as silica, porous glass and hydroxyethyl methacrylates. A variety of chemical reactions, most usually involving cyanogen bromide, are used to "activate" or react with hydroxyl groups on the matrix surface in order to provide attachment sites for the ligand. A flexible spacer arm molecule is often employed between the matrix and ligand in order to reduce steric hindrance.

Affinity ligands can be employed that involve a variety of different interactions, with the limiting factor in resolution being non-specific binding. Specific interactions that have been used include the following:

(a) Biospecific Interaction - This involves biological site recognition. Specific binding pairs can include enzymes with substrates, inhibitors or coenzymes; hormones with receptors; and antibodies with antigens. The greatest amount of attention has been focused on such biologically specific interactions, especially those involving antigen-antibody pairs which are termed immunospecific interactions.

(b) Dye-Ligands - Reactive triazine dyes are at present the most commonly employed affinity interaction ligand, but suffer from lack a of reproducability.

(c) Hydrophobic Interaction - Ligands consist essentially of hydrocarbon chains bound to a gel matrix, which interact with hydrophobic regions of different proteins to varying extents.

(d) Metal Chelation - The ligand forms a complex with the target molecule.

(e) Reversible Covalent - This involves formation of disulfide bonds that are reversible.

While affinity techniques are applied extensively in laboratory scale systems, they have been infrequently used at a production level and even

then at a small throughput scale with only a few liters of media employed. This is due to the small quantities of the product materials involved, but is also a reflection of the inherent limitations of this separation process. While offering potential, affinity techniques are still beset by a process and materials problems.

Some of the problems associated with other types of chromatographic methods also occur, such as column channeling and plugging, high pressure drops, low throughputs, difficulty in scaling up and product dilution, as well as other unique problems. Affinity media tends to be expensive, because of the necessity of activation and the addition of costly ligands as in the case of antibodies and this represents a major limiting factor.

The activation process used to derivatize the matrix is costly and employs a toxic chemical (i.e. cyanogen bromide), which is undesirable. Only limited activation of the packing matrix is achieved, such that a very small proportion of the column packing surface is active. The packing is often non-uniform.

The ligands themselves may be sensitive, especially in the case of biospecific protein ligands and thus, interfering substances and contaminants that could otherwise inactivate the ligand, such as lipids and protease enzymes, must be removed. Progressive losses in activity are usually experienced through successive cycles of adsorption and desorption. This can occur as a result of ligand inactivation, or leakage of insufficiently bound ligand, which can potentially contaminate products.

The stronger and more specific binding involved requires the use of much harsher pH or ionic strength conditions to desorb bound species and reactivate the column and such conditions may be detrimental to both the product and the ligand.

While affinity techniques have begun to make a major impact on bioprocessing separations, their potential is far from being realized. In a separation sequence, such techniques could be applied at any step, however, they are rarely employed first. With improvements, it is likely that affinity techniques will tend to be used

earlier to take more advantage of their effectiveness at low concentrations.

(4) High Performance Liquid Chromatography

High performance liquid chromatography (or HPLC) is usually considered separately from other chromatographic techniques. It differs, more in the mode of operation of the system, rather than in the kind of solute-packing interactions, since it can incorporate the different kinds of media described in the previous sections.

HPCL is distinguished by the use of longer columns with smaller (ca 10 micron), more rigid, dense packings operated with relatively high flow rates at much higher pressures than in traditional techniques. It can provide faster processing with better resolution.

While HPLC is more of a laboratory and preparative technique, it is applicable to the production of products on a very small scale.

(a) Future Developments

Chromatography is a rapidly developing technology with improved media, as well as new configurations and process concepts, being developed.

Affinity techniques have been the centre of much work given both the potential and problems involved. As summarized briefly in an article by Clonis (1987), the trend in media has been toward rigidity, which is important in overcoming problems inherent with compressible gels, as well as toward reducing non-specific adsorption and bleeding of ligands. Other imperatives in affinity include use of milder conditions to effect desorption, such as the use of more specific elution methods and pulsing of elution buffers and improvement in the tolerance of non-protein fouling or alternately the development of processes to selectively remove such contaminants.

The trend in chromatography process systems has been toward integrated packages, especially those that have been pre-approved for regulatory purposes. A variety of new

process configurations have also been developed. A new proposed configuration for columns is a radial flow system. Such a system, which features flow from the exterior walls toward the centre, may offer the potential of improved flow capacity. Another proposed development to overcome the flow limitations of gel columns, which are restricted both by gel compressibility and diffusional kinetics, is the use of macroporous membranes as support matrices for either ion exchange or affinity groups, as mentioned previously in the membrane section.

As also mentioned under the membrane section, an interesting process concept being investigated by a variety of researchers, including those in Canada, is the combination of macrosolute bound affinity particles with ultrafiltration, in order to selectively control permeation through the membrane. Other approaches employing non-immobilized ligands have also been developed. Another new process concept is the reversible attachment of liquid micelles within a column thereby exploiting the liquid-liquid interaction to selectively extract components into micelles that are themselves then eluted from the column.

Of more general concern in chromatography is still the batch, cyclic nature of the process. There is considerable potential to improve the efficiency of all such processes through the development of quasi-continuous methods.

Chromatography is a powerful separation and purification technique, well suited to bioprocessing needs. As such, it is an area of intense development. While there is a great deal of competition, there is still an enormous scope for technical improvement and new process concept development.

Of the various downstream technologies discussed, those which represent the least opportunities appear to be in areas related to capital intensive hardware, such as centrifuges and homogenizers. More potential is seen in areas involving exploitation of chemical and biological interactions, specifically extraction - chromatography processes.

V. MARKET ANALYSIS: A TECHNICAL PERSPECTIVE

A. Mammalian Cell Culture

Increasing interest in products made directly from mammalian cells, will maintain demand for mammalian cell culturing techniques.

The culturing of mammalian cells is a rapidly advancing field with scope for incremental technical improvements. To a certain degree, some Canadian companies have commenced working on the development of novel processes and systems.

The technology development is expensive with substantial technical risk attached. Major players, specifically Endotronics, have experienced severe financial losses.

Vertical integration between the equipment fabricator and the product manufacturer is occurring. A number of the major developers of especially advanced mammalian cell systems are involved in contract product manufacturing. These include the following:

Invitron
Celltech
Damon Biotech
Bioresponse
Endotronics
Charles River Biotechnical Services

These firms represent a significant component of the production of some mammalian products, such as monoclonal antibodies and several of these firms are moving toward the production of high value biological chemicals such as tPA.

The potential effect of this vertical integration is that a significant proportion of the potential equipment market in general is being satisfied "in-house" with less inclination to purchase outside.

