EVALUATION OF PROCESS SIMULATORS FOR AN INTEGRATED BIOPROCESS DESIGN

by

Sabina Ahmad Rouf

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Evaluation of Process Simulators for an Integrated Bioprocess Design

ABSTRACT

Several alternate flowsheets for the production of tissue plasminogen activator (t-PA) in Chinese hamster ovary (CHO) cells were simulated using "BioProcess Simulator™" (BPS) of Aspen Technology Inc. The flowsheet developed for typical conditions reported for laboratory-scale production and purification of t-PA was defined as the base case. The alternate flowsheets differed in the fermentation media, type and mode of operation of the bioreactor and scale-up strategy. A trial and error method was used to design the downstream processing train so that the required purity and specified overall recovery are met within a predetermined batch time. The simulator was also used to carry out economic evaluation of process alternatives. Overall economic performance was measured in terms of return on investment (ROI) and gross margin (GM). The flowsheet that used a fed-batch mode of operation for the bioreactor was found to result in the largest ROI. This was followed by a multiple bioreactor flowsheet, where the downstream train was much smaller compared to the base case. A two-stage cell culture process increased the ROI of the process by 44% whereas, the ROI increased only marginally by replacing the stirred tank bioreactor with an airlift bioreactor.

Simulation of the base case was also carried out with "SuperPro Designer®" of Intelligen Inc. The simulation was found to be simpler and faster using the short cut unit operation models. However, due to flexibility and thoroughness of the simulation, as well as analytical ability, BPS was found to be more suitable for a detailed process design. On the other hand, contrary to experimental observations, the rigorous models of

BPS for some units (e.g. chromatography) generated results which were found to be insensitive to variations in process parameters.

To backup the simulation results, a sensitivity study of the process performance to uncertain parameters and variables was carried out using BioProcess Simulator™. The membrane separation units were found to be most sensitive to flow velocity and exponent 'b', that accounts for dependence of gel resistance on Reynolds number. Both of these quantities affect the thickness of the boundary layer, thereby reducing gel resistance. For the chromatography columns, solute capacity was found to be the critical parameter. The performance of the affinity chromatography was sensitive to the choice of the Langmuir isotherm constant, whereas the ion exchange column was sensitive to charge on the t-PA molecule. Resolution in the gel filtration column was most significantly affected by column length and to a lesser extent, by load volume and flow rate within the range studied. Among the economic parameters, ROI was found to be most sensitive to changes in equipment cost, followed by raw materials cost. A large increase in ROI was observed with improvements in overall recovery and could serve as an incentive to optimize the operation of the processes.

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CHAPTER 1

INTRODUCTION

1.1. Design of Large Scale Process for Biopharmaceuticals

As biotechnology moves from the research laboratory to the marketplace (Table 1.1.1), the development of large-scale production processes for biopharmaceuticals becomes inevitable. Biopharmaceuticals, particularly glycosylated proteins, are mostly produced in animal cells because of the fidelity with which animal cells can produce authentically processed human proteins. The success of the overall production process depends to a great extent on the successful integration of the expression system, the cell culture system and the processing methods applied to produce the finished product (Cartwright, 1994).

Table 1.1.1: Therapeutic product status (Shamel and Chow, 1989)

On Market	Year of Entry
human insulin (h insulin)	'83
human growth hormone (h GH)	'85, '87
alpha interferon (A-INF)	'86
OKT3	'86
hepatitis-B vaccine (HEP-B VAC)	'86
tissue plasminogen activator (t-PA)	'87
Erythropoietin (EPO)	'89

The purity requirement of biopharmaceuticals is very stringent. In general, the final product should be free of infectious agents and meet the specified limits for other contaminants such as DNA, cell and media proteins, endotoxin, etc. (Anicetti et al., 1989). In addition, chemical contaminants such as antifoam agents, antibiotics, leached chromatographic ligands and so on should be eliminated from the finished product. Downstream processing, therefore, serves as the primary guardian for safety, purity, potency and consistency of the product as required by regulatory agencies.

Purification of products from animal cells to the required purity is extremely challenging because some of the impurities have properties similar to the required product. The use of complex medium provides a high level of background protein, often globally similar in physicochemical properties to the product. Generation of closely related product variants can further complicate the situation. Product variants can form due to differential glycosylation in which changing culture conditions during the fermentation run result in the synthesis of a preponderance of different glycoforms at different stages. Variants may also result from partial proteolytic cleavage due either to the variable activity of cellular processing enzyme, the induction of extracellular protease, or to cell lysis. Interaction between the product and macromolecular medium components can give rise to high molecular weight complexes. These complexes, although inactive, can co-purify with the product (Cartwright, 1994).

Since the required protein is usually of a labile nature, only mild purification techniques can be applied. Therefore, the maintenance of a sterile atmosphere to exclude infectious agents and produce apyrogenic product becomes difficult. In addition, the level at which animal cells secrete proteins is low in most cases and the harvested supernatant contains only a low concentration of the secreted product. Purification techniques work less efficiently in this situation and if the product is present at too low a concentration or as a very low percentage of the total protein, purification may not be practical either technically or economically.

Downstream processing, therefore, plays a determinant role in achieving the purity and assuring the safety of biopharmaceutical products derived from animal cells. For high value products, downstream processing accounts for at least 70 to 80% of the overall costs. Hence, it is usually the effectiveness of downstream processing which determines the economic viability of such a process (Cartwright, 1994).

There is no algorithm or procedure by which one can proceed, step by step, to develop a foolproof large-scale design. The number of options for protein purification is so large that one cannot say that there is only one right method or one best process. Each protein is different and these differences preclude the formal development of an algorithm for designing downstream processes (Wheelwright, 1991) although the strategy is similar.

The design of bioprocesses is complicated by its impact on the competitive advantage. Anything that provides an edge over competitors or serves to differentiate the product from similar products is a source of advantage. There are three main sources of competitive advantage in the manufacture and sale of high value products: first to market, high quality and low cost (Figure 1.1). Each of these offers advantages that can be realized only at the expense of the others. The optimal process is that which gives the best performance with respect to the desired advantage. Early market penetration is often critical for economic success of a new biopharmaceutical product. The company

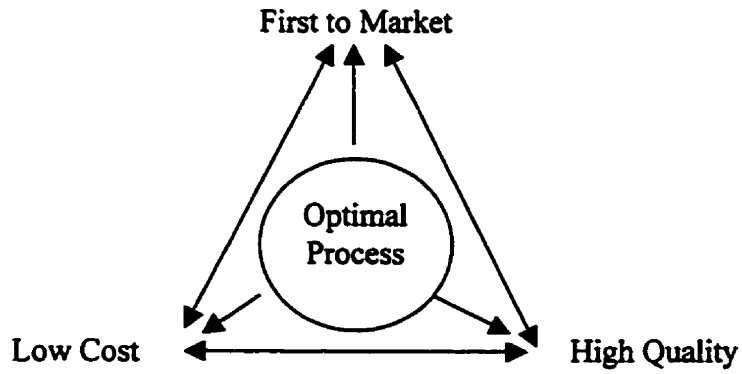

Figure 1.1: The competitive advantage for optimal bioprocess design

that is the first to market a new protein enjoys a substantial advantage over the competitors, particularly if being first to market includes being first with patent protection. The company that finds itself in second place may manage only one-tenth the sales of the company that got there first. With this difference, it obviously makes little sense to spend years developing the lowest cost process if long term development means losing the race. On the other hand, if the product is not unique or if it can exist in many forms, then a greater advantage may be obtained by differentiation of quality than by being first to market.

Many products must share the market with competitors for various reasons, such as insufficient patent protection to keep all competition off the market. In such cases being first to market may not be enough to ensure competitive advantage and product differentiation may be obtained by varying the characteristics of the product. A protein that is more pure, is more active, has greater lot-to-lot consistency or is improved by some other measure of performance may outshine the competition. Similarly, a company that can configure its product in more alternatives to suit individual customers, shows greater flexibility and may gain an advantage.

As a product acquires competitors, price becomes a greater issue with customers and lower cost products gain an advantage. Of course, the lower the cost is from the beginning, the higher the margin and the greater the profit. Thus the company whose process designer has initially developed a lower cost process prevails over the company that must redesign to lower its cost. Regulatory restrictions impose such a high cost on process change that good original design provides a competitive edge.

Once the competitive edge has been identified, it must be maintained as the criterion for evaluation of process design because it defines the goal. The most profitable process is the one that minimizes time to market, maximizes a differentiating characteristic or minimizes cost within the constraints of product specifications and available resources.

In a regulated industry changes in manufacturing methods tend to be expensive as they often require repeated studies of safety and efficacy as well as delays resulting from seeking approval for the changes. Builder et al. (1988) estimates, for a licensed product, a minimum of two years from the time of conception of a process change until the point when a license amendment may be granted. The cost for the entire effort could be millions of dollars. Thus design may be difficult to alter once a validated process has been established. Rushing in with an incomplete or ill-defined process may cause considerable difficulties once the product is on the market (Wheelwright, 1991).

Process design and scale up may be divided into three distinct steps: selection of unit operations, optimization of operating parameters at the small scale and increase of equipment size while maintaining the same operating parameters. The design of a large-scale protein purification process is an evolutionary method as shown in Figure 1.2.

The most common approach to downstream process design is to adapt a standard purification strategy. The advantage of copying an existing process is that the development time is less. If the protein at hand is similar in nature, characteristics and properties to other proteins for which purification process exists, then copying the existing method may be very straightforward and simple. But every protein is different and there is no guarantee that this will produce acceptable material. The product may also be unrepresented in the literature. In these cases, another method of process development must be looked for.

The second method, both in terms of most common application and in order of complexity, is called "heuristics " or rules of thumb. Heuristics serve as guidelines only for approximate design. The purpose of heuristics is to narrow the list of possible separation steps based on general experience.

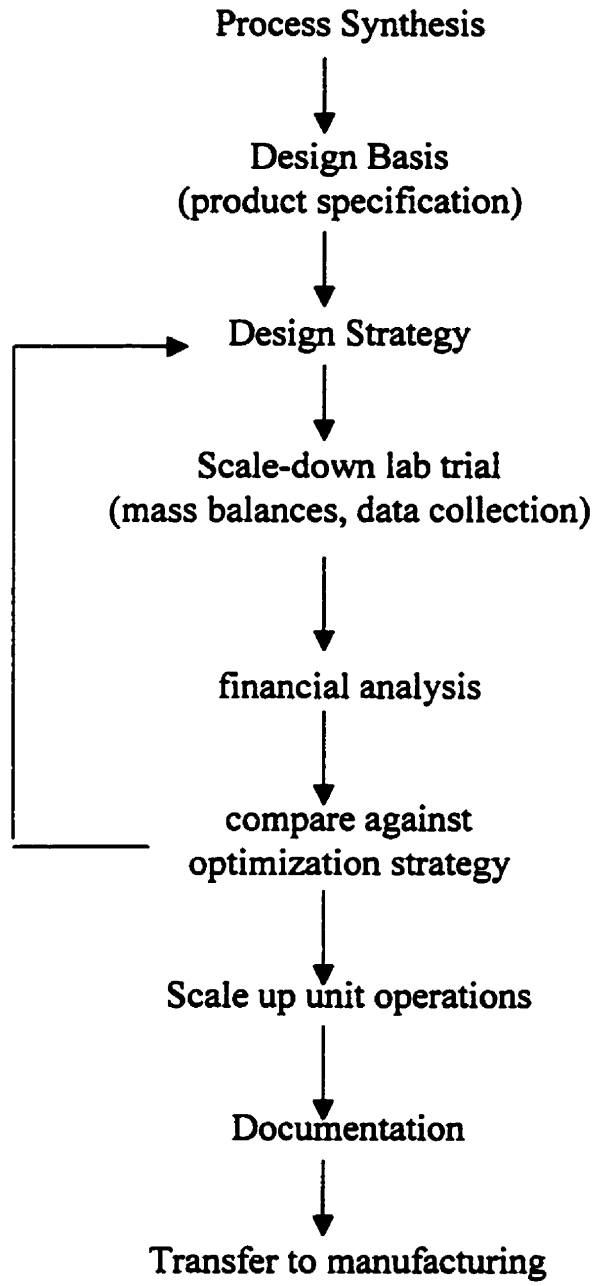

Figure 1.2: Process synthesis flowchart (Wheelwright, 1991)

A third approach in the process design is the use of expert systems. The idea here is to follow the pattern of thought of the expert designer through a series of if-then postulates. Expert systems are based on models describing the expert; they do not necessarily follow a strict algorithm but rather rely more on qualitative judgments for choosing among the competing alternatives. Leser and Asenjo (1994) have developed an expert system to assist the rational selection of large-scale protein purification processes. This knowledge-based expert system is capable of selecting protein recovery, separation and purification processes by using a fundamental database containing characteristics of the protein molecules. The development of the database for Baculovirus-derived proteins has also been described (Turner et al., 1994). The rationale for choosing high-resolution separation methods for multicomponent mixtures is based on equations describing heuristics and the behavior of proteins in chromatographic separations. Siletti and Stephanopoulos (1986) also carried out some earlier work in this area.

The fourth design method is the use of algorithm or mathematical models. The use of a model allows investigation of the effects of operating condition changes on the process output. The availability of computer simulation techniques for process design can greatly facilitate process design using mathematical models. Flowsheet simulation offers the opportunity to significantly shorten the time required for process development (Cooney et al., 1988). Computer simulation can be used as a useful tool to compare process alternatives on a consistent basis and to synthesize and analyze interactively a large number of project ideas in a short time. It can help to screen project ideas by identifying the most promising ones. It can be used to interpret pilot plant data and substantially reduce the time and cost for pilot plant development (Grob, 1993). Interactions exist among the different stages of a process and early evaluation greatly facilitates successful process design. Performing process design in an integrated manner also results in great savings in time and cost (Zhou et al., 1997). For example, before finalizing the cell culture system, the impact of medium composition or possible cell lysis on the quality of the product stream destined for purification, could be taken into account. Another

advantage of using process simulation is that all aspects of a process (e.g. yields, scheduling, economics etc.) are accounted for simultaneously and when changes are made to one aspect of the process the impact on all other areas is automatically determined (Shanklin et al., 1999).

Process optimization by simulation can reduce manufacturing costs by up to 30 percent depending on the process, largely by improving product recovery yields (Evans, 1988). Process development and simultaneous economic evaluation by a simulator will result in faster, rigorous and consistent economic and technical justification analysis for the process.

The only difficulty with using models to describe highly complex systems is that a highly complex model may be required. However, even a simple fundamental model will contribute in the development of heuristics and empiricism.

The fifth approach can be defined as a hybrid of the preceding methods. Process synthesis may consist of following an existing example, modifying it by means of heuristics, following a modified expert system that relies mainly on heuristics or having an algorithm for part of the design and using these techniques to combine the unit operation in a stepwise fashion.

1.2. The Present State of Bioprocess Simulation

The use of computer aided-design packages for process simulation, cost estimation, equipment sizing and process optimization is widespread for chemical processes. The advantages include reduction of time taken to develop a process design and increase in productivity, efficiency and the level of control of the selected process option (Gritsis and Titchener-Hooker, 1989).

Computer-assisted techniques in biochemical process industries, however, have not found general application although the benefits achievable by process simulation in this area have been stressed by several investigators (Evans and Field, 1986; Evans, 1988; Gritsis and Titchener-Hooker, 1989; Grob, 1993; Petrides, 1994; Aelion and Petrides, 1994; Petrides et al., 1996; Zimmermann, 1997). There are some significant differences between chemical and biochemical processes that necessitated special features for biochemical process simulators. First, biochemical processes use living organisms to synthesize the desired product. The microbial kinetics is much less understood and requires the development of specific biochemical models. Determination of their rate of growth, metabolism of substrate and product formation requires an understanding of the cell physiology and biochemistry. The effect of overall process environment and operating parameters on the cell and its production are much more complicated compared to a chemical process. For example, the level and range of shear force within a bioreactor can have a profound effect upon the rate of growth and productivity of some organisms (e.g. *Penicillium chrysogenum*) (Gritsis and Titchener-Hooker, 1989).

A second feature of biochemical processes is the heavy use of batch or semi-continuous processes (Evans and Field, 1986; Evans, 1988). It is further complicated by the combination of batch, semi-continuous and truly continuous modes of operation within the same overall process. For example, the bioreactor is frequently operated in a batch mode whereas a chromatography column in a cyclic mode. Differential equations must be used to describe the time-dependent batch biochemical processes. Overlap between batch and semi-continuous steps can prove difficult to accurately simulate (Shanklin, 1999). Specialized mathematical routines and substantial programming effort is required to generate suitable simulators to solve the systems of differential and algebraic equations (for continuous or equilibrium unit operations), which result from the mixed mode of operation, over different time domains. The specification of the sequence of operation within a unit operation also complicates such a process. For example, the

sequencing of loading, washing and elution stage of a chromatography column requires the specification of an independent variable for each step.

Finally, many unit operations unique to biochemical engineering are poorly understood. There are predictive models for these operations but quantitative models frequently do not exist (e.g. homogenization). A bioprocess simulator must be flexible enough to accommodate improved and more sophisticated models, as they are developed (Evans and Field, 1986; Evans, 1988). Experimental data and curve fitting provides the simplest model for simulation purposes. Empirical models may be developed with parameters and constants estimated from experimental data (or from literature sources). The most sophisticated models, derived from first principles, often require detailed thermodynamic data on all the components. Under this situation prediction of relatively simple parameters such as viscosity, becomes difficult. Physical property information is lacking for many typical materials in a bioprocess.

Nevertheless, several investigators pursued the development of bioprocess simulators adopting two alternative routes. The first approach was to modify an existing package of conventional chemical process simulator. The alternate route has been the use of research information and data in order to develop models, which describe individual biochemical engineering unit operations and processes.

The unique requirements of a bioprocess simulator, outlined in the literature (Evans and Field, 1986; Evans, 1988; Cooney et al., 1988) and necessitated by the unique features of bioprocesses, are the following:

Unit operation model: A bioprocess involves unit operations, many of which are not available in conventional chemical process simulators. Moreover, there are often insufficient data available to develop predictive models based on first principles. Therefore, a bioprocess simulator must provide models at various levels of rigor.

Initially, the simulator will rely on empirical and semi empirical models requiring user supplied data. Each increase in rigor will require more fundamental data. The user may select the suitable model depending on the need and availability of data. The simulator must also be flexible enough to accommodate for improved and more sophisticated models, as they become available.

Physical properties: In contrast to chemical process simulators that deal with liquid and vapor only, a biochemical process involves biomaterials such as cells, cell debris, organic and inorganic solids, proteins and polymers. For best simulation results these biomaterials must be characterized according to biological activity, viscosity, solubility, diffusivities etc. The physical properties systems should be developed on a rigorous framework to provide compatibility with unit operation models that are more dependent on physical properties, such as precipitation. The bioprocess simulator should contain a collection of methods and models to calculate thermodynamic and transport properties of conventional and non-conventional (e.g. proteins, cells, polysaccharides etc.) components. This will enable the unit operation models to determine property dependent performance and also help to characterize the process streams.

Batch and cyclic processes: The mixed mode of operation that is typical of biochemical processes require development and solution of steady state as well as dynamic models of unit operations. Dynamic models are usually in the form of non-linear differential equations, which must be integrated over a period of time whereas the steady state models are algebraic. The bioprocess simulator must be capable of handling these mixed types of equations and properly integrate the batch processes with the continuous ones.

Besides the above requirements a bioprocess simulator should also be equipped with a fast and effective method for equipment sizing and costing, data regression system and an interactive user interface (Evans and Field, 1986).

The first attempt at bioprocess simulation was carried out with Aspen Plus (a chemical process simulator) to test its ability to handle biochemical flowsheet (Acetone-Butanol-Ethanol process) simulation. (Marlatt and Datta, 1986). This was followed by Bhattacharya and Motard (1986), who carried out steady state simulation of the same process with ASEPN PLUS modified by addition of some appropriate modules. They also studied economics of several process alternatives.

Its applicability was next explored for simulation and optimization of recovery of penicillin G from fermentation broth (Evans, 1988). Since the recovery process involves conventional unit operations such as filtration, solvent extraction, crystallization, drying etc., no difficulty was encountered in simulating this process with ASPEN PLUS. The authors also reported the optimization of the solvent extractor performance by manipulating the pH and solvent flow rate. The optimum pH thus calculated was found to match the actual pH used in the process. ASPEN PLUS was used again to demonstrate the economic impact of alternative processing strategies. An alternate recovery process that uses a second extractor to back extract the penicillin to remove impurities was evaluated against the standard process. Simulated results showed that the two extractor model would increase the net recovery cost by 30 percent.

The same group also simulated the downstream processing of a proteolytic enzyme (Cooney et al., 1988). The flowsheet for protease recovery involved vacuum filtration followed by ultrafiltration to concentrate the product and then spray drying. Since models for ultrafiltration and spray drying are not available in ASPEN PLUS, user defined blocks were written for these units. The effect of wash water flow rate to the filter and the quantity of water removal by the ultrafilter were also studied.

With these successful results, modification of ASPEN PLUS simulator to a complete bioprocess simulator (Aspen BioProcess Simulator™) was carried out by including typical biochemical unit operations such as chromatography, membrane separation etc.

The architecture and flow of information in this simulator is shown in Figure 1.3. The developers tried to meet most of the aforementioned unique requirements of bio-simulators in Aspen BioProcess Simulator™.

Most of the biochemical unit operation blocks are available in BioProcess Simulator™ (Grob, 1993). There are options for adding user-supplied subroutines to modify the unit operation models. There are dynamic models in addition to steady state ones for some of the unit operation blocks that normally operate in batch mode (e.g. fermenter, membrane separator etc.). To combine the batch operation with the continuous mode, the simulator time averages the output of the batch blocks. The average performance is then used to treat the batch operation as a "pseudo continuous" unit operation. The simulator accepts user supplied parameter estimation methods. It also accepts user-defined properties. Built in methods are available for the calculation of density and enthalpy with user supplied coefficients.

The application of BioProcess Simulator™ has been demonstrated in the area of process development. As an exercise in bioprocess simulation, Petrides et al. (1989) simulated the conceptual design of down stream processing of porcine growth hormone (pGH) using BioProcess Simulator™. A plant was designed to produce 6000 kg/year of purified pGH. pGH is produced as an intracellular product in recombinant *E. coli*. Therefore cell disruption, solubilization, protein refolding etc. were considered essential part of the downstream process. Its purification also included typical biochemical operation such as high resolution stages (e.g. ion-exchange chromatography). The performance characteristics of the unit operation blocks were drawn from laboratory and pilot plant data. BioProcess Simulator™ was also used to calculate process economics. A total investment of $31,158,781 was estimated for this process.

BioProcess Simulator™ has recently (March, 1997) evolved into a new product called Batch Plus. Feedback from customers such as Novartis Pharmaceuticals Corp. (E.

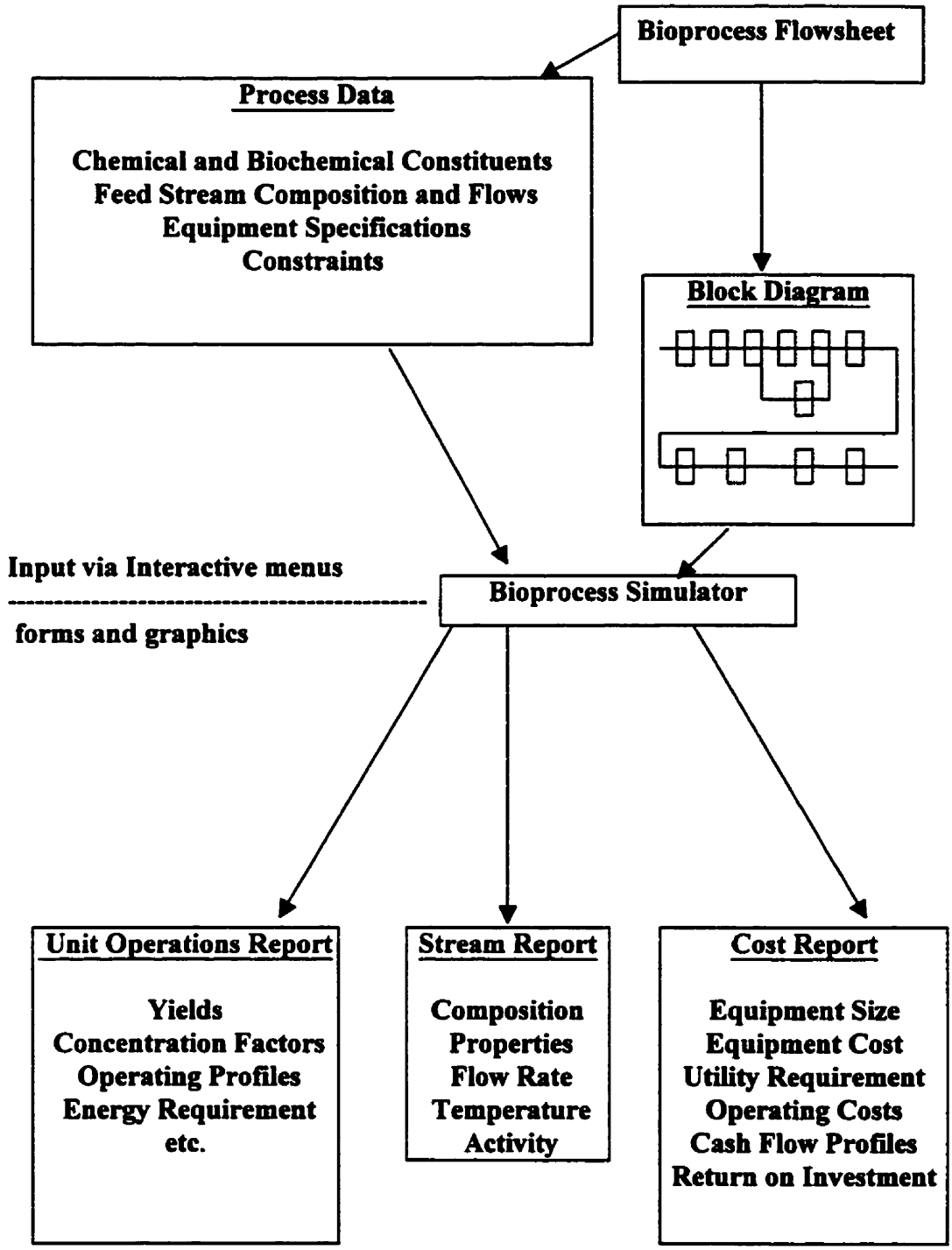

Figure 1.3: Architecture and flow of information in bioprocess simulator (Evans, 1988)

Hanover, NJ), Eli Lilly & Co. (Indianapolis, IN), Genentech (S. San Francisco, CA) and Merck (Whitehouse Station, NJ) fueled evolution of BioProcess Simulator™ (Zimmermann, 1997). Batch Plus takes the user step-wise from laboratory scale to pilot stage to full-scale commercial production. It can be used for a wide variety of unit operations and generates cost analysis for each phase of scale-up. An integrated element of Batch Plus' cost evaluation is environmental impact assessment. It provides calculations of projected atmospheric emissions, as well as the contents of solid and liquid waste streams (Zimmermann, 1997). Batch Plus is geared to large pharmaceutical companies with complex recipes.

The adaptation of an existing flowsheeting program (e.g. Aspen Plus) to develop a biochemical process simulator (such as Aspen BioProcess Simulator™) has been criticized by some as being inefficient (Gritsis and Titchener-Hooker, 1989). According to Gritsis and Titchener-Hooker (1989), the simulator may still preserve certain attributes originating from its chemical engineering parenthood which make it either difficult to use or inappropriate in some biochemical processing tasks.

With this opinion in mind, a research group at the University College London (UCL) used research information and data to develop models that describe individual biochemical engineering unit operations and processes (Gritsis and Titchener-Hooker, 1989). These were solved using numerical techniques and simulation tools capable of carrying out dynamic simulation, such as SPEED UP. Models for precipitation, centrifugation and ultrafiltration were developed and were used to simulate a separation train consisting of these three consecutive unit operations. They also optimized the performance of the ultrafiltration unit.

Later, they extended their models to simulate the purification of alcohol dehydrogenase (ADH) (Zhou et al., 1997; Lu et al., 1994). Two new unit operation models for fermenter and homogenizer were included in this simulation. They used g-PROMS, a new equation

oriented general modeling and simulation package for combined discrete and continuous process, to simulate the primary separation stages.

A pilot scale verification of the simulated results was carried out by Clarkson et al. (1994). It was found that interaction between the unit operations resulted in poor level of agreement for some streams. The models were improved to account for these interactions (e.g. particle breakage during centrifugation that reduces the efficiency of precipitation step). This group however, has not yet reported development of any model for high-resolution steps (i.e. chromatography), which have become an essential part of down stream processing of high value products. Their activity also seems to be confined to academic studies.

BioPro Designer® appears to be the second commercial bioprocess simulator to follow Aspen BioProcess Simulator™. Its development was initiated at the Biotechnology Process Engineering Center (BPEC) of MIT and it was later commercialized by Intelligen Inc. It is claimed to be an attempt in integration of various activities of process design such as process synthesis, analysis and evaluation into a coherent package (Petrides, 1994). This package uses algorithmic knowledge mainly in the form of short cut models for material and energy balances for the unit operations. It is also capable of carrying out equipment sizing and costing. All the unit operation models in BioPro are algebraic when specified for steady state (Aelion and Petrides, 1994). Dynamic models are also available for batch or semibatch operation (Petrides, 1994). It performs process scheduling to handle the effect of batch and semi-continuous operation on the estimation of material balance and sizing of equipment. The program estimates the cycle times of the various process steps given the starting time of each unit operation and generates a Gantt chart that graphically depicts the operation and turnaround times of each unit operation. BioPro Designer® has been used at New Jersey Institute of Technology as a teaching tool for process design. Senior undergraduate and graduate students used BioPro to build biochemical processes, consider structural alternatives, perform

sensitivity analysis on process parameters and conditions and analyze and evaluate several integrated processes for citric acid, sodium nitriloacetate etc. They also examined economic and environmental impacts of process modifications. The accuracy of the economic report was within ± 25%. The feedback of the students on using BioPro Designer® was positive (Aelion and Petrides, 1994). BioPro Designer® has also been used for simulation and economic evaluation of biosynthetic human insulin production (Petrides et al., 1995; Petrides et al., 1996). Ernst et al. (1997) used SuperPro Designer®, which is a combination of BioPro Designer® and EnviroPro Designer® both from Intelligen Inc., for analysis and cost estimation of production of heparinase I expressed in *E. coli*.

Biotechnology Design Simulator (BDS) from Life Sciences International (LSI, Philadelphia) is another batch-oriented product (Zimmermann, 1997). It can be used for scheduling of batch operations and resource utilization as a function of time (Petrides et al., 1996). BDS can integrate behaviors of vendor equipment models, such as specific chromatography column, into a given simulation. This is a feature which most of the other packages lack (Zimmermann, 1997). BDS can also integrate human decision-making guidelines into simulations, such as how shift changes affect technicians and managers. It considers environmental factors such as VOC emissions.

A novel bioprocess simulator called SIMBIOS is currently under development (Simon et al., 1994). It is being developed as a joint collaboration between Systems engineering Department of Research Institute of Chemical Engineering H.A.S., in Hungary and the Chemical Engineering Department of Graz University of Technology in Austria. There are two levels of calculation in this system: a) linearized models of unit operations for fast estimation of mass balances and b) in-depth calculation by rigorous, non-linear models. The first level of calculation is completed with a correction step. If the results are out of confidence limits then correction is carried out by rigorous models. When

developed, the simulator will also perform sensitivity of output variables with respect to input values.

Besides the integrated process simulators described above, there are several simulating tools available that simulate the fermentation process only. CAMBIO is such a software for dynamic modeling and simulation of biochemical processes (Farza and Cheruy, 1993). CAMBIO works by first developing a functional diagram of the reactions involved using a set of design symbols and mnemonic icons. From the functional diagram, a dynamic material balance equation of the process is generated automatically by taking advantage of the knowledge in the diagram. These equations are further modified by user supplied reaction rate expressions. The dynamic behavior of the process is immediately visualized by interfacing automatically with specialized software. Farza and Cheruy (1993) reports modeling of an anaerobic digestion process using CAMBIO.

Another bio-simulator called BIOSIM has been developed for simulation of biological fermenter and cell culture reactors (Cadman and Davison, 1989). The simulator consists of a model library, a model builder, a dynamic simulator and a data comparator. These are integrated to a single menu-driven system that works in PC. The introductory menu allows the user to review the available models, modify them or to develop a new model. Provided the required parameters, initial conditions and time of simulation, the simulator automatically determines and varies the integration time step in order to meet relative error criteria. A model parameter estimator is currently under development for BIOSIM. It has been used to model production of α-amylase from *Bacillus amyloliquefaciens*.

1.3. Scope and Objective

The overall objective of this research was to explore the possibility of designing large scale integrated bioprocesses using available biosimulation packages. Two bioprocess simulators were selected for this study:

i) BioProcess Simulator™ (BPS) from Aspen Technology and
ii) SuperPro Designer® from Intelligen Inc.

Production of tissue plasminogen activator (t-PA) from Chinese Hamster Ovary (CHO) cells was selected as a model process for this study. t-PA is a high value product for human use; therefore, downstream processing is an important part of the overall process. Since process information is proprietary, five alternate flowsheets were developed for this process based on information from technical literature, published laboratory scale production and purification methods for t-PA combined with engineering judgment and experience with other recombinant products. The flowsheet based on conventional fermentation norms for mammalian cell culture and standard purification methods for biopharmaceuticals was designated as the base case flowsheet. The alternate flowsheets were variations of the base case and differed in the medium type and mode of operation of the bioreactor, unit operation in downstream processing and scale up strategy. The first alternative to the base case was designed to evaluate the concept of two-stage cell culture. Use of serum and serum free medium was considered in the growth and production bioreactor respectively. For this flowsheet, the affinity chromatography columns of the base case were also replaced with ion exchange columns. The mode of operation of the bioreactor was switched from batch to fed-batch for the second alternative while in the third alternative the stirred tank type bioreactor of the base case was substituted by an airlift bioreactor. Since cell culture bioreactor cycles can last up to a week, in the fourth alternative, an economically sized single purification train was designed to process the product from multiple equal sized bioreactors instead of the

single large bioreactor of the base case. For comparison, the base case flowsheet was simulated using both simulators. Simulation of the other four flowsheets was carried out with BioProcess Simulator™ only since it allows detailed analysis of the simulated process.

Simulation of bioreactor is found to be as effective as the kinetic model supplied. BioProcess Simulator™ allows addition of user supplied kinetic models so that the success of simulation of the bioreactor was a matter of finding appropriate kinetics, formulated to account for the required details. Therefore, in this study more emphasis was placed on the design of downstream processing as a part of the integral process. The high purity requirement of biotherapeutics signifies the importance of downstream processing for the t-PA production process. Process development with BioProcess Simulator™ was a trial and error approach. Repeated simulation runs were carried out to design flowsheets that satisfy the predetermined values of purity, recovery and batch time.

An advantage of using a process simulator for process development is that it allows economic evaluation of the simulated process as a part of the package. Economics of the simulated flowsheets were evaluated by combining information from both, BioProcess Simulator™ and SuperPro Designer®. Economic performance was measured in terms of Return on Investment (ROI) and Gross Margin (GM).

Since the simulated flowsheets are based on information from literature, there were some parameters for which estimates could not be found. BioProcess Simulator™ was used to carry out sensitivity study of the simulated processes to uncertain parameters and variables to determine which of the unknown parameters are critical. This study also indicated the areas where research should be focused to improve process performance.

The feature of BioProcess Simulator™ that allows addition of user supplied kinetic models was utilized to compare some unstructured models for mammalian cells. The kinetic models were added as FORTRAN subroutines to the main bioreactor model. BioProcess Simulator™ generates concentration profiles for substrates and products according to the supplied kinetics to be used for time dependent specific growth rate calculation. The model also allowed user defined variables and parameters to be varied to study the effect of these quantities.

In summary, the specific goals of the research were:

- to critically evaluate the applicability of two commercially available bio-simulation packages for development of large scale integrated bioprocesses. To date, evaluation of the bioprocess simulators has been reported by the developers only. This research will evaluate the simulators from a more independent user's perspective.

- to study some innovative concepts in bioprocess design such as two stage cell culture, fed-batch operation and multiple bioreactor scale up for large scale process with the aid of the simulation packages and

- to examine the applicability of the simulator for economic evaluation and sensitivity analysis, that improves reliability of the results obtained.

1.4. Contributions of the Research

The significant contributions of this research are:

- Identified the strengths and weaknesses of Aspen BioProcess Simulator™ for bioprocess simulation. Although, BioProcess Simulator™, equipped with rigorous unit operation models and model analysis tools, appears to be a comprehensive

flowsheeting package at a first glance, some of the unit operation models in the simulator were found not to function properly.

- Reported on first comparative study of BioProcess Simulator™ and SuperPro Designer® for bioprocess simulation at process level.

- Developed a comprehensive process and economic "investigative basis" for further research in bioprocess simulation study by others.

- Developed a detailed process description including operating and design parameters of a proprietary process. This information was used in simulation, identification of critical parameters and economic evaluation of the process.

- Demonstrated the economic potential of novel process concepts.

1.5. Organization of the Thesis

The Introduction (Chapter One), that provides background for the research, is followed in Chapter Two by a Literature Review on the selected simulation packages (Aspen BioProcess Simulator™ and SuperPro Designer®) and the process (production of t-PA from CHO cells). Chapter Three describes the simulation methodology used with each simulator. The procedure adopted for economic evaluation, sensitivity analysis and kinetic study are also discussed. The design basis for the simulated processes is presented in Section 3.5. A general process description for large-scale biopharmaceutical production (Section 3.6) is included in this chapter whereas, a brief process specific description of each process flowsheet is presented in Chapter Four (Section 4.1.2, 4.2.2, 4.3.2, 4.4.2 and 4.5.2).

Chapter Four is the main section of the thesis, which presents and discusses the simulation results and results of the economic evaluation of each process. Each of the alternate processes is compared with the base case. A sensitivity study of process specific uncertain variables is presented at the end of each section to show how the process performance would change due to uncertainties arising from these sources. All the simulation results are further summarized at the end of this chapter for ease of comparison.

Chapter Five addresses sensitivity study to parameters and variables common to all the processes. Chapter Six is an extension of application of BioProcess Simulator™ to comparison of unstructured kinetic models. The conclusions and recommendations for future work are presented in Chapter Seven.

CHAPTER 2

LITERATURE REVIEW

2.1. THE SIMULATORS

Aspen BioProcess Simulator™ (Aspen Technology Inc., 1995) and SuperPro Designer® (Intelligen Inc., 1994) appear to be the most popular commercial simulators for bioprocess so far. Table 2.1.1 summarizes the basic features of BioProcess Simulator™ and SuperPro Designer®. Both of these simulators were used to simulate the base case of the t-PA production process. A detailed comparison of their performance at process level has been carried out in section 4.1.3 and 4.1.5. The economic evaluators of the simulators have been compared in section 3.2.

2.1.1. Aspen BioProcess Simulator™

Aspen BioProcess Simulator™ was developed by modification of its parent Aspen Plus, a third generation process simulator for chemical process industries (Bhattacharya and Motard, 1986). BioProcess Simulator™, therefore shares the well-developed simulation features of Aspen Plus, such as model analysis tools. Model analysis tools include process optimization, case study, data fit and sensitivity study of the process parameters and variables to process performance (Aspen Technology Inc., 1995). The 'Design Specification' feature allows BioProcess Simulator™ to design a unit operation block according to the required specification. Initially developed for steady state process,

BioProcess Simulator™ has several batch unit operation blocks typical to bioprocessing. The ordinary differential equations describing the batch operation are solved using Gear's method (also known as Backward Differentiation Formula). Users can manipulate the integration parameters such as integration step size, magnitude of integration error etc. There are several convergence methods (e.g. Wegstein's, Secant, Broyden, SQP etc.) available for recycle streams. The convergence parameters are also adjustable. Aspen allows user specified convergence options, user selected tear streams and calculation sequence for the convergence block.

Besides these tailorable calculation methods, BioProcess Simulator™ has the capability of modifying the report options in several ways. BioProcess Simulator™ package allows customized reports to be printed out. In addition to block and stream reports, it can be specified to generate property report, utility report etc. Another important feature of BioProcess Simulator™ is the flowsheeting option. It allows addition of subroutines which are essential requirements for bioprocess simulation (Shanklin et al., 1999).

BioProcess Simulator™ package uses an expert system to assist setting up the flowsheet. The expert system prevents over- and under-specification of the flowsheet (Grob, 1993).

Aspen is capable of handling process streams of any complexity, including solid flows and slurries. The built-in data banks contain over 1500 components (Grob, 1993). It also allows the definition of new data structures to store and manipulate data on new and unusual species and streams (Bhattacharya and Motard, 1986). Using the provisions to store new property models, the requirements of biomass, nutrients, bioproducts and electrolytes typical of biochemical processes can be incorporated. Aspen BioProcess Simulator™ is equipped with costing and economic evaluation routines and data banks that allow process evaluation and technological assessment on a common basis. It generates economic report in terms of capital cost, operating cost and profit, by calculating equipment, labour, material and utility cost.

BioProcess Simulator™ has been used for process development by many pharmaceutical companies such as Novartis Pharmaceuticals Corp. (E. Hanover, NJ), Eli Lilly & Co. (Indianapolis, IN), Genentech (S. San Francisco, CA), Merck (Whitehouse Station, NJ) etc.

2.1.2. SuperPro Designer®

SuperPro Designer® on the other hand was developed specifically for bioprocesses with the ability to assess environmental impact. It consists of three main components: user interface, synthetic component and analytical component. The synthetic component contains experiential knowledge mainly in the form of heuristics that result in the synthesis of a feasible flowsheet. The analytic component consists of algorithmic knowledge in the form of models for the unit operations that enable analysis and evaluation of the synthesized flowsheet. Its unit operation models allow simulation of integrated biochemical, pharmaceutical, food, specialty chemical as well as waste recycling, treatment and disposal processes. Some new models for product formulation and packaging (e.g. extrusion, blow and injection molding, filling, labeling, boxing etc.), solids handling (e.g. silo, hopper etc.) and transportation have been added to the latest version (v3.0) of SuperPro Designer® (Petrides, 1998).

It carries out environmental impact assessment and VOC emission calculations as a part of the simulation. The unit operation block models are based on short-cut algebraic equations. It is, therefore, useful when detailed information is not available for a process. These equations can be specified for steady state, batch and semibatch operation. Many of the unit operation models can be used either in design or in rating mode as required. Super Pro carries out material and energy balances for integrated flowsheets. It estimates equipment sizes and purchase costs. It is equipped with an economic evaluator capable of analyzing detailed economic report. SuperPro handles a mixed mode of unit operations by scheduling batch and semicontinuous processes. It has built-in library for

process equipment, heating/cooling agents and construction material. It has built-in database for chemical components. A user expandable mixture database for buffers etc. has been added to version 3.0 of SuperPro Designer® (Petrides, 1998). It also allows addition of new components to its component database if required. Physical properties can be modified and economic data updated.

SuperPro uses Weigstein's method of numerical convergence of material and energy balances. It allows modification of convergence parameters such as maximum number of iterations, value for acceptable tolerance etc. (Intelligen Inc., 1994). Like Aspen BioProcess Simulator™, it also allows scaling up or down of all input stream flows by a user-specified factor.

The Pro Designers are intermediate level simulators, ideal for a "first crack" at a design and can operate with minimal data, using initial default values (Zimmermann, 1997). They are limited to the Windows platform. The user-friendly design shortens the learning time and allows designers to quickly exploit the software's functions.

Material balances in both SuperPro and Aspen BioProcess Simulator™ are estimated using the sequential modular approach (Petrides, 1994). The various unit operations in a flow sheet are sequenced according to their calculation order. Composition and flow rate of outlet streams are calculated given the inlet stream and some specific engineering information for the units.

SuperPro is used as a design tool by companies such as Smith Kline Beecham, E. Merck (Germany), Bristol-Myers Squibb, Eli Lilly, Rhone-Poulenc Rorer, Genzyme, John Brown, Merck & Company, Novo Nordisk (Denmark), Pfizer and Fluor Daniel etc. (Intelligen Inc., 1994).

Table 2.1.1: Features of Aspen BioProcess Simulator™ and SuperPro Designer®

	Aspen BioProcess Simulator™	SuperPro Designer®
Unit Operation Blocks	commonly used blocks (e.g. fermenter, chromatography, centrifuge, ultrafiltration etc.)	common blocks as well as blocks for specialized operation (e.g. product formulation, packaging, solids handling etc.)
Model Equations	rigorous algebraic and ordinary differential equations for batch operations	shortcut, algebraic models
Input Requirements	detailed information required for each block	less input information required
Capabilities	Simulation Optimization of units and flowsheet Design Specifications Sensitivity Study Case Studies	Simulation Economic Evaluation Process Scheduling Environmental Impact Assessment
Data Banks	component, physical property	component, physical property, construction material, mixture
Modifications	allows addition of subroutines e.g. growth rate, mass transfer correlation etc.	not possible with this version

2.2. THE PROCESS

2.2.1. Process Selection

Selection of a process for simulation was dictated by several criteria. First of all, it was decided to simulate an integrated process rather than separate units since study of an integrated process allows for the exploration of the interactions among the unit operation blocks and to identify the effect of upstream sections on downstream processing. For the necessity of process validation, it was also decided that the selected process should be an existing and established one. Importance was given on processes that involves typical biochemical unit operation blocks such as membrane separation and chromatography. The economic importance of the process was another criteria. Since a major advantage of process simulation is to reduce the process development cost, processes that involve a huge sum of money for development were searched for.

Recombinant protein therapeutics have enormous economic potential (Figure 2.1.1) (Dunnill, 1987). Genetic engineering has been successful in producing large quantities of pharmaceuticals (e.g. human growth hormone, alpha-interferon, t-PA, insulin etc.) that were otherwise scarce. It has increased productivity, almost in a dramatic fashion of many pharmacologically active products and made them available commercially. Approximately 22% of the $9 billion domestic sales of prescription drugs in US in 1984 came from products for which genetics and fermentation biotechnology played a significant role (Elander, 1989). It is reported that, in 1995, the annual sales of diagnostics and therapeutics resulted in over $5 billion dollars (Cooney, 1995). Leading biotech drugs broke over $1 billion in annual sales in 1997 (Business Cover Story Biopharmaceuticals, 1998). Although the selling price of these products is high, the costs of commercial recombinant DNA work are also considerably higher. Schering Plough invested $6 million in a pilot scale bioprocessing and purification facility, Genentech raised $32 million for clinical test and development of t-PA, Eli Lilly invested $60

million in facilities to produce human insulin, to mention a few (Elander, 1989). This represents an area where the benefits of process simulation can be fully utilized.

Production of recombinant therapeutics by genetic engineering is also complicated by the fact that recombinant cells are very difficult to work with because of their fragility and genetic instability. Moreover, the very high purity requirement of the product makes downstream processing a very significant part of the overall process.

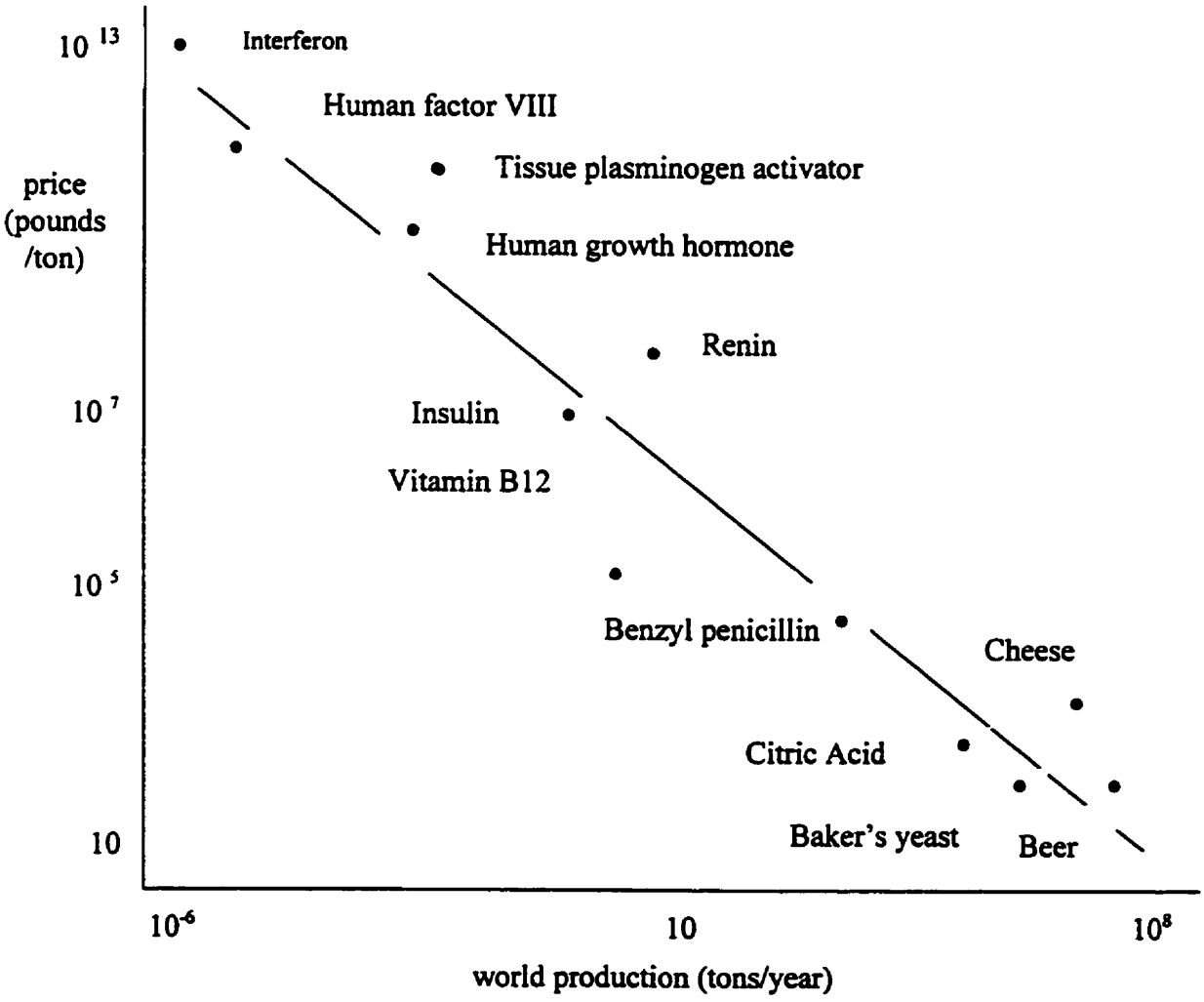

Figure 2.2.1: World Production levels and prices of the products of fermentation (Dunnill, 1987)

In light of the above reasons, production of t-PA from recombinant CHO cells was selected to be a suitable process. Production of t-PA represents by far the largest scale production of a recombinant protein in animal cells. Tissue Plasminogen activators are a group of proteolytic enzyme that finds application in the treatment of cardiovascular and cerebrovascular obstructions (i.e. heart attack and stroke). The fact that, in the western world, these represent a major cause of death in men and women over the age of 40, underlines the importance of this cellular product. During the last two decades, serious efforts were made to characterize and produce this enzyme in large scale. It finally became commercially available when Genentech developed and obtained FDA approval for its large scale production in 1987 (Lubiniecki et al., 1989). The cell line has been patented while the production method has not been made public. Genentech produces t-PA at a purity of greater than 99.999%. Worldwide annual sales of t-PA resulted in $261 million in 1997 (Business Cover Story Biopharmaceuticals, 1998). The current selling price of a gram of t-PA is about $16,000 (Cooney, 1999).

2.2.2. Literature Review on Selected Process

2.2.2.1. INTRODUCTION

2.2.2.1.(a). Function of t-PA

Thrombosis is the blockage of blood circulation by deposited fibrin clot or thrombus and this is a major cause of death in the western world. Plasminogen activators are thrombolytic agents. These are a group of proteolytic enzymes that act on plasminogen and convert it to plasmin. Plasmin is a powerful fibrinolytic agent, which is capable of initiating fibrinolytic process to breakdown fibrin (Figure 2.2.2).

There are two recognized classes of human Plasminogen activators, tissue type (t-PA) and urokinase type (u-PA). Differences in biological and immunological properties between these two types have been observed. There is fortuitous plasminogen activator

of bacterial origin - streptokinase. Streptokinase is an extracellular protein released from various strains of *Streptococci.* Streptokinase acts by forming a complex with plasminogen first in order to activate it.

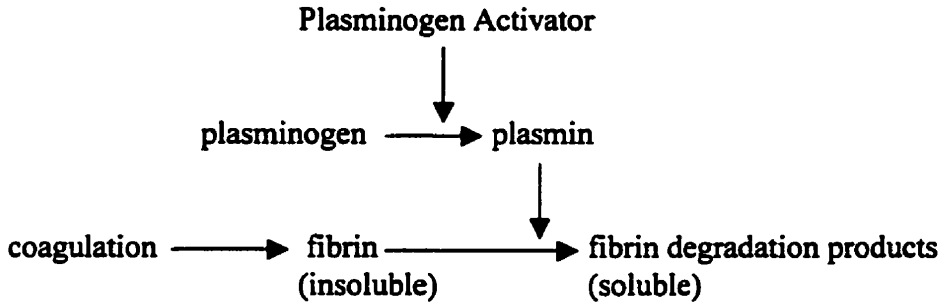

Figure 2.2.2: Fibrinolysis

Commercially available Urokinase was first isolated from human urine. During the mid-to-late-1970's, development was underway to produce Urokinase from normal human cell cultures. However, the combination of low product yields and high medium costs impeded the commercialization of this process. During the early 1980's it was reported that a related enzyme, tissue plasminogen activator (t-PA), offers potential clinical advantage over Urokinase (Bliem, 1988).

t-PA is very specific in its action. It binds relatively strongly to fibrin clots preferentially activating plasminogen entrapped in blood clots. It does not alter the circulating plasminogen and other blood clotting factors. u-PA on the other hand, is relatively non-specific and activates both circulating and fibrin bound plasminogen. This indiscriminate activation leads to serious risk of hemorrhage. With Streptokinase antigenic reactions are common in addition to increased danger of hemorrhage (Butler, 1987). In view of these facts, t-PA is regarded as more effective and safer thrombolytic agent compared to others. The recent activities are devoted to develop second generation t-PA which are even more effective and longer lasting (Section 2.2.2.5).

Plasminogen activators are very widespread in the body. They occur in most body fluids (e.g. blood, lymph, urine, tears, saliva etc.) and in a wide variety of tumors and tissues.

2.2.2.1.(b). Structure of t-PA

t-PA is a serine protease with a molecular weight of approximately 70,000 Da. It is a glycoprotein containing 7% sugar and has an isoelectric point of 7.5.

It is composed of a single polypeptide chain containing 527 amino acids. It is synthesized within the cell as a polypeptide chain and released as a single chain enzyme.

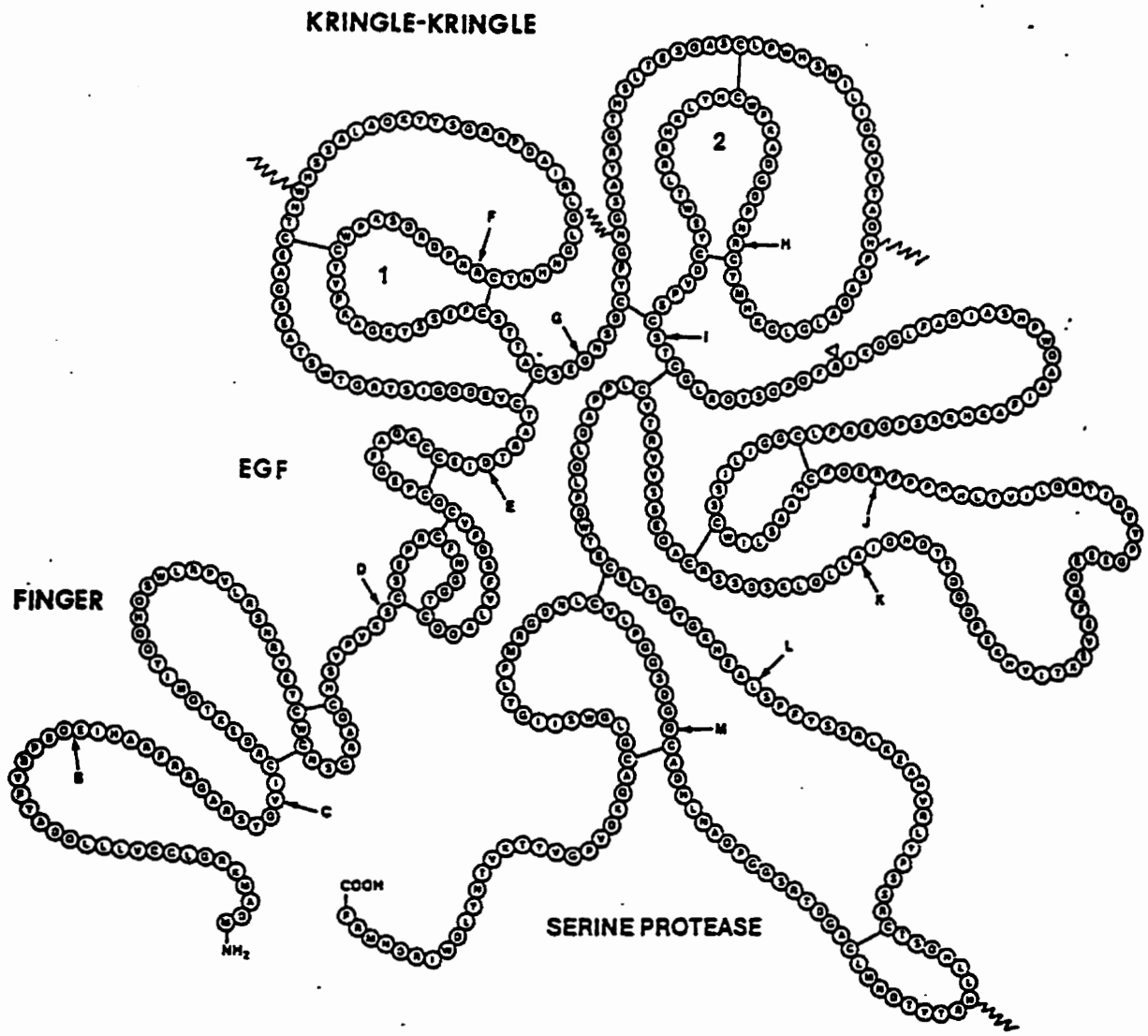

Figure 2.2.3. Structure of t-PA molecule (Klausner, 1986; Pohl et al., 1984)

Upon limited proteolytic action by plasmin in the extracellular medium, the molecule is cleaved and converted to a two-chain activator linked by an inter-chain di-sulphide bond. The t-PA molecule is composed of a number of discrete regions (Figure 2.2.3). A finger domain near the N-terminus, an epidermal growth factor (EGF) domain that is common to several serine proteases, a di-sulfide looped kringle-kringle structure similar to constructs found in plasminogen and urokinase and a carboxy-terminal serine protease domain.

There are four glycosylation sites in t-PA molecule at positions 118, 186, 218 and 448 (Griffiths and Electricwala, 1987). The site at 218 never appears to be glycosylated (Datar et al., 1993). The middle site (186) is not glycosylated most of the time. Thus, depending on the number of carbohydrate groups t-PA can be divided into two types: Type I (those containing three carbohydrate groups) and type II (with two carbohydrate groups).

2.2.2.2. Production of t-PA

The potential therapeutic application of t-PA as fibrinolytic drugs for the treatment of cardiovascular obstructions (i.e. heart attack), cerebrovascular obstructions (i.e. stroke) and pulmonary thrombotic obstructions motivated the efforts for its large scale production. Clinical study at the beginning was limited by its insufficient supply. Its clinical use required a specific and efficient production method to give highly purified protein in high yield. Early attempts to produce large amount of t-PA from animal cells were unsuccessful (Table 2.2.1). Production of t-PA from Bowes melanoma cell line in relatively large amount enabled its clinical trials. Current production methods using recombinant technology results in high level of t-PA production.

The t-PA gene has been expressed in different systems such as mammalian cells, bacterial, yeast and insect cells. Except mammalian cells, all the other hosts were found to be unsuitable because t-PA was produced intracellularly and it was either

hyperglycosylated or hypoglycosylated. The level of production was also low in most cases (Lemontt et al., 1985; Sarmientos et al., 1989; Upshall et al., 1987; Oka et al., 1990; Schleuning, 1987).

The current commercial method of tPA production uses mammalian cells as host. It has been shown that t-PA essentially identical to natural t-PA can be produced in recombinant animal cells. A variety of animal cell lines have been reported to be successful for cloning and expression of t-PA. These include rat myeloma, mouse C127 fibroblasts, CHO cells and human melanoma cells (Lubiniecki et al., 1989). Available data suggest that CHO cells are generally better in terms of productivity than most other host cell lines studied (Table 2.2.2) although direct comparisons are difficult to make. While of comparable productivity to CHO cells, C127 cells are anchorage dependent. Scale up of C127 cells might, therefore prove more difficult than for CHO cells. All recombinant systems reported were found substantially more productive than non-recombinant systems such as Bowes melanoma (Table 2.2.1).

Table 2.2.1: Comparative yields of t-PA melanoma, epithelial and recombinant DNA cell lines expressing melanoma t-PA (Griffiths and Electricwala, 1987)

Cell line	production rate (pg/cell/day)	production rate (IU/ 10^6 cells /day)
Bowes melanoma	0.31	18
r Bowes melanoma	3.10	180
Epithelial cell (GPK)	0.38	3
GPK (+ azacytidine)	1.26	10
GPK (+ concanavalin A)	5.80	46
r CHO	0.86	50
r CHO (+ methotrexate)	8.61	500

Most of the expression vectors used for t-PA production employed strong constitutive promoters and enhancers such as virus-derived or immunoglobulin regulatory elements to drive t-PA expression. Gene amplification technique was used to maximize the expression. Amplification by methotrexate (MTX) to increase yield of t-PA was first achieved by Genentech (Levinson et al., 1984) and has since been used in various constructs. However, the presence of MTX results in some less desirable consequences in addition to the increased production level. Several researchers have observed prolonged doubling time of the cells and in some cases, increased cell fragility with less efficient cell growth in stirred bioreactors.

Table 2.2.2: Reported yields of recombinant t-PA in animal cells (Lubiniecki et al., 1989)

Cell Type	System	Peak Titer (mg/L)	Specific productivity mg/10^9 cells/day	Group
rat	fed batch air lift	40	4 *	Celltech
myeloma	same	52	4 *	
CHO/SV40	petri dish	--	26 - 49	Genentech
CHO/SV40	perfused matrix	65	20	
CHO/Ad2	petri dish	--	10	Genetics Inst.
Mouse	petri dish	0.3	20	Integrated
C 127	perfused micro carrier fermentor	55	25 *	Genetics
Human melanoma	25 cm^2 flask	8	3.1	Beecham
Bowes ** melanoma	25 cm^2 flask	1	0.3	
	perfused micro carrier fermentor	0.1	0.1	

* averaged over entire fermentation

** non-recombinant cells shown for comparison

For the recombinant cell lines, it still remains to be established whether the stimulants used to increase productivity of animal cell culture can further increase the yield of tPA from these cells (Electricwala, 1987). Chen et al. (1998) observed that hyperosmolality leads to an increase in t-PA production by a CHO cell line. The specific rate of rt-PA production was found to increase with medium osmolality in the range of 330 to 450 mOsm, reaching approximately 1.6 times that obtained at physiological osmolality.

Along with DNASE, production of t-PA represents the largest scale production of recombinant proteins in animal cells (Cooney, 1999). Genentech, who pioneered production of t-PA in MTX (methotrexate)-amplified CHO cells, have retained CHO suspension culture as the commercial production method. Two production plants using this technology at 10,000 litre fermentation scale have been built at Genentech in the USA and at Boehringer Ingleheim in Germany (Lubiniecki et al., 1989). No major problems were encountered in scale-up of the system and the two plants were commissioned in 1987. Since then, quantitatively stable production of qualitatively consistent tPA has apparently been achieved.

Since the market for t-PA looks impressive, research in this area is literally clogged with competitors (Klausner, 1986). Several companies have evaluated high cell density bioreactors, which avoid the use of excessively large bioreactors, as an alternative approach to producing the biomass required for t-PA production at this scale. In these bioreactors, cell densities exceeding 10^8 per ml have been achieved instead of the few million per ml obtainable in classical suspension culture. An example is the 24 litre scale fluidized bed reactor for t-PA production developed by Verax Corporation. Recombinant CHO cells were entrapped in a collagen-based matrix in a reactor. The reactor was continuously perfused with a serum free medium at a feed rate of 330 litres per day. A steady state t-PA level of 65 μg/ml was obtained, corresponding to a daily output of 21.4 g of t-PA. This level of production was maintained stable for at least 10 days (Tung et

al., 1988). Similar quantities of t-PA have been obtained in other high-density mammalian cell culture systems based on perfused hollow fibre reactors (Tyo et al., 1988) and on perfused ceramic matrix (Berg and Bodeker, 1988). Choi et al. (1995) used a depth filter perfusion system for continuous production of t-PA from recombinant CHO cells. The system was equipped with a 40 μm polypropylene depth filter for cell immobilization. Final cell density with oxygen control was $1.8x10^7$ cells/ml of the total working volume and maximum t-PA productivity was 2.63 mg/L/day. Stable operation was possible for more than 20 days.

The difficulties of process control and reproducibility in high density culture systems caused by non-homogeneous condition in the reactor led to a compromise where cells in free suspension were maintained in a perfused reactor. Production of a t-PA like thrombolytic from CHO cells in a continuous perfusion bioreactor has been reported (Kallstrom et al., 1991). Critical nutrients were added and waste products were removed by the continuous perfusion stream. The cell density in the continuously perfused culture increased from $2X10^6$ cells/ml in batch culture to $10X10^6$ cells/ml by altering the medium flowrates. Optimal production occurred at cell density of approximately $5X10^6$ cells/ml. Production potential was found to be determined by the availability of nutrients and energy rather than inhibitor dilution. Volumetric productivity of the t-PA variant, compared to a batch culture, increased 15 to 18-folds using high perfusion rates (4 reactor volume/day).

Microcarrier culture of CHO cell for tPA production has also been extensively studied. Semi-continuous culture of CHO cells on microcarriers of gelatin and dextran has been evaluated (Nilsson et al., 1988). Significantly higher specific and total production rates were observed on gelatin microcarriers compared to dextran. Addition of 6-aminohexanoic acid to the medium was found to improve the specific production rate in both cases. Effect of medium components on the productivity of tPA by fibroblast cells on microcarrier has been studied (Takagi et al., 1994c). The same group developed an

algorithm for on-line determination of optimum time for switching from growth phase to production phase of tPA producing fibroblast cells on microcarrier. The optimum switching time was determined to be the time at which the tPA production activity of the cells in the growth culture became highest. The tPA production activity was found to be a function of cell concentration and oxygen consumption rate (Takagi and Ueda, 1994b). They also studied an oxygen supply strategy for microcarrier culture of the same cell line for t-PA production. An air spray system was used to maintain high oxygen transfer rate while the carriers were suspended by addition of soluble starch to the medium accompanied by slow agitation (Takagi et al., 1994a). Celltech developed a microcarrier process at 40 L scale for tPA production from mouse C127 cells. tPA concentration in this perfusion reactor rose as high as 55 mg/L (Rhodes and Birch, 1988). This seems to be the largest microcarrier culture for tPA production reported so far.

A negative feedback system was observed by some during the synthesis of t-PA in cell culture. When the extracellularly released t-PA level reached a certain concentration in the medium, the rate of cellular synthesis was found to decrease. This method of synthetic regulation, which is very unusual for an extracellularly released product, suggests that some of the t-PA may re-enter the cells, which was confirmed by various observations (Kadouri and Bohak, 1983). t-PA concentrations around 50 mg/L were found to be toxic to cells. In monolayer cultures, cells were found to detach from substrate as these levels were approached (Kaufman et al., 1985). For maximum productivity, the concentration of extracellular t-PA must be kept low to minimize the negative feedback of its biosynthesis. This could be done by either using a high ratio of medium volume to cell number or perfusing the system continuously. A more economical alternative would be to recycle the medium through a column capable of in-line extraction of t-PA (Kadouri and Bohak, 1983).

2.2.2.3. Purification of t-PA

2.2.2.3.(a). Animal Cell Culture

Information is not available on the large scale purification method of tPA that uses recombinant cells for its production. In laboratory, t-PA has been isolated from many organs and cells. This section considers the purification scheme used in laboratory scale purification of tPA.

Many of the methods used for purification of t-PA are lengthy and employed very harsh conditions. Chaotropic solutions of potassium thiocyanate or buffers of low pH have been used in some cases. These methods resulted in the recovery of a few milligrams of enzyme from several kilogram of starting material. Table 2.2.3 (Griffiths and Electricwala, 1987) lists several purification methods used to recover t-PA from different sources together with their molecular weight and specific activity. The specific activities listed in this table are as quoted in the literature. They are expressed in different units and when expressed in the same units (against a reference International standard of human urokinase) they show wide variation with respect to different sources. The molecular weight of purified plasminogen activators also varies considerably ranging from 35,000 to 85,000 Da. Such differences might have been caused by the diverse range of species, tissues and cell lines that have been used as a source of t-PA. This may also be a result of limited proteolysis of the native protein. This table considered only those methods were the enzyme was characterized and purified to homogeneity.

It is observed from the table that the major step in the purification scheme in all the cases (except the first one) is chromatographic separation. The methods differ in the different type of chromatographic material used e.g. metal chelate, Con-A, n-Butyl agarose, Octyl Sepharose, Hydroxyapatite, lectin or lysine/arginine etc. (Table 2.2.3 and Table 2.2.4). Some methods used an extraction, precipitation or a fractionation step before chromatography. The final step in all the cases is gel filtration. t-PA produced from human embryonic lung cells (HEL cells) has been shown to be purified by a two step

method (Brouty-Boye et al., 1984) involving affinity chromatography on fibrin/Celite to remove the non-fibrin binding proteins such as urokinase-like plasminogen activator followed by gel filtration to eliminate protein contaminants of high molecular weight.

Table 2.2.3: Laboratory scale purification of tissue plasminogen activator (Griffiths and Electricwala, 1987)

Source (Ref.)	Specific activity	Molecular weight	Purification method
hog ovaries (Griffiths and Electricwala, 1987)	100-175,000 units/mg	60,000	NH_4SCN extraction acid precipitation zinc fractionation sephadex G-200 gel filtration
human blood vessel perfusate (Binder et al., 1979)	10-40,000 CTA units/mg	70-75,000	$(NH_4)_2SO_4$ fractionation reverse $(NH_4)_2SO_4$ solubilization octyl-sepharose chrom. sephadex G-75 gel filtration sephadex G-150 gel filtration
human uterus (Ranby et al., 1982)	16,000 IU/mg	64-69,000	acetate buffer extraction $(NH_4)_2 SO_4$ precipitation zinc-chelate agarose chrom. n-butyl agarose chrom. con-A agarose chrom. sephadex G-150 chrom.
human blood vessel perfusate (Allen and Pepper, 1981)	50,000 CTA units/mg	56,000	polyethylene glycol precipitation hydroxyapatite chrom. sepharose 6B gel filtration

			lysine/fibrin agarose chrom.
pig heart (Wallen et al., 1982)	250,000 IU/mg	64,000	$(NH_4)_2SO_4$ fractionation adsorption on fibrin sephacryl S-300 chrom. arginine-sepharose chrom. sephacryl S-200 chrom.
human melanoma cell (Rijken and Collen, 1981)	90,000 IU/mg	72,000	zinc chelate agarose chrom. con-A agarose chrom. sephadex G-150 gel filtration
human neuroblastoma cell (Noll et al., 1985)	934,000 units/mg	37,500-66,500	$(NH_4)_2SO_4$ precipitation affi-gel blue chrom. p-aminobenzaaminidine sepharose chrom.
rat brain tumour cell (Bykowska et al., 1981)	-	60,000	zinc chelate agarose chrom. con-A agarose chrom. sepharose G-150 gel filtration
human embryonic lung (Brouty-Boye et al., 1984)	10-40,000 CTA units/mg	56,000 & 83,000	fibrin celite affinity chrom. ultrogel ACA 44 gel filtration
human melanoma cell (Wallen et al., 1983)	220,000 IU/mg	72,000	immunoaffinity chrom. arginine-sepharose chrom. sephadex G-150 gel filtration
RSV infected chick (Kacian and Harvey, 1985)	477,000 units/mg	48,000	fibrin celite affinity chrom. p-aminobenzaaminidine sepharose chrom. ultrogel ACA 22 gel

			filtration
human and guinea pig epithelial cell (Electricwala and Atkinson, 1985)	6,000 & 12,500 IU/mg	62,000	zinc chelate agarose chrom. con-A agarose chrom. ultrogel ACA 44 gel filtration
human melanoma cell (Einarsson et al., 1985)	200,000 IU/mg	65,000	immunosorbent chrom. arginine sepharose chrom. sephadex G-150 gel filtration
human melanoma cell (Kruithof et al., 1985)	80-100,000 IU/mg	67,000	SP sephadex chrom. sephadex G-100 gel filtration

Purification of t-PA from Bowes melanoma cell line, that secretes elevated amounts of t-PA compared to other cells, usually involves affinity chromatography and a gel filtration step. One preparation used immunoadsorption on antibodies as a major step in purification (Wallen et al., 1982). With the availability of monoclonal antibodies to melanoma t-PA , immunoaffinity chromatography has been used in another case as a single step purification method (Reagan et al., 1985). This was the only method among those tested that was able to separate tPA from uPA completely (Schaumann et al., 1990). tPA has also been purified using its affinity for more or less specific serine protease inhibitors. Thus p-benzamidine Sepharose has also been used to bind tPA (Harakas et al., 1988).

It is worth pointing out that all of these purification methods were carried out in laboratory scale. Many laboratory scale purification methods are not truly scaleable to manufacturing levels and should be avoided. Methods involving ammonium sulfate precipitation at an early, large volume stage, preparative reverse phase HPLC or preparative electrophoresis are inappropriate for large scale (Tolbert, 1988).

Table 2.2.4: Different Chromatographic media used for lab-scale purification of t-PA

Type of Chromatography	Chromatographic Medium Used	Function
Hydrophobic Int.	Octyl - Sepharose	----------
Affinity	Zinc-chelate Agarose	binds all plasminogen activator
Hydrophobic Int.	n-Butyl Agarose	----------
Affinity	Con-A Agarose	separates u-PA and t-PA fraction
Hydrophobic Int.	Hydroxyapatite	----------
Affinity	Lysine/Fibrin Agarose	biospecificity for t-PA
Affinity	Arginine Sepharose	positive charge
Affinity	Affi-gel Blue	----------
Affinity	p- aminobenzaminidine	serine protease inhibitor
Affinity	Fibrin Celite	removes non-fibrin binding protein
Affinity	Immunoaffinity Chrom.	monoclonal antibody to t-PA
Affinity	Immunosorbent Chrom.	antibody to t-PA
Ion-Exchange	Sp- Sephadex	----------

Purification on the laboratory scale tends to be based on analytical techniques, using expensive and sophisticated methods (Electricwala, 1987). Highly purified tPA has been obtained by these methods without much importance being paid to the overall recovery. On the laboratory scale, the objective is to achieve high yield but the main emphasis is on purity. Whereas on the industrial scale, high yield reproducibility together with the maintenance of high purity are desirable. Thus, on the large scale, the factors governing the purification scheme are somewhat different. Some of the major differences between analytical and large scale purification methods have been presented in Table 2.2.5.

Techniques such as ultrafiltration, ion exchange chromatography, immunoaffinity or other types of affinity chromatography and gel filtration have been successfully used in

large scale purification. Scale up of chromatographic methods can often present a problem.

Table 2.2.5: Differences between Analytical and Large Scale purification (Electricwala, 1987)

	Analytical	Large - Scale
Sample Size	small	large
Chromatography flow rate	slow for high resolution	fast for high
Time required	not important	throughput
Yield	poor to high acceptable	important
Use of expensive technique (s)	acceptable to achieve	high yield required
	purity	limited acceptance

In a laboratory scale, small flow rate is maintained through chromatographic columns to achieve high resolution in contrast to fast flow rates on large scale, which are necessary to handle the large throughput. Higher flow rates are handled on an industrial scale by using larger diameter columns or stacked columns. Similarly laboratory scale gradient elution is replaced by step elution on the industrial scale because gradient elution is difficult to perform reproducibly.