A last consideration, is that superior technical advancements have not necessarily proven themselves cost effective. There are, for instance, problems with many of the more advanced membrane and matrix core systems, such that if scale-up to larger production rates is desired it can only be achieved through the expensive replication of units.

Superior technology is no guarantee of economic competitiveness, as shown by one major Canadian user of mammalian cell cultures, who has remained with the older roller bottle technology over more advanced perfusion and dense cell immobilized systems strictly on the basis of economics.

B. Plant Cell Culture

There are few players involved in the plant cell area and less competition. There is a good scientific research base for this type of technology, with several Canadian research organizations recently involved in cell culture drug production projects. Economics will again drive this technology. Opportunity is viewed as long term, with potential markets in pharmaceutical and natural foodstuffs production. These areas must be extensively researched prior to development.

C. Fungal Fermentation

The intensely competitive market for microbial fermentation systems presents Canadian manufacturers with immense difficulties, especially given the size, technical reputations and marketing strength of the international competitors. It is important to note that many of the larger international firms have in the past themselves faced financial difficulties. It is safe to say that the fermentor business provides little margin for error. The equipment is by nature very expensive and a single major project going bad is virtually sufficient to devastate even a large manufacturing company.

The microbial fermentor market is the most established, the largest and the most competitive. It is seen as a mature situation, with opportunity for Canadian manufacturers potentially in two niche areas.

Computer control systems and sensor development may provide lucrative areas for Canadian manufacturers, but the developer should proceed with caution.

D. Large Scale Industrial Fermentation

Given present depressed world oil prices, the economics of producing ethanol and other chemicals by fermentation has been marginal. In the last few years, only a single alcohol plant has been sold by a Canadian company, (St. Lawrence Starch to Minnesota Corn Processors), a corn wet miller in Minnesota. The sale was the result of long and extended discussions. This lack of feasibility combined with a general over-capacity in traditional fermentation activities, such as brewing and distilling, suggests that there are no steady markets available for the production of new fermentation facilities.

Notwithstanding the present softness of the crude oil market, the price of light crude must ultimately rise as a result of diminishing supplies. It can be anticipated that fermentative methods of production will become increasingly economical. While there is little general incentive for new fermentative production, there is still niche specific potential for successful projects on the basis of additional considerations.

Such factors include waste utilization, by-product beneficiation; or favorable taxation policies, such as those recently introduced in Saskatchewan. Another general opportunity for ethanol, will be in its application as an octane enhancer replacement in leaded fuel phase down. Firms involved in process development and equipment will have to rely on the exploitation of such individual new opportunities, as well as the application of appropriate, cost efficient retrofit technologies.

E. Membrane Systems

Membranes represent an interesting opportunity area in biotechnology equipment. Given the relatively limited understanding of membrane systems, there is a wide potential for technical improvement and application, either alone or in conjunction with other separation or bioreactor activities. There is also a large number of Canadian companies involved either directly or in related areas, as well as an extensive research base in Canada. In addition, membrane systems are beginning to find application in other industries, such as chemical and food processing. There are good possibilities for the application of systems to other purposes.

One important priority, is the development of better industry performance standards. This development, in itself, could represent a major technical and marketing advantage.

F. Other Downstream Processing

1. Sterilization

Continuous media sterilization systems specific to biotechnology applications have only been available for a very few years. Since the technology is new, there may be potential for the technical improvement and development of new systems.

A more detailed specific analysis of this sector would be required, based on a broader understanding of the market direction in general.

2. Centrifugation

Centrifuges are not considered good candidate technology for Canadian manufacturers. They are sophisticated and thus manufacturing and development costs are high. Competing companies have established reputations and well organized marketing organizations. The market for such expensive equipment is small, with only five advanced biotechnology separators having been sold in Canada over the last four years.

3. Cell Disruption

Homogenization, which dominates cell disruption, is a well developed technology with marginal technical and operational improvements likely. Few of these types of units have been sold here in Canada for bioprocessing uses, a reflection of the relatively small market involved.

Given that mechanical cell disruption by whatever means is energy and capital intensive, with high maintenance requirements and potential damage to products, there is motivation for replacement with enzymatic or other biological techniques. The necessity for any cell disruption technique can be eliminated altogether by using organisms that secrete products or altering organisms to induce secretion. Cell disruption is considered unattractive for exploration by Canadian manufacturers.

4. Extraction

While some aspects of this technology are quite old, there is good scope for new and improved technologies. This area may also offer possible applications in other industries such as food processing.

5. Chromatography

Chromatography is an area of intense development. While there is much competition, there is still enormous scope for technical improvement. One possible limitation, but also a potential opportunity, is that large scale chromatographic techniques have not had much success in areas outside bioprocessing and there may be much less potential for application of processes and equipment to other industries.

Of the various downstream technologies discussed, those which represent the least opportunities appear to be in areas related to capital intensive hardware, such as centrifuges and homogenizers. More potential is seen in areas involving exploitation of chemical and biological interactions, specifically extraction and chromatography processes.

VI. CHALLENGES AND OPPORTUNITIES: Primary Source Data

A. The Canadian Equipment Market

1. The total market size for fermentors, downstream processing equipment and related controls in Canada will remain relatively small for the next five years. In itself, it does not appear to be sufficiently dynamic to support an equipment manufacturing industry devoted to its service. Consequently, manufacturers concentrating solely on the Canadian market will have to look to other, probably related, industries to provide a large part of their revenue.

2. The market information available, both in Canada and the United States, is very limited. How can equipment development take place with so little market intelligence? Canadian manufacturers need considerable additional information regarding the U.S. market particularly and international markets generally.

3. The market for biotechnology equipment is highly fragmented. Market fragmentation is exacerbated by user equipment purchase behaviour. The complexities of process development, coupled with the highly proprietary nature of the process, militate against open tender calls and frank discussion of specific needs. In some instances, process developers have preferred to develop in-house, buying equipment on a component by component basis. Thus, they have protected the privacy of the process system itself. Others develop close working relationships and allegiances with established equipment suppliers, effectively shutting out new entrants in the marketplace. For the relatively small Canadian supplier, breaking into such purchasing patterns is a time-consuming and expensive proposition. The early identification of opportunities, probably at the conceptual stage of system development, becomes critical. Creating industry awareness of one's manufacturing capabilities and product specifications is an important task in this effort.

4. Will the industry become increasingly fragmented as processes, applications and end-products become more specialized? Or will a few processes and products gain such obvious superiority that they and the companies that own them, come to dominate the marketplace? Will the biotechnology industry emulate the computer industry? Are we headed toward a proliferation of small firms, or will the future of biotechnology rest in the hands of a few organizations? Each scenario has opportunities and risks. If specialization is the future, then market niches should be easier to identify. If industry concentration occurs, then

marketing efforts can be focussed on a few major accounts. Picking the winning process technologies on which to focus will, in either event, become the most important decision for the equipment developer.

5. At present, the greatest purchasers of biotechnology equipment are government operations: the ARC Pilot Plant and the NRC's BRI Pilot Plant are the largest in Canada. The availability of these facilities could obviate equipment purchases for some firms.

B. Specific Equipment Requirements

From the primary data collected in the survey of biotechnology equipment users in Canada, we have found that the following items of equipment will likely be purchased within the next three years. This is not an exhaustive inventory, but a sample that we believe to be representative of buying activity nationally in the medium term.

1. Mammalian Cell Culture

- 12- 500 gallon tanks and related downstream processing equipment

- pilot scale/commercial scale plant for the production of interferon B

- Instruments: filter press, spectrophotometer, UV monitors

- automated protein purification equipment, automated fraction collectors, pumps for moving 100 litre volumes of material

2. Plant Cell Culture

- small batch bioreactorss, totally 1000 litres of capacity, with related pumps, controls and instruments

3. Fungal Fermentation

- substantial expansion in fermentation and ultra-filtration in one instance

- two or three 20,000 litre fermentors with prep vessels, packaging system and control systems within three years in another instance.

4. Membranes

- systems are under consideration by several firms

5. Other Downstream Processing Equipment

- see comments under Fermentation

C. Canadian Equipment Users Interviewed

1. A large majority of respondents have some plans for additional capital equipment purchases in the next two or three years. There is no evidence that these purchases will be made in a concentrated period of time.

2. Capital equipment investment will vary from a few instruments in some firms to full production scale facilities in others.

3. There is a significant movement toward scale-up in the industry, whether from lab scale to pilot scale or pilot scale to production scale.

4. There are only a handful of companies that are at the point of going to production scale to capture international markets. These companies include Brookside Farms, Fox Wellcome, Iotech and Philom Bios. Of these, the former two are involved in mammalian cell culture, the latter two in fungal fermentation.

5. On the positive side, there is a great deal of optimism among process users that they will be able to develop their markets. There is a healthy, aggressive entrepreneurial spirit in the industry, especially among those small companies that sense they can take on leadership roles in their technologies. All of the firms we spoke with are focussed on the international marketplace.

6. On the negative side, many of these firms are concerned about the availability of venture capital to take their endeavors beyond lab scale. Some feel that government regulations and regulatory procedures will force them to sell their products elsewhere.

7. There is general consensus among biotechnology entrepreneurs that the Canadian government is meeting the challenge of funding biotechnology at the research level. As the results of this research progress to commercialization, the question

in the minds of many seems to be: will the government extend its support to the trial and production stages of the endeavour? Will there be an industrial strategy for biotechnology? The nature of the support requested is varied; streamlining of regulatory approval procedures, regulations consistent with those of competing countries, funding and market information have been cited. The streamlining of regulatory approval alone could create a favorable climate for national and offshore technology owners, with attendant benefits for domestic equipment manufacturers.

8. Equipment purchasers are overwhelmingly concerned with the reliability of the equipment they buy. Due to the costs associated with downtime and the slow part replacement schedules, most respondents considered reliability more important than initial price.

9. Of those users who intend to purchase downstream processing equipment, several mentioned membranes as a possibility. Others are looking at conventional separation techniques.

D. Canadian Manufacturers of Equipment

1. The manufacturers interviewed appear to treat their biotechnology equipment product lines as one segment of their business. It is likely that this situation will continue and should continue, with other products in their portfolios providing the cash flow to finance marketing and product development activities in bio-technology oriented equipment.

2. Equipment research and development expertise in biotechnology will be founded in the research and development of technologies for related applications in such areas as the food, dairy and pharmaceutical industries.

3. Competition in the equipment supplier marketplace is daunting. There are several multi-national firms with established track records, full product lines and extensive distribution systems. These organizations have sufficient hold on the market that it is difficult to envision a frontal attack by a Canadian firm. The research and development investment capital required for significant product line extension and distribution is substantial. Without clearly defined demand and competitive ability, funds for such efforts will be limited. It is therefore unlikely that a full-service company of the scope of an Alfa-Laval will be forthcoming.

4. At the same time, foreign suppliers have several notable weaknesses. Equipment users complain of poor service performance and long delivery times for replacement parts from these firms. Original equipment and replacement parts are very expensive and subject the buyer to duty and currency fluctuations. Some interviewees stated that Canadian suppliers also tend to be expensive and slow to deliver service or parts.

5. Canadian equipment suppliers will likely best compete in carefully selected market niches. By identifying specific limitations and problems in traditional and state of the art processes, they can exploit opportunities that optimize the use of their expertise and that focus their limited R & D dollars.

6. Several specific areas in which Canadian efforts might prove successful include biosensors, including microsensors and microprocessor-based control systems.

Worldwide demand for biosensors is expected to increase dramatically in the next 12 years: from 1987 sales of $38 MM, market size should rise to $450 MM by the year 2000. [High Technology Business, "Biosensor Business Nets Growth," April 1988, p.9]

7. Canadian firms will continue to compete successfully in the manufacturing of traditional stainless steel vessels. This is well established technology and there are a large number of competitors nationally and internationally. However, demand will continue to be spotty.

8. In whatever product categories Canadian firms compete, they must adopt or maintain a global perspective. Market opportunities must be identified on a world basis, particularly in the United States.

9. With regard to the encouragement of a Canadian equipment manufacturing capability, it has been suggested by several sources that the government has an opportunity to play a major role. Government equipment procurement policies could be designed to favour national manufacturers. One source suggested that as the government encourages federally funded projects to buy Canadian, so it too could favour indigenous sources, even if initially at a premium price. This would assist domestic firms in establishing a track record with their products.

10. Financing remains a critical issue in biotechnology. The uncertainty of R & D results, the high cost of clinical testing, the risk of regulator disapproval, to name a few factors, make biotechnology a risky business. Given the

relatively low cost of the research portion of the process, it is likely that a number of products, promising at the research stage, will lure their owners into development without sufficient capital to complete the process. To the equipment supplier, the risks associated with doing business with the undercapitalized user are potentially devastating. In the event of business failure, expensive equipment, custom made and unpaid for, even if physically recoverable, will be difficult to re-sell. The equipment supplier must therefore choose market niches carefully, not just on the basis of market size, but on the financial resources available to the equipment users in the niche.

11. There will be an increase in the amount of used equipment available as biotechnology equipment users go out of business. There is steady trade now, in used equipment in the dairy and food industries.