The time required for purification is not important on the laboratory scale. But on the industrial scale it affects the cost of the final product and, if the enzyme is unstable, the yield diminishes with time. The time required by gel filtration is dramatically reduced by using 'fast flow' matrices such as Sephacryl on the industrial scale.

2.2.2.3.(b). Recombinant Cells

t-PA is produced as soluble, mature protein in animal cells. In an appropriately selected medium, purification from animal cells presents no particular problem. The purification procedure developed (Rijken and Collen, 1981) for cultured human melanoma cell line

has been adopted by others to purify recombinant t-PA produced in *E. coli*, yeast, and mammalian cells (Heinrikson and Tomasselli, 1991). This method employs three column chromatographic steps. The first two steps are affinity chromatography on zinc chelate agarose and concanavalin A respectively. The third step is a gel filtration on Sephadex G- 150. Zinc chelate chromatography is preferred over ultrafiltration and precipitation for initial enrichment of the conditioned medium because it results in minimal loss of enzyme activity.

2.2.2.4. Analytical Methods

t-PA's complex physicochemical properties, higher molecular mass and variation in glycosylation pattern increases the possibility of the formation of undesirable product variants that can serve as a major impurity in the final product. It is probable that a highly purified preparation of t-PA will contain a large number of variants because of its inherent heterogeneity (e.g. the carbohydrate side chains) or because of degradative reactions such as deamidation. The two 'natural' variants of t-PA are type I and type II glycosylated forms (Section 2.2.2.1). In addition, t-PA can exist in a two-chain form as a result of proteolytic cleavage. The relative amount of these variants in a product depends on the culture conditions and the recovery processes.

The variation in glycosylation results in charge heterogeneity. Therefore, isoelectric focusing (IEF) gel electrophoresis can be used to differentiate between the variants. Gel permeation chromatography (GPC) can be used to determine the amount of 2-chain form in a given t-PA preparation. IEF can also be used to examine many degradative reactions. Degradation forms resulting from aggregation can be analyzed with size exclusion chromatography (Anicetti, 1989). Other analytical techniques used for the detection of contaminants in t-PA are presented in Table 2.2.6.

Table 2.2.6: Impurities and contaminants of concern and analytical techniques frequently employed for their detection in the QC of biotechnology-derived therapeutic proteins (Garg, 1991)

Impurities or Contaminants	Analytical technique
Proteinaceous contaminants (e.g. host cell proteins, other protein impurities)	SDS-PAGE electrophoresis, HPLC, immunoassays (ELISA etc.)
Endotoxin	Rabbit pyrogen test, LAL*
DNA	DNA dot-blot hybridization
Proteolytic degradation products	IEF**, SDS-PAGE, HPLC, N- and C-terminus analysis
Presence of mutants and other residues	Tryptic mapping, amino acid analysis
Deamidated forms	IEF
Microbial contamination	Sterility testing
Virus	Viral Susceptibility test
Mycoplasma	21 CFR method (Code of Federal Regulations)
General Safety	21 CFR 610.11

* Limulus amoebocyte lysate

** Isoelectric focusing

2.2.2.5. Recent Trends

Clinical experience with t-PA has been that the early claims of efficacy of t-PA were over emphasized in terms of the potential clinical usefulness of t-PA. The effectiveness of three thrombolytics- recombinant t-PA, microbial streptokinase (SK) and anisoylated plasminogen activator-streptokinase complex (APSAC) were compared in the third International Study of Infarct Survival (ISIS-3). Reports showed that the mortality for patients receiving t-PA was 10.3 percent, compared with 10.5 percent for APSAC and 10.6 percent for SK (Bluestone, 1992). The report also revealed that 3 out of 1,000 patients receiving SK suffered stroke, compared to six receiving APSAC and seven

receiving t-PA (Bluestone, 1992). However, fewer reports of allergy, hypotension and bleeding complexities were associated with the use of t-PA.

Therefore, researchers are looking for an improved t-PA, the so-called second-generation t-PA. The aim is to develop rival substances, t-PA mutants to improve the activity of t-PA and examine how adjuncts could increase t-PA's efficacy.

Three approaches are being considered for the development of a second-generation t-PA: 1) protein engineering, 2) modification of the cell line and 3) synergism strategies.

2.2.2.5.(a). Protein engineering

Protein engineering appears to be the most elegant way of creating a second-generation, improved t-PA. The objective is to modify the different domains of the t-PA molecule to obtain an improved product (Table 2.2.7). The finger, kringle and the epidermal growth factor regions are drawing the greatest attention. Modified t-PA without the kringle structure has been found to be much more stable in vitro, in the presence of inhibitors (Klausner, 1986). This finding shows the possibility of increasing the half-life of t-PA and thereby improving its therapeutic effectiveness. The finger domain looks like the fibrin-binding site of the t-PA molecule. Investigators are looking for methods for exposing the fibrin-binding sites more and engineering additional fibrin-binding sites into the molecule. Emphasis is placed on decreasing the size of the molecule by eliminating inactive parts and reducing the required dosage. Since the interactions between the different regions of the t-PA molecules are not yet clearly understood and changing one region of t-PA can have profound effect on the activity of another, only painstaking research will provide evidence on these novel possibilities achievable by protein engineering.

Several second-generation t-PA have already been developed. Reteplase has only two of the five protein domains, the kringle-2 and protease domain. The alterations have

increased the half-life of the protein in human plasma from 3–6 minutes to 16-18 minutes. The absence of F domain has made reteplase more effective in disrupting clots but it has also increased the possibility of unwanted bleeding (Davidson, 1997). In another second-generation t-PA, NPA, developed by Genetics Institute (Framingham, MA) and undergoing clinical trials by Bristol-Myers Squibb (BMS, New York), the F and E domains have been removed and amino acids in the molecule have been changed at three different sites (Davidson, 1997).

Table 2.2.7: Overview of approaches to development of second-generation t-PA (Cartwright, 1992)

	Biological effects			
t -PA domains deleted	Fibrin binding	Fibrin enhancement of plasminogen activation	Binding to lysine sepharose	Initial plasma half-life
F	reduced	unchanged	unchanged	increased
E	reduced	unchanged	NA	increased
F, E	reduced	unchanged	unchanged	increased
F, E, K1	reduced	reduced	unchanged	increased
F, E, K2	abolished	abolished	unchanged	NA
K1, K2	greatly reduced	abolished	abolished	NA
F, E, K1, K2	abolished	abolished	abolished	NA
Glycosylation site (s)	unchanged	unchanged	unchanged	increased

2.2.2.5.(b). Cell line

Perhaps the most important parameter for t-PA modification is the cell line. A new cell line has been used to prepare a kidney plasminogen activator (k-PA), which seems to be a precursor of urokinase. While t-PA circulates in the blood in an active state, k-PA

circulates as an inactive zymogen. It becomes active only when it comes into contact with bound plasmin at the site of a blood clot and breaks down the clot (Klausner, 1986). Various companies have reportedly achieved high production of t-PA using new types of cell line such as transformed myeloma cells. The role of glycosylation in t-PA activity is going to be explored in detail to enable investigators design an improved t-PA molecule. Enhancement of t-PA activity by application of glycoprotein remodeling technology will be considered. The possibility of producing a longer-acting t-PA molecule by its modification with polyethylene glycol (PEG) will be examined.

2.2.2.5.(c). Synergism Strategies

Instead of trying to improve the t-PA molecule itself, a number of commercial concerns are working on products that could be complementary to t-PA. Secretion of a recombinant hybrid plasminogen activator (t-PA/u-PA) by mouse myeloma cells has been reported (Pierard et al., 1989). 10 mg of t-PA with 3 mg of k-PA has been reported to be used successfully for heart attack patients. Synergism has reduced the normal dosage of pure t-PA from 60 -100 mg to a fraction of it. Another set of molecules that could be synergistic with t-PA are protein S and protein C. Protein S is a cofactor to the anticoagulant protein C. Protein S localizes protein C's activity to where it is needed and these two have been found in vitro to accelerate the action of t-PA. Researchers are also looking at monoclonal antibodies to increase t-PA's performance by steering it by the antibody to the vicinity of blood clots. They are also looking at genetically re-engineered antibodies to engineer the thrombolytic agent into the antibody or to create an antibody with dual specificity, both to the clot and the thrombolytic agent.

Nitromed (Boston, MA) has developed a nitrosylated t-PA variant by combining the fibrinolytic activity of t-PA with the antiplatelet effect of nitric oxide (Davidson, 1997).

2.2.2.6. Concluding Remarks

tPA has played a significant role in the establishment of cultured recombinant animal cells as a practical and proven technology for drug manufacture. Development of its production process has involved study of a large number of species, cell lines, and stimulants. To increase the production level, various expression system and promoters have been evaluated for recombinant t-PA. The recent trend of research in this field is directed towards the development of improved tPA mutants.

CHAPTER 3
METHODOLOGY

3.1. SIMULATION

The input files containing the process information supplied to the simulator to simulate the base case flowsheet described in Chapter 4 have been compiled in Appendix A. The corresponding output file showing the results of the simulation run can be found in Appendix B. In the simulations carried out, variables and parameters supplied and obtained as results, were cross checked with literature values where available, to ensure that they do not represent physically unrealistic situations. The information that served as guidelines for the choice of these quantities have been discussed under 'Process Description' in section 3.6.

3.1.1. Aspen BioProcess Simulator™

The following information is required to simulate any process with Aspen:

- components present in the process,
- properties of the components,
- flowsheet of the process,
- stream composition and flowrate and
- specifications for the unit operation blocks.

BioProcess Simulator™ can be used through a graphical user interface (GUI) called the Model Manager. With Model Manager, the required information is supplied to the simulator by filling out forms or menus. Model Manager performs degree of freedom calculation so that none of the blocks are over- or under-specified. After successful completion of the prompted forms, the user is asked to submit the input file to the simulation engine. A simulation run with BioProcess Simulator™ typically creates 24 files, four of which are important to the end user (*.input, *.report, *.history and *.backup, where * represents a wildcard of the file name). The input information supplied to model manager is summarized in the *.input file. The results are stored in the *.report file and the errors and warnings generated after the simulation are saved in the *.history file. The simulation run is saved as a *.backup file for future use. BioProcess Simulator™ can also be used on Unix platform. With Unix platform, the input information are written down as an *.input file instead of filling out forms.

COMPONENTS: The components present in the entire flowsheet, either as reactants or products, has to be defined in the simulation. BioProcess Simulator™ allows three types of component - conventional, non-conventional and biocomponent. Conventional components are molecular components such as solvents, solutes and gases. Examples of conventional components are water, oxygen, lactic acid, ethanol, ammonia etc. Non-conventional components are heterogeneous solids such as cells, cell debris and complex media components such as serum. In most cases, properties are not available for non-conventional component in the Aspen BioProcess Simulator™ data bank. Biocomponents are large biological molecules. This is a special type of conventional component that uses property models better suited for proteins and polysaccharides. An example is t-PA.

A total of thirteen components (glucose, glutamine, Hams F-12 medium, t-PA, BSA, CHO cells, oxygen, nitrogen, CO_2, elution buffers 1,2 and 3, equilibration and wash

buffers) were specified for most of the simulations reported in chapter 4. Glucose and glutamine contained in Ham's F-12 medium were specified as separate components. Glucose and glutamine free Ham's F-12 medium is essentially inorganic salt, of which 87% is NaCl and 9.3% is $NaHCO_3$ (Butler, 1987). Therefore, Ham's F-12 medium was specified as NaCl. The other conventional components specified are water, the eluants and wash buffers for the chromatographic columns, oxygen, nitrogen and carbon dioxide. The buffers were specified as solutions containing different concentrations of mainly HCl, NaCl and NH_4HSO_4 as described in literature. t-PA and BSA were specified as biocomponent (i.e. protein). CHO cell was defined as non-conventional component.

PROPERTIES: BioProcess Simulator™ has built-in library of models for property estimation of conventional components. Properties of bovine serum albumin (BSA) are available in the databank. Properties of components declared as biocomponents (i.e. FBS and t-PA) were defaulted to those of BSA. BioProcess Simulator™ calculates only two properties for non-conventional components - density and enthalpy. The user can supply other properties if required. The biodensity model BIODEN and bioenthalpy model BIOENTH were specified for the calculation of density and enthalpy of the cells. The BIODEN model calculated density as a second order polynomial of temperature ($\rho = a + bT + cT^2$). For CHO cells the density was set equal to that of water ($a = 1 g/cm^3$, b and c = 0). Enthalpy of non-conventional components is calculated from heat of formation (ΔH°_f) and a temperature-dependent heat capacity. The heat of formation is calculated from a hypothetical total combustion reaction (Biomaterial + Oxygen = Carbon Dioxide + Water + Nitrogen). Calculation of heat of combustion is based on 106 kcal of energy released per mole of oxygen consumed (Bailey and Ollis, 1986). Since heat capacity of solids is relatively temperature independent, the heat capacity was set equal to 1 cal/g-K (Aspen Plus, 1995).

For t-PA and Glutamine, additional property data specifying molecular weights were supplied.

FLOWSHEET: The simulator has to be supplied with information on flowsheet connectivity. All the unit operation blocks present in the flowsheet and their input and output streams were specified in this step and the simulator (model manager) generated a process flow diagram from the information supplied. Five different flowsheets were simulated for the t-PA process. These flowsheets differed in the fermentation media used, type and mode of operation of the bioreactor and the scale-up strategy. Details of the simulated flowsheets are presented in Chapter Four.

STREAMS: The compositions and flowrates of all feed streams entering the process must be specified. The feed streams identified for the t-PA production process, are the batch charge and the air-CO_2 mixture to the bioreactor and the wash, equilibration and regeneration buffer streams to the chromatography columns. The batch bioreactor model BFERM, requires one batch charge and at least one or more continuous feeds. A flowrate for the batch charge has to be defined since Aspen is designed as a steady state simulator. This flowrate, however, does not affect the flowrate of the product streams from the bioreactor. The media contains 90% Ham's F-12 medium, 10% Foetal Bovine Serum (FBS) and 4 mM Glutamine. The molar concentration of FBS was calculated to be 0.0015 mol/L, assuming a density equal to water and molecular weight of 66,000 (that of BSA). Water was defined as the solvent for the batch charge stream. Concentration of glucose in the batch charge was specified as 10 mmol/L and glutamine as 5.12 mmol/L (4 mmol/L free glutamine plus 1.123 mmol/L from Ham's F-12 medium).

For this aerobic fermentation, sterile air was provided to the bioreactors at an average rate of 0.08 VVM by an axial compressor through an air filter. This stream was specified to contain 5% CO_2. The specified flowrate is adjusted by the model to maintain the specified K_La and an air flowrate profile is generated in the report file. The equilibration buffer used in laboratory with Lysine Sepharose 4B, is 0.02 M phosphate, 0.15 M

sodium chloride and 0.01% Tween 80 (Dodd et al., 1986). For washing, a mixture of 0.02 M Tris, 0.5 M sodium chloride, 0.5 M L-arginine and 0.01% Tween 80 was employed. For ion exchange, the equilibration buffer was 0.1 M sodium acetate, 0.25 M sodium chloride, 0.05% sodium nitrite and 0.01% Tween 80. This buffer was also used to wash the column (Kruithof et al, 1985). The flowrate of the buffers were specified such that the superficial velocities were within the range reported in the literature (Section 3.6).

UNIT OPERATION BLOCKS: The input information required to simulate the different unit operation blocks is different. The typical values for operating variables and parameters for each of the blocks were obtained from the literature and supplied to the simulator. Detailed description of the process can be found in Section 3.6.

Bioreactor: The model equations for bioreactor (BFERM) in Aspen BioProcess Simulator™ simulate fermentation of microorganisms in stirred tank reactor in batch, fed-batch and continuous mode of operation. To apply the model equations to mammalian cell cultures, the unit for cell concentration had to be manipulated. In BioProcess Simulator™ the available unit for cell concentration is mass concentration (e.g. mg/L, g/L etc.). For mammalian cells it is customary to express cell concentration in terms of no. of cell /ml. Therefore, the cell concentration was converted to mass concentration for simulation by assuming that the weight of $3.8X10^9$ cells is equal to one gram dry weight (Zeng and Deckwer, 1995).

The required input information to simulate the bioreactor are inoculum concentration and volume, volume charge, substrate and biomass ID, heat transfer parameters, growth and product formation kinetics, k_La and power correlations. There are some optional input requirements (temperature, pressure, down-time etc.) which could be supplied by the user if the default values set by the simulator are not acceptable. Other optional inputs are required to avail the optional features (to plot the time profiles, to handle time-

varying feed streams, to control a variable by manipulating an inlet stream etc.) of the model.

The report file of BFERM generates result on mass and energy balance, dynamic composition in the fermenter, cell viability profile, substrate utilization and biomass formation rates, flowrates in vent and heat duty profile. Out of these, the dynamic composition of the different components is of major importance.

The model also carries out viability calculations provided a constant deathrate, although, in most practical situations deathrate is not a constant. For viability calculations the cells have to be supplied with a BIOSTATE attribute meaning that its state can change with time and passage from one unit operation to the other.

BFERM model has built-in correlations for the calculation of a) gas-liquid mass transfer coefficient, b) agitation power input, c) unaerated power and d) viscosity. It also contains built-in biomass growth reaction rate equations. In addition it allows users to construct their own correlation or add a new correlation as a subroutine. Instead of product and biomass yields, kinetics in the form of subroutines are also accepted for growth and product formation. The input variables in the user kinetics are the concentration of the different components, mass fraction of the different elements, temperature, pressure and total volume. The subroutine also allows user defined variables so that variables other than the ones supplied by the main program can be incorporated into the kinetic expression.

Membrane Separators: BioProcess Simulator™ uses the MEMBRANE and BMEMBRANE models to simulate continuous and batch or fed-batch operation of microfiltration and ultrafiltration units. The MEMBRANE model calculates the compositions and flowrates of both permeate and retentate stream exiting the continuous filtration unit. A recycle stream, an associated pump and a process stream cooler are

integral parts of the model. BMEMBRANE calculates the composition and flow rates of time-averaged permeate stream for batch operation of the same units. It calculates retentate composition as a function of time and also calculates concentration, recovery and pumping work. This model can simulate several different membrane operations such as concentration, continuous diafiltration and discontinuous diafiltration. Both the models have built-in flux models and models for calculating physical properties such as viscosity and diffusivity. It also accepts user supplied flux models.

Microfiltration: The inputs required to simulate the microfiltration unit are the feed, retentate and permeate pressure, recycle ratio or cross flow velocity, membrane geometry (type, area, length and diameter of the fibers etc.) and flux model with parameters. For the resistance in series model, the flux parameters are membrane resistance and gel resistance as a function of transmembrane pressure, Reynolds number and key component concentration ($Rg = Rg_0 (\Delta p)^a (Re)^b (C_b)^c$).

The model also calculates extent of protein denaturation if the fraction of protein denatured is provided.

During the simulation of the microfiltration, a balance had to be made between the concentration factor (feed/retentate ratio) and the recovery of t-PA. A low concentration factor reduced the amount of fluid (permeate) to be treated by the expensive affinity chromatography columns downstream but on the other hand lowered recovery of t-PA in permeate.

Ultrafiltration: The purpose of the ultrafiltration unit in the flowsheet simulated was to reduce the amount of fluid going to the next down stream unit. In contrast to microfiltration, retentate is the stream of interest in ultrafiltration where t-PA is concentrated by removal of water.

BioProcess Simulator™ simulates ultrafiltration using the same model (MEMBRANE) as for microfiltration. t-PA was defined as the key component for this unit. In the absence of cells, a slightly higher value for the transmembrane pressure could be used in this unit. Since there are no non-conventional components (i.e. cells or cell debris) present in the feed streams, the input streams to this unit were specified as conventional.

Chromatography: BioProcess Simulator™ has a general model for simulating affinity, ion exchange, reverse phase and hydrophobic interaction chromatography. The model applies to adsorption, wash and elution stages of operation. The model is based on the rate theory of mass transfer and accounts for film and pore diffusional mass transfer resistances for each solute of interest. It assumes equilibrium exists at each point along the length of the chromatography column (Aspen Technology, 1995).

There are some variations in the input requirements for these different types of chromatography. The main information required to simulate an affinity column with BioProcess Simulator™ are column length, diameter, particle diameter, velocity and end points of loading, washing and elution stages, elution profile, solute properties such as molecular weight, adsorbent capacity and adsorption isotherm constants etc.

The model calculates concentrations of products and contaminants for the eluate throughout each stage of operation. The stages are terminated by user supplied criteria based on product or contaminant concentrations. The model reports the number of columns required to the nearest integer number and the effective column usage. It also reports the recovery and purity of the components of interest. The model uses extended Langmuir isotherm to calculate binding equilibrium for multi-component systems and also allows the user to specify an adsorption isotherm. It allows the user to either use the built-in correlation or specify methods for estimating the mass transfer coefficients.

The affinity adsorbent (ligand) recommended by Pharmacia (Catalogue 1990/91) for serine protease such as t-PA is lysine sepharose. The loading, washing and elution velocities are found to vary over wide range in literature. The initial guesses were updated by looking at the response (t-PA concentration at column outlet) and pressure drop across the column. The fraction of eluate to be collected as product was also determined from the elution profile.

Gel Filtration: The model for gel-filtration reports the composition of the eluate as a function of elution volume. It estimates pressure drop across the bed and can plot concentration profiles to generate chromatograms. The model uses steric-volume theory to estimate the elution volume for each component. It uses the plate model theory to calculate the extent of peak spreading. The input information required for simulation of gel filtration with BioProcess Simulator™ are column dimensions, gel parameters, operating conditions (sample volume, flow rate etc.) and molecular weight of solutes for the calculation of elution volume. BioProcess Simulator™ has a built-in data bank that contains parameters for thirteen gels. There are also provisions to enter parameters for user-defined gels.

Since debugging of the complete process becomes complicated and cumbersome, the individual unit operation blocks were simulated first. When there were no syntax error, convergence problem or simulation difficulty with the separate blocks, they were put together to generate the complete flowsheet. However, for some cases (e.g. diafiltration) the errors causing the simulation problem were not easily identifiable and therefore could not be remedied.

The synthesis (selection of number and size) of the final flowsheet was a trial and error process, since it was found that the performance of the membrane separation and chromatography blocks were interrelated. The operating variables were varied within

limits to synthesize a flowsheet that meets the pre-specified overall recovery within the batch time outlined in the design basis (Section 3.5).

BioProcess Simulator™ time averages the output of the batch blocks to combine the batch operation with the continuous mode. The average performance is then used to treat the batch operation as a "pseudo continuous" unit operation.

3.1.2. SuperPro Designer®

The basic approach to simulation with SuperPro Designer® (SPD) is the same as described for Aspen BioProcess Simulator™.

Components: The components present in the flowsheet are introduced with SuperPro Designer®. Components can either be selected, if present, from the built-in data bank or defined as a new component for the specific design case. The new component can be added to the component databank to be available for another design case. For the simulated process t-PA, biomass (CHO cell), glutamine and ammonium bicarbonate (elution buffer) were added as user supplied components.

Properties: Each component in the data bank has two types of properties associated with it - Basic component properties (e.g. molecular weight, particle size, density, heat capacity, critical temperature and pressure etc.) and environmental component properties (e.g. COD, BOD, TS, TOC, diffusivity in air and water etc.). The built-in property databank contains default properties for the components present. These properties can be modified or updated. Each component property is employed in certain unit operation models. For the new components defined for the design case, formula and molecular weights were supplied to SuperPro Designer®. Particle size was supplied for CHO cell.

Flowsheet and Streams: The flowsheet is created by selecting the different unit operations and connecting the input/output streams. Editing of the flowsheet thus

generated, is very simple. SuperPro Designer® specifies the flowrate and composition of the feed streams in the dialog box accompanying each stream in the flowsheet.

Unit Operation Block: Information was supplied for the different unit operation blocks in the flowsheet.

Fermenter: This model simulates a stirred fermenter using a simple stoichiometric reactor model. The main reaction data required are reaction stoichiometric coefficients and extent of reaction. Other required information are liquid to total height and height to diameter ratio, agitation rate, fermentation temperature and heat transfer agent. In rating mode, liquid volume and number of units need to be specified. The output data are height, diameter, total volume of the fermenter, power, heating or cooling and residence time if used in rating mode.

Membrane Filters: Identical modelling equations are used to simulate microfilter and ultrafilter by SuperPro. The model simulates the performance of a semicontinuous unit. The required input data are the rejection coefficient, average filtrate flux, maximum particle concentration in retentate, denaturation percentage, temperature, heat transfer agent etc. For microfilter, membrane pore size and for ultrafilter molecular weight cut-off must be provided. Operation in rating mode also requires specification on membrane area and number of units. The output generated is power, heating or cooling, concentration factor and membrane area.

Chromatographic Columns: SuperPro uses identical modelling equations for affinity chromatography and ion exchange. The only difference is in the default values of some variables (e.g. resin unit cost). The model can be used either to calculate the cycle time by specifying the linear velocities for loading, elution and washing-regeneration or vice versa. The required input for the model are resin binding capacity, binding percentage and recovery yield for each component, total eluant required, amount of buffers required

for different stages etc. Simulation in rating mode would also require specification on column length, diameter and number of unit. The main objective of this chromatographic column model is to estimate the number and size of columns required to carry out the desired purification, most parameters affecting purification and overall material balances being specified by the user.

Gel Filtration: The model for gel filtration is very similar to the model of the chromatographic column in SuperPro Designer®. This model requires specification on recovery yield of each component and sample volume as a percentage of column volume. The output generated is the same as for the previous model.

Scheduling information was supplied for the different steps of the process using the Gantt Chart menu. After successful completion of the simulation, the options under the task menu was used to generate the stream report (*.SR). If information on raw material, revenue and waste streams are supplied, the other options under the task menu can be used, which are: perform economic calculation, generate economic evaluation report (*.EER), itemized cost report (*.ICR), environmental impact report (*.EIR) and input data report (*.IDR).

Input information required to carry out simulation with Aspen BioProcess Simulator™ and SuperPro Designer® are different because they use different unit operation models (rigorous and short cut models). Most of the information required by BioProcess Simulator™ were available in the literature or could be estimated from the built-in correlations. On the other hand, in SuperPro, some of the information required by some of the unit operation blocks can only be obtained from experiments. For example, simulation of the chromatographic column required the knowledge of binding percentage and recovery of each component. For these situations, results from BioProcess Simulator™ simulations were used as input to SuperPro.

The purity requirement of therapeutic pharmaceuticals like t-PA is very stringent. In general, the final product should be free of infectious agents and meet the limits specified for other contaminants such as DNA, cell and media proteins, endotoxin etc. (Anicetti, *et al.*, 1989). In this simulation, it was not possible to follow the removal of DNA and endotoxin. Instead, clearance of only foreign proteins was investigated. Among these contaminating proteins, the clearance of bovine serum albumin (BSA) was considered to be critical, since t-PA and BSA have very close molecular weights (about 68,000 and 65,000 respectively). Validated processes have shown that a combination of two chromatographic steps normally results in the desired reduced level of retrovirus and DNA (Brady et al., 1990). Therefore, two chromatographic steps were included in the flowsheet and it was assumed that the desired level of removal of these contaminants is achieved by the processes.

For calculation of t-PA purity from simulation, it was noted that, the basic rules for preserving significance states for addition and subtraction, a figure in a sum or difference is significant only if all figures in the same position relative to the decimal point in the numbers being added or subtracted are significant. For multiplication and division, the number of significant figures in a product or quotient is the same as in the one of the quantities being multiplied or divided which has the fewest (Reilly, 1992). Therefore, statistically, to calculate a purity of up to six significant figures (99.9999%), all the inputs to the simulator should have been specified up to the same extent. In reality, none of the inputs (e.g. flowrate, composition etc.) can be determined to such accuracy. However, processes, based on flowsheets similar to the ones simulated, are found to be capable of producing t-PA of the required purity (Cooney, 1999). Therefore, this issue was not considered critical in this research.

3.2. ECONOMIC EVALUATION

Economic evaluation provides an idea of the total cost involved in a process. It can identify the economically inefficient steps of a process so that attention can be focused to

improve/optimize those steps. Economic evaluation is also necessary to compare process alternatives. It helps to eliminate alternatives with weak economic potential before too much research effort has been invested. Process alternatives have been compared on economic basis for several biochemicals such as penicillin, ethanol, reducing sugars, single cell protein etc. (Evans, 1988; Maiorella et al., 1984). Among recombinant products, Datar et al. (1993) compared the economics of t-PA production in CHO cells and *E. coli.* They also carried out economic evaluation of production of human insulin in recombinant *E. coli*. Short cut material balance estimates were used in both the cases to size the equipment.

Economic evaluations are subject to assumptions regarding the process. Previously different conclusions were reported as to the economic viability of the processes because of the diversity of the basic assumptions made in the studies (Bhattacharya and Motard, 1986). For example, Datar and Rosen (1990) and Petrides et al. (1995) both carried out economic evaluation for intracellular biosynthetic human insulin (BHI) production in *E. coli* by the proinsulin method. Datar and Rosen (1990) based their equipment sizing on step yield and mass balance whereas the latter case was based on simulation with BioPro Designer®. The only difference between the flowsheets considered were the three additional chromatographic steps included by Petrides et al. (1995) for their plant designed for 1500 kg of BHI per year compared to the 1000 kg plant of Datar and Rosen (1990). However, the conclusions reached by the two groups were completely different. The purchased equipment cost estimated by Petrides et al. (1995) was 4.4 times that reported by the other group. Breakdown of the annual operating cost showed that for the first case (Datar and Rosen, 1990) labor was the dominant contributor (23.37%) and contribution by raw materials and utilities were 18.91% and 13.95% respectively. In contrast, for the latter case (Petrides et al., 1995) the major contributor (48.2%) was DFC-dependent items (depreciation, maintenance material, insurance, local taxes and factory expense). Combined raw material and consumables accounted for 37.9% while 5% contribution was made by labor-dependent items and only 0.3% by utilities. A

higher degree of uniformity can be expected by carrying out economic evaluation with a process simulator.

ASPEN PLUS was used for economic evaluation of penicillin production (Evans, 1988). Economics of producing porcine growth hormone in recombinant *E. coli* was investigated by Petrides et al. (1989) using BioProcess Simulator™. Ernst et al. (1997) used SuperPro Designer® for economic evaluation of a process for production of recombinant heparinase I expressed in *E. coli*.

Aspen BioProcess Simulator™ and SuperPro Designer® are both equipped with economic evaluators. Both of these simulators follow the same basic approach for economic evaluation and contain modifiable cost factors and correlations and provide default estimations. However, the economic evaluator of Aspen BioProcess Simulator™, which has been derived from the chemical process simulator Aspen Plus, is more geared towards chemical processes. Its calculation mode is rigorous and requires a lot of data from real projects to make appropriate use of the available options. SuperPro Designer® on the other hand, has an economic evaluator that is specifically developed for bioprocesses. It is simple and easy to use. For preliminary screening of these conceptual flowsheets and in the absence of detailed information, economic evaluation with SuperPro was thought to be sufficient in this case.

Economic evaluation consisted of three stages - capital cost estimation, operating cost estimation and profitability analysis.

Capital Cost Estimation: The number and size of equipment for calculation of purchased equipment cost were obtained from simulation by BioProcess Simulator™. SuperPro Designer® was used in rating mode so that the size and number of the different equipment obtained from the two simulators were the same. Estimation of equipment purchase cost was obtained from SuperPro Designer®. SuperPro generates an equipment

list along with the size and number of each item as a part of economic evaluation. Then it uses the built-in power law model ($C/Co = [Q/Qo]^n$) to calculate the purchase cost of the equipment (C_{PE}). For example, the following power law is used to calculate the purchase cost of fermenter by SuperPro:

$$C / 65{,}296 = Q^{0.5} / 750 \qquad (3.1)$$

SuperPro Designer® also accepts user supplied updated cost or cost model for purchase cost estimation. A 20% allowance (Petrides et al., 1989) was made in the purchased equipment cost to account for unlisted equipment such as heat exchanger, pump, storage tank etc. The total (C_{TC}) and fixed capital (C_{FC}) was then estimated by multiplying the purchase equipment cost from SuperPro Designer® by appropriate cost factors. The cost factors used are 4.6 for fixed capital investment (C_{FC}) and 5.5 for total capital investment (C_{TC}). These estimates are of "budget authorization" grade only and reflect the experience in the engineering and construction of similar projects (Datar et al., 1993).

Modifiable built-in cost factors in SuperPro Designer® could have also been used to estimate the capital cost by combining Direct Fixed Capital (DFC) and working capital. DFC is estimated from its different components (piping, instrumentation, insulation, electrical facilities, buildings, yard improvement, auxiliary facilities, engineering, construction, contractor's fee and contingency) whereas, estimate of working capital from SuperPro Designer® covers cost for 30 days of labor, raw material, utilities and waste treatment. However, since the economics of the simulated processes were evaluated for comparison purposes only, the "budget authorization" estimates were considered sufficient.

In BioProcess Simulator™, equipment is divided into five major equipment types for cost estimation. These equipment types are: heat transfer equipment, vessels and tanks, pumps and compressors, towers and user models. The user has to specify the sizing and

costing data. Several equipment types are then combined to represent a common set of equipment, such as a distillation column consisting of a tower, heat exchanger, pump etc. This method is inconvenient for estimation of purchase price of typical biochemical unit operation blocks (e.g. bioreactor, membrane separators, chromatography column etc.) and therefore estimates from SuperPro Designer® were used.

Operating Cost Estimation: Operating costs were estimated by combining information from both the simulators. Estimates on amount of raw materials, process chemicals and other consumables required by the process were obtained from simulation with BioProcess Simulator™. The amount of raw materials, particularly the buffers required for the chromatography columns, was convenient to calculate from the results reported by BioProcess Simulator™ since it generates elution profiles. SuperPro Designer® requires specification on the amount of buffers required as a multiple of the column volume and in literature, these amounts are found to vary over a wide range (Clonis, 1990) (Section 3.6.5). The number of labor hours on the other hand, was obtained from SuperPro Designer®. BioProcess Simulator™ also estimates base labor hours, however, since the models in SuperPro Designer® are particularly suitable for bioprocesses, these estimates were considered to be more reliable. This estimate included labor for warehousing (2000 hr/batch), packaging (2000 hr/batch) quality control (1000 hr/batch) and others (2000 hr/batch). The unit labor rate considered was $18/hour and the prices of the media and buffers as follows (Datar et al., 1993):

serum free medium	$25/L
10% serum containing medium	$18/L
diafiltration stream	$5/L
elution buffer for affinity chrom.	$10/L
equilibration buffer	$5.5/L
elution buffer for gel chrom.	$28.75/L
wash buffer for gel chromatography	$5.5/L

The replacement frequency and unit cost for the chromatographic media and membranes were considered as following (SuperPro Designer®, 1996):

I.Affinity Chromatography:	replacement frequency	200 cycles
	unit cost of resin	\$2000/L
II.Ion-Exchange:	replacement frequency	200 cycles
	unit cost of resin	\$400/L
III.Gel Chromatography:	replacement frequency	300 cycles
	unit cost of resin	\$200/L
IV.Membrane Separation:	replacement frequency	2000 oper. hour
	membrane unit cost	\$200/m^2

Realizing the variability in these prices, sensitivity studies were carried out to the overall economics of the process to labor (Section 4.3.7), serum free medium price (Section 4.1.6), 10% serum containing medium price (Section 4.2.6), price of diafiltration stream (Section 4.2.6) and replacement frequencies (Section 4.1.6).

The total operating cost was calculated by summing up the components, estimates of which were obtained by applying cost factors. Modifiable cost factors are available in the built-in evaluator of SuperPro Designer® for several cost components of operating cost such as fringe benefits, supervision, operating supplies, laboratory expense, insurance, local taxes, factory expense and administration overhead. Datar et al. (1990) used cost factors to evaluate the economics of human growth hormone production from recombinant *E. coli*. These cost factors were found to be more appropriate for calculation of capital investment and operating cost of recombinant protein production in absence of

detailed information and were adapted here. A FORTRAN program was written for this purpose. The cost factors used can be found in Appendix C.

Profitability Analysis: The economics of the flowsheet was evaluated in terms of Return on Investment (ROI) defined as the ratio of (net profit after tax + depreciation) to total capital investment and Gross Margin (GM) defined as the ratio of pretax profit to annual product sales. Other measures such as Unit Production Cost (UPC) defined as the ratio of total expense to annual production and Cost of Sales (COS) defined as the ratio of (direct cost + indirect cost + depreciation) to revenue have also been used by some authors (Datar et al., 1993).

The economic evaluators of SuperPro Designer® and BioProcess Simulator™ also generates cash flow analysis tables which contain the net cash flow for each year of the expected life time of the project taking into account loan payments for DFC, working capital, up front royalties and up front R & D, if any. Cash flow analysis tables were not considered for the sake of simplicity.

The economics of the process under study (t-PA from CHO cells) is expected to differ from bulk biochemical production processes due to several reasons. Research and development costs for this process should be significantly higher because of the inclusion of clinical trials and government approval costs, which are generally very high. The next important item is utility. Pharmaceutical plants require special kinds of process utilities such as water for injection, pharmaceutical water, clean steam, different classes of air (class 10,000 air for purification suites, class 1000 and class 100 air for fill and finish areas) etc. in addition to plant utilities. Cost for these process utilities will be significantly higher than the plant utilities normally required. Also the requirement to maintain safety measures to prevent release of the recombinant cells to the environment has to be considered. For plants producing pharmaceuticals from recombinant animal cells, a containment level of at least GLSP (Good Large Scale Practice) is recommended

(Miller and Bergmann, 1993). The maintenance of this safety level will add to the waste treatment cost substantially. All these costs which can vary over a wide range depending on the process and thereby incorporate uncertainty in the estimated economics will be investigated in Chapter Five.

3.3. SENSITIVITY STUDY

The 'sensitivity study' feature of BioProcess Simulator™ was used to carry out sensitivity to uncertain parameters and variables. There are three steps in the development of a sensitivity block. First of all, the dependent variables to be studied have to be defined. There is a built-in exhaustive list of variables in Model Manager, from which the user can choose the variables of interest. The next step is to declare the variables to be tabulated for each independent variable. The final step is to define the independent variables and to specify the interval over which sensitivity study is to be carried out. The user also has to specify the number of points to be studied within the interval or the increment for each step. Sensitivity study appears as a separate block in the input file (Appendix F).

3.4. STUDY OF KINETICS

To study the unstructured kinetic models for mammalian cells, each model was written down as a FORTRAN subroutine. The subroutines were added to the main fermenter model. The parameters of the model could be varied using a sensitivity block. For most cases, the variation of the parameters over a reasonable interval changed the performance of the bioreactor so significantly that simulation did not converge. The effect of specific growth rate was studied by simply replacing the expression in the subroutine with a new expression.

3.5. DESIGN BASIS

The calculation of the design basis for the process to be simulated is shown in Table 3.5.1. The process is based on 2.8 million annual cases of heart attack and stroke worldwide. Treatability with t-PA depends on the patients' age and the time elapsed since onset of the heart attack. Typically about 40% or only 1.1 million cases are found to be treatable by t-PA. The process is designed for 10% market penetration. A complete dosage of 100 mg, therefore, requires the production of 11,000 gram of purified t-PA per year. For an assumed recovery of 40% and product titer of 33.5 mg t-PA/L (Datar et al., 1993), 820,000L plant capacity is estimated. In laboratory, t-PA concentrations of higher than 33.5 mg/L have been observed. However, amplification with MTX in higher producers resulted in increased doubling time and also in some cases, increased fragility (Cartwright and Crespo, 1991). t-PA concentrations around 50 mg/L were also found to be toxic to cells. In monolayer cultures, cells were found to detach from substrate as these levels were approached (Kaufman et al., 1985). Since these changes will prevent efficient cell growth in stirred bioreactors, higher t-PA concentrations were not assumed for the design basis. However, in reality, the product titer may improve with time without toxicity to the cells (Cooney, 1999).

A 20% margin was allocated for unforeseen events such as product contamination etc. This is a rather conservative margin since contamination loss of even 5% is considered reasonable (Nelson, 1988). The bioreactor volume thus calculated was found to be 30,000L for 35 batches requiring 10 days each. Since a single 30000 L bioreactor for CHO cell cultivation is impracticable with the current state of technology, the total bioreactor volume was divided into five equal sized bioreactors. The use of multiple bioreactors also provides a buffer against unforeseen losses due to contamination, equipment breakdown and other problems (Section 4.5.1). One of these five bioreactors with the associated downstream units was simulated for the base case (Section 4.1), two stage cell culture (Section 4.2) and the airlift bioreactor based flowsheets (Section 4.4).

The bioreactor volume was recalculated for the fed-batch cases (Section 4.3) and for the single versus multiple bioreactor study (Section 4.5) it was further divided into six 1000 L bioreactors.

Table 3.5.1: Design Basis (Datar, R.V., 1993, Chisti, Y., 1993)

Worldwide cases	2.8 million/year
Treatable cases (40%)	1.1 million/year
Market penetration (10%)	110,000 / year
Amount of t-PA per dose	100 mg
Production of purified t-PA	11,000 g/year
Recovery Yield	40 %
Product titer before purification	33.5 mg/L
Plant capacity	820,000 L/year
Over design for Contingency	20%
Plant capacity	1025,000 L
Time to complete one batch	10 days
No. of Batches per year	35
Batch Volume	30,000 L
Fermenter Liquid Volume	5 X 6000 L
Total volume	5 X 8500 L

3.6. DEVELOPMENT OF GENERAL PROCESS DESCRIPTION

Commercial production of t-PA under the name Activase® (common name Alteplase) began in Novenber, 1987, by Genentech, Inc., San Francisco, CA. t-PA is produced in CHO cells. The master working cell bank is tested for stability and the absence of exogenous bacteria, mycoplasma, fungi and viral agents associated with mammalian cell culture. Molecular identity test for t-PA includes tryptic mapping, specific activity and protein content analysis. The final product is tested for appearance, sterility, safety,

pyrogenicity, identity, purity, potency, pH, formulation chemicals content, moisture and fill volume (Sofer and Nystrom, 1991).

A typical biotherapeutic production plant involves several sections in the downstream processing unit (Figure 3.6.1). A unit by unit process description has been developed for the complete process of t-PA production based on information from literature. The flowsheet for this process is based on the one outlined by Datar et al. (1993). The flowsheet consists of fermentation followed by microfiltration for cell separation and ultrafiltration for broth volume reduction. The permeated broth is then passed through two sets of affinity chromatography and ultrafiltration units. The final step is a gel filtration. Scale up of inoculum with seed fermenters has been omitted from the flowsheet since this operation is similar to the operation of the production bioreactor.

For t-PA, the gel filtered product is sterile filtered and then lyophilized. Validation steps for this process include demonstrating removal of foreign proteins and cell culture additives as well as inactivation and removal of model retroviral particles (Sofer and Nystrom, 1991).

Following is a general process description developed for the process simulated.

3.6.1. Bioreactor:

The first step in the design of a fermentation process is to decide on the type of the bioreactor. Commercially available bioreactors range from simple stirred tank to complicated membrane perfusion systems. The various designs have all proven successful, to a greater or lesser extent, in culturing mammalian cells. Table 3.6.1 compares the different bioreactor systems in terms of operability, ease of scale-up and productivity (Kearns, 1990).

The simple operation and ease of scale-up of stirred tank and airlift bioreactors give them preference over other systems for large-scale operation. Stirred tank reactors are the most dominant type of cell culture bioreactors in industry today (Smith, 1994). This is mainly due to the experience and knowledge gained in this area from the traditional fermentation industry. Many large-scale mammalian cell cultures use stirred tank reactors since they are perceived as being reliable and well understood. The largest reported cell culture processes in stirred suspension are operated in Japan at 20,000 L scale for tobacco cells (Arathoon and Birch, 1986). Wellcome Biotechnology Ltd. produces interferon from Namalwa cells at 8000 L scale in a magnetically driven stirred tank reactor (Phillips et al., 1985; Pullen et al., 1985). Similar technology is used to produce Foot and mouth Disease vaccines from baby hamster kidney cells at 3000 L scale by Wellcome (Pullen et al., 1985).

Table 3.6.1: Comparison of Bioreactor Systems (Kearns, 1990)

	Operation	Scale-up	Productivity
Stirred Tank Reactor	simple	yes	low
Airlift Reactor	simple	yes	low
Hollow Fiber Reactor	Complex	insufficient data	high
Ceramic Matrix (Opticell, Charles River)	Complex	insufficient data	intermediate
Glass Bead Reactor	Complex	no	intermediate
Fluidized Bed Reactor (Verax)	Complex	insufficient data	high
Membroferm (Sulzer)	Complex	insufficient data	high
Stirred Tank/Airlift Reactor with Dialysis or Perfusion	Complex	yes	high

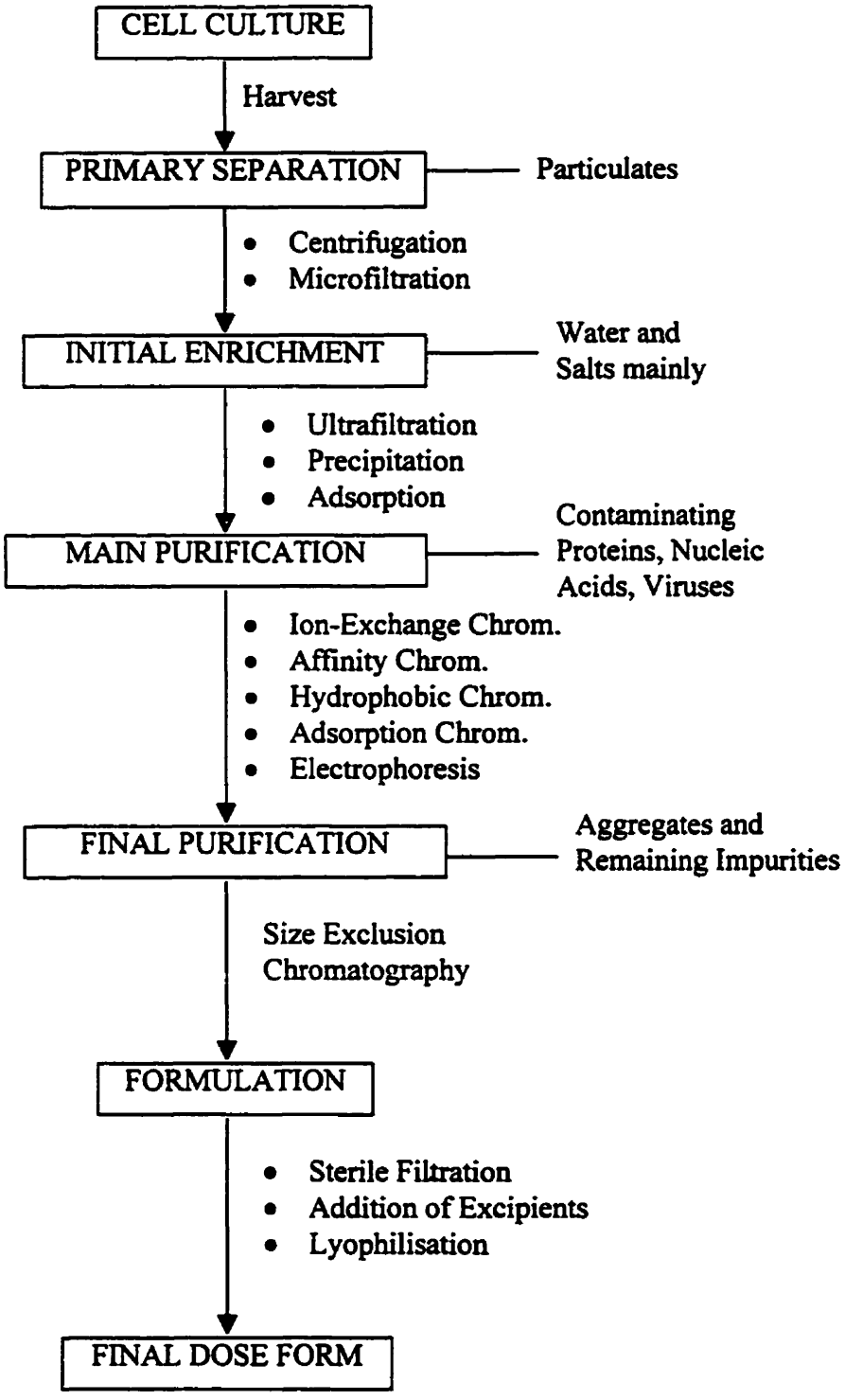

Figure 3.6.1: Generalized flowsheet for downstream processing steps applied to recombinant protein from animal cells

However, a major drawback of stirred tank reactors in case of mammalian cell culture is that they often produce high shear, which is detrimental for these fragile cells. Also maintenance of sterile conditions in the bioreactor is a problem because of leakage through the moving parts.

Airlift bioreactors, on the other hand, produce a gentle, low shear environment suitable for tissue culture and genetically engineered microorganisms (Chisti, 1989). Airlift design is simple since it removes the need for motors and agitators associated with agitated reactors. In addition, hydrodynamic and mass-transfer characteristics of the airlift reactor are predictable with increase in scale (Arathoon and Birch, 1986). There are several examples too of its use in large-scale culture of mammalian cells. The largest airlift reactor (1000 L) is used by Celltech for production of monoclonal antibody from hybridoma. Celltech has also designed a 10,000 L airlift reactor for the same purpose (Birch et al., 1987).

The choice between these two types of reactors is dictated by the cell line. The characteristics of cell line that are of concern are cell biology, genetic stability, growth pattern, growth kinetics, oxygen requirements, shear sensitivity and nutrient requirement (Nelson, 1988). It is found that CHO cells tend to form aggregates in suspension in conventional medium (Boraston et al., 1992). This is in contrast to hybridoma, which occur naturally as anchorage independent cells (lymphocyte part) and can be grown in airlift reactors. Our experience with CHO cells revealed that these aggregates can be broken down by stirring the suspension at low rpm (below 200 rpm). At this low speed of stirring no observable cell damage occurred to CHO cells (Dutton, 1998). For comparison, a rotational speed of 150 rpm (impeller tip speed of 0.35 m/s) was found to have no detrimental effect on hybridoma cells (Chisti, 1993). Instead, cells could be more prone to damage in the air-bubble-rich environment in airlift reactors due to bubble break up (Handa et al., 1987). The largest commercial producer of t-PA, Genentech, uses

suspension culture of CHO cells in stirred tank reactors. Therefore, stirred tank appears to be a better option than airlift for this particular cell line.

Next, a choice had to be made regarding the fermentation mode - suspension or microcarrier culture. Since CHO cells could be both anchorage dependent and independent (Boraston et al., 1992), both kind of culture is possible with this cell line. All of the large-scale cell culture process mentioned uses suspension technology. The commercial process for t-PA also uses suspension culture (Lubiniecki et al., 1989). Examples of large-scale micro carrier culture are lacking, one of the difficult aspects in scale up being the transfer of cells from one culture to the next (Arathoon and Birch, 1986). The largest scale of micro carrier culture seems to be the production of viruses at 1000 L (Reuveny, 1990). So suspension culture appears to be the preferred mode of fermentation in this case. Experience with CHO cells show that they form aggregates in suspension of serum containing medium. These aggregates can be broken down by stirring at 150 rpm (Dutton, 1998) or even by adjusting the amino acid and inorganic salt content of the medium so that they can grow as single-cell suspension (Boraston et al., 1992). Therefore, the production of t-PA in stirred tank reactor in suspension culture seems feasible.

The upstream section of the bioreactor consists of medium preparation and inoculation. Currently there are two approaches to media preparation: one is to prepare stock solutions of component mixtures of the defined basal medium. The basal medium is essentially a mixture of carbohydrate, amino acids, salts, vitamins and various other biochemicals. The carbohydrate used is usually glucose. Substitution of glucose with galactose, fructose, maltose and starch in the medium has been studied but no advantage was observed (Macmillan et al., 1987). Similarly the amino acid commonly employed in the media preparation is glutamine although some investigators have reported that asparagine and glycyl-glutamine, with a much longer half-life, could also serve the same purpose for CHO cells (Dyring et al., 1994; Kurano et al., 1990; Holmlund et al., 1992).

At the time of use the final medium is then prepared from frozen stock solutions. The second, more common approach is to prepare a batch of medium ready to use from powder, which is frequently purchased from medium manufacturers. These powder mixtures are then dissolved in appropriate amount of USP/WFI grade water and other supplements are added to this solution to complete the medium. Subsequently, the medium is filter sterilized, and may be then quarantined at 40° C for a period of up to three weeks, depending upon the holding capacity of the plant (Bliem, 1988).

The cell culture production process begins with the preparation of a seed stock culture from the working cell bank. For inoculum preparation, a production plant may have a battery of bioreactors of progressively increasing volume connected in series. Each of these bioreactors provides inoculum for the next larger reactor in the train by aseptic transfer of the inoculum culture. It is difficult to generalize about scaleup rates since it depends on the type of culture, its growth rate, its viability and whether the cells will be suspended or grown on microcarriers (Beck et al., 1987). For the 1000 L airlift bioreactor used by Celltech for MAb production, the inoculum culture was grown in roller bottles (ca. 1 litre) and then transferred aspetically to a 10 L inoculum bioreactor. When cell density reached a level of 10^6/ml, the contents were transferred to a 100 L bioreactor. This in turn, provided inoculum for the sterile, medium-filled 1000 L bioreactor (Birch et al., 1987). The inoculum usually constitutes between 5-25% of the working volume of the bioreactor (Chisti, 1992). Amplification of an ampoule of cells (about 10^7 cells) to the level required for seeding a bioreactor (~$5X10^9$ to 10^{11} cells) takes several weeks (Bliem, 1988). After completion of the fermentation process the separated cells are sent to a kill tank for total inactivation.

The oxygen demand of animal cells is low in comparison to microbial cells. The oxygen uptake rate of mammalian cell lines has been shown to be in the range of (2-10) X 10^{-12} g/cell.h and the measured oxygen demand, (0.045-0.47) mmol oxygen/liter-hour at 10^6 cells/ml (Fleischaker and Sinskey, 1981). Oxygenation or aeration in stirred-tank

bioreactors can be achieved by three methods, individually or in combination: membrane aeration, surface aeration or direct sparge aeration. The available surface area per unit volume limits the use of surface aeration to unsparged stirred-tank bioreactors of between 10 L and 100 L depending on the culture's oxygen uptake rate and density. For membrane aeration, silicon tubing is generally used because of its high oxygen permeability and autoclavability. However, when scaling up to very large volumes, the amount of silicon tubing required for efficient oxygen transfer increases proportionately and may become limiting (Nelson, 1988). Air sparging, although it causes cell damage or foaming, and sometimes both, is the most direct method for supplemental oxygenation and is used in most large bioreactors. However, there is an upper limit on the partial pressure of dissolved oxygen due to toxicity to mammalian cells (Young and Dean, 1987) and the recommended air flow (containing 5% CO_2) rate to stirred reactors for cell culture is less than 0.1 VVM (Arathoon and Birch, 1986). The inlet air and also the exhaust gases from bioreactors are filter sterilized. Either absolute (such as 0.1 to 0.4 μm hydrophobic filters) or depth filters are employed (Chisti and Moo-Young, 1991). Often two filter cartridges in series are used at both locations. The first filter serves as a prefilter and protects the final filter. Bioreactors are typically maintained at 2 to 10 psi above atmospheric pressure (Beck et al., 1987). The dissolved oxygen concentration maintained in the laboratory scale bioreactors (2 litres) for CHO cell culture is found to be between 40% and 60% (Hansen and Emborg, 1994; Kurano et al., 1990, Lakhotia et al., 1992) and the fermentation temperature is 37^0 C (Dyring et al., 1994; Kurano et al., 1990; Nilsson et al., 1988).

The literature is replete with correlations for oxygen mass-transfer (k_La) coefficients from the scale-up of microbial fermentation. These correlations for k_La use the gassed volumetric power input and the superficial gas velocity as parameters. These correlations are based on a well-dispersed gas phase and the high power input that are typical of microbial systems. Such correlations are not appropriate for mammalian cell culture systems because of the extremely low agitation and aeration rates that are required

because of the shear sensitivity of these cells (Nelson, 1988). Therefore, the basic approach to k_La calculation was adopted to get an estimate of the oxygen mass-transfer coefficient for the simulated bioreactor (Appendix D). The calculated value of k_La was found to be about 6 h^{-1}.

The possible limiting factors in the scaling up of cell culture bioreactors include physical parameters such as oxygen tension or turbulence, biological parameters such as substrate utilization or strain stability and biochemical parameters such as product half-life in the cell culture environment. A technique that underlines most of the current scale-up strategies employed in microbial and lately in animal and plant technology is the dimensional analysis. Dimensional analysis in cell culture mainly concerns the use of the dimensionless power number. The power number describes the dynamic mixing forces of liquid agitation using impellers (Bliem and Katinger, 1988). Where oxygen transfer is not a limitation, scaling up is achieved by maintaining a similar fluid turnover, that is impeller pumping rate per unit bioreactor volume, at the two scales (Chisti, 1993).

The aspect ratio for stirred tank bioreactors are usually maintained around 1 for better bulk mixing. The impeller diameter to bioreactor diameter ratio is kept around 0.3 (Chisti and Moo-Young, 1991). Agitation through mechanical seals is considered risky and hence magnetically coupled agitators are used in large-scale stirred tank bioreactors (Phillips et al., 1985; Chisti, 1993). Top-drive systems are preferred by some because the mechanical seals are out of the liquid, preventing them from damaging cells or contributing toxic trace contaminants. On the other hand, bottom-drive systems are favored by others because of both, the large size of the agitator motors and agitator shafts that are necessary in top-drive systems (Nelson, 1988). Baffles are safely eliminated from mammalian cell culture The power input per volume for cell culture processes lies in the region of 0.005-0.1 W/kg in contrast to microbial processes which usually lie in the region of 1-5 W/kg (Bliem and Katinger, 1988). Operating speeds for cell culture agitators are normally kept below 100 rpm (Beck et al., 1987). Scaled up agitation and

power input requirement for cell culture bioreactors can be calculated by maintaining similar fluid turnover, that is similar impeller pumping rate per unit bioreactor volume at the two scales (Chisti, 1993). This approach resulted in an agitation rate of 77 rpm and power input of 13 W/m^3 for the process to be simulated (Appendix D). Scaled up agitation and power input requirement can also be calculated by maintaining a constant Kolmogorov length scale (Nelson, 1988). For an impeller diameter of 0.6 m this approach resulted in an agitation rate of 0.57 s^{-1} (obtained by trial and error approach) (Appendix D). The resulting power input was found to be too small (1.15 W/m^3) and below the range (0.005-0.1 W/kg) reported by Bliem and Katinger (1988).

Many impeller types have been proposed for mammalian cells, however, there is no single best recommendation for the impeller of choice. Marine type propellers have worked well under specific practical conditions up to several thousand litres. Another type that has been found satisfactory in tissue culture vessels up to 500 L is the Elephant Ear Impeller (Charles and Wilson, 1994). Besides these other types of impellers such as sail-type agitator (Tolbert and Feder, 1983), paddle and coiled stirrer vibromixer (Lynn and Acton, 1975; Hu and Dodge, 1985), "scull" type agitator (Hu and Dodge, 1985) etc. have also been applied for cell culture.

A partially structured kinetic model developed for hybridoma (Phillips, 1991) and adapted for CHO cells (Dutton, 1998) was used for the simulated bioreactors. Growth and product formation kinetics were supplied as user defined subroutines. The batch time was assumed to be 200 hours. The parameters for this model were obtained from experiments carried out with CHO cells (Dutton, 1998). However, with the specific cell line used in the experiments, the maximum t-PA titre was found to be only about 10.5 mg/L, which is much lower than that considered in the design basis (33.5 mg/L) and reported for a different cell line (Datar et al., 1993). The bioreactor, therefore, was simulated independently of the downstream units and the product titre in the stream entering the first block of the downstream train was assumed to be the same as

considered in the design basis. The kinetic equations for this model can be found in Appendix G. Several other unstructured kinetic model from literature for hybridoma and CHO cells have been investigated in Chapter Six.

3.6.2. Cooling Down of Broth:

The cell culture broth has to be cooled down to 4° C to prevent denaturation of the product and minimize the possibility of contamination. This cooling down is usually carried out in the bioreactor by circulating coolant through the jacket (Charles and Wilson, 1994).

3.6.3. Primary Separation

Unit operations recommended for primary separation are centrifugation and microfiltration.

Microfiltration has several advantages over other methods of harvesting. In contrast to centrifugation, where supernatant can be heavily contaminated with colloids and small particles, microfiltration completely removes suspended solids and produces a fully clarified product stream (Antoniou et al., 1990). It allows operation in closed system without generating aerosols, which is an essential feature for processing genetically engineered microorganisms and cells. In closed systems it results in high yields (>95%). Although centrifugation offers high processing speed and can be scaled up, it subjects the cells to high shear environment, which can be avoided by using microfiltration. These systems generally have greater than 99.9% retention of cells (Zahka and Leahy, 1985). A 15-fold concentration of mammalian cells was attained by van Reis et al. (1991) during cross-flow filtration in industrial scale at an average yield of >99%.

3.6.4. Initial Enrichment

Ultrafiltration, precipitation and adsorption are among the methods recommended for initial enrichment. Precipitation although widely applicable, is a relatively slow method with poor yield. It is difficult to carry out at large scale and is inefficient in dilute protein

solutions. The requirement to add a precipitant and its subsequent removal is also disadvantageous from both process design as well as economic standpoint (Cartwright, 1994).

Adsorption, which is a simple technique for large scale separation, has the disadvantage that new process parameters has to be developed for each individual product and again the component adsorbed has to be subsequently recovered from the solid phase.

Ultrafiltration is almost universally employed for initial enrichment because of its rapid throughput and versatility (Cartwright, 1994). Ultra and Microfiltration for primary separation and initial enrichment can be carried out by one of three types of membrane system - hollow fiber, plate and frame or spiral cartridge. Hollow fiber and plate and frame membranes are more easily cleaned than spiral cartridges (Scott et al., 1987). Hollow fibers have the highest surface area to volume ratio among filtration modules. The low pressure drops and flow rate make this type of membranes most economical in terms of energy consumption. However, the small diameter fibers are susceptible to plugging and the purchase and replacement cost of these type of membrane is very high (Cheryan, 1986). The only report on industrial scale harvest of t-PA using microfiltration used hollow fiber type of membrane (Reis, 1991).

Gentle process conditions are recommended for initial harvest of product so that the cells are subjected to only mild forces (Rudolph and MacDonald, 1994). This is to prevent the cells from lysing, which can result in increased level of contaminating proteins and nucleic acids. Most of the data for simulation were chosen to maintain conditions similar to those maintained by Reis et al. (1991). To keep the transmembrane pressure around 5 kPa (0.05 atm) and axial pressure drop around 25 kPa, the pump pressure was calculated to be 1.25 atm and permeate and retentate pressures 1.08 and 1 atm respectively. This results in a transmembrane pressure of 0.045 atm (0.67 psi). This is within the range of transmembrane pressure (<1 psi) recommended by Rudolph and MacDonald (1994)

although transmembrane pressures up to 2 psi have been applied for harvesting animal cells in pilot scale (~1 m^2) (Maiorella et al., 1990). The flux observed at 4^0C with cell harvesting is between 30 to 60 L/m^2-h and at 30^0C is between 60 to 120 L/m^2-h. A much higher transmembrane pressure (20 to 40 psi) is employed for concentration of protein resulting in permeate flux between 10 to 100 L/m^2-h at 4^0C (Rudolph and MacDonald, 1994). For recombinant human growth hormone, transmembrane pressure up to 0.65 atm has been used (Maa and Hsu, 1995). The maximum solids concentration that can be handled by the cell harvesting filter is 10^8 cells/ml and 10 g/L or 20% for clarification and concentration of proteins. Length and diameter of the fibres were chosen to be 0.435 m and $6X10^{-4}$ m respectively. The system described by Reis (1991) used 180 m^2 of membrane area at a feed rate of $3.3X10^4$ L/h. The permeate flux was 50 L/m^2-h. A membrane pore size of 0.2 μm was used by Reis (1991) for microfiltration of CHO cells. This pore size appears satisfactory because although mammalian cells have an approximate size of 15 – 20 μm (Scharer, 1996), cell debris as small as 0.4 μm could be present (Bowen, 1993).

The resistance in series flux model was chosen for the system. Membrane resistance and parameters for calculation of gel resistance were obtained from several sources (Tamer, 1995, Lee, 1989, Wood, 1988). In the calculation of gel resistance ($Rg = Rg_0 (\Delta p)^a (Re)^b (C_b)^c$), the exponent on pressure (a) was set to 0.13 instead of 0.7 as reported for pressure in kPa, to take into account the different unit of pressure (N/m^2) for this particular block in BioProcess Simulator™. The exponents b and c were set to -0.7 and 0.2 as obtained from literature. All of the above works carried out to obtain the exponents for gel resistance calculation used *E. coli*. The only report (Reis et al., 1991) on filtration of t-PA containing broth does not mention the calculation of resistance. The data presented was used to get an estimate of the total resistance, which turned out to be $6.6X10^{11}$ m^{-1}. The total resistance for the simulated filtration blocks, calculated by combining contributions from membrane and gel resistance, were found to be similar in magnitude.

The observed range of Reynolds Number for hollow fiber membranes is between 500 to 3000 (Cheryan, 1986). The critical average wall shear rate, above which cell damage is observed, was found to be about 3000 s^{-1} for crossflow microfiltration of animal cells (Maiorella, 1990).

Tangential flow ultrafiltration was found to result in at least ten-fold reduction in volume within an hour of harvesting the bioreactor with virtually quantitative recovery of a monoclonal antibody (Birch et al., 1987). While Harshbarger et al. (1995) reported that volume reductions of 50:1 are common for protein purification using ultrafiltration.

It is observed that protein is not denatured by shear force during microfiltration except in gas-liquid interface at elevated temperature (around 40° C) and high pH >8 (Rudolph and MacDonald, 1994). Since the temperature of the downstream train is assumed to be 4° C, the percentage of protein denatured is expected to be very low for the simulated processes.

Membrane filters are sanitized by treatment with hypochlorite (bleach) or sodium hydroxide solutions (Inampudi et al., 1995). Membranes should be cleaned immediately following use. Protein residue on membranes should not be allowed to come in contact with air since removal of dried protein is very difficult (Wheelwright, 1991). Membranes are kept wet with water or buffer to prevent precipitation of protein. Residual proteins are removed by washing with sodium hydroxide and in severe cases with protein digestive enzyme. For killing vegetative bacteria Berglof et al. (1988) recommends 0.1M NaOH for an hour of contact time.

3.6.5. Main Purification

Chromatographic steps are the ones recommended for main purification of recombinant protein from animal cells. Among these, affinity chromatography has the advantage of being very bio-specific.

Affinity Chromatography:

The first step in the design of an affinity chromatography process is the choice of affinity ligand and support matrix. Table 2.4.6 lists some of the affinity ligands used for laboratory scale purification of t-PA. A catalogue from Pharmacia (Pharmacia LKB Biotechnology) was consulted to choose a ligand and a matrix that will result in high capacity for t-PA. The catalogue lists Lysine Sepharose 4B and Arginine Sepharose 4B as suitable adsorbents for affinity purification of t-PA. It also lists a capacity of >0.6 mg plasminogen/ml gel for Lysine Sepharose 4B. For plasminogen activator its capacity was assumed to be 0.5 mg/ml gel (Scharer, 1996).

Next, an equilibrium relationship in the form of an isotherm needs to be established for t-PA Lysine sepharose system. There is no information in the literature about the type of equilibrium relationship that exists between t-PA and any ligand. According to Arnold et al. (1985), many affinity systems show a hyperbolic Langmuir type equilibrium relation. Hedman et al. (1987) also found that for several combinations of stationary phase and proteins, Langmuir isotherm is a suitable equation to describe experimental equilibrium measurements. Langmuir isotherm is also found to be most commonly used in the study of both biospecific affinity chromatographic processes (Anspach et al., 1989; Arve and Liapis, 1987; Chase, 1984) and ion exchange chromatography (Cowan et al., 1989; Gosling et al., 1989). So, it was assumed that the equilibrium relationship in this case can be described by Langmuir type isotherm. There are only few papers that have considered the adsorption isotherm constants of proteins on ligands. The Langmuir adsorption isotherm constant reported for a monoclonal antibody on Sepharose 4B is 162

$X10^3$ cm^3/g (Arnold et al., 1985). A value of 120 $X10^3$ cm^3/g was assumed for adsorption isotherm constant in the simulation.

Most of the chromatographic methods described in the literature are laboratory scale. Differences between analytical and large-scale purification methods have been shown in Table 2.4.7. Sofer and Nystrom (1989) outlined the procedure for scaling up of laboratory scale chromatographic techniques. Experimentally determined optimum column length, linear flowrate and sample concentration are kept the same in the scale up of chromatographic columns. The diameter, volumetric flowrate and sample loading are increased in the larger scale. In large columns, the weight of the adsorbent material can cause distortion of the packing in the middle. Therefore, the column height is restricted to a maximum of 50 cm when scaling up (Scopes, 1994). The height to diameter ratio is smaller than 2 and sometimes below 1.0 (Yamamoto et al., 1988), so that the throughput can be maximized without resulting in excessive pressure drop. The recommended ratio of diameter to height for adsorption chromatography columns for process-scale work is approximately 2 to 4 (Bonnerjea and Terras, 1994). In simulation, the dimension of the column (diameter = 40 cm and height = 80 cm) was selected such that D/L is 2. The column height was restricted to below 50 cm.

For column chromatography, it is a established practice to use relatively large diameter particles (approximately 100 μm) in the first column in order to achieve a high volumetric throughput at a moderate column inlet pressure. Smaller particles have preferentially been used for the final polishing purification steps (Hedman et al., 1991). The range of particle diameter defined by Pharmacia for chromatographic application varies between 45 and 160 μm. Particle sizes usually used in column chromatography are larger than 40 μm (Chisti and Moo-Young, 1990). A particle diameter of 100 μm was used in the simulation.

Step elution is recommended in scaled-up chromatographic processes instead of continuous concentration gradient to simplify operation (Bonnerjea, 1994). There is no guideline as to the magnitude of velocity to be used for loading the column. Modern process-scale gels can withstand high linear flow velocities of several hundred and even a thousand centimeters per hour (Bonnerjea, 1994). However, the kinetics of the adsorption and desorption of the product to the gel may limit the actual flow rate to a much lower figure, at least for the binding and elution steps. Higher flow rates can be used during the washing, regeneration and reequilibration steps to decrease the overall cycle time. The loading velocity reported in the literature is found to vary between 6 to 270 cm/h. 1 to 5 times the column volume of wash fluid and 4 to 12 times column volume of elution fluid have been reported to be used in analytical scale chromatographic processes (Clonis, 1990). The different equilibration and elution buffers used in laboratory scale affinity chromatography of t-PA are KSCN, ammonium bicarbonate, guanidinium hydrochloride, sodium chloride, phospahte buffer etc.

In simulation, a conservative value of 40% of the total capacity was assumed for loading of the chromatography column in the loading stage. On preparative scale, column loading of 85% of total capacity has been used for purification of monoclonal antibody with Protein A Sepharose (Birch et al., 1987). This single step affinity purification resulted in high purity (>95%) and very high yield (>90%). The recovery of biological activity in chromatographic columns varies from process to process. Typical figures for recovery are around 80 to 85% (Johansson et al., 1986).

Even high resolution chromatography can not achieve the required purity for pharmaceutical applications in a single operation and several chromatographic steps in succession are usually required for most processes (Bonnerjea and Terras, 1994).

Chromatography systems are not considered true closed systems with respect to aseptic processing. The primary concern in regeneration and sterilization of chromatography

columns are buildup of protein, DNA and endotoxins. There are various agents for microbial inactivation such as phosphoric acid, ethanol, hibitane digluconate associated with benzyl alcohol, ethylene oxide, formaldehyde, hypochlorites and hydrogen peroxide (Bengio et al., 1995). The choice of the right agent is dependent on both, the source of suspected contamination and the chemical resistance of the packing. Sodium hydroxide is the most widely used agent in industrial chromatography processing for the clean-in-place of media and equipment. However, when sporulated forms of microorganism are suspected sodium hydroxide is not sufficient for total inactivation and peracetic acid can totally inactivate such spores in minutes (Bengio et al., 1995).

3.6.6. Final Purification

This step consists of size exclusion or gel filtration chromatography for removal of aggregates and remaining impurities.

Gel Filtration:

The final step in the down stream processing of t-PA is gel filtration. It is often used as a final stage in many purification schemes to remove trace amounts of impurities and self-aggregates of the product. Gel filtration, however, is a low-capacity technique and not well suited to industrial scale use (Bonnerjea and Terras, 1994). For gel filtration, resolution is a function of column length, ratio of sample volume to column volume, linear flow velocity and sample concentration. Many matrices for gel filtration are weak and compressible when packed in large columns. Column lengths of approximately 1 meter are feasible on process scale with rigid gels (Bonnerjea and Terras, 1994). This restricts the diameter to about 20 cm. For wide-diameter gel filtration columns (diameter/length $\approx$ 3.0) required to obtain high throughput, columns connected in series or stacked columns can be used. For simulation of gel filtration, a 147 L column with a height to diameter ratio of 1.5 was assumed.

The ratio of sample volume to column volume is usually between 1 to 5% for gel filtration columns (Wheelwright, 1991). For impurities of largely differing molecular

weights, the sample volume can be increased up to 10% of the column volume (Bonnerjea and Terras, 1994). A sample volume, 5% of the column volume was assumed for simulation. The protein concentration that can be handled by gel filtration lies between 10 to 50 g/L (Bonnerjea and Terras, 1994).

Flow velocity for gel-filtration is usually an order of magnitude lower than the flow velocities characteristic of adsorption chromatography. The recommended flow velocity is within 3-30 cm/h (Bonnerjea and Terras, 1994). According to Wheelwright (1991), the optimal flowrate for maximum resolution is approximately 2 ml/cm^2-h. Sofer (1986) maintained a velocity of 13 cm/h for large-scale gel filtration of insulin in a 96 L column. The flow rate for this simulation is assumed to be 16 cm/h.

The gel filtration media used for laboratory scale purification of t-PA are Sephadex G-100, G-150 and G-200, with Sephadex G-150 being the most popular. The useful molecular weight fractionation range of Sephadex G-150 for globular proteins is $5x10^3$ to $1.5x10^5$ (Pharmacia LKB Biotechnology). Fast flow matrices such as Sephacryl S-200, which is also fairly rigid, may be a good choice for large scale application of gel filtration (Glick, 1997). The elution buffers used in laboratory scale gel filtration of t-PA are ammonium bicarbonate, sodium acetate, phosphate buffer etc.

CHAPTER 4

SIMULATION OF PROCESSES

4.1. Simulation of Base Case with BPS and SuperPro Designer®

4.1.1. INTRODUCTION

The base case flowsheet was developed using the typical conditions found in the laboratory scale production and purification of t-PA and published in the literature. Both Aspen BioProcess Simulator™ (BPS) and SuperPro Designer® were used to simulate this flowsheet. A description of the flowsheet follows.

4.1.2. PROCESS DESCRIPTION

The downstream processes of the base case flowsheet consist of two alternate steps of ultrafiltration and affinity chromatography, followed by a final step of gel chromatography (Figure 4.1.1). The required process information and operating conditions, required as input for simulation, were gathered from literature. The kinetics for the bioreactor was developed in our laboratory. The simulated bioreactor is a stirred tank type with the CHO cells grown in suspension culture. This is the mode of operation

adopted by Genentech for large-scale production of t-PA (Cartwright et al., 1992). The bioreactor of this flowsheet uses serum free medium. The serum free medium used in our laboratory consists of HB-CHO basal mixture and a proprietary supplement (Immucor Canada Inc.). The actual composition of the serum free medium is not known except that it contains 320 mg/L of total protein. The bioreactor is inoculated with 1000 L of inoculum having a concentration of $2X10^5$ cells/ml. Liquid is assumed to occupy 80% of the bioreactor volume. The ratio of liquid height to total diameter (L/D) is assumed to be 1.5 and the impeller diameter d_i, to tank diameter $d_i/D = 0.3$ (Chisti, 1993). The contents of the bioreactor are aerated with 0.02 VVM sterile air.