VII. CHALLENGES AND OPPORTUNITIES: Secondary Data Sources

Biotechnology is a world-scale enterprise. Companies wishing to compete in the industry must adopt a global perspective. While there is obvious merit in establishing a market presence domestically, for Canadian firms success will be fundamentally determined by performance in the international arena.

There is general agreement that the biotechnology market is enormous, and that sales in the next ten years will increase by orders of magnitude. There is less general agreement on precise sales figures for different product classifications.

Consensus on what will define the parameters of the industry is also elusive. For some, biotechnology will be absorbed by long-established industries such as medicine, chemicals and agriculture. If, and as, biotechnology becomes a working part of other industries, equipment manufacturers will have to continuously re-target their markets, and be sensitive to the differences in needs between companies in these traditional areas and today's "new biotechnology firm".

Concurrently, the possibilities in biotechnology continue to expand, with potential such as that recognized by Britain's Department of Trade and Industry in awarding to Biotechnica Ltd. a prize for its Biotag. "Today, the security measures taken to protect documents, bank bonds, and other sensitive items are largely electronic; tomorrow they could be biological."

[Bio/Technology, Vol. 5, March 1987, "Pushing Probes to Market" by Jennifer Van Brunt and Arthur Klausner, p. 215.]

A. World Markets for Biotechnology Products

Predictions of market size vary from "ball-park" industry estimates to specific figures for individual products. For example, industry sales have been projected as moving from $1 billion in 1987 to $50 to $100 billion by the year 2000. [High Technology Business, March 1988, "Cash Crisis Creates Biotech Alliances" by Francesca Lunzer, p. 20]

William A. Cochrane, President of Connaught Laboratories Ltd., offered more specific figures in "biotechnology and the Canadian Pharmaceutical Industry" [Business Quarterly, Autumn 1985, Vol. 50, #3, pp.92]:

Biotechnology and Pharmaceutical Products Market Opportunity

Year	Sales	
	Biotech. Pharm. Products	All Biotech Pdts.
1980	$5.0 Billion	$21.0 Billion
1990	$14.4 Billion	$43.0 Billion
2000	$43.0 Billion	$65.0 Billion

Biotechnology and Immunodiagnostic Products Market Opportunity

Geographic Area	Sales Millions $ U.S.			Growth Rate	
	1980	1985	1990	1980/85	1985/90
U.S.A.	384	830	1,890	17%	18%
Europe	252	610	1,445	19%	19%
Rest of World	300	830	2,030	22%	19%
TOTAL	936	2,270	5,365	19%	19%

In the same article, Canadian sales levels for biotechnologically produced pharmaceutical/biological products are estimated to be $860 million in 1990 and $2.3 billion by 1995.

Market analyses of therapeutics and diagnostics have been further detailed in a report from Boston Biomedical Consultants:

BIOTECHNOLOGY PRODUCT SALES IN 1990 (in millions of dollars)

THERAPEUTICS (of a $65-billion market)	$2,945
Anti-cancer	1,300
Interleukins	500
Interferons	475
Tumor necr. fctr	150
Colony stim.fctr	150
B-Cell growth fctr	25
Blood proteins	930
t-PA	500
Superoxide dismut.	300
Erythropoietin	130
Hormones	480
Growth factors	200
Human insulin	125
Factor VIII	100
Human growth horm	55
Vaccines	235
Hepatitis B	100
AIDS	75
Herpes	60
DIAGNOSTICS IN VITRO (of a $8.9-Billion market)	1,314
Nucleic acid probe	157
Infectious dis.	134
Genetic screen.	23
Monoclonal antiby	1,157

It is interesting to note that the combined revenue from biotechnology-based therapeutics and diagnostics in 1990 represents only slightly over 5% of total sales for that market.

For the equipment manufacturer, the implications of these statistics are uncertain and potentially misleading. The relationship between quantity/dollar value of end-product and demand for equipment is non-linear. In most of these areas, high dollar value of end-product can be produced by relatively small-capacity pieces of equipment. As sales figures for biotechnology end-products continue to climb rapidly, it can be expected that the growth in demand for equipment will be less steep and could quickly plateau.

B. Industry Trends

There are indications that the industry, with cash poverty widespread among the smaller organizations, is beginning to shake-out. This is not particularly caused by the manifest superiority of some products or processes, but by the high cash requirements of product development. Small companies are turning to large companies to finance development in exchange for product rights.

Francesca Lunzer, in "Cash Crisis Creates Biotech Alliances," refers to a prediction by Roger Shamel, president of Consulting Resources, who once estimated that consolidation could mean a reduction in the number of companies from 300 to about 150 "via merger and acquisition in the next 10 years. But that was before October 19 [1987]. 'I've revised my view since the crash,' he says. 'Now I think we'll see some firms fail." [High Technology Business, April 1988, p.20.

For small companies, the future may be even more uncertain. "By the end of the next decade," predicts Nanette Newell, "fewer than 10% of existing small companies are likely to exist as independent entities." Newell suggests that in most cases, production and sales will be taken over by large firms. [Business Quarterly, Autumn 1985, Vol. 50, #3. Nanette Newell, "The Next Decade in Biotechnology" pp.89.]

There are several significant implications in these trends for equipment manufacturers:

1. there will be fewer established companies in the industry as smaller organizations merge with larger ones;

2. there will be more corporate concentration: equipment suppliers will have to deal with corporate bureaucracies;

3. purchasing departments will be able to write their own deals because of purchasing power;

4. barriers to entry will increase in some technologies as large firms create efficient infrastructures, limiting competition;

5. there will be small companies fighting guerrilla wars and trying to outflank their large competitors: if they develop superior technology, they will be a good potential market for equipment, but they will be looking for significant cost-reducing items from equipment suppliers.

The strategic focus of the industry is turning to manufacturing and marketing. In a survey of 135 U.S. biotech firms, Dibner, Hamilton and Vila found that product development is currently rated significantly more important than research, manufacturing or marketing. Manufacturing and marketing are now at about the same level as research. "In five years, biotech executives expect marketing to be of highest importance, followed by product development and manufacturing. They project that research, once most important to the firm, will be the least crucial activity by 1993." [Bio/Technology, Vol.6, March 1988, "The Maturing of Biotech Companies: Shifting Emphasis from Science to Business," by M.D. Dibner, W.F. Hamilton, and J. Vila, p.276.]

The increasing attention given to manufacturing and marketing is closely tied with the movement toward creating vertically integrated biotechnology firms. G. Steven Burrill and Allison W. Smith of Arthur Young state that vertical integration is the declared goal of many companies, though few have achieved it and in all likelihood few will. Regarding production strategy, "a key element of vertical integration is the ability to manufacture. The survey revealed that companies of all sizes and markets consider in-house production capability to be a top priority." [Bio/Technology, Vol. 5, November 1987, "The Keys to Commercialization in Biotechnology," p.1146.] This is because of the strategic advantages of keeping proprietary process technologies secret. This has implications for equipment manufacturers. It may mean less industry-wide economies of scale than would be possible if large contract production houses became the norm; and this means greater equipment demand.

There have been limited instances of mergers, acquisitions or alliances between equipment manufacturers and equipment users.There are several examples, however, of large biotech companies merging with equipment manufacturers, including Cetus Instruments with Perkin-Elmer and Genentech with Hewlett-Packard.

Where might the smaller equipment supplier fit in? For many suppliers, it will probably be at the process development/system integrator stage. An ongoing relationship can enhance product/process improvements, which should improve the competitive strength of the equipment user.

C. Finance

In spite of the October 1987 market downturn, with the consequent dramatic reduction in the availability of risk capital, biotechnology remains a hot venture capital interest.

In an exclusive survey of 209 respondents, High Technology Business found that 64 invested the greatest dollar amounts in 1987 in biotechnology. This was only bettered by computers at 94 and followed by medical products at 63.

For 1988, the projected greatest dollar investment by industry will be medical products at 66, followed by biotechnology and computers at 62. "Medical equipment and biotechnology hold perhaps the strongest appeal for venture capitalists this year." However, "the outlook for biotechnology... is decidedly mixed: venture capitalists complain about a glut of new companies, high capital requirements and long paybacks." [High Technology Business, March 1988, "Where Venture Capital is Investing Now" by Fredric Paul, pp. 22.]

Continued optimism among venture capitalists, which likely is mirrored in the attitude of traditional lending institutions, is encouraging and by implication indicates continuing demand for biotechnology equipment.

D. Competitors in the Equipment Market

There appears to be differentiated demand in some volume for off-the-shelf fermentors and bioreactors. In "A Closer Look at Fermentors and Bioreactors," Jennifer Van Brunt provides charts of bench-scale and pilot-scale fermentors and bioreactors, with descriptions of their systems. This is provided as a representative sample only, but "to the uninitiated, the sheer number of choices can be overwhelming." [Bio/Technology, Vol. 5, November 1987, pp. 1134-1138.] Van Brunt cites 17 different suppliers of bench-scale fermentors and bioreactors and 11 suppliers of pilot-scale fermentors and bioreactors. The systems included in the table are representative of a wide range of systems offered by all the manufacturers.

The market in new tools for different techniques is also dynamic. Over 70 new tools for chromatography were offered to the marketplace in the 18 months ending March 1987. [Bio/Technology, Vol. 5, March 1987, pp. 252,254.]

Biosensor technology is the subject of considerable interest at present. Although in fermentation, there is some uncertainty about the value of on-line monitoring because of the time involved in most fermentations, some product developers predict a strong demand. Gary Steele, President of Molecular Devices (Palo Alto, CA) says "We have just scratched the surface of on-line monitoring. When you come out of the reactor, you have a product, by-products and contaminants. During early process development, you need to validate the process. And once you have that, quality control assurances are necessary. Biosensor technology will impact each stage of that system." [Bio/Technology, Vol. 5, May 1987, Jennifer Van Brunt, "Biosensors for Bioprocesses," p. 438.

E. Biotechnology Equipment Market: Sales Projections

Projecting the size of the biotechnology equipment market even within the next five years is a difficult and inexact process. Even with the assumption of a significant margin of error, however, the growth in demand for capital equipment is substantial.

In "Biotechnology Instrumentation Market" [American Biotechnology Laboratory, Vol. 4, No. 2, p. 6, 1986] Steven Delco provides the following data:

Biotechnology Instrument Market
Project Sales (millions of dollars)

Instrument	1985	1986	1989
HPLC systems	46.0	55.2	99.3
HPLC columns	12.45	15.93	31.87
HPLC detectors	20.93	27.21	53.05
DNA synthesizers	12.8	14.5	21.70
Peptide synthesizers	15.0	24.1	52.6
UV-VIS spectrophotometers	15.6	17.9	24.0
Gel electrophoresis	31.0	36.3	56.2
Laboratory fermentor	16.5	18.31	25.72
Robotics/automation	14.0	17.5	35.4
Software/hardware	29.0	36.2	48.3
Total	213.28	263.15	458.14

The different time periods and equipment categories described by Mark Pine in "Demand for Biotechnology Equipment and Supplies Continues to Grow" [Genetic Engineering News, Vol. 7, No. 1, January 1987, pp. 32,46] make for a useful comparison and indicate the same general trends:

Composite Market Projections - Biotechnology Equipment and Supplies

($ in millions)

	1986	1991	1996
Biological Incubators	43	51	54
Ultracentrifuges	45	52	67
Liquid Chromatography			
High Pressure (HPLC)	170	330	470
Low Pressure/Gravity	36	53	140
Electrophoresis Products	32	68	90
DNA Synthesizers	8.9	19.2	34.0
Fermentation & Culturing			
Suspension Systems (Conventional Fermentors)			
Laboratory	17	37	58
Pilot Plant	18	49	76
Full Scale Industrial	13	16	23
Novel Cell Culturing	6	24	26
Ultrafiltration Equipment	60	93	145
Laboratory Filtration	76	95	110
Laboratory Chemicals			
General Biochemicals	91	130	183
Specialized Biochemicals	29	75	130
Support Media	65	100	150
Research Radiochemicals	27	29	25

F. Finding Competitive Advantage

There are no Canadian biotechnology equipment manufacturers large enough to compete head to head with some of the large multinationals in the industry. None possesses the financial strength or distribution network requisite to establishing a comparable presence in the international marketplace. The strategic options available to Canadian firms then, are to outflank the large competitors or to engage in guerrilla warfare. In the former case, domestic companies must fill a need in a significantly large segment of the market that is currently unsatisfactorily served by competitors; in the latter, they must adopt a hit-and-run approach that targets specialized products to specific, smaller user groups. In an article on strategic planning for marketing in the U.S., Ed Weymes recommends a flanking or bypass manoeuvre [Business Quarterly, Fall 1987, Vol. 52, Number 2, pp. 101-103].

The choice will depend to some extent on the financial, product and management strengths of the firm.

Both strategic options involve the identification and exploitation of market niches. Finding niches in a youthful industry in a state of flux is easier said than done. What may appear to be a promising process or technique one day can be undermined the next by an announcement from a multinational or a small lab. For the equipment manufacturer, it means aiming at a moving target in many instances. Success is going to be determined to a great extent by the accuracy and specificity of the market intelligence the company can access. This is critical at the product strategy planning stage and throughout product development. It means knowing not just the general directions of the equipment users and manufacturing competitors, but the details of their efforts. This requires an on-going commitment to market research and it remains a critical component for success.