Permeate and retentate pressures for the membrane filtration units were chosen to yield a transmembrane pressure of <1 psi, as recommended by Rudolph and MacDonald (1994) and Maiorella et al. (1991) for harvesting animal cells. Resistance in series model was chosen as the flux model for the system. Membrane resistance and gel resistance parameters were obtained from several sources (Tamer, 1995; Lee, 1989; Wood, 1988).

The affinity ligand recommended for purification of t-PA is Lysine Sepharose, which has a capacity of >0.6 mg plasminogen per ml gel (Pharmacia LKB Biotechnology). It is widely used in laboratory scale purification of t-PA because of its biospecificity (Clonis, 1990). A Langmuir type equilibrium relationship was assumed for the system since this relationship has been found satisfactory for many ligand-protein combinations (Arnold et al., 1985). The Langmuir adsorption isotherm constant was assumed to be $120X10^3$ cm^3/g. This value seems reasonable when compared to the reported value of $162X10^3$ cm^3/g for anti-benzenearsonate monoclonal antibody on Sepharose 4B (Arnold et al., 1985). Simulation was carried out for a 200 L column having a height to diameter ratio of 0.5 and a superficial loading velocity of about 40 cm/h.

Sephadex G-100, G-150 and G-200 are the gel filtration media used in the laboratory scale purification for t-PA, with Sephadex G-150 being most popular. Gel filtration was

simulated in a 147 L column with a length to diameter ratio of 1.5. The sample volume was taken as 5% of column volume as recommended (Wheelwright, 1991) and the flow velocity was 16 cm/h.

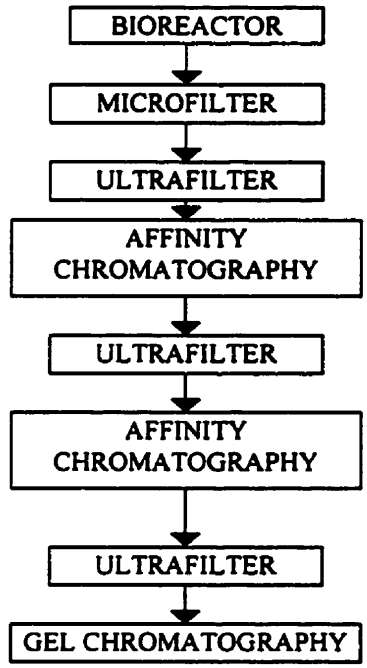

Figure 4.1.1: Base case flowsheet for t-PA production

The process is designed to produce 11,000 g of purified t-PA per year (Section 3.5), which is the same considered by Datar et al., (1993), so that the economics of the simulated flowsheets could be compared to their reported values. To meet this production level, five 6000 L bioreactors are required. One of these five bioreactors with its associated downstream processing units was simulated.

4.1.3. SIMULATION AND RESULTS

For simulation purposes, the concentration of BSA-like protein in the serum free medium was considered equal to that of serum containing medium. The total protein concentration of serum is about 100 mg/ml (Macmillan et al., 1987). 60% of these protein were

considered to be BSA-like (Scharer, 1996) and then a sensitivity study (Section 4.1.6) was carried out to see how lower concentration of BSA in the serum free medium affects the process. Required process information about flowsheet and streams, composition of each stream, properties and values of operating variables and parameters of the different unit operation blocks, were supplied through input files.

Summary of the simulation results is presented in Table 4.1.1 (BPS simulation) and 4.1.2 (SuperPro Simulation). Detailed results can be found in Appendix B. The base case requires five sets of equipment (Table 3.5.1) to meet the total production of 30,000 L. The overall recoveries of the flowsheet are 45.7% according to BPS and 45.97% according to SuperPro Designer®. These are well above the recovery yield (40%) assumed in the design basis. The batch time (218 hours or about 9.08 days) is also within the time (10 days) assumed. This allows for a turnaround time of about 17 hours for the bioreactors. A new feature of version 4.0 (to be released in early 1999) of SuperPro Designer® (Intelligen Inc., 1994) would calculate time taken for unit procedures taking place in the bioreactor such as charging of medium, sterilization, clean-in-place etc. This will allow an accurate calculation of turnaround time for the bioreactors. Turnaround times of 8 hours have been found satisfactory for recombinant bacteria at 23,000 L scale (Charles and Wilson, 1994).

Table 4.1.1: Simulation results for base case flowsheet with BPS

Block	Size	Time (hour)	Recovery (%)	Purity (%)	t-PA (g)
Bioreactor	6000 L	200	-	0.55	192
Microfilter	80 m^2	1	93.3	0.55	177
Ultrafilter	60 m^2	1	83	7.65	147.4
Affinity Chrom.	200 L X 3	3.7	73.6	99.98	108.5
Ultrafilter	10 m^2	0.5	94	-	102.0
Affinity Chrom.	85 L	3.2	90.687	99.9977	92.5
Ultrafilter	2.5 m^2	0.5	94.13	-	87.07
Gel Filtration	147 L X 6	7	99.997	99.9998	87.07

The change in volume of the broth as it moves through the processing train is shown in Figure 4.1.2. Broth volume increases after each chromatographic step and therefore an ultrafiltration step is required to reduce the volume so that the throughput to the next column is not too large. In the membrane separation units, lower retentate volume is obtained at the expense of recovery in the block. The ultrafiltration units were simulated such that the reduction in broth volume does not cause the retentate to become too concentrated in t-PA. There is an upper level set within the model in BPS. Operation at a concentration factor resulting in t-PA concentration higher than this generates a warning in the report and history file. No such feature was observed with SuperPro Designer®. In the chromatography columns, compromise had to be made between recovery and degree of purification. A more narrowly cut fraction resulted in a higher degree of purification at the expense of reduced recovery.

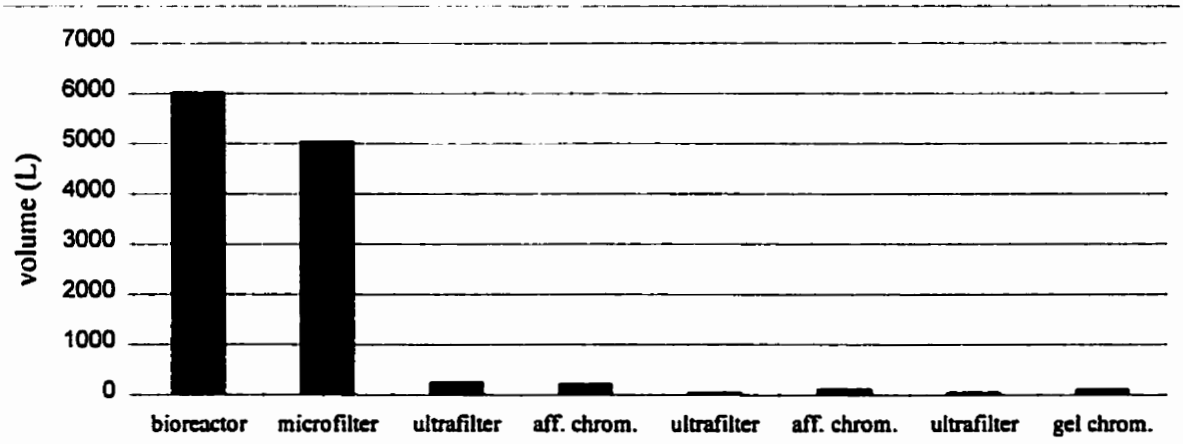

Figure 4.1.2.: Broth volume at different points of purification

As a supporting feature of BPS, among other variables, it calculates the pressure drop in the chromatographic columns and generates a warning when the specified process conditions cause any column to operate at subatmospheric pressure. This situation was observed in the gel filtration column with the first simulation run and the pressure was fixed subsequently.

A comparison of the results obtained with the two simulators shows that almost identical figures are obtained by feeding the information from BPS to SuperPro Designer® for simulating the process. However, there are some limitations of SuperPro Designer® that were encountered while using the simulator for this particular process. The "Fermentor" model that simulates stirred tank bioreactor in SuperPro Designer® was not used in the simulation because the model requires stoichiometric coefficients, which are not constant quantities for this process. BPS, in contrast, generates time profiles for all the reactants and products in the bioreactor according to the kinetics provided. For the chromatography columns, the recoveries reported by BPS were used directly as input to SuperPro Designer®. This resulted in exactly the same recovery in these columns. However, for the membrane separation units, the rejection coefficients specified for the BPS models had to be modified because their definition is different for the two simulators. In BPS rejection coefficient (R_i) is used to calculate the solute concentrations in the permeate (C_{pi}) relative to bulk retentate concentration (C_{bi}) and is defined as:

$$C_{pi} = C_{bi}\,(1-R_i) \qquad (4.1.1)$$

Table 4.1.2: Simulation results for base case flowsheet with SuperPro Designer®

Block	Stream	Size	Time (hour)	Recovery (%)	t-PA (g)
Bioreactor	S - 101	6000 L	201	-	192.0
Microfilter	S - 102	80 m^2	1	93.3	179.2
Ultrafilter	S - 105	60 m^2	1	83.2	149.1
Affinity Chrom.	S - 117	200 L X 3	3.7	73.6	109.7
Ultrafilter	S- 120	10 m^2	0.5	94.0	103.2
Affinity Chrom.	S - 109	85 L	3.2	90.7	93.6
Ultrafilter	S - 133	2.5 m^2	0.5	94.3	88.3
Gel Filtration	S - 125	147 L X 6	7	99.997	88.27

whereas, in SuperPro Designer® it is used to estimate the fraction of a component (F_i) remaining in the retentate as follows (McGregor, 1986) :

$$F_i = \frac{1}{CF + R_i (1-CF)} \qquad (4.1.2)$$

where CF is concentration factor defined as the ratio of volumetric flowrate of the feed to that of the concentrate. The rejection coefficient was further adjusted to maintain all the variables, which are common to the models in both the simulators, at the same values so that the two simulators could be compared. When used in rating mode, the membrane separation model in SuperPro Designer® requires specification on rejection coefficient (R_i), average filtrate flux, maximum concentration of the key component in retentate and membrane area among other variables. The simulator in this mode adjusts the concentration factor, which in turn determines the retentate volume, to calculate the fractions of components remaining in retentate (F_i) and to meet the specification on the maximum concentration of the key component in the retentate. A readjustment of the rejection coefficient causes the simulator to readjust the CF calculated such that the above requirements are satisfied. The rejection coefficient was adjusted until a CF close to the one reported by BPS was obtained. Tables 4.1.3 and 4.1.4 show the rejection coefficients used and the maximum t-PA concentration reported for the membrane separation units in the simulated process for the two simulators.

Table 4.1.3: Rejection coefficients (R_i) used for simulating the membrane separation units with the simulators

Block	BPS	SuperPro Designer®
Microfilter	1.0	1.0
Ultrafilter I	0.99	0.94
Ultrafilter II	0.98	0.965
Ultrafilter II	0.98	0.965

From Table 4.1.1 it is also found that BPS estimates that this flowsheet is capable of producing t-PA of required purity (99.9998%). With the current version of SuperPro Designer® (v 2.0) it was not possible to obtain t-PA purity up to four decimal points at

the scale used. SuperPro Designer® reports result up to five decimal points and do not have an engineering format for reporting numbers. With the scale used, a 99.9999% purity of t-PA with respect to BSA would mean that the concentration of BSA is below 10^{-6} units of concentration. A problem was also encountered while supplying specification for the chromatography columns due to the same reason. Simulation of the affinity chromatography columns with SuperPro Designer® requires the binding percentage and yield of each protein. This information was obtained from BPS. The yield of BSA in the first column was found to be about $2x10^{-6}$. However, the model for chromatography columns in SuperPro Designer® only accepts yields as a percentage of up to two decimal points. In the absence of engineering format it was not possible to reproduce yield of BSA with SuperPro Designer® at this scale. However, since it was possible to almost exactly reproduce the recovery and yield of t-PA, one can expect that a simulation with scaled up numbers would show that the required purity of t-PA is also met with SuperPro Designer® simulation.

Table 4.1.4: Maximum t-PA concentration in retentate reported for the membrane separation units by the simulators

Block	BPS	SuperPro Designer®
Ultrafilter I	0.64	0.64
Ultrafilter II	2.88	3.00
Ultrafilter III	4.75	5.0

4.1.4. ECONOMIC EVALUATION

Economic evaluation was carried out by combining information from both the simulators. Estimation of purchased equipment cost and operating labor was obtained from SuperPro Designer®. Size and number of the equipment were obtained from both BPS and SuperPro Designer® (Section 3.2). SuperPro Designer® was used in rating mode so that the size and number of the different equipment obtained from the two simulators were the same. Datar and Rosen (1990), while evaluating economics of a process producing human growth hormone from *E. coli*, used cost factors which appears to be more

appropriate for calculation of capital investment and operating cost of recombinant protein production process and were adapted here. A FORTRAN program was written for this purpose. The cost factors used can be found in Appendix C.

The economics of the flowsheet was evaluated in terms of Return on Investment (ROI) and Gross Margin (GM). The results were compared with those of Datar et al. (1993), who carried out a comparative study of t-PA production in animal cell and bacterial fermentation. The reported economics is for a process producing t-PA from CHO cells using the same purification scheme as the base case but separate bioreactors for growth and production using serum containing medium in both. An alternate flowsheet that uses two stage cell culture as outlined by Datar et al. (1993), is simulated and compared in the next section (Section 4.2). The equipment in their flowsheet were sized based on production demand, step yields and mass balances. The batch time was assumed to be 5-7 days. In their calculation, the selling price of t-PA was taken to be $22,000/g and the production rate, 11,000 g/year so that revenue (R) is 242 X 10^6 dollars. These same figures are used for economic evaluation of the base case so that the two processes can be compared. A more realistic selling price of t-PA would be about $16,000/g (Cooney, 1999). Table 4.1.5 shows the calculation of the economic parameters for the two cases.

The ROI for the base case is about 11% lower than the one considered by Datar et al. (1993). However, their calculations are based on 47% overall recovery whereas the simulated process is based on 40%. The estimated equipment purchase cost for the base case is 28% higher. Datar et al. (1993) do not mention the size and number of any equipment in their flowsheet except for the bioreactor (7000 L). The purchased equipment costs in their case are 1993 prices calculated from grass-roots and in some cases obtained from vendors and updated in-house files. The prices obtained from SuperPro are 1994 prices.

Table 4.1.5: Comparative economic analysis (in millions of dollars)

	Base Case	Datar et al. (1993)
Purchased Equipment Cost, C_{PE}	14.2	11.12
Fixed Capital, C_{FC} = (4.6XC_{PE})	65.32	51.16
Total Capital, C_{TC} = (5.5XC_{PE})	78.1	61.40
Revenue, R	242	242
Annual Operating Cost, AOC	101	117
Gross Profit, GP = (R-AOC)	141	125
Net Profit, NP (40% tax)	84.6	75
Net Cash Flow, (NP + Dep.)	90.45	81
Return on Investment, ROI	116%	130%
Gross Margin, GM = (GP/R)	58%	52%

A breakdown of the equipment purchase cost (Figure 4.1.3) for the base case shows that bioreactors account for about 14% of the total cost and among the downstream units, gel filtration columns (27%) are the major contributors followed by the chromatographic columns (25%). In contrast, Datar et al. (1993) reported that, for their flowsheet, bioreactors account for 54% of the C_{PE}, whereas recovery equipment contributes only 24%. This discrepancy is most likely due to the higher overall recovery (47%) assumed for their flowsheet, which reduced the size of the recovery units. The large bioreactor volume (42,000 L) used by their two-step culture process could have also partly contributed to this incongruity. It was not possible to compare the number and size of the recovery units in these flowsheets.

Of the annual operating cost (AOC), process chemicals and media was found to account for 42% and labor, about 5.2% of the total cost (Figure 4.1.4). At an estimated price of $25/L, cost of serum free medium accounts for about 62% of the total process chemicals and media cost. It is clear that the profitability of this flowsheet is very sensitive to this price. Process chemicals and media cost is actually found to be lower for the base case. Datar et al. (1993) considered the use of serum containing medium in their bioreactors instead of serum free medium. Even then, fermentation material accounted for 75% of

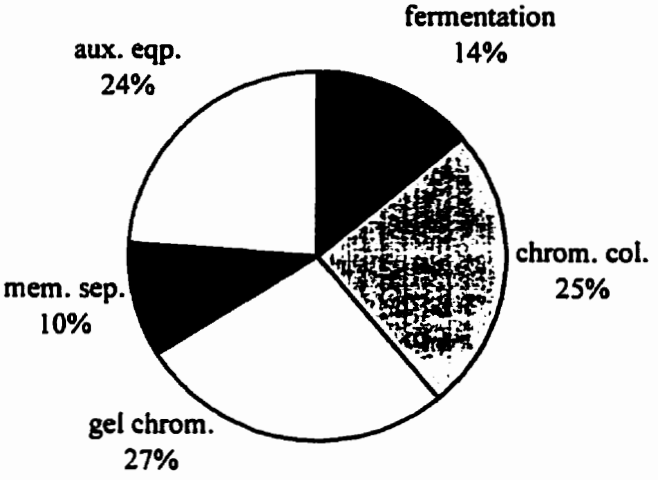

Figure 4.1.3: Breakdown of equipment purchase cost for base case

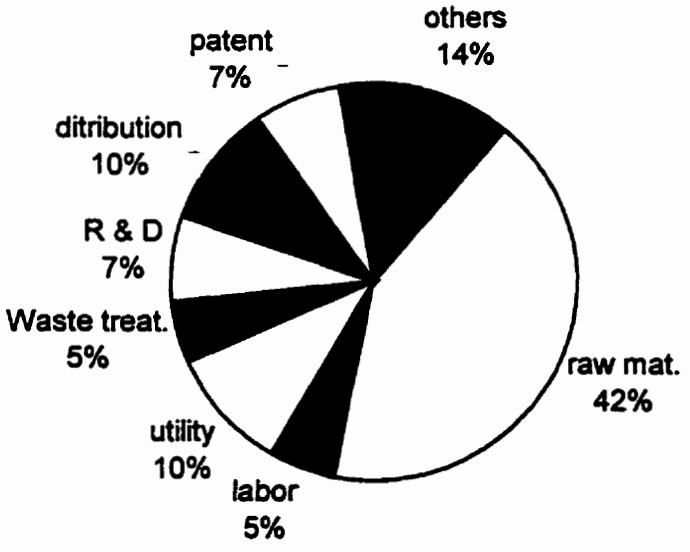

Figure 4.1.4: Breakdown of annual operating cost

their material price, which for the base case is found to be 62%. Their flowsheet, therefore, was found to be very sensitive to serum price and a sensitivity study for that case showed that, a 70% increase in the serum price would bring about 35% reduction in the ROI of the process. The estimated labor costs are found to be close and are higher for the other flowsheet probably because of the larger number of bioreactors (Figure 4.1.4). The combined contribution of tax/insurance and plant overhead in the case of Datar et al. (1993) is found to be $4.68 million which is close to the number ($4.5 million) obtained for the base case. Estimation of contributions from utilities, waste treatment and patent/royalties using cost factors (Datar and Rosen, 1990) yielded smaller numbers. Contribution from other sources combined came to $22.22 million for the base case and $36.27 million for the other flowsheet.

4.1.5. COMPARISON OF BPS AND SUPER PRO DESIGNER

The short-cut unit operation models of SuperPro make simulation of bioprocess simple and easy. However, for some models the required input information was not easily available. For example, to simulate a bioreactor with SuperPro, the extent of reaction must be known. This is a quantity not common in the jargon of biotechnology. Instead, BatchPro Designer (Intelligen, Inc., 1997), another tool from Intelligen Inc., appears to be more suitable for bioprocess simulation, especially the bioreactor, since it is equipped with rigorous and versatile reaction modules.

Most unit operation models in SuperPro are very simplistic. The majority of the unit operation models use a constant linear multiplier to convert a component inlet-stream to the outlet stream composition. Therefore, reaction yields and separation rates are rarely a function of process stream properties or operating conditions (Shanklin, 1999). This straightforward approach in modelling taken by SuperPro results in loss of its analytical ability. For example, the microfilter model in SuperPro requires the specification of permeate flux directly rather than calculating the flux from specified resistance, pressure

drop and fluid viscosity as is done by Aspen BPS. With an appropriate model, this fundamental approach adopted by BPS allows the user to explore the effect of change in transmembrane pressure or increased resistance due to fouling. It is also not possible to access the built-in unit operation models of SuperPro Designer® to modify or improve them, a feature identified as one of the unique requirements of a bioprocess simulator (Shanklin, 1999).

The output generated by SuperPro is not very informative. SuperPro cannot generate graphical outputs and therefore, unlike BPS, it is not possible to follow the changes in the variables of the process with this simulator.

The simplicity of simulation with SuperPro with small amounts of process information versus the analytical ability of BPS suggests that these two simulators should be used simultaneously. Since simulation of most of the units is simpler with SuperPro, it can be used initially to screen process alternatives in a short time and with minimal effort. Once a process has been chosen, it can be analyzed using the more complex approach of BPS to find the optimum operating conditions and predict the changes that might result from changing parameters of the process.

Table 4.1.6: Comparison of SuperPro and BPS at process level

	SuperPro	Aspen BPS
Required Data	few	many
Output Data	concise	informative
Input File Size	82 KB [a]	9 KB [b]
Error Diagnostics	undetermined	reasonable
Suitability	preliminary screening	detailed analysis

[a]contains thermodynamic and other properties, information for economic evaluation, scheduling data besides components, flowsheet connectivity and block specifications
[b]contains only the name of the components, databanks to be used, flowsheet connectivity and unit operation block specifications

It is to be noted that BPS seems to have reached a level of dormancy where no more development activity is planned over the current version. This is most probably due to the

development of Batch Plus (Zimmermann, 1997) by Aspen Technology. Development of Batch Plus was based on feedback from pharmaceutical companies using BPS. On the other hand, efforts continue to improve and add new features to SuperPro Designer®. Handling of visual objects and interfacing with other applications are much easier with later versions of the software. New features have been added to enhance presentation of the flowsheet and formatting of the stream reports. New unit operation models (e.g. custom mixer, splitter, batch evaporator, extractor, inclined plate clarifier etc.) have been included and updating of the economic models (e.g. equipment cost data, estimation of labor requirement etc.) have been carried out (Intelligen Inc., 1996; Intelligen Inc., 1998). Modifications of the existing models of SuperPro Designer® (e.g. fermentors, chromatography columns, filters etc.) are going on and some new variables (e.g. aeration rate in the bioreactor model) have been added to the existing models.

Some of the future modifications in SuperPro are going to deal with the very practical aspects of bioprocessing. Handling of unit procedure is going to be implemented in version 4.0 of the software to be released in 1999 (Petrides, 1998). This will enable calculation of time for bioreactor operations such as charging of medium, sterilization, clean-in-place etc. Some bioprocess specific operations such as a Centritech centrifuge for modeling removal of mammalian cells under gentle conditions, will be added and more refined scheduling of batch operation down to task-level activities are going to be included (Intelligen Inc., 1997). A new class of stream to represent flow of discrete entities, such as bottles, boxes etc. has already been added to the later versions and unit operations for molding, filling, boxing etc. have been implemented to model product formulation and packaging. To model the novel concept of multiple and overlapping batches (Section 4.5), provision has been made in version 3.0 to allow process steps to share equipment in batch recipes. Calculation of resources such as utilities, labor, raw materials etc., for multiple and overlapping batches to eliminate resource bottlenecks is going to be implemented.

4.1.6. SENSITIVITY STUDY

This section reports on sensitivity of the base process to process specific variables. A more general sensitivity study for unit operation blocks and economic parameters and variables, applicable to all the simulated processes, is presented in Chapter Five. The purpose of this section is both, to investigate how any change in the particular variable would affect the process performance and also to find out by how much the simulation results are affected if the estimate used for the variable is not correct.

1. Price of Serum Free Medium

The price of serum free medium is a variable that is expected to change due to the endless effort to develop low cost serum free medium. Sensitivity study to serum free medium price shows that 50% reduction in this price will result in 12% increase in the ROI of the base process and 70% increase will bring about 12.5% reduction (Figure 4.1.5). Therefore, although the base process is sensitive to serum free medium price, it is not as sensitive as the other flowsheet (Datar et al., 1993) is to serum price (Previous Section).

2. Percentage of BSA in Serum Free Medium

In the base case simulation, it was assumed that the concentration of BSA like protein in the serum free medium is equal to its concentration in serum containing medium (Section 4.1.3). Two simulation runs were carried out to study the sensitivity of the base process to lower BSA like protein concentration in the medium. The main effect was observed in the performance of the affinity chromatography columns. Table 4.1.7 compares the recovery and purity of t-PA in these columns as concentration of BSA like protein goes down in the medium.

It appears from the table that a 50% reduction of BSA concentration in the medium would result in significant improvement in recovery in the first affinity chromatography column. A further reduction would improve purity but would not result in proportionate higher recovery. This provides a huge incentive to use low protein medium and not just

serum free medium for t-PA production. With 30% BSA like protein in the medium, it appears that completely pure t-PA can be obtained with a single chromatography column. However, BPS reports the purity in the chromatography columns up to three decimal places and therefore, a purity of 100% as reported by BPS only means that it exceeds 99.999%. For t-PA the required purity is 99.9999%. Which means the process may still require another chromatography column. In Section 3.1.1 it was also mentioned that removal of infectious agents and other contaminants such as DNA, endotoxin etc. require a combination of two chromatography steps. Therefore, although the purity of t-PA with respect to BSA in a 30% BSA containing medium reaches more than 99.999% with a single chromatography column, the requirement of another chromatography step from the process can not be eliminated. The regulatory authorities also require a combination of two different chromatography steps for these processes, so that the gel filtration column for the polishing step should also be present in this flowsheet (Cooney, 1999).

Table 4.1.7: Comparison of recovery and purity with changing BSA concentration

BSA Concentration	Recovery (%)	Purity (%)
100%	73.6	99.98
50%	84.56	99.999
30%	87.6	>99.999

The volume of product recovered from the chromatography column in both cases is 75 L per column. Therefore the sizes of the subsequent down stream units will not be affected by switching to a low BSA medium.

3. Combination of Affinity Chromatography and Ion-Exchange

Since ion-exchange resins are cheaper than affinity media (Sofer, 1986; Moo-Young and Chisti, 1990), it was further investigated whether a combination of affinity and ion-exchange process rather than two affinity columns as considered for the base case, would meet the required purity and recovery as outlined in the design basis. A simulation run

was carried out which revealed that such a combination would result in 93.93% recovery and 99.9977% purity in the ion-exchange column.

Table 4.1.8: Simulation results with BPS for combined affinity and ion-exchange chromatography flowsheet

Block	Size	Time (hour)	Recovery (%)	Purity (%)	t-PA (g)
Bioreactor	6000 L	201	-	0.55	192
Microfilter	80 m^2	1	93.3	0.55	177
Ultrafilter	60 m^2	1	83	7.65	147.4
Affinity Chrom.	200 L X 3	3.7	73.6	99.98	108.5
Ultrafilter	10 m^2	0.5	94	-	102.0
Ion-exchange	113 L	4.44	93.935	99.9977	95.8
Ultrafilter	4 m^2	1.8	84.88	-	81.3
Gel Filtration	147 L X 3	7	99.996	99.9998	81.3

The elution peak from the ion-exchange column is skewed with a long tail. The product recovery is achieved in 215L of volume, whereas, with an affinity column (base case) the volume of product is 100L. Therefore, the use of an ion-exchange column would require larger sizes of downstream units following the column. Alternatively, using larger membrane area and longer batch time for ultrafiltration would reduce the volume to be treated by the gel filtration column (Table 4.1.8.), which are much more expensive then the membrane separation units. The total batch time in this case therefore, would be 220.5 hours compared to 218 hours for the base case.

Table 4.1.9: Comparative economic analysis (in millions of dollars)

	Combined Chromatography	Base Case
Purchased Equipment Cost, C_{PE}	9.89	14.2
Fixed Capital, C_{FC} = (4.6XC_{PE})	45.5	65.32
Total Capital, C_{TC} = (5.5XC_{PE})	54.4	78.1
Revenue, R	242	242
Annual Operating Cost, AOC	94.64	101
Gross Profit, GP = (R-AOC)	147.36	141
Net Profit, NP (40% tax)	88.4	84.6
Net Cash Flow, (NP + Dep.)	94.4	90.45
Return on Investment, ROI	174%	116%
Gross Margin, GM = (GP/R)	61%	58%

Table 4.1.9 presents the overall economics for this process and compares it to the base case. As anticipated, combination of chromatography (affinity and ion exchange) process is found to be a very attractive alternative to the base case. The reduced equipment purchase cost results from the use of fewer number of gel filtration (3 instead of 6) columns. Throughput to the gel filtration column could be reduced to a smaller volume with this process at the cost of lower recovery since the recovery in the ion-exchange column (93.9%) is higher than the affinity column (90.7%) in the base case. Therefore, the overall recovery is still above than that required (40%). The lower annual operating cost resulted mainly from the lower replacement cost of the ion exchange resin ($400/L) compared to affinity chromatography media ($2000/L). However, the main concern with this flowsheet is whether a combination of an affinity chromatography column and an ion-exchange column followed by gel filtration, would be able to reduce the endotoxins, DNA and other contaminants to the desired level. This can only be investigated by experimental studies.

4. Replacement Frequency of Chromatographic Resin and Membrane

Replacement of chromatographic resin and ultrafiltration and microfiltration membranes accounts for about 18% of the raw material cost for this process. Though this represents a small fraction of the overall cost, the figures used in its calculation varied widely depending on the source. The lifetime for chromatographic resins (affinity and ion exchange) suggested by SuperPro Designer®, is 200 cycles, and for gel filtration media, is 300 cycles. For membrane separation units, the lifetime is assumed to be 2000 operating hours. The replacement costs from this source are $2000/L for affinity chromatography, $400/L for ion exchange, $200/L for gel filtration and $200/m^2 for membranes. The figures for these reported by Petrides et al. (1989) is slightly different. They considered replacement frequency of 400 cycles for ion exchange and gel filtration media and replacement cost of $450/L for ion exchange, $400/L for gel filtration and $250/m^2 for ultrafiltration membranes.

In the base process, only 35 cycles are carried out with the chromatographic columns per year. Therefore, with this process it may not be necessary to replace the resins every year. However, the lifetime of the resins depends on the particular resin itself and it has also been noted that the position of the column in the purification sequence also influences the lifetime of the resin (Sofer and Nystrom, 1989). Since the sample contains more contaminants towards the beginning of the sequence, the first separation medium used generally requires more frequent replacement than the others. A single replacement of the resins was considered in the annual operating cost calculation. Similarly, for the membranes, single replacement was considered although the operating hour per year is much less than that recommended for replacement. Therefore, a sensitivity study was carried out to determine by how much uncertainties involved in the calculation from this source will effect the overall process performance.

Figure 4.1.6 shows that, if replacement takes place once every 175 cycles (i. e. close to the recommended replacement frequency), and the cost for the replacement is distributed equally over the whole period, the ROI will improve by 4% (case 2). If replacement cost is not distributed (case 1), the ROI of the process for the year in which replacement does not take place will increase by 5%.

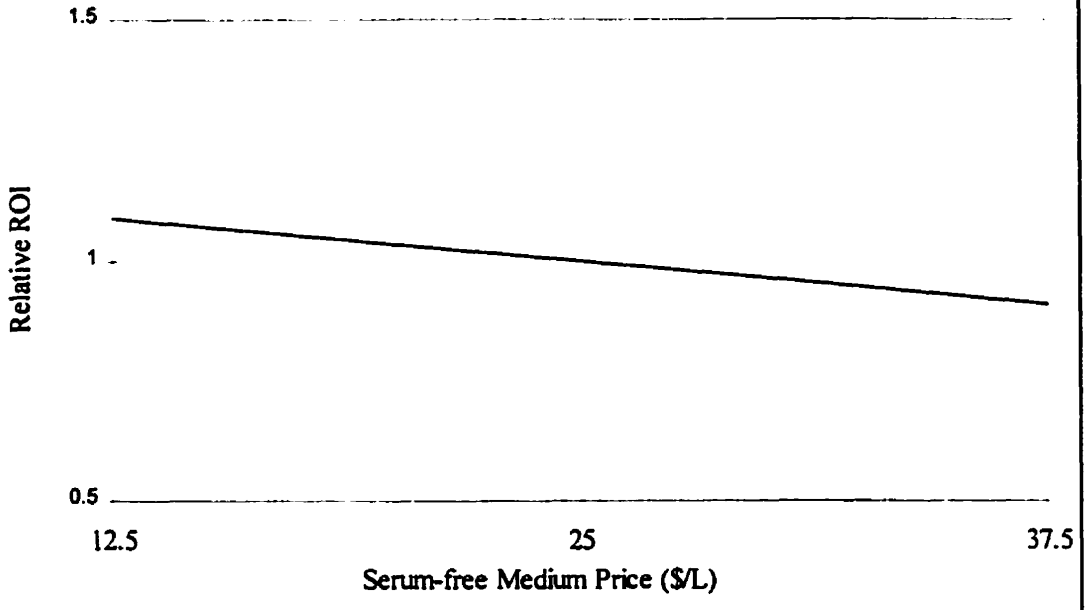

Figure 4.1.5: Sensitivity to Serum-free Medium Price

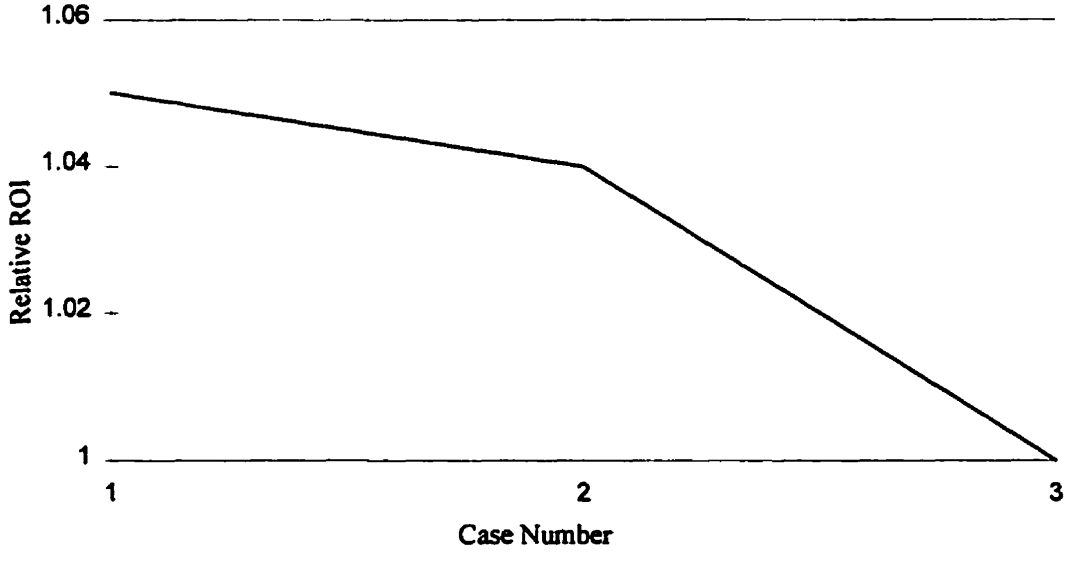

Figure 4.1.6: Sensitivity to Replacement Frequency

4.2. Process Alternative: Two Stage Cell Culture

4.2.1. INTRODUCTION

This alternative to the base case flowsheet uses the concept of two-stage cell culture. Separate bioreactors are used for growth and production of the cells where the growth bioreactor is supplied with serum containing medium and the production bioreactor with serum free medium. Serum contains some growth promoting substance, which are difficult to replicate in serum free medium. The main advantage of two-stage cell culture is the greatly simplified downstream processing in absence of background protein in the medium. Since downstream processing accounts for about 70 to 80% of the overall cost for high value products like t-PA, such a flowsheet seems worth investigating.

4.2.2. PROCESS DESCRIPTION

Although several investigators (Dutton, 1998; Lubiniecki et al., 1989) have reported that CHO cells tend to form aggregates in suspension culture in medium containing serum, such medium has been used by several other investigators (Kurano et al., 1990; Leelavatcharamas et al., 1994; Hansen and Emborg, 1994; Lakhotia et al., 1992; e.g. 10% fetal calf serum, 5-10% FCS, 3% fetal bovine serum etc.) for suspension culture of CHO cells in stirred bioreactors. A tangential-flow filtration system has been devised to produce t-PA by two-stage cell culture that reduces levels of serum coming from the seed train before inoculating the production vessel (Ogez and Builder, 1990). Datar et al. (1993) also carried out economic evaluation of a two-stage cell culture process, based on experimental observation, for production of t-PA from CHO cell.

In the alternate flowsheet, shown in Figure 4.2.1 along with the base case flowsheet, the growth bioreactor is supplied with medium containing 10% serum whereas the production bioreactor is charged with serum free medium. The concentration of BSA-like protein in the serum free medium is assumed to be 10% of that in the serum containing medium. Because of this low background protein concentration, the downstream process of this flowsheet uses ion exchange chromatography for purification. Ion-exchange chromatography is assumed to be sufficient to reach the desired purity. In laboratory, it has been demonstrated that purification of t-PA from serum free medium using a single ion-exchange column followed by gel filtration resulted in a purification factor of 58 and an overall yield of 61% (Kruithof et al., 1985).

With the two step cell culture flowsheet, broth from the growth bioreactor is reduced to about 50% of its original volume by microfiltration. Therefore, the number of production bioreactors is half of that of growth bioreactors (Datar et al., 1993) and the number of associated downstream units is half of that required by the base process. Before introduction to the production bioreactor, the cells from the growth bioreactor are washed to remove the bulk of the serum proteins. This cell washing is carried out in the microfilter. In laboratory, phosphate buffered saline is used to wash cells grown in serum containing medium prior to transferring them to serum free medium (Kruithof et al., 1985).

Takagi and Ueda (1994) reported on on-line determination of optimum time for switching from growth phase to production phase for t-PA production for anchorage-dependent diploid fibroblast cells of human embryo lung (HEL) in microcarrier culture. The optimum switching time was determined to be the time at which the t-PA production activity of the cells in growth culture became highest. t-PA production activity of the cells was estimated by on-line regression analysis using physiological data of the current state, including oxygen consumption rate and cell concentration, as well as data from past batches. The optimum switching time was found to vary with growth culture batches.

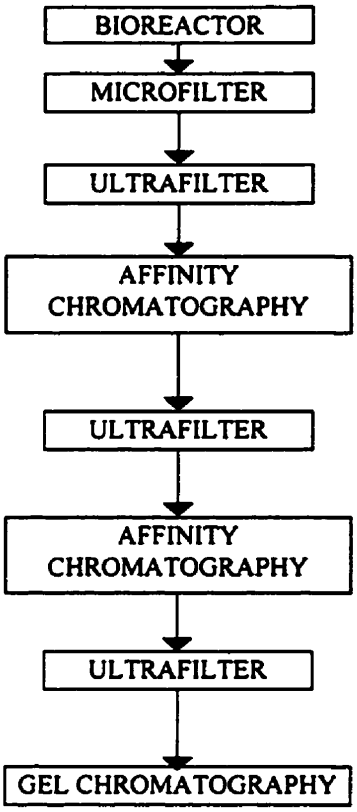

Fig. 4.2.1.(a) Process flowsheets for t-PA production from CHO cells

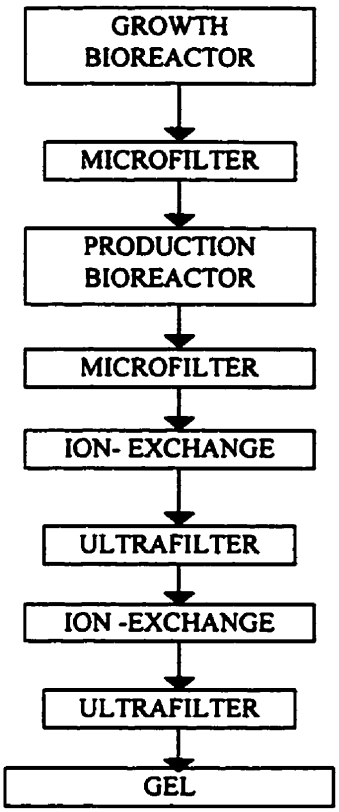

Fig. 4.2.1.(b) Alternate flowsheet for t-PA production form CHO cells

Simulation of this process requires information about process parameters and operating conditions. The simulated bioreactors are stirred tank type with the CHO cells grown in suspension culture. The batch charge to the growth bioreactor consists of 10% Fetal Bovine Serum (FBS), 90% Ham's F-12 medium and 4mM Glutamine. The bioreactor is inoculated with 1000 L of inoculum having a concentration of $2X10^5$ cells/ml. Ham's F-12 medium is replaced with HB-CHO basal mixture and a proprietary supplement (Immucor Canada Inc.) in serum free medium for the production bioreactor. The contents of the bioreactor are aerated with 0.02 VVM sterile air.

The operating variables and parameters for the different units of the down stream train are the same as for the base case (Section 4.1.2). The affinity chromatography columns of the base case are, however, substituted with ion-exchange chromatography columns in this

flowsheet. The ion-exchange columns in the alternate flowsheet are simulated under similar conditions as for the affinity chromatography columns. The ion-exchange resins used in the laboratory scale purification of t-PA are strong cations such as SP Sephadex C-50 and Mono S (Kruithof et al., 1985, Prouty, 1993). The total ionic capacity of SP Sephadex C-50 is found to be between 2.0 - 2.6 mmol/g (Pharmacia LKB Biotechnology). An estimated surface area of 1 cm^2/cm^3 was used in the simulation. The operation was simulated at a pH of 4.5, close to the isoelectric point of BSA (5.0) as described in the laboratory scale purification methods. In laboratory, the ion-exchange column is equilibrated with a mixture of 0.1M sodium acetate, 0.25M sodium chloride, 0.01% Tween 80 and 0.05% NaN_3 at pH 4.5. This same buffer is used to wash the column and t-PA is eluted by a sodium chloride gradient from 0.25 to 1M in the same buffer (Kruithof et al., 1985). The other laboratory method uses two steps with ion exchange for purification of t-PA (Prouty, 1993). A shallow gradient of NaCl (0.1 to 0.157 M) at pH 5 is used to elute the protein in 35 mM acetic acid.

For ion-exchange, column length has no major impact on resolution (Chisti and Moo-Young, 1990). Therefore, ion exchange columns tend to be short, up to 1m in height. Columns are scaled up in diameter to accommodate the quantity of matrix needed for the separation, holding constant height. Cross-linking procedures have resulted in matrices for ion exchange with improved rigidity and flow characteristics. Separation efficiency was not affected by increasing velocity up to 280 cm/s in a 150 L DEAE-Sepharose CL-6B Fast Flow column (Berglof and Cooney, 1989). The column was scaled up from laboratory to large scale by maintaining the same elution conditions since resolution was found to be largely dependent upon elution conditions.

4.2.3. SIMULATION AND RESULTS

Table 4.2.1 shows the Aspen BPS simulation results for this flowsheet. From the table it is found that this flowsheet is capable of producing t-PA of required purity (99.9998%). The overall recovery of the flowsheet is 46.3%. This is well above the recovery yield

(40%) assumed in the design basis. The time required for completion of one batch for this flowsheet is 221 hours or about 9.2 days. This is also within the batch time (10 days) assumed in the basis. This flowsheet requires five sets of equipment for the first two steps and then only three sets afterwards because of the assumption stated in the previous section. The cell concentration in the production bioreactor therefore, is twice the concentration in the growth bioreactor and the product concentration is also double.

A cell wash stage, to remove serum from the broth of growth bioreactor, was attempted using the diafiltration option of the batch membrane separation model of BPS. However, the model with diafiltration never converged. The model performed without problem until the diafiltration step was reached and then stopped. The error message retrieved from the history file reported that the solver encounters divisions by zero with further calculation. Repeated trials with this model turned out to be unsuccessful. Therefore, a reasonable volume was assumed for the diafiltration stream to calculate the raw material cost. A sensitivity study to the diafiltration stream volume was also performed (Section 4.2.6).

Table 4.2.1: Simulation results for alternate flowsheet

Block	Size	Time (h)	Recovery (%)	Purity (%)	t-PA (g)
Growth Bioreactor	6000 L	80	-	-	-
Microfilter	80 m^2	1	-	-	-
Production Bioreactor	5000 L	121	-	6.86	390.0
Microfilter	70 m^2	1	84.5	6.86	329.55
Ultrafilter	50 m^2	1	84.76	-	279.32
Ion-Exchange Chrom.	200 L X 2	5	83.45	99.93	233.0
Ultrafilter	15 m^2	0.7	92.37	-	215.3
Ion-Exchange Chrom.	113 L	4	87.16	99.999	187.66
Ultrafilter	5 m^2	0.4	96.26	-	180.65
Gel Filtration	147 L X 5	6.9	99.997	99.9998	180.64

The elution profile of t-PA from the ion exchange column was different than the one observed from the affinity chromatography column of the base case. The t-PA peak was

found to have an elongated tail. This tailing could have been a result of large particle diameter, high flow rate and low molecular diffusivity of the solute (Yamamoto, 1978, 1979). Tailing of elution curve is also observed with ion exchange chromatography when increased sample concentration causes the column to operate in the nonlinear region of the isotherm (Kato et al., 1982). With the sample concentrations (0.3 g/L and 0.7 g/L in the first and second column respectively) handled in the ion exchange columns of this flowsheet, the second explanation seems improbable. Even then, simulations were carried out with lower t-PA concentration. Strangely enough, the peaks were found to have longer tails and lower heights at progressively lower concentrations and sharpening of the peak was observed at higher concentrations. For single component Langmuir isotherms, tailing of the peak increases as the nonlinearity increases (Yamamoto et al., 1988; Lin et al., 1989). The second coefficient of the isotherm is a measure of nonlinearity, which in the BPS model is denoted by "kload". A sensitivity study of the ion exchange model, however, revealed that, the performance of the column was insensitive to kload (Chapter Five). Simulations were also carried out with lower flow rate (half of the design case flow rate). No improvement in the elution profile, however, was observed by changing this variable.

A strong self-sharpening effect is exhibited by the elution profile as mass transfer coefficient becomes large (Lin et al. 1989). To increase the mass transfer coefficient, simulations were carried out with smaller particle diameter (up to 60 μm of particle diameter). Although the reported mass transfer coefficient increased, there was no change in the elution profile. To improve the molecular diffusivity, simulation was carried out with a lower molecular weight (up to one-tenth of the actual molecular weight) of t-PA just for observation purposes. Again, the elution curve was unaltered. These observations suggest that this phenomenon could be a result of limitation of the ion-exchange model in BPS. This may also result from the choice of parameters (e.g. capacity, adsorption isotherm constant etc.) for this model, for most of which no close estimates were available from literature. Sensitivity study to these parameters was carried out and the

results are presented in Chapter Five. Solute capacity and charge were found to be critical parameters for this model.

Because of this tailing elution curve, the volume of product from the ion exchange columns was much larger compared to the affinity columns (300 L compared to 67 L). This required larger membrane separation units following both the ion exchange columns. The volume of the second ion exchange column was also found to be bigger (113 L) compared to the second affinity chromatography column (85 L). However, as discussed in the following sections, the contribution of membrane separation units to the total equipment purchased cost is not significant and the ion exchange columns are also much cheaper than affinity columns. Therefore, the economics of the process was not significantly affected by the larger product volume from the columns.

4.2.4. ECONOMIC EVALUATION

The economics of this flowsheet was evaluated using the estimates of purchased equipment cost and operating labor from SuperPro Designer® for calculation of total capital investment and annual operating cost. Cost factors (Datar and Rosen, 1990) used in this calculation can be found in Appendix C. Table 4.2.2 summarizes the economics of the simulated process.

Breakdown of equipment cost (Figure 4.2.2) for this case shows that contribution from fermentation equipment accounts for about 30% of the total cost. This is due to the eight bioreactors used for growth and production of the cells. Ion-exchange chromatography columns account for about 18% followed by membrane separation and gel filtration columns, which contribute almost equally (16% and 15% respectively). A breakdown of the annual operating cost shows that contribution from raw material (41%) dominates the total cost. Of the raw material cost 84% comes from fermentation media for the eight bioreactors (Figure 4.2.3). The gel filtration columns contribute by 7.4% while the ion-exchange columns account for only 5.5% and the diafiltration stream, 2.3%. A sensitivity

study was carried out to diafiltration stream since simulation of diafiltration did not converge (Section 4.2.6).

Table 4.2.2: Economic analysis (in millions of dollars)

	Two Stage Cell Culture
Purchased Equipment Cost, (C_{PE})	8.8
Fixed Capital Investment, $C_{FC} = (4.6XC_{PE})$	40.48
Total Capital Investment, $C_{TC} = (5.5XC_{PE})$	48.4
Revenue, R	176
Annual Operating Cost, AOC	92.90
Gross Profit, GP = (R-AOC)	83.1
Net Profit, NP (40% tax)	49.86
Net Cash Flow (NP + Dep.)	55.86
ROI (Net Cash Flow/ C_{TC})	115%
Gross Margin, GM = (GP/R)	47%

4.2.5. COMPARISON WITH BASE CASE

The two-stage cell culture flowsheet is compared to the base case on the basis of Return on Investment (ROI) and Gross Margin (GM). The economics of the base case is recalculated assuming the selling price of t-PA to be $16,000/g as suggested by Cooney (1999). Table 4.2.3 shows the calculation of the economic parameters for the flowsheets. As it appears from the table, the two-stage cell culture flowsheet has higher ROI and GM because of both lower total capital investment (which is a consequence of lower purchased equipment cost) and lower annual operating cost.

Table 4.2.3: Comparative economic analysis (in millions of dollars)

	Base Case	Two Stage Culture
Purchased Equipment Cost, (C_{PE})	14.2	8.8
Fixed Capital Investment, $C_{FC} = (4.6XC_{PE})$	65.32	40.48
Total Capital Investment, $C_{TC} = (5.5XC_{PE})$	78.1	48.4
Revenue, R	176	176
Annual Operating Cost, AOC	101	92.90
Gross Profit, GP = (R-AOC)	75	83
Net Profit, NP (40% tax)	45	49.86
Net Cash Flow (NP + Dep.)	51	55.86
ROI (Net Cash Flow/ C_{TC})	65%	115%
Gross Margin, GM = (GP/R)	43%	47%

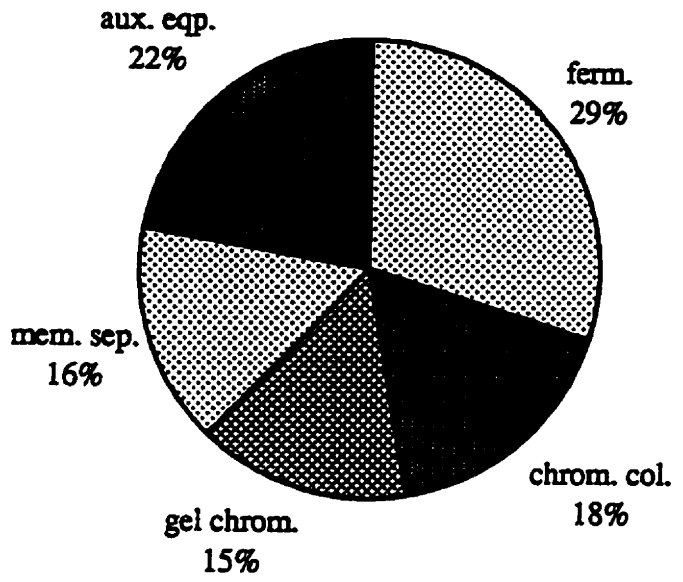

Figure 4.2.2: Breakdown of equipment purchase cost

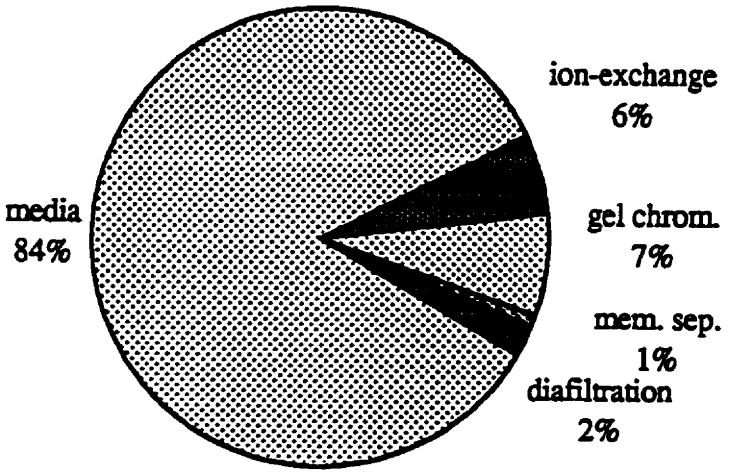

Figure 4.2.3: Breakdown of raw material cost

The two stage cell culture flowsheet uses fewer (three) sets of equipment following cell separation compared to the base case (five). This results in significant savings ($5.4 million) in the equipment purchase cost and consequently lower capital investment for this flowsheet. The expensive affinity columns of the base case have been replaced with cheaper ion-exchange columns in this flowsheet. The purchase cost obtained from SuperPro for these columns are almost the same, however, the replacement cost for the resins are quite different ($2000/L versus $400/L). Therefore, the contribution (20%) from affinity columns to raw material for base case is much higher compared to the contribution (5.5%) from ion-exchange columns. The contribution of media, on the other hand, increased from 62% for the base case to 84% for the two-stage cell culture flowsheet.

For both the cases, raw material accounts for the major portion (about 40%) of annual operating cost for the processes. The operating cost for the two-stage cell culture flowsheet is found to be lower which is mainly due to the use of lower priced serum containing medium in the growth bioreactor and also replacement of affinity columns by the ion-exchangers. Serum containing medium for the five growth bioreactors accounts for 59% of the media cost compared to 41% by the serum free media for the three production bioreactors.

4.2.6. SENSITIVITY STUDY

1. Sensitivity to Serum Price

Since serum price is a variable that can easily change, sensitivity of the flowsheet to serum price was studied to investigate by how much the process can benefit from a lower serum price. Figure 4.2.4 shows that a 50% decrease in this price will result in about 6% increase in the ROI of the process. Therefore, the process is fairly sensitive to serum price.

2. Diafiltration Stream

The diafiltration stream contributes very insignificantly (2.3%) to the total raw material price of the process. However, since the simulation of the microfiltration model with the diafiltration option did not converge, a sensitivity study was carried out to determine whether this would effect the process significantly. Figure 4.2.5 shows that even a 4-fold increase in the volume of the diafiltration stream will only lower the ROI by 1.8%. Therefore, the effect of this stream on the overall process economics seems very insignificant. However, a larger diafiltration volume would also mean increased batch time or increased membrane area for a fixed batch time for the process.

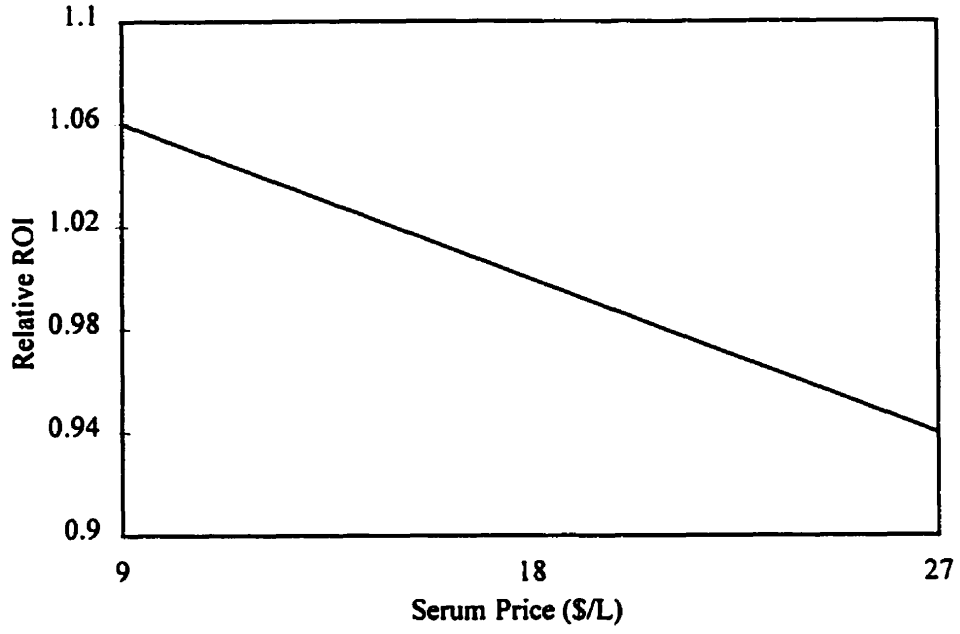

Figure 4.2.4: Sensitivity to serum price

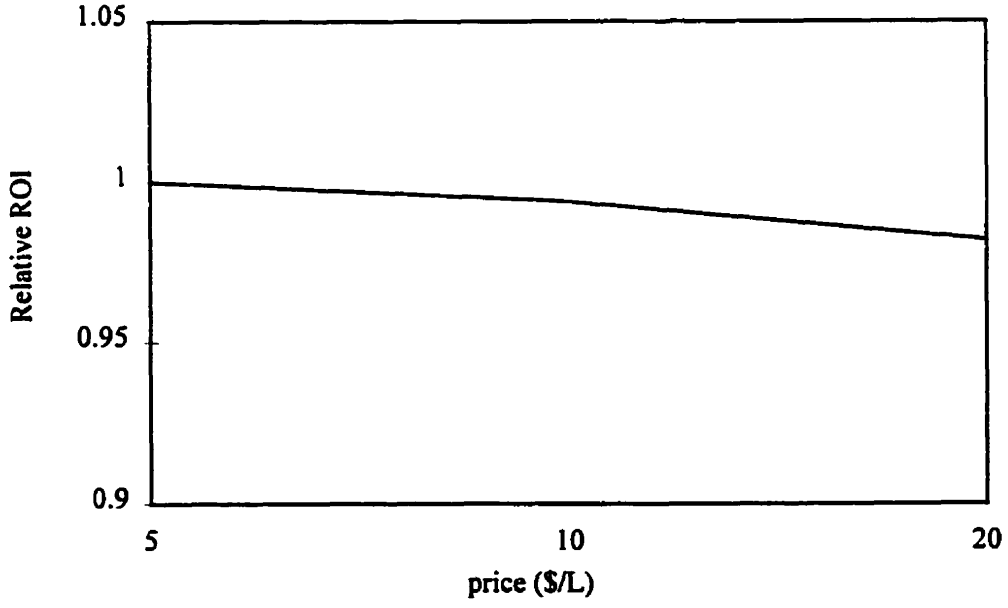

Figure 4.2.5: Sensitivity to price of diafiltration stream

4.3. Process Alternative: Fed-batch Mode of Operation

4.3.1. INTRODUCTION

Animal cell cultivation technology has faced some unresolved issues for the past forty years, which include low product concentration, low viable cell density, short production phase, toxic byproduct accumulation and depletion of essential nutrients. Metabolic waste products, lactate and ammonia, have significant effect on cell growth and metabolite production. Lactate is mainly produced from glucose metabolism and ammonium is excreted when amino acids are metabolized by the cells. Accumulation of waste metabolites at a high rate can be avoided through controlled feeding of nutrients, glucose and glutamine. The control of nutrient level can be realized only by a continuous dynamic feeding using fed-batch mode of operation. A sound strategy for fed-batch culture must consider both, providing sufficient nutrients and minimizing the production of undesired metabolites and the increase of osmolality (Zhou et al., 1995). Experiments have shown that fed-batch operation can result in up to 8-fold increase in product concentration compared with conventional batch culture in the same bioreactor. This feeding strategy can also be used to feed specially formulated supplemental medium, which in one case resulted in 17-fold increase in product concentration (Xie and Wang, 1996). Fed-batch mode substantially increases the final product concentration while keeping the simplicity of operation and of quality control to comply with FDA regulations (Tremblay et al., 1992). Genetic drift and contamination problems are also minimized since the culture lasts about twice as long as a regular batch culture (Tremblay et al., 1992). Although FDA has not yet approved fed-batch operation for production of biological from animal cell culture, the high product concentration achievable with this

mode of operation makes investigation of this process worthwhile for large-scale operation.

4.3.2. PROCESS DESCRIPTION

Process information for the bioreactor was obtained from literature reporting fed-batch operation mainly with hybridoma (Zhou et al., 1995; Kurokawa et al., 1994). Inoculum cell concentration in these experiments were varied between $1.2x10^5$ to $4x10^5$ cells/ml. Zhou et al. (1995) reported the production of IgG in mouse-mouse hybridoma by fed-batch culture. They used a continuous feed consisting of 15.5-fold salt free (without inorganic components) concentrated medium, 258.3 mM glucose and 93 mM glutamine to maintain glucose concentration in the medium at about 0.15g/L. Glutamine concentration was not controlled. The maximum total and viable cell concentration reached were $1.36x10^7$ and $1.25x10^7$ cells/ml respectively. Lactic acid production was significantly reduced. The antibody concentration reached was about 58 mg/L, which is 7.25 times the concentration achieved by batch culture of the same cell line. Over a period of 230 hour, 135 ml of the continuous feed was added to the original volume of 500 ml. Both glucose and glutamine concentration were maintained at 1.1 mM (0.2 g/L) and 0.685mM (0.1 g/L) respectively by Kurokawa et al. (1994) in a fed-batch culture of hybridoma to obtain a monoclonal antibody concentration of 172 mg/L. The concentration of glucose and glutamine in the feed were 55.55 mM (10 g/L) and 34.24 mM (5 g/L) respectively. The final cell concentration reached after 120 hour was $4.1x10^6$ cells/ml.

The above observations were simulated for a stirred tank type bioreactor operated in fed-batch mode with the CHO cells grown in suspension culture. Serum free medium was used in the bioreactors of this flowsheet.

The downstream process considered was similar to the one used in the base case so that the flowsheets could be compared on the basis of mode of operation of bioreactor only.

Details of the downstream units can be found under Section 4.1.2. Cell separation from broth was achieved by microfiltration, followed by two alternate steps of ultrafiltration and affinity chromatography for main purification. The final step was polishing by gel filtration.

4.3.3. SIMULATION AND RESULTS

Two fed-batch processes described by Kurokawa et al. (1994) (case A) and Zhou et al. (1995) (case B) were simulated. For simulation purposes, two continuous feed streams were defined, one contains glucose and the other containing glutamine only. The "Feedback Control" option of Aspen BioProcess Simulator™ was used to control the concentration of glucose and glutamine in the bioreactor by manipulating the flowrate of the continuous feed streams.

In case A, both glucose and glutamine concentrations in the medium were controlled. The initial working volume of the bioreactor was specified to be 3000 L and the concentration of glucose and glutamine in the media to be 1.48 mM and 0.92 mM respectively. For this case, the concentrations of these substrates in the continuous feed were 0.055 M and 0.034 M and the set points were 1.11 mM (0.2 g/L) and 0.685 mM (0.1 g/L) respectively. These are the same concentrations as maintained by Kurokawa et al. (1994) in their experiment, where an adaptive control algorithm was used to control glucose and glutamine concentration. Kurokawa et al. (1994) did not compare the product concentration achieved by the fed-batch scheme to batch operation. Instead they demonstrated that simultaneous control of both glucose and glutamine concentrations is advantageous for production of pharmaceuticals by animal cell culture and lower the substrate concentration level maintained higher the product concentration achieved. For simulation purposes it was assumed that the product concentration reached in this case was four times the concentration reached in the batch bioreactor. Also, the batch time for the bioreactor for simulation purposes was considered to be 200 hours instead of 120 hours as reported. Since a smaller batch time would allow more batches to be carried out

annually, the process can be carried out in a smaller sized plant. This will result in higher ROI, thereby masking the effect of higher product concentration on ROI alone (Section 4.3.7).

Only glucose concentration in the culture media was controlled in case B. The initial working volume of the bioreactor in this case was specified to be 2800 L and the concentration of glucose and glutamine in the media to be 1.39 mM and 0.3 mM respectively. For this case, the concentrations of these substrates in the continuous feed were 0.2583 M and 0.098 M and the desired level of glucose concentration in the media was 0.0833 mM (0.15 g/L). These are again the same concentrations as maintained by Zhou et al. (1995). In laboratory, flowrate adjustment for controlling glucose concentration was achieved in response to oxygen uptake rate (OUR), which is stoichiometrically related to rate of glucose consumption. For simulation purposes, a product concentration of eight times was considered and the batch time was assumed to be 200 hours instead of 230 hours for reasons already explained.

The simulation results obtained by Aspen BioProcess Simulator™ for the fed-batch bioreactors show the final bioreactor volume and the amount of the continuous feed streams required. Aspen calculates the amount of the continuous streams as a multiple of the actual flowrate of the feed streams as defined in the input files rather than generating a time profile for the flowrate of the continuous feed streams. However, if an optimum feed profile is known, it can be supplied to the simulator using the optional "Feed-Profile" form since BioProcess Simulator™ accepts time varying feed profile in the form of the following polynomial:

$$F/F_{ref} = \sum A_i \{t/t_{ref}\}^{Bi} \qquad (4.3.1)$$

The batch time in the bioreactor can be divided into several intervals and the polynomial can vary from interval to interval.

Table 4.3.1 shows the final bioreactor volumes and the actual amount of feed added as calculated by Aspen for the two bioreactors.

Table 4.3.1: Simulation results for the fed-batch bioreactors

	Initial Volume (L)	Final Volume (L)	Glucose Feed Calc./Actual	Glutamine Feed Calc./Actual
Case A	3000	4250	2.6	4.3
Case B	2800	4115	1.5	-

Simulation of the downstream train was carried out by supplying the simulator with information presented under process description section. Concentration of t-PA in the broth from the bioreactor was specified as either four times (Case A) or eight times (Case B) the concentration from the batch bioreactor (33.5 mg/L) (Section 3.5). For the downstream train, several solutions (size and number of the units) can be obtained by changing the membrane area, cycle time and pump capacity for the membrane separation units within practical limits. These changes result in different concentrations and volumes of product coming out from these units and going to the next unit, which is the chromatography column. The volume and number of the columns change to handle the different throughputs and different concentrations, which cause the column to operate at a different point of the isotherm. The interaction that was observed between these two units has been discussed in detail in Section 4.3.4.

The design basis (Section 3.5) considered for the base case had to be modified for the fed-batch processes so as to obtain the same annual production of t-PA. The bioreactor volume was recalculated to take advantage of the higher product titre concentration, which also reduced the size of the downstream units. The new bioreactor volumes were based on 40% overall recovery. However, it was difficult to reproduce the desired overall recovery and therefore, different bioreactor volumes were also considered for case B. The interaction between ultrafiltration and chromatography columns allows for numerous alternate solutions. The development (i.e. selection of size for the unit operation blocks) of the flowsheet therefore, was a trial and error process so that the target annual

production of t-PA (13750 g/year with a 20% product margin) is met within the given time constraint (~10 days).

Several alternate flowsheets that use different size of the unit operation blocks were developed from simulation for case A and case B. Table 4.3.2 and Table 4.3.3 show result for two different flowsheets for Case A, which differ in the size of the unit operation blocks and recoveries therein. With the first flowsheet, an overall recovery of 43.2% is obtained while with the second one the recovery is only 21.7%. Two sets of the equipment for process 1 and four sets of equipment for process 2 are required to meet the required production level. For the equal sized bioreactors of the two processes, microfilters of different size (65X2 m^2 and 60X4 m^2 for process 1 and process 2 respectively) were simulated for cell separation. Initial enrichment of the microfiltered broth was carried out with ultrafilters of equal sized membrane (50 m^2) with twice the number of units for process 2 compared to process 1. These differences in the upstream units reduced the retentate volume from about 740LX2 and a t-PA concentration of 0.6 g/L for process 1, to 296LX4 and t-PA concentration of 1.2 g/L for process 2. The result was the greatly differing recovery (about 81% and 49%) of the more than 99.9% pure t-PA in the first affinity chromatography column. The concentration of t-PA in the feed to this column was found to be critical to its performance.

Although, the total size of the first three units for process 2 were found to be much larger than process 1, the total number of chromatography columns required by the two processes were not very different. Process 1 required 20 columns, 186 L each to achieve this recovery compared to 16 columns of almost equal volume for process 2. The column in process 1 also took 50 minutes more to complete one cycle of operation than that for process 2. The product volume from the columns were 272 L x 20 for process 1 and 100 L x 16 for process 2. Reduction of the larger product volume from the chromatography column of process 1 required a larger ultrafilter (60X2 m^2 compared to 10X4 m^2) and longer filtration time (0.95 hour compared to 0.75 hour). The size of the following unit

operation blocks were not very significantly different although larger number (or size) was required for all of these blocks for process 2.

Table 4.3.2: Simulation results for case A (4 X batch) process 1

Block	Size	Time (h)	Rec. (%)	t-PA (g)	[t-PA] g/L	Broth Volume (L)
BR	4250L X 2	200	-	569.5	0.134	4250 X 2
MF	65 m^2 X 2	1.0	79.2	451.0	0.1338	3371.7 X 2
UF I	50 m^2 X 2	1.25	93.3	420.8	0.6033	738.7 X 2
AFF I	186L X 20	4.5	80.9	340.45	0.125	272X20
UF II	60 m^2 X 2	0.95	94.0	320.0	1.9516	175.5 X 2
AFF II	146L X 6	2.97	97.97	313.5	0.574	182X6
UF III	20 m^2 X 2	1.0	78.3	245.9	5.8289	44 X 2
GEL	147L X 12	6.9	99.9	245.2	1.2526	32.575X12

Table 4.3.3: Simulation results for case A (4 X batch) process 2

Block	Size	Time (h)	Rec. (%)	t-PA (g)	[t-PA] g/L	Broth Volume (L)
BR	4250 L X 4	200	-	569.5	0.134	4250 X 4
MF	60 m^2 X 4	1.0	74.3	423.14	0.1339	3161 X 4
UF I	50 m^2 X 4	1.0	84.0	355.43	1.2	295.96 X 4
AFF I	190L X 16	3.65	48.88	173.74	0.434	100X16
UF II	10 m^2 X 4	0.75	91.3	158.62	1.379	115 X 4
AFF II	147L X 8	2.97	86.29	136.88	0.57	120X8
UF III	10 m^2 X 4	0.35	90.4	123.74	4.02	30.77 X 4
GEL	147L X 8	6.9	94.0	116.3	1.25	23.2X8

Table 4.3.4 summarizes the overall performance of the two processes for case A, developed by trial and error approach with BioProcess Simulator™.

Table 4.3.4: Comparison of simulated alternate flowsheets for case A

	Process 1	Process 2
Bioreactor Volume	4250 L x 2	4250 L x 4
Overall Recovery in each Bioreactor	43.2%	21.7 %
Downstream Processing Time	18.6 hour	16.6 hour
Product Purity	99.9999 %	99.99986 %
t-PA/batch	492g	494g
[t-PA] in Chromatography Column	0.6g/L	1.2g/L
No. of Chromatography Columns	186 x 20 147 x 6 147 x 12	190 x 16 147 x 8 147 x 16

Table 4.3.5: Simulation results for case B (8 x batch) process 1

Block	Size	Time	Rec.	t-PA (g)	[t-PA] g/L	Broth Volume (L)
BR	3745 X 2	200	-	2007	0.268	3745 X 2
MF	60 X 2	1.0	76.6	1538	0.2665	2885.3 X 2
UF I	45 X 2	1.0	92.06	1415	1.51	468 X 2
AFF I	195 X 12	3.65	39.96	565	0.589	80 X 12
UF II	10 X 2	1.2	92.77	525	4.9	53.55 X 2
AFF II	135 X 2	2.84	95.5	501	2.09	120 X 2
UF III	5 X 2	0.55	95.4	478	14.34	16.67 X 2
GEL	147 X 4	6.9	86.2	418	4.54	23 X 4

For case B, three different flowsheets were constructed from simulation. Out of these, process 1 and process 2 meet the desired production level of t-PA, while process 3 underproduces by 23%. The overall recovery for process 1 is 21.2%. Therefore, two sets of equipment are required by process 1 to meet the production level (Table 4.3.5). The overall recoveries for process 2 and process 3 are 38% and 53% respectively. Process 2

uses much smaller bioreactor volume and membrane area compared to process 1 (Table 4.3.6). However, to handle the large retentate volume dilute in t-PA, the process requires fifteen chromatography columns and also achieves high recovery of t-PA in the columns.

The different trials that were carried out with process 2 to meet the desired production level of t-PA are summarized in Table 4.3.7. The overall recovery of this process was found to be determined by the recovery in the first affinity column as observed for case A. The volume of retentate from UF I was varied by varying either the membrane area or the operating time of this batch ultrafiltration block. The resulting recoveries, of more than 99.99% pure t-PA, in the AFF II block were found to increase with decreasing t-PA concentration in the feed (i.e. the retentate) to the column. An additional chromatography column was required for every 80 L of retentate volume for 40% loading (design case) of the 200 L columns.

Table 4.3.6: Simulation results for case B (8 x batch) process 2

Block	Size	Time (h)	Rec. (%)	t-PA (g)	[t-PA] g/L	Broth Volume (L)
BR	4250	200	-	1139.0	0.268	4250
MF	80	1.0	86.2	981.82	0.267	3676.3
UF I	55	1.1	95.5	937.64	0.745	1260.5
AFF I	200 X15	3.73	71.8	673.53	0.56	80 X 15
UF II	30	1.3	78.4	527.8	1.835	287.55
AFF II	190 X 3	3.36	98.0	517.25	0.907	190 X 3
UF III	10	1.4	87.83	454.0	21.4	21.2
GEL	147 X 3	6.9	99.89	453.2	4.52	33.38 X 3

Process 3 uses half the size of equipment as process 2. It was tried to maintain a high recovery with this flowsheet so that the t-PA recovered would meet the desired amount.

However, the resulting overall recovery (55%) was not high enough to meet the desired production level (Table 4.3.8).

Table 4.3.7: Recovery and productivity versus t-PA concentration in the affinity column

Retentate Volume (L)	[t-PA] (g/L)	Recovery (%)	Productivity (g)
897.5	1.047	53.13	499
1040	0.9	60.986	571
1260.5	0.7456	71.83	675
1361.2	0.6959	75.77	717.7
1361.6	0.6898	76.09	714.6
1480.5	0.638	80.39	760.5
1608	0.59	84.6	804
1748	0.5423	89.774	851
1899	0.50	90.33	859.4
2062	0.4646	91.78	879

Table 4.3.8: Simulation results for case B (8x batch) process 3

Block	Size	Time	Rec.	t-PA (g)	[t-PA] g/L	Broth Volume (L)
BR	2125	200	-	569.5	0.268	2125
MF	40	1.0	85.84	488.86	0.265	1843
UF I	20	1.0	97.456	476.42	0.5867	812
AFF I	201 X10	3.73	85.51	407.39	0.51	80 X 10
UF II	20	1.25	83.227	339.06	6.74	50.3
AFF II	124	2.75	99.0	335.67	2.517	133.3
UF III	5	0.50	97.0	301.4	10.8	28
GEL	147 X 4	6.9	99.998	301.4	3.1	24.25 X 4

4.3.4. INTERACTION BETWEEN UNITS:

Simulation revealed that interaction exists between the membrane separation units and the chromatography columns in the downstream train which limits the recovery of t-PA in the chromatography columns.

For a fixed filtration time, the retentate volume depends on the membrane area of the ultrafiltration units. The retentate volume in turn determines the size and number of column of the chromatography units (Figure 4.3.1). As the membrane area is increased, retentate volume goes down and therefore, the number of chromatography columns required is reduced. However, as retentate volume is decreased, the concentration of t-PA in the retentate increases. As the rate at which BSA permeates out from the filtration units is much higher than that of t-PA, increasing membrane area results in higher ratio of t-PA to BSA in the retentate. The result of this concentration overload (Ghodbane and Guiochon, 1988) in the chromatography columns is broadening and interference of the elution peaks.

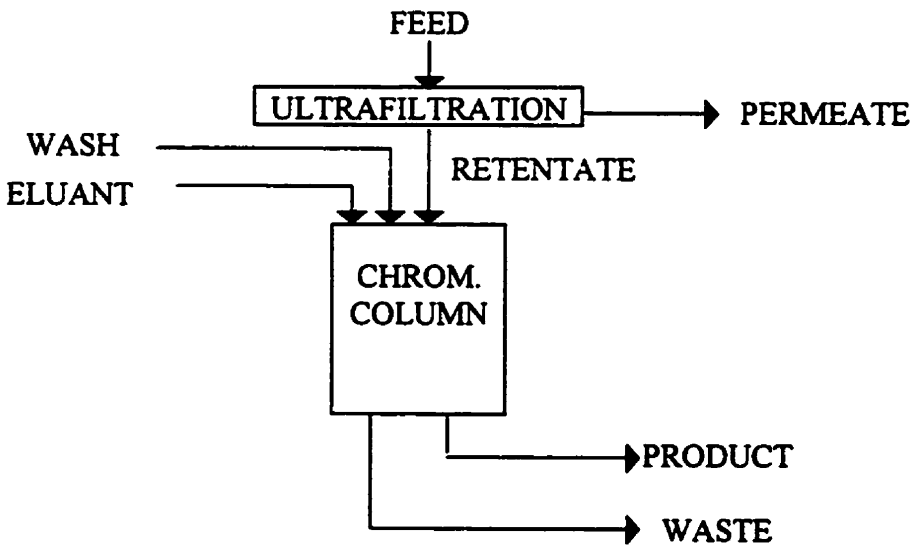

Figure 4.3.1: Interaction between ultrafiltration and chromatography

Figure 4.3.2 shows two elution profiles for t-PA and BSA from the columns at two different ratios of the proteins. For a ratio of 10 (Figure 4.3.2.(a)), the large t-PA peak interferes with BSA and since t-PA is required at very high purity, the amount of t-PA represented by the area under this peak is wasted. Only the amount of t-PA under the area of the second smaller peak is recovered. So, in this case the recovery of t-PA is low. For the other case (Figure 4.3.2.(b)), where this ratio is 0.1, a small peak of t-PA is obtained, which represents almost pure t-PA and therefore, in this case recovery is very high.

In simulating the chromatography column with BioProcess Simulator™, it was found that increase in t-PA concentration only increases the area under the interfering t-PA peak, while the other peak remains unchanged. Since, t-PA is required at very high purity, the t-PA associated with the first peak is wasted. Therefore, although concentration of t-PA is increased by the fed-batch process, the recovery in the chromatographic columns does not increase. Ghodbane and Guichon (1988) simulated a chromatographic column for a binary mixture. The product concentration was varied over a wide range in this simulation. In this case, the recovery was found to increase with increasing concentration, although the effect diminished with increasing sample size unless very high concentrations were reached (greater than relative concentration of 0.8). The purity of the product was 99%. However, in this case Langmuir isotherm constants assumed for the components were very close (2.38 and 2.56) unlike the case considered here. The presence of a split elution peak for t-PA and the increase in the area of the smaller interfering peak with higher concentration could be an artifact of the simulator. It is impossible to draw any conclusion from observations since the only works reported in literature for affinity chromatography are carried out for analytical purpose using small sample size and low concentrations.

For the hypothetical case shown in Figure 4.3.3, the retentate volume was fixed at 100 L. Under this situation, although, lower recovery is obtained at higher t-PA to BSA ratio, production of t-PA, which is the product of recovery and t-PA concentration, actually

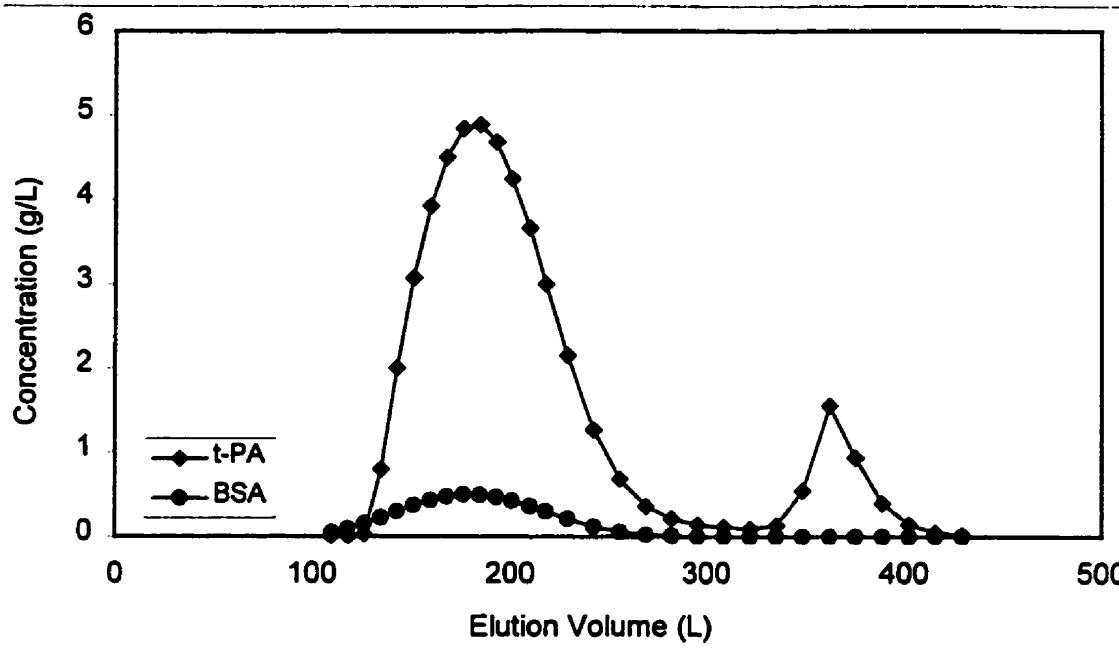

Figure 4.3.2.(a): Elution profile for t-PA/BSA = 10

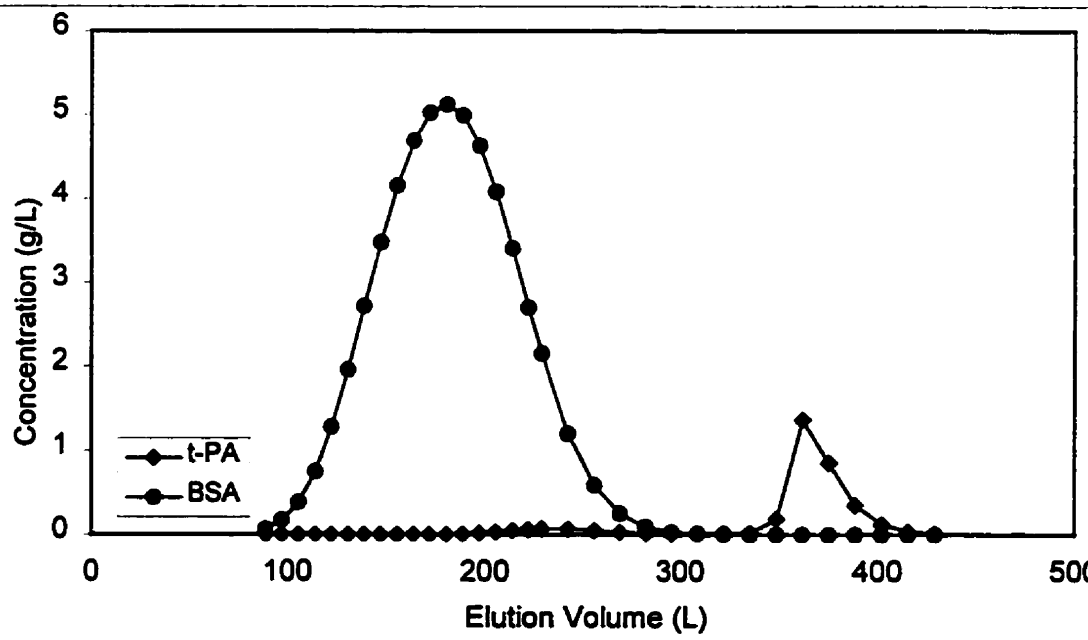

Figure 4.3.2.(b): Elution profile for t-PA/BSA= 0.1

increases up to a certain point. Thereafter, the production increases only marginally but recovery decreases very significantly. Under these conditions the optimum ratio, at which the column should be operated, lies around 0.2. At this point, recovery is about 50% and concentration of t-PA in retentate is about 1.2 g/L. The production curve was also found to reach a plateau in preparative liquid chromatography of a binary mixture with increasing sample size but not with increasing concentration (Ghodbane and Guichon (1988)). This effect was not pronounced in the base case simulation where concentration of t-PA is much lower. This places a limitation on the operation of the ultrafiltration units. They can be operated up to a certain low concentration factor (feed volume/retentate volume) and therefore, the retentate volume is still going to be large. This will necessitate a larger number of columns for purification as already observed in the previous section.

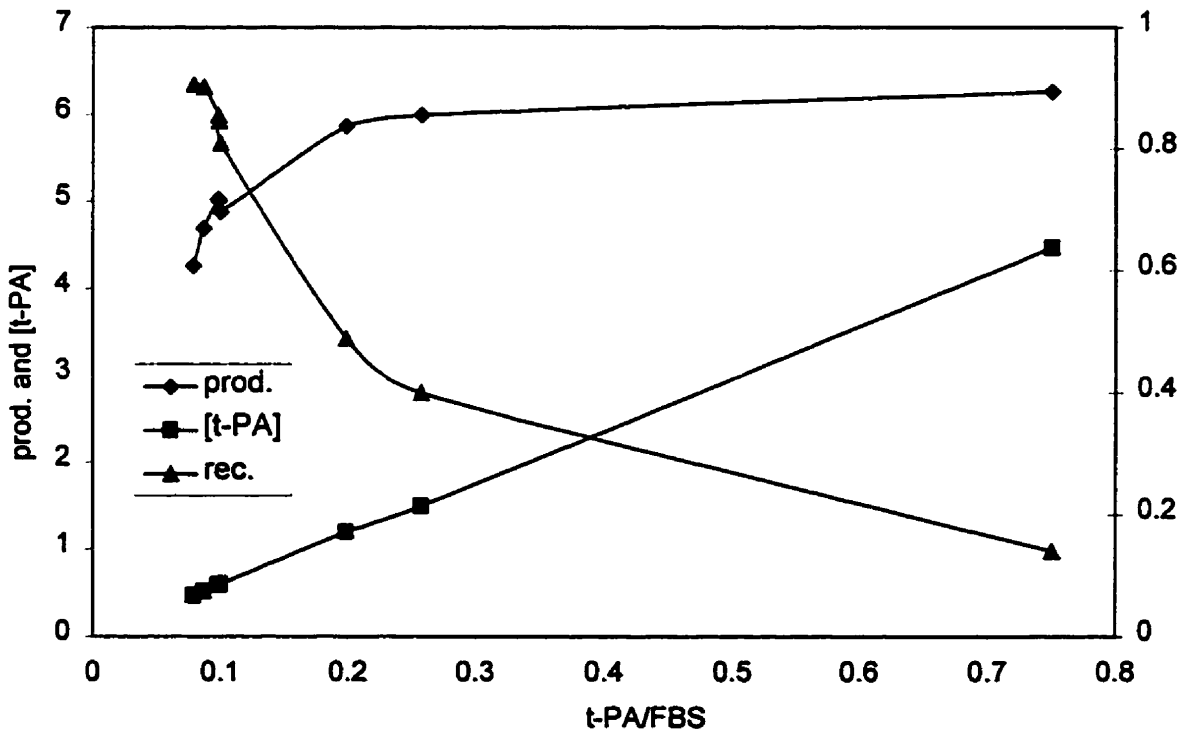

Figure 4.3.3: Productivity and recovery of t-PA in affinity chromatography column

4.3.5. ECONOMIC EVALUATION

The economics of the fed-batch processes were evaluated by combining information from the two simulators, BioProcess Simulator™ and SuperPro Designer®, for reasons explained in Section 3.2. Table 4.3.9 summarizes the economic performance of the processes for case A.

Table 4.3.9: Summary of economic performance of fed-batch process (case A)

Case A	C_{PE} (million)	C_{TC} (million)	AOC (million)	ROI (%)	GM (%)
Process 1	10.0	55.0	59.86	138	66
Process 2	11.16	61.38	66.36	117	62

Breakdown of purchased equipment costs (Figure 4.3.4. (a, b)) for the processes of case A shows that for process 1, about 77% of the contribution comes from downstream units compared to 70% for process 2. Out of this, chromatography columns account for about 73% and 64% for the processes respectively. Therefore, although process 2 requires twice the bioreactor volume and much larger membrane area, contribution from cost of chromatographic columns is still large and C_{PE} of process 2 is only 10.4% higher than process 1. Return on investment (ROI) for process 1 is also about 15% higher.

The annual operating cost (AOC) for process 2 is 9.8% higher than process 1. For this cost (AOC) the greatest contribution comes from raw material (around 43%). A further breakdown of the raw material cost (Figure 4.3.5. (a, b)) shows that, for process 1, the media accounts for 30% compared to 70% for downstream units. For process 2, which uses four sets of equipment, the scenario is reversed and in this case media and other material for the two bioreactors account for 59% compared to 41% by downstream units. It is also to be noted that, between these two processes, although process 2 has twice the bioreactor volume and membrane area, the numbers of chromatographic columns are very close. The contribution of the continuous feed is 1.4% and 3% for process 1 and process 2 respectively. Although this represents a very small percentage, a sensitivity study to the price of the continuous feed cost was carried out (Section 4.3.7).

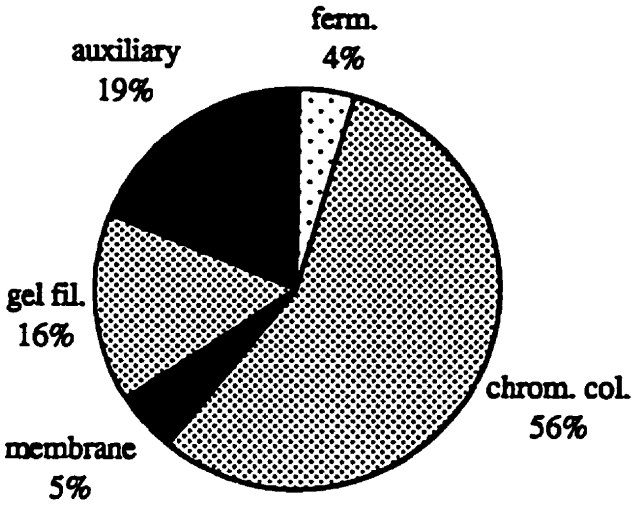

Figure 4.3.4.(a): Breakdown of equipment purchase cost for process 1 of case A fed-batch process

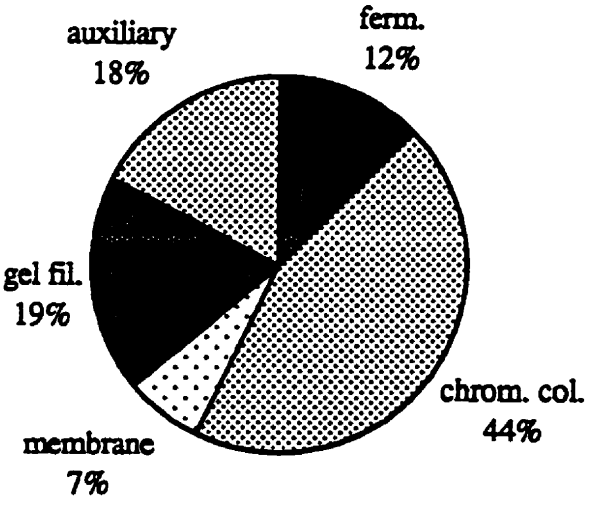

Figure 4.3.4.(b): Breakdown of equipment purchase cost for process 2 of case A fed-batch process

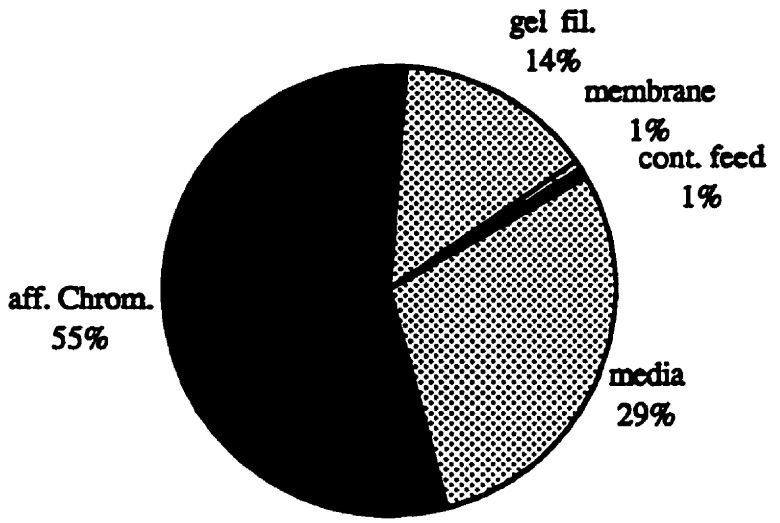

Figure 4.3.5.(a): Breakdown of raw material cost for process 1 of case A fed-batch process

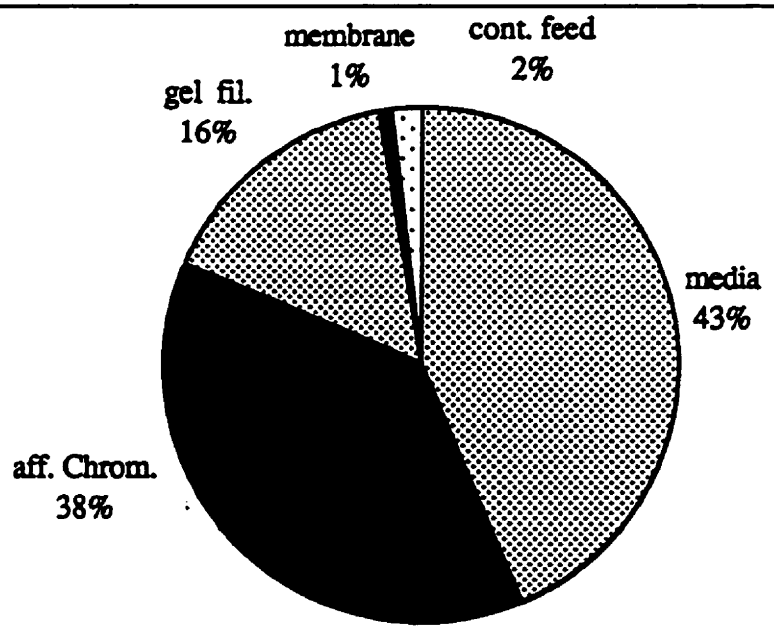

Figure 4.3.5.(b): Breakdown of raw material cost for process 2 of case A fed-batch process

Table 4.3.10: Comparison of alternate flowsheet simulation for case B

	Process 1	Process 2	Process 3
Bioreactor Volume	3745 L x 2	4250 L	2125 L
Overall Recovery	21.16 % x 2	39.8%	53 %
Downstream Processing Time	17.1 hour	18.8 hour	18.1 hour
t-PA/batch	425g	453g	302g
[t-PA] in Chrom.	1.5	0.745	0.588
Rec. in AFF I	39.96 %	71.8 %	85.5 %
No. of Chrom. Col.	195 x 12 135 x 2 147 x 4	200 x 15 190 x 3 147 x 3	200 x 10 200 147 x 4

In case B, lowest purchased equipment cost is calculated for process 3, which requires lowest number of chromatography columns. Breakdown of C_{PE} (Figure 4.3.6.(a, b)) for process 1 shows again that downstream units are the significant contributors to this cost. Affinity Chromatography columns account for 68% of the cost for process 2, which uses largest number of columns for the first chromatographic step. Contribution from bioreactor is highest for process 1 (11.8% compared to 4.2% and 7% for process 2 and process 3 respectively) which requires the largest combined bioreactor volume. For the other units the contributions are more or less congruent.

Breakdown of annual operating cost (AOC) again reveals, that raw material is the largest (around 42%) contributor in this case. Contribution from labor is higher (around 7%) in this case than case A (about 5.5%). Since fed-batch processes

Table 4.3.11: Summary of economic performance of fed-batch process (Case B)

Case B	C_{PE} (million)	C_{TC} (million)	AOC (million)	ROI (%)	GM (%)
Process 1	6.38	35.09	38.7	252	78
Process 2	6.9	37.95	40.5	230	77

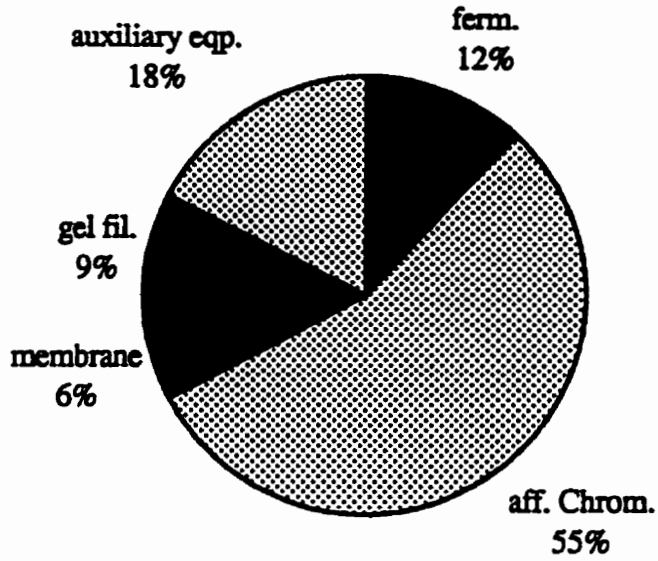

Figure 4.3.6.(a): Breakdown of equipment purchase cost for process 1 of case B fed-batch process

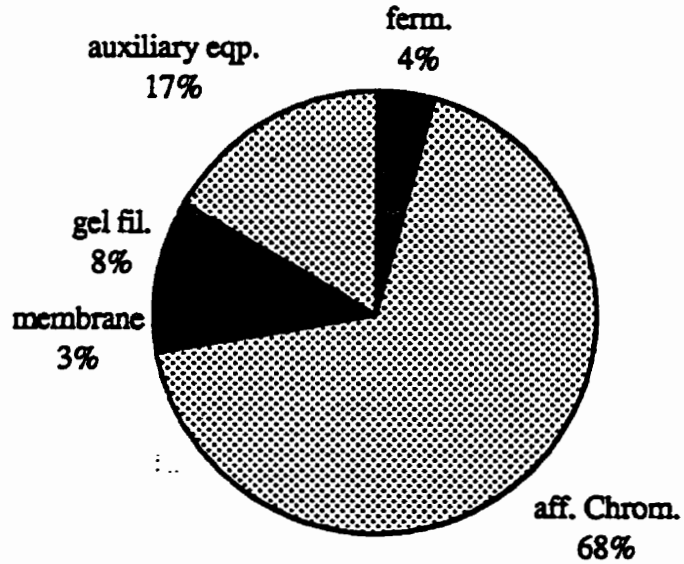

Figure 4.3.6.(b): Breakdown of equipment purchase cost for process2 of case B fed-batch process

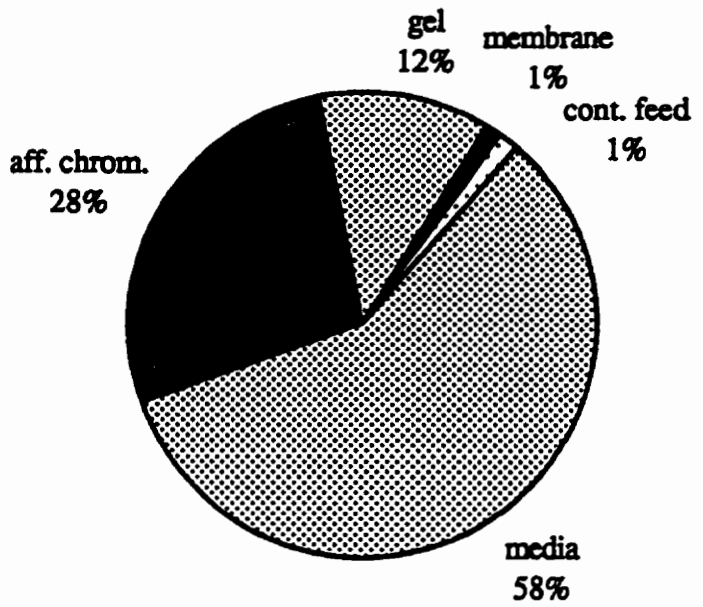

Figure 4.3.7.(a): Breakdown of raw material cost for process 1 of case B fed-batch process

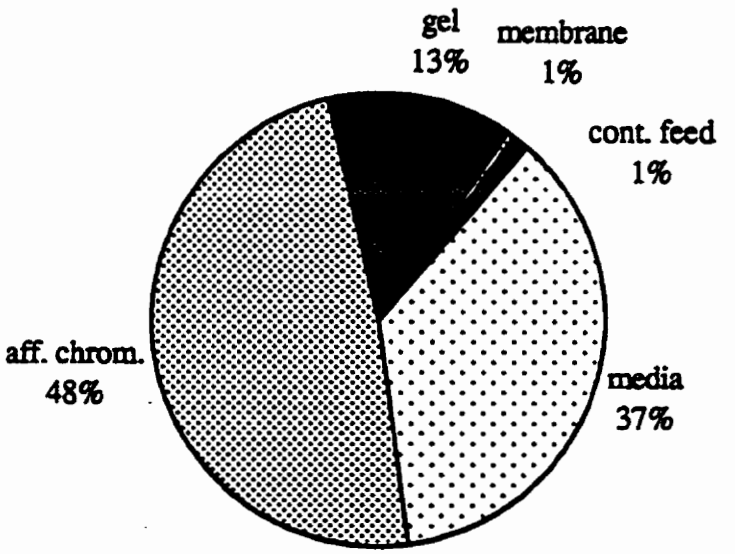

Figure 4.3.7.(b): Breakdown of raw material cost for process 2 of case B fed-batch process

may involve more labor force for preparation of continuous feed, sterilization, and maintenance of cell culture for a time span that is roughly twice as long as batch culture (Tremblay et al., 1992), a sensitivity study was carried out on labor cost for these processes (Section 4.3.7). Combined contribution from sources such as operating supplies, plant overhead and administration (calculated as a function of operating labor) and maintenance supplies, tax and insurance (calculated as a function of fixed capital investment) and depreciation etc. is found to be of similar magnitude (around 12%) as raw material. In this calculation fixed contributions were assumed from utilities, waste treatment, research and development, distribution and patent (Datar and Rosen, 1990).

Breakdown of raw material cost (Figure 4.3.7. (a,b)) show that media and inoculum accounts for 58% of the cost for process 1 that uses largest combined bioreactor volume. Affinity chromatography column materials are the largest contributors for process 2 and 3, accounting for about 45% of the total cost. Contribution from continuous feed is around 1% in all the cases.

4.3.6. COMPARISON WITH BASE CASE

In Table 4.3.12, the simulation results for the fed-batch cases are compared with the base case. The results for process 1 of both cases are shown since they have higher ROI than the other processes. It is noted from the table that switching from batch to fed-batch mode of operation results in significant reduction in the sizes of the unit operation blocks, particularly for the first three blocks (i.e. bioreactor, microfilter and ultrafilter) because of the higher product concentration. However, the number and size of the affinity chromatography columns are very similar.

The design basis (Section 3.5) considered for the base case had to be modified for the fed-batch processes so as to obtain the same annual production of t-PA. The bioreactor volume was recalculated to take advantage of the higher product titre concentration, which reduced the size of the downstream units (Table 4.3.13). The improvement in economics therefore resulted from a smaller plant size rather than higher revenue.

Table 4.3.12: Comparison of flowsheet simulation results

Block	Batch		Fed-batch 1		Fed-batch 2	
	Size	Rec. (%)	Size	Rec. (%)	Size	Rec. (%)
Bioreactor	30000 L	-	4250 L X 2	-	3745 L X 2	-
Microfilter	80 m^2 X 5	93.3	65 m^2 X 2	79.2	60 m^2 X 2	76.6
Ultrafilter	60 m^2 X 5	83.0	50 m^2 X 2	93.3	45 m^2 X 2	92.06
Affinity Chromatography	200 L X 15	73.6	185 L X 20	80.9	195 L X 12	39.96
Ultrafilter	10 m^2 X 5	94.0	60 m^2 X 2	94.0	10 m^2 X 2	92.77
Affinity Chromatography	85 L X 5	90.7	145 L X6	97.97	135 L X 2	95.5
Ultrafilter	2.5 m^2X 5	94.1	20 m^2 X 2	78.3	5 m^2 X 2	95.4
Gel Chromatography	147 L X 20	99.99	147 L X 12	99.9	147 L X 4	86.2

Table 4.3.13 summarizes economics of the base case and process 1 of case A and case B.

Table 4.3.13: Comparison of economic performance of batch and fed-batch processes

	Batch	Case A (4 X batch)	Case B (8 x batch)
Bioreactor Volume	30000 L	4250 L X 2	3745 L X 2
Recovery	45.3%	43.2%	42%
Batch Time	~9 days	~9 days	~9 days
No. of Batches/year	35	35	35
t-PA /Batch	435g	490g	425g
t-PA/year	15225 g	17150	14875 g
C_{PE}	14.2	10.0	6.38
AOC	101	59.86	38.7
ROI	65%	138%	252%

A comparison of the purchased equipment cost shows that the contribution of cost related to fermentation equipment goes down from 14% to about 4% as the process switches from batch to fed-batch mode of operation. Cost related to gel chromatography columns

also decreases because of the greatly reduced throughput. In contrast, cost associated with affinity chromatography columns increases from 25% to 57% for case A and then to 60% for case B.

Comparison of the annual operating cost shows that raw material and media is the largest cost component in all the cases with the contribution being about 43%. Contribution from labor related items is found to be higher for the fed-batch processes (about 7% compared to 5% for batch).

A comparison of raw material cost reveals that for the batch process, fermentation media accounts for about 60% compared to 40% for downstream units. In the case of fed-batch processes, the scenario is reversed. For these processes, media accounts for about 40% compared to about 60% for downstream units. The significance of downstream units in determining the overall cost of pharmaceutical therapeutics is already known (Cartwright, 1994). By moving from batch to fed-batch mode of operation the balance of cost contribution between fermentation and downstream units seems to move further up towards the purification units.

4.3.7. SENSITIVITY STUDY

I) Sensitivity to Continuous Feed Cost

Although continuous feed cost represents a very small percentage (around 1%) of total raw material price, a sensitivity study was carried out. It is anticipated that, the current efforts of formulating stoichiometrically balanced, nutrient fortified media (Xie and Wang, 1994; Zhou et. al, 1995) to achieve even higher product concentration may result in several folds increase in this price.

As shown in Figure 4.3.8, a 10-fold increase in the price of this stream over the base price of $10.0/L, would only lower the ROI of the process by 3.5%. Therefore, fed-batch processes will remain an economically attractive alternative to batch operation even if the price of the continuous feed increases significantly.

Another uncertain quantity in the simulation of the fed-batch processes is the volume of the continuous feed added. The effect of increased cost of the stream will still be insignificant. However, the necessity of larger plant size to handle the increased throughput will significantly lower ROI of the process.

II) Sensitivity to Labor Cost

Fed-batch processes in principle should involve more labor force than a batch process for preparation of continuous feed, its sterilization and maintenance of cell culture which lasts roughly twice as long as a regular batch culture (Tremblay et al., 1992). In general, labor cost is found to be the largest cost component, accounting for more than 50%, for bioreactors (Wilkinson, 1987). For chromatography columns, labor cost tends to run far ahead of supplies and amortized equipment (Wheelwright, 1987). Therefore, a sensitivity study was carried out to labor cost for the fed-batch processes. In addition to cost for supervision (A), maintenance (B) and operating labor (C), this study included cost of other labor dependent items of annual operating cost such as operating supplies (10% of C), laboratory charges (25% of C), plant overhead (60% of (A+B+C)) and administration (25% of plant overhead) (Datar and Rosen, 1990).

Although, labor cost accounts for about 5.5% and 7% of the annual operating cost for case A and case B respectively of the fed-batch processes, it was found that a 50% increase in the labor cost will bring about 9% reduction in ROI of the process (Figure 4.3.9). Therefore, increase in the base unit labor rate ($18/hour) or the labor-hour may have significant effect on the economics of the process and the possibility of minimizing labor cost by automation of the process should be investigated.

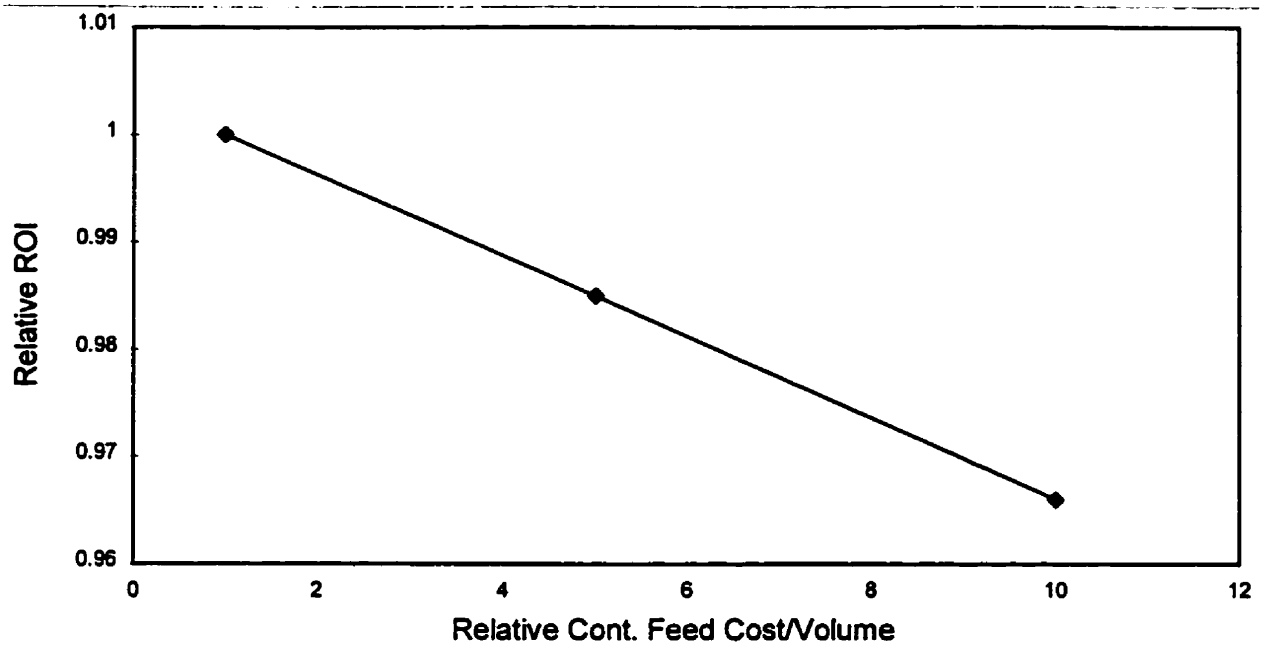

Figure 4.3.8: Sensitivity to Cont. Feed Cost/Volume

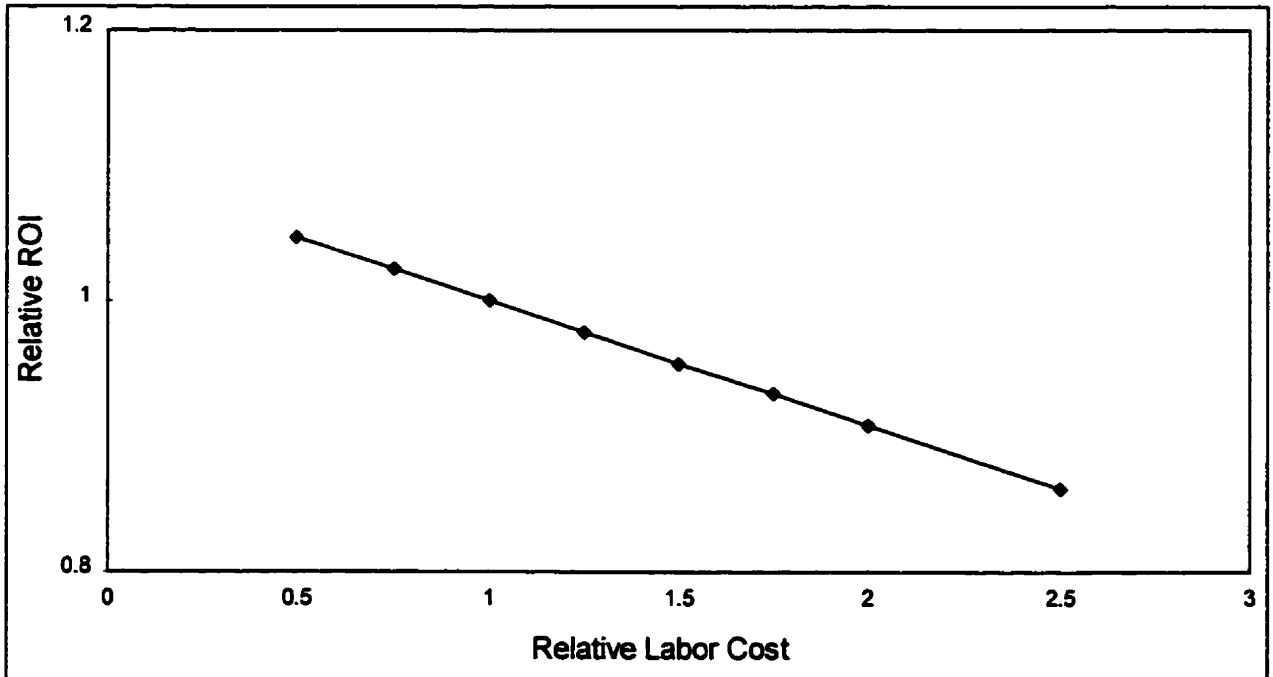

Figure 4.3.9: Sensitivity to Labor Cost

III) Batch Time

Another variable that has significant effect on the ROI of the process, is the batch time. A breakdown (Figure 4.3.10) of the batch time for case A fed-batch process shows that the operation of the bioreactor occupies 93% of the total time required for the complete batch. In this simulation, equal batch time (~9 days) was considered for the batch and fed-batch processes. However, in general, fed-batch cultures lasts roughly twice as long as regular batch cultures (Tremblay et al., 1992). For fed-batch cultures itself, achievement of higher product titre using fed-batch operation may require longer culture time. For example, case A (4X) required 120 hours compared to 230 hours for case B (8X) in the laboratory (experiments carried out with different cell lines).

The effect of shorter batch time is larger number of batches per year, which results in smaller plant size. And a reduction in plant size results in higher ROI. For a fixed plant size, reduction of turn around time increases the revenue from the plant by allowing more batches to be carried out per year. This served as one of the major motivations for process improvement by Celltech for their 1000 L airlift bioreactor which resulted in reduced turn around time of 1.5 days instead of 4 days (Wilkinson, 1987).

Sensitivity study (Figure 4.3.11) of case A fed-batch process shows that a 50% reduction in batch time would result in about 100% increase in ROI for the process. This is a huge incentive to reduce the culture time of fed-batch operation to reach the same product concentration by manipulating other variables such as metabolic pathways of the same cell line or switching to different cell line etc. However, larger number of batches per year will require more frequent replacement of materials such as chromatographic resins, ultrafiltration and microfiltration membranes etc. This will also require more raw materials, media, labor force, operating supplies, utilities etc., which will increase the annual operating cost. This increased cost was also considered in this study.

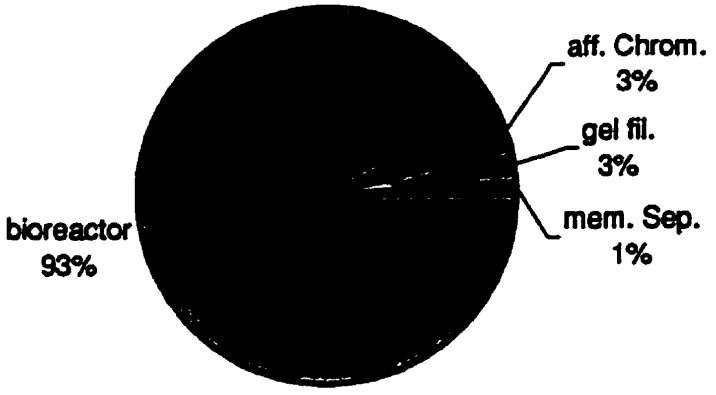

Figure 4.3.10: distribution of batch time for the fed-batch processes

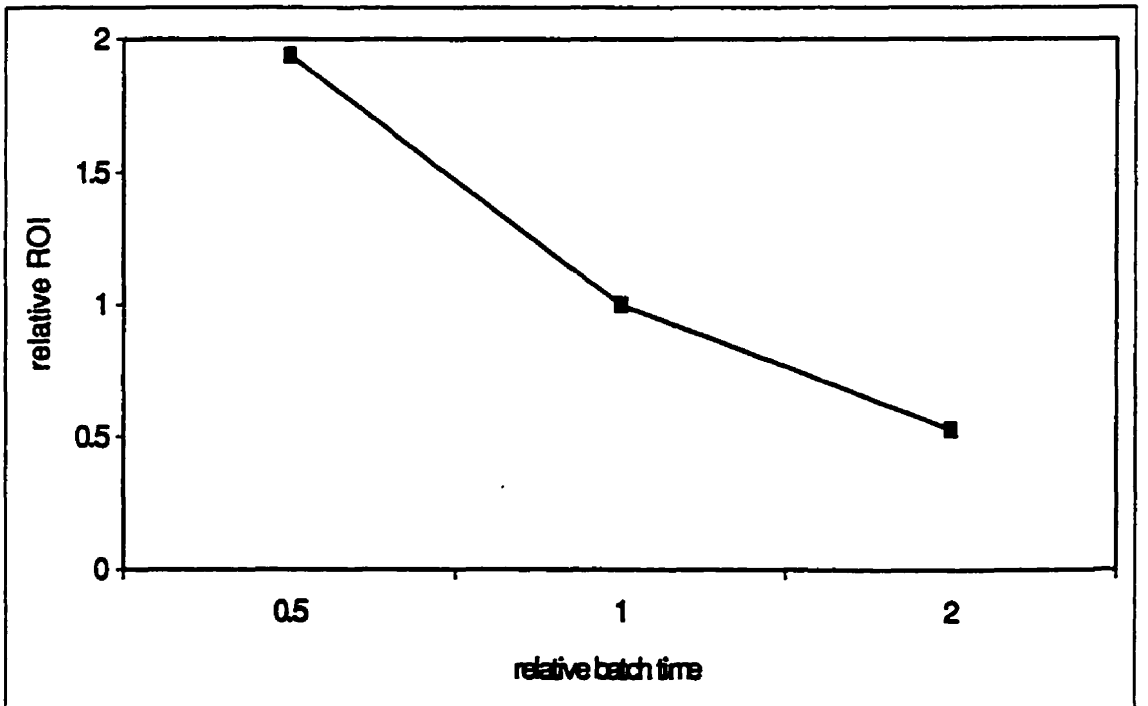

Figure 4.3.11: Sensitivity of ROI to batch time

4.4. Process Alternative: Airlift Bioreactor

4.4.1. INTRODUCTION

This alternate process to the base case uses airlift bioreactor instead of the stirred tank type used in the base case. The airlift type of bioreactor has been used for many years for the cultivation of microbial cells and lately for hybridoma (Rhodes and Birch, 1988) and CHO cells (Arathoon and Birch, 1986). It produces a gentle, low shear environment suitable for tissue culture and genetically engineered microorganisms (Chisti, 1989). The main advantage of the airlift design is its simplicity in construction and operation. It removes the need for motors and agitators associated with stirred reactors. In addition, hydrodynamic and mass transfer characteristics of the airlift reactor are predictable with increase in scale (Arathoon and Birch, 1986) and therefore it is relatively easy to scale up.

The basic principle of airlift bioreactor is that, it uses gas mixtures to provide mixing. Gas mixtures are introduced into the culture from a sparge tube at the base of a central draught tube. This causes a reduction in the bulk density of the liquid in the draught tube compared with the outer zone of the vessel, which sets the culture in circulation. In addition to providing mixing, the gas mixtures also supply oxygen to the culture. Means of directing flow other than draught tubes are also used. Celltech developed airlift bioreactors up to 1000 L scale for production of monoclonal antibody (Rhodes and Birch, 1988). A 10,000 L scale airlift bioreactor was also designed for the same purpose.

4.4.2. PROCESS DESCRIPTION

The downstream processes of this flowsheet consist of the same steps used in the base case flowsheet, two alternate steps of ultrafiltration and affinity chromatography,

followed by a final step of gel filtration. The required process information and operating conditions, required as input for simulation, were gathered from literature. These are the same as described for the base case and can be found under Section 4.1.2.

The simulated bioreactor is an internal loop, draft tube type airlift reactor with the CHO cells grown in suspension culture. It is inoculated with 1000 L of inoculum having a concentration of $2X10^5$ cells/ml. A concentric draft tube internal loop airlift reactor was simulated for the process since the 1000 L airlift reactor operated by Celltech for hybridoma culture is of the same type. Table 4.4.1 compares the performance of the internal loop airlift reactors with external loop reactors. The external loop configuration results in high shear, which could be detrimental to animal cells.

The presence of a draft tube in the internal loop airlift reactors and the ratio of downcomer area to riser area (A_d/A_r) was found to have no effect, in general, on the overall gas holdup (ε) in the bioreactor (Chisti, 1989). However, the ratio (A_d/A_r) is found to influence the liquid circulation and mixing in airlift reactors. This ratio is the principal factor, which was found to control the total circulation path flow resistance in airlift reactors. There was no definite general agreement on the influence of this geometric term on circulation except that interstitial liquid velocity, V_L was found to be dependent on (A_d/A_r) in the following manner:

$$V_L \alpha (A_d/A_r)^{\nu} \qquad (4.4.1)$$

where ν was found to vary between 0.2 and 0.77 (Chisti, 1989). For the bioreactor simulated, the ratio of downcomer area (A_d) to riser area (A_r) was assumed to be 1.25 (Chisti, 1996). This ratio results in a downcomer diameter (d_D) to column diameter (d_c) ratio of about 0.53. For internal loop bioreactors, largest liquid circulation rates are reported for diameter ratio of between 0.6 and 0.5 (Weiland, 1984; Miyahara et al., 1986;

Jones, 1985) and efficient mixing and oxygen transfer between 0.8 and 0.9 (Weiland, 1984). The diameter ratio for the simulated bioreactor, therefore, is within the range for large liquid circulation rate but below the range for high oxygen transfer. For animal cells, since oxygen demand is low in comparison to microbial cells (Fleischaker and Sinskey, 1981) the specified diameter ratio should be satisfactory.

Table 4.4.1.Relative performance of external and internal loop airlift bioreactors (Chisti, 1989)

Parameter	External-loops	Internal-loops
$k_L a_L$	lower	higher
ε	lower	higher
ε_r	lower	higher
ε_d	lower	higher
U_{Lr}	higher	lower
Circulation time, t_c	lower	higher
Liquid Reynolds nos. (shear)	higher	lower
Heat transfer	probably higher	probably lower

The liquid height (h_L) to total diameter (d_t) ratio is found to effect the liquid circulation velocity and mixing time in airlift bioreactors (Ganzeveld et al., 1995). A higher aspect ratio results in higher liquid circulation velocity and longer mixing time. Since the diameter ratio for the simulated bioreactor is chosen to give a high liquid circulation velocity, the aspect ratio was assumed to be 3.0 to achieve better mixing (Chisti, 1996). This resulted in a total diameter (d_t) of 1.36m and liquid height (h_L) of 4.1m for the reactor. The relationship [$A_r + A_d = \pi(d_t)^2/4$] yielded a downcomer area (A_d) of 0.81 m^2 and a riser area (A_r) of 0.65 m^2. The riser height to riser diameter ratio was assumed to be 3.5. A higher ratio (5) was used in the concentric draft tube airlift reactor described by Chisti (1989). This ratio results in a higher riser height than the liquid height, h_L, for the simulated bioreactor and therefore, had to be lowered.

An estimate of the superficial gas velocity in the riser (U_{Gr}) was obtained from the following equation for draft tube internal loop airlift bioreactor (Bello et al., 1985):

$$K_L a_D = 0.76[1+A_d/A_r]^{-2}(U_{Gr})^{0.8} \qquad (4.4.2)$$

where K_L was assumed to be 5 h^{-1} as in the base case. This gave a value of 0.00287 m/s for U_{Gr}. The above equation is applicable up to a gas-liquid dispersion height (h_D) of 1.8 m. For the simulated bioreactor h_D is greater than 4m (exact height to be calculated later). However, none of the other equations was found to be more suitable for the simulated case and therefore the above equation was chosen for U_{Gr} calculation.

The volumetric flowrate was calculated to be:

$$\begin{aligned} Q_{Gr} &= (U_{Gr})(A_r) \\ &= 112 \text{ L/min} \end{aligned}$$

Which is equivalent to (112L/min)/(6000L) = 0.0186 VVM. Birch et al. (1987) have measured oxygen transfer coefficients in airlift reactors and shown that the transfer rate needed to support a hybridoma culture of average maximum population density (ca. 2 $x10^6$ cells/ml) at 1000 L scale is about 2.5 h^{-1} which corresponds to about 0.01 VVM.

In airlift reactors power supply originates from two sources: i) isothermal expansion of the gas as it moves up the reactor and ii) the kinetic energy of the gas injected into the reactor. It has been demonstrated that the contribution of kinetic energy to total power input is a very small percentage and for most practical situations does not exceed 1.5% (Chisti, 1989). The power delivered to the fluid, therefore, was calculated from the following relationship:

$$P_G/V_L = \rho_L g\, U_{Gr} /(1+ A_d/ A_r) \qquad (4.4.3)$$

and found to be 12.5 W/m^3. This quantity was calculated to be 13 W/m^3 for the stirred bioreactor of the base case.

The heat transfer coefficient (h_f) was obtained from the following correlation proposed by Chisti (1989) for concentric draft tube internal loop systems:

$$h_f = 13.34(1+ A_r /A_d)^{-0.7} [U_{Gr} /(1+ A_d/ A_r)]^{0.275} \quad (4.4.4)$$

and found to be 1.4 kw/m^2-^{0}c. For the base case the heat transfer coefficient was found to be 540 kcal/m^2-hr-^{0}c.

The total gas holdup, ε and the gas holdup in the downcomer (ε_d) and the riser area (ε_r) were calculated following a trial and error approach using the following equations:

The gas holdup correlation due to Hills (1976):

$$\varepsilon_r = (U_{Gr})/[0.24 + 1.35 (U_{Gr} + U_{Lr})^{0.93}] \quad (4.4.5)$$

which can be used to calculate ε_r for any given gas velocity using an assumed value for liquid velocity. The downcomer gas holdup is then obtained using the following equation for draft tube internal loop airlift (Bello et al., 1985):

$$\varepsilon_d = 0.89\varepsilon_r \text{ and} \quad (4.4.6)$$
$$\varepsilon = (A_r\varepsilon_r + A_d\varepsilon_d)/(A_r + A_d) \quad (4.4.7)$$

The superficial liquid velocity, U_{Lr}, can be calculated from the following simplified equation (Chisti et al., 1988; Kubota et al., 1978) for the concentric tube type internal loop airlift devices once estimate of the gas holdups are obtained:

$$U_{Lr} = \frac{[2gh_D(\varepsilon_r - \varepsilon_d)]^{0.5}}{[K_B (A_r /A_d)^2]^{0.5}[1/(1-\varepsilon_d)^2]^{0.5}} \quad (4.4.8)$$

In the above equation, A_b is the free area for liquid flow under the draft tube, h_D is the gas-liquid-solid dispersion height and K_B is the frictional loss coefficient for bottom zone of the reactor. The coefficient K_B, for almost all the several different types of internal-loop airlift can be calculated from the following correlation (Chisti, 1989):

$$K_B = 11.4 (A_d/A_b)^{0.79} \quad (4.4.9)$$

and the gas-liquid-solid dispersion height, h_D can be obtained from the following relationship:

$$h_D = h_L/(1-\varepsilon) \qquad (4.4.10)$$

An initial value of zero was assumed for U_{Lr}, the superficial liquid velocity in the riser, and then it was incremented by a small number (0.01) and compared to the updated value obtained from the above equation. This procedure was continued until no significant (tolerance of 0.001) difference was found between the two. The final values obtained are the following:

$U_{Lr} = 0.096$ m/s
$\varepsilon = 0.0064$
$\varepsilon_r = 0.007$
$\varepsilon_d = 0.006$
$h_D = 4.53$ m

The values of ε_r and ε_d obtained are found to be within the range observed for hybridoma culture in an internal loop airlift bioreactor (Ganzeveld et al., 1995).

4.4.3. SIMULATION AND RESULTS

BioProcess Simulator™ does not have any model for airlift bioreactor. Therefore, the model in the SuperPro Designer® was used to simulate the bioreactor for this flowsheet. This model simulates an airlift bioreactor using a simple stoichiometric reactor model. The user has to specify the stoichiometry and the extent of the overall reaction. The modelling equations of the airlift bioreactor in SuperPro Designer® are identical to the stirred tank bioreactor. The specific input data required to simulate the airlift bioreactor are the ratios of riser height to riser diameter and the riser area to downcomer area. The outputs from the model are riser height and diameter and downcomer volume.

Using the above model a single 6000 L bioreactor was simulated in rating mode. Glutamine was defined as the limiting reactant with the stoichiometries being the values that would result in the final concentrations of the reactants and products in the broth, as observed with the BioProcess Simulator™ simulation.

The reported outputs of simulation are as following:

Riser Height, R_h	= 3.81 m
Riser Diameter, R_d	= 1.09 m
Riser Volume, R_v	= 3.56 m^3
Downcomer Volume, D_v	= 4.44 m^3
Total Volume, V_T	= 8.0 m^3
Liquid Volume, V	= 6.0 m^3

The details of the flowsheet can then be summarized in the following table, where the downstream units are simulated using BioProcess Simulator™ and are the same as for the base case.

Table 4.4.2: Simulation results for airlift reactor flowsheet

Block	Size	Time (hour)	Recovery (%)	Purity (%)	t-PA (g)
Bioreactor	6000 L	200	-	0.55	192
Microfilter	80 m^2	1	93.3	0.55	177
Ultrafilter	60 m^2	1	83	7.65	147.4
Affinity Chrom.	200 L X 3	3.7	73.6	99.98	108.5
Ultrafilter	10 m^2	0.5	94	-	102.0
Affinity Chrom.	85 L	3.2	90.687	99.9977	92.5
Ultrafilter	2.5 m^2	0.5	94.13	-	87.07
Gel Filtration	147 L X 6	7	99.997	99.9998	87.07

4.4.4. ECONOMIC EVALUATION

The overall economics of the process is summarized in Table 4.4.3. Of the purchased equipment cost (Figure 4.4.1), the affinity chromatography columns and the gel filtration units combined contribute by 55%. Contribution from the bioreactors and the membrane

separation units are pretty close (11% and 10% respectively), while the auxiliary equipment accounted for 24%.

Table 4.4.3: Economic analysis of the process (in millions of dollars)

Purchased Equipment Cost, C_{PE}	13.6
Fixed Capital, C_{FC} = (4.6XC_{PE})	54.4
Total Capital, C_{TC} = (5.5XC_{PE})	74.8
Revenue, R	176
Annual Operating Cost, AOC	101
Gross Profit, GP = (R-AOC)	75
Net Profit, NP (40% tax)	45
Net Cash Flow, (NP + Dep.)	51
Return on Investment, ROI	68%
Gross Margin, GM = (GP/R)	43%

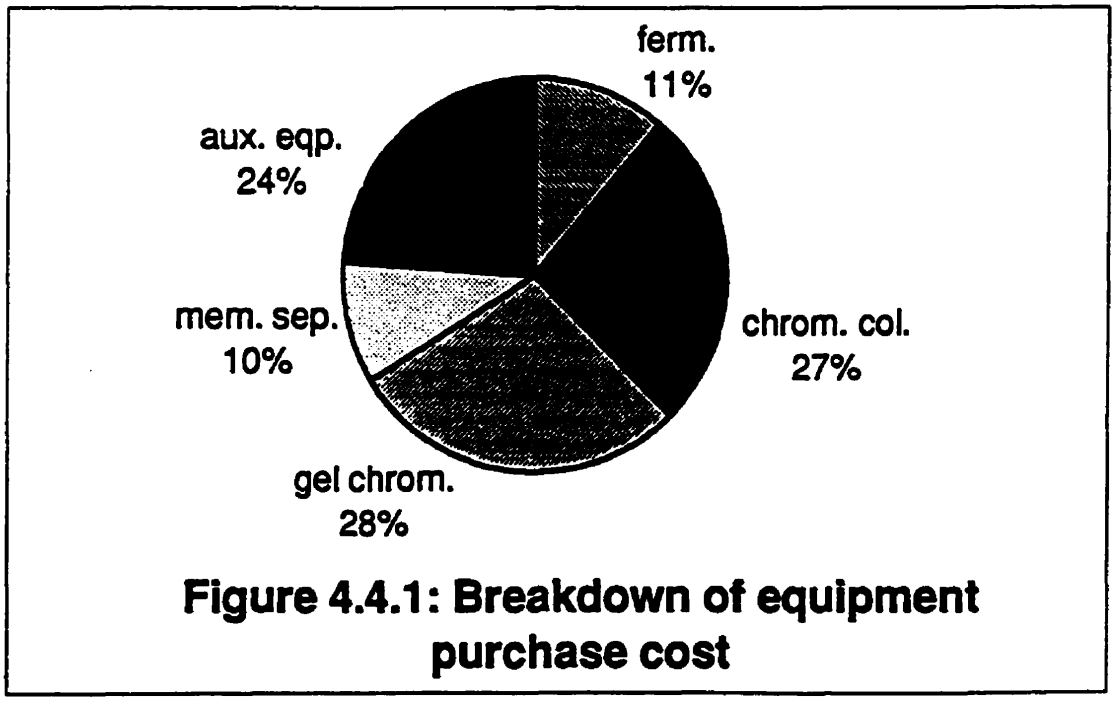

Figure 4.4.1: Breakdown of equipment purchase cost

Calculation of the annual operating cost is the same as for the base case. The ROI of the process is 68% with a gross margin of 43%.

4.4.5. COMPARISON WITH BASE CASE

The effect of replacing the stirred tank bioreactor of the base case with an airlift type reactor was observed in the lower purchase equipment cost for this flowsheet. The purchase cost of an equal sized airlift bioreactor was found to be 75% of the stirred tank bioreactor according to the SuperPro Designer® built-in cost model because of the simplicity in its construction. The lower C_{PE} resulted in 4.4% lower total capital investment for this flowsheet, which improved the ROI by an almost equal amount, other costs remaining unchanged.

Table 4.4.4: Comparative economic analysis (in millions of dollars)

	Airlift	Base Case
Purchased Equipment Cost, C_{PE}	13.6	14.2
Fixed Capital, $C_{FC} = (4.6XC_{PE})$	62.56	65.32
Total Capital, $C_{TC} = (5.5XC_{PE})$	74.8	78.1
Revenue, R	176	176
Annual Operating Cost, AOC	101	101
Gross Profit, GP = (R-AOC)	75	75
Net Profit, NP (40% tax)	45	45
Net Cash Flow, (NP + Dep.)	51	51
Return on Investment, ROI	68%	65%
Gross Margin, GM = (GP/R)	43%	43%

With airlift bioreactors, the reactor type per se, had no effect on growth kinetics or antibody production rate (Birch et al., 1987; Arathoon and Birch, 1986). However, because of its simple construction airlift bioreactors help to maintain aseptic condition and operation of the bioreactor, the effect of which was not possible to compare with that of a stirred tank bioreactor. Power and labor cost are also expected to be cheaper for this type of reactor compared to a stirred tank type.

On the other hand, the presence of bubbles in the medium in an air lift bioreactor was found to cause more cell damage to some cell line then providing a gentle environment in the absence of agitators. Numerous hybridoma cell lines have been successfully grown in airlift bioreactors while others could not be. Handa et al. (1987) tried to elucidate the relationship between gas-liquid interface and the survival of mammalian cells. They found that cell viability and survival in the presence of air bubbles depended on three factors- cell type, bubble size and superficial gas velocity. Some cell lines were found to be more sensitive to the presence of gas bubbles than others, even though no gross morphological difference was observed. Increasing the superficial gas velocity, as well as the presence of small bubbles, were found to be detrimental to cell survival. A later study by the same authors (Handa et al., 1989) revealed two mechanisms of cell damage. One due to rapid oscillations caused by bursting bubbles and the other due to damage caused by shearing in draining liquid films (or lamellae) in foams. They also established that cell death was associated with the region of bubble disengagement at the medium surface and therefore, short bubble columns, as well as small bubbles and high bubble frequencies were detrimental to cells.

However, it was also verified by the same authors (Handa et al. 1988) that increasing bubble column height, which reduces the proportion of the time spent by the cells in the "destructive zone" at the medium surface, caused a progressively increased retention of cell viability. Addition of several non-ionic surfactant such as Pluriol PE 6800 had concentration dependent protective effect on decreasing cell growth caused by the presence of bubbles. Therefore, with a little modification it should still be possible to use airlift bioreactors for large scale culture of mammalian cells. However, the removal of the surfactant from the broth and its subsequent effect on downstream processing needs to be investigated.

4.5. Process Alternative: Single versus Multiple Bioreactor

4.5.1. INTRODUCTION

An issue that has received mixed opinion in the scaling up of bioreactors is whether to increase the size or the number when moving to industrial scale. Celltech designed a 10,000 L bioreactor for production of monoclonal antibody from animal cell where it was decided to increase the size rather than the number of the bioreactor. The justification being to benefit from the economies of scale (Birch et al., 1987). If scale-up consists of increasing the number of units without increasing the unit-size, then both capital and labor costs increase linearly with increase in scale. However, if scale up is achieved by increasing the unit-size the capital cost increases approximately to the 0.6 power (Figure 4.5.1).

In contrast, Horvath (1989) pointed out that having several large bioreactors rather than one or two very large ones could be advantageous due to several reasons. Multiple bioreactors offer flexibility of operation, ease of start up, inoculum preparation, cleaning, sterilization etc. Manufacturers are able to match production with market demand adopting a modular approach. In large-scale batch culture, contamination or equipment malfunction can ruin an entire run and waste several days as downtime for maintenance and repair. The modular design will minimize production disruption because the operation of the contaminated bioreactor can be terminated without disturbing the others.

In the above discussions the effect of bioreactor scale up on downstream units, which for high value products account for about 80% of the production cost, was not considered. A major advantage of a modular approach to scale up could be the size reduction of the downstream processing equipment. Proper scheduling of the downstream units would allow the same sets to be used with the different bioreactors and this should result in major savings in capital cost. It was also pointed out by Harshbarger et al. (1995) that,

since a cell culture bioreactor cycle can last up to a week, multiple bioreactor trains can be used to feed an economically sized, single purification train. In the absence of dedicated facilities or excess facility capacity, the production of even more than one product using the same equipment, which requires additional cost for validated cleaning and sanitizing procedures between each product manufacturing run, has been found economically feasible (Sofer and Nystrom, 1991). In USA, approximately 80% of the new biotherapeutics are expected to come from companies seeking approval for multi-product facilities that will use the same purification equipment for purification of multiple products (McCormick, 1991). The labor cost can also be kept low using multiple bioreactors by sequencing the operation of the bioreactors so that the same labor force can be used to operate the bioreactors at different points of time of the same batch.

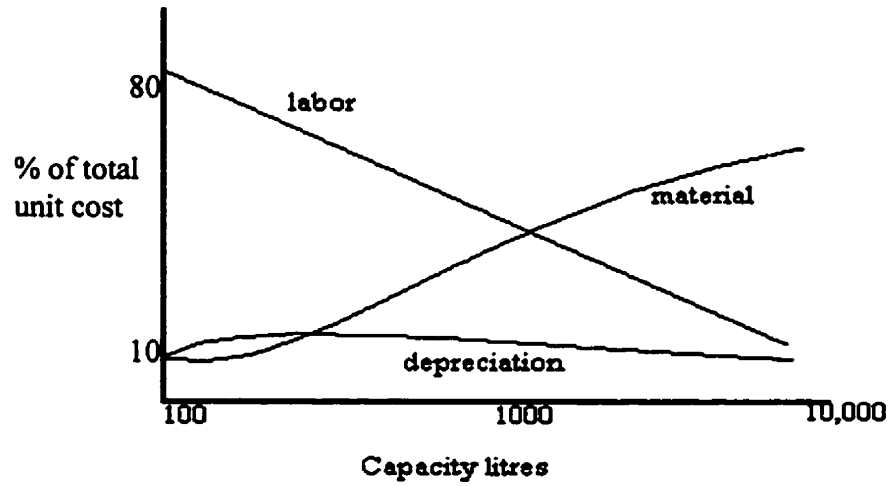

Figure 4.5.1: Effect of scale on relative cost of components

4.5.2. PROCESS DESCRIPTION

The downstream processes of this flowsheet consist of two alternate steps of ultrafiltration and affinity chromatography, followed by a final step of gel chromatography. The simulated bioreactor was stirred tank type with the CHO cells grown in suspension culture. Serum free medium was used in the bioreactor, which consists of HB-CHO basal mixture and a proprietary supplement (Immucor Canada

Inc.). This medium contains 320 mg/L of total protein. The bioreactor was inoculated with 160 L of inoculum having a concentration of $2X10^5$ cells/ml.

The operating conditions for the membrane filtration units were the same as described for the base case (Section 4.1.2). Simulation of affinity chromatography was carried out for an 85 L column having a height to diameter ratio of 0.5 and at a superficial loading velocity of about 40 cm/h. A 30 L column was used for the second chromatography. Gel filtration was simulated in a 75 L column having a length to diameter ratio of 1.5. The sample volume was taken as 5% of column volume as recommended (Wheelwright, 1991) and the flow velocity was 16 cm/h.

4.5.3. SIMULATION AND RESULTS

The 6000L bioreactor of the base case flowsheet was divided into six equal sized bioreactor for this flowsheet and one of the six bioreactors with its associated downstream units was simulated using BioProcess Simulator™. The simulation results for the flowsheet is presented in Table 4.5.1.

Table 4.5.1: Summary of simulation results

Block	Size	Time (h)	Recovery (%)	Broth Volume (L)
Bioreactor	1000 L	200	-	1000
Microfilter	15 m^2	1	82.6	804
Ultrafilter	10 m^2	1	79.7	30.2
Affinity Chromatography	85 L	3.3	74.4	42.2
Ultrafilter	2 m^2	0.4	95.0	10.8
Affinity Chromatography	30 L	2.5	98.8	30
Ultrafilter	1 m^2	0.5	94.3	3.2
Gel Chromatography	75 L	5.65	99.99	13

The overall recovery for this flowsheet was found to be 43.4% with a batch time of 215 hours and a final product purity of 99.9998%. In the downstream train longest time is taken to polish the product with gel filtration. To utilize the same sets of downstream

equipment by the six bioreactors, the operation of the bioreactors has to be scheduled so that there is no overlap in the usage of equipment.

4.5.4. SCHEDULING

Simulation results have shown that the bioreactor requires 200 hours to complete one batch compared to 15 hours to purify the product. Therefore, product from all the bioreactors will be ready for purification well before the purification of the broth from the first bioreactor is complete. This will cause a lineup in the purification section while the products from the other bioreactors in storage tanks await purification. This kind of operation has several disadvantages. First of all, large volumes (at least 5000 L in this case) of expensive storage tanks will be required to maintain the broth in aseptic condition for a long time (60 hours for the sixth bioreactor). Possibilities of product degradation will be high in presence of proteases and other compounds in the stored unpurified broth. This type of operation will also increase the batch time significantly (275 hours in contrast to 215 hours) to exceed the time pre-set in the design basis.

An alternative approach would be to schedule the operation of the bioreactor so that there is no lineup for the downstream units. If the bioreactors are inoculated every five hours (or every four hours with an hour allocated to cool down the broth), the different units will be occupied for the time interval as shown in Figure 4.5.2. All the membrane separation units (1,2,4 and 6) will be ready for the next batch well ahead of time. For the affinity chromatography columns, the cycle time shown includes time for loading, washing and elution of the column. After that there will be about an hour and 45 minutes (1.7 hours) left to regenerate the first column (unit 3) for the next batch. This time should be sufficient to regenerate a single 85 L column. For the second chromatography

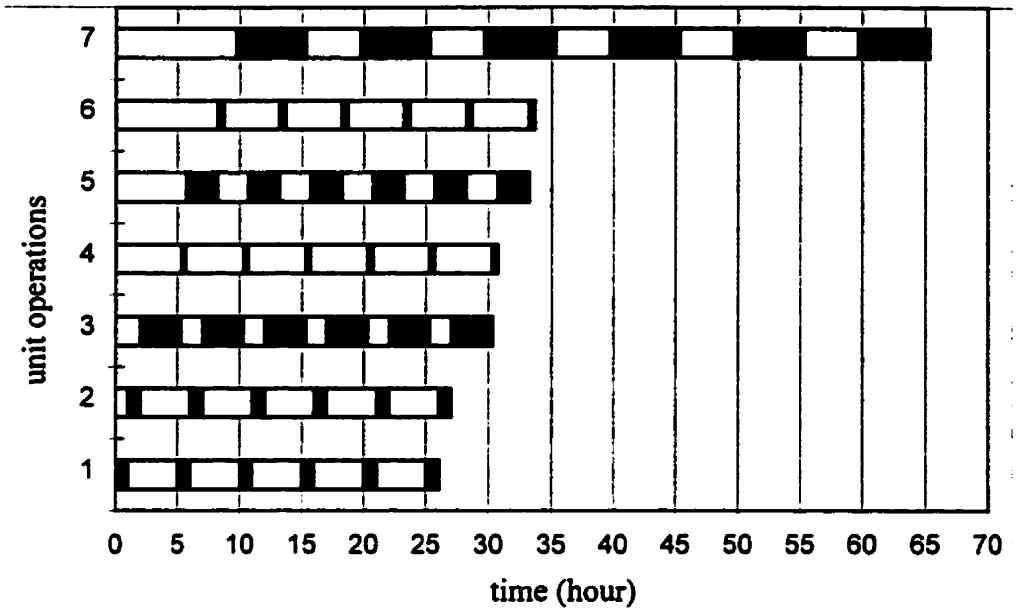

Figure 4.5.2: Scheduling of the unit operation blocks

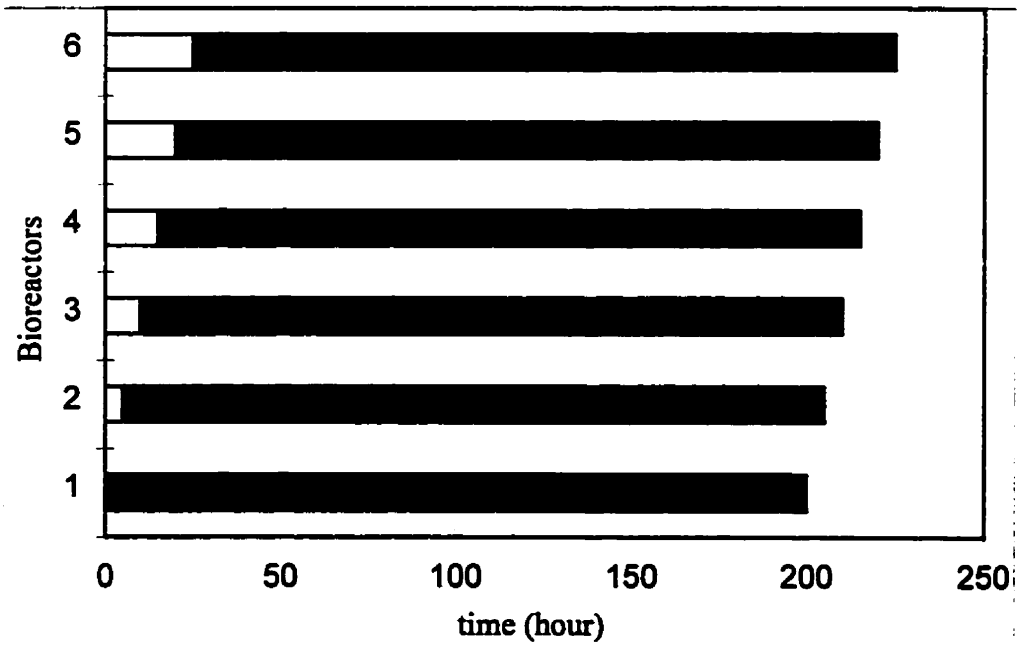

Figure 4.5.3: Scheduling of the Bioreactors

column (unit 5) there will be an hour and 30 minutes to regenerate the column, which again should be sufficient for a 30 L column. However, polishing (unit 7) of the first batch of product from the first bioreactor would not be complete when the second batch of product gets ready for gel filtration (Figure 4.5.3). There would be about 40 minutes left of the polishing step for the first batch. There could be two solutions to this problem depending on the situation. An additional gel filtration column can be added to the flowsheet so that the product from the second bioreactor can use the unoccupied column. Alternatively if there is no restriction on the batch time, the small volume (3.2 L in this case) of almost pure product from the ultrafiltration step can be stored and later purified using the same gel filtration column.

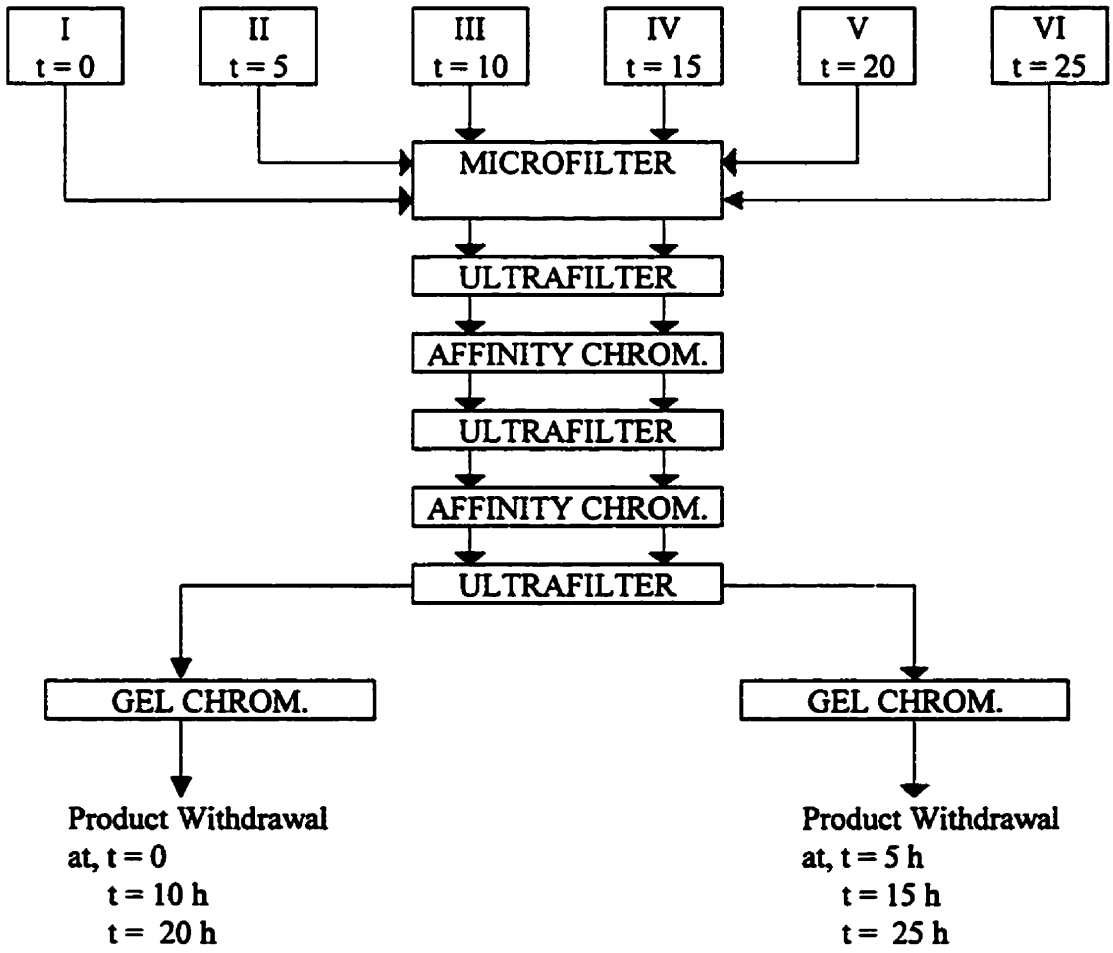

Figure 4.5.4: Flowsheet for first approach

The second approach above would be considered under sensitivity (Section 4.5.7) as an alternative. If the first approach is adopted, the batch time will be 240 hours (215 +25). In laboratory, t-PA has been found to be stable for more than 350 hours in serum-free medium and up to 250 hours in medium containing 5% fetal calf serum (FCS) (Dyring et al., 1994). The flowsheet for this case is shown in Figure 4.5.4.