Niche marketing reduces the Canadian company's dependence on distribution systems and marketing/sales forces. It allows the concentration of marketing dollars and energy, with the potential to see a higher return per marketing dollar spent than does the broad product line strategy. It is this factor that Kenneth G. Hardy notes first in his study on successful Canadian manufacturers operating in the U.S.: "The products produced by these firms were specialty or niche products, that is to say, narrowly defined products and/or narrowly defined applications...The essence of niche marketing is to search out the market for narrow windows of opportunity that because of their high degree of specialization, protect the firm from direct competition." [Business Quarterly, "Key Success Factors For the Small/Medium Sized Canadian Manufacturers Doing Business in the United States," March 1987, Vol. 51, Number 4, pp.68].

Finding niches will require the selling of perceptible and demonstrable product improvements. As Steven Delco writes, "Major advances in technology must be made; for example, systems for large-scale purification and preparation must truly meet the needs of the manufacturing environment in the areas of purity and reproducibility...We need fewer 'me too' items and more cooperative efforts by industry leaders to develop systems that work." "Biotechnology Instrumentation Market" [American Biotechnology Laboratory, Vol. 4, No. 2, p. 6, 1986].

Mark Pine suggests some niches that will grow dramatically: "Relatively few suppliers have entered the market for novel cell culturing equipment. Much of the growth in this market will occur in the pilot plant systems, which accommodate most of the products of technology. Novel cell culturing equipment will also be applied toward the coming mammalian cell products. Ultrafiltration, one aspect of downstream processing, will outperform other filtration segments as the purification schemes for these products become more complex." [Op. Cit. p.46] Whether these areas could become profitable for Canadian manufacturers will depend on a variety of factors intrinsic to individual companies.

VIII. CONCLUSIONS

Biotechnology is entering a new, highly competitive phase. Manufacturers of biotechnology equipment must accurately assess their own strengths and weaknesses in the context of international competition.

The primary and secondary research conducted for this report indicates that the Canadian market per se, while healthy, is too small to support a significant manufacturing capability. The sales volume of biotechnology equipment in Canada will unlikely exceed several million dollars annually in the next five years.

There will be limited but steady demand for fermentation vessels at lab and pilot scales. This segment of the industry is characterized by established firms with established technologies. As the industry scales up to commercial production levels, there will be steady, high growth for "production scale" fermentors; however, these will be predominantly for high value products and systems will be relatively small. Competitive advantage is and will be defined in terms of technological improvement, proven product reliability, cost and replacement parts service.

Opportunities in the fermentation industry will arise predominantly from technological improvements that offer greater process control, decreased labour or energy inputs and/or increased throughput. In this regard, there will be opportunities for developers of biosensors and computer-based control systems.

In the area of downstream processing, the next five years will see a greater use of membrane technology applications. Ultrafiltration appears to offer particularly significant opportunities.

The greatest opportunities for growth are markets in the United States and possibly Europe. While there is a growing demand for biotechnology equipment in Canada, the market is too small to sustain a manufacturing industry.

Canadian companies must face the multinational goliaths of the industry with a strategy that maximizes manoeuvrability and builds upon current strengths. It is therefore imperative that equipment development be targeted and sold to very specific market niches that offer the greatest profit potential, least risk and least competitive activity.

Canadian companies are not gathering enough market intelligence to safely guide development and manufacture. It is imperative that Canadian companies who want to compete within the international framework begin to accumulate very specific information regarding target niche markets.

IX. RECOMMENDATIONS

1. Canadian manufacturers must focus their competitive strengths on carefully selected market niches that are appearing in the industry internationally. Depending on their in-house expertise and corporate mission, there are opportunities in membrane technology, biosensors and computer-based control systems.

2. Manufacturers need to develop a closer working relationship with equipment users, particularly at the process development stage. A significant amount of this effort should be focussed on equipment users in the United States.

3. Manufacturers need to establish a marketing presence in the United States. This will entail building an effective distribution channel and appropriate promotional programs. The creation of a market intelligence gathering network is also necessary.

4. There is a national need for a coherent and detailed industrial strategy for biotechnology as it moves from research into commercialization on a large scale. A continuing dialogue needs to be nurtured between researchers, producers and manufacturers that centres on developing internationally competitive biotechnology products and processes.

5. A marketing and competition oriented approach to the biotechnology business needs to be fostered at the research level, as it is with equipment users and manufacturers. An emphasis on products and processes with high commercial potential should be encouraged by government and research institutions.

It is recommended that firms and or the industry concentrate on identifying potential market niches that offer a strategic fit with their manufacturing, distribution and financial capabilities. Exploiting profitable market segments will require a well-researched assessment of the potential for specific technologies and an accurate analysis of competitor strengths and weaknesses.

Success for the Canadian equipment manufacturer will depend on how well foreign markets and particularly the U.S., are penetrated.

The success of this penetration is highly dependant on an a broader understanding of the World Biotechnology market direction as a whole. It is the authors contention that such information is crucial to successful strategic competition within the whole Biotechnology industry in Canada.

Canadian companies are not going to succeed manufacturing low value equipment. High value niche products, designed and produced as a result of accurate market intelligence will offer Canadian companies the most likely route for market penetration, profit and long term product acceptance.

Canadian companies must become more proactive in defining their development directions. They must begin to define markets first, prior to embarking on expensive research and development programs.

X. APPENDIX A: DIRECTORY OF BIOTECHNOLOGY EQUIPMENT MANUFACTURERS

A. General Comments

The development of a list of manufacturers and potential manufacturers, is in itself somewhat complex given the diverse kinds of activities in which companies are involved. Some differentiation has been attempted to distinguish the different types of involvement.

The first category is that of SYSTEMS MANUFACTURERS, which applies to companies who manufacture and market a complete system package. The next category is that of PROCESS DEVELOPERS, which applies to companies developing a process or piece of equipment but who are not intent on manufacturing it themselves. The next category is that of FABRICATORS who manufacture equipment only, possibly also including design and engineering and who are not involved in the design of the process or marketing of the equipment product.

The next category is that of COMPONENT MANUFACTURER who produce equipment components and may also market them. Finally is the category of ENGINEERING firms who would be involved in the design of systems as a service but not necessarily in the marketing of the system.

An attempt was made to limit the entries on the list to Canadian companies or foreign firms who actually do manufacturing or process development here.