Scheduling was simple for the above process where overlapping occurred in only one unit at the end of the process. For more complex situations, optimization packages such as GAMS/Minos (Brooke and Kendrick, 1992) can be used. SuperPro Designer® (version 3.0) allows simulation of process steps to share equipment in batch recipes. This feature could be used to schedule the downstream section under more complicated cases of overlapping. The new Gantt charts of SuperPro Designer® (v 4.0) (to be released in early 1999) would also lend itself for scheduling calculation of multiple batches (Petrides, 1998).

4.5.5. ECONOMIC EVALUATION

The overall economics of this process has been summarized in Table 4.5.2. The revenue in this case is one fifth of that for the base case which requires five of the 6000 L bioreactors simulated.

Table 4.5.2: Economic analysis of the process (in millions of dollars)

Purchased Equipment Cost, C_{PE}	1.63
Fixed Capital, $C_{FC} = (4.6XC_{PE})$	7.5
Total Capital, $C_{TC} = (5.5XC_{PE})$	8.965
Revenue, R	35.2
Annual Operating Cost, AOC	16.05
Gross Profit, GP = (R-AOC)	19.15
Net Profit, NP (40% tax)	11.49
Net Cash Flow, (NP + Dep.)	12.3
Return on Investment, ROI	137%
Gross Margin, GM = (GP/R)	54%

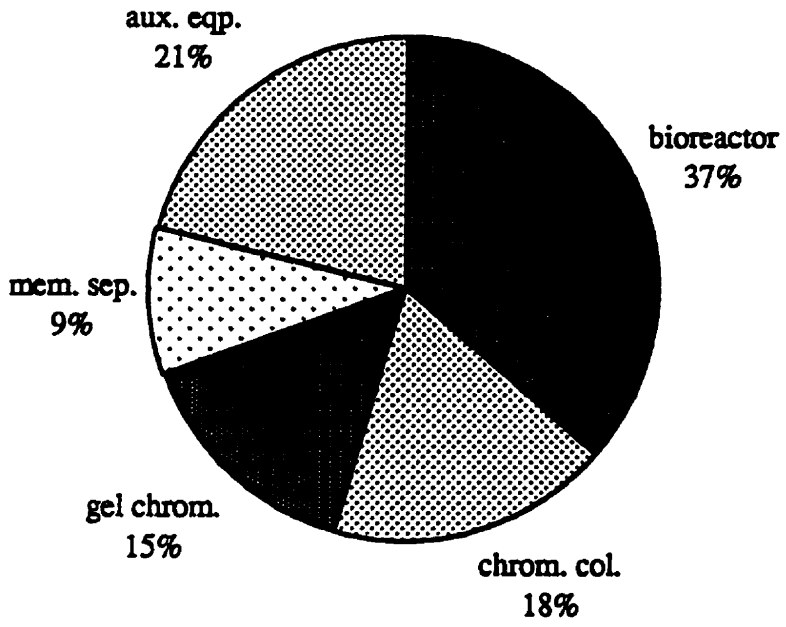

Figure 4.5.5: Breakdown of equipment purchase cost

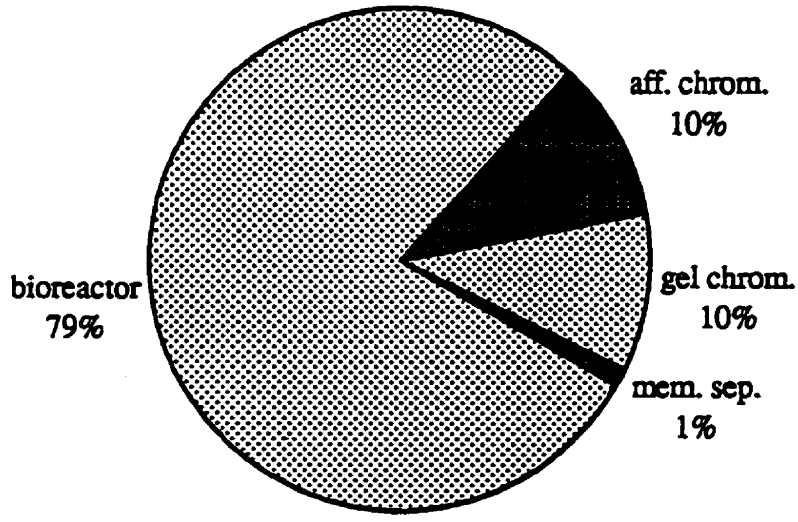

Figure 4.5.6: Breakdown of raw material cost

The equipment purchase cost for this flowsheet is dominated (36.3%) by the cost of six 1000 L bioreactors followed by chromatography (18.3%) and gel filtration (15%) columns (Figure 4.5.5). The contribution of upstream units, such as inoculum bioreactors, air compressors, filters, blending tank for media preparation etc. and other auxiliary equipment such as pumps, heat exchangers, storage tanks etc. contribute by 21%.

Process chemicals and media contribute by 41.6% to the annual operating cost (Figure 4.5.6) whereas, labor costs account for 10%. Fermentation media for the six bioreactors is found to be the major contributor (79%) to the raw material cost. Affinity and gel filtration columns contribute almost equally (10.4% each), whereas, contribution from the membrane separation units is negligible. A more practical approach to the calculation of the resources (i.e. utilities, raw materials, labor) for this multiple and overlapping batch process would be by using the new feature of version 4.0 of SuperPro Designer® to be released in early 1999. This feature will allow elimination of resource bottlenecks for multiple/overlapping batch processes by calculating resource demand (Petrides, 1998).

The return on investment (ROI) of the process is 137% with a gross margin (GM) of 54%.

4.5.6. COMPARISON WITH BASE CASE

The multiple bioreactor case is a scaled down version of the base case where a single 6000 L bioreactor out of the five bioreactors of the base case was further divided into six 1000 L bioreactors. The combined performance of the multiple bioreactors, is therefore, compared to the performance of a single bioreactor of the base case.

The high ROI for the multiple bioreactor case resulted from savings in the equipment purchase cost. The ability to share the same set of equipment by the six bioreactors resulted in the greatly reduced size of the downstream units. A comparison of the contribution from the different sources of the equipment purchase cost shows that

bioreactors contribute by only 14% for single bioreactor case compared to 36.3% for the multiple bioreactor case. The major contributors for the single bioreactor case are the chromatography columns (52%) which in the latter case contribute by about only 33.3%.

Table 4.5.3: Comparative Economic Analysis (in millions of dollars)

	Multiple Bioreactors	Single Bioreactor
Purchased Equipment Cost, C_{PE}	1.63	2.84
Fixed Capital, $C_{FC} = (4.6XC_{PE})$	7.5	13.06
Total Capital, $C_{TC} = (5.5XC_{PE})$	8.965	15.62
Revenue, R	35.2	35.2
Annual Operating Cost, AOC	16.05	20.2
Gross Profit, GP = (R-AOC)	19.15	15
Net Profit, NP (40% tax)	11.49	9
Net Cash Flow, (NP + Dep.)	12.29	10.2
Return on Investment, ROI	137%	65%
Gross Margin, GM = (GP/R)	54%	43%

The contribution of labor costs to annual operating cost is doubled (10%) for the multiple bioreactor case. This is due to the extra labor required to operate and maintain the six bioreactors. It might be possible to schedule the labor in such a way so as to use the same force to maintain the different bioreactors and achieve some savings from this source. The contribution from raw material was found to be similar for both the cases (about 42%). Of the raw material, media accounts for about 79% for the multiple bioreactor case compared to 62% for the other. Buffers and resin for the affinity chromatography columns of the base case accounts for twice as much as for the multiple bioreactor case (20%). Contribution from gel filtration is also higher for the base case (17.5%) compared to this case (10.45%).

The multiple bioreactor case would require more frequent replacement of the chromatographic resins and membranes since it carries out six times the cycles with the columns and six times longer operation with the membranes. A sensitivity study to the replacement frequency (Section 4.1.6) was carried out for the base case where it was found that the ROI of the base case would increase by about 4% if replacement takes

place every five years instead of every year. Therefore, even with the much higher (five times) replacement frequency, the ROI of the multiple case would remain high enough compared to the base case.

4.5.7. SENSITIVITY

An alternative to the two-gel filtration column flowsheet shown in Figure 4.5.4 was outlined in Section 4.5.4. According to this alternate approach, retentate from the final ultrafiltration unit has to be stored until the column becomes available for processing the broth from the successive bioreactors. The retentate from the second bioreactor has to be stored for 0.65 hour and therefore the retentate from the last bioreactor has to be stored for a total of 3.25 hours. This will increase the batch time by the same amount and for 350 operating days per year, the number of batches that can be carried out will be 34 instead of 35 (Section 3.5). The completion of an additional batch would require 5 more days of operation with this flowsheet.

The flowsheet for this process is shown in Figure 4.5.7. The economy of the process under this situation have been summarized in Table 4.5.4.

Table 4.5.4: Economic analysis of the alternative flowsheet for the multiple bioreactor process (in millions of dollars)

Purchased Equipment Cost, C_{PE}	1.5
Fixed Capital, C_{FC} = (4.6 X C_{PE})	6.9
Total Capital, C_{TC} = (5.5 X C_{PE})	8.25
Revenue, R	34.2
Annual Operating Cost, AOC	15.45
Gross Profit, GP = (R-AOC)	18.75
Net Profit, NP (40% tax)	11.25
Net Cash Flow, (NP + Dep.)	11.85
Return on Investment, ROI	144%
Gross Margin, GM = (GP/R)	55%

Replacement of one of the gel filtration column by a storage tank in the alternate flowsheet reduces the purchased equipment cost by 8%. The omission of raw material

cost accounting for buffer for one of the columns and its media replacement, reduces the annual operating cost by 0.6 million dollars. The revenue on the other hand is also lower for this case since the longer batch time does not allow the 35 batches set in the original design basis. The overall effect is 5% improvement of the ROI of the process with almost unchanged gross margin.

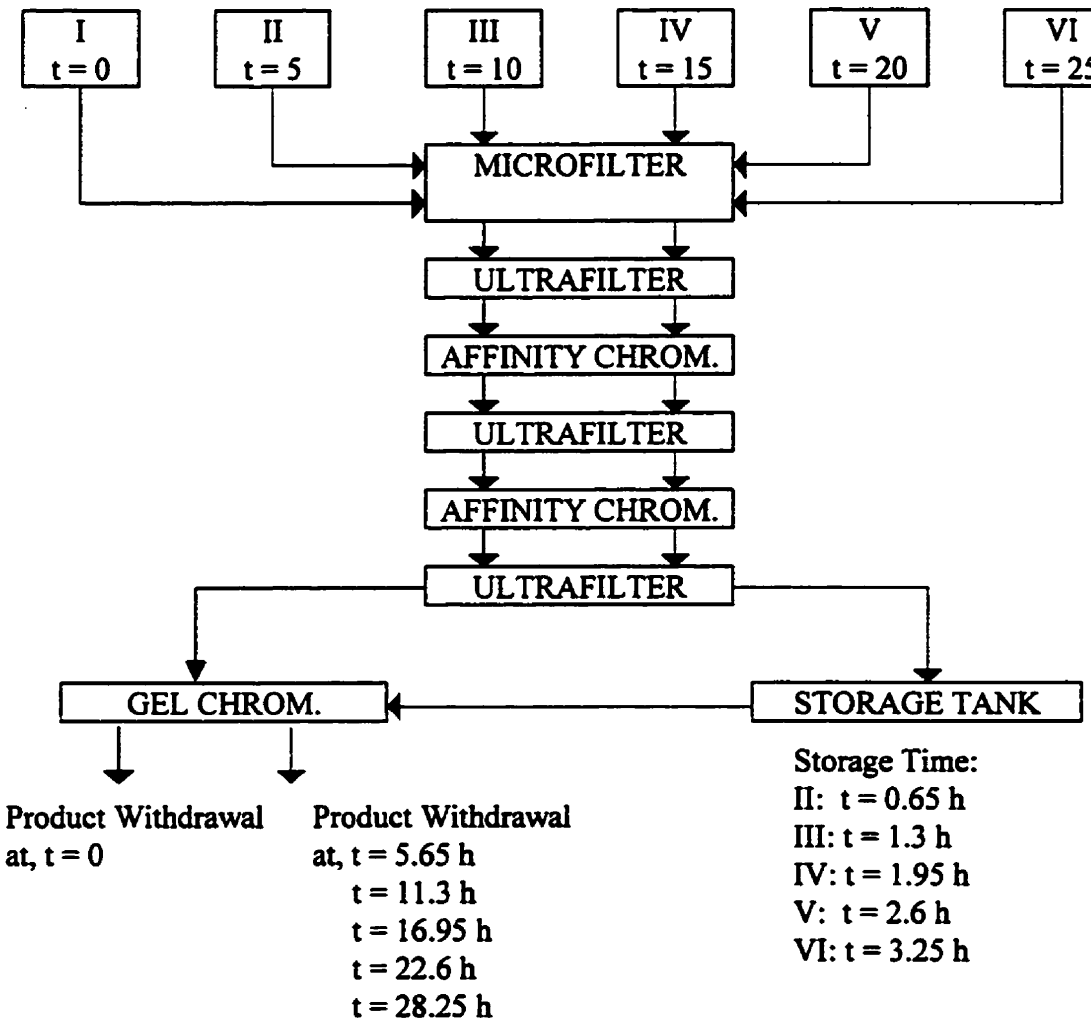

Figure 4.5.7: Flowsheet for alternate approach

Therefore, if storing the product for a maximum of up to 3.25 hours before gel filtration does not result in any deleterious effect, this flowsheet becomes an attractive alternative to the first case.

4.6. Summary of Results

The simulation results for the flowsheets and results of economic evaluation are summarized in this section so that they can be easily compared.

4.6.1. Flowsheet Simulation

The following tables compare the size of the individual unit operation blocks in the flowsheet, recoveries therein and batch time. The overall recovery for all the flowsheets are found to be above the assumed value of 40% and the total batch time, less than that (10 days or 240 hours) specified in the design basis (Section 3.5).

Table 4.6.1: Size of the unit operation blocks in the simulated flowsheets

Units	Base Case	Two Stage Cell Culture	Fed-batch (A)	Fed-batch (B)	Airlift Bioreactor	Multiple Bioreactor
Growth Bioreac.	-	6000 L X 5	-	-	-	-
MF	-	80 m^2 X 5	-	-	-	-
Prod. Bioreac.	6000 L X 5	5000 L X 5	4250L X 2	3745 X 2	6000 L X 5	1000 L X 6 X 5
MF	80 m^2 X 5	70 m^2 X 5	65 m^2 X 2	60 X 2	80 m^2 X 5	15 m^2 X 5
UF	60 m^2 X 5	50 m^2 X 5	50 m^2 X 2	45 X 2	60 m^2 X 5	10 m^2 X 5
AFF. I	200 L X 15	200 L X 10	186L X 20	195 X 12	200 L X 15	85 L X 5
UF	10 m^2 X 5	15 m^2 X 5	60 m^2 X 2	10 X 2	10 m^2 X 5	2 m^2 X 5
AFF. II	85 L X 5	113 L X 5	146L X 6	135 X 2	85 L X 5	30 L X 5
UF	2.5 m^2 X 5	5 m^2 X 5	20 m^2 X 2	5 X 2	2.5 m^2 X 5	1 m^2 X 5
GEL	147 L X 30	147 L X 25	147L X 12	147 X 4	147 L X 30	75 L X 5

Table 4.6.2: Recoveries in the individual unit operation blocks in the downstream train

Units	Base Case	Two Stage Cell Culture	Fed-batch (A)	Fed-batch (B)	Airlift Bioreactor	Multiple Bioreactor
MF	93.3	84.5	79.2	76.6	93.3	82.6
UF	83	84.76	93.3	92.06	83	79.7
AFF. I	73.6	83.45	80.9	39.96	73.6	74.4
UF	94	92.37	94.0	92.77	94	95.0
AFF. II	90.7	87.16	97.97	95.5	90. 7	98.8
UF	94.13	96.26	78.3	95.4	94.13	94.3
GEL	99.997	99.997	99.9	86.2	99.997	99.99
Overall	45.7	46.3	43.0	41.0	45.7	43.3

Table 4.6.3: Batch time in hours for the individual unit operation blocks of the simulated flowsheets

Units	Base Case	Two Stage Cell Culture	Fed-batch (A)	Fed-batch (B)	Airlift Bioreactor	Multiple Bioreactor
Growth Bioreac.	-	80	-	-	-	-
MF	-	1	-	-	-	-
Prod. Bioreac.	200	120	200	200	200	200
MF	1	1	1	1	1	1
UF	1	1	1.25	1	1	1
AFF. I	3.7	5	4.5	3.65	3.7	3.3
UF	0.5	0.7	0.95	1.2	0.5	0.4
AFF. II	3.2	4	2.97	2.84	3.2	2.5
UF	0.5	0.4	1	0.55	0.5	0.5
GEL	7	7	7	7	7	5.65
Total	216.9	220	218.7	217	216.4	214.4

Table 4.6.4: Purity in the chromatography columns in the downstream train

Units	Base Case	Two Stage Culture	Fed-batch (A)	Fed-batch (B)	Airlift Bioreactor	Multiple Bioreactor
CHROM. I	99.98	99.978	99.994	99.98	99.98	99.990
CHROM. II	99.998	99.999	99.999	99.998	99.998	99.998
GEL	99.9998	99.9996	99.9999	99.9998	99.9998	99.9997

4.6.2. Economic Evaluation

The economic performances of the simulated flowsheets are summarized in this section. "Budget authorization" grade estimates of the total capital investment (C_{TC}) for the processes were obtained by multiplying the total equipment purchase cost (C_{PE}) by appropriate cost factor. This estimate was considered satisfactory for comparison purposes. Calculation of annual operating cost (AOC) is explained in Appendix C. The overall economics of the process was measured in terms of return on investment (ROI), defined as the ration of (net profit after tax + depreciation) to total capital investment and Gross Margin (GM), defined as the ratio of pretax profit to annual product sales. Table 4.6.5 summarizes the economic performance of the simulated flowsheets.

Table 4.6.5: Summary of economic performance of the alternate flowsheets

	Base Case	Two Stage Cell Culture	Fed-batch (A)	Fed-batch (B)	Airlift Bioreactor	Multiple Bioreactor
C_{PE} (million)	14.2	8.8	10.0	6.38	13.6	8.15
C_{TC} (million)	78.1	48.4	55.0	35.1	74.8	44.8
AOC (million)	101	92.9	59.86	38.7	101	80.25
ROI (%)	65	115	138	252	68	137
GM (%)	43	47	66	78	43	54

From the table, it is observed that all the alternate flowsheets have higher ROI than the base case. ROI of the process marginally increases by replacement of the stirred tank bioreactor of the base case with an airlift type. This increase results from the lower purchase cost of the airlift bioreactor compared to a stirred tank reactor because of the simplicity of construction of the former. Two-stage cell culture process increases the ROI of the base case by 77% partially due to replacement of the expensive affinity chromatography columns in the downstream train by ion-exchange columns. ROI

increased most significantly by switching from batch to fed-batch mode of operation. 112% increase in ROI is observed for fed-batch case (A) where the product concentration is four times of that for the batch process (base case). Highest ROI is calculated for case (B) of fed-batch process with eight times the product concentration. The multiple bioreactor case is also found to be a very attractive alternative to the base case. Since downstream processing accounts for about 70 to 80% of the overall production cost for biotherapeutics, using an economically sized single purification train for this process brought about the huge increase in ROI.

The contribution from fermentation and recovery equipment to total capital investment was found to differ depending on the process (Figure 4.6.1.(a) and 4.6.1.(b)). Fermentation equipment were found to be the major contributors for the two stage cell culture process, that uses separate bioreactors for growth and production, and multiple bioreactor case, with the largest contribution made by the latter (36%). Largest contribution (95%) by recovery equipment to total equipment purchase cost was observed for fed-batch case (A), followed by airlift bioreactor and fed-batch case (B), for which cases the fractions contributed (88%) were very close. Contribution by raw material and media cost to the annual operating cost was found to be the most significant for all the flowsheets simulated. As observed for the total equipment purchase cost, contribution from fermentation media were much higher for the two stage cell culture process and multiple bioreactor case, compared to raw materials for recovery section (Figure 4.6.2. (a)). However, in this case, contribution for the two-stage cell culture process was larger than the multiple bioreactor case. For the raw materials associated with recovery units, contribution was highest (70%) for the fed-batch case (A), as expected, followed by fed-batch (B) (Figure 4.6.2. (b)).

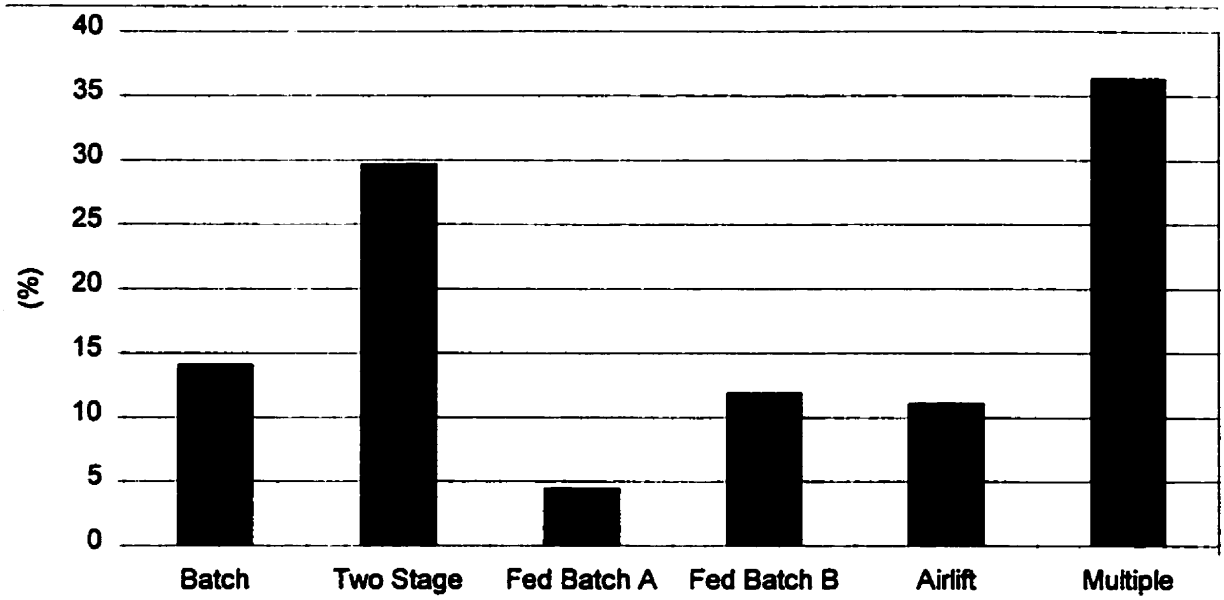

Figure 4.6.1.(a): Comparison of Equipment Purchase Cost (Fermentation)

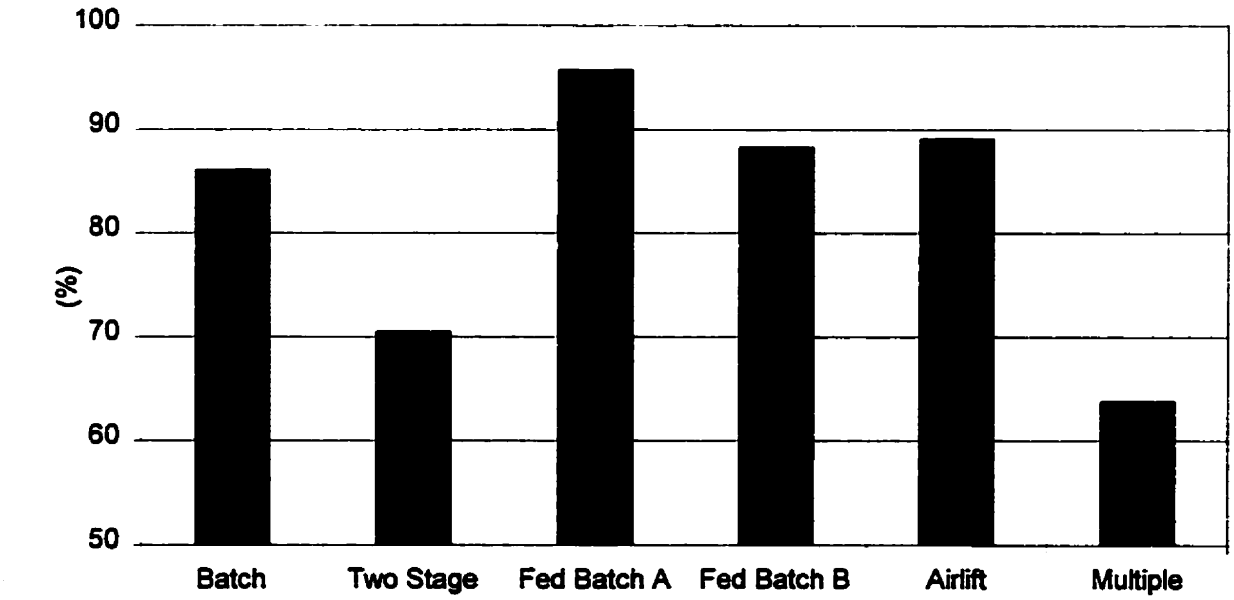

Figure 4.6.1.(b): Comparison of Equipment Purchase Cost (Recovery)

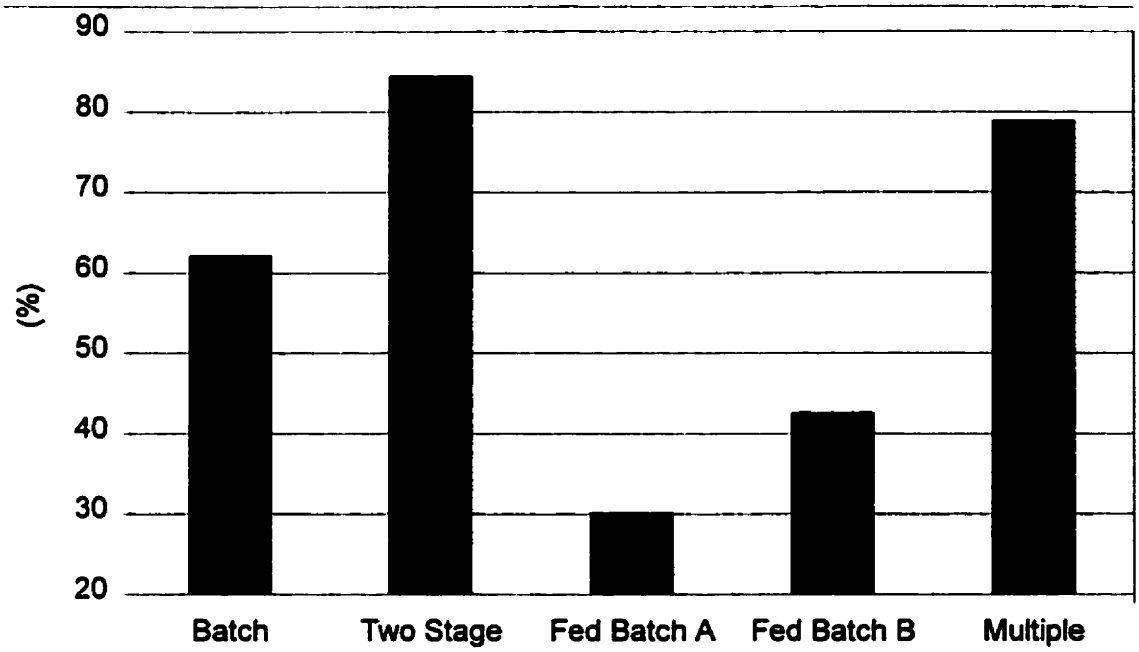

Figure 4.6.2.(a): Comparison of Raw Material Cost (Fermentation)

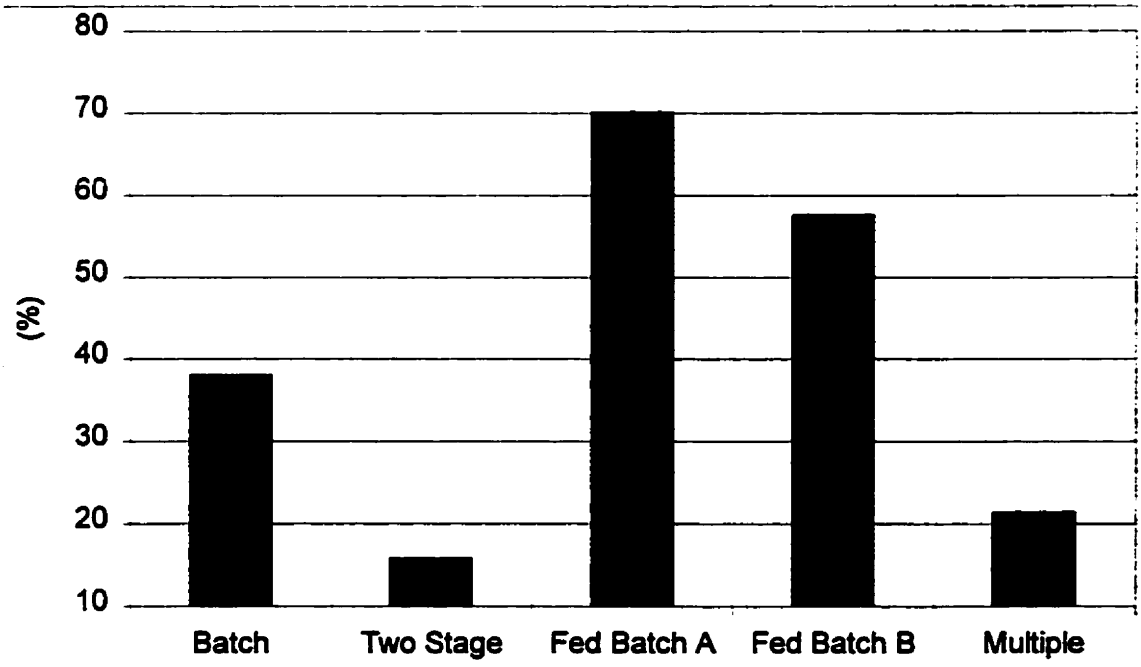

Figure 4.6.2.(b): Comparison of Raw Material Cost (Recovery)

CHAPTER 5

SENSITIVITY STUDY

This chapter presents results of sensitivity study of the simulated processes to uncertain parameters and variables. This parametric sensitivity study was carried out using BPS. Some uncertainty was also involved in the calculation of overall economics of the processes. In the second part of this chapter, sensitivity of overall economics, measured in terms of ROI, to uncertain quantities in its calculation are presented.

5.1. UNIT OPERATION BLOCKS

The parameters and operating variables used to simulate the unit operation blocks were mostly obtained from literature. For all the cases a range of values rather than a fixed number was obtained and in some cases estimates were used due to lack of knowledge. One of the advantages of designing a process using BPS is that it allows study of sensitivity of a simulated process to uncertain parameters. The flowsheet analysis feature of BPS allows virtually all the variables and parameters of the models to be studied that are specified as input. This feature of BPS was utilized in the following sections to identify the critical parameters of the different unit operation blocks and to determine to

what extent process performance will be affected due to reasonable variation in the value of the parameter. In this study, only those parameters were selected that are relevant to the specific model chosen for each block. The parameters were varied over a range of ± 50% where a range could not be found from literature. All the sensitivity study was carried out for the unit operation blocks of the base case (Section 4.1).

5.1.1. Bioreactor

The parameters and variables allowed to vary for sensitivity study of this model are:

1. Oxygen Transfer: minimum dissolved oxygen, maximum VVM, minimum k_La, final dissolved oxygen, initial oxygen saturation, dissolved oxygen concentration, percent of dissolved oxygen etc.

2. Agitation: power, maximum agitation power, power by volume, driver efficiency, length of impellers, number of impellers, rotational speed, impeller diameter, impeller width, number of blades, number of baffles, baffle width etc.

3. Reaction rate: nitrogen/ash usage, C, H, O, N imbalance, substrate, carbon dioxide, oxygen, hydrogen and nitrogen source utilization rate, mumax, K_s, K_o, K_d etc.

4. Geometry: maximum volume, final volume, bioreactor diameter.

5. Heat Transfer: cooling duty, coolant temperature, heat transfer coefficient, heat transfer area.

6. Inoculum: concentration, volume, volume charge.

7. Time: cycle time, batch time, idle time, down time.

The variables studied for the bioreactor are biomass/substrate/N source rate and biomass/substrate concentration by varying the following parameters and variables:

Table 5.1.1. Variables studied for sensitivity of the bioreactor block

Varied Variables	Suggested Range	Reference
Inoculum Concentration	$1X10^5$ -$2X10^5$ cells/ml	
Inoculum Volume	5% to 25% of the liquid volume	Chisti, (1992)
Volume Charge	-	-
Minimum Oxygen Level	40%	Hansen and Emborg, (1994)
K_La		
Kinetic Parameters	-	-

Sensitivity study for the bioreactor was not very informative. The results showed changes that occur at the initial conditions or at a certain point of the fermentation cycle. With sensitivity study it was not possible to follow the changes during the entire course of fermentation.

- **Inoculum Concentration:** The inoculum concentration was varied between 3 $X10^{-2}$ and $8X10^{-2}$ gm/L. In BPS the available unit for cell concentration is mass concentration (e.g. mg/L, gm/L etc.). The actual inoculum concentration in cells/ml for mammalian cell was therefore, converted to mass concentration for simulation by assuming that the weight of $3.8X10^9$ cells is equal to one gram (Zeng and Deckwer, 1995). Variation in the inoculum concentration had significant effect on the growth rate and concentration of the biomass (Appendix F). 43% reduction in inoculum concentration brought about similar magnitude of reduction in biomass production rate and biomass concentration.

- **Inoculum Volume:** The inoculum usually constitute between 5-25% of the working volume of the bioreactor (Chisti, 1992). The inoculum volume was varied between 500 L (8.3%) to 1500 L (25%) for sensitivity study. The result of variation in inoculum volume was similar to inoculum concentration. However, in this case the substrate (glucose) concentration was also affected. 50% reduction in inoculum volume increased the substrate concentration by 9%.

- **Volume Charge:** The volume charge or the batch charge to the bioreactor was varied between 2000 L to 5000 L. 20% reduction in charge volume resulted in an equal amount of reduction in both glucose and glutamine consumption rate, since according to the supplied kinetics (Appendix G) these rates are dependent on the initial concentration of the substrates. The reduced consumption rate increased glucose concentration by 4%. Due to concentration effect at smaller volume, biomass growth rate increased slightly while the cell concentration increased by 20%.

- **Minimum Oxygen Level:** The minimum dissolved oxygen level required in the bioreactor was specified as a fraction of solubility at the fermentation temperature of 37°C. The bioreactor model, BFERM was supposed to calculate the minimum air flow rate required to keep the dissolved oxygen concentration above this level. At 37°C, the solubility of oxygen is found to be 0.195 mmol O_2/L (Bailey and Ollis, 1986). The dissolved oxygen concentration maintained in the laboratory scale bioreactors (2 litres) for CHO cell culture is found to be between 40% and 60% (Hansen and Emborg, 1994; Kurano et al., 1990, Lakhotia et al., 1992). The oxygen concentration therefore, should be between 0.078 and 0.117 mmol O_2/L. However, it was found that the simulation of the bioreactor does not converge at oxygen concentrations even as high as 0.715 mmol O_2/L. The input file, that simulates the bioreactor successfully, was only modified by changing the oxygen flowrate to reduce the dissolved oxygen level from 6.4 mmol O_2/L. The simulation would not converge after this single modification. The sensitivity study by varying the minimum oxygen level in the bioreactor, therefore had no effect on the dissolved oxygen percentage which was at 100% (Appendix F).

- **K_La:** For reasons already explained in the previous section, sensitivity study with K_La had no effect on the related variables of the bioreactor (Appendix F).

- **Kinetic Parameters:** Among the kinetic parameters, the preferential substrate utilization constant (G_S), specific death rate (k_d) and specific growth rate (μ_{max}) were varied. The simulator was able to vary the user defined kinetic parameters and the response to sensitivity study was determined by the kinetics supplied to the simulator as subroutine. 80% increase in G_S resulted in 23% reduction in glucose uptake rate, which in turn increased the glucose concentration by 38%. Biomass growth rate fell by 18% and the cell concentration by 13% when the specific death rate became 3.5 times the base value ($1.0 X10^{-3} h^{-1}$). A 25% increase in the specific growth rate over the base value on the other hand, increased the biomass growth rate by 120% and the cell concentration by 75%.

5.1.2. Membrane Separators

The parameters and variables of the membrane separator model that can be varied for sensitivity study are the following:

1. Membrane (circular type): total membrane area, fiber or tube length, fiber or tube diameter, number of channels in parallel, number of cartridges in series, membrane resistance.

2. Pressure: pump pressure, permeate side pressure, retentate pressure, retentate pressure drop.

3. Pump: pump efficiency, drive efficiency, pump horse power, electric horse power.

4. Cooler: temperature at cooler outlet, cooler duty, maximum duty.

5. Physical Property: viscosity, diffusivity, density.

6. Flow: friction factor, setpoint velocity, Reynolds number, Reynolds number for transition from laminar to turbulent region, Schmidt number.

7. Operation: rejection coefficient of key component, rejection coefficients for other components, fraction of key component denatured, volume ratio (concentration factor), gel-resistance, exponents on transmembrane pressure, Reynolds number and key component concentration in the equation for gel resistance calculation ($R_g=R_{g0}(P)^a\ (Re)^b\ (C_b)^c$), gel resistance parameter (R_g0) and decay coefficient (K) in the fouling equation ($J = Jo\ X\ e^{-Kt}$).

8. Tank: temperature and pressure in tank, initial and maximum tank volume, retentate concentration in tank.

Of the above parameters and variables the following were studied for sensitivity study of the microfilter block:

Table 5.1.2. Variables studied for sensitivity of the microfilter block

Varied Variables	Suggested Range	Reference
Setpoint Velocity	0.5-2.5 m/s	Cheryan, (1986)
Key Component Rejection	0.8 - 1.0	-
Denaturation Fraction	1% to 5%	-
Viscosity	1 to 1.65 cp	BPS simulation results
Membrane Resistance	0.55 to 4.7X10^7 s^2/cm^2	Cheryan, (1986)
Rgelo	3.51- 1.53X10^6 Pa-s/m	Lee, (1989)
a	0.746 and 0.692	Lee, (1989)
b	-0.77	Tamer (1995)
c	0.18 and 0.2	Lee, (1989); Wood, (1988)

- **Setpoint Velocity:** The setpoint velocity through the tube of the membrane was varied between 0.8 m/s and 2.4 m/s which represents a range of 50% to 150% of the

velocity (1.6 m/s) defined for the design case. A 50% reduction in the velocity resulted in 10% reduction of the permeate flux (Figure 5.1.1) and 45% reduction of the volume ratio (feed volume/retentate volume) or in other words the volume of the retentate increased by 80% over the design case. The fraction of key component (CHO cells) retained by the membrane, as a result, increased and this increased the gel resistance by 50% due to the dependence of gel resistance on Reynolds number and key component concentration, C_b ($R_g=R_g0(\Delta P)^a (Re)^b (C_b)^c$). The shear rate, however, remained unaffected by variation in set point velocity. The model for microfiltration uses an average velocity through the unit for calculation of shear rate, which may not have changed significantly within the range employed for sensitivity study to affect the shear rate.

- **Key Component Rejection:** Rejection coefficient (r) for the key component was set to 1.0 for the design case. For sensitivity study, this coefficient was varied between 80% and 100% of the value. At r = 0.8, the volume ratio increased by 40% (or the volume of the retentate became 70% of the design case) and the gel resistance improved (decreased) by 16% over the design case.

- **Denaturation Fraction:** The optional feature of MEMBRANE model was used to study the effect of including a protein denaturation fraction for t-PA in the simulation. Surprisingly enough, no effect was observed on the material balance of t-PA by inclusion of the fraction protein denatured. The key component concentration also remained unaffected (Appendix F).

- **Viscosity:** Sensitivity study was used to explore the effect of changing viscosity due to increased protein concentration during filtration. The upper limit of viscosity was chosen as the highest value observed in simulation, which was around 1.65 cP. At the upper limit, permeate flow reduced to about 48% of the value observed for a viscosity of 1cP (Figure 5.1.1) and the shear rate ($\gamma = \Delta Pr/2L\mu$) varied between $3.93X10^7$ hr^{-1} to $1.57X10^7$ hr^{-1} between the viscosities of 0.8 cP and 2.0 cP. Since the range of shear rate

observed with hollow fiber membranes covers $1.44X10^7$ hr $^{-1}$ to $5.04X10^7$ hr $^{-1}$ (Cheryan, 1986), the operation of the simulated membranes were within the range.

- **Membrane Resistance:** A wide range of membrane resistance is observed for hollow fiber membranes depending on the material of construction. The materials that have been used for micro and ultra filtration membranes are mainly polymers such as polysulfone, cellulose acetate, cellulose nitrate, polyamide, polypropylene, acrylonitrile copolymer, nitrocellulose etc. Depending on the material, these membranes differ in thermal resistance, operating pH range, resistance to sanitizing chemicals etc. The range of membrane resistance reported by Cheryan (1986) for hollow fiber membranes is found to vary by an order of magnitude. For sensitivity study the base case resistance was varied between 10 times and 0.1 times of the design case resistance (2.0×10^{11} m^{-1} or 0.51×10^7 s^2/cm^2). Ten fold increase in the membrane resistance reduced the permeate flux to 12.5% of its base case value. A similar decline was observed in the volume ratio (feed volume/retentate volume) resulting from higher retentate volume due to reduced permeate flux. A 3-fold increase reduced the permeate flux to 42.5% of the base case value.

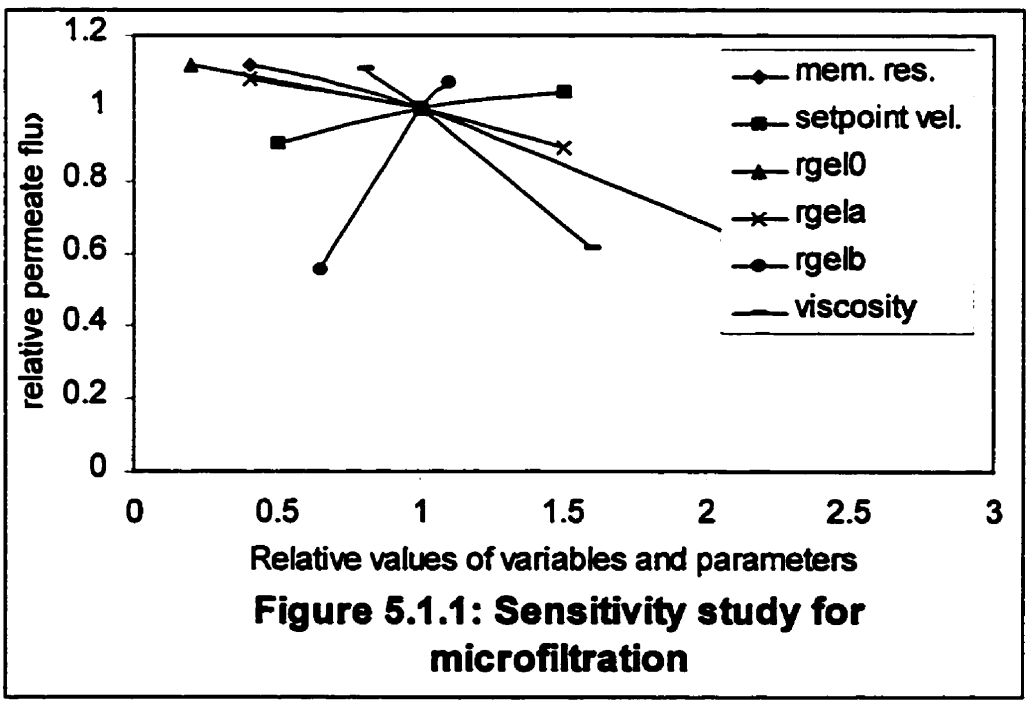

Figure 5.1.1: Sensitivity study for microfiltration

- **Rgelo:** Rgelo is a parameter in the gel resistance equation $R_g = R_{g0}(\Delta P)^a (Re)^b (C_b)^c$. The value of this parameter was assumed to be 4.5 $X10^{12}$ m^{-1} for the base case. For this value the gel resistance was found to be 5.42$X10^{10}$ m^{-1}. Rgelo was varied between 1$X10^{12}$ and 9$X10^{12}$ m^{-1}. An 80% reduction in Rgelo reduced the gel resistance by 50% and this increased permeate flux by about 12% (Figure 5.1.1). A 100% increase in Rgelo increased the gel resistance by 72% and reduced permeate flux by 13%. The value assumed for Rgelo in the base case is a bit higher than that reported for *E. coli* (Lee, 1989). The combined resistance from gel and membrane (2.54$X10^{11}$ m^{-1}), however, was found to be of similar magnitude (6.6$X10^{11}$ m^{-1}) as reported by Reis et al. (1991) for t-PA containing broth.

- **a:** The dependence of gel resistance on transmembrane pressure is described by the equation $R_g = R_{g0}(\Delta P)^a (Re)^b (C_b)^c$. The values obtained for the exponent 'a' from literature are 0.746 and 0.692 for filtration of *E. coli* (Lee, 1989). In absence of an estimate for CHO cell or t-PA, a value of 0.7 was used in the simulation. This value, however, was reported for the case where pressure was measured in kPa. For simulation, this value had to be converted to pressure units of N/m^2, the unit used by BPS for this particular variable. The converted value (0.13) was varied between 0.05 and 0.2 for sensitivity study. A 50% increase in 'a' brought about a similar scale of increase in the gel resistance and permeate flux was reduced to 90% of its base value (Figure 5.1.1).

- **b:** The exponent 'b' relates the dependence of gel resistance on Reynolds Number in the equation $R_g = R_{g0}(\Delta P)^a (Re)^b (C_b)^c$. The value of exponent 'b' obtained from literature for filtration of *E. coli* is -0.77 (Tamer, 1995). Since an estimate could not be found for CHO cell or t-PA, a sensitivity study was carried out by varying 'b' between -0.5 and -0.85. Simulation did not converge over the ± 50% range initially intended. The gel resistance was found to be very sensitive to the value of 'b'. At the upper limit of -0.85, which represents a 10% increase, gel resistance was reduced to 70% of its base value.

- **c:** The exponent 'c' relates the dependence of gel resistance on key component concentration in the equation $R_g=R_{g0}(\Delta P)^a (Re)^b (C_b)^c$. The values obtained for exponent 'c' from literature are 0.18 (Lee, 1989) and 0.20 (Wood, 1988) again for filtration of *E. coli*. In absence of an estimate for CHO cell or t-PA, a value of 0.2 was used in the simulation. For sensitivity study this value was varied between 0.1 and 0.3 with a step size of 0.05. The process was found to be relatively insensitive to variations in 'c'.

For the ultrafiltration block, the batch model for membrane separation BMEMBRANE was employed. This model has a holding tank for the batch charge where continuous feeds (e.g. diafiltration stream) if present, are added, and part of the retentate recycled. It is necessary to recirculate the retentate in order to maintain a significantly higher flow rate of the process feed than permeate. High velocity parallel to the filtration surface prevents accumulation of the retained components on the surface. The following variables and parameters were studied for sensitivity of this block.

Table 5.1.3. Variables studied for sensitivity of the ultrafilter block

Varied Variables	Suggested Range	Reference
Decay Coefficient	0.12 to 0.64 hr^{-1}	BPS simulation
Recycle Ratio	10 to 20	BPS simulation
Pump Capacity	75 L/hr to 100 L/hr	BPS simulation

- **Decay Coefficient:** Decay coefficient (K) is a parameter in the fouling model $J = Jo \times e^{-Kt}$ proposed by Shepard and Thomas (1970), where Jo is the initial flux before fouling. In batch ultrafiltration and microfiltration, typical flux-time data show a steady decline in flux. Part of this decline is attributed to an increase in viscosity but part is due to fouling of the membrane pores. Fouling is caused by the deposition and accumulation of submicron particle on the membrane surface and/or the crystallization and precipitation of solutes on the surface and within the membrane pores. Fouling is a time dependent phenomenon and is also partially concentration dependent. Fouling of membranes can be minimized by pretreatment of membrane, modification or

pretreatment of feed and adjustment of operating conditions such as feed velocity and pressure etc. Fouled membranes can be cleaned by either physical means (e.g. water rinsing, gas-liquid cleaning, electric vibration etc.) or chemical means (treatment with detergents, alkali, acids, oxidants etc.).

Three stages of flux decline have been identified by Fane and Radovich (1986) for the lifetime of an ultrafiltration membrane. Stage I is associated with solvent (water)-flux decline, where low concentrations of contaminants, dust, bacteria etc. obstruct the largest pores of the membrane. The second stage is due to concentration polarization and initiation of fouling. Stage III is the period when solute molecules bind to the membrane forming a deposit that results in irreversible flux decline. BPS has three built-in models for fouling. The above model was chosen for this simulation because it is easy to obtain an estimate of the fouling coefficient (K) for this model although this model is a simplistic representation of a complex phenomenon

J (flux) versus t (time) data is generated by the BMEMBRANE model of BPS and an estimate of decay coefficient (K) was obtained by simply plotting ln J versus t. For the ultrafilters of the base case, K was found to vary between 0.12 to 0.64 hr^{-1}. Sensitivity study revealed that about 70% variation in K changed the flux by about 6% only.

- **Recycle Ratio:** Both the batch and continuous models for membrane separation units in BPS has a recycle stream that recycles a portion of the retentate. This is necessary to maintain a high liquid velocity parallel to the filtration surface that prevents accumulation of retained components. Recycle ratio of 15 was specified for the design case. 33% increase in this value had insignificant effect on tank volume and tank concentration. However, friction factor, f went down by 43% and power of the recycle pump increased by about 45%. Permeate flux was found to increase by about 10%.

- **Pump Capacity:** Increased pump capacity reduces the filtration time for batch ultra and micro filtration blocks. The capacity of the recycle pump was varied over a range of 75% to 150% of the base value of 100 L/hr. A 50% increase in the pump capacity increased pump power by about the same magnitude as expected. This variation in pump capacity reduced the tank volume by 15% and increased permeate flux by about 22%. The reduced tank volume increased the concentration of the key component in the tank by 15%.

5.1.3. Affinity Chromatography

The variables and parameters of the model which are allowed to vary for sensitivity study are the following:

1. Column: length and diameter, total volume, number of column and void fraction.
2. Resin: diameter, porosity, tortuosity.
3. Rate theory parameters: diffusivity, mass transfer coefficient, capacity, isotherm adsorption constant kload.
4. Operation: cycle time, superficial velocity, linear velocity, pressure drop, regeneration time, volume, concentration, molarity and pH of eluant etc.

The following parameters and variables were studied for this block:

Table 5.1.4. Variables studied for sensitivity of the affinity chromatography block

Varied Variables	Suggested Range	Reference
Capacity	>0.6 mg plasminogen/ml gel	Pharmacia LKB Biotechnology
Kload	$162X10^3$ cm^3/g	Arnold et al. (1985)

Capacity: Lysine Sepharose 4B has been used in laboratory scale purification (Einarsson et al., 1985; Dodd et al., 1986) of t-PA among other affinity ligands (Table 2.2.4). Clonis (1990) pointed out that Lysine Sepharose 4B found application in the above process because of its biospecificity for t-PA. The major chromatographic material supplier Pharmacia lists only Lysine Sepharose 4B as a ligand for plasminogen and quotes a

capacity of >0.6 mg plasminogen/ml gel. Since plasminogen activator functions by binding to plasminogen (Figure 2.2.2) and the binding efficiency has to be less than 100%, a conservative estimate of 0.5 mg plasminogen activator/ml gel was assumed in all the simulations. However, since the actual capacity of the ligand is not known, in the sensitivity study carried out, the capacity was varied over a range of 50% to 150% of the design case capacity. A strong dependence of recovery in the affinity chromatography column was observed on the capacity of the ligand for t-PA. 60% reduction in the capacity over the design case reduced the recovery in the column by about 63%. The purity also went down by 0.2%, which is significant since the purity level dealt with in the column is greater than 99.9%. A 20% increase resulted in 17% higher recovery. This result has significant impact on the overall economics (ROI) of the process. 20% increase in the capacity will increase the overall recovery of the process from 40% to 47%. This will improve the ROI of the process by 28% as shown in Figure 5.1.2.

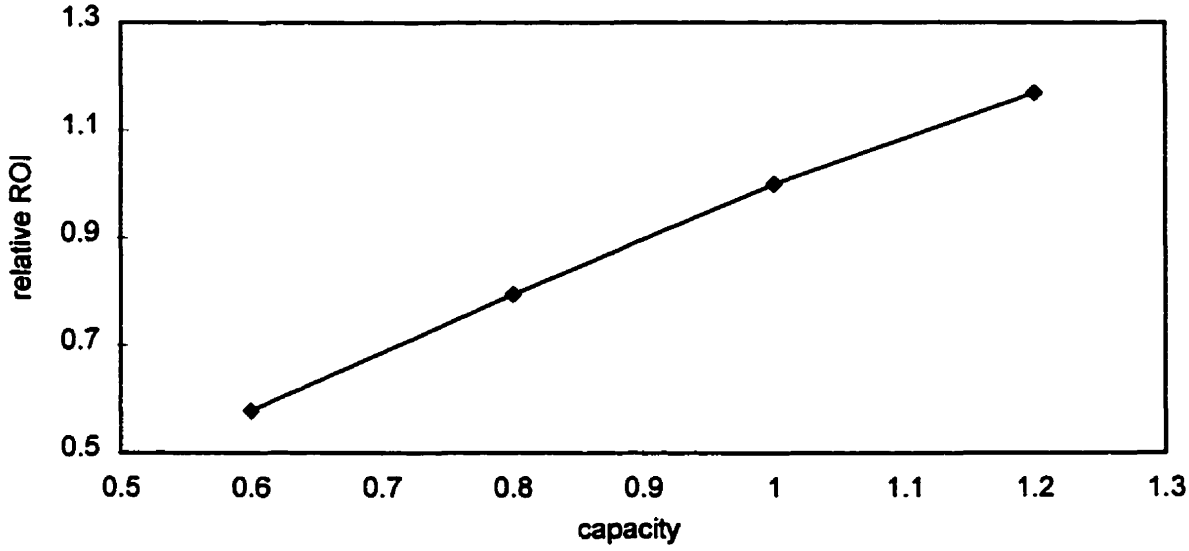

Figure 5.1.2: Sensitivity of ROI to capacity of affinity ligand

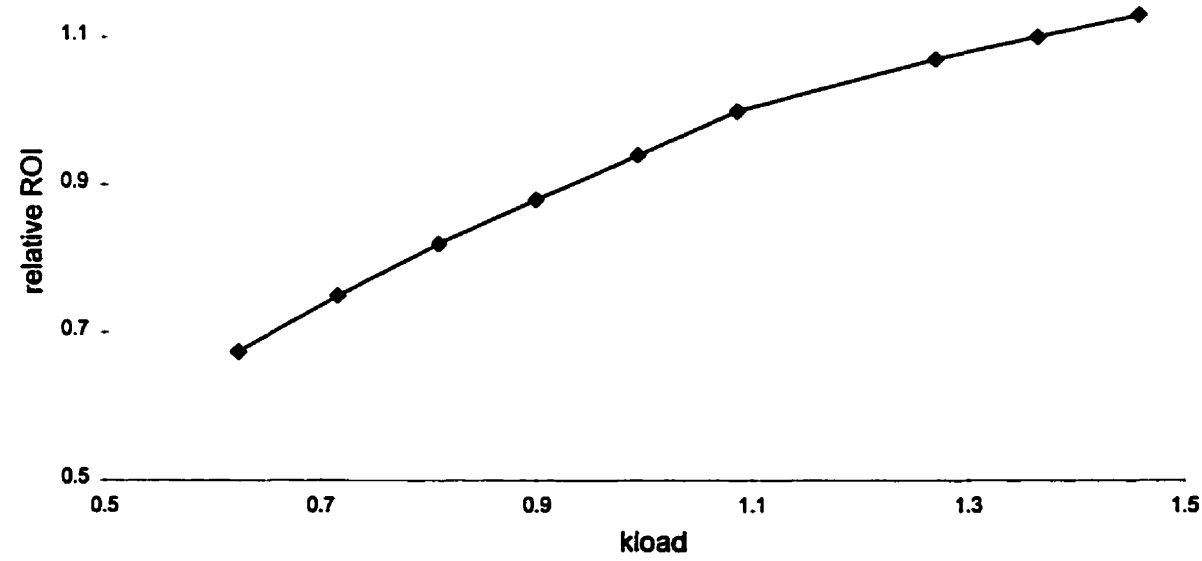

Figure 5.1.3: Sensitivity of ROI to kload (Langmuir isotherm constant)

• **Kload:** kload is the equilibrium constant at load conditions for the Langmuir isotherm. The only value of kload reported in the literature for a similar system is $162x10^3$ cm^3/g for anti-benzenearsonate monoclonal antibody on Sepharose 4B (Arnold et al., 1985). In base case this value for t-PA on Lysine Sepharose 4B was assumed to be 120 cm^3/g . For sensitivity study a range of 75 cm^3/g to 175 cm^3/g was investigated. 45% increase in kload improved the recovery in the column by 14% over the base case. Therefore the column performance is also sensitive to kload. However, the response was not as significant as the change in capacity of the ligand (Figure 5.1.3).

• **Cycle:** Affinity adsorbents lose their initial capacity with increasing number of cycles with the column. Hamman and Calton (1985) proposed the following equation that accounts for the loss of capacity of affinity adsorbents:

$$\text{Total Protein Isolated} = C_o \frac{[1 - e^{-0.693n/n1/2}]}{[1 - e^{-0.693/n1/2}]} \qquad (5.1)$$

where, C_o is the capacity of first cycle, n is the number of cycles and $n_{1/2}$ is the number of cycles at which the column capacity is one-half the initial capacity. For $n_{1/2} = 8$, Figure 5.1.4 shows how the column capacity decrease with increasing number of cycles (n).

Cycle half-life ($n_{1/2}$) can vary over a wide range for affinity adsorbents. It is usually found to be a function of the method used to immobilize the adsorbent on to the matrix, the reagents used to elute the adsorbed species and buffers to wash and regenerate the column, proteolytic and denaturing agents in the process stream and thermal denaturation with time (Hamman and Calton, 1985). Figure 5.1.5 shows the total amount of protein purified as a function of half-life for $n = 2 \text{ X } n_{1/2}$.

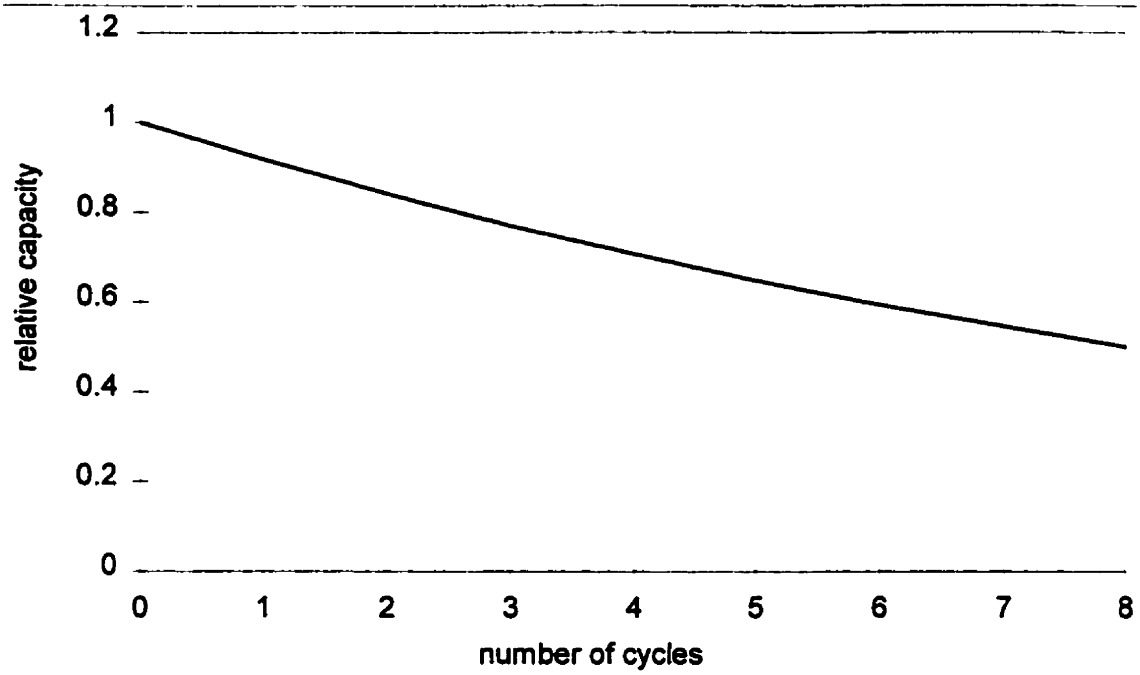

Figure 5.1.4: Loss of capacity with number of cycles

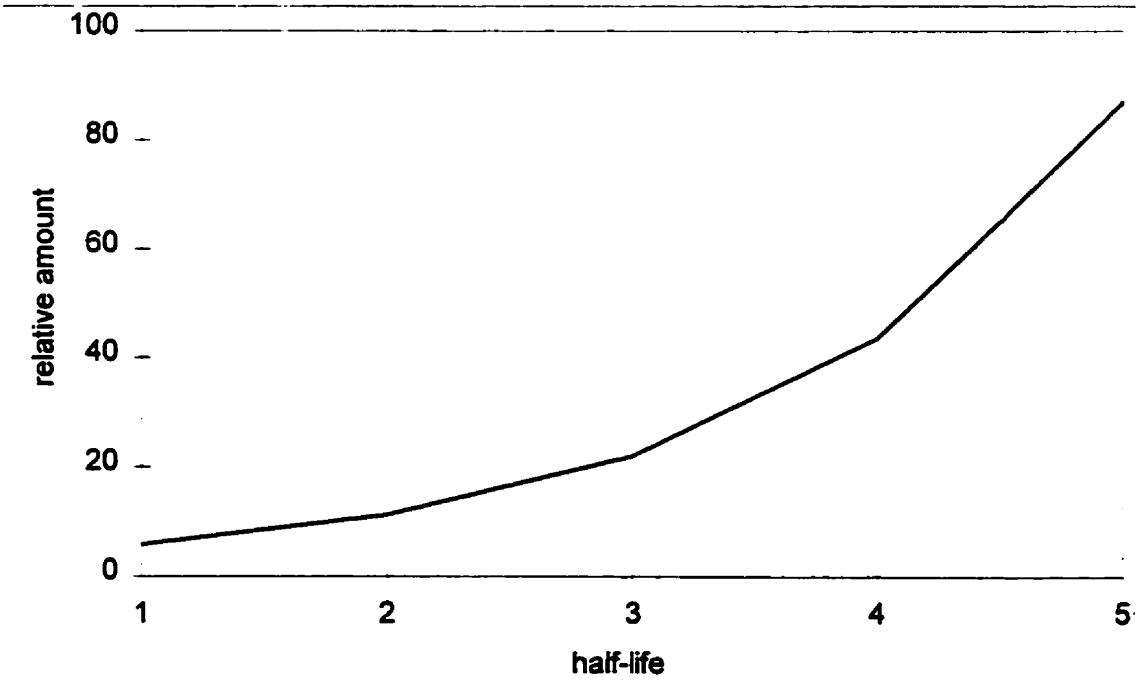

Figure 5.1.5: Protein purified as a function of cycle half-life

5.1.4. Ion-Exchange Chromatography

The parameters and variables allowed to vary by BPS for the ion-exchange chromatography model are the same as for the affinity chromatography column except for the resin. The parameters allowed to vary for the resin in this case are specific surface area, total ionic capacity, charge and charge slope.

The following table lists the parameters studied for the ion-exchange block.

Table 5.1.5. Variables studied for sensitivity of the ion exchange block

Varied Variable	Suggested Range	Reference
Specific Surface Area	1 cm^2/cm^3	Aspen Plus, User's Guide (1995)
Total Ionic Capacity	2.0-2.6 mmol/g	Pharmacia LKB Biotechnology (1990/91)
Charge	14.3	Appendix H (Scharer, 1996)
Capacity	?	
Kload	?	

- **Specific Surface Area:** For specific surface area, a value of 1 cm^2/cm^3 was used in the simulation of the ion-exchange column. In BPS this is defined as the ratio of the total internal and external surface area of the adsorbent to column volume. This value was obtained from the example problem for the same model included in the BPS manual (Aspen Plus, Release 9.2). Since estimate of this parameter for the particular resin (SP Sephadex) could not be found from any other source, a sensitivity study was carried out by varying specific surface area over a range of 3 to 0.05 cm^2/cm^3. Variation in the value of the specific surface area was found to have no effect on the recovery, purity and point concentration (at a specific point in the elution curve) of t-PA in the column (Appendix F). Specific surface area is most probably used to get an estimate of surface potential (ψ_o), which is required to calculate the equilibrium constant (K_i) for this model.

- **Total Ionic Capacity:** The total ionic capacity for SP Sephadex has been quoted between 2.0 to 2.6 mmol/g by Pharmacia LKB Biotechnology (Catalogue, 1990/91). The capacity quoted in manufacturer's catalogue (usually expressed in terms of density of charged groups per unit weight of the medium), is not necessarily an indicator of the protein binding capacity. Only a small proportion of this capacity may be actually accessible to the protein depending on the hydrodynamic size of the protein and the support pore diameter (Chisti and Moo-Young, 1990). In BPS model, this quantity is defined as total ionic capacity of adsorbent per unit column volume, in units of kmol/m^3. A value of 1.3 kmol/m^3 was used for total ionic capacity in the simulation, which was calculated assuming a voidage of 60%. Again no effect of this parameter was observed on recovery, purity and point concentration of t-PA with sensitivity study (Appendix F). Instead, the solute capacity was found to be a critical parameter for the model.

- **Solute Capacity:** No estimate was found in the literature for adsorption isotherm constants for t-PA and SP Sephadex system. Loading capacity is found to vary with ionic strength and pH of solution because these parameters affect the dimensions of the hydrophillic, porous support media. Yamamoto et al. (1988) list protein adsorption capacity for some anionic exchangers which were found to lie between 22 to 116 mg/ml and vary significantly depending on pH. The total capacity reported for BSA (127 mg/ml Sephadex A50 and 12 mg/ml DEAE Sepharose) were found to differ largely depending on the resins (Graham et al., 1987). A conservative estimate of 7 mg/ml was used in the simulation for t-PA. Solute capacity, which is a parameter in the multi-component Langmuir equilibrium isotherm model, was found to be a critical parameter in determining the recovery in the column. The base capacity was varied between a range of ± 50% for sensitivity study. A 50% change in the solute capacity resulted in about 17% decrease in the recovery of t-PA in the ion-exchange column. Therefore, as already observed for the affinity chromatography column, the performance of the ion-exchange column is also found to be very sensitive to solute capacity.

- **Kload:** Unlike solute capacity the other isotherm parameter, kload, was found to create no effect on the recovery, purity or point concentration in the column. For kload, a value of 45 m^3/kg for t-PA was used in the simulation. This value has been reported as 33g/mg Sephadex A50 and 35 g/mg DEAE Sepharose for BSA. For sensitivity study, kload for t-PA was varied between 5 and 100 m^3/kg (Appendix F).

- **Charge:** An estimate for charge on t-PA molecule at pH 5 was obtained following the method recommended by Scharer (1996) (Appendix H). For BSA, the operating pH of the column is very close to its isoelectric point and therefore, an estimate of –0.8 was used. However, the charges on these molecules are very sensitive to the ionic strength of the solution and therefore a sensitivity study was performed. The recovery in the column was found to be reasonably sensitive to the charge on t-PA molecule. A 50% decrease reduced the recovery by 7% whereas a 50% increase in the charge increased the recovery by only 3.5%.

5.1.5. Gel Chromatography

For this model the parameters and variables for sensitivity study are:

1. Column: length and diameter, total volume, void volume and column voidage.

2. Gel: diameter, porosity and void fraction.

3. Plate theory parameters: HETP, A, B, C, V_E, K_{AV}, N, Lambda, Z, alpha and beta.

4. Operation: superficial velocity, linear velocity, pressure drop, cycle time, load volume, cycle volume and flow rate.

The parameters and variables studied for this model are the following:

Table 5.1.6. Variables studied for sensitivity of the gel filtration block

Varied Variable	Suggested Range	Reference
Length	0.2 to 1 m	Bonnerjea and Terras, 1994
Load Volume	1% to 10% of column volume	Wheelwright,1991; Bonnerjea and Terras, 1994
Flow Rate	3-30 cm/h	Bonnerjea and Terras, 1994

- **Length:** Resolution of gel filtration column is a function of column length among other variables. On a process scale, individual columns of up to 1 meter in length are feasible with modern gels that have some rigidity (Bonnerjea and Terras, 1994). In these cases, the diameter (to about 20 cm) and linear flow velocities are restricted to avoid compression of the gel bed. If a greater column length is required in order to achieve the necessary resolution, or a wider diameter column is necessary to obtain a high throughput, columns connected in series (stacked columns) are used. For sensitivity study column lengths between 0.2 to 1 meter were considered. Resolution dropped by 63% at a column length of 0.2 m (Figure 5.1.6), which is about 27% of the length (0.75m) for the design case. For the other extreme (i.e. 1 m), resolution increased by 22% and the pressure drop became about 0.4 atm compared to 0.3 atm for the design case.

- **Load Volume:** Load volume is another variable that dictates resolution of a gel filtration column. The load volume or the ratio of sample volume to column volume is usually between 1 to 5% for gel filtration columns (Wheelwright, 1991). For impurities of largely differing molecular weights, the sample volume can be increased up to 10% of the column volume (Bonnerjea and Terras, 1994). At 2% load volume, the resolution is found to improve by 32% and for the upper limit of 10%, it drops to 33% of the resolution for the base case (load volume of 5%) (Figure 5.1.6).

- **Flow Rate:** The resolution of gel filtration column is also a function of flow rate. The recommended flow velocity for gel filtration column is within 3-30 cm/h (Bonnerjea and Terras, 1994). According to Wheelwright (1991), the optimal flowrate for maximum resolution is approximately 2 ml/cm^2-h. Sofer (1986) maintained a velocity of 13cm/h for large-scale gel filtration of insulin in a 96L column. The flowrate for this simulation was assumed to be 16 cm/h. For the simulated gel filtration column (50 cm diameter), this flow velocity corresponds to 32 L/h. The range for sensitivity study was between 7.5L/h to 75 L/h. Towards the lower limit, resolution is found to improve by about 38% over the design case and it drops by 17% as the flow rate is increased to reach the upper limit (Figure 5.1.6). However, at the lower bound the time required for filtration becomes too large. 36.6 hours of gel filtration time is required at the flow rate of 6.0 L/h which is 5.3 times of the cycle time (6.9 hour) for the design case.

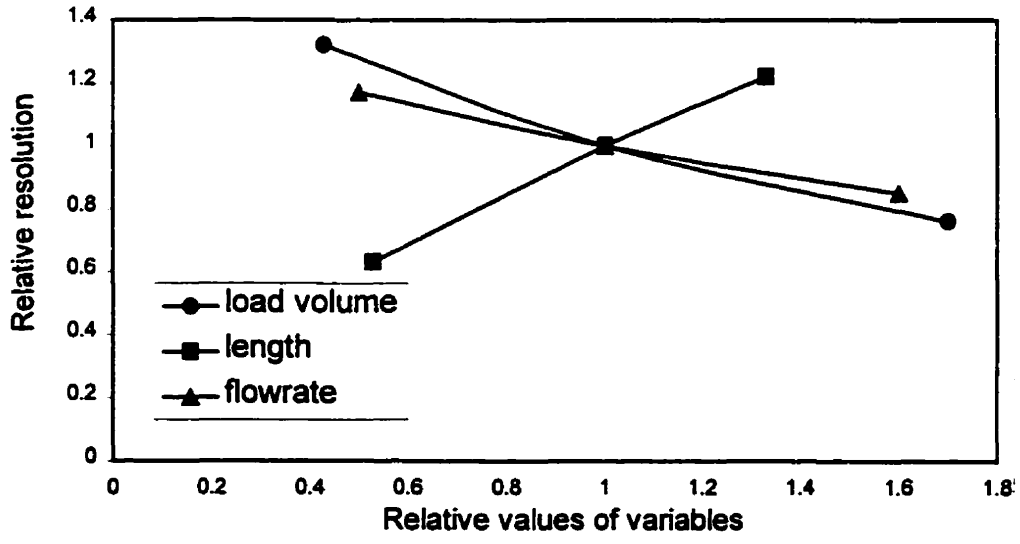

Figure 5.1.6: Sensitivity study for gel filtration column

Sensitivity Study of Combined Variables:

BPS allows a combination of maximum five variables or parameters for sensitivity study. This feature of BPS was utilized to study the combined effect of the variables on the resolution of the gel filtration column. The following table summarizes the effect observed with the variables set at values resulting in poorest resolution.

Table 5.1.7. Combined effect of the variables of the gel filtration column on resolution

Length (m)	Load Volume (% of Column Volume)	Flow Rate (L/hr)	Resolution
0.2	-	-	0.28
-	15	-	0.50
-	-	60	0.63
0.2	-	60	0.24
0.2	15	-	0.17
-	15	60	0.45
0.2	15	60	0.15

For the above case and with the range of variables studied, the length of the column is found to effect the resolution most significantly. The effect of flow rate is found to be least critical. A combination of length and load volume reduced the resolution by about 40% than length alone. Inclusion of flow rate reduced the resolution by further 12%.

5.2. ECONOMIC EVALUATION

In economic evaluation of the process, uncertainty was involved in some estimates. Ambiguity was present with some cost factors utilized in the calculation and above all, the specific nature of the process required further analysis with respect to some critical issues.

Labor Cost: For economic evaluation of the simulated processes, estimate of labor cost was obtained from the built-in estimator of SuperPro Designer®. It was later pointed out that labor requirement values used in the first few versions of SuperPro Designer® were

on the low side, which were corrected to reflect more realistic estimates in the subsequent versions (Intelligen Inc., 1996).

In addition, it is found that labor cost is the largest cost component, in general, accounting for more than 50%, for bioreactors (Wilkinson, 1987). Also for chromatography columns, labor cost tends to run far ahead of supplies and amortized equipment (Wheelwright, 1987). Therefore, a sensitivity study of the overall economics of the base process to labor cost was carried out. In this study, in addition to cost for supervision (A), maintenance (B) and operating labor (C), cost of other labor dependent items of annual operating cost such as operating supplies (10% of C), laboratory charges (25% of C), plant overhead (60% of (A+B+C)) and administration (25% of plant overhead) were also included (Datar and Rosen, 1990).

Although, labor cost accounts for only about 5.2% of the annual operating cost for the base case, it was found that a 50% increase in the labor cost will bring about 5% reduction in ROI of the process (Figure 5.2.1). Therefore, increase in the base unit labor rate ($18/hour) or the labor-hour may have significant effect on the economics of the process and the prospect of minimizing labor cost by automation of the process should be investigated.

Waste Treatment and Environmental Regulations: For plants producing biochemicals from recombinant organisms, safety measures to prevent release of the recombinant cells to the environment have to be considered. Discharges containing viable recombinant organisms must be inactivated by a validated inactivation process prior to release from a closed system. The two most frequent techniques used for this purpose are thermal inactivation and chemical inactivation. All the waste streams from the plant are routed to a contained drain kill tank system. These streams include condensate from the vessel, all process lines and associated steam traps. Exhaust gas from the bioreactor is cyclone separated and sterile filtered using 0.22 micron membranes before being released to the

atmosphere. National Institutes of Health (NIH) has defined four physical containment levels in the 'Guidelines for Research Involving Recombinant DNA Molecules' (NIH, 1991). The containment boundary for extracellular product encompasses the bioreactor, kill tank and cell harvesting followed by cross flow microfiltration through 0.22 micron membrane (Giorgio and Wu, 1986). For plants producing pharmaceuticals from recombinant animal cells, a containment level of at least GLSP (Good Large Scale Practice) is recommended (Miller and Bergmann, 1993). Each of the physical containment level has specific design requirement regarding facility design (e.g. architectural finishes, washing facilities, garments, ventilation etc.), equipment design (e.g. for biological safety cabinets, exhaust gases, rotating seals, systems for monitoring integrity of containment, transfer steps etc.) and response plans for accidental spills. The maintenance of this safety level will add up to the waste treatment cost substantially.

Waste treatment cost is calculated as 5% of the annual operating cost for the simulated processes as suggested by Datar and Rosen (1990). 50% increase in this cost component will reduce the ROI of the process by about 2% and 100% increase by about 3% (Figure 5.2.1). Therefore, the process is found to be fairly insensitive to this cost for the calculation strategy adopted to obtain annual operating cost.

Utilities: Pharmaceutical plants require special kind of process utilities such as water for injection (WFI), pharmaceutical water, clean steam, different classes of air etc. WFI has low limits of ionic, microbial and endotoxin contamination. It is used primarily for purification buffers and for any final clean-in-place (CIP) rinse. It is also used to prepare cell culture media to minimize trace components. Process air is classified according to the number of 0.5 μm diameter particle tolerable per ft^3. Class 10,000 air is recommended for purification suites whereas class 1000 and class 100 air for fill and finish areas (Harshbarger et al. 1995). The cost of production of these process utilities and distribution through special piping (e.g. process piping, hygienic utility piping etc.) will be significantly higher than those normally required plant utilities. For the simulated

processes, cost of utility was calculated as a fixed percentage (10%) of the annual operating cost (Datar and Rosen, 1990). A sensitivity study (Figure 5.2.1) revealed that 50% increase in this cost would bring down the ROI of the process by about 3% and 100% increase by about 6%.

Equipment Purchase Cost: Estimates of equipment purchase cost for the simulated processes were obtained from the built-in models of SuperPro Designer®. Sensitivity study was carried out to observe the effect of change in this price on the ROI of the process. Even a 25% increase in this price will lower the ROI of the process by almost 21%, 50% increase by 35% and 75% increase by 45% (Figure 5.2.1). Therefore, as anticipated, the process is found to be very sensitive to this price and any change in the price of the major contributors to this cost should be readily included for economic evaluation.

Cost of Raw Material: price of raw material and media was found to be the major contributor to the annual operating cost for all the processes. Therefore, sensitivity to this price was deemed important. 25% increase in this price was found to reduce ROI of the process by 9% and 50% increase by 18% (Figure 5.2.1). Therefore, the process is also fairly sensitive to the price of raw material and media.

Overall Yield: The design of the simulated processes is based on an overall recovery of 40%. Sensitivity study was carried out to find how much improvement in ROI of the process could be expected if optimization of operation of the downstream units and development of novel purification methods increase the overall recovery. If overall recovery increases to 50%, the ROI of the process will improve by 37%. ROI increase by 74% if the overall recovery becomes 60% (Figure 5.2.2). Therefore, there is a huge incentive for optimization of the downstream units for new plants before they are put to operation. However, for the existing plants, revalidation of the process steps can offset the gain in ROI.

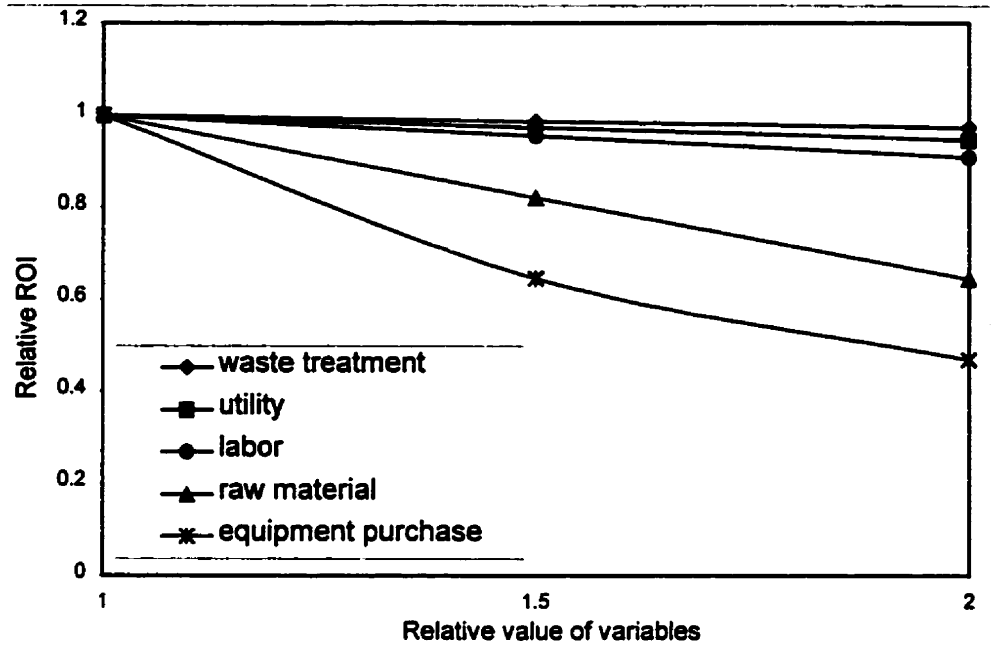

Figure 5.2.1: Sensitivity to Economic Variables

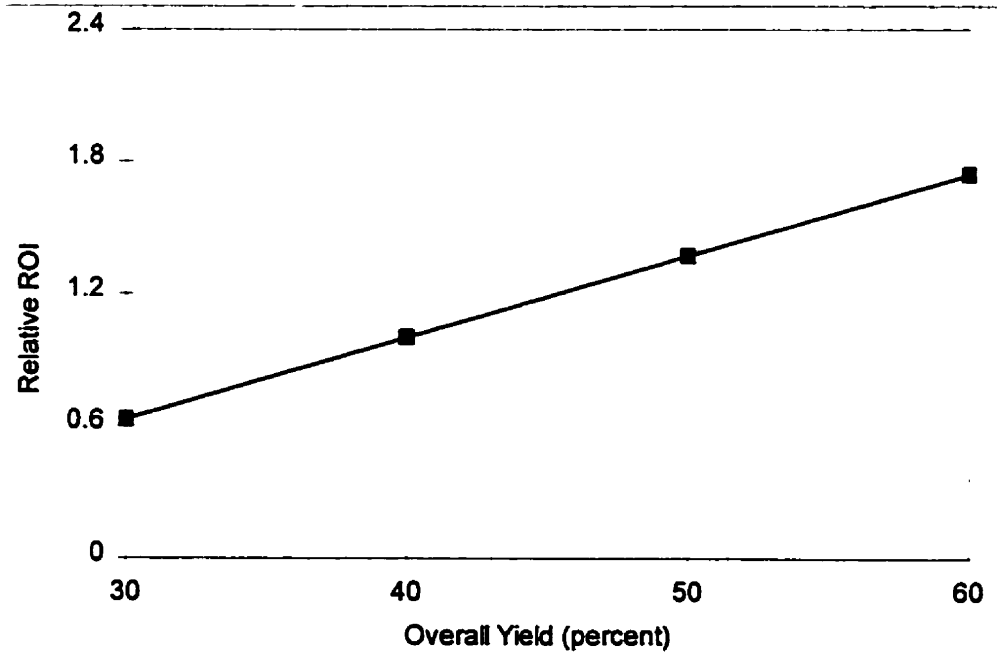

Figure 5.2.2: Sensitivity to overall yield

Clinical Studies and FDA Approval: Clinical Studies for achieving FDA approval represents a major cost in bringing a recombinant therapeutic, like t-PA, into the market. $32 million was raised by Genentech for clinical test and development of t-PA (Elander, 1989). In the five phase conceptual layout described by Bliem (1988) for establishing an industrial scale cell culture process, the major cost was shown to be associated with the final phase, accounting for clinical studies and marketing program. The cumulative cost for the development of a therapeutic product in 1988 was likely to lie in the region of $50 to $150 million (Bliem, 1988). The high cost and consequently, the high risk associated with the development and commercialization of pharmaceuticals was emphasized in another report, where values of up to $350 million have been reported as the average cost of a successful drug substance (Business Week, October 17, 1994, p.204).

For production of porcine growth hormone from *E. coli* this cost was estimated to be $25 million by Petrides et al. (1989), which they amortized over ten years. For the processes simulated, this enormous cost was amortized over the life of the product and was handled simply by calculating it (R & D cost) as a nominal percentage (7%) of the overall cost. The effect of R&D cost on the selling price is shown in Figure 5.2.4. For a total cost of $150 million, the selling price of t-PA has to be increased by about $800 per gram. For a more realistic figure of $250 million dollar, the selling price is increased by $1,700 per gram to maintain the same ROI. If the selling price is held constant on the other hand, ROI of the process will be reduced to 94.6% of its base value for R&D cost of $150 million and further to 88.5% for $250 million (Figure 5.2.3).

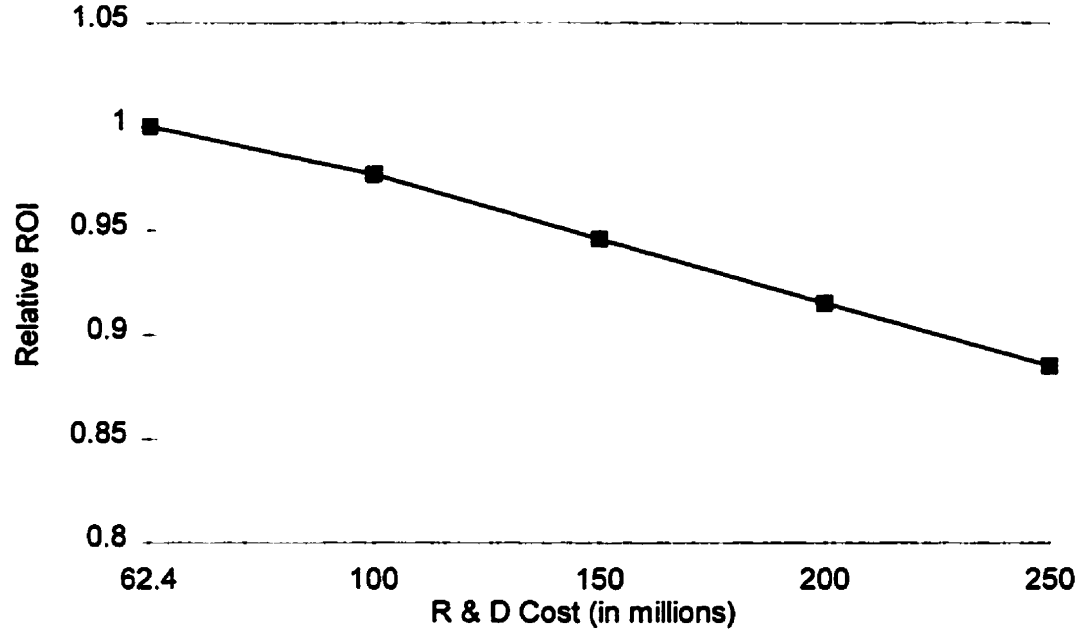

Figure 5.2.3: Sensitivity to research and development cost

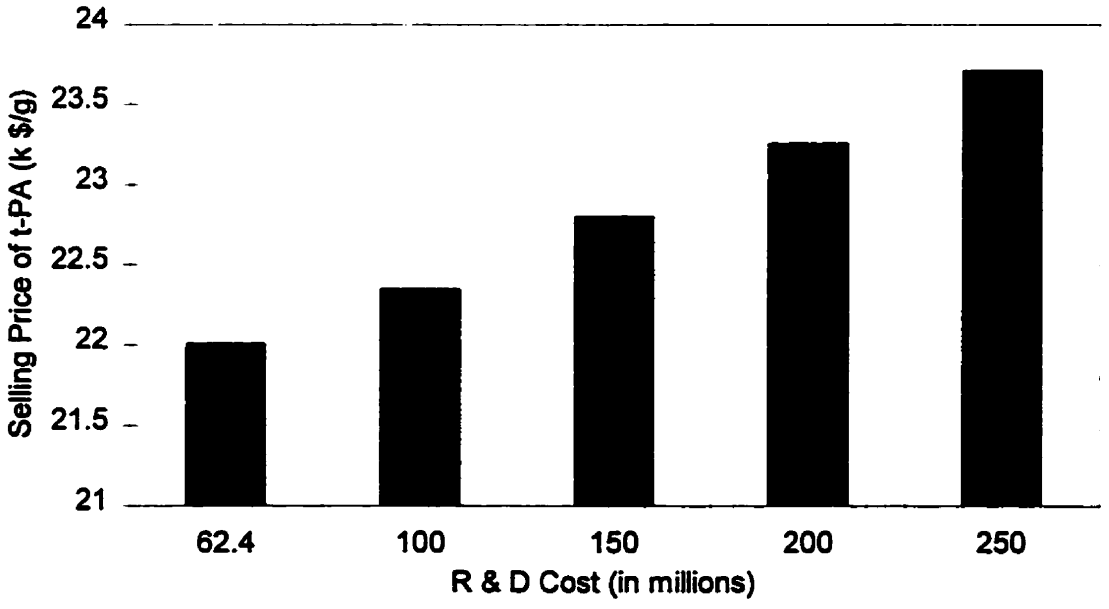

Figure 5.2.4: Sensitivity to research and development cost at fixed ROI for base case

CHAPTER 6

STUDY OF MAMMALIAN CELL KINETICS

Several unstructured kinetic models for animal cells were investigated using Aspen BioProcess Simulator™ (BPS). The kinetic equations were added as subroutines to the "Fermenter" model. The simulator solves the systems of differential equations and generates concentration profiles for substrates and product. Since BioProcess Simulator™ allows the parameters of the models to be varied over specified range, it is very convenient to carry out kinetic modelling study using this simulator. Models with different specific growth rate and rate expressions were selected for this study.

6.1.SPECIFIC GROWTH RATES

The following specific growth rate (μ) expressions were obtained from literature and considered for the kinetic study:

a) $\mu = \mu_{max} (G)/(K+G)$ (Newland et al., 1994)

b) $\mu = \mu_{max} [(G)(S)]/[(K+G)(K'+S)]$ (Tremblay et al., 1993)

c) $\mu = \mu_{max} [(S)(K_L)]/[(K+S)(K_L+L)]$ (Kurokawa et al., 1994)

d) $\mu = \mu_{max} [(G)(K_L)(K_A)]/[(K+G)(K_L+L)(K_A+A)]$ (Bree et al., 1988)

e) $\mu = \mu_{max}$ (Serum)/(K+Serum) (Dalili and Ollis, 1989)

f) $\mu = \mu_{max}$ [(Serum)(G)]/[[$(K_S)_o X^{\beta}$+Serum] (Glacken et al., 1989)

[(K+G)(1+A^2/K_A)(1 +L^2/K_L)]]

g) $\mu = \mu_{max}$ [$(K_S)(K_A)$]/[$(K_S+S)(K_A+A)$] (Kurano et al., 1990)

where, S represents the concentration of Glucose in the medium,

G is concentration of Glutamine,

X is biomass concentration,

A is concentration of Ammonia and

L is concentration of Lactate

In the above expressions the limiting substrates considered are glucose, glutamine and serum components (Dalili and Ollis, 1989). Different combinations of the limiting substrates have been assumed in different cases. The expressions also include inhibition by metabolic waste products lactate and ammonia and in one case even by the substrate glucose (Kurano et al., 1990). Lactate inhibition, however, was not observed up to concentrations of 40 mM for a hybridoma cell line (Glacken et al., 1988) and up to 28 mM for CHO cells (Kurano et al., 1990). Monod model was used to express substrate limitation in all the cases. The above specific growth rate expressions were all developed for different cell lines of hybridoma except for expression (g), which was developed for CHO.

The specific growth rate model (d) proposed by Bree et al. (1988) was analyzed using BioProcess Simulator™. The effect of adding each term to the growth expression was studied as shown in Figure 6.1.1. In this case inhibition by ammonia was found to be

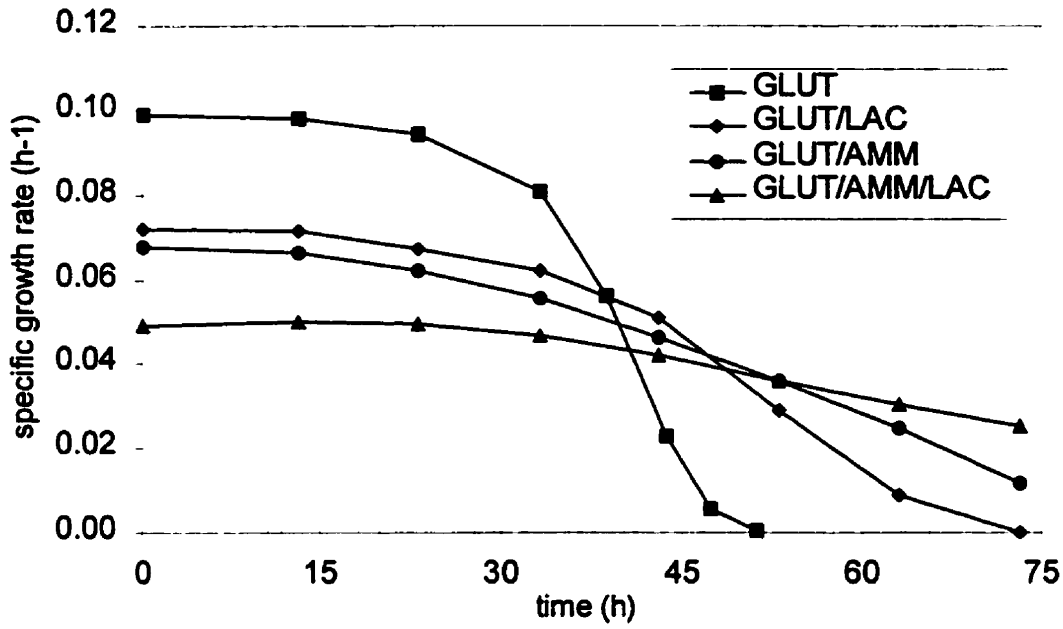

Figure 6.1.1: Analysis of growth rate expression using BPS

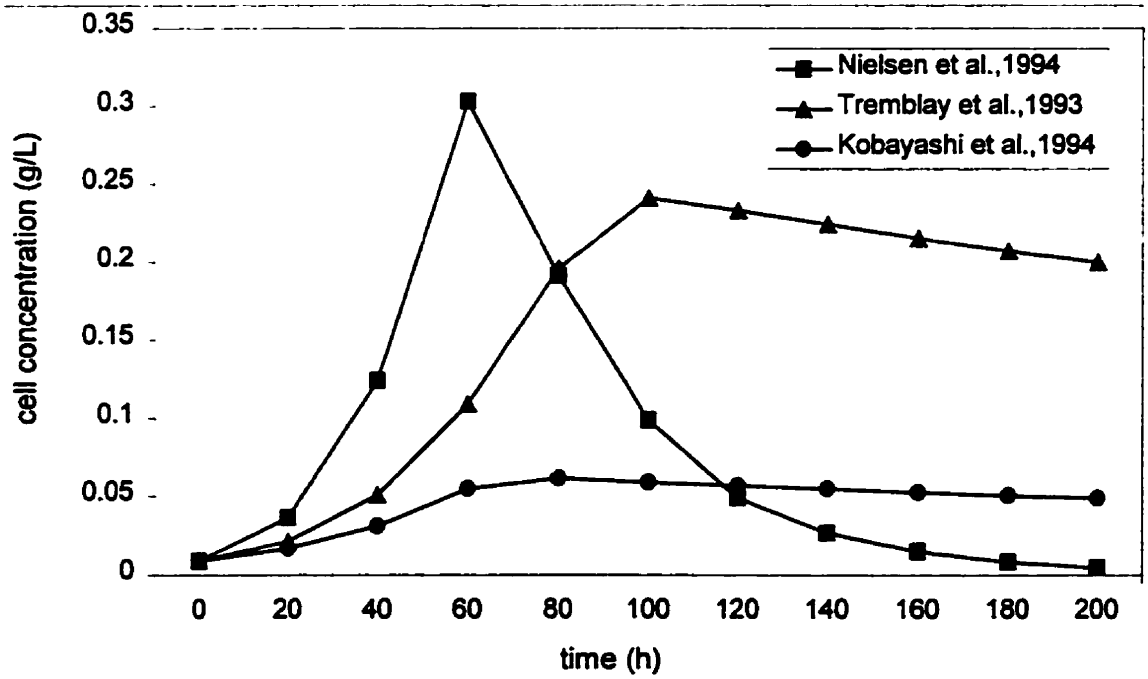

Figure 6.1.2: Cell concentration for different specific growth rate expressions

slightly stronger than lactate inhibition and including both the terms reduced the specific growth rate significantly. This type of study can be useful to develop appropriate growth rate expressions.

Alternatively, the simulator can also be used to screen growth rate expressions. The rate expression in the subroutine can be easily replaced with another and the concentration profile thus generated can be compared to the experimental data. Three different growth rate expressions were considered for the kinetic model proposed by Newland et al. (1994). Replacement of the growth rate expression of this model by that formulated by Tremblay et al. (1993) reduced the maximum cell concentration since this model considers glutamine as a limiting substrate in addition to glucose (Figure 6.1.2). When the rate expression for μ was replaced with the one used by Kurokawa et al. (1994), cell concentration went down significantly. This model includes lactate inhibition in addition to limitation by glucose. The lactate inhibition constant is large while the yield coefficient, $Y_{L/X}$, is very large and the yield coefficient, $Y_{X/S}$, is very small compared to others (Tables 6.1.4 and 6.2.1).

When the kinetic parameters μ_{max} and the different saturation and inhibition constants (K, K_L, K_A, K_S) for the above expressions were compared, they were found to vary over a wide range (Table 6.1). Maximum value of μ_{max} (3.0 d^{-1}) was observed for model (d) that is about four folds higher than the reported minimum (0.8 d^{-1} for (b)).

A sensitivity study was carried out using BioProcess Simulator™ to observe the effect of change in μ_{max}. Simulation did not converge with wide variations in this parameter since the performance of the bioreactor was very sensitive to this change and the ordinary differential equation solver of BioProcess Simulator™ could not converge due to large variations of bioreactor performance. Table 6.1.2 shows the result obtained with a smaller range.

Table 6.1.1: Reported maximum specific growth rate for the different models

μ_{max} (d^{-1})	Reference
1.42	Kurokawa et al., (1994)
0.8	Newland et al., (1994)
1.09	Tremblay et al.,(1993)
3.0	Bree and Dhurjati (1988)
0.92	Glacken et al., (1989)
1.32	Dalili and Ollis (1989)
1.78	Kurano et al., (1990)

Table 6.1.2: Result of sensitivity study to maximum specific growth rate

μ_{max}. (1/s)	Xrate (kg/h)	Srate (kg/h)	Xconc (g/L)	Sconc (g/L)
1.0 E-06	2.0E-04	-6.7E-03	1.04E-02	1.54
3.17 E-06	7.78E-04	-2.3E-02	1.45E-02	1.425
5.33 E-06	1.51E-03	-4.36E-02	1.95E-02	1.285
7.5 E-06	2.36E-03	-6.76E-02	2.53E-02	1.12
9.26 E-06	3.1E-03	-8.85E-02	3.04E-02	0.98

Sensitivity study was also carried out to study the changes in K_s saturation constant for glucose. Results are presented in the table below.

Table 6.1.3: Result of sensitivity study to saturation constant for glucose (K_S)

K_S (g/L)	Xrate (kg/h)	Srate (kg/h)	Xconc (g/L)	Sconc (g/L)
1.0 E-02	3.27E-03	-9.3E-02	3.13E-02	0.956
3.25 E-02	3.175E-03	-9.04E-02	3.08E-02	0.97
5.5 E-02	3.09E-03	-8.8E-02	3.03E-02	0.985
7.75 E-02	3.01E-03	-8.58E-02	2.98E-02	0.998
0.1	2.93E-03	-8.37E-02	2.94E-02	1.01

Table 6.1.4. Reported saturation and inhibition constants for the different models

Saturation Constant for Glucose (K_S):	
0.05 g/L	Kurokawa et al., (1994)
0.18 g/L	Tremblay et al.,(1993)
Saturation Constant for Glutamine (K_G):	
0.0876 g/L	Newland et al., (1994)
0.0438 g/L	Tremblay et al.,(1993)
0.1168 g/L	Bree et al., (1988)
0.0219 g/L	Glacken et al., (1989)
Inhibition Constant for Lactate (K_L):	
1.25 g/L	Kurokawa et al., (1994)
0.72 g/L	Bree et al., (1988)
Inhibition Constant for Ammonia (K_A):	
0.01785 g/L	Bree et al., (1988)
0.135 g/L	Kurano et al., (1990)
Saturation Constant for Serum (K_{Serum}):	
26.5%	Glacken et al., (1989)
1.59%	Dalili and Ollis (1989)

6.2. RATE EXPRESSIONS

The different rate expressions used in the above models are shown below. For the substrate glucose, two rate expressions were found, the difference being the maintenance term. Maintenance was considered in only two cases (Kurokawa et al., 1994; Bree et al., 1988), however, contribution from this term was very small in both the cases. A wide variation in the yield coefficient, $Y_{X/S}$, was observed. Figure 6.2.1 shows the glucose

profile for different values of the yield coefficient for the kinetic model (c) (Kurokawa et al., 1994).

a) Glucose: i) $dS/dt = -(\mu x)/Y_{X/S}$ (6.2.1)

ii) $dS/dt = -(\mu x)/Y_{X/S} - mx$ (6.2.2)

In all the models considered, lactate production rate was expressed in terms of the yield coefficient, $Y_{L/X}$. Again, the yield coefficient was found to vary over a wide range, the maximum being 125 times (Kurokawa et al., 1994) the smallest reported (Bree et al., 1988).

b) Lactate: i) $dL/dt = (\mu x)/Y_{L/X}$ (6.2.3)

Three different rate expressions were observed for glutamine and ammonia. The reported yield coefficients, $Y_{X/G}$ and $Y_{A/X}$ and the specific production rate q_G and q_A were found to be more or less congruent.

c) Glutamine: i) $dG/dt = -(\mu x)/Y_{X/G}$ (6.2.4)

ii) $dG/dt = -q_G \alpha\, x - k.G$ (6.2.5)

iii) $dG/dt = -(\mu x)/Y_{X/G} - (1/Y_P)dP/dt - (1/Y_{AG})dA/dt$ (6.2.6)

d) Ammonia: i) $dA/dt = (\mu x)/Y_{A/X}$ (6.2.7)

ii) $dA/dt = q_A \alpha\, x + k.G$ (6.2.8)

iii) $dA/dt = q_A \alpha\, x + k.G + k_U C_{U,0}\, e^{-kut}$ (6.2.9)

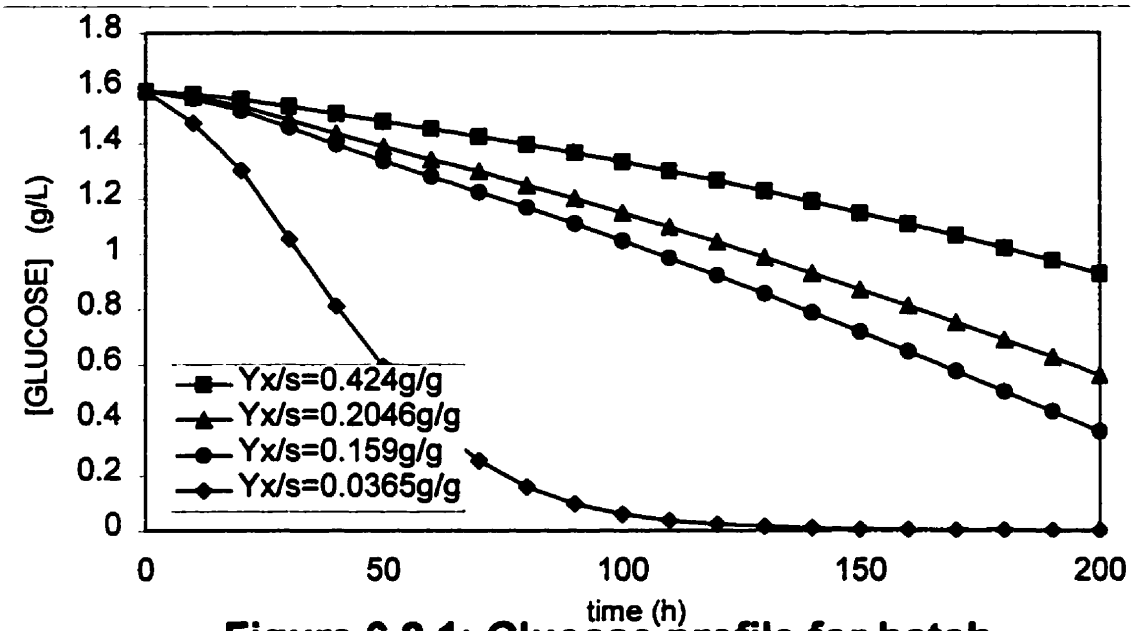

Figure 6.2.1: Glucose profile for batch fermentation as a function of yield constant (Yx/s)

Table 6.2.1: Reported kinetic parameters for mammalian cell cultures

Reported Value	In Units of g/g	Reference
Yield (Cell/Glucose), $Y_{X/S}$:		
1.39 X 10^8 cells/g	0.0365	Kurokawa et al., (1994)
0.14 cell/pmol	0.2046	Newland et al., (1994)
1.09 X 10^8 cells/mmol	0.159	Tremblay et al.,(1993)
2.9 X 10^5 cells/ml/mM	0.424	Bree et al., (1988)
Yield (Lactate/Cell), $Y_{L/X}$:		
1.3 X 10^8 g/cells	49.4	Kurokawa et al., (1994)
-	5.647	Tremblay et al.,(1993)
-	0.393	Bree et al., (1988)
Yield (Cell/Glutamine), $Y_{X/G}$:		
0.36 cell/pmol	0.6488	Kurokawa et al., (1994)
3.8 X10^8 cells/mmol	0.685	Tremblay et al.,(1993)
9.434 X 10^5 cells/ml/mM	1.7	Bree et al., (1988)
Yield (Ammonia/Glutamine), $Y_{A/G}$:		
0.85 mol/mol	0.099	Tremblay et al.,(1993)
0.66 mol/mol	0.077	Bree et al., (1988)
Yield (Ammonia/Cell), $Y_{A/X}$:		
-	0.144	Tremblay et al.,(1993)
-	0.045	Bree et al., (1988)
Specific Glutamine Degradation Constant (k_G):		
4.8 X 10^{-3} h^{-1}	-	Glacken et al., (1989)
2.0 X 10^{-3} h^{-1}	-	Kurano et al., (1990)
2.7 X 10^{-3} h^{-1}	-	Newland et al., (1994)
Specific Glutamine Consumption Rate (q_G):		
0.0388 g/g-h	-	Glacken et al., (1989)
0.04 g/g-h	-	Kurano et al., (1990)
Specific Ammonia Production Rate (q_A):		
3.23 X 10^{-3} g/g-h	-	Glacken et al., (1989)
8.33 X 10^{-4} g/g-h	-	Kurano et al., (1990)

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1. CONCLUSIONS

7.1.1. Flowsheet Analysis

A large increase in ROI was observed for the flowsheets where the bioreactors were operated in fed-batch mode. Two cases of fed-batch operation were simulated assuming the product concentration to be four times and eight times the concentration for the base case. Another flowsheet, which also resulted in a high ROI is the multiple bioreactor flowsheet. Since cell culture bioreactor cycles can last up to a week, an economically sized single purification train can be used to process the product from multiple bioreactors. The single bioreactor of the base case was divided into multiple equal sized bioreactors and the downstream train was designed to handle broth from one of the bioreactors. In this case, the size of the downstream train was much smaller than the base case. Since for high value products like t-PA, downstream processing accounts for about 70 to 80% of the overall production cost, significant saving was realized from the smaller downstream train.

Although there are simplifying assumptions and simplifications involved with any simulation, the difference in economic performance observed for these flowsheets were so significant that it was reasonable to draw conclusions based on the simulation results.

7.1.2. Simulation

Simulation experience with BioProcess Simulator™ and SuperPro Designer® led to the conclusion that these two simulators should be used in conjunction to develop a new process. Since simulation of most of the units is simpler with SuperPro, it can be used initially to screen process alternatives in a short time and minimum effort. Once a process has been chosen, it can be analyzed using the more complex approach of BioProcess Simulator™ to find the optimum operating conditions and predict the process performance with changing parameters.

Simulation with SuperPro Designer® was straightforward once the required input information were obtained. Some simulation problems were, however, encountered while working with BioProcess Simulator™ as highlighted below.

- **Bioreactor:** The simulation for the bioreactor was found not to converge at low air flowrates. Simulation converged with air flowrates corresponding to dissolved oxygen levels of 6.4 mmol/L. This is much higher than the solubility of oxygen (0.224 mmol/L) at the fermentation temperature of 37°C. In laboratory studies reported, the dissolved oxygen concentrations maintained for t-PA production were between 25% to 40% of saturation, i.e. 0.056 to 0.089 mmol/L. The simulation did not converge even at dissolved oxygen concentrations as high as 0.715 mmol/L. Therefore, with the fermenter model of BioProcess Simulator™, it was not possible to investigate the effect of k_La, air flow rate or dissolved oxygen concentration.

- **Affinity Chromatography:** The presence of a split elution peak for t-PA and the increase in the area of the smaller interfering peak with higher concentration could be an artifact of the simulator. For the simulated fed-batch processes, this behavior affected the performance of the affinity columns very significantly. Since t-PA is required at very

high purity, the t-PA associated with the first peak is wasted and therefore although the fed-batch process increased concentration of t-PA, the recovery was low. The results reported in literature for affinity chromatography are carried out for analytical purposes using small sample size and low concentration. Therefore, it was not possible to verify these observations and it remains to be investigated experimentally.

Ion-Exchange Chromatography: The elution profile of t-PA from the ion-exchange column was found to have an elongated tail. According to chromatography theory, tailing of elution peaks can occur as a result of large particle diameter, high flow rate and low molecular diffusivity of the solute (Yamamoto, 1978; 1979). Tailing of the elution curve was also observed with ion-exchange chromatography when increased sample concentration causes the column to operate in the nonlinear region of the isotherm (Kato et al., 1982). Simulations with lower t-PA concentrations, however, did not improve the elution profile. The peaks were found to have longer tails and lower heights at progressively lower concentrations (50% and 25% of the design case) and sharpening of the peak was observed at higher concentrations (125% and 225% of the design case). Elution profile remained unchanged for simulations with lower (half of the design case flow rate) and higher flow rates (double of the design case flow rate). Although simulation with smaller particle diameter (up to 60 μm of particle diameter) changed the reported mass transfer coefficient, the elution profile did not change. To test for higher molecular diffusivities, simulation was carried out with a lower molecular weight (one-tenth of the actual molecular weight) of t-PA for observation purposes. Again, the elution curve was unaltered. These observations led to the conclusion that the tailing t-PA peak could be due to limitation of the ion-exchange model in BioProcess Simulator™. This may also have resulted from the choice of parameters. Solute capacity was found to be a critical parameter for this model for which no close estimate was available from literature.

- **Membrane Separation:** In the two-stage cell culture flowsheet, the cells from the serum containing media of the growth bioreactor are washed to get rid of the serum proteins before being transferred to the production bioreactor. Simulation of the cell wash stage was attempted using the diafiltration option of the batch membrane separation model of BioProcess Simulator™. However, the model with diafiltration never converged. The model performed without difficulties until the diafiltration step was reached and then stopped. The error message from the history file reported that the solver encounters divisions by zero with further calculation. Repeated trials with this model were unsuccessful. (The support group of Aspen Technology was informed of this. The problem was followed up for six months, but they were unable to provide any solution).

7.1.3. Economic Evaluation

Economic evaluation with SuperPro Designer® was found to be simple and satisfactory for flowsheet comparison purposes. The economic evaluator of BioProcess Simulator™ requires detailed information, which were not available at the conception level. "Budget Authorization" grade estimate of the total capital investment was obtained by multiplying the total equipment purchase cost by appropriate cost factors. The contribution from fermentation and recovery equipment to the total equipment purchase cost was found to vary depending on the flowsheet. Fermentation equipment was found to be the major contributors for the two-stage cell culture and the multiple bioreactor case. However, for annual operating cost, the major contributor for all the cases was raw material cost, although contributions from media and recovery material were found to vary significantly. The media cost was found to be the largest contributor for the two-stage and multiple bioreactor case. Economic performances of the flowsheet were measured in terms of Return on Investment (ROI) and Gross Margin (GM). The highest ROI and GM were calculated for case B fed-batch process (8 X product concentration

for batch), followed by multiple bioreactor case and case A fed-batch having almost equal ROI.

7.1.4. Sensitivity Analysis

Conclusions of sensitivity analysis of uncertain parameters and variables for the following units are given below.

- **Membrane Separation Units:** The critical variable identified for this unit is set point velocity. A 50% reduction in velocity reduced permeate flux by 10%. This was a result of increased gel resistance estimated by the equation ($R_g = R_g0(\Delta P)^a (Re)^b (C_b)^c$). Gel resistance ($R_g$) increased as a result of both lower Reynolds numbers (Re) and higher key component concentration (C_b). Among the uncertain parameters, the exponent 'b' denoting the dependence of gel resistance on Reynolds number was found to be the most critical one. Over ± 50% range of 'b', the changes in operating conditions were so significant that simulation did not converge. At the upper limit of -0.85, which represents only a 10% increase, gel resistance was reduced by 30% of its base value. For other parameters, e.g. R_g0 and 'a', 80% and 50% reduction respectively, reduced the gel resistance by 50%. Enhancing flux by increasing tangential velocity can result in undesirable effects. Large pressure drops are generated at higher velocity in common membrane modules resulting in higher transmembrane pressure (TMP). This can cause compaction of the layer of cells retained at membrane surface. Another drawback of high velocity is the higher shear rate which affects the thickness of the boundary layer, but on the other hand can damage sensitive mammalian cells (Nagata et al., 1989). Using shear filters or rotary filters is one way of decoupling shear generation and TMP with minor damage to shear sensitive cells (Frenander and Jonsson, 1996). In these filters, high tangential flow rates are achieved by rotating metal disks. These rotary filters not only improve the flux but also the protein transmission.

- **Affinity Chromatography Column:** The most critical parameter was found to be the solute capacity followed by Langmuir isotherm constant. A 20% increase in capacity resulted in 17% higher recovery whereas 45% increase in Langmuir isotherm constant (kload) improved the recovery in the column by 14% over the base case.

- **Ion-exchange Chromatography:** For this column the critical parameters were solute capacity and charge on t-PA molecule. A 50% change in the solute capacity resulted in about 17% decrease in the recovery of t-PA in the ion-exchange column. 50% increase in the charge increased the recovery by 3.5%. However, 50% reduction reduced the recovery by 7%.

- **Gel Filtration:** The variable that was found to result in most significant effect, within the range studied, on the resolution of the gel filtration column is column length. This was followed by load volume (as a percent of column volume) and flow rate respectively.

- **Economics:** The overall economics of the processes was found to be most sensitive to equipment purchase cost, raw material cost and overall recovery yield of the process. Among other variables, labor cost and cost of utility reduced the ROI of the processes by about 5%. The effect of research and development (R & D) cost on ROI was not simple to determine since this cost can vary over a wide range. For R & D cost of 250 million dollars (US), the ROI was found to be reduced by 10% from its base value.

- **Individual Processes:** The base case process was found to be very sensitive to the percentage of bovine serum albumin (BSA) in the medium. ROI of the fed-batch processes were very sensitive to the batch time, a major part of which is occupied by the bioreactor.

7.2. RECOMMENDATIONS

- Experiments should be carried out to validate the performance of the individual unit operation blocks. Although overall congruency of economics of the simulated processes with published trends indirectly validated the results, validation of the performance of the individual models will place more confidence on the results obtained.

- Performances of some of the models in BioProcess Simulator™ were not satisfactory. These models should be reviewed and the solution algorithms checked. Besides the problems already mentioned, the ion-exchange chromatography model was found to be insensitive to eluant molarity, elution volume, specific surface area and total ionic capacity. Similarly, the membrane separation model was not able to register the inclusion of denaturation fraction in simulation.

- Since fed-batch mode of operation was found to possess very high economic potential, the possibility of using other high-density culture systems, such as perfusion culture for large scale production of high value products should be investigated.

- The possibility of using novel methods such as perfusion chromatography. membrane affinity separation (Asenjo, 1994; Spalding, 1991), dynamic membrane filtration (DMF) (Frenander and Jonsson, 1996) and new materials such as HyperD affinity matrix (Boschetti, 1994) for large scale purification of proteins should be considered.

- Finally, the critical process parameters and variables should be determined experimentally for a particular system to confer more significance to the simulation results. The assumptions and the choice of correlation and equations can only be justified by comparison with practical situations. Similarly chromatographic media, ligand, microfiltration and ultrafiltration membrane, etc. should be chosen by carrying out

experiments with the actual system. For example, Sephadex A50 has very high capacity for BSA, which is about ten times of that for DEAE Sepharose. However, due to the very open gel-like structure of this resin it is found unsuitable for large-scale operation (Graham et al., 1987). Therefore, experimentation and simulation should be considered complementary rather than independent approaches to reliable process design.

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Appendix A

Aspen BPS Input File for Simulation of Base Case

This Appendix contains Aspen BPS input file used to simulate the base case. The flowsheet was simulated in two sections – bioreactor and downstream processing. Results of the simulation run are summarized in Chapter 4 and detailed results are presented in Appendix B. For brevity, input files for the other simulated flowsheets are omitted. The simulation runs were performed using version 9.2.

A.1. SIMULATION OF BIOREACTOR

```
TITLE 'Simulation for t-PA production'

IN-UNITS MET VOLUME-FLOW='L/HR' ENTHALPY-FLO='KCAL/HR' &
    HEAT-TRANS-C='KCAL/HR-SQM-K' TEMPERATURE=C DELTA-T=C &
    HEAD=METER UA='KCAL/HR-K' HEAT=KCAL FLUX='L/SQM-HR' &
    MOLE-CONC='MOL/L'

DEF-STREAMS MIXNC ALL

RUN-CONTROL MAX-TIME=10000

DATABANKS PURECOMP / AQUEOUS / SOLIDS / INORGANIC / BPS &
    / NOASPENPCD

PROP-SOURCES PURECOMP / AQUEOUS / SOLIDS / INORGANIC / &
    BPS

COMPONENTS
  GLUCOSE C6H12O6 GLUCOSE /
  GLUTAMIE BSA GLUTAMIE /
  FBS BSA FBS /
  T-PA BSA T-PA /
  OXYGEN O2 OXYGEN /
  CO2 CO2 CO2 /
  NITROGEN N2 NITROGEN /
  WATER H2O WATER /
  HAM NACL HAM /
  CHOCELL • CHOCELL

BIO-COMPS GLUTAMIE FBS T-PA

FLOWSHEET
  BLOCK FERMENT IN=F-1 F-2 OUT=F-4 F-3

PROPERTIES BPSIDEAL

NC-COMPS CHOCELL BIOSTATE BIOCOMP

NC-PROPS CHOCELL ENTHALPY BIOENTH / DENSITY BIODEN

PROP-DATA
  PROP-LIST ATOMNO / NOATOM
  PVAL GLUTAMIE 6 1 7 8 / 5 10 2 3
```

```
PROP-DATA
  PROP-LIST ATOMNO / NOATOM
  PVAL T-PA 6 1 8 7 16 / 2486 4950 1365 775 40

STREAM F-1
  SUBSTREAM MIXED TEMP=37 PRES=1 VOLUME-FLOW=750 SOLVENT=WATER &
  NPHASE=1 PHASE=L
  MOLE-CONC GLUCOSE 10.0E-03 / GLUTAMIE 5.10E-03 / FBS &
  0.0015 / HAM 0.2

STREAM F-2
  SUBSTREAM MIXED TEMP=25 PRES=1
  MOLE-FLOW OXYGEN 0.029 / CO2 7.35E-03 / NITROGEN 0.21

BLOCK FERMENT BFERM
  PARAM METHOD=USER-RATES INOC-CONC=0.0526 &
    INOC-VOL=1000 VOL-CHARGE=5000 KLA=7.0 POWER=30 <WATT/CUM> &
    FERM-TEMP=37 PRES=1 NPRODUCTS=1 DOWN-TIME=5 MIN-O2=0.40 &
    MAX-TIME=200.0 GASSED-VOL=1.15 &
    DUTY-METHOD=OUR
  N-SOURCE GLUTAMIE
  SUBSTRATE GLUCOSE
  BIOMASS CHOCELL
  SUBROUTINE KINETICS=K-TPA
  PRODUCTS T-PA
  USER-VECS NREAL=12
  REAL-KINET 1.67 0.0008 0.00026 7.2E-07 -1.94E-06 0.006 7.79 &
     4.167E-06 1.138E-05 0.025 20.0 25.5
  STOP-INT CRITNO=1 INT-NO=1 VARIABLE=ABS-TIME &
    STOP-VALUE=200
  COOLANT TEMP=10 U=525 <WATT/SQM-K> AREA=13.78
  PLOT PLOTNO=1 COMP-LIST=GLUCOSE GLUTAMIE
  PLOT PLOTNO=2 COMP-LIST=CHOCELL
  PLOT PLOTNO=3 COMP-LIST=OXYGEN
  PLOT PLOTNO=4 COMP-LIST=T-PA
  COMP-ATTR 1 CHOCELL BIOSTATE ( 0.75 0.25)
  COMP-ATTR 2 CHOCELL BIOCOMP (53 7.3 19 12 8.7 80 )

STREAM-REPOR NOMOLEFLOW MASSFLOW BPS-REPORT=YES
```

A.2. SIMULATION OF DOWNSTREAM PROCESSING

```
TITLE 'Purification of t-PA '

IN-UNITS MET VOLUME-FLOW='L/HR' ENTHALPY-FLO='KCAL/HR' &
    HEAT-TRANS-C='KCAL/HR-SQM-K' TEMPERATURE=C DELTA-T=C &
    HEAD=METER HEAT=KCAL MOLE-CONC='MOL/L'

DEF-STREAMS MIXNC ALL

RUN-CONTROL MAX-TIME=10000

DATABANKS PURECOMP / AQUEOUS / SOLIDS / INORGANIC / BPS &
    / ASPENPCD

PROP-SOURCES PURECOMP / AQUEOUS / SOLIDS / INORGANIC / &
    BPS / ASPENPCD

COMPONENTS
  CHOCELL * CHOCELL /
  WATER H2O WATER /
  TPA BSA TPA /
  HAM NACL HAM /
  BSA BSA BSA /
  GLUC C6H12O6 GLUC /
  ELUENT1 HCL ELUENT1 /
  ELUENT2 HCL ELUENT2 /
  ELUENT3 NH4HSO4 ELUENT3/
  WASH NACL WASH

BIO-COMPS TPA BSA

FLOWSHEET
  BLOCK MICROF IN=FEED OUT=PERM1 RET1
  BLOCK UF IN=PERM1 OUT=PERM2 RET2
  BLOCK AFF1 IN=RET2 ELUTE1 WASH OUT=PROD1 WASTE1
  BLOCK UF1 IN=PROD1 OUT=PERMB1 RETB1
  BLOCK AFF2 IN=RETB1 WASH2 ELUTE2 OUT=PROD2 WASTE2
  BLOCK UF2 IN=PROD2 OUT=PERMB2 RETB2
  BLOCK GEL IN=RETB2 ELUANT OUT=PROD WASTE

PROPERTIES BPSIDEAL
  PROPERTIES NRTL
```

```
NC-COMPS CHOCELL BIOCOMP

NC-PROPS CHOCELL ENTHALPY BIOENTH / DENSITY BIODEN

PROP-DATA
  PROP-LIST ATOMNO / NOATOM
  PVAL TPA 6 1 8 7 16 / 2486 4950 1365 775 40

PROP-DATA
  PROP-LIST ATOMNO / NOATOM
  PVAL ELUENT1 6 1 8 7 35.5 / 19 18 0 3 1

PROP-DATA
  PROP-LIST ATOMNO / NOATOM
  PVAL ELUENT2 6 1 8 7 35.5 / 19 18 0 3 1

PROP-DATA
  PROP-LIST ATOMNO / NOATOM
  PVAL WASH 6 1 8 7 / 1 5 3 1

PROP-SET MASSCONC MASSCONC UNITS='GM/L' SUBSTREAM=MIXED PHASE=L

STREAM ELUTE1
  SUBSTREAM MIXED TEMP=4 PRES=1.2
  MASS-FLOW WATER 375 / ELUENT1 120

STREAM ELUTE2
  SUBSTREAM MIXED TEMP=4 PRES=1.2
  MASS-FLOW WATER 375 / ELUENT2 120

STREAM ELUANT
  SUBSTREAM MIXED TEMP=4 PRES=1.2 MASS-FLOW=5.5 SOLVENT=WATER &
    NPHASE=1 PHASE=L
  MASS-CONC ELUENT3 3.5

STREAM WASH
  SUBSTREAM MIXED TEMP=4 PRES=1.2
  MASS-FLOW WATER 247.5 / WASH 2.0

STREAM WASH2
  SUBSTREAM MIXED TEMP=4 PRES=1.2
  MASS-FLOW WATER 247.5 / WASH 2.0

STREAM FEED
  SUBSTREAM MIXED TEMP=4 <C> PRES=1
```

```
  MASS-FLOW WATER 5388/TPA 0.192/HAM 62.4/BSA 41.2/GLUC 1.1
  SUBSTREAM NC TEMP=4 <C> PRES=1
  MASS-FLOW CHOCELL 0.24
  COMP-ATTR CHOCELL BIOCOMP ( 53 7.3 19 12 8.7 80 )

BLOCK MICROF MEMBRANE
  PARAM PRES=1.25 P-PERM=1.08 TCOOL=4 <C> VISC=1
  GEOMETRY TYPE=CIRCULAR AREA=80.0 LENGTH=.435 DIAM=6.E-04 &
    NSERIES=1 NMEM=2
  PDROP MODEL=FIXED P-RET=1.0
  FLUX MODEL=RESIS-SERIES
  KEY-COMP CID=CHOCELL
  SOLVENT CID=WATER
  CONV-RECYCLE SETPOINT-VEL=1.6
  RESIS-SERIES RESIS-MEM=2.0E+11 RGEL0=4.5E+12 RGELA=0.13  &
    RGELB=-0.77 RGELC=0.2

BLOCK UF MEMBRANE
  PARAM PRES=1.275 P-PERM=1.08 TCOOL=4 <C> VISC=1
  GEOMETRY TYPE=CIRCULAR AREA=60.0 LENGTH=.435 DIAM=6.E-04 &
    NSERIES=1 NMEM=2
  PDROP MODEL=FIXED P-RET=1.0
  FLUX MODEL=RESIS-SERIES
  KEY-COMP CID=TPA REJECT=0.99
  SOLVENT CID=WATER
  CONV-RECYCLE SETPOINT-VEL=1.6
  RESIS-SERIES RESIS-MEM=2.0E+11 RGEL0=4.5E+12 RGELA=0.13  &
    RGELB=-0.77 RGELC=0.2

BLOCK AFF1 CHROM
  TYPE TYPE=AFF
  PARAM LENGTH=0.4 DIAMETER=.8 REGEN-TIME=1.5 PART-DIAM=10E-05 &
    PROFILE=STEP
  PARAMB NELPROF=1 NSPLIT=1
  STAGE STAGE-ID=LOAD FLOW=200 REL-VOL=0.4 STAGE-PH=7
  STAGE STAGE-ID=WASH FLOW=250 REL-VOL=0.85 STAGE-PH=7
  STAGE STAGE-ID=ELUTE FLOW=200 MAXVOL=500 STAGE-PH=7 &
    END-CID=TPA END-CONC=0.001
  SOLUTES SOLUTE-CID=TPA MW=68000 CAPACITY=0.5 KLOAD=120  &
    EXPON=-10
  SOLUTES SOLUTE-CID=FBS MW=65000 CAPACITY=0.001 KLOAD=5  &
    EXPON=-0.0
  PLOT-CONC PLOTNO=1 PLOT-CID=TPA FBS PLOT-STAGE=LOAD
  PLOT-CONC PLOTNO=2 PLOT-CID=TPA FBS PLOT-STAGE=WASH
  PLOT-CONC PLOTNO=3 PLOT-CID=TPA FBS PLOT-STAGE=ELUTE
```

```
  PROFILE PROF-NO=1 ELUANT-CID=ELUENT1 ELUTE-VOL=0 &
    ELUANT-CONC=226.7
  SPLIT-BASE SPLIT-BASIS=VOLUME
  SPLIT-VOL SPLIT-VOL-NO=1 SPL-VOL-SID=PROD1 VOL-START=380 &
    VOL-STOP=450

BLOCK UF1 BMEMBRANE
  DESCRIPTION
  OPER-SEQ NMODULES=1
  TANK-PARAMS TANKT=4 MAX-VOLUME=225 VINTANK=200
  MODEL-PAR MODULE-ID=1 P-PERM=1.08 PUMP-PRES=1.25 TCOOL=4
  GEOMETRY GEO-MODULEID=1 TYPE=CIRCULAR AREA=10 LENGTH=0.435 &
    DIAM=6E-4 NSERIES=3 NMEMBRANE=1
  PDROP PRES-MOD-ID=1 MODEL=FIXED P-RET=1.0
  FLUX FLUX-MOD-ID=1 FLUX-MODEL=RESIS-SERIES KEY-CID=TPA &
    REJECT=0.98 SOLVID=WATER RESIS-MEM=2E11 RESIS-GEL=4.5E12 &
    RGELA=0.13 RGELB=-0.77 RGELC=0.2
  PROFILE PRINT-TIME=5 <MIN> MAX-NPOINT=25 MAX-TIME=1.0
  SEQ-PARAM STAGE-ID=1 START=VOL START-VAL=200 STOP-VAR=TIME &
    STOP-VAL=0.5 PUMP-CAPACTY=400.0
  INTG-PARAM INT-TOL=0.01
  SPLIT SP-STAGE-ID=1 SPLIT-ROWID=1 SPLIT-FRAC=1.0
  MODULE-REC VEL-STAGE-ID=1 MODULE-NO=1 XRECYCLE=15.0
  CONNECTIVITY CONN-ROWID=1 MODULE-LIST=1
  PLOT-BMEM1 PLOTNO=1 OPTIONS= ACONCK RFOUL UNIT-LIST=1

BLOCK AFF2 CHROM
  TYPE TYPE=AFF
  PARAM LENGTH=0.3 DIAMETER=.6 REGEN-TIME=1.5 PART-DIAM=10E-05 &
    PROFILE=STEP
  PARAMB NELPROF=1 NSPLIT=1
  STAGE STAGE-ID=LOAD FLOW=100 REL-VOL=0.4 STAGE-PH=7
  STAGE STAGE-ID=WASH FLOW=150 REL-VOL=0.20 STAGE-PH=7
  STAGE STAGE-ID=ELUTE FLOW=100 MAXVOL=500 STAGE-PH=7 &
    END-CID=TPA END-CONC=0.001
  SOLUTES SOLUTE-CID=TPA MW=68000 CAPACITY=0.10 KLOAD=80 &
    EXPON=-10
  SOLUTES SOLUTE-CID=FBS MW=65000 CAPACITY=0.00 KLOAD=0.00 &
    EXPON=-0.01
  PLOT-CONC PLOTNO=1 PLOT-CID=TPA FBS PLOT-STAGE=LOAD
  PLOT-CONC PLOTNO=2 PLOT-CID=TPA FBS PLOT-STAGE=WASH
  PLOT-CONC PLOTNO=3 PLOT-CID=FBS PLOT-STAGE=WASH
  PLOT-CONC PLOTNO=4 PLOT-CID=TPA FBS PLOT-STAGE=ELUTE
  PLOT-CONC PLOTNO=5 PLOT-CID=FBS PLOT-STAGE=ELUTE
  PROFILE PROF-NO=1 ELUANT-CID=ELUENT2 ELUTE-VOL=0 &
```

```
    ELUANT-CONC=226.7
  SPLIT-BASE SPLIT-BASIS=VOLUME
  SPLIT-VOL SPLIT-VOL-NO=1 SPL-VOL-SID=PROD2 VOL-START=55 &
    VOL-STOP=110

BLOCK UF2 BMEMBRANE
  DESCRIPTION
  OPER-SEQ NMODULES=1
  TANK-PARAMS TANKT=4 MAX-VOLUME=75 VINTANK=55
  MODEL-PAR MODULE-ID=1 P-PERM=1.08 PUMP-PRES=1.275 TCOOL=4
  GEOMETRY GEO-MODULEID=1 TYPE=CIRCULAR AREA=2.5 LENGTH=0.435 &
    DIAM=6E-4 NSERIES=1 NMEMBRANE=1
  PDROP PRES-MOD-ID=1 MODEL=FIXED P-RET=1.0
  FLUX FLUX-MOD-ID=1 FLUX-MODEL=RESIS-SERIES KEY-CID=TPA &
    REJECT=0.98 SOLVID=WATER RESIS-MEM=2E11 RESIS-GEL=4.5E+12 &
    RGELA=0.13 RGELB=-0.77 RGELC=0.2
  PROFILE PRINT-TIME=5 <MIN> MAX-NPOINT=25 MAX-TIME=1
  SEQ-PARAM STAGE-ID=1 START=VOL START-VAL=0 STOP-VAR=TIME &
    STOP-VAL=0.5 PUMP-CAPACTY=110
  INTG-PARAM INT-TOL=0.01
  SPLIT SP-STAGE-ID=1 SPLIT-ROWID=1 SPLIT-FRAC=1.0
  MODULE-REC VEL-STAGE-ID=1 MODULE-NO=1 XRECYCLE=25.0
  CONNECTIVITY CONN-ROWID=1 MODULE-LIST=1
  PLOT-BMEM1 PLOTNO=1 OPTIONS= ACONCK RFOUL UNIT-LIST=1

BLOCK GEL GELCHROM
  PARAM FLOW-RATE=32 LOAD-VOL=7 LENGTH=0.75 DIAMETER=.5 &
    GEL-NAME=S-200HR
  SOLUTE CID=TPA MW=68000
  SOLUTE CID=FBS MW=96000
  SOLUTE CID=GLUC MW=180
  SOLUTE CID=HAM MW=55
  SOLUTE CID=ELUENT1 MW=236
  SOLUTE CID=ELUENT2 MW=236
  SOLUTE CID=ELUENT3 MW=67
  SPLIT SPLIT-NO=1 STREAM-ID=PROD CID=TPA GTLT=GT CONC=.001
  SPLIT SPLIT-NO=2 STREAM-ID=PROD CID=FBS GTLT=LT CONC=.000008
  PLOT PLOTNO=1 CID=TPA FBS

STREAM-REPOR NOMOLEFLOW MASSFLOW PROPERTIES=MASSCON
```

Appendix B

Summary of Result for the Simulation Runs

This appendix contains report file from both Aspen BPS and SupePro Designer for simulation of the base case flowsheet. The report file from BPS (*.rep) was edited to retain only the relevant design information. The report file for SuperPro included in this appendix is for stream report (*.sr) only.

B.1.Aspen BPS

```
 BLOCK:  AFF1     MODEL: CHROM
 ------------------------------
   PROCESS STREAM:         RET2
   BUFFER STREAM:          ELUTE1
   ELUANT STREAM:          WASH
   OUTLET STREAM(S):       PROD1        WASTE1
   PROPERTY OPTION SET:    BPSIDEAL


                          ***  INPUT DATA  ***
  COLUMN AND GEL PARAMETERS:

   TYPE OF CHROMATOGRAPHY                                  AFFINITY
   COLUMN LENGTH          METER                               .40000
   COLUMN DIAMETER        METER                               .80000
   VOID FRACTION                                              .35000
   GEL DIAMETER           METER                           .10000E-03
   GEL POROSITY                                               .60000
   GEL TORTOUSITY                                            2.0000
   REGENERATION TIME      HR                                 1.5000


STAGE OPERATION PARAMETERS:
                                      LOAD          WASH          ELUTE

   FLOWRATE              L/HR        200.0000      250.0000      200.0000
   VOLUME                L             ---           ---           ---
   RELATIVE VOLUME                     0.4000        0.8500        ---
   MAXIMUM VOLUME        L             ---           ---         500.0000
   PH                                  7.0000        7.0000        7.0000
   COMPONENT ID                        ---           ---           TPA
   LIMITING CONC.        GM/L          ---           ---         1.0000-03
   TIME STEP             HR        1.6667-02     1.6667-02     1.6667-02
   NUMBER OF POINTS                    100           100           100

  ELUTION PROFILE:
   ELUTION COMPONENT                 ELUENT1
   TYPE OF PROFILE                     STEP
                      VOLUME       ELUANT COMP CONCEN
                       L                GM/L
                   .00000E+00          226.70

  SOLUTE CHARACTERISTICS:
   COMPONENT                           CT            KFEED         EXPONENT
                                       GM/L
   TPA                                 0.5000        120.0000      -10.0000
   FBS                             1.0000-03           5.0000        0.0

   COMPONENT                           MW          BULK-DIFF       PORE-DIFF
                                                   SQCM/SEC        SQCM/SEC
   TPA                             6.8000+04           ---           ---
   FBS                             6.5000+04           ---           ---
```

SPLIT CRITERIA:

SPLIT NO.	OUTLET STREAM	VOL START L	VOL STOP L
1	PROD1	.38E+03	.45E+03

*** DATA FROM DATA BANK ***

GEL CHARACTERISTICS:

VOID FRACTION		.35000
GEL DIAMETER	METER	.10000E-03
GEL POROSITY		.60000
GEL TORTOUSITY		2.0000

*** RESULTS ***

BED VOLUME	L	201.06
CYCLE TIME	HR	3.8188
TIME FRACTION IN USE		.92561
NUMBER OF COLUMNS		12

STAGE RESULTS:

		LOAD	WASH	ELUTE
VOLUME	L	80.4247	175.0000	243.3333
SUPERFICIAL VELOC.	CM/HR	39.7887	49.7359	39.7887
LINEAR VELOCITY	CM/HR	113.6821	142.1026	113.6821
PRESSURE DROP	ATM	0.1050	0.1313	0.1050

SOLUTE CHARACTERISTICS:

COMPONENT	BULK-DIFF SQCM/SEC	PORE-DIFF SQCM/SEC
TPA	3.4761-07	1.0428-07
FBS	3.5288-07	1.0586-07

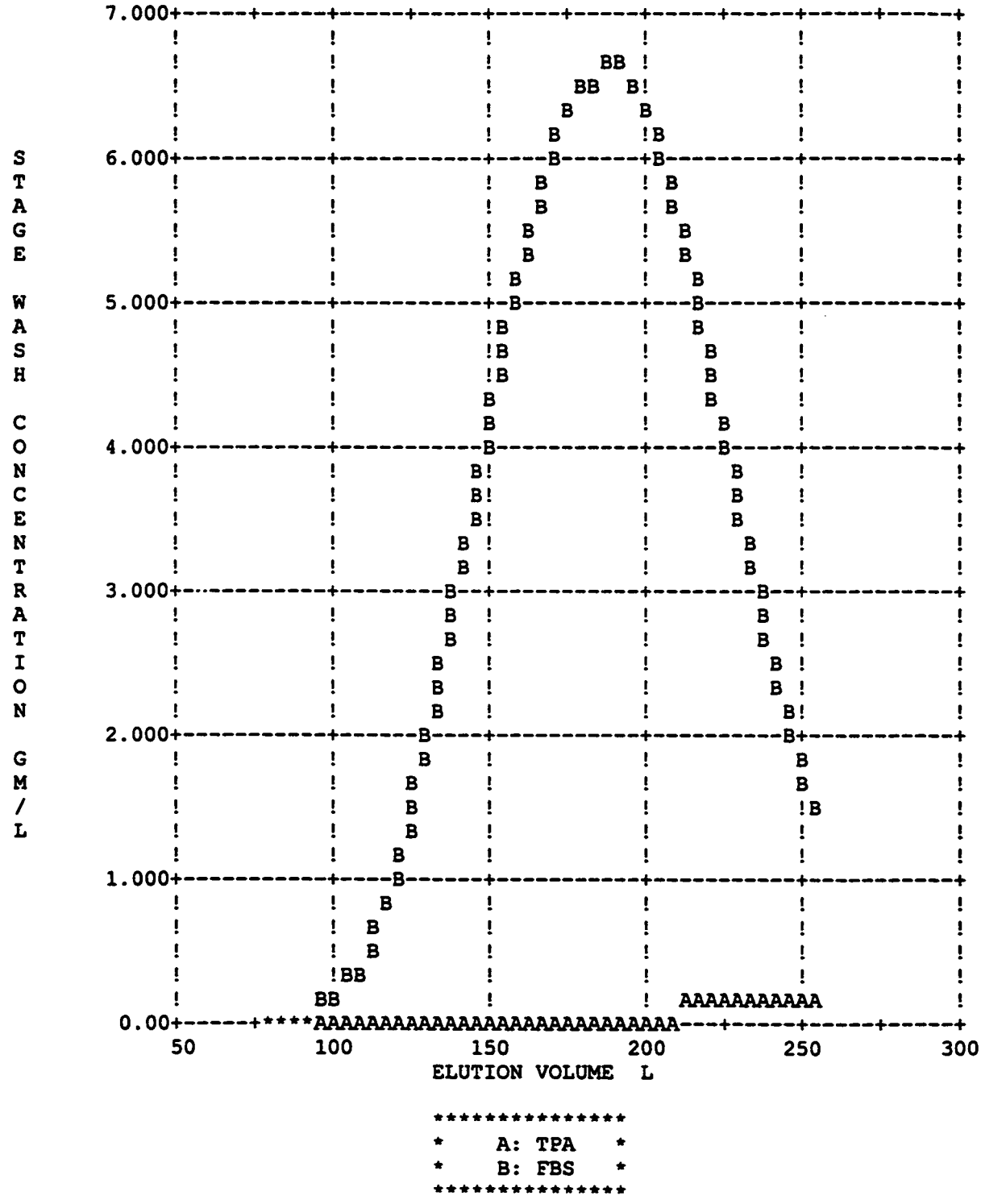
COMPONENT	TRANS-LOAD	TRANS-WASH GM/S-SQCM-GM/CC	TRANS-ELUTE
TPA	6.4401-05	6.4686-05	6.4401-05
FBS	6.5357-05	6.5647-05	6.5357-05

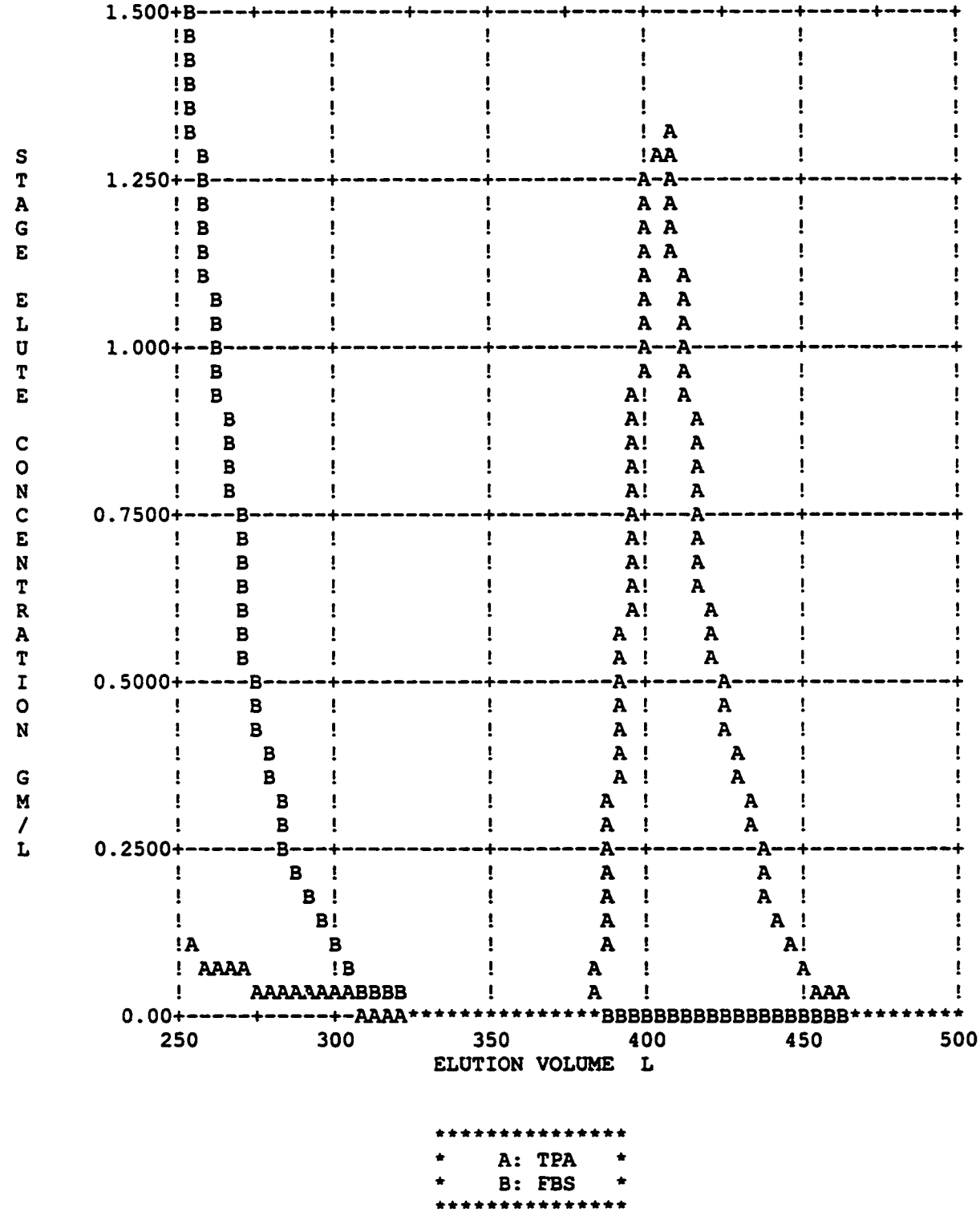
STREAM	COMPONENT	CONC. FACTOR	RECOVERY	PURITY
PROD1	TPA	.84340	.74299	.99997
	FBS	.19971E-05	.17783E-05	.28559E-04
WASTE1	TPA	.41484E-01	.22385	.18353E-01
	FBS	.18396	1.0034	.98165

*** BATCH RESULTS FOR SOLUTES ***

STREAM	VOLUME L
RET2	80.42
ELUTE1	175.0
WASH	243.3
PROD1	70.00
WASTE1	428.8

=TOTAL-IN= 498.8
=TOTAL-OUT= 498.8




STAGE ELUTE CONCENTRATION GM/L
1.500
1.250
1.000
0.7500
0.5000
0.2500
0.00
250
300
350
400
450
500
ELUTION VOLUME L
A: TPA
B: FBS

BLOCK: AFF2 MODEL: CHROM

PROCESS STREAM:	RETB1	
BUFFER STREAM:	WASH2	
ELUANT STREAM:	ELUTE2	
OUTLET STREAM(S):	PROD2	WASTE2
PROPERTY OPTION SET:	BPSIDEAL	

*** INPUT DATA ***

COLUMN AND GEL PARAMETERS:

TYPE OF CHROMATOGRAPHY		AFFINITY
COLUMN LENGTH	METER	.30000
COLUMN DIAMETER	METER	.60000
VOID FRACTION		.35000
GEL DIAMETER	METER	.10000E-03
GEL POROSITY		.60000
GEL TORTOUSITY		2.0000
REGENERATION TIME	HR	1.5000

STAGE OPERATION PARAMETERS:

		LOAD	WASH	ELUTE
FLOWRATE	L/HR	100.0000	150.0000	100.0000
VOLUME	L	---	---	---
RELATIVE VOLUME		0.4000	0.2000	---
MAXIMUM VOLUME	L	---	---	500.0000
PH		7.0000	7.0000	7.0000
COMPONENT ID		---	---	TPA
LIMITING CONC.	GM/L	---	---	1.0000-03
TIME STEP	HR	1.6667-02	1.6667-02	1.6667-02
NUMBER OF POINTS		100	100	100

ELUTION PROFILE:

ELUTION COMPONENT ELUENT2

TYPE OF PROFILE STEP

VOLUME L	ELUANT COMP CONCEN GM/L
.00000E+00	226.70

SOLUTE CHARACTERISTICS:

COMPONENT	CT GM/L	KFEED	EXPONENT
TPA	0.1000	80.0000	-10.0000
FBS	0.0	0.0	-1.0000-02

COMPONENT	MW	BULK-DIFF SQCM/SEC	PORE-DIFF SQCM/SEC
TPA	6.8000+04	---	---
FBS	6.5000+04	---	---

SPLIT CRITERIA:

SPLIT NO.	OUTLET STREAM	VOL START L	VOL STOP L
1	PROD2	55.	.11E+03

*** DATA FROM DATA BANK ***

GEL CHARACTERISTICS:

VOID FRACTION		.35000
GEL DIAMETER	METER	.10000E-03
GEL POROSITY		.60000
GEL TORTOUSITY		2.0000

*** RESULTS ***

BED VOLUME	L	84.823
CYCLE TIME	HR	3.0060
TIME FRACTION IN USE		.98835
NUMBER OF COLUMNS		8

STAGE RESULTS:

		LOAD	WASH	ELUTE
VOLUME	L	33.9292	17.5000	105.0000
SUPERFICIAL VELOC.	CM/HR	35.3677	53.0516	35.3677
LINEAR VELOCITY	CM/HR	101.0507	151.5761	101.0507
PRESSURE DROP	ATM	6.6610-02	9.9920-02	6.6610-02

SOLUTE CHARACTERISTICS:

COMPONENT	BULK-DIFF SQCM/SEC	PORE-DIFF SQCM/SEC
TPA	3.6558-07	1.0967-07
FBS	3.7112-07	1.1134-07

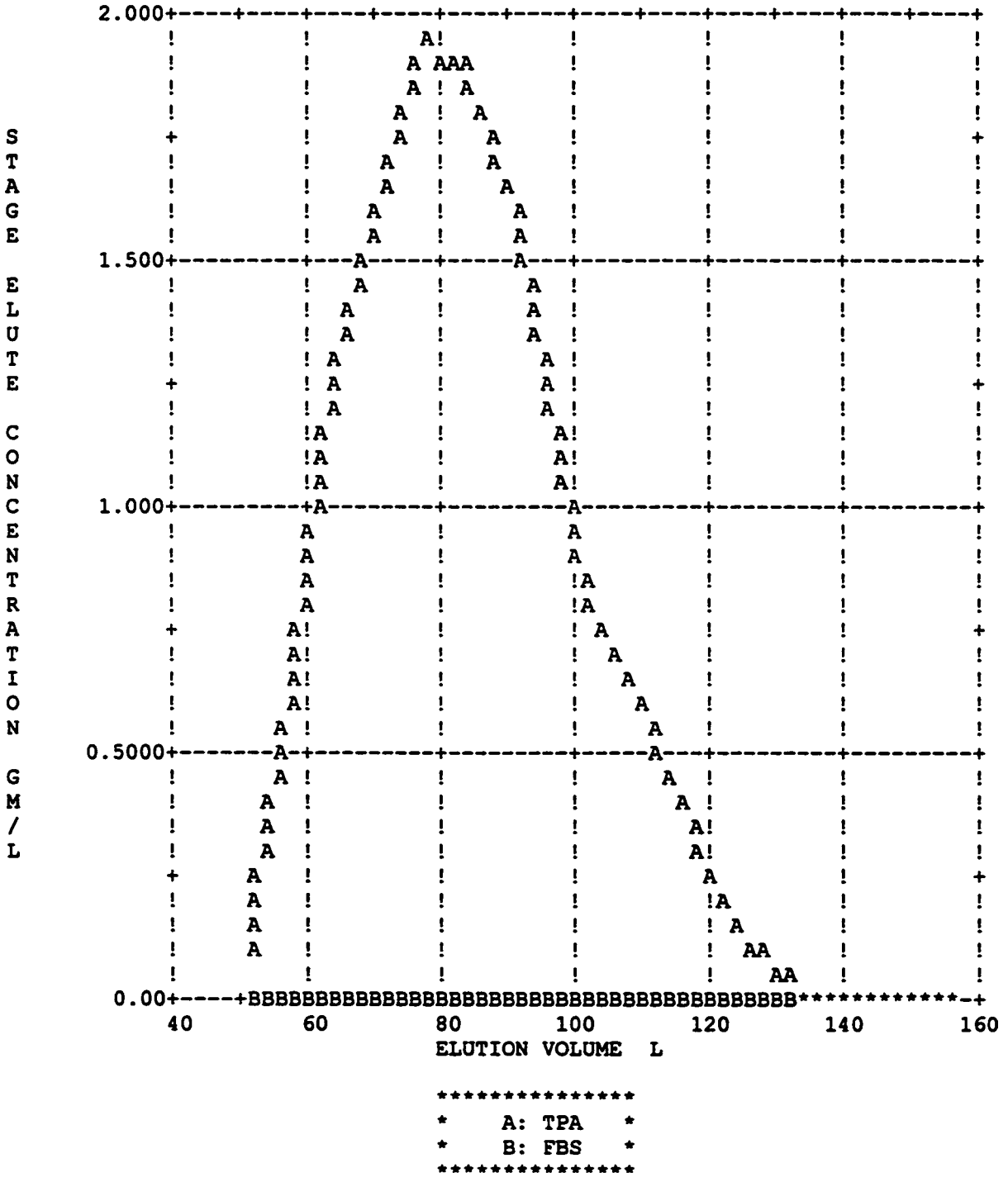
COMPONENT	TRANS-LOAD	TRANS-WASH GM/S-SQCM-GM/CC	TRANS-ELUTE
TPA	6.7491-05	6.8048-05	6.7491-05
FBS	6.8491-05	6.9059-05	6.8491-05