There are a variety of other subsidiaries of foreign manufacturers and Canadian companies that act as agents for foreign firms. These were not listed, unless their activities were substantially beyond the marketing of foreign based technologies. In this respect, it is important to note that such international firms cannot be disregarded as potential Canadian manufacturers. Some such firms have considered performing some of their manufacturing tasks in Canada.

B. Fermentation Systems

(Including microbial systems, mammalian cell culture, plant cell culture, fungal and high volume/low value fermentation systems)

The numbers of systems manufacturers is very small, as are the numbers of fabricators with direct experience in fermentation systems. A number of companies with potential as manufacturers was included under the category of Fabricators. The additions to this list are somewhat speculative being compiled from available information lists of fabricators. In addition, the companies listed under the category of process developers was limited to those who have expressed some intention to market the developed technologies.

1. Systems Manufacturers

WHE Process Systems
100 - Klondike Drive
Weston, Ontario M9L 1X3
(416) 744-4155
Mr. Paul Hallman
V.P. Sales
Produce "Biotrol" fermentor - S.S. external loop cyclone fermentor.
Based on design by Dawson at NRC. Not yet released for sale.
Agent for Cherry-Burrell ?? and what else ?
Stainless steel fabrication capability.
Primary business activity is in Food applications.

Pegasus Industrial Specialties
4490 - Sheppard Avenue East
Scarborough, Ontario M1S 4J9
(416) 298
Mr. Ron Trent
Vice-President
Primary business is glass piping and fabrication and as agent for Corning, also involved with distillation, extraction, adsorption columns and evaporators.
Former agent for Chemap AG (Swiss Fermentor Manufacturer).
Produce "Versitec" fermentor - inexpensive glass laboratory fermentor.
Recently involved in development of mammalian cell culturing system in collaboration with D.Armstrong of NRC.

2. Process Developers

Mammalian CELL SYSTEMS

ChemBioMed Inc.
Edmonton, Alberta
(403) 450-6800
Mr. Jack Cuyler
Senior V.P. Operations
Have been working on three phase fluidized bed system for mammalian cell.
Culture in collaboration with L.Behie of the University of Calgary.
This system has also been applied to fungi and could also have application to plant tissue.

LOW VALUE/HIGH VOLUME PROCESSES - PRIMARILY FOR ETHANOL

St. Lawrence Starch
Mississauga, Ontario
Ethanol production system based on starch substrates
Based on SLR hydrolysis reactor system.

Iotech Corporation
Ottawa, Ontario
Developing ethanol from cellulose process based on enzymatic hydrolysis of cellulose substrate (Fungal cellulase enzyme).

Biohol Developments
(Joint venture of Weston and St. Lawrence Starch).
Developing ethanol production process based on bacterium Zymmomonas mobilis.

Wardrop Engineering
Winnipeg, Manitoba
Developing ethanol from cellulose process based on acid (HF) hydrolysis of cellulose substrate.

3. Assemblers/Fabricators

Zeton Inc.
4189 - Harvester Road
Burlington, Ontario L7L 4M3
(416) 632-3123
Mr. Sandy Watt
President
Design and build custom process systems primarily for pdu and pilot but also production. They are not primarily developers of processes and build to suit needs of clients.

A-L Stainless
113 - Park Street South
Peterborough, Ontario K9J 3R8
Mr. Dave Shepard
President
Former stainless steel fabrication facilities of Alfa-Laval, now Canadian owned. Design and construct stainless steel vessels up to large sizes. Primary activities are in food and dairy applications, including large sterile tanks used in aseptic food applications.

Falco
Lachine (Montreal), Quebec
Fabricator primarily involved in construction of dairies.

Ellet Copper and Brass
1575 Kingsway Avenue,
P.Q., B.C. V3C 4E5
Custom fabricator involved in brewery and food applications.

TCI-Superior Div Mueller Canada Inc.
6500 Northwest Drive
Mississauga, Ontario L4V 1K4
(416) 677-9000
Brian Smith
Vice President, Technical Services and Business Development
Are part of US company, but do manufacturing on an international basis for parent firm. Have been involved with the design and construction of a fermentor for Allelix, however, they are not primarily involved in process development aspects.

Ebco Industries Ltd.
7851 Alderbridge Way
Richmond, B.C. V6X 2A4
(604) 278-5578
Jack Sample
Technology Manager

With respect to ARC 15,000 L project:

Maloney Steel Ltd.
8825 - Shepard Avenue S.E.
Calgary, Alberta
Primarily fabricator for oil and gas.
Are building 15,000L Bioengineering design fermentor for ARC.

Other major fabricators who put in bids for this job (aside from Ellet and Falco):

Dacro Industries
2201 - 7th Street
Nisku, Alberta
Primarily fabricator for oil and gas.

Cessco
Primarily fabricator for oil and gas.

From "Food in Canada" 1988 Buyer's Guide:

Fermentors:

Dominion Bridge Process Equipment
Toronto, ON (416)-477-4948

AO Wilson Process Equipment Ltd. ?? unknown, may only be an agent

ETMW Enterprises ?? unknown, may only be an agent

Coppersmiths Canam Inc. ?? unknown, may only be an agent

Processor Vessels:

Bedarco McGruer Inc.
5730 - Place Turcot,
Montreal, Quebec
(514) 933-7551
Custom metal fabricator making process vessels for food applications.

HB Metal Manufacturing

Knight Custom Manufacturing

JH Lock and Sons

Mordhurst Automation INc.

O'Connor Tanks

PAP Process Engineering Services

Vats:

Ruge Manufacturing and Consulting

VESSELS AND FERMENTORS OF ALTERNATIVE MATERIALS

Canbar Inc.
Waterloo, Ontario
(519) 886-2880
Have supplied plastic storage vessels to University of Waterloo.
Also supply processor vessels to food industry.

Gemini Biochemical Research
Calgary Alberta
Ian Forrester
President
Small contract research firm.
Has built 2000L fermentor vessel from fiber glass, presently being used for non-sterile microbial growth applications.

4. Component Manufacturers

Prochem
Ontario
Kurt Schnieder
Manuafactures low shear/good mixing impellers applied to chemical and biotech applications.

C. Membrane Systems

1. Systems Manufacturer (including membranes)

Zenon Environmental Inc.,
Hamilton, Ontario
OEM for Filmtec
Primarily involved in water purification and environmental abatement techniques.
Presently, only Canadian company actually manufacturing their own membranes. They produce membrane system packages. They are also involved in research at BRI (J.Luong) using membranes in concern with affinity techniques.

2. Systems Manufacturers (excluding membrane)

Petwa,
Calgary, Alberta
They produce membrane system packages and are primarily involved in water purification.