STREAM	COMPONENT	CONC. FACTOR	RECOVERY	PURITY
PROD2	TPA	.55075	.90362	1.00000
	FBS	.55266	.91655	.28970E-07
WASTE2	TPA	.30881E-01	.93438E-01	1.00000
	FBS	.35495E-01	.10856	.33183E-07

*** BATCH RESULTS FOR SOLUTES ***

STREAM	VOLUME L
RETB1	33.93
WASH2	17.50
ELUTE2	105.0
PROD2	55.00
WASTE2	101.4

=TOTAL-IN= 156.4
=TOTAL-OUT= 156.4



BLOCK: GEL MODEL: GELCHROM

PROCESS STREAM:	RETB2	
ELUANT STREAM:	ELUANT	
OUTLET STREAM(S):	PROD	WASTE
PROPERTY OPTION SET:	BPSIDEAL	

*** INPUT DATA ***

ELUTION FLOW RATE	L/HR	32.000
LOADING VOLUME	L	7.0000
COLUMN LENGTH	METER	.75000
COLUMN DIAMETER	METER	.50000
NUMBER OF PLOT POINTS		200
GEL NAME		SEPHACRYL S-200 HR

COMPONENT	MW	VE (L)	KAV
TPA	6.8000+04	---	---
FBS	9.6000+04	---	---
ELUENT3	67.0000	---	---

** SPLIT CRITERIA **

STREAM	COMPONENT	SPECIFICATION	CONC GM/L
PROD	TPA	GREATER THAN	.10000E-02
PROD	FBS	LESS THAN	.80000E-05

*** DATA FROM GEL DATA BANK ***

GEL NAME		SEPHACRYL S-200 HR
VOID FRACTION		.36000
GEL DIAMETER	METER	.47000E-04

COMPONENT	A METER	B SQCM/SEC	C HR	KAV
TPA	.92120E-04	.45218E-06	.14295E-02	.22046
FBS	.92120E-04	.40112E-06	.18196E-02	.16606
ELUENT3	.92120E-04	.67990E-05	.16707E-04	1.0000
WASH	.92120E-04	.64342E-05	.17654E-04	1.0000

*** RESULTS ***

SUPERFICIAL VELOCITY	CM/HR	16.297
LINEAR VELOCITY	CM/HR	45.271
PRESSURE DROP	ATM	.30431
TOTAL GEL VOLUME	L	147.26
VOID VOLUME	L	53.014

INJECTION FREQUENCY	L	220.89
CYCLE TIME	HR	6.9029
TIME FRACTION IN USE		.98358
NUMBER OF COLUMNS		29
NUMBER OF PLOT POINTS USED		182

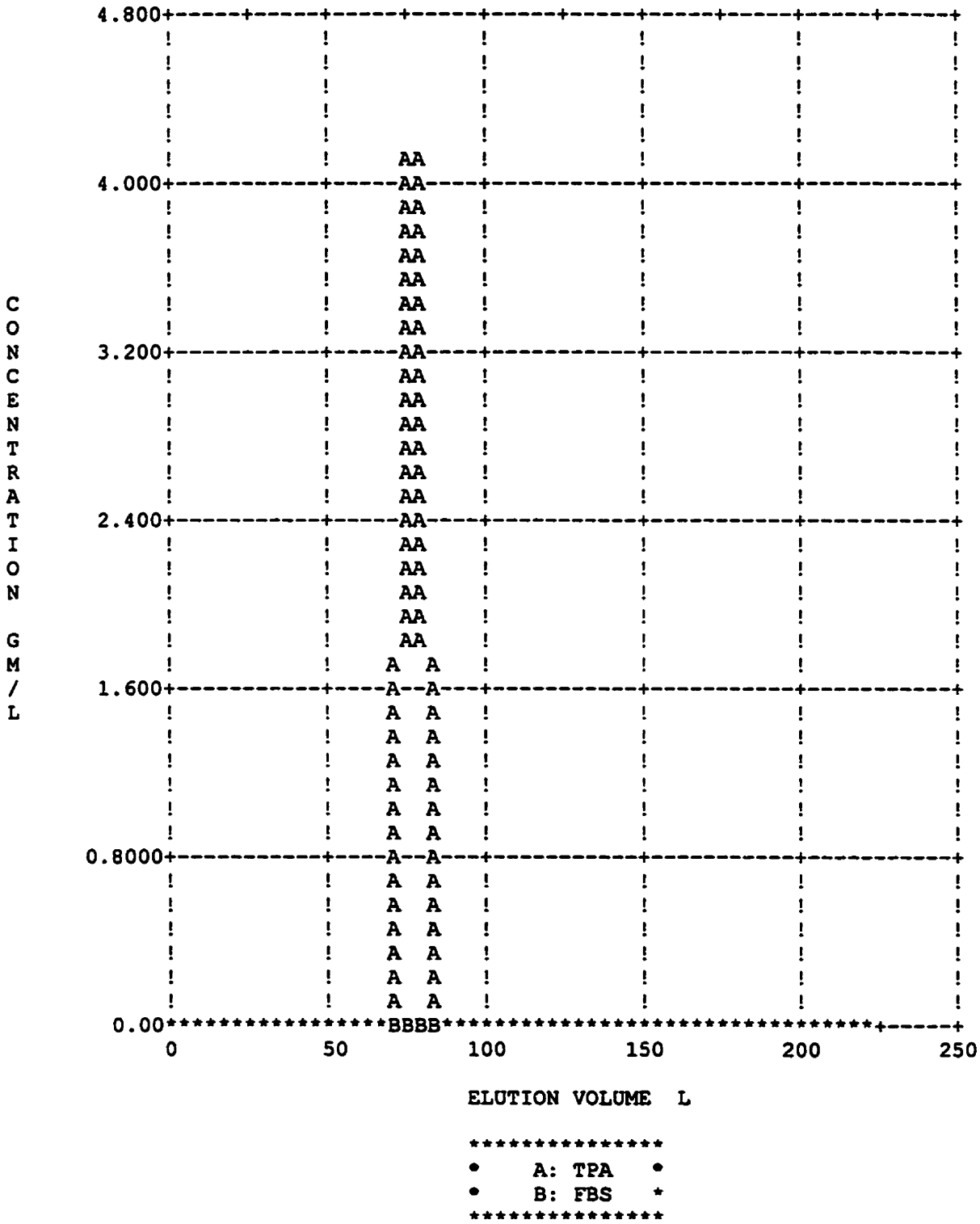
COMPONENT	VE L	NPLATE	HETP METER
TPA	73.792	1014.0	.73964E-03
FBS	68.665	818.62	.91618E-03
ELUENT3	147.26	7136.7	.10509E-03
WASH	147.26	7127.3	.10523E-03

STREAM	COMPONENT	CONC. FACTOR	RECOVERY	PURITY
PROD	TPA	.29988	.99997	.28878
	FBS	.29489	.10000E+36	.72125E-08
	WASH	.00000E+00	.00000E+00	.00000E+00
WASTE	TPA	.12373E-05	.34918E-04	.15958E-05
	FBS	.59158E-03	.10000E+36	.19380E-10
	WASH	.35434E-01	1.0000	.81154E-01

COMPONENT	COMPONENT	RESOLUTION
FBS	TPA	.31199
FBS	WASH	5.1409
TPA	WASH	4.8580

STREAM	COLLECTED FRACTION NO.	START VOLUME L	STOP VOLUME L
PROD	1	65.622	88.963
WASTE	1	.00000E+00	65.622
	2	88.963	220.89

** CONCENTRATION PROFILE **



```
BLOCK:  MICROF   MODEL: MEMBRANE
 ------------------------------
   INLET STREAM:           FEED
   RETENTATE STREAM:       RET1
   PERMEATE STREAM:        PERM1
   PROPERTY OPTION SET:    BPSIDEAL

                      ***  MASS AND ENERGY BALANCE  ***
 REAL COMPONENTS    (KG/HR    )          IN           OUT         PRODUCED
 TOTAL                                 5493.13      5493.13       .000000E+00

 ENTHALPY   (KCAL/HR )              -.206224E+08  -.206223E+08   46.3167
 DUTY       (KCAL/HR )                                          -46.3167


                           ***  INPUT DATA  ***

   MEMBRANE GEOMETRY
    TYPE OF MEMBRANE                                            FIBER
    MEMBRANE AREA          SQM                                  80.000
    MEMBRANE LENGTH        METER                                .43500
    NO CHANNELS IN SERIES                                          1
    FIBER DIAMETER         METER                                .60000E-03

   OPERATING CONDITIONS
    PUMP  PRESSURE         ATM                                  1.2500
    PERMEATE PRESSURE      ATM                                  1.0800
    VELOCITY SETPOINT      M/SEC                                1.6000
    OUTLET TEMPERATURE     C                                    4.0000
    PUMP EFFICIENCY                                             .65000
    DRIVER EFFICIENCY                                           1.0000
    VISCOSITY              CP                                   1.0000
    FLOW TRANSITION RE                                          1000.0

   PRESSURE DROP SPECIFICATIONS
    SPECIFICATION METHOD                                        FIXED
    RETENTATE PRESSURE     ATM                                  1.0000

   FLUX MODEL OPTION AND PARAMETERS
    MODEL NAME                                                  RESIS-SERIES
    MEMBRANE RESISTANCE    1/METER                              .20000E+12
    GEL RESISTANCE PARAM0                                       .45000E+13
    GEL RESISTANCE PARAMA                                       .13000
    GEL RESISTANCE PARAMB                                      -.77000
    GEL RESISTANCE PARAMC                                       .20000


   KEY COMPONENT PROPERTIES
    KEY COMPONENT                                               CHOCELL
    REJECTION COEFF.                                            1.0000

   REJECTION COEFFICIENTS
```

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 CHOCELL                                                    1.0000

                          ***  RESULTS  ***

 PERFORMANCE  RESULTS :

 COMPONENT          CONCENTRATIONS          CONCENTRATION     FRACTION
                 RETENTATE      PERMEATE       FACTOR         RETAINED
                             GM/L

 CHOCELL          .66903         .00000E+00      14.996          1.0000

 CALCULATED FLOW PARAMETERS:

  NUMBER OF CHANNELS                                        97566.
  FEED/RETEN VOL RATIO                                      14.996
  RETENTATE FLOW RATE  L/HR                                 358.73
  PERMEATE  FLOW RATE  L/HR                                 5020.8
  PERMEATE  FLUX       L/SQM-SEC                            .17433E-01
  CALC. RECYCLE RATIO                                       29.008
  REYNOLDS NUMBER                                           980.27
  GEL RESISTANCE       1/METER                              .61548E+11
  FRICTION FACTOR                                           .66830E-02

 CALCULATED PUMP AND COOLER DUTIES:

  PUMP BRAKE HP        KW                                   1.7475
  PUMP ELECTRIC HP     KW                                   1.7475
  COOLER DUTY          KCAL/HR                              1456.3

BLOCK:  UF       MODEL: MEMBRANE
------------------------------
  INLET STREAM:          PERM1
  RETENTATE STREAM:      RET2
  PERMEATE STREAM:       PERM2
  PROPERTY OPTION SET:   BPSIDEAL

                    ***  MASS AND ENERGY BALANCE  ***
REAL COMPONENTS   (KG/HR   )        IN          OUT         PRODUCED
TOTAL                             5126.83       5126.83      .000000E+00

ENTHALPY   (KCAL/HR )          -.192473E+08  -.192473E+08    4.09335
DUTY       (KCAL/HR )                                       -4.09335

                          ***  INPUT DATA  ***

 MEMBRANE GEOMETRY
  TYPE OF MEMBRANE                                          FIBER
  MEMBRANE AREA        SQM                                  60.000
  MEMBRANE LENGTH      METER                                .43500
  NO CHANNELS IN SERIES                                        1
  FIBER DIAMETER       METER                                .60000E-03
```

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OPERATING CONDITIONS
 PUMP  PRESSURE        ATM                                    1.2750
 PERMEATE PRESSURE     ATM                                    1.0800
 VELOCITY SETPOINT     M/SEC                                  1.6000
 OUTLET TEMPERATURE    C                                      4.0000
 PUMP EFFICIENCY                                              .65000
 DRIVER EFFICIENCY                                            1.0000
 VISCOSITY             CP                                     1.0000
 FLOW TRANSITION RE                                           1000.0

PRESSURE DROP SPECIFICATIONS
 SPECIFICATION METHOD                                         FIXED
 RETENTATE PRESSURE    ATM                                    1.0000

FLUX MODEL OPTION AND PARAMETERS
 MODEL NAME                                                   RESIS-SERIES
 MEMBRANE RESISTANCE   1/METER                                .20000E+12
 GEL RESISTANCE PARAM0                                        .45000E+13
 GEL RESISTANCE PARAMA                                        .13000
 GEL RESISTANCE PARAMB                                       -.77000
 GEL RESISTANCE PARAMC                                        .20000

USER FLUX ROUTINE NAME                                        MISSING
INTEGER VECTOR LENGTH                                            0
REAL VECTOR LENGTH                                               0

KEY COMPONENT PROPERTIES
 KEY COMPONENT                                                TPA
 REJECTION COEFF.                                             .99000

REJECTION COEFFICIENTS
 TPA                                                          .99000

                          ***  RESULTS  ***

PERFORMANCE  RESULTS :

COMPONENT          CONCENTRATIONS          CONCENTRATION     FRACTION
                RETENTATE      PERMEATE       FACTOR         RETAINED
                          GM/L

TPA               .63798         .62602E-02      17.874         .83278

CALCULATED FLOW PARAMETERS:

 NUMBER OF CHANNELS                                           73175.
 FEED/RETEN VOL RATIO                                         21.463
 RETENTATE FLOW RATE   L/HR                                   233.92
 PERMEATE  FLOW RATE   L/HR                                   4786.8
 PERMEATE  FLUX        L/SQM-SEC                              .22161E-01
 CALC. RECYCLE RATIO                                          23.221
 REYNOLDS NUMBER                                              980.42
 GEL RESISTANCE        1/METER                                .62899E+11
 FRICTION FACTOR                                              .73502E-02
```

CALCULATED PUMP AND COOLER DUTIES:

PUMP BRAKE HP	KW	1.4479
PUMP ELECTRIC HP	KW	1.4479
COOLER DUTY	KCAL/HR	1240.9

BLOCK: UF1 MODEL: BMEMBRANE

INLET STREAMS:	PROD1
RETENTATE STREAM:	RETB1
PERMEATE STREAM:	PERMB1
PROPERTY OPTION SET:	BPSIDEAL

*** INPUT DATA ***

OPERATION SEQUENCE

NUMBER OF MODULES		1
NUMBER OF ROWS		1
NUMBER OF STEPS		1
DOWN TIME	HR	.00000E+00

MODULE CONNECTIVITY

ROW 1 MODULE NO 1

TANK PARAMETERS

TANK TEMPERATURE	C	4.0000
TANK PRESSURE	ATM	1.0000
MAXIMUM TANK VOLUME	L	225.00
BATCH VOLUME	L	200.00

FEED PUMP SPECIFICATIONS

FEED PUMP PRESSURE	ATM	1.0000
FEED PUMP EFFICIENCY		.65000

MEMBRANE GEOMETRY

MODULE NO.		1
TYPE OF MEMBRANE		FIBER
MEMBRANE AREA	SQM	10.000
MEMBRANE LENGTH	METER	.43500
FIBER DIAMETER	METER	.60000E-03
NO OF MEM PER CHANNEL		1
NO CHANNELS IN SERIES		3.0000
NO IN PARALLEL		4065.3

OPERATING CONDITIONS

MODULE NO.		1
RECYCLE PUMP PRES	ATM	1.2500

PERMEATE PRESSURE	ATM	1.0800
COOLER TEMPERATURE	C	4.0000
RECYCLE PUMP EFF.		.65000
DRIVER EFFICIENCY		1.0000
VISCOSITY MODEL		SYSTEM
DIFFUSIVITY MODEL		SYSTEM

PRESSURE DROP SPECIFICATIONS

MODULE NO.		1
SPECIFICATION METHOD		FIXED
AXIAL PRESSURE DROP	ATM	.25000

FLUX MODEL OPTION AND PARAMETERS

MODULE NO.		1
MODEL NAME		RESISTANCE
MEMBRANE RESISTANCE	1/METER	.20000E+12
RGEL COEFFICIENT		.45000E+13
RGEL CONSTANT A		.13000
RGEL CONSTANT B		-.77000
RGEL CONSTANT C		.20000

KEY COMPONENT PROPERTIES

MODULE NO.		1
KEY COMPONENT		TPA
REJECTION COEFF.		.98000

STAGE PARAMETERS

STAGE NUMBER		1
STOP VARIABLE		TIME
STOP VALUE	HR	.50000
BLEED RATIO		.00000E+00
PUMP CAPACITY	L/HR	400.00
RECYCLE RATIO		
MODULE NO 1		15.000
SPLIT FRACTION		
ROW NO 1		1.0000
CONTROL		NO
INLET STREAM		NONE

PRINTING PARAMETERS

OUTPUT TIME INTERVAL	HR	.83333E-01
MAXIMUM BATCH TIME	HR	1.0000
MAX NO OF OUTPUT PTS		25

*** RESULTS ***

CUM FEED PUMP WORK	KW-HR	.00000E+00

MAX FEED PMP POWER	KW	.00000E+00
CYCLE TIME	HR	.50000
MAXIMUM TANK VOLUME	L	200.00

INLET VOLUME BALANCE

BATCH VOLUME	L	200.00
CONT DIAFIL VOLUME	L	.00000E+00
CONT FEED VOLUME	L	.00000E+00
DISC DIAF VOLUME	L	.00000E+00
TOTAL INLET VOLUME	L	200.00

OUTLET VOLUME BALANCE

RETENTATE VOLUME	L	44.604
PERMEATE VOLUME	L	155.39
BLEED VOLUME	L	.00000E+00
TOTAL OUTLET VOLUME	L	200.00

MODULE BASED RESULTS

MODULE NO.		1
NO IN PARALLEL		4065.3
PERMEATE VOLUME	L	155.39
PERMEATE MASS	KG	158.08
CUM RCYCLE PUMP WORK	KW-HR	.51618E-01
MAX RCYCLE PMP POWER	KW	.10317
CUM COOLER DUTY	KCAL	326.54
MAXIMUM DUTY	KCAL/HR	1789.8
MINIMUM FLUX	L/SQM-SEC	.80847E-02

INLET MATERIAL BALANCE

OUTLET MATERIAL BALANCE

	PERMEATE	RETENTATE	BLEED	TOTAL OUTPUT
COMPONENTS	KG	KG	KG	KG
WATER	157.10	45.104	.00000E+00	202.21
TPA	.33621E-02	.10556	.00000E+00	.10892
FBS	.31413E-05	.30474E-08	.00000E+00	.31444E-05
WASH	.97104	.11760	.00000E+00	1.0886
TOTAL	158.08	45.327	.00000E+00	203.41

COMPONENT	CONCENTRATION FACTOR	RECOVERY

STAGE NUMBER 1

WATER	1.0002	.22306
TPA	4.3455	.96913
FBS	.43456E-02	.96916E-03
WASH	.48437	.10803

CONCENTRATION PROFILES

TIME	CONCENTRATION TANK
HR	GM/L
COMPONENT TPA	
.00000E+00	.54460
.83333E-01	.62166
.16667	.72751
.25000	.88192
.33333	1.1300
.41667	1.6021
.50000	2.3665

TIME	CONCENTRATION TANK
HR	GM/L
COMPONENT FBS	
.00000E+00	.15722E-04
.83333E-01	.15736E-04
.16667	.15785E-04
.25000	.15336E-04
.33333	.15708E-04
.41667	.14191E-04
.50000	.68321E-07

PROFILES OF TANK PROPERTIES

TIME	TANK VISCOSITY	TANK VOLUME
HR	CP	L
.00000E+00	1.5393	200.00
.83333E-01	1.5400	172.81
.16667	1.5409	145.63
.25000	1.5421	118.44
.33333	1.5443	91.234
.41667	1.5483	64.222
.50000	1.5491	44.604

MODULE BASED PROFILES

MODULE NO. 1

TIME	VELOCITY	RECYCLE RATIO
HR	M/SEC	
.00000E+00	1.5058	15.000
.83333E-01	1.5060	15.000
.16667	1.5063	15.000
.25000	1.5065	15.000
.33333	1.5068	15.000
.41667	1.5069	15.000
.50000	1.5099	15.000

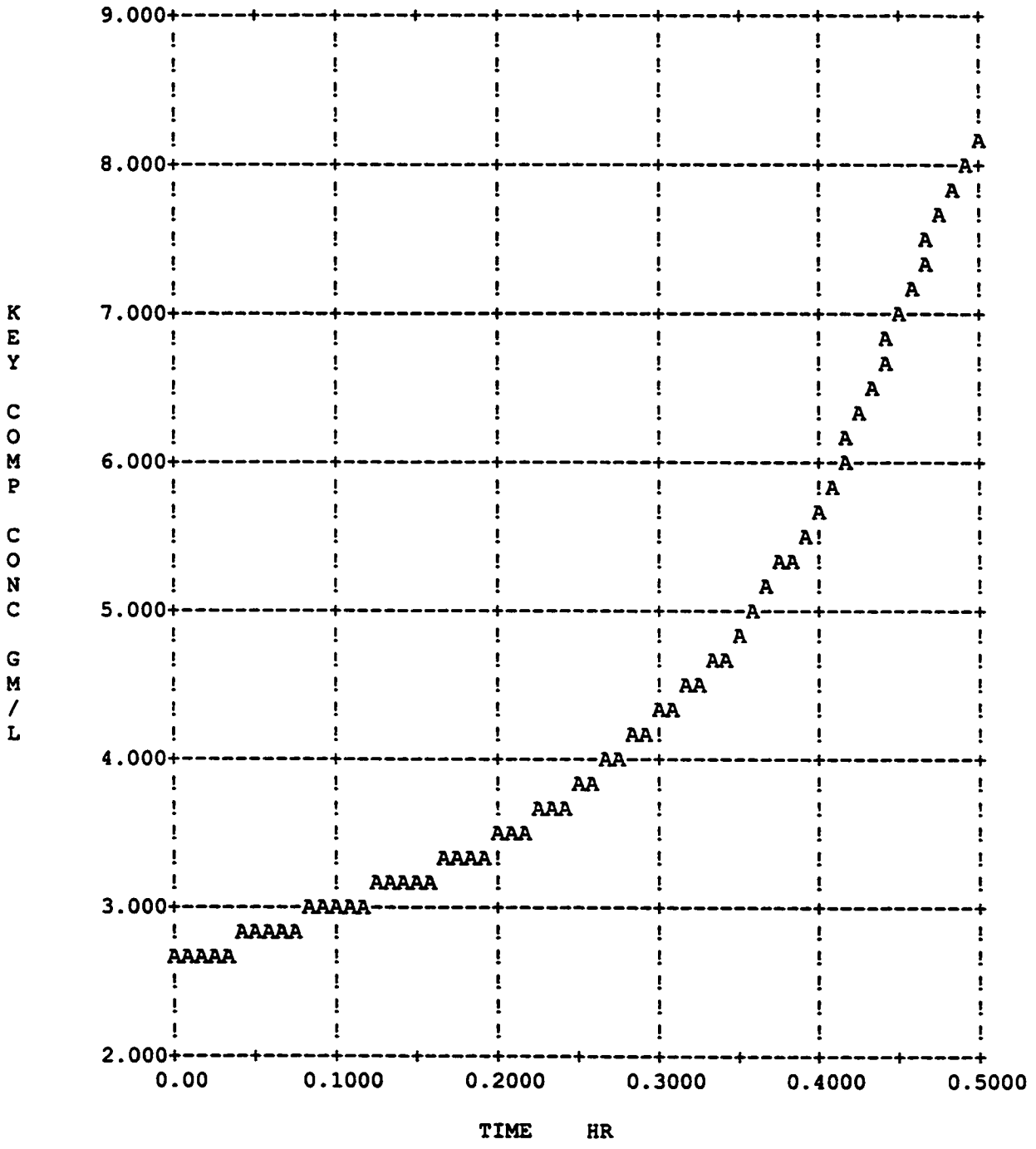
MODULE NO. 1

TIME	FLUX	PERMEATE FLOWRATE	PERMEATE VOLUME
HR	L/SQM-SEC	L/HR	L
.00000E+00	.91269E-02	328.57	.00000E+00
.83333E-01	.90412E-02	325.48	27.186
.16667	.89383E-02	321.78	54.373
.25000	.88136E-02	317.29	81.564
.33333	.86369E-02	310.93	108.81
.41667	.83781E-02	301.61	135.81
.50000	.80847E-02	291.05	155.39

PERMEATE CONCENTRATION

COMPONENT TPA

TIME	UNIT 1
.00000E+00	.54529E-01
.83333E-01	.60277E-01
.16667	.67710E-01
.25000	.77435E-01
.33333	.93299E-01
.41667	.11998
.50000	.16179


KEY
COMP
CONC
GM/L
9.000
8.000
7.000
6.000
5.000
4.000
3.000
2.000
0.00
0.1000
0.2000
0.3000
0.4000
0.5000
TIME HR

```
  BLOCK:  UF2      MODEL: BMEMBRANE
------------------------------------
  INLET STREAMS:          PROD2
  RETENTATE STREAM:       RETB2
  PERMEATE STREAM:        PERMB2
  PROPERTY OPTION SET:    BPSIDEAL

                          ***  INPUT DATA  ***

  OPERATION SEQUENCE

   NUMBER OF MODULES                                      1
   NUMBER OF ROWS                                         1
   NUMBER OF STEPS                                        1
   DOWN TIME              HR                      .00000E+00

  MODULE CONNECTIVITY

   ROW    1   MODULE NO   1

  TANK PARAMETERS

   TANK TEMPERATURE       C                       4.0000
   TANK PRESSURE          ATM                     1.0000
   MAXIMUM TANK VOLUME    L                       75.000
   BATCH VOLUME           L                       55.000

  FEED PUMP SPECIFICATIONS

   FEED PUMP PRESSURE     ATM                     1.0000
   FEED PUMP EFFICIENCY                           .65000

  MEMBRANE GEOMETRY

   MODULE NO.                                             1
   TYPE OF MEMBRANE                                FIBER
   MEMBRANE AREA          SQM                     2.5000
   MEMBRANE LENGTH        METER                   .43500
   FIBER DIAMETER         METER                   .60000E-03
   NO OF MEM PER CHANNEL                          1
   NO CHANNELS IN SERIES                          1.0000
   NO IN PARALLEL                                 3048.9

  OPERATING CONDITIONS

   MODULE NO.                                             1
   RECYCLE PUMP PRES      ATM                     1.2750
   PERMEATE PRESSURE      ATM                     1.0800
   COOLER TEMPERATURE     C                       4.0000
   RECYCLE PUMP EFF.                              .65000
   DRIVER EFFICIENCY                              1.0000
```

VISCOSITY MODEL		SYSTEM
DIFFUSIVITY MODEL		SYSTEM

PRESSURE DROP SPECIFICATIONS

MODULE NO.		1
SPECIFICATION METHOD		FIXED
AXIAL PRESSURE DROP	ATM	.27500

FLUX MODEL OPTION AND PARAMETERS

MODULE NO.		1
MODEL NAME		RESISTANCE
MEMBRANE RESISTANCE	1/METER	.20000E+12
RGEL COEFFICIENT		.45000E+13
RGEL CONSTANT A		.13000
RGEL CONSTANT B		-.77000
RGEL CONSTANT C		.20000

KEY COMPONENT PROPERTIES

MODULE NO.		1
KEY COMPONENT		TPA
REJECTION COEFF.		.98000

STAGE PARAMETERS

STAGE NUMBER		1
STOP VARIABLE		TIME
STOP VALUE	HR	.50000
BLEED RATIO		.00000E+00
PUMP CAPACITY	L/HR	110.00
RECYCLE RATIO		
MODULE NO 1		25.000
SPLIT FRACTION		
ROW NO 1		1.0000
CONTROL		NO
INLET STREAM		NONE

PRINTING PARAMETERS

OUTPUT TIME INTERVAL	HR	.83333E-01
MAXIMUM BATCH TIME	HR	1.0000
MAX NO OF OUTPUT PTS		25

*** RESULTS ***

CUM FEED PUMP WORK	KW-HR	.00000E+00
MAX FEED PMP POWER	KW	.00000E+00
CYCLE TIME	HR	.50000
MAXIMUM TANK VOLUME	L	55.000

INLET VOLUME BALANCE

BATCH VOLUME	L	55.000
CONT DIAFIL VOLUME	L	.00000E+00

CONT FEED VOLUME	L	.00000E+00
DISC DIAF VOLUME	L	.00000E+00
TOTAL INLET VOLUME	L	55.000

OUTLET VOLUME BALANCE

RETENTATE VOLUME	L	14.431
PERMEATE VOLUME	L	40.536
BLEED VOLUME	L	.00000E+00
TOTAL OUTLET VOLUME	L	54.967

MODULE BASED RESULTS

MODULE NO.		1
NO IN PARALLEL		3048.9
PERMEATE VOLUME	L	40.536
PERMEATE MASS	KG	41.289
CUM RCYCLE PUMP WORK	KW-HR	.22875E-01
MAX RCYCLE PMP POWER	KW	.45734E-01
CUM COOLER DUTY	KCAL	309.79
MAXIMUM DUTY	KCAL/HR	1554.3
MINIMUM FLUX	L/SQM-SEC	.76970E-02

OUTLET MATERIAL BALANCE

COMPONENTS	PERMEATE KG	RETENTATE KG	BLEED KG	TOTAL OUTPUT KG
WATER	40.957	14.560	.00000E+00	55.518
TPA	.39879E-02	.68537E-01	.00000E+00	.72525E-01
WASH	.32736	.12170	.00000E+00	.44906
TOTAL	41.289	14.751	.00000E+00	56.039

COMPONENT	CONCENTRATION FACTOR	RECOVERY
STAGE NUMBER 1		
WATER	.99953	.26226
TPA	3.6016	.94501
WASH	1.0328	.27101

CONCENTRATION PROFILES

TIME HR	CONCENTRATION TANK GM/L
COMPONENT TPA	
.00000E+00	1.3186
.83333E-01	1.4906

.16667	1.7195
.25000	2.0387
.33333	2.5175
.41667	3.3131
.50000	4.7492

PROFILES OF TANK PROPERTIES

TIME	TANK VISCOSITY	TANK VOLUME
HR	CP	L
.00000E+00	1.5512	55.000
.83333E-01	1.5530	48.194
.16667	1.5552	41.377
.25000	1.5573	34.560
.33333	1.5615	27.748
.41667	1.5684	20.932
.50000	1.5811	14.431

MODULE BASED PROFILES

MODULE NO. 1

TIME	VELOCITY	RECYCLE RATIO
HR	M/SEC	
.00000E+00	.90771	25.000
.83333E-01	.90762	25.000
.16667	.90754	25.000
.25000	.90746	25.000
.33333	.90722	25.000
.41667	.90674	25.000
.50000	.90573	25.000

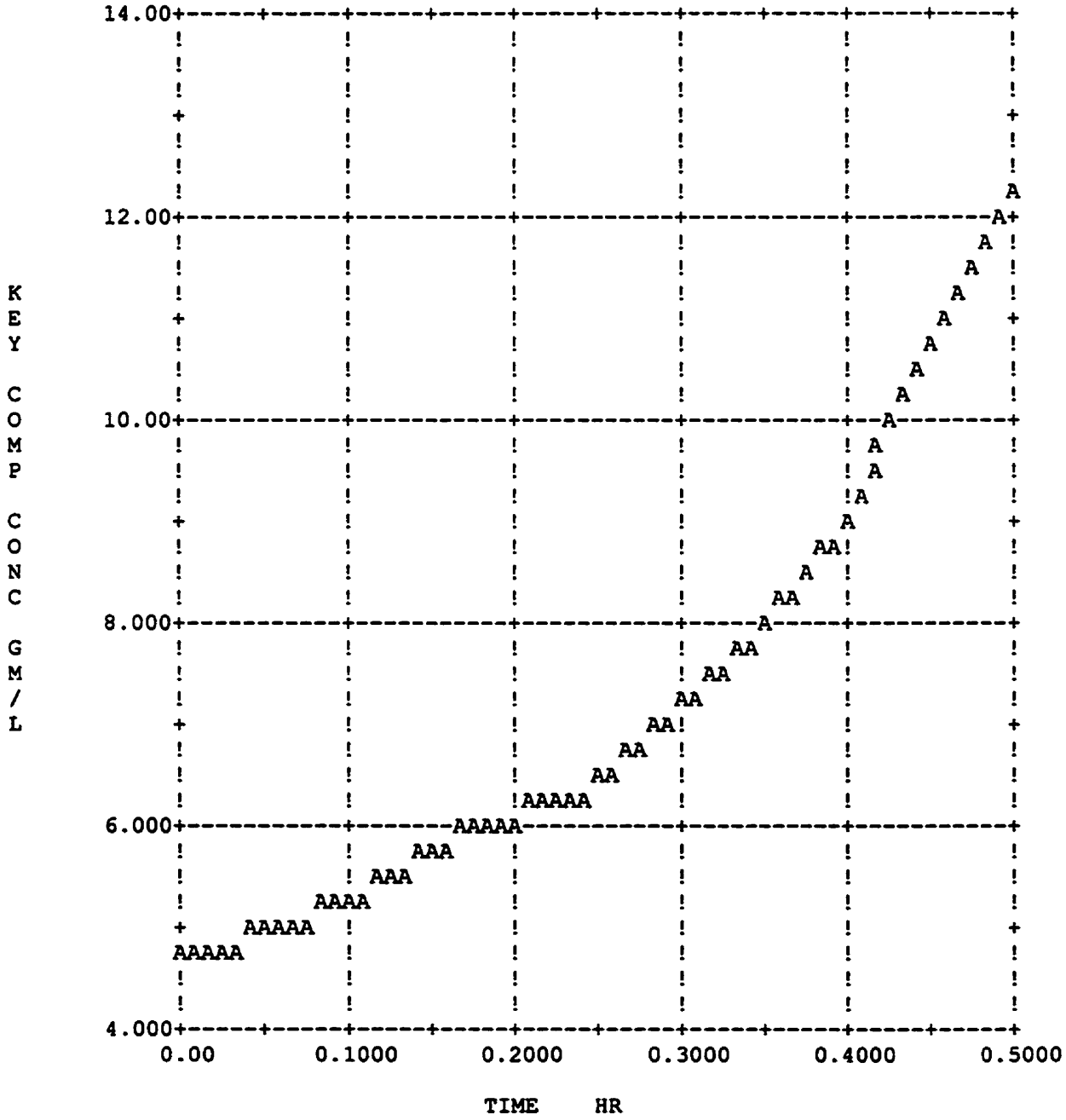
MODULE NO. 1

TIME	FLUX	PERMEATE FLOWRATE	PERMEATE VOLUME
HR	L/SQM-SEC	L/HR	L
.00000E+00	.90458E-02	81.412	.00000E+00
.83333E-01	.89140E-02	80.226	6.8125
.16667	.87710E-02	78.939	13.626
.25000	.86280E-02	77.652	20.439
.33333	.84115E-02	75.703	27.253
.41667	.81164E-02	73.047	34.065
.50000	.76970E-02	69.273	40.536

PERMEATE CONCENTRATION

COMPONENT TPA

TIME	UNIT 1
.00000E+00	.94734E-01
.83333E-01	.10581
.16667	.11834
.25000	.13087
.33333	.15327
.41667	.18784
.50000	.24553



ELUANT ELUTE1 ELUTE2 FEED PERM1

STREAM ID	ELUANT	ELUTE1	ELUTE2	FEED	PERM1
FROM :	----	----	----	----	MICROF
TO :	GEL	AFF1	AFF2	MICROF	UF
CLASS:	MIXNC	MIXNC	MIXNC	MIXNC	MIXNC
TOTAL STREAM:					
KG/HR	892.3104	657.5000	335.5530	5493.1320	5126.8301
KCAL/HR	-3.3936+06	-1.9078+06	-1.0437+06	-2.0622+07	-1.9247+07
SUBSTREAM: MIXED					
PHASE:	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID
COMPONENTS: KG/HR					
WATER	889.2170	498.1060	272.9406	5388.0000	5028.9284
TPA	0.0	0.0	0.0	0.1920	0.1792
HAM	0.0	0.0	0.0	62.4000	58.2415
FBS	0.0	0.0	0.0	41.2000	38.4543
GLUC	0.0	0.0	0.0	1.1000	1.0266
ELUENT1	0.0	159.3939	0.0	0.0	0.0
ELUENT2	0.0	0.0	62.6123	0.0	0.0
ELUENT3	3.0934	0.0	0.0	0.0	0.0
WASH	0.0	0.0	0.0	0.0	0.0
TOTAL FLOW:					
KMOL/HR	49.3859	28.0819	15.3205	300.1538	280.1507
KG/HR	892.3104	657.5000	335.5530	5492.8920	5126.8301
L/HR	883.8331	509.0066	276.1903	5379.2110	5020.7672
STATE VARIABLES:					
TEMP C	4.0000	4.0000	4.0000	4.0000	4.0090
PRES ATM	1.0000	1.0000	1.0000	1.0000	1.0800
VFRAC	0.0	0.0	0.0	0.0	0.0
LFRAC	1.0000	1.0000	1.0000	1.0000	1.0000
SFRAC	0.0	0.0	0.0	0.0	0.0
ENTHALPY:					
CAL/MOL	-6.8714+04	-6.7936+04	-6.8126+04	-6.8702+04	-6.8702+04
CAL/GM	-3803.0553	-2901.5445	-3110.4462	-3754.1806	-3754.1716
KCAL/HR	-3.3936+06	-1.9078+06	-1.0437+06	-2.0622+07	-1.9247+07
ENTROPY:					
CAL/MOL-K	-40.0441	-39.5946	-39.7466	-40.0466	-40.0460
CAL/GM-K	-2.2162	-1.6910	-1.8147	-2.1883	-2.1882
DENSITY:					
MOL/CC	5.5877-02	5.5170-02	5.5471-02	5.5799-02	5.5798-02
GM/CC	1.0095	1.2917	1.2149	1.0211	1.0211
AVG MW	18.0681	23.4136	21.9021	18.3002	18.3002

MIXED SUBSTREAM PROPERTIES:

*** LIQUID PHASE ***					
MASSCONC GM/L					
WATER	1006.0915	978.5847	988.2341	1001.6338	1001.6254
TPA	0.0	0.0	0.0	3.5693-02	3.5693-02
HAM	0.0	0.0	0.0	11.6002	11.6001

FBS	0.0	0.0	0.0	7.6591	7.6590
GLUC	0.0	0.0	0.0	0.2044	0.2044
ELUENT1	0.0	313.1471	0.0	0.0	0.0
ELUENT2	0.0	0.0	226.7000	0.0	0.0
ELUENT3	3.5000	0.0	0.0	0.0	0.0

PERM2 PERMB1 PERMB2 PROD PROD1

STREAM ID	PERM2	PERMB1	PERMB2	PROD	PROD1
FROM :	UF	UF1	UF2	GEL	AFF1
TO :	----	----	----	----	UF1
CLASS:	MIXNC	MIXNC	MIXNC	MIXNC	MIXNC
TOTAL STREAM:					
KG/HR	4887.9181	316.1564	82.5774	97.4101	207.0690
KCAL/HR	-1.8351+07	-1.1987+06	-3.1266+05	-3.6998+05	-7.8518+05
SUBSTREAM: MIXED					
PHASE:	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID
COMPONENTS: KG/HR					
WATER	4794.7399	314.2076	81.9147	96.9354	205.8498
TPA	2.9967-02	6.7243-03	7.9758-03	0.1370	0.1108
HAM	55.5152	0.0	0.0	0.0	0.0
FBS	36.6543	6.2827-06	3.4815-09	3.4234-09	3.2010-06
GLUC	0.9786	0.0	0.0	0.0	0.0
ELUENT1	0.0	0.0	0.0	0.0	0.0
ELUENT2	0.0	0.0	0.0	0.0	0.0
ELUENT3	0.0	0.0	0.0	0.3375	0.0
WASH	0.0	1.9420	0.6547	0.0	1.1082
TOTAL FLOW:					
KMOL/HR	267.1044	17.4657	4.5552	5.3836	11.4404
KG/HR	4887.9181	316.1564	82.5774	97.4101	207.0690
L/HR	4786.8467	310.7835	81.0722	96.4500	203.6026
STATE VARIABLES:					
TEMP C	4.0103	4.1053	3.9619	4.0000	4.0000
PRES ATM	1.0800	1.0800	1.0800	0.6956	0.8686
VFRAC	0.0	0.0	0.0	0.0	0.0
LFRAC	1.0000	1.0000	1.0000	1.0000	1.0000
SFRAC	0.0	0.0	0.0	0.0	0.0
ENTHALPY:					
CAL/MOL	-6.8702+04	-6.8629+04	-6.8637+04	-6.8722+04	-6.8632+04
CAL/GM	-3754.2836	-3791.3450	-3786.2488	-3798.1091	-3791.8452
KCAL/HR	-1.8351+07	-1.1987+06	-3.1266+05	-3.6998+05	-7.8518+05
ENTROPY:					
CAL/MOL-K	-40.0454	-40.0496	-40.0298	-40.0707	-40.0796
CAL/GM-K	-2.1883	-2.2125	-2.2081	-2.2146	-2.2143
DENSITY:					
MOL/CC	5.5800-02	5.6199-02	5.6187-02	5.5818-02	5.6190-02
GM/CC	1.0211	1.0172	1.0185	1.0099	1.0170
AVG MW	18.2996	18.1015	18.1280	18.0936	18.0997

MIXED SUBSTREAM PROPERTIES:

*** LIQUID PHASE ***

MASSCONC GM/L					
WATER	1001.6489	1011.0174	1010.3918	1005.0330	1011.0373
TPA	6.2602-03	2.1636-02	9.8379-02	1.4211	0.5446
HAM	11.5974	0.0	0.0	0.0	0.0
FBS	7.6573	2.0216-05	4.2943-08	3.5494-08	1.5722-05
GLUC	0.2044	0.0	0.0	0.0	0.0
ELUENT1	0.0	0.0	0.0	0.0	0.0
ELUENT2	0.0	0.0	0.0	0.0	0.0
ELUENT3	0.0	0.0	0.0	3.5000	0.0
WASH	0.0	6.2489	8.0758	0.0	5.4432

PROD2 RET1 RET2 RETB1 RETB2

STREAM ID	PROD2	RET1	RET2	RETB1	RETB2
FROM :	AFF2	MICROF	UF	UF1	UF2
TO :	UF2	----	AFF1	AFF2	GEL
CLASS:	MIXNC	MIXNC	MIXNC	MIXNC	MIXNC
TOTAL STREAM:					
KG/HR	147.4048	366.3018	238.9120	90.6536	29.5011
KCAL/HR	-5.5746+05	-1.3750+06	-8.9638+05	-3.4387+05	-1.1120+05
SUBSTREAM: MIXED					
PHASE:	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID
COMPONENTS: KG/HR					
WATER	146.0328	359.0715	234.1885	90.2073	29.1206
TPA	0.1907	1.2795-02	0.1492	0.2111	0.1370
HAM	0.0	4.1584	2.7262	0.0	0.0
FBS	5.5862-09	2.7456	1.8000	6.0948-09	3.4815-09
GLUC	0.0	7.3306-02	4.8059-02	0.0	0.0
ELUENT1	0.0	0.0	0.0	0.0	0.0
ELUENT2	0.0	0.0	0.0	0.0	0.0
ELUENT3	0.0	0.0	0.0	0.0	0.0
WASH	1.1812	0.0	0.0	0.2352	0.2434
TOTAL FLOW:					
KMOL/HR	8.1210	20.0031	13.0463	5.0102	1.6195
KG/HR	147.4048	366.0618	238.9120	90.6536	29.5011
L/HR	144.6711	358.4858	233.9242	89.2468	28.9248
STATE VARIABLES:					
TEMP C	4.0000	4.0000	4.0000	4.0000	4.0000
PRES ATM	0.9000	1.0000	1.0000	1.0000	1.0000
VFRAC	0.0	0.0	0.0	0.0	0.0
LFRAC	1.0000	1.0000	1.0000	1.0000	1.0000
SFRAC	0.0	0.0	0.0	0.0	0.0
ENTHALPY:					
CAL/MOL	-6.8643+04	-6.8702+04	-6.8706+04	-6.8633+04	-6.8662+04
CAL/GM	-3781.7634	-3754.1812	-3751.8632	-3793.2211	-3769.3149
KCAL/HR	-5.5746+05	-1.3743+06	-8.9638+05	-3.4387+05	-1.1120+05
ENTROPY:					
CAL/MOL-K	-40.0485	-40.0466	-40.0574	-40.1611	-40.1081
CAL/GM-K	-2.2064	-2.1883	-2.1874	-2.2196	-2.2018
DENSITY:					
MOL/CC	5.6134-02	5.5799-02	5.5772-02	5.6139-02	5.5991-02

GM/CC	1.0189	1.0211	1.0213	1.0157	1.0199
AVG MW	18.1510	18.3002	18.3125	18.0936	18.2159

MIXED SUBSTREAM PROPERTIES:

*** LIQUID PHASE ***

MASSCONC GM/L					
WATER	1009.4128	1001.6340	1001.1297	1010.7623	1006.7690
TPA	1.3186	3.5693-02	0.6379	2.3655	4.7389
HAM	0.0	11.6000	11.6544	0.0	0.0
FBS	3.8613-08	7.6590	7.6949	6.8292-08	1.2036-07
GLUC	0.0	0.2044	0.2054	0.0	0.0
ELUENT1	0.0	0.0	0.0	0.0	0.0
ELUENT2	0.0	0.0	0.0	0.0	0.0
ELUENT3	0.0	0.0	0.0	0.0	0.0

STREAM ID	PROD2	RET1	RET2	RETB1	RETB2
WASH	8.1648	0.0	0.0	2.6354	8.4148
SUBSTREAM: NC	STRUCTURE: NON CONVENTIONAL				
COMPONENTS: KG/HR					
CHOCELL	0.0	0.2400	0.0	0.0	0.0
TOTAL FLOW:					
KG/HR	0.0	0.2400	0.0	0.0	0.0
STATE VARIABLES:					
TEMP C	MISSING	4.0000	MISSING	MISSING	MISSING
PRES ATM	0.9000	1.0000	1.0000	1.0000	1.0000
VFRAC	MISSING	0.0	MISSING	MISSING	MISSING
LFRAC	MISSING	0.0	MISSING	MISSING	MISSING
SFRAC	MISSING	1.0000	MISSING	MISSING	MISSING
ENTHALPY:					
CAL/GM	MISSING	-3108.9182	MISSING	MISSING	MISSING
KCAL/HR	MISSING	-746.1510	MISSING	MISSING	MISSING
DENSITY:					
GM/CC	MISSING	1.0000	MISSING	MISSING	MISSING
AVG MW	1.0000	1.0000	1.0000	1.0000	1.0000
COMPONENT ATTRIBUTES:					
CHOCELL BIOCOMP					
CARBON	MISSING	53.0000	MISSING	MISSING	MISSING
HYDROGEN	MISSING	7.3000	MISSING	MISSING	MISSING
OXYGEN	MISSING	19.0000	MISSING	MISSING	MISSING
NITROGEN	MISSING	12.0000	MISSING	MISSING	MISSING
ASH	MISSING	8.7000	MISSING	MISSING	MISSING
MOISTURE	MISSING	80.0000	MISSING	MISSING	MISSING

WASH WASH2 WASTE WASTE1 WASTE2

STREAM ID	WASH	WASH2	WASTE	WASTE1	WASTE2
FROM :	----	----	GEL	AFF1	AFF2
TO :	AFF1	AFF2	----	----	----
CLASS:	MIXNC	MIXNC	MIXNC	MIXNC	MIXNC
TOTAL STREAM:					
KG/HR	863.4371	46.8860	824.4014	1267.5733	293.5332

KCAL/HR	-2.6721+06	-1.7753+05	-3.1348+06	-4.8059+06	-1.0205+06
SUBSTREAM: MIXED					
PHASE:	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID
COMPONENTS: KG/HR					
WATER	697.2089	46.5102	821.4022	1259.5157	267.4090
TPA	0.0	0.0	4.7863-06	3.3406-02	1.9726-02
HAM	0.0	0.0	0.0	4.9434	0.0
FBS	0.0	0.0	5.8125-11	1.8061	6.6165-10
GLUC	0.0	0.0	0.0	8.7145-02	0.0
ELUENT1	160.4495	0.0	0.0	0.0	0.0
ELUENT2	0.0	0.0	0.0	0.0	25.8400
ELUENT3	0.0	0.0	2.7558	0.0	0.0
WASH	5.7787	0.3758	0.2434	1.1874	0.2644
TOTAL FLOW:					
KMOL/HR	39.2097	2.5864	45.6217	70.0138	14.9169
KG/HR	863.4371	46.8860	824.4014	1267.5733	293.5332
L/HR	707.7615	46.0317	816.3079	1247.0897	266.7977
STATE VARIABLES:					
TEMP C	4.0000	4.0000	4.0000	4.0000	4.0000
PRES ATM	1.0000	1.0000	0.6956	0.8686	0.9000
VFRAC	0.0	0.0	0.0	0.0	0.0
LFRAC	1.0000	1.0000	1.0000	1.0000	1.0000
SFRAC	0.0	0.0	0.0	0.0	0.0
ENTHALPY:					
CAL/MOL	-6.8148+04	-6.8636+04	-6.8711+04	-6.8640+04	-6.8410+04
CAL/GM	-3094.6749	-3786.3070	-3802.4323	-3791.3270	-3476.5008
KCAL/HR	-2.6721+06	-1.7753+05	-3.1348+06	-4.8059+06	-1.0205+06
ENTROPY:					
CAL/MOL-K	-39.6049	-40.0240	-40.0428	-40.0824	-39.9621
CAL/GM-K	-1.7985	-2.2079	-2.2159	-2.2139	-2.0308
DENSITY:					
MOL/CC	5.5400-02	5.6189-02	5.5888-02	5.6142-02	5.5911-02
GM/CC	1.2199	1.0185	1.0099	1.0164	1.1002
AVG MW	22.0209	18.1274	18.0703	18.1046	19.6778

MIXED SUBSTREAM PROPERTIES:

*** LIQUID PHASE ***

MASSCONC GM/L					
WATER	985.0900	1010.3949	1006.2406	1009.9639	1002.2913
TPA	0.0	0.0	5.8634-06	2.6787-02	7.3937-02
HAM	0.0	0.0	0.0	3.9640	0.0
FBS	0.0	0.0	0.0	1.4482	0.0
GLUC	0.0	0.0	0.0	6.9878-02	0.0
ELUENT1	226.7000	0.0	0.0	0.0	0.0
ELUENT2	0.0	0.0	0.0	0.0	96.8524
ELUENT3	0.0	0.0	3.3759	0.0	0.0
WASH	8.1648	8.1648	0.2981	0.9521	0.9913

B.2.SuperPro Designer

STREAM REPORT

Batch Time = 215.5 h

STREAM NAME		S-102	S-111	S-115	S-114	S-105
SOURCE		MF-101	V-102	MF-101	INPUT	P-101
DESTINATION		UF-101	P-103	OUTPUT	C-101	C-101
STREAM PROPERTIES						
ACTIVITY	U/ml	0.0	0.0	0.0	0.0	0.0
TEMP	°C	4.0	4.0	4.0	4.0	6.2
PRES	bar	1.0	1.0	1.0	1.0	2.0
DENSITY	g/l	1000.4	1000.0	1001.5	1000.0	1000.4
COMPONENT FLOWRATES (kg/Batch)						
Biomass		0.0000	0.0000	8.3959	0.0000	0.0000
Carb. Dioxide		0.0000	0.0000	0.0000	0.0000	0.0000
Elute 1		0.0000	0.0000	0.0000	301.5000	0.0000
Elute 2		0.0000	0.0000	0.0000	0.0000	0.0000
Elute 3		0.0000	0.0000	0.0000	0.0000	0.0000
Glucose		1.0275	0.0000	0.0734	0.0000	0.0477
Glutamine		0.0000	0.0000	0.0000	0.0000	0.0000
Ham's F-12		58.2376	0.0000	4.1624	0.0000	2.7038
Nitrogen		0.0000	0.0000	0.0000	0.0000	0.0000
Oxygen		0.0000	0.0000	0.0000	0.0000	0.0000
Proteins		37.5838	0.0000	2.6862	0.0000	1.7449
t-PA		0.1792	0.0788	0.0128	0.0000	0.1491
Wash		0.0000	0.9162	0.0000	0.0000	0.0000
Water		4918.4621	22.9060	351.5379	1206.0000	228.3507
TOTAL (kg/batch)		5015.4901	23.9011	366.8687	1507.5000	232.9962
TOTAL (m3/batch)		5.0133	0.0239	0.3663	1.5075	0.2329

STREAM NAME		S-122	S-101	S-104	S-113	S-110
SOURCE		INPUT	R-101	V-101	C-103	V-104
DESTINATION		C-103	MF-101	P-101	OUTPUT	UF-103
STREAM PROPERTIES						
ACTIVITY	U/ml	0.0	0.0	0.0	0.0	0.0
TEMP	°C	4.0	37.0	4.0	4.3	4.0
PRES	bar	1.0	1.0	1.0	1.1	1.0
DENSITY	g/l	1000.0	1000.5	1000.4	1000.0	1000.0
COMPONENT FLOWRATES (kg/Batch)						

Biomass	0.0000	8.3959	0.0000	0.0000	0.0000
Carb. Dioxide	0.0000	0.0000	0.0000	0.0000	0.0000
Elute 1	0.0000	0.0000	0.0000	0.0000	0.0000
Elute 2	0.0000	0.0000	0.0000	0.0000	0.0000
Elute 3	306.7917	0.0000	0.0000	306.7917	0.0000
Glucose	0.0000	1.1009	0.0477	0.0000	0.0000
Glutamine	0.0000	0.0000	0.0000	0.0000	0.0000
Ham's F-12	0.0000	62.4000	2.7038	0.0000	0.0000
Nitrogen	0.0000	0.0000	0.0000	0.0000	0.0000
Oxygen	0.0000	0.0000	0.0000	0.0000	0.0000
Proteins	0.0000	40.2700	1.7449	0.0000	0.0000
t-PA	0.0000	0.1920	0.1491	0.0000	0.0936
Wash	0.0000	0.0000	0.0000	17.9078	3.8200
Water	1165.8083	5270.0000	228.3507	1613.5028	95.5000
TOTAL (kg/batch)	1472.6000	5382.3588	232.9962	1938.2022	99.4136
TOTAL (m3/batch)	1.4726	5.3796	0.2329	1.9382	0.0994

STREAM NAME		S-118	S-106	S-108	S-119	S-123
SOURCE		INPUT	V-103	P-102	INPUT	INPUT
DESTINATION		C-101	UF-102	C-102	C-102	C-102
STREAM PROPERTIES						
ACTIVITY	U/ml	0.0	0.0	0.0	0.0	0.0
TEMP	°C	4.0	4.0	4.7	4.0	4.0
PRES	bar	1.0	1.0	1.3	1.0	1.0
DENSITY	g/l	1000.0	1000.0	1000.0	1000.0	1000.0
COMPONENT FLOWRATES (kg/Batch)						
Biomass		0.0000	0.0000	0.0000	0.0000	0.0000
Carb. Dioxide		0.0000	0.0000	0.0000	0.0000	0.0000
Elute 1		0.0000	0.0000	0.0000	0.0000	0.0000
Elute 2		0.0000	0.0000	0.0000	12.7333	0.0000
Elute 3		0.0000	0.0000	0.0000	0.0000	0.0000
Glucose		0.0000	0.0000	0.0000	0.0000	0.0000
Glutamine		0.0000	0.0000	0.0000	0.0000	0.0000
Ham's F-12		0.0000	0.0000	0.0000	0.0000	0.0000
Nitrogen		0.0000	0.0000	0.0000	0.0000	0.0000
Oxygen		0.0000	0.0000	0.0000	0.0000	0.0000
Proteins		0.0000	0.0002	0.0000	0.0000	0.0000
t-PA		0.0000	0.1097	0.1032	0.0000	0.0000
Wash		92.7692	7.7308	1.3187	0.0000	14.6923
Water		2319.2308	193.2692	32.9680	63.6667	367.3077
TOTAL (kg/batch)		2412.0000	201.1099	34.3898	76.4000	382.0000
TOTAL (m3/batch)		2.4120	0.2011	0.0344	0.0764	0.3820

STREAM NAME		S-126	S-112	S-125	S-127	S-128
SOURCE		INPUT	P-103	C-103	INPUT	R-101
DESTINATION		C-103	C-103	OUTPUT	R-101	OUTPUT
STREAM PROPERTIES						
ACTIVITY	U/ml	0.0	0.0	0.0	0.0	0.0
TEMP	°C	4.0	5.1	4.6	37.0	37.0
PRES	bar	1.0	1.5	1.3	1.0	1.0
DENSITY	g/l	1000.0	1000.0	1000.0	1000.7	1.2
COMPONENT FLOWRATES (kg/Batch)						
Biomass		0.0000	0.0000	0.0000	0.0082	0.0000
Carb. Dioxide		0.0000	0.0000	0.0000	0.0000	0.0105
Elute 1		0.0000	0.0000	0.0000	0.0000	0.0000
Elute 2		0.0000	0.0000	0.0000	0.0000	0.0000
Elute 3		0.0000	0.0000	0.0000	0.0000	0.0000
Glucose		0.0000	0.0000	0.0000	5.2400	0.0000
Glutamine		0.0000	0.0000	0.0000	4.3800	0.0000
Ham's F-12		0.0000	0.0000	0.0000	62.4000	0.0000
Nitrogen		0.0000	0.0000	0.0000	0.0000	0.2100
Oxygen		0.0000	0.0000	0.0000	0.0000	0.0700
Proteins		0.0000	0.0000	0.0000	40.2700	0.0000
t-PA		0.0000	0.0788	0.0788	0.0606	0.0000
Wash		28.3192	0.9162	11.3277	0.0000	0.0000
Water		707.9808	22.9060	283.1923	5270.0000	0.0000
TOTAL (kg/batch)		736.3000	23.9011	294.5988	5382.3588	0.2905
TOTAL (m3/batch)		0.7363	0.0239	0.2946	5.3786	0.2525

STREAM NAME		S-129	S-130	S-131	S-117	S-132
SOURCE		G-101	AF-101	INPUT	C-101	C-101
DESTINATION		R-101	G-101	AF-101	V-103	OUTPUT
STREAM PROPERTIES						
ACTIVITY	U/ml	0.0	0.0	0.0	0.0	0.0
TEMP	°C	40.0	25.0	25.0	4.0	4.8
PRES	bar	1.9	1.0	1.0	1.0	1.3
DENSITY	g/l	0.0	1.2	1.2	1000.0	1000.0
COMPONENT FLOWRATES (kg/Batch)						
Biomass		0.0000	0.0000	0.0000	0.0000	0.0000
Carb. Dioxide		0.0105	0.0105	0.0105	0.0000	0.0000
Elute 1		0.0000	0.0000	0.0000	0.0000	301.5000
Elute 2		0.0000	0.0000	0.0000	0.0000	0.0000

Elute 3	0.0000	0.0000	0.0000	0.0000	0.0000
Glucose	0.0000	0.0000	0.0000	0.0000	0.0477
Glutamine	0.0000	0.0000	0.0000	0.0000	0.0000
Ham's F-12	0.0000	0.0000	0.0000	0.0000	2.7038
Nitrogen	0.2100	0.2100	0.2100	0.0000	0.0000
Oxygen	0.0700	0.0700	0.0700	0.0000	0.0000
Proteins	0.0000	0.0000	0.0000	0.0002	1.7447
t-PA	0.0000	0.0000	0.0000	0.1097	0.0393
Wash	0.0000	0.0000	0.0000	7.7308	85.0385
Water	0.0000	0.0000	0.0000	193.2692	3560.3123
TOTAL (kg/batch)	0.2905	0.2905	0.2905	201.1099	3951.3863
TOTAL (m3/batch)	0.0000	0.2428	0.2428	0.2011	3.9513

STREAM NAME	S-109	S-121	S-103	S-116	S-107
SOURCE	C-102	C-102	UF-101	UF-101	UF-102
DESTINATION	V-104	OUTPUT	OUTPUT	V-101	OUTPUT
STREAM PROPERTIES					
ACTIVITY U/ml	0.0	0.0	0.0	0.0	0.0
TEMP °C	4.0	4.3	4.0	4.0	4.0
PRES bar	1.0	1.1	1.0	1.0	1.0
DENSITY g/l	1000.0	1000.0	1000.4	1000.4	1000.0
COMPONENT FLOWRATES (kg/Batch)					
Biomass	0.0000	0.0000	0.0000	0.0000	0.0000
Carb. Dioxide	0.0000	0.0000	0.0000	0.0000	0.0000
Elute 1	0.0000	0.0000	0.0000	0.0000	0.0000
Elute 2	0.0000	12.7333	0.0000	0.0000	0.0000
Elute 3	0.0000	0.0000	0.0000	0.0000	0.0000
Glucose	0.0000	0.0000	0.9798	0.0477	0.0000
Glutamine	0.0000	0.0000	0.0000	0.0000	0.0000
Ham's F-12	0.0000	0.0000	55.5338	2.7038	0.0000
Nitrogen	0.0000	0.0000	0.0000	0.0000	0.0000
Oxygen	0.0000	0.0000	0.0000	0.0000	0.0000
Proteins	0.0000	0.0000	35.8389	1.7449	0.0002
t-PA	0.0936	0.0096	0.0301	0.1491	0.0066
Wash	3.8200	12.1910	0.0000	0.0000	6.4121
Water	95.5000	368.4423	4690.1114	228.3507	160.3013
TOTAL (kg/batch)	99.4136	393.3763	4782.4939	232.9962	166.7201
TOTAL (m3/batch)	0.0994	0.3934	4.7804	0.2329	0.1667

STREAM NAME	S-120	S-124	S-133
SOURCE	UF-102	UF-103	UF-103
DESTINATION	P-102	OUTPUT	V-102

STREAM PROPERTIES

ACTIVITY	U/ml	0.0	0.0	0.0
TEMP	°C	4.0	4.0	4.0
PRES	bar	1.0	1.0	1.0
DENSITY	g/l	1000.0	1000.0	1000.0

COMPONENT FLOWRATES (kg/Batch)

Biomass	0.0000	0.0000	0.0000
Carb. Dioxide	0.0000	0.0000	0.0000
Elute 1	0.0000	0.0000	0.0000
Elute 2	0.0000	0.0000	0.0000
Elute 3	0.0000	0.0000	0.0000
Glucose	0.0000	0.0000	0.0000
Glutamine	0.0000	0.0000	0.0000
Ham's F-12	0.0000	0.0000	0.0000
Nitrogen	0.0000	0.0000	0.0000
Oxygen	0.0000	0.0000	0.0000
Proteins	0.0000	0.0000	0.0000
t-PA	0.1032	0.0147	0.0788
Wash	1.3187	2.9038	0.9162
Water	32.9680	72.5940	22.9060
TOTAL (kg/batch)	34.3898	75.5125	23.9011
TOTAL (m3/batch)	0.0344	0.0755	0.0239

OVERALL MATERIAL BALANCE (kg/Batch)
(Hours per Batch = 216)

COMPONENT	IN	OUT	(OUT-IN)
Biomass	0.008230	8.395930	8.387700
Carb. Dioxide	0.010500	0.010500	0.000000
Elute 1	301.500000	301.500000	0.000000
Elute 2	12.733333	12.733333	0.000000
Elute 3	306.791667	306.791667	0.000000
Glucose	5.240000	1.100900	-4.139100
Glutamine	4.380000	0.000000	-4.380000
Ham's F-12	62.400000	62.400000	0.000000
Nitrogen	0.210000	0.210000	0.000000
Oxygen	0.070000	0.070000	0.000000
Proteins	40.270000	40.270000	-0.000000
t-PA	0.060600	0.192000	0.131400
Wash	135.780769	135.780769	0.000000
Water	11099.994231	11099.994231	0.000000
TOTAL	11969.449330	11969.449330	0.000000

Appendix C

Economic Evaluation

This appendix contains the cost factors used to carry out calculation of total capital investment and annual operating cost of the simulated processes. The appendix also shows the calculation of equipment purchase cost and raw material and media cost for each process considered in Chapter 4.