WHE Process Systems,
refer to above
Assembling membrane systems specifically for biotech and food applications.

Seprotech Systems Inc.,
Ottawa, Ontario
Remnants of former Memtek Corporation which had financial problems and went out of business.
Were primarily involved in maple syrup business.

Danny Douvoe
Quebec
Puts together membrane systems

3. Process Developers

Continental Pharma Cryosan Inc.,
Montreal, Quebec
(514) 935-4004
Robert Heft
They are involved here in Canada in the development of a membrane system incorporating heparinase.

Wardrop Engineering Inc.
77 - Main Street,
Winnipeg, Manitoba R3C
Hadi Husain
Involved in work on membrane processes involving whey, substrate concentration, and ethanol product separation.

OTHER COMPANIES INVOLVED IN MEMBRANES TO AN UNKNOWN EXTENT

BTC (Ballard Technologies Corporation), North Van.
Alkaline water electrolysis

Revotek, Ottawa, Ontario

Electrohome Limited, Kitchener, Ontario
May not be in membrane business any more.

Retech Capital Applied Research and Technology, Victoria, B.C.

Industrial Labs of Canada, Tillsonburg Ontario

Radioimmunoassays Inc. Process development

Calgon

D. Downstream (and upstream) Processing

1. Systems Manufacturers

St. Lawrence Reactors
Mississauga, Ontario
Mr. Per Assarsson
President
Company is a subsidiary of St. Lawrence Starch, a large corn wet miller and also producer of ethanol.
They have developed a tubular starch hydrolysis reactor system which they use in their own plant and are marketing, primarily for ethanol production systems. System during operation also provides continuous sterilization of media and have sold a small system to U of Waterloo for exactly this purpose.

2. Process Developers

Winnipeg Rh Institute
Winnipeg, Manitoba
Bert Friesen
Executive Director
Scale up of blood plasma separation using chromatographic methods
Process being marketed to China by US consulting firm Davey-McKee

Export Packers/STC Laboratories
Winnipeg, Manitoba
Les Carvalho
Technical Manager
Primarily involved in egg processing for food applications.
Have developed separation systems for extraction of valuable enzymes from egg albumin. Some of these technologies will be marketed.

3. Engineering Companies

SNC Group
Montreal, Quebec
May be involved in fermentation process design for Ontario paper company.

Montreal Engineering
Montreal, Quebec
May have been involved in design and construction of fermentors for Iotech.

Stanley Engineering, Edmonton, Alberta
Engineering consulting firm
Did a retrofit design of a 7500 L fermentor for ARC.

XI. APPENDIX B: ORGANIZATIONS AND INDIVIDUALS CONTACTED

ABI Biotechnology
Winnipeg, Manitoba
(204) 477-
John Langstaff — Research Director

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Scarborough, Ontario
(416) 299-6101
Sam Lombardo

Alberta Research Council,
Biotechnology Department,
Edmonton, Alberta
(403) 450-5303
Ella Kershavarz — Research Scientist
Alan Jones — Research Scientist
Manoj Kole — Research Scientist
Doug Wilson — Project Engineer

Allelix Inc.
Mississauga, Ontario
(416) 677-0831
Nigel Smart

Bio-International Inc.,
Toronto, Ontario
(416) 927-8067
Masa Misawa

Bio-Mega Inc.
2100 Rue Cunard
laval, Quebec H3S 2G5
(504) 682-4640
Jacques Gauthier — President
Greg Constantino — Head, Biotechnology

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170, 6815- 8th Street N.E.
Calgary, Alberta T2E 7H7
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Bill Scowcroft — Vice-President, Research & Development

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Clearbrook, B.C. V2T 3T8
(604) 852-5940
Hugh Wiebe — President

Canber Industries Ltd.

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Allan McInnes President

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David Gannon

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Willowdale (Toronto), Ontario
(416) 667-
Don Gerson V.P. Vaccine Manufacturing
Connaught Research Institute
John Vose

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Gloucester, Ontario K1G 3N3
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Brian Foody President

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London, Ontario
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Dr. Boyland Production Manager

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Gerard Andre Head, Biochemical Engineering

National Research Council,
Plant Biotechnology Institute,
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Fox Wellcome Project

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Graham Mowatt President

Pegasus Industrial Specialties

Scarborough, Ontario
(416) 298-3141
Ron Trent Vice President

Philom Bios Inc.
#15 Innovation Blvd.
Saskatoon, Sask. S7N 2X8
(306) 665-6211
John V. Cross President

Quadra Logic Technologies Ltd.
2660 Oak Street
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Doug Kilburn Professor

University of Waterloo
Department of Chemical Engineering
Waterloo, Ontario
Murray Moo-Young Professor

Vencap Equities Alberta Ltd.
1980 Manulife Place
10180- 101st. Street
Edmonton, Alberta T5J 3S4
(403) 420-1171
Bill McKenzie

VIDO
Veterinary Infectious Disease Organization
124 Veterinary Road
Saskatoon, Sask. S7N 0W0
(306) 966-7465
Steve Acres President

Zenon Environmental
Burlington, Ontario
Tony Tonelli V.P. Membranes

XII. APPENDIX C: MARKET SURVEY SAMPLE QUESTIONNAIRE

COMPANY NAME:

Contact:

1. In which of the following areas of interest is your company involved:

As an:	Equipment User	Equipment/Process Developer
Animal Cell Culture Systems	X	
Plant Cell Culture Systems		
Fungal Fermentation Systems		
Fermentation Process Systems for Low Value, High Volume		
Membrane Bioprocessing	X	
Other Downstream Processing (e.g. chromatography, extraction etc.)	X	X

2. Are you using any other major types of process equipment or systems not identified above?

4. Are you presently developing Equipment or Processes applicable to biotechnology? If so:

5. Under which of the above categories?

6. Do you plan to commercialize it?

7. Have you used Equipment or Processes made or developed in Canada? If so, please describe.

8. What factors would be important to you in deciding whether to buy Equipment or Processes made or developed in Canada.?

9. What processing equipment do you think you will require within the next five years?

10. What current Process Needs do you have that are not adequately fulfilled by existing available equipment or processes?

11. For each of the identified areas in which you are involved (whether as an equipment user or developer), please answer the following questions:

    A. Is your effort in this area a significant factor in your company's mission? If so, please describe that significance.

    B. What would you describe as the most important strengths of your present production efforts?

    C. What would you describe as your most important priorities for improvement? (i.e. yield improvement, increased throughput,energy or labour cost reductions, etc.)

12. Of the areas being considered in this study, which do you think offer(s) the best opportunity for Canadian manufacturers?

13. Are there other potential opportunity areas not included in the identified areas of interest?

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