C.1.CAPITAL COST (C_{TC})

Table C.1.1: 'Budget Authorization Grade'estimate of capital cost (Datar et al., 1993)

Total Purchased Equipment Cost	C_{PE}
Direct Installed Cost, C_{DI}	3.8 X C_{PE}
Fixed Capital Investment, C_{FC}	4.6 X C_{PE}
Total Capital Investment, C_{TC}	5.5 X C_{PE}

Table C.1.2: Detailed estimate of fixed capital and comparison of cost factors

	Super Pro	Petrides et al., (1989)	Datar and Rosen (1990)
Equipment Purchase	PC	PC	PC
Installation	0.12 x PC	0.40 x PC	
Piping	0.35 x PC	0.35 x PC	
Instrumentation	0.40 x PC	0.40 x PC	
Insulation	0.03 x PC	0.03 x PC	
Electrical Facilities	0.10 x PC	0.10 x PC	
Buildings	0.30 x PC	0.45 x PC	0.29 x PC
Yard Improvement	0.15 x PC	0.15 x PC	0.17 x PC
Auxiliary Facilities	0.40 x PC	0.40 x PC	
Total plant direct cost (TPDC)	1.85 x PC	2.28 x PC	2.76 x PC
Engineering	0.25 x TPDC	0.25 x TPDC	
Construction	0.35 x TPDC	0.35 x TPDC	
Total plant indirect cost (TPIC)	0.60 x TPDC	0.60 x TPDC	2.18 x PC
Total plant cost (TPC)	(TPDC+ TPIC)	(TPDC+TPIC)	
A.Contractor's fee	0.05 x TPC	0.05 x TPC	
B.Contingency	0.10 x TPC	0.10 x TPC	0.30 x PC
Direct fixed Capital (DFC)	TPC+A+B	TPC +A+B	TPC+A+ B

Table C.1.3: Calculation of Total Equipment Purchase Cost for Base Case Flowsheet

Equipment	Size	Quantity	Unit Price	Price ($)
Blending Tanks	6500 L	1	10,335	10,335
	1500 L	1	3,529	3,529
	300 L	1	3,529	3,529
Axial Compressor	5 kW	1	43,303	43,303
Absolute Filter	0.01 m^3/s	1	4,154	4,154
Microfilter	80 m^2	1	79,818	79,818
Inoculum Fermenters	1000 L	1	75,397	75,397
	200 L	1	65,296	65,296
	40 L	1	65,296	65,296
Production Bioreactor	8000 L	1	184,684	184,684
Microfilter	80 m^2	1	79,818	79,818
Holding Tank	5500 L	1	10,335	10,335
Ultrafilter	60 m^2	1	71,690	71,690
Holding Tank	300 L	1	3,529	3,529
Affinity Chromatography Column	200 L	3	240,000	720,000
Holding Tank	250 L	1	3,529	3,529
Ultrafilter	10 m^2	1	25,375	25,375
Holding Tank	40 L	1	3,529	3,529
Affinity Chromatography Column	85 L	1	113,114	113,114
Holding Tank	100 L	1	3,529	3,529
Ultrafilter	2.5 m^2	1	16,741	16,741
Holding Tank	30 L	1	3,529	3,529
Gel Filtration Column	147 L	6	129,202	775,212
TOTAL			$\$2.365X10^6$	
Unlisted Equipment (20%)			$473055	
SUBTOTAL			$\$2.838\ X10^6$	
Total Equipment Purchase for the Entire Plant		5	$\$14.2\ X10^6$	

Table C.1.4: Calculation of Total Equipment Purchase Cost for Two Stage Fermentation Flowsheet

Equipment	Size	Quantity	Unit Price	Price ($)
Blending Tanks	6500 L	8	10,335	82,680
	1500 L	8	3,529	28,232
	300 L	8	3,529	28,232
Axial Compressor	5 kW	8	43,303	346,424
Absolute Filter	0.01 m^3/s	8	4,154	33,232
Inoculum Fermenters	1000 L	8	75,397	603,176
	200 L	8	65,296	522,344
	40 L	8	65,296	522,344
Microfilter	80 m^2	5	80,000	400,000
Growth Bioreactor	8000 L	5	185,000	925,000
Production Bioreactor	6500 L	3	169,000	507,000
Microfilter	70 m^2	3	74,000	222,000
Holding Tank	4100 L	3	9,000	27,000
Ultrafilter	50 m^2	3	67,000	201,000
Holding Tank	40 L	3	4,000	12,000
Ion Exchange Column	200 L	3	240,000	720,000
Holding Tank	630 L	3	4,000	12,000
Ultrafilter	15 m^2	3	32,000	96,000
Ion Exchange Column	113 L	3	134,000	402,000
Holding Tank	250 L	3	4,000	12,000
Ultrafilter	3 m^2	3	17,000	51,000
Holding Tank	60 L	3	4,000	12,000
Gel Filtration Column	147 L	12	129,202	$1.55X10^6$
TOTAL			$7.3 $X10^6$	
Unlisted Equipment (20%)			$1.46 $X10^6$	
SUBTOTAL			$8.8 $X10^6$	

Table C.1.5: Calculation of Total Equipment Purchase Cost for Fed-Batch Case (A) Flowsheet

Equipment	Size	Quantity	Unit Price	Price ($)
Blending Tanks	6500 L	2	10,335	20,670
	1500 L	2	3,529	7,058
	300 L	2	3,529	7,058
Axial Compressor	5 kW	1	43,303	43,303
Absolute Filter	0.01 m^3/s	1	4,154	4,154
Microfilter	80 m^2	1	79,818	79,818
Inoculum Fermenters	1000 L	1	75,397	75,397
	200 L	1	65,296	65,296
	40 L	1	65,296	65,296
Production Bioreactor	4250 L	2	155,000	310,000
Microfilter	65 m^2	2	70,000	140,000
Holding Tank	3400 L	2	7,660	15,320
Ultrafilter	50 m^2	2	67,000	134,000
Holding Tank	750 L	2	4,000	8,000
Affinity Chromatography Column	185 L	20	238,000	$4.76X10^6$
Holding Tank	2720 L	2	7,000	14,000
Ultrafilter	60 m^2	2	72,000	144,000
Holding Tank	80 L	2	4,000	8,000
Affinity Chromatography Column	145 L	6	147,000	882,000
Holding Tank	550 L	2	4,000	8,000
Ultrafilter	20 m^2	2	38,000	76,000
Holding Tank	45 L	2	4,000	8,000
Gel Filtration Column	147 L	12	129,000	$1.55X10^6$
TOTAL			$8.41X10^6	
Unlisted Equipment (20%)			$1.68 X10^6	
SUBTOTAL			$10.08 X10^6	

Table C.1.6: Calculation of Total Equipment Purchase Cost for Fed-Batch Case (B) Flowsheet

Equipment	Size	Quantity	Unit Price	Price ($)
Blending Tanks	6500 L	2	10,335	20,670
	1500 L	2	3,529	7,058
	300 L	2	3,529	7,058
Axial Compressor	5 kW	1	43,303	43,303
Absolute Filter	0.01 m^3/s	1	4,154	4,154
Microfilter	80 m^2	1	79,818	79,818
Inoculum Fermenters	1000 L	1	75,397	75,397
	200 L	1	65,296	65,296
	40 L	1	65,296	65,296
Production Bioreactor	3745 L	2	146,000	292,000
Microfilter	60 m^2	2	71,690	143,380
Holding Tank	3000 L	2	7,077	14,154
Ultrafilter	45 m^2	2	62,565	125,130
Holding Tank	500 L	2	3,644	7,288
Affinity Chromatography Column	195 L	12	239,116	$2.87X10^6$
Holding Tank	500 L	2	3,644	7,288
Ultrafilter	10 m^2	2	25,375	50,750
Holding Tank	60 L	2	3,644	7,288
Affinity Chromatography Column	135 L	6	144,877	869,262
Holding Tank	130 L	2	3,529	7,058
Ultrafilter	5 m^2	2	16,741	33,482
Holding Tank	20 L	2	3,529	7,058
Gel Filtration Column	147 L	4	129,000	516,000
TOTAL			$5.32X10^6	
Unlisted Equipment (20%)			$1.06 X10^6	
SUBTOTAL			$6.38 X10^6	

Table C.1.7: Calculation of Total Equipment Purchase Cost for Airlift Bioreactor

Equipment	Size	Quantity	Unit Price	Price ($)
Blending Tanks	6500 L	1	10,335	10,335
	1500 L	1	3,529	3,529
	300 L	1	3,529	3,529
Axial Compressor	5 kW	1	43,303	43,303
Absolute Filter	0.01 m^3/s	1	4,154	4,154
Microfilter	80 m^2	1	79,818	79,818
Inoculum Fermenters	1000 L	1	56,550	56,550
	200 L	1	48,970	48,970
	40 L	1	48,970	48,970
Production Bioreactor	8000 L	1	138,520	138,520
Microfilter	80 m^2	1	79,818	79,818
Holding Tank	5500 L	1	10,335	10,335
Ultrafilter	60 m^2	1	71,690	71,690
Holding Tank	300 L	1	3,529	3,529
Affinity Chromatography Column	200 L	3	240,000	720,000
Holding Tank	250 L	1	3,529	3,529
Ultrafilter	10 m^2	1	25,375	25,375
Holding Tank	40 L	1	3,529	3,529
Affinity Chromatography Column	85 L	1	113,114	113,114
Holding Tank	100 L	1	3,529	3,529
Ultrafilter	2.5 m^2	1	16,741	16,741
Holding Tank	30 L	1	3,529	3,529
Gel Filtration Column	147 L	6	129,202	775,212
TOTAL			$2.266X10^6$	
Unlisted Equipment (20%)			$453,300	
SUBTOTAL			$2.72 $X10^6$	
Total Equipment Purchase for the Entire Plant		5	$13.6 $X10^6$	

Table C.1.8: Calculation of Total Equipment Purchase Cost for Multiple Bioreactor Scaleup

Equipment	Size	Quantity	Unit Price	Price ($)
Blending Tanks	6500 L	1	10,335	10,335
	1500 L	1	3,529	3,529
	300 L	1	3,529	3,529
Axial Compressor	5 kW	1	43,303	43,303
Absolute Filter	0.01 m^3/s	1	4,154	4,154
Microfilter	15 m^2	6	29,000	174,000
Inoculum Fermenters	1000 L	1	75,397	75,397
	200 L	1	65,296	65,296
Production Bioreactor	1330 L	6	75,000	450,000
Microfilter	15 m^2	1	29,000	29,000
Holding Tank	850 L	1	4,000	4,000
Ultrafilter	10 m^2	1	25,375	25,375
Holding Tank	100 L	1	4,000	4,000
Affinity Chromatography Column	85 L	1	133,825	133,825
Holding Tank	60L	1	4,000	4,000
Ultrafilter	2 m^2	1	17,000	17,000
Holding Tank	15 L	1	4,000	4,000
Affinity Chromatography Column	30 L	1	93,000	93,000
Holding Tank	30 L	1	4,000	4,000
Ultrafilter	1 m^2	1	17,000	17,000
Holding Tank	10 L	1	4,000	4,000
Gel Filtration Column	75 L	2	93,537	187,074
TOTAL			$1.35X10^6$	
Unlisted Equipment (20%)			$271,150	
SUBTOTAL			$1.63 X10^6$	

C.2.ANNUAL OPERATING COST (AOC)

Table C.2.1: Estimation of Operating Cost (AOC) (Datar et al., 1990)

Raw Material and Media	
Opearting Labor	A
Supervision Labor	0.25 X A
Maintenance Labor	0.06 X C_{FC}
Opearting Labor + Supervision Labor + Maintenance Labor = C	
Operating Supplies	0.10 X A
Maintenance Supplies	0.04 X C_{FC}
Laboratory	0.25 X A
Patent	0.07 X AOC
Waste Treatment	0.05 X AOC
Plant Overhead, D	0.6 X C
Tax	0.02 X C_{FC}
Insurance	0.005 X C_{FC}
Depreciation	0.10 X C_{FC}
Administration	0.25 X D
Distribution	0.10 X AOC
R & D	0.07 X AOC

Table C.2.2: Comparison of cost factors for annual operating cost estimation

	Super Pro	Petrides et al., (1989)	Datar and Rosen (1990)
LABOR DEP-ITEMS			
Operating Labor (A)	$19/hour		$18/hour
Maintenance Labor (B)	0.03 x DFC	----------	
Fringe Benefits (C)	0.40 x (A + B)	0.22 x A	
Supervision (D)	0.20 x (A + B)	0.18 x A	0.25 x A
Operating Supplies	0.10 x A	0.10 x A	0.10 x A
Laboratory	0.15 x A	0.15 x A	0.25 x A
DFC DEP- ITEMS			
Depreciation	0.10 x DFC	0.10 x DFC	
Maintenance Material	0.03 x DFC	0.06 x DFC	0.06 x DFC
Insurance	0.01 x DFC	0.01 x DFC	0.005x DFC
Local Taxes	0.02 x DFC	0.02 x DFC	
Factory Expense	0.05 x DFC	0.05 x DFC	
Administration and Overhead	0.60 x (A+B+C)	----------	0.75 x (A+B+D)
Utilities	0.01 x AOC	0.01 x AOC	0.10 x AOC
Waste treatment	0.02 x AOC	0.05 x AOC	0.05 x AOC
Research and Development	0.11 x AOC	0.14 x AOC	0.07 x AOC

C.2.1.Calculation of Raw Material and Media Cost for Base Case:

I. Media: 6000 L X \$25/L X 5 X 35 = \$26.25 X 10^6

II. Buffers:

a) Affinity elution buffer: (3X154.2 + 105) X 5 X 35 X \$10/L
= \$0.993 X 10^6

b) Affinity equilibration buffer: (3 X 243.3 + 17.5) X 5 X 35
X \$5.5/L=0.72X10^6

c) Gel elution buffer: (225 X 6) X 5 X 35 X \$28.75/L = \$6.8 X 10^6

TOTAL = 8.512 X 10^6

III. Replacement:

a) Affinity Chromatography media: (200L X 5 X 3 + 85 X 5) X \$2000/L
= \$6.85 X 10^6

b) Gel filter media: (147 L X 4 X 5) X \$200/L = \$588,000

c) Membranes: (80+60+10+2.5) m^2 X 5 X \$200/m^2 = \$152,500

TOTAL = \$7.6 X 10^6

SUBTOTAL = \$42.35 X 10^6

Table C.2.3: Estimation of Operating Cost (AOC) (Datar et al., 1990)

Raw Material and Media		42.35
Opearting Labor	A	1.515
Supervision Labor	0.25 X A	0.38
Maintenance Labor	0.06 X C_{FC}	3.312
Opearting Labor + Supervision Labor + Maintenance Labor = C		5.207
Operating Supplies	0.10 X A	0.1515
Maintenance Supplies	0.04 X C_{FC}	2.208
Laboratory	0.25 X A	0.38
Patent	0.07 X AOC	
Waste Treatment	0.05 X AOC	
Plant Overhead, D	0.6 X C	3.124
Tax	0.02 X C_{FC}	1.104
Insurance	0.005 X C_{FC}	0.276
Depreciation	0.10 X C_{FC}	6.0
Administration	0.25 X D	0.78
Distribution	0.10 X AOC	
R & D	0.07 X AOC	

0.39 AOC + 61.58 = AOC

AOC = \$101 X 10^6

C.2.2.Calculation of Raw Material and Media Cost for Two-stage Fermentation:

I. Bioreactor:

a) Growth Bioreactor: 6000 L X \$18/L X 5 X 35 = \$18.9 X 10^6

b) Production Bioreactor: 5000 L X \$25/L X 3 X 35 = \$13.125 X 10^6

c) Diafiltration stream: 1000 L X 5 X 35 X \$5/L = \$0.875 X 10^6

TOTAL = 32.9 X 10^6

II. Buffers:

a) Ion-exchange elution buffer: (2 X 500 + 301.2) X 3 X 35 X \$10/L = \$1.366 X 10^6

b) Ion-exchange equilibration buffer: (2 X 83.3 + 24) X 3 X 35 X \$5.5/L = 0.11 X 10^6

c) Gel elution buffer: (197 X 4) X 3 X 35 X \$28.75/L = \$2.38 X 10^6

TOTAL = 3.856 X 10^6

III. Replacement:

a) Ion-exchange Chromatography media: (200L X 2 X 3 + 113 X 3) X \$400/L= \$6.85 X 10^6

b) Gel filtration media: (147 L X 5 X 3) X \$200/L = \$440,000

c) Membranes: (80 X 5 + 70 X 3 + 50 X 3 + 15 X 3 + 5 X 3)m^2 X\$200/m^2 = \$164,000

TOTAL = \$1.22 X 10^6

SUBTOTAL = \$37.98 X 10^6

Table C.2.4: Estimation of Operating Cost (AOC) (Datar et al., 1990)

Raw Material and Media		37.98
Opearting Labor	A	1.8
Supervision Labor	0.25 X A	0.45
Maintenance Labor	0.06 X C_{FC}	2.87
Opearting Labor + Supervision Labor + Maintenance Labor = C		5.12
Operating Supplies	0.10 X A	0.18
Maintenance Supplies	0.04 X C_{FC}	1.9
Laboratory	0.25 X A	0.45
Patent	0.07 X AOC	
Waste Treatment	0.05 X AOC	
Plant Overhead, D	0.6 X C	3.07
Tax	0.02 X C_{FC}	0.957
Insurance	0.005 X C_{FC}	0.24

Depreciation	0.10 X C_{FC}	6.0
Administration	0.25 X D	0.767
Distribution	0.10 X AOC	
R & D	0.07 X AOC	

$$0.39\ AOC + 56.67 = AOC$$
$$AOC = \$92.9 \times 10^6$$

C.2.3.Calculation of Raw Material and Media Cost for Fed-batch Case A:

I.Bioreactor:

a) Media: 4000 L X \$25/L X 2 X 35 = $\$7.0 \times 10^6$

a) Inoculum: 800 X 2 X 35 X \$10/L = \$560,000

b) Continuous feed: (300 X 2 X 35 X \$10/L) = \$210,000

TOTAL = 7.77×10^6

II. Buffers:

a) Affinity elution buffer: (3750 + 580) X 2 X 35 X \$10/L = $\$3.03 \times 10^6$

b) Affinity equilibration buffer: (2267 + 162.5) X 2 X 35 X \$5.5/L = 0.935×10^6

c) Gel elution buffer: (220 X 6) X 2 X 35 X \$28.75/L = $\$2.656 \times 10^6$

TOTAL = 6.62×10^6

III. Replacement:

a) Affinity Chromatography media: (1860 + 438) X 2 X \$2000/L = $\$9.2 \times 10^6$

a) Gel filter media: (147 L X 6 X 2) X \$200/L = \$352,800

c) Membranes: (65+65+50+60+20) m^2 X 2 X \$200/m^2 = \$104,00

TOTAL = $\$9.34 \times 10^6$

SUBTOTAL = $\$23.73 \times 10^6$

Table C.2.5: Estimation of Operating Cost (AOC) (Datar et al., 1990)

Raw Material and Media		23.73
Opearting Labor	A	0.68
Supervision Labor	0.25 X A	0.17
Maintenance Labor	0.06 X C_{FC}	2.8
Opearting Labor + Supervision Labor + Maintenance Labor = C		3.64
Operating Supplies	0.10 X A	0.068
Maintenance Supplies	0.04 X C_{FC}	2.2
Laboratory	0.25 X A	0.17
Patent	0.07 X AOC	
Waste Treatment	0.05 X AOC	
Plant Overhead, D	0.6 X C	2.18
Tax	0.02 X C_{FC}	1.1
Insurance	0.005 X C_{FC}	0.275
Depreciation	0.10 X C_{FC}	2.6
Administration	0.25 X D	0.545
Distribution	0.10 X AOC	
R & D	0.07 X AOC	

$$0.39\ AOC + 36.52 = AOC$$
$$AOC = \$59.86 \times 10^6$$

C.2.4.Calculation of Raw Material and Media Cost for Fed-batch Case B:

I.Bioreactor

a) Media: 3000 L X \$25/L X 2 X 35 = \$5.25 X 10^6
b) Inoculum: 700 L X \$10/L X 2 X 35 = \$0.49 X 10^6
c) Continuous Feed: 150 L X \$10/L X 2 X 35 = \$ 0.105 X 10^6

TOTAL: \$5.845 X 10^6

II. Buffers:

a) Affinity elution buffer: (145.8 X 6 + 190) X 2 X 35 X \$10/L = \$745,360
b) Affinity equilibration buffer: (236.7 X 6 + 29.17) X 2 X 35 X \$5.5/L = \$558,000
c) Gel elution buffer: (220 X 2) X 2 X 35 X \$28.75/L = \$0.88 X 10^6

TOTAL = \$2.19 X 10^6

III. Replacement:

a) Affinity Chromatography media: (195L X 6 X 2 + 135 X 2) X \$2000/L = \$5.22 X 10^6

b) Gel filter media: (147 L X 2 X 2) X \$200/L = \$117,600
c) Membranes: (60+60+45+10+5) m^2 X 2 X \$200/m^2 = \$72,000

TOTAL = \$5.41 X 10^6

SUBTOTAL = \$13.44 X 10^6

Table C.2.6: Estimation of Operating Cost (AOC) (Datar et al., 1990)

Raw Material and Media		13.44
Opearting Labor	A	1.24
Supervision Labor	0.25 X A	0.31
Maintenance Labor	0.06 X C_{FC}	1.88
Opearting Labor + Supervision Labor + Maintenance Labor = C		3.43
Operating Supplies	0.10 X A	0.124
Maintenance Supplies	0.04 X C_{FC}	1.254
Laboratory	0.25 X A	0.31
Patent	0.07 X AOC	
Waste Treatment	0.05 X AOC	
Plant Overhead, D	0.6 X C	2.0
Tax	0.02 X C_{FC}	0.627
Insurance	0.005 X C_{FC}	0.156
Depreciation	0.10 X C_{FC}	2.0
Administration	0.25 X D	0.50
Distribution	0.10 X AOC	
R & D	0.07 X AOC	

0.39 AOC + 27.27 = AOC
AOC = \$44.7 X 10^6

C.2.5.Calculation of Raw Material and Media Cost for Multiple Bioreactor Scaleup:

I. Media: 1000 L X \$25/L X 6 X 35 = \$5.25 X 10^6

II. Buffers:
a) Affinity elution buffer: (181.3 + 39.42) X 6 X 35 X \$10/L = \$0.463 X 10^6
b) Affinity equilibration buffer: (126.7 + 5.83) X 6 X 35
X \$5.5/L=0.15 X 10^6
c) Gel elution buffer: (113 X 2) X 3 X 35 X \$28.75/L = \$0.68 X 10^6

TOTAL = 1.298 X 10^6

III. Replacement:

a) Affinity Chromatography media: (85 L + 30 L) X \$2000/L
= \$230,000

b) Gel filter media: (75 X 2) X \$200/L = \$30,000

c) Membranes: (15+15+11+2+1) m^2 X \$200/m^2 = \$8,800

TOTAL = \$0.27 X 10^6

SUBTOTAL = \$6.82 X 10^6

Table C.2.7: Estimation of Operating Cost (AOC) (Datar et al., 1990)

Raw Material and Media		6.82
Opearting Labor	A	0.986
Supervision Labor	0.25 X A	0.246
Maintenance Labor	0.06 X C_{FC}	0.395
Opearting Labor + Supervision Labor + Maintenance Labor = C		1.63
Operating Supplies	0.10 X A	0.098
Maintenance Supplies	0.04 X C_{FC}	0.26
Laboratory	0.25 X A	0.246
Patent	0.07 X AOC	
Waste Treatment	0.05 X AOC	
Plant Overhead, D	0.6 X C	0.976
Tax	0.02 X C_{FC}	0.13
Insurance	0.005 X C_{FC}	0.033
Depreciation	0.10 X C_{FC}	0.6
Administration	0.25 X D	0.244
Distribution	0.10 X AOC	
R & D	0.07 X AOC	

0.39 AOC + 11.03 = AOC

AOC = \$16.08 X 10^6

Appendix D

K_La, Agitation and Power Input Requirement

This appendix presents the calculation of K_La and power requirement used for simulation of the bioreactor. An estimate of K_La is obtained following the basic approach in its calculation. Power requirement is calculated using two approaches – similar fluid turnover and constant Kolmogorov length scale.

D.1. Calculation of K_La using Basic Approach

1. Determination of Bubble diameter, d_B:

$$d_B = \left[\frac{6\sigma d_0}{g(\rho_L - \rho_G)} \right]^{1/3}$$

2. Determination of Bubble type:

$$d^* = d_B \left[\frac{\mu_L^2}{\rho_L g(\rho_L - \rho_G)} \right]^{-1/3}$$

rigid bubble : $d^* \leq \sim 10$
mobile bubble : $d^* \geq \sim 50$

3. Calculation of Re:

Bubble rise velocity, U_b : 16 cm/s

$$Re = \frac{d_B U_b \rho_L}{\mu_L} = 400$$

4. Choose correlation:

$$Sh = 0.95\ Re^{1/2}\ Sc^{1/3} \quad [10<Re<10^4]$$

$k_L = 0.0048$ cm/s

5. Determination of 'a':

$$a' = \frac{6H}{d_B(1 - H)}$$

$$H = \frac{U_G}{U_b} = \sim 0.02$$

$a' = 0.349\ cm^{-1}$

6. Determination of k_La' :

$k_La' = 6.3\ h^{-1}$

D.2. Calculation of Agitation and Power Input Requirements

D.2.1. Approach A: Similar Fluid Turnover (Chisti, 1993)

Table D.2.1: Comparison of aspect ratio

Reactor Size (L)	Length/Diamter (L/D)	Impeller Diameter, d_i (m)	d_i/D
300	1.0	0.21	~ 0.29
6000	1.2	0.621	0.3

If oxygen transfer rate is not a limiting factor scale-up can be carried out by maintaining a similar fluid turnover, that is impeller pumping rate per unit bioreactor volume, at the two scale. The specific pumping rate of the impeller is:

$$Q/V \sim (N\, d_i^3)/V$$

For the 300 L bioreactor, $(N\, d_i^3)/V = 0.051$

For the 6000 L bioreactor,

$$(N'\, d_i'^3)/V' = 0.051$$

$$N' = 1.28\ s^{-1} = 77\ rpm$$

Power Input = $N_p(N')^3(d_i')^5\rho = 77.5$ W

Power Input per Unit Volume = 13 W/m^3

D.2.2. Approach B: Constant Kolmogrov Length Scale (Nelson, 1988)

Viscosity, $\mu = 1.2 \times 10^{-3}$ Pa.s

Density, $\rho = 1000\ kg/m^3$

Kinematic Viscosity, $\nu = \mu/\rho = 1.2 \times 10^{-6}\ m^2/s$

For the base case (Chisti, 1993):

$N_0 = 1.67\ s^{-1}$

$Re_{i,0} = 61375$

$N_p = 0.4$

$\varepsilon_0 = P/V = 3.36 \times 10^{-3}$

$L_0 = [\nu^3/\varepsilon_0]^{1/4} = 130\ \mu m$

Agitation rate for sclaed up vessel:

$$N_1^3 = N_0^3\ [N_p(Re_{i\cdot 0})/N_p(Re_{i,1})]\ (d_{i,0}/d_{i,1})^2$$

Table D.2.2: Results of trial and error runs

Assumed Agitation Rate	Impeller Reynolds Number	Calculated Agitation Rate
0.38	118605	0.283
0.283	88330	0.724
0.724	226073	0.53
0.53	165311	0.59
0.59	183492	0.568
0.568	177220	0.574
0.574	179286	0.572
0.5722	178595	0.573
0.573	178825	0.5727
0.5727	178748	0.5728
0.5728	178774	0.5728

Power Input $= N_p(N')^3(d_i')^5\rho = 6.94$ W

Power Input per Unit Volume $= 1.15\ W/m^3$

Appendix D

K_La, Agitation and Power Input Requirement

This appendix presents the calculation of K_La and power requirement used for simulation of the bioreactor. An estimate of K_La is obtained following the basic approach in its calculation. Power requirement is calculated using two approaches – similar fluid turnover and constant Kolmogorov length scale.

E.1. BIOREACTOR

```
SENSITIVITY S-1
  DEFINE XRATE BLOCK-VAR BLOCK=FERMENT VARIABLE=BIOMASS-RATE &
    SENTENCE=BIOMASS-RATE ID1=50
  DEFINE SRATE BLOCK-VAR BLOCK=FERMENT VARIABLE=SUBSTRT-RATE &
    SENTENCE=SUBSTRT-RATE ID1=50
  DEFINE XCONC BLOCK-VAR BLOCK=FERMENT VARIABLE=BIOMASS-CONC &
    SENTENCE=BIOMASS-CONC ID1=50
  DEFINE SCONC BLOCK-VAR BLOCK=FERMENT VARIABLE=SUBSTRT-CONC &
    SENTENCE=SUBSTRT-CONC ID1=50
  DEFINE NRATE BLOCK-VAR BLOCK=FERMENT VARIABLE=NSOURCE-RATE &
    SENTENCE=NSOURCE-RATE ID1=50
  DEFINE MINO2 BLOCK-VAR BLOCK=FERMENT VARIABLE=MIN-DISOX &
    SENTENCE=RESULTS
  DEFINE MINKLA BLOCK-VAR BLOCK=FERMENT VARIABLE=MIN-KLA &
    SENTENCE=RESULTS2
  DEFINE FO2 BLOCK-VAR BLOCK=FERMENT VARIABLE=FINAL-DISOX &
    SENTENCE=RESULTS2
  DEFINE ISATO2 BLOCK-VAR BLOCK=FERMENT VARIABLE=INIT-SAT-O2 &
    SENTENCE=RESULTS2
  DEFINE DO2PER BLOCK-VAR BLOCK=FERMENT VARIABLE=DO2-PERCENT &
    SENTENCE=DO2-PERCENT ID1=50
  TABULATE 1 "XRATE"
  TABULATE 2 "SRATE"
  TABULATE 3 "XCONC"
  TABULATE 4 "SCONC"
  TABULATE 5 "NRATE"
  TABULATE 6 "MINO2"
  TABULATE 7 "MINKLA"
  TABULATE 8 "FO2"
  TABULATE 9 "ISATO2"
  TABULATE 10 "DO2PER"
  VARY BLOCK-VAR BLOCK=FERMENT VARIABLE=INIT-SAT-VAL &
    SENTENCE=DISSOLVED-O2
  RANGE LOWER="0.0195E-03" UPPER="0.0975E-03" NPOINT="5"
  VARY BLOCK-VAR BLOCK=FERMENT VARIABLE=INOC-CONC &
    SENTENCE=PARAM
  RANGE LOWER="0.03" UPPER="0.25" NPOINT="5"
  VARY BLOCK-VAR BLOCK=FERMENT VARIABLE=INOC-VOL &
    SENTENCE=PARAM
  RANGE LOWER="500" UPPER="2000" NPOINT="4"
  VARY BLOCK-VAR BLOCK=FERMENT VARIABLE=VOL-CHARGE &
```

```
    SENTENCE=PARAM
  RANGE LOWER="2000" UPPER="4000" NPOINT="3"
  VARY BLOCK-VAR BLOCK=FERMENT VARIABLE=MIN-O2 SENTENCE=PARAM
  RANGE LOWER="0.15" UPPER="0.5" NPOINT="5"
  VARY BLOCK-VAR BLOCK=FERMENT VARIABLE=KLA SENTENCE=PARAM
  RANGE LOWER="2" UPPER="8" NPOINT="5"
  VARY BLOCK-VAR BLOCK=FERMENT VARIABLE=VALUE-LIST &
    SENTENCE=REAL-KINET ELEMENT=1
  RANGE LOWER="0.5" UPPER="4.0" INCR="0.5"
  VARY BLOCK-VAR BLOCK=FERMENT VARIABLE=VALUE-LIST &
    SENTENCE=REAL-KINET ELEMENT=4
  RANGE LOWER="0.001" UPPER="0.0035" NPOINT="5"
  VARY BLOCK-VAR BLOCK=FERMENT VARIABLE=VALUE-LIST &
    SENTENCE=REAL-KINET ELEMENT=6
  RANGE LOWER="0.003" UPPER="0.009" NPOINT="5"
  VARY BLOCK-VAR BLOCK=FERMENT VARIABLE=VALUE-LIST &
    SENTENCE=REAL-KINET ELEMENT=7
  RANGE LOWER="5" UPPER="10" NPOINT="5"
  VARY BLOCK-VAR BLOCK=FERMENT VARIABLE=VALUE-LIST &
    SENTENCE=REAL-KINET ELEMENT=8
  RANGE LOWER="0.01" UPPER="0.02" NPOINT="5"
  VARY BLOCK-VAR BLOCK=FERMENT VARIABLE=VALUE-LIST &
    SENTENCE=REAL-KINET ELEMENT=9
  RANGE LOWER="0.03" UPPER="0.05" NPOINT="5"

STREAM-REPOR NOMOLEFLOW MASSFLOW BPS-REPORT=YES
```

E.2. CONTINUOUS MEMBRANE SEPARATION UNITS

```
SENSITIVITY S-1
  DEFINE PF BLOCK-VAR BLOCK=MICROF VARIABLE=PERM-FLUX &
    SENTENCE=RESULTS
  DEFINE SR BLOCK-VAR BLOCK=MICROF VARIABLE=SHEAR-RATE &
    SENTENCE=RESULTS
  DEFINE FP BLOCK-VAR BLOCK=MICROF VARIABLE=FLOW-PERM &
    SENTENCE=RESULTS
  DEFINE VR BLOCK-VAR BLOCK=MICROF VARIABLE=VOL-RATIO &
    SENTENCE=RESULTS
  DEFINE VR BLOCK-VAR BLOCK=MICROF VARIABLE=VOL-RATIO &
    SENTENCE=RESULTS
  DEFINE CF BLOCK-VAR BLOCK=MICROF VARIABLE=CONC-FACTOR &
    SENTENCE=CONC ID1=TPA
  DEFINE FR BLOCK-VAR BLOCK=MICROF VARIABLE=FRAC-RET &
    SENTENCE=RETAINED ID1=TPA
```

```
  DEFINE RG BLOCK-VAR BLOCK=MICROF VARIABLE=RESIS-GEL &
    SENTENCE=RESULTS2
  DEFINE PC BLOCK-VAR BLOCK=MICROF VARIABLE=PRM-CONC &
    SENTENCE=PERMEATE ID1=TPA
  DEFINE KC BLOCK-VAR BLOCK=MICROF VARIABLE=KEYCONC &
    SENTENCE=RESULTS
  TABULATE 1 "PF"
  TABULATE 2 "SR"
  TABULATE 3 "FP"
  TABULATE 4 "VR"
  TABULATE 5 "CF"
  TABULATE 6 "FR"
  TABULATE 7 "RG"
  TABULATE 8 "PC"
  VARY BLOCK-VAR BLOCK=MICROF VARIABLE=FRACTION &
    SENTENCE=DENATURE ID1=TPA
  RANGE LOWER="0.05" UPPER="0.2" NPOINT="2"
  VARY BLOCK-VAR BLOCK=MICROF VARIABLE=RGELB &
    SENTENCE=RESIS-SERIES
  RANGE LOWER="-0.5" UPPER="-.85" NPOINT="2"
  VARY BLOCK-VAR BLOCK=MICROF VARIABLE=RGELC &
    SENTENCE=RESIS-SERIES
  VARY BLOCK-VAR BLOCK=MICROF VARIABLE=RGELC &
    SENTENCE=RESIS-SERIES
  RANGE LOWER="0.1" UPPER="0.3" NPOINT="2"
  VARY BLOCK-VAR BLOCK=MICROF VARIABLE=RGEL0 &
    SENTENCE=RESIS-SERIES
  RANGE LOWER="1E+12" UPPER="9E+12" NPOINT="5"
```

E.3.BATCH MEMBRANE SEPARATION UNIT

```
SENSITIVITY S-1
  DEFINE TV BLOCK-VAR BLOCK=UF VARIABLE=TVOLVALUE &
    SENTENCE=TVOL-RES ID1=5
  DEFINE F BLOCK-VAR BLOCK=UF VARIABLE=PLUXVALUE &
    SENTENCE=PFLUX ID1=5 ID2=1
  DEFINE TC BLOCK-VAR BLOCK=UF VARIABLE=VALUE &
    SENTENCE=TCONC-RES ID1=5 ID2=TPA
  DEFINE PP BLOCK-VAR BLOCK=UF VARIABLE=P2-POWER &
    SENTENCE=RESULTS2 ID1=1
 DEFINE PP BLOCK-VAR BLOCK=UF VARIABLE=P2-POWER &
    SENTENCE=RESULTS2 ID1=1
;;  DEFINE VEL BLOCK-VAR BLOCK=UF VARIABLE=VELVALUE &
;;    SENTENCE=VEL-RES ID1=10 ID2=1
;;  DEFINE FF BLOCK-VAR BLOCK=UF VARIABLE=FRICFAC &
```

```
;;      SENTENCE=PDROP ID1=1
;;   DEFINE PRET BLOCK-VAR BLOCK=UF VARIABLE=P-RET &
;;      SENTENCE=PDROP ID1=1
   TABULATE 1 "TV"
   TABULATE 2 "F"
   TABULATE 3 "TC"
   TABULATE 4 "PP"
;  TABULATE 5 "VEL"
;  TABULATE 6 "FF"
;  TABULATE 7 "PRET"
   VARY BLOCK-VAR BLOCK=UF VARIABLE=XRECYCLE &
     SENTENCE=MODULE-REC ID1=1 ID2=1
   RANGE LOWER="10" UPPER="20" NPOINT="5"
   VARY BLOCK-VAR BLOCK=UF VARIABLE=PUMP-CAPACTY &
     SENTENCE=SEQ-PARAM ID1=1
   RANGE LOWER="75" UPPER="150" INCR="50"
   VARY BLOCK-VAR BLOCK=UF VARIABLE=DCOEF SENTENCE=FOULING &
     ID1=1
   RANGE LOWER="3.33E-05" UPPER="1.766E-04" NPOINT="5"
;;   VARY BLOCK-VAR BLOCK=UF VARIABLE=VOLUME SENTENCE=SEQ-PARAM &
;;      ID1=1
;;   RANGE LOWER="500" UPPER="2000" INCR="500"
   VARY BLOCK-VAR BLOCK=UF VARIABLE=RGELC SENTENCE=FLUX &
     ID1=1
   VARY BLOCK-VAR BLOCK=UF VARIABLE=RGELC SENTENCE=FLUX &
     ID1=1
   RANGE LOWER="0.15" UPPER="0.3" NPOINT="4"

STREAM-REPOR NOMOLEFLOW MASSFLOW PROPERTIES=MASSCONC
```

E.4. AFFINITY CHROMATOGRAPHY COLUMN

```
 SENSITIVITY S-1
   DEFINE REC BLOCK-VAR BLOCK=AFF VARIABLE=RECOV &
     SENTENCE=PERFORMANCE ID1=PROD ID2=TPA
   DEFINE PP BLOCK-VAR BLOCK=AFF VARIABLE=PERF-PURITY &
     SENTENCE=PERFORMANCE ID1=PROD ID2=TPA
   TABULATE 1 "REC"
   TABULATE 2 "PP"
   VARY BLOCK-VAR BLOCK=AFF VARIABLE=CAPACITY SENTENCE=SOLUTES &
     ID1=TPA
   RANGE LOWER=".1" UPPER="0.7" NPOINT="7"
   VARY BLOCK-VAR BLOCK=AFF VARIABLE=KLOAD SENTENCE=SOLUTES &
   RANGE LOWER=".1" UPPER="0.7" NPOINT="7"
```

```
  VARY BLOCK-VAR BLOCK=AFF VARIABLE=KLOAD SENTENCE=SOLUTES &
    ID1=TPA
  RANGE LOWER="75" UPPER="175" NPOINT="10"
  VARY BLOCK-VAR BLOCK=AFF VARIABLE=KLOAD SENTENCE=SOLUTES &
    ID1=FBS
  RANGE LOWER="1.5" UPPER="50" NPOINT="10"

STREAM-REPOR NOMOLEFLOW MASSFLOW PROPERTIES=MASSCONC
```

E.5. ION-EXCHANGE CHROMATOGRAPHY COLUMN

```
SENSITIVITY S-1
  DEFINE REC BLOCK-VAR BLOCK=IEX VARIABLE=RECOV &
    SENTENCE=PERFORMANCE ID1=PROD ID2=TPA
  DEFINE PP BLOCK-VAR BLOCK=IEX VARIABLE=PERF-PURITY &
    SENTENCE=PERFORMANCE ID1=PROD ID2=TPA
  DEFINE CS BLOCK-VAR BLOCK=IEX VARIABLE=RES-CHASLOPE &
    SENTENCE=SOLUTE-RES ID1=TPA
  DEFINE X BLOCK-VAR BLOCK=IEX VARIABLE=CONCEN &
    SENTENCE=CONC-PROFILE ID1=TPA ID2=75
  TABULATE 1 "REC"
  TABULATE 2 "PP"
  TABULATE 3 "CS"
  TABULATE 4 "X"
  VARY BLOCK-VAR BLOCK=IEX VARIABLE=KLOAD SENTENCE=SOLUTES &
    ID1=TPA
  RANGE LOWER="5" UPPER="100" NPOINT="2"
  VARY BLOCK-VAR BLOCK=IEX VARIABLE=ELU-MOLARITY &
    SENTENCE=PROFILE ID1=1
  RANGE LOWER="0.05" UPPER="1.0" NPOINT="2"
  VARY BLOCK-VAR BLOCK=IEX VARIABLE=ELUTE-VOL SENTENCE=PROFILE &
    ID1=1
  RANGE LOWER="200" UPPER="700" NPOINT="2"

STREAM-REPOR NOMOLEFLOW MASSFLOW PROPERTIES=MASSCONC
```

E.6. GEL FILTRATION COLUMN

```
SENSITIVITY S-1
  DEFINE R BLOCK-VAR BLOCK=GEL VARIABLE=RECOVERY &
    SENTENCE=PERFORMANCE ID1=PROD ID2=TPA
  DEFINE P BLOCK-VAR BLOCK=GEL VARIABLE=PURITY &
    SENTENCE=PERFORMANCE ID1=PROD ID2=TPA
  DEFINE RES BLOCK-VAR BLOCK=GEL VARIABLE=RESOLUTION &
```

```
    SENTENCE=RESOLUTION ID1=TPA ID2=FBS
  DEFINE C BLOCK-VAR BLOCK=GEL VARIABLE=CONC-FACTOR &
    SENTENCE=RESOLUTION ID1=TPA ID2=FBS
  DEFINE C BLOCK-VAR BLOCK=GEL VARIABLE=CONC-FACTOR &
    SENTENCE=PERFORMANCE ID1=PROD ID2=TPA
  DEFINE DELP BLOCK-VAR BLOCK=GEL VARIABLE=DELP &
    SENTENCE=RESULTS
  DEFINE N BLOCK-VAR BLOCK=GEL VARIABLE=NCOLUMN &
    SENTENCE=RESULTS
  DEFINE TIME BLOCK-VAR BLOCK=GEL VARIABLE=CYCLE-TIME &
    SENTENCE=RESULTS
  DEFINE USE BLOCK-VAR BLOCK=GEL VARIABLE=COLUMN-USE &
    SENTENCE=RESULTS
  TABULATE 1 "R"
  TABULATE 2 "P"
  TABULATE 3 "RES"
  TABULATE 4 "C"
  TABULATE 5 "DELP"
  TABULATE 6 "N"
  TABULATE 7 "TIME"
  TABULATE 8 "USE"
  VARY BLOCK-VAR BLOCK=GEL VARIABLE=LENGTH SENTENCE=PARAM
  RANGE LOWER="0.2" UPPER="1.0" INCR="0.2"
  VARY BLOCK-VAR BLOCK=GEL VARIABLE=LOAD-VOL SENTENCE=PARAM
  RANGE LOWER="3" UPPER="15" INCR="3"
  VARY BLOCK-VAR BLOCK=GEL VARIABLE=FLOW-RATE SENTENCE=PARAM
  RANGE LOWER="6" UPPER="60" INCR="10"

STREAM-REPOR NOMOLEFLOW MASSFLOW PROPERTIES=MASSCONC
;
;
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Appendix F

Results of Sensitivity Study

F.1. Bioreactor

VARY 1 FERMENT PARAM INOC-CON	XRATE	SRATE	XCONC	SCONC	NRATE
GM/L	KG/HR	KG/HR	GM/L	GM/L	KG/HR
3.0000-02	8.3251-03	-6.8846-02	3.6249-02	0.7688	-2.9029-02
e 8.5000-02	8.3251-03	-6.8846-02	3.6249-02	0.7688	-2.9029-02
0.1400	3.8625-02	-6.8867-02	0.1681	0.7683	-2.8982-02
0.1950	5.3743-02	-6.8882-02	0.2338	0.7681	-2.8972-02
0.2500	6.8825-02	-6.8898-02	0.2994	0.7679	-2.8962-02
5.2600-02	1.4587-02	-6.8851-02	6.3512-02	0.7687	-2.9023-02

e ERRORS OCCURRED FOR VALUES IN THIS ROW.

VARY 1 FERMENT PARAM INOC-VOL	XRATE	SRATE	XCONC	SCONC	NRATE
L	KG/HR	KG/HR	GM/L	GM/L	KG/HR
500.0000	7.2532-03	-6.8795-02	3.4464-02	0.8383	-2.8972-02
1000.0000	1.4587-02	-6.8851-02	6.3512-02	0.7687	-2.9023-02
e 1500.0000	1.4587-02	-6.8851-02	6.3512-02	0.7687	-2.9023-02
2000.0000	2.9222-02	-6.8940-02	0.1089	0.6591	-2.9075-02
1000.0000	1.4587-02	-6.8851-02	6.3512-02	0.7687	-2.9023-02

e ERRORS OCCURRED FOR VALUES IN THIS ROW.

VARY 1 FERMENT PARAM VOL-CHAR	XRATE	SRATE	XCONC	SCONC	NRATE
L	KG/HR	KG/HR	GM/L	GM/L	KG/HR

2000.0000	1.5210-02	-2.7704-02	0.1327	0.6196	-1.1771-02
e 3000.0000	1.5210-02	-2.7704-02	0.1327	0.6196	-1.1771-02
4000.0000	1.4601-02	-5.5105-02	7.6319-02	0.7385	-2.3236-02
5000.0000	1.4587-02	-6.8851-02	6.3512-02	0.7687	-2.9023-02

e ERRORS OCCURRED FOR VALUES IN THIS ROW.

VARY 1 FERMENT PARAM MIN-O2	MINO2	MINKLA	FO2	ISATO2	DO2PER
	MOL/L	1/HR	MOL/L	MOL/L	
0.1500	6.3956-03	MISSING	6.3956-03	6.3999-03	100.0255
0.2375	6.3956-03	MISSING	6.3956-03	6.3999-03	100.0255
0.3250	6.3956-03	MISSING	6.3956-03	6.3999-03	100.0255
0.4125	6.3956-03	MISSING	6.3956-03	6.3999-03	100.0255
0.5000	6.3956-03	MISSING	6.3956-03	6.3999-03	100.0255
0.3500	6.3956-03	MISSING	6.3956-03	6.3999-03	100.0255

VARY 1 FERMENT PARAM KLA	MINO2	MINKLA	FO2	ISATO2	DO2PER
1/HR	MOL/L	1/HR	MOL/L	MOL/L	
2.0000	6.3956-03	MISSING	6.3956-03	6.3999-03	100.0255
3.5000	6.3956-03	MISSING	6.3956-03	6.3999-03	100.0255
5.0000	6.3956-03	MISSING	6.3956-03	6.3999-03	100.0255
6.5000	6.3956-03	MISSING	6.3956-03	6.3999-03	100.0255
8.0000	6.3956-03	MISSING	6.3956-03	6.3999-03	100.0255
5.0000	6.3956-03	MISSING	6.3956-03	6.3999-03	100.0255

VARY 1 FERMENT REAL-KIN VALUE-LI	XRATE	SRATE	XCONC	SCONC	NRATE
(G_s)	KG/HR	KG/HR	GM/L	GM/L	KG/HR

0.5000	1.3921-02	-3.6352-02	6.0636-02	0.1215	-2.8721-02
1.0000	1.4252-02	-6.9473-02	6.2062-02	0.4645	-2.8742-02
1.5000	1.4508-02	-7.0499-02	6.3167-02	0.7070	-2.8962-02
2.0000	1.4646-02	-6.4894-02	6.3762-02	0.8676	-2.9086-02
2.5000	1.4527-02	-5.8666-02	6.3240-02	0.9804	-2.9235-02
3.0000	1.4660-02	-5.3017-02	6.3815-02	1.0631	-2.9175-02
3.5000	1.4706-02	-4.8155-02	6.4014-02	1.1265	-2.9209-02
4.0000	1.4741-02	-4.4008-02	6.4163-02	1.1765	-2.9230-02
1.6700	1.4587-02	-6.8851-02	6.3510-02	0.7687	-2.9022-02

VARY 1 FERMENT REAL-KIN VALUE-LI	XRATE	SRATE	XCONC	SCONC	NRATE
(k_d)	KG/HR	KG/HR	GM/L	GM/L	KG/HR
1.0000-03	1.6607-02	-6.8853-02	6.9413-02	0.7687	-2.9022-02
1.6250-03	1.5789-02	-6.8852-02	6.7039-02	0.7687	-2.9022-02
2.2500-03	1.5008-02	-6.8851-02	6.4753-02	0.7687	-2.9022-02
2.8750-03	1.4264-02	-6.8850-02	6.2552-02	0.7687	-2.9022-02
3.5000-03	1.3555-02	-6.8850-02	6.0432-02	0.7687	-2.9022-02
2.6000-03	1.4587-02	-6.8851-02	6.3510-02	0.7687	-2.9022-02

VARY 1 FERMENT REAL-KIN VALUE-LI	XRATE	SRATE	XCONC	SCONC	NRATE
(μ_{max})	KG/HR	KG/HR	GM/L	GM/L	KG/HR
3.0000-02	5.7501-03	-6.8843-02	3.5088-02	0.7688	-2.9027-02
3.5000-02	8.8724-03	-6.8846-02	4.5785-02	0.7687	-2.9024-02
4.0000-02	1.3444-02	-6.8850-02	6.0100-02	0.7687	-2.9022-02
4.5000-02	2.0143-02	-6.8856-02	7.9423-02	0.7687	-2.9021-02
5.0000-02	2.9790-02	-6.8861-02	0.1050	0.7686	-2.9002-02
4.1000-02	1.4587-02	-6.8851-02	6.3510-02	0.7687	-2.9022-02

F.2. Continuous Membrane Separation

VARY 1 MICROF CONV-REC SETPOINT	PF	SR	FP	VR	CF
M/SEC	L/SQM-SEC	1/HR	L/MIN		

0.8000	1.6176-02	3.1446+07	77.6458	5.1501	0.9998
1.2000	1.7266-02	3.1446+07	82.8755	7.1482	1.0000
1.6000	1.7933-02	3.1446+07	86.0768	9.3747	0.9999
2.0000	1.8388-02	3.1446+07	88.2609	11.9042	1.0006
2.4000	1.8722-02	3.1446+07	89.8666	14.8502	1.0051
1.6000	1.7933-02	3.1446+07	86.0768	9.3747	0.9999

VARY 1 MICROF CONV-REC SETPOINT M/SEC	FR	RG 1/METER	PC GM/L
0.8000	0.1941	8.1872+10	3.3212-02
1.2000	0.1399	6.4085+10	3.3210-02
1.6000	0.1066	5.4264+10	3.3211-02
2.0000	8.4061-02	4.7972+10	3.3208-02
2.4000	6.7686-02	4.3541+10	3.3198-02
1.6000	0.1066	5.4264+10	3.3211-02

VARY 1 MICROF KEY-COMP REJECT	PF L/SQM-SEC	SR 1/HR	FP L/MIN	VR	CF
0.8000	1.8571-02	3.1446+07	89.1384	13.3519	1.0059
0.8500	1.8452-02	3.1446+07	88.5691	12.3755	0.9999
0.9000	1.8311-02	3.1446+07	87.8923	11.3859	1.0000
0.9500	1.8140-02	3.1446+07	87.0703	10.3778	0.9986
1.0000	1.7933-02	3.1446+07	86.0768	9.3747	0.9999
1.0000	1.7933-02	3.1446+07	86.0768	9.3747	0.9999

VARY 1 MICROF KEY-COMP REJECT	FR	RG 1/METER	PC GM/L
0.8000	7.5341-02	4.5530+10	3.3195-02
0.8500	8.0798-02	4.7109+10	3.3211-02

0.9000	8.7828-02	4.9011+10	3.3211-02
0.9500	9.6227-02	5.1362+10	3.3215-02
1.0000	0.1066	5.4264+10	3.3211-02
1.0000	0.1066	5.4264+10	3.3211-02

VARY 1 MICROF RESIS-SE RESIS-ME 1/METER	PF L/SQM-SE C	SR 1/HR	FP L/MIN	VR	CF
8.0000+10	2.0069-02	3.1446+07	96.2851	1378.4674	1.0013
5.6000+11	7.6219-03	3.1446+07	36.5849	1.6120	1.0000
1.0400+12	4.2358-03	3.1446+07	20.3317	1.2674	1.0000
1.5200+12	2.9306-03	3.1446+07	14.0666	1.1709	1.0000
2.0000+12	2.2399-03	3.1446+07	10.7516	1.1256	1.0000
2.0000+11	1.7933-02	3.1446+07	86.0768	9.3747	0.9999

VARY 1 MICROF RESIS-SE RESIS-ME 1/METER	FR	RG 1/METER	PC GM/L
8.0000+10	7.2641-04	1.4719+11	3.3210-02
5.6000+11	0.6203	3.8230+10	3.3211-02
1.0400+12	0.7889	3.6457+10	3.3211-02
1.5200+12	0.8540	3.5892+10	3.3211-02
2.0000+12	0.8884	3.5615+10	3.3211-02
2.0000+11	0.1066	5.4264+10	3.3211-02

VARY 1 MICROF RESIS-SE RGEL0	PF L/SQM-SE C	SR 1/HR	FP L/MIN	VR	CF

8.0000+11	2.0050-02	3.1446+07	96.2402	839.5688	1.0277
2.0600+13	1.1710-02	3.1446+07	56.2085	2.4000	1.0000
4.0400+13	8.3321-03	3.1446+07	39.9938	1.7096	1.0000
6.0200+13	6.4886-03	3.1446+07	31.1453	1.4776	1.0000
8.0000+13	5.3189-03	3.1446+07	25.5308	1.3604	1.0000
4.5000+12	1.7933-02	3.1446+07	86.0768	9.3747	0.9999

VARY 1 MICROF RESIS-SE RGEL0	FR	RG 1/METER	PC GM/L
8.0000+11	1.2241-03	2.7412+10	3.3209-02
2.0600+13	0.4166	1.8938+11	3.3210-02
4.0400+13	0.5849	3.4724+11	3.3211-02
6.0200+13	0.6767	5.0271+11	3.3211-02
8.0000+13	0.7350	6.5725+11	3.3211-02
4.5000+12	0.1066	5.4264+10	3.3211-02

VARY 1 MICROF RESIS-SE RGELA	PF L/SQM-SE C	SR 1/HR	FP L/MIN	VR	CF
5.0000-02	1.9408-02	3.1446+07	93.1598	30.1564	1.0002
0.1000	1.8587-02	3.1446+07	89.2155	13.4960	1.0005
0.1500	1.7431-02	3.1446+07	83.6690	7.5953	1.0000
0.2000	1.5950-02	3.1446+07	76.5591	4.8674	1.0000
0.1300	1.7933-02	3.1446+07	86.0768	9.3747	0.9999

VARY 1 MICROF RESIS-SE RGELA	FR	RG 1/METER	PC GM/L
5.0000-02	3.3167-02	3.4932+10	3.3210-02
0.1000	7.4136-02	4.5318+10	3.3209-02
0.1500	0.1316	6.1581+10	3.3210-02
0.2000	0.2054	8.5873+10	3.3211-02
0.1300	0.1066	5.4264+10	3.3211-02

VARY 1 MICROF RESIS-SE RGELC	PF	SR	FP	VR	CF
	L/SQM-SEC	1/HR	L/MIN		
0.1000	1.7612-02	3.1446+07	84.5382	8.1540	1.0027
0.1500	1.7785-02	3.1446+07	85.3702	8.7716	1.0001
0.2000	1.7933-02	3.1446+07	86.0768	9.3747	0.9999
0.2500	1.8057-02	3.1446+07	86.6742	9.9532	1.0001
0.3000	1.8165-02	3.1446+07	87.1897	10.5130	1.0000
0.2000	1.7933-02	3.1446+07	86.0768	9.3747	0.9999

VARY 1 MICROF RESIS-SE RGELC	FR	RG	PC
		1/METER	GM/L
0.1000	0.1229	5.8891+10	3.3198-02
0.1500	0.1140	5.6368+10	3.3210-02
0.2000	0.1066	5.4264+10	3.3211-02
0.2500	0.1004	5.2511+10	3.3210-02
0.3000	9.5120-02	5.1018+10	3.3211-02
0.2000	0.1066	5.4264+10	3.3211-02

VARY 1 MICROF RESIS-SE RGELB	PF	SR	FP	VR	CF
	L/SQM-SEC	1/HR	L/MIN		
-0.5000	9.9674-03	3.1446+07	47.8433	1.9862	1.0000
-0.6000	1.3407-02	3.1446+07	64.3528	3.0108	1.0000
-0.7000	1.6352-02	3.1446+07	78.4900	5.3935	1.0001
-0.8000	1.8477-02	3.1446+07	88.6889	12.5689	1.0008
-0.8500	1.9212-02	3.1446+07	92.2172	23.2864	1.0153
-0.7700	1.7933-02	3.1446+07	86.0768	9.3747	0.9999

VARY 1 MICROF RESIS-SE RGELB	FR	RG	PC
		1/METER	GM/L
-0.5000	0.5034	2.5746+11	3.3211-02
-0.6000	0.3321	1.4010+11	3.3211-02
-0.7000	0.1854	7.8840+10	3.3210-02
-0.8000	7.9629-02	4.6775+10	3.3208-02
-0.8500	4.3601-02	3.7333+10	3.3188-02
-0.7700	0.1066	5.4264+10	3.3211-02

VARY 1 MICROF PARAM VISC CP	PF L/SQM-SE C	SR 1/HR	FP L/MIN	VR	CF
0.8000	1.9989-02	3.9307+07	95.8993	211.4549	1.0032
1.0000	1.7933-02	3.1446+07	86.0768	9.3747	0.9999
1.2000	1.5039-02	2.6205+07	72.1866	3.9868	1.0000
1.4000	1.2769-02	2.2461+07	61.2912	2.7479	0.9999
1.6000	1.1025-02	1.9654+07	52.9196	2.2183	1.0000
1.8000	9.6583-03	1.7470+07	46.3597	1.9272	1.0000
2.0000	8.5638-03	1.5723+07	41.1062	1.7440	1.0000
1.0000	1.7933-02	3.1446+07	86.0768	9.3747	0.9999

VARY 1 MICROF PARAM VISC CP	FR	RG 1/METER	PC GM/L
0.8000	4.7444-03	8.5136+10	3.3210-02
1.0000	0.1066	5.4264+10	3.3211-02
1.2000	0.2508	5.2658+10	3.3210-02
1.4000	0.3638	5.5061+10	3.3212-02
1.6000	0.4507	5.8484+10	3.3211-02
1.8000	0.5188	6.2275+10	3.3211-02
2.0000	0.5733	6.6215+10	3.3211-02
1.0000	0.1066	5.4264+10	3.3211-02

VARY 1 MICROF TPA DENATURE FRACTION	PF	SR	FP	VR	CF
	L/SQM-SEC	1/HR	L/HR		
1.0000-02	1.7502-02	3.1446+07	4410.5165	11.1335	9.2796
3.2500-02	1.7502-02	3.1446+07	4410.5165	11.1335	9.2796
5.5000-02	1.7502-02	3.1446+07	4410.5165	11.1335	9.2796
7.7500-02	1.7502-02	3.1446+07	4410.5165	11.1335	9.2796
0.1000	1.7502-02	3.1446+07	4410.5165	11.1335	9.2796
5.0000-02	1.7502-02	3.1446+07	4410.5165	11.1335	9.2796

VARY 1 MICROF TPA DENATURE FRACTION	FR	RG	PC	KC	VISC
		1/METER	GM/L	GM/L	CP
1.0000-02	0.8334	6.0519+10	7.2487-03	0.3624	1.0000
3.2500-02	0.8334	6.0519+10	7.2487-03	0.3624	1.0000
5.5000-02	0.8334	6.0519+10	7.2487-03	0.3624	1.0000
7.7500-02	0.8334	6.0519+10	7.2487-03	0.3624	1.0000
0.1000	0.8334	6.0519+10	7.2487-03	0.3624	1.0000
5.0000-02	0.8334	6.0519+10	7.2487-03	0.3624	1.0000

VARY 1 MICROF TPA DENATURE FRACTION	DENS	RGELO	RETC
	GM/CC		GM/L
1.0000-02	1.0215	4.5000+12	0.3676
3.2500-02	1.0215	4.5000+12	0.3676
5.5000-02	1.0215	4.5000+12	0.3676
7.7500-02	1.0215	4.5000+12	0.3676
0.1000	1.0215	4.5000+12	0.3676
5.0000-02	1.0215	4.5000+12	0.3676

F.3. Batch Membrane Separation

VARY 1 UF 1 MODULE-R XRECYCLE	TV	F	TC	PP
	L	L/SQM-SEC	GM/L	KW
10.0000	40.0887	6.0554-03	2.2837	23.3818
12.5000	38.0796	6.5420-03	2.3938	28.1661
15.0000	36.5972	6.9537-03	2.4849	30.8773
17.5000	35.5808	7.2963-03	2.5633	39.4109
20.0000	34.6222	7.5789-03	2.6161	44.6769
15.0000	36.7172	6.9561-03	2.4772	33.6792

FF
0.1326
8.7917-02
6.3414-02
4.7053-02
3.6325-02
6.2754-02

VARY 1 UF 1 SEQ-PARA PUMP-CAP L/HR	TV	F	TC	PP
	L	L/SQM-SEC	GM/L	KW
75.0000	40.6508	5.8952-03	2.2397	24.9729
100.0000	36.7075	6.9546-03	2.4788	33.8038
125.0000	34.2617	7.7760-03	2.6974	42.3297
150.0000	31.7742	8.4605-03	2.8571	50.0608
100.0000	36.2087	6.9427-03	2.5252	33.5646
150.0000	31.7742	8.4605-03	2.8571	50.0608
100.0000	36.2087	6.9427-03	2.5252	33.5646

VARY 1 UF 1 FOULING DCOEF	TV L	F L/SQM-SEC	TC GM/L	PP KW
5.5000-04	36.7161	4.1670-03	2.4861	34.0038
1.7875-03	36.5002	9.7596-04	2.5150	40.9393
3.0250-03	37.0309	2.6965-04	2.4882	34.2830
4.2625-03	36.7788	8.5952-05	2.5093	55.8922
5.5000-03	42.4230	1.0053-05	2.1839	50.0541
1.4444-03	36.7780	1.5202-03	2.4923	34.6527

VARY 1 UF 1 FLUX RGELA	TV L	F L/SQM-SEC	TC GM/L	PP KW
8.7500-02	33.5408	1.7736-03	2.7126	31.0465
0.1156	35.4554	1.5723-03	2.5750	33.5654
0.1437	38.2161	1.4105-03	2.4061	34.1675
0.1718	40.1026	1.2246-03	2.2988	32.2883
0.2000	43.1390	1.0934-03	2.1466	33.8579
0.1300	37.0813	1.5235-03	2.4769	34.5555

F.4. Affinity Chromatography

VARY 1 AFF TPA SOLUTES KLOAD	REC	PP
75.0000	0.6082	0.9995
86.1111	0.6499	0.9995
97.2222	0.6869	0.9995
108.3333	0.7190	0.9995
119.4444	0.7514	0.9996
130.5555	0.7845	0.9996
141.6666	0.7908	0.9996

152.7777	0.8202	0.9996
163.8888	0.8396	0.9997
175.0000	0.8566	0.9997
120.0000	0.7525	0.9996

VARY 1 AFF TPA SOLUTES CAPACITY GM/L	REC	PP
0.1000	0.1235	0.9976
0.2000	0.2802	0.9990
0.3000	0.4382	0.9994
0.4000	0.6027	0.9995
0.5000	0.7581	0.9996
0.6000	0.8916	0.9997
0.7000	0.0	MISSING
0.5000	0.7525	0.9996

F.5. Ion exchange Chromatography

VARY 1 IEX PARAM SPEC-ARE SQCM/CC	REC	PP	CS	X GM/L
5.0000-02	0.8335	0.9997	-6.6176-05	9.6495-02
0.5416	0.8334	0.9997	-6.6176-05	9.6792-02
1.0333	0.8332	0.9997	-6.6176-05	9.5458-02
1.5250	0.8332	0.9997	-6.6176-05	9.5458-02
2.0166	0.8332	0.9997	-6.6176-05	9.5458-02
2.5083	0.8332	0.9997	-6.6176-05	9.5458-02
3.0000	0.8332	0.9997	-6.6176-05	9.5458-02
1.0000	MISSING	MISSING	MISSING	MISSING

VARY 1 IEX PARAM TOT-CAP MOL/L	REC	PP	CS	X GM/L

1.0000-02	0.8335	0.9997	-6.6176-05	9.6495-02
0.5644	0.8334	0.9997	-6.6176-05	9.6792-02
1.1188	0.8332	0.9997	-6.6176-05	9.5458-02
1.6733	0.8332	0.9997	-6.6176-05	9.5458-02
2.2277	0.8332	0.9997	-6.6176-05	9.5458-02
2.7822	0.8332	0.9997	-6.6176-05	9.5458-02
3.3366	0.8332	0.9997	-6.6176-05	9.5458-02
3.8911	0.8332	0.9997	-6.6176-05	9.5458-02
4.4455	0.8332	0.9997	-6.6176-05	9.5458-02
5.0000	0.8332	0.9997	-6.6176-05	9.5458-02
1.3000	0.8332	0.9997	-6.6176-05	9.5458-02

VARY 1 IEX TPA SOLUTES CHARGE	REC	PP	CS	X GM/L
7.0000	0.8493	1.0000	-1.4706-05	0.0
14.3000	0.9156	1.0000	-3.0637-05	2.6838-06
21.0000	0.9476	0.9999	-4.6569-05	9.7342-05

VARY 1 IEX 1 PROFILE ELU-MOLA MOL/L	REC	PP	CS	X GM/L
5.0000-02	0.8335	0.9997	-6.6176-05	9.6495-02
0.2875	0.8334	0.9997	-6.6176-05	9.6792-02
0.5250	0.8332	0.9997	-6.6176-05	9.5458-02
0.7625	0.8332	0.9997	-6.6176-05	9.5458-02
1.0000	0.8332	0.9997	-6.6176-05	9.5458-02
0.5000	0.8332	0.9997	-6.6176-05	9.5458-02

VARY 1 IEX 1 PROFILE ELUTE-VO	REC	PP	CS	X

L				GM/L
200.0000	0.8338	0.9997	-6.6176-05	9.6428-02
325.0000	0.8338	0.9997	-6.6176-05	9.6863-02
450.0000	0.8335	0.9997	-6.6176-05	9.5404-02
575.0000	0.8335	0.9997	-6.6176-05	9.5404-02
700.0000	0.8335	0.9997	-6.6176-05	9.5404-02
0.0	0.8332	0.9997	-6.6176-05	9.5458-02

VARY 1 IEX TPA SOLUTES KLOAD	REC	PP	CS	X GM/L
5.0000	0.8335	0.9997	-6.6176-05	9.6495-02
15.5555	0.8334	0.9997	-6.6176-05	9.6792-02
26.1111	0.8332	0.9997	-6.6176-05	9.5458-02
36.6666	0.8332	0.9997	-6.6176-05	9.5458-02
47.2222	0.8332	0.9997	-6.6176-05	9.5458-02
57.7777	0.8332	0.9997	-6.6176-05	9.5458-02
68.3333	0.8332	0.9997	-6.6176-05	9.5458-02
78.8888	0.8332	0.9997	-6.6176-05	9.5458-02
89.4444	0.8332	0.9997	-6.6176-05	9.5458-02
100.0000	0.8332	0.9997	-6.6176-05	9.5458-02
45.0000	0.8332	0.9997	-6.6176-05	9.5458-02

VARY 1 IEX TPA SOLUTES CAPACITY GM/L	REC	PP	CS	X GM/L
2.0000	0.6690	0.9999	-6.6176-05	0.9911
5.2500	0.8253	0.9998	-6.6176-05	0.7514
8.5000	0.7708	0.9997	-6.6176-05	9.7296-04
11.7500	0.6099	0.9995	-6.6176-05	0.0
15.0000	0.4346	0.9994	-6.6176-05	0.0
7.0000	0.8332	0.9997	-6.6176-05	9.5458-02

F.6. Gel Filtration chromatography

F.6.1. Single Variable

VARY 1 GEL PARAM LENGTH METER	R	P	RES	C	DELP ATM
0.2000	0.4758	0.3620	0.2777	0.3891	8.0729-02
0.4000	0.8754	0.4142	0.4748	0.4849	0.1617
0.6000	0.9896	0.3687	0.6409	0.4005	0.2427
0.8000	0.9995	0.3193	0.7880	0.3217	0.3237
1.0000	0.9999	0.2821	0.9218	0.2694	0.4047
0.7500	0.9989	0.3306	0.7527	0.3386	0.3034

VARY 1 GEL PARAM LENGTH METER	N	TIME HR	USE
0.2000	8.0000	1.8407	0.9176
0.4000	15.0000	3.6815	0.9788
0.6000	23.0000	5.5223	0.9575
0.8000	30.0000	7.3631	0.9788
1.0000	37.0000	9.2038	0.9920
0.7500	28.0000	6.9029	0.9832

VARY 1 GEL PARAM LOAD-VOL L	R	P	RES	C
3.0000	0.9999	0.1837	0.9954	0.1544
6.0000	0.9996	0.2973	0.8015	0.2901
9.0000	0.9920	0.3868	0.6709	0.4326
12.0000	0.9391	0.4433	0.5768	0.5461
15.0000	0.8175	0.4642	0.5059	0.5943
7.0000	0.9989	0.3306	0.7527	0.3386

VARY 1 GEL PARAM LOAD-VOL L	N	TIME HR	USE
w 3.0000	65.0000	6.9029	0.9882
6.0000	33.0000	6.9029	0.9732
9.0000	22.0000	6.9029	0.9732
12.0000	17.0000	6.9029	0.9446
15.0000	13.0000	6.9029	0.9882
7.0000	28.0000	6.9029	0.9832

VARY 1 GEL PARAM FLOW-RAT L/HR	R	P	RES	C	DELP ATM
6.0000	0.9999	0.3914	1.0367	0.4411	5.6899-02
16.0000	0.9999	0.3547	0.8828	0.3770	0.1517
26.0000	0.9994	0.3386	0.7926	0.3510	0.2465
36.0000	0.9984	0.3257	0.7298	0.3313	0.3414
46.0000	0.9967	0.3148	0.6823	0.3150	0.4362
w 56.0000	0.9947	0.3051	0.6443	0.3011	0.5310
w 60.0000	0.9939	0.3015	0.6311	0.2960	0.5690
32.0000	0.9989	0.3306	0.7527	0.3386	0.3034

VARY 1 GEL PARAM FLOW-RAT	N	TIME	USE
6.0000	147.0000	36.8155	0.9988
16.0000	56.0000	13.8058	0.9832
26.0000	34.0000	8.4958	0.9965
36.0000	25.0000	6.1359	0.9788
46.0000	20.0000	4.8020	0.9575
w 56.0000	16.0000	3.9445	0.9832
w 60.0000	15.0000	3.6815	0.9788
32.0000	28.0000	6.9029	0.9832

F.6.2. Combined Variable

VARY 1 GEL PARAM LENGTH METER	VARY 2 GEL PARAM LOAD-VOL L	VARY 3 GEL PARAM FLOW-RAT L/HR	R	P	RES
0.2000	3.0000	6.0000	0.9561	0.4275	0.5948
0.2000	3.0000	16.0000	0.9265	0.3839	0.4983
0.2000	3.0000	26.0000	0.9041	0.3350	0.4432
0.2000	3.0000	36.0000	0.8882	0.2993	0.4054
0.2000	3.0000	46.0000	0.8785	0.2727	0.3771
0.2000	3.0000	56.0000	0.8746	0.2522	0.3548
0.2000	3.0000	60.0000	0.8747	0.2452	0.3470
0.2000	6.0000	6.0000	0.5518	0.4629	0.3857
0.2000	6.0000	16.0000	0.5531	0.4267	0.3427
0.2000	6.0000	26.0000	0.5540	0.3824	0.3157
0.2000	6.0000	36.0000	0.5545	0.3498	0.2960
0.2000	6.0000	46.0000	0.5549	0.3254	0.2807
0.2000	6.0000	56.0000	0.5553	0.3060	0.2681
0.2000	6.0000	60.0000	0.5556	0.2994	0.2636
0.2000	9.0000	6.0000	0.3678	0.4629	0.2854
0.2000	9.0000	16.0000	0.3688	0.4267	0.2611
0.2000	9.0000	26.0000	0.3696	0.3826	0.2451
0.2000	9.0000	36.0000	0.3705	0.3504	0.2331
0.2000	9.0000	46.0000	0.3716	0.3264	0.2235
0.2000	9.0000	56.0000	0.3731	0.3077	0.2154
0.2000	9.0000	60.0000	0.3737	0.3013	0.2126
0.2000	12.0000	6.0000	0.2759	0.4629	0.2265
0.2000	12.0000	16.0000	0.2766	0.4267	0.2109
0.2000	12.0000	26.0000	0.2772	0.3826	0.2004
0.2000	12.0000	36.0000	0.2779	0.3503	0.1923
0.2000	12.0000	46.0000	0.2787	0.3257	0.1857
0.2000	12.0000	56.0000	0.2798	0.3045	0.1801
0.2000	12.0000	60.0000	0.2803	0.2959	0.1781
0.2000	15.0000	6.0000	0.2207	0.4588	0.1877
0.2000	15.0000	16.0000	0.2213	0.3914	0.1769
0.2000	15.0000	26.0000	0.2217	0.2744	0.1694
0.2000	15.0000	36.0000	0.2223	0.1756	0.1636
0.2000	15.0000	46.0000	0.2230	0.1159	0.1588
0.2000	15.0000	56.0000	0.2238	8.2809-02	0.1547
0.2000	15.0000	60.0000	0.2242	7.3970-02	0.1532
0.6000	3.0000	6.0000	0.9999	0.2858	1.3364
0.6000	3.0000	16.0000	0.9999	0.2377	1.0680

0.6000	3.0000	26.0000	0.9999	0.2101	0.9256
0.6000	3.0000	36.0000	0.9999	0.1914	0.8322
0.6000	3.0000	46.0000	0.9999	0.1774	0.7642
0.6000	3.0000	56.0000	0.9999	0.1664	0.7118
0.6000	3.0000	60.0000	0.9999	0.1626	0.6938
0.6000	6.0000	6.0000	0.9999	0.3867	0.9505
0.6000	6.0000	16.0000	0.9995	0.3616	0.8064
0.6000	6.0000	26.0000	0.9978	0.3438	0.7225
0.6000	6.0000	36.0000	0.9950	0.3295	0.6642
0.6000	6.0000	46.0000	0.9915	0.3174	0.6202
0.6000	6.0000	56.0000	0.9879	0.3066	0.5852
0.6000	6.0000	60.0000	0.9864	0.3012	0.5731
0.6000	9.0000	6.0000	0.9853	0.4824	0.7376
0.6000	9.0000	16.0000	0.9705	0.4520	0.6477
0.6000	9.0000	26.0000	0.9579	0.4301	0.5924
0.6000	9.0000	36.0000	0.9467	0.4126	0.5527
0.6000	9.0000	46.0000	0.9367	0.3980	0.5219
0.6000	9.0000	56.0000	0.9277	0.3853	0.4969
0.6000	9.0000	60.0000	0.9243	0.3807	0.4881
0.6000	12.0000	6.0000	0.8246	0.5098	0.6026
0.6000	12.0000	16.0000	0.8215	0.4821	0.5412
0.6000	12.0000	26.0000	0.8168	0.4618	0.5021
0.6000	12.0000	36.0000	0.8117	0.4454	0.4733
0.6000	12.0000	46.0000	0.8066	0.4315	0.4505
0.6000	12.0000	56.0000	0.8016	0.4194	0.4317
0.6000	12.0000	60.0000	0.7996	0.4149	0.4251
0.6000	15.0000	6.0000	0.6606	0.5101	0.5093
0.6000	15.0000	16.0000	0.6615	0.4838	0.4648
0.6000	15.0000	26.0000	0.6622	0.4651	0.4356
0.6000	15.0000	36.0000	0.6624	0.4503	0.4138
0.6000	15.0000	46.0000	0.6623	0.4379	0.3963
0.6000	15.0000	56.0000	0.6619	0.4271	0.3817
0.6000	15.0000	60.0000	0.6617	0.4232	0.3764
1.0000	3.0000	6.0000	0.9999	0.2483	1.8990
1.0000	3.0000	16.0000	0.9999	0.2024	1.4876
1.0000	3.0000	26.0000	0.9999	0.1769	1.2758
1.0000	3.0000	36.0000	0.9999	0.1599	1.1392
1.0000	3.0000	46.0000	0.9999	0.1475	1.0411
1.0000	3.0000	56.0000	0.9998	0.1378	0.9660
1.0000	3.0000	60.0000	0.9998	0.1344	0.9404
1.0000	6.0000	6.0000	0.9999	0.3501	1.4108
1.0000	6.0000	16.0000	0.9999	0.3018	1.1703
1.0000	6.0000	26.0000	0.9999	0.2725	1.0351
1.0000	6.0000	36.0000	0.9999	0.2519	0.9434
1.0000	6.0000	46.0000	0.9999	0.2375	0.8751
1.0000	6.0000	56.0000	0.9998	0.2292	0.8214

w	1.0000	6.0000	60.0000	0.9998	0.2260	0.8028
	1.0000	9.0000	6.0000	0.9999	0.4055	1.1222
	1.0000	9.0000	16.0000	0.9999	0.3609	0.9646
	1.0000	9.0000	26.0000	0.9999	0.3423	0.8708
	1.0000	9.0000	36.0000	0.9996	0.3307	0.8050
w	1.0000	9.0000	46.0000	0.9990	0.3209	0.7547
w	1.0000	9.0000	56.0000	0.9981	0.3123	0.7144
w	1.0000	9.0000	60.0000	0.9977	0.3091	0.7003
	1.0000	12.0000	6.0000	0.9999	0.4472	0.9317
	1.0000	12.0000	16.0000	0.9992	0.4251	0.8204
	1.0000	12.0000	26.0000	0.9974	0.4091	0.7515
	1.0000	12.0000	36.0000	0.9947	0.3960	0.7020
w	1.0000	12.0000	46.0000	0.9913	0.3849	0.6634
w	1.0000	12.0000	56.0000	0.9876	0.3752	0.6321
w	1.0000	12.0000	60.0000	0.9861	0.3715	0.6211
	1.0000	15.0000	6.0000	0.9925	0.5010	0.7964
	1.0000	15.0000	16.0000	0.9826	0.4762	0.7136
	1.0000	15.0000	26.0000	0.9737	0.4579	0.6610
	1.0000	15.0000	36.0000	0.9656	0.4431	0.6223
w	1.0000	15.0000	46.0000	0.9582	0.4306	0.5919
w	1.0000	15.0000	56.0000	0.9513	0.4196	0.5668
w	1.0000	15.0000	60.0000	0.9487	0.4156	0.5579
	0.7500	7.0000	32.0000	0.9989	0.3306	0.7527

w WARNINGS OCCURRED FOR VALUES IN THIS ROW.

F.7. Summary of Results

Table F.7.1: Result of sensitivity study for the bioreactor

Varied Variable	Change	Biomass Production Rate	Substrate Consump. Rate	Cell Conc.	Substrate Conc.	Glutamine Consump. Rate
Inoculum Conc.	43% ↓	43% ↓	-	43% ↓	-	-
Inoculum Volume	50% ↓	50.3% ↓	-	45.7% ↓	9% ↑	-
Volume Charge	20% ↓	0.1% ↑	20% ↓	20% ↑	4% ↓	20% ↓

Table F.7.2: Result of sensitivity study for microfilter

Varied Variable	Change	Permeate Flux	Shear Rate	Volume Ratio	Gel Resistance
Setpoint Velocity	50% ↓	10% ↓	-	45% ↓	50% ↑
Key Comp. Rejection	20% ↓	3.5% ↑	-	40% ↑	16% ↓
Membrane Resistance	3 X ↑	57.5% ↓	-	80% ↓	-
Rgel0	80% ↓	12% ↑	-	-	50% ↓
a	50% ↑	10% ↓	-	50% ↑	50% ↑
b	10% ↑	7% ↑	-	250% ↑	30% ↓
Viscosity	65%↑	48% ↓	37% ↓	-	-

Table F.7.3: Results of sensitivity study for ultrafilter

Varied Variable	Change	Tank Volume	Flux	Tank Concentration	Friction Factor	Pump Power
Decay Coefficient	70% ↑	-	6% ↓	-	-	-
Recycle Ratio	33% ↑	6% ↓	9% ↑	5.6%↑	43% ↓	45%↑
Pump Capacity	50% ↑	15% ↓	22% ↑	15% ↑	-	48%↑

Table F.7.4: Result of sensitivity study for affinity chromatography

	Change	Recovery	Purity
Capacity	60% ↓	63% ↓	0.2% ↓
Kload	45%↑	14% ↑	0.01%↑

Table F.7.5: Summary of sensitivity study for ion exchange chromatography

Varied Variable	Change	Recovery	Purity	Charge Slope
Charge	50% ↓	37% ↓	-	54% ↑
Solutes Capacity	50% ↓	17% ↓	0.023%	-

Table F.7.6: Summary of sensitivity study for gel filtration

Varied Variable	Variation	Purity	Resolution	Concentration Factor $[t\text{-}PA]_{prod}/[t\text{-}PA]_{feed}$	Pressure Drop
Length	47% ↓	?	37% ↓	?	47% ↓
Load Volume	57% ↓	45% ↓	32% ↑	55% ↓	-
Flow Rate	50% ↓	7% ↑	17% ↑	11% ↑	50% ↓

Table F.7.7: Summary of sensitivity study of economic parameters

	Labor	Waste Treatment	Utility	C_{PE}	Raw Mat.	Recovery	R&D
Change	50% ↑	50% ↑	50% ↑	50% ↑	50% ↑	50% ↑	4X
ROI	5% ↓	2% ↓	3% ↓	35% ↓	18% ↓	74% ↑	11.5% ↓

Appendix G

Kinetic Model

G.1.Growth and Product formation kinetics:

Glucose:

$$\frac{dS_1}{dt} = -\frac{1}{G_s\ S_1}\frac{S_2\,[\,G_s\,S_1\,X_v\,(\,A\,\mu_{max} + B\,)\,]}{[9G_sS_1 + 2\,S_2\,]}$$

$$\frac{dS_1}{dt} = -\frac{[X_v\,(A\,\mu_{max} + B\,)\,]}{D} \quad \text{when } S_2 \approx 0$$

Glutamine:

$$\frac{dS_2}{dt} = -\frac{[G_s\,S_1\,X_v\,(\,A\,\mu_{max} + B\,)\,]}{[\,9G_s\,S_1 + 2\,S_2]} - k_{GLN}\,S_1$$

$$\frac{dS_2}{dt} = 0 \text{ when } S_1 \text{ X } S_2 = 0$$

Viable cell:

$$\frac{dX_v}{dt} = (\,\mu_{max} - k_dPo_2\,)\,X_v$$

$$\frac{dX_v}{dt} = -\,k_d.X_v \quad \text{when } S_2 \approx 0$$

Product: $\frac{dP}{dt} = \alpha X_v + \beta \mu X_v$

$\frac{dP}{dt} = \alpha X_v \quad \text{if } VI < E$

Viability Index: $VI = \int_0^t X_v \, dt$

where, preferential substrate utilization constant $G_s = 1.67$

extent of non-growth association $\alpha = 0.006$

extent of growth association $\beta = 7.79$

specific growth rate (h -1) $\mu = 0.015$

maximum specific growth rate (h -1 $\mu_{max} = 0.041$

specific rate of cell lysis (h-1) $k_l = 0.007$

apparent specific death rate (h-1) $k_d = 0.0026$

specific biosynthetic energy rate $A = 0.0008$

specific maintenance energy rate $B = 0.00026$

glucose oxidation $D = 20$

product changeover point $E = 13.5$

chemical degradation of glutamine constant $k_{GLN} = 0.013$

percentage of oxygen in the air mixture $Po_2 = 10$

Appendix H

Charge on t-PA Molecule

Let K_{INT} = Intrinsic dissociation constant; characteristic dissociation constant of a specific side chain residue

n_i, n_j = number of residues per protein molecule

α_i, α_j = fraction of residue that lost a proton

Group	No. in t-PA molecule	pK_{INT}	Charge after proton loss	Type
α-COOH	1	3.75	-	j
side chain – COOH (Asp, Glu)	28, 26	4.6	-	j
phenoloc – OH (Tyr)	24	9.6	-	j
sulphydryl – 5H (Cysh)	0			
imidazole (His)	16	7.0	0	i
α - NH2	1	7.8	0	i
side chain – NH2 (Lys)	21	10.2	0	i
guanidyl (Arg)	35	> 12.00	0	i

The required equations are:

$$\mu = 0.5\ \Sigma c_i Z_i^2 \qquad \text{(A.H.1)}$$

$$W = 3.57\ [1/b - (0.33\mu^{0.5})/(1+(2.5+b)\ 0.33\mu^{0.5})] \qquad \text{(A.H.2)}$$

$$pH = pK_{INT\,(i)} + \log \alpha_i/(1-\alpha i) - 0.868\ Z\ W \qquad \text{(A.H.3)}$$

$$Z = \Sigma n_i\ (1-\alpha_i) - \Sigma n_j\ \alpha_j \qquad \text{(A.H.4)}$$

In this calculation, it was assumed that, b =15 A° and μ = 0.05. W was calculated from equation A.H.2 and was found to be 0.124. Then a Z_{old} was assumed and α_i and α_j were calculated from equation A.H.3. Next, a Z_{new} was calculated from equation A.H.4 using the α values obtained. This trial and error approach was continues until convergence was obtained. Table A. H.1 shows the result of the various trials.

No. of trial	Z_{old}	Z_{new}
1	1.0	30.6
2	2.0	28.3
3	3.0	26.3
4	4.0	24.54
5	5.0	2305
6	6.0	21.78
7	7.0	20.68
8	7.5	20.18
9	10.0	18.0
10	12.0	16.4
11	14.0	14.7
12	14.5	14.3

So, the charge on t-PA molecule was taken as 14.5 for simulation of the ion-exchange columns